

PROCEEDINGS

INTERNATIONAL FOOD CONFERENCE 2016

“INNOVATION OF FOOD TECHNOLOGY
TO IMPROVE FOOD SECURITY AND HEALTH”

Surabaya, 20-21 October 2016

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Proceeding
International Food Conference 2016
INNOVATION OF FOOD TECHNOLOGY TO
IMPROVE FOOD SECURITY AND HEALTH

October 20 – 21, 2016
Universitas Katolik Widya Mandala Surabaya
Surabaya – Indonesia

Organized by:

Faculty of Agricultural Technology, Widya Mandala Catholic University Surabaya
Perhimpunan Penggiat Pangan dan Nutrasetical Indonesia (P3FNI)
Indonesian Association of Food Technologists (PATPI) Chapter Surabaya

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Asosiasi Profesi Keamanan Pangan Indonesia (APKEPI)
Pergizi Pangan Indonesia
Indonesian Society for Lactic Acid Bacteria (ISLAB)

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PREFACE

Honorable and Distinguished Guests, Ladies and Gentlemen,

First of all, I would like to thank God the Almighty Father, for pouring His grace and blessings upon our lives. Especially, on this very special occasion, Thursday, 20th October 2016, we all gather here on the 2nd International Food Conference 2016 with the following theme “Innovation of Food Technology to Improve Food Security and Health”. This great scientific event is held by the Faculty of Agricultural Technology, Widya Mandala Catholic University Surabaya (WMCUS), in collaboration with the Indonesian Association of Food Technologist (PATPI) Surabaya Chapter, P3FNI and is also supported by the Indonesian Society for Lactic Acid Bacteria (ISLAB), Pergizi Pangan Indonesia, and Asosiasi Profesi Keamanan Pangan Indonesia (APKEPI).

Therefore please allow me to express my sincerest gratitude and highest appreciation to all aforementioned parties which have actively expressed their strong care, commitment, and enthusiasm in handling various issues related to health promotion and well-being of the society through food consumption. This is, indeed, aligned with the theme of 56th Anniversary of WMCUS, namely “Together with all nation’s components, the University is strongly committed to establish a competitive Indonesian Golden Generation”.

I believe this scientific meeting will provide a great opportunity for researchers and industry practitioners to disseminate and discuss their latest research innovation and findings in the areas of food technology, health, and food securities. This will result in strategy formulation to overcome problems related to the above fields. I hope this meeting may also expand and strengthen the collaboration between academia and industry practitioners.

Through this important event, food technology may be proven to become one of important contributing factors in promoting the quality of human lives. Ultimately, our nation’s competitiveness will be enhanced and Indonesia will be more respected by other nations in the global era. May we continuously strive for excellence in our professional lives to serve the community at large so we may become the sign of God’s presence and love.

May God bless us all !

Surabaya, 20th October 2016

Rector

Drs. Kuncoro Foe, G.Dip.Sc., Ph.D.

INTRODUCTION TO THE SEMINAR

Honorable guests, ladies and gentlemen

First of all I would like to welcome you all in this beautiful city of Surabaya, Indonesia. We are delighted to have you here to meet and to share our knowledge, research, and discuss latest trend in the area of food technology and nutrition. The topics of our International Food Conference 2016 is “Innovation in food technology to improve food security and health” and this year is the second edition of the conference after successful first edition in 2011.

As we already aware that the field of Food Technology is growing rapidly and its development is making a great impact on the health and wellbeing of the society. Food technology covers wide range of area starting from the simplest food preservation such as sun drying, post harvest handling to reduce losses, to the advanced nanotechnology for functional food application. Therefore food technology has become one of the most important contributors in human life. Nowadays, food technology are not only intended to fulfill the foods needed for daily consumptions, but has also been an important factor playing role in combating health problems in the world. Research on health problems of the society has been polarized into two groups which are health problems because of malnutrition and health problems due to over nutrition and unbalanced dietary and lifestyle habit.

The aim of this conference is to provide forum for researcher and industries to disseminate their latest research innovation in food technology, health, and food security, create opportunities for researcher to discuss health and food security problems around the world as well as the strategy to manage such problems and also Strengthen the collaboration between universities and industries by designing an event for researcher and industries to gather and discuss opportunities for collaborations.

The participants including invited speakers are coming from different countries such as Australia, Malaysia, Vietnam, Italia, Nigeria, and Indonesia. There are total of 81 papers presented in both oral and poster presentation.

We would like to express our sincere gratitude to all of the invited speakers Ibu Tetty Sihombing from BPOM, Prof Son Radu, Dr. Peter Sopade, Prof. Endang Sutriswati, Prof. Hany Widjaja, Ir. Indah Kuswardani, Prof. Rindit Pambayun, Prof. Achmad Subagio, Prof. Anang Legowo, Dr. Tyas Utami, Dr. Agustin Wardani, Prof. Nuri Andarwulan, Mr. Lino Paravano, Prof Hardinsyah, and Prof. Marsono. We would like to express our gratitude to P3FNI and PATPI Surabaya for the assistance in preparation for this conference.

We would also like to thank our sponsors that made this event possible. Last but not least, I would like to thank all members of organizing committee for their full supports and commitments in preparing this conference. I wish that all of us will have a fruitful discussion and for all of you having a pleasant stay in Surabaya. Thank you.

Warm regards

Ignasius Radix AP Jati
Chair of Organizing Committee IFC 2016

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Institut für Biologische Chemie und Ernährungswissenschaft



Designing program to calculate micronutrient status and recommendation: A case of Indonesia

Hans K Biesalski
Institute of Biological Chemistry and Nutrition
University of Hohenheim
Germany

Introduction

Food Security

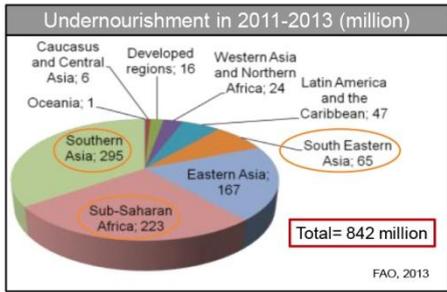
Condition when all people, all time, have access to sufficient, safe, and **nutritious food**

DIMENSION



2

- What happened when food insecurity occurs?



3

Hidden Hunger

Condition where intake of micronutrients and vitamin are below their requirements

Most common deficiencies:

IRON

- Anemia
- Cognitive development
- educability

ZINC

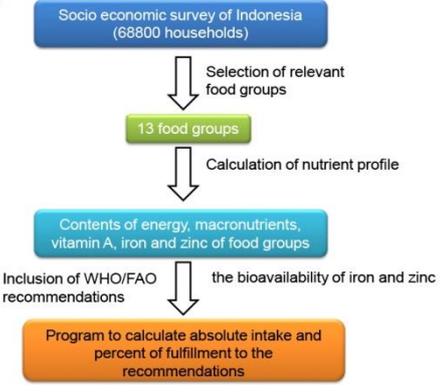
- Immune function
- Growth
- Development

VITAMIN A

- Blindness
- Child and maternal mortality

4

Flowchart



5

Calculator of Inadequate Micronutrient Intake (CIMI)

6

DATA INPUT

Country: Population:

Food consumption in grams

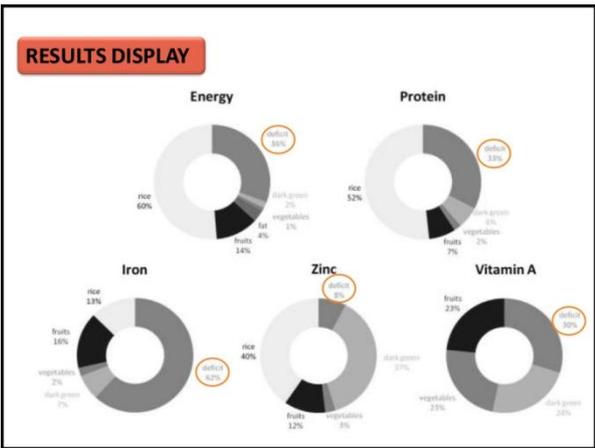
Rice	<input type="text" value="181"/>	Pulses and Nuts	<input type="text" value="0"/>
Other grains	<input type="text" value="0"/>	Dark Green leafy veggies	<input type="text" value="40"/>
Other starchy staples	<input type="text" value="0"/>	Other vegetables	<input type="text" value="0"/>
Meat including chicken	<input type="text" value="0"/>	Fruits	<input type="text" value="0"/>
Eggs	<input type="text" value="0"/>	Fats and Oils	<input type="text" value="5"/>
Fish	<input type="text" value="0"/>	Pre and Pro Vitamin A sources	<input type="text" value="0"/>
Dairy	<input type="text" value="0"/>		

Calculate Status

RESULTS DISPLAY

	intake	% of total energy intake	recommended intake	% of recommended intake
energy (kcal)	1944.52	-	3050	63.8
proteins (g)	41.10	8.5	62	66.3
fats (g)	38.86	17.7	-	-
carbohydrate (g)	368.58	73.8	-	-

	intake	recommended intake	% of recommended intake
iron (mg)	10.40	27.4	37.9
zinc (mg)	6.42	7	91.8
vitamin A (retinol) (µg)	71.29	-	-
beta-carotene (µg)	2079.21	-	-
retinol equivalent (conversion factor 1.6)	416.53	600	69.4
retinol equivalent (conversion factor 1.12)	243.28	-	-



Validation

- Location: West Timor (children), East Java (adult-female)
- Methods: 24h recall

	Children	Female Adult
N	118	124
Age (Years)	2,47 ± 1,17	36,1 ± 8,3

- Comparison with Nutrisurvey <http://www.nutrisurvey.de/>

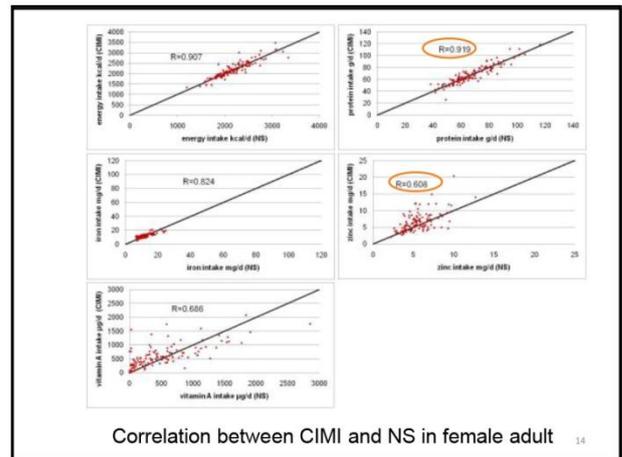
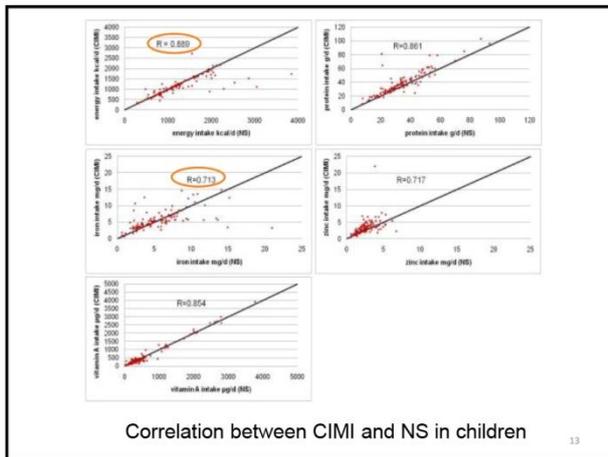
Average intake calculated by CIMI and NS

	Children (n = 118)		Adult female (n = 124)	
	NS	CIMI	NS	CIMI
Energy	1274.92	1175.8	2175.61	2190.82
Protein (g/d)	35.03	39.26	67.92	68.07
Iron (mg/d)	5.59	5.56	11.62	11.78
Zinc (mg/d)	2.63	3.46	5.49	6.40
vitamin A (µg/d)	613.01	602.16	508.56	602.99

Percentage of subject below the threshold of inadequate intake

	Children (n = 118)		Adult female (n = 124)	
	NS	CIMI	NS	CIMI
Energy < 2/3 RNI	13.5%	15.2%	1.6%	4.03%
Protein < 2/3 RNI	1.69%	0%	0%	0.81%
Iron < 2/3 RNI	38.1%	31.3%	16.9%	12.1%
Zinc < 2/3 RNI	36.4%	17.8%	29.8%	24.1%
vitamin A < 2/3 RNI	31.3%	33.9%	45.9%	27.4%

BIOAVAILABILITY	Children (n = 118)		Adult female (n = 124)	
	NS	CIMI	NS	CIMI
Iron < 2/3 RNI	38.1%	19.49%	16.9%	89.52%
Zinc < 2/3 RNI	36.4%	43.22%	29.8%	37.90%



- Conclusion**
- CIMI provide accurate results
 - CIMI calculate the absolute intakes, and percent of nutrient fulfillment compared to the recommendation
 - The result can be used as recommendation to improve the nutritional status of Indonesian.
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UNIVERSITI SAINS MALAYSIA

Mycotoxins Contamination in Food and Feed

Latiffah Zakaria
 School of Biological Sciences
 Universiti Sains Malaysia
 Penang, Malaysia


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WHERE IS USM?



Penang Island
 Mainland-
 •Trans Kerian
 •Kubang Kerian

Peninsular Malaysia
 KUALA LUMPUR
 GEORGETOWN
 BUTTERWORTH
 KOTA BHARU
 KUALA TERENGGANU
 Kuantan
 Kelang
 Port Dickson
 Gemas
 Toman
 Melaka
 Muar
 Batu Pahat
 Johor Bahru
 Singapore
 Sumatra Indonesia


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PENANG, MALAYSIA



email: dean_bio@usm.my
 Website : bio.usm.my

Mycotoxin Occurrence

Several factors contribute to mycotoxin contamination / production in Malaysia :

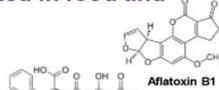
- tropical region
- heavy rainfall
- high temperature (28 – 30°C)
- high relative humidity (70% – 90%)

>> conducive growth of toxigenic fungi and mycotoxin production

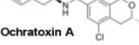
UAM **Mycotoxin**

Important mycotoxins detected in food and feed in Malaysia:

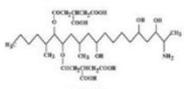
- aflatoxins (AFs)
- ochratoxin A (OTA)
- fumonisins (FUMs)
- deoxynivalenol (DON)
- zearalenon (ZEN)



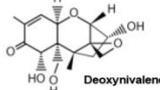
Aflatoxin B1



Ochratoxin A



Fumonisins



Deoxynivalenol

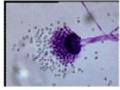
Mycotoxin

Mycotoxigenic fungi :

- *Aspergillus*
- *Penicillium*
- *Fusarium*

> commonly present in the environment

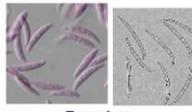
> can grow under variable environmental conditions and variety of substrates



Aspergillus



Penicillium



Fusarium

Occurrence of Mycotoxins in Food and Feed

- Cereals and Grains
- Nuts and Nut Products
- Spices
- Cocoa beans
- Milk and Egg
- Animal feed



Occurrence of Mycotoxin in Cereals and Grains

- Rice > staple food in Malaysia
- wheat, barley, corn > imported from China, Argentina, Thailand, India, Indonesia

Aflatoxins (AFs)

- detected in stored paddy, rice, rice flour, wheat flour, corn-based product



Occurrence of Mycotoxin in Cereals and Grains

- AFs > contaminated starch-based food from retail markets (Abdullah et al., 1998)
- using HPLC

> **rice samples** > 3.69 – 77.50 ng/g of AFG₁ & AFG₂

> **wheat flour** > AFB₁ (25.62 ng/g), AFB₂ (11.25 – 252.50 ng/g), AFG₁ (25 – 289.38 ng/g), AFG₂ (16.25 – 436.25 ng/g)

Abdullah N, Nawawi A. & Othman I. (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. *Mycopathology* 143 : 53 – 58

Occurrence of Mycotoxin in Cereals and Grains

- AFs > **corn-based products** (Hong et al., 2010)
- AFB₁ and AFB₂ using HPLC

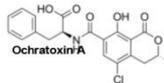
- AFB₁ and AFB₂ > detected in 45% corn-based products
> 0.2 – 101.8 ppb



Hong L.S, Yusof N.I.M. & Ling H.M. (2010). Determination of aflatoxins B1 and B2 in peanuts and corn based products. *Sains Malaysiana* 39 : 731 – 735.

Occurrence of Mycotoxin in Cereals and Grains

- OTA - detected in cereal > rice, oat, maize, barley and wheat (Rahmani et al., 2010)
- HPLC and fluorescence detection



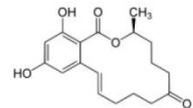
- six rice samples (31) > contaminated with OTA (0.05 – 5.32 ng/g)
- one barley sample (11) > 0.03 ng/g
- one wheat sample (6) > 0.01 ng/g
- one oat sample (4) > 0.07 ng/g

Rahmani A, Jinap S. & Soleimany F. (2010). Validation of the procedure for the simultaneous determination of aflatoxins ochratoxin A and zearalenone in cereals using HPLC-FLD. *Food Additive and Contaminants A* 27:1683 – 1693.

Occurrence of Mycotoxin in Cereals and Grains

- ZEN - detected (Soleimany et al., 2011) :
- > three (30) rice samples - 2.4 – 6.11 ng/g
- > three (10) wheat samples - 2.98–6.73 ng/g
- > one maize (5) sample – 5.17 ng/g

- RP-HPLC with a photodiode array and fluorescence detector

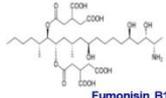


Soleimany F., Jinap S, Rahmani A. & Khatib A (2011) Simultaneous detection of 12 mycotoxins in cereals using RP-HPLC-PDA-FLD with PHRED and a post-column derivatization system, *Food Additives & Contaminants: Part A*, 28:4, 494-501

Occurrence of Mycotoxin in Cereals and Grains

FUM - detected in rice, wheat, barley, oat, maize samples

Mycotoxin contamination in cereal samples in Malaysian market (ng/g)					
	Rice (50)	Wheat (20)	Barley (10)	Oat (10)	Maize (10)
FB1 (ng/g)	7(41.3–53.2)	3 (42.5–69.1)	3 (45.5–97.7)	3 (49.5–177.3)	3 (48.2–209.3)
FB2 (ng/g)	3 (40.1–61.5)	3 (42.0–75.3)	3 (43.1–72.5)	1 (57.3)	3 (58.7–113.5)



Soleimany F, Jinap S. & Abas F. (2012). Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. *Food Chemistry* 130:1055 –1060.

Occurrence of Mycotoxin in Cereals and Grains

AFs, OTA, ZEA and FUM - detected in cereals and grains

Production of mycotoxins in cereals:

➤ highly dependent on environmental factors (temperature and moisture content), pre-harvest and/or postharvest

➤ thus, changes in the weather conditions - mycotoxins will be affected (Milani, 2013)

Milani, J.M. (2013). Ecological conditions affecting mycotoxin production in cereals: a review. *Veterinarni Medicina*, 58 (8): 405–411

Occurrence of Mycotoxin in Cereals and Grains

Best way to reduce risk :

- prevent fungal development and mycotoxin biosynthesis

- harvest grain at maturity and low moisture content

- stored under cool and dry conditions

Occurrence of Mycotoxin in Nuts and Nut Products

Peanuts – ingredient in a variety of food / dishes
- majority are imported > China, India, Vietnam

- Consumption of contaminated groundnuts > outbreak AF poisoning in pigs farms in Melaka in 1960



Occurrence of Mycotoxin in Nuts and Nut Products

The studies found > peanuts and peanuts products are contaminated with AFs (AFB₁, AFG₁)

Mycotoxin levels (ng/g) in nuts		
Products	AFs	Reference
Raw shelled peanut	40	MARDI (1985)
Raw shelled peanut	0.85 - 762.05	Sulaiman et al. (2007)
Raw shelled peanut	0.2 - 101.8	Hong et al. (2010)
Raw shelled peanut	5.25 - 15.33	Reddy et al. (2011)
Peanut candy and peanut bar	9 - 180	Ali et al. (1999)
Coated nut product	113 - 514	Leong et al. (2010)
Roasted groundnut in shell	29.7 - 179	Leong et al. (2010)
Peanut products	0.33 - 273.63	Leong et al. (2011)
Honey peanuts	1.47 - 6.25	Reddy et al. (2011)
Roasted peanuts	3.21 - 8.91	Reddy et al. (2011)

1) MARDI (1985). Annual report. Food Technology Division, MARDI, Serdang, Selangor.
 2) Sulaiman M, Yee C, Hamidi A, & Yatin A. (2007). The occurrence of aflatoxins in raw shelled peanut samples from three districts of Perak, Malaysia. *EJEF Chemistry* 6 : 2045-2052
 3) Hong L.S, Yusof N.I.M. & Ling H.M. (2010). Determination of aflatoxins B1 and B2 in peanuts and corn based products. *Sains Malaysiana* 39(5) :731-73.
 4) Reddy K, Farzana N.I. & Salleh B. (2011). Occurrence of *Aspergillus* spp. and aflatoxin B1 in Malaysian foods used for human consumption. *Journal of Food Science* 76:T99-104.

Occurrence of Mycotoxin in Nuts and Nut Products

The only study of OTA in nuts > Afsah-Hejri et al. (2012)

> raw peanuts – 2.82 – 7.41 ng/g.



Afsah-Hejri L, Jinap S. & Mirhoseini H. (2012). Ochratoxin A quantification: newly developed HPLC conditions. *Food Control* 23:113-119.

Occurrence of Mycotoxin in Nuts and Nut Products

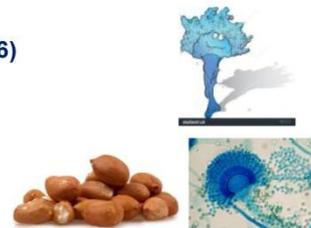
Contamination of peanuts by *Aspergillus* :
 - preharvest stages > peanuts in direct contact with soil
 - cross contamination > processing, transportation, storage



Occurrence of Mycotoxin in Nuts and Nut Products

Contamination of peanuts by *Aspergillus* spp.:

- *A. niger* (n = 54)
- *A. tubingensis* (n = 6)
- *A. flavus* (n = 20)
- *A. chevalieri* (n = 18)
- *A. amstelodami* (n = 16)
- *A. aculeatus* (n = 4)
- *A. sydowii* (n = 4)
- *A. fumigatus* (n = 2)



Occurrence of Mycotoxin in Spices

Malaysia > spices are used as ingredients in daily cooking
 > main producer of black and white peppers – one of the major export commodities

Different types of spices have been reported to be contaminated with AFs and OTA
 - white and black peppers
 - chilli



Occurrence of Mycotoxin in Spices

Studies have shown > AFB1 and OTA detected in sample of spices

Mycotoxin levels (ng/g) in spices			
Products	AFs	OTA	Reference
White pepper seed	0.2 - 4.6	18 - 2.4	Jalili et al. (2009, 2010)
White pepper powder	0.1 - 4.6	0.21 - 3.4	Jalili et al. (2010)
Black pepper seed	0.1 - 4.8	0.15 - 13.58	Jalili et al. (2010)
Black pepper powder	0.7 - 4.9	0.23 - 12.64	Jalili et al. (2010)
Chilli and pepper	0.58 - 4.64	-	Reddy et al. (2011)
Cumin powders	1.89 - 4.64	-	Reddy et al. (2011)
Chilli	0.2 - 79.71	0.2 - 101.24	Jalili & Jinap (2012)

- 1) Jalili M., Jinap S., Adzahan N. 2009. Survey of aflatoxins in retail samples of whole and ground black and white peppercorns. *Food Additive and Contaminants B* 2:178-82.
- 2) Jalili M. & Jinap S. & Radu S. (2010). Natural occurrence of ochratoxin A contamination in commercial black and white pepper products. *Mycopathologia* 170 : 251-258.
- 3) Jalili M. & Jinap S. (2012). Reduction of mycotoxins in white pepper. *Food Additive and Contaminants A* 29:1947-1958
- 4) Reddy K, Farhana NI, Salleh B. 2011. Occurrence of *Aspergillus* spp. and aflatoxin B1 in Malaysian foods used for human consumption. *Journal of Food Science* 76:T99-104

Occurrence of Mycotoxin in Spices

Mycotoxin contamination of spices :

- white and black peppers > due to traditional processing and storage methods
- chilli > long and improper storage conditions



Occurrence of Mycotoxin in Cocoa bean

- Cocoa beans > source of cocoa powder
- beans are susceptible to fungal spoilage during and after fermentation
- > the first stage in preparation for cocoa production

Fungi can grow and produce mycotoxin during cocoa processing



M. Sánchez-Hervás, J.V. Gil, F. Bisbal, D. Ramón, P.V. Martínez-Culebras (2008). Mycobiota and mycotoxin producing fungi from cocoa beans. *International Journal of Food Microbiology* 125: 336-340

Occurrence of Mycotoxin in Cocoa bean

Reports by MARDI :

- surveyed of dried cocoa beans in 1986 (Selangor and Perak)
- 11% - 13.8% (contaminated)

- reports in 1981 – 1984, and 1985 – 1995
> AF contamination



MARDI. (1986). Annual report. Food Technology Division, MARDI, Serdang, Selangor.

Occurrence of Mycotoxin in Herbal Medicine

Traditional herbal medicine

- jamu and makjun
- readily available and consumed regularly to promote health

Conventional methods of collection, storage, and marketing usually promote growth of several toxigenic fungi
> susceptible to mycotoxin contamination



Roy AK, Chourasia HK, Kumari N. (1987). Association of mycoflora with some crude herbal drugs of Bhutan. Indian Botanical Reports 6:48-9.

Occurrence of Mycotoxin in Herbal Medicine

Herbal products commonly consumed and available in the markets > presence of aflatoxins (Ali et al., 2005)

- AFB1 - 0.26 µg/kg (70%)
- AFB2 - 0.07 µg/kg (61%)
- AFG1 - 0.10 µg/kg (30%)



Ali N, Hashim N, Saad B, Safan K, Nakajima M, & Yoshizawa T. (2005). Evaluation of a method to determine the natural occurrence of aflatoxins in commercial traditional herbal medicines from Malaysia and Indonesia. *Food and Chemical Toxicology* 43 : 1763-1772.

Occurrence of Mycotoxin in Milk and Eggs

Reports on mycotoxins contamination:

- fresh milk and eggs from wet markets (samples from Selangor, Negeri Sembilan and Melaka)

> only fresh milk from Selangor – AFM1 (0.24 ng/g)

> 20% egg – AFs (0.16 – 0.41 ng/g)



Abidin H. & Mat Isa A. (1992). Aflatoxin in animal produce. Proceedings of the National MPA Seminar Agriculture Sector. Vol. II. Ministry of Science Technology and the Environment, Malaysia. p 574-575.

Occurrence of Mycotoxin in Milk and Eggs

Mycotoxins in milk and eggs :

- related to the type and quality of animal feed
- when animals ingest the contaminated feeds
- > some toxins can be metabolized and remain in milk and eggs



Occurrence of Mycotoxin in Animal Feed

Animal feed > most of the raw ingredients (cereal grains, soybean meal and corn meal) are imported - Thailand, China, India, Argentina, U.S.A., Australia, and Canada.
> corn – common feed ingredient

Common mycotoxin detected > AFs
- reports on OTA, ZEN and FUM



Occurrence of Mycotoxin in Animal Feed

Mycotoxins contamination of animal feed :

- feed ingredients are susceptible to fungal growth
- > due to inadequate conditions of harvesting, storage, handling, processing and transportation



Occurrence of Mycotoxin in Animal Feed

Reports on mycotoxins contamination of animal feed :

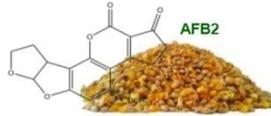
- 42 animal feeds analyzed, eight (corn [2], copra meal, wheat, palm kernel, corn germ meal, poultry feed) > total AFs (6.51 - 101.9 ng/g) (Khayoon et al., 2010)



Khayoon W.S, Saad B, Yan C.B, Hashim .NH, Ali A.S.M, Salleh M.I. & Salleh B. (2010). Determination of aflatoxins in animal feeds by HPLC with multifunctional column clean-up. *Food Chemistry* 118:882 – 886.

Occurrence of Mycotoxin in Animal Feed

Imported poultry feed :
> elevated concentrations of AFB2 detected in the corn samples
> 0.2 to 101.8 ng/g - exceed FDA levels of 20 ng/g (Hong et al., 2010)

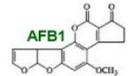


Hong L.S., Yusof N.I.M. & Ling H.M. (2010). Determination of aflatoxins B1 and B2 in peanuts and corn based products. Sains Malaysiana 39(5):731-735.

Occurrence of Mycotoxin in Animal Feed

- AFB1 was detected in 81.2% of corn samples ranging from 1.0 to 135 ng/g (80 corn grains from various states)

- levels of AFs in 22.5% of the samples > above an international regulatory limits (20 ng/g) for animal feeds (Reddy and Salleh 2011)



Reddy K. & Salleh B. (2011). Co-occurrence of moulds and mycotoxins in corn grains used for animal feeds in Malaysia. Journal of Animal and Veterinary Advances 10:669-673.

Conclusion

There are mycotoxins contamination in various types of food products and feed ingredients

- consequent exposure to community / population
> human and animal health hazards

- It is important to raise awareness on occurrence of mycotoxins in food and feed commodities

Relevant authorities should play an important role to deliberate issues of contamination

Thank You

Organizing Committee



Food Processing and Product Properties, Focusing On Glycaemia

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The University of Queensland, AUSTRALIA*

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Abstract

Rice, cassava, maize, bananas, sweetpotatoes, potatoes, soybeans, peanuts, and mung beans are the topmost food crops in Indonesia, in terms of production. Like other food raw materials, they are processed into various products using thermal and non-thermal techniques, in association with moisture, enzymes and microorganisms. These processing techniques influence product properties to various extents that determine uses. With starch being a common component of the food crops, we present here the dependence of starch digestibility and glycaemic properties on heat-moisture, heat-moisture-mechanical, pressure, and biotechnological processing techniques. Glycaemic properties are quantified as glycaemic indices (GIs) and loads (GLs), and high GI foods are associated with various lifestyle diseases that are increasing in Indonesia in particular and globally in general. Processes that gelatinize starch produce high GI foods, in the absence of starch retrogradation that increases resistant starch type 3. Fermented products are unique in their contents of organic acids, which inhibit amylolysis and produce low GI foods. Various studies are presented to challenge food processing and utilization in Indonesia to diversify raw materials and include pigmented varieties and cultivars for desirable product glycaemic properties.

Keywords: Indonesian food crops, heat-moisture, heat-moisture-mechanical, high pressure processing, fermentation, glycaemic index

Background

On production (Table 1), rice, cassava, maize, bananas, sweetpotatoes, potatoes, soybeans, peanuts, and mungbeans are the top nine Indonesian food crops (BPS, 2016; FAOSTAT, 2016). When combined with animal products, fruits and vegetables and other plant materials (e.g. coconut), they form various foods in Indonesia that are consumed by its 252 million people, judging by the 2014 national population estimate (BPS, 2016). Prior to use, these food materials are processed using heat, moisture, pressure, shear, biotechnology, and their combinations, as typified with banana processing in Fig. 1. Boiling, steaming, popping, microwaving, baking, roasting, extrusion, high pressure, fermentation, and germination are notable food processes. These processes can be preceded by operations or processes such as milling, soaking and parboiling. When processed, food systems undergo structural and nutritional modifications, amongst others, to extents that are dependent on uses. Starch, a polymer of glucose, is a major nutrient, and food processing gelatinizes it, de-polymerizes it, and on cooling, the gelatinized starch can retrograde under favorable conditions. Upon digestion, starches or carbohydrates modulate blood glucose according to the glycaemic properties of the products, which with other product properties, are influenced by food processing.

Glycaemic properties are dependent on starch digestibility properties, and are quantified by the glycaemic index (GI), related to which is the glycaemic load (GL) that incorporates carbohydrate quantity and quality (Atkinson *et al.*, 2008; Taylor *et al.*, 2015). Relative to a reference food (e.g. glucose, white wheat bread or rice), the GI is defined as the incremental area under the blood glucose-time curve of the food divided by that of the reference (Sugiyama *et al.*, 2003; Atkinson *et al.*, 2008). With glucose as the

reference, foods are classified (Atkinson *et al.*, 2008; Nayak *et al.*, 2014) as low ($GI \leq 55\%$, $GL \leq 10$), intermediate or medium ($GI 56$ to $< 70\%$, $GL 11$ to < 20) and high ($GI \geq 70\%$, $GL \geq 20$) GI (Fig. 2A). High GI foods are associated with diabetes, obesity, cardiovascular diseases and associated health and nutritional issues. Although complex, and involves an inter-play of many food and non-food factors, food glycaemic properties are critical to human wellbeing, and understanding food processing effects on the GI is important in designing how to manage these lifestyle diseases in Indonesia and globally. This is more important in Indonesia, where diabetic individuals increase at about 250,000 yearly, and are projected to reach 12 million in 2030 from about 7 million in 2010. This will put Indonesia amongst the top 10 countries in the world with the highest diabetic 20- to 79-year olds (Shaw *et al.*, 2010; Whiting *et al.*, 2011). Globally, diabetes is increasing, and about 54% more adults (20-79 years) are projected in 2030 than in 2010 to be diabetic, culminating in a possible increase (US\$114 billion) in global health bills to US\$490 billion during the 20-year period (Zhang *et al.*, 2010).

Table 1. The topmost produced Indonesian food crops

Crop	Bahasa	2012	2013	2014	2015
Rice, paddy ^a	<i>Padi</i>	69,056,126	71,279,709	70,846,465	75,397,841
Cassava ^a	<i>Ubi Kayu</i>	24,177,372	23,936,921	23,436,384	21,801,415
Maize ^a	<i>Jagung</i>	19,387,022	18,511,853	19,008,426	19,612,435
Bananas ^b	<i>Pisang</i>	6,189,052	5,359,115	na ^c	na
Sweetpotato ^a	<i>Ubi Jalar</i>	2,483,460	2,386,729	2,382,658	2,297,634
Potatoes ^b	<i>Kentang</i>	1,094,232	1,124,282	1,316,016	na
Soybeans ^a	<i>Kedelai</i>	843,153	779,992	954,997	963,183
Peanuts ^a	<i>Kacang Tanah</i>	712,857	701,680	638,896	605,449
Mung beans ^a	<i>Kacang Hijau</i>	284,257	204,670	244,589	271,463

^aBPS (2016)

^bFAOSTAT (2016)

^cna = not available

Digestibility properties are also studied using *in vitro* procedures, and various protocols have been published (Chen & Sopade, 2013; Minekus *et al.*, 2014; Villemejeane *et al.*, 2016), which are used to define very rapidly (VRDS), rapidly (RDS) and slowly digestible (SDS), and resistant (RS) starches (Fig. 2B). Depending on the states of the starches in foods, their interactions with other food nutrients, the extents of gelatinization and retrogradation, and whether modified, there are five (RS1 – RS5) RS classes (Birt *et al.*, 2013). A parameter of the *in vitro* time-course starch digestibility, hydrolysis index relative to white wheat bread, has been used to estimate the GI (eGI), with a reasonable degree of accuracy (Sopade, 2016). Therefore, using results from *in vivo* and *in vitro* studies, and concentrating on food crops that are available in Indonesia, this review discusses how food processing affects starch digestibility and glycaemic properties. The review broadly classifies food processing to discuss into, (a) heat-moisture, (b) heat-moisture-mechanical, (c) pressure, and (d) biotechnological treatments.

Heat-moisture food processing

Boiling, steaming or pressure cooking of soaked food crops is a high moisture (excess water) heat treatment of food crops, and can be at temperatures below, at or above 100°C. Although baking ovens are usually at temperatures above 100°C, evaporation and cooling of added water keep the crumbs below 100°C. Roasting, toasting and frying mainly heat-treat with produce moisture. Heat-moisture treatments yield products that vary in starch gelatinization, and this defines starch digestibility and GIs, as gelatinization and digestibility are directly related (Mahasukhonthachat *et al.*, 2010). However, cooling or post-process handling temperatures influence starch retrogradation (and RS), and consequently glycaemic properties.

Irrespective of the processing, starch characteristics play an important part in the GI as they influence gelatinization and digestibility. Starch is a glucose polymer with two main fractions, amylose (straight) and amylopectin (branched), and food crops differ in these fractions. Even within a crop, there are varietal or cultivar differences in the amylose:amylopectin ratios reflecting in waxy, regular or normal and high amylose genotypes (Kaur *et al.*, 2016). High amylose genotypes (e.g. long grain rice) gelatinize at a higher temperature and digest less (low GI) than the other two, while waxy (e.g. glutinous) genotypes are generally easier to gelatinize and digest the most (high GI) of the three. For example, Boer *et al.* (2015) reported that Basmati rice of 20-25% amylose content had a GI of 57/67 (European/Chinese), while Jasmine rice of a low amylose content (waxy genotype) had a GI of 68/80. Apart from showing differences in the GI of rice genotypes, these authors revealed the ethnicity factor in GI measurements, and the need to study Indonesian foods under Indonesian conditions to supplement existing database on GIs of foods. Starch crystallinity and crystalline patterns, which can vary within and between food crops, also influence digestibility and GI. Waxy genotypes are generally more crystalline, and while cereals (e.g. rice) exhibit crystalline pattern A, roots and tubers (e.g. cassava) pattern B, and legumes (e.g. soybeans) pattern C (Chen & Sopade, 2013). Generally, cereals digest the most (high GI), and legumes the least, and pronounced starch-protein interactions in the latter enhance their low GI properties (Tinus *et al.*, 2011). Indonesians reportedly consume about 130 kg of white rice (a cereal, high GI) per person per year, which is more than twice the global (60 kg/person/year) average (Budijanto & Yuliana, 2015). This has led to a massive import bill to meet the demand, but there have been well-intentioned national campaigns (e.g. One Day, No Rice', 'No Rice Day' and 'One Meal, No Rice') and food diversification programmes to manage this. There are government initiatives to increase the consumption of roots and tubers (and possibly legumes/pulses and bananas) from 10 to 40 kg per capital per year (Winarno *et al.*, 2012; Subejo & Padmaningrum, 2013), and this will also lead to the consumption of relatively low GI foods. Bananas (and plantains) are reported (Zhang & Hamaker, 2012; Odenigbo *et al.* 2013) to have specialized starch structures that are resistant to digestion, and increasing their consumption will add to low GI Indonesian foods.

The effects of starch characteristics on digestibility and glycaemic properties apply to all processed food crops, so also are the beneficial micronutrients and polyphenols in pigmented genotypes that are reported (Singh *et al.*, 2010) to be amylase inhibitors. Sui *et al.* (2016) reduced the GI of breads by adding anthocyanin-rich black rice extract powders. Polyphenols in Indonesian pigmented rice varieties have been reported (Murdifin *et al.*, 2015), and Waramboi *et al.* (2012) measured differences in

starch digestibility and eGI of pigmented and non-pigmented raw Australian and Papua New Guinean sweetpotatoes. Although they did not investigate starch digestibility, Jusuf *et al.* (2006) studied Indonesian pigmented sweetpotatoes, and found that when added to wheat flour to make baked goods, acceptable products with higher antioxidant properties were produced. Hence, pigmented genotypes offer advantages for glycaemic and antioxidants properties of products.

Heat-moisture-mechanical food processing

Extrusion is the most popular of the processing techniques in this category, and it is used for a variety of products. It is a process in which moistened food materials are plasticized and cooked in a tube (extruder) by a combination of pressure, heat and mechanical shear, before propulsion through a die (Sopade and Le Grys, 1990). The temperature developed during the process gelatinizes starch and denatures proteins, and the sudden drop in temperature and pressure at the die, flashes off water leading to porous products. The various transformations during extrusion are dependent on screw configuration, moisture level, temperature, feed rate, screw speed and feed composition, and both single- and twin-screw extruders are used in the food industry (Sopade and Le Grys, 1990; Brennan *et al.*, 2013; Sopade, 2016).

Extrusion is a versatile process, and most food materials can be processed individually or combined. Expansion properties vary depending on feed materials and processing conditions, which also determine functional and digestibility properties. Because they are generally more gelatinized, extrudates digest more (Fig. 3) and have a higher GI than non-extrudates (Waramboi *et al.*, 2012; 2014). Specifically, starch digestibility and GI increase with temperature, decrease with feed rate and moisture, and unchanged by screw speed (Sopade, 2016). For example, the inverse relationship between starch digestibility and extrusion moisture is reported (Mahasukhonthachat *et al.*, 2010) to be due to increase in heat capacity of the melt, depression of the melt glass transition temperature, reduction in the melt temperature, and enhanced starch retrogradation and RS. With other product properties within acceptable limits, high-moisture extrusion is beneficial in producing low GI extrudates. Low GI extrudates have also been produced (Brennan *et al.*, 2013) by adding high-fiber components to the feed, which inhibit amylolysis and/or increase digesta viscosity to slow down enzyme-substrate interactions. There are possibilities for Indonesian food crops to be combined with other plant materials for low GI extrudates. With a high demand for rice in Indonesia, rice analogues, with better health and nutritional properties, from non-rice materials can be produced with extrusion, but the acceptability of such products will need to be studied and ascertained. Budi *et al.* (2015) reported extrusion of rice analogues from maize and other materials, and although glycaemic properties were not investigated, it is believed that the importance of these properties in food and nutrition security of Indonesia will add them to future studies. Budijanto & Yuliana (2015) extensively discussed the potentials of hot extrusion in producing desirable rice analogues

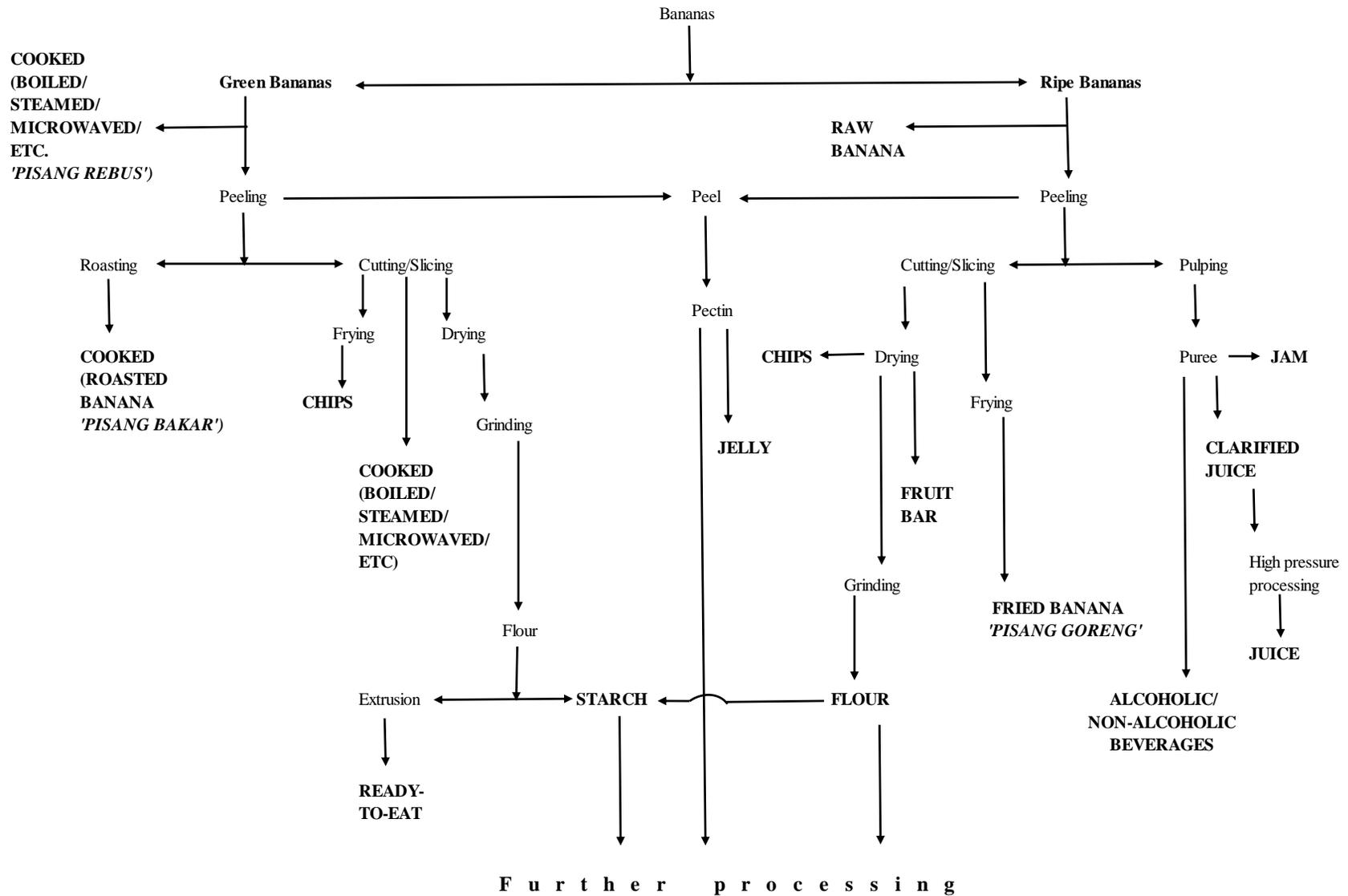


Figure 1. Banana processing and products

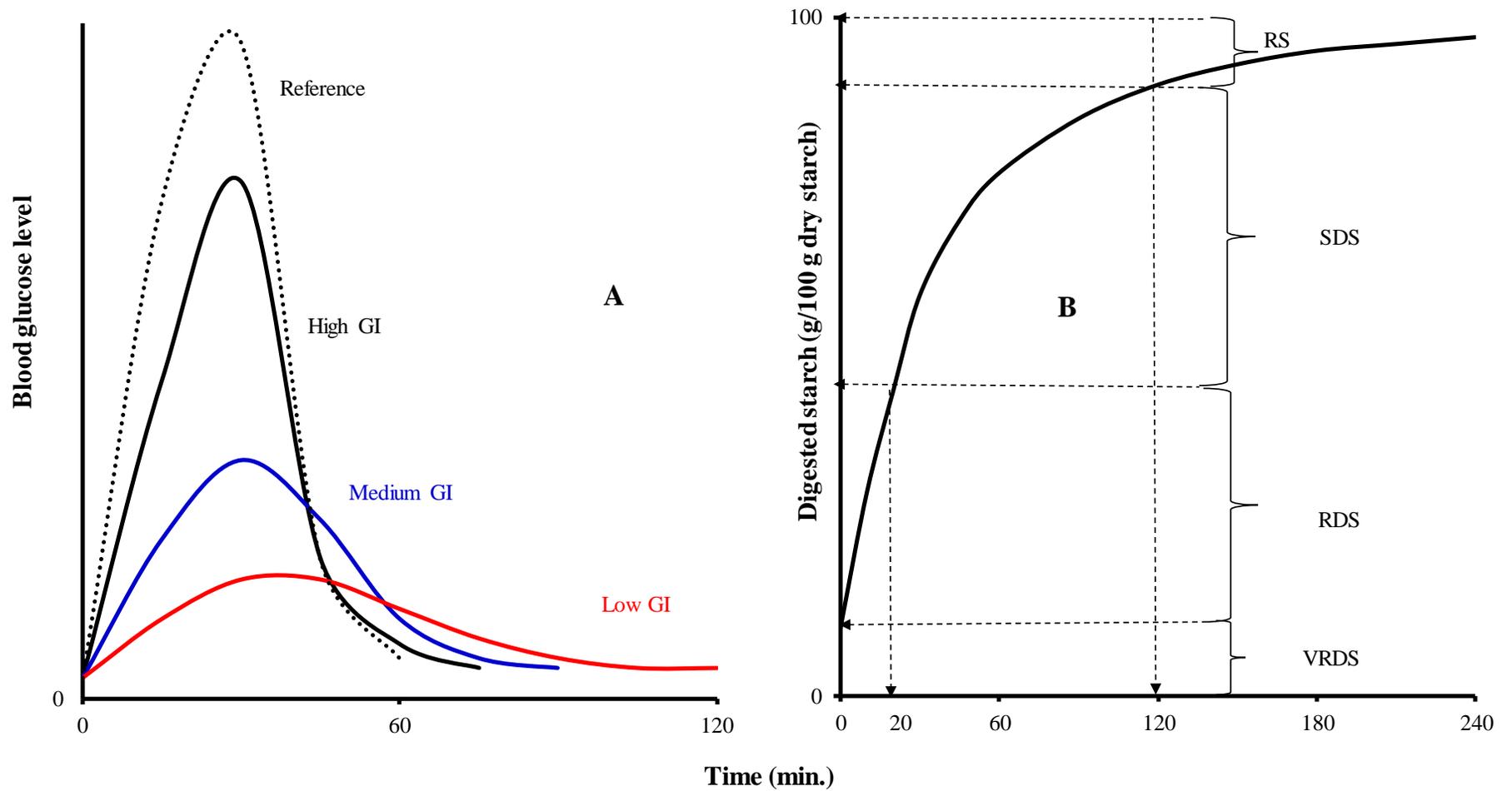


Figure 2. Typical measures of glycaemic properties. (A – blood glucose-time profiles [*in vivo*]; B – starch digestogram [*in vitro*])

for Indonesia with acceptable glycaemic and aesthetic properties. Incidentally, extruders are manufactured to cover a wide range of capital commitments, from low to high costs to suit all budgets, and need to be further or to continue to be explored to process Indonesian food crops for good properties.

Pressure food processing

High pressure processing (HPP) is a non-thermal technique that subjects food materials to high pressures (100 – 900 MPa) to primarily inactivate spoilage and pathogenic microorganisms and some enzymes that adversely affect food quality (San Martín *et al.*, 2002). Additionally, HPP gelatinizes starch, denatures proteins and changes various properties of foods including glycaemic properties (Tian *et al.*, 2014). HPP of rice starches produces more SDS than conventional thermal processes. The advantages of HPP lie in the desirable effects of high pressures on non-covalent bonds and food structures, and although it is used more for liquid foods, it has been used for pastes and solid foods. The technique is generally more expensive, and not as widely applied as others.

Biotechnological food processing

Fermentation, germination and sprouting are the main biotechnological processes for food crops. Fermentation uses natural or added microorganisms, while natural enzymes in food crops are mainly responsible for germination or sprouting. Germinated grains can be kilned (dried) to produce malt. Fermented products are produced by solid-state (no 'free' water) or submerged ('free' water) technique (Ray and Sivakumar, 2009), and popular fermented products include *tempeh* (or *tempe*), *tape* and sourdoughs (Ray and Sivakumar, 2009; Alming *et al.*, 2012; Wolter *et al.*, 2014). Both soybean *tempeh* and cassava *tape* are possibly the most popular fermented products in Indonesia. Apart from these main materials for these fermented products, cereals and other legumes have been used to produce *tempeh*, while *tape* can be made from other roots and tubers (Ray and Sivakumar, 2009; Alming *et al.*, 2012). Sourdough breads are not as popular as these two products in Indonesia, and the organisms involved in the fermentation of cereals, legumes and roots and tubers, ranging from *Rhizopus* to *Lactobacillus* spp., are detailed elsewhere (Ray and Sivakumar, 2009; Kamal-Eldin, 2012).

A common attribute of fermented products is the production of organic acids during the fermentation process. These acids favourably contribute to the sensory, health and nutritional properties of products (Lukito, 2001). Specifically, various studies revealed that fermentation essentially inhibits starch digestibility of, and produces low GI products (Alming *et al.*, 2012; Wolter *et al.*, 2014). The high acidity of fermented products is thought to inhibit amylolysis, coupled with reduced gelatinization and enhanced retrogradation that increase RS2 and RS3 in the products (Wolter *et al.*, 2014; Sopade, 2016). It appears that desirable glycaemic outcomes of fermentation can be enhanced by using appropriate pigmented varieties and cultivars of food crops. This will diversify food products, and contribute to food and nutrition security in Indonesia.

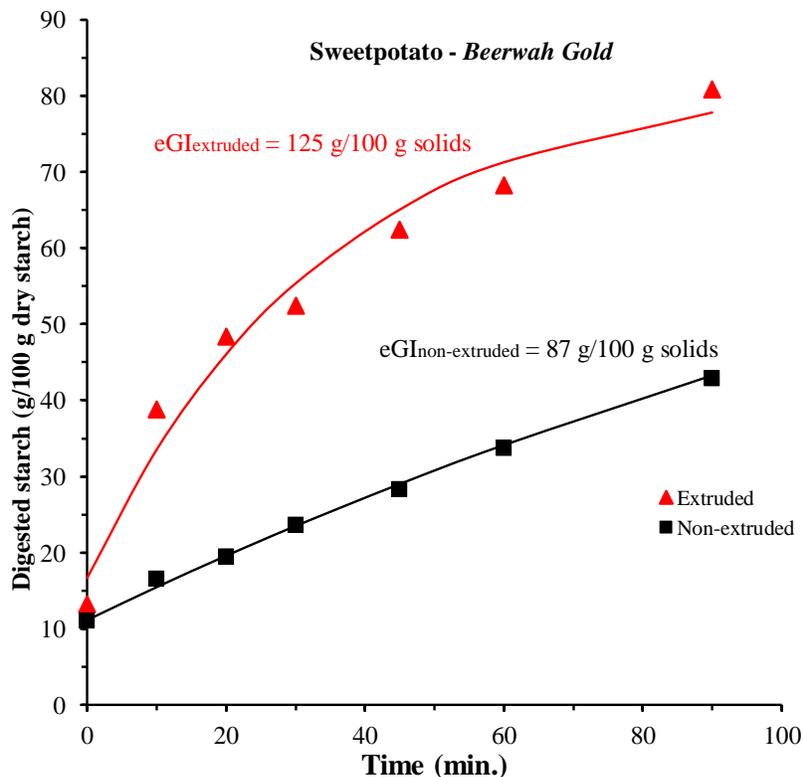


Figure 3. Starch digestograms of an extruded and non-extruded sweetpotato cultivar (Adapted from Waramboi *et al.*, 2014)

Conclusions

By design, food processing defines various properties of food products, and processing techniques can be chosen for selected product properties and uses. With special reference to glycaemia, low GI foods are the preference to manage lifestyle diseases (e.g. ‘diabesity’) that are associated with high and rapid increases in blood glucose upon consumption of starch- or carbohydrate-containing foods. The various thermal and non-thermal processes that are applied to foods are discussed to reveal that, in the absence of inhibitors, processes that gelatinize starches with minimal retrogradation or resistant starches, increase starch digestibility and produce high GI foods. Organic acids that are produced during fermentation typify fermented products as low GI foods because of their favorable amylolysis inhibitory properties. Pigmented varieties and cultivars of Indonesian food crops can contribute to the production of low GI foods, by virtue of their phenolic constituents that are also amylolysis inhibitors.

References

- Alminger, M.L., Eklund-Jonsson, C., Kidman, S. & Langton, M. 2012. Starch microstructure and starch hydrolysis in barley and oat tempe during *in vitro* digestion. *Food Digestion*, 3: 53-62.
- Atkinson, F.S., Foster-Powell, K. & Brand-Miller, J.C. 2008. International tables of glycemic index and glycemic load values: 2008. *Diabetes Care*, 31: 2281–2283.
- Birt, D.F., Boylston, T., Hendrich, S. *et al.* 2013. Resistant starch: Promise for improving human health. *Advances in Nutrition*, 4: 587–601.
- Boers, H.M., ten Hoorn, J.S. & Mela, D.J. 2015. A systematic review of the influence of rice characteristics and processing methods on postprandial glycaemic and insulinaemic responses. *British Journal of Nutrition*, 114: 1035–1045.
- BPS. 2016. Badan Pusat Statistik. <http://www.bps.go.id/index.php>. Accessed on 2 August 2016

- Brennan, M.A., Derbyshire, E., Tiwari, B.K. & Brennan, C.S. 2013. Ready-to-eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. *International Journal of Food Science and Technology*, 48: 893–902.
- Budi, F.S., Hariyadi, P., Budijanto, S. & Syah, D. 2015. Effect of extrusion temperature and moisture content of corn flour on crystallinity and hardness of rice analogues. AIP Conf. Proc. 1699, 030001-1–030001-9. <http://dx.doi.org/10.1063/1.4938286>
- Budijanto, S. and Yuliana, N.D. 2015. Development of rice analog as a food diversification vehicle in Indonesia. *Journal of Sustainable Agriculture*, 10: 7-14.
- Chen, G. & Sopade, P.A. 2013. *In vitro* starch digestion in sweet potato (*Ipomoea batatas* L.) flours. *International Journal of Food Science and Technology*, 48: 150-156.
- FAOSTAT. 2016. Food and Agriculture organisation of the United Nations. FAOSTAT Database. <http://faostat3.fao.org/home/E>. Accessed on 2 August 2016.
- Jusuf, M., Hilman, Y., Ginting, E. & Setiawan, A. 2006. Selection of sweetpotato clones with high anthocyanin content in Indonesia. *ACTA Horticulturæ*, 703: 165-170.
- Kamal-Eldin, A. 2012. Fermented cereal and legume products. In: *Fermentation Effects on Food Properties* (Edited by B.M. Mehta, A. Kamal-Eldin and R.Z. Iwanski). Boca Raton, USA: CRC Press. Pp. 209-226.
- Kaur, B., Ranawana, V. & Henry, J. 2016. The glycemic index of rice and rice products: A review, and table of GI values, *Critical Reviews in Food Science and Nutrition*, 56: 215-236.
- Lukito W. 2001. Candidate foods in the Asia-Pacific region for cardiovascular protection: nuts, soy, lentils and tempe. *Asia Pacific Journal of Clinical Nutrition*, 10: 128-133.
- Mahasukhonthachat, K., Sopade, P.A. & Gidley, M.J. 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science*, 51: 392-401.
- Minekus, M., Alminger, M., Alvito, P. et al. 2014. A standardised static *in vitro* digestion method suitable for food – an international consensus. *Food and Function*, 5: 1113–1124.
- Murdifin, M., Pakki, E., Rahim, A., Syaiful, S.A., Ismail, Evary, Y.M. & Bahar, M.A. 2015. Physicochemical properties of Indonesian pigmented rice (*Oryza sativa* Linn.) varieties from South Sulawesi. *Asian Journal of Plant Sciences*, 14: 59-65.
- Nayak, B., Berrios, J.D.J. & Tang, J. 2014. Impact of food processing on the glycemic index (GI) of potato products. *Food Research International*, 56: 35–46.
- Odenigbo, A.M., Asumugba, V.U., Ubbor, S. & Ngadi, M. 2013. *In vitro* starch digestibility of plantain and cooking-banana at ripe and unripe stages. *International Food Research Journal*, 20: 3027-3031.
- Ray, R.C. & Sivakumar, P.S. 2012. Traditional and novel fermented foods and beverages from tropical root and tuber crops: review. *International Journal of Food Science and Technology*, 44: 1073–1087.
- San Martín M.F., Barbosa-Cánovas, G.V. & Swanson, B.G. 2002. Food processing by high hydrostatic pressure. *Critical Review in Food Science and Nutrition*, 42: 627–45.
- Shaw, J.E., Sicree, R.A. & Zimmet, P.Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, 87: 4-14.
- Singh, J., Dartois, A. & Kaur, L. 2010. Starch digestibility in food matrix: a review. *Trends in Food Science and Technology*, 21: 168-180.
- Sopade, P.A. & LeGrys, G.A. 1990. Extrusion cooking of starch-containing food systems: A review. *Nigerian Food Journal*, 8: 18-31.
- Sopade, P.A. (2016). Cereal processing and glycaemic response. *International Journal of Food Science and Technology* (in press).
- Subejo, Padmaningrum D. 2013. Tackling food security problem in Indonesia. The Jakarta Post. 26 November 2013. <http://www.thejakartapost.com/news/2013/11/26/tackling-food-security-problem-indonesia.html>. Accessed on 2 August 2016.
- Sugiyama, M., Tang, A.C., Wakaki, Y. & Koyama W. 2003. Glycemic index of single and mixed meal foods among common Japanese foods with white rice as a reference food. *European Journal of Clinical Nutrition*, 57: 743–752.
- Sui, X., Zhang, Y. & Zhou, W. 2016. Bread fortified with anthocyanin-rich extract from black rice as nutraceutical sources: Its quality attributes and *in vitro* digestibility. *Food Chemistry*, 196: 910–916.
- Taylor, J.R.N., Emmambux, M.N. & Kruger, J. 2015. Developments in modulating glycaemic response in starchy cereal foods. *Starch/Stärke*, 67: 79–89.
- Tian, Y., Li, D., Zhao, J., Xu, X. & Jin, Z. 2014. Effect of high hydrostatic pressure (HHP) on slowly digestible properties of rice starches. *Food Chemistry*, 152: 225–229
- Tinus, T., Damour, M., van Riel, V. & Sopade, P.A. 2012. Particle size-starch-protein digestibility relationships in cowpea (*Vigna unguiculata*). *Journal of Food Engineering*, 113: 254-264.
- Villemejeane, C., Denis, S., Marsset-Baglieri, A., Alric, M., Aymard, P. & Michon, C. 2016. *In vitro* digestion of short-dough biscuits enriched in proteins and/or fibres using a multi-compartmental and dynamic system (2): Protein and starch hydrolyses. *Food Chemistry*, 190: 164–172.
- Waramboi, J.G., Gidley, M.J. & Sopade, P.A. 2012. Kinetics of starch digestion in sweetpotato flours from Papua New Guinean and Australian cultivars. *Carbohydrate Polymers*, 87: 461-470.
- Waramboi, J.G., Gidley, M.J. & Sopade, P.A. 2014. Influence of extrusion on expansion, functional and digestibility properties of whole sweetpotato flour. *LWT-Food Science and Technology*, 59: 1136-1145.
- Whiting, D.R., Guariguata, L., Weil, C. & Shaw, J. 2011. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Research and Clinical Practice*, 94: 311-321.
- Winarno B., Ismail M., Hilman H. & Hariyadi P. 2012. RI needs to diversify its staple foods. The Jakarta Post. 12 November 2012. <http://www.thejakartapost.com/news/2012/11/12/ri-needs-diversify-its-staplefoods.html>. Accessed on 2 August 2016.
- Wolter, A., Hager, A.-S., Zannini, E. & Arendt, E.K. 2014. Influence of sourdough on *in vitro* starch digestibility and predicted glycemic indices of gluten-free breads. *Food and Function*, 5: 564–572.
- Zhang, P. & Hamaker, B.R. 2012. Banana structure and digestibility. *Carbohydrate Polymers*, 87: 1552-1558.
- Zhang, P., Zhang, X., Brown, J., Vistisen, D., Sicree, R., Shaw, J. & Nichols, G. 2010. Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, 87: 293-301.

The Role and Mechanism of Resistant Starch (RS) in Reducing Plasma Glucose Concentration

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Abstract

Resistant starch (RS) is the sum of starch and products of starch degradation not absorbed in the small intestine. Although chemically RS different with Non Starch Polysaccharides (NSP) the most constituent of dietary fibre (DF), the physiological effect of RS and DF is not different. Some physiological effect of RS has been proven in some studies such as reduction of cholesterol and glucose absorption and prevention of colorectal cancer. The physiological effects are due to the chemical and physical properties of the RS. The health effects of RS to human depend on the RS itself and fermentation of the RS. RS is an undigestible starch, this starch is not good source of glucose, in other world RS has a small bioavailability of glucose. From the physical properties, RS is very viscous in the digesta; so it will inhibit the glucose absorption in the small intestine. RS was also believed to increase the insulin sensitivities. RS is resistant by amilolytic enzymes in the small intestine and will escape to the colon. Fermentation of RS by colonic bacteria produces short chain fatty acid (SCFA). These volatile fatty acids also have potential contribution in reducing plasma glucose concentration. It has been suggested that there were three mechanisms of reducing blood glucose by SCFA. Acetate and propionate increase the buffering capacity and it will decrease fatty acid and resulted in decreasing of lipid storage with the end effect of increasing insulin sensitivity. Acetate and butyrate also increase the Adenosine Monophosphate Kinase (AMPK) resulted in increasing glucose uptake and therefore decreasing the blood glucose concentration. The last mechanism butyrate increase the activity of glucose transporter (GLUT4), resulted in the increasing of insulin sensitivity and decreasing of the blood glucose concentration.

Keyword: resistant starch, fermentation, SCFA, insulin sensitivity, glucose transporter (GLUT4).

Introduction

Historically, it was believed that starch, the primary complex carbohydrate and energy source of any plant foods was completely digested in the small intestine. However, since early of 1980 it was recognised that starch digestion may be incomplete. A significant fraction of starch may escape digestion in the small intestine of human and pass through to the colon. This fraction is called resistant starch and is fermented in the colon in exactly the same way as non starch polysaccharides and with the same end products (Englyst and Cummings, 1987). Therefore, resistant starch (RS) is defined as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (Asp, 1992). As the starch is not completely digested it was suggested that starches was classified according to the susceptibility to digestion. Englyst *et al.* (1992) proposed a nutritional classification of starches based on the the rapidity with which glucose is released from a food source under specified laboratory conditions. Based on the susceptibility to digestion total starch (TS) is divided into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Total starch (TS) is measured as the yield of glucose from finely milled or homogenised sample of the food in which the starch is completely gelatinised at 100° C, treated with potassium hydroxide to ensure complete dispersion of the starch to an amorphous, digestible form and then enzymatically digested with pancreatin and amyloglucosidase. RDS

consists mainly of amorphous and dispersed starch. It is typically found in high amounts in starchy foods which have been cooked by moist heat e.g. bread and potatoes. It is measured chemically as the starch which is converted to the constituent glucose molecules in 20 minutes of enzyme digestion. SDS, like RDS is expected to be completely digested in the small intestine but, for one reason or some reasons, it is digested more slowly. It is measured chemically as the starch converted to glucose after a further 100 minutes enzyme digestion (total 120 minutes digestion). RS is the starch which may potentially resist digestion in the small intestine. It is measured chemically as the difference total starch (TS) and the sum of RDS plus SDS.

Formerly, it was only 3 types of RS including physically inaccessible starch (RS-1), resistant starch granules (RS-2) and retrograded starch (RS-3) (Englyst *et al.*, 1992). RS-4 (modified starch) was then introduced as other type of RS. The RS4 formed by cross-linking, is also known to be stable when treated by enzyme or acid (Mun and Shin, 2006). The description of RS was shown in Table 1. RS1: The starch is physically inaccessible to digestion due to intact cell wall. RS2: Native starch granules protected from digestion by the structure of the starch granule. RS3 retrograded starch, was formed as an effect of processing and RS4 is chemically modified starch, do not occur naturally but are created to be resistant to digestion. In the last five years it is also known other type of RS, called RS5. Basically, RS5 is a complex of amylo-lipid.

Table 1. Classification of type of resistant starch (RS), food source, and factors affecting their resistance to digestion in the colon

Type of RS	Description	Food sources	Resistance minimized by
RS1	Physically protected	Whole or partly milled grains and seeds, legumes	Milling, chewing
RS2	Ungelatinized resistant granules with type B crystallinity, slowly hydrolyzed by α -amilase	Raw potatoes, green bananas, some legumes, high amylose corn	Food processing and cooking
RS3	Retrograded starch	Cooked and cooled potatoes, bread, cornflakes, food products with repeated moist heat treatment	Processing conditions
RS4	Chemically modified starches due to cross-linking with chemical reagents.	Food in which modified starches have been used (for example, bread, cakes)	Less susceptible to digestibility in vitro

Nugent (2005)

The presence of RS can be influenced by some factor including processing (cooking, cooling), the type of starch (amylose or amylopectin or the ratio of amylose-amylopectin), the physical state of the starch (degree of hydration, particle size) and the presence of other components such as dietary fibre, protein and lipids (Anonymous, 1990).

Physicochemical properties and physiological effect of RS

According to the definition of dietary fibre (Anonymous, 2001) RS is part of dietary fibre. Part of the physicochemical properties of RS is similar to that of non starch polysaccharides (NSP). The physicochemical properties of NSP includes incorporation into plant cell wall, increased viscosity, increase water holding capacity, increased particle size, and increases susceptibility to bacterial

fermentation (Anonymous, 1990). Amongst these properties, the important physicochemical properties which may influence on the physiological effects are increases the viscosity, water holding capacity and fermentation.

Viscosity is affected by concentration, temperature, ionic concentration, pH, hydrophilic or hydrophobic properties, particle size, and association with protein. Cooking can alter the viscous nature of a polysaccharide solution by rearranging the physical configuration. The addition of purified viscous polysaccharides to meals or drinks of glucose reduces the rate of absorption and decreases post-prandial plasma glucose concentrations. It seems likely that the effect is brought about by the resistance of the viscous sols to the flow induced by gastrointestinal motility (Blackburn *et al.*, 1984). The viscosity of most polysaccharide sols is lost in the colon when the colonic bacteria ferment the polysaccharide and produce short chain fatty acids (SCFA). However, the bacterial enzymes necessary for fermentation of the more closely packed substances, such as xanthan gum, are not present in all people, and there may be insufficient time available for the fermentation of others to take place. Thus the viscous nature of the luminal content may influence colonic mixing and transit (Anonymous, 1990).

The water holding capacity of polysaccharides is a measure of the ability of a polysaccharide to immobilise water within its matrix. Such water will influence the metabolic activity of the polysaccharide along the gut. The amount of trapped water present in polysaccharide depends on the source of polysaccharide, mode of preparation and the method of measurement (Anonymous, 1990). Viscous polysaccharides have a greater water holding capacity, and may dilute and disperse intestinal contents causing distension and encouraging propulsive motor activity. In the case of highly viscous solutions this effect would be counter-balanced by the inhibitory effect of an increased luminal viscosity on propulsion. In the colon, the correlation between the water holding capacity and the biological effect of the polysaccharides on faecal bulking is affected by the fermentation of the polysaccharide. Polyphenolic materials in the polysaccharides make them resistant to fermentation by virtue of the cross linkages.

A review on health properties of resistant starch done by Nugent (2005) reported that there are nine potential physiological effects of resistant starch including (1) improves glycaemic and insulinaemic responses, (2) improved bowel health, (3) improved blood lipid profile, (4) prebiotic and culture protagonist, (5) increased satiety and reduced energy intake, (6) increased micronutrient absorption, (7) adjunct to oral rehydration therapies, (8) synergistic interactions with other dietary components, e.g. dietary fibres, proteins and lipids, (9) thermogenesis. The physiological effect of improving glycaemic and insulinaemic responses is related to diabetes, impaired glucose and insulin responses and the metabolic syndrome which are the most highly prevalence diseases. How are this effect happened will be described in this paper.

Short chain fatty acids (SCFAs)

Important physiological effects of RS are due not to the RS itself but to the end-product of the bacterial fermentation. Short chain fatty acids (SCFA) are the metabolic products of anaerobic bacterial fermentation of dietary fibre including non starch polysaccharides, oligosaccharides and resistant starch. The principal SCFA are acetate, propionate and butyrate, while isobutyrate, valerate and isovalerate make a minor contribution (Fleming and Arce, 1986, Cummings and Bingham, 1987). Fermentation of dietary

fibre also yields gases (CO₂, CH₄ and H₂) and an increased of bacterial mass. Pathway of SCFA production in the colon can be seen in Figure 1.

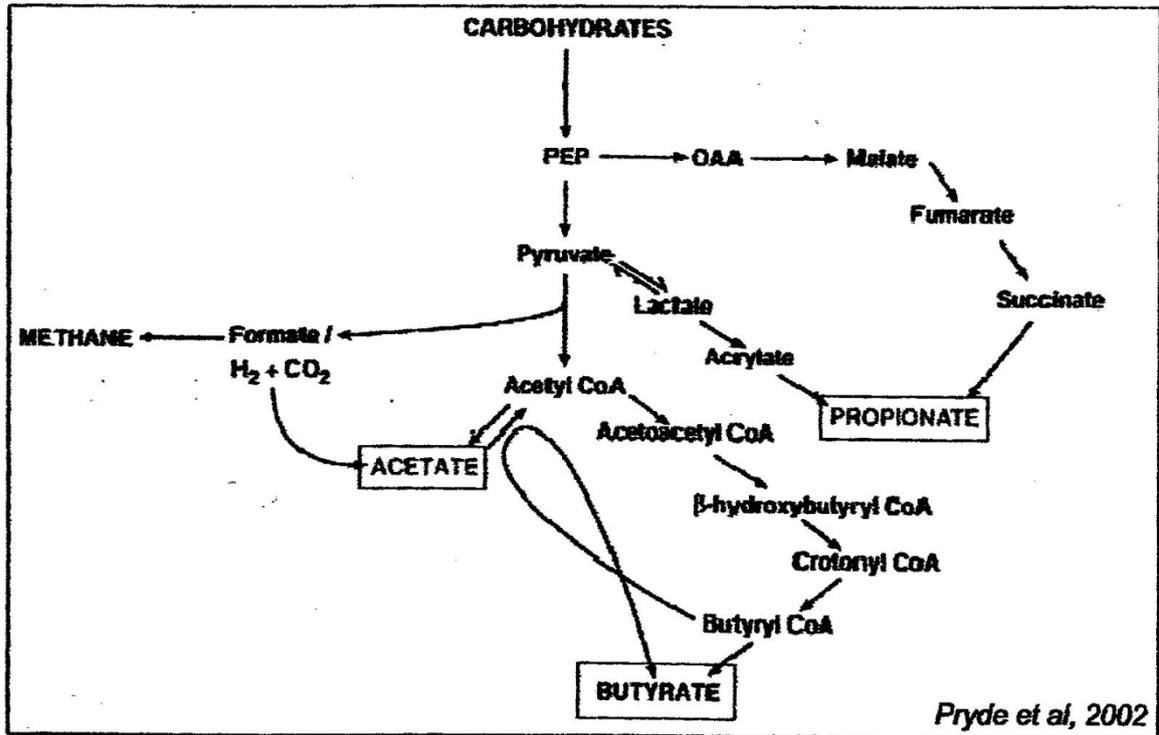


Figure 1. Pathway of SCFA production in the colon

The production and proportion of SCFA depend on type of the undigestible and particle size of material passed through to the colon (Marsono and Topping, 1995). It was believed that RS was potential in producing butyrate. SCFA have been proposed as mediators of some of the physiological effects of fibre e.g. regulation of hepatic carbohydrate and lipid metabolism (Chen *et al.*, 1984) and contributors to metabolisable energy in rats (Illman *et al.*, 1982) and in pigs (Topping *et al.*, 1985). Acetate can inhibit cholesterolgenesis and is lipolitic in rats; in humans it can decrease the availability free fatty acids (FFA). High concentrations of FFA are associated with decreased insulin sensitivity and impaired glucose uptake. Propionate is an effective inhibitor of fatty acid and cholesterol synthesis *in vitro* (Beynen *et al.*, 1982; Berggren *et al.*, 1996). Butyrate, one of the major end products of RS fermentation has been hypothesized to have protective against colorectal cancer by suppressing abnormal cell growth (Cummings and Bingham, 1987) and by interfering with the promotion phase of carcinogenesis (Bright-See, 1988). Butyrate was also reported to have a beneficial effect for the diabetics in reducing plasma glucose concentration by increasing the insulin sensitivity (Gao *et al.*, 2009, Canfora *et al.*, 2015). Other important consequences of SCFA production in the colon are: (1) decreased pH in the colon which may affect microbial metabolism and (2) increased contribution of bacterial cells to fecal bulk (Schneeman, 1986).

Possible mechanism of resistant starch (RS) in reducing plasma glucose concentration

It is mentioned previously that improve glycaemic and insulinaemic responses is one of the important physiological effect of RS. The effects are related to diabetes, impaired glucose and insulin responses and the metabolic syndrome. Based on the physicochemical properties and the production of SCFA during fermentation, it is suggested that the mechanism how RS reducing plasma glucose concentration are due to the RS itself and fermentation end product, SCFA.

1. Resistant starch has a low bioavailability of glucose
Resistant starch is undigestible starch OR only partially digested in the small intestine. It means RS has a low bioavailability of glucose, resulted in less marked rises of blood glucose or flat response glucose
2. High intakes of RS increase the viscosity of the digesta or intestinal content. Viscous digesta tend to delay mouth to caecum transit. It could reduce the rate of digestion of starchy food. It will also result in less marked rises of blood glucose or flat response glucose or lower the plasma glucose concentration.
3. Viscous digesta will reduce epithelial access and decrease the absorption glucose, resulted in lower plasma glucose concentration.
4. It has been reported that RS decrease glucose concentration by increasing insulin sensitivity, through alteration in fatty acid flux. Increasing the fatty acids flux resulted in development of insulin resistant. SCFA produced during RS fermentation inhibit adipose tissue lipolysis. (Robertson *et al.*, 2005, Johnston *et al.*, 2010).
5. Fermentation of RS produces SCFA. The presence of SCFA inhibit lipolysis in the adipose tissue and reduce the concentration of fatty Acid, resulted in the increasing of insulin sensitivity (Gao *et al.*, 2009))
6. It is also proposed that SCFA increase the activity of glucose transporter GLUT4, resulted in increasing glucose uptake (Conforra *et al.*, 2015)
7. Butyric acid increase the activity of glucose transporter (GLUT4), resulted in increasing of insulin sensitivity and decrease glucose concentration (Conforra *et al.*, 2015)

Conclusion

Resistant starch, the undigestible starch in the diet escapes digestion in the small intestine and passes through to the colon. The important physical properties of RS which may have important physiological effects are high viscosity and water holding capacity. Fermentation of RS in the colon produces SCFA and also yields gases (CO₂, CH₄ and H₂). Of the SCFA acetate, propionate and butyrate are the principal products. The possible mechanisms of lowering plasma glucose concentrations are due to the RS itself and to the end-product of the bacterial fermentation in the colon. RS has a low bioavailability of glucose, delay carbohydrates digestion and inhibit glucose absorption. In addition RS increase the insulin sensitivity. SCFAs contributes a significant role in lowering plasma glucose concentration by increasing insulin sensitivity and glucose transporter (GLUT4) resulted in increasing glucose up take to the muscle.

References

- Anonymous. 1990. Complex Carbohydrates in Foods. *The report of the British Nutrition Foundation's task force*. Chapman and Hall, London.
- Anonymous. 2001. AACC Report: The definition of dietary fiber. *Cereal Foods World* 46(3):112-126.
- Asp, N-G. 1992. Resistant Starch. *Eur. J. Clin. Nutr.* 46 (suppl 2): S1.
- Berggren, A.M., Nyman M.G.L. and Lundquist. 1996. Influence of orally and rectally administered propionate on cholesterol and glucose metabolism in obese rats. *Br. J. Nutr.* 76:287-94.
- Beynen A.C., Buecler K. and Van Der Molen A.J. 1982. The effects of lactate and acetate on fatty acid and cholesterol biosynthesis by isolated rat hepatocytes. *Int. J. Biochem.* 14:165-9.
- Blackburn, N.A., Redfern, J.S., Jarjis, M., Holgate, A.M., Hanning, I., Scarpello, J.H., Johnson, I.T., Read, N.W. 1984. The mechanism of action of guar gum in improving glucose tolerance in man. *Clin. Sci.*, 66 (3): 329-36
- Bright-See, E., 1988. Dietary fiber and cancer. *Nutr. Today*, July/August: 4-10.
- Canfora E.E., Jocken J.W., Blaak E.E. 2015. Short-Chain fatty acids in control of body weight and insulin sensitivity. *Nature Rev Endocrin.* 11: 577-591.
- Chen, J.W.L., Anderso, J.W. and Jennings, D. 1984. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fat rats. *Proc. Soc. Exp. Biol. Med.*, 75: 215-218.
- Cummings, J.H. and Bingham, S.A. 1987. Dietary fibre, fermentation and large bowel cancer. *Cancer Surveys* 6: 601-621.
- Englyst H.N., Kingman S.M., and Cummings, J.H. 1992. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 46 (suppl 2): S33-S50.
- Fleming, S.E., and Arce, D.E. 1986. Volatile fatty acids: their production, absorption, utilization, and roles in human health. *Clin. Gastroenterol.* 15: 787-814.
- Gao Z., Yin, J, Zhang J., Ward RD., Martin RJ., Lefevre M., Cefalu WT., Ye J. 2009. Butyrate improves insulin sensitivity and increase energy expenditure in mice. *Diabetes* 58(7): 1509-17.
- Illman, R.J., Trimble, R.P., Snoswell, A.M. and Topping, D.L. 1982. Daily variations in the concentrations of volatile fatty acids in the splanchnic blood vessel of rats fed diets high in pectin and bran. *Nut. Rep. In.* 26: 349-446.
- Johnston KL., Thomas EL., Bell JD, Frost GS., Robertson MD. 2010. RS improves insulin sensitivity in metabolic syndrome. *Diabet Med* 27 (4): 391-7.
- Marsono, Y. and Topping, D.L. 1999. Effects of particle size of rice on resistant starch and SCFA of the digesta in caestomised pigs. *Indonesian Food and Nutr. Progr* 6 (2):44-50
- Mun S-H and Shin, M. 2006. Mild hydrolysis of resistant starch from maize. *Food Chem.* 96: 115-121.
- Nugent A.P. 2005. Health properties of resistant starch. *British Nutrition Foundation Nutr. Bull.* 30: 27-54.
- Pryde, S.E., Duncan, S.H., Hold, G.L., Stewart, C.S., Flint, H.J. 2002. The microbiology of butyrate formation in the human colon. *FEMS Microbiology Letters* 217: 133-139.
- Robertson, M.D., Bickerton, A.S., Dennis, A.L., Vidal, H., and Frayn, K.N. 2005. Insulin sensitivity effects of dietary RS and effects on skeletal muscle and adipose tissue metabolism. *Am. J. Clin Nutr.* 82(3): 559-567.
- Schneeman, B.O. 1986. Dietary fibre: Physical and chemical properties, methods of analysis, and physiological effects. *Food Tech.* Feb. 1986:104-110.
- Topping, D.L., Illman, R.J., Taylor, M.N. and McIntosh, G.H. 1985. Effects of wheat bran and porridge oats on hepatic portal venous volatile fatty acids in pig. *Ann. Nutr. Metab.* 29: 325-309.

Equations Models to Estimate Basal Metabolic Rate Based on Body Weight and Height of Indonesian Young Adults

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Abstract

Basal metabolic rate (BMR) can be measured directly and indirectly. Direct measurement of BMR required an expensive tool and tedious effort. This study is aimed at developing an indirect and simple equation model to estimate basal metabolic rate based on body weight and height of Indonesian young adults. For this study, 98 Indonesian young adults (51 males and 47 females) aged 19-29 years old come from various city of Indonesia. Each subject was measured his/her body weight (BW), body height (BH), body fat (BF), body water (BW), body muscle (BM), and BMR using bioelectrical impedance (BIA) method by InBody 230 body composition analyzer. Two equation models were developed based on BW, BH, Sex, and Age to estimate the BMR. The results showed that the two equation models, firstly using BW, BH, and Sex as independent variables, secondly using BW, BH, Sex, and Age as independent variables were strongly correlated with BMR ($r_1^2 = 0.946$, $r_2^2 = 0.945$). The estimated BMR from this equation model was lower than estimated BMR from Oxford equation model by Henry for global wide adults, and higher than estimated BMR from the equation model by Ismail for Malaysians.

Keywords: basal metabolic rate, bioelectrical impedance, energy basal metabolism, equation model, Indonesian adults

Introduction

Energy requirement of each person is required in order to assess individual energy intake. Energy requirement for an adult is the summation of his/her basal energy or basal metabolic rate (BMR) and energy activity. The large component of energy requirement is BMR, which is the minimum amount of energy required to keep body functioning, including the heart, lungs and temperature regulation (FAO/WHO/UNU 2003).

For healthy people, BMR is affected by body composition and body size, including fat free mass, fat mass, BW and BH, as well as age, sex, body temperature, diet, hormone, and climate (FAO/WHO, 1985; FAO/WHO/UNU, 2003; Johnstone *et al.*, 2005; Sabounchi *et al.*, 2013). The higher the body temperature the higher the BMR. People with low food intake and low animal protein intake have lower BMR. BMR of young adults is greater than old adults. Females have been found to have lower BMR than males. Western people on going to tropics show a fall in BMR. Higher thyroid hormone raises the BMR (Johnstone *et al.*, 2005)

Energy requirement can be measured directly and indirectly. Direct measure of BMR requires a special apparatus, such as to measure the oxygen consumption of the body (Nieman *et al.*, 2003). During the test the subject should be seated or reclining comfortably in a quiet room set at a neutral temperature, and not speaking, reading or watching television. It is required an expensive tool, time consuming, and tedious effort. One of the methods to measure indirectly the BMR is by formulating a predictive equation model to estimate BMR based on primary data of BMR.

Some have developed an indirect measure of BMR based on the data resulted from the direct

measure of BMR through a predictive regression model for BMR using diverse independent variables, mostly body weight (BW), body height (BH), body mass index (BMI), age, and sex. About a century ago, the first equation model to estimate BMR was developed by Harris and Benedict (1918). According to Sabouchiet *et al.*, (2013), there were 248 BMR estimation equations had been developed for specific population and race, age and sex groups. Few of them done through the application of the meta-analysis method with a large sample size, in which the results can be applied globally, such as the Harris-Benedict revised equation (Roza and Shizgal, 1984), the Schofield equation (FAO/WHO/UNU, 1985), the IOM equation (2002), and the Henry or Oxford equation (FAO/WHO/UNU, 2003;Henry, 2005).

The Harris-Benedict equation, the Schofield equations were derived from database contained relatively few subjects from the tropical region and Asia, but not for the IOM equation and Henry equation. Previous energy requirement formulated for Indonesians by the Ministry of Health of Indonesia (MOHI), was derived from the Schofield equation (FAO/WHO/UNU, 1985), which was overestimated for Asian people. Current energy requirement formulated for Indonesians by MOHI were derived from Henry equation and IOM equation (Hardinsyah *et al.*, 2012), but some Indonesian nutritionists commented that there results are still overestimated. Even they are lower than the results from the Schofield equation.

In developing countries, such as Indonesia, the apparatus for measuring directly or indirectly BMR is not commonly available, except in the high class hospital in urban areas. Therefore, the predictive equation model to estimate BMR with high validity is required. Up till now there is no studies have been done to develop a simple predictive equation model of BMR for Indonesians. The only study on this field in Indonesia was done among predominantly obese Javanese women aged 40-60 yrs based on body fat which is invasive and not simple to measure (Salma *et al.*, 2016). Therefore, this study is aimed at developing an indirect and simple equation model to estimate basal metabolic rate based on body weight and height of Indonesian young adults.

Methods

Subjects

The subjects of this study are 98 Indonesian young adults aged 19-29 years old come from various parts of Indonesia (Java, Sumatra, Kalimantan, Sulawesi and Nusa Tenggara). The subjects consist of both males and females namely 51 males and 47 females.

Data Collection and Measurement

Each subject was measured his/her body height (BH), body weight (BW), body fat (BF), body water (BW), body muscle (BM), and BMR. The BH of the subjects were measured stature metre. The BW, BF, BW, BM, BMR of the subjects were measured using body composition analyzer of bioelectrical impedance (BIA) method by InBody 230.

Statistical Analysis

Although fat free mass, fat mass, waist and hip circumference are significant predictors of BMR, these variables not commonly available because required a special apparatus to measure them. According to FAO/WHO/UNU (2001), Henry (2005) and Sabounchi *et al.*, (2013), estimating BMR from BW and BH BW are strongly feasible and practical. Therefore, the present study used BW, BH, Sex and Age variables to estimate BMR. Two equation models were developed based on BW and BH to estimate the BMR. Since the age group of the subjects on this study is very narrow and sample sizes were not large enough, then the variables of age and sex were included into independent variables to estimate BMR.

Results and discussion

The subjects of the present study were Indonesian young adults aged 19-29 years old. The males body weight (BW) was heavier about 6.5 kg than females; and the males body height (BH) was higher about 11.1 cm than females, but the body mass index (BMI) of males was similar to females (Table 1).

The mean BW, BH and BMI of this study were similar to the mean BW, BH and BMI of the previous study based on the national survey data of Indonesia but for adults aged 20-39 yrs. The mean BW, BH and BMI of males was 58.7 kg, 163.4 cm and 22.0 kg/m², respectively; and the mean BW, BH and BMI of females was 53.2 kg, 152.5 cm and 22.9 kg/m², respectively (Hardinsyah *et al.*, 2012). A study among Malaysian young adults (18-30 yrs) done by Ismail *et al.* (1998) also had lower results of BW namely 58.6 kg for males and 49.8 kg for females but similar results of mean BH namely 1.64 m for males and 1.54 m for females.

Table 1. Characteristic of the subjects

No.	Variables	Unit	mean±SD (median)		
			Males	Females	Total
1.	Age	Year	21.53±2.14 (21.00)	21.53±1.97 (22.00)	21.53±2.06 (22.00)
2.	Body Weight (BW)	Kg	58.22±6.60 (57.20)	51.73±6.87 (51.30)	55.11±7.43 (54.35)
3.	Body Height (BH)	Cm	165.81±5.69(165.80)	154.73±6.06 (154.70)	160.50±8.06 (160.50)
		M	1.66±0.06 (1.48)	1.54 ± 0.06 (1.55)	1.61±0.08 (1.61)
4.	Body Mass Index (BMI)	kg/m ²	21.25±1.83(21.20)	21.60±1.97 (21.10)	21.41±1.90 (21.15)

The results of the regression equation models to estimate BMR for Indonesian young adults was shown in Table 2. Considering the values of determination coefficients, regardless the AGE variable, the two BMR equation models were very valid ($r^2 = 0.946$, $r^2 = 0.945$). The inclusion of AGE variable into the BMR equation model did not affect the validity. This occurred because the age range of the subject was not wide, only from 19 to 29 yrs. In practice, the first BMR equation model without AGE variable is the simplest.

Compared to the previous studies that developing BMR equation model from BW and BH (Henry, 2005) and from BW and AGE (Ismail *et al.*, 1998), the present study had significantly higher determination coefficient (r^2), which implies it is more valid equation model. This can be explained by

two reasons. First, the BH variable was not included in the BMR equation model of Ismail (Ismail *et al.* 1998). While according to Sabounchi *et al.* (2013) and McMurray (2014), BW and BH was predominantly determined BMR. Second, different methods used to measure BMR of the different studies. Ismail study measured BMR using Douglas bag. Henry study used secondary data of BMR with a larger sample size from Caucasian and different method of BMR measurement (Henry, 2005). Since Indonesia is a neighboring country of Malaysia and Indonesians body composition are similar to Malaysians, and Indonesians is part of the Asians, then this study also comparing the results of the BMR equation model of the present study with BMR equation model of Henry (Henry, 2005) for global populations include Asian, and the BMR equation model of Ismail for Malaysians (Ismail *et al.*, 1998). By applying all three BMR equation models into the subjects of this study, the mean BMR of each of the BMR equation models was produced, as shown in Table 3.

Table 2. Two alternative regression equation models to estimate BMR (kcal)

Regression models of the present study to estimate BMR	r^2
1. $BMR_1 = -57.195 - 150.895 \text{ SEX} + 6.433 \text{ BW} + 737.575 \text{ BH}$	0.946
2. $BMR_2 = -19.050 - 151.643 \text{ SEX} + 6.481 \text{ BW} + 728.070 \text{ BH} - 1.134 \text{ AGE}$	0.945
Regression models of the previous studies to estimate BMR by Henry and by Ismail	r^2
$BMR_{\text{Henry}} = 113 + 14.4\text{BW} + 313\text{BH}$ (for males 18-30 yrs)	0.584
$BMR_{\text{Henry}} = -282 + 10.4\text{BW} + 615 \text{ BH}$ (for females 18-30 yrs)	0.524
$BMR_{\text{Ismail}} = (3.083 + 0.047\text{BW} - 0.035\text{AGE}) \times 239.006$ (for males 18-60 yrs)	0.291
$BMR_{\text{Ismail}} = (1.985 + 0.054\text{BW} - 0.027\text{AGE}) \times 239.006$ (for females 18-60 yrs)	0.262

*BW in kilogram and BH in meter

Table 3. Comparing BMR values from present study and study for Asians (Henry) and Malaysians (Ismail)

BMR equation models	BMR (kcal)	
	Mean \pm SD (Median)	
	Males	Females
Kcal/person/day		
I. The presents study models (Hardinsyah et al, 2016)		
Model 1	1389.49 \pm 76.31 (1399.00)	1115.06 \pm 82.64 (1111.00)
Model 2	1389.51 \pm 76.23 (1399.00)	1115.00 \pm 82.92 (1110.00)
II. Henry equation models		
	1470.45 \pm 106.86 (1466.00)	1207.55 \pm 101.88 (1211.00)
III. Ismail equation models		
	1210.86 \pm 74.84 (1206.00)	1003.09 \pm 91.17 (998.00)
Kcal/kg BW/day		
I. The presents study models (Hardinsyah et al, 2016)		
Model 1	1389.49 \pm 76.31 (1399.00)	1115.06 \pm 82.64 (1111.00)
Model 2	1389.51 \pm 76.23 (1399.00)	1115.00 \pm 82.92 (1110.00)
II. Henry equation models		
	1470.45 \pm 106.86 (1466.00)	1207.55 \pm 101.88 (1211.00)
III. Ismail equation models		
	1210.86 \pm 74.84 (1206.00)	1003.09 \pm 91.17 (998.00)

The estimated mean BMR from the present study was lower about 7% than estimated mean BMR from the Oxford equation model by Henry for global wide adults include for Asians. Comparing with BMR equation model by Ismail for Malaysians, the estimated mean BMR from the present study was higher about 13%. It was realized that the Ismail BMR equation model had very lower validity among those equation shown in Table 3 ($r^2=0.291$ for males and $r^2=0.262$ for females), as well as the study was done about 20 years ago in which the body composition of Malaysians at that time may not the same as now.

The weakness of this study was that the small sample size and limited age range (19-29 yrs) of the subjects of the study. Further studies are required with a larger sample size for both younger age groups and older age groups. Regardless the weakness, this is the first study to develop a practical indirect method to estimate BMR for Indonesian young adults with a strongest validity.

Conclusion

The BMR of young Indonesian adults validly and practically estimated indirectly by BW (kg) and BH (m) and Sex with R^2 values of 0.946. The estimated BMR from this equation model was lower than estimated BMR from Oxford equation model by Henry for global wide adults and higher than estimated BMR from the equation model by Ismail for Malaysians. Further studies required to estimate BMR indirectly, validly and practically for younger ages and older ages.

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References

- FAO/WHO/UNU. 1985. *Energy and protein requirements* : Report of a Joint FAO/WHO/UNU Expert Consultation Rome 5- 17 October 1981. FAO. Rome
- FAO/WHO/UNU. 2003. *Human energy requirements*. Report of a Joint FAO/WHO/UNU Expert Consultation. Rome, 17–24 October 2001. FAO. Rome
- Hardinsyah, Anies Irawati, Djoko Kartono, Sri Prihatini, Itni Linorita, Lucy Amilia, Mutia Fernanda, Ezria E Adyas, Desi Yudianti, Clara M Kusharto, Yayat Heryatno. 2012. *Pola konsumsi pangan dan gizi penduduk indonesia berdasarkan data Riset Kesehatan Dasar 2010*. Departemen Gizi Masyarakat FEMA IPB dan Badan Penelitian dan Pengembangan Kesehatan. Kemenkes RI
- Hardinsyah, Riyadi H, Tambunan V. 2012. "Kecukupan Energi, Protein, Lemak dan Karbohidrat". *Proceedings Widya Karya Nasional Pangan dan Gizi*. LIPI. Jakarta.
- Harris J, Benedict F. 1918. "A Biometric Study of Human Basal Metabolism". *PNAS* 4 (12): 370–3. Bibcode:1918PNAS.4.370H. PMC 1091498. PMID 16576330. doi:10.1073/pnas.4.12.370
- Hayter JE1, Henry CJ. 1993. "Basal metabolic rate in human subjects migrating between tropical and temperate regions: a longitudinal study and review of previous work". *Eur J Clin Nutr.* ;47(10): pp. 724-34.
- Henry, CJK. 2005. "Basal metabolic rate studies in humans: measurement and development of new equations". *Public Health Nutrition*, 8 (7):. 1133-1152. DOI: <http://dx.doi.org/10.1079/PHN2005801>. Published online: 01 January 2007.
- Ismail MN, Ng KK, Chee SS, Roslee R, and Zawiah H. 1998. "Predictive equations for the estimation of basal metabolic rate in Malaysian adults". *Mal J Nutr* ;4: 81-90.
- Johnstone, AM, Murison, SD, Duncan, JS, Rance, KA and Speakman, JR. 2005. "Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine" *Am J Clin Nutr*; 82(5): 941-948.
- McMurray RG, Soares J, Caspersen CJ, McCurdy T. 2014. "Examining variations of resting metabolic rate of adults: a public health perspective". *Medicine and Science in Sports and Exercise*. 46(7): 1352–1358.

- Mifflin, MD, St Jeor, ST, Hill, LA, Scott, BJ, Daugherty, SA, Koh, YO. 1990. "A new predictive equation for resting energy expenditure in healthy individuals". *The American journal of clinical nutrition* 51 (2): 241–7. PMID 2305711.
- Nieman, DC, Trone, GA, Austin, MD. 2003. "A new handheld device for measuring resting metabolic rate and oxygen consumption". *J Am Diet Assoc.* 10: 588-593.
- Roza, AM, Shizgal, HM. 1984. "The Harris Benedict equation reevaluated: resting energy requirements and the body cell mass". *The American Journal of Clinical Nutrition.* 40: 168–182.
- Sabouchi, NS, Rahmandad, H, and Ammerman, A. 2013. "Best Fitting Prediction Equations for Basal Metabolic Rate: Informing Obesity Interventions in Diverse Populations". *Int J Obes (Lond)*. 2013 Oct; 37(10): 1364–1370. Published online 2013 Jan 15. doi: 10.1038/ijo.2012.218.
- Salma S, Sudargo T, Rahmawati NT. 2016. Correlation Between Body Fat Percentage (BFP) and Basal Energy Expenditure (BEE) in Javanese Women Aged 40-60 Years Old in Sleman, Yogyakarta, Indonesia. *Pakistan Journal of Nutrition*, 15(3): 260-263
- Soares MJ, Francis DG, Shetty PS. (1993). "Predictive equations for basal metabolic rates of Indian males". *Eur J Clin Nutr.*47(6): 389-94.

**The difference on under nutrition children risks
in Mojowarno subdistrict, Jombang Regency
(Studies in Under and Normal Nutrition Children, Mother Nutritional Knowledge Level,
Energy-Protein Consumption and Protein Foods Quality)**

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Abstract

This study aimed to determine the difference between under-nutrition children risks in Mojowarno Subdistrict, Jombang Regency. This study involved a group of under-nutrition and a group of normal nutrition children to determine the risk of mother nutritional knowledge level, energy-protein consumption and protein foods quality. This of research included in the observational study with case control design. Where the case is a group of under-nutrition children and the control group is normal nutrition children based on BB/U-index. Each group consisted of 30 respondents. This study used a questionnaire to determine the level of nutritional knowledge of mothers of each group, 24 hours food recall to obtain data on the level of energy consumption and protein as well as the quality of food protein (SAA and MCT). Data were analyzed using Odds Ratio Test and Independent T-Test as well as the calculation of protein quality in a way that is theoretically Amino Acid Score (SAA) and Theoretical Digest Quality (MCT). Data analysis showed statistically significant differences in the level OR of mother nutritional knowledge level, energy-protein consumption among under and normal children status. There were no significant differences at the level of the OR energy consumption and protein based nutritional knowledge level of the mother and there were no significant differences in the average quality of protein (SAA and MCT) among under and normal children status in Mojowarno, Jombang. However descriptively there is a difference between groups of under and normal children status caused by slight variations in the diversification of food sources of protein consumed. The level of mother nutritional knowledge level, energy and protein consumption level have a higher risk to under-nutrition children than normal nutrition children. The diversity of food that consumed determines the value of protein quality in food.

Keywords: Under Nutrition, Normal Nutrition, Mother Nutritional Knowledge Level, Energy and Protein Consumption and Protein Quality of Food.

Introduction

Nutritional problems in Indonesia and in developing countries, in general, is still dominated by the problems of Protein Energy Under-nutrition, Anaemia Iron Deficiency, Iodine Deficiency Disorder, Vitamin A Deficiency and the problem of obesity, especially in big cities (Supariasa, 2012). Nutritional problems that arise have an impact on the quality of Human Resources (Khomsan, 2012).

Riskesdas 2013 shows that nationally 19.6% of the Indonesian population are under-nutrition. The number of under-nutrition in East Java Province was 18.1%. Although the figure is still below the national average, but when compared with the MDG targets by 2015 that is 15.5%, the incidence of under-nutrition in East Java Province is still to be revealed. Preliminary data study in Jombang Health Office said that the incidence of under-nutrition was 7.04%. Although the figure is still below the national average and the province, but there is one district, namely Mojowarno, the number of under-nutrition children under five years old was 56.3%. Classification of poor households in Jombang 2014 Mojowarno included in the ten districts with the poorest households (Inayah, 2014). Based on the background above, this research aims were to investigate whether there are differences in the risk of under-nutrition children

under five years old in Mojowarno Jombang by studies on groups of under-nutrition and normal nutrition with mother nutritional knowledge, energy and protein consumption and protein quality of food.

Materials and Methods

This study used a questionnaire to determine the level of nutritional knowledge of mothers of each group, 24 hours food recall to obtain data on the level of energy consumption and protein as well as the quality of food protein, that is Amino Acid Score and Protein Digest Quality (SAA and MCT). This kind of research included in the observational study with case control design. Where the case is a group of under-nutrition children and the control group is normal nutrition children based on BB/U-index.

Statistical Analysis

Data were analyzed using Odds Ratio Test and Independent T-Test as well as the calculation of protein quality in a way that is theoretically Amino Acid Score (SAA) and Theoretical Digest Quality (MCT).

Result and discussion

Mojowarno located on the edge of Jombang. On the east by the Ngoro District, west bordering Diwek and the north bordering the District of Mojoagung.



Figure 1. The Distribution of Mother Nutritional Knowledge Level

Figure 1 shows that the number of mothers with sufficient nutrition knowledge level more in the normal-nutrition group in the amount of 27 respondents (90%), whereas in the group of under-nutrition children by 17 respondents (56.7%). A number of mothers with low (less) knowledge level found in the under-nutrition group, 13 respondents (43.4%) and 3 respondents (10%) in the group of normal-nutrition. The results of the questionnaires, the level of nutrition knowledge of mother obtained an average value of a group of under-nutrition children is 58 and a group of normal-nutrition is 73.

It shows that the level of nutrition knowledge of mothers of under-nutrition children group is lower than the group of normal-nutrition and most levels of maternal nutrition knowledge lacking match group under-nutrition children. Lack of Mother Nutritional Knowledge Level can lead to an error in preparing food for infants, affecting the nutritional status of children.

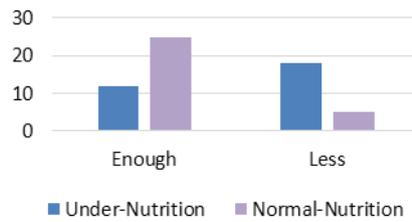


Figure 2. The Distribution of Energy Consumption Level

Figure 2 shows that the group of under-nutrition children as much as 15 respondents (50%) identified as the level of energy consumption enough and 15 other respondents categorized the level of energy consumption is less. Whereas in the group normal-nutrition level of energy consumption by category pretty much as 25 respondents (83.3%) and 5 respondents (16.7%) are in the poor category. This illustrates that the level of energy consumption by category pretty much more common in infants with normal-nutrition. The average value of energy consumption levels in the group of under-nutrition children classified as less in the amount of 79.3% (892 kcal), while the group is quite normal-nutrition that is equal to 106.8% (1201.3 kcal). The results show that the energy consumption level group of under-nutrition children still under Figures Nutritional Adequacy, lack of energy consumed from food sources of energy can cause the body to obtain energy from food resources of protein's fat, if it lasts for a long time, the body will lose body mass is causing nutritional status of children becomes less.

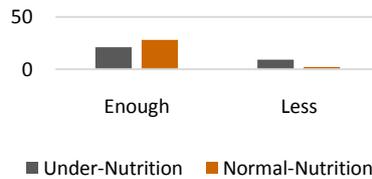


Figure 3. The Distribution of Protein Consumption Level

Figure 3 shows that the level of protein intake as much as 29 respondents (96.6%) group is in the category of nutritional well enough, while the under-nutrition group was 21 respondents (70%). The level of protein intake by as much as 9 less category of respondents (30%) was from the group of under-nutrition children, while one respondent (3.4%) from the group of normal-nutrition. The average value of protein consumption level group of under-nutrition children and nutrition were 118.3% (30.8 grams) and 155.4 % (40.4 grams). Although the average value of protein consumption level both groups is sufficient, but the level of protein consumption with enough categories to be more prevalent in the group of normal-nutrition. Useful as a precursor of protein formation of enzymes and hormones which both affect the body's metabolism which can result in the nutritional status of children.

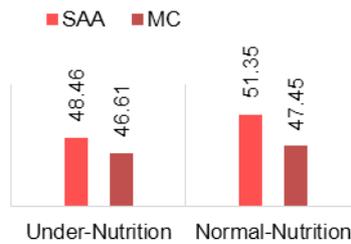


Figure 4. The Distribution of Amino Acid Score and Theoretical Digest Quality (MCT)

Figure 4 shows the ratio between the SAA and MCT under-nutrition toddler group and a group of normal-nutrition. The average value of SAA in the group of normal-nutrition and a group of under-nutrition children, were 51.35 and 48.46 respectively. The value of 51.35 indicates that amino acids are utilized the body from being absorbed in the group of normal-nutrition and amino acids at 48.46 which utilized the body from being absorbed in the group of under-nutrition children. In addition, the average value of MCT on normal-nutrition group and the group of under-nutrition children are 47.43 and 46.61. 47.43 This shows that the protein can be absorbed by the body of the protein consumed in the group of normal-nutrition and 46.61 absorbable proteins from being consumed by a group of under-nutrition children.

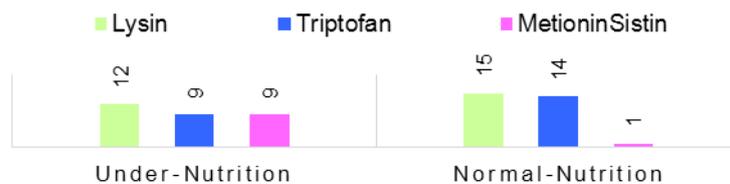


Figure 5. The Respondents Distribution Based on Kind of Amino Acid

Figure 5 shows the limiting amino acids in the group of normal-nutrition. 30 respondents on normal-nutrition group are 15 respondents with a limiting amino acid lysine, 14 respondents with a limiting amino acid tryptophan and 1 respondent with limiting amino acid methionine-cystine. Whereas in the group of under-nutrition children from 30 respondents there were 12 respondents with a limiting amino acid lysine, 9 respondents with tryptophan amino acid and 9 respondents with methionine-cystine amino acid.

Lysine is an amino acid found in many foodstuffs nuts and animal foods such as cheese, egg, fish, milk and red meat. Tryptophan is found in beef, chicken, tuna and egg. Lysine is one of nine essential amino acids needed for growth and repair of tissues. Lysine included in the amino acid that is important and necessary once in the growth and development of children. This amino acid is very useful for bone growth and development in children, helps absorption of calcium and maintaining nitrogen balance in the body, but it is also necessary to produce lysine antibodies, enzymes and the formation of collagen tissue repair as well.

Tryptophan in the body are used to produce niacin (vitamin B) is important for healthy digestion, skin, and nerves. The food menu is not diverse will result in the consumption of protein foods that are low quality and will result in nutritional problems.

Table 1. The Difference of Under-Nutrition Risk Based On Mother Nutritional Knowledge Level

Mother Nutritional Knowledge Level	Nutrition Status (weight for age)				OR	CI
	Under		Normal			
	n	%	n	%		
Less	13	43,3	3	10	6,882	1,707 – 27,752
Enough	17	56,7	27	90		
Total	30	100	30	100		

Table 1 lists the results of statistical tests, showed a significant odds ratio value is OR = 6.8 (CI = 1.707 to 27.75). It states that mothers with low nutritional knowledge level have a risk 6.8 times have under-nutrition children compared to mothers with sufficient levels of nutrition knowledge, so that the risk of incidence of under-nutrition children under five become smaller, mother must have sufficient levels of nutritional knowledge namely in terms of setting food for the family, especially toddlers.

The results of the questionnaires showed that most mothers of under-nutrition children do not know the food source of energy, only three people from 30 respondents who answer correctly the question of food sources of energy, while in the group of normal-nutrition as much as 11 respondents answered the question. Where these questions illustrate the mother's knowledge related to food source of energy when food sources of energy are less consumed, it can lead in solving the energy source of protein and fat that can inhibit the growth and development of infants.

The lack of nutritional knowledge of parents, especially the mother is one of the causes of under-nutrition in young children, therefore the level of nutrition knowledge of mothers influence the incidence of under-nutrition in young children, to avoid any incidence of under-nutrition in infants, mothers should have enough knowledge about how to organize eating toddler. The lack of nutrition knowledge mislead about how the use of certain food and how to arrange a family member who is sick because the mother is the one who is closest to the child must have knowledge of nutrition because childhood is an important period of growth and development which at this time determine the quality of future generations. The family is an important element in the care of children given the children of the family. A child's life can be determined by the family environment or family lifestyle, this can be seen when the child support was excellent, the growth and development of children is relatively stable, but if the family doesn't have good support, then the child will have problems that could influence the child psychologically (Hidayat, 2009).

These results are consistent with research conducted by Nainggolan and Zuraida (2012) that there is a significant relationship between knowledge of nutrition and nutritional status of children and is the most powerful influence on nutritional status of children in Puskesmas Rajabasa, Beautiful Village Rajabasa Kingdom, Bandar Lampung, These results are also consistent with the results of research by Kurniawati (2011) that the low level of knowledge of risk 3,003 times greater against low nutritional status of infants at Baledono Village, District Purworejo, Purworejo, so the level of nutrition knowledge of mothers have an influence on the nutritional status of children.

Table 2. The Difference of Under-Nutrition Risk Based On Energy Consumption Level

Energy Consumption Level	Nutrition Status (weight for age)				OR	CI
	Under		Normal			
	n	%	n	%		
Less	15	50	5	16,7	5,000	1,510 – 16,560
Enough	15	50	25	83,3		
Total	30	100	30	100		

Table 2 is the result of a statistical test that showed a significant odds ratio value is $OR = 5$ ($CI = 1.510$ to 16.560). It states that toddlers with less energy consumption levels had 5 times the risk of under-nutrition infants compared with the level of energy consumption sufficiently, so that the risk of incidence of under-nutrition children under five to be small, the toddler must have a sufficient level of energy consumption.

Food consumption is a directly caused the incidence of under-nutrition if the child does not get energy intake from food sources sufficient energy so the body needs energy from protein and fat, if it happens continuously, it can cause the body to lose body mass and raises events under-nutrition. Therefore, the level of energy consumption has an influence on the nutritional status of infants. This is in line with the results of research by Lutifiana (2013) which strongly influence the energy consumption of nutritional status of children.

The energy needs of a healthy person can be defined as the level of energy intake that can be metabolized from food that will balance energy output, coupled with additional needs for growth (Arisman, 2004). Energy needs can occur when energy consumption through food is less than the energy required. The body will have a negative energy balanced. Consequently, weigh less than ideal body weight. If they occur in children will inhibit the growth of children (Almatsier, 2009).

The impact of the lack of energy and protein intake in the daily diet in a sustainable manner can cause Protein Energy Under-nutrition , lack of food consumption of energy sources can lead to the mobilization of food reserves to produce energy for the sake of saving lives through catabolic process, in case of stress catabolic (infection) then needs protein will increase causing protein deficiency relative that can cause nutritional problems in infants such as kwashiorkor, marasmic-kwashiorkor (Dewi *et al.*, 2013).

Table 3. The Difference of Energy Consumption Level Based On Mother Nutritional Knowledge

Mother Nutritional Knowledge Level	Energy Consumption Level		Total	OR	CI
	Less	Enough			
Less	9	7	16	2,755	0,852 – 9,912
Enough	14	30	44		
Total	23	37	60		

Table 3 is the result of a statistical test that shows the value Odds Ratio = 2.755. It states that mothers with the knowledge level of under-nutrition are at risk of experiencing 2.755 less energy consumption levels than mothers with sufficient levels of nutritional knowledge. However, the statistical test results showed that the risk was not significant ($CI = 0.852$ to 9.912), meaning that the rate of maternal nutrition knowledge lacking not affect the level of energy consumption toddlers. Age mothers in this study range from 18-50 years, increasing a person's age, the more experience gained for child care, in this case, the provision of proper food to children, in addition to the results of the recall also mentioned

that the repetition menus found in both groups. This is consistent with the statement Priyanto (2009) and Notoadmojo (2003) which states that factors including age may determine the individual's knowledge because of the experience gained, so that knowledge is increasing and becoming more prepared to organize the child's diet.

Table 4 is a statistical test result that shows significant Odds Ratio values are OR = 10.5 (CI = 1.227 to 90.662). It states that the toddler with the level of protein intake is less at risk 10.5 times compared under-nutrition toddler with enough protein consumption level, so that the risk of incidence of under-nutrition children under five to be small, the toddler must have sufficient levels of protein

Table 4. The Difference of Under-Nutrition Risk Based On Protein Consumption Level

Protein Consumption Level	Nutrition Status (weight for age)				OR	CI
	Under		Normal			
	n	%	n	%		
Less	8	26,7	1	3,3	10,545	1,227 – 90,662
Enough	22	73,3	29	96,7		
Total	30	100	30	100		

consumption. Toddler with protein consumption level is less at risk of under-nutrition due to inadequate intake of protein that acts as a regulator of metabolism and defends against infection. Protein functions as regulator in the body and as precursor of protein enzymes and hormones. While both works as a regulator of metabolism in the body. The function is to defend the body against disease-causing microbes is because protein is an antibody-forming material, where these antibodies react with antigens (germs), so that the antigen can't be active anymore (Muchtadi, 2009).

Table 5. The Difference of Protein Consumption Level Based On Mother Nutritional Knowledge

Mother Nutritional Knowledge Level	Protein Consumption Level		Total	OR	CI
	Less	Enough			
Less	5	11	16	2,897	0,736 – 11,254
Enough	6	38	44		
Total	11	49	60		

Table 5 is the result of a statistical test that shows the value Odds Ratio = 2.897. It states that mothers with the knowledge level of under-nutrition are at risk of experiencing 2,897 protein consumption level of less than mothers with sufficient levels of nutritional knowledge. However, the statistical test results showed that the risk was not significant (CI = 0.736 to 11.254). This means that the knowledge level of maternal nutrition the less not affect the level of protein consumption toddlers. Age of mothers in the study ranged from 18-40 years, the more experience gained for child care. In this case, the provision of proper food to children, in addition to the results of the recall also mentioned that the repetition menus found in both groups. This is consistent with the statement Proyanto (2009) and Notoadmodjo (2003) which states that factors including age may determine the individual's knowledge because of the experience gained, so that knowledge is increasing and becoming more prepared to organize the child's diet.

Table 6. The Average of Protein Quality of Food (Amino Acid Score) Based On Nutrition Status (weight for age)

Nutrition Status	Total	\bar{x} AAS	<i>p-Value</i>
Under	30	48,465	0,94
Normal	30	51,350	

Statistical test results Independent T -Test with $\alpha = 0.05$ was obtained p-value of 0.94 for the difference in average consumption of protein quality is based on Amino Acid Score is p-value (0.94) > 0.05. It concluded that there was no significant difference in the average consumption of protein quality based on the SAA between under-nutrition children and normal-nutrition. The average value of SAA in the group of normal-nutrition and a group of under-nutrition children were 51.35 and 48.46 respectively. The value of 51.35 indicates that amino acids are utilized the body from being absorbed in the group of normal-nutrition and amino acids at 48.46 which utilized the body from being absorbed in the group of under-nutrition children. The results show the number of food sources of protein consumed by both groups is almost the same, while the results of statistical tests Independent T-Test for the type of food consumed showed that milk consumption both groups had a significant difference, namely p-value (0.00) < 0.05. The number of respondents who consumed each type of food source of these proteins which may cause slight variations in the average SAA is 48.469 in the group of under-nutrition children and 51.350 in the group of normal-nutrition because the group normal-nutrition to eat more types of food sources of protein and dairy consumption on both nutritional groups was higher than the group of under-nutrition, as well as the number of respondents who eat certain types of food protein sources mentioned above more on normal-nutrition group.

Tabel 7. The Average of Protein Quality of Food (Theoretical Digest Quality/MCT) Based On Nutrition Status (weight for age))

Nutrition Status	Total	\bar{x} MCT	<i>p-Value</i>
Under	30	46,615	0,183
Normal	30	47,450	

Statistical test results Independent T-Test with $\alpha = 0.183$ p-value obtained for the difference in average consumption of protein quality is based on the Theoretical Digest Quality (MCT), namely p-value (0.183) > 0.0. It concluded that there was no significant difference in the average consumption of protein quality based MCT among under-nutrition children and normal-nutrition. The average value of MCT on normal-nutrition group and the group of under-nutrition children are 47.43 and 47.61. 47.43 This shows that the protein can be absorbed by the body of the protein consumed in the group of normal-nutrition and 46.61 absorbable proteins from being consumed by a group of under-nutrition children.

Average quality based MCT protein or proteins adsorbed on normal-nutrition in the amount of 47.450 whereas in the group of under-nutrition at 46.615. This indicates that the value of MCT toddler Mojowarno not meet the quality standards of the Indonesian population digested food consumption which ranged from 85-92 (Hardinsyah, 1989). Eggs are an ingredient of food with the digestive quality of 100%, meat and fish 97%, but the number of respondents who consumed these foods in the two groups did not differ greatly and statistical tests showed no significant difference in both groups. Protein function (Winarno, 2004) are as enzymes, transporters and storage devices, escapement, the mechanical support,

the body's defense or immune system, media propagation of nerve impulses, controlled growth. Given the importance of protein for the human body is necessary to note the intake of protein into the body so that the food consumed preferably is a protein with a high-quality protein that can supply the amino acids needed by the body up to the age of the children which is a period of growth and development is rapidly going on, so need to consume foods with a high digestibility quality that can be utilized by the body optimally.

Conclusion

The level of mother nutritional knowledge level, energy and protein consumption level have a higher risk to under nutrition children than normal nutrition children. The diversity of food that consumed determines the value of protein quality in food.

References

- Adriani, M. dan Wirjatmadi, B. 2012. *Peranan Gizi dalam Siklus Kehidupan*. Jakarta : KENCANA PRENADA MEDIA GROUP.
- Adriani, M. dan Wirjatmadi, B. 2013. *Pengantar Gizi Masyarakat*. Jakarta : KENCANA PRENADA MEDIA GROUP.
- AKG. 2013. *AKG 2013*.
- Almatsier, Sunita. 2009. *Prinsip Dasar Ilmu Gizi*. Jakarta : PT Gramedia Pustaka Utama.
- Arisman. 2004. *Gizi dalam Daur Kehidupan*. Jakarta : EGC.
- Baliwati, Y.F., Ali Khomsan dan Meti Dwiriani. 2004. *Pengantar Pangan dan Gizi*. Jakarta : Penebar Swadaya.
- BPS Kabupaten Jombang. *Jombang dalam Angka 2014*.
- Dewi, A.B.F.K.D., Pujiastuti N., dan Fajar I. 2013. *Ilmu Gizi untuk Praktisi Kesehatan*. Yogyakarta : Graha Ilmu.
- Fajar, Ibnu et al. 2009. *Statistika untuk Praktisi Kesehatan*. Yogyakarta : Graha Ilmu.
- Hardinsyah dan Drajat Murtianto. 1989. *Menaksir Kecukupan Energi dan Protein serta Penilaian Mutu Gizi Konsumsi Pangan*. Jakarta : Wirasari.
- Helmyati, Siti et al. 2009. *Statistika untuk Praktisi Kesehatan*. Yogyakarta : Graha Ilmu.
- Hidayat, A. Aziz Alimul. 2009. *Pengantar Ilmu Keperawatan Anak 1*. Jakarta : Salemba Medika.
- Inayah, R., Otok B.W., dan Purnami S.W. 2014. *Klasifikasi Rumah Tangga Miskin di Kabupaten Jombang berdasarkan Faktor-faktor yang Mempengaruhi dengan Pendekatan CART (Classification and Regression Tress)*. Jurnal Sains dan Seni PomITS, 2 (3).
- Keputusan Menteri Kesehatan Republik Indonesia No. 1995/MENKES/SK/XII/2010 Tentang Standar Antropometri Penilaian Status Gizi Anak.
- Khomsan, A. 2012. *Ekologi Masalah Gizi, Pangan, dan Kemiskinan*. Bandung : Alfabeta.
- Khotimah, N.N., Siregar, R. dan Mardiana. 2012. *Hubungan Pengetahuan Gizi Ibu dan Pola Makan Balita dengan Status Gizi Balita (12 – 59 Bulan) di Wilayah Kerja Puskesmas Gandus Kecamatan Gandus – Palembang Tahun 2010*. Jurnal Pembangunan Manusia, 2 (6).
- Kurniawati, Erni. 2011. *Hubungan Tingkat Pengetahuan Ibu tentang Gizi dengan Status Gizi Balita di Kelurahan Baledono, Kecamatan Purworejo, Kabupaten Purworejo*.
- Linder, MC. 2010. *Biokimia Nutrisi dan Metabolisme dengan Pemakaian Secara Klinis*. Jakarta : UI Press.
- Muchtadi, Deddy. 2009. *Prinsip Teknologi Pangan Sumber Protein*. Bandung : Alfabeta.
- Nainggolan J dan Remi Zuraida. 2012. *Hubungan antara Pengetahuan dan Sikap Gizi Ibu dengan Status Gizi Balita di Wilayah Kerja Puskesmas Rajabasa Indah Kelurahan Rajabasa Raya Bandar Lampung*. Bandar Lampung : Fakultas Kedokteran Universitas Lampung.
- Notoatmodjo, Soekidjo. 2005. *Metodologi Penelitian Kesehatan*. Jakarta : PT Rineka Cipta.
- Piliang, W.G. dan Djojoseobagio S. 1996. *Fisiologi Nutrisi*. Jakarta : Penerbit Universitas Indonesia.
- Purwanto. (2007). *Instrumen Penelitian Sosial dan Pendidikan*. Yogyakarta : Pustaka Pelajar.
- Prakoso, Bayu Adi. 2015. *Perbedaan Tingkat Konsumsi Energi, Protein, Vitamin A dan Perilaku Kadarzi pada Anak Balita Stunting dan Non Stunting di Desa Kopen Kecamatan Teras Kabupaten Boyolali*. Surakarta : Program Studi S1 Gizi Fakultas Ilmu Kesehatan Universitas Muhammadiyah Surakarta.
- Rahim, Fitri Kurnia. 2014. *Faktor Resiko Underweight Balita Umur 7 – 59 Bulan*. Jurnal Kesehatan Masyarakat, 9 (2): 115 – 121.
- Riskesdas. 2013. *Riskesdas 2013*.
- Sajogyo, et al. 1986. *Menuju Gizi Baik yang Merata*. Yogyakarta : Gajah Mada University Press.
- Saepudin, Malik. 2011. *Metodologi Penelitian Kesehatan Masyarakat*. Jakarta : CV. Trans Info Media.
- Santoso, Budi et al. 2014. *Studi Diet Total : Survei Konsumsi Makanan Individu Provinsi Jawa Tengah 2014*.
- Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan, Jakarta.
- Sediaoetama, Achmad Djaeni. 2010. *Ilmu Gizi*. Jakarta : Dian Rakyat.
- Soetjiningsih. 1995. *Tumbuh Kembang Anak*. Surabaya : EGC.
- Suhardjo. 2003. *Berbagai Cara Pendidikan Gizi*. Jakarta : Bumi Aksara.
- Supariasa, I.D.N., Bakri, B. dan Fajar, I. 2012. *Penilaian Status Gizi*. Jakarta : EGC.
- Wijayanti, Endah Kusuma. 2011. *Hubungan Pengetahuan Gizi Ibu tentang Kadarzi dengan Tingkat Konsumsi Energi dan Protein serta Status Gizi Batita di Kelurahan Tanjungrejo Janti Kota Malang*. Malang : Karya Tulis Ilmiah, Gizi – Politeknik Kesehatan Kemenkes Malang.
- Winarno, FG. 2004. *Kimia Pangan dan Gizi*. Jakarta : PT.Gramedia Pustaka Utama.

Variation of Seaweed Addition on the Nutritional Content of *Cendol*

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Abstract

Cendol is one of traditional food favored by people, especially who live in West Sulawesi. *Cendol* made from modified rice flour by add seaweed and processed it to be porridge. Some studies reported that seaweed contains many significant amounts of fat, protein, vitamins and minerals essential to fulfilling nutrient needs. The objective of this study was to analyze the nutritional contents (carbohydrate, protein, fat, vitamin A, fiber, and Fe) of modified seaweed *cendol*. The method was randomized design with two times repetition in the laboratory by analyzing 100 grams of each sample. Modified seaweed *cendol* samples were 0%, 10%, 20%, 30% of seaweed addition, respectively. The result showed the highest nutrient content was in 20% of seaweed addition that contains carbohydrate, Fe and Vitamin A was 27.64%, 9.94%, and 0.098%, respectively. Different results for fat and fiber content. The higher percentage of seaweed addition, the higher nutrient content of fat and fiber was 0.09% and 0.78%, respectively. While the highest protein content (2.45%) was in 10% of seaweed addition.

Keywords: *cendol*: seaweed; nutritional content

Introduction

Indonesia is known as a maritime country that has a huge sea area that contains a lot of potential of biological resources and diversity. Indonesia's long beach is approximately 81,000 km and the most potential biological resources are seaweed. It is potential developing because it has a high economic value.

West Sulawesi province as one of the seaweed cultivation showed an increasing yield annually since 2011 - 2014 are 35,000, 63,000, 94,500 and 141,700 tons respectively. Seaweed contains many essential nutrients that are necessary for the human body, such as protein, carbohydrate, crude fiber, as well as energy. It contents low fat and high crude fiber causes seaweed is good to be consumed daily. Seaweed chemically made up of ash (29.97%), protein (5.91%), fat (0.28%), carbohydrates (63.84%), total dietary fiber (78.94%) and iodine (283.93%).

Seaweed also contains vitamins, such as Vitamin A, B Vitamins (thiamine, riboflavin, Vitamin B₆, and Vitamin B₁₂), Vitamin C, Vitamin D, Vitamin E, and minerals. Excessive of seaweed as a food ingredient does not cause obesity. As medicines, it boosts immunity and good for healthy skin². Along with the development of science and technology, the use of seaweed has developed in various sectors such as agriculture (as organic fertilizer and as one of the culture media in the tissue culture); livestock sector (as the livestock feeds to produce tasty meat); medical (as a bacterial culture medium); pharmaceutical (as a maker of suspension, emulsifier, tablet, plaster and filter); industrial (as the additive in the textile, paper, ceramic, photography, insecticide, wood protector and fire prevention).

One of the utilizations of seaweed is a modification of traditional food or drink that is often used for snacks which is *cendol*. It has been widely popular with the community because of the chewy texture. It is generally made of rice flour, tapioca flour, *hunkwe* flour or other types of flour.

In order to improve the variety and formulation, *cendol* is made of additional variations of seaweed. The aim is to increase the nutrients contents such as Fe, Vitamin A, fiber, carbohydrate, protein and fats of *cendol* that can be used as an alternative food to prevent and address the nutritional problem in the community. Furthermore, *cendol* can become an alternative food to prevent the nutritional problem, particularly in West Sulawesi.

The purpose of this study was to determine the nutrient content (Fe, Vitamin A, fiber, carbohydrate, protein, and fats) of *cendol* with variations of additional seaweed porridge (0%, 10%, 20% and 30%). Furthermore, it can become an alternative food to prevent the nutritional problem in West Sulawesi.

Material

The materials used in this study consisted of the ingredients of modified *cendol* and chemicals material. The ingredients of *cendol* were rice flour, tapioca flour, water, coconut milk, screw pine leaf, salt, milk powder, sugar, palm sugar, and seaweed porridge (Table 1). While the chemicals material was got from the Center for Health Laboratory in Makassar as the location testing for nutrient content of *cendol*.

Table 1. Ingredients of Modified Cendol

Ingredient (gr)	Additional Variation			
	0 (%)	10(%)	20(%)	30(%)
Rice flour	100	100	100	100
Tapioca flour	50	50	50	50
Seaweed porridge	0	15	30	45
Milk	20	20	20	20
Salt	2	2	2	2
Screw pine leaf	1	1	1	1

Study Design

The study design was a completely randomized design. The sample in this study was *cendol* with the additional of seaweed porridge as much as 10%, 20%, 30% and 0% as the control with two times repetition for each sample. The weight of *cendol* was 100 grams for each of the various additions. Analysis for nutrient content (Fe, Vitamin A, fiber, carbohydrate, protein, and fat) was performed at the Center for Health Laboratory in Makassar during period time of June - October 2015.

Cendol Process Procedures

The processing procedure of modified seaweed *cendol* was based on the standard recipes of *cendol*. The processing procedure was once dissolved the rice flour, tapioca flour, salt, milk, food coloring then mixed them all. After the dough was mixed well and then cook it while stirring until popped and lumpy. Pour the dough into the molds of *cendol* that was placed over a container of ice water. Press the dough of *cendol* until it out of the molds and then set aside.

Prepare shredded coconut as much as 2 coconuts. Add 2 liters of water, squeeze once to obtain the

coconut milk. Filter the coconut milk and boiled. During the process of boiling of coconut milk, it should be stirred continuously. Prepare as much as 1 kg of brown sugar. Mush it until smooth. Then 1-liter water was added and after that boiled in order to avoid clotting of sugar during boiling. It must be stirred until boiled. Filter the sugar and then cooled. Pour *cendol* into a glass, add the brown sugar syrup and coconut milk, and add ice cubes. *Cendol* is ready served.

Process of Seaweed Porridge Production

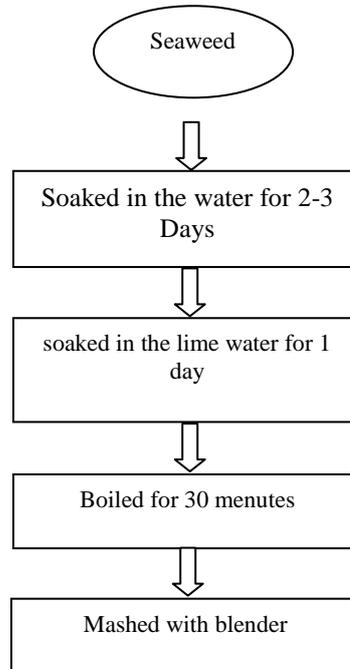


Figure 1. Chart of Seaweed Porridge Production

Analysis of Nutrient Contents

The analysis of nutrient content was conducted by the Center for Health Laboratory, Makassar. Procedure was done based on the nutrient analysis protocol. Such as atomization method to measure Fe content, spectrophotometry for Vitamin A content, titrimetry for carbohydrates, protein and fiber content. Fat was examined by Gravimetric method.

Result and Discussion

Result

Nutrient content testing was conducted in the Center for Health Laboratory, Makassar. Nutrient content of cendol showed varied results for each variation of 0%, 10%, 20% and 30% of addition seaweed porridge. The average results for the nutrient content (Fe, Vitamin A, fiber, carbohydrates, fat, and proteins) had increased as shown in Table 2.

Discussion

Content of Fe

In this study, the data showed the highest content of Fe was 9.94 % found in the sample with 20% addition of seaweed and decreased up to 7.03% in a sample with 30% of addition. It could be affected by the processing that caused the reduction of the nutrient content in *cendol* of additional seaweed. Based on research conducted by Norziah in Penang Malaysia showed that iron content in seaweed of *Gracilaria sp* was 95.6 mg per 100 g dry weight, while the iron content in seaweed of *Sargassum sp* was 68.21 mg per 100 g. Generally, minerals are not significantly affected by the chemical and physical treatment during processing. However, the presence of oxygen, some minerals such as Fe can be oxidized or reduced that affect the biological value.

Table 2. Nutritional content of Cendol with Variation of Seaweed Addition

Sample	1st Test (ug/g)	2nd Test (ug/g)	Average (ug/g)
Fe			
0%	5,89	6,74	6,32
10%	11,12	8,35	9,74
20%	9,84	10,04	9,94
30%	6,86	7,20	7,03
Carbohydrate			
0%	12,78	12,00	12,39
10%	23,25	22,95	23,1
20%	28,13	27,15	27,64
30%	25,57	24,40	24,99
Vitamin A			
0%	0,007	0,005	0,006
10%	0,012	0,015	0,135
20%	0,016	0,18	0,098
30%	0,022	0,027	0,023
Protein			
0%	0,84	1,03	0,935
10%	2,42	2,48	2,45
20%	1,89	2,04	1,97
30%	1,88	2,03	1,96
Fat			
0%	0,02	0,02	0,02
10%	0,03	0,03	0,03
20%	0,05	0,05	0,05
30%	0,09	0,09	0,09
Fiber			
0%	0,2	0,17	0,19
10%	0,22	0,23	0,23
20%	0,33	0,31	0,32
30%	0,78	0,78	0,78

Content of Carbohydrate

The highest carbohydrate content was 27.64 grams found in the sample with 20% addition of seaweed but the value decreased up to 24.99 grams on the addition of 30%. It was in line with the previous study done by Holdt and Kraan (2011) that the total carbohydrate content in seaweed was from 20% up to 76%. Carbohydrates are organic compounds consisting of crude fibers and free materials without nitrogen. Carbohydrates made up of carbon, hydrogen, and oxygen in different proportions. Simple carbohydrates are generally more soluble in water comparing fat or protein. In this study, the *cendol* was made of flour as the main material. The heating process can affect the physical structure of raw starch granules make it more soluble in water and easily hydrolyzed by the enzyme and changes structure in the starch called as gelatinization. This study is in line with the previous research conducted by Hudaya. The study showed that additional seaweed flour in *tahu sumedang* increased the carbohydrate content up to 4.03%. It was because of the carbohydrates in seaweed consists of two common forms that are fibril, usually in form of cellulose, and amorphous state usually in form of *gelatin*.

Content of Vitamin A

This study found that the highest content of vitamin A was in the sample with 10% addition of seaweed as many as 0.098 grams. Vitamin A is a fat-soluble vitamin, while seaweed contain a few of fat to dissolve the vitamin A in all variant of *cendol* with seaweed addition. Besides, vitamin A would easily be damaged by heating, especially at high temperatures. A research done by Handayani reported the activity of vitamin A was calculated based on the levels of β -carotene using retinol equivalent. The level of β -carotene determined the activity of vitamin A, therefore the seaweed could have vitamin A active.

Content of Protein

The functions of proteins are as builder substance and body maintenance. During the digestion process, proteins will be converted into an amino acid and absorbed by the body. In general, the protein content of food will determine the quality of the food⁷. The highest protein content was in the sample with 20% addition of seaweed as many as 1.97 grams but decreased up to 1.96 grams in 30% addition. The protein in food will be denatured when heated at a moderate temperature of 60-90°C for one hour or more. Denaturation is a structural change of protein which only full primary structures were left in the denaturation state. The lower value of protein content in *cendol* could be due to the heating during processing which uses high temperature. Heating process will cause the protein to be degraded. The study conducted by Hudaya found that decreasing of protein content in the tofu with addition of seaweed flour was caused due to the nitrogen compounds that volatile when processing

Content of Fat

Food source of fat can be derived from the animals called animal fat and from a vegetable called as plant fat. Plants generally stored its food reserve in the form of carbohydrates particularly in polysaccharides, whereas the animals reserve in form of fat in fat tissues. The different form of food reserve causes the plant fats generally has a lower percentage. In this study, *cendol* with 30% of seaweed addition contained 0.09 grams of fat was the highest fat content among others. Fat content in seaweed was

very low, less than 1% hence seaweed is safe to consume in large quantities. Low-fat content causes the seaweed be used as one of the major food sources for a low-fat diet. However, a certain amount of fat is needed for the human body. Fat is an organic compound that is insoluble in water but soluble in organic solvents. Fat is the greatest source of the energy providing competing to protein and carbohydrates. In this study found fat is the lowest nutrient content among all variant of sample test. Hence the additional seaweed in *cendol* had no effect increasing the concentration of each addition. It is in line with the study done by Hatta R that there was an increased fat content in *dodol* substituted with seaweed

Content of Fibre

Fibre has a positive nature for nutrition and metabolism in human body. Dietary fibre is the component of plant tissue that is resistant toward hydrolysis process by enzymes in the stomach and intestines. The highest fibre content in this study was 0.78 grams at 30% seaweed addition. The fibre content in *cendol* increases in line with increasing concentration of seaweed in the *cendol*. It is because of seaweed has high fibre content that would increase the fibre content of *cendol*. This finding was in line with a study done by Handayani that seaweed cake obtained improved results in each substitution of seaweed.

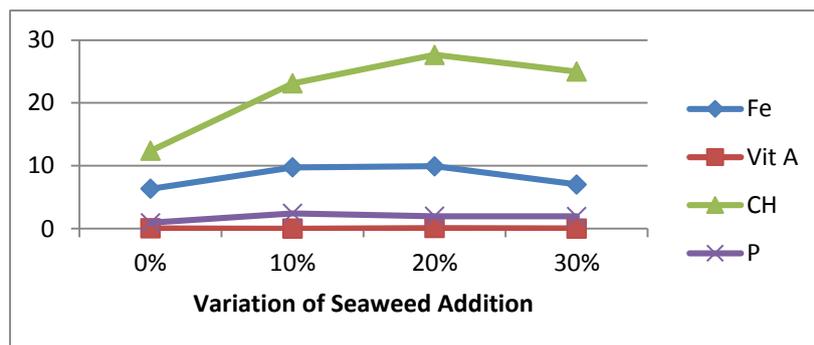


Figure 1. Fe, Carbohydrate, and Protein Contents

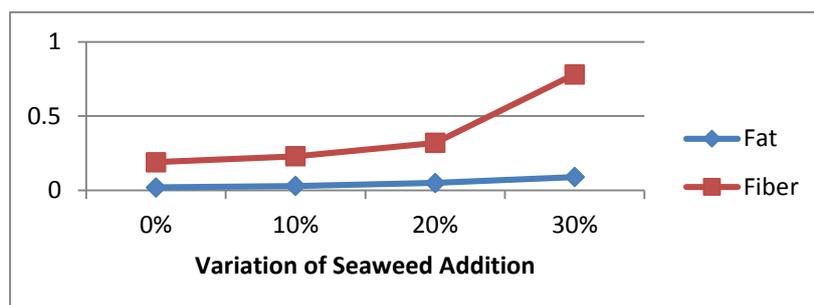


Figure 2. Fat and Fiber Contents

Variation of Increasing Nutrient Content of *Cendol*

Nutrient content of *cendol* increased by the 20% addition of seaweed but declined in Fe, vitamin A, carbohydrates and protein content for another additional concentration of seaweed. The additional concentration of a substance/compound will increase the reaction rate but after the concentration is increased, the rate would reach a saturation point and will not grow anymore. It will not affect the rate of reaction after additional concentration. It was seen in the sample with 20% addition of seaweed that the

content of Fe, carbohydrate, and protein which reached its highest level (Figure 1). Whereas, the nutrient contents of *cendol* was increased in accordance to the variation of the seaweed. The higher the concentration, the increasing content of fat and fibre (Figure 2)

Conclusion

The nutrient content of *cendol* with addition of seaweed was increased. The highest content of fat and fibre (0.99% and 0.78%) were found at 30% additional of seaweed. Furthermore, for Fe, vitamin A, carbohydrate, and protein were 9.94%, 0.135%, 27.64% and 2.45%, respectively found in the sample with 20% addition of seaweed.

Acknowledgement

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References

- Andansari SA, Sari RD, Roesyady A. 2014. Konversi rumput laut menjadi monosakarida secara hidrotermal. *Jurnal Teknik Pomits*, 3(2):2337-3539.
- Astawan M, Koswara S, Herdiani F. 2004. Pemanfaatan rumput laut (*Eucheuma Cottonii*) untuk meningkatkan kadar iodium dan serat pangan pada selai dan dodol. *Jurnal Teknologi dan Industri Pangan*, XV(1) : 61-9.
- Batista-Gonzalez, A. E., Silva, A. M. D. O. E., Vidal-Novoa, A., Pinto, J. R., Mancini, D. A. P., & Mancini-Filho, J. 2012. Analysis of in Vitro and in Vivo Antioxidant Properties of Hydrophilic Fractions From the Seaweed Halimeda Monile L. *Journal of Food Biochemistry*, 36(2), 189–197. <http://doi.org/10.1111/j.1745-4514.2010.00525>.
- Clenia M. 2008. Analisis rasio biaya sumberdaya domestik usaha budidaya rumput laut di Indonesia. *Jurnal Bisnis & Ekonomi*, 15(1), 31–38.
- Chandra AA. 2008. Pembuatan cendol dari beberapa bentuk sediaan rumput laut (*Eucheuma Cottonii*). *Jurnal pangan dan gizi*, 2(2): 67-74.
- Handayani R, Aminah S. 2011. Variasi substitusi rumput laut terhadap kadar serat dan mutu organoleptik cake rumput laut (*Eucheuma Cottonii*). *Jurnal pangan dan gizi*, 2(2):67-74.
- Harmayani E, Murdiati A. 2011. Karakteristik pati ganyong (*Canna Edulis*)& pemanfaatannya sebagai bahan pembuatan cookies & cendol. *Agritech Journal*, 31: 297.
- Hatta R. 2012. Studi pembuatan dodol dari rumput laut (*Eucheuma Cottonii*) dengan penambahan kacang hijau (*Phaseolus Aureus*). *Jurnal Tekno Pangan*, 15(1), 21–30.
- Holdt, S. L. and Kraan, S. 2011. Bioactive compounds in seaweed: Functional food applications and legislation. *J. Appl. Phycol.* 23: 543–597.
- Hudaya NR. 2008. Pengaruh penambahan tepung rumput laut untuk peningkatan kadar iodium dan serat pada tahu sumedang. *Jurnal Tekno Pangan*, 15(1):21–30.
- Kartika, S. R. 1998. Pedoman uji indrawi bahan pangan. Yogyakarta: Gajah Mada University Press.
- Polat S, Ozogul Y. 2013. Seasonal proximate and fatty acid variations of some seaweeds from the northeastern mediterranean coast. *Oceanologia*, 55(2): 375–391. <http://doi.org/10.5697/oc.55-2.375>.
- Mappiratu. 2009. Kajian teknologi pengolahan karaginan dari rumput laut *Eucheuma Cottonii* skala rumah tangga. *Media Litbang Sulteng*, 2(1), 1–6. Retrieved from <http://download.portalgaruda.org/article.php?article=11183&val=759>.
- Santoso J, Yoshie Y, Suzuki T. 2004. Mineral, fatty acid and dietary fiber compositions in several Indonesian seaweeds. *Jurnal Ilmu-Ilmu Perairan dan Perikanan Indonesia*, 11(1): 45-51.
- Sunita, A. 2009. Prinsip dasar ilmu gizi. PT. Gramedia Pustaka Utama, Jakarta.
- Yang, Y. J., Nam, S.-J., Kong, G., & Kim, M. K. 2009. A case-control study on seaweed consumption and the risk of breast cancer. *British Journal of Nutrition*, 103: 1345–1353. <http://doi.org/10.1017/S0007114509993242>.

Chemical Constituents of Ginger (*Zingiber officinale* Roscoe) Essential Oil and Its Potency as Natural Preservative on Fresh Chicken Meat

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Abstract

Ginger (*Zingiber officinale* Roscoe) possesses antibacterial activity related to its phenols, flavonoids, terpenoids, and essential oils constituents. The purpose of this research was to determine the chemical compounds of ginger essential oil and analyze their potency as natural preservative on fresh chicken meat. Ginger essential oil was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Potency of ginger essential oil as preservative on fresh chicken meat was determined based on its inhibitory activity against the bacterial growth on meat. The major constituents of ginger essential oil were geranyl acetate (24.60%), cineole (17.89%), camphene (12.95%), geraniol (8.48%), and zingiberene (7.95%). Ginger essential oil at concentration of 1250 µg/ml can be used as natural preservative of fresh chicken meat for 12 days and capable of prolonging shelf life of up to 6 days at temperature of 3-7°C.

Keywords: chemical constituents, essential oil, fresh chicken meat, ginger, natural preservative

Introduction

Food-borne illness is a growing public health problem in Indonesia, as well in other developing countries (Jahan, 2012; Newell et al., 2010). *Vibrio cholerae* and *Salmonella typhimurium* are the common causes of food-borne illness, along with food poisoning caused by *Escherichia coli*, *Bacillus* spp., and *Staphylococcus aureus* (Agustina et al., 2013; Lesmana et al., 2002; Vollaard et al., 2004). One of alternatives used to combat this problem is utilization of preservatives acting as antibacterial. Plant derived products are often considered to be safer and more effective against the bacteria by functioning on multi-targeted sites and thus be useful to control of food-borne illness (Han and Bhat, 2014; Voon et al., 2011).

Ginger (*Zingiber officinale* Roscoe) is one of nine featured Indonesian medicinal plants (Widyawati, 2007) and native to South East Asia. This herb is commonly cultivated in Indonesia for its medicinal value. Its rhizome is usually used as ingredient in folk medicine (jamu) for treating stomach discomfort, tumours, relieving rheumatic pains, and as a post partum. Ginger has been reported possessing antimicrobial activity against *Bacillus licheniformis*, *B. spizizenii*, *B. subtilis*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas stutzeri* and *Candida albicans* (Bellik, 2014; Rialita et al., 2015; Sivasothy et al., 2011b). In continuation of our study on the utilization of essential oil of Indonesian spices as preservatives related to their antimicrobial activity (Hamad et al., 2016a; Hamad and Hartanti, 2015; Hamad et al., 2016b), we investigated the chemical constituents of ginger essential oil and analyzed its potency as natural preservative on fresh chicken meat based on its activity inhibiting bacterial

growth on tofu using spectrophotometric method.

Materials and Methods

Plant materials

Fresh rhizomes of ginger were purchased from a local market at Purbalingga, Central Java, Indonesia. Plant materials were dried and pulverized to fine powders.

Hydrodistillation

Dried rhizomes were subjected to hydrodistillation using a Clevenger apparatus for 4 h for the isolation of essential oils according to the recommended method (Guenther, 1961). The rendement of the extracted essential oil was 0.643% (w/v). The essential oil were dehydrated over anhydrous sodium sulphate and stored at 0°C in air-tight glass vial until used for further analysis.

Analysis of chemical compositions

The volatile composition of plant extracts were analyzed using GC-MS system (Agilent 6980N GC System coupled to Agilent 5973 inert MSD detector), equipped with a ZB-5 capillary column (30 m x 0.25 mm x 0.25 µm). The carrier gas was helium at flow rate of 1.3 ml/min, and 2 µL of sample was injected. The electron impact technique (70 eV) was used. The injector and detector temperatures were 250°C and 230°C.

Potency of essential oil of ginger as fresh chicken meat

The inhibitory of bacterial growth on fresh chicken meat by ginger essential oils were conducted according to previously reported method (Hamad and Hartanti, 2015; Hayouni et al., 2008) with a slight modification. The fresh chicken meat used in this study was obtained from local market at Tambak Sogra, Purwokerto. The meat was cut in size of 1 x 1 x 1 cm. Each piece was immersed in boiling water for 2 min, in order to reduce the number of the microorganisms attached to the surface of the meats. Meats were soaked in 100ml of broth containing 3 different concentrations of ginger essential oil under sterile condition, they were 250, 1250, and 6250 µg/ml, respectively. The meats in the broths were stored in refrigerator at temperature of 5±2 °C. On day 3, 6, 9, 12, and 15 the optical densities of broths were observed at the wavelength of 600 nm.

Sensory evaluation was conducted subjectively to evaluate the odour, colour, texture, and occurrence of slime on the meats during storage.

Statistical analysis

Means separation of optical densities of each group in the same day was accomplished by Duncan's multiple range tests. Significance was evaluated at $p < 0.05$. Statistical analysis was conducted by the general procedures of SPSS Statistics v.13 (SPSS Inc.).

Result and discussion

The chemical composition of ginger essential oil obtained by GC-MS analysis and their relative

concentrations quantified by normalization areas through examining the chromatogram shown in Table 1. There were 14 identified compounds with total concentration of 99.99%. The major constituents of ginger essential oil were geranyl acetate (24.60%), cineole (17.89%), camphene (12.95%), geranial (8.48%), zingiberene (7.95%), and neral (6.90%). The result of our study was different from previously reported data. Essential oil of ginger grown in Pakistan produced essential oil with camphene, α -terpineol, and p-cineole as major constituents (El-Ghorab et al., 2010). Another report showed that camphene, geranial, geranyl acetate, and neral were the dominant compounds of ginger essential oils from Negeri Sembilan, Malaysia (Sivasothy et al., 2011a). This phenomenon can be explained by a report that showed that composition of essential oils from a particular species of plant can differ between harvesting seasons and geographical source (Burt and Reinders, 2003).

Table 1. Chemical constituents of ginger essential oil

No	Constituent Name	Retention time (minute)	Concentration (%)
1	β -ocimene	12.834	4.34
2	Camphene	13.705	12.95
3	Mircene	15.919	2.82
4	Cineole	17.972	17.89
5	1- methylbutylacetate	18.462	1.44
6	Neral	27.130	6.90
7	Geranial	28.367	8.48
8	Cytronelylacetate	31.116	1.11
9	Geranyl acetate	32.442	24.60
10	A-curcumene	35.703	3.03
11	Zingiberene	36.164	7.95
12	Farnesene	36.470	3.58
13	β -bisabolene	36.558	1.28
14	β -sesquiphellandrene	37.074	3.62

In our previous study, the MIC of ginger essential oil against *E. coli* and *V. cholera* were 500 μ g/mL (Hamad et al., 2016a), hence we use that data to provide 3 different concentrations of ginger essential oil to preserve fresh chicken meat, they were 250, 1250, and 6250 μ g/ml, respectively. The optical density at the wavelength of 600 nm of the broth used to soak the tofu was used as the indirect method to estimate the number of bacteria grown in meat. The measured optical density of the broth on day 3, 6, 9, 12 and 15 are presented in Table 2. The result showed that ginger essential oil at concentration of 1250 and 6250 μ g/ml were capable of inhibiting the growth of bacteria on the fresh chicken meat until day 15. It is shown by significantly different OD 600 nm of those concentrations of clove essential oil from that of negative control of the respective observation day on day 3, 6, 9, 12, and 15 ($p < 0.05$). This suggested that ginger essential oil at those concentrations could be used as natural preservative of fresh chicken meat for at least 15 days at temperature of 5 ± 2 °C. The profile of inhibition of bacterial growth on chicken meat during 15 days storage is shown in Figure 1.

The sensory evaluation of fresh chicken was performed to analyze the the odour, colour, texture, and occurrence of slime on the meats during storage. This data can be used to determine the shelf life of meat preserved with ginger essential oil (Table 3). The colour of chicken meat at all groups was white and remained unchanged during 15 days of storage. On day 3 and 6, the odour and texture of meats in negative control group were moderate and firm, respectively. The colour and texture of meats treated with ginger essential oil were comparable to those of negative control group, yet the odour was stronger with

the hint of ginger aroma. The meat in negative control showed a changing sensory characteristic in day 9, which its texture turned stinky and there was slightly observable slime formation.

Table 2. The indirect enumeration of bacteria on fresh chicken meat treated with ginger essential oil

Concentration of ginger essential oil ($\mu\text{g/mL}$)	OD 600 nm				
	Day 3	Day 6	Day 9	Day 12	Day 15
0	0.055 \pm 0.046 ^a	0.046 \pm 0.003 ^{ab}	0.302 \pm 0.063 ^a	0.469 \pm 0.052 ^a	0.537 \pm 0.010 ^a
250	0.056 \pm 0.005 ^a	0.050 \pm 0.005 ^a	0.247 \pm 0.007 ^a	0.476 \pm 0.004 ^a	0.530 \pm 0.013 ^a
1250	0.029 \pm 0.169 ^b	0.036 \pm 0.003 ^c	0.128 \pm 0.006 ^b	0.354 \pm 0.042 ^b	0.436 \pm 0.025 ^b
6250	0.015 \pm 0.001 ^b	0.039 \pm 0.003 ^{bc}	0.090 \pm 0.006 ^b	0.320 \pm 0.006 ^b	0.396 \pm 0.008 ^c

Note: different subset indicated that the mean of OD was significantly different at $p < 0.05$

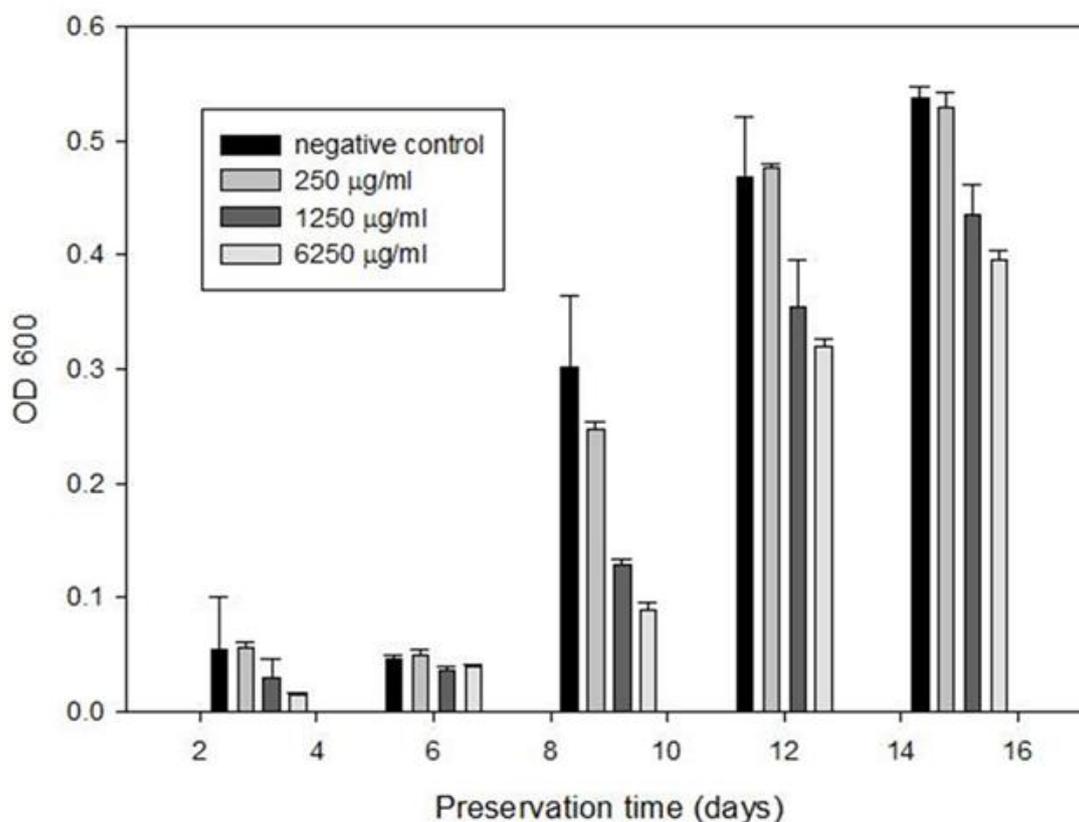


Figure 1. Profile of bacterial growth on fresh chicken meat treated with ginger essential oil

Meat treated with ginger essential oil at concentration of 250 $\mu\text{g/ml}$ group showed a changing organoleptic characteristic in day 12, which its texture turned stinky. The formation of slime in this group was also observed in day. Hence, treatments with ginger essential oil at this concentration capable of preserving chicken meat for 6 days and prolonging its shelf life for 3 days in temperature of 5 ± 2 °C. The meat treated with ginger essential oil at concentration of 1250 $\mu\text{g/ml}$ started to develop slime formation at day 12, with another aspects analyzed was remain unchanged. Hence, ginger essential oil at concentration of 1250 $\mu\text{g/ml}$ capable of prolonging its shelf life for 6 days in temperature of 5 ± 2 °C. At higher concentration (6250 $\mu\text{g/ml}$), a slime formation was observed at day 15. This result suggested that the higher concentration of ginger essential oil used, the longer shelf life of fresh chicken meats were.

Table 3. The result of sensoric analysis of fresh chicken meat preserved with ginger essential oil

Variable		Organoleptic			
Time (days)	Concentration ($\mu\text{m/ml}$)	Odor	Color	Slime	Texture
3	0	Moderate	White	None	Firm
	250	Strong	White	None	Firm
	1250	Strong +	White	None	Firm
	6250	Strong ++	White	None	Firm
6	0	Moderate	White	None	Firm
	250	Strong	White	None	Firm
	1250	Strong +	White	None	Firm
	6250	Strong ++	White	None	Firm
9	0	Moderate	White	Slightly	Friable
	250	Strong	White	None	Firm
	1250	Strong +	White	None	Firm
	6250	Strong ++	White	None	Firm
12	0	Moderate	White	Noticeable	Friable
	250	Strong	White	Slightly	Friable
	1250	Strong +	White	Slightly	Firm
	6250	Strong ++	White	None	Firm
15	0	Stinky	White	Noticeable	Friable
	250	Strong	White	Noticeable	Friable
	1250	Strong +	White	Slightly	Friable
	6250	Strong ++	White	Slightly	Firm

Table 4. The group of chemical constituents of ginger essential oil used in this study

Groups	Chemical compounds	Presentage (%)
Aldehyde	Neral, geranial	15,38
Esther	1- methylbutyl acetate, citronelyl acetate, geranyl acetate	27,15
Ether	Cineole	17,89
Hidrokarbon	β -ocimene, camphene, myrcene, α -curcumene, zingiberene, farnesene, β -bisabolene, β -sesquiphellandrene	39,58

Our result confirmed the previous reports that ginger was capable of being used as natural food preservative. It has been confirmed that the used of water extract of ginger at concentration of 15% prolonged the shelf life of beef and tilapia for 24 hours without changed their sensory characteristic (Purwani and Muwakhidah, 2008). Further study reported that it inhibited microbial growth on tilapia at concentration of 50% (Purwani *et al.*, 2009).

The antimicrobial activity of ginger essential oil was responsible for its potency to be used as natural preservative. Its potency as fresh chicken meat's preservative is related to its chemical constituents. It has been reported that aldehyde or phenol essential oils possessing the highest antibacterial activity, followed by alcohol essential oils. Ketone or ester and acetate essential oils had much weaker activity, while hydrocarbon ones were usually inactive (Bassolé and Juliani, 2012). Ginger

essential oil used in our study was consisted of hydrocarbons, but also contained considerable fraction of ester, ether, and aldehyde terpenes (Table 4). Hence, the major constituents responsible for its potency for preserving fresh chicken meat were neral and geranial.

Conclusion

Ginger essential oil mainly contained geranyl acetate, cineole, camphene, geranial, zingiberene, and neral. It is at concentration of 1250 µg/ml capable of prolonging fresh chicken meat shelf life for 6 days in temperature of 5±2 °C. This potency was related to its considerable fraction of aldehyde constituents, neral and geranial.

References

- Agustina R., Sari T.P., Satroamidjojo S., Bovee-Oudenhoven I.M.J., Feskens E.J., Kok F.J. 2013. Association of food-hygiene practices and diarrhea prevalence among Indonesian young children from low socioeconomic urban areas. *BMC Public Health* 13.
- Bassolé I.H.N., Juliani H.R. 2012. Essential oils in combination and their antimicrobial properties. *Molecules* 17:3989-4006.
- Bellik Y. (2014) Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of *Zingiber officinale* Roscoe. *Asian Pac J Trop Dis* 4:40 - 44.
- Burt S.A., Reinders R.D. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Let. Appl. Microbiol.* 36:162-167.
- El-Ghorab A.H., Nauman M., Anjum F.M., Hussain S., Nadeem M. 2010. A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *J. Agric. Food Chem.* 58:8231–8237.
- Hamad A., Alifah A., Permadi A., Hartanti D. 2016a. Chemical constituents and antibacterial activities of crude extract and essential oils of *Alpinia galanga* and *Zingiber officinale*. *Int. Food Res. J.* 23:837-841.
- Hamad A., Hartanti D. 2015. The Use of Essential Oil of Clove (*Syzygium aromaticum*) as Tofu's Natural Preservative. *Farmasains* 2:289-294.
- Hamad A., Mahardika M.G.P., Istifah, Hartanti D. 2016b. Antimicrobial and volatile compounds study of four spices commonly used in Indonesian culinary. *J. Food Pharm. Sci.* 6:1-5.
- Han C.V., Bhat R. 2014. In vitro control of food-borne pathogenic bacteria by essential oils and solvent extracts of underutilized flower buds of *Paeonia suffruticosa* (Andr.). *Ind. Crops Prod.* 54: 203-208.
- Hayouni E.A., Chraief I., Abedrabba M., Bouix M., Leveau J.-Y., Mohammed H., Hamdi M. 2008. Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat. *Int. J Food Microbiol.* 125:242–251.
- Jahan S. 2012. Epidemiology of Foodborne Illness, in: B. Valdez (Ed.), *Scientific, Health and Social Aspects of the Food Industry* InTech, Rijeka. pp. 321-342.
- Lesmana M., Subekti D.S., Tjaniadi P., Simanjuntak C.H., Punjabi N.H., Campbell J.R., Oyoyo B.A. 2002. Spectrum of vibrio species associated with acute diarrhea in North Jakarta, Indonesia. *Diagn. Micr. Infect. Dis.* 43: 91-97.
- Newell D.G., Koopmans M., Verhoef L., Duizer E., Aidara-Kane A., Sprong H., Opsteegh M., Langelaar M., Threlfall J., Scheutz F., Giessen J.v.d., Kruse H. 2010. Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microbiol.* 139:S3–S15.
- Purwani E., Hapsari N.W.S., Rauf R. 2009. Respon hambatan bakteri gram positif dan negatif pada ikan nila (*Oreochromis niloticus*) yang diawetkan dengan ekstrak jahe (*Zingiber officinale*) *J. Kesehatan* 2: 61-70.
- Purwani E., Muwakhidah. 2008. Efek berbagai pengawet alami sebagai pengganti formalin terhadap sifat organoleptik dan masa simpan daging dan ikan. *J. Penelitian Sains Teknol.* 9:1-4.
- Rialita T., Rahayu W.P., Nuraida L., Nurtama B. 2015. Aktivitas antimikroba minyak esensial jahe merah (*Zingiber officinale* var. *Rubrum*) dan lengkuas merah (*Alpinia purpurata* K. Schum) terhadap bakteri patogen dan perusak pangan. *Agritech.* 35:43-52.
- Sivasothy Y., Chong W.K., Hamid A., Eldeen I.M., Sulaiman S.F., Awang K. 2011a. Essential oils of *Zingiber officinale* var. *rubrum* Theilade and their antibacterial activities. *Food Chem.* 124:514-517.
- Sivasothy Y., Chong W.K., Hamid A., Eldeen I.M., Sulaiman S.F., Awang K. 2011b. Essential oils of *Zingiber officinale* var. *rubrum* Theilade and their antibacterial activities. *Food Chemistry* 124:514 - 517.
- Vollaard A.M., Ali S., Vanasten H.A.G.H., Ismid I.S., Widjaja S., Visser L.G., Surjadi C.H., Dissel J.T.v. 2004. Risk factors for transmission of foodborne illness in restaurants and street vendors in Jakarta, Indonesia. *Epidemiol. Infect.* 132:863-872.
- Voon H.C., Bhat R., Rusul G. 2011. Flower extracts and their essential oils as potential antimicrobial agents for food uses and pharmaceutical applications. *Compr. Rev. Food Sci. F* 11:34-55.
- Widyawati T. 2007. Aspek Farmakologi Sambiloti (*Andrographis paniculata* Nees). *Majalah Kedokteran Nusantara* 40.

Instant Tiwul Made of *Colocasia esculenta* (L.) Schott as A Current Functional Food Development for Hypercholesterolemic Patients

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Abstract

Colocasia esculenta (L.) Schott contains macronutrient, micronutrient and bioactive compounds, which are less used. *Colocasia esculenta* (L.) Schott's strengths are its water soluble polysaccharide and its rather low Glycemic Index (GI). Due to the high potency of Indonesian traditional food to be developed into a functional food, so the instant tiwul is made. This research aims to determine the physical and chemical quality of instant tiwul as a functional food product and the effect of instant tiwul provision towards cholesterol level on rats. The research stages include instant tiwul production, physical evaluation, macronutrient and recommended dietary allowances evaluation, and in vivo evaluation on hypercholesterolemic male wistar rats. The in vivo research data result was obtained from the T test evaluation. The result shows the physical characteristic of instant tiwulis preferred by the volunteer with 71.75% value. The color evaluation of instant tiwul results on lightness degree of 36.8%, redness degree (a*) of 16.1% and yellowish degree (b*) of 13.7%. The cooking time is 9 minutes and 45 seconds. The result of carbohydrate proximity evaluation is 84.87%; fat is 0.17%; protein is 2.78%; crude fiber is 2.84%; moisture is 7.57%; and carbon is 4.61%. The in vivo evaluation shows the hypercholesterolemic lowering activity of 36.40%. In conclusion, the instant tiwul made of *Colocasia esculenta* (L.) Schott has macronutrient compounds functioned as a functional food and having effect on hypercholesterolemic wistar rats.

Keywords: Hypercholesterolemia, Functional Food, Fiber, *Colocasia esculenta* (L.) Schott, Instant Tiwul

Introduction

The local food potencies in Indonesia are very large, some of them are tubers. Tubers can grow well almost in all regions of Indonesia. One of the tuber variety is *Colocasia esculenta* (L.) Schott. *Colocasia esculenta* (L.) Schott has a high protein content, vitamin B₁, P and Fe and low cholesterol contents. Besides its macro and micro nutrients, *Colocasia esculenta* (L.) Schott has bioactive compounds as water soluble polysaccharide and rather low Glycemic Index (GI). According to the research at the University of Sidney, *Colocasia esculenta* contains 54 IG. The secondary metabolite composition of *Colocasia esculenta* (L.) Schott was reported containing 2.65% of flavonoid, 1.01% of alkaloid, 0.70% of saponin and 1.06% of tannin. According to Nurcahya (2013), research with DPPH analysis on prolifenol and flavonoid total contribution showed *Colocasia esculenta* antioxidant activity is higher than cassava. *Colocasia esculenta* has a potency to increase human immune system towards diseases caused by free radical reactions as cancer, heart disease, diabetes, and aging.

Tiwul is a traditional food that was consumed in the past. Nowadays tiwul is rarely consumed by people due to its physical form although it is good for health, so tiwul can be used as a functional food. The global demand on delicious, affordable and healthy food makes tiwul becoming a potency for traditional food developers. Junk food slowly replaces traditional food, so the understanding on the traditional food significance to human's health is important to make this traditional food play a role as a functional food.

One of ways to deal with hypercholesterolemia is by using food made of *Colocasia esculenta*

which has high fiber content. According to Alinnor *et al.* (2010) some of fibers in tubers can lower the blood cholesterol without absorbing the bile acid. Fiber has a binding effect on organic compounds as bile acid and cholesterol, so it can lower fatty acid content in the digestive tract. The binding of bile by fiber also causes the bile acid to coming out from the enterohepatic circulation because the bile acid that is secreted to the intestine cannot be absorbed but it is secreted with the feces. Besides, research by Saputro (2015), stated that there is a water soluble polysaccharide content inside tubers. However, the exact amount of it has not been found yet. The water soluble polysaccharide is a water soluble fibre that is defined as component inside a plant that is not degraded enzymatically becoming sub units that can be absorbed in the gastro and small intestine. This water soluble polysaccharide also has some benefits for the body to cure degenerative diseases (Rosyida, 2011).

Based on the potential quality, raw material availability, and treatment, further evaluation is needed on the physical and chemical quality of instant tiwul and the effect of *Colocasia esculenta* (L.) Schott instant tiwul provision towards cholesterol level on wistar (*Rattus norvegicus*) rats in vivo.

Materials and Methods

Material used in this research was *Colocasia esculenta* (L.) Schott taken from Materia Medica Batu. Materials used in the chemical analysis of this research were salt (NaCl), aquadest, H₂SO₄ 1.25 %, NaOH 1 M, K₂S₂O₄, HgO, HCl 0.1 N, red metal indicator, petroleum ether. Materials for in vivo evaluation were wistar rats, BR-1 rat food, and quail egg yolk, which is used to increase the cholesterol level.

1. *Colocasia esculenta* (L.) Schott Flour Production

Tools and materials were prepared. Tubers bentol was washed with clean water. Cut of 1-2 mm thickness to make it easy during destruction process. Tubers were soaked with NaCl 2% to get rid of oxalate calcium (itchy) in bentol within an hour. Tuber was dried under the sun. Dried bentol was mixed using blender and filtered with 60 mesh filter.

2. Instant Tiwul Production

Weigh 100 g of tuber bentol flour. Add slowly 150 ml of warm water. Stir until resulting on a rather wet granule. Steam the dough for 25 minutes. Lift the dough and oven it using 50°C for 24 hours.

3. Organoleptic Properties

Organoleptic evaluation was done using Hedonic Method

25 semi-skilled panelists were chosen (4th grade of AKAFARMA students of Putra Indonesia Malang). 50 gr of sample was given to each of them. Then, the panelists were asked to give judgement by filling out the given questionnaire. The observed parameters were colour, aroma, texture, and taste.

4. Colour Reader Analysis

The instant tiwul was added into a transparent plastic. a* (redness degree), b_□ (yellowish degree) and L* (lightness) rider target was determined. The result on color reader was noted down.

5. Cooking Time Analysis

Prepare tools and materials. Weigh 2 g of instant tiwul. Prepare 50 ml of boiling water. Add

the instant tiwul and boiling water to a beaker glass. Wait until the instant tiwul was cooked and count the time using a stopwatch.

6. Proximate Analysis

Proximate analysis among other, Crude Fiber analysis by Difference SNI 01-2891 – 1992, Protein analysis with Kjeldahl Macro Method (AOAC, 1970), Fat analysis with Soxhlet Method (Woodman, 1941), Ash Content analysis (SNI 01-2891-1992), and Moisture analysis with Gravimetric Method (AOAC, 1970).

7. Bioassay of Cholesterol Lowering Effect of Instant Tiwul

BR1 food provision per rat for a week is 20 gram/ rat/ day. This food provision is as an adaptation condition. High cholesterol food was given in the amount of 8 gram of quail egg yolk for 7 days After 7 days the cholesterol level check up was conducted. Two groups were made with 8 rats in each group. The first treatment group was given the instant tiwul, whereas the second treatment group was given BR1. The instant tiwul provision was conducted for 14 days. The lowering effect on blood cholesterol level check up was conducted in the first and second week using the easy touch.

8. Statistical Analysis

The obtained data from the physical and chemical evaluation were analyzed descriptively. The panelist evaluation was calculated based on the deviation standard. The data from the in vivo evaluation were analyzed using the T test evaluation to determine the comparison of instant tiwul provision on hypercholesterolemic male wistar rats.

Result and discussion

Organoleptic Properties

Organoleptic is also known assensory evaluation. The organoleptic characteristic of food product and material is the first thing observed by the consumers before they evaluate the nutrition content. The following Table 1 is the instant tiwul organoleptic observation result.

Table 1. Instant Tiwul Made of *Colocasia esculenta* (L.) Schoot Organoleptic Result

Parameter	Result
Form	Coarse-grained
Aroma	<i>Colocasia esculenta</i> (L.) Schoot
Colour	Reddish brown
Taste	A bit sweet

On the organoleptic observation, instant tiwul was in a coarse form due to the moisture that it is almost similar to granule in the treatment process. The resulted colour is brown due to the color of the powder used which is brownish white. Moreover, heat process in the instant tiwul production also contributes to the brown color. Aroma and taste of instant tiwul is still specific to *Colocasia esculenta* (L.) Schoot because *Colocasia esculenta* (L.) Schoot was solely used as a raw material without the other powder addition. *Colocasia esculenta* (L.) Schoot also has a bit sweet taste and *Colocasia esculenta* (L.) Schoot aroma.

Voluntary Acceptance Analysis

The instant tiwul made of *Colocasia esculenta* (L.) Schoot research result was tested in hedonic scale which consists of taste, colour, texture and aroma evaluations. From the voluntary acceptance evaluation result on the cooked instant tiwul, these data are obtained (Table 2).

Table 2 . Instant tiwul Made of *Colocasia esculenta* (L.) Schoot Organoleptic Result

No	Parameter	Result	% Average
1.	Aroma	72%	
2.	Colour	75%	71.75%
3.	Texture	75%	
4.	Taste	65%	

Based on Table 2, the average score of voluntary acceptance evaluation was 71.75%. This value shows that instant tiwul made of *Colocasia esculenta* (L.) Schoot is or accepted by panelists. Based on report by Soekarto (1985), when the obtained average data is $50 < x \leq 75$ it can be concluded that the society likes to the instant tiwul made of Instant tiwul made of *Colocasia esculenta* (L.) Schoot.

Colour Reader Analysis

The colour reader evaluation of instant tiwul made of *Colocasia esculenta* (L.) Schoot was conducted to determine the resulted lightness level. It was conducted using the colour reader. This tool can differentiate colour based on 3 values that are L* (Lightness), a* (Redness) and b* (Yellowness). The result can be seen in the following Table 3.

Table 3. Instant Tiwul Made of *Colocasia esculenta* (L.) Schoot and Cassava Colour Reader

Colour	Instant tiwul Made of <i>Colocasiaesculenta</i> (L.) Schoot	Instant tiwul Made of Cassava
L*	36.8	40.2
a *	16.1	15.1
b *	13.7	18.4

In this research, it is obtained 36.8% of lightness (L*), 16.1% of redness (a*) due to enzymatical process and 13.7% of yellowish (b*) due to the instant tiwul colour that tends to yield reddish brown color. Meanwhile, on the instant tiwul made of cassava purchased in the market has 40.2% of lightness (L*) due to the addition of other material. Less of redness compared to the instant tiwul made of *Colocasia esculenta* (L.) Schoot was observed, which is 15.1%. It is happened due to the more lightness colour of instant tiwul made of cassava compared to the lightness colour of instant tiwul made of *Colocasia esculenta* (L.) Schoot.

Cooking Time Analysis

In the instant tiwul made of *Colocasia esculenta* (L.) Schoot, the cooking time was 9 minutes 45 seconds, whereas in the instant tiwul made of cassava was 15 minutes 34 seconds of cooking time. Thus can be concluded that cooking time of the instant tiwul made of *Colocasia esculenta* (L.) schoot was

faster than the instant tiwul made of cassava.

Proximate Analysis

Proximate analysis is the testing of food compounds that is consisted of macro and micro nutrients to ensure the food safety. The proximate analysis was conducted to determine the quality and quantity of a food product. It contains carbohydrate, crude fiber, protein, fat, moisture, carbon, and colour evaluation. The following is the proximate analysis result table of instant tiwul made of *Colocasia esculenta* (L.) Schoot.

Table 4. The Proximate Analysis

Parameter	Result
Protein (%)	2.78
Fat (%)	0.17
Carbohydrate (%)	84.87
Moisture (%)	7.57
Carbon (%)	4.61
Crude Fiber (%)	2.84

From the result, it is known that the protein content of instant tiwul is 2.78%. Whereas according to Hasan *et al.* (2001), the instant tiwul made of cassava contains 1.45% of protein. It shows that the instant tiwul made of *Colocasia esculenta* (L.) Schoot contains a very high protein content. Fat is a macronutrient component in a lipid group that dissolves in the non polar solvent. From the observation, the instant tiwul made of *Colocasia esculenta* (L.) Schoot contains 0.17% of fat. According to Hasan *et al.* (2001), the fat content in tiwul made of arrowroot tuber is 0.27%, in tiwul made of elephant foot yam is 0.27%, and in tiwul made of cassava is 0.24%. The fat content in all of tiwul is low but the content in the tiwul made of *Colocasia esculenta* (L.) Schoot is lower than that is 0.17% so this tiwul is good for the diet therapy due to its low fat content.

Carbohydrate is a macronutrient component that functions as an energy source in the body. From the observation, the instant tiwul made of *Colocasia esculenta* (L.) Schoot contains 84.87% of fat. According to Hasan *et al.* (2001), the carbohydrate content in tiwul made of arrowroot tuber is 72.91%, in tiwul made of elephant foot yam is 74.33%, and in tiwul made of cassava is 82.70%. It proves the carbohydrate content of instant tiwul made of *Colocasia esculenta* (L.) Schoot is higher than those in the instant tiwul made of arrowroot tuber, elephant foot yam and cassava, so it can be used as a rice substitution. According to Septian (2012), in 100 gram of rice, the carbohydrate content is 7.9 gram. The instant tiwul made of *Colocasia esculenta* (L.) Schoot also has a rather low GI that is 54. According to Nurcahya (2013), *Colocasia esculenta* (L.) Schoot is one of tubers that can be used as a rice substitution for diabetic patients due to its quite high fiber and protein that can lower the blood glucose level so that it is very good for obesity patients.

Water is a micronutrient component in the food material that influences appearance, texture, taste and endurance. From the observation, the instant tiwul made of *Colocasia esculenta* (L.) Schoot contains 7.57% of moisture. According to Hasan *et al.* (2011), the moisture content in tiwul made of

arrowroot tuber is 13.50%, in tiwul made of elephant foot yamis 13.80%, and in tiwul made of cassava is 13.50%. According to Winarno (1992), the moisture in a product can increase the endurance. With 7.57% of moisture means that the instant tiwul made of *Colocasia esculenta*(L.) Schoot can be kept in a longer time. It is in accordance to Suratmo (1992) statement that the food material that has a moisture of less than 10% can be kept longer because the microbes cannot grow and the enzymes are not active. The moisture affects the packaging effectivity and the endurance. The higher the moisture, the material will be susceptible to damage.

Ash is an anorganic compound resulted from the rest of an organic burning. The carbon determination is used as a parameter of nutrient content to determine whether a treatment process is good or not and to understand the type of material used. The instant tiwul made of *Colocasia esculenta* (L.) Schoot contains 4.61% of carbon. According to Hasan *et al.*(2011), the carbon content in tiwul made of arrowroot tuber is 3.90%, in tiwul made of elephant foot yam is 3.51%, and in tiwul made of cassava is 0.89%. The instant tiwul made of *Colocasia esculenta*(L.) Schoothas a quite high carbon content due to the environment factor or the growth place.

The crude fibre is very important in the food product quality evaluation because this number is an index and determining the food nutrient. Besides, the crude fiber content can be used to evaluate a treatment process. The instant tiwul made of *Colocasia esculenta* (L.) Schoot contains 2.84% of crude fibre. According to Hasan *et al.*(2011), the crude fibre content in tiwul made of cassava is 1.45%. It shows that the instant tiwul made of *Colocasia esculenta* (L.) Schoot contains a higher crude fibre content compared to the instant tiwul made of cassava, so this instant tiwul can be used as a cholesterol therapy.

Blood Cholesterol Level Treatment

The hypercholesterolemic effect evaluation was done to the rats with food with the addition of quail egg yolk. The cholesterol lowering effect average between rats with the BR food provision and rats with the instant tiwul provision can be seen in Figure 1.

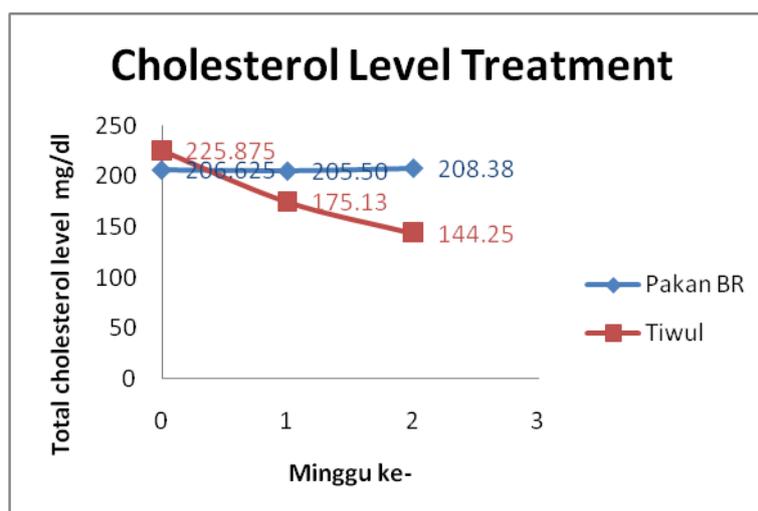


Figure 1. Blood Cholesterol Lowering Effect

The cholesterol lowering effect in this research used the instant tiwul that was compared to the standard BR food. The tiwul instant provision is as a functional food with the aim to determine whether it

can lower the cholesterol level on rats or not; with the assumption of instant tiwul having food fiber and the expectation of water soluble polysaccharide existence as its bioactive compound. To increase the cholesterol level the rats was given food that was added with egg yolk for a week. Then, the treatment was conducted by dividing the rats into 2 treatment groups, using BR and the instant tiwul.

The rats with the instant tiwul provision experience the cholesterol level reduce. The instant tiwul used to lower the cholesterol was made of *Colocasia esculenta* (L.) Schoot powder. *Colocasia esculenta* (L.) Schoot is a tuber with a bioactive compound that functions to lower the cholesterol level. Active compound existed in *Colocasia esculenta* (L.) Schoot is water soluble polysaccharide that is a food fiber. In the previous research *Colocasia esculenta* (L.) Schoot is known of having a water soluble polysaccharide content after it is evaluated with the HPLC.

In the previous research *Colocasia esculenta* (L.) Schoot is known of having a water soluble polysaccharide content Jenkinset *et al.* (2001) research shows that the water soluble polysaccharide, especially fiber that has a high viscosity, consistently reducing total cholesterol and LDL level. Prasetyo,(2015) stated the dissolved fiber will increase the digestive tract viscosity so it will inhibit the cholesterol to reach the intestine epithel. Besides, according to Tensiska (2008) in Maligan *et al.* (2011), the water soluble polysaccharide in the colon will be fermented resulting the Short Chain FattyAcid (SCFA) as propionate and butyrate acids. The SCFA affects the fat control,which is propionate acid will be metabolized in the liver and reducing the cholesterol synthesis. Food fibre, according to Estiasih (2012), is also proven of having a lowering effect on the rats' plasma lipid.

The statistic evaluation result that was obtained in the T test evaluation shows sig value of 0.003 on cholesterol level that is less than 0.05. It shows that the decision, which is taken, is H1 accepted. The evaluation result showing H1 accepted and H0 rejected shows that there is an effect of instant tiwul provision on the hypercholesterolemic rats.

Conclusion

Organoleptic of instant tiwul made of *Colocasia esculenta* (L.) Schoot is a brown granule with *Colocasia esculenta* (L.) Schoot aroma and taste. On the instant tiwul made of *Colocasia esculenta* (L.) Schoot sample it is obtained that the carbohydrate proximate analysis of 84.87%; fat of 0.17%; protein of 2.78%; crude fiber of 2.84%; moisture of 7.57%; and carbon of 4.61%.The in vivo evaluation shows the hypercholesterolemic lowering activity of 36.409% for 2 weeks consumption of instant tiwul made of *Colocasia esculenta* (L.) Schoot.

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References

- Alinnor, I.J., and C.O. Akalezi. 2010. Proximate and Mineral Composition of Dioscorea rotundata (white yam) and Colocasia esculenta (white cocoyam). Pakistan Journal of Nutrition 9(10): 998 -1001
- Danuri, H., 2008. Optimizing Angkak Pigments and Lovastatin Production By Monascus purpureus. Hayati Journal of Biosciences, 15(2), pp.61-66.
- Estiasih, T., Harijono, Weny B.S., and Atina R. 2012. Hypoglycemic Activity of Water SolublePolysaccharides of Yam (Dioscorea

- hispidadents) Prepared by Aqueous, Papain, and Tempeh Inoculum Assisted Extractions. World Academy of Science, Engineering and Technology. 323-329
- Hasan, I. 2011. Indeks Glikemik Oyek Dan Tiwul Dari Umbi Garut. Jurnal teknologi dan industri hasil pertanian, no 1.
- Jenkins, D. J. A., Wolever, T. M. S., and Jenkin, A. L. 1999. Viscous Fibers Health claims and Strategies Nutrient Absorption and Metabolism, in Modern Nutrition in Health and Disease, 9thed, 679-698. Baltimore, Maryland, USA.
- Khoiri, A. 2013. Sifat Tekstural dan Cooking Quality Mie Bebas Gluten dari Tepung Sukun. Seminar Nasional, 835 - 843.
- Nurchaya, H. 2013. Budidaya dan Cara Olah Talas untuk Makanan dan Obat. Yogyakarta: Pustaka Baru Press.
- Prasetyo Sonny Saputro, E. 2015. Pengaruh Polisakarida Larut Air (PLA) Dan Serat Pangan Umbi- Umbian Terhadap Glukosa Darah. Jurnal Pangan dan Agrindustri, 3.
- Rosyida, N. 2011. Efek Hipokolesterolemik Polisakarida Larut Air dari Gadung (*Dioscorea hispida* Dennst.) yang Diekstrak dengan Berbagai Metode. Skripsi. Jurusan Ilmu dan Teknologi Pangan Fakultas Teknologi Pertanian Universitas Brawijaya. Malang
- Saputro, P. S., & Estiasih, T. 2015. Pengaruh Polisakarida Larut Air (PLA) dan Serat Pangan Umbi-umbian Terhadap Glukosa Darah : *Jurnal Pangan dan Agroindustri*, 756-762
- Septian, I. 2012. Indeks Glikemik Berbagai Produk Tiwul Berbasis Singkong (*Manihot Esculenta* Crantz) Pada Orang Normal . Tesis, Bogor : Program Pasca Sarjana, Institut Pertanian Bogor.
- Soekarto T. 1985. Pengujian Organoleptik Untuk Industri Pangan dan Hasil Pertanian. Bhratara Karya Aksara. Jakarta.
- Suratmo, 1992. Analisa Bahan Pangan Nabati. IPB Press, Bogor.
- Tensiska. 2008. Serat Makanan. Jurusan Teknologi Industri Pangan Fakultas Teknologi Industri Pertanian Universitas Padjadjaran.
- Winarno, F. 2002. Kimia Pangan dan Gizi. Jakarta: Gramedia Pustaka Utama.

The Effect of Calcium Carbonate (CaCO₃) Concentration on the Physicochemical and Organoleptic Properties of Black Rice-Lady Finger Banana Breakfast Cereal

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Abstract

One of the cereals which is potentially processed to be a breakfast cereal is black rice. Black rice (*Oryza sativa L. indica*) is one of the rice varieties commonly grown in Indonesia. The utilization of black rice is not as high as white rice. However, black rice possesses some antioxidative phytochemical compounds, such as anthocyanins, phenolic compounds, and flavonoids which can inhibit free radical and therefore lower the risk of cancer. The usage of lady finger banana (*Musa acuminata cv. Lady Finger*) is to improve the taste and aroma of the cereal and also to utilize Indonesia's local commodity. Water absorption capacity is an essential parameter for breakfast cereal. Calcium carbonate (CaCO₃) can interact with cereal starch granules and affect starch gelatinization. Starch gelatinization itself affects physicochemical and organoleptic properties of breakfast cereals. CaCO₃ at levels 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% were investigated for its effect on rehydration rate, water absorption capacity, moisture content, and organoleptic properties of black rice-lady finger banana breakfast cereal. It was hypothesized that CaCO₃ significantly affects physicochemical and organoleptic properties of breakfast cereals.

Keywords: flakes, black rice, banana, lady finger, calcium carbonate

Introduction

Breakfast cereal can be manufactured from many kinds of cereals, such as corn, wheat, and oat. One of the cereals which is potentially processed into a breakfast cereal is black rice. Black rice (*Oryza sativa L. indica*) is one of the rice varieties commonly grown in Indonesia which is not utilized as much as white rice. However, black rice possesses some phytochemical compounds, such as anthocyanins (Hiemori *et al.*, 2009), phenolic compounds (Zhou *et al.*, 2004), and flavonoids (Nakornriab *et al.*, 2008) which can inhibit free radicals. Therefore, the risk of cancer can be minimized by consuming black rice. Beside black rice, lady finger banana (*Musa acuminata cv. Lady Finger*) is another Indonesia food crop that can be consumed more. Lady finger banana is usually eaten as fresh fruits rather than processed into food products. The utilization of lady finger banana will improve the taste and aroma of the cereal of its compound.

Water absorption capacity is an essential parameter for breakfast cereals. Santiago-Ramos *et al.* (2015) stated that Ca²⁺ ions from CaCO₃ (calcium carbonate) can interact with cereal starch granules and affects starch gelatinization and starch ability to bind water. Therefore, the physicochemical and organoleptic properties of the breakfast cereal can be affected too. The purpose of this research was to identify and understand the effect of calcium carbonate (CaCO₃) concentration on the physicochemical and organoleptic properties of a breakfast cereal based on black rice.

Materials and Method

Materials

Black rice (*Oryza sativa var. java*) in the form of black rice flour was obtained from Lingkar Organik, Jogjakarta, Indonesia. Lady finger banana originally from Lumajang, Indonesia was obtained from a local vegetable seller in Surabaya, Indonesia. Other materials to produce instant cereal were commercial goods sold in supermarkets, such as granulated sugar (Gulaku), salt (Kapal Lajar), and mineral water (Aquase).

Chemicals

Calcium carbonate used for cereal production was purchased from Merck (Darmstadt, Germany).

Production of Lady Finger Banana Puree

Lady finger banana fruit was blanched using steam for 5 minutes then peeled in order to remove the skin. The fruit flesh then was blended using food processor (Miyako Ch-501 pf Ap, China) for 1 min.

Production of Black Rice-Lady Finger Banana Breakfast Cereal

Black rice flour was first passed through 100 mesh sieves. Black rice flour was then mixed with the banana puree and mineral water and mixed until homogenized. The mixture then was added with granulated sugar, salt, and calcium carbonate and mixed until homogenized again. The usage level of calcium carbonate was 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%; and 0.6% based on the total weight of the black rice flour, banana puree, and mineral water. The mixture then was heated for 45 sec. at 80-85°C. After heating, the mixture was pressed using a flakes presser (Akebonno, China) for 45 seconds at 150°C before oven-dried (105°C; Nayati, Japan) for 10 min. The manufacture of the cereal was done in three replications.

Water Absorption Capacity Analysis

Water absorption capacity of each sample was analyzed using the method described by Rangana (1979) cited in Dewi (2012). The breakfast cereal was first weighed and then dipped in 80°C water for 20 sec. After dipping the cereal, the final weight was measured and the difference between the two weights was calculated then divided by the initial weight to calculate the percent water absorption capacity. The ratio of the cereal sample:water was 1:6.

Re-hydration Rate Analysis

Re-hydration rate of each sample was analyzed using method as described by Rangana (1979) cited in Dewi (2012). Same with the water absorption analysis, breakfast cereal was first weighed and then dipped in 80°C water for 20 sec. The ratio of cereal sample:water was 1:6. After dipping the cereal, the final weight was calculated. The difference between final and first weights was calculated and the re-hydration rate was stated in g/20 s.

Statistical Analysis

All experiments were done in three replications. Statistical analysis used in this research were ANOVA (Analysis of Variance) test and DMRT (Duncan's Multiple Range Test), with SPSS Statistics version 23.

Results and Discussion

Water absorption capacity and re-hydration rate of the cereal samples showed same trend. Within the concentration of 0-0.2% CaCO₃, the water absorption capacity and re-hydration rate increased. The maximum water absorption capacity and re-hydration were at the concentration of 0.2% CaCO₃. Started from the concentration of 0.3-0.6% CaCO₃, the trend reversed.

Table 1. Water Absorption Capacity and Re-hydration Rate of Cereal Samples with Various Concentration of Calcium Carbonate (CaCO₃)

Concentration of CaCO ₃ (%)	Water Absorption Capacity (%)	Re-hydration Rate (g/20 s)
0	90.58 ± 1.43 ^b	7.13 ± 0.63 ^a
0.1	92.22 ± 1.34 ^b	7.60 ± 0.10 ^{a,b}
0.2	128.79 ± 1.46 ^c	11.13 ± 0.67 ^d
0.3	120.78 ± 1.54 ^d	9.45 ± 0.27 ^c
0.4	110.63 ± 1.32 ^c	9.23 ± 0.18 ^c
0.5	110.06 ± 2.28 ^c	8.20 ± 0.18 ^b
0.6	84.93 ± 1.91 ^a	7.64 ± 0.29 ^{a,b}

Note: Mean values with the same letters are not significantly different at $\alpha = 5\%$

The water absorption capacity and re-hydration rate decreased and these agree with the results of Bryant and Hamaker (1997). These authors reported that the high pH of the system possibly caused ionization of starch hydroxyl groups. Therefore, there is also an opportunity of interaction between Ca²⁺ and starch molecules. At the concentration of 0-0.2% CaCO₃, the crystalline regions of starch granules are possibly disrupted by alkali. Pineda-Gomez *et al.* (2011, 2012) as described by Santiago-Ramos *et al.* (2015) stated that the ions in alkali solutions diffused into the amylose-rich amorphous regions and breaking the intermolecular bonds. At higher concentration of CaCO₃, Ca²⁺ ions stabilized the starch granules and increase the rigidity. There is also a possibility of crosslinking between Ca²⁺ ions and starch granules, so that the water absorption capacity and re-hydration rate decreased in higher concentration (0.3-0.6%) of CaCO₃. Oosten (1982) as described by Bryant and Hamaker (1997) stated that Ca²⁺ and other divalent cations bound tightly with starch molecules causes decrease in water holding capacity.

Another parameters analysis conducted by Bryant and Hamaker (1997) showed relatively similar trends with the water absorption capacity trend. The amount of digestible starch had its peak on the 0.2% lime. This phenomenon may be due to the disruption of crystalline regions of starch granules, so that enzymes had greater access to the starch molecules. Soluble starch after cooking and assayed as glucose in the supernatant peaked at 0.2% lime for the isolated starch.

Further Research

As a health-promoting food product, further research should be done. Antioxidant activity of the cereal sample should be assayed since black rice contained anthocyanins, such as cyanidin-3-glucoside and peonidin-3-glucoside (Zawistowski *et al.*, 2008); phenolic compounds (Zhou *et al.*, 2004), and flavonoids (Nakornriab *et al.*, 2008). Beside its health benefits, the organoleptic properties are also essential parameters to be tested. Nowadays, breakfast cereals are a valuable component of people's busy life, so the potential to manufacture the black rice-lady finger banana cereal into commercially should be explored upon ascertaining its desirable food properties, such as organoleptic properties. Its starch digestibility properties are also important.

Conclusion

Within the concentration of 0-0.2% CaCO₃, the water absorption capacity and re-hydration rate increased. On the contrary, the water absorption capacity and re-hydration rate decreased within the concentration of 0.3-0.6% CaCO₃. Further research should be done regarding the health benefits and other properties of the black rice-lady finger banana breakfast cereal.

References

- Bryant, C.M. and B.R. Hamaker. 1997. Effect of Lime on Gelatinization of Corn Flour and Starch. *Cereal Chem.* 74(2):171–175.
- Dewi, M. S. H. 2012. Black Glutinous Rice Flakes Characteristics with Various Boiling and Drying Temperature. *Thesis*, Faculty of Agricultural Technology Widya Mandala Catholic University Surabaya, Surabaya.
- Hiemori, M., E. Koh, and A.E. Mitchell. 2009. Influence of Cooking on Anthocyanins in Black Rice (*Oryza sativa* L. japonica var. SBR). *J. Agric. Food Chem.* 57:1908–1914.
- Nakornriab, M., T. Sriseadka, and S. Wongpornchai. 2008. Quantification of Carotenoid and Flavonoid Components in Brans of Some Thai Black Rice Cultivars Using Supercritical Fluid Extraction and High-Performance Liquid Chromatography-Mass Spectrometry. *J. Food Lipid* 15:488–503
- Santiago-Ramos, D., J. de Dios Figueroa-Cardenas, J.J. V´eles-Medina, R. Reynoso-Camacho, M. Ramos-Gomez, M. Gaytan-Martinez, and E. Morales-Sanchez. 2015. Effects of Annealing and Concentration of Calcium Salts on Thermal and Rheological Properties of Maize Starch During an Ecological Nixtamalization Process. *Cereal Chem.* 92(5):475–480.
- Zhou, Z.K., K. Robards, S. Helliwell, and C. Blanchard. 2004. The Distribution of Phenolic Acids in Rice. *Food Chem.* 87:401–406.

Texture of Cookies with Pregelatinization Time and Substitution Level of Red Kidney Bean Flour

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Abstract

The use of red kidney bean flour in cookies making is as wheat flour substitution. Wheat flour substitution used pre-gelatinized red kidney bean flour to reduced starchy taste because of ungelatinized starch. Pre-gelatinized time of red kidney bean flour influenced the swelling of the starch granule. Wheat flour substitution in cookies cannot be done until 100% because red kidney bean flour does not have gluten which is contributes in cookies texture. Pre-gelatinized time and substitution level of red kidney bean flour involved the objective (breaking force) and subjective texture (preference level of breaking force and mouthfeel preference level). This research aim was to identify the effect of pre-gelatinization time and substitution level of red kidney bean flour to the cookies texture and determined the correlation between objective and subjective. Experimental design used factorial randomized block design. First factor was pre-gelatinization levels of red kidney bean flour (10, 12.5 and 15 minutes of steaming time). Second factor was red kidney bean flour substitution level. The treatment was replicated three times. The results showed that pre-gelatinized time and substitution level of red kidney bean flour influenced the breaking force, preference level of breaking force and mouthfeel preference level. There was close enough correlation (0.73) between breaking force and breaking force preference level, but no correlation between breaking force and mouthfeel of cookies.

Keywords: cookies, red kidney bean flour, substitution level, pre-gelatinization level, texture

Introduction

Red kidney bean (*Phaseolus vulgaris* L.) is one of local food stuff with productivity of 103,376 tons in 2013 (Statistics Indonesia, 2014). Butt *et al.* (2010) explain that starch content in red kidney bean (39.45%) can become energy source for human body and protein content (24%) can be used for cell regeneration. Its productivity does not follow with proper utilization. Red kidney bean is commonly used as ingredient in soup, ice cream, and porridge. Making red kidney bean flour is one of the alternatives that can improve its utilization because it is easily applicable in food products, such as cookies.

Cookies is a kind of bakery product that preferred by many people, from kids to olders because it is simple and has long shelf life. It is usually eaten as snack in many occasion. This is supported by the high consumption levels of cookies in cities and villages (Rosmisari, 2006), which is 0.50 and 0.40 kg/capita/year.

Red kidney bean flour is used as wheat flour substitution. Cookies texture is a very important parameter in cookies characteristics. Subtitution of wheat flour with red kidney bean will change the texture characteristics in cookies. Cookies with good texture characteristics can improve its consumers acceptability.

Although consist of starch and protein, red kidney bean flour can not be used to substitute 100% wheat flour because it lacks gluten formation proteins. Gluten protein is very important in the formation of cookies texture. Apotiola and Fashakinly (2013) reported that substitution of wheat flour up to 20%

with 10% cocoyam and 10% soybean flour can be used without affecting the sensory qualities of texture of the cookies. Incorporating sweet potato flour to wheat flour for 40% in cookies (Singh *et al.*, 2008; Srivastava *et al.*, 2012). Konuma *et al.* (2012) also reported that substituting wheat flour with sago starch for 40%. This research use 50% and 60% red kidney bean to substitute wheat flour.

Substituting wheat flour in cookies requires red kidney bean to be pregelatinized before use. Pregelatinization process is supposed to reduce the starchy taste in cookies due to the improperly gelatinized starch. Pregelatinization time of red kidney bean is one factor that can influence red kidney bean starch to be swollen. Its swollen level influences texture of the cookies. This research used pregelatinization time of 10 minutes, 12.5 minutes, and 15 minutes.

Substitution of wheat flour with red kidney bean and pregelatinization time of red kidney bean will change the texture characteristics in cookies. Cookies texture will be analyzed by objective and subjective tests. Objective texture will be evaluated by analysis of the breaking force by using texture analyzer and subjective test will be evaluated by analysing the preference level of breaking force and mouthfeel preference level. Objective texture test can not be used to describe the texture changes effects because of the wheat flour substitution with red kidney bean flour and pregelatinization time of red kidney bean flour and also the otherwise. Therefore there is the need to evaluate the correlation between objective-subjective. This research aimed to identify the effect of pregelatinization time and substitution level of red kidney bean flour to the cookies texture and determine the correlation between objective and subjective.

Materials and Methods

Pregelatinized Red Kidney Bean Flour Making

Red kidney bean was steeped in water (1:10) for 10 hours then the shell was peeled. Peeled red kidney bean was then steamed at 85-90°C and drained to reduce the water content and reduce its temperature. Steamed red kidney bean grinded to increase the surface area during roasting. It was roasted (90°C) for 24 minutes and continued with re-grinded and sieved through 80 mesh sieve. Pregelatinized red kidney bean flour was then packed and sealed in 0.8mm PP plastic bag and then put and stored into a food container (topless) before use.

Cookies Making

Cookies was made by mixed margarine (40.5%), eggs (18%), icing sugar (54%), and salt (0.5%) with mixer for 3 minutes. Mixed flour of medium wheat flour and pregelatinized red kidney bean flour (100%), and baking powder (0.9%) were added and mixed until form a homogen and well mixed cookies dough. Cookies dough then moulded and shaped (3.7 cm × 1.5 cm × 0.4 cm) and then bake with oven at 175°C for 15 minutes. Note: the percentage based on total flour weight.

Research Design

This research used Factorial Randomized Block Design with two factors. First factor was pregelatinization time levels of red kidney bean flour, consisted of 10, 12.5 and 15 minutes of steaming time. Second factor was pregelatinized red kidney bean flour substitution level through wheat flour, consisting of 50% and 60%. The treatment was replicated three times. Data were analyzed by analysis of varians (ANOVA) at $\alpha=5\%$ to evaluate the significant effect on every parameter and followed with *Duncan's Multiple Range Test* (DMRT) at $\alpha = 5\%$ to determine the significant level of treatments. Data continued

with subjective-objective correlation test to evaluate the correlation between objective and each subjective test of the cookies texture.

Determination of Water Content(AOAC, 1990)

Moisture content of red kidney bean flour and cookies was done using thermogravimetry methods. The water was evaporated through drying in the oven at 105°C. Drying was considered done when it reached constant weight, then calculated using wet based calculation. Moisture content was described as amount of water (g) per 100 g sample.

Determination of BreakingForce (Turksoyet al., 2007 with modification)

BreakingForce was analyzed using Texture Profile Analyzer TA-XT Plus with compression in the center of sample until it broke into two pieces. Breaking force showedthe maximum force which is needed to break a product. Probe used was three point bend rig (small) probe. Cookies height was also measured (cm). Breaking force described as maximal force needed to break cookies (g) per height of the cookies (cm). Settings used for texture analyzer were:

Mode	: measure force in compression
Option	: return to start
Pre-test speed	: 1.50 mm/s
Test speed	: 0.5 mm/s
Post-test speed	: 10.00 mm/s
Target mode	: distance
Distance	: 5.00 mm
Trigger type	: auto (force)
Trigger force	: 25.00 g
Break mode	: off
Stop plot at	: start position
Tare mode	: auto
Control oven	: disabled
Frame deflection correction	: off (XT2 compatibility)
Data acquisition rate	: 400 pps
Probe return correction	: 20 mm
Speed return	: 10 mm/s
Cookies distance	: 2 cm

Determination of Preference Sensory Test (Turksoy et al., 2007 with modification)

Preference sensory test was done using a seven Hedonic Scale Scoring with score start from 1 (very dislike) until 7 (very like). This test used 100 untrained panelists. Parameters that analyzed were the preference on texture of cookies on breaking force and mouthfeel. The panelist determined the preference of breaking force of cookies by analyzing the breaking force of cookies when was bitten by incisor. Mouthfeel preference was determined by analyzing the sandiness sensation in mouth while eating the cookies. Cookies sample used for sensory tests were prepared one day before the day of testing. Cookies packedand sealed in 0.8mm PP plastic bag then put and stored into a food container (topless) were used.

Results and discussion

The most important cookies characteristics is breaking force. Breaking force was analyzed using Texture Analyzer TA-XT plus. Breaking force was used to evaluate the effect of substitution of pregelatinization red kidney bean flour level and pregelatinization time of red kidney bean flour level. The highest breaking force was at 10 minutes pregelatinization red kidney bean flour and 60% pregelatinized red kidney bean flour substitution treatment. The lowest breaking force was at 15 minutes pregelatinization red kidney bean flour and 50% pregelatinized red kidney bean flour substitution treatment. The results showed that there was significantly different breaking force in cookies, as shown in Table 1. Table 1 showed that breaking force reduced as pregelatinization time of red kidney bean flour increased and also as substitution level of red kidney bean flour increased.

Table 1. Cookies Characteristics with Substitution Level and Pre-gelatinization Time of Red Kidney Bean Flour

Pregelatinization time	Substitution level	Breaking force (g/cm)
10 minutes	50%	2784.4191 ^e
	60%	2103.1402 ^c
12.5 minutes	50%	2461.2179 ^d
	60%	1950.1157 ^b
15 minutes	50%	2174.9352 ^c
	60%	1641.1895 ^a

Note: mean with different letter indicate significant difference at $\alpha = 5\%$

As combination of pregelatinization time and substitution level of red kidney bean flour increased, it reduced the breaking force of cookies. This happened probably because gluten from wheat flour content reduced when the substitution level of pregelatinized red kidney bean flour increased. Starch granules size became bigger when pregelatinization time of red kidney bean flour increased.

Gluten play an important role in cookies texture formation. Cookies texture was made from complex matrix between gluten protein and starch. Substitution of wheat flour with pregelatinized red kidney bean flour did not contribute to the texture formation. Reduced amount of wheat flour made the gluten protein content to decrease, therefore the matrix formation weaker. The combination with different pregelatinization time of red kidney bean flour affected the matrix. Longer pregelatinization time of red kidney bean flour made the starch granules to become more swollen and its structure more brittle, although bigger in size. Interaction between gluten strand and red kidney bean starch granules made the complex matrix to become weaker, therefore became easy to break and breaking force being smaller.

Substitution of wheat flour with other types flour commonly made the cookies texture harder because of many kinds of the components in the flour, such as starch, protein, fiber that did not contribute to the formation of cookies texture. Aziah *et al.* (2012) in making cookies used mungbean and chickpea combined with corn flour (1:1), which resulted in increased hardness (53.00 ± 1.80 N and 61.87 ± 0.34 N) than control (41.50 ± 1.74 N). Singh *et al.* (2008) also reported that as concentration of sweet potato flour to wheat flour increased the fracture strength force increased. Otherwise, Konuma (2012) reported that substituting 40% wheat flour with sago starch in cookie made the hardness to decrease from 1.920 ± 0.385 kg to 1.693 ± 0.308 kg.

Texture or cookies with combination of pregelatinization time and substitution level of red kidney bean flour was also evaluated with sensory test. The preference test of the cookies used two texture parameters, breaking force and mouthfeel. The result showed that there was significant difference for breaking force and mouthfeel preference in cookies, as shown in Table 2.

Table 2. Sensory Characteristics of Breaking Force and Mouthfeel of Cookies with Substitution Level and Pre-gelatinization Time of Red Kidney Bean Flour

Pre-gelatinization Time	Substitution Level	Breaking Force	Mouthfeel
10 Minutes	50%	4.95 ^c	3.98 ^b
	60%	5.20 ^c	3.38 ^a
12.5 Minutes	50%	5.24 ^c	4.77 ^{de}
	60%	4.53 ^b	4.32 ^c
15 Minutes	50%	4.35 ^b	5.05 ^e
	60%	3.32 ^a	4.58 ^{cd}

Note: Means with different letter in the same column indicate significant difference at $\alpha = 5\%$. Score 1 shows very dislike to score 7 shows very like

The highest preference of breaking force was at 10 minutes pregelatinization of red kidney bean flour and 60% pregelatinized red kidney bean flour substitution treatment. The lowest breaking force was at 15 minutes pregelatinization of red kidney bean flour and 60% pregelatinized red kidney bean flour substitution treatment. Combination of 10 minutes pregelatinization red kidney bean flour with 50% and 60% pregelatinized red kidney bean flour substitution treatment also combination of 12.5 minutes pregelatinization red kidney bean flour and 50% pregelatinized red kidney bean flour substitution treatment were not significantly different. This condition explained that both pregelatinization time and substitution of red kidney bean influenced the preference of breaking force. Related research also reported that using sago starch reduced the texture preference being smooth (Konuma, 2012). Singh (2008) reported that using a higher proportion of sweet potato flour reduced the texture preference from 4.6 (crisp) to 3.2 (crumbly). Otherwise, substituting 50% wheat flour with 30% cocoyam flour and 10% soybean flour reduced texture preferences than control, from 4.30 ± 0.67 (good) to 3.10 ± 0.99 (neutral) (Apotiola and Fashakinly, 2013).

Both pregelatinization time and substitution of red kidney bean influenced the preference of breaking force. This trend was supported with breaking force (Table 1). This showed that increasing level of substitution and pregelatinization time made cookies texture break easily and panels did not prefer this characteristic of cookies. This condition also supported the result of correlation analysis which showed that there was close enough correlation (0.73) between breaking force and breaking force preference level. The results of correlation analysis showed that the preference analysis on broken force can represent objective broken force analysis of cookies.

Mouthfeel was also one of important texture characteristics of cookies. The highest preference of mouthfeel was at 15 minutes pregelatinization of red kidney bean flour and 50% pregelatinized red kidney bean flour substitution treatment. The lowest breaking force was at 10 minutes pregelatinization of red kidney bean flour and 60% pregelatinized red kidney bean flour substitution treatment. There was an increased trend of mouthfeel preference as pregelatinization time became longer and concentration

substitution of red kidney bean higher until the 15 minutes pregelatinization red kidney bean flour and 50% pregelatinized red kidney bean flour substitution treatment, then it reduced at 15 minutes pregelatinization of red kidney bean flour and 60% pregelatinized red kidney bean flour substitution treatment. This implied that, although substitution of red kidney bean increased, the presence of pregelatinization on red kidney bean made its starch to support complex matrix formation and then reduced the sandiness level. Preference of mouthfeel had no correlation through breaking force as shown by the result of correlation analysis (-0.13).

Recommended treatment was determined by panels preference of breaking force and mouthfeel throughout the treatments. As shown in Table 2, the highest breaking force preference was at 12.5 minutes pregelatinization time and 50% pregelatinized red kidney bean flour substitution treatment, but not significantly different with 10 minutes pregelatinization time combined with 50% and 60% pregelatinized red kidney bean flour substitution treatments. While the preference of mouthfeel was highest at 15 minutes pregelatinization time and 50% pregelatinized red kidney bean flour substitution treatment. This treatment was not significantly different with 12.5 minutes pregelatinization time and 50% pregelatinized red kidney bean flour substitution treatment. Therefore, the recommended treatment was combination of 12.5 minutes pregelatinization time of red kidney bean and 50% substitution of wheat flour with pregelatinized red kidney bean flour treatment.

Conclusion

The results showed that pregelatinized time and substitution level of red kidney bean flour influenced the breaking force, preference level of breaking force and mouthfeel preference level. There was close enough correlation (0.73) between breaking force and breaking force preference level, but no correlation between breaking force and mouth feel of cookies. The recommended treatment was therefore pregelatinized time of red kidney bean for 12.5 minutes combined with 50% substitution level of red kidney bean flour.

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References

- Apotiola, Z.O. and Fashakinly, J.F. 2013. "Evaluation of Cookies from Wheat Flour, Soybean Flour, and Cocoyam Flour Blends". *Food Science and Quality Management* vol. 14, pp. 17-21.
- Aziah, A.A.N., Noor, A.Y.M., and Ho, L.H. 2012. "Physicochemical and organoleptic properties of cookies incorporated with legume flour". *International Food Research Journal* Vol. 19, no. 4, pp. 1539-1543.
- AOAC. 1990. *Official Methods of Analysis 14th Edition*. Washington D.C.: Association of Analytical Chemists.
- Statistic Indonesia. 2014. *Vegetables Production in Indonesia 1997-2013*. http://www.bps.go.id/tab_sub/view.php?kat=3&tabel=1&daftar=1&id_subyek=55%20¬ab=70. (10 Juni 2015).
- Butt, M. S. and Batool, R. 2010. "Nutritional and Functional Properties of Some Promising Legumes Protein Isolates". *Pak. Jurnal. Nutrition* vol. 9, no. 4, pp. 373-379.
- Konuma, H., Rolle, R., and Boromthananat, S. 2012. "Adding value to underutilized food resources: substituting wheat flour with sago starch in cookie formulation". *Journal of Agricultural Technology* vol. 8, no. 3, pp. 1067-1077.
- Rosmisari, A. 2006. *Review: Tepung Jagung Komposit, Pembuatan dan Pengolahannya*. Seminar Nasional Teknologi Inovatif Pascapanen Pengembangan Pertanian, Bogor.
- Singh, J., Singh, N., Sharma, T.R., and Saxena, S.K. 2003. "Physicochemical, rheological and cookie making properties of corn and potato flour". *Food Chemistry* vol. 83, pp. 387-393.
- Singh, J., Singh, N., Sharma, T.R., and Saxena, S.K. 2008. "Effect of incorporating sweetpotato flour to wheat flour on the quality characteristics of cookies". *African Journal of Food Science* vol. 2, pp. 065-072.

- Srivastava, S., Genitha, T.R., and Yadav, V. 2012. "Preparation and quality evaluation of flour and biscuit from sweet potato". *J. Food Process Technol.* Vol. 3, no.12, pp 1-5.
- Turksoy, S., Keskin,S.,Ozkaya, B., and Ozkaya, H. 2011. "Effect of black carrot (*Daucus carota* L. Ssp. *Sativus* var.*atrorubens* Alef.) fiber addition on the composition and quality characteristics of cookies".*Journal of Food, Agriculture & Environment* Vol 9, no. 3&4, 57-60.

Safety Evaluation of *Lactobacillus plantarum* Mut-7 as a Potential Indigenous Probiotic Strain Using *Sprague Dawley* Rats as a Model

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Abstract

Probiotic strains from fermented food have been isolated for many years, and an investigation into the safety properties of these probiotic strains is needed. *Lactobacillus plantarum* Mut-7 is an indigenous strain with probiotic potential isolated from fermented cassava (gatot). The aim of this study was to evaluate the potential probiotic of an orally administered probiotic strain *L. plantarum* Mut-7 and to clarify the safety of this strain for human use. A high dose (10^{11} CFU/rats/day) of *L. plantarum* Mut-7 was forced feeding to *Sprague dawley* rats for 28 days. The growth of animals, organ weight, hematology, GOT and GPT activity, and intestinal morphology were investigated. Translocation test and molecular detection based on BOXAIR-PCR of bacterial strain in blood and tissues (heart, liver, lung, spleen, and kidney) were indicated as safety parameter. Digesta and fecal bacterial count were used as indicator for in vivo gastrointestinal survival. The results indicated no adverse effects on the rats, no illnesses or deaths and viable cells of LAB were recovered from the blood and tissue samples have low similarity coefficient < 75% with *L. plantarum* Mut-7. After ingestion of *L. plantarum* Mut-7 10^{11} CFU/rat/day, the count of LAB in digesta and feces reached 10^7 - 10^8 CFU/ml and the count of *L. plantarum* in digesta and faeces reached 10^4 CFU/ml, indicating that *L. plantarum* Mut-7 was able to survive in the gastrointestinal tract transit of rats in vivo. We might then conclude that *L. plantarum* Mut-7 is safe for human consumption as a probiotic strain.

Keywords: safety evaluation, probiotic, L. plantarum, gastrointestinal tract

Introduction

Probiotics are live, non pathogenic micro-organisms that confer beneficial and health functions on the host when administered in adequate amounts (FAO/WHO, 2002). Probiotics are mostly from the lactic acid bacteria group (LAB) such as *Lactobacillus spp*, but are also from the *Bifidobacterium* group, *Saccharomyces*, *Streptococcus* and *Lactococcus*. However, some species of *Lactobacillus* and *Bifidobacterium* are not natural microflora but have the ability to adapt to conditions in the gastrointestinal tract and to withstand the acidic conditions of the stomach/digestive tract bile salts, and to survive pathogens (Emmawati, 2014; Rahayu *et al.*, 2015).

LAB have received increasing attention in recent years and have been shown to be useful to humans. They have proven useful for food fermentation and have been ingested safely with traditional fermented foods (Zhou *et al.*, 2000). This fermented food is produced using a spontaneous inoculant to produce flavor over extended periods of time. One of the local strains of *Lactobacillus plantarum* Mut-7 was identified by Rahayu (2003) as the indigenous LAB. Mainly applied to functional foods, it has health benefits, when added to pineapple/papaya juice it can decrease cholesterol levels while increasing the viability of living cells by 10^9 CFU- 10^8 CFU after being stored for 3 months and is able to survive in acidic conditions (Bieber *et al.*, 2003) and it has the ability as a fermentation starter culture on tamarind leaves,

fruit and bark, and to reduce the content of saponin, followed by improving nutrition, especially protein (Sariri et al, 2012).

L. plantarum Mut-7 has the ability as a starter culture and probiotic, but needs to be evaluated for safety before it can be considered safe for use in the food industry at large. Although *Lactobacillus spp* has gained GRAS status (Generally Recognized As Safe) (Gharaei and Eslamifar, 2011), specific bacterial strains differ from one another (Kemgang et al., 2014). Rahayu et al. (2011), Rahayu et al. (2015), Bieber et al. (2008), Emmawati (2014) tested for the potential of an indigenous probiotic in vitro and it is known that *L. plantarum* Mut-7 shows anti microbial activity by inhibiting *E. coli*, *S. dysenteriae*, *S. choleraecius* and *Shigella flexneri*, is resistant to stomach acid and bile salts and has different genetic profiles to others in the plantarum group. A safety evaluation of the probiotic strain *Lactobacillus plantarum* Mut-7 was done using Sprague Dawley rats as a model. The animals were given a dose of 10^{11} CFU / rat *L. plantarum* Mut-7 cells suspension for 28 days in this research to examine the capability of the probiotic strain, physiological changes such as: changes in general physical condition, weight change of organs, hematological levels, SGOT and SGPT, morphology of the gastro intestinal tract and analysis of bacteria in the blood, lungs, heart, spleen, liver, and kidneys. Therefore, the purpose of this study was to provide information regarding the security of long-term consumption of *L. plantarum* Mut-7 strains as probiotics.

Materials and Methods

Bacterial Strains

Indigenous strain identified as a potential probiotic LAB strains *L. plantarum* Mut-7 from division Food & Nutrition Culture Collection (FNCC) Pusat Studi Pangan dan Gizi, Universitas Gadjah Mada were used in this study. Stock strain were anaerobically propagated in Pepton-Glucose-Yeast extract (PGY) broth (*Oxoid, UK*) and then concentrated by centrifugation. The cell pellets were re suspended in 10% skim milk (*Lactona*) at concentration 10^{11} CFU/ml.

Animals

Female albino Norwegian rats (*Rattus norvegicus*) *Sprague dawley* aged four weeks, were housed individually in stainless steel cages. A 12 h light-dark cycle and a temperature 25°C were maintained in this study. Animals were offered 15 g AIN-93 M modification Reeves et al. (1993) without used tert-butylhydroquinon based diet and water ad libitum. Ethical clearance number 331/KEC-LPPT/X/2015.

Experimental design

Sprague dawley rats were adapted for one week in experimental condition. Twenty four (Federer, 1991) rats were randomly assigned into four different group. The treatment group initial conditions (P.0) after the adaptation period on day 8 were euthanazed humanely, the control group (P.1) after 7 day adaptation period followed standard based diet on day 29 were euthanazed humanely, skim milk group (P.2) , after 7 day adaptation period followed by feeding standards and intervention skimmed milk 1ml 10 % during 28 days on day 29 were euthanazed humanely, and the probiotics group (P.3) after 7 day adaptation period followed by feeding standards and intervention 1ml cell suspension of *L. plantarum* Mut-7 10^{11} CFU/rats/ day in skimmed milk 10 % during 28 on day 29 were euthanazed humanely. The study was performed according to the limit test described in the OECD guideline for the testing of chemicals No. 407: Acute Oral Toxicity- Fixed Dose Procedure. Activity, behaviour of each rats were

observed and recorded daily, while feed intake and body weight were measured weekly. Their blood and tissue (kidney, liver, lung, spleen, and heart) samples were collected aseptically for microbiology laboratory analysis.

Haematology

Blood samples were obtained by orbital sinus on the eyes and collected into EDTA treated tubes. Analysis used hematology analyzer for the analysis of white blood cells (WBC) (leukocytes, lymphocytes, and neutrophils), the number of red blood cells (RBC), platelet count (PLT), hematocrit (HCT), hemoglobin (HGB), the mean corpuscular volume average (MCV), the number of mean corpuscular hemoglobin (MCH) , and the concentration of mean corpuscular hemoglobin (MCHC). Hematology analyzer based on the principle of flow cytometer, the method of measuring the number and properties of the cells are coated by the flow of liquid through the narrow opening. Thousands of cells flowed through the gap such that the cells can pass one by one, then the number of cells and the size was calculated (Zhou *et al.*, 2000).

Blood biochemistry

Analysis of the activity of serum glutamic oxalocetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) in blood samples used centrifugation to obtain serum. Serum reacted with enzyme reagents and reagent starter (reagent kit) using the tool MicroLab 300 , read the absorbance by spectrophotometric method wavelength 340 nm (Steppe *et al.*, 2014).

Histology

The gross anatomy of organs of each rat was checked and recorded. Organ weight index was expressed as the actual organ weight (mg) divided by body weight (g). Ileum , cecum and colon analyzed histopathology with microscopic and quantitative analyzes such as high villi, high epithelium, and thick mucous used Hematoxilin eosin (HE) analysis was based on the method performed by Muntiha (2001).

Bacterial translocation

Liver, kidneys , lungs, heart, spleen, and blood were used for bacterial translocation analysis conducted by streak plate with enrichment to the de Man Rogosa Sharpe (MRS) broth (Oxoid, UK) medium for 1 hour and 24 hours . Growth with streak plate method on MRS agar (Oxoid, UK), 3 g CaCO₃, 1 ml NaN₂, 1 L aquades, 20 g sorbitol, 10 g bacteriological peptone (*Oxoid, UK*), 10 g beef extract (*Oxoid, UK*), 5 g yeast extract (*Oxoid, UK*), 5 g CH₃COONa, 2 g K₃PO₄, 0,1 g MgSO₄, 0,05 g MnSO₄, 0,02 g bromocresol purple, and 2 ml antibiotic (*Ciprofloxacin invus IV 0,2%*) incubated at 37⁰C for 48 hours . The growth of bacterial colonies on MRS agar suspected as LAB and the growth of bacterial colonies on LPSM suspected *Lactobacillus plantarum* (Modification Zhou *et al.*, 2000; Athennia *et al.*, 2015).

PCR based Technique ((rep-PCR) with BOXA1R primer)

Twenty six potential bacterial translocations in MRS medium were recovered from blood and organ and nine strain of *L. plantarum* (FNCC, UGM) were evaluated using Repetitive sequence based polymerase chain reaction (rep-PCR) with BOXA1R primer. Total genomic DNA of suspected cultures and purified test strain were isolated using DNA kit from Geneaid PrestoTM mini. The DNA fragments isolated were electrophoresis agarose gel 1,5% and then amplified using Repetitive sequence-Polymerase Chain Reaction (Rep-PCR) (de Bruijn, 1992). The following isolate continued with identification by

genomic analysis 16S rRNA.

LAB and L. plantarum population in digesta and feces.

Analysis of the population of lactic acid bacteria and *L. plantarum* in digesta and feces refers to the modification of the method Frias *et al.* (2009), Yakabe *et al.* (2009), Zhou *et al.* (2000), and Athenia *et al.* (2015). Animals were analyzed with a spread plate method, samples of feces and digesta as much as 1 gram inserted into a tube containing 9 ml of PBS solution, mixed by vortex and made certain dilution series. Each series dilute 0.1 ml were taken and leveled to seep in MRS agar and *Lactobacillus plantarum* Selective Medium (LPSM).

Statistical analysis

The statistical analysis used one-way ANOVA analysis with a confidence level of 95 % and continued with Duncan Multiple Range Test to determine the significance of difference if there is a significantly different results in $P>0,05$ using SPSS 17 software.

Results and discussion

Physiological conditions of the animals

All of the animals survived the 28 day study period. Physiological conditions were consistent and showed no adverse effects during the consumption of the probiotics. This consistency in physiological conditions was used as an indicator of infection caused by microbial metabolite production and release of cytokines that affect nerve signalling in the central nervous system (Kanra *et al.*, 2006). The results in Table 1 showed that no abnormal changes in feed intake (data not shown), body weight, and organ weight index were recorded.

Table 1. Mean of body weight per group (g) during the intervening 28 days

Group	Mean of body weight per group (g) per weeks (Mean±SEM)				
	0	1	2	3	4
P.1	209,83 ^a ±3.17	220,50 ^a ±3.22	231,33 ^a ±4.12	242,00 ^a ±3.24	248,00 ^a ±5.09
P.2	210,33 ^a ±4.58	221,33 ^a ±5.19	231,33 ^a ±5.26	239,83 ^a ±6.19	245,50 ^a ±6.48
P.3	205,83 ^a ±5.47	212,67 ^a ±5.66	223,17 ^a ±5.34	235,83 ^a ±5.36	243,50 ^a ±4.64

Note: Different superscript notation in the same column indicate significant difference ($P>0,05$) + SEM= standard error. The treatment group initial conditions (P.0), the control group (P.1), skim milk group (P.2), and the probiotics group (P.3).

Table 2. Organ weight index per group

	Organ weight index (weight organ/ weight animals)x100) (rerata±SEM)				
	Jantung	Limpa	Paru	Liver	Ginjal
P.0	0,55 ^a ±0,06	0,68 ^a ±0,13	0,89 ^a ±0,37	3,07 ^a ±0,37	0,75 ^a ±0,16
P.1	0,55 ^a ±0,06	0,59 ^a ±0,07	0,90 ^a ±0,35	2,87 ^a ±0,38	0,80 ^a ±0,10
P.2	0,52 ^a ±0,04	0,47 ^a ±0,05	0,76 ^a ±0,10	2,53 ^a ±0,02	0,80 ^a ±0,02
P.3	0,56 ^a ±0,03	0,50 ^a ±0,11	0,77 ^a ±0,14	2,52 ^a ±0,04	0,79 ^a ±0,10

Note: Different superscript notation in the same column indicate significant difference ($P>0,05$) + SEM= standard error. The treatment group initial conditions (P.0), the control group (P.1), skim milk group (P.2), and the probiotics group (P.3).

One of the biochemical measurements (Table 3) used as a marker of damage to hepatocytes is realized by measuring serum enzyme glutamic oxaloacetic transaminase (SGOT) and serum enzyme glutamic pyruvic transaminase (SGPT). It was higher than the normal value for all groups, showing that elevated levels of SGOT and SGPT had caused by hepatocyte damage which had occurred since the first conditions (P.0). Oxidative stress can also lead to high levels of SGOT and SGPT due to an accumulation of metabolites in the body disturbing the balance of free radicals and antioxidants. It can be expected that the increase in SGOT and SGPT levels above the normal value was not caused by the consumption of *L. plantarum* Mut-7 for 28 days.

Table 3. Mean of blood haematology

Parameter	Group (Mean±SEM)				Ref
	P.0	P.1	P.2	P.3	
WBC (x10 ³ /μL)	7,7 ^a ±1,08	7,5 ^a ±0,85	8,1 ^a ±0,53	7,2 ^a ±0,95	5,3
RBC (x10 ⁶ /μL)	6,8 ^a ±0,31	7,2 ^a ±0,37	7,9 ^a ±0,46	7,3 ^a ±0,29	8,2
HGB (g/dL)	13,5 ^a ±0,46	14,0 ^a ±0,58	14,7 ^a ±0,48	13,9 ^a ±0,34	14,6
HCT (%)	41,1 ^a ±1,35	41,9 ^a ±1,87	44,2 ^a ±1,86	42,3 ^a ±1,08	44,6
MCV (fL)	60,3 ^a ±1,24	57,9 ^a ±0,83	56,3 ^a ±1,26	58,4 ^a ±0,86	54,4
MCH (pg)	19,8 ^a ±0,53	19,4 ^a ±0,47	18,7 ^a ±0,60	19,2 ^a ±0,51	17,78
MCHC (g/dL)	32,8 ^a ±0,55	33,5 ^a ±0,53	33,2 ^a ±0,37	32,9 ^a ±0,51	32,72
PLT (x10 ⁴ /μL)	67,3 ^a ±12,82	60,4 ^a ±4,78	77,7 ^a ±9,86	68,4 ^a ±6,47	87,0
LYM (%)	65,7 ^a ±3,96	55,5 ^a ±6,06	70,5 ^a ±3,99	67,6 ^a ±4,67	76,8
LYM# (x10 ² /μL)	50,6 ^a ±6,32	40,7 ^a ±5,98	57,5 ^a ±5,93	47,0 ^a ±4,03	41,6
NEUT (%)	34,3 ^a ±3,96	44,5 ^a ±6,06	29,5 ^a ±3,99	32,4 ^a ±4,67	24,1
NEUT# (x10 ² /μL)	27,3 ^a ±6,27	34,3 ^a ±6,08	23,1 ^a ±2,89	24,8 ^a ±6,76	10,3
SGOT (x10U/l)	12,7 ^a ±9,06	9,8 ^a ±6,85	17,1 ^b ±1,66	14,96 ^{ab} ±1,16	
SGPT (x10U/l)	7,45 ^a ±8,61	7,12 ^a ±6,37	8,65 ^a ±6,49	7,64 ^a ±6,77	

Note: Different superscript notation in the same row indicate significant difference (P>0.05) + SEM = standard error . Ref (reference (Steppe et al., 2014)), WBC (leukosit), LYM (lymphocyte), NEUT (neutrophil), RBC (red blood cells), PLT (platelet count), hematocrit (HCT), hemoglobin (HGB), the mean corpuscular volume average (MCV), the number of mean corpuscular hemoglobin (MCH) , and the concentration of mean corpuscular hemoglobin (MCHC), serum enzyme glutamic oxaloacetic transaminase (SGOT) and serum enzyme glutamic pyruvic transaminase (SGPT). The treatment group initial conditions (P.0), the control group (P.1), skim milk group (P.2), and the probiotics group (P.3).

Histology

The results showed that after administration of *L. plantarum* Mut-7 there was no inflammation that triggers infection. Inflammation is characterized by changes in the structure of the ileum in the form of reddish spots, enlarged globules of mucosa, thinning of the mucosa, the intestinal villi becoming longer, thickening of the bowel wall and widening of the space between the villi and crypts. (Shackelford and Elwell, 1999). The microscopy of ileum, caecum, and colon (figure not shown) showed the epithelial cells to be in a healthy status. The mechanism of bacterial attachment (adhesion) first took place in the mucous layer as a bond between the structure of the bacteria and specific receptors on the surface of the epithelial cells that glyco-conjugate (attach to the side of the oligosaccharide chains of membrane microvilli) (Owehand and Salminen, 2003). The ability of a bacterial strain in doing attachment to the mucosal layer varies depending on the type of strain. Pathogenic bacteria that will cause erosion and damage to the mucosal lining of the epithelial cell surface. The results showed that no damage and no changes in the structure can be determined by quantitative analysis in Table 4.

Table 4. Gut mucosal histology measurements of rats

Parameter	Group (Mean ± SEM)			
	P.0	P.1	P.2	P.3
Ileum villi height	310,35 ^a ±51,35	250, 45 ^a ±13,51	293,08 ^a ±60,56	308,06 ^a ±33,33
Ileum epithelial cell height	17,65 ^a ±02,26	17,43 ^a ±02,37	18,11 ^a ±01,72	17,13 ^a ±01,91
Ileum number of villi	21,17 ^a ±01,05	24,67 ^a ±01,78	21,33 ^a ±01,78	26,67 ^a ±01,58
Caecum mucosa thickness	244,50 ^a ±17,90	257,9 ^a ±20,28	220,54 ^a ±08,66	213,69 ^a ±21,06
Caecum epithelial cell height	17,38 ^a ±01,36	17,63 ^a ±01,17	16,64 ^a ±01,26	18,53 ^a ±01,51
Colon mucosa thickness	210,59 ^a ±09,65	225,89 ^a ±15,43	209,83 ^a ±10,51	231,44 ^a ±14,97
Colon epithelial cell height	21,97 ^a ±02,88	17,82 ^a ±01,65	21,51 ^a ±08,01	20,05 ^a ±01,41

Note: The treatment group initial conditions (P.0), the control group (P.1), skim milk group (P.2), and the probiotics group (P.3).

Bacteria analysis on organ and blood

The results of microbiological analysis (Table 5) using MRS with the addition of CaCO₃ detected bacterial colonies in the blood both in initial conditions and in the probiotic group. Also it detected bacterial colonies in the heart, spleen, liver, lungs and kidneys of all groups. Colonies of bacteria were detected suspected as LAB. Previous research detected the presence of bacteria on animal research

happened to Zhou *et al.* (2000), lymph nodes , spleen , liver , kidneys , and blood was detected in all treatment and control groups. Athenia *et al.* (2015) has done evaluation candidate probiotic *L. plantarum* Dad-13 the same result also occurs in bacteria detected MRS media suspected as LAB. The results of microbiological analysis using LPSM media showed no colonies detected on the LPSM media and that the LAB colony *L. plantarum* does not come from the intervention of *L. plantarum* Mut-7 given as 10^{11} CFU/rat/day. These results are consistent with the theory that Lactobacillus cannot experience translocation by itself, but can occur when the intestinal mucosa is interrupted or when the immune system is unable to control translocation of pathogenic microorganisms into the bloodstream, causing sepsis or local infection (Kemgag *et al.*, 2014) , Research conducted by Frias *et al.* (2009) states that translocation is associated with the immune system and damage to the mucosa. When damage occurs to mucosal epithelial cells, secretion (IgA) occurs in the immune system, which clinically affects the probiotic strain.

Table 5. Incidence of bacterial detected on blood and organ

	MRS media (LAB)				LPSM media (<i>L. plantarum</i>)			
	P.0	P.1	P.2	P.3	P.0	P.1	P.2	P.3
Blood	2/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6
Heart	2/6	1/6	3/6	4/6	0/6	0/6	0/6	0/6
Liver	4/6	5/6	4/6	5/6	0/6	0/6	0/6	0/6
Lungs	4/6	5/6	2/6	4/6	0/6	0/6	0/6	0/6
Kidney	3/6	2/6	2/6	3/6	0/6	0/6	0/6	0/6
Spleen	3/6	1/6	5/6	1/6	0/6	0/6	0/6	0/6

Note: a/b= the positive animals/ total examined animals. The treatment group initial conditions (P.0), the control group (P.1), skim milk group (P.2), and the probiotics group (P.3).

Table 6. The Population of LAB and *L. plantarum* at the end of treatment

	LAB count (Log CFU/ml)						<i>L. plantarum</i> count (Log CFU/ml)					
	Digest			Faeces			Digest			Faeces		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
P.0	5,32	4,08	6,79	5,77	3,78	6,90	nd	nd	nd	nd	nd	nd
P.1	4,64	3,00	6,16	5,29	4,32	6,00	nd	nd	nd	nd	nd	nd
P.2	5,77	4,46	6,38	6,37	5,33	7,48	nd	nd	nd	nd	nd	nd
P.3	7,18	6,25	8,23	8,16	7,29	8,48	4,85	3,65	5,58	4,90	3,81	6,48

Note: The treatment group initial conditions (P.0), the control group (P.1), skim milk group (P.2), and the probiotics group (P.3).

Molecular detection of isolate detected in blood and organ

The numbers of bands resulting from the analysis by BOX-PCR in 26 bacterial isolates are 353 bands with an average of 13 bands / isolates (Figure 1). The observation of the pattern of bands (fingerprint) of each isolate with *L. plantarum* Mut-7 none of the isolates in each treatment group showed similarities fingerprint patterns with *L. plantarum* Mut-7. 26 isolates and *L. plantarum* Mut-7 based on fingerprint patterns BOX-PCR analysis (Figure 2) has similarity level (S.L.) between 54% - 90%. Referring to the value similarity level, according to Nucera *et al.* (2013) Rep-PCR is used as a method of genotyping is > 95% or 93% (Gevers *et al.*, 2001) it can be stated that among 26 isolates, there were no resemblance identical genes with bacterial strain *L. plantarum* Mut-7. Phylogenetic analysis based on BOXA1R-PCR banding types of 26 culture isolated from blood and organ of rats showed that all isolates have low similarity coefficient < 75% with *L. plantarum* Mut-7. Ten (10) isolates of 26 isolates from rats organ were further analysis using 16S rRNA genes. The result showed that they are belong to *Lactobacillus* (8 species) and *Enterococcus* (2 species), none of *Lactobacillus* strains belong to *L. plantarum*. These results supported the safety data of *L. plantarum* Mut-7 as probiotic bacteria.

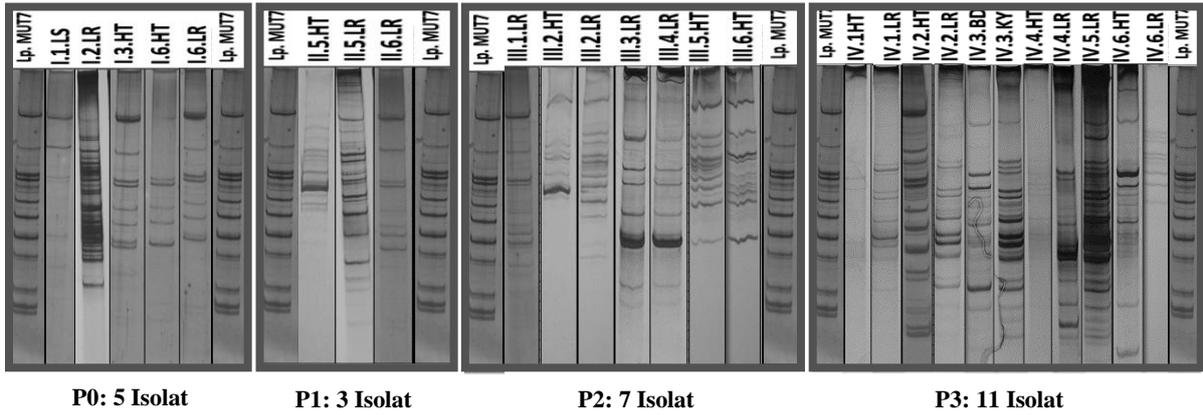


Figure 1. Amplification of polyacrylamide gel electrophoresis (PAGE) 8% from 26 bacterial isolates with BOX- PCR

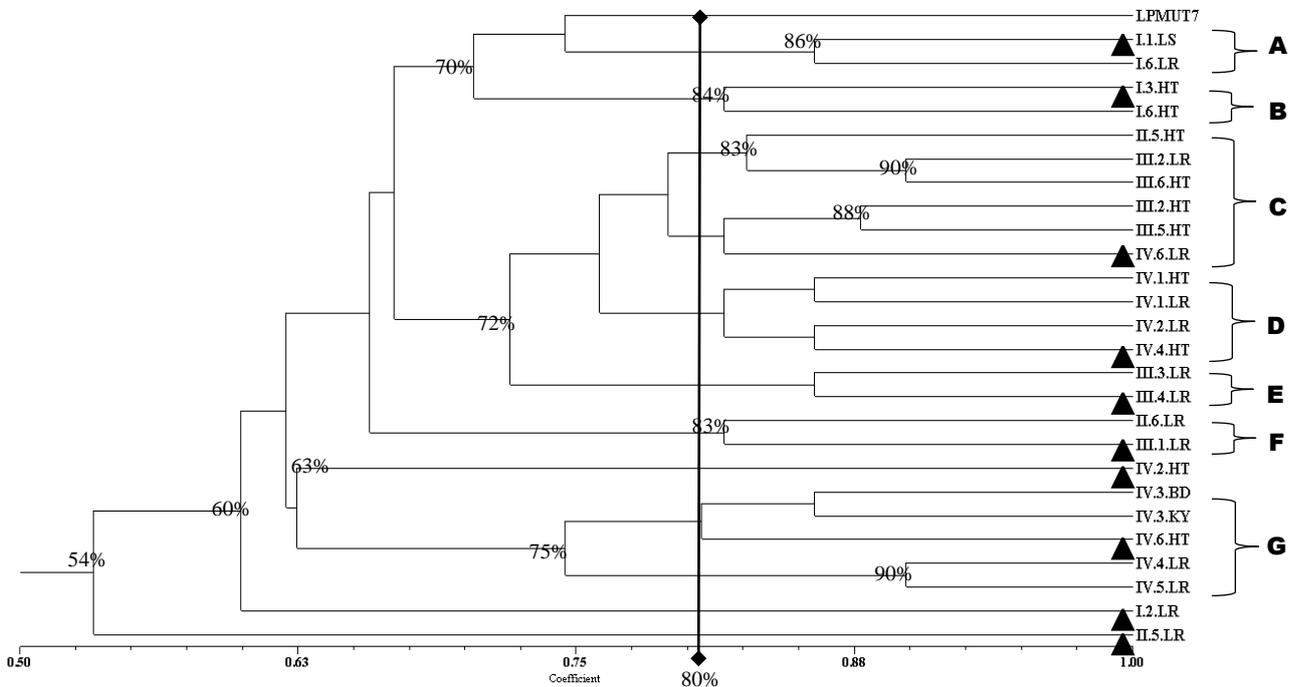


Figure 2. Dendrogram of 26 isolate origin in blood and organs using rep-pcr with BOX A1R primer

Digesta and faecal bacterial count

Digesta and faecal bacterial counts (Table 6.) were used as indicator for in vivo gastrointestinal survival. After consumption of *L. plantarum* Mut-7, LAB population in the probiotic group increased, showing that *L. plantarum* Mut-7 can survive from gastro-intestinal tract into the faeces. These results were similar to those of Athennia et al. (2015), where the number of LAB in digesta was 10^7 CFU / ml and in faeces 10^8 CFU / ml after administration of *L. plantarum* Dad-13. Survival rates of *L. plantarum* as probiotic bacteria in the gastro-intestinal tract can be seen in Table 1. These results are in accordance with the terms of probiotics (WHO / FAO, 2002), which state that in order to give beneficial effects to the

host, microorganisms added must remain alive. *L. plantarum* only detected in probiotic group with an average growth of 10^{4-5} CFU / ml and promotes the growth of LAB in the digestive tract of probiotic group. These results are consistent with the studies made by Rahayu et al. (2015) where, in the in vitro testing of *L. plantarum* Mut-7, it is able to survive in pH 2 stomach acid, a 3% concentration of bile salts and to inhibit pathogenic bacteria

Conclusion

L. plantarum Mut-7 has probiotic capability, does not have a significant influence on changes in body weight, feed intake, the index organ weights, hematology levels, SGOT, SGPT, histology, and not detected in the organs.

References

- Athennia, A., Tyas, U., Y. Marsono., Endang S, R. 2015. Evaluasi Keamanan Kandidat Probiotik: Efek Pemberian *Lactobacillus plantarum* Dad 13 Dosis Tinggi pada Performa Tikus *Sprague Dawley*. Tesis S2. Program Pasca Sarjanan UGM, Yogyakarta. *Unpublish*.
- Chowdhury, A., Hossain, M. N., Fakkrudin, M., Billah, M.M., and Ahmed, M.M. 2012. Screening of *Lactobacillus* spp. From Buffalo Yoghurt for Probiotic and Antibacterial Activity. *J. Bacteriol Parasitol*, p.3-8.
- De bruijn, J. Frans. 1992. Use Of Repetitive (Repetitive Extragenic Palindromic and Enterobacterial Repetitive Intergeneric Consensus) Sequences and The Polymerase Chain Reaction To Fingerprint The Genomes of *Rhizobium Meliloti* Isolates and Other Soil Bacteria. *Appl. Environment Microbial*. 58 (7): 2180-2187.
- Emmawati, A. 2014. Study of Antiinfection Properties of Lactic Acid Bacteria Isolated from Mandai (Dissertation), Graduate School, Bogor Agricultural University, Indonesia
- FAO/WHO. 2002. Probiotics in Food: Health and Nutritionals Properties and Guidelines for Evaluation. WHO FAO of the united nations, Rome.
- Frias, R., Ouwehand, A., Spillmann, T., Vankerckhoven, V., Trautwein, M. W., Salminen, S., and Guemonde, M. 2009. Effect of Clinical and Probiotic *Lactobacillus rhamnosus* Strains on Intestinal Permeability and Bacterial Translocation in Healthy and Colitic Rats. *Food Research International Journal* 42(2009), p.636-640.
- Gevers, D., 2001. Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiology Letters*, 205, p..31-36
- Gharaei F, and Eslamifar M . 2011. Isolation and Applications of one strain of *Lactobacillus paraplantarum* from tea leaves (*Camellia sinensis*). *AmJ Food Technol* 6, p.429-434.
- Kanra G1, Kara A, Demiralp O, Contorni M, Hilbert AK, Spyr C, and Viviani S. 2006. Safety and immunogenicity of a new fully liquid DTPw-HepB-Hib combination vaccine in infants. *Hum Vaccin* 2(4): 155-60.
- Kemgag, S.T., Kapila, S., Shanmugam, V.P., dan Kapila, R. 2014. Cross-talk between probiotic lactobacilli and host immune system. *J Appl Microbiol* 117, p. 303-19.
- Kemgag, S.T., Kapila, S., Shanmugam, V.P., Reddian, S., and Kapila, R. 2016. Fermented milk with probiotic *Lactobacillus rhamnosus* S1K3 (MTCC5957) protects mice from salmonella by enhancing immune and nonimmune protection mechanisms at intestinal mucosal level. *Journal of Nutritional Biochemistry* 30, p. 62-73.
- Lestari LA, Harmayani E, dan Sugahara T. 2010. Immunomodulatory Effect of Heat-Killed Indigenous Probiotic in HB4C5 cell-line and Balb/c Mice. In Proceeding of the 2nd International Symposium on Probiotic and Prebiotic, Jakarta, 4-5 August.
- Lestari LA, Setyabudi FS, Julia M, dan Amalia LD. 2003. Effect of Synbiotic Yogurt Made with Indigenous Probiotic *Lactobacillus plantarum* Mut7 and Sweet Potato Fiber (*Ipomoea batatas*) in Healthy Children. *International Reaserch Journal of Microbiology* 4(3): 98-102.
- Muntiha, M., 2001, *Teknik Pembuatan Preparat Histopatologi dari Jaringan Hewan dengan Pewarnaan Hematoksin dan Eosin, Temu Teknis Non Peneliti*, p.156-163.
- Nucera, D. M., Lomonaco, S., Costa, A., Morra, P., & Grassi, M. A. 2013. Diagnostic Performance of rep-PCR as a Rapid Subtyping Method for *Listeria monocytogenes*. p. 868-871. <http://doi.org/10.1007/s12161-012-9496-1>
- Ouwehand, A. C. and Salminen, S.2003. A review In vitro adhesion assays for probiotics and their in vivo relevance. *Microbial Ecology in Health and Disease* 15 (4): 175-184.
- Rahayu, E., S. 2003. Lactic acid bacteria in fermented foods of Indonesian origins, *Agritech* , 23, p.75-84
- Rahayu, E., S., Yogeswara, A., Mariyatun, Windiarti, L., Utami, T., and Watanabe, K. 2015. Molecular characteristics of Indigenous probiotic strains from Indonesia. *International Journal of Probiotics and Prebiotics* 10(4): 109-116.
- Reeves, P., G., Nielsen, F., H., and Fahey, G., C. 1993. AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. United States, Urbana.
- Sariri, A., K., Mulyono, A., M., W., and Tari, A., I., N. 2012. Effectiveness of *Lactobacillus plantarum* Mut-7 Agents Fermentation to Reduce Trembesi (*Albizia saman*) Saponins Content. Universitas Veteran Bangun Nusantara, Sukoharjo
- Shackelford CC and Elwell MR. 1999. Small and Large Intestine, and Mesentary. In : RR Maronpot, GA Boorman, BW Gaul, Editor. *Pathology of the Mouse Reference and Atlas*. Vienna: Cache River Press. p. 81-115
- Steppe, M., Nieuwerburgh, F., V., Boyen, F., Eeckhaut, V., Deforce, D., Haesebrouck, F., Ducatelle, R., and Immerseel, F., V. 2014. Safety assessment of the butyrate-producing *Butyricoccus pullicaecorum* strain 25-3T, a potential probiotic for patients with inflammatory bowel disease, based on oral toxicity tests and whole genome sequencing. *Food and Chemical Toxicology* 72(14): 129-137.
- Yakabe, T., Moore, E. L., Yokota, S., Sui, H., Nobuta, Y., Fukao, M., Palmer, H., and Yajima, N. 2009. Safety assessment of *Lactobacillus brevis* KB290 as a probiotic strain. *Journal of Food and Toxicology* 38, p.156-166.

- Zhou, J.S., Shou, Q., Rutherford, K.J. Prasad, J., Gopal, P.K., and GilL., S.H. 2000. Acute Oral Toxicit and Bacterial Translocation Studies on Potentially Probiotic Strain of Lactic Acid Bacteria. *Food and Chemical Toxicology* 38, p.156-166.
- Zhu, J., Zhao, L., Guo, H., Jiang, L., and Ren, F. 2011. Immunomodulatory effects of novel *Bifidobacterium* and *Lactobacillus* strains on murine macrophage cells. *Af J Microbiol Res* 5(1): 8-15.

The Effects of Washing on the Chemical Properties and Bacteria Content of Fermented Cassava (a Case Study in the Growol Rural Industry, Kalirejo Village, Kokap, Kulon Progo, DIY)

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Abstract

Growol is made from cassava in consecutive stages: peeling and washing, soaking in water for 4-5 days (during which time spontaneous fermentation takes place), washing of the fermented cassava, pressing, milling and steaming. Growol is a staple food in Kulon Progo Regency-DIY, especially in Kalirejo village in the Kokap District. Growol contains bacteria *Lactobacillus* sp which have functional properties as a pro-biotic. These bacteria grow during the fermentation or soaking of the cassava and produce lactic acid, which gives a sour taste on the growol. Usually, fermented cassava is washed as many as 2 or 3 times, but a higher frequency of washing can remove *Lactobacillus* sp, thus reducing the pro-biotic effect of the growol. The purpose of this research was to determine the effects of varying the washing frequency of fermented cassava on its chemical properties, on the total bacteria, and on the presence of *Lactobacillus* bacteria in both the fermented cassava and washing water. The effects of washing the fermented cassava were assessed by washing at frequencies of: 0, 1, 2, and 3 times. The fermented cassava was separated from the soaking water and was then washed. Both fermented cassava and washing water were analyzed for starch content, reducing sugar, titratable acidity, pH, and total bacteria plate count, then given a qualitative test of *Lactobacillus* bacteria. The result showed that the more frequently the cassava was washed, the lower the levels of reducing sugar and titratable acidity in the fermented cassava, and the greater the quantity of starch being transferred into the filtrate. The amount of reducing sugar in the fermented cassava before washing was $0.0311 \pm 0.0007\%$, becoming $0.0189 \pm 0.0006\%$ after being washed 3 times, titratable acidity decreased from $1.69 \pm 0.01\%$ to $0.34 \pm 0.01\%$ and the pH went from 3.39 to 3.79. Total bacteria in the fermented cassava reduced from $9.8 \cdot 10^6$ cfu/g to $3.6 \cdot 10^6$, but *Lactobacillus* was still present. Therefore, to maintain the typical flavour of growol and to increase the yield, the fermented cassava should be washed a maximum of 2 times.

Keywords : fermented cassava, pro-biotic, functional food.

Introduction

Cassava (*Manihot esculenta* Crantz) is used as a staple food in several regions of Indonesia, for example the cassava-based food *tiwul* is consumed in the district of Gunung Kidul and *growol* in the Kulon Progo district of DIY. This is due to the high carbohydrate content of the cassava tubers, especially starch. Susilawati *et al.* (2008) state that the starch content of cassava is 13.94-19.79% depending on growing site conditions. In addition to rice, the carbohydrate in cassava is a useful energy source for human activities.

The *growol* making process takes place in several stages: cassava peeling, slicing, soaking (to incur spontaneous fermentation) for 3 - 5 days, washing and separation of cassava fibres, milling, pressing and steaming (Anonymous, 2015). Furthermore, *growol* is molded into conical shapes, each weighing about 5 kg, before being wrapped in banana leaves. Several decades ago, the people of Kalirejo village, Kokap,

Kulon Progo district, DIY consumed *growol* as a main meal every day both morning and evening, while rice was consumed once daily at noon. There are at least two hamlets in Kalirejo village which still produce *growol*. These are named Sangon I and Sangon II. But nowadays the consumption of *growol* in that village is shrinking due to a decrease in the production of cassava as a raw material and because of a shift to rice as a staple food, especially among the younger generation. In addition, the typical sour taste of *growol*, which is rather bland without any salty or sweet taste, is less popular among young people. But if used as a local staple, *growol* could have an advantage as a pro-biotic functional food, because of its *Lactobacillus* bacteria content.

Functional food is a natural or processed food containing one or more compounds which benefit specific physiological functions and health (BPOM, 2005). Moreover, a functional food does not have the contra-indications or side effects of other nutrients on the metabolism when consumed at proper or recommended levels. The compound groups which are considered to have certain physiological functions in functional food include foods that contain lactic acid bacteria. These functional foods are called probiotics that are living organisms which can provide the host with beneficial health effects when consumed in a sufficient quantities (FAO/WHO, 2002), by improving the balance of the intestinal microflora in the digestive tract (Shitandi *et al.*, 2007). Pro-biotics generally come from lactic acid bacteria (LAB), particularly the genera *Lactobacillus* and *Bifidobacterium*, which form part of normal flora found in the human gastro-intestinal tract (Sujaya *et al.*, 2008). The lactic acid bacteria in *growol* grow during soaking of the cassava when spontaneous fermentation occurs at that stage. The predominant lactic acid bacteria grown on *growol* are *Lactobacillus plantarum* and *Lactobacillus casei* subsp. *Rhamnosus* (Putri *et al.*, 2012).

The physiological effect of *growol* is its ability to prevent diarrhoea (Lestari, 2009) due to cell activity in the lactic acid bacteria (*Lactobacillus casei* subsp. *Rhamnosus* TGR2) and its secondary metabolites which act against pathogenic bacterial cells. Furthermore, it has been stated that the anti-microbial activity result from its ability to inhibit the growth of *Staphylococcus aureus* FNCC 0047, *E.coli* FNCC 0091, *Morganella morganii* FNCC 0122, *Salmonella typhimurium* FNCC 0050 and *Bacillus cereus* FNCC 0057. Moreover, the extracellular metabolites of *Lactobacillus casei* subsp. *Rhamnosus* are stable at room temperature, heat resistant to 98°C for 30 minutes, have pH 3-8 and can endure treatment of 15 minutes heating at 121°C and cooling for 21 days at 4°C (Rahayu *et al.*, 1995 in Lestari, 2009). Hence, *growol* that is processed through the steaming stage for 15 minutes at 100°C maintains its pro-biotic effect.

Problem raised is that *growol*, which contains lactic acid bacteria, should be washed before further processing. Generally, the washing frequency used to be 2 or 3 times (Sutanti *et al.*, 2013), in order to retain the typical sour flavor of the *growol*. But nowadays, *growol* producers wash the *growol* as many as 4 or 5 times for better taste which is preferred by consumers, yet one of the characteristic of LAB presence in the product which then could give the probiotic effect is the sour flavor. Therefore, to retain *growol's* high potential as a functional food, it is necessary to study and evaluate the effects of the frequency of washing the fermented cassava on its chemical properties and bacterial content as intermediate components during *growol* production. Furthermore, it is important to increase the

knowledge of the communities in making their local staple food *growol* a pro-biotic by means of improvements to the washing techniques of the *growol*.

Materials and Methods

Materials

The material used for making *growol* was locally grown cassava, obtained from farmers in Kalirejo village and the surrounding areas. These included varieties of Martapura, Ketan, Randu and Kapipirin (Kalirejo's *growol* producer, 2015). The fresh cassava was purchased within 48 hours of harvesting.

Chemicals

The chemicals used for analysis of the starch content, reducing sugar and titratable acidity i.e. hydrochloric acid, sodium hydroxide, glucose standard, arsenomolybdate, and Nelson Somogyi reagent (cupric sulfate, sodium carbonate, sodium sulfate, and K-Na-tartrate) with the qualification of pro analysis were from Merck.

Methods

This study evaluated the effects of washing the fermented cassava on its chemical properties (starch content, reducing sugar, titratable acidity, moisture and pH), and total content of bacteria and *Lactobacillus* qualitatively.

Evaluation of the effects of washing fermented cassava, chemical analysis and microbiological testing

Processing the *growol* referred to Sutanti *et al.* (2013). Cassava was peeled, cut ± 5 cm length, washed, then soaked in water at a ratio of 1: 3 cassava : water (w/v) for 4 days (fermentation), separation of the fermented cassava from the washing water, then further washing of the fermented cassava, pressing, milling and steaming. To determine the effects of washing the fermented cassava on the chemical properties, total bacteria and *Lactobacillus*, sampling of the fermented cassava (sediment) and washing water was carried out periodically thus : 0 (before washing), after first, second and third washes. Fermented cassava and washing water from each wash were analyzed for their chemical properties, which were: starch, using Direct Acid Hydrolysis method; reducing sugar, using Nelson Somogyi method and titratable acidity and moisture content by the gravimetric static method (AOAC, 1990). The microbiological testing for total bacteria was carried out by total microbial Pour Plate method and a qualitative *Lactobacillus* test was conducted in the Yogyakarta Health Laboratory. As supporting data, the degree of acidity (pH) was determined by using a Schoot type CG842 pH meter.

Design Experiment

The research was conducted using Completely Randomized Design (Sugiyono, 2004) with the factor of washing of the fermented cassava. The data results were processed by using SPSS 13.0 for window.

Result and discussion

Chemical Properties of Fermented Cassava and its Filtrate

Results of chemical analysis of fermented cassava are shown in Table 1. Carbohydrate is the main component of the solid part of *growol*. The carbohydrate content of the Kalirejo's *growol* is 41.95% (Wariyah and Sri Luwihana, 2015). The raw material of *growol* is cassava, so carbohydrate forms the

main component in growol. According to Putri *et al.* (2012), soaking of cassava for 1-5 days during *growol* processing allows a fermentation process involving amylolytic lactic acid bacteria and crude starch. The lactic acid bacteria that have the ability to use starch as its substrate is known as amylolytic lactic acid bacteria. The metabolites formed from this fermentation process are lactic acid, reducing sugar, and starch. Therefore, these components were analyzed as an indicator of starch hydrolysis and indications of the formation of lactic acid were based on titratable acidity and pH.

Table 1. Starch content, reducing sugar and titratable acidity of fermented cassava

Washing frequency (times)	Starch (% wb)	Reducing sugar (% wb)	Titratable acidity (% wb)	pH	Moisture (%wb)
0	9.11±0.03 ^a	0.0311±0.0007 ^c	1.69±0.01 ^e	3.39	75.27±0.49 ^e
1	11.95±0.34 ^b	0.0291±0.0002 ^c	0.82±0.02 ^d	3.63	62.65±0.17 ^d
2	14.72±0.45 ^c	0.0287±0.0026 ^a	0.37±0.01 ^c	3.71	57.20±1.31 ^c
3	14.00±0.17 ^c	0.0189±0.0006 ^c	0.34±0.01 ^b	3.79	60.10±0.18 ^b
Pressed Fermented cassava	16.31±0.84 ^d	0.0235±0.0007 ^b	0.22±0.02 ^a	3.99	52.78±0.13 ^a

Mean values in a column with similar superscript are not significantly different at $\alpha=0.05$, n =

Physically, the fermented cassava retained its form and white color due to its starch content, but it felt soft and smelled sour. Table 1 shows cassava fermentation, which is indicated by amylolytic activity and the formation of lactic acid, led to the presence of starch, reducing sugar, acid and pH changes. After soaking for 4 days, the fermented cassava was separated from the soaking water. It was then washed three times. Analysis of the fermented cassava showed the fermented cassava constituents of starch, reducing sugar and lactic acid at 9.11±0.03%, 0.0311±0.0007% and 1.69±0.01%, respectively. After washing, the starch content of the fermented cassava was higher, which meant that its purity had increased. In contrast, the reducing sugar and titratable acidity was lower. This was due to the dissolution of the reducing sugars and acids into the washing water. This condition caused an increase in the pH of the fermented cassava, or a higher degree of acidity. The degree of acidity or pH of the fermented cassava before washing was 3.39 and after the third washing became 3.79. The impact of dissolving the reducing sugar and acid into the washing water was to decrease the typical sour flavor of *growol*. According to the *growol* producers, this was intentionally reduced because *growol* consumers prefer that flavor.

Meanwhile, Table 2 shows the result of the soaking water and washing water analyses. The soaking water separated from the fermented cassava before washing contained reducing sugar, acid (as lactic acid) and solid of 0.0195±0.001%, 1.51±0.025% and 1.30 ± 0.05%, respectively; and had a pH of 3.63. After the fermented cassava had been washed, the washing water, which had not previously been acidic (pH ± 7.0) became acidic with a pH of 4.95. This demonstrated that the greater the frequency of washing, the more the reducing sugar and acid were dissolved into the washing water. Similarly, the total solids dissolved were also greater during increased washing. In the third wash, the total solids in the washing water reached 5.77 ± 0.05%. The solids remaining in the fermented cassava comprised mostly starch, so the solid loss during washing meant a loss of starch. These conditions caused a decrease in the *growol* yield. Therefore greater washing frequency resulted in a reduction in the typical flavor of *growol* and a lower product yield. The biggest disadvantage of excessive washing was that the extra-cellular metabolites produced by pro-biotic *Lactobacillus* were also reduced. Figure 1 shows the fermented cassava washing process.

Table 2. Reducing sugar, titratable acidity, pH and total solid of filtrate/washing water

Washing frequency (times)	Reducing sugar (% wb)	Titratable acidity (%wb)	pH	Total solid (%wb)
0	0.0195 ±0.001 ^c	1.51±0.025 ^c	3.63	1.30 ± 0.05 ^c
1	0.0198 ±0.001 ^c	0.54±0.009 ^b	3.68	0.52 ± 0/01 ^b
2	0.0054 ±0.000 ^b	0.02±0.004 ^a	4.99	0.40 ± 0.02 ^a
3	0.0043 ±0.000 ^a	0.03±0.001 ^a	4.95	5.77 ± 0.05 ^d

Mean in a column with similar superscript are not significantly different at $\alpha = 0.05$; n=



Figure. 1 The fermented cassava washing process

TPC and Lactobacillus in the precipitate and filtrate of fermented cassava

Lactic acid bacteria can produce extra-cellular amylase and ferment starch directly into lactic acid. This is because fermentation with amyolytic lactic acid bacteria combined the two processes, namely the enzymatic hydrolysis of the carbohydrates (starch) and fermentation by using sugar to produce lactic acid (Reddy *et al.*, 2008). There were 5 isolates of amyolytic lactic acid bacteria in the fermented cassava, dominated by *Lactobacillus plantarum* and *Lactobacillus rhamnosus* (Putriet *al.*, 2012). Qualitative test of bacteria in the soaking water and in the fermented cassava from Kalirejo'sgrowolisshownin Table 3.

Table 3. Total bacteria and *Lactobacillus*

Washingfrequency(times)	Total bacteria(cfu/g)		<i>Lactobacillus sp.</i>	
	Fermented cassava	Filtrate	Fermented cassava	Filtrate
0	9.8 ±10 ⁶	1.8 ±10 ⁷	+	+
1	7.2±10 ⁶	2.0±10 ⁷	+	+
2	8.1±10 ⁶	2.2±10 ⁷	+	+
3	9.2±10 ⁶	3.2±10 ⁷	+	+
Pressed fermented cassava	3.6 ±10 ⁷			

Table 3 shows that the fermented cassava and soaking water contained bacteria at 9.8.10⁶cfu/g and 1.8.10⁷cfu/g (1 ml= 0.96 g), respectively. According to Putri *et al.* (2012) the total bacteria in fermented cassava was 1.10^{7.5}cfu/g and in the soaking water 1.10⁸cfu/ml. These differences were largely due to variations in sampling times and fermentation conditions. The fermented cassava was separated from the soaking water then washed up to 3 times using clean water. The result showed that the bacteria in the fermented cassava decreased, albeit not significantly, before increasing again. Total bacteria in both the soaking water and washing water tended to increase during the washing process. This result showed that bacterial growth occurred during the wait for the next wash. The quantitative test of *Lactobacillus* identified the presence of *Lactobacillus* in the fermented cassava and soaking water. Analogous to this research was that done by Putri *et al.* (2012) which found that the dominant bacteria in the soaking water and fermented cassava were *Lactobacillus sp.* namely *Lactobacillus plantarum* and *Lactobacillus casei* subsp. *Rhamnosus*. Growol made using a process of fermentation for 4 days and washing 3 times still

contains 4.7×10^3 cfu/g lactic acid bacteria. (Wariyah and Luwihana, 2015). Yet according to Suharni (1984) in Lestari (2009), the amount of lactic acid bacteria per gram of *growol* is 1.64×10^8 cfu. The low bacteria content in Kalirejo's *growol* was influenced by several factors such as: variations in fermentation times from 1-5 days, and the washing frequency of the fermented cassava. Repeated washing could lead to depletion of bacteria which had ended up in the washing water, thereby reducing the pro-biotic effect of the *growol*.

Conclusion

The result showed that the greater the frequency of washing, the lower the measures of reducing sugar and titratable acidity in the fermented cassava. In addition to this, more starch was transferred to the filtrate or washing water. The reducing sugar in the fermented cassava before washing was 0.031% but it decreased to become $0.0189 \pm 0.0006\%$ after being washed 3 times. The titratable acidity of $1.69 \pm 0.01\%$ then decreased to $0.34 \pm 0.01\%$ and the pH went up from 3.39 to 3.79. Total bacteria in the fermented cassava reduced from 9.8×10^6 cfu/g to 3.6×10^6 cfu/g, but still contained *Lactobacillus*. Therefore, to maintain the typical flavor of *growol* and to increase the yield, washing of fermented cassava should be done no more than twice.

Acknowledgement

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References

- Anonim. 2015. KulonProgo Tempo Dulu. www.kotawates.com/growol-citarasa-kulon-progo-tempo-dulu.html. Downloaded on August 11, 2015.
- AOAC. 1990. Official methods of analysis of AOAC international. 16th Edition. Washington D.C.: Agricultural Chemicals, Contaminant, Drug.
- BPOM, 2005. Ketentuan Pokok Pengawasan Pangan Fungsional. jdih.pom.go.id/produk/peraturankepalabpom/PER_KBPOM-No_HK.00.05.52.0685_tahun_2005. Downloaded on April 14, 2014.
- FAO/WHO. 2002. Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food, London.
- Lestari, L.A. 2009. Potensi Probiotik Lokal sebagai Makanan Fungsional Pencegah Diare. <http://gizikesehatan.ugm.ac.id/2009/06/potensi-probiotik-lokal-sebagai-makanan-fungsional-pencegah-diare/>. Downloaded on September 14, 2016.
- Putri, W.D.R, Haryadi, Marseno, D.W., Nur Cahyanto, M. 2012. Isolation and Characterization of Amyolytic Lactic Acid Bacteria during Growol Fermentation, an Indonesian Traditional Food, *Jurnal Teknologi Pertanian* Vol. 13, 1 : 52-60.
- Reddy, G., Altaf, M.D., Naveena, B.J., Venkateshwar, M., and Kumar, E.V. 2008. Amyolytic bacterial lactic acid fermentation, a review. *Biotechnology Advances* 26: 22-34.
- Shitandi, A., Alfred, M. and Symon, M. 2007. Probiotic characteristic of lactococcus strain from local fermented *Amaranthushybridus* and *Solanum nigrum*, African Crop Science Conference Proceedings, 8:1809- 1812.
- Sujaya, I. N., Ramona, Y., Widarini, N.P., Suariani, N.P., Dwipayanti, N.M.U., Nocianitri K.A. and Nursini, N.W. 2008. Isolasi dan Karakteristik Bakteri Asam Laktat dari Susu Kuda Sumbawa, *J. Vet.* 9 (2) : 52 – 59.
- Sugiyono. 2004. Metode Penelitian Bisnis, Alfabeta, Bandung.
- Susilawati, A., Nurjanah, S. and Putri, S. 2008. Cassava (*Manihot esculenta*) Physical and Chemical Properties of Different Plantation Location and Harvesting Ages. *Jurnal Teknologi Industri dan Hasil Pertanian*, Vol. 13, 2:59-72.
- Sutanti, A., Sri Luwihana D. dan Kanetro, B. 2013. The Effect of Pre Treatment and Cowpea Flour Concentration on Physical Properties and Preference Level of Oyek. *Jurnal Agri Sains* Vol. 4, 7: 11-22. <http://lppm.mercubuana-yogya.ac.id/wp-content/uploads/2013/12/JURNAL-AGRISAINS-VOL.-4-No.-7.-SEPTEMBER-2013-COMPRESS.pdf>. Downloaded on September 7, 2016.
- Wariyah, Ch. and Sri Luwihana, 2015. "Improvement of Growol as a probiotic-Functional Food (case Study at Kalirejo, Kokap, Kulon Progo, DIY)", Proceeding of 1st International Seminar on "Natural Resources Biotechnology : from Local to Global", Faculty of Biotechnology, Atmajaya University of Yogyakarta, ISSN : 2460-8238, September 8th – 9th, page 150-156.

Effect of Egg Reduction and Xanthan Gum Concentration on the Physicochemical Properties of Reduced Fat Rice Cake

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Abstract

Egg plays an important role as a liquid contributor and forms the volume, texture, flavor, and color of reduced fat rice cake. The high price of eggs can make high production cost of reduced fat rice cake. The reduction of eggs in cake making will reduce the cake production cost. Eggs reduction will affect on the characteristics of reduced fat rice cake, so it's needed another material to improve the characteristic of reduced fat rice cake. One material that can be used is xanthan gum. Xanthan gum can make the distribution of trapped air to be uniform when batter mixing, increase volume and softness of cake. This study used a factorial randomized block design with two factors, namely egg reduction which consisted of four levels, i.e. 20%, 30%, 40%, 50% and xanthan gum concentration which consisted of two levels, i.e. 0.2% and 0.4%. This experiment was replicated four times. Data were analyzed using Analysis of Variance at $\alpha = 5\%$. If the ANOVA showed a significant effect, data were analyzed by Duncan's Multiple Range Test at $\alpha = 5\%$ to determine the level of treatments that gave a significant difference. The results showed that interaction of egg reduction and xanthan gum concentration provided significant effect on moisture content, specific volume and hardness of reduced fat rice cake. Xanthan gum concentration provided significant effect on springiness and egg reduction showed significant effect on cohesiveness of reduced fat rice cake.

Keywords: reduced fat rice cake; egg reduction; xanthan gum; physicochemical properties

Introduction

The development of cardiovascular disease is rapid and approximately 40% of deaths associated with the disease. This leads to increase public awareness of health and need for low-fat foods because of the association between high-fat diet with cardiovascular disease, hypertension and certain cancers, especially colon cancer.

Cake is one of the food products made with materials such as flour, fat, sugar and eggs. Rice cake is made by replacing wheat flour with rice flour. Saputra (2013) reported that rice cake contains high amount of fat (margarine) which approximately 81.8% of the weight of flour and has a fat content of 16.84%. According to Pomeranz and Schellenbenger (1971), fat plays an important role in cake quality, i.e. volume, taste, texture, aroma, colour, shelf life, reduce crumb cake, contribute to the softness and moistness and make the cake easier to swallow.

Reduced fat rice cake is made by substituting whole margarine (fat) with a fat replacer. Fat replacer is a material that can replace some or all of fat in food products that aimed to reduce the fat content and calories in food products, but does not change the taste or texture of the food product (Hui, 2006 and Rudolph *et al.*, 1994 in Swanson, 1996). One fat replacer that has been used in the reduced fat rice cake making is steamed red kidney bean. Trisnawati and Sutedja (2014) showed that the replacement

of all margarine can be performed with steamed red kidney beans. The reduced fat rice cake had good volume development, good softness and moistness score close to neutral with a fat content of 5.18%. The use of steamed red kidney beans in fresh crushed form is not practical and will lead to a short shelf life so it is developed into red kidney bean flour. Trisnawati and Sutedja (2014) mentioned that reduced fat rice cake with red kidney bean flour as a fat replacer has a better score for pore uniformity, softness and moistness.

The fat content in reduced fat rice cake can be reduced to less than 3% so that it can be classified as low-fat product. Such efforts can be done by reducing the use of eggs. Eggs are by weight, the largest materials in the reduced fat rice cake, which is 472.7% of the weight of flour. Stadelman and Cotterill (1990) stated that whole egg contains 10.5 to 11.8% of fat, while the yolk contains 31.8 to 35.5% of fat. According to Arozarena *et al.* (2001), the egg had a role in the formation of cake characteristics, namely volume and texture. Hussain and Al-Oulabi (2009) stated that egg whites or whole eggs play a role in the stable foam formation that is containing a lot of air when mixed with sugar. When stable foam is mixed with flour, the foam will form a batter that contains air and has a function like leavening agent. Egg yolks can contribute to flavour and colour while the lecithin can act as an emulsifier.

Moreover, the price of eggs is quite high, ranging from IDR 22,000.00 up to IDR 25,000.00 per kg of eggs. These prices are determined by the shelf life and availability of egg in the market. Fresh eggs from farms are generally sold at higher price than the old eggs. The availability of eggs and the higher demand will also cause an increase in the price of eggs which leads to the high production cost of rice cake. Reduction of the number of eggs can lower the production cost of rice cake.

The egg reduction in reduced fat rice cake will affect the quality of rice cake. In order to maintain the quality, other ingredient is needed to replace the function of the eggs. The decrease in rice cake quality can be solved with the use of hydrocolloid, one of which is xanthan gum. According to Phillips and Williams (2000), xanthan gum is able to assist uniform distribution of trapping air when mixing cake batter, increase the volume of development and the softness of texture. Gomez (2007) mentioned that the xanthan gum is used at a concentration of 1% by weight of flour in cake making. Ashwini *et al.* (2009) stated, the use of xanthan gum at 0.5% in the eggless cake making can produce the highest batter viscosity compared with other hydrocolloids and the eggless cake have a quality that equal to the control cake.

This study aimed to determine the effect of egg reduction, xanthan gum concentration and their interaction on the physicochemical characteristics of reduced fat rice cake. Physicochemical properties consisted of moisture content, specific volume, and texture which include hardness, springiness and cohesiveness.

Materials and Methods

Materials

Reduced fat rice cake making materials consisted of rice flour, eggs, sugar, Na-CMC (Natrium Carboxymethyl Cellulose), xanthan gum, skim milk powder, baking powder and red kidney beans. The materials were obtained from the local market.

Preparation of red kidney bean flour

300 g of red kidney beans were soaked for 10 hours in 1 : 5 ratio of red kidney beans : water. The

red kidney beans were peeled and steamed for 15 minutes at 85 – 90°C. Steamed red kidney beans were crushed and dried in a oven at 70°C for 5 hours. Then dried red kidney beans were sieved to 80 mesh size.

Reduced fat rice cake making

The basic formula of reduced fat rice cake was 180 g of white eggs, 65 g of egg yolks, 65 g of sugar, 55 g of rice flour, 2.75 g of baking powder, 2.2 g of Na-CMC, 5.5 g of skim milk powder, 15 g of red kidney bean flour and 30 g of water. Reduction eggs were done by reducing the egg white and egg yolk in accordance with the treatment. i. e. 20%, 30%, 40% and 50%. Xanthan gum is added by 0.2% (0.11 g) and 0.4% (0.22 g).

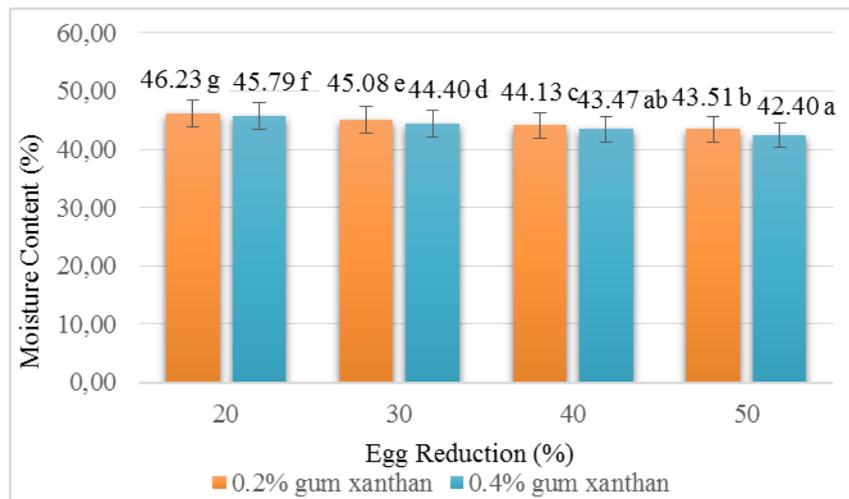
Experimental design and statistical analysis

Experimental design was Completely Randomized Block Design with two factors. The first factor was egg reduction that consisted of four levels, namely 20%, 30%, 40%, and 50%. The second factor was gum xanthan concentration that consisted of two levels, namely 0.2% and 0.4% from rice flour weight. The experiment was conducted with four replications. Data were analyzed using Analysis of Variance at $\alpha = 0.05$ and Duncan Multiple Range Test at $\alpha = 0.05$ if there was significant difference between treatments.

Result and discussion

Moisture content

The moisture content of reduced fat rice cake was determined by thermogravimetry method. The moisture content of reduced fat rice cake ranged from 42.40% to 46.23%. ANOVA result at $\alpha = 0.05$ showed that the interaction of egg reduction and the xanthan gum concentration significantly affect the moisture content of reduced fat rice cake. The relationship between the egg reduction and xanthan gum concentration with a moisture content of reduced fat rice cake and the result of DMRT at $\alpha = 0.05$ are shown in Figure 1.



Means accompanied by the same letter on the same line do not present a statistically significant difference ($\alpha=0.05$) according to DMRT's test.

Figure 1. Relationship of Egg Reduction and Xanthan Gum Concentration with Moisture Content of Reduced Fat Rice Cake

The moisture content decreased at any egg reduction and xanthan gum concentration because of the high moisture content of eggs, especially egg whites. According to the USDA (2010), the moisture content of eggs is 76.15%, so egg reduction leads to reduce water availability in reduced fat cake batter. Eggs also contain proteins that can bind free water in the rice cake batter. The reduced of egg protein caused the water-binding agent in rice cake batter is reduced so that it increased the amount of free water in rice cake batter. Free water was evaporated when baking so that the moisture content of reduced rice cake decreased.

The fat content of egg yolk is 26.54% (USDA, 2010) and lecithin is about 10% (Amendola and Rees, 2003). Lecithin is an emulsifier which acts to form a stable emulsion system in a cake batter. The decrease of fat and lecithin content caused the emulsion system of rice cake become unstable.

Xanthan gum is able to trap water and forms a gel matrix. According Arabshirazi *et al.* (2012), xanthan gum has a structure containing hydrophilic group such as hydroxyl and carboxylate. Miller and Hosenev (1993) also stated that the addition of xanthan gum can improve moisture retention. Increasing concentrations of xanthan gum can help to trap free water and weakly bound water, so the moisture content of reduced fat rice cake getting lower.

Specific volume

The specific volume of reduced fat rice cake was affected by batter volume that formed after mixing. The greater the volume of batter would increase the specific volume of cake. ANOVA results at $\alpha = 0.05$ indicated that the interaction of egg reduction and xanthan gum concentration significantly affected on specific volume of reduced fat rice cake. The relationship between egg reduction and xanthan gum concentration with specific volume of reduced fat rice cake and the results DMRT at $\alpha = 0.05$ in Figure 2.

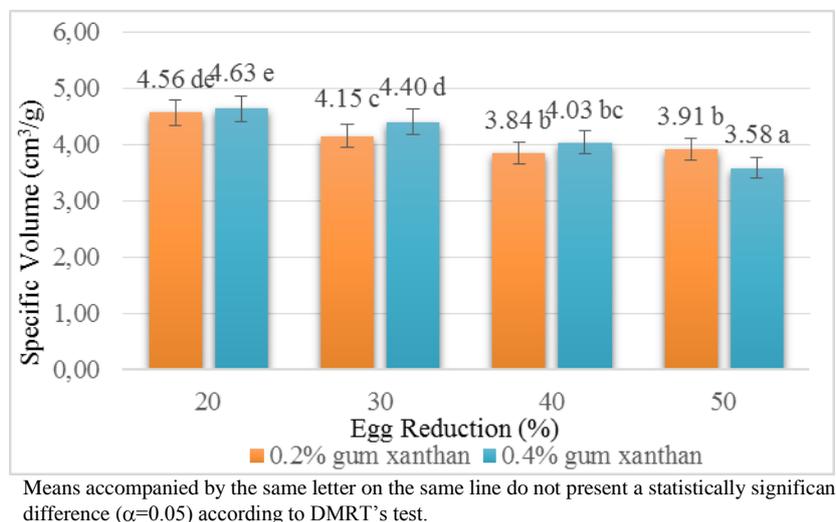


Figure 2. Relationship of Egg Reduction and Xanthan Gum Concentration with Specific Volume of Reduced Fat Rice Cake

Figure 2 showed the greater egg reduction would decrease specific volume of reduced fat rice cake. The increasing of xanthan gum concentration could increase the specific volume of reduced fat rice cake. Egg reduction resulted in lower egg protein (albumin), fats and emulsifiers. These three components

have a function in the formation of foam when mixing. Reduced albumin protein caused less of air trapped in the batter. Egg yolks contain fat that acts to maintain the foam stability. According to Chevallier *et al.* (2000), the fat can form a layer that will provide additional protection to the surface of the foam layer, so that foam will not easily collapse when baking. Egg yolks also contain emulsifiers, namely lecithin. The existence of lecithin is reduced due to egg reduction lead to decrease the foam stability. The decreasing of foam stability caused the trapped air is difficult to be maintained so that the foam collapses easily. All of these decreased the specific volume of reduced fat rice cake.

Xanthan gum is capable to trap free water and weakly bound water in the cake batter. The higher xanthan gum concentration caused more trapped water thus increased the viscosity of rice cake batter. Demirkesen *et al.* (2010) stated that the increasing of xanthan gum concentration affect on batter viscosity. This can increase the stability of the cake batter so that the batter is able to maintain the trapped air. Thus the specific volume of reduced fat rice cake is generally increase at a higher xanthan gum concentration.

Figure 2 also showed that the 50% of egg reduction and 0.4% of xanthan gum concentration resulted in a specific volume of the reduced fat rice cake that is lower than the 0.2% concentration. In this treatment, the egg reduction is very high, so the foam that formed became less. The higher xanthan gum concentration caused the cake batter is too viscous that it can not produce a bigger specific volume of reduce fat rice cake.

Hardness

Hardness value is indicated by the peak value graph (force) after the product is pressed for the first time (Roshental, 1999). The higher the hardness values, means the greater the force applied product against the force exerted so that the product is harder. ANOVA results at $\alpha = 0.05$ indicated that the interaction of egg reduction and the xanthan gum concentration significantly affected on the hardness of reduced fat rice cake. The relationship between the egg reduction and the xanthan gum concentration with hardness of reduced fat rice cake and the results DMRT at $\alpha = 0.05$ in Figure 3.

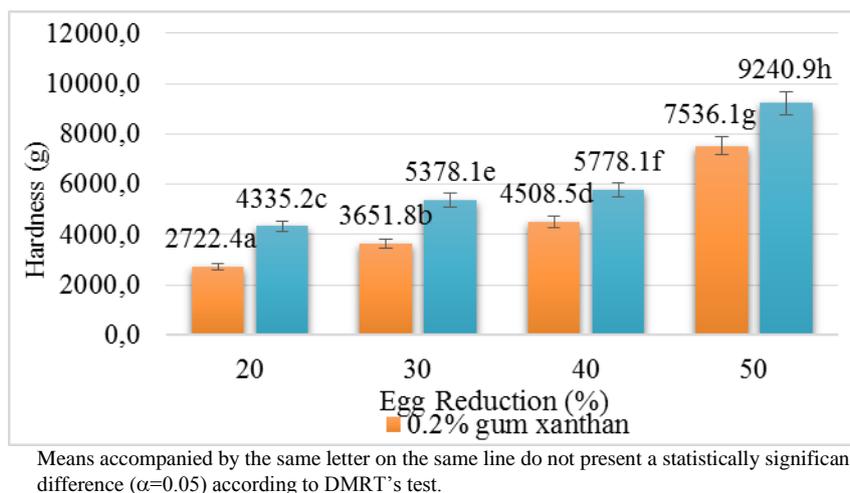


Figure 3. Relationship of Egg Reduction and Xanthan Gum Concentration with Hardness of Reduced Fat Rice Cake

Egg whites contain proteins that able to trap air that incorporated when mixing to form a foam

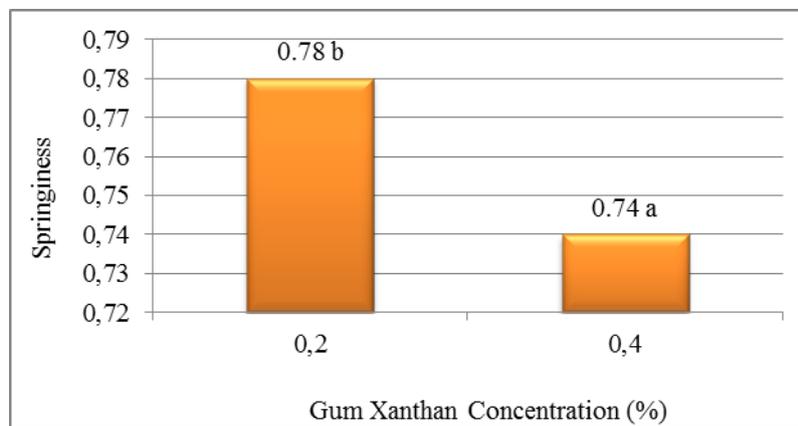
(Charley, 1982). The amount of egg white is sufficient to provide trapping a lot of air. According to Charley (1982), the yolk contains lecithin which acts to form a stable emulsion system in a cake batter. Egg yolks also contain fat components that can help trapping of air by forming a coating on the surface of the foam so that the foam is not easily collapsed and unstable. The optimum condition caused the rice cake batter is able to expand with the optimum so that the pore walls of rice cake are thin, so the reduced fat rice cake has a low hardness.

The egg reduction causes a decrease in the protein components that contribute to air trapping. Fat and emulsifier components contained in the yolk also reduced. The expansion was not optimum that lead the cake's pore wall become thick. This causes an increase in hardness of reduced fat rice cake with the increasing of egg reduction.

The use of xanthan gum leads to reduce free water due to the ability of xanthan gum in water entrapment. This causes the rice cake batter becomes too viscous at the higher xanthan gum concentration. The batter is too viscous resulting in more dense batter and less expands when baking. The pore walls of rice cake are also increasing massively so the cake becomes harder and has the higher hardness values.

Springiness

Springiness is the ability of a food product to return to normal condition after a given pressure (Roshental, 1999). Springiness higher value indicates that the product is more elastic. ANOVA results at $\alpha = 0.05$ showed that the xanthan gum concentration significantly effect on reduced fat rice cake springiness. The relationship between the xanthan gum concentration with springiness of reduced fat rice cake and the results DMRT at $\alpha = 0.05$ in Figure 4.



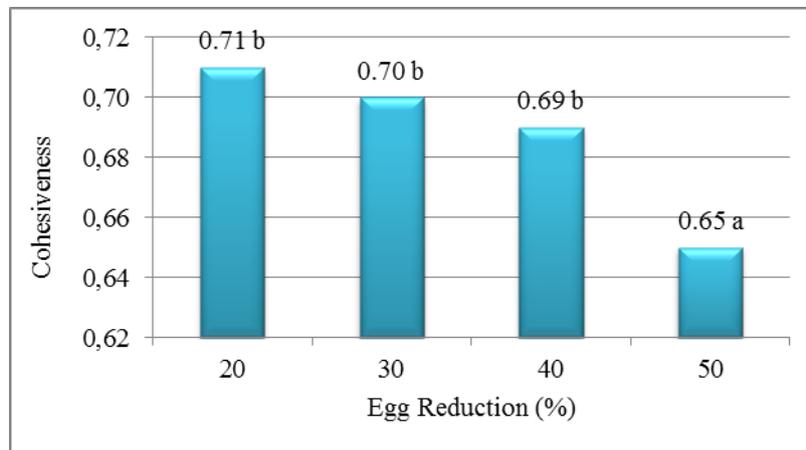
Means accompanied by the same letter on the same line did not present a statistically significant difference ($\alpha=0.05$) according to DMRT's test.

Figure 4. Relationship of Xanthan Gum Concentration with Springiness of Reduced Fat Rice Cake

Figure 4 showed that increasing the xanthan gum concentration caused decreasing low-reduced fat rice cake's springiness. This is due to the rice cake batter is too viscous, so when baking the structure of reduced fat rice less elastic and less expansion. According to Arabshirazi *et al.* (2012), the addition of xanthan gum can cause the batter structure firmer and reduce the degree of its relaxation. This condition caused the reduced fat rice cake with a higher xanthan gum concentration has a lower ability to return to its original shape after pressured. This is indicated by the lower value of springiness.

Cohesiveness

Moskowitz (1999) stated that cohesiveness is the compactness of each component in a product that will form the texture of the product. ANOVA results at $\alpha = 0.05$ showed that egg reduction treatment significantly effect on reduced fat rice cake cohesiveness. The relationships of egg reduction with cohesiveness of reduced fat rice cake and results DMRT at $\alpha = 0.05$ can be seen in Figure 5.



Means accompanied by the same letter on the same line did not present a statistically significant difference ($\alpha=0.05$) according to DMRT's test.

Figure 5. Relationship of Egg Reduction with Cohesiveness of Reduced Fat Rice Cake

Figure 5 showed that the lower cohesiveness of reduced fat rice cake along with the reduction of eggs concentration. Egg whites have the ability to bind water because of its protein content. Egg reduction caused the decrease of protein content so that rice cake batter become less viscous. Reduced component of egg white proteins also cause a reduction in protein gel matrix that formed so that the structure of reduced fat rice cake becomes less compact. Reduced number of egg yolks also responsible for the reduction in lecithin as an emulsifier and fat components that contribute to the formation of emulsions. This leads to interactions between components in a cake is getting weaker, so cohesiveness values were lower.

Conclusion

The interaction between egg reduction and xanthan gum concentration significantly affect the moisture content, specific volume and hardness. The xanthan gum concentrations significantly affect the springiness, while the egg reductions significantly affect the reduced fat rice cake cohesiveness. The egg reduction as well as the xanthan gum concentration from 20%: 0.2% to 50%: 0.4% resulted in an increase in the value of hardness, but caused a decrease in moisture content and specific volume. Xanthan gum concentration caused a decrease in the value of springiness, while the egg reduction resulted in a decrease of reduced fat rice cake cohesiveness.

The egg reduction and xanthan gum concentration had a significant effect on the texture. Need further study on the effect of the egg reduction and xanthan gum concentration on the sensory characteristics of reduced fat rice cake in order to observe the maximum limit egg reduction and the xanthan gum concentration that appropriate to consumer acceptance.

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References

- Allshouse, J., Frazao, B. and Turpening, J. 2002. "Are Americans turning away from lower fat salty snacks?" *Food Review International*, Vol. 25 No. 3, pp. 38-43.
- Amendola, J. and Rees, N. 2003. *Understanding Baking: The Art and Science of Baking, 3rd Edition*, John Wiley and Sons, Inc., USA.
- AOAC. 1990. *Official Methods of Analysis 14th Edition*, Association of Analytical Chemists, Washington D.C.
- Arabshirazi, S., Movahhed, S. and Nematti, N. 2012. "Evaluation of addition of xanthan and hydroxypropylmethyl cellulose gums on chemical and rheological properties of sponge cake", *Annals of Biological Research*, Vol. 3 No. 1, pp. 589-594.
- Ashwini, A., Jyotsba, R. and Indrani, D. 2009. "Effect of hydrocolloids and emulsifier on the rheological, microstructural and quality of eggless cake", *Food Hydrocolloids*, No. 23, pp. 700-707.
- Charley, H. 1982. *Food Science, Second Edition*, John Wiley and Sons, Inc., New York
- Chevallier, S., Colonna, P., Della Valle, G. and Lourdin, D. 2000. "Contribution of major ingredients during baking of dough systems", *Journal of Cereal Science*, No. 31, pp. 241-252.
- Demirkesen, I., Mert, B., Sumnu, G. and Sahin, S. 2010. "Rheological properties of gluten-free bread formulations", *Journal of Food Engineering*, No. 96, pp. 295-303.
- Gomez, M., Ronda, F., Caballero, P. A., Blanco, C. A. and Rosell, C. M. 2007. "Functionality of different hydrocolloids on the quality and shelf-life of yellow layer cakes", *Food Hydrocolloids*, No. 21, pp. 167-173.
- Lopez, A. C. B., Accacia, J. G. P. and Roberto, G. C. 2004. "Flour mixture of rice flour, corn, and cassava starch in the production of gluten free white bread", *J. of Braz. Arch. of Biol. and Technol*, Vol. 47 No. 1, pp. 63-70.
- Miller, R. A. and Hosenev, R. C. 1993. "The role of xanthan gum in white layer cakes", *Cereal Chemistry*, Vol. 70 No.5, pp. 585-588.
- Moskowitz, H. R. 1999. *Food Texture: Instrumental and Sensory Measurement*, Marcel Dekker, Inc., New York
- Phillips, G. O. and Williams, P. A. 2000. *Handbook of Hydrocolloids*, CRC Press., New York
- Roshental, A. J. 1999. *Food Texture Measurement and Perception*, Aspen Publisher, Inc., Maryland.
- Saputra, R. B. S. 2013. "Karakteristik fisikokimia dan organoleptik cake beras dengan proporsi margarine dan kacang merah kukus", *Skripsi S-1*, Fakultas Teknologi Pertanian, Universitas Katolik Widya Mandala Surabaya, Surabaya.
- Sudarmadji, S., Haryono, B. dan Suhardi. 1997. *Prosedur Analisa untuk Bahan Makanan dan Pertanian*, Liberty, Yogyakarta.
- Sutedja, A. M. dan Trisnawati, Ch. Y. 2013. "Karakteristik sensoris dan mikrostruktur cake beras rendah lemak". *Laporan Penelitian PPPG Research Project 2012*, Pusat Penelitian Pangan dan Gizi, Lembaga Penelitian dan Pengabdian Masyarakat, Universitas Katolik Widya Mandala Surabaya, Surabaya.
- Trisnawati, Ch. Y. dan Sutedja, A. M. 2008. "Peningkatan kualitas rice cak dengan penambahan Na-CMC dan defatted rice bran", *Laporan Penelitian PPPG Research Project 2007*, Pusat Penelitian Pangan dan Gizi, Lembaga Penelitian dan Pengabdian Masyarakat, Universitas Katolik Widya Mandala Surabaya, Surabaya.
- Trisnawati, Ch. Y. and Sutedja, A. M. 2014. "Utilization of mung bean and red kidney bean as fat replacer in rice cake" in *Proceeding International Conference Food for a Quality Life in Jakarta, 2014*, Southeast Asian Food & Agricultural Science & Technology (SEAFASST) Center, Bogor Agricultural University, Bogor, pp. 39 – 50.
- USDA. (2007). <http://digilib.unila.ac.id/3319/13/13.%20BAB%20II.pdf> (accessed 20 July 2015).
- USDA. 2010. "Nutrient value and weight for edible portion", <http://ndb.nal.usda.gov> (accessed 5 May 2016).

Quercetin Encapsulation from Extract as a Natural Antioxidant with Emulsion Solvent Evaporation Method

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Abstract

Strobilanthes crispus' extract contains 54.5% quercetin. Quercetin had to pass encapsulation process in order to protect itself from external factors and improve the stability of these molecules. The Encapsulation method used is the emulsion solvent evaporation method. This research is conducted to find the optimum condition for quercetin encapsulation using PDLLA or Chitosan-TPP as encapsulator and PVA or Tween 80 as stabilizer. This study showed, PDLLA is better than chitosan-TPP because PDLLA formed emulsion in oil phase. Meanwhile, the mixture of chitosan-TPP formed suspension. PVA as stabilizer led to higher entrapment of molecules than Tween 80. The particles which made by 1% PDLLA as encapsulator and 0.1% PVA as stabilizer gave the best percentage of loading capacity (49.79%) and encapsulation efficiency (99.08%). The size of particles is in the range of 16.24 μm to 112.7 μm (microparticles), affected by molecular weight of encapsulator used. PDLLA with low molecular weight will be deprotonated easily and reduce pH. Low pH condition can trigger particles aggregations that cause the particles' size become larger. The antioxidant activities of *Strobilanthes crispus*' extract can reach one third of ascorbic acid's antioxidant activities from vitamin C supplements.

Keywords: Antioxidant; Encapsulator; Emulsion; Stabilizer; and Quercetin.

Introduction

Antioxidants are substances that can reduce the activity of free radicals, thus preventing cell damages. Free radicals can occur in the body in small amounts as a result of metabolic processes, as well as from outside the body, such as motor vehicle fumes. *Strobilanthes crispus* has many active substances such as flavonoids as antioxidants (e.g quercetin), with a high antioxidant activity (Liza, 2014). Quercetin is located within *Strobilanthes crispus* extract obtained by sonication. For its full antioxidant activities, the extracts obtained can not be taken for granted, but needs to be encapsulated so that it can easily disperse spread in the blood, is more accurate in reaching the target cells, and prevent any adverse effects of gastrointestinal fluids damage due to external factors. Encapsulation method used is the emulsion solvent evaporation method, which was used in the present study. The objectives of this study was to investigate the optimum condition for quercetin encapsulation by varying the type of encapsulator (PDLLA and chitosan-TPP), type of stabilizer (Tween 80 and PVA), concentration of stabilizer, and concentration of encapsulator to extract particle encapsulation based on loading capacity and encapsulation efficiency percentage. Particles containing quercetin produced will be analyzed for the presence of quercetin using FTIR spectrophotometer, morphological analysis with FE-SEM, and, by using DPPH method to determine the antioxidant activities, with Vitamin C as a standard because it is a commercially used as an antioxidant.

Materials and Methods

Materials

The extraction solvent was 70% ethanol, while G60 F254 Silica TLC plate, DPPH, methanol, ethyl acetate, pure quercetin, and poly (D, L-Lactide) or PDLLA obtained from Sigma. Chitosan made from shrimp shells was obtained from Chimultiguna. PVA and Tween 80 were obtained from Bratachem.

Extraction of *Strobilanthes crispus* with Sonication Method

Strobilanthes crispus leaf powder was mixed (1:10, w/v) with the ethanol and sonicated with a Selma S40H Ultrasonicator at a frequency of 37 kHz for 50 min at 25°C. The precipitate was separated from filtrate using a vacuum pump. The filtrate was dried after evaporation (Rotary Evaporator) to a low water content. Prior to drying, the evaporated extract was stored (3°C) in a dark container in a refrigerator to prevent light oxidation.

Analysis of Quercetin's Presence with TLC Method and Quercetin's Concentration in Extract

The stationary phase used was a silica plate (2 x 7 cm), while the stationary phase was a mixture of methanol and ethyl acetate at a ratio of 4:1 (Pratiwi, 2015). The reference solution used was pure quercetin dissolved in 70% ethanol and Rf was calculate as follow:

$$R_f = \frac{\text{distance from the starting point to the center of the spot}}{\text{distance the starting point to the solvent front}} \quad (1)$$

The concentration of quercetin in the *Strobilanthes crispus* extract was determined using a UV-VIS spectrophotometer at wavelength of 210 nm from the standard curve of the pure quercetin.

Antioxidant Activity Test of *Strobilanthes crispus*' Extract with DPPH Method

One millilitre (1 mL) of various concentrations of the extract solution was mixed with 2 mL of methanol and 1 mL of DPPH. The antioxidant activity was recorded at the concentration of 50% inhibition with respect to time of incubation.

Manufacture of *Strobilanthes crispus* Particle by Emulsion Solvent Evaporation Method

In the encapsulation processes, there are organic and the continuous phases. The organic phase in the present study consisted of quercetin in the extract, encapsulator, and organic solvents, whereas the continuous phase was stabilizer and distilled water. The organic phase was prepared by mixing 1% (w/v) extract in 4 mL of methanol and 3% (w/v) encapsulator (PDLLA or chitosan-TPP) in 4 mL of chloroform and then stirred for homogeneity. The concentration (% w/v) of stabilizer (Tween 80 or PVA) was prepared in 80 mL of distilled water. The concentration (% w/v) of the stabilizer in the continuous phase was varied: 0, 0.05, 0.2, and 0.5.

The phases were sonicated as above and then mixed with a magnetic stirrer for 24h at room temperature before centrifuging for 20 min at 10°C and 13.000 rpm (Pratiwi, 2015) and freeze dried.

Calculation of Loading Capacity Percentage and Encapsulation Efficiency Percentage

Loading capacity and efficiency of encapsulation were calculated as follow:

$$\% \text{ Loading capacity quercetin} = \frac{\text{mass of encapsulated quercetin}}{\text{mass of particles from encapsulation}} \times 100\% \quad (2)$$

$$EE(\%) = \frac{W_{\text{added drug}} - W_{\text{free drug}}}{W_{\text{added drug}}} \quad (3)$$

where $W_{\text{added drug}}$ is an amount of quercetin in the extract is added to the process of encapsulation, and $W_{\text{free drug}}$ is the number of quercetin on the extract remaining in the supernatant after centrifugation (Campos et al., 2001).

Analysis of Particles' Functional Group with FTIR Spectrophotometer

Chemical characterization of the functional groups was done by FTIR spectroscopy, to asses the bonds to the encapsulation. The encapsulates were mixed with KBr powder at 5-10%, ground and pressed (MINI HAND PRESS, 10 ton force) to make KBr pellets that was used in the spectrophotometer.

Analysis of Particles' Morphology with FE-SEM

FE-SEM Morphology test was done with the purpose of knowing the physical properties of the samples. The encapsulates were stuck on 1 cm diameter brass pieces by two – sided tapes before coating with a beam of thin platinum layer (coating) for 30 seconds at a pressure below 2 Pa and strong current of 30 mA. The FE-SEM was used, and micrograph taken at a voltage of 10 kV electron.

Particle's Antioxidant Activity Test with DPPH Method

The antioxidant activity of the samples was done with DPPH method, with vitamin C as a positive control.

Result and discussion

Preparation of Strobilanthes crispus' Extract with Sonication Method

About 50 kg of *Strobilanthes crispus* leaves powder which produced about 2.5 g heavy crude extract with a yield value of 5% (w/w). Yield values obtained was lower compared with the results obtained previously which was 10.67% (w/w), although using the same methods and stages (Pratiwi, 2015). Differences in yield value can be affected by several factors, including the age of the plant, genetic and environmental factors grow crops (Nurcholis W., 2008). The older the plant, the more secondary metabolites it contains (Lusianawati, 2013). In addition to the factors mentioned earlier, the present study used a powder : solvent ratio of 1 : 10, while the previous study (Liza, 2014) used 1:20, which could have extracted more. A 1:10 ratio was used in this study because it was judged to be enough as the leaf powder was properly soaked. With a higher ratio, more solvent would need to be evaporated which may not necessary be more efficient in the present study.

Detection and Concentration of Quercetin in the Extract

TLC's test for standard quercetin give Rf value of 0.948, while the *Strobilanthes crispus* leaves extract give Rf value of 0.942 that can be seen at Figure 1. Extract's Rf value has a value very close to the Rf value of standard quercetin which indicates that the *Strobilanthes crispus* leaves extract is containing quercetin.

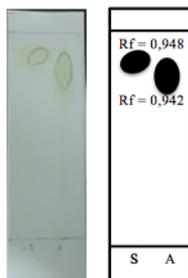


Figure 1. (a) TLC Result, (b) TLC Illustration Result

The calibration curve of pure quercetin that read using UV-Vis Spectrophotometer at a wavelength of 210 nm produces Equation (4).

$$\text{Absorbance} = 0.0081 (\text{quercetin's concentration}) - 0.0092 \tag{4}$$

Making *Strobilanthes crispus*' extract solution with a concentration of 10 ppm is done and then the solution was identified by UV-Vis Spectrophotometer at a wavelength of 210 nm. UV-Vis Spectrophotometer absorbance value of extract solution is 0,035. Therefore, it can be concluded that the level of quercetin in *Strobilanthes crispus* leaves extract is 54.5% (w/w).

Antioxidant Activity of the extract with DPPH Method

Antioxidant activity test of *Strobilanthes crispus*' extracts done using DPPH Method because quercetin is an antioxidant that is an ion scavenger. This stage produces a graph that gives the relation between inhibition percentage and the concentration of *Strobilanthes crispus* leaves extract which can be seen in Figure 2.

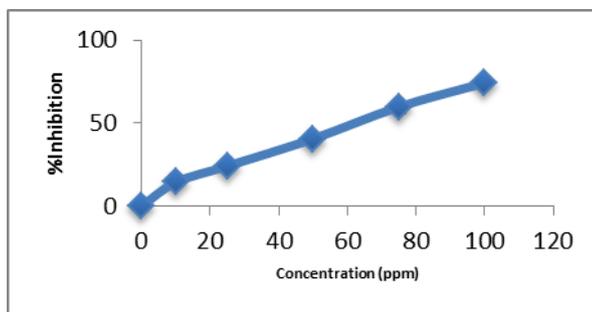


Figure 2. Graph of antioxidant inhibition percentage against concentration

Inhibition percentage of antioxidant shows the number of DPPH radical compounds that can be converted into non-radical compounds by using antioxidants at a certain concentration, so that the higher the concentration of antioxidants, then the percent inhibition will also increase. The antioxidant power is determined by the number IC₅₀ which is a value indicating the concentration of extract that can inhibit the oxidation reaction by 50%. IC₅₀ value of *Strobilanthes crispus* leaves extract is at a concentration of 63.54 ppm, so *Strobilanthes crispus* leaves extract is included in the class of powerful antioxidants. In addition, we also want to know how long the antioxidants will last. The results can be seen in Figure 3.

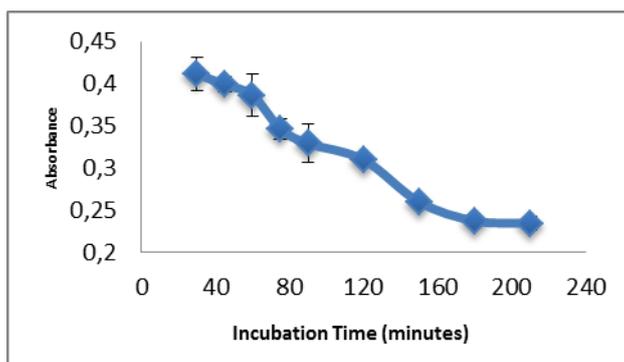


Figure 3. Graph of absorbance against incubation time

The antioxidant activity of *Strobilanthes crispus* leaves extract can last up to 210 minutes or about 3 hours 30 minutes, before the antioxidants in the extract completely reacted with DPPH radical compounds.

Determine the Type of Encapsulator and Stabilizer for Quercetin Encapsulation from Strobilanthes crispus Leaves Extract

This variety will produce 4 pieces combined with the type of encapsulation and type of stabilizer different, but with the same composition, namely the A-I to use PDLA-Tween 80, A-II for the use of chitosan-TPP-Tween 80, A-III for use PDLA- PVA, and A-IV for use Chitosan-TPP-PVA.

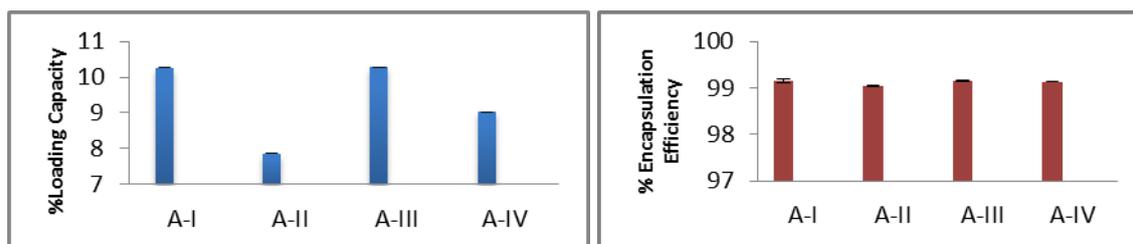


Figure 4. %LC dan %EE in the amount of stabilizer

Use of Chitosan-TPP provides lower quercetin loading capacity and encapsulation efficiency. The first cause is in the mix, the chitosan-TPP does not form an emulsion, but forming a suspension, so that the process of encapsulation is not going well. Another cause is the pH of the mixture is not sufficiently acidic to the encapsulation process using chitosan-TPP because ordinary encapsulation with chitosan-TPP using ionic gelation method that provides the acidic conditions. Chitosan-TPP requires an acidic pH because in the acidic pH, bonding TPP (tripolyphosphate) has a good performance to keep the chitosan matrix formed will not decay, so that the active substance can be encapsulated well (Pratiwi F. M., 2014). Value of % LC and % EE from the use of PVA is higher than Tween 80, compared with the same type of encapsulator. The cause is PVA has emulsifying ability better than Tween 80. The PVA was able to make the emulsion becomes more stable and able to reduce the possibility of active substances that are not encapsulated as compared to the use of Tween 80 (Vineeth et al. 2014).

Determine the amount of Stabilizer for Quercetin Encapsulation from Strobilanthes crispus' Extract

B-I is a combination using PVA 0,05% (w/v), B-II is a combination using PVA 0,1% (w/v), B-III is a combination using PVA 0,2% (w/v), and B-IV is a combination using PVA 0,5% (w/v).

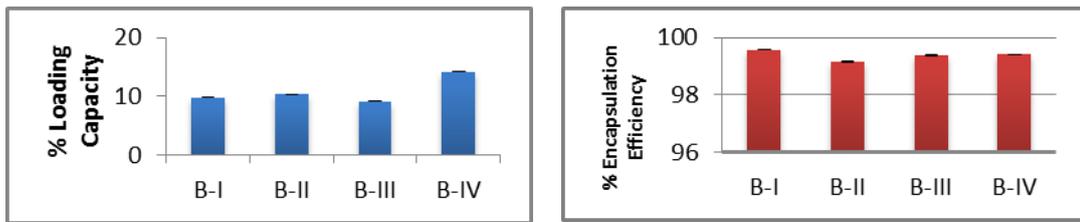


Figure 5. %LC and %EE in the amount of Stabilizer

Fluctuating value of %LC will generate a graph with a similar trend, but one thing it can be concluded that the optimum value of %LC on all existing combination lies in the combination of B-II, although the highest value of %LC is the combination of B-IV. These conclusions based on the tests conducted. The process of making the particles after centrifugation step on a combination of B-IV is very difficult to do because of the structure of the particles of a combination of B-IV is very smooth and form structures such as flour, so that the particles stick to the centrifuge tubes. This can happen because of the increased amount of stabilizer will cause a decrease in particle size and decrease the possibility of formation of aggregates, resulting in differences in the structure of the combination of B-IV with other combinations.

The increase in the value of %LC in combination B-I combination B-II may be caused by a combination of B-II lower the surface tension greater than the combined B-I and provide appropriate conditions for the encapsulation of the particles because the emulsion is formed into a more stable and resulted in a decrease in the number of particles retrieved. As with the continued increase in the number of combinations of stabilizers B-II into a B-III provides the downward trend in the value of %LC. This can be due to an increase in the amount of stabilizer in combination B-II to combination B-III will lead to an increase in viscosity of the solution, so that the particles will get into more and the value of %LC becomes lower. Based on research at this stage, the amount of PVA as stabilizer is best in combination II or 0.1% PVA in distilled water. These elections are not adjusted for the best value %LC is the combination of B-IV, but the %EE occurs best in combination B-I. This is done with a combination of reasons B-II also has a value of %LC is quite big compared to the combination of B-IV and the amount of stabilizer that gives the optimum value of %LC. Other reasons underlying the selection of a combination of B-II is a combination of B-IV generates particle structure that resembles flour, making it difficult to remove all the particles of centrifuge tubes so that the value of %LC can be ascertained the truth. While the combination of B-III is not selected even if the value of %EE of a combination of B-III high as the value of %EE in all four combinations are very high and everything is still above 99% with little difference, while the value of the optimum %LC achieved in combination B-II, thus combination B-II were selected for the study on the the following variation.

Determine the amount of Encapsulator for Quercetin Encapsulation from Strobilanthes crispus' Extract

C-I is a combination using 1% PDLLA, C-II is a combination using 2% PDLLA, C-III is a combination using 3% PDLLA, and C-IV is a combination using 5% PDLLA.

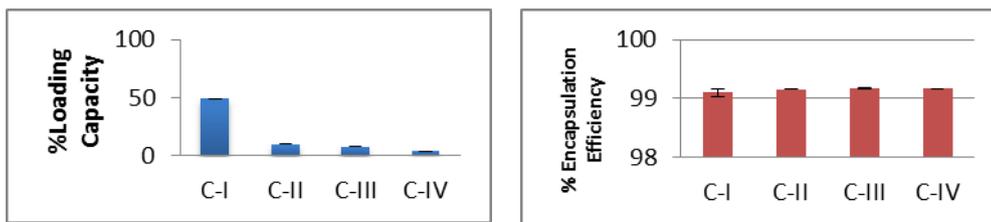


Figure 6. %LC dan %EE in the amount of encapsulator

%EE showed an increase on a combination of C-I to CIII, but the value of C-IV has the same value with C-III. Although it showed constant rising trend and at the end, the value of %EE has a high value reaches 99%. Therefore, only %LC used as a parameter to chose the best combination. The best amount of encapsulator for the encapsulation of quercetin from *Strobilanthes crispus* leaves extract is using 1% (w/v) PDLLA. %LC quercetin on the use of 1% (w/v) PDLLA reached 49.348%; means that there are 49.35 mg of quercetin in 100 mg of particles.

Characterization of Encapsulated Functional Group Particles with FTIR Spectrophotometer

Clusters which are important in identifying the presence of quercetin is a group of O-H, C-H, C = O, -C-C-, and C-O ether (Liza, 2014). Encapsulation process was successful because quercetin has been encapsulated with PLDAA which can be evidenced by the quercetin group at particle encapsulation yields.

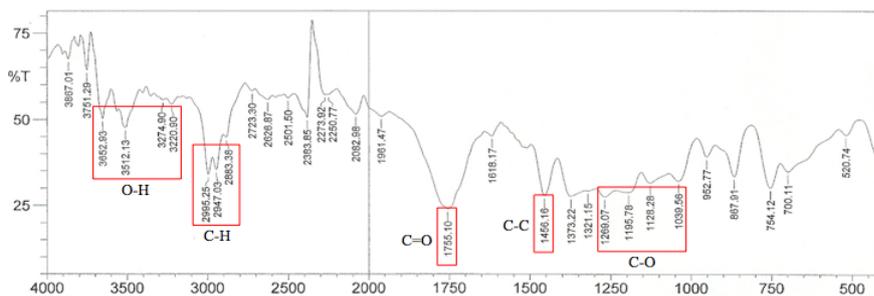


Figure 7. Particles' FTIR result

Characterization of Particles' Morphology with FE-SEM

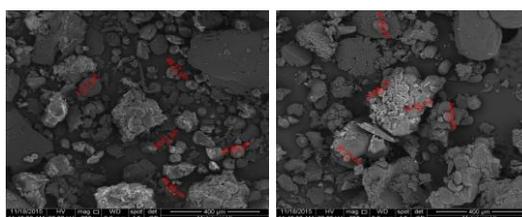


Figure 8. Particles' FE-SEM result

The size of the particles obtained is in the range of 16 μm to 113 μm with a standard deviation of 26.67. Particles obtained are not yet nanometer-sized but micrometer-sized, and this can occur for several reasons. The first reason is PDLLA used in this study has a molecular weight of 10,000 Da. The molecular weight of PDLLA is much smaller compared to the research conducted by Kumari *et al.* (2011). Kumari *et al.* (2011) using PDLLA with a molecular weight of 75,000 to 120,000 Da. The molecular weight of encapsulator used has a major role to the size of the particles to be generated in the encapsulation process.

PDLLA as encapsulation is stabilized particle which tends to be more electrostatic than the steric and electrostatic stabilization occurring due to the existence of negative charge repulsion. The negative charge is provided by a carboxylic acid group that had been deprotonized (-COO⁻), causing changes in the degree of acidity or pH. Therefore, the pH is a parameter of the encapsulation process that uses PDLLA and will affect the stability of the emulsion is formed. Low pH conditions that can occur due to reduced deprotonized group at the end of the PDLLA and produce deprotonized carboxylic acid groups which spread freely in the emulsion. Deprotonized carboxylic acid groups are derived from the formation of hydrogen bonds between the hydroxyl groups coming from acid groups and the carbonyl group of the ester group on the polymer backbone. The lower the molecular weight value, the likelihood of this process to produce greater deprotonized carboxylic acid groups, so lead to lower pH value. Low pH conditions can lead to aggregation of the particles and will increase the size of the particles formed (Palacio *et al.*, 2011).

Encapsulated Antioxidant Particles' Test

To generate a percentage of inhibition or the same antioxidant activity, the required amount of quercetin in *Strobilanthes crispus* leaves extract should be more than quercetin in particle encapsulation yields. This shows that encapsulation is able to modify and enhance the antioxidant activity of quercetin than in extract form without encapsulation (Gouin, 2004).

Conclusion

Strobilanthes crispus' crude extract obtained from the extraction process by the sonication method will generated a yield value by 5% from a 1:10 (w/v) ratio of the *Strobilanthes crispus* leaf powder to the solvent used (70% ethanol). The *Strobilanthes crispus* extract contained quercetin as evidenced in the chromatography (R_f = 0.948 for pure quercetin and R_f = 0.942 for the extract). UV-Vis spectrophotometry revealed the extract had about 54.5% (w/w) of quercetin. The extract can be classified as a powerful antioxidant with IC₅₀ value of 64 ppm. Particles with optima percentage loading capacity (49%) and encapsulation efficiency (99%) were obtained with 1% (w/v) PDLLA as the encapsulator and 0.1% (w/v) PVA as efficiency (99%) were obtained with 1% (w/v) PDLLA as the encapsulator and 0.1% (w/v) PVA as the stabilizer, with a particle size that ranged from 16 µm to 113 µm. FTIR spectroscopy revealed the presence of O-H, C-H alkanes, C=O carbonyl, C-C aromatic, and C-O ether in the encapsulator. The optimum encapsulate could be used in the ratio 3:1 of vitamin C for the same percentage inhibition.

References

- A.M.D.Campos,A.Sa´nchez,M.J.Alonso. 2001. Chitosannanoparticles:anewvehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A, Int. J. Pharm. 224 (2001): 159–168.
- Gouin S. 2004. Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci Tech 15:330–347.
- Liza. 2014. Additional Extraction Technique for Seed Polyphenols. In R. R. Watson, *Polyphenols in Plants : Isolation, Purification and Extract Preparation*. Elsevier, London, p. 313.
- Lusianawati. 2013. Aktivitas Ekstrak dan Nanopartikel Ekstrak Kulit Kayu Mahoni sebagai Inhibitor Enzim HMG-KoA Reduktase [tesis]. Bogor: Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor.
- Nurcholis W. 2008. Profil Senyawa Penciri Bioaktifitas Tanaman Kunyit pada Agrobiotik Berbeda [tesis]. Bogor: Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor.
- Palacio, Juliana, Victor H. Orozco, dan Betty L.Lopez. 2011. *Effect of the Molecular Weight on the Physicochemical Properties of Poly(Lactic acid) Nanoparticles and on the Amount of Ovalbumin Adsorption*. Colombia: Universidad de Antioquia.

- Pratiwi, F. M. 2014. *Nanoenkapsulasi Ekstrak Daun Strobilanthes crispus (Strobilanthes crispus) dengan Metode Gelasi Ionik untuk sediaan Obat Antihiperkolesterolemia*. Makara Sains, Universitas Indonesia.
- Pratiwi, Dian. 2015. *Isolasi dan Identifikasi senyawa quercetin dari Ekstrak Daun Strobilanthes crispus (S.Crispus) sebagai Antioksidan Alami*. Universitas Indonesia
- Vineeth, P. et al. 2014. *Influence of Organic Solvents on Nanoparticle Formation and Surfactants on Release Behavior In-Vitro using Costunolid as Model Anticancer Agent*. India: Institute of Chemical Technology.

The Risk Factors of Incidence of Stunting on Children (1-2 Years) in Region of District Health Center Kokar, Alor Regency NTT

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Abstract

This study aimed to determine the risk factors for the incidence of stunting in children (1-2 years) in the region of district health center Kokar, Alor Regency, East Nusa Tenggara. This study involved a group of *stunting* and a group of normal nutrition. This research is included in the observational study with case control design. The case is a group of *stunting* (short and very short) children and the control group is normal nutrition children based on TB/U-index. Each group consisted of 20 respondents. This study used a questionnaire to determine the age gestation, body length of birth, immunization history, mother education level, family income, mother nutritional knowledge level, infectious disease of each group, 24 hours food recall to obtain data on the level of protein consumption. Data were analyzed using Odds Ratio Test. The result shows that statistically significant differences found in the level OR of Family income, Mother Nutritional Knowledge Level, Infectious disease, and Protein Consumption among *stunting* and normal children status. There were no significant differences at the level of the OR Age gestation, Body length of birth, Immunization history, and Mother education level among *stunting* and normal children status in district health center Kokar, Alor Regency, East Nusa Tenggara.

Keywords: Stunting, Normal Nutrition, Age gestation, Body length of newborn, Immunization history, Mother education level, Family income, Mother Nutritional Knowledge Level, Infectious Disease and Protein Consumption.

Introduction

The main challenge in the development of a nation is to develop the quality of human resources, which are healthy, intelligent and productive. In 2003, the Human Development Index (HDI) of Indonesia is still low, is ranked 112 out of 174 countries, lower than neighboring countries. Low HDI is highly influenced by the low nutritional status and health status of the population. It is seen from the high infant mortality rate which is 35 per thousand live births and the mortality rate of children under 5 years old is 58 per thousand live births. More than half the deaths of infants and toddlers are caused by the poor nutritional status of children under five (Azwar, 2004).

Based on the results of the Health Research in Indonesia in 2013, it is known that the prevalence of stunting nationwide is 37.2 %, which is composed of 18.0 % and 19.2 % very short, which means it has an increase of 1.6 % in 2010 (35.6 %) and 2007 (36.8 %) and in 2013, in NTT (Nusa Tenggara Timur) has a short percentage of stunting or above the national prevalence which is 50.1 % , and the highest when compared to other province. Data from Alor District Health Office in 2014, the prevalence of stunting or short in toddler is 25.9 % and 11.3 % 14.6 % for Very Short and Short categories, respectively. In the district of Alor Northwestern (Kokar), prevalence of stunting was 34.5 %, which comprised of 19.1 % and 15.4 % for Very Short and Short, respectively. Based on the description above, the aims of this research is to investigate the risk factors for the incidence of stunting in children (1-2 years) in the region of district health center kokar, or regency NTT.

Materials and Methods

Data on mothers education level, knowledge of nutrition, family income, the incidence of infectious diseases, immunization history, length and weight of newborn, gestational age was obtained using questionnaire while protein consumption level data obtained through the 2 x 24 -hour recall. This kind of research included in the observational study with case control design. The case is stunting toddler group (short and very short) and the control group is toddlers with normal nutritional status based on the index TB / U in Puskesmas Kokar , Alor district and each group consisted of 20 respondents in the same proportion .

Statistical Analysis

Data were analyzed using Odds Ratio Test

Result and Discussion

Kokar Health Center located in the districts of Alor Northwest, with an area of 65.09 km² , with borders of Flores Sea region of Northern South East of the village of South Alila South side Village Small Alor West side of the Flores Sea. PHC Kokar has six work areas are villages Adang, Alaang village, Alila village, village Aimoli, large Alor village, and village Oamate

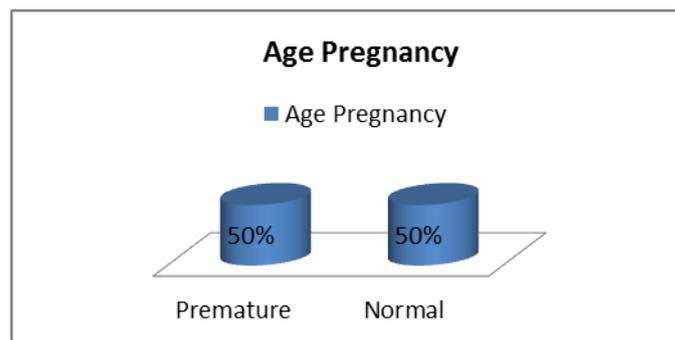


Figure. 1 Distribution of Pregnancy Age

The figure shows that the percentage of infants born within age pregnancy term is 50 % of 20 children and 50 % of preterm birth is 20 children .

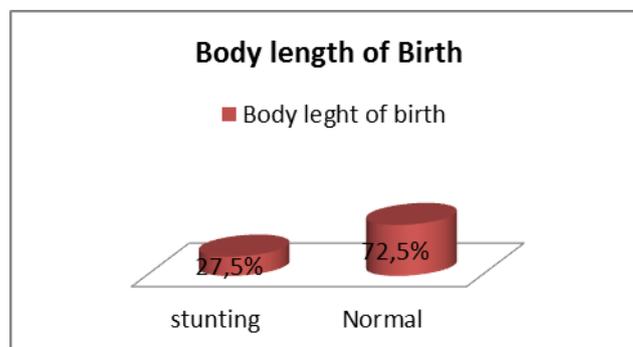


Figure 2. Distribution of Long Firm Birth

Figure 2 shows the percentage of long- normal birth weight infants which is 72.5 % (29 children), while the short length birth weight of infants are 27.5 % (11 children). According to Marmi *et al.* (2012), newborn infant (neonate) is a new baby to experience the process of birth, aged 0-28 days. BBL requires an adjustment in the form of physiological maturation, adaptation (adjusting from intrauterine life to extra uterine life) and a tolerance for the BBL to require a good life, normal infants born weighing 2500-4000 grams and 48-52 cm length of the body.

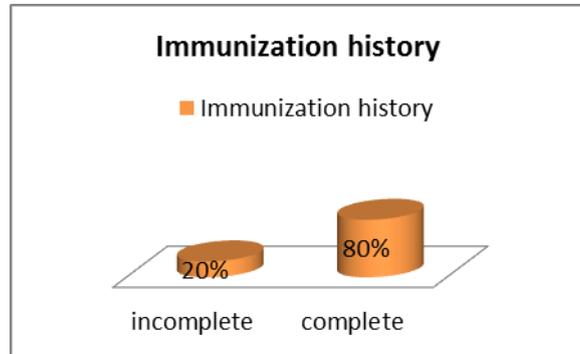


Figure 3. Distribution Immunization history

Figure 3 showed the percentage of children under the age of five that were immunized completely was higher than children without complete immunization. Immunization is a process to enhance the body's immune defense system and protect against invading microorganisms (bacteria and viruses) that can cause infections before these microorganisms have a chance to invade our bodies. With immunization, the body will be protected from infection (Marmi, 2012).

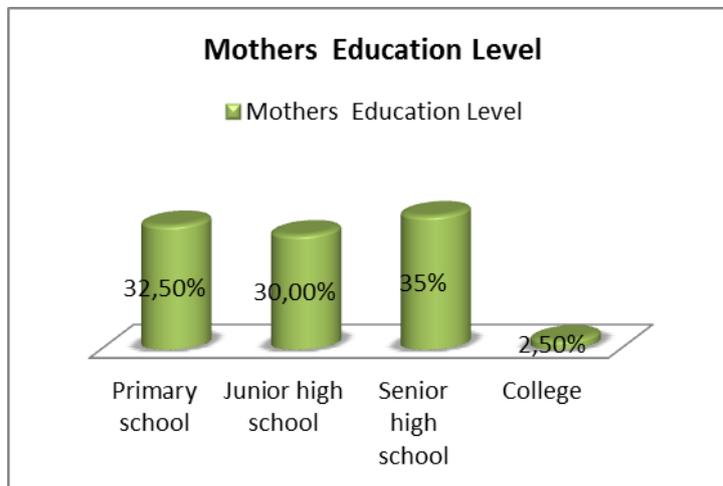


Figure 4. Distribution Mothers Education Level

Figure 4 shows that the number of respondents who have low education levels (62.5%), which consists of Primary School (32.5%), junior high school (30.0%), have completed high school/vocational (35.0%), undergraduate (2.5%). Education is essential to eliminate the factors of social behavior and culture which often become obstacle to the improvement of health of the community (Soekirman, 2000).

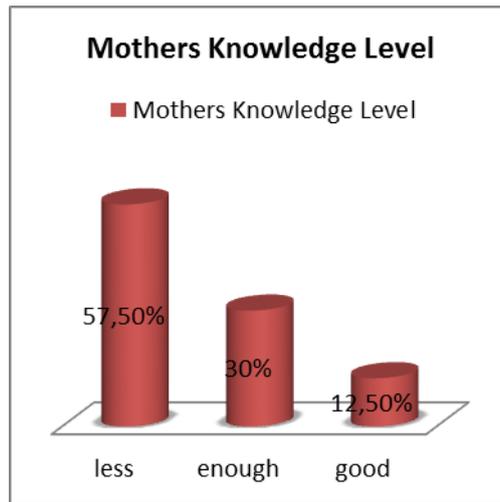


Figure 5 . Mothers Knowledge Level

Figure 5 is the general level of nutrition knowledge of mothers, it can be seen that the level of less nutrition knowledge of mothers reach 57.5 %, only 30 % have enough knowledge and 12.5 %, have good knowledge.

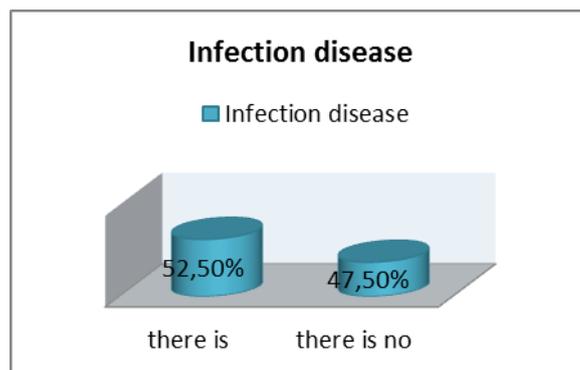


Figure 6. Infection disease

Figure 6 shows the percentage of respondents who had no incidence of infectious diseases was 52.5 %, consisting of ARI 20 %, diarrhea 20 %, Diarrhea + ISPA 12.5 %, and the percentage of respondents that has no infectious diseases were 47.5 %.

Table 1. Distribution of Protein Consumption

Protein Consumption Level	Amount	
	n	%
Deficit	10	25.0
Less	8	20.0
Moderate	17	42.5
Good	5	12.5
Total	40	100.0

Table 1 shows the percentage of children under five protein consumption levels. It can be seen that the number of children who have less protein consumption level is 60% and children who have a good level of consumption is 40 %.

Children dominant protein intake comes from fish of the sea, the NTT province has great potential fishery resources, it is supported by the vast region dominated parts of the ocean. Alor

Northwestern (Kokar) is a district with the second highest fish production of 4.3 tons/year, but Alor consumption rate is still below the national average, while we know that protein is needed for growth and development, regulation of fluid balance in the body, in addition to protein also serves for the formation of antibodies to prevent infection. So, low protein consumption level will affect the function of the protein in the body of children.

Table 2. Distribution of Age Pregnancy between children with Stunting and Normal Nutritional Status

Age Pregnancy	Amount				Total		OR (CI 95%)
	Stunting		Normal		n	%	
	N	%	n	%			
Abnormal	6	15,0	4	10,0	10	25,0	1,714 (0,4007.340)
Normal	14	35,0	16	40,0	30	75,0	
Total	20	50,0	20	50,0	40	100,0	

Table 3. Distribution of Body Length of Birth between children with Stunting and Normal Nutritional Status

Nutritional Status	Amount				Total		OR (CI 95%)
	Stunting		Normal		n	%	
	N	%	n	%			
Abnormal	5	12,5	6	15,0	11	27,5	0,778 (0,1933.130)
Normal	15	37,5	14	35,0	29	72,5	
Total	20	50,0	20	50,0	40	100,0	

Good nutrition is important to support the growth of a child born with a short length of body in order to get the length of a normal body with age. Babies born with a short body length usually caused by the mother who has less nutrition intake during pregnancy, so that the growth of the fetus in the womb is not optimum. Alor regency is one of the regions with the largest fish producer in the province with 4.3 tons / year, as consequences the level of protein intake of infants in the study site on average comes from animal foods (sea trout). Consumption of 40% protein will be sufficient for baby born with short body length because it will help to support the body metabolism thus the baby will regain its normal body length

Odds Ratio statistical tests showed that infants with short body length having 0.778 times risk for the occurrence of stunting than the length of children with a normal birth weight.

Food consumption can affect a person's nutritional status, nutritional status is good or optimal nutrition status occur when the body gets enough nutrients that are used efficiently, thus allowing the optimum physical growth, brain development, employability and health (Almatsier, 2006). This does not rule out the possibility that infants are born with a short body length caused by the good consumption of food, it will have an impact on the nutritional status of children so that children who were born with short body length do not become stunted.

Table 4. Distribution of immunization history between Children's with Stunting and Normal Nutritional Status

Immunization history	Amount				Total		OR (CI 95%)
	Stunting		Normal		n	%	
	n	%	n	%			
imcomplete	6	15,0	2	5,0	8	20,0	3,857 (0,67322.109)
complete	14	35,0	18	45,0	32	80,0	
Total	20	50,0	20	50,0	40	100,0	

Immunization is a process for making the immune system, immunization is needed for prevention of infection and for immune (antibody). If a toddler is not getting complete immunization will result in reduced immunity and are prone to infections, it also will affect the level of food consumption. Children who have infectious diseases tend to eat less than it needs, in addition to the ability of households to access health services related to the availability of health services and the economy's ability to pay a service charge for income communities in the study which is low is still a lot that is 57.5 %

Odds Ratio statistical tests showed that infants with a history of incomplete immunization have a risk of 3.8 times for the occurrence of stunting compared to toddlers who have a complete immunization history. This is supported by research from Citaningrum (2012) which states that toddlers who do not get basic immunization has a chance to encounter incidence of stunting 2.128 higher than infants who receive primary immunization.

Table 5. Distribution of Mothers Education Level Among Children's with Stunting and Normal Nutritional Status

Mothers Education Level	Amount						Total N	OR (CI 95%)
	Stunting		Normal		Total			
	n	%	n	%	N	%		
low	10	25,0	13	32,5	23	57,5	0.538 (0.1511.917)	
high	10	25,0	7	17,5	17	42,5		
Total	20	50,0	20	50,0	40	100,0		

Education is an activity or process of learning to develop or enhance certain capabilities so that the educational goals that can stand alone and education levels also contribute to determining whether someone is easy to absorb and understand the knowledge they gained (Intan, 2010). In the table above can be seen that the percentage of mothers with low levels of education is 57.5 %, this is because of the study sites the number of educational facilities such as the number of elementary, junior high/equivalent is still limited and do not have a high school / equivalent, access to education facilities is still difficult, for example if it is to achieve educational facilities Senior high school then have to travel for an hour (BPS Regency Alor, 2013), this could be one cause of maternal education level is low in the region of Puskesmas Kokar higher than women with the level of higher education.

Statistical test results showed that the odds ratio lower maternal education level had 0.538 times risk for the occurrence of stunting than toddlers who have mothers with higher education levels. This is not in line with the research Rosha *et al.* (2012) that the level of education is a risk factor stunting where children whose mothers had a low education level of 1.56 times at risk of becoming stunted than infants who have mothers with higher education levels.

Table 6. Distribution of Family Income between Children's with Stunting and Normal Nutritional Status

Family Income	Amount						Total N	OR (CI 95%)
	Stunting		Normal		Total			
	n	%	n	%	N	%		
low	13	32,5	5	12,5	18	45,0	5.571 (1.42021.660)	
high	7	17,5	15	37,5	22	55,0		
Total	20	50,0	20	50,0	40	100,0		

Adequate intake related to the quality and quantity of food provided, it is influenced by economic status. The low economic status affects the inability to obtain sufficient food and quality due to low

purchasing power (Anugraheni and Kartasurya, 2012). Income levels in the low research sites affected by the job, most of the mothers are housewives and also laborers. Low-income levels affect purchasing power, so families with low-income will affect food supply with quality and quantity, so that will have an impact on the level of consumption of different and much lower than the family income level is high. It can also be seen in families with low-income levels based on the recall of food consumption varies less protein source which consumes only fish while for families with higher income levels are more varied, namely fish, eggs, milk, and tempe.

Odds Ratio Test statistics show that families with a lower income level had 5.571 times risk for the occurrence of stunting than families with higher income levels, it is in line with research Husein and Nuryanti (2013), that a family with a low-income level has 11.8 times higher risk his great stunting. The family economic status will affect the ability of family nutrition and the ability to access health care. Kids in families with low economic level have a greater risk of stunting due to poor nutrition fulfillment capabilities, increasing the risk of malnutrition. Family economic status expressed by categories of income per capita. Per capita income is the total income earned big family in the last month and then divided by the number of family members (Kusuma and Nuryanto, 2013).

Table 7. Distribution of Nutrition Knowledge Level of Capital between Children's with Stunting and Normal Nutritional Status

Nutrition Knowledge Level	Amount				Total		OR (CI 95%)
	Stunting		Normal		n	%	
	n	%	n	%			
Less	15	37,5	8	20,0	23	57,5	4.500 (1.16617.373)
Good	5	12,5	12	30,0	17	42,5	
Total	20	50,00	20	50,0	40	100,0	

Mother's knowledge about food nutrition is very influential on the development of infant nutritional, where the lack of knowledge of the mother would nutritious food, and do not understand how to provide the right food, can cause poor nutrition intake (Solihin, 2003).

Mother's nutrition knowledge is an important element in providing a balanced and nutritious food for family members. Mother's role is important in supporting efforts to address nutritional issues, particularly in terms of the nutritional intake of the family, ranging from the preparation of food, the selection of groceries, to the food menu.

Based on the results of the questionnaire that was given to the respondents, showed that on average respondents still do not understand the functioning of the food in the body and what good source of protein to help the growth of children, this shows that people are still difficult to obtain information about health and nutrition, this will impact on the level of consumption, it is known that there are many mothers who have low levels of knowledge about nutrition, so it can be seen also at the level of protein consumption toddlers in the study site is also low. Statistical test Odds Ratio showed that mothers with nutritional knowledge have less risk 4,500 times for the occurrence of stunting in infants than mothers who have a good knowledge, this is in line with research Nasikhah and Margawati (2012), that the knowledge of maternal nutrition is risk factor of stunting where the value of OR was 2.92 times. Provision of food at the household level is influenced by the knowledge, attitudes and behavior, especially mothers about nutrition and health. The way a person thinks or knowledgeable about the food and the view will be expressed in the form of the act of eating and food choices.

Table 8. Distribution of Infection Disease incidence between Children's with Stunting and Normal Nutritional Status

Infection disease	Amount				Total		OR (CI 95%)
	Stunting		Normal		N	%	
	n	%	n	%			
There Is	14	35,0	7	17,5	21	52,5	4.333 (1.15016.323)
There is no	6	15,0	13	32,5	19	47,5	
Total	20	50,0	20	50,0	40	100,0	

Besides nutritional problems caused by the lack of nutrient intake, it can also occur due to poor environmental sanitation and personal hygiene. Thus, it will lead to the emergence of infectious diseases, especially diarrhea and ARI (Acute Respiratory Infection). Both of these diseases are the two sequences disease that most often affect children under five in developing countries. Both of these infectious diseases are also associated with the occurrence of shock- growth and high rates of infant mortality. Nutrition issue in infants and children under five in Indonesia from infectious diseases are closely related to environmental sanitation, an infectious disease that is often suffered by toddlers generally include diarrhea, sore throat, ISPA (Hidayat and Fuada, 2011).

Infectious diseases are often associated with poor environmental sanitation and low PHBs, East Nusa Tenggara is a province with lowest access to improved sanitation facilities at 30.5 % and households with low PHBs that is 20 % which is below the national average

Table 9. Distribution between Children's Protein Consumption of Children's with Stunting and Normal Nutritional Status

Protein Consumption	Amount				Total		OR (CI 95%)
	Stunting		Normal		n	%	
	n	%	n	%			
Less	16	40,0	9	22,5	25	62,5	4.889 (1.19919.942)
good	4	10,0	11	27,5	15	37,5	
Total	20	50,00	20	50,0	40	100,0	

Food intake is often low quantity and quality. Good quality of food intake is an important component in the diet of children because it contains a source of macro nutrients (carbohydrates, fats, proteins) and micronutrients (zinc, calcium) all play a role in the growth of children. Protein intake is a risk factor for stunting. Children with a low protein intake have 11.8 times risk for stunting to occurs. Bone growth initiated by the synthesis of cartilage, which then undergoes ossification, cartilage synthesis requires sulfur in large quantities, because one of the main constituents is sulfur. The body gets most of the sulfur through the catabolism of amino acids, which are the necessary protein needed for the child's growth process (Al - Anshori and Nuryanto, 2013).

Based on the results of the recall 2x4 hours, it was found that there are still many children under five with low levels of protein intake, it is caused by poor nutrition knowledge of mothers due to the lack of information in health and nutrition, the incidence of infectious diseases which result in a decrease of appetite will leads to lack of food intake. Low level of family income also affects the purchasing power of families to purchase quality food for the child to meet the protein intake. The low protein consumption level of children will affect the protein function responsible for growth and development leads to stunting. Besides, protein consumption of control group more varies such as, fish, eggs, milk, tempe compared with the case group who consumed only single source of protein from fish.

Odds Ratio statistical tests showed that infants with low protein consumption levels have 4,889 times risk for the occurrence of stunting than a toddler with good protein consumption level. This is possible because although the intake of carbohydrates, fats, zinc and calcium is sufficient, but protein deficiency is more important in the incidence of stunting in children. It can occur due to the lack or poor quality protein containing essential amino acids.

Conclusions

Based on the results and discussion can be concluded that:

1. Children with mothers pregnancy age (1-2 years) with stunting in preterm birth is 30 % whereas the normal Children on the pre term birth risk factor of 20 % to 1,714 times .
2. Body length of birth Children (1-2 years) with a body length of stunting with short birth is 25 % whereas the normal Children born with a body length short of 30%, with a risk factor of 0.778 times.
3. Children Immunization history (1-2 years) with stunting were getting incomplete immunization is 30 % whereas the normal Children getting incomplete immunization is 10 % with a risk factor of 3.857 times.
4. Mother's education level of Stunting Children (1-2 years) with a low education level is 50 % and whereas normal Children with low maternal education level is 65 % with a risk factor of 0.538 times.
5. The level of family income of Stunting Children (1-2 years) families with a low-income level are 65 % whereas the normal Children families with a low-income level are 25 % with a risk factor of 5.571 times .
6. The level of mothers nutrition knowledge of Children (1-2 years) with stunting mother Children premises knowledge level of malnutrition is 75 % while in normal Children mother knowledge level is 40 % less by a factor of risk 4.5 times .
7. Children with The incidence of infectious diseases (1-2 years) is 52 % (ARI 20 %, diarrhea 20 %, Diarrhea ARI + 12.5 %) and no infection was 47.5 %. In Stunting Children with the incidence of infectious diseases is 70 % while in normal Children with the incidence of infectious diseases is 35 % with a risk factor of 4,333 times .
8. Children protein consumption level (1-2 years) , the level of deficit protein consumption is 25 %, the level of less protein consumption is 35 %. In stunting Children and consumption levels are 80 % less protein while normal Children with less protein consumption level is 45 % with a risk factor of 4,889 times.

References

- Al-Anshoridan Nuryanto. 2013. Faktor Resiko Kejadian *Stunting* pada Anak Usia 12-24 bulan. Undip, Semarang.
- Almatsier, S. 2001. *Prinsip Dasar Ilmu Gizi*. PT gramedia pustaka utama, Jakarta.
- Anugraheni dan Kartasurya. 2012. *Faktor Resiko Kejadian Stunting pada Anak Usia 12-36 bulan*. Undip, Semarang.
- Apriantono A. 2005. Revitalisasi Pertanian dan Pemantapan Ketahanan Pangan Rumah Tangga. Prosiding temu ilmiah. Kongres XIII PERSAGI: 11-20.
- Asrinah, Dkk. 2010. *Asuhan Kebidanan Masa Kehamilan*. Yogyakarta: Graha Ilmu.
- Azwar, A. 2004. Aspek Kesehatan dan Gizi dalam Ketahanan Pangan. Dalam Widya Karya Nasional Pangan dan Gizi VIII.17-19 mei 2003, Jakarta.
- Baliwati, dkk. 2004. *Pengantar Pangan dan Gizi*. Penebar swadaya, Jakarta.
- BPS. 2009. *Kabupaten Alor dalam Angka*. (ONLINE) diakses pada tanggal 31 Maret 2015.
- Citaningrum. 2012. *Kejadian Stunting pada Anak Berumur di bawah Lima Tahun di Provinsi Papua Barat*. FKUI.
- Depkes RI. 2002. *Pedoman Pemberantasan Infeksi Saluran Pernapasan Akut*. Kementerian Kesehatan Republik Indonesia.
- Depkes RI. 2005. *Diitjen bina kesehatan masyarakat .Buku kesehatan ibu dan anak*. Jakarta.
- Depkes RI. 2012. *Pedoman Pengendalian Infeksi Saluran Pernapasan Akut*. Kementerian Kesehatan Republik Indonesia.
- Depkes RI. 2013. *Laporan Hasil Riset Kesehatan Dasar Indonesia tahun 2013*. Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan RI, Jakarta.
- Dewi, A.B.F. Kurnia dkk. 2013. *Ilmu Gizi Untuk Praktisi Kesehatan*. Graha Ilmu, Yogyakarta.

- Dewi, Intan Candra. 2010. *Hubungan Pengetahuan, Sikap dan Persepsi Ibu dengan Pemenuhan Kecukupan Gizi Balita*. TESIS. Universitas Sebelas Maret Surakarta.
- Dinas Kesehatan Kabupaten Alor. 2014. *Laporan Pemantauan Status Gizi Balita melalui Operasi Timbang di Posyandu*.
- Dinas Kesehatan Kabupaten Alor. 2015. *Surveilens terpadu penyakit berbasis puskesmas*.
- Fajar, Ibnu dkk. 2009. *Statistika untuk Praktisi Kesehatan*. Graha Ilmu, Yogyakarta.
- Fitri. 2012. *Berat Lahir Sebagai Faktor Dominan Terjadinya Stunting pada Balita (12-59 bulan) di Sumatera*. TESIS. Fakultas Kesehatan Masyarakat Universitas Indonesia.
- Hanni, Ummi dkk. 2011. *Asuhan Kebidanan pada Masa Kehamilan Fisiologis*. Salemba Medika, Jakarta.
- Hidayat, TS dkk. 2011. *Hubungan Sanitasi Lingkungan, Morbiditas dan Status Gizi Balita di Indonesia*.
- Husaini MA, et.al. 2000. *Development Effects of Short-term Supplementary Feeding in Nutritionally at risk Indonesian Infant*. Am. J. Clin. Nutr, 45.
- Jahari A.B. 2002. *Penilaian Status Gizi dengan Antropometri*. Kongres Nasional dan temu ilmiah persatuan ahli gizi Indonesia XII. Jakarta.
- Khomson, Ali. 2003. *Pangan dan Gizi untuk Kesehatan*. PT Raja grafindo Pers.
- Kusharisupeni. 2002. *Peran Status Kelahiran terhadap Stunting pada Bayi*. Jurnal Kedokteran, 23: 73-80.
- Kusuma KE dan Nuryanto. 2013. *Faktor Resiko Kejadian Stunting pada Anak Usia 2-3 tahun*. Undip, Semarang.
- Meilyasari, Friska dan Isnawati, Muflihah. 2014. *Faktor Resiko Kejadian Stunting pada Balita Usia 12 Bulan di Desa Purwokerto Kecamatan Patebon Kabupaten Kendal*. Undip, Semarang.
- Nasikhah, Roudhotun. 2012. *Faktor Resiko Kejadian Stunting pada Balita Usia 24 – 36 Bulan di Kecamatan Semarang Timur*. Undip, Semarang.
- Notoatmodjo, Soekidjo. 2003. *Ilmu Kesehatan Masyarakat*. PT Rineka cipta, Jakarta.
- Notoatmodjo, Soekidjo. 2007. *Kesehatan Masyarakat Ilmu dan Seni*. PT Rineka cipta, Jakarta.
- Pudjiadji. 2005. *Ilmu Gizi Klinis Anak*. FKUI, Jakarta.
- Picauly, I dan Toy, SM. 2013. *Analisis Determinan dan Pengaruh Stunting terhadap Prestasi Belajar Anak Sekolah di Kupang dan Sumba Timur, NTT*. Jurnal Gizi dan Pangan, Maret 2013, 8(1): 55-62.
- Rahayu, L.S dan Sofyaningsih, M. 2011. *Pengaruh BBLR (Berat Badan Lahir Rendah) dan Pemberian ASI Eksklusif terhadap Perubahan Status Stunting pada balita di Kota dan Kabupaten Tangerang Provinsi Banten*. FK Universitas Muhammadiyah, Banten.
- Rahardjo, Marmidan Kuku. 2012. *Asuhan Neonatus, Bayi, Balita dan Anak Prasekolah*. Pustaka Pelajar, Yogyakarta.
- Rencana Aksi Daerah-Pangandan Gizi. 2012. *Provinsi Nusa Tenggara Timur*.
- Rosha, Bunga Ch. 2012. *Analisis Determinan Stunting Anak 0-23 Bulan pada Daerah Miskin di Jawa Tengah dan Jawa Timur*. *Penel Gizi Makan* 2012, 35(1): 34-41.
- Santoso, S. dan Ranti L. A. 2005. *Kesehatan dan Gizi*. PT. Rineka Cipta: Jakarta.
- Sawadogo, et.al. 2006. *An Infant and Child Feeding Index Is Associated with the Nutritional Status of 6-to 23-Month-Old Children in Rural Burkina Faso. Community and International Nutrition*. [Online]. 136., p. 656—663. <http://jn.nutrition.org/content/136/3/656.full.pdf> [diakses 18 April 2015].
- Soegianto, S, 2002. *Ilmu Penyakit Anak—Diagnosa dan Penatalaksanaan*. Salemba Media, Jakarta.
- Soekirman. 2000. *Ilmu Gizi dan Aplikasinya untuk Keluarga dan Masyarakat*. Direktorat Jenderal Pendidikan Tinggi Depdiknas RI, Jakarta.
- Solihin. 2003. *Ilmu Gizi Klinis pada Anak*. Balai Penerbit FKUI, Jakarta.
- Suhardjo. 2003. *Berbagai cara Pendidikan Gizi*. PT Rineka cipta. Jakarta.
- Suharjo. 2003. *Pemberian Makan pada Bayi dan Anak*. Kanisius, Yogyakarta.
- Suharyono, 1991. *Diare Akut Klinik dan Laboratorium*. Jakarta: Rineka Cipta.
- Suharyono, dkk, 1998. *Gastrologi Anak Praktis*, FK UI, Gaya baru, Jakarta.
- Suirakoa, I Putu. 2011. *Perbedaan Konsumsi Energi, Protein, Vitamin A, dan Frekuensi Sakit karena Infeksi pada Anak Balita Status Gizi Pendek (Stunted) dan Normal di Wilayah Kerja Puskesmas Karangasem I*. *Jurnal Ilmu Gizi*. Volume 2 Nomor 1. Februari 2011: 74 – 82.
- Sulistyawati, A. 2014. *Asuhan Kebidanan Pada Masa Kehamilan*. Salemba medika, Jakarta.
- Sunarti, 2012. *Pro Kontra Imunisasi*. Hanggar Kreator, Yogyakarta.
- Supariasa, dkk. 2001. *Penilaian status Gizi*. EGC, Jakarta.
- Triton, PB. 2006. *Mengasuh dan Perkembangan Balita*. Yogyakarta.
- WHO. 2007. *Infection prevention and control of epidemic-and pandemic-prone acute respiratory diseases in healthcare*. WHO Interim Guidelines.

Lipase from *Mucor miehei* as Biocatalysts for The Hydrolysis – Esterification Sequential Reaction of Coconut Oil in Natural Flavor Production

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Abstract

Healthy flavor additives from natural materials are increasingly in demand due to their use in food industry. Biocatalyst can be used in the food industry. Biocatalyst can be used to make a natural flavor product that is environmentally friendly, including mild reaction condition. Lipase from *Mucor miehei* biocatalyst is widely used in the food industry, especially for fats and oil processing. The purpose of this study is to perform the enzymatic reaction of lipase from *Mucor miehei* in sequential hydrolysis followed by esterification of coconut oil with citronellol to produce natural flavoring. Lipase was used as a biocatalyst in the hydrolysis reaction of coconut oil to produce medium chain of free fatty acid (FFA) with activity of 4.94 U/ml. Coconut oil with high content of lauric acid was used as a substrate for the further enzymatic reaction with citronellol to produce natural flavor. The hydrolysis was carried out by using oil to water ratio, produced FFA and glycerol. Furthermore, reaction between FFA and citronellol was carried out in various molar ratio and reaction time. The conversion of 51.13% as natural fruity flavor, citronellylauric, was achieved at 40°C for 10 h. The concentration FFA and citronellol were analyzed using titration method and GC-FID.

Keywords: citronellol; coconut oil; esterification; hydrolysis; lipase; *Mucor miehei*

Introduction

Lipase has increasingly been used in paper industry, detergents, oils and fats, pharmacy, cosmetics, and food industry. Lipase can be extracted from plants, animals and microorganism, including *Candida rugosa*, *Candida antarctica*, *Thermomyces lanuginosus*, *Mucor miehei*, *Pseudomonas mendocina*, and *Pseudomonas Alcaligenes* (Sharma *et al.*, 2013). Lipase (triacylglycerol acylhydrolases, E.C 3.1.1) from various microbial source are widely used in industrial application to assist the hydrolysis, esterification, and transesterification reaction. Lipase has ability to catalyze the hydrolysis of triacylglycerol into glycerol and free fatty acid. Lipase promotes the esters bonds cleavage to produce glycerol and fatty acids in the hydrolytic reaction. The flavor synthesis using lipase was carried through the esterification process between alcohol and carboxylic acid. Paludo (2015) reported that ethyl butyrate was obtained from esterification between ethanol and butyric acid in n-hexane as the solvent. Patel (2015) reported that flavor synthesise using caprilic acid and ethanol with various alkanes solvent (Patel *et al.*, 2015). While Ferraz (2015) reported that fatty acid can be synthesized using lipase from *Pennicillium crustosum* to assist the reaction between geraniol and propionic acid (Ferraz *et al.*, 2015). Nowadays, mostly people concern about healthy food especially consumption of flavor derived from natural materials as the source for the synthesis of alcohol and acid ester with solvent-free system. In this study, acid products can be obtained from natural materials, such as coconut oil containing 92% triglycerides. Hydrolysis involves reaction between acid and water into Free Fatty Acid (FFA). Ester flavor can be derived through esterification reaction between FFA and alcohol, such as isolated citronellol. The purpose of the study is to obtain new natural flavor using coconut oil as the substrate through sequential process containing of two reaction, hydrolysis and esterification by *Mucor miehei* crude lipase.

Materials and Methods

Materials

Coconut oil was purchased from local market (Sewon, Bantul, Indonesia) which has commercial name Laitco, was used as the substrate in hydrolysis reaction to produced FFA. Stock culture of *M. miehei* was obtained and identified by Biochemical Technology Laboratory, Chemical Engineering Department, Institut Teknologi Sepuluh Nopember, Indonesia. Citronellol was purchased from Sigma-Aldrich, China.

Chemicals

Potato Dextrose Agar (PDA), Tween-80, KH₂PO₄, FeSO₄.7H₂O, olive oil, phosphoric buffer solution gum Arabic solution, NaOH, peptone, acetone, ethanol and all other chemicals were purchased from local market.

Solid state Fermentation and Extraction of Lipase

The stock culture of *Mucor miehei* was obtained from Biochemical Technology Laboratory of Institut Teknologi Sepuluh Nopember used as the inoculum. The culture was maintained on Potato Dextrose Agar (PDA) and was incubated at 37°C for 7 days. Sub-culturing was carried out once in 3 weeks and the culture was stored at 40°C. The *Mucor miehei* strain was cultivated in medium containing peptone, KH₂PO₄, FeSO₄.7H₂O, olive oil, palm oil, dried coconut grout, and water. The medium was sterilized at 121°C for 15 min. The cells were maintained in incubator at 37°C for 5 days. The crude lipase was recovered by extraction method with buffer phosphate in incubator shaker at 150 rpm, 37°C, for 135 minutes. Filtration was conducted to obtain the supernatant (Moentamaria, 2015).

Crude lipase activity assay

Lipase activity was determined by titration method using olive oil emulsion as the substrate. The consumption of acid was determined by titration with NaOH 0.05 mol L⁻¹. Oil emulsion containing 25 mL of olive oil and 7% Arabic gum solution. Then, 5 mL of oil emulsion was added into 2 mL of 0,1 M phosphoric buffer solution (pH 7,0) and 1 mL of lipase was incubated with shaking at 37 °C for 30 minutes. One unit of enzymatic activity (U) was defined as the amount of enzyme necessary to consume 1µmol of fatty acid per minute, under the described assay conditions (Ferraz et al., 2015).

Free fatty acid (FFA) determination

FFA content in oil usually determined by titration with a standard alkaline solution, such as NaOH with specific concentration or normality (N). FFA concentration in coconut oil and product of hydrolysis was defined the as lauric acid, can be expressed using modified equation which was given by Rukunudin (1998) as follow:

$$\text{FFAml} \times \text{sample weight} \times (1)$$

Hydrolysis and esterification sequential reaction

The hydrolysis reactions in solvent-free system was carried out in erlenmeyer which contains 10 g of coconut oil with ratio oil-water ratio (wt/wt) of 1:0.6 ; 1:1; 1:3, 1:5 (Khaskheli et al., 2015, Li et al.,

2015, Sharma *et al.*, 2013). The reaction was maintained at 40°C in various reaction times of 5, 10, 15, and 20 h. The FFA as the top layer product was separated using separation tunnel. The acid value, saponification value, and % FFA of the product were determined. Furthermore, the esterification reaction was carried out in similar erlenmeyer which contains FFA with citronellol in molar ratio of 1:1. Both of hydrolysis and esterification sequential reactions used crude lipase of 4.94 U/ml. The remaining citronellol was confirmed using GC-FID analysis (HP 5890), further the conversion of the substrate can be determined.

Result and discussion

Characterization of coconut oil as substrate

Coconut oil mostly contained medium chain triglycerides (MCT). The physiochemical properties of coconut oil were determined as shown in Table 1.

Properties	Values
Density	0.880 g/cm ³
Acid value	0.5585 mg KOH/g of oil
Saponification value	260,716 mg KOH/ g of oil
FFA	0.2 %

Based on the results, coconut oil which used in this study is included in edible oil grade. Furthermore, the oil can be used as the substrate of natural flavour production. GC-MS analysis results confirmed that the coconut oil contains fatty acid as lauric acid (C12) 57.78% and myristic acid (C14) 18.78%.

Identification of microorganism growth condition

Mucor miehei which grown in PDA medium has the mycelium with glaucous color. The incubation has been optimized at 37°C. Figure 1 shows the morphological characterization of *Mucor miehei* using optical microscopy analysis.

Hydrolysis reaction of coconut oil

Coconut oil contains triglycerides of 92% as saturated fatty acids. FFA can be calculated as lauric acid with the maximum value of 0.3%. Triglycerides consist of 70% lower chain saturated fatty acids has been known as medium chain fatty acids (MCFAs). Coconut oil also called as lauric oil due to the composition of the oil consists mainly 48% of lauric acid (12:0) (Sun, 2012; Krishna, 2010; Guston, 2002). Figure 2 shows the mechanism of hydrolysis-esterification sequential reaction. First path shows that reaction between triglyceride and water to into FFA and glycerol. Hydrolysis was carried out in various oil-water ratio (wt./wt.) of 1:0,6; 1: 1; 1:3; 1:5 with concentration of crude lipase of 4.94 U/mL. Previous studies reported that the FFA contains 4.8 g including in the initial FFA (Sun, 2012; Krishna, 2010; Guston, 2002). The amount of water addition plays an important role in hydrolysis reaction, especially for enzymatic reaction in organic medium.

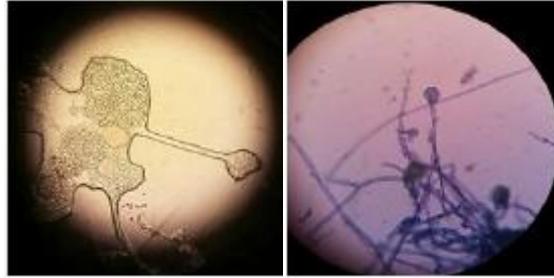


Figure 1. (a) *Mucor miehei* with magnification of 100x in microscope, (b) *Mucor miehei* staining

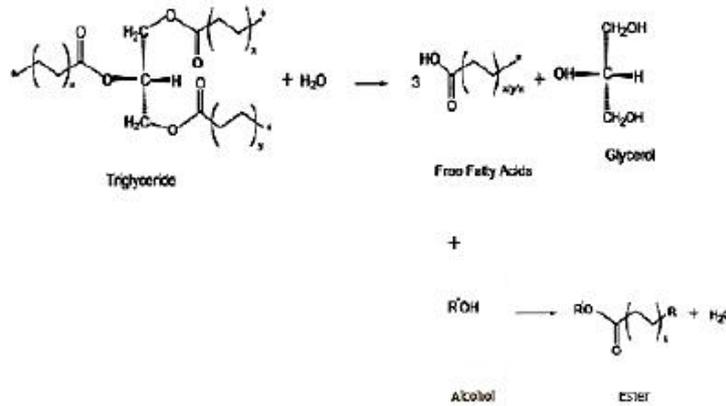


Figure 2. Hydrolysis-esterification sequential reaction mechanism. (modified from Gupta *et al.*, 2013)

The mixture of oil-water system consisting of two layers, and the region between both of the layers called as the interface. Lipases are only activated in this region. Water excess provides the enzyme surface in the water layer become thicker due to the diffusivity of enzyme activated site in the substrate and product.

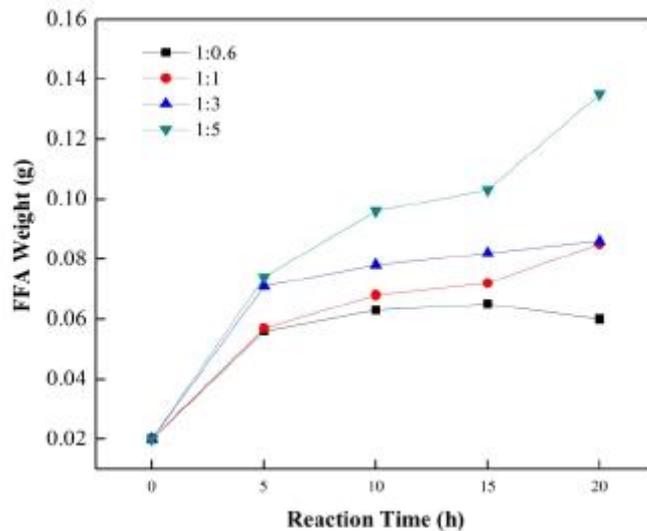


Figure 3. Effect of oil-water ratio (wt./wt.) on hydrolysis of coconut oil

As shown in Figure 3, the FFA amount of 0.155 g was achieved at oil-water ratio of 1:5 for 20 h. The increasing of the FFA reached 5.77 times from the initial FFA due to the role of lipase to provide the

(RCOO -) bond breakage of the triglycerides and hydrogen ion. Further, the obtained FFA was used as the substrate for esterification reaction. The reaction involves the FFA and isolated citronellol using lipase as the biocatalysts in the solvent-free system. In this study, solvent-free system was applied due to the edible grade of the natural flavour product.

Esterification reaction of coconut oil

In this study, we have developed the natural flavour ester synthesis by *Mucor miehei* lipase as the biocatalysts. Lipase assists the reaction between FFA and isolated citronellol. The second path of Figure 2 shows the mechanism of esterification reaction. The reaction was maintained in various reaction time of 5, 10, 15 and 20 h. The conversion of 51.15% was achieved at 40°C for 10 h. Abbas (2003) reported that ethyl caproate, methyl caproate and allyl caproate ester have been synthesized successfully using *Mucor sp* lipases. At the same reaction time of 24 h, the conversion of ethyl caproate, methyl caproate and allyl caproate ester were 99, 97, and 91%, respectively. The lipases selectivity to the desired ester product depend on the length of alcohol and acid chains. Coconut oil substrate takes longer reaction time due to the FFA content as lauric acid which has C12 chain. Lauric acid have longer carbon chain than caproic acid, thus the conversion is low as well as the longer carbon chain. For the synthesis of ethyl caproate, the highest conversion was achieved of 99% due to the utilization of ethanol as the alcohol substrate. Citronellol has longer carbon chain than ethanol, thus the conversion quite low. The high conversion can be achieved with longer reaction time. The amount of lipase also plays important role in the reaction due to the sufficiently of enzyme active site which has ability to bind with the substrate.

Conclusion

Natural flavor ester from coconut oil is *Mucor miehei* lipase can be developed. Enzymatic method was carried out in hydrolysis-esterification sequential reaction. In the hydrolysis reaction, crude lipase produced from *Mucor miehei* in amount of 4.94 U / ml was used to synthesize coconut oil and water into FFA and glycerol. The result of hydrolysis reaction was obtained at oil-water ratio of 1:5 for 20 h, which obtains FFA increasing of 5.77 times. The FFA which produced by hydrolysis reaction and citronellol were used as the substrate of esterification resulting in a conversion of 51.13%. The reaction was maintained in the free solvent system. The product has been confirmed as the ester-based citronellyl laurate.

References

- Abbas, H. and Comeau, L. "Aroma synthesis by immobilized lipase from *Mucor sp.*", *Enzyme and Microbial Technology*. Vol. 32 No. 5, p. 589-595.
- Ferraz, L. I. R., Possebom, G., Valandro Alvez, E., Luiz Cansian, R., Paroul, N., De Oliveira, D. & Treichel, H. 2015. "Application of Home-Made Lipase in The Production of Geranyl Propionate by Esterification of Geraniol and Propionic Acid in Solvent Free" System. *Biocatalysis and Agricultural Biotechnology*, 4, p. 44.
- Gunstone, J. D. 2002. "Vegetable oils in Food Technology: Composition, Properties and Uses Blackwell Publishing-CRC Press (Canada)
- Gupta, S, Bhattacharya, A, Murty, C.N. 2013. "Tune to immobilize lipases on polymer membranes: Techniques, factors and prospects". *Biocatalysis and Agricultural Biotechnology*, vol 2, p 171-190
- Khaskheli, A. A., Talpur, F. N., Ashraf, M. A., Cebeci, A., Jawaid, S. & Afridi, H. I. 2015. "Monitoring The *Rhizopus Oryzae* Lipase Catalyzed Hydrolysis of Castor Oil by ATR-FTIR Spectroscopy". *Journal of Molecular Catalysis B: Enzymatic*, 113, p. 56.
- Krishna, G.A.G., Gaurav, R., Ajit, S.B. et al. (1). "Coconut coconut oil: chemistry, production and its applications -a review *Indian Coconut Journal*", p 15-27.

- Li, D., Qin, X., Wang, J., Yang, B., Wang, W., Huang, W. & Wang, Y. 2015. "Hydrolysis of Soybean Oil to Produce Diacylglycerol by A Lipase from *Rhizopus Oryzae*". *Journal of Molecular Catalysis B: Enzymatic*, 115, p. 43.
- Moentamaria, D., Irfan, Z. and Udjiana, S. (15). "Immobilized Lipase Technology for Making Natural Flavor", *International Journal of Engineering Research and Development*, Vol. 11, p. 30–35
- Paludo, N., Aves J.S, Altman C, Ayub Marco, A.Z, Lafuente, RF, Rafael C, Rodrigues. 2015. " The combined Use of Ultrasound and Molecular Sieves Improves The Synthesis os Ethyl Butyrate Catalyzed by Immobilized *Thermomyces lanuginosus* Lipase". *Ultrasonic Sonochemistry*, Vol 22, p 89-94
- Patel, V., Gajera, H., Gupta, A., Manocha, L. & Madamwar, D. 2015. "Synthesis of Ethyl Caprylate in Organic Media Using *Candida Rugosa* Lipase Immobilized on Exfoliated Graphene Oxide: Process Parameters and Reusability Studies". *Biochemical Engineering Journal*, 95, p. 62.
- Rukunudin, I.H., White, P.J., Bern, C.J. and Bailey, T.B. 1998. "A Modified Method for Determining Free Fatty Acids from Small Soybean Oil Sample Sizes". *JAOCS*, Vol. 74 No. 5, p. 564.
- Sharma, A., Chaurasia, S. P. & Dalai, A. K. 2013. "Enzymatic Hydrolysis of Cod Liver Oil for The Fatty Acids Production. *Catalysis Today*", 207, p. 93.
- Sun, J., Yu, B., Curran, P. and Liu, S.-Q. (1). "Lipase-catalysed transesterification of coconut oil with fusel alcohols in a solvent-free system". *Food Chemistry*, Vol. 14 No.1,p. 89–94.

Effect of Unsaponifiable Fraction from Palm Fatty Acid Distillate on LDL/HDL Ratio of Hypercholesterolemia Rats

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Abstract

Physical refining of palm oil by deodorization produces palm fatty acid distillate (PFAD) that contains some bioactive compounds such as vitamin E, phytosterol, and squalene. These bioactive compounds were accumulated in unsaponifiable fraction (USF). This study aims to identify the effects of USF against LDL/ HDL ratio which tested to hypercholesterolemic rats using *in vivo* method. The rats were divided into 4 groups that administered by USF of 0, 200, 500, 1000 mg/kg bw/day. As comparison, one group of rats was fed by commercial squalene supplement of 90 mg/kg bw/day. One group of normal rats was used as a control. The design applied in the study is True Experimental Design: Post Test Only Control Group Design. While the selection of the objects of the study is for grouping and treating them applying CRD (Complete Randomized Design). The measurement of the total cholesterol uses CHOD-PAP method. LDL analysis applies Friedward method. The data result of the lipid profile analysis were performed using SPSS 16 software for statistical test analysis of variance (ANOVA) and were continued using LSD comparison test (Least Significant Difference) or DMRT (Duncan Multiple Range Test) with a confidence interval of 5%. The result of the study showed that by giving USF shows an apparent effect ($\alpha=0.05$) to the average of change in the level LDL/ HDL ratio. The best hypocholesterolemic effect was found on USF with dose of 1000 mg/kg bw/ day.

Keywords: hypercholesterolaemia; LDL/HDL ratio; palm fatty acid distillate; unsaponifiable fraction

Introduction

Oil palm as the producer of palm oil and palm kernel is one of the excellent crop plantations to be major non-petrol foreign exchange source. One stage of the manufacturing process of palm oil is the process of steam distillation in the purification process. From this process the byproduct obtained is palm fatty acid distillate (PFAD). Byproducts of this deodorization process contains 10000 ppm vitamin A. PFAD advantages as a source of vitamin E is mostly in the form of vitamin E tocotrienols (70%) and the rest is tocopherol (30%) (Musalmah *et al.*, 2005)

Unsaponifiable fraction (USF) is the result of saponification process which still contains several multicomponent bioactive compounds. According to Estiasih (2014), bioactive compounds found in the USF is vitamin E, phytosterols and squalene. Vitamin E is a vitamin that dissolve well in fat and protects the body from free radicals. Vitamin E acts to prevent the oxidation of LDL-cholesterol and plaque formation. Vitamin E is a fat peroxide chain breaker on the membrane and Low Density Lipoprotein (LDL). Vitamin E also strengthens the walls of blood capillaries and prevent blood cell damage due to toxins (Papas, 2008).

Plant sterols, or phytosterols have anti-atherogenic properties. Phytosterol consumption in large quantities may interfere with the absorption of cholesterol so that it will increase its excretion (Hui 1996). Several studies on the effects of squalene to cholesterol reduction has been done. The available evidence suggests that the human body some squalene containing food is absorbed and converted into cholesterol

but this increase of cholesterol synthesis is not associated with elevated levels of cholesterol in the blood but instead in feces containing high cholesterol (Standberg, 1990).

Based on the potential benefits, the availability of treatment and the potential development of byproducts PAFD from previous research, it is necessary to carry further testing on the effects hypocholesterolemic of the USF from PAFD which was tested on Wistar rats (*Rattus norvegicus*) in vivo.

Materials and Methods

Materials

USF (table 1) (Estiasih, 2014), wistar male rats with body weight of 150-200 g and age of 8-12 weeks, AIN-93M standard diet with slight modification, atherogenic diets, commercial squalene supplement, and serum blood lipid profile analysis kit (Diasys).

Table 1. The concentration of bioactive compounds of USF

Bioactive Compounds	Ppm	%
Vitamin E	19.600	1,96
Phytosterols	5.500	0,55
Squalene	323.000	32,3

Bioassay of Cholesterol Lowering Effect of USF

The protocol of bioassay had been approved for ethical clearance No. 190-KEP-UB from Animal Care and Use Committee, Brawijaya University. As many as 24 Wistar male rats were used in this study. Each rat was caged individually and adapted to laboratory environment for 7 days. During adaptation, rats were fed by standard diet of AIN-93 M with slight modification (Table 2) (Reeves, 1993). Rats were divided into 6 groups and each group comprised of 4 rats. One group was normal rats without USF treatment. Other four groups were hypercholesterolaemia rats treated by USF 0, 200, 500, 100 mg/kg bw/day and one group was hypercholesterolaemia rats treated by commercial squalene at dose of 90 mg/kg bw/day. All groups of hypercholesterolaemia rats were fed by atherogenic diet (Table 2).

Table 2. Composition of modified AIN-93M and atherogenic diet

Component	Composition (g/kg diet)	
	Modified AIN-93M [1]	Atherogenic Diet
Maize starch	620, 692	620, 692
Casein	140	116,528
Sucrose	100	100
Soybean oil	40	40
CMC	50	50
AIN mineral mix	35	35
AIN vitamin mix	10	10
L-cystin	1,8	1,8
Choline bitartrate	2,5	2,5
TBHQ	-	-
Cholic acid	-	2
Cholesterol crystal	-	20
Tallow	-	185

Rats were treated by USF and squalene administration for 4 weeks, and body weight of each rat was measured daily as well as the quantity of feed consumption. Treatment by USF administration was conducted after total cholesterol level of rats reached >130 mg/dL. Lipid profile was analyzed at week 0, 1, 2, 3, and 4 to measure LDL cholesterol, and total cholesterol of serum blood. Blood was taken by *retro orbital plexus* method and centrifuged at 4000 rpm for 10 min to separate blood serum. Blood serum total cholesterol and HDL cholesterol levels were measured by method of CHOD-PAP, GPO-PAP.

Meanwhile, blood serum LDL level was calculated based on the data of total cholesterol and HDL cholesterol.

Statistical analysis

This study was designed using in-vivo experimental methods. The design of experiment used is True Experimental Design: Post Test Only Control Group Design. While the selection of the object of research for grouping and treatment selection is using a CRD (completely randomized design) with 5 treatment groups and one control group. Data from the lipid profile analysis (total cholesterol, LDL cholesterol, HDL cholesterol) is performed using a statistical test analysis of variance (ANOVA) followed by Duncan comparison test with a confidence interval of 5%.

Result and discussion

The Effect of USF on Blood Serum HDL Cholesterol Level

The effect of USF doses on blood serum HDL cholesterol level was shown in Figure 1.

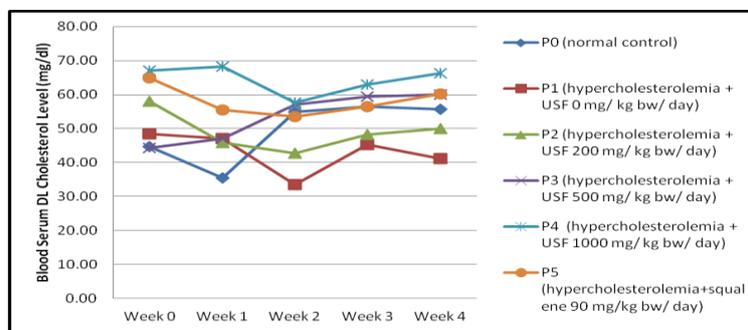


Figure 1. Blood serum HDL cholesterol level after USF administration at various dose

The mean levels of HDL in mice are shown in Figure 1 and it shows that the value of HDL in each treatment group declined in the first week but increased in the following week until the end of experimental period. Results of ANOVA indicates that the factor of the treatment is not significantly affecting ($p = 0.574$) increasing levels of HDL in blood serum ($\alpha = 0.05$), however, HDL levels of all groups of mice appears to be rising. Normal HDL levels in mice is > 50 mg / dl. Further test results of mean accumulation in HDL levels is presented in Table 3.

Table 3. Mean accumulation of HDL level

Treatment Group	HDL Level (mg/dl)		
	Week 1	Week 4	Mean Difference (%)
P0 (negative control)	35,47	55,78	19,88 a
P1 (positive control)	47,01	41,19	17,53 a
P2 (HC+USF 200 mg/ kg bw)	45,78	50,01	15,86 a
P3 (HC+USF 500 mg/ kg bw)	46,98	60,03	26,32 a
P4 (HC+USF 1000 mg/kg bw)	68,24	66,23	1,36 a
P5 (HC+squalene 18 mg/ 200 g bw)	55,57	60,17	7,80 a

*note : (+) increase (-) decrease

* Figures followed by different letters indicate Duncan test result is significantly different at 5%

Table 3 shows that increasing HDL serum after the conditions of hypercholesterolemia (week 1) until the end of the treatment period (week 4) respectively from the largest, the treatment group of

hypercholesterolemia mice with administration of 500 mg USF / kg (P3) (26.32%), negative control treatment group (P0) (19.88%), positive control treatment group (P1) (17.53%), hypercholesterolemia mice with administration of 200 mg USF / kg (P2) (15,86%), hypercholesterolemia mice with administration of 18 mg USF supplemental squalene / 200g BB (P5) (7.8%) and hypercholesterolemia mice with administration of 1000mg USF / kg (P4) (1.36%).

HDL (High Density Lipoprotein) is known as good fats. HDL carries free cholesterol from peripheral tissues to the liver. Cholesterol is converted to ester cholesterol and is partially transferred to VLDL through the help of the enzyme CETP and also returned to the liver by IDL and LDL. Hearts utilize cholesterol by conversion into bile salts or through direct into bile (Mayes, 2003).

Decreased HDL performed in this study is using high-cholesterol feed induced to experimental animals. This process does not lead to significant decreased levels of HDL to each group of experimental animals resulting in increased HDL levels by the end of the study became insignificant.

According to Pastore (2003), a high-carbohydrates diet and saturated fatty acid diet would cause a decrease in HDL cholesterol. More specifically, this research mentions that a high-carbohydrates diet and polyunsaturated fatty acid diet would cause a decrease in Apolipoprotein A-1, which is the main constituent of HDL.

The Effect of USF on Blood Serum HDL Cholesterol Level

The effect of USF doses on blood serum LDL cholesterol level was shown in Figure 2.

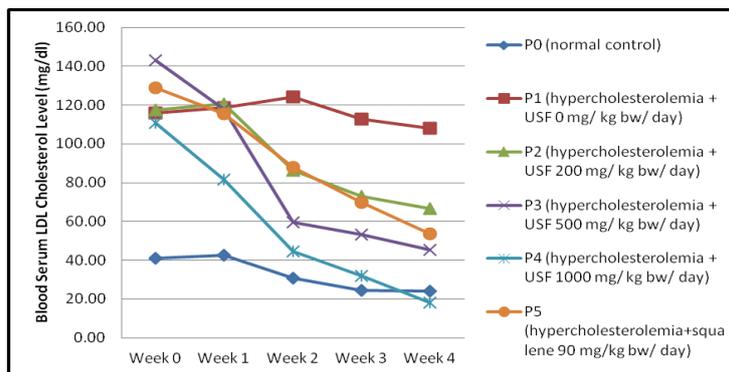


Figure 2. Blood serum HDL cholesterol level after USF administration at various dose

Figure 2 shows that the administration of treatment in all groups except for the positive control can reduce LDL levels to normal. According Papas (2008), LDL cholesterol levels of the mice under normal conditions would range between 10-25 mg / dl. Based on the graph of LDL analysis result there is a difference of LDL level from each treatment group in every week. In the group of normal mice (P0) and hypercholesterolemia mice (P1) the difference of LDL levels every week is not very substantial. Whereas in the group of hypercholesterolemia mice with administration of 200 mg USF / kg administration (P2), group of hypercholesterolemia mice with by administration of 500 mg USF / kg (P3), group of hypercholesterolemia mice with administration of 1000 mg USF / kg (P4) and group of hypercholesterolemia mice with squalene supplementation of 90 mg / kg (P5) depletion of LDL levels occurs every week. Figure 2 also shows that 4 weeks treatment can reduce LDL serum for all treatment

groups except normal control and a positive control that almost did not show a significant difference. This is due to little addition of palm oil to the normal control and positive control which is 0.5 ml, hence there are no factors that can lower blood LDL levels in mice. Results of analysis of variance stated that treatment factors have a significant effect ($p = 0.000$) to decrease serum cholesterol levels ($\alpha = 0.05$). Further test results of mean accumulation of cholesterol is shown in Table 4.

Table 4. Mean accumulation of LDL Level

Treatment Group	LDL Level (mg/dl)		
	Week 1	Week 4	Mean Differene (%)
P0 (negative control)	42,57	24,13	-69,97 ab
P1 (positive control)	118,53	108,05	-7,17 a
P2 (HC+USF 200 mg/ kg bw)	120,51	66,54	-76,79 ab
P3 (HC+USF 500 mg/ kg bw)	117,84	45,42	-214,84 b
P4 (HC+USF 1000 mg/kg bw)	81,70	18,10	-513,19 c
P5 (HC+squalene 18 mg/ 200 g bw)	115,43	53,64	-140,45 ab

*note : (+) increase (-) decrease

* Figures followed by different letters indicate Duncan test result is significantly different at 5%

Table 4 shows that the reduction in serum LDL levels after the conditions of hypercholesterolemia (week 1) until the end of the treatment period (week 4) respectively from the largest, treatment group of hypercholesterolemia mice with administration of 1000mg USF / kg (P4) (513.19%), hypercholesterolemia mice with administration of 500mg USF / kg(P3) (214.84%), hypercholesterolemia rats with squalene supplementation of 90 mg / kg (P5) (140.45%) and hypercholesterolemia mice with administration of 200mg USF / kg (P2) (76.79%). All treatment groups except normal and positive control group showed a decrease in LDL levels, but only the treatment group P4 which is hypercholesterolemia mice with administration of 1000mg USF / kg whose LDL values are considered fall within normal limits.

A significant decrease in LDL level is suspected because of the amount of vitamin E in the product USF. Previous clinical studies have been conducted by Hanum *et al.* (2008) using supplemental vitamin E. In this study tocopherol and tocotrienol supplementation in wheat were randomly assigned to healthy subjects for 2 consecutive months. The goal was to determine the bioavailability of vitamin E and its effect on the arteries in healthy subjects. An increase or decrease in the arteries' plaque is the reference prediction of cardiovascular events. The results of this study showed that tocotrienol supplementation given with bioavailability for 6 hours have a tendency to decrease plaque formation in all treatment group (Hanum *et al.*, 2008).

Zureik (2004) in his study reports that the effects found after supplementation with a daily dose of anti-oxidants (120 mg vitamin C, vitamin E 30 mg, 6 mg of b-carotene, 100 mg of selenium and zinc 20 mg) is no thickening of artery intima / plaque and this supplementation did not cause damage to the arteries. Daniel (1991) has also proved that vitamin E supplementation of 18 mg, 42 mg and 240 mg can lower total cholesterol in the blood serum of between 5% - 35.9%.

Vitamin E contained in USF of PAFD, carried by LDL thus protects it from oxidative modification, which is incorporated into other components of the vascular system, including endothelial cells, smooth muscle cells, platelets and immune cells, and has been shown to modulate various inflammatory processes involved in atherogenesis. Vitamin E inhibits the production of proinflammatory cytokines by endothelial cells and immune cells (Cannon *et al.*, 1991, Devaraj *et al.*, 1996, Wu *et al.* 1999). Vitamin E suppress the expression of adhesion molecules on endothelial cells and ligands on monocytes and reduce

their interactions, which is the main process that is important in the formation of fat initiation and atherogenesis.

The Effect of USF on Ratio of LDL/ HDL Cholesterol Level

Based on the examination of various lipid profile, a sign of risk factors for coronary heart disease (CHD) examination is now has been developed. Signs of CHD risk factors include: total cholesterol, low density lipoprotein (LDL) cholesterol (LDL-c), high density lipoprotein (HDL) cholesterol (HDL-c), the ratio of LDL / HDL-c, triglycerides, high sensitive C reactive protein (hsCRP), Lipoprotein (a) [Lp (a)], Homocysteinemia and small dense (sd) LDL (Kreisberg, 2003).

Cholesterol levels alone do not accurately predict the risk of CHD, since it is a total value of different types of cholesterol namely LDL-c, HDL-c, very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL). Thus, the determination of treatment delivery is based on the levels of HDL-c, LDL-c and triglycerides (Greenberg, 2001). The discovery of CHD risk sign examination means that early diagnosis has been developed and treatment can be performed more quickly and precisely.

According Silalahi (2002), Changes in the ratio of LDL cholesterol / HDL is the most predictive value in the incidence of atherosclerosis and coronary heart disease, compared with only high levels of total cholesterol and LDL cholesterol. Results of analysis of variance stated that treatment factors have a significant effect ($p = 0.04$) towards a decrease in blood serum cholesterol levels ($\alpha = 0.05$). Further test results mean the accumulation of cholesterol can be seen in Table 5.

Table 5. Mean Accumulation of LDL/HDL Ratio

Treatment Group	LDL/HDL Ratio		
	Week 1	Week 4	Mean Difference (%)
P0 (negative control)	0,92	0,43	-112,13 a
P1 (positive control)	2,39	2,62	8,81 a
P2 (HC+USF 200 mg/ kg bw)	2,03	1,33	-52,60 a
P3 (HC+USF 500 mg/ kg bw)	3,23	0,76	-327,31 ab
P4 (HC+USF 1000 mg/kg bw)	1,65	0,27	-504,91 b
P5 (HC+squalene 18 mg/ 200 g bw)	1,99	0,89	-123,07 a

*note : (+) increase (-) decrease

* Figures followed by different letters indicate Duncan test result is significantly different at 5%

Table 5 shows that the ratio of LDL / HDL were significantly different in treatment contained administration of 1000mg USF / kg (P4). P4 treatment gives substantial difference to P0, P1, P2, P3 and P5 treatment. Among various lipid parameters, the ratio of LDL / HDL cholesterol is found to be a powerful factor that is associated with the incidence of atherosclerosis in the blood vessels (Yukihiko, 2012).

Goldstein in his research showed a correlation between elevated serum lipid levels with the incidence of coronary heart disease (CHD) and atherosclerosis. It is concluded that the most predictive relationship is the ratio of LDL / HDL. This association can be explained by the role of the proposed LDL transports cholesterol to the tissues and the role of HDL as cholesterol scavenger. The lower the LDL / HDL, the risk of the disease due to high cholesterol is getting smaller (Mayes, 1999).

Conclusion

Based on the discussion of the effect of treatment on the lipid profile of mice, it can be seen that the treatment can give a very good effect on the improvement of hypercholesterolemic mice blood lipid

profile. The dose given in this study was 1000 mg / kg mice or equal to 200 mg / 200 gram mice / day. When converted in adult humans weighing 70 kg, the recommended dose is 11 111 mg / day or 11 g / day, with a conversion value from humans to rats of 0,018

References

- Cannon, J. G., Meydani, S. N., Fielding, R. A., Fiatarone, M. A., Meydani, M., Farhangmehr, M., Orencole, S. F., Blumberg, J. B. & Evans, W. J. 1991. Acute phase response in exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis. *Am. J. Physiol*, 260: R1235–R1240.
- Daniel. TS et al. 1991. Effect of a Palm Oil- Vitamin E Concentrate on The Serum and Lipoprotein Lipids in Humans. *Am J Clin Nutr* 199 1 ; 53: 10275-305.
- Devaraj, S., Li, D. & Jialal, I. 1996. The effects of alpha tocopherol supplementation on monocyte function: decreased lipid oxidation, interleukin 1b secretion, and monocyte adhesion to endothelium. *J. Clin. Investig.* 98: 756–763.
- Estiasih., et. al. 2014. The Effect of Unsaponifiable Fraction from Palm Fatty Acid Distillate on Lipid Profile of Hypercholesterolaemia Rats. *Journal of Food and Nutrition Research*, 2014, Vol. 2, No. 12, 1029-1036
- Greenberg RS, Daniels SR, Flanders W. 2001. Diagnostic testing. In: *Medical epidemiology*. 3rd ed. New York: Lange Medical Books/McGraw-Hill.
- Hanum Ghulam Rasool, A.; Rahman, A.R.A.; Yuen, K.H.; Wong, A.R. 2008. Arterial compliance and vitamin E blood levels with a self emulsifying preparation of tocotrienol rich vitamin E. *Arch. Pharm. Res.* 31, 1212–1217.
- Hui YH. 1996. *Bailey's Industrial Oil and Fat Products (5th ed vol.3)*, Edible Oil and fat Products: Products and Application Technology. John Wiley&Sons, Inc. New York.
- Kreisberg R, Oberman A. 2003. Medical management of hyperlipidemia/ dyslipidemia. *J Clin Endocrin Met*; 88: 2445-61.
- Mayes, P.A. 1996. Lipid Transport and Storage. Di dalam Murray R. K., D. K. Granner., P. A. Mayes., dan V. W. Rodwell (eds). *Harper Biochemistry* 24th ed. Prentice Hall International, Inc, London
- Murray RK, Graaanner DK, Mayes PA, Rodwell VM. 2003. *Biokimia Harper*. terjemahan oleh Alexander dan Andry Hartono; Jakarta: EGC
- Musalmah, M., M. Y. Nizam, A. H. Fairuz, A.H. Noor Aini, A.I. Azian, M.T. Gapor, and W.Z. Wan Ngah. 2005. Comparative effects of palm vitamin E and α tocopherol on healing and wound tissue antioxidant enzyme levels in diabetic rats. *Lipids* 40: 575-580.
- Ngatijan. 2006. *Metode Laboratorium dalam Toksikologi*. Yogyakarta : Bagian Farmakologi dan Toksikologi, Fakultas Kedokteran, Universitas Gadjah Mada.
- Papas, A. M. 2008. Vitamin E: A New Perspective. *Nutri News* 9(1): 1-8
- Pastore R. 2003. Getting to the heart of the matter. akses 26 april 2014 <http://metabolism.com/healthbytes>.
- Silalahi J, Tampubolon SD. 2002. Asam lemak trans dalam makanan dan pengaruhnya terhadap kesehatan. *Jurnal Teknologi Pangan* ;8(2):184-188
- Strandberg TE, Tilvis RS, Miettinen TA. 1990. Metabolic variables of cholesterol during squalen feeding in humans: comparison with cholestyramine treatment. *J Lipid Res* 31:1637-1643.
- Wu, D., Koga, T., Martin, K. R. & Meydani, M. 1999. Effect of vitamin E on human aortic endothelial cell production of chemokines and adhesion to monocytes. *Atherosclerosis* 147: 297–307.
- Yukihiko Momiyama, et.al. 2012. Associations between LDL/HDL-cholesterol ratio and thoracic and abdominal aortic atherosclerosis assessed by MRI. *Journal of Cardiovascular Magnetic Resonance* 2012, 14(Suppl 1):P72
- Zureik, M., Galan, P., Bertrais, S., Mennen, L., Czernichow, S., Blacher, J., Ducimetiere, P., Hercberg, S., 2004. Effects of long-term daily low-dose supplementation with antioxidant vitamins and minerals on structure and function of large arteries. *Arterioscler. Thromb. Vasc. Biol.* 24 (8), 1485–1491.

Soft Bran of Sorghum Potential for High Fiber Supplement Food

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Abstract

Soft bran of sorghum produced from the second polishing of rice sorghum, which is the outermost part of the grain portion encased by husks. This research is aimed to examine the potential of soft bran sorghum as a food supplement, and research used was Randomized Block Design (RBD): 1-factor RBD experiments with 3 replications. Factor Types of Sorghum Rice Bran (B) were B₁ : soft bran of red sorghum (*Sorghum bicolor*) and B₂ : soft bran of white sorghum (KD 4). Observations: organoleptic test with parameters of taste, color, flavor, and appearances, and chemical analysis of moisture content, carbohydrate, protein, fat, and fiber. Data analysis: organoleptic test used Friedman test, while chemical analysis used variance analysis continued by Duncan test 5%. Conclusions: 1) Rendement of sorghum grain was 6 tons/ha, sorghum rice was 4,2 tons/ha, sorghum flour was 4,2 tons/ha, soft bran of sorghum was 0,6 tons/ha, bran of the sorghum grain was 1,2 tons/ha; 2) The best treatment for soft bran of sorghum (B₂/ soft bran of white sorghum (KD 4)): water content was 11%, carbohydrates was 71%, protein was 11.5%, fat was 2.1%, and fiber was 11.2%; 3) Soft bran of sorghum could be used as food supplements: bakery, cookies, soup mix, pureed, and supplement drinks.

Keywords: sorghum, soft bran, supplement, fiber

Introduction

Many kinds of alternative food sources could potentially be developed to support the diversification program and food security of the Indonesian, one of them is sorghum (*Sorghum Sp*). Sorghum as a world's food source is ranked 5th after wheat, rice, corn and barley. When soil moisture is not a limiting factor, average sorghum yields can reach 5-6 ton/ ha. In Indonesia sorghum has long been known by farmers, especially in Java, NTB and NTT, specifically in East Java, the largest areas of sorghum production are in Lamongan, Bojonegoro, Blitar, Bangkalan, Sampang, and Lumajang.

Soft bran of sorghum is the outermost part of the grain portion encased by husks. Grains are fruits and seeds of a variety of the true cereal crops, such as rice, wheat, and sorghum. The term soft bran is mainly associated with rice, because this cereal is known as the cultural heritage. However, rice bran can be obtained also from corn, wheat, and sorghum.

The anatomy of soft bran consists of aleurone and pericarp layer. Aleurone is the outermost layer of the endosperm, while pericarp is the deepest part of the husk. The separation process of soft bran from the other part of rice is known as milling (polishing) to increase their shelf life, as well as to bleach them. The majority of the community equalizes between rice bran and bran although, soft bran and bran is different. Bran is rice milling waste/pulverization of the first rice. Meanwhile, rice bran is a residual of

pulverized soft bran / rice milling of the second (rice husk). Soft bran of sorghum is produced from the second polishing of rice sorghum which is the outermost part of the grain portion encased by husks.

During this time soft bran of sorghum has not been fully utilized, therefore further research is needed. The aims of this research are to analyze the potential of soft bran of sorghum as a food supplement.

Materials and Method

Research used Randomized Block Design (RBD): 1-factor RBD experiments with 3 replications. Factor Types of soft bran sorghum (B) were B1: soft bran of red sorghum (*Sorghum bicolor*) and B2: soft bran of white sorghum (*KD 4*). Observations: organoleptic test with parameters of taste, color, flavor, and appearances, and chemical analysis of moisture content, carbohydrate, protein, fat, and fiber. Data analysis were organoleptic test used Friedman test, while chemical analysis used variance analysis continued by Duncan test 5%.

Results and Discussion

Soft bran of sorghum yield

Sorghum yields are presented on Table 1. It can be seen that the production of Soft bran sorghum 10% of the total grain sorghum, it indicates that the Soft bran of sorghum potential to be developed further, and Soft bran of sorghum yield demonstrates the potential to be used as a food supplement.

Table 1. Sorghum yields

Sorghum Production	Yield
Sorghum grain	6 ton/ha
Sorghum rice	4,2 ton/ha
Sorghum flour	4,2 ton/ha
Sorghum soft bran	0,6 ton/ha
Sorghum bran	1,2 ton/ha

Physical Test of Sorghum Rice Bran

The result of physical observations for Soft bran of sorghum is presented on Table 2. In Table 2, difference on the color of Soft bran of sorghum is recorded. It will become the advantages of Soft bran of sorghum which can produce product with different variety of color.

Table 2. Color Test of Sorghum Soft bran

Treatment	Color
B1	Reddish white
B2	Yellowish white

Organoleptic Test of Sorghum Rice Bran

The result of organoleptic test is presented on Figure 1 and Table 3.

The result of percentage calculation of organoleptic test with parameters of color, flavor and appearance used treatment for factors types of sorghum soft bran (B) were B1: Soft bran of red sorghum (*Sorghum bicolor*) and B2: Soft bran of white sorghum (*KD 4*), the meaning of scores were 1: dislike, 2: somewhat like, 3: neutral, 4: like, dan 5: really like.

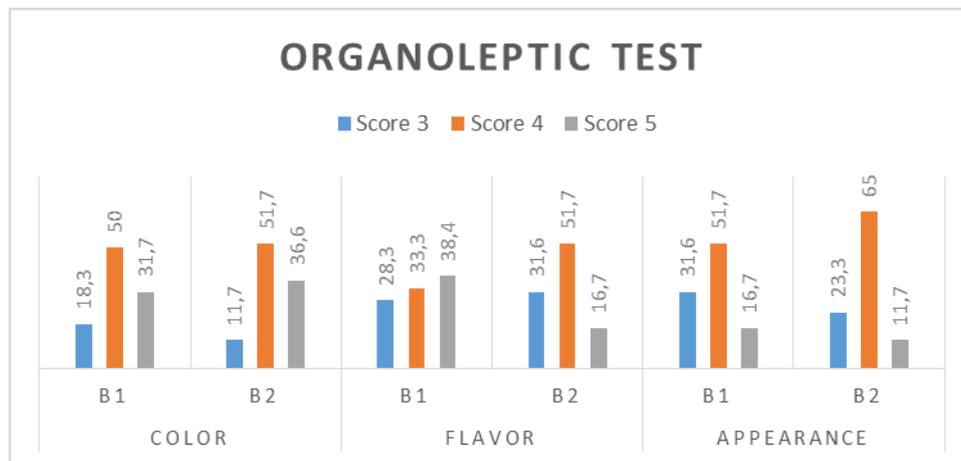


Figure 1. The Result of Organoleptic Test of Sorghum Soft Bran

Table 3. The Result of Friedman Test of Sorghum Rice Bran

Test Statistics ^a			
Parameter	Warna	Aroma	Kenampakan
N	60	60	60
Chi-Square	1.190	0.167	0.00
df	1	1	1
Asymp. Sig.	.275	.683	1.00

a. Friedman Test

It was showed that the highest score for color parameter was on B2 treatment (B2 was white soft bran / KD4 with percentage 36.6% score 5 (really like), flavor parameter was on B1 treatment (red soft bran / *Sorghum bicolor*) with percentage 38.4% score 5 (really like); and the highest score for appearance parameter was on B1 treatment (red soft bran / *Sorghum bicolor*) with percentage 31.6% score 5 (really like). As shown on Table 2, it seemed that physically soft bran color was different, it was an advantage of sorghum soft bran, and on Table 3, it seemed that the result of friedman test for sorghum soft bran product demonstrated that color parameter (Sig 0,275 > 0,05), flavor parameter (Sig 0,683 > 0,05), and appearance (Sig 1,000 > 0,05), which was meant that the parameters of color, flavor, and appearance were not significantly different, for those parameters resulted from factor types of sorghum soft bran, showed that all could be accepted by the panelists, although there was difference for the color of the sorghum soft bran i.e. red soft bran which was resulted from red sorghum / *Sorghum bicolor* and white soft bran which was resulted from white sorghum /KD4, it was an advantage of both types of sorghum soft bran resulted.

Chemical Test of Sorghum Soft Bran

The result of chemical test is presented in Figure 2. The moisture content was (10,9-13,60%), carbohydrate was (69,56-71,53%), protein was (10-11,52%), fat was (1,57-2,8%), and fiber was (11,21-12,01%). The result of variance analysis shows that the moisture content was (Sig 0,847 > 0.05), carbohydrate was (Sig 0,424 > 0.05), protein was (Sig 0,653 > 0.05), fat was (Sig 0,626 > 0.05), crude

fiber was (Sig 0,082 > 0.05), it can be concluded that moisture content, carbohydrate, fat, and crude fiber were not significantly different. Moisture content of sorghum soft bran was < 14%. This condition is as a requirement for safety moisture content, so it is to prevent the growth of microbes, and the high content of carbohydrate, protein, fat become an advantage of sorghum soft bran, as well as high fiber content. Dietary fiber is one of the non nutrition nutritional substances that can not be digested by human digestive enzymes. The dietary fiber does not produce energy and nutrition. Dietary fiber contained in many plants, spread from the roots, stems, leaves, flowers, fruits until the seeds. The presence of fiber in the diet is highly recommended in the diet, although it has no nutritional value, because of dietary fiber in charge of maintaining the health of the digestion and can prevent various diseases. Nutrient content for soft bran of rice, corn, and sorghum is presented in Table 4.

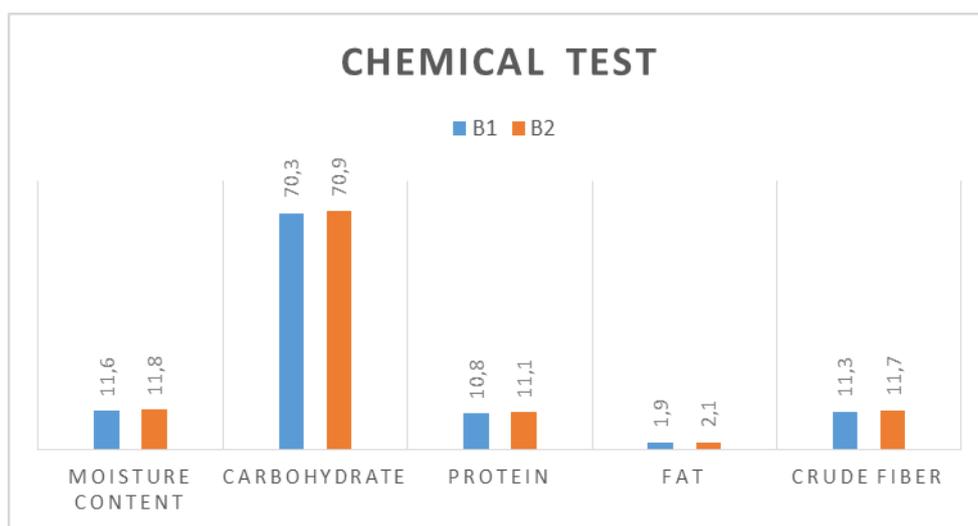


Figure 3. The Result of Chemical Test of Sorghum Soft Bran

The carbohydrate and protein of sorghum soft bran and soft bran of rice and corn can be seen in Table 4. The sorghum soft bran protein and carbohydrate content are similar. While the fat content are lower for sorghum soft bran.

Table 4. The comparison between nutritional value of Soft bran of rice, corn, and sorghum

Component	Soft bran of rice	Soft bran of corn	Soft bran of red sorghum	Soft bran of white sorghum
Carbohydrate	54,6 g	64,5 g	70,3 g	71 g
Protein	12,6 g	9 g	10,8 g	11,1 g
Fat	14,8 g	8,5 g	1,9 g	2,1 g

Source: Depkes (1996) dan Noerhartati (2015-2016)

Probability Test

The result of probability test calculations of sorghum soft bran is presented in Figure 4. Probability analysis performed to determine the odds of each ground state. Ground state to include the quality of color, flavor, appearance, moisture content, carbohydrate, protein, fat, and fiber content. Probability value showed the importance of a ground state, the greater the probability of the value of the ground state, the more important the ground state. On behalf of sorghum soft brand products, the parameter of appearance was (21%) and it was considered as the most important parameter compared to the other parameters, i.e.

color was (17%), protein was (14%), carbohydrate was (13%), flavor was (12%), moisture content was (9%), fat was (7%), and fiber content was (7%).

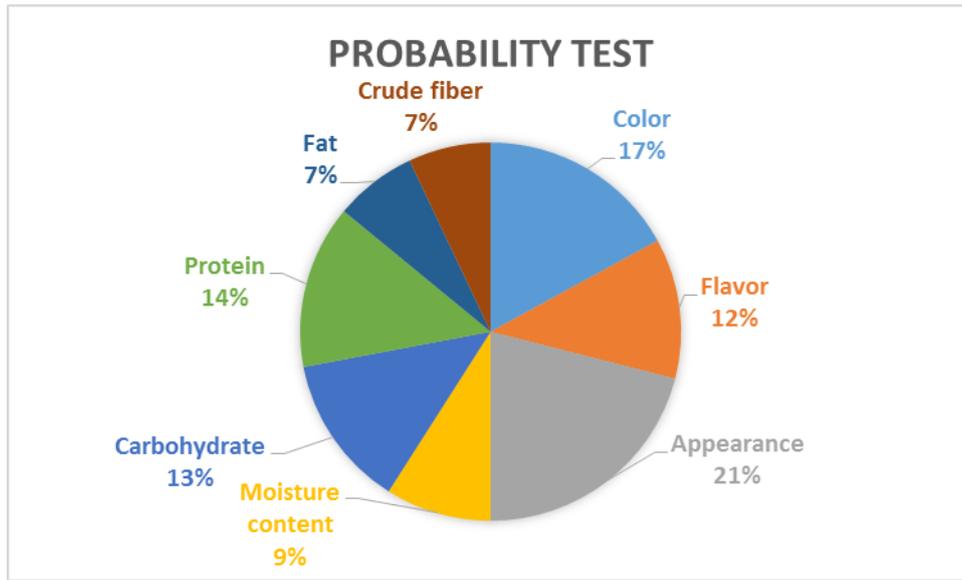


Figure 4. Probability test chart of sorghum soft bran

Alternative Selection

The result of alternative selection calculation is presented on Figure 5. Alternative processes are nothing compared to determine the optimal process. Based on the result of alternative selection for selected treatment was treatment B2 (B2 was soft bran of white sorghum / KD4), with the value of calculation result was 25.79, with the value of moisture content was 11%, carbohydrate was 71%, protein was 11,5%, fat was 2,1%, and fiber was 11,2%.



Figure 5. Graph of alternative selection of sorghum soft bran

Soft bran of sorghum as a food supplement

Soft bran of sorghum can be used as a food supplement: bakery, cookies, soup mix, pureed, and supplement drinks. The flow diagram of sorghum soft bran preparations as a food supplement is presented on Figure 6. The use of sorghum soft bran as a food supplement, in the following manner: Sorghum soft bran resulted is previously mashed, then done the sifting, in order that rice bran produced has a uniform size. It is continued to the process of roasted. Soft bran of sorghum is later ready to be used as a food supplement.

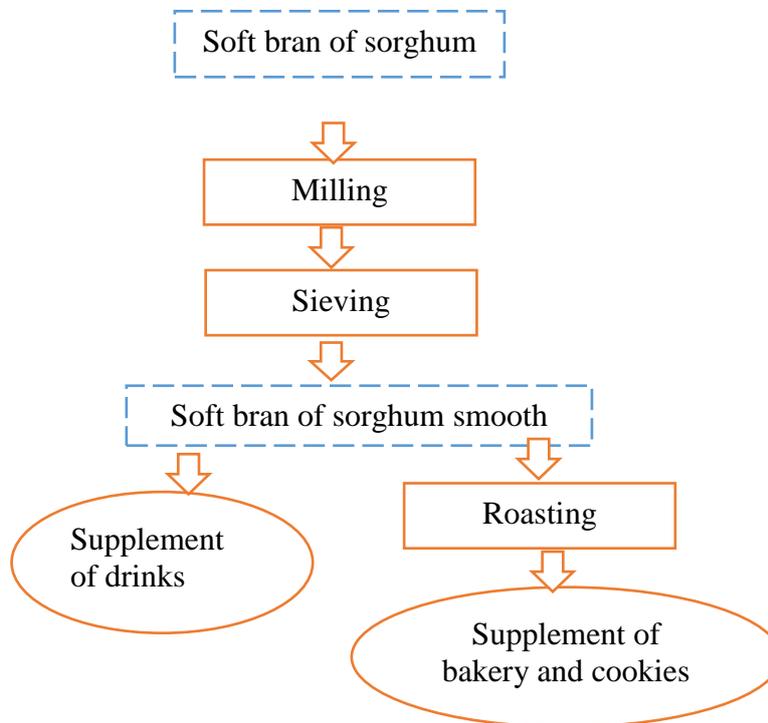


Figure 6. Flow diagram of sorghum soft bran preparations as a food supplement

Conclusion

- 1) Rendement of sorghum grain was 6 ton/ha, sorghum rice was 4,2 ton/ha, sorghum flour was 4,2 ton/ha, sorghum soft bran was 0,6 ton/ha, sorghum bran was 1,2 ton/ha;
- 2) The best soft bran of sorghum was (B2/soft bran of white sorghum/*KD4*), with moisture content was 11%, carbohydrate was 71%, protein was 11,5%, fat was 2,1%, and crude fiber was 11,2%;
- 3) Soft bran of sorghum could be used as a food supplement: bakery, cookies, soup mix, pureed, and supplement drinks.

References

Depkes RI. 1996. Lists of Food Composition. Bhartara Publisher. Jakarta.pp. 5-7
Endang Noerhartati, 2009. Lecture Module of Industrial Materials Second Edition. Study Program TIP, Faculty of Engineering, UWKS, Januari 2009. pp. 8-15
_____, 2009. Lecture Module of Quality Control Technology First Edition. Study Program TIP, Faculty of Engineering, UWKS, Februari 2010. pp. 32-40

- _____, 2010. Various Industrial Products Made from Wheat and sorghum. Papers of Business and Technology Meeting". East Java Provincial Agriculture Office 2728 Juli, 2010 – Batu Malang. pp. 14-24
- _____, 2010. Assorted Cookies Made from Sorghum Flour (*Sorghum bicolor*). Papers of "Liputan Si Unyil TV TRANS 7". Collaboration of Agricultural technology Study Program Faculty of Engineering Universitas Wijaya Kusuma Surabaya with Liputan Si Unyil TV TRANS 7. (2 September 2010). pp. 5-17
- _____, 2012. PostHarvest Technology and Processing of Wheat and Sorghum Commodities. Coordination Meeting on Food Development Alternatives" East Java Provincial Agriculture Office. Surabaya. pp. 15-25
- _____, 2012. Various Food Products Processing Technology of Wheat and Sorghum. An Event on Production and Productivity Improvement of Other Cereals Commodities in East Java in the Year of 2012. East Java Provincial Agriculture Office. Malang. pp. 15-26
- _____, 2014. Alternative Food Ingredients from Sorghum (*Sorghumsp*): Grains, Flour, Assorted Cookies and Sorghum Molasses of Tree Trunk. Media Ilmu. Sidoarjo. pp. 5-25
- _____, 2014. Business Plan of Sorghum Agroindustry. Facilitation Partnership Activity and Food Alternative Technology Adoption. East Java Provincial Agriculture Office Surabaya. pp. 14-24
- _____, 2014. Business Incubator of Sorghum Pastry. Facilitating the Sorghum Development Activity. East Java Provincial Agriculture Office. Lamongan. pp. 15-25
- Illaningsyah, F., Sri I, Sri P, Iim A, and Fajarwati. 2013. The Effect of Supplementation Isolate Sorghum Protein to Chemical, Biological and Organoleptic Properties of Biscuits Sorghum. Proceedings of the National Seminar. PATPI 2013. Jember. pp. 5166
- Kentz, N.L. 1970. *Technology of Cereal*. Pergamon Press. Sydney. pp. 54-60.
- Metz, S.A. 1970. *Cereal Technology*. The AVI Publishing Company. USA. pp. 172-195
- National Sorghum Producers. 2007. *Grain Sorghum Production Handbook*. University of Arkansas Division of Agriculture. North Inerstate 27 lubbock. Texas (www.sorghumgrowers.com) (accessed 14 July 2009)
- National Sorghum Producers. 2007. *Sorghum Grower*. University of Arkansas Division of Agriculture. North Inerstate 27 lubbock. Texas (www.sorghumgrowers.com) (accessed 14 July 2009)
- Pramudya, B. and S. Budijanto. 2001. Excavation of Potential Local Food for Food Diversification at the National Workshop on Local Food Development. Food Security Board East Java Province. Surabaya. pp. 40-46
- Rahayuningsih, 2008. Utilization of Sweet Potato Flour as Flour Substitution in Wet Noodle Making. Proceedings of the National Seminar on Agroindustry Based on Local Resources to Support Food Security ISBN 9789791366281. pp. 131-140
- Revitriani, M, Endang RW, and Diana P. 2013, The Concentration Studies on Kimpul (*xanthomasagitifolium*) Flour in Making Wet Noodle. J. REKA Agroindustry. I (1) 2013:2836. pp. 28-36
- Rob. Mudjisihono and Suprpto, 1987. Cultivation and Processing of Sorghum. PenebarSwadaya. Jakarta Pusat. pp. 55-70
- Sirappa, M.P. 2003. Prospect of Sorghum Development in Indonesian as Alternative Commodity for Food, Feed, and Industrial Uses. Indonesian Journal of Agricultural Science, 22(4) pp. 6-12
- Stonestreet, N.J.M.S Alawi and SR. Bean. 2010. Sorghum Proteins: The Concentration, Isolation, modification and food applications of Kafirin. J. Food Science Vol. 75 (5): pp. 90104
- Taylor. J.R. Schober T.J and Bean, S.R. 2006. Novel Food and NonFood Uses for Sorghum and Millets. J. Cereal Science, 44(3): pp. 252271
- Yousif, A., D.Nhepera and S.Johson. 2012. Influence of Sorghum Flour Addition on Flat Bread In Vitro Starch Digestibility, Antioxidant, Capacity and Consumer Acceptability Food. Ford. Chem. 134 (2012): pp. 880887

Anti-Hypercholesterolemia Effect of Black Rice Bran in Male Wistar Rat

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Abstract

This study was designed to evaluate the efficacy of Black Rice Bran (BRB) to mitigate the onset of hypercholesterolemia in Wistar rats fed with atherogenic diets. The rat was fed with the experimental diets during a 12-h period for 16 weeks. The body weights were measured every week. At the end of week-16, total of cholesterol, HDL, LDL and triacylglycerol (TAG) in their blood were measured immediately. The rats were sacrificed to remove the heart and liver, then analyzed the total cholesterol (TC) content in those organs. The rats fed with diet contained 0.5% Cholesterol and Cholic Acid exhibited more severe hypercholesterolemia than others fed with diet contained only 0.5% cholesterol. The inclusion of the BRB in the diets significantly ($p < 0.05$) decreased the level of TC, LDL and TAG of rats plasma fed control diets that either contained or were absent in bile salt ($p < 0.05$). There were no differences in HDL-level for all treatments. Conclusion: supplementation of atherogenic experimental diets with BRB decreased lipid levels for hypercholesterolemic rats, grace of bioactive components present in BRB, ex: anthocyanin, oryzanol, and fiber

Keywords: Black Rice Bran; Anti-Hypercholesterolemia; Wistar Rat

Introduction

Rice is Indonesian staple food and rice bran is a by-product of rice milling. White rice brand and rice bran oil are reported to have beneficial effect for human health (Cara *et al.*, 1992; Kahlon *et al.*, 1992; Ausman *et al.*, 2005). Ling *et al.* (2001) and Xia *et al.* (2003) showed that the outer layer of black rice has functional effect. This layer was source of fibre, oil, flavonoids, polyphenols and anthocyanidins. Pigmented rice contained two main of anthocyanin, those are Cyanidin-3-Glucoside and Peonidin-3-Glucoside (Hu *et al.*, 2003; Abdel-Aal *et al.*, 2006).

Anthocyanin is natural phenolic that colouring a lot of fruits and vegetables (Murota *et al.*, 2002; Kerckhoffs *et al.*, 2002; Lee *et al.*, 2006; Wu *et al.*, 2006). Anthocyanin reduced the risk of coronary heart and atherosclerosis disease risk through its antioxidant, anti-platelet and anti-inflammation activities (Hu *et al.*, 2003; Xia *et al.*, 2003); then anthocyanin rich-diets are good for our health (Ling *et al.*, 2001; Xia *et al.*, 2003; Galvano *et al.*, 2004).

Some researchers reported that black rice and or the outer layer of this rice significantly reduced lipid level in the blood (Ling *et al.*, 2002), plaque of atherosclerotic in hypercholesterolemia rabbit, and Apo-E in the rat (Ling *et al.*, 2001; Xia *et al.*, 2003). Lichtenstei *et al.* (1994), Suh *et al.* (2005), Juliano *et al.* (2005) and Ausman *et al.* (2005) showed the positive effect of rice brand oil or γ -Oryzanol on human health. The oil contained some fatty acids like Oleic, Linoleic, and Palmitic, and also Sterol and Oryzanol (Lichtenstein *et al.*, 1994; Chen & Cheng, 2006). γ -Oryzanol had antioxidant activity too (Juliano *et al.*, 2005; Suh *et al.*, 2005). Based on such research, the aim of this study is to investigate the effect of black rice bran on cholesterol level of rats.

Materials and Methods

Materials

The rat used was male white rat Wistar (*Rattus norvegicus*) age of 5 weeks with weight in average of 120-150 g and in good health. The rats were obtained from Laboratory of PUSVETMA Surabaya. Research sample is dry simplicial from black rice bran (*Oryza sativa L. indica*), that bought from farmer in Kepanjen area, Malang, East Java, Indonesia. Test kit was used for measurement of the total cholesterol, HDL, LDL and triacylglycerol (TAG).

Method

This research used Block Random Design with Feed Diets Varieties as experimental factor; those are No cholesterol (NK) as negative control; Cholesterol 0,05% (P) as positive control, Cholesterol + BRB (P+BR), Cholesterol+Cholic Acid (C), Cholesterol+Cholic Acid+BRB (C+BR). The experiment was repeated 5 times respectively.

The rats was handled based on Manual "Perawatan dan Penggunaan Hewan Coba" Vol. 1 from Dewan Perawatan Hewan (1993). The rats were settled in stainless steel cage and maintained under controlled condition: in temperature at 25°C, under light (14:10: dark cycle). The rats were acclimatization for a week. The rat divided into 5 groups of experiments based on the feed diets (NK, P, P+BR, C, C+BR). The feed composition for each group of experiment was prepared as described in Table 1.

Table 1. Feed Diet Composition for Each Experiment

Feed Diet Composition (g/100g)	Group Experiments*				
	NK	P	P + BR	C	C+BR
Casein	25,5	25,5	25,5	25,5	25,5
Corn starch	47,0	46,50	43,50	46,00	43,25
Sucrose	3,00	3,00	3,00	3,00	3,00
Alphacellulose	5,00	5,00	5,00	5,00	5,00
Vitamin mix	3,00	3,00	3,00	3,00	3,00
Choline chloride	0,20	0,20	0,20	0,20	0,20
Methionine	0,30	0,30	0,30	0,30	0,30
Mineral mix	3,00	3,00	3,00	3,00	3,00
Canola oil	10,0	7,00	7,00	7,00	7,00
Butter	-	3,00	3,00	3,00	3,00
Cholesterol	-	0,50	0,50	0,50	0,50
Black Rice Bran	-	-	3,00	-	3,00
Cholesterol	-	0,50	0,50	0,50	0,50
Cholic Acid	-	-	-	0,05	0,05

* The experiments: No cholesterol (NK); Cholesterol 0,05% (P), Cholesterol + BRB (P+BR), Cholesterol+Cholic Acid (C), Cholesterol+Cholic Acid+BRB (C+BR)

Period of experiment was 16 weeks. The rat was feed daily based on the group of experiments. The body weight was measured once in a weeks. At the end of experiments (weeks-16), the rats were sacrificed and the bloods were collected in heparinized tubes. Meanwhile heart and liver were removed. The tubes were centrifuged on 4°C at 1000 rpm for 5 minutes. The hearts and livers were rinsed in 0,9% NaCl at 4°C, air-dried, and then weighed. Total Cholesterol (TG), HDL, LDL and TAG of blood plasma were measured using test kit (Sigma, St. Louis, MO, USA). Cholesterol content in the hearts and livers were analysed based on Folch *et al.* (1957).

Statistical analysis

Data was analysed statistically using ANOVA at $\alpha = 0,05\%$, then analysed further using BNT. All the analysis was performed using SPSS 20.

Result and discussion

The Effect on Weight of the Winstar Rat Body, Heart, and Liver

There was no significant difference between group of experiment for body weight, heart weight, liver weight, and feed efficiency ratio (FER) (Table 2). It means the addition of black rice bran at 3% in feed did not give a significant effect. Xia *et al.* (2003) and Ling *et al.* (2002) researches showed that the addition of black rice bran on experiment feed, for rat and or rabbit, did not increased the body weight of the animals.

Table 2. The Experiment Effect on Weight of Winstar Rat Body, Heart, and Liver

Experiments	Body weight (g)	FER*	Heart weight (g)	Liver weight (g)
NK	297,6	0,483	1,07 ± 0,04	12,62 ± 0,33
P	308,3	0,467	1,03 ± 0,02	19,39 ± 0,85
P + BR	306,4	0,470	1,10 ± 0,05	18,60 ± 0,22
C	246,8	0,491	1,13 ± 0,05	19,99 ± 0,35
C + BR	258,9	0,462	1,06 ± 0,02	15,30 ± 0,91

* FER= Feed Efficiency Ratio

The C+BR-Rats had lower liver weight compared to P+BR and C-Rats. Black rice bran contained soluble dietary fibre, unsaturated fatty acid, and sterol. The dietary fibre bound fatty acids, cholesterol, and bile salt in the intestine, thus decreased fat absorption (Cara *et al.*, 1992; Vissers *et al.*, 2000; Vaskonen *et al.*, 2002; Xia *et al.*, 2003). Those will decrease fat content in the liver, especially for BRB-Rats.

The Effect on Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Triacylglycerol (TAG) of The Winstar Rat

The addition of BRB significantly decreased Total Cholesterol (TC), LDL-C and TAG content in the rats. Data in Table 3 showed that TC of P+BR-rats and C+BR-rats were significantly lower ($p < 0,05$) than TC of P-rats and C-rats respectively. It also happened for LDL-C and TAG of P-BR-rats and C+BR-rats, if compared to TC of P-rats and C-rats respectively. There was no significant effect of BRB addition on HDL-C content of the rats.

Table 3. Experiment Effect on Total Cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), and Triacylglycerol (TAG) of Winstar Rat

Experiments	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TAG (mmol/L)
NK	1,79 ± 0,10	1,11 ± 0,09	0,68 ± 0,09	0,51 ± 0,04
P	2,51 ± 0,16	0,91 ± 0,06	1,65 ± 0,08	0,78 ± 0,08
P + BR	2,18 ± 0,11	0,92 ± 0,08	1,27 ± 0,05	0,49 ± 0,05
C	4,20 ± 0,12	0,73 ± 0,12	3,47 ± 0,12	0,55 ± 0,03
C + BR	2,52 ± 0,13	0,81 ± 0,14	1,71 ± 0,14	0,43 ± 0,02

The decrease of Total Cholesterol Content in the rats may be affected by oryzanol, anthocyanin and dietary fibre in BRB. The soluble dietary fibre can increase hypocholesterolemic effect (Fernandez *et*

al, 1997; Mekki *et al.*, 1997; Behall *et al.*, 2004). Bifidobacterium in the intestine will ferment the fibre and Short Chain Fatty Acid (SCFA), then decreases cholesterol in the blood and other tissues (Hara *et al.*, 1999).

The oryzanol decreases the cholesterol absorption (Lichtenstein *et al.*, 1994; Cicero & Gaddi, 2001; Berger *et al.*, 2005; Suh *et al.*, 2005). Oryzanol significantly decreased Total Cholesterol, LDL, and VLDL content in the hypercholesterolemia rats (Suh *et al.*, 2005) and in hypercholesterolemia man (Berger *et al.*, 2005).

Frank *et al.* (2002) reported that Cyanidin-3-O-Glucoside did not influenced fat profile of hypercholesterolemia rats, but Nielsen *et al.* (2005) proved that anthocyanin extracted from blackcurrant significantly decreased total cholesterol, LDL, VLDL, and TAG in the blood of hyperlipidaemia-inherited rabbits.

The Effect on Cholesterol Level in The Heart and Liver of Winstar Rat

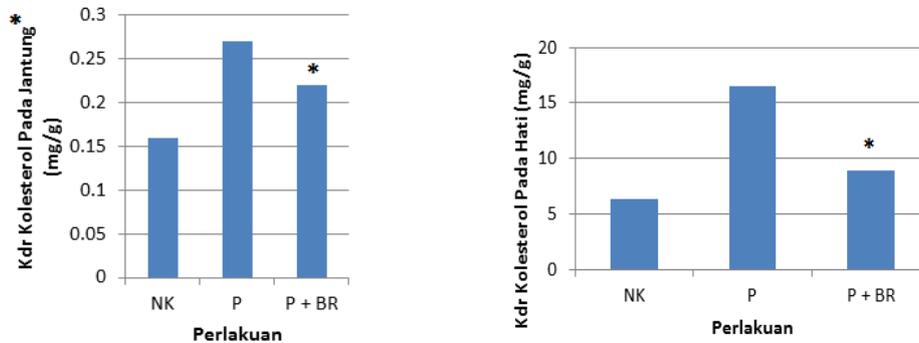


Figure 1. Cholesterol Content in The Heart and Liver of Winstar Rat Feed with Diet Contained Cholesterol and or BRB comparing to No Cholesterol Diet.

* Significantly different at $\alpha < 0,05$, ** Significantly different at $\alpha < 0,01$
 NK: No Cholesterol, P: cholesterol 0,05%, P+BR = Cholesterol+BRB

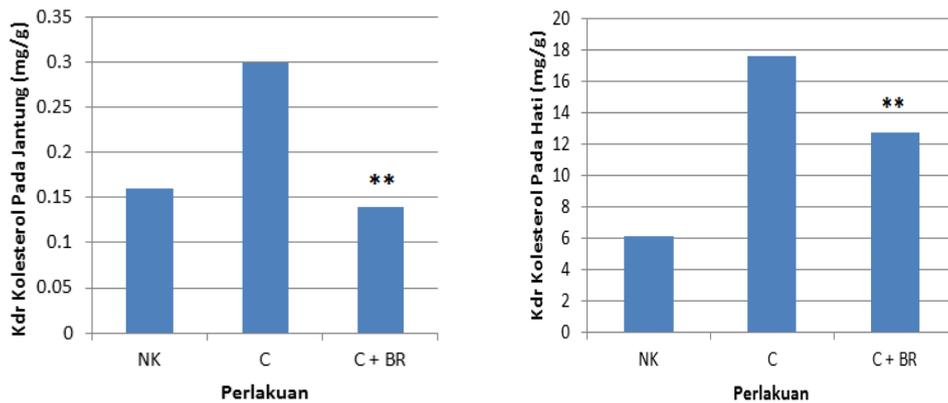


Figure 2. Cholesterol Content in The Heart and Liver of Winstar Rat Feed with Diet Contained Cholesterol and Cholic Acid and or BRB comparing to No Cholesterol Diet.

* Significantly different at $\alpha < 0,05$, ** Significantly different at $\alpha < 0,01$
 NK: No Cholesterol, C: cholesterol 0,05%+Cholic Acid, C+BR = Cholesterol+Cholic Acid+BRB

Cholesterol accumulation in the liver and heart of the rats treated no BRB feed (P and C) were significantly lower than the ones in the rats treated with BRB feed (P+BR and C+BR) (Figure 1 and 2). The cholesterol in those P and C-rats were significantly higher than control (NK). It may be influenced

by the anthocyanin, oryzanol and soluble dietary fibre in the BRB, which play important role in the decrease of cholesterol absorption (Lichtenstein *et al.*, 1994; Cicero & Gaddi, 2001; Berger *et al.*, 2005; Suh *et al.*, 2005) in the blood and other tissues (Hara *et al.*, 1999). It was supported by Xia *et al.* (2003) that shown the importance of BRB in the decrease of LDL and TAG in the hypercholesterolemia rats (Table 2). The dietary fibre in the BRB can bind the bile salt then inhibit fatty acid absorption (Ausman *et al.*, 2005).

Conclusion

Supplementation of atherogenic experimental diets with Black Rice Bran (BRB) decreased lipid levels in plasma, heart, and liver of the rat; and also LDL, total cholesterol, and triglyceride in plasma of the Wistar rat. It needs to study further, the effect of BRB on human.

References

- Abdel-Aal, E.-S., Young, J. C., & Rabalski, I. 2006. "Anthocyanin Composition in Black, Blue, Pink, Purple, and Red Cereal Grains". *Journal of Agricultural and Food Chemistry* 54: 4696–4704.
- Ausman, L. M., Rong, N., & Nicolosi, R. J. 2005. "Hypocholesterolemic Effect of Physically Refined Rice Bran Oil: Studies of Cholesterol Metabolism and Early Atherosclerosis in Hypercholesterolemic Hamsters". *Journal of Nutritional Biochemistry* 16: 521–529.
- Behall, K. M., Scholfield, D. J., & Hallfrisch, J. 2004. "Diets Containing Barley Significantly Reduce Lipids in Mildly Hypercholesterolemic Men and Women". *American Journal of Clinical Nutrition* 80: 1185–1193.
- Berger, A., Rein, D., Schafer, A., Monnard, I., Gremaud, G., Lambelet, P., & Bertoli, C. 2005. Similar Cholesterol-Lowering Properties of Rice Bran Oil, with Varied c-Oryzanol, in Mildly Hypercholesterolemic Men". *European Journal of Nutrition* 44: 163–173.
- Cara, L., Dubois, Ch., Borel, P., Armand, M., Senft, M., Portugal, M., Pauli, A.-M., Bernard, P.-M., & Lairon, D. 1992. "Effect of Oat Bran, Rice Bran, Wheat Fiber, and Wheat Germ on Postprandial Lipemia in Healthy Adults". *American Journal of Clinical Nutrition* 55: 81–88.
- Chen, C.-W., & Cheng, H.-H. 2006. "A Rice Bran Oil Diet Increases LDL-Receptor and HMG-CoA Reductase mRNA Expressions and Insulin Sensitivity in Rats with Streptozotocin/Nicotinamide-Induced Type-2 Diabetes". *Journal of Nutrition* 136:1472– 1476.
- Cicero, A. F. G., & Gaddi, A. 2001. "Rice Bran Oil and c-Oryzanol in The Treatment of Hyperlipoproteinaemias and Other Conditions". *Phytotherapeutic Research* 15: 277–289.
- Fernandez, M. L., Vergara-Jimenez, M., Conde, K., Behr, T., & Abdel-Fattah, G. 1997. "Regulation of Apolipoprotein B Containing Lipoproteins by Dietary Soluble Fiber in Guinea Pigs". *American Journal of Clinical Nutrition* 65: 814–822.
- Folch, J., Lees, M., & Sloane-Stanley, G. H. 1957. "A Simple Method for The Isolation and Purification of Total Lipids from Animal Tissues". *Journal of Biological Chemistry* 226: 497–509.
- Frank, J., Kamal-Eldin, A., Lundh, T., Ma'ath, K., To'o'ron, R., & Vessby, B. 2002. "Effect of Dietary Anthocyanins on Tocopherols and Lipids in Rats". *Journal of Agricultural and Food Chemistry* 50: 7226–7230.
- Galvano, F., La Fuci, L., Lazzarino, G., Fogiliano, V., Ritieni, A., Ciappellano, S., Battistini, N. C., Tavazzi, B., & Galvano, G. 2004. "Cyanidins: Metabolism and Biological Properties". *Journal of Nutritional Biochemistry* 15: 2–11.
- Ha, T.-Y., Songyi, H., Kim, S.-R., Kim, I.-H., Lee, H.-Y., & Kim, H.-K. 2005. "Bioactive Components in Rice Bran Oil Improve Lipid Profiles in Rats Fed a High-Cholesterol Diet". *Nutrition Research* 25: 597–606.
- Hara, H., Haga, S., Aoyama, Y., & Kiriya, S. 1999. "Short Chain Fatty Acids Suppress Cholesterol Synthesis in Rat Liver and Intestine". *Journal of Nutrition* 129: 942–948.
- Hu, C., Zawistowski, J., Ling, W., & Kitts, D. D. 2003. "Black Rice (*Oryza sativa* L. indica) Pigmented Fraction Suppresses both Reactive Oxygen Species and Nitric Oxide in Chemical and Biological Model System". *Journal of Agricultural and Food Chemistry* 51: 5271–5277.
- Juliano, C., Cossu, M., Alamanni, M. C., & Piu, L. 2005. "Antioxidant Activity of gamma-Oryzanol: Mechanism of Action and Its Effect on Oxidative Stability of Pharmaceutical Oils". *International Journal of Pharmacy* 299: 146–154.
- Kahlon, T. S., Chow, F. I., Sayre, R. N., & Betschart, A. A. 1992. "Cholesterol-Lowering in Hamster Fed Rice Bran at Various Levels, Defatted Rice Bran and Rice Bran Oil". *Journal of Nutrition* 122: 513–519.
- Kerckhoffs, D. A. J. M., Brouns, F., Hornstra, G., & Mensink, R. P. 2002. "Effect on The Human Serum Lipoprotein Profile of b-Glucan, Soy Protein and Isoflavones, Plant Sterols and Stanols, Garlic and Tocotrienols". *Journal of Nutrition* 132: 2494–2505.
- Lee, S.-H., Sohn, Y. S., Kang, K. K., Kwon, J. W., & Yoo, M. 2006. "Inhibitory Effect of DA-9201, an Extract of *Oryza sativa* L., on Airway Inflammation and Bronchial Hyper-Responsiveness in Mouse Asthma Model". *Biological and Pharmaceutical Bulletin* 29: 1148–1153.
- Lichtenstein, A. H., Ausman, L. M., Carrasco, W., Gaultieri, L. J., Jenner, J. L., Ordovas, J. M., Nicolosi, R. J., Goldin, B. R., & Schaefer, E. J. 1994. "Rice Bran Oil Consumption and Plasma Lipid Levels in Moderately Hypercholesterolemic Humans". *Arteriosclerosis and Thrombosis* 14: 549–556.
- Ling, W. H., Cheng, Q. X., Ma, J., & Wang, T. 2001. "Red and Black Rice Decrease Atherosclerotic Plaque Formation and Increase Antioxidant Status in Rabbits". *Journal of Nutrition* 131: 1421–1426.
- Ling, W. H., Wang, L. L., & Ma, J. 2002. Supplementation of The Black Rice Outer Layer Fraction to Rabbits Decreases Atherosclerotic Plaque Formation and Increases Antioxidant Status". *Journal of Nutrition* 132: 20–26.

- Mekki, N., Dubois, C., Charbonier, M., Cara, L., Senft, M., Pauli, A. M., Portugal, H., Gassin, A. M., Lafon, H., & Lairon, D. 1997. "Effects of Lowering Fat and Increasing Dietary Fiber on Fasting and Postprandial Plasma Lipids in Hypercholesterolemic Subjects Consuming a Mixed Mediterranean-Western Diet". *American Journal of Clinical Nutrition* 66: 1443–1451.
- Murota, K., Shimizu, S., Miyamoto, S., Izumi, T., Obata, A., Kikuchi, M., & Terao, J. 2002. "Unique Uptake and Transport of Isoflavone Aglycones by Human Intestinal Caco-2 cells: Comparison of Isoflavonoids and Flavonoids". *Journal of Nutrition* 132: 1956–1961.
- Suh, M. H., Yoo, S.-H., Chang, P.-S., & Lee, H. G. 2005. "Antioxidative Activity of Microencapsulated c-Oryzanol on High Cholesterol-Fed Rats". *Journal of Agricultural and Food Chemistry* 53: 9747–9750.
- Vaskonen, T., Mervaala, E., Krogerus, L., & Karppanen, H. 2002. "Supplementation of Plant Sterols and Minerals Benefits Obese Zucker Rats Fed an Atherogenic Diet". *Journal of Nutrition* 132: 231–237.
- Vissers, M. N., Zock, P. L., Meijer, G.W., & Katan, M. 2000. "Effect of Plant Sterols from Rice Bran Oil and Triterpene Alcohols from Sheanut Oil on Serum Lipoprotein Concentrations in Humans". *American Journal of Clinical Nutrition* 72: 1510–1515.
- Wu, X., Pittman, H. E., & Prior, R. L. 2006. "Fate of Anthocyanins and Antioxidant Capacity in Contents of The Gastrointestinal Tract of Weanling Pigs Following Black Raspberry Consumption". *Journal of Agricultural and Food Chemistry* 54: 583–589.
- Xia, M., Ling, W. H., Kitts, D. D., & Zawistowski, J. 2003. "Supplementation of Diets with Black Rice Pigment Fraction Attenuates Atherosclerotic Plaque Formation in Apolipoprotein E Deficient Mice". *Journal of Nutrition* 133: 744–751.

Nanoencapsulation of *Andrographis paniculata* Extract As Inhibitors Enzyme α -Glucosidase And In-Vitro Release Study

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Abstract

Andrographis paniculata is one of the medicinal plants that became the top priority to be developed in Indonesia and declared as bio-pharmacy drug ingredients that are safe. BPOM incorporate this plant as seed plants to be developed in the pharmaceutical industry phytopharmaca. Chitosan as main active compound has been shown to have many pharmacological activities, one of which is as α -glucosidase enzyme inhibitors which has clinical potential as an antitumor, antiviral, antidiabetic, and immunoregulator agents. This study aims to do nanoencapsulation of *Andrographis paniculata* leaf extract to increase its active compound bioavailability and get a release profile through synthetic fluids media simulation. Nanoencapsulation with ionic gelation method result the highest encapsulation efficiency and loading capacity values of 73.47% and 46.29% at 2%: 1% of chitosan:STPP ratio. The optimum α -glucosidase inhibition of 37.17% was obtained at 16% concentration. Burst release at gastric pH conditions indicate that most of the drug (in this study is an *Andrographis paniculata* leaf extract) adsorbed on the surface of the nanoparticles.

Keywords: *Andrographis paniculata* leaf extract, α -glucosidase inhibitors, nanoencapsulation, release profile

Introduction

Sambiloto (*Andrographis paniculata*) is one of 13 leading commodities Indonesian medicinal plants by the government through the Directorate General POM [1]. The active ingredient contained in sambiloto are diterpene lactones and flavonoids [2]. Diterpene lactone found in sambiloto is chitosan, 14-deoxy-11,12-didehydroandrographolide, neoandrographolide, and 14-deoksiandrographolide, while the two flavonoids were identified 5,7,2', 3'-tetrametoksiflavanon and 5-hydroxy-7, 2', 3'-trimetoksiflavanon [3]. Chitosan is the most active and important compounds are most commonly found in sambiloto. Andrographolide have the ability as an antidiabetic, hepatoprotective, cardioprotective, anti-inflammatory, antiangiogenic, antithrombotic, and the antiviral agent [4]. Diterpene compounds have the ability as an inhibitor of α -glucosidase enzyme, wherein the enzyme α -glucosidase inhibitors have clinical potential that can serve among other things as an antitumor, antiviral, antidiabetic, and agents immunoregulator [5] [6].

Various pharmacological activity generated sambiloto makes this plant has a very good potential to be used as a cure various diseases, including diabetes and hepatitis B. However, andrographolide as the main active compound in sambiloto has properties that are unstable in acidic and alkaline conditions on digestion extreme and has a biological half-life is very short (2 hours), so we need a method to be able to deliver an active compound into the body. The application of nanotechnology is one method that can help

the problems of the application of nanotechnology ini. The advantages of which can increase the surface area, increasing solubility, improving bioavailability in oral administration, and protection of the drug from degradation so that it can sustain the release of active compounds in the long term.

The polymer can be selected to facilitate the preparation of nanoparticles are in the form of a water-soluble polymer. One of the water-soluble polymer is chitosan. Chitosan has the properties of an ideal, that is biocompatible, biodegradable, non-toxic and not expensive [7]. In addition, chitosan is a polysaccharide second in terms of its availability in nature and included as polielektrolitkationik [8]. The usage of chitosan as enkapsulator has been widely used before, including the encapsulation of vitamin C [9] and encapsulation tea [10]. However, the use of chitosan as enkapsulator also has the disadvantage of mechanical properties of chitosan are fragile and should be stabilized by cross-linker (crosslinker) polianion. Crosslinker polianion the most widely used is sodium tripolyphosphate (STPP) because it is not toxic and has multivalent. In addition, the use of tripolyphosphate to the formation of chitosan gel can improve the mechanical properties of the particles formed as tripolyphosphate has a high negative charge density so that interaction with chitosan polikation will be greater [11]. The existence of cross-linker to make chitosan formed is still not decayed so that the active substances contained can not be separated before reaching the target when passing through the highly acidic pH.

Based on this background, the study aims to do nanoencapsulation the sambiloto leaf extract with chitosan-STPP and see its benefits as an inhibitor of α -glucosidase enzyme. Variations in the concentration ratio of chitosan: STPP would be done to see the composition that produces encapsulation efficiency and optimum loading capacity. Test of inhibition of the enzyme α -glucosidase performed in vitro with the substrate p-nitrophenyl- α -D-glukopiranosida. In this case it is important to review the release profile of the extract of sambiloto leaf that has dinanoencapsulation with a coating of chitosan in order to determine the performance of the drug in the body. In this study, the release profile test performed in vitro using synthetic fluids media Simulated Gastric Fluid (SGF) pH 1.2 which shows the condition of the hull and Simulated Intestinal Fluid (SIF) pH 7.4 which shows the condition of the intestine.

Materials and Methods

Materials

Materials used include leaf dry sambiloto (*Andrographis paniculata*), chitosan, sodium tripolyphosphate (STPP), tween 80, acetic acid (Merck), the enzyme α -glucosidase (Sigma-Aldrich), p-nitrophenyl- α -D-glukopiranosida(Sigma Aldrich), bovine serum albumin (Merck), a standard andrographolide (Sigma-Aldrich), ethanol technical, methanol, chloroform (Merck), KH₂PO₄ (Merck), NaOH (Merck), Na₂CO₃ (Merck), dimethyl sulfoxide (DMSO) (Merck), and distilled water.

Andrographispaniculata Extract

Extraction is done using ethanol 70% with sonication. A total of 50 g of dried leaves sambiloto is diluted with a ratio of 1:10 (w / v) (do two circulation). Extraction with sonicator lasted for 60 minutes at a frequency of 40 kHz and a temperature of 30⁰C filtrate obtained was concentrated using a vacuum rotary evaporator to form a thick extract.

Nanoencapsulation Andrographis paniculata Extract

A total of 0.5 g of chitosan was dissolved in 50 mL of acetic acid 1% (w / v), and then 0.15 g of extract of sambiloto leaf is added and stirred with a magnetic stirrer until homogeneous. In another container, 0.25 g of STPP was dissolved in 25 mL of distilled water, and 200 mL of 0.1% Tween 80 (v / v) is added to a solution of STPP and stirred with a magnetic stirrer. Chitosan extract solution then dripped by syringe into a solution of STPP accompanied by magnetic stirring for 30 minutes. The solution was then centrifuged at 10,000 rpm for 15 minutes. The precipitate and then freeze dry cleaning to get nanokapsul. Variations chitosan: STPP by 1.5%: 1% and 2%: 1%. Loading capacity calculations performed on the resulting dry nanokapsul and encapsulation efficiency of calculation andrographolide levels in nanokapsul formed using High Performance Liquid Chromatography (HPLC). Particle size and morphology were identified using a Field Emission Scanning Electron Microscopy (FE-SEM).

Preparation of Test Solution

pH 6.8 phosphate buffer solution made from a solution of 0.1 M KH₂PO₄ 0.1 N NaOH solution and substrate solution was prepared by dissolving 0.0315 g p-nitrophenyl- α -D-glukopiranoksida in 20 mL of phosphate buffer. Carrier enzyme solution is prepared by dissolving 6 mg BSA in phosphate buffer to 6 mL, then an enzyme solution prepared by dissolving α -glukosidase to a solution of BSA to obtain a concentration of 0.3 U / mL, 0.15 U / mL, 0.075 u / mL, and 0.0375 u / mL. Na₂CO₃ solution made by dissolving 1.06 g Na₂CO₃ in 100 mL of phosphate buffer. A sample of extract of sambiloto leaf, nanokapsul sambiloto leaf extract, and standard andrographolide dissolved in DMSO and then diluted in phosphate buffer to obtain a concentration variation of 0.5%, 1%, 2%, 4%, 8%, 12%, 16% and 18%.

α -Glukosidase inhibition test [12]

The test sample of extract of sambiloto leaf, nanokapsul sambiloto leaf extract, and standard andrographolide done by adding 10 mL samples with different concentrations at 490 mL and 250 mL of phosphate buffer substrate solution. The mixture was then incubated for 5 min at 37 ° C. After incubation, to the mixture was added 250 mL of an enzyme solution with a concentration of 0.0375 U / mL, the mixture was incubated another 15 minutes at 37 ° C. The reaction was stopped by the addition of 2000 mL of Na₂CO₃. The mixture had an absorbance was measured by UV-Vis spectrophotometer at a wavelength of 400 nm. Testing of control samples was done by adding 2000 mL of Na₂CO₃ in the sample solution and the substrate that has been incubated, then the addition of 250 mL of an enzyme solution with a concentration of 0.0375 U / mL. The mixture had an absorbance was measured by UV-Vis spectrophotometer at a wavelength of 400 nm.

Calculation of Percent Inhibition

Percent inhibition of each sample was analyzed from the absorbance of the yellow color that appears on p-nitrophenyl formed at a wavelength of 400 nm. Percent inhibition was calculated by the equation:

$$\% \text{ Inhibition} = \frac{A_B - A_S}{A_B} \quad (1)$$

where AB is reduced by absorbance absorbance blank blank control, and the US is the absorbance of the

sample is reduced by absorbance control samples.

Making of Synthetic Fluid Media

Media buffer pH 1.2 was prepared by dissolving 4.0824 g KH₂PO₄ with distilled water to 300 mL, then as much as 0.8 g NaOH diluted with distilled water to 200 mL. Media buffer pH 7.4 was prepared by diluting 1.7 mL of 37% HCl to 100 ml with distilled water, then as much as 6 g KCl diluted with distilled water to 400 mL.

Profile Test Release

A total of 1 g matrix nanocapsul chitosan concentration of the three variations: STPP was dissolved in 50 mL of buffer pH 1.2, and then incubated at 37°C. Data is collected every one hour by taking 10 mL and then perform centrifuge at 2000 rpm for 10 minutes. Supernatant was then taken to measure its absorbance. Substitution media buffer of pH 1.2 to pH 7.4 is done on the clock 3 with filter media solution pH 1.2 with paper Whatmann No. 42 with the aid of a vacuum pump. Nanoencapsul solids were filtered and diluted with media buffer pH 7.4. Data retrieval then do like the previous procedure.

Result and discussion

Andrographis paniculata Extract.

Andrographispaniculata extract is performed three times to produce an average yield of 16% which is almost the same as Yonanda study [13] that is equal to 15.88% and vary by study Mathew, et al. [14] using methanol and generate value yield of 39.8%. This is because the solvent with low viscosity, easier to pour. The use of percolation extraction methods by Dewi [15] produces a yield of 17.09%.

Nanoencapsulation Andrographis paniculata Extract.

Table 1 shows the results of the calculation encapsulation efficiency (EE), loading capacity (LC), and the size of particles obtained from extracts of leaves *sambiloto*. Calculation of nanoparticle encapsulation efficiency is done by calculating the concentration chitosan encapsulated using HPLC results data. The highest encapsulation efficiency was obtained in the variation of chitosan: STPP 2%: 1% with levels of 4.33 mg encapsulated andrographolide. Loading capacity is calculated by the amount of dry *sambiloto* nanoencapsul formed after freeze drying, and the highest yield was obtained also in the variation of chitosan: STPP 2%: 1%.

Table 1. Encapsulation efficiency, loading capacity and particle size

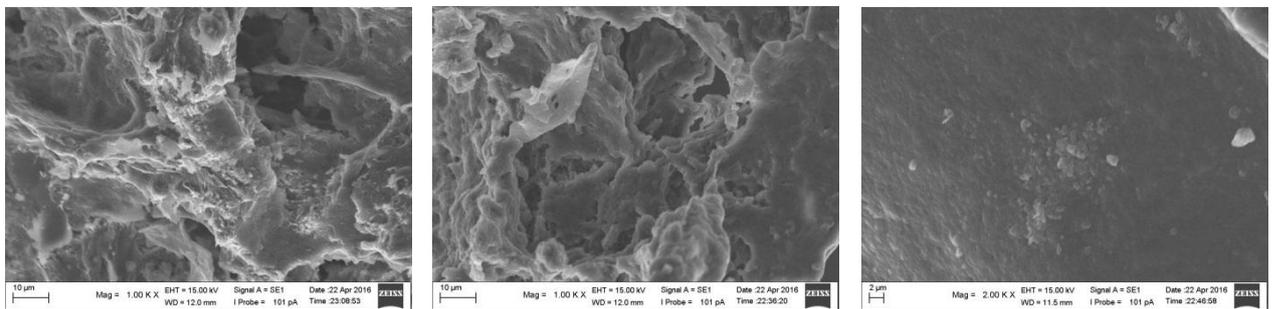
No	Variation Chitosan:STPP	EE	LC	Particle Size (nm)		
				Min	Max	Average
1	1%:1%	55,89%	30,62%	480,4	837,6	677,4
2	1%:1,5%	28,79%	11,09%	526,5	893,4	768,2
3	2%:1%	73,50%	46,29%	557,9	892,6	689,4

High chitosan concentration which makes the number of coated andrographolide caused more surface area of the particles increases so that the greater the value of its efficiency. STPP higher concentration makes at least andrographolide that adsorbated because STPP function as a crosslinking agent that strengthens the matrix of nanoparticles make the diffusion rate of the active ingredient

(andrographolide) decreases.

The particle size and the largest average namely 893.4 nm is obtained in the variation 2, which uses the highest concentration of STPP. This is because the use of STPP high cause solidification droplet quickly when the ionic gelation reaction takes place [16]. Addition of chitosan concentration and STPP would increase the size of the particles [17], and in this study the addition of chitosan concentration on three variations do not produce too big effect on the particle size.

Figure 1 shows the particles produced variations 1 and 2 are not spherical (not round), agglomerated, porous, and the surface is porous and not smooth. The more spherical particle and the more angles at the particles can cause the formation agglomerations [18]. The resulting particle shape variation third round was not perfect with a smooth surface. The higher the concentration of chitosan, the form of particles produced will be spherical (rounded) [19].



(a) (b) (c)

Figure 1. Results FE-SEM (a) Variation 1 (chitosan:STPP 1%:1%) (b) Variation 2 (chitosan:STPP 1%:1,5%) dan (c) Variation 3 (chitosan:STPP 2%:1%)

α-Glukosidase inhibition test.

The result of α -glucosidase inhibition of sambiloto leaf extract, nanoencapsul sambiloto leaf extract, and standard andrographolide shown in Figure

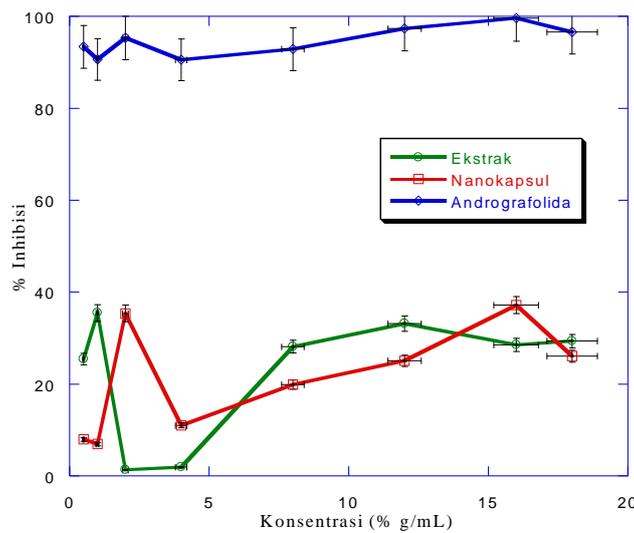


Figure 2. Percent inhibition α -glucosidase

Percent maximum inhibition and optimum concentration of each sample required to inhibit α -

glucosidase enzyme is different. The crude extract of sambiloto leaf yield optimum percent inhibition of 33.17% at a concentration of 12%. Optimum nanoencapsulation extract of chitosan generating percent inhibition were slightly higher at 37.17%, but the concentration required to achieve percent inhibition greater than crude extracts at 16%.

Profile Test Release

The test results release profile shown in Figure 2 generates percent 3. Variasi The cumulative release of the 74.83% at 7, followed by the first variation of 19.92% and 10.45% of the variation 3 at the same hour. This result is inversely proportional to the results of encapsulation efficiency and loading capacity that generate maximum value in the variation 3. It shows that the concentration ratio of chitosan: STPP on third variation of 2%: 1% (g / mL) resulted in a strong cross-linking between the two and characteristics strong matrix so difficult split and make the release (release) drug lasts longer.

The release profile of nanoencapsulation extract of sambiloto leaf depicts the burst release on the condition of the stomach and intestines sustained released on conditions. Characteristics of chitosan unstable under acidic conditions led to the electrostatic repulsion between molecules on the surface of chitosan nanoparticles that produces the drug adsorbed diffusion, thereby making high levels of drug release from the matrix [21] [22]. The events burst release indicates that some drugs (in this study is an extract of sambiloto leaf) adsorbed on the surface of the nanoparticles [23].

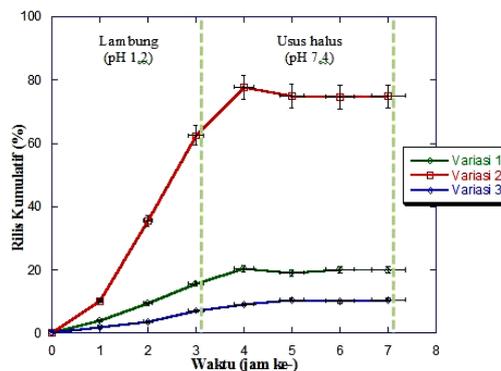


Figure 3. The release profile of sambiloto nanoencapsule leaf extract

Sustained release profile caused by the properties of chitosan as a main ingredient matrix properties insoluble at pH below 6.0, but has resistance at pH above that value, and the hydrogen bonds between the molecules of chitosan nanoparticles create stronger which causes diffusion into the molecule becomes more difficult, followed by release of the drug lasts longer [21].

Conclusion

The application of nanotechnology is proven to help the problems of sambiloto which is limited to the delivery of active compounds into the body by maintaining the release in the long term.

References

- [1] Herdiani, Elvina. (2012). *Potensi Tanaman Obat Indonesia*. [online] Terdapat di <http://www.bbpp-lembang.info/index.php/arsip/artikel/artikel-pertanian/585-potensi-tanaman-obat-indonesia> [diakses 11 September 2015].
- [2] Akbar, Shahid. (2011). *Andrographis paniculata*: A Review of Pharmacological Activities and Clinical Effects. *Alternative Medicine Review* Vol.16, Number 1.
- [3] Chao, Wen-Wan & Lin, Bi-Fong. (2012). Hepatoprotective Diterpenoids Isolated from *Andrographis paniculata*. *Scientific Research* 3,136-143.
- [4] Hidalgo, Maria A., Hancke, Juan L., Bertoglio, Juan C., & Burgos, Rafael A. (2013). Chitosan a New Potential Drug for the Long Term Treatment of Rheumatoid Arthritis Disease. *Intech*.
- [5] Dai, Gui-Fu., Xu, Hai-Wei., Wang, Jun-Feng., Liu, Feng-Wu., & Liu, Hong-Min. (2006). Studies on the Novel α -Glucosidase Inhibitory Activity and Structure-Activity Relationships for Chitosan Analogues. *Bioorganic & Medicinal Chemistry Letters* 16 : 2710-2713.
- [6] Xu, Hai-Wei., Dai, Gui-Fu., Liu, Gai-Zhi., Wang, Jun-Feng., dan Liu, Hong-Min. (2007). Synthesis of Chitosan Derivatives: A New Family of α -Glucosidase Inhibitors. *Bioorganic & Medicinal Chemistry* 15 (2007) 4247-4255.
- [7] Tiyafoonchai, W. (2003). Chitosan nanoparticles: A promising system for drug delivery. *Naresuan University Journal* 11, (3): 51-66.
- [8] Wu, Y., Yang, W., Wang, C., Hu, J., Fu, S. (2005). Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. *International journal of pharmaceutics* 295(1-2):235-45.
- [9] Desai, K. G. H. and Park, H. J., (2005). Encapsulation of vitamin C in tripolyphosphate crosslinked chitosan microspheres by spray drying. *J. Microencapsulation*, 22, 179-192.
- [10] Hu, B., Pan, C., Sun, Y., Hou, Z., Ye, H., Hu, B. And Zeng, X. X. (2008). Optimization of fabrication parameters to produce chitosan tripolyphosphate nanoparticles for delivery of tea catechins. *J. Agric. Food Chem.*, 56, 7451-7458.
- [11] Aini, Desna Qurratul. (2015). *Uji Pelepasan Terkendali Ekstrak Daun Keji Beling (Strobilanthes crispus) untuk Sediaan Obat Antihiperkolesterolemia*. Skripsi. Depok: Universitas Indonesia.
- [12] Aisyah, Nurul. (2014). *Ekstraksi Akar Ilalang (Imperata cylindrica) serta Aktivitas Inhibisinya terhadap α -Glukosidase yang Berfungsi dalam Pembentukan Virus Hepatitis B*. Skripsi. Depok: Fakultas Teknik Universitas Indonesia.
- [13] Yonanda, Rona Utama. (2011). *Formulasi Ekstrak Sambilito (Andrographis paniculata) dan Brotowali (Tinospora crispa) sebagai Inhibitor α - Glukosidase dan Analisis Sidik Jari Menggunakan Teknik Kromatografi*. Institut Pertanian Bogor, Bogor, 9-10.
- [14] Mathew, Arhewoh., Falodun, Abiodun., Okhamafe, Augustine O., Bao, Yang., Sheng, Qui. (2011). Ultrasonic Assisted Extraction and radical scavenging activity of some selected medicinal plants. *Journal of Pharmacy Research*, 4(2), 408-410
- [15] Dewi, Veronica. (2013). *Enkapsulasi Ekstrak Sambilito (Andrographis paniculata) dengan Kasein Susu sebagai Sediaan Antidiabetes*. Skripsi. Depok: Universitas Indonesia.
- [16] Mardiyati, Etik., Muttaqien, Sjaikhurizal EL., & Setyawati, D. R. (2012). *Sintesis Nanopartikel Chitosan-Tripolyphosphate dengan Metode Gelasi Ionik: Pengaruh Konsentrasi dan Rasio Volume terhadap Karakteristik Partikel*. Dipresentasikan pada Prosiding Pertemuan Ilmiah Ilmu Pengetahuan dan Teknologi Bahan, BPPT Serpong.
- [17] Nanda, Rabindra K., Patil, Subodh S., and Navathar, Dipak A. (2012). Chitosan Nanoparticles Loaded with Thiocolchicoside. *Der Pharma Chemica* 4(4):1619-1625
- [18] Saputra, Gia. (2015). *Karakterisasi Nanoencapsulation Chitosan-Ekstrak Etanol 70% Daun Sirih (Piper betle Linn) dengan Metode Gelasi Ionik*. Naskah Publikasi. Pontianak: Universitas Tanjungpura.
- [19] Ko, J.A., et al. (2002). Preparation and Characterization of Chitosan Microparticles Intended for Controlled Drug Delivery. *International Journal of Pharmaceutics* 249:165-174.
- [20] Nagarajan, E., et al. (2015). Development and Evaluation of Chitosan Based Polymeric Nanoparticles of an Antiulcer Drug Lansoprazole. *Journal of Applied Pharmaceutical Science* Vol. 5 (04), pp. 020-025.
- [21] Dhanasingh, Selvakumar & Nallaperumal, Shunmuga Kumar. (2010). Chitosan/Casein Microparticles: Preparation, Characterization and Drug Release Studies. *World Academy of Science, Engineering and Technology* Vol:4 08-23.
- [22] Dounighi, Mohammadpour., et al. (2012). Preparation and In Vitro Characterization of Chitosan Nanoparticles Containing *Mesobuthus eupeus* Scorpion Venom as an Antigen Delivery System. *The Journal of Venomous Animals and Toxins Including Tropical Diseases* Vol. 18, Issue 1, 44-52.
- [23] Nesalin, J. Adlin Jino & Smith, A. Anton. (2012). Preparation and Evaluation of Chitosan Nanoparticles Containing Zidovudine. *Asian Journal of Pharmaceutical Sciences* 7 (1): 80-84.

Production Of *Aspergillus Oryzae* Crude Enzyme Based on Tofu Waste and Tapioca Dregs for Biotransformation of Salvianolic Acid B

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Abstract

The prevalence of heart disease showed that 7.4 million death in the world in 2012, caused by coronary heart disease. Common oral medication was done using nitrate tablets or sprays and showed fast effects but just in short term, so it cannot be used for sustainable medications. *Salvia przewalskii* contains Przewalskinic Acid A which shows strong antioxidant activities and gives potential effects in protecting brain and heart damage. This strong active compound is rare and the content in *S. Przewalskii* herb is very low. Beside the extraction method from *Salvia przewalskii*, Przewalskinic Acid A can be obtained by biotransformation of Salvianolic Acid B from *Salvia milthiorrhiza* using crude enzyme from *Aspergillus oryzae*. Salvianolic Acid B was extracted three times using methanol for 24 hours then resulted the average extraction yield was 5.31% (w/w). Antioxidant activity assay using DPPH resulted that Salvianolic Acid B scavenged DPPH potently with an IC50 7.97 ± 0.16 ppm. After reacted by crude enzyme, Salvianolic acid B shows increase of antioxidant activity 20.2% became 6.36 ± 0.11 ppm in optimum incubation time of *Aspergillus oryzae* and 15.8% became 6.71 ± 0.19 ppm in optimum concentration of nitrogen source. Specific activity of enzyme in variation of incubation time is 0.0068 U/mg, whereas, specific activity of enzyme in tofu waste concentration as nitrogen source variation is 0.0059 U/mg.

Keywords: crude enzyme; incubation time; nitrogen concentration; Przewalskinic Acid A; Salvianolic Acid B

Introduction

The usage of enzyme was proved in various sectors of industries, such as lipase in food industries, pectinase in beverage industry, and penicillin amidase in pharmacy sector (Chaplin, 2014). Enzyme can be produced by animal, plant, and microorganisms, but enzyme production for commercial purposes mostly used microorganisms such as bacteria and yeast. It because yeast easily grows in various medium included waste and the growth of yeast is easily controlled.

Raw material of tofu waste is soybean, which has nitrogen content that enough for yeast which is around 3% (b/b). Tofu waste and tapioca dregs has enough carbon content, which are 41,71 g/100 g and 40,87 g/100 g, respectively (Sumanti, 2003), therefore tofu waste and tapioca dregs can be used as medium for *Aspergillus oryzae* growth.

Aspergillus oryzae are mostly used in food industry for fermented soybean, sauce, beer, etc. This yeast is classified as GRAS group (*Generally Regarded As Safe*) by Food and Drug Administration (FDA) (Tailor *et al*, 1979, Abe *et al*, 2002) and mostly used for commercial usage (Chen, 2007).

There were several factors which influence *Aspergillus oryzae* growth, such as nutrition in medium, ratio of carbon and nitrogen, temperature, strain, pH, aeration, nitrogen concentration, incubation time. Those factors were controlled to produce optimum condition for yeast growth, so yeast could produce secondary metabolite such as enzyme.

Materials and Methods

Materials

A reference substance of Salvianolic Acid B was obtained from Sigma Aldrich, Germany. Plates

of silica gel (60-F254) were purchased from Merck, Germany. Potato Dextrose Agar was obtained from Hi-Media, Merck, SD Fine and SRL, India. Tofu waste and tapioca dregs were obtained from tofu and tapioca home industry in Bogor, Indonesia. Dried *Salvia milthiorrhiza* root was obtained from traditional market in Jakarta, Indonesia.

Microorganism

The *Aspergillus oryzae* was obtained from Indonesian Culture Collection (INACC) LIPI (Bogor, Indonesia) which was isolated from fermented soybean. The solid medium used from maintaining the microorganism was Potato Dextrose Agar (PDA) [3% (w/v) and demineralised water].

Preparation of Tofu Waste and Tapioca Dregs

Tofu waste and tapioca dregs were dried using oven at 80°C for 48 h. Then, tofu waste and tapioca dregs were crushed, and then the powder was sieved.

Analysis of Nitrogen Content in Tofu Waste and Tapioca Dregs

Nitrogen content in tofu waste and tapioca dregs was analyzed using SNI. 01-2891-1992 (7.1 methods). Briefly, 0.5 g sample was mixed with 5 ml H₂SO₄ then were destructed at 300°C until the solution is clear. The mixture was cooled and diluted with aquadest then 20 ml of NaOH 40% was added. After that, the solution was distilled and HCl was added. NaOH was then added to the mixture and titration was performed

Analysis of Carbon Content in Tofu Waste and Tapioca Dregs.

Carbon content was analyzed using Walkley and Black method. Briefly, 0,5 g of tofu waste and tapioca dregs were mixed with 5 ml aquadest in graduated glass. Then, K₂Cr₂O₇ 2N and H₂SO₄ were added to the mixture and was incubated for 30 min. The absorbance was measured by spectrophotometer with wavelength of 651 nm. Standard curve (50, 100, 150, 200, and 250) ppm was made by dilute 1.25 g glucose into 100 ml aquadest. Then, 5 ml K₂Cr₂O₇ 2N and H₂SO₄ were added to the mixture then was incubated for 24 h. The absorbance was measured with spectrophotometer UV-Vis in 651 nm.

Extraction of Salvianolic Acid B from Salvia milthiorrhiza root

Extraction of Salvianolic Acid B from *S. milthiorrhiza* root was done by extracting of dried root of *S. milthiorrhiza* powder with methanol (1:5) for 24 h at room temperature. The extract was filtered under vacuum through filter paper. Then the crude extract containing Salvianolic Acid B was removed the ester using diethyl ether and petroleum ether (1:1), and was dissolved in water (300 ml). The extract was filtered under vacuum then was combined and concentrated by freeze drying to obtain Salvianolic Acid B.

Preparation of Enzyme Inducer

10 g *Salvia milthiorrhiza* root was crushed and added with 60 ml water, boiled for about 1 h, then filtered to remove precipitate, and adjusted to a final volume 20 ml. The filtrate was used as an

inducer for enzyme production.

Cultivation of Aspergillus oryzae in Submerged Fermentation

Aspergillus oryzae was inoculated on PDA medium, and then the spore was harvested and inoculated into 100 ml Erlenmeyer flask. The Erlenmeyer flasks were incubated under agitation (140 r/min) at 37°C. Experiments were done in duplicate and the results were expressed as the mean values. Micronutrients; CaCl₂·2H₂O, FeSO₄·7H₂O, MnSO₄·2H₂O, CuSO₄·5H₂O, and buffer; Na₂HPO₄, KH₂PO₄ were added to the culture to support the yeast growth.

Incubation Time of Aspergillus oryzae

Incubation time was optimized by incubating various flasks at 37°C for 48, 72, 96, 120, 144 h at submerged condition. Enzyme assay was carried out after purification and biotransformation.

Nitrogen Source Concentration

In order to obtain the liquid medium containing different nitrogen concentration, the concentration of tofu waste in the medium was 0.0 g/L; 0.2 g/L; 0.4 g/L; 0.8 g/L; 2 g/L; 4.0 g/L; 8.0 g/L; 10.0 g/L. Respectively. All the flasks were incubated at 37°C on shaker at 140 rpm. At regular intervals, enzyme assay was performed.

Enzyme Purification

The culture was centrifuged to separate biomass with supernatant. Then, enzyme supernatant was precipitated by (NH₄)SO₄ (70% saturation) and stored at 4°C for 12 h. Precipitated enzyme was collected by centrifugation, dissolved in 0.02 M acetate buffer (pH, 1/10 volume of initial culture), then dialyzed in the same buffer for 24 h. The dialyzed enzyme solution was centrifuged to remove the insoluble impurities and diluted with the same buffer to half the volume of the initial culture. The crude enzyme was stored at 4°C for subsequent experiments.

Preparation of Przewalskinic Acid A

To produce Przewalskinic Acid A, (1 ml) crude enzyme of *Aspergillus oryzae* [12] was mixed with the same volume of Salvianolic acid B (16 mg/ml) then reacted for 14 hours at temperature 40°C. Enzymatic reaction was stopped using water-saturated-n-butanol (2 ml), then the mixture was centrifuged to remove insoluble material. Then, supernatant was concentrated by freeze drying to obtain reaction product containing Przewalskinic Acid A.

Thin Layer Chromatography Analysis of Salvianolic Acid B

TLC analysis was performed on silica gel plates developed with ethyl acetate/formic acid (10:1, v/v) [13]. Visualization of TLC plates was performed by spraying with 5% FeCl₃ reagent, followed by heating at 80°C for 3 minutes. Then, TLC results of Salvianolic Acid B and Przewalskinic Acid A were compared.

Antioxidant Activity of Przewalskinic Acid A Assay using DPPH

The antioxidant activity of plant extract was measured using DPPH, a stable free radical. Extract solution was dissolved in methanol using ultra-sonicator. 1 ml extract solution was mixed with 1 ml DPPH, and 2 ml methanol, and then the mixture was incubated for 30 minutes at temperature 37°C in the dark. The scavenging activity was measured by noting the decrease in absorbance at 515-516 nm as compared to DPPH control using VersaMax ELISA Microplate Reader instrument. The analysis was done in triplicate. Inhibition of free radical DPPH in percent (DPPH scavenged) was calculated from the absorption according to the following equation:

$$\text{DPPH Scavenged \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Dose response curve was plotted between % inhibition and concentrations. IC50 values defined as the concentration (in ppm) of the extract required to deplete the amount of DPPH radical by 50%. Then, IC50 value of Salvianolic Acid B and Przewalskinic Acid A were compared. Crude Enzyme Activity Assay using HPLC.

HPLC analysis was performed on a Shimadzu HPLC LC20 instrument

A Hypersil HyPurity C18 column (Φ 4.6 × 250 mm, 5 μ m) was used to analyze the enzymatic reaction products, and a mobile phase of methanol (solvent A)/distilled water containing 1.0 acetic acid (solvent B) at flow rate of 1.0 ml/min was used as follows: 0-5 min, A was 5%, 5-15 min, A was 15%, 15-30 min A was 50%, 30-40 min, A was 90%; monitoring at 286 nm. The injection volume was 20 μ l and the column temperature was set at 35°C. One unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 nmol Przewalskinic acid A per minute under optimum condition.

Result and discussion

Based on the analysis of carbon and nitrogen content, from Table 1, it is known that tapioca dregs contained high carbon and low nitrogen, whereas tofu waste contained adequate carbon and higher nitrogen than tapioca dregs. Therefore, tofu waste was potentially used for nitrogen source and the two of them were potentially used as carbon source.

Table 1. Carbon and Nitrogen Content in Tofu Waste and Tapioca Dregs

Materials	Carbon Content (g/100 g)	Nitrogen Content (g/100g)
Tofu Waste	30.25	0.19
Tapioca Dregs	26.50	3.00

In proximate analysis, found that tapioca dregs had higher carbon content and higher nitrogen content was in tofu waste (Sumanti *et al.*, 2005). Tapioca dregs from industry contained 14,54% heavy fiber (Nurhayati *et al.*, 2006), whereas tofu waste contained 3,76% heavy fiber (Suprapti, 2005). Tapioca dregs contained higher heavy fiber than tofu waste, and its carbon was hard to degrade with

microorganism (Utari, 1997). Tapioca dregs medium was good to combined with tofu waste which easier to degrade with *Aspergillus oryzae*.

Extraction of Salvianolic Acid B from Salvia milthiorrhiza

The result of extraction from *Salvia milthiorrhiza* root showed two layers, the upper layer was filtrate and the bottom layer was the dregs of *Salvia milthiorrhiza*. The filtrate contained methanol solvent, and extract color was brown. Brown color showed that the solvent can extract the active compound in *Salvia milthiorrhiza* root propitiously.

The extraction process resulted in yield extract of 5.31 g and the yield value was 5.31% (b/b). This result was lower than Tomahayu (2014) with the same method and solvent. The yield value was 9.61%. It could be caused by quality of the *Salvia milthiorrhiza* root, size of the extracted powder, and age of plant (Putri et al., 2014). Besides that, the difference of drying method and solvent ratio resulted in the difference of yield extract.

Cultivation in Submerged Fermentation

During cultivation process, there was alteration of medium color compared to medium control without yeast. Control medium without yeast was white and turbid whereas medium which cultured 120 h, was altered to orange. This was caused by metabolism activity of *Aspergillus oryzae* which utilized tofu waste and tapioca dregs as nutrition for growth.

Incubation Time

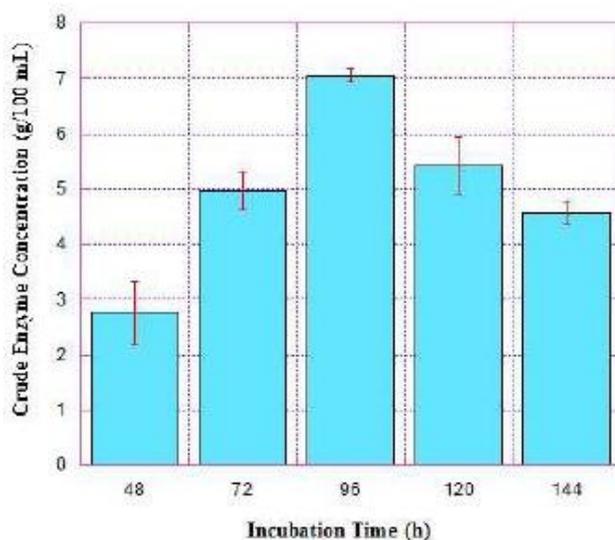


Figure 1. Effect of Incubation Time of *Aspergillus oryzae* on Concentration of Crude Enzyme

In variation of incubation time, the optimum incubation time which gave highest enzyme concentration was 96 h, as seen in Figure 1. In 48 h incubation time, *Aspergillus oryzae* tend to produce low concentration of enzyme because in range of 24-48 h of incubation, yeast cell started to sprout with the growth of mycelia was 28%. In 72 h of incubation, the growth of mycelia was 92%. *Aspergillus oryzae* completely sprouted in 96 h of incubation (Retnowati, 2009). At 96 h, yeast was at the peak of exponential phase (*doubling time*) and started to enter stationer phase. At exponential phase, yeast cell

cleaved actively. During cleaved process and peak of exponential phase, hydrolysis protein was used for protein formation for endospore and vegetative cell, there for it would enhance the protein content and enzyme in cell (Prestidge *et al.*, 1971, James *et al.*, 1985).

Stationary phase of *Aspergillus oryzae* was known at 120 h to 144 h. At that phase, cell started to deficiency in nutrition and the growth rate was slow therefore cell enter death phase. Same result was also found by Abdulameer *et al.* (2015), the activity of inulase enzyme in 120 h of incubation affected the reduction of enzyme activity 45,69% from 140 U/mg to 76,3 U/mg. The increase of incubation time will decrease enzyme production. It might be caused by deficiency of nutrient, accumulation of toxic substances (Kheng and Omar, 2004).

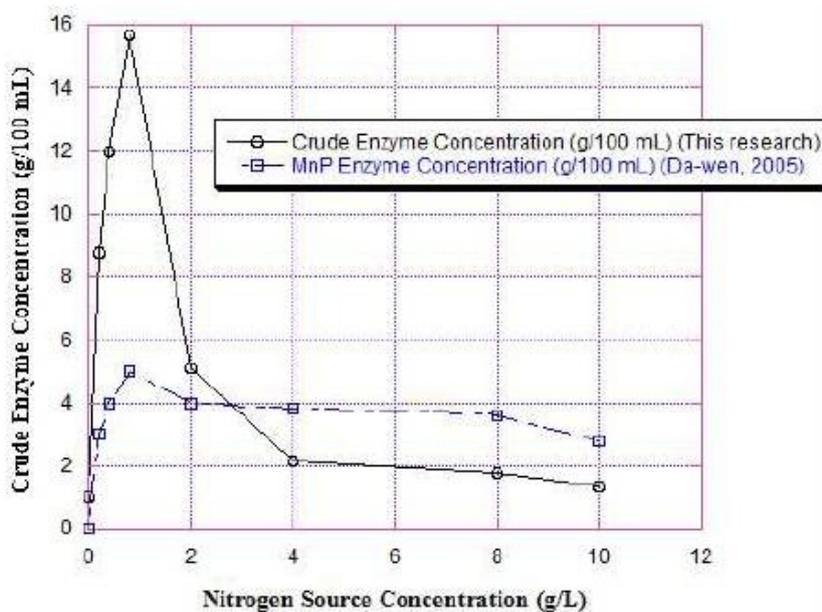


Figure 2. Effect of Nitrogen Source Concentration of *Aspergillus oryzae* on Concentration of Crude Enzyme

Figure 2, shows that production of *Aspergillus oryzae* enzyme affected by nitrogen concentration in medium as stated by Okafor *et al.* (2013), that the ability of yeast in produce secondary metabolites was highly influenced by amount of nutrient. Nitrogen was the most important element for living thing especially yeasts, because nitrogen was main component in every kind of protein and nucleic acid. Yeast could use various nitrogen source (organic and inorganic) in certain range as structural component and functional in cell (peptide and protein), polyamine, and nucleic acid. Yeast had ability in break the N2 covalent bond to release free nitrogen. Primary structure of protein was formed by N-tip and ended with C-tip (Buxbaum, 2007).

Medium which contained low nitrogen content cannot supply for the requirement of enzyme formation, whereas the excess of nitrogen could disrupt yeast growth and in some case can decelerate of enzyme production (Kole *et al.*, 1988). Yeast could produce secondary metabolite if the supply of nitrogen was minimum of $\pm 1 \times 10^{-3}$ M (Griffin, 1981). *Aspergillus oryzae* could produce crude enzyme because the nitrogen level was in 4.29×10^{-3} M, thus above the minimal level. The enhancement of nitrogen content occurred until nitrogen level of 17.14×10^{-3} M (0.8 g/L). In nitrogen content of $42.85 \times$

10⁻³ M, enzyme production decrease significantly. This means, enzyme content was excess and disrupt yeast growth (Kole *et al.*, 1988). In Figure 2, the addition of tofu waste compared to addition of ammonium tartrate, showed similar result. The increase in ammonium tartrate was not responsible for the increase of MnP enzyme concentration in *Phanerochaete chrysosporium*. Concentration of ammonium tartrate which gave the highest enzyme concentration was 0.8 g/L, after 0.8/L and caused the reduction of MnP enzyme. It was caused by complicated metabolism of nitrogen in filament yeast; therefore disrupt metabolism process (Feldman, 2012).

The increase of tofu waste concentration in medium made medium more condensed and difficult to agitate therefore leads to the reduction of aeration. Medium with addition of 0.2 g/L was more clear compared to addition 10 g/L. So, it could be concluded that there was the difference in aeration. Aeration played role in protein and amino acid composition in yeast. Vananuvat and Kinsella (1975) found that yeast which cultivated in *batch* culture with high agitation speed tend to produce higher enzyme concentration than with lower agitation speed. Rahardjo *et al.* (2005) also found that low aeration caused low oxygen content in medium. Low oxygen content was act as inhibitor for yeast growth and production of hydrolytic enzyme in *Aspergillus oryzae*. High concentration of tofu waste caused more condensed medium so aeration obstruction increased, therefore affected the reduction of *Aspergillus oryzae* enzyme.

Result of Thin Layer Chromatography

TLC was performed in plate silica gel with spraying chromogenic reagent FeCl₃ followed by heating for 3 minutes. After heating, on the plate appeared black marks. The marks showed the R_f for standard of Salvianolic Acid B, Salvianolic Acid B from extraction, and Przewalskinic Acid A were, 0.928, 0.957, and 0.972, respectively. There were little differences in R_f values of standard of Salvianolic Acid B, Salvianolic Acid B from extraction, and Przewalskinic Acid A, and it showed that the three samples contained phenol, qualitatively.

DPPH Assay for Antioxidant Activity

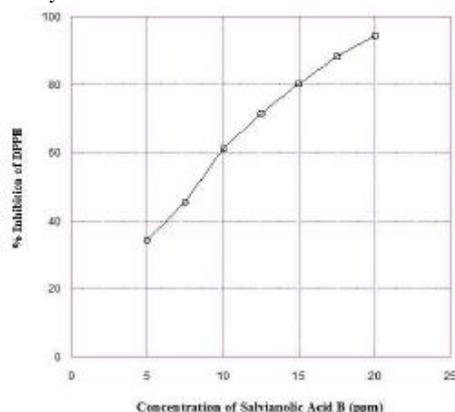


Figure 3. Percent Inhibition of DPPH of Salvianolic Acid B from Extraction

Figure 3, show that the increases of salvianolic acid B concentration affect the increase of percent inhibition of DPPH. Percent inhibition defined as the amount of free radical DPPH which successfully alter into non radical compound in certain concentration of antioxidant. From the graph, it is known that IC₅₀ of Salvianolic acid B was 7.97 ± 0.16 ppm. This value was near to IC₅₀ from previous

research (Lin, 2014), of 7.75 ppm with methanol and water for extraction. Therefore, Salvianolic acid B was classified into strong antioxidant (Ariyanto, 2006). Salvianolic acid B was reacted with crude enzyme in optimum condition for enzymatic reaction for 14 h. Reaction between extract-enzyme was stopped by addition of alcohol to denature enzyme. Insoluble material was separated with centrifugation, then supernatant was concentrated with freeze dryer to get reaction product. Przewalskinic acid A which gets from reaction from crude enzyme in optimum incubation time was evaluated by DPPH assay and gave result as seen in Figure 4.

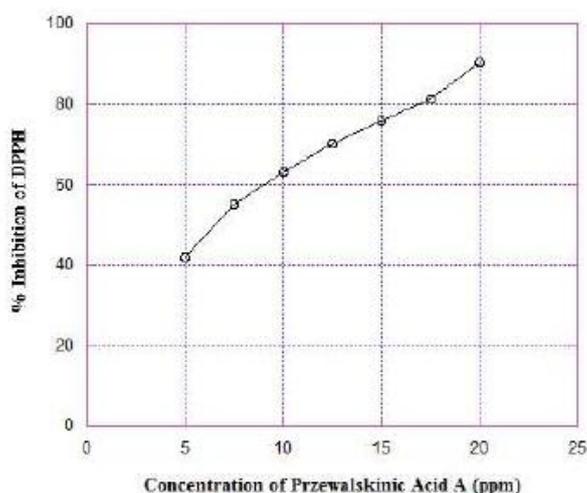


Figure 4. Percent Inhibition in DPPH Assay of 96 h Incubation Time

Figure 4, shows that the increase of Przewalskinic acid A concentration caused the increased in percent inhibition of DPPH. IC50 value was the amount of antioxidant which can reduce 50% free radical, DPPH. From Figure 4, known that IC50 for Przewalskinic acid A in optimum incubation time (96 h) was 6.36 ± 0.11 ppm. That value shows enhancement of 20.2% antioxidant activity compared to Salvianolic acid B. Przewalskinic acid A as the result of biotransformation with crude enzyme in

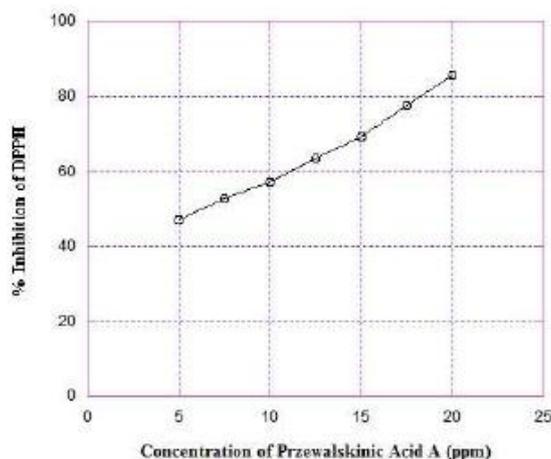


Figure 5. Percent Inhibition in DPPH Assay of 0.8 g/L Tofu Waste Crude Enzyme Activity in Optimum Incubation Time

Retention time of Salvianolic acid B was in range of 35-37 min [Figure 6. (a)], whereas retention time of Przewalskinic acid A was in 32 min (Liu, 2014). Figure 6, shows the reduction *peak* of Salvianolic acid B from extraction [Figure 6. (a)], compared to *peak* in 32 min in after reacted by crude

enzyme of *Aspergillus oryzae* [Figure 6 (b)]. From Figure 6. (b), seen that appeared new *peak* in 27-32 min range which known as Przewalskinic acid A. This chromatogram indicated reduction of Salvianolic acid B content which later converted into Przewalskinic acid A (Liu, 2014). From HPLC data known that 1 g Salvianolic acid B was converted into 0.00041% into 0.0041 mg Przewalskinic acid A, then given product formation rate was 0.0068 nmol/mL/min, therefore activity of enzyme was 0.0000274 U/ml. Specific activity of crude enzyme in optimum incubation time was 0.0068 U/mg. Specific enzymatic activity of crude enzyme was classified into low compared to specific enzymatic activity of inulinase from *Aspergillus niger* AN20 which was incubated 96 h. Specific enzymatic activity of inulinase enzyme from *Aspergillus niger* AN20 was 140.5 U/mg (Abdulameer et al, 2015). This difference could be caused by the impurities content and enzyme molecule which denaturated (Scopes, 1982).

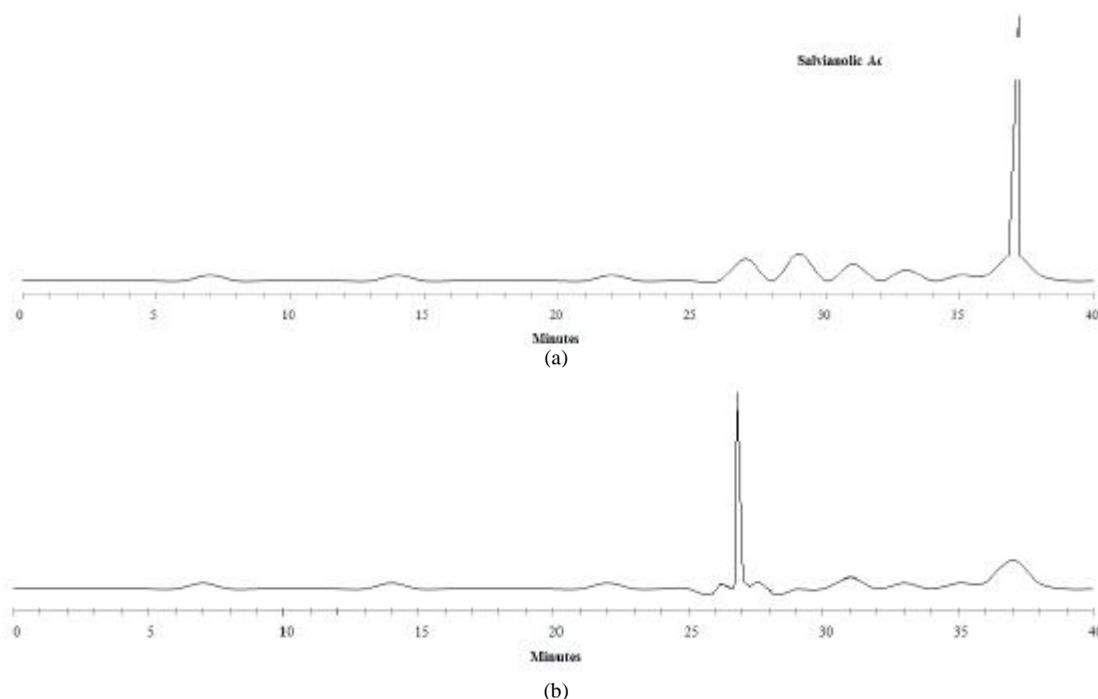


Figure 6. (a) Chromatogram of Salvianolic Acid B from Extraction, (b) Chromatogram of Przewalskinic Acid A from Optimum Incubation Time

Crude Enzyme Activity in Optimum Nitrogen Source Concentration

From Figure 7 (a) and (b), known there was reduction *peak* of Salvianolic acid B from extraction [Figure 7 (a)], compared to *peak* in 32 min after reacted by crude enzyme of *Aspergillus oryzae* [Figure 7 (b)]. From [Figure 7 (b)], seen that appeared new *peak* in 27-32 min range which known as Przewalskinic acid A. This chromatogram indicated reduction of Salvianolic acid B content which later converted into Przewalskinic acid A (Liu, 2014). From HPLC data known that 1 g Salvianolic acid B was converted into 0.00036% into 0.0036 mg Przewalskinic acid A, then given product formation rate was 0.0059 nmol/mL/min, therefore activity of enzyme was 0.0000237 U/ml. Specific activity of crude enzyme in optimum nitrogen source concentration was 0.0059 U/mg. Specific enzymatic activity of crude enzyme was classified into low compared to specific enzymatic activity of amylase from *Aspergillus oryzae*, 54.54 U/mg. This difference could be caused by the impurities content and enzyme molecule which denaturated (Scopes, 1982). Kind of nitrogen source also affected activity and enzyme concentration

which produced (Hamilton *et al*, 1999). Nitrogen source from *yeast extract* gave high enzymatic activity of amylase, 12 U/ml whereas medium from soybean gave enzymatic activity 7 U/ml.

It was caused by *yeast extract* was pure nitrogen source compared to tofu waste. Tofu waste contained many impurities and nitrogen content in tofu waste was bonded with other organic compound such as carbohydrate and mineral. Nitrogen which bonded with other organic compound will form more complex compound therefore more difficult to degrade by yeast and convert into extracellular enzyme (Scopes, 1982).

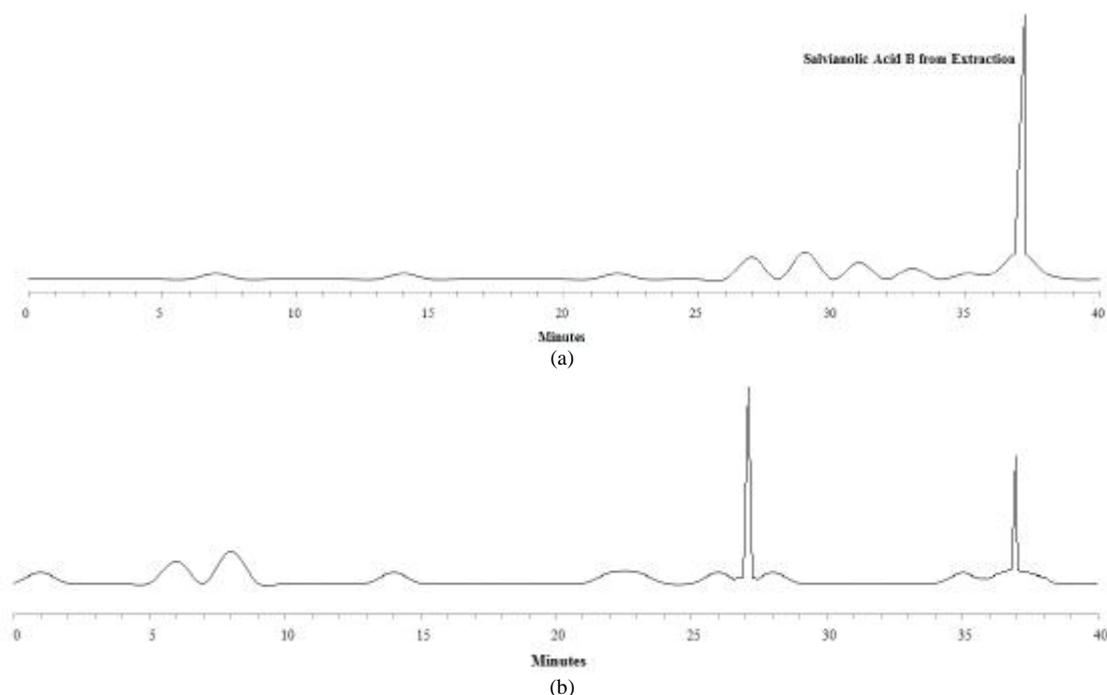


Figure 7. (a) Chromatogram of Salvianolic Acid B from Extraction, (b) Chromatogram of Przewalskinic Acid A from Optimum Nitrogen Source Concentration

Conclusion

Incubation time of *Aspergillus oryzae* which produce highest crude enzyme was at 96 h with specific enzymatic activity was 0.0068 U/mg. At optimum incubation time, enzyme gave 20.2% enhancement of antioxidant activity. Concentration of tofu waste which gave highest crude enzyme was 0.8 g/L with specific enzymatic activity was 0.0059 U/mg. In optimum nitrogen concentration, enzyme gave 15.8% enhancement of antioxidant extract.

References

- Abdulameer, Zainab W., Bahaa N. Essa, Ghazi M. Aziz. 2015. Optimum condition for Inulinase production by *Aspergillus niger* using solid state fermentation. *Baghdad Science Journal*. 12 (2):307-316.
- Abe, Keietsu. 2002. *Aspergillus: Biology and Genomics*. Norfolk, UK: Caister Academic Press.
- Ariyanto, R.. 2006. Uji Aktivitas antioksidan: Penentuan Kandungan Fenolik dan Flavonoid Total Fraksi Kloroform dan Fraksi Air Ekstrak Metanolik Pegagan (*Centella asiatica L. Urban*). *Skripsi*. Fakultas Farmasi Universitas Gadjah Mada.
- Buxbaum, Engelbert. 2007. *Fundamentals of Protein Structure and Function*. Springer Science & Business Media.
- Chaplin, Martin. 2014. *Enzyme Technology*. Cambridge University Press.
- Chen, CW. 2007. Proteomic Analysis of Extracellular Proteins from *Aspergillus oryzae* Grown under Submerged and Solid State Culture Conditions. *Applied and Environmental Microbiology*. p. 3448-3457, Vol. 72, No. 5.
- Da-wen, GAO. 2010. Membrane fouling in an anaerobic membrane bioreactor: Differences in relative abundance of bacterial species in the membrane foulant layer and in suspension. *Journal of Membrane Science* 364:331-338.
- Feldman, Horst. 2012. *Yeast: Molecular and Cell Biology, 2nd Revised and Greatly Enlarged Edition*. Munchen, Germany: Wiley-Blackwell.

- Griffin, H.D. 1981. *Fungal Physiology*. New York: John Wiley and Sons, Inc.
- Hamilton, Lynn M., Chaterine T. Kelly, William M. Fogarty. 1999. Production and properties of the raw starch-digesting (α -amylase) of *Bacillus sp.* IMD 435. *Process Biochemistry*. (35):27-31.
- James W. and J. Mandelstam, "Protease production during sporulation of germination mutants of *Bacillus subtilis* and the cloning of a functional *gerE* gene." *Journal of General Microbiology*, vol. 131, no. 9, pp. 2421–2430, 1985.
- Kheng, P. P. and Omar, I. O. 2004. Inulinase production by a local fungal isolate. *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. *Songklanakarin J. Sci. Technol.* 27(2): 326-336.
- Kole, MM., Draper I, Gerson DF. 1988. Protease production by *Bacillus subtilis* in oxygen-controlled glucose fed-batch fermentation. *Appl Microbiol Biotechnol.* 28:404–8.
- Lin, Yun-Lian., Chia-Hung Wu, Miao-Hua Luo, Yeh-Jeng Huang, Chuen-Neu Wang, Ming-Shi Shiao, Yi-Tsau Huang. 2005. In Vitro Protective Effects of Salvianolic Acid B on Primary Hepatocytes and Hepatic Stellate Cells. *Journal of Ethnopharmacology*. (105): 215-222.
- Liu, Chunying., Yinghua Jin, Hongshan Yu, Chankai Sun, Shuiping Zhou, Yonghong Zhu, Longquan Xu, Chong Yu, Fengxie Jin. 2014. Preparation of przewalskinic acid A from salvianolic acid B using a crude enzyme from an *Aspergillus oryzae* strain. *J Ind Microbiol Biotechnol.* Vol 41:887–892.
- Nurhayati; Sjojan, O dan Koentjoko. 2006. Kualitas Nutrisi Campuran Bungkil Inti Sawit dan Onggok yang difermentasi Menggunakan *Aspergillus niger*. *J.Indon. Trop. Anim. Agric.* 31 [31 September 2006]
- Okafor, George. U. O., F. O. Tasie., dan Anyamene. 2013. Studies Low- cost Substrate and Other Cultural Conditions for Optimal Amylase Yield from *Aspergillus oryzae* SR2. *Americal Journal of Food Technology*. (2):1-11.
- Prestidge L., V. Gage, and J. Spizizen. 1971. Protease activities during the course of sporulation on *Bacillus subtilis*. *Journal of Bacteriology*. Vol. (107) No. 3, pp. 185–213, 1971.
- Putri, Dea Alvicha, Sumpono, Ginting, Sura Menda. *Pengaruh Metode Ekstraksi Dan Konsentrasi Terhadap Aktivitas Jahe Merah (Zingiber Officinale Var Rubrum) Sebagai Antibakteri Escherichia Coli*. TESIS. Universitas Bengkulu.
- Rahardjo, Yovita S.P., Susana Sie, Frans J. Weber, Johannes Tramper, Arjen Rinzema. 2005. *Effect of Low Oxygen Concentration on Growth and Alpha Amylase Production of Aspergillus oryzae in Model Solid-State Fermentation Systems*. *Biomolecular Engineering*. (21):163-172
- Retnowati, Yuliana., Wirnangsi D. Uno., Syam Kumaji., Yulianti Humokor. 2009. *Pertumbuhan Kapang Monascus purpureus, Aspergillus flavus dan Penicillium SP Pada Media Beras, Jagun dan Kombinasi Beras Jagung*. Gorontalo: Universitas Negeri Gorontalo.
- Scopes, RK. 1982. *Protein Purification. Principles and Practices*. New York, pp:282-293.
- Sumanti, Debby M, Carmencita Tjahjadi, Marleen Herudiyanto, Tati Sukarti, "Mempelajari Mekanisme Produksi Minyak Sel Tunggal dengan Sistem Fermentasi Padat pada Media Onggok-Ampas Tahu dengan Menggunakan Kapang *Aspergillus terreus*." *Jurnal teknologi dan industri pangan*, vol.XVI (2005), hal. 51-61.
- Suprapti, Lies. 2005. *Pembuatan Tahu*. Yogyakarta: Kanisius.
- Taylor, MJ and Richardson. 1979. *Aspergillus: Biology and Genomics*. Norfolk, UK: Caister Academic Press.
- Utari, Rita. 1997. Seleksi Kapang Mucor Untuk Produksi Minyak Mengandung Asam Gamma Linolenat dengan Sistem Fermentasi Padat pada Media Onggok-Ampas Tahu dan Onggok-Dedak Padai. Skripsi. FATETA. IPB Bogor.
- Vananuvat, P dan J.E. Kinsella. 1975. Amino Acid Composition of Yeast *S. Fragilis*. *J. Agr. Food Chem* 23:595.

Utilization of Pregerminated Jackbean and Soybean for Increasing The Protein Content of Instant *Tiwul*

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Abstract

Instant *tiwul* is made principally from cassava flour. This product contains low in protein without addition of ingredients which high in protein content such as legumes. Therefore, the objective of this research was to improve the quality of instant *tiwul* especially its protein content using pregerminated jackbean, pregerminated soybean, tapioca and skimmed milk.

The results indicated that the best quality of instant *tiwul* was obtained by using weight proportion of cassava flour-pregerminated jackbean-tapioca of 70:20:10 with 4% skimmed milk addition. The Product had good sensory characteristics (texture, flavor, hedonic value). The instant *tiwul* prepared by substituting pregerminated beans flour and skimmed milk addition contained protein and crude fat in the range of 6-12% and 0.70-3.47% dry weight repectively. The cooked instant *tiwul* still had a specific aroma of jackbean and soybean as well.

Keywords : Instant *tiwul*; cassava flour; pregerminated jackbean, pregerminated soybean; skimmed milk

Introduction

Tiwul is one of traditional Javanese food made from cassava that is mashed then steamed (Andrarini, 2004). *Tiwul* steaming results are usually eaten with grated coconut. *Tiwul* have low nutritional value especially protein and fat amount 1,2 g / 100 g of and 1.5 g / 100 g (Andoko and Parjimo, 2007). As a main food in some districts, *Tiwul* made from cassava needs to be improved content results in protein with the addition of flour substitution in high protein content such as peanut flour, tubers, cereals and fish flour to increase in nutrients (Suarni, 2004; Wardayani *et al.*, 2008; Ade-Omowaye *et al.*, 2008). Rukmini and Naufalin (2015) have examined instant *tiwul* using flour substitution germ of cereals to increase the protein content. In this study, flour substitutes used were pre-germinated jackbean and soybean flour.

Jackbeans protein content is quite high at around 18- 25%, the fat content is between 0,2- 3.0% and the carbohydrate content is 50-60% (Mesen and Somaatmadja, 1993). Jackbean has a great potential to be an alternative food source of protein for the balance of amino acids and high bioavaibility (Windrati *et al.*, 2010). Soybeans have a high protein content of 35-38% and fat content of 16-20% (Afandi, 2001).

Beans are high protein sources but have non-nutrition substances. The process of pregermination cause biochemical changes that include increased levels of reducing sugars, increase the concentration of free amino acids, reduced levels of fat, among others into fatty acids monoglycerides and reduced anti-nutrition substances such as phytic acid and oligosaccharides cause flatulence as raffinosa, stakiosa and verbakosa (Koswara, 1992). Pregermination, at the start of germination is carried out for 24 hours or early shoots to sprout (2-5mm). To further increase the protein content of instant *tiwul* added skimmed milk (low fat) 2-6%. Skimmed milk is the milk part is left after cream is taken partially or entirely. Skimmed milk contains all the nutrients of milk except fat and vitamins that are fat soluble (Buckle *et al.*, 1987).

The main process of instant *tiwul* is gelatinization starch in the presence of water in the dough mixture of flour and their heat during steaming (steam blanching). Instant *tiwul* is *tiwul* steaming results are then dried to reduce the moisture content. For instant products, instant *tiwul* should be ready to cook (fast). That is easily rehydrated and has physicochemical properties such as instant *tiwul* steaming results mainly chewy texture and no retrogradation of starch (hardening). According to Taiwo and Adeyemi (2009), rehydrating the dried food product is affected by several factors such as chemical composition, drying technique, medium composition and temperature for rehydration. The addition of hydrocolloids to the dough can improve the physicochemical properties of the product including increased water binding capacity and inhibit starch retrogradation (Alam *et al.*, 2009; Subari *et al.*, 2011). Instant *Tiwul* is in dried product and the product must be rehydrated into products ready for consumption before being dried (Opara *et al.*, 2013).

The main purpose of research is to produce instant dry *tiwul* (ready to cook dried instant *tiwul*) with high rehydration capacity, high protein content and delicious flavor, aroma and chewy texture. These objectives achieved by attention to the treatment applied to the base material (cassava) and material substitution (jackbean and soybeans), specifies the percentage of skimmed milk to produce the optimal instant *tiwul* with high protein content.

Materials and Methods

Materials and Tools

Materials used includes cassava, soybeans, jackbeans (Bogor), skimmed milk, sugar, salt, baking soda, powdered agar, STPP, vanillin and chemicals for analysis according to the procedure used. Tools used includes analytical balance, electric oven, blender, stainless steel sieve of 60 mesh and 80 mesh, granular mold, household cooking equipment and glassware for analysis.

Preparation of cassava flour

Fresh cassava peeled, sliced with a slicer ± 2 mm and washed, then soaked in baking soda and water is added until a pH of about 9 for 15 minutes. After 15 minutes, cassava is washed with water to pH ± 7 and dried on cabinet dryer with a temperature of 50°C until a broken dry ± 24 hours. Dried cassava is milled and sieved to 80 mesh sieve to produce cassava flour (Rukmini *et al.*, 2014).

Preparation of pregerminated jackbean flour

Jackbean poured with boiling water and soaked for 8 hours sealed and observed until have been pre germination or grow 2-3 mm in that time. Then pregerminated jackbean was added to the filter or ordinary cloth. Then do boiling in 3% NaOH solution for 7-8 minutes. Washed and kneaded until neutral. Do steam blanching for 30 minutes, then chopped to shrink the size of a pea to be faster in the drying process. Dried in a cabinet dryer temperature 55°C until dry fractures (24 hours). Crushed and sieved to 80 mesh size sieve to produce pregerminated jackbean flour (Rukmini *et al.*, 2014).

Preparation of pregerminated soybean flour

Soybean washed and soaked for 12 hours and then washed again. Settling on a banana leaf and closed for 12 hours until pregermination or potential stems grow 2-3 mm. Then washed and boiled for 15 minutes and then cooled and peeled the husk. The next process is steamed for 15 minutes and drained. Dried in a dryer cabinet temperature of 55°C until dry fractures (24 hours). Crushed and sieved to 80 mesh size sieve to produce pregerminated soybean flour (Rukmini *et al.*, 2014).

Experimental Design

Experimental factorial study using a randomized block design. Areas of study consists of types flour substitution (K) is K1 = pregerminated jackbean flour and K2 = pregerminated soybean flour, the proportion of cassava flour - flour substitution (pregerminated of bean flour) - Tapioca (P, b/b), namely P1 = 75:15:10, 70:20:10 and P2 = P3 = 65:25:10 ; addition of skimmed milk (M; w / w of the total flour) that M0 = 0%, M1 = 2%, M2 = 4% , M3 = 6%. Of the three factors obtained by factorial experimental design as much as 2 x 3 x 4 = 24. Each experiment was repeated 2 times.

Statistical analysis

The analysis conducted on instant *tiwul* include moisture content, ash content, total protein content, soluble protein content and fat content (Sudarmadji et al., 1997), rehydration coefficient (Audu and Arema, 2011). The analysis conducted on cooked instant *tiwul* include sensory analysis (Setyaningsih et al., 2010). Analysis of the color, aroma, taste and preferences conducted sensory test method scoring (1-4) by 15 semi-trained panelists. Data were analyzed by Fischer (test F) and followed by Duncan's Multiple Range Test (DMRT).

Result and discussion

Pshychochemical Characteristics of *Tiwul* Instan is presented in Table 1.

Table 1 Pshychochemical data treatments of Instan *Tiwul*

Treatment	Moisture	Ash	Rehidration Coefficient	Soluble Protein
K1P1M1	3.285	0.980	3.170	0.0274
K1P1M2	2.885	1.165	3.885	0.0310
K1P1M3	3.315	1.295	3.235	0.0326
K1P2M1	3.420	1.270	3.930	0.0313
K1P2M2	3.190	0.979	3.565	0.0356
K1P2M3	2.825	1.290	2.975	0.0339
K1P3M1	4.055	1.310	3.280	0.0307
K1P3M2	3.230	1.430	3.415	0.0373
K1P3M3	3.065	1.425	3.360	0.0440
K2P1M1	3.615	1.037	3.760	0.0338
K2P1M2	3.350	0.849	5.535	0.0393
K2P1M3	3.700	1.020	3.840	0.0740
K2P2M1	3.705	1.250	3.415	0.0648
K2P2M2	3.610	0.945	3.240	0.0667
K2P2M3	3.920	1.049	2.940	0.0555
K2P3M1	3.795	1.265	3.435	0.0684
K2P3M2	4.320	1.225	3.225	0.0714
K2P3M3	3.735	1.250	2.810	0.0697

Information :

P ₁ = 75:15:10	K ₁ = pregerminated jackbean flour	M ₀ = skimmed milk 0%
P ₂ = 70:20:10	K ₂ = pregerminated soybean flour	M ₁ = skimmed milk 2%
P ₃ = 65:25:10		M ₂ = skimmed milk 4%
		M ₃ = skimmed milk 6%

A. Moisture content

The study had no significant effect on moisture content instant *tiwul*. The moisture content of instant *tiwul* substituted pregerminated soybean flour ranged from 2.83 to 4.06% mm lower than the moisture content instant *tiwul* substituted pregerminated jackbean flour that ranged from 3.35 to 4.32% bb. The higher proportion of flour substitution (pregerminated jackbean or soybean flour) were added, the moisture content will be higher. Low levels of water caused high storability of instant *tiwul*.

B. Ash Content

The ash content indicates mineral content, the purity and cleanliness of a material (Nuri et al., 2011). The study treatment did not significantly affect the ash content of instant *tiwul*. The results of the analysis of the ash content on *tiwul* instant substituted pregerminated jackbean flour ranged from 0.79 to 1.22% bk, while the instant *Tiwul* with pregerminated soybean flour ranged from 1.00 to 1.43% bk. This is similar to research Oluwamukomi et al. (2010), wheat-cassava biscuits substituted soybean flour 10% has ash content of 1.34%.

C. Rehydration coefficient

Rehydration coefficient indicates a high binding power or water rehydration of instant *tiwul* when its re-cooked. The high coefficient means instant *tiwul* easily bind water, therefore, when cooking with steam or added with water becomes soft texture, cooked *tiwul* is easily chewed.

The results of the analysis of the coefficients rehydration of instant *tiwul* substituted pregerminated jackbean flour has tended to be higher range (3.84 to 4.25) than using pregerminated soybean flour (3.41 to 4.17). It's possible because stripping jackbean is boiled in 3% NaOH solution (lye peeling) for 7-8 minutes. In alkaline conditions the protein is more soluble in water (Fennema, 1996). The presence of soluble protein amount of water causes the protein in the dry *tiwul* easily binds water molecules when *tiwul* put into the water when the coefficient rehydration analysis. Rukmini and Naufalin research results (2015), instant *tiwul* substituted cereals flour has a coefficient in the range of 3 - 4. At this range, instant *tiwul* easily cooked or have a shorter cooking time.

D. Protein content solubility

The results of the levels of soluble protein analysis showed instant *tiwul* substituted pregerminated soybean flour have dissolved higher protein content than the instant *tiwul* substituted pregerminated jackbean flour. At the time of pre-germination, the protein would be hydrolyzed by the enzyme protease into peptides and amino acids, while the starch hydrolyzed by the enzymes amylase, among others, into glucose, maltose, dextrin. Soluble protein content of instant *tiwul* with highest proportion of flour substitution additions can be seen in Figure 1.

E. Protein and Fat Contents of Instant *Tiwul*

Determination of the best combination of instant *tiwul* based on the highest levels of soluble protein. Instant *tiwul* substituted pregerminated soyben flour had higher levels of protein and fat contents when compared to the instant *tiwul* substituted pregerminated jackbean flour. Levels of protein and fat content instant *tiwul* substituted of pregerminated soyben flour P3M3 (proportion 65:25:10; 6% skimmed milk) are continuously 12.04% and 3.47% bk, while the levels of protein and fat content instant *tiwul* substituted pregerminated jackbean flour (the proportion of 65:25:10; 4% skimmed milk) are continuously

6.24% and 0.70% bk. Fat and Protein content of instant *tiwul* on the treatment with proportion 65:25:10 and 6% skimmed milk can be seen in Figure 2.

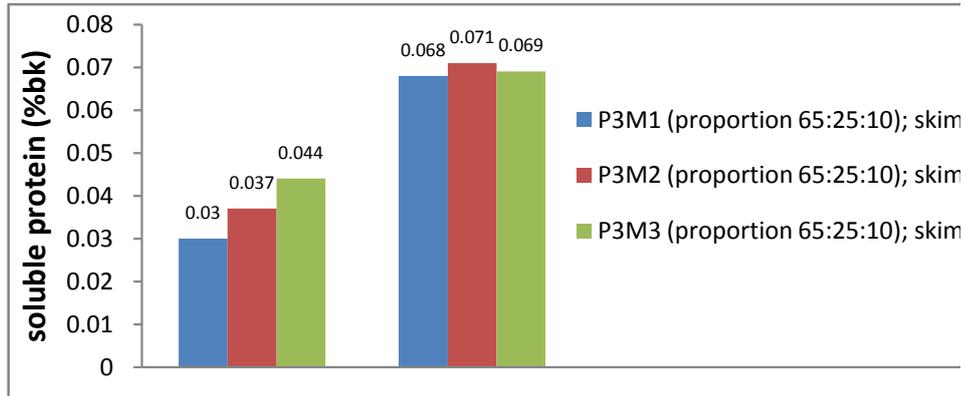


Figure 1. Soluble protein of instan *tiwul* on treatment (proportion of cassava flour - flour substitution (pregermination of bean flour) - Tapioca (P, b/b) 65:25:10)

Instant *tiwul* results of the use of pregerminated of beans flour above shows that its produced high protein instant *tiwul*, because the instant *tiwul* produced without high-protein flour substitutes containing only 1.65% protein (Suhardi and Suhardjo, 2006). For comparison, the protein content of rice ranges from 6-8%. In addition, higher levels of soybean protein *Tiwul* with pre-germination caused by soybean protein content higher than the jackbean continuously 37.3% and 27.4% (Agbedo and Aletor, 2005).

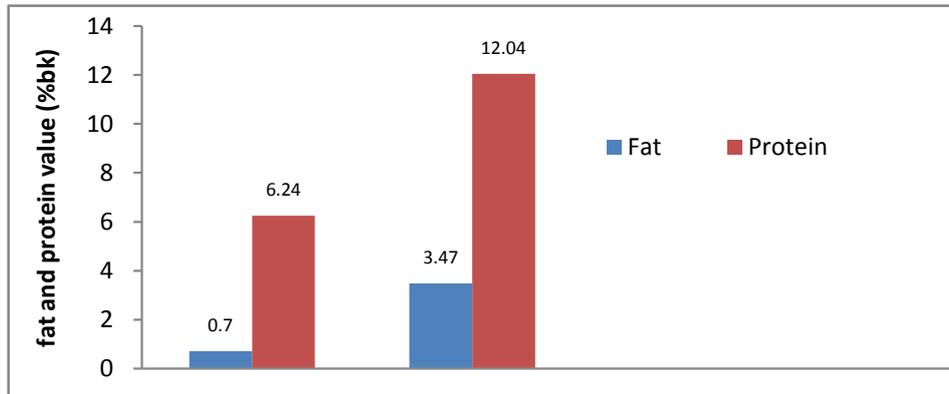


Figure 2. Fat and Protein value of instan *tiwul* on the treatment proportion of cassava flour - flour substitution (pregermination of bean flour) - Tapioca (P, b/b) 65:25:10 and 6% skimmed milk

Sensory Characteristics of Instant *tiwul* can be seen in Table 2.

A. Texture

Chewy texture of cooked instant *Tiwul* is an important sensory properties giving specific characteristics to cooked *Tiwul*. This is provided by gelatinization of starch and protein hydration. Besides the addition of powdered agar and STPP will help the formation of relatively chewy texture. The study did not affect the value texture instant *tiwul*. Instant *tiwul* substituted pregerminated jackbean flour has texture value range 1.40 – 2.87 (not chewy – chewy), while instan *tiwul* substituted pregerminated soybean flour has texture value range 1.73 – 2.7 (rather chewy – chewy).

Table 2 Sensory data treatments of Instant *Tiwul*

Treatments	Color	Flavour	Aroma	Texture	preference
K1P1M1	1.365	2.635	2.670	2.335	2.670
K1P1M2	1.530	2.465	2.565	2.400	2.435
K1P1M3	1.865	2.000	2.870	1.435	1.800
K1P2M1	2.165	2.470	2.665	2.070	2.400
K1P2M2	1.335	2.800	2.865	2.870	2.835
K1P2M3	1.135	2.635	2.665	2.500	2.565
K1P3M1	2.100	2.165	2.830	1.730	2.100
K1P3M2	1.600	2.370	2.600	2.300	2.365
K1P3M3	2.370	2.300	2.600	2.000	2.200
K2P1M1	2.030	2.495	2.595	2.265	2.565
K2P1M2	1.765	2.395	2.865	1.965	2.400
K2P1M3	1.830	2.400	2.500	1.765	2.430
K2P2M1	1.865	2.635	2.865	2.600	2.630
K2P2M2	2.130	2.865	2.830	2.465	2.660
K2P2M3	2.480	2.695	2.795	2.700	3.130
K2P3M1	1.130	2.230	2.465	1.800	2.295
K2P3M2	1.365	2.500	2.670	1.730	2.400
K2P3M3	1.130	2.595	2.365	2.530	2.430

Information :

P1 = 75:15:10	K1 = pregerminated jackbean flour	M0 = skimmed milk 0%
P2 = 70:20:10	K2 = pregerminated soybean flour	M1 = skimmed milk 2%
P3 = 65:25:10		M2 = skimmed milk 4%
		M3 = skimmed milk 6%

B. Aroma of Bean Substitutions

The study did not affect the value of bean aroma. The average value of beans aroma ranged from 2.3 to 2.87 that the range rather smell to rotten smell. The highest average value of bean aroma obtained from the combination treatment of types of flour (soybean); the proportion of cassava flour: soy flour; tapioca 75:15:10 and the addition of 6% skimmed milk (K1P1M3). The smell arising from that instant *tiwul* unpleasant smell coming from flour substitution (jackbean and soybeans). According Theerakulkait et al. (1995), unpleasant smell is caused by the activity of lipoxxygenase enzyme found naturally in the materials (soybeans). Lipoxxygenase enzyme is removed by using hot water (temperature 80-100°C).

C. Colors

The colors of instant *tiwul*, raw and cooked, is caused by non-enzymatic Maillard browning reaction. Maillard reaction is the reaction of aldehydes and ketones (sugars reduction) with amino compounds such as amino acids, peptides and proteins (Meyer, 1973; Fennema, 1996). The study did not affect the color instant *tiwul* that ranged from 1.1 to 2.4 (yellow-brown - brown). Instant *tiwul* substituted pregerminated of soybean flour has a darker color (brown) than substituted with pregerminated jackbean flour. These are due to the higher protein content of soybeans than jackbean. According to Agbede and Aletor (2005), the protein content of the jackbean and soybeans continuously 27.4% and 37.3%. Amino acids and reducing sugars are formed during the germination of soybean than the pre-formed on the pre-germination of jackbeans.

D. Flavor of Instant Tiwul

Flavor Instant cooked *tiwul* be formed due to the Maillard reaction. Duncan Multiple results showed that the proportion (P; tapioca flour tapioca substitutes) affect the flavor of instant *tiwul*. Values range instant *tiwul* flavor ranges from 2-3 (delicious). Kusmiadi (2008) stated, foods containing carbohydrates and protein will be non-enzymatic browning. Some of carbonyl compounds of non-enzymatic browning reaction easily react with free amino acids. This reaction causes the degradation of amino acids to aldehydes, ammonia and carbon dioxide, known as Strecker degradation. Aldehyde compounds contribute to the formation of flavor during browning reaction (Fennema, 1996). The availability of amino acids as a result of pre-germination process of beans Strecker degradation.

E. Preference of Instant Tiwul

A preference is the acceptance test involves assessment of a person of a nature or quality which causes a person prefer (Soekarto, 1985). It is a combination of all the variables determined by the sensory panelists. The study did not affect cooked instant *tiwul*. The average value of cooked instant *tiwul* ranged from 1.8 to 2.6 (little bit prefer - preferably). This is influenced by the use of skimmed milk that will increase the preference of instant *tiwul*. The best sensory value of instant *tiwul* substituted pregerminated jackbean and soybean flour can be seen on figure 3. Treatment that produce highest value of sensory was proportion (70:20:10 and 6% skimmed milk).

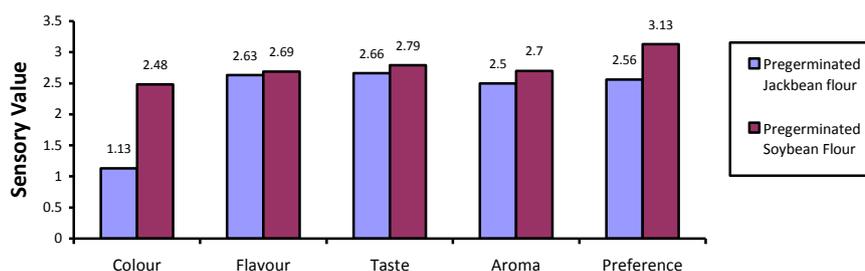


Figure 3. The best sensory value of instant *tiwul* substituted pregerminated jackbean and soybean flour on proportion of cassava flour - flour substitution (pregermination of bean flour) - Tapioca (P, b/b) 70:20:10 and 6% skimmed milk

Conclusion

1. Types of substitutes pregerminated soybean flour produce instant *tiwul* with higher level of protein solubility contents while pregerminated jackbean flour produce instant *tiwul* with higher rehydration coefficient values.
2. The best proportion of cassava flour- pregerminated beans flour-tapioca 70:20:10 b/b because produce the good sensory values (textures, flavors and preferences).
3. Cooked instant *tiwul* have the typical bean aroma both jackbean or soybean
4. Instant *tiwul* with substitute of beans pre germination flour contains protein 6 - 12 %bk and fat 0,70 – 3,47% bk.

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References

- Ade-Omowaye BIO, Akinwande BA, Bolarinwa IF, Adebisi AO. 2008. Evaluation of Tigernut (*Cyperus esculentus*)-wheat Composite Flour and Bread. *Afric J Food Sci.* 2:87-91
- Afandi, S. 2001. Mempelajari Pembuatan Tepung Kedelai (Glycine Max Merr) Amerika Serikat dan Analisis Mutu Tepung yang Dihasilkan. *Skripsi.* Fakultas Teknologi Pertanian, Institut Pertanian Bogor (Tidak Dipublikasikan)
- Agbede, J.O and V.A. Aletor. 2005. Studies of The Chemical Composition and Protein Quality Evaluation of Differently Processed *Canavalia ensiformis* and *Mecuna Pruriens* Seed Flours. *J. of Food Composition and Analysis* 18:89-103
- Alam F, Siddiqui A, Lufti Z, Hasnain A. 2009. Effect of Different hydrocolloids on Gelatinization behavior of hard wheat flour. *Trakia J Sci.* 7(1):1-6
- Andarwulan, N., D. Rahayuning, dan S. Koswara. 2004. Formulasi *flakes triple mixed* ubi jalar-kecambah-wheat germ sebagai produk sarapan fungsional untuk anak-anak (On-line). <http://www.iptek.net.id/ind/pustakapangan-/pdf/prosiding/oral/GB26NURIANDARWULAN-BOGORRevised.pdf> diakses 26 Agustus 2010.
- Andoko, A. dan Parjimo. 2007 *Budi Daya Ubi kayu: Ubi Jalar.* Agromedia Pustaka, Jakarta.
- Andrarini. 2004. Faktor-Faktor yang Mempengaruhi Konsumsi *Tiwul* di Pedesaan dan Perkotaan di Kabupaten Gunung Kidul Provinsi Daerah Istimewa Yogyakarta. *Skripsi.* IPB, Bogor.
- Buckle, K. A., R. A. Edwards, G. H. Fleet, and M. Wooton. 1987. *Ilmu Pangan.* Penerbit Universitas Indonesia, Jakarta. Hal 269-304.
- Fennema, O.R. 1996. *Food Chemistry.* Third Ed. Marcell Decker, Inc, New York.
- Koswara, S. 1992. *Teknologi Pengolahan Kedelai Menjadi Makanan Bermutu.* Pustaka Sinar Harapan, Jakarta
- Mesen, V. dan Somaatmadja S. 1993. *Proses Sumber Daya Nabati Asia Tenggara I.* Gramedia Pustaka Utama, Jakarta.
- Oluwamukomi MO, Oluwalana IB dan Akinbowale OF. 2011, Physicochemical and Sensory Properties of Wheat-Cassava Composite Biscuit Enriched With Soy Flour. *African Journal of Food Science* Vol. 5 (2) pp. 50 - 56
- Opara CC, Aqueze CC dan Abraham A. 2013. Dehydration and Rehydration of fufu. *Greener J Sci Eng Technol Res.* 3(2): 68-75
- Rukmini HS, Naufalin R dan Agustia FC. 2014. Penggunaan Kacang Koro dan Kedelai Pra Germinasi Untuk Meningkatkan Kandungan Protein *Tiwul* Instan. Laporan Penelitian Strategis Nasional, DP2M Dikti.
- Rukmini HS dan Naufalin R. 2015. Formulasi *Tiwul* Instan Tinggi Protein Melalui Penambahan Lembaga Serealia dan Konsentrat Protein Kedelai. *Jurnal Teknologi Industri Pertanian.* Vol 25. No.3
- Suarni. 2004. Pemanfaatan Tepung Sorgum untuk Produk Olahan. *J Litbang Pert.* 23 (4) : 145-151
- Subarie D, Babie J, Aekar D, Pilizota V, Kopjar M, Ljubas I, Ivanovska S. 2011. Effect of galactomannan Hydrocolloids on gelatinization and retrogradation of tapioca and corn starch. *Croat J Food Sci Technol* 3(1):26-31
- Suhardi dan Suhardjo. 2006. Teknologi Produksi *Tiwul* Instan dari Tepung ubi Kayu Komposit. Info Teknologi Pertanian No. 27. Balai Pengkajian Teknologi Pertanian Jawa Timur.
- Taiwo, KA dan Adeyemi O. 2009. Influence of Blanching on the drying and Rehydration of Banana Slices. *Afric J Food Sci.* 3 (10): 307-31
- Theerakulkait C, Barrett DM dan Mc Daniel MR. 1995. Sweet corn germ enzymes affect odor formation. *J Food Sci.* 60 (5): 1034-1040
- Wardayani, NIA., Susanti I, Aviana T, Herman AS. 2008. Potensi Ubi-umbian dan Serealia dalam Menunjang Diversifikasi Pangan Berbasis Sumber Daya Lokal. *J Riset Indus.* 2 (1): 35-43
- Windrati, S. W., Nafi, A., Augustine, D. P. 2010. Sifat Nutrisional Protein RichFlour (PRF) Koro Pedang (*Canavalia ensiformis L.*), *AGROTEK* 4(1) = 18-26.

Competitive Advantage of Purse Liquid Sugar as Natural Sweeteners Materials

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Abstract

Sugar is one of the basic needs of society, particularly its role as a sweetener. It is necessary to find an alternative sweetener other than sugar cane, which include developing a glucose syrup (liquid sugar) from the starch. Purse (*Xanthosoma sagittifolium*) as one type of root crops has a great opportunity to be developed because it has high carbohydrate content (34.2 g/100 g). The purpose of this research was to determine the best enzymatic hydrolysis process in the production of purse liquid sugar.

Research design was a randomized block design (RBD) factorial pattern with 3 times repeated. The first factor is the volume of the enzyme (E) with three levels, namely: E1 = 1 ml, E2 = 2 ml, and E3 = 3 ml. The second factor is the hydrolysis temperature (S) with four levels, namely: S1 = 70°C, S2 = 80°C, S3 = 90°C and S4 = 100°C. The parameters tested were °Brix, water content, ash content, reducing sugar, and organoleptic test on taste, color and aroma. The best process is the process which has highest expected value based on Expected Value Method. Furthermore, the products produced from the selected process were calculated on the caloric value and the Glycemic Index (GI).

The results of this research showed that: (1) Treatment interaction of enzyme volume and hydrolysis temperature causes a significant differences in parameters of °Brix, moisture content, and reducing sugar, but did not cause significant differences in the parameter of ash content; and (2) Based on the expected value method, the best process is hydrolysis process by adding 3 ml enzyme and hydrolysis temperature of 100°C (E3S4), which produces liquid sugar of purse with 73.73% water content, 0.24% ash content, 25.17 °Brix, 23.43 % reducing sugar, 106 calories and value IG is 80.63.

Keywords: Enzymatic, hydrolysis, liquid sugar, purse

Introduction

Sugar is one of the basic needs of society, particularly its role as a sweetener. Along with the increase in per capita income and population, the needs for sugar have also increased. The increased of sugar production in the country is not able to compensate for the increase in sugar consumption, so imports of sugar become reasonable choice. To reduce the import of sugar, the sugar production in the country should be encouraged. While also seeking other alternative sweeteners as sugar substitutes, including by developing glucose syrup (liquid sugar) from the starch. Glucose syrup is defined as a clear liquid and viscous whose main components are glucose derived from the hydrolysis of starch. Glucose syrup is the result of starch hydrolysis process, which is derived from a variety of carbohydrate sources such as cassava, sweet potato, sago and corn (Hidayat, 2006).

Purse (*Xanthosoma sagittifolium*) as one kind of plant tubers have a great opportunity to be developed because it has many benefits and can be cultivated easily. Purse can be developed as a source of non-rice carbohydrate (Azwar, 2010). The price of purse is very cheap. Meanwhile, underutilization and high carbohydrate content (34.2 g/100g), make the opportunity for purse to be developed as a raw material for making of glucose syrup.

Hydrolysis method is a method to get glucose syrup from tubers starch. Hydrolysis method can be done by acid hydrolysis, enzymatic hydrolysis and a combination of both. Acid hydrolysis has a

fundamental difference with enzymatic hydrolysis. Acid hydrolysis which typically use a strong acid (HCl), will break the chain of starch at random, while the enzymatic hydrolysis will decide of starch chains specifically on certain branching. Enzymes that can be used in the hydrolysis of starch to produce glucose syrup is α -amylase and glucoamylase (Februadi, 2011). α -amylase enzyme playing a key role in the hydrolysis of starch, glycogen and α -1, 4-glucan. Glucoamylase enzym capable of hydrolyzing the α -1,4 bond in the chain amylose, amylopectin, glycogen, and pullulan. This enzyme can also attack the bond α -1,6 at the branching point. This means that the starch can be decomposed completely into glucose. Hydrolysis using a strong acid would only produce glucose syrup with a dextrose equivalent (DE) value of 55, while the enzymatic hydrolysis will produce higher DE value.

Research of Budiyanto *et al.* (2005) showed that the enzymatic hydrolysis of cassava using the α -amylase enzyme is done at enzym concentrations of 0.6-1.2 ml/kg of starch, temperature of 90-100°C during 20-60 minutes, while the enzyme amiloglukosidase performed at enzyme concentrations of 0.8-1.2 ml/kg of starch, the temperature of 60°C, pH of 4.0-4.6 during 72 hours. Risnoyatiningasih (2011) showed that the enzymatic hydrolysis of yellow sweet potato starch at a temperature of 60°C using the amylase enzym 2 ml and glucoamylase 0.1 ml produce glucose content with a conversion of 66.08%.

The purpose of this research was to determine the best enzymatic hydrolysis process in the production of purse liquid sugar.

Materials and Methods

Materials and Tools

The materials used are purse tubers, α -amylase and glucosidase enzymes, NaOH 1%, and materials for chemical analysis. The tools used are erlenmeyer, measure instruments, and tools for chemical analysis

Research Design

The design of research was a randomized block design (RBD) factorial pattern with 3 times repeated. The first factor is the volume of the enzyme (E) with three levels, namely: E1 = 1 ml, E2 = 2 ml, and E3 = 3 ml. The second factor is the hydrolysis temperature (S) with four levels, namely: S1 = 70°C, S2 = 80°C, S3 = 90°C and S4 = 100°C.

Observation Parameters

The parameters tested were °Brix, moisture content, ash content, reducing sugar, and organoleptic test on taste, color and aroma. The selected process will then be calculated caloric value and the Glycemic Index (GI).

Alternative Selection

Alternative selection was done to determine the best treatment in the manufacturing process of purse liquid sugar by enzymatic hydrolysis. The best process is the alternative process which has highest expected value based on Expected Value Method. For product of purse liquid sugar, quality parameters used for selecting the best alternative is water content, ash content, reducing sugar content, as well as organoleptic on taste, aroma, and color.

Data Analysis

Analysis of organoleptic data which is ordinal data using Friedman test, whereas analysis of the chemical test data performed by analysis of variance, if there is significant continued with Duncan test with 95% confidence level.

Results and Discussion

1. Purse Starch Processing

Purse starch is produced from the purse tubers with the stages of the process, namely sorting, stripping, downsizing, soaking in a saturated salt solution, washing, milling, extortion, filtration, sedimentation, drying, flouring and packaging. The yield generated is 20.33% as shown in Table 1.

Table 1. Yield of Purse Starch

No	Purse Tubers	Purse Starch	Yield (%)
1	35	7,2	20,57
2	35	6,8	19,43
3	30	6,3	21,00
Average			20,33

Taste

Based on the result of Frequency Analysis, the percentage of score gained for taste parameter are shown in Table 2 and Figure 1.

Table 2.Score Gained Percentage for Taste Parameter of Purse Liquid Sugar

Score	Score Gained (%)											
	E1S1	E1S2	E1S3	E1S4	E2S1	E2S2	E2S3	E2S4	E3S1	E3S2	E3S3	E3S4
1	13,3	10,0	6,7	3,3	3,3	6,7	0,0	0,0	0,0	0,0	0,0	0,0
2	50,0	50,0	50,0	50,0	53,3	50,0	53,3	56,7	43,3	56,7	50,0	50,0
3	36,7	36,7	43,3	46,7	43,3	43,3	40,0	36,7	46,7	40,0	40,0	40,0
4	0,0	3,3	0,0	0,0	0,0	0,0	6,7	6,7	10,0	3,3	10,0	10,0
5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Based on the results of Friedman test for score ofpurse liquid sugar taste shows that there are no significant differences among treatments, with a calculated F value of 12.669.

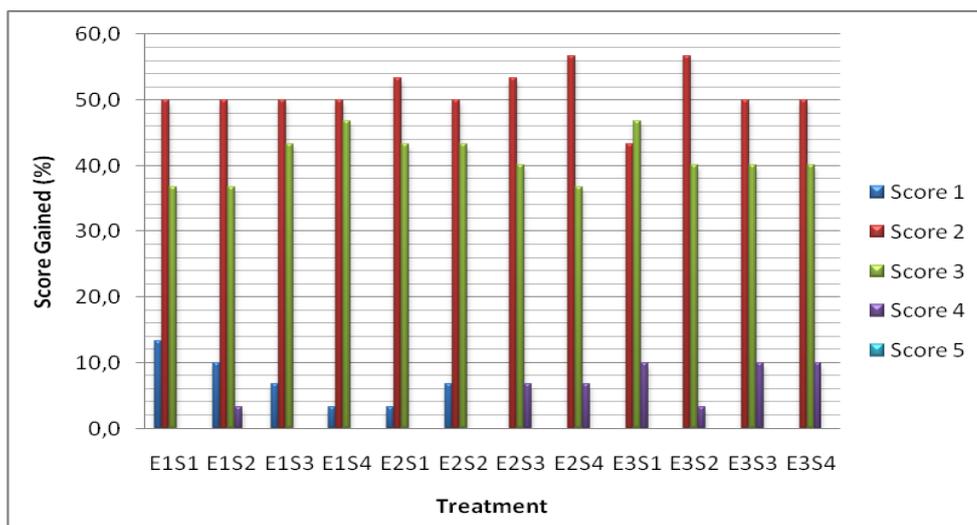


Figure 1. Graph ofScore Gained Percentage for Taste Parameter of

Purse Liquid Sugar

Aroma

Based on the result of Frequency Analysis, the percentage of score gained for aroma parameter are shown in Table 3 and Figure 2.

Table 3. Score Gained Percentage for Aroma Parameter of Purse Liquid Sugar

Score	Score Gained (%)											
	E1S1	E1S2	E1S3	E1S4	E2S1	E2S2	E2S3	E2S4	E3S1	E3S2	E3S3	E3S4
1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2	40,0	43,3	33,3	40,0	46,7	43,3	53,3	56,7	50,0	43,3	63,3	66,7
3	50,0	43,3	56,7	53,3	50,0	50,0	40,0	36,7	36,7	56,7	36,7	33,3
4	10,0	13,3	10,0	6,7	3,3	6,7	6,7	6,7	13,3	0,0	0,0	0,0
5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Based on the results of Friedman test for score of purse liquid sugar aroma shows that there are no significant differences among treatments, with a calculated F value of 14.039.

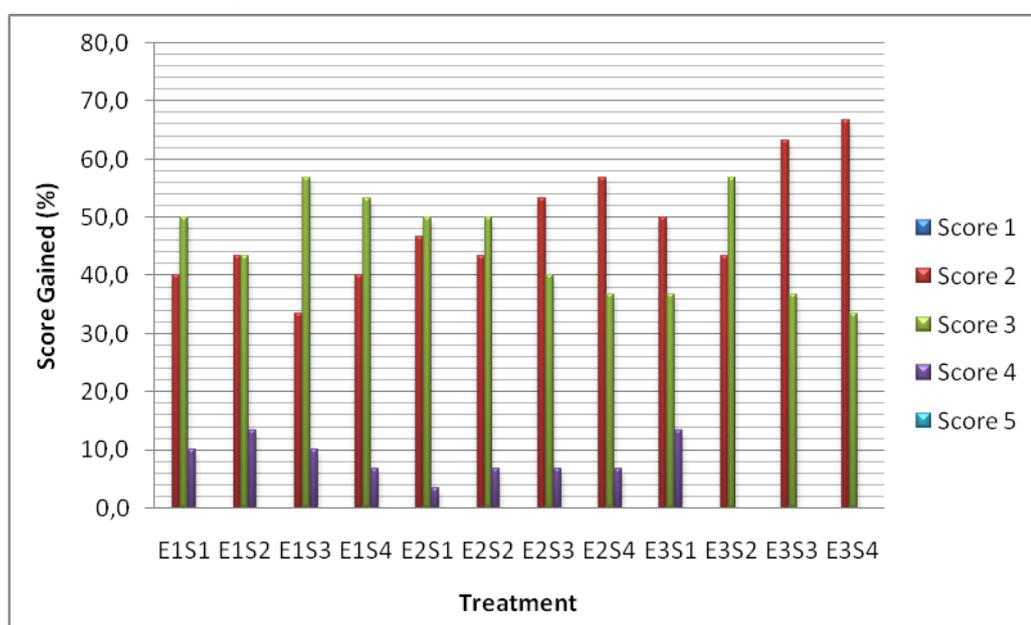


Figure 2. Graph of Score Gained Percentage for Aroma Parameter of Purse Liquid Sugar

Color

Based on the result of Frequency Analysis, the percentage of score gained for color parameter are shown in Table 4 and Figure 3.

3. Chemistry Contents

°Brix

°Brix measurement is performed to determine the degree of sweetness of purse liquid sugar from enzyme hydrolysis. Data and graph of °Brix purse liquid sugar from enzyme hydrolysis is shown in Table 5 and Figure 4.

Table 4. Score Gained Percentage for Color Parameter of Purse Liquid Sugar

Score	Score Gained (%)											
	E1S1	E1S2	E1S3	E1S4	E2S1	E2S2	E2S3	E2S4	E3S1	E3S2	E3S3	E3S4
1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2	20,0	20,0	10,0	10,0	16,7	13,3	16,7	20,0	20,0	13,3	16,7	10,0
3	56,7	53,3	70,0	66,7	66,7	70,0	60,0	60,0	56,7	63,3	50,0	53,3
4	23,3	26,7	20,0	23,3	16,7	16,7	23,3	20,0	23,3	23,3	33,3	36,7
5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Based on the results of Friedman test for score of purse liquid sugar color shows that there are no significant differences among treatments, with a calculated F value of 5.215.

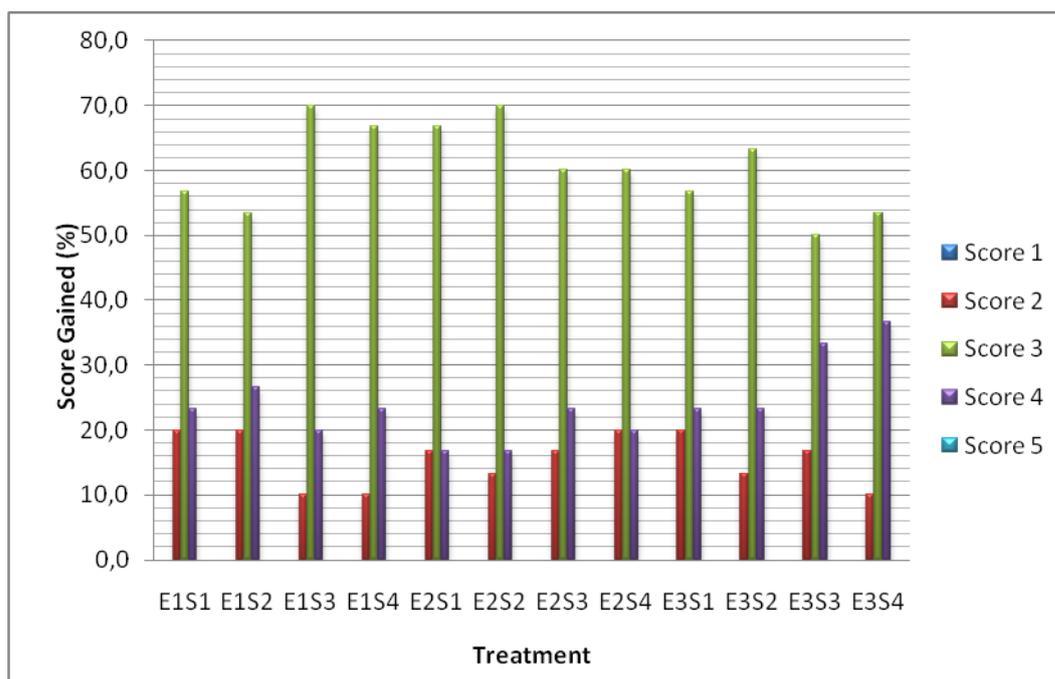


Figure 3. Graph of Score Gained Percentage for Color Parameter of Purse Liquid Sugar

Table 5. °Brix of Purse Liquid Sugar

Treatments	S1	S2	S3	S4	Average
E1	21,83 c	23,83 bc	21,83 c	26,67 a	23,54
E2	23,83 bc	23,33 bc	24,67 a	25,33 ab	24,29
E3	22,00 c	24,33 b	24,50 b	25,17 ab	24,00
Average	22,56	23,83	23,67	25,72	23,94

In Figure 4 it appears that the same enzyme concentration, the higher the hydrolysis temperature showed that °Brix tends to increase. This is in accordance with the opinion of Suhartono (1989) that the α-amylase enzyme works in the temperature range 90-100°C. Added by Hartiati and Yoga (2014), that the taro potato starch hydrolysis by the enzyme concentration of 1.0 ml/kg at a temperature of 95°C to produce dextrose equivalence (DE), the highest 34.26%.

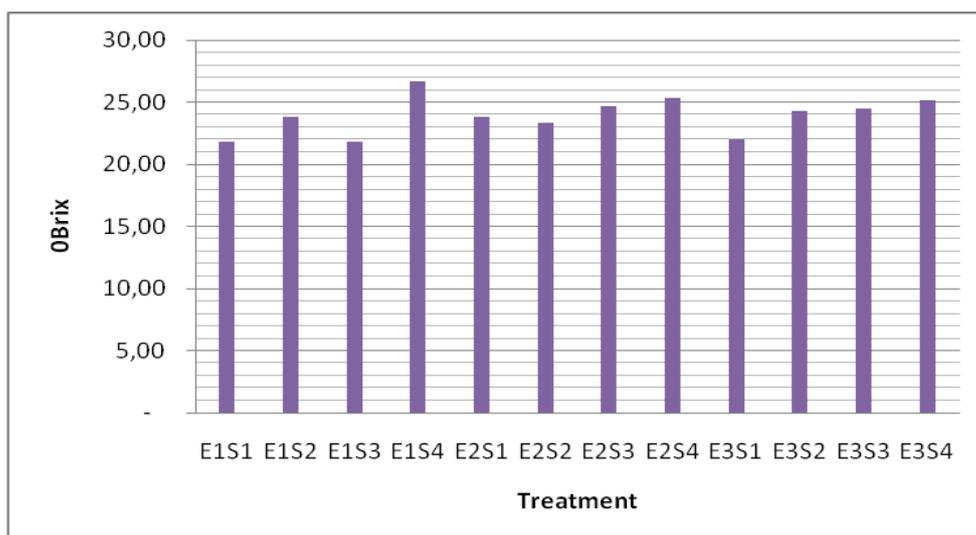


Figure 4. Graph of °Brix of Purse Liquid Sugar

Results of analysis of variance showed that there were significant differences in the treatments interaction to °Brix of purse liquid sugar is generated, as shown in Table 6.

Table 6. Notation of Duncan Test for Treatments Interactions

Treatments	°Brix
E1S1	21,83 c
E1S2	23,83 bc
E1S3	21,83 c
E1S4	26,67 a
E2S1	23,83 bc
E2S2	23,33 bc
E2S3	24,67 b
E2S4	25,33 ab
E3S1	22,00 c
E3S2	24,33 b
E3S3	24,50 b
E3S4	25,17 ab

Description: Different letters indicate significant differences

Moisture Content

Moisture content measurement is performed to determine the moisture content of purse liquid sugar from enzyme hydrolysis with the treatment enzyme volume and temperature hydrolysis. The results of measurements of moisture content of purse liquid sugar can be seen in Table 7 and Figure 5.

Table 7. Moisture Content of Purse Liquid Sugar (%)

Treatments	S1	S2	S3	S4	Average
E1	77,47	76,51	73,36	72,05	74,85
E2	76,61	75,72	73,31	73,66	74,83
E3	75,88	74,94	74,30	73,73	74,71
Average	76,65	75,72	73,66	73,15	74,80

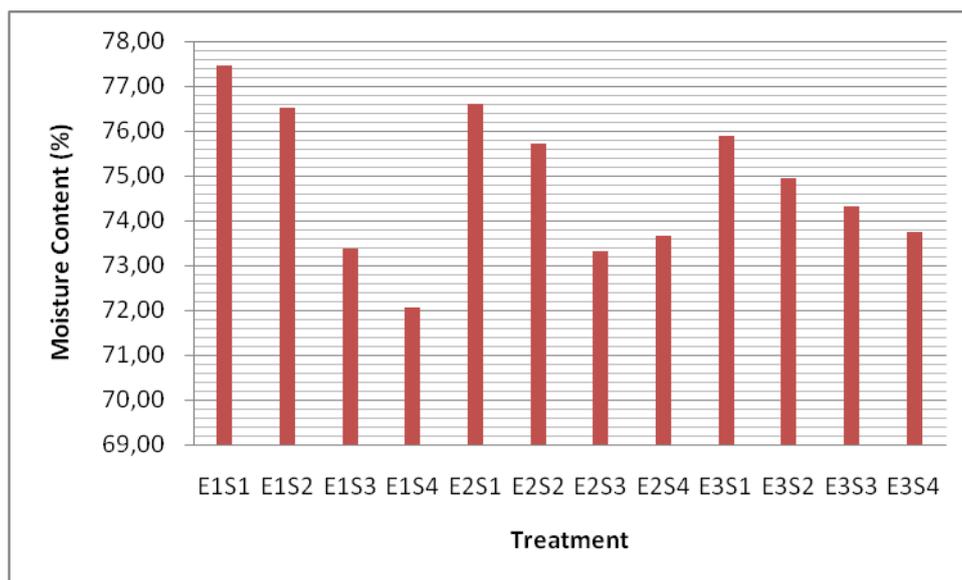


Figure 5. Graph of Purse Liquid Sugar MoistureContent

Results of analysis of variance showed that there were significant differences in the treatments interaction. In Table 8 it appears that at the same concentration, along with increased in hydrolysis temperature, moisture content tends to decrease. This is possibly due to the longer time the hydrolysis will cause more water is evaporated.

Table 8. Notation of Duncan Test for Treatments Interactions

Treatments	Moisture Content (%)
E1S1	77,47 f
E1S2	76,51 ef
E1S3	73,36 ab
E1S4	72,05 a
E2S1	76,61 ef
E2S2	75,72 de
E2S3	73,31 ab
E2S4	73,66 bc
E3S1	75,88 de
E3S2	74,94 cd
E3S3	74,30 bc
E3S4	73,73 bc

Description: Different letters indicate significant differences

Ash Content

Ash content measurement is performed to determine the ash content of purse liquid sugar from enzyme hydrolysis with the treatment enzyme volume and temperature hydrolysis. The results of measurements of ash content of purse liquid sugar can be seen in Table 9 and Figure 6.

Results of analysis of variance showed that there were no significant differences among the treatments.

Reducing Sugar Content

Reducing sugar content measurement is performed to determine the reducing sugar content of purse liquid sugar from enzyme hydrolysis with the treatment enzyme volume and temperature hydrolysis. The results of measurements of reducing sugar content of purse liquid sugar can be seen in Table 10 and Figure 7.

Table 9. Ash Content of Purse Liquid Sugar (%)

Treatments	S1	S2	S3	S4	Average
E1	0,26	0,24	0,23	0,27	0,25
E2	0,29	0,25	0,24	0,24	0,26
E3	0,31	0,26	0,25	0,24	0,27
Average	0,29	0,25	0,24	0,25	0,26

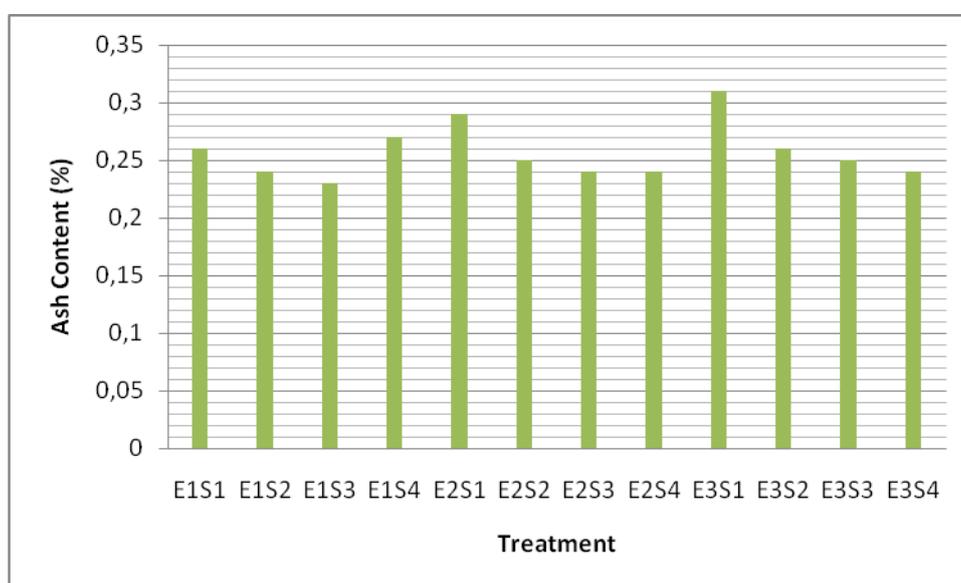


Figure 6. Graph of Purse Liquid Sugar Ash Content

Table 10. Reducing Sugar Content of Purse Liquid Sugar (%)

Treatments	S1	S2	S3	S4	Average
E1	14,88	16,97	14,42	23,63	17,48
E2	18,73	17,04	18,69	21,08	18,89
E3	14,05	18,19	19,92	23,43	18,90
Average	15,89	17,40	17,68	22,71	18,42

Results of analysis of variance showed that there were significant differences in the treatments interaction. In Table 11 it appears that the same enzyme concentration, the higher the hydrolysis temperature showed that reducing sugar content tends to increase. This is in accordance with the opinion of Suhartono (1989) that the α -amylase enzyme works in the temperature range 90-100°C, thereby reducing sugar content produced are also getting bigger.

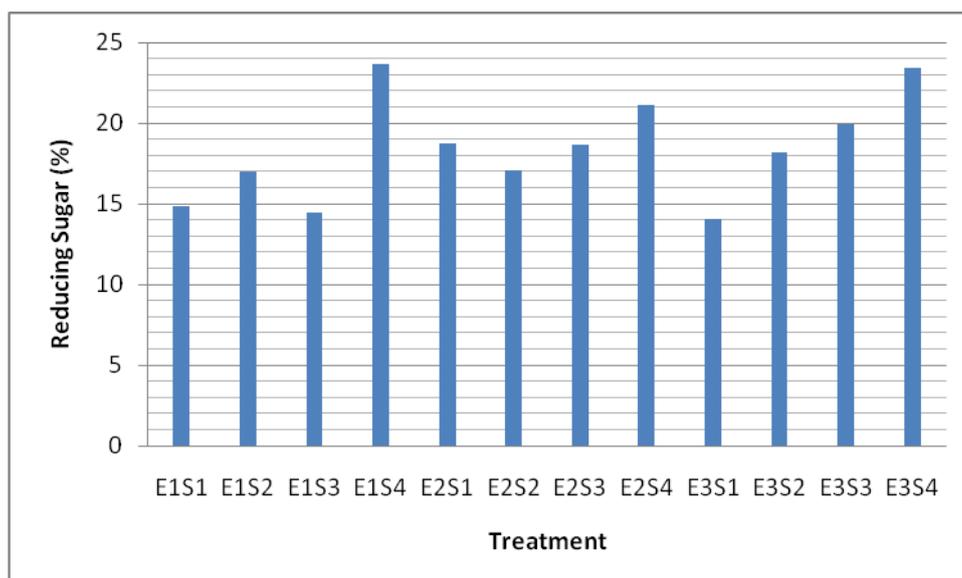


Figure 7. Graph of Purse Liquid Sugar Reducing Sugar Content

Table 11. Notation of Duncan Test for Treatments Interactions

Treatments	Reducing Sugar Content (%)
E1S1	14,88 f
E1S2	16,97e
E1S3	14,42f
E1S4	23,63a
E2S1	18,73cd
E2S2	17,04e
E2S3	18,69cd
E2S4	21,08b
E3S1	14,05f
E3S2	18,19de
E3S3	19,92bc
E3S4	23,43a

Description: Different letters indicate significant differences

Alternative Selection

Alternative selection is done with the aim of selecting the best treatment of some existing treatments. Decision-making is a process of systematically selecting the best treatment. Determining the weight of the interests of each parameter is done by using Analytical Hierarchi Process. As for determining the selection of the best treatment based Expected Value Method.

Based on the expected value method, the best process is hydrolysis process by adding 3 ml enzyme and hydrolysis temperature of 100⁰C (E3S4) with expected value of 8.58.

Calories Value and Glycemic Index

Calories value of purse liquid sugar from enzymatic hydrolysis process chosen is 106 calories with IG value of 80.63.

Conclusion

Treatment difference of enzyme volume and the heating temperature on the enzyme hydrolysis causes no significant difference in the parameters of taste, aroma and color of the purse liquid sugar product. While, treatment interaction between enzyme volume and heating temperature on the enzyme hydrolysis causes a significant differences in parameters of °Brix, water content, and reducing sugar, but did not cause significant differences in the parameter of the ash content. Based on the expected value method, the best process is hydrolysis process by adding 3 ml enzyme and hydrolysis temperature of 100°C (E3S4), which produces liquid sugar of purse with 73.73% water content, 0.24% ash content, 25.17 °Brix, 23.43 % reducing sugar, 106 calories and value IG is 80.63.

REFERENCES

- Azwar, D.; and R. Erwanti. Pembuatan Sirup Glukosa Dari Kimpul (*Xanthosoma violaceum* Schott) Dengan Hidrolisa Enzimatis. <http://www.eprints.undip.ac.id>. Downloaded 11 Mei 2010
- Apriantono, A., D Fardiaz, NL Puspitasari, and Budiyo. 1989. Petunjuk Laboratorium Analisa Pangan. PAU Food and Nutrition. IPB. Bogor.
- Budiyo, A. Martosuyono, P., and Richana N. 2005. Optimasi Proses Produksi Tepung Kasava dari Pati Ubi Kayu Skala Laboratorium. Bulletin of the Center for Postharvest. Jakarta.
- Hartiati, A. and Yoga, IWG. 2014. Proses Liquefikasi Pati Ubi Talas Menggunakan Enzim α -Amilase. Proceedings of the National Seminar on Technology. University of Udayana. Denpasar.
- Hidayat, 2006. Analisis Studi Kelayakan Agroindustri Sirup Glukosa di Kabupaten Lampung Tengah. Skripsi. Departement of Agriculture. University of Lampung. Lampung
- Lutony, T.L., 1993. Tanaman Sumber Pemanis. Penebar Semangat. Jakarta.
- Puspitasari, D., Rahayuningsih, T., and Rejeki, FS. 2012. Karakteristik dan Formulasi Tepung Komposit Kimpul-Kacang Tunggak untuk Pengembangan Biskuit Non Terigu. Hibah Bersaing Research Report. University of Wijaya Kusuma Surabaya. Surabaya.
- Risnoyatuningsih, S. 2011. Hidrolisis Pati Ubi Jalar Kuning Menjadi Glukosa Secara Enzimatis. Chemical Engineering, University of Pembangunan Nasional Jawa Timur. Surabaya.
- Sudarmaji, S., Haryono and Suhardi. 1990. Analisa Bahan Makanan dan Pertanian. Liberty. Yogyakarta.
- Sudarmaji, S., Haryono and Suhardi. 1994. Prosedur Analisa Untuk Bahan Makanan dan Pertanian. Liberty. Yogyakarta.

Optimisation Process of Twin Screw Extruder Technology for Instant Artificial Rice Production

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Abstract

Artificial rice is granules exactly like rice, and it can be produced using a variety of methods from various carbohydrate sources, one of which is corn flour. Artificial rice production process can be conducted through several methods including granulation system and extrusion. This study was aimed at optimising the process parameters in designing process technology for instant artificial rice made from corn flour. This study included three phases: the production of corn starch, optimisation stages of instant artificial rice production and characterization of the products produced. Optimisation process was performed using a central composite design with response surface method. In the first stage, optimal conditions of moisture content of 60 %, temperature between 90 °C and 100°C, sodium-alginate of 1%, GMS of 2% and maximum steaming length of 6 minutes were obtained. Based on the optimisation results with RSM, optimal temperature condition reached is 96°C with a steaming period of 5 minutes.

Key words: influences, optimisation, instant artificial rice

Introduction

Studies in the field of artificial rice production have been conducted respectively by Mishra *et al.* (2012); Wang *et al.* (2011); Budijanto *et al.* (2011); Herawati *et al.* (2011); Herawati and Widowati (2009); Lisnan (2008); Samad (2003); US Paten No. 3,620,762; 3,628,966, patent Jepang HEI 4-13986, 3-69267, US Paten No. 4,129,900; Paten No 5,211,977. However, the cost of production of this artificial rice cannot compete with that of paddy rice, thus, its development has faced several constraints. One of the break throughs that can be explored and developed and expected to increase the added value of the artificial rice is by producing instant artificial rice.

Instant rice is rice that can be prepared instantly within 2 to 15 minutes with only a simple preparation. Instant rice is characterized by its porous granules. The more porous structure, the penetration of hot water to the granules when rehydrated will be accelerated. Once cooked, the instant rice must comply with that of paddy rice in terms of taste, aroma, and texture (Luh, 2000; Rewthong *et al.*, 2010). Decent instant rice only takes 5 minutes to cook mean while minutes rice is a product that can be served by brewing the rice with hot water for 1 to 2 minutes.

Wenger and Huber (1988) produced a patent instant rice which is processed using low shear extrusion, with 60 to 80% of a mixture of rice and rice flour with the addition of water as much 20 to 40% and the use of temperature of 150 to 210 °F with an optimum preparation period of 5 to 10 minutes. A patent instant rice-like product has been available with a mixture of rice flour of 95 to 100%, starch complex of 0 to 0.75%, and gum of 0 to 0.25% (Scelia *et al.*, 1986).

There is instant rice formulated from rice flour mixed with emulsifiers and hydrocolloids as a thickening agent with a single screw type extrusion method (Wang *et al.*, 2011). Hydrocolloid can be added to 0.2 to 2.5% before the extrusion process to produce artificial rice (Mishra *et al.*, 2012). The addition of alginate can serve as a binder, and the added levels affect the product viscosity (Cox and Cox, 1993). Technological process for artificial rice production can be conducted using non-rice flour such as corn flour. Optimization of technological process of instant artificial rice processing can be improved by using white corn flour as a main raw material thus resembles the color that of paddy rice. Optimization of process parameters and characterization of instant artificial rice products are essential. This study aims to optimize the process parameters to produce technological process for the production of instant artificial rice made from corn flour.

Material and Methods

Corn Flour Technology Production

The first phase of this research included the step of making corn flour (methods of Dry Mill and Alkaline Cooked Mill) (Johnson, 1991). Based on the results of the production of corn flour using both methods, characteristics of the flour were analyzed, and they include: analyses of water content, ash, fat, protein (AOAC, 1998), total starch (Dubois *et al.*, 1956), amylose (IRRI, 1978), dietary fiber (Asp *et al.*, 1983) and amylograph profile (Wang *et al.*, 2011).

Artificial Rice Instant Process Production

The next stage of the process was using hydrocolloid containing sodium-alginate of 1%, GMS emulsifier of 2%, 10% starch, and white corn flour. Based on the composition, an analysis was performed to determine the critical parameters. Following this, optimization of the results of the parameters was conducted. The design was prepared using Response Surface Design with CCD method (Central Composite Design) by means of two factors: the extruder temperature (with the lower limit of 90 °C and upper limit of 100 °C) and steaming period (the upper limit of 5 minutes and lower limit of 1 minute).

Table 1. Central Composite Design DX 7 Rice Artificial Instant Optimization Process

Std	Run	Factor A	Factor B
		(Temperature, °C)	(Steaming time, min)
6	1	102	3
1	2	90	1
5	3	88	3
9	4	95	3
4	5	100	5
10	6	95	3
3	7	90	5
2	8	100	1
11	9	95	3
8	10	95	6
7	11	95	0

Analysis

The analysis used for optimization included the cooking time (Bergman *et al.*, 2004; Roberts, 1972), degree of gelatinization (Wootton and Munk, 1971), WAI (Water Absorption Index) (Anderson *et al.*, 1969), EI (Expansion Index) (Kumagai *et al.*, 1987) and Complexing Index (Guraya *et al.*, 1997). Based on the analysis results, ANOVA was conducted and its confidence level of 95% was also analyzed. The results were overlaid and an optimization process was conducted to obtain the optimum condition.

Cooking Time Analysis

The optimum cooking time condition defined as cooking time which was needed of 1 gram rice to absorbed 2.5 g of water (Bergman *et al.*, 2004). The analysis included: a 100 g instant artificial rice was added to 250 gram of boiled water, and cooked in a *Rice Cooking Miyako MCM-606 B/ 3 in one* rice cooker (with a capacity of 0.63 L, 350 watt power, 220 Vac-50 Hz). The cooking time was analyzed as total time to complete cooking process until timer set ended.

Degree of Gelatinization

Degree of gelatinization was measured using method by Wootton and Munk (1971). Briefly, two grams of the sample was macerated with 100 mL distilled water in a Warring blender. The suspension was centrifuged at 500 rpm for 10 min; in duplicates a 1 mL of solution were diluted with water to 10 mL and treated with 0.1 mL iodine solution. The absorbance of these samples were read at 600 nm with spectrophotometer (Model 2903, Perkin-Elmer Co. Ltd.), against a reagent blank. A further suspension of the product (2 g) was prepared in 95 mL distilled water (instead of 100 mL distilled water) as described earlier. To this suspension, a 5 mL of 10 M aqueous solution of potassium hydroxide was added and the mixture was allowed to stand for 5 min with a gentle agitation. The alkaline suspension was centrifuged and 1 mL of duplicate aliquots was treated with 1 mL of 0.5 m hydrochloric acid and diluted with water to iodine solution (0.1 mL) and the absorbance was measured as described earlier. The degree of starch gelatinization was calculated as:

$$\text{Degree of gelatinization (\%)} = \frac{A_1}{A_2} \times 100\%$$

A₁ and A₂ are absorbance of the iodine complex prepared from the aqueous suspension before and after alkali solubilization

Water Absorption Index

The WAI were measured using a technique developed for cereals (Anderson *et al.*, 1969). The ground extrudate was suspended in water at room temperature for 30 min, gently stirred during this period, and then centrifuged at 3,000 x g for 15 min. The supernatant was decanted into an evaporating dish of known weight. The WAI is the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. Determinations were made in duplicates.

Expansion Index

The diameter ratio of instant artificial rice against the diameter of die was used to express the expansion index (Kumagai *et al.*, 1987). Ten samples were used for each product to calculate the mean with the following formula :

$$EI = \frac{D_p}{D_d} \times 100$$

D_p is instant artificial rice diameter and D_d is the diameter.

Complexing Index

The amount of lipid required to saturate the starch was assessed by allowing excess lipid to react with starch in dilute solution as described by Blazek and Copeland (2009). The Complexing Index of starch in the instant artificial rice was measured by method of Guraya *et al.* (1997). Two grams of potassium iodide and I₂ were dissolved and macerated in overnight. One hundred milliliters of water was added to the solution. Five gram of sample were then mixed with 25 mL of destilated water for 2 min and centrifuged in 3,000 rpm for 15 min. The supernatant of 500 µL was then added with 15 ml destilated water and 2 mL of iodine solution. The absorbance of a UV-Vis beam at 690 nm was read and the Complexing Index was then calculated with the following formula:

$$CI(\%) = \frac{A_{bc} - A_{bs}}{A_{bc}} \times 100\%$$

A_{bc} = Control absorbance; A_{bs} = Sample absorbance; A mixture native flour as control

Texture The characteristics of the texture of instant artificial rice measured (Wang *et al.*, 2011) included hardness, stickiness, elasticity, cohesiveness, and chewiness. A compression test was performed using XT2i Texture Measuring Machine (Stable Microsystems, UK) on instant artificial rice, followed by compression-withdraw cycle at 50% deformation. The speed of compression head was adjusted to 2 min/s. The hardness value is a maximum peak force during the first compression. Adhesive force is a maximum negative peak force after the first compression.

Whiteness Index Whiteness index was analyzed using chromameter CR-300 model (Prasert and Swannaporn, 2009). The a, L and b values were counted with the following formula:

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5}$$

Results and discussions

Corn Flour

Corn flour is the main ingredient/raw material in the production of instant artificial rice. Important characteristics that were analyzed included proximate, total starch, amylose, and dietary fiber. The proximate, amylose and dietary fiber levels of corn flour with Alkaline-cooked processing method

showed higher levels than those of Dry Mill method due to the water content of alkaline cooked corn flour was higher than that of Dry mill processing method.

Table 2. Proximate Analysis of Corn Flour

Analysis	Dry Mill Corn Flour(%)	Alkali Cooked Mill Corn Flour(%)
Moisture Content	8.21	9.86
Ash Content	0.24	0.30
Fat Content	0.65	0.68
Protein Content	8.92	9.26
Starch Content	73.97	72.05
Amylose Content	23.01	25.72
Dietary Fiber Content	4.63	5.80

Overall, the two methods showed no significant differences; as a result, in the following stages of processing, corn flour material will be used Dry Mill method. However, the results was different from previous study conducted by Riyani (2007) using several types of local corn varieties. Based on the analysis, the composition of *Srikandi Putih* corn flour using Dry mill method contained 7.34% of water, 1.45% of ash, 10.77% of protein, 6.49% of fat, 38.33% of amylose, and 58.59% of starch in dry basis. While, in the use of alkaline-cooked method, the composition of flour contained 8.70% of water, 1.87% of ash, 10.37% of protein, 6.99% of fat, 35.74% of amylose and 52.16% of starch. The significant differences in the analysis results conducted by Riyani (2007) are possible due to the existence of several factors such as cultivation condition, seed derivatives, and drying process condition.

Furthermore, based on the analysis on amilograph profile, analysis on the highest viscosity, gelatinization temperature (Tgel), and viscosity at 50°C was conducted.

Table 3. Amylograph Profile of Dry Mill and Alkaline-Cooked Corn Flour

Sample	Tgel (°C)	Peak Visc.(BU)	93°C Visc. (BU)	93°C/ 20 min Visc.(BU)	50°C Visc. (BU)	50°C/ 20 min Visc. (BU)
Dry Mill	81	-	100	190	420	+ 230
Alkali Cooked Mill	84	-	120	240	450	+ 210

Instant Artificial Rice

Based on the results, significant results were shown on process parameters of cooking time, degree of gelatinization, WAI, expansion index and complexing index based on ANOVA analysis, thus, they were used as parameters for process optimization.

Table 4. Significant respond coefficients of regression equation

	Cooking Time	Degree of Gelatinization	WAI	Expansion Index	Complexing Index
Model	0.0020	0.0005	0.0021	0.0040	0.0001
A. Temperature	0.0024	0.0021	0.0296	0.0009	0.4015
B. Steaming	0.0106	0.0004	0.0015	0.1056	< 0.0001
AB	-	0.0302	-	0.7061	-

Lack of Fit	0.0019	0.0062	0.0238	0.0013	0.0003
R ²	0,788984	0,908436	0,785657	0,942491	0,895955

A= Temperature, B = Steaming

Based on ANOVA R² values analysis, the results showed significance due to treatments. The expansion index analysis had the highest value. Because all have shown significance, all parameter responses then be used in the optimization of instant artificial rice.

Cooking Time

Cooking time analysis showed linear patterns resulted in which the cooking time becoming shorter when the temperature and steaming time increased (Figure 1).

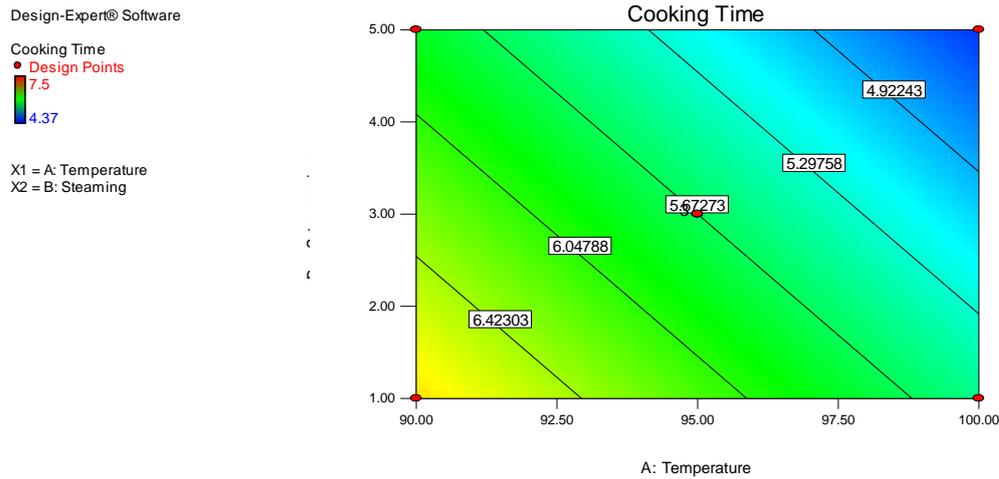


Figure 1. Cooking Time of Instant Artificial Rice

Degree of Gelatinization

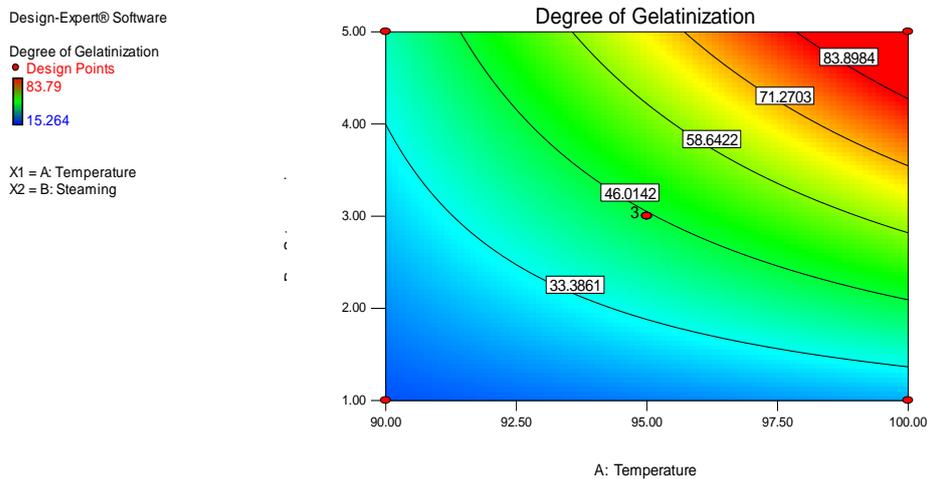


Figure 2. Degree of Gelatinization Instant Artificial Rice

The degree of gelatinization profile showed that it increased as temperature and steaming time also increased. This evidence supports theory explained by Koide *et al.* (1999) that the degree of gelatinization increases as the extruder temperature also increases. In this study, temperature of 100 °C

with a 5 minute steaming period produced the highest degree of gelatinization i.e. 83.79%. The degree of gelatinization increased from 50 to 60% at temperature of 80 °C, and reached 90% at temperature of 120°C (Koide *et al.*, 1999). However, results are influenced by the use of the extruder type and raw materials.

Water Absorption Index

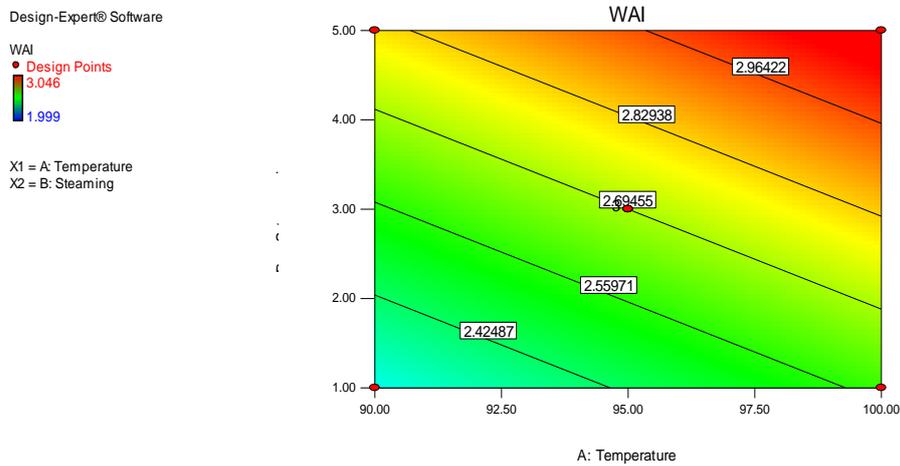


Figure 3. Water Absorption Index of Instant Artificial Rice

WAI is an analysis that can measure the maximum amount of water that can be absorbed by a product. Govindasamy et al. (1996) explained that barrel temperature and screw speed affects the WAI value of the extrudate produced. Based on optimization analysis, to increase extruder temperature and combination of steaming process could improve WAI.

Expansion Index

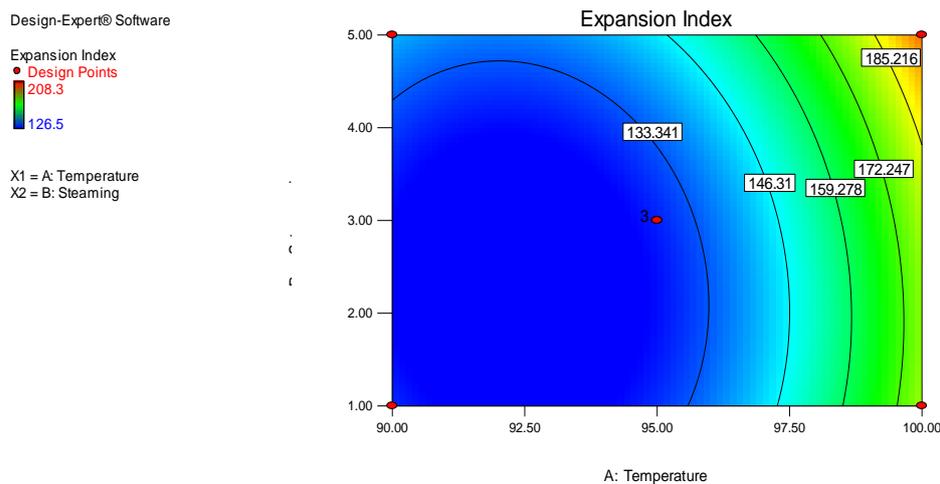


Figure 4. Expansion index of Instant Artificial Rice

Expansion index (EI) is a parameter to determine how much the product can puff and to whether the product starts to undergo excessive puffiness, and this can eventually lead to formation of puff-

products such as cereal snacks. In artificial rice production, the product is expected to puff just adequately, otherwise, it resemble an extrusion puff product. This parameter is important, because it is expected that the product still has the characteristics like cooked rice, and not cereal breakfast products. In this case, EI was analyzed and optimized based on the lowest EI. Kumagai *et al.* (1987) conducted an analysis on extrusion products by comparing the diameter of the product and diameter of extruder die.

Complexing Index

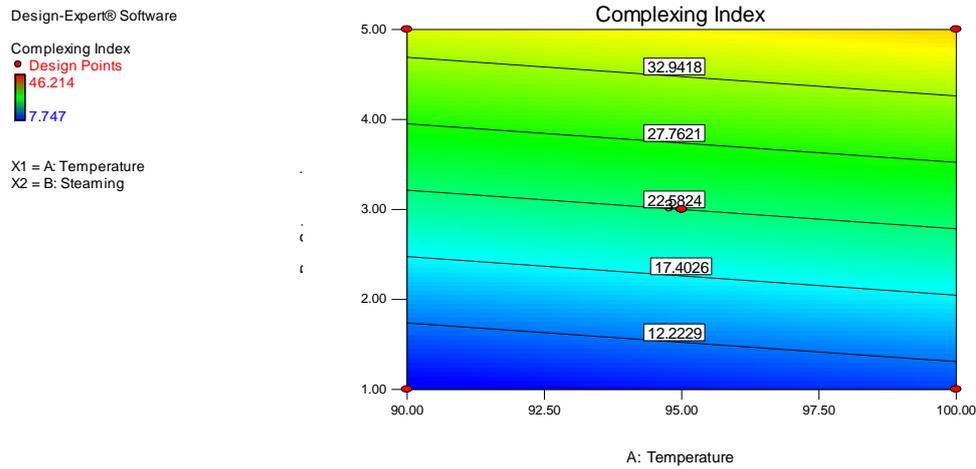


Figure 5. Complexing Index of Instant Artificial Rice

To perform the optimization process, the five parameters were evaluated to obtain desirable value of 0.837. Based on the results of the optimization process, optimum conditions were at temperature of 96 °C and 5 min steaming period.

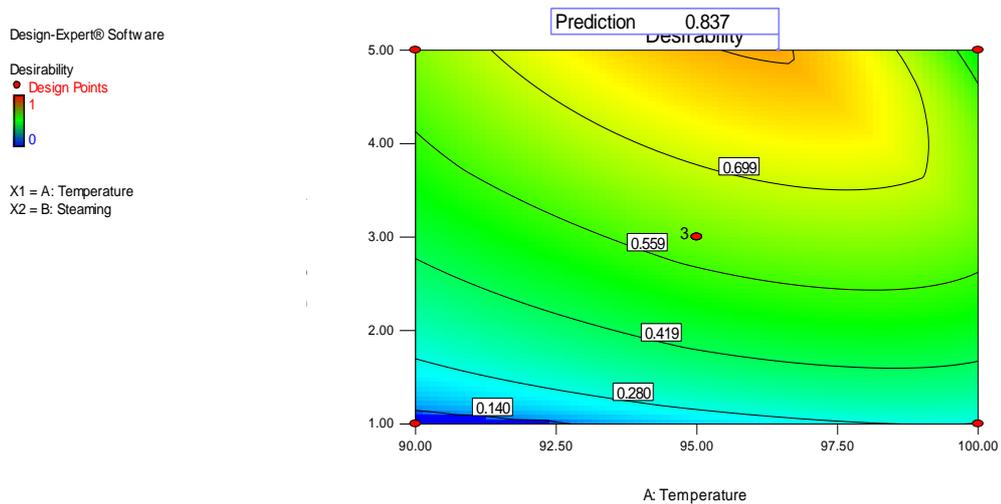


Figure 6. Overlay From Five Resps Parameters To The Desirable Optimized Condition

Texture Profile

Table 5. Texture Profile Analysis of Instant Artificial Rice

Method	Hardness	Chewiness	Cohesiveness	Elasticity
100 C : 1 M	139.1	76.96	0.560	0.987
102 C: 3 M	118.1	79.11	0.675	0.992
100 C: 5 M	118.9	71.88	0.606	0.997
90 C : 1 M	265.8	147.75	0.567	0.980
88 C: 3 M	203.5	90.08	0.491	0.901
90 C : 5 M	203.9	75.16	0.392	0.941
95 C: 3 M	328.1	205.09	0.642	0.974
95 C: 3 M	315.55	203.22	0.663	0.972
95 C: 3 M	314.35	205.67	0.669	0.978
95 C: 0 M	594.5	431.10	0.741	0.979
95 C: 6 M	255.3	175.73	0.705	0.976

Wang *et al.* (2011) analyzed the texture of instant artificial rice product made from rice flour. The value of hardness becomes an important parameter, because the quality of the rice product produced is expected to have almost the same quality as that of the paddy rice. Wang *et al.* (2011) produced the value of hardness with hydration time of 5 min as much as 213.64 g and 330.47 g with hydration time of 3 min. The value of stickiness is also an important parameter since the product which is not too hard or sticky becomes the parameter in optimizing the products.

Whiteness Index

Table 6. Whiteness Index of Instant Artificial Rice

Method	L	a	b	Whiteness index
100 C : 1 M	71.402	0.190	13.286	68.443
102 C: 3 M	70.722	0.308	13.344	67.809
100 C: 5 M	69.276	0.470	13.144	66.573
90 C : 1 M	72.328	0.448	14.544	68.730
88 C: 3 M	71.884	0.510	14.710	68.257
90 C : 5 M	70.980	0.448	14.878	67.385
95 C: 3 M	73.366	0.242	14.418	69.712
95 C: 3 M	72.878	0.24	14.566	69.209
95 C: 3 M	72.826	0.294	14.074	69.393
95 C: 0 M	76.264	0.306	14.450	72.198
95 C: 6 M	73.132	0.228	14.964	69.240

Based on the analysis results of whiteness index, values ranging from 66.573 to 72.198 were obtained while the analysis result showed that the uniformity of instant artificial rice size ranged from 0.203 to 0.281. Process parameters affect texture characteristics, whiteness index and size uniformity. With the combination of process temperature and steaming time, a variety of characteristics of whiteness index, uniformity of size, chewiness, elasticity, and cohesiveness were obtained.

Conclusion

Technological process of artificial rice production can be conducted through a number of methods, some of which include granulation system and extrusion. In the extrusion method, moisture content of materials, extruder temperature, and screw rotation speed influence the process parameters. In the first stage, the optimum condition with 60% of moisture content, temperature between 90°C to 100°C, sodium-alginate of 1%, GMS of 2% and maximum steaming of 6 min were obtained. Based on this optimization using RSM, the optimal temperature condition was 96°C with a steaming period of 5min. Production of artificial rice is an alternative form of opportunities in the development of food diversification concept using a number of raw material sources of non-rice through rice-based product development.

References

- Anderson, R. A., Conway, H. F., Pfeifer, V. F., Griffin, E. L., 1969. Gelatinization of corn grits by roll and extrusion cooking. *Cereal Science Today*, 14, p. 4–12.
- AOAC, 1998. Official Methods of Analysis of the Association Analytical Chemistry Inc, Washington D.C.
- Asp, N. G., 1983. Rapid enzymatic assay of insoluble and soluble dietary fiber. *J Agric Food Chem.* 31: 476-482.
- Blazek, J., Copeland, L., 2009. Effect of monopalmitin on pasting properties of wheat starches with varying amylose content. *Carbohydrate Polymers* 78, 131-136.
- Budijanto, S., 2010. Inaktivasi Enzim Lipase untuk Stabilisasi bekatul (Maksimum FFA 5%) 4 Varietas padi Sebagai bahan Ingridient Pangan Fungsional yang dapat Disimpan 6 bulan. KKP3T Research Report. Indonesia Agency for Agriculture Research and Development.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P.A., Smith, F., 1956. Calorimetric method for determination of sugars and related substance. *Analytical Chemistry*, 28, p. 350-356.
- Govindasamy, S., Campanella, O.H., Oates, C.G., 1996. High Moisture Twin-Screw Extrusion of Sago Starch: 1 Influence on Granule Morphology and Structure. *Carbohydrate Polymers*, 30, p. 215-286.
- Guraya, H., Kadan, R., and Champagne, E., 1997. Effect of rice starch–lipid complexes on in vitro digestibility, complexing index, and viscosity. *Cereal Chemistry*, 74, p. 561–565.
- Herawati, H., Widowati, S., 2009. Karakteristik Beras Mutiara dari Ubi Jalar (*Ipomea batatas*). *Buletin Teknologi Pascapanen Pertanian*, 5(1), p. 39-48.
- Herawati, H., Arif, A., Oktaviani, K., Widowati, S., 2011. Karakteristik beras artifisial berbasis ubi kayu dan kedelai. Disampaikan pada Seminar Nasional Teknologi Pascapanen 2011, Bogor.
- Johnson, L. A., 1991. Corn: production, processing and utilization. In: K. J. Lorenz dan K. Kulp (eds). "Handbook of Cereal Science and Technology" Marcell Dekker, Inc. New York.
- Kumagai, H., Byeong-Heon, L., Toshimasa, Y., 1987. Flour treatment to improve the quality of extrusion-cooked rice flour products. *Agricultural Biological Chemistry* 51 (8), p. 2067–2071.
- Lisnan, 2008. Pengembangan Beras *Artificial* Dari Ubi Kayu (*Manihot esculenta Crant.*) Dan Ubi Jalar (*Ipomoea batatas*) Sebagai Upaya Diversifikasi Pangan. Skripsi. Institut Pertanian Bogor.
- Luh, B. S., 1991. "Rice, Vol I: Production". New York, Van Nostrand Reinhold. P: 51-88.
- Luh, Bor.S. 1980. Rice: Production and Utilization. *Food Technologist*. Department of Food Science and Technology. University of California. Avi Publishing Company, Inc. United States of America. 294-299 p.
- Mishra, A., Mishra, H. N., Rao, P. S., 2012. Preparation of Rice Analogues Using Extrusion Technology. *International Journal Food Sci. & Tech.*, Volume 47, Issue 9 : 1789–1797
- Prasert, A. W., Suwannaporn, P., 2009. Optimization of instant jasmine rice process and its physicochemical properties. *Journal of Food Engineering*, 95, p. 54–61.
- Rewthong, O., Soponronnarit, S., Taechhapairoj, C., Tungtrakul, P., Prachayawasakorn, S., 2010. Effects of Cooking and Pretreatment Methods on texture and Starch Digestibility of Instant Rice. *Journal of Food Engineering*, 103, p. 258-264.
- Riyani, 2007. Teknologi produksi dan karakterisasi tepung jagung varietas unggul nasional. Skripsi. Fakultas Teknologi Pertanian, IPB, Bogor.
- Roberts, L. R., 1972. Quick cooking rice. In Houston D. F. (ed.) "Rice chemistry and technology". American Association of Cereal Chemist., St Paul, Minnesota, p: 1-10.
- Samad, Y., 2003. "Pembuatan Beras Tiruan (Artificial Rice) Dengan Bahan Baku Ubi kayu Dan Sagu". Prosiding Seminar Teknologi Untuk Negeri 2003, Vol. II, p. 36-40 /Humas-Bppt/Any.
- Scelia, R. P., Hegedus, E., Giacone, J., Bruins, H. B., Benjamin, E. J., 1986. Extruded quick cooking rice like product. *European Patent*, 0226375.
- Wang, J. P., An, H. Z., Jin, Z. Y., Xie, Z. J., Zhuang, H. N., Kim, J. M., 2011. Emulsifiers and Thickeners on Extrusion-Cooked Instant Rice Product. *Journal of Food Science Technology*, published online 28th may 2011.
- Wenger, M. L., Huber, G. R., 1988. Low shear extrusion process for manufacture of quick cooking rice. US Patent 4.769.251.
- Wooton, M. D. W., Munk, N., 1971. A rapid method for the estimation of starch gelatinization in processed foods. *Food Technol. Aust.*, 1, p. 612-615.

Gluten Free Noodle and Pasta Process Production Technology

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Abstract

Noodle product originating from China. Noodle can be classified into five types: raw or fresh noodle, wet noodle, dried noodle, fried noodle and instant noodle. While the pasta is shaped mold throughout extrusion technology process production. Noodle and pasta are usually processed from wheat as raw based material. This is done because of gluten protein of wheat flour that has a high elasticity. Thus, the noodle becomes elastic and has a nice texture. Along with the increasing number of diseases or celiac disorder digestive tract, developed gluten-free noodle. One of the obstacles in the processing of gluten-free noodle that is decreasing the power of elasticity, which causes brittle and cracked noodle. The existence of some process modifications and the addition of food additives is very important to produce optimized noodle and pasta. In the manufacture of noodle, to do the modification process prior gelling combined with the addition of hydrocolloids to get the highest elasticity as noodle from gluten free based raw material. While other studies have also produced noodle with the addition of GMS (Glycerol monostearate) to improve the elasticity and texture of the product. Likewise with the pasta-making process technology, can be added by using the GMS or other type of hydrocolloid. In this paper, we try to do reviewed on the technology of processing noodle and pasta gluten free by using gluten free based raw materials.

Keywords: technology, mie, pasta, gluten free

Introduction

Noodles are very popular food in Indonesia and generally in Asia. Nearly 40% use of wheat in Asia is destined for the manufacture of noodle products. Potential production of noodles in Indonesia is quite large. This boosted the level of demand of wheat for noodle-making is increasing. Wheat gluten containing components namely glutenin responsible for the elasticity of the resulting noodles.

Gluten in noodle products have a negative effect among others that can cause celiac diseases or gastrointestinal disturbances. To suppress the onset of the celiac disease, one of the alternatives that can be done is by using other alternative raw materials for the main raw material in the manufacture of noodles and other pasta products. Increased celiac or inflammatory bowel disease caused by gluten (Capriles and Areas 2014). Some indications of the disease such as the existence of malabsorption, diarrhea, weight loss and osteoporosis as well as other allergic symptoms (Sollid and Lundir 2009). The existence of the disease is more widespread in America and Europe that have high levels of consumption of wheat products is quite high, which is based on the data 1 100 to 1 300 of the population have this disease (Rowers 2005; Rubio-Tapia *et al.*, 2012). This is triggering the development of processed food products based on gluten-free flour Packaged Facts (2013).

The use of non-wheat flour has characteristics that are not as good elasticity of the gluten protein. To produce quality noodles and pasta products optimally, it needs modification process on flour and other process steps. The addition of food additives such as STTP (sodium tripolyphosphate) can improve the elasticity of the resulting noodles. Some non-wheat noodle processing technology were also

recently published (Suhendro et al. (2000); Purwani et al. (2006); Sugiyono et al. (2010); Sugiyono et al. (2011); Purwandari et al. (2014); Mulyadi et al. (2014); Cai et al. (2016)).

Some gluten-free pasta processing technology were also reported such as research conducted by Marti et al. (2011); Mulyawanti et al. (2015); LaRossa et al. (2015). Based on the development of noodles technology, this paper aims to explore studies regarding the modification of technology on powder manufacturing technology, formula composition of noodles and pasta as well as the stages of the process used.

Gluten free flour

Indonesia has a lot of potential local sources of carbohydrates to be used as raw material for making flour. Some local sources of carbohydrates among others are cassava, sweet potato, sorghum, sago, Dioscorea esculenta, arrowroot, taro, breadfruit, and sweet potatoes. Sorghum flour processing technology were reported by Suhendro et al. (2000); Schober et al. (2007); Schober et al. (2014); Winger et al. (2014); Trappey et al. (2015). Milling methods can be done to reduce levels of tannin attached to grain sorghum, in order to obtain flour that does not taste astringent (Widowati, 2015).

Processing technology for cassava flour, can be done using fermentation by using LAB (Lactic Acid Bacteria) as published by Subagio (2006; 2007); Hersoelistyorini et al. (2015); Kurniati et al. (2012). By using these technologies, acquired characteristics more closely resembling of wheat flour. Technology modification of non wheat flour can be performed using food additives. Cai et al. (2016) using rice flour with a combination of physical treatment as well as the use of food additives. One of the viscosity profile of the flour before the process of making noodles and pasta, can be observe using RVA (Rapid Visco Analyzer) and obtained as shown in Figure 1.

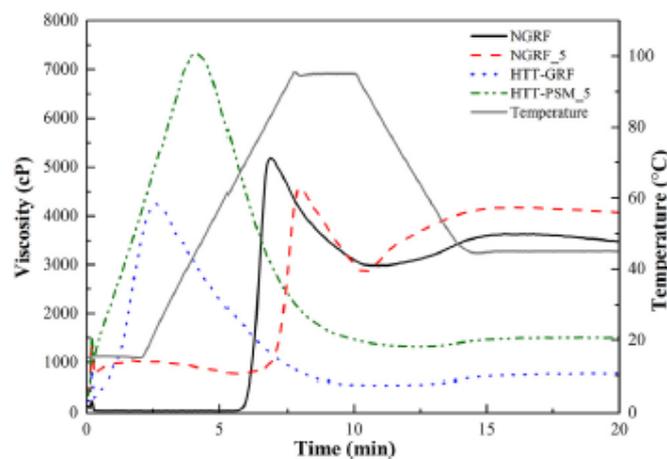


Figure 1. Profile RVA (Rapid Visco Analyzer) From Rice Flour and Rice Flour Modified for Producing Noodles (Cai et al. 2016)

The addition of salt and egg proteins affecting the quality of peak viscosity, setback and final viscosity of rice flour (Imaningsih 2012; Herawati and Sunarmani 2016). Analysis of the characteristics of pasta and noodles can be seen from the quality of the batter or dough. The viscosity and elasticity of the dough will determine the quality of noodles and pasta produced. The quality of the resulting dough with a modification as shown in Figure 2 (Cai et al., 2016).

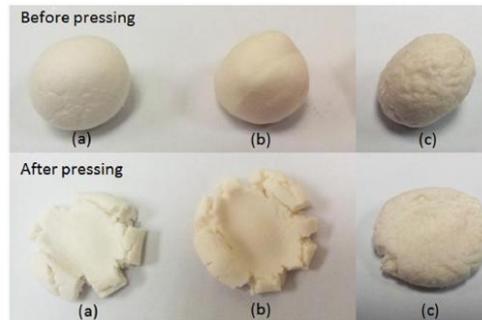


Figure 2. Effect of Modifications To The Characteristics Dough For Making Noodles (Cai *et al.* 2016)

Noodle production

The development of local sources of carbohydrates require modification technology to produce quality good elasticity so that the resulting products are the optimal noodles. Mulyadi *et al.* (2014) and Sugiono *et al.* (2011) using a modified form of the addition hidokoloid CMC (Carboxy Methyl Cellulose) to produce optimal quality of the resulting noodles (Herawati and Sunarmani 2016).

Cai *et al.* (2016) used a combination of the heating process and the use of hydrocolloid that is Xanthan gum, to produce better quality on elasticity of noodles. Some other studies using other carbohydrate sources such as *ubikayu* or *gatotan* conducted by Purwandari *et al.* (2014). The use of hydrocolloids such as CMC (carboxy Methil Cellulose) were done by Mulyadi *et al.* (2014) and Sugiyono *et al.* (2011). Several research on process technology production of gluten-free noodles as listed in Table 1.

Table 1. Production Process Technology Gluten Free Noodles

No	Flour Type	Modification Technology	Source
1	Rice	Heating and hydrocolloid addition	Cai <i>et al.</i> (2016)
2	Fermented Cassava	Pre gelatinization	Purwandari <i>et al.</i> (2014)
3	Sweet Potato	Hydrocolloid and yellow egg addition	Mulyadi <i>et al.</i> (2014); Sugiyono <i>et al.</i> (2011)
4	Sorgum	Reformulation	Suhendro <i>et al.</i> (2000)
5	Corn	Extrusion and Callendaring modification	Muhandri <i>et al.</i> (2011); Subarna <i>et al.</i> (2012); Subarna dan Muhandri (2013)

The use of hydrocolloid can increase the viscosity of the resulting noodles. To increase the elasticity of the noodle dough, food additives such as monoglycerides and STTP (Sodium tripolyphosphate) can be used. STTP can bind water so that the mixture of water contained in the dough does not easily evaporate. Potassium carbonate or sodium carbonate is added, with the aim of forming a bond, thus increasing the elasticity and extensibility of noodles produced and also improve the texture of noodles.

Pasta production

Technology production process gluten-free pasta can be processed from several flour. Based on the research results, pasa gluten-free can be prepared from rice flour (Marti *et al.*, 2011); sweet potato

flour (Mulyawanti *et al.*, 2015); cornstarch (Larrosa *et al.*, 2015; 2016); and rice flour combined with cornstarch (Sanguenetti *et al.*, 2015), as shown in Table 2.

Table 2. Production Process Technology Gluten-Free Pasta

No	Flour Type	Modification Technology	Source
1	Rice	Extrusion method	Marti <i>et al.</i> (2011)
2	Sweet Potato	Cold extrusion combined with green bean addition	Mulyawanti <i>et al.</i> (2015)
3	Corn	Formulation and cooking modification	Larrosa <i>et al.</i> (2015)
4	Corn	Hydrocolloid addition	Larrossa <i>et al.</i> (2016)
5	Rice and Maizena	Hydrocolloid addition	Sanguenetti <i>et al.</i> (2015)

To increase the viscosity and elasticity of the pasta product, multiple sources of carbohydrates with their local modification of the process as well as the presence of some food additives can be used. Larrossa *et al.* (2016) and Sanguenetti *et al.* (2015) using a class of hydrocolloids for food additives. While Mulyawanti *et al.* (2015) using additional green bean flour to increase the protein content of the pasta product.

Characteristics of noodle and pasta

The analysis can be performed to determine the quality of the pasta noodle products produced such as cooking loss, yield, water absorption capabilities, the ability to bind oil, texture, organoleptic parameters, as well as the profile of the product microstructure. Characteristics of noodles produced from cassava flour after fermentation can be seen in Table 3.

Analysis of the characteristics of noodles and pasta are produced not only in the form of texture characteristics, but also include sensory aspects. Some characteristic of texture that can be observed include: extensibility, tensile strength, hardness, adhesiveness and chewiness.

Purwandari *et al.* (2014) conducted a sensory analysis of the fermented cassava noodle. Some important parameters to determine the sensory quality noodle products among which firmness, elasticity, color, flavor, surface smoothness (Choo and Aziz, 2010); firmness, cohesiveness, and stickiness (Li *et al.*, 2012); hardness, slipperiness, chewiness, and elasticity (Yuan *et al.*, 2008); color, flavor, texture, and mouth feel (Yousif *et al.*, 2012).

Other analysis that can be used to observe the quality of pasta is with texture profile analysis using landfill and oil absorption power. Based on the analysis of texture and absorption ability of the oil to the pasta with the treatment use of hydrocolloid (Table 4).

Noodle made from blend of potato and rice flour had better acceptance as relatively low hardness and chewiness (Sandhu *et al.*, 2010) as compared to noodle made from each type of flour (Purwandari *et al.* 2014). While the addition of hydrocolloid with the trademark of Nutrim% can improve the quality of the resulting noodles (Inglett *et al.*, 2005). While the characteristics of profile microstructure resulting from Larrossa *et al.* (2015) are shown in Figure 3.

Table 3. Effect of Addition of Water and Flour Type Of Cooking Loss and Water Absorption Of Fermented Cassava Flour Noodles

Flour: water, in pregelatinized flour	Pre-gelatinized flour, flour in dough	Cooking Loss (%)*	Water Absorption*
1:7	5:5	4,761b	0,922b
1:7	5:5,5	3,199a	0,824ab
1:7	5:6	3,626a	0,833ab
1:8	5:5	3,172a	0,847b
1:8	5:5,5	3,116a	0,838b
1:8	5:6	3,241a	0,806ab
1:9	5:5	3,368a	0,823ab
1:9	5:5,5	3,433a	0,830ab
1:9	5:6	3,129a	0,722a

*: Same letter in a coloumn indicates no significant (P>0,05) difference (Purwandari *et al.* 2014)

Table 4. Results of Analysis TPA (Texture Profile Analyzer) and Oil Absorption Percentage Of Gluten-Free Pasta

Pasta Sampel	TPA Test				Percent Absorbed Oil (db)
	Hardness (N)	Adhesivness (Ns)	Springiness	Cohesivness	
GG(1,5)	9,13bc	4,06b	0,106b	0,14d	9,50a
XG(1,5)	7,92c	7,20a	0,121b	0,17c	6,98c
GG(2,5)	11,80a	3,70b	0,115b	0,18c	8,22c
XG(2,5)	10,48ab	4,26b	0,153b	0,23b	7,48c
Control	8,17c	-1,45c	0,340a	0,26a	14,43a

Means with different letters within each column are statistically significantly according to Duncan's Multiple Range Test

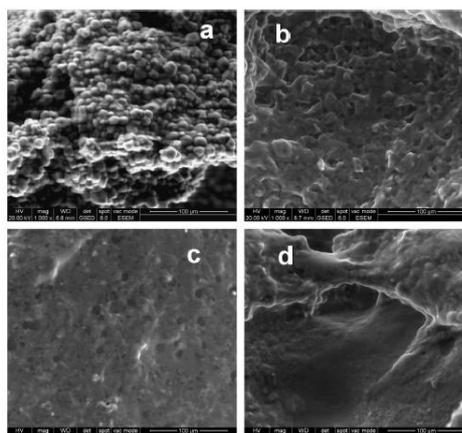


Figure 3. Profile SEM (Scanning Microscope Electrone) Of Corn Pasta Cooking With Modifications (a) 0 min, (b) 5 minutes, (c) 10 minutes, (d) 15 minutes (Larossa *et al.* 2015)

Cooking process technology affects the profile microstructure of the resulting noodles. Based on the research results Larossa *et al.* (2015), with the treatment of cooking 0, 5, 10 and 15 minutes influence the outcome of SEM (Figure 3).

Conclusion

Noodles and pasta products can be processed using the local sources of carbohydrates as raw materials. Some local sources of carbohydrates such as: rice flour, cassava, sweet potato, maize and sorghum. The existence of some process modifications and the addition of food additives is very important to produce noodle and pasta. In the manufacture of noodle and pasta, to do the modification process prior gelling combined with the addition of hydrocolloids to get the highest elasticity as noodle from gluten free based raw material. While other studies have also produced noodle and pasta with the addition of hydrocolloid to improve the elasticity and texture of the product.

References

- Cai J., Chiang J.H., Tan M.Y.P., Saw L.K., Xu Y., Ngan-Loong M.N. 2016. Physicochemical properties of hydrothermally treated glutinous rice flour and xanthan gum mixture and its application in gluten-free noodles. *Journal of Food Engineering* 186: 1-9.
- Capriles, V. D., & Areas, J. G. (2014). Novel approaches in gluten-free breadmaking: interface between food science, nutrition, and health. *Comprehensive Reviews in Food Science and Food Safety*, 13, 871-890.
- Choo, C. L. and Aziz, N. A. A. 2010. Effects of banana flour and β -glucan on the nutritional and sensory evaluation of noodles. *Food Chemistry* 119: 34-40.
- Herawati H. And Sunarmani. 2016. Gluten free flour and its challenges in uses. Paper presented at the international conference "Food Innovations ASEAN Economic Community (AEC) Challenges" Jakarta International Expo - Kemayoran, Indonesia, September 21-23th 2016.
- Herawati H. And Sunarmani. 2016. Teknologi proses pengolahan produk roti gluten free. *Prosiding Seminar Nasional dalam Rangka Dies Natalis ke-53 Fakultas Pertanian Universitas Sriwijaya, Palembang* 14 September 2016.
- Hersoelistyorini, W., Dewi, S. S., Kumoro, A. C. 2015. Sifat fisikokimia dan organoleptik tepung mocaf (modified cassava flour) dengan fermentasi menggunakan ekstrak kubis. *The 2nd University Research Coloquium*, ISSN 2407-9189, 10-17.
- Imanningsih, N. 2012. Profil gelatinisasi beberapa formulasi tepung-tepungan untuk pendugaan sifat pemasakan. *Penel Gizi Makan* 2012, 35(1): 13-22.
- Inglett, G. E., Peterson, S. C., Carriere, C. J. and Maneepun, S. 2005. Rheological, textural, and sensory properties of Asian noodles containing and oat cereal hydrocolloid. *Food Chemistry* 90:1-8.
- Kurniati, L.I., Aida, N., Gunawan, S., Widjaja, T. Pembuatan mocaf (modified cassava flour) dengan fermentasi menggunakan *Lactobacillus plantarum*, *Saccharomyces cerevisiae* dan *Rhizopus oryzae*. *JURNAL TEKNIK POMITS* Vol. 1, No. 1: 1-6.
- Larrosa V., Lorenzo G., Zaritzky N., Califano A. 2015. Dynamic rheological analysis of gluten-free pasta as affected by composition and cooking time. *Journal of Food Engineering* 160: 11-18.
- Larossa V., Lorenzo G., Zanitsky N., Califano A. 2016. Improvement of the texture and quality of cooked gluten-free pasta. *LWT - Food Science and Technology* 70 : 96-103.
- Li, P-H., Huang, C-C., Yang, M-Y. and Wang, C-C.R. 2012. Textural and sensory properties of salted noodles containing purple yam flour. *Food Research International* 47 (2): 223-228.
- Marti A, Seetharaman K., Pagani M.A. 2010. Rice-based pasta- Acomparision between conventional pasta-making and extrusion-cooking. *J. Cereal Sci* 52: 440-409. gluten-free pasta from rice flour. *Carbohydr Polym* 84: 1069-1074.
- Marti A, Pagani M.A., Seetharaman K. 2011. Understanding starch, organization in
- Muhandri T., Ahza A.B. Syarief R. Sutrisno. 2011. Optimasi proses ekstrusi mi jagung dengan metode respon permukaan. *J. Teknol dan Industri Pangan*. 22: 97-104.
- Mulyadi A. F., Wijana S., Dewi I.A., Putri, W.I. 2014. Organoleptic Characteristics of Dry Noodle Products from Yellow Sweet Potato (*Ipomoea batatas*) (Study on Adding Eggs and CMC). *Jurnal Teknologi Pertanian* Vol. 15 No. 1: 25-36.
- Mulyawanti I., Budijanto S., Sedarnawati Y. 2016. Optimasi formula dan struktur mikroskopik pasta bebas gluten berbahan dasar puree ubi jalar ungu dan tepung kacang hijau. *Jurnal Agritech* Vol 36, No 01.
- Purwandari U., Hidayati D., Tamam B., Arifin S. 2014. Gluten-free noodle made from gathotan (an Indonesian fungal fermented cassava) flour: cooking quality, textural, and sensory properties. *International Food Research Journal* 21(4): 1615-1621.
- Purwani E.Y., Widaningrum, Thahir R., Muslich. 2006. Effect of moisture threatment of sago starch on its noodle quality. 2006. *Indonesia J. Agri Sci* 7(1):8-14.
- Rowers M. 2005. Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease? *Gastroenterology*, 128, S47-S51.
- Rubio-Tapia, A., Ludvigsson, J. F., Brantner, T. L., Murray, J. A., & Everhart, J. E. (2012). The prevalence of celiac disease in the United States. *American Journal of Gastroenterology*, 107, 1538-1544.
- Sanguenetti A.M., Secchi N., Caro A.D., Fadda C., Paolo A.M., Catzeddu P., Piga A. 2015. Gluten-free fresh filled pasta: The effects of xanthan and guar gum on changes in quality parameters after pasteurisation and during storage. *LWT - Food Science and Technology* 64: 678-684.
- Schober, T. J., Bean, S. R., & Boyle, D. L. (2007). Gluten-free sorghum bread improved by sourdough fermentation: biochemical, rheological, and microstructural background. *Journal of Agricultural and Food Chemistry*, 55, 5137-5146.
- Schober, T. J., Messerschmidt, M., Bean, S. R., Park, S. H., & Arendt, E. K. (2005). Gluten-free bread from sorghum: quality differences among hybrids. *Cereal Chemistry*, 82, 394-404.
- Sollid, L. M., & Lundin, K. E. A. (2009). Diagnosis and treatment of celiac disease. *Mucosal Immunology*, 2, 3-7.

- Subagio. 2006. Industrialisasi Modified Cassava Flour (Mocaf) sebagai Bahan Baku Industri Pangan untuk Menunjang Diversifikasi Pangan Pokok Nasional. Dalam Hal Lala F. *et al.* 2013. Uji Karakteristik Mie Instan Berbahan-Baku Tepung Terigu dengan Substitusi Mocaf. *J Biopros Kom Trop.* 1(2): 11 – 20.
- Subagio, A. 2007. Industrialisasi Modified Cassava Fluor (Mocaf) sebagai Bahan Baku Industri Pangan untuk Menunjang Diversifikasi Pangan Pokok Nasional. Jember: Fakultas Teknologi Pertanian, Universitas Jember.
- Subarna, Muhandri T., Nurtama B., Fierliyanti A.S. 2012. Peningkatan mutu mi kering jagung dengan penerapan kondisi optimum proses dan penambahan monoglisierida. *J. Teknol dan Industri Pangan* 23: 146-152.
- Subarna dan Muhandri T. 2013. Pembuatan mi jagung kering dengan metode kalendring. *J. Teknol dan Industri Pangan.* 24(1):75-80.
- Sugiyono, Setiawan, E., Syamsir, E., Sumekar, H. 2011. Pengembangan produk mi kering dari tepung ubi jalar (*Ipomea batatas*) dan penentuan umur simpan dengan metode isotherm sorpsi. *J. Teknol dan Industri Pangan.* Vol XXII(2):164-170.
- Suhendro E.L., Kunez F.C., Mc Donough C.M., Rooney L.W., Waniska R. D. 2000. Cooking characteristics and quality of noodles from food shorgum. *Cereal Chem* 77(2): 96-100.
- Sugiyono, Sarwo E. W., Koswara S., Herodian S., Widowati S., Santosa B.A.S. 2010. Pengembangan produk mie instan dari tepung hotong (*Setaria italica Beauw*) dan pendugaan umur simpannya dengan metode akselerasi. *J. Teknol Industri Pangan* 21(1):45-50.
- Trappey, E. F., Khouryieh, H., Aramouni, F., & Herald, T. (2015). Effect of sorghum flour composition and particle size on quality properties of gluten-free bread. *Food Science and Technology International*, 21, 188-202.
- Wenger, M., Khouryieh, H., Aramouni, F., & Herald, T. J. (2014). Sorghum flour characterization and evaluation in gluten-free flour tortilla. *Journal of Food Quality*, 37, 95-106.
- Yousif, E. I., Gadallah, M. G. E. and Sokour, A. M. 2012. Physico-chemical and rheological properties of modified corn starches and its effect on noodle quality. *Annals of Agricultural Science* 57 (1): 19-27.
- Yuan, M-L., Lu, Z-H., Cheng, Y-Q. and Li, L-T. 2008. Effects of spontaneous fermentation on the physical properties of corn starch and rheological characteristics of corn starch noodle. *Journal of Food Engineering* 85: 12-17.

Inactivation of *Staphylococcus aureus* GMP4 on Tofu with Pasteurization Process in Gamatahu KP4 UGM Yogyakarta

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Abstract

Tofu is a food that serves as one of main source of protein in Indonesia with total consumption 24% of the total protein consumption. Tofu is easily contaminated by microbes due to its high a_w and protein content. *Staphylococcus aureus* is a bacterium that are restricted on the tofu because it is a type of pathogenic bacteria and potentially present in the process of tofu making either in raw materials, processing equipment, or processing labor. The contaminant bacteria can be decreased by pasteurization treatment. The research was conducted by inoculating *Staphylococcus aureus* GMP4 which was isolated from the home industry of tofu with a value of $D_{60^\circ\text{C}}=2.72$ minutes and $z=18.87^\circ\text{C}$ into tofu packaging at 9 logCFU/gram then pasteurized for 15,30 and 45 minutes. The results showed that after 15 minutes pasteurization, the number of bacteria was decreased to 4.2 log CFU/g. The same results were also obtained in 30 and 45 minutes of pasteurization, in which the both times showed a decreased number of bacteria to <2 log CFU/g. Pasteurization does not have a significant effect on the texture of tofu, among others: hardness, chewiness and springiness ($p > 0.05$) but significant effect on gumminess ($P < 0.05$). Color variable with parameter L^* , b^* and ΔE^* were not affected either by pasteurization ($p > 0.05$) but the parameter a^* was significantly affected ($p < 0.05$). Sensory quality, among others: taste, aroma, texture, after taste and preference of tofu showed no significant differences during the 15, 30 and 45 minutes ($p > 0.05$) of pasteurization.

Keywords : Tofu, *Staphylococcus aureus*, inactivation, pasteurization, pasteurizer.

Introduction

Based on data obtained from the National Socio-economic Survey (Susenas) in 2013, the amount of tofu consumption was 7.039kg/capita/year or 24% from total protein consumption of Indonesian society. Due to the high market demand of the tofu, many micro and small scale industries were established. Most of home industries do not pay much attention to the hygiene during tofu processing which may cause contamination in the processing of tofu, one of them being microbiological contamination (Rahayu *et al.*, 2012).

Restricted bacterial contamination during the manufacturing process of tofu on Guidelines for The Assessment of Microbiological Quality of Processed Foods issued by Food and Drug Administration (FDA) Philippines in 2013 were *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* is a bacterium that is restricted on the tofu because it is a type of pathogenic

bacteria and potentially present in the process of making tofu with poor hygiene practices either in raw materials or processing equipment (Asao *et al.*, 2003). This bacterium is also potentially present on personnel involved in the processing of tofu because *S. aureus* is a natural colonizer of the skin and mucous membranes of warm-blooded animals and humans (Irlinger, 2008).

In a home industry of tofu in Yogyakarta was found total bacteria *S. aureus* $1,0 \times 10^1$ CFU/gm (Mailia *et al.*, 2015). The initial amount of this bacterium in the end product of tofu can increase rapidly because tofu is a highly perishable food with relatively high moisture content (86%), pH (5,8-6,2), water activity (0,96) and protein(6-8,4%) (Rahayu *et al.*, 2012; Wang, 1979).

S. aureus is a non-spore-forming bacterium that is resistant to heat and potentially, can survive in the process of making tofu. Thermal resistance of this species has been widely studied, and a higher thermoresistance of *S. aureus* among the pathogens that can be found in milk (*Cronobacter sakazakii*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella spp.*) has been reported (Pearce *et al.*, 2012). The D-values at 70°C for *S. aureus* in egg products were higher (of about 0.4 min) with respect to *Salmonella ssp.* and *L. monocytogenes* (Li, Sheldon, and Ball, 2005). Similarly (Kennedy *et al.*, 2005) observed that *S. aureus* has a greater D-value than *L. monocytogenes* and should be used as the target microorganism in designing mild thermal treatments for food.

The way to decrease pathogenic bacteria including *S. aureus* is pasteurization treatment, and this method is suitable to be applied on tofu because of its semi-solid structure. Pasteurization of milk at 66°C for 15 seconds can reduce the number of *S. aureus* > 6.7 log cycle (Pearce *et al.*, 2012). Pasteurization in food products can decrease or increase food quality and it can be seen from the sensory properties such as texture, color and organoleptic quality (Arnoldi, 2001). Optimal pasteurization process needs to be identified to reduce unwanted quality loss and to maximize the quality improvement (Holdsworth, 2004). According to Lewis and Heppel (2000), pasteurization process is called mild heat treatment because it does not cause material damage to both chemical composition and sensory if the temperature and time are well regulated. Therefore, it is necessary to design a pasteurization method that can be applied in the processing of tofu. The importance of this study is to determine the effectiveness of pasteurization process to control *S. aureus* GMP 4 growth and the effect of pasteurization on the quality of packaged tofu.

Materials and Methods

Materials

This study used tofu Gamatahu from the Gamatahu factory, KP4 UGM, Yogyakarta. This product is packaged using plastic polypropylene (PP) with a thickness of 0.3 mm, which contains 10 pieces of tofu and 100ml water per packaging.

Bacterial strains used and preparation of inocula

The strain *S. aureus* GMP4 is the most heat-resistant of *S. aureus* bacterium isolated from the home industry of tofu in Sudagaran, Yogyakarta (Mailia *et al.*, 2015). This strain was maintained as freeze stock at -40°C in the Food and Nutrition Study Center, Universitas Gadjah Mada. Before the experiments, the strain was sub-cultured in Nutrient broth (NB; Oxoid, UK) for 24 h at 37°C.

The culture was activated by streaking a loopfull of *S. aureus* GMP4 from Nutrient broth to the agar slant and incubated at 37°C for 48h. A loopfull of *S. aureus* was transferred from the nutrient agar slant to 100 mL of BHI broth and incubated at 37°C for 24h as the stock culture (Montanari et al., 2015). For the preparation of the inoculum, *S. aureus* was prepared by inoculating 1 mL of stock culture into nutrient broth (500 mL) and incubated for 24 h at 37°C with shaking at 200rpm. Cell enumeration was conducted by plating using a nutrient agar medium. The plates were incubated at 37°C for 24 h and from the enumeration, cell count of 8,9 log CFU/ml was obtained.

Inoculation

Inoculation process was preceded by the process of making tofu. In this process, the soybean soaked for 2 hours, milled, boiled for 20 minutes, and filtered to take the soybean extract. *Kecutan* was added into soybean extract as clotting agent to make tofu. Finished tofu was cut into small squares and put into plastic containers PP. Before the sealing, 100 ml inoculum of *S. aureus* GMP4 was poured into the packaging instead of water with concentration of *S. aureus* cells 8.9 log CFU / ml.

Pasteurization

Pasteurization method used in this study is hot water dipping using retort pasteurizer without heat supply. This pasteurizer has two separate tubes. The inner tube is tofu tube with a diameter of 48 cm and height of 60 cm and made of stainless steel plate with a thickness of 0.8 mm. The function of tofu tube is to place tofu during pasteurization. The pasteurization process was carried out using pasteurizer TPM 7060 made by Department of Agricultural Engineering, Universitas Gadjah Mada. The method used in this pasteurization was hot water dipping (Sachindra *et al.*, 1997). The water inside pasteurizer was heated to boiling point using steam as heat source. After the temperature dropped to 80°C, tofu in the packaging was pasteurized for 15, 30 and 45 minutes. During pasteurization, tofu and water temperature were observed using thermocouple (Lutron, TM-946) with 4 channels.

Microbial enumeration

The enumeration of *S. aureus* before and after pasteurization treatment for 15, 30 and 45 minutes were carried out using Baird Parker agar (BPA; Oxoid, UK) medium and egg yolk tellurite emulsion as supplement (Oxoid, UK). Tofu in the packaging was crushed and diluted to a dilution of 6. Exactly 0.1 ml was taken and spread onto the surface of the medium and incubated at 35°C for 48 hours. After 48 hours, *S. aureus* enumeration was carried out. The characteristics of the colonies were circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by an opaque zone and frequently with outer clear zones; colonies have buttery to gummy consistency when touched with inoculating needle and continued with test of coagulase, catalase, and mannitol (Jackson *et al.*, 2001).

Color measurement

The surface color of the tofu was measured using a chromameter (Konica Minolta, Japan). From this measurement we will acquire L* data (lightness, ranging from zero (black) to 100 (white), a*

(ranging from +60 (red) to -60 (green)), b^* (ranging from +60 (yellow) to -60 (blue)), and ΔE^* (total color change).

Texture measurement

Textural properties of tofu were measured by subjecting the sample to a compression test using texture analyzer (Brookfield LFRA texture analyzer, USA). Data were analyzed using software along with the instrument. Texture profile analysis was carried out on the cylindrical shaped tofu using LFRA texture analyzer with trigger point 20g and the test speed of 1mm/s to obtain hardness, chewiness, springiness and gumminess values.

Sensory Test

Sensory test was carried out by 50 panelists with age range from 18-35 years. Sensory parameters which were tested are the taste, texture, aftertaste, aroma and preferences. Descriptions for each score were: 5 = very like, 4 = like, 3 = rather liked, 2 = dislike, and 1 = strongly dislike.

Statistical Test

Color, texture and organoleptic data were evaluated using SPSS 16. For color and texture measurement were evaluated in triplicate, analyzed using one-way ANOVA followed by Post Hoc Test. Organoleptic test was analyzed by Kruskal-Wallis test.

Result and Discussion

Tofu and Water Temperature during Pasteurization

Pasteurization method used in this study is hot water dipping using retort pasteurizer without heat supply. This pasteurizer has two separate tubes. The inner tube is tofu tube with a diameter of 48 cm and height of 60 cm and made of stainless steel plate with a thickness of 0.8 mm. The function of tofu tube is to place tofu during pasteurization. From this research was obtained that the average tofu temperature during pasteurization is rising from 60.83°C at the start to 70.53°C at the end of the pasteurization, while the average water temperature is dropping from 79.38°C at the start to 69, 43°C at the end of pasteurization (Figure 1).

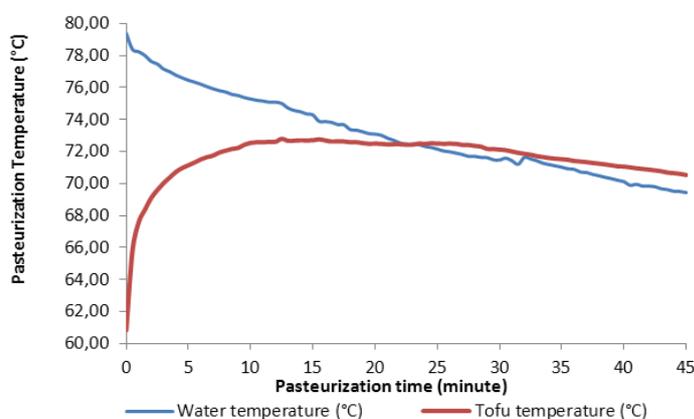


Figure 1. Water and tofu temperature during pasteurization

The increase in tofu temperature occurs due to heat transfer from the water into the tofu packaging by convection and conduction. Heat transfer from the water in the pasteurizer to packaging plastic occurs by convection, as it passes through the plastic packaging thickness, the heat transfer occurs by conduction, heat transfer from the water in the packaging toward tofu occurs by convection and the last is the heat transfer in tofu which occurs by conduction (Holdsworth and Simpson, 2007).

Effects of pasteurization on the number of *S. aureus* GMP4

During pasteurization, the food is heated in a controlled temperature, and held at that temperature for a set time period. The lethality associated with the pasteurization process is based on the holding period. The traditional batch pasteurization process is accomplished using holding time of 30 minutes at 63°C. By using a reference temperature of 63°C (Singh and Heldman, 2009).

The bacteria used in this study was *S. aureus* GMP4 which have a value $D_{60^\circ\text{C}} = 2.72$ min and $Z = 18.87^\circ\text{C}$. From z-value, we can estimate the number of bacteria after pasteurization of non isothermal process using lethality (L) value (Ball, 1928).

$$L = 10^{[(T-T_{\text{ref}})/z]} \quad (1)$$

In which T is any lethal temperature and Tref is reference temperature in the pasteurization process (63°C).

Peleg (2005) developed a method to calculate and record theoretical microbial survival curves during thermal processing of foods and pharmaceutical products simultaneously with the changing temperature and demonstrate that the method can be used to calculate non isothermal survival curves with widely available software such as Microsoft Excel.

The efficacy of thermal preservation processes of foods and pharmaceutical products is currently calculated in terms of an equivalent time at a reference temperature known as the Fo-value (Holdsworth and Simpson, 2007). The calculation of the Fo-value is based on the assumption that thermal inactivation of microbial cells and spores follows first-order kinetics. The Fo-value reached after any time t is calculated by:

$$F_o(t) = \int_0^t L \cdot dt \quad (2)$$

where T(t) is the temperature after time t at the point of interest. The Fo-value is calculated graphically or numerically using equation (2).

From D-value, we can calculate D-value at reference temperature 63°C (Do) using equation (3)

$$D_t = D_o \cdot 10^{[(T_{\text{ref}}-T)/z]} \quad (3)$$

For convection-heating process, all bacteria in these products are subjected to essentially the same amount of lethal heat. In other words, product at all points in the container receives heat processes

of essentially the same F value. It is considered that heating at the geometrical center of the container approximates closely the effective mean heating throughout the container (Stumbo, 1973). If the Fo-value reached after pasteurization time (Fo (t)), D-value at reference temperature (Do), and initial number spores or vegetative cells of the organism of concern in the entire container (a) has known, it can be calculated the number spores or vegetative cells of the organism in the entire container has received a process of designated F value (b) using equation (4)

$$F_o(t) = D_o (\log a - \log b) \quad (4)$$

From the estimated calculation based on D and Z values, the estimated total count of *S. aureus* bacteria after 15, 30 and 45 minutes pasteurization were -13.47 log CFU/g, -38.81 log CFU/g, and -60.72 log CFU/g, respectively. Whereas, the results of this study showed that the total count of *S. aureus* bacteria after 15 minutes pasteurization was 4.2 log CFU/g and <2 log CFU/g after 30 and 45 minutes pasteurization.

From this result, there are differences in the heat resistance of *S. aureus* GMP4 between estimate calculation and research results, it can be caused by the observation of D and z values which use a constant temperature (isothermal) while in this study is using varying temperatures (non isothermal) with relatively high temperature rise on tofu at the beginning of the pasteurization process that can lead to heat shock. *S. aureus* has DnaK heat shock system that will generate heat shock protein (Hsp) which is the key of *S. aureus* survival at higher temperature (Muga and Moro, 2008).

The number of bacteria also affect the heat resistance. The amount of inoculum used in this research is much more than the one used in the heat resistance test of *S. aureus* GMP4. In the same environmental conditions, the more inoculum present, the higher the heat resistance of the bacteria *L. monocytogenes* and *S. aureus* (Besse et al., 2004; Koutsoumanis and Sofos, 2004; Tango et al., 2016).

At conditions close to the boundary, higher numbers of cells increase the probability of one or more cells in the population to grow. Thus, large inocula allow growth initiation under conditions where the growth of small inocula is usually inhibited (McKellar, 2000; Baranyi, 1998). Another possible reason for the effect of inoculum size on the thermal resistant of *S. aureus* could be cell-to-cell communication (quorum sensing) via specific diffusible signal molecules known as autoinducers and the concentrations of autoinducers exceed gene induction thresholds only when cells reach high densities (Fuqua et al., 1994; Redfield, 2002). Bacterial cells produce extracellular signal molecules and sense the concentration of these molecules on the cell surface. If the concentration of a signal molecule exceeds a threshold value, genes responsive to the molecule are induced (Redfield, 2002; Winzer et al., 2002). Inoculum size is also affect relative lag time (RLT) of *S. Aureus*, RLT values were decreased with the increasing number of *S. aureus* inoculum (Tango et al., 2016).

Another factor which affect bacteria thermal resistance is the pH of treatment medium. Under isothermal conditions, *S. aureus* ATCC 25923 and *S. aureus* ATCC 13565 show a higher heat resistance at pH 4,0 than at pH 7,4. In contrast, under nonisothermal treatments both strains were more heat resistant when treated at pH 7,4 than at pH 4,0 (Hassani et al., 2006). Soymilk with pH of 6.5 to 7.2 is used as

media in the heat resistance test of *S. aureus* GMP4 under isothermal treatment, while in this study, tofu is used as the media (pH 4.5-5.3) under non isothermal treatment.

Effect of pasteurization on color

From the color parameters were assessed, L* (lightness, ranging from zero (black) to 100 (white)), b* (ranging from +60 (yellow) to -60 (blue)), and ΔE^* (total colour change) showed no significant differences in all four treatment ($p > 0,05$). Only parameter a* (ranging from +60 (red) to -60 (green)) ($p < 0,05$) that changed significantly. The changing trend of tofu color was observed for all of the pasteurization treatment (Table 1). There is a decrease in tofu brightness (L*), redness (a*) and ΔE , but there is an increase in tofu yellowness (b*) during the pasteurization treatment.

Table 1. Averaged colour parameters of tofu at different pasteurization time

	Color L*	Color a*	Color b*	Color ΔE^*
Control	88,62 ^{ab}	4,80 ^c	11,63 ^a	69,83 ^{ab}
Pasteurization 15 minutes	88,93 ^b	4,50 ^a	11,20 ^a	70,06 ^b
Pasteurization 30 minutes	88,83 ^{ab}	4,53 ^a	11,17 ^a	69,96 ^{ab}
Pasteurization 45 minutes	88,43 ^a	4,70 ^b	11,73 ^a	69,65 ^a

*Data with the same letter within the same column were not significantly different ($P > 0,05$).

The same result regarding the decrease in brightness (L*) was also showed in a study of deep-fried tofu (Baik and Mittal, 2002). The decrease in L* with time was most probably as a result of maillard browning and caramelization. The rate of the Maillard reaction depends on its chemical environment such as water activity, pH and chemical composition of the food; however, the most predominant factor on the velocity of the reaction is temperature (Carabasa and Ibarz, 2000).

The change in total color (ΔE^*), increase in yellowness (b*) and redness parameter (a*) that changed significantly could be attributed to the high temperature of the tofu surface during pasteurization. High temperature initiated nonenzymatic browning reactions such as Maillard reaction and the caramelization of sugars. Maillard reactions include those involving reducing sugars, aldehydes, and ketones with amines, amino acids, peptides, and protein (Hutchings, 1999). In tofu, the reactants could have been reducing sugars and amino acids. Caramels and melanoidan pigments were probably generated by the heat treatment of carbohydrates such as sucrose, glucose, or inverted sugar in the tofu (Baik and Mittal, 2002).

Effect of pasteurization on texture

The textural properties of semisolid or solid food include hardness, cohesiveness, springiness, adhesiveness (or stickiness), chewiness, and gumminess. From the observation of hardness was obtained that there is a greatly increase in hardness on unpasteurized tofu and after pasteurization 15 minutes. But from the observation of all four samples, there was no significant differences in hardness ($p > 0,05$). Hardness is the force required to attain a given deformation of the material (Bourne, 1982). Hardness is

the most important tofu textural characteristic on which market classes of tofu are based. Tofu products do not have a set of officially approved standards of identity (Yuan and Chang, 2007).

Table 2. Averaged texture parameters of tofu at different pasteurization time

	Hardness	Chewiness	Springiness	Gumminess
Control	756,50 ^a	4633,02 ^a	10,96 ^a	418,55 ^a
Pasteurization 15 minutes	1136,33 ^b	4731,80 ^a	11,32 ^a	627,20 ^b
Pasteurization 30 minutes	978,00 ^{ab}	6316,52 ^a	11,22 ^a	562,74 ^b
Pasteurization 45 minutes	1038,00 ^{ab}	6954,71 ^a	11,27 ^a	617,12 ^b

*Data with the same letter within the same column were not significantly different (P >0.05).

For chewiness parameter there was a considerable rise in line with the increase of pasteurization time, but chewiness is not a significant factor in tofu products, it is applied to solid foods only (Szczesniak and Bourne1995). Pasteurization also does not have a significant effect on the springiness of tofu. Springiness is described as the rate in which a deformed material recovers to its original condition after the removal of deforming force (Bourne, 1982).

From this research, pasteurization have a significant effect on the gumminess of tofu (P <0.05). Gumminess is defined as the energy to disintegrate semisolid food to a state ready for swallowing (Bourne, 1982). From the data can be seen that there is a significant increase in gumminess in line with the increase of pasteurization time, this result is match with the increase in hardness of tofu with pasteurization treatment because gumminess is calculated as the product of hardness × cohesiveness (Bourne, 1982).

Effect of pasteurization on sensory quality

From the sensory quality test, the taste, texture, aftertaste, aroma and preference showed no significant differences in all four samples (p>0,05).

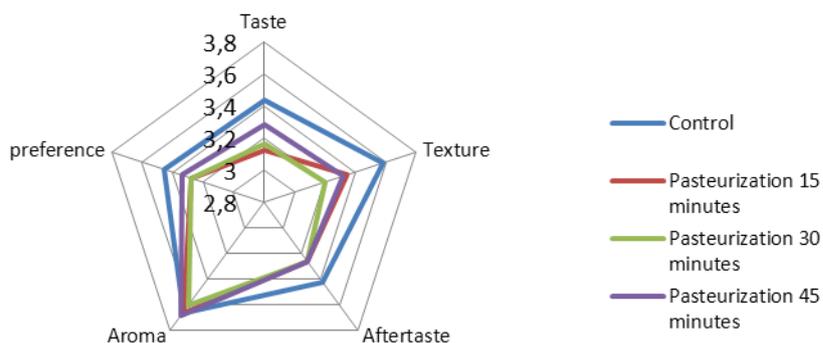


Figure 2. Graphics of sensory test results

From the results of the organoleptic test, panelists preferred tofu which is not pasteurized for taste, texture, aftertaste, and preferences parameter, but for the aroma parameter, panelists prefer tofu that pasteurized for 45 minutes (Figure 2).

For texture parameter, the texture from unpasteurized tofu (control) is the most preferred. This is suitable with the test results of texture in this study. From the data can be seen that there is an increase in gumminess and hardness in line with the increase of pasteurization time. So pasteurized tofu is less favored because it is more chewy and hard.

In this study, the result of sensory test for aroma is different from the other parameters. For the aroma, 45 minutes pasteurize tofu is preferred by the panelists. The odor components in soymilk are primarily the oxidation products of unsaturated lipids catalyzed by lipoxygenases and hydroperoxide lyase. The denaturation temperature of lipoxygenases is approximately 80°C (Wilkins *et al.*, 1967). Hydroperoxide lyase can be completely inactivated at 70 °C, which is lower than that of lipoxygenases (Omura and Takechi, 1990). The actual sources of beany off-flavor in soymilk have not been definitely characterized. Instead, there are a host of compounds which have been identified that may contribute to the soy odor (Wilkins and Lin, 1970). Hexanal is the most commonly studied since it gives a beany sensory and grassy flavor in soymilk (Omura and Takechi, 1990; Ma *et al.*, 2002). Soymilk had the lowest beany odor after 20 minutes of cooking (Yuan and Chang, 2007).

Conclusion

The tofu pasteurization treatment using pasteurizer for 15 minutes with a starting temperature of water in the pasteurizer 80 ° C can already be said to be effective because it can reduce the number of *S. aureus* GMP4 by 5 log cycle. From the estimated calculation based on D and z values, the estimated total count of *S. aureus* GMP4 after 15 minutes pasteurization was -13.47 log CFU/g. The difference is probably due to differences in inoculum size and pH media. The pasteurization treatment overall does not degrade the quality of tofu if seen from the color, texture, and organoleptic test.

References

- Anonim, 2013. Revised Guidelines For The Assessment of Microbiological Quality of Processed food by Department of Health Food and Drug Administration. Philipinnes.
- Arnoldi, A., 2001. Thermal processing and food quality: Analysis and control. In P. Richardson (Ed.), Thermal Technologies in Food Processing. Boca Raton, Florida: CRC Press. p. 138–159.
- Asao, T., Kumeda, Y., Kawai, T., Shibata, T., Oda, H., Haruki, K., Nakazawa, H., and Kozaki, S., 2003. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiol Infect* 130, 33–40.
- Baik, O. D., and Mittal, G. S., 2002. Kinetics of tofu color changes during deep-fat frying, *Lebensm.-Wiss. U.-Technol.* 36, 43–48.
- Ball, C. O., 1928. Thermal process time for canned foods. *Bull. Natl. Res. Council*, 7, Part 1, No. 37. p 76.
- Baranyi, J., 1998. Comparison of stochastic and deterministic concepts of bacterial lag. *Journal of Theoretical Biology* 192: 403–408.
- Besse, N. G., Audinet, N., Barre, L., Cauquil, A., Cornu, M., and Colin, P., 2006. Effect of the inoculum size on *Listeria monocytogenes* growth in structured media. *International Journal of Food Microbiology*, 110, 43–51.
- Bourne, M. C., 1982. Food textures and viscosity : concept and measurement. NewYork: AcademicPress.
- Carabasa, M., & Ibarz, A., 2000. Kinetics of colour development in aqueous glucose systems at high temperatures. *Journal of Food Engineering* 44, 181–189.
- Fuqua, W.C., Winans, S.C., Greenberg, E.P., 1994. Quorum sensing in bacteria: the LuxR/LuxI family of cell density responsive transcriptional regulators. *Journal of Bacteriology* 176, 269–275
- Hassani, M., Cebria ´n, G., Man ´as, P., Condo ´n, S., Paga ´n, R., 2006. Induced thermotolerance under nonisothermal treatments of a heat sensitive and a resistant strain of *Staphylococcus aureus* in media of different pH. *Letters in Applied Microbiology* 43, 619–624.
- Holdsworth, S. D., 2004. Optimising the safety and quality of thermally processed packaged foods. In P. Richardson (Ed.), Improving the thermal processing of foods. Boca Raton, Florida: CRC Press. p. 1–31.
- Holdsworth, D., and Simpson, R., 2007. Thermal Processing of Packaged Foods (Second Edition). Springer. New York USA. p. 45–48.
- Hutchings, J. B. (1999). Food color and appearance (Second edition). Aspen Publishers Inc. Gaithersburg. p. 610.
- Irlinger, F. (2008). Safety assessment of dairy microorganisms: coagulase-negative staphylococci. *International Journal of Food Microbiology*, 126, 302-310.
- Jackson, J.G., Merker, I.R., and Bandler, R., 2001. Bacteriological Analytical Manual. U.S. Food & Drug Administration

- Kennedy, J., Blair, I. S., McDowell, D. A., & Bolton, D. J., 2005. An investigation of the thermal inactivation of *Staphylococcus aureus* and the potential for increased thermotolerance as a result of chilled storage. *Journal of Applied Microbiology*, 99, 1229-1235.
- Koutsoumanis, K. P., and Sofos, J. N., 2005. Effect of inoculum size on the combined temperature, pH and aw limits for growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 104, 83-91.
- Lewis, M., dan Heppel, N., 2000. *Continuous Thermal Processing of Foods Pasteurization and UHT Sterilization*. Aspen Publishers, Inc. Gaithersburg, Maryland.
- Li, X., Sheldon, B. W., and Ball, H. R., 2005. Thermal resistance of *Salmonella enterica serotypes*, *Listeria monocytogenes*, and *Staphylococcus aureus* in high solids liquid egg mixes. *Journal of Food Protection*, 68, 703-710.
- Ma, H., Guan, C. Y., He, X. L., Zhang, G. Z., and Din, A. L., 2002. Effects of lipoxygenase null genes of soybean in controlling bean-flavor of soymilk and soy flour. *Agric. Sci. China*, 1 (9), 965-971.
- Mailia, R., Yudhistira, B., Pranoto, Y., Rochdyanto, S., and Rahayu, E.S., 2015. Ketahanan Panas Cemar *Escherichia Coli*, *Staphylococcus Aureus*, *Bacillus Cereus* Dan Bakteri Pembentuk Spora Yang Diisolasi Dari Proses Pembuatan Tahu Di Sudagaran Yogyakarta. *Agritech*, Vol. 35, No. 3, 300-308.
- Montanari, C., Serrazanetti, D I., Felis, G., Torriani, S., Tabanelli, G., Lanciotti, R., and Gardini, F., 2015. New insights in thermal resistance of staphylococcal strains belonging to the species *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* and *Staphylococcus aureus*. *Food Control* 50, 605-612.
- Muga, A., and Moro, F., 2008. Thermal adaptation of heat shock proteins. *Curr. Protein Pept. Sci.* 9, 552-566.
- Omura, Y., and Takechi, H., 1990. Effect of hot water treatment on flavor of soymilk. *Nippon Shokuhin Kogyo Gakkaishi*, 37, 278-280.
- Pearce, L. E., Smythe, B. W., Crawford, R. A., Oakley, E., Hathaway, S. C., & Shepherd, J. M., 2012. Pasteurization of milk: the heat inactivation kinetics of milk-borne dairy pathogens under commercial-type conditions of turbulent flow. *Journal of Dairy Science*, 95, 20-35.
- Peleg, M., Normand, and Corradini, M. G., Generating microbial survival curves during thermal processing in real time, *Journal of Applied Microbiology* 98, 406-417.
- Rahayu, E.S., Rahayu, S., Sidar, A., Purwadi, T., and Rochdyanto, S., 2012. *Teknologi Proses Produksi Tahu*. Penerbit Kanisius, Yogyakarta.
- Redfield, R.J., 2002. Is quorum sensing a side effect of diffusion sensing? *Trends in Microbiology* 10, 365- 370.
- Sachindra, N. M., Sakhare, P. Z., and Rao, D. N., 1998. Reduction in Microbial Load on Buffalo Meat By Hot Water Dip Treatment. *Meat Science*, 48, No. 1/2, 149-157.
- Singh, R. P., and Heldman, D. R., 2009. *Introduction to Food Engineering*. Elsevier. UK. p. 426
- Stumbo, C. R., *Thermobacteriology in Food Processing*. Academic Press. USA. p. 157-159
- Szczesniak, A. S., and Bourne, M. C., 1995. *Letters. J Food Sci* 60, (6):vii.
- Tango, C. N., Khan, I., Park, Y. S., and Oh, D. H., 2016. Growth of *Staphylococcus aureus* in cooked ready-to-eat ground fish as affected by inoculum size and potassium sorbate as food preservative. *Food Science and Technology* 71, 400-408.
- Wang, H.L., Mustakas, G.C., Wolf, W.J., Wang, LC., Hesseltine, C.W., dan Bagley, E.B., 1979. *Soybeans as Human Food - Unprocessed and Simply Processed*. U.S. Department of Agriculture Science and Education Administration, Washington, D.C. p 54.
- Wilkens, W. F., and Lin, F. M., 1970. Gas chromatographic and mass spectral analyses of soybean milk volatiles. *J. Agric. Food Chem.*, 18, 333-336.
- Wilkens, W. F., Mattick, L. R., Hand, D. B., 1967. Effect of processing method on oxidative off-flavors of soybean milk. *Food Technology* 21, 1630-1633.
- Winzer, K., Hardie, K.R., Williams, P., 2002. Bacterial cell-to-cell communication: sorry, can't talk now—gone to lunch. *Current Opinion in Microbiology* 5, 216- 222.
- Yuan, S., and Chang S. K. C., 2007. Texture Profile of Tofu as Affected by Instron Parameters and Sample Preparation, and Correlations of Instron Hardness and Springiness with Sensory Scores. *Journal of Food Science* 72, 136-145.

Optimization Alginat Extraction From Brown Algae (*Sargassum polycystum*)

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Abstract

Sargassumpolycystum is one of brown algae that grown spread only at certain area in Indonesia, including West Papua seashore. There is only few information about the economic value and bioactive compounds of the algae. This research focus on alginat extraction from *S. polycystum* using isopropanol at three concentration levels (85, 90, and 95%) that will give the best characteristics. Extraction using 85% isopropanol gave the highest yield of gelatin (27.57%), water content (1.52%), and ash content (6.11%). Extraction using 85% isopropanol gave the alginat that showed the lowest viscosity (672.29 cps) comparing to 90% isopropanol extract (708.71 cps) and 95% isopropanol extract (749.24 cps). Because of viscosity is the important characteristic of gelatin, thus the best extraction was given by 95% isopropanol (gelatin yield: 24.66%, water content: 0.62%, ash content: 3.95%, and viscosity 749.24 cps).

Keyword: Sargassumpolycystum, Alginat, Extraction, Isopropanol, Viscosity

Introduction

Indonesia as the island nation has a have territorial waters with the second longest coastline in the world. It is 81,290 km. in length of coastline. The area of the sea in the vast Indonesia holds a great potential of natural resources of marine biodiversity for great developed. One of the Indonesian natural resources of marine biodiversity a very potentially in order to meet the needs in domestic and export was seaweed.

Seaweeds are a potential renewable resource in the marine environment where about 6,000 species have been identified and grouped into different classes: green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae) algae. Seaweeds are extensively used as food by coastal peoples, particularly in East Asia such as China and Vietnam, but also in some parts of Europe including Scotland. This is because seaweeds are known to provide an excellent source of bioactive compounds such as carotenoids, dietary

fibre, essential fatty acids, vitamins and minerals (3, 16). Sargassum and Turbinaria are widespread tropical genera, and more than 400 species of Sargassum exist. The seaweed would be worth a higher economical if processed into products between the form of agar-agar, karaginan, and alginat and prepared food products consumption. as well as natural resources other marine flora, production of phytochemicals such as Alginic acid, agar-agar, carrageenan, and like iodine, which are widely used in several industries involved in the manufacture of certain food materials, fertilizers and pharmaceuticals. Brown algae are a promising object in the food industry and prophylactic and clinical medicine. The word alginate is a generic term, meaning the various derivatives of alginic acid that either occur naturally in certain brown seaweeds (alginophytes) are produced from the natural derivatives.

Alginate is the major structural component of the brown algal cell and mainly consists of β -D mannuron acid and α -L glucuronic acid units (14). In a wide range of industrial application, alginates are essential component as thickening, gelling or stabilizing agents and in some cases, immunostimulatory agents (6, 16). The preparation of alginic acid prevents chemically induced cancerogenes chemically induced cancerogenes' and ulcerogenes's. Alginates, extracted from brown seaweed with an acid and an alkali, are used in a wide range of applications, particularly in the food, industrial, and pharmaceutical fields because of their water holding and gel forming capacities and ability to form and stabilize emulsions (18, 14). Extracts of alginate play a key role in the food industry, food, textiles, health and cosmetics. In the food industry, alginates were used to stabilize mixtures dispersions and emulsions, which increase viscosity and forms gel, such as jam and jellies (24). Alginate can be used in the manufacture of soft capsules and is consumed as a beverage for lowering blood sugar level. In the textile industry, alginate was used as an additive for textile dye (13, 16).

Specific to the waters of Manokwari of West Papua, have not done research on alginate content of Brown algae so that needs to be examined based on the use of the alginate content of isopropanol at some concentration by looking at the amount of redman, viscosity and grey levels.

Materials and Methods

Materials

Sargassum polycystum samples was taken at Manokwari intertidal region of West Papua during low tide, then cleaned to remove dirt and sediments that stick further dried for

7 days. Sample mashed with a blender, then the sample is ready for maceration

Chemicals

Some of the equipment and chemicals used in this study as Beaker Glassware, Vortex, Glass Measure, Ovens, Filter Paper, Pumpkin Measure, mixer, digital scales, pH meters, and Brookfield, Viscometer, distilled, Na₂CO₃ 7.5%, CaOCl₂, isopropyl Alcohol, NaOH 0.5%, 0.5% HCl, CaOCl₂ 1%, Isopropanols.

Extraction of samples

The extraction began (17) be modified, that is Sargassum polycystum (30 gr) be mashed (5 minute) soaking HCl 0,5 % (ratio 10:1, v/w). soaking, NaOH 0,5% 30 minute (ratio 10:1, v/w). Filtration extract, Na₂CO₃ 7,5 % (ratio 10:1, 50° C, 2 hours). Filtrat bleaching with CaOCl₂ 1% (ratio 10:1, v/w) and *Pickling* HCL 5% (pH 2,8), 5 hours (ratio 10:1). sediment formed added NaOH 10% pH 10 for 5 hours (10:1, v/w). Disentrifuge 5 minute obtainable supernatan. Presipitat to purification with isoprophanols concentration 85 %, 90 %, dan 95 %, fisdrying ± 60 ° C with 17 hours for get salt Alginate.

1. Yield was expressed as weight percentage for alginate with weight of the seaweed samples (1)
2. Water Content was obtained by gravimetric method (1), by drying the sample of alginate at the temperature 105⁰ C.
3. Ash content was obtained by gravimetric method (1), by burning the sample in hot air oven at a temperature of 200⁰ C till it became ash.
4. The viscosity of alginate dispersion was determined using the method of James (1995), An aliquot of 250 ml of 1% alginate solution was heated at 50°C until it turned to gel and ready to determine its viscosity by viscotester.

Statistical analysis

Do not use statistical analysis but the results described descriptively

Result and Discussion

A. Yield Rendemen

Data can be show for the highest yield value owned by HCl 27.57% NaOH = 26.23 and then the smallest 24.66 of Na₂CO₃ (Figure 1)

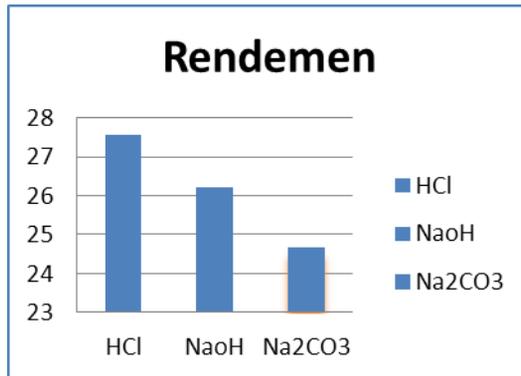


Figure 1. Total Rendemen

Yield on a solution of HCl has the highest value since the HCl will break down the cell walls of seaweed. This research was the use of HCl on a alginate, will break down the cell walls to ease extraction, because HCl is a strong acid and will be ionized. On the stem of seaweed (thallus) functions as a place of hoarding food substances reserves because the process of nutrient uptake from the environment occur more at the thallus. Allegedly is one of the factors that cause a alginate most high yield deposits. The content of the Brown seaweed from a alginate depending on the age, species and habitats (4, 7, 23). Monosaccharide synthesis occurs in the leaves and the next most translocation to other plant parts for synthesized into a polymer the longer the extraction of the higher yield (14). This indicates the evidence, that the longer soaking thallus with a alginate yield values of HCl, of ekstraksi increases, because HCl will break down the cell walls of seaweed, breaking the cell walls to ease extraction, because HCl is a strong acid and can be ionized perfectly (6,14).

B. Water Content

Highest water levels there are 85% isopropanol 1.52 then followed by 90% = 0.73 and 95% of the smallest 0.62 (Figure.2).

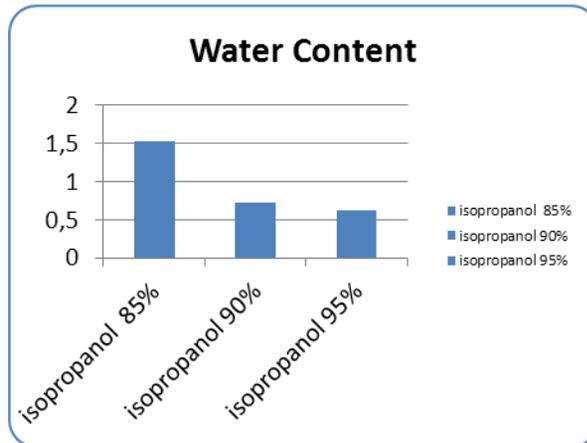


Figure 2. Water Content

Moisture content in the extract can be seen in Figure 2. Below of pictures can be explained that for the highest water levels there are 85% isopropanol = 1.52, 90% = 0.73 and 95% = 0.62. The value above indicates that the use of 95% isopropanol yielded the lowest water levels (0,62%) and the highest water levels (1.52%) resulting in the use of 85% isopropanol. Filtrate water levels after the alleged extracts by isopropanol solution that is getting stronger with the use of isopropanol concentration, so that the moisture content of the extract alginate will be lower. Therefore, the more concentrated isopropanol concentration, the higher the water withdrawn. That the use of isopropanol can precipitate alginat flawlessly than with ethanol (25).

Moderately ethanol has more polar than with isopropanol, where the more polar a liquid then approaching nature water which are highly polar, then ethanol is more difficult to draw water. Figure 2 shown that the higher the concentration of isopropanol, water levels will be low. Suspected as more and more water is being drawn from a alginat then water levels will decrease. More concentrated isopropanol concentration then increases the amount of hydroxyl makin so much stronger in the water binding. In addition, allegedly caused by a section of the thallus water content is free which can be easily fastened with hydrogen bonding and other types of acids has hydrophobic properties so that it can bind to the water so that the longer is extracted then the more water that is stuck (15,16).

C. Ash Content

Research results show that levels of ash in the highest concentration of 85% isopropanol = 6.11, 95% , 90 %3.40 and the smallest 3.95 on concentration of 95% (Figure 3).

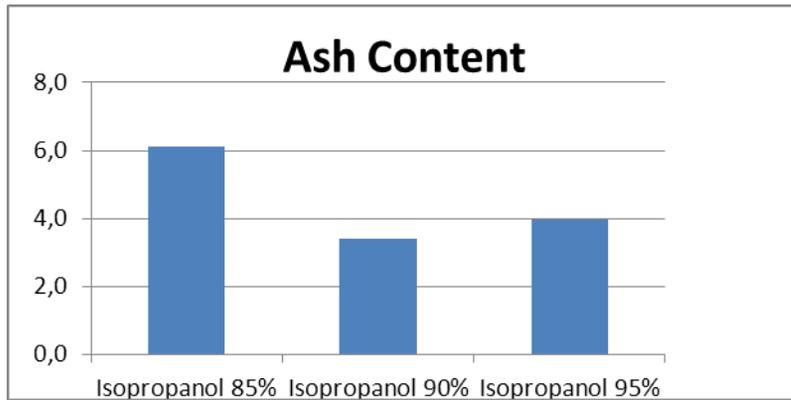


Figure 3. Ash Content

Ash on the product indicating the purity of the products affected by the mineral content of raw materials. Brown algae including materials containing minerals is quite high as Na, Cl, K, Ca, Mg, Fe and S (14, 24). The high levels of ash, because of the use of strong acid resulting in the addition of alkaline pH needs to be neutralized (NaOH). The high-NaOH means the amount of the resulting alginate salts increases. Technically, the high levels of ash in the research allegedly also comes from the remains of the rock (mineral) is still attached to on the seaweed because of less washing clean so carried away at the time of extraction. The use of bleach (Ca resources) is added in the process of bleaching (22,24). The stronger the acid is used to cause the more of its cell wall seaweed, that with the extraction of more and more material that can be removed from the plant tissue. Research results show that levels of ash in the highest concentration of 85% isopropanol = 6.11, 90 % = 3.40 and on concentration of 95% = 3.95 cps. The grey levels is one of the criteria that determine the quality of the alginate produced. Average levels of ash treatment result addition of isopropanol is between 34.01% – 35.25%. The high levels of ash in the extract of a alginate allegedly because of sargassum seaweed that grows in coastal waters are affected by both poor ocean water due to pollution and other contaminants. Whereas the levels of ash that is allowed according to food chemical codex between 13 – 27%. Allegedly because at the time of the formation of aalginat salts, the use of NaOH is capable of destroying organic compounds into inorganic compounds. This data is supported by the research of (26), that grey levels of this type of Sargassum sp range between 30 – 35%.

D. Viscosity

Viscosity results showed that 95% isopropanol have highest value = 749.24 cps = 90% = 708.71 cps, and lowest 85% = 672.29 cps (Figure 4).

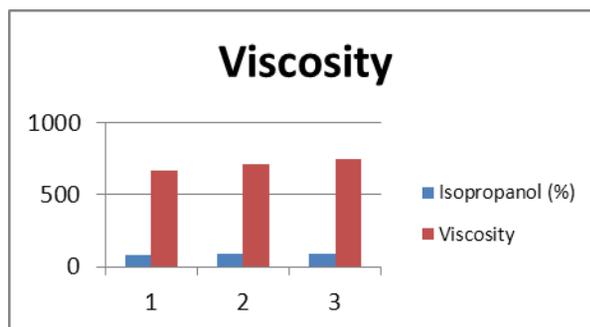


Figure 4. Viscosity

Viscosity results showed that 95% isopropanol have highest value = 749.24, isopropanols = 90% = 708.71 cps and 85% = 672.29 cps. This is presumably because the more water is drawn in by isopropanol during a purification, so that when testing the viscosity of alginate produced is able to bind more water (not quickly saturated) and increase viscosity. This is in accordance with the nature of the functions of isopropanol that draws water from a mixture of hydrophilic system (12). From the graph in Figure 4 also obtained a description of their viscosity increasing trend along with the increasing value of brightness. This is presumably due to its astringent properties owned fucoxanthin brown brown algae (21). Observed from the formula submitted by (5), appears that the dye is a major constituent of the carbon chain. compounds that have a molecular formula thus is hydrophobic, so many brown dye will resist and reduces water combine with alginate thus giving nature viscus. As a result, the viscosity of the alginate becomes lower. Instead the reduced dye brown algae mean less material resisted joining the water and viscosity alginate when dissolved in water greater. That the increased of viscosity depending on the presence of guluronat acid and manuronat acid (4). This is in support of (26) that the use of raw materials should in dry conditions because it did not spend too much chemical substances. The higher the concentration of isopropanol added resulted in the higher viscosity. This is allegedly related to the withdrawal of water content by isopropanol is more effective. (12) suggests that the use of isopropanol on a alginate salt drying function to the withdrawal of water from a alginate extract suspension. With fresh, raw materials extraction is easier because then not needed of rehydration process on tissue cells of dried seaweed

Conclusion

Use of the isopropanol concentration of 95%, the highest viscosity value (749.238-cps) if compared with the concentration of 85 and 90%.a alginate Content contained in *Sargassumpolycystum* based on the amount of yield (27.57, 26.23 26.23%), and the moisture content (1.52, 0.73, 0.62%), and the levels of ash (6.11, and 3.40, 3.39%), indicating a trend of increasingly high percentage of isopropanol treatment then the amount obtained is increasingly well (percentage). In particular the value of viscosity (672.290, 708.711, 749.238 and cps), the higher the concentration isopropanol treatment then the value the better percentage rise.

References

- AOAC. 1990. Official Methods of Analysis of The Assosiation of Official Analytical Chemists. 15th ed. Washington., p 456-579
- Belitz. H.D and W.Grosch.(1987). Food Chemistry. Springer Verlag Berlin, New York, Paris, Tokyo
- Chandini SK, Ganesan P, Bhaskar N (2008) In vitro antioxidant activities of three selected brown seaweeds of India. Food Chem 107: p. 707–713
- Chapman. V. J. and D.J. Chapman. (1980). Seaweed and Their Uses. Third Editions.Chapman and Hall
- Glasby. J. S. (1982). Encyclopedia of TheTerpenoids.A Willey- Interscience Publication. Chichester New York, Brisbane, Toronto, Singapore
- Gliksman. M. (1969). Gum Technology in The Food Industry. Academic Press. New York
- Gopinathan CP, Panigrahy R. 1983. Seaweed resources. CMFRI Bull; 34: p. 47-51
- Hernandez-Carmona G, McHugh DJ, Lopez-Gutierrez F (2000) Pilot plant scale extraction of alginates from *Macrocystispyrifera*. 2: Studies on extraction conditions and methods of separating the alkaline-insoluble residues. J ApplPhycol 11: p. 493–502
- James CS (1995). Analytical Chemistry of Foods.Blackie Academic and Profesional. London, pp. 95-102
- Johnson. R.C., Muller. T.S., Roman, J.R., Costello, W.J and Jones, K.W. (1990) Effects OfAlgin/Calسيوم and Adipic Acid Concentrations on muscle-Juncture Formation. Journal Of Food Science 55. No. 4 : p. 906-910
- Larsen B, Salem DMSA, Sallam MAE, Mishrikey MM, Beltagy AI (2003) Characterisation of The Alginates From Algae Harvested at The Egyptian Red Sea coast. Carbohyd Res 338:p. 2325–2336
- Mairamo. A.L. (1977).Sulfated Seaweed Polisaccharide Food Coloid AVL, Westport Connecticut
- McCormick Ali E. Alginate-Lifecaster's Gold. Art casting J 2001;
- McHugh DJ. 1987. Production, properties and uses of alginates. FAO Fish Technol; 288: p.58-115
- McHugh DJ, Hernandez-Carmona G, Arvizu-Higuera DL, Rodriguez-Montesinos YE (2001). Pilot plant Scale Extraction of Alginates From*Macrocystispyrifera*. 3: Precipitation, Bleaching and Conversion of Calcium Alginate to Alginic acid. J Appl Phycol13:p. 471–479
- McHugh DJ. 2003. A Guide to The Seaweed Industry. FAO Fisheries Technical Paper. 441. P. 27-49
- Mushollaeni W. 2007. Extraction of Alginate from Brown Seaweeds *Sargassum*, and *Turbinaria*. Young Lecturer Experiment Paper, p 20-33.
- Pilnic, W. and Rombouts, F., 1985. Polysaccharides and Food Processing, Carbohydr. Res.,vol. 142, p.93–105
- Reen, D. Uses .W. (1986). Uses of Marine Algae in Biotechnology and Industry. LokakaryaBioteknologiRumpputlaut.SekretariatDewanRisetnasional, Jakarta.
- Rioux LE, Turgeon SL, Beaulieu M (2007) Characterisation of polysaccharides extracted from brown seaweeds. CarbohydPolym 69: p. 530–537
- Rohfritsch A, Payri C, Stiger V, Bonhomme F (2007) Molecular and morphological relationships Between Two Closely Related Species,*Turbinariaornata* and *T. conoides* (*Sargassaceae*, *Phaeophyceae*). BiochemSystEcol 35:p. 91–98
- Sriamornsak P, Thirawong N, Korkerd K (2007) Swelling, erosion and release behavior of alginate-based matrix tablets. Eur J Pharm Biopharm 66: p. 435–450
- Taylor. W. R. (1979). Marine Algae of The Eastern Tropical and Subtropical coast of Theamericans. The University of Michigan Press. MichiganD
- Toft., K, Grasdalen H, Smidsrod O. 1986. Synergistic Geletion of Alginates and Pectins. ACS Symposium Series;310: p. 117-132

- Yani M. Modification and Optimation Processing of Alginate Extraction from JenisTurbinaria sp. Agricultural Faculty of Bogor Agricultural Institute, Bogor, 1998. pp 20-35.
- Yunizal J, Murtini T, Basmal J, Nasran S, Marsiana E, Abdulrokim I, Darwin, Maryadi, Sahid. Technology of Extraction Alginate from Brown Seaweeds. Technical Paper. Jakarta, pp. 25-40

Effect of Carrageenan Concentration on Physicochemical and Organoleptic Characteristics of Mulberry (*Morusnigra* L.) Sheet Jam

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Abstract

Mulberry sheet jam is a modified jam product that is compact, has high plasticity and low stickiness. Mulberry sheet jam is more practical, in terms of preparation and storage, compared to regular jam. Mulberry sheet jam needs gelling agents, such as agar and carrageenan, to give it compact and dense texture. The research design used here is Randomized Block Design (RBD) with one factor, which is carrageenan concentration (P) consisting of six levels, 0.5% (P1); 0.75% (P2); 1.00% (P3); 1.25% (P4); 1.50% (P5); 1.75% (P6) based on the mulberry puree. The experiment was carried out four times. Parameters that were tested include water content, texture, color, syneresis, sensory evaluation, and antioxidant activity. Different carrageenan concentration affected moisture content, texture (hardness, cohesiveness, adhesiveness), and also organoleptic score (taste, color, and texture) of mulberry sheet jam. Higher carrageenan concentration decreased moisture content, syneresis level, and adhesiveness, in the other hand hardness and cohesiveness increased. Mulberry sheet jam color is reddish purple (a mixture between blue and red with low intensity). The choice of the best treatment was based on the biggest area of spider web chart that consisted of organoleptic test and antioxidant activity. Best concentration of carrageenan was 1.0% with 42.59% water content; 817.88 g hardness; 0,170 cohesiveness; -716,76 g.s; and acceptance score of taste 5.1122; color 5.0695; texture 5.0878; and antioxidant activity 5.3998 with standard score 1-7.

Keywords: mulberry sheet jam; carrageenan

Introduction

Jam is an intermediate moisture product which has a chewy texture. Along with technology development, jam is modified into sheet jam which has soft solid texture, not sticky, and glossy appearance (Yenrimaet *al.*, 2009). The difference between common jam and sheet jam are the sheeting process and the use of gelling agent. One of potential fruit to be processed into sheet jam is mulberry. Based on the color, mulberry can be separated into three types which are white mulberry, red mulberry, and black mulberry. Black mulberry (*Morusnigra* L.) was chosen because of its sour and a little bit astringent flavor. Besides this fruit has specific flavor and impressive purplish red color which indicate the existence of anthocyanin pigments. Anthocyanin is defined as a natural antioxidant component.

Gelling agents which is added are carrageenan and agar bar. Both are extracted from red seaweed. Agar has a role in forming firm gel (Bureyet *al.*, 2008). Carrageenan can be used as thickeners, gelling agent, stabilizers, and prevent syneresis process (Wade and Weller, 2006). The addition of carrageenan concentration should be appropriate to produce a better characteristics of sheet jam. Carrageenan concentration below 0.5% produce less compact texture of sheet jam while carrageenan concentration above 1.75% may decrease the mulberry flavor. Because of that reason, the difference concentration of

carrageenan may affect physicochemical characteristics which are water content, color, texture attributes (hardness, adhesiveness, cohesiveness), syneresis level, and sensorial properties of mulberry sheet jam.

Materials and Methods

Materials

Black mulberry from WismaSamadi at Bintang Kejora garden, Pacet, JawaTimur. Black mulberry was sorted, washed and kept in freezer. Other mulberry sheet jam materials were procured from supermarkets which are granulated sugar (premium gulaku), agar bar (AA), carrageenan (C-Tech DG MA-03), mineral water (aquase), and oriented polypropylene (OPP) plastic.

Chemicals

Methanol solution and DPPH (1,1 –diphenil-2 picrylhydrazyl) reagent.

Mulberry sheet jam production

Frozen mulberry was thawed about 30 minutes. After that, mulberry was mixed with mineral water (ratio 5:1). Mulberry puree then blended with different carrageenan concentration. The concentration levels of carrageenan were 0.5%; 0.75%; 1.0%; 1.25%; 1.5%; and 1.75% from mulberry puree and keep for 15 minutes. Then, mixed with granulated sugar (60% w/w) and agar bar (1% w/w). The mixture then was heated and stirred at 85-95°C for 15 minutes. Mulberry jam was packed into OPP plastic and flatted with rolling pin. Then, jam sheet was stored in room temperature on closed box. A part of mulberry sheet jam was formed in tube mold (2x2cm) for texture analysis.

pH analysis

pH was measured using pH meter. Mulberry puree was placed on beaker glass then pH was read when stable.

Water content analysis

Water content was measured using oven vacuum method (AOAC, 1995). Mulberry sheet jam sample was weighed 0.5g then stored inside oven vacuum at 70°C and 25 cmHg reduce pressure till 8 hours then cooled down for 10 minutes and weighed. After that, the sample was re-heated in oven for 2 hours and the procedure repeated until the weight of sample became constant.

Color analysis

Color analysis was carried out using Minolta color reader (Xrite, 2015) to define L (lightness), a* (redness), b* (yellowness), C (Chroma), dan °h (hue degree).

Texture analysis

Texture was analyzed using texture profile analyzer including three parameters which are hardness, adhesiveness, and cohesiveness (Bourne, 1978). Mulberry sheet jam was formed on tube mold (2x2cm). A cylindrical probe (d=40mm) was used to compress the samples during two cycles of test with 3 s of time interval between two cycles. The speed of this analyze was 20 mm/s (pretest), 0.5 (test), and 10 mm/s (post test).

Syneresis level analysis

Syneresis level was measured for 4, 8, and 12 days. Mulberry sheet jam was stored in closed box and the samples weighed after 4, 8, and 12 days storage. After that, the free water on the surface was removed using filter paper. Syneresis level was expressed in percentage by subtracting the first weight

from the final weight ,then divided by the first weight and multiplied by 100 (Imeson, 2010).

DPPH radical scavenging capacity

Antioxidant activity was analyzed in three steps. First, DPPH (40mg/mL) solution was made from 4mg DPPH powder in 100mL methanol. The control solution was made from 5g sheet jam sample (not including mulberry puree) and methanol. After that, the sample solution was made from 5g mulberry sheet jam and methanol. Finally, the antioxidant activity was evaluated using spectrophotometric method (Sashikumar *et al.*, 2009). The absorbance was measured in 515 or 517nm wave length. Antioxidant activity was stated in percentage based on this formula:

$$\% \text{ antioxidant activity} = \{(1 - \text{sample absorbance}) / \text{control absorbance}\} \times 100\%$$

Sensory evaluation

Sensory evaluation was done by 100 untrained panels of Widya Mandala Catholic University student. The sample was carried out after a maximum of 3 days of jam manufacture. Taste, color, and texture of mulberry sheet jam were evaluated following seven point of hedonic scale (7=like very much, 6= like moderately, 5= like slightly, 4= neither like nor dislike, 3= dislike slightly, 2= dislike moderately, 1= dislike very much). The samples were presented in the same size and condition.

Statistical analysis

All experiments were done in four replications. Data collected from this research then analyzed using ANOVA (Analysis of Variance) test with $\alpha = 5\%$ and DMRT (Duncan's Multiple Range Test). DMRT was used if there was significant difference. DMRT test was used to separate which mulberry sheet jam sample gave significant difference. Statistical software used for these tests was SPSS Statistics version 23.

Result and Discussion

pH Analysis

pH analysis was used to control the process stabilization. It was done to ensure that all of the mulberry puree which used in research was in the same condition. Form the 4 replication in this research the average value of mulberry puree pH range from 3.55-3.61.

Moisture Content

Moisture content was indicated the amount of evaporated water especially free binding water. In the case of moisture content, the increases of carrageenan concentration will decrease the moisture content. Carrageenan helps to maintain water inside the sheet jam matrix (Imeson, 2010). Carrageenan as a hydrocolloid has hydrophilic molecules which can prevent the free water to evaporate during the heating process. Based on the research, water content of mulberry sheet jam range from 39.35-44.64%. Tabel 1. illustrated the moisture content of mulberry sheet jam. Higher addition of carrageenan concentration will decrease the moisture content of mulberry sheet jam.

Color

Table 2. illustrated the color attributes of mulberry sheet jam which are included lightness (L), redness (a*), yellowness (b*), chroma (C), and hue (°h). The lightness value decrease due to the increases

of carrageenan concentration. Carrageenan concentration had a direct relationship to moisture content, whereas an increase in carrageenan concentration had an inverse correlation to moisture content. Decrease of moisture content will also decrease the color intensity of mulberry sheet jam. Lower moisture content will cause decreasing amount of free water which prove that molecules inside are getting closer. It means more difficult to reflect the light without water so that the product looks darker (MacDougall, 2002).

Table 1. Moisture Content of Mulberry Sheet Jam

Carrageenan Concentration (%)	Water Content (% wb)
1.75	39.35 ± 0.37 ^a
1.50	40.25 ± 0.38 ^b
1.25	41.14 ± 0.28 ^c
1.00	42.59 ± 0.31 ^d
0.75	43.93 ± 0.43 ^e
0.50	44.64 ± 0.37 ^f

Note: a. The average value ± deviation standard from 4 replications

b. Different *superscript* shows the significant different at $\alpha = 5\%$.

Table 2. Color Properties of Mulberry Sheet Jam

Carrageenan Concentration	L	a*	b*	C	$^{\circ}h$
0.50%	25.9±0.4	1.4±0.9	-0.4±0.2	1.5±1.3	359.6±0.4
0.75%	25.7±0.4	1.1±0.6	-0.4±0.1	1.1±0.6	359.6±0.2
1.00%	25.5±0.4	1.0±0.4	-0.5±0.1	0.9±0.5	359.5±0.2
1.25%	25.4±0.3	0.9±0.4	-0.6±0.0	0.9±0.4	359.4±0.2
1.50%	25.3±0.4	0.8±0.3	-0.6±0.1	0.8±0.3	359.4±0.1
1.75%	25.1±0.7	0.7±0.2	-0.6±0.2	0.7±0.4	359.3±0.1

From the $^{\circ}h$ we may conclude that all sample has red purple color. Hue is defined by the color description (Table 3.). The samples score are between 359.2 until 359.9 which means that mulberry sheet jam has red purple color. It is in line with the basic color of black mulberry which is red purple.

Table 3. Color Description

$^{\circ}h$	Color Description
18-54	Red (R)
54-90	Yellow Red (YR)
90-126	Yellow (Y)
126-192	Yellow Green (YG)
162-198	Green (G)
198-234	Blue Green (BG)
234-270	Blue (B)
270-306	Blue Purple (BP)
306-342	Purple (P)
342-18	Red Purple (RP)

Source: Hutchings (1999)

Texture Properties

Texture of mulberry sheet jam was analyzed at room temperature after 24 hours of preparation after the jam was fully set. The texture measurement including three parameters which are hardness, cohesiveness, and adhesiveness. Hardness increased with increased of carrageenan concentration. Based on the research, the value of hardness range from 721.65-1675.0925g; adhesiveness value range from -1397.0575-(-539.3550)g.s; cohesiveness value range from 0.16-0.22. Carrageenan is able to bind water and help the jam to maintain the form of sheet jam. (Philips and Williams, 2009). It proves that increased carrageenan concentration will create a harder texture of mulberry sheet jam. Adhesiveness shows the

stickiness of the product. The increased of carrageenan concentration will decreased the adhesiveness of mulberry sheet jam. It shows that the main function of carrageenan as thickener in this product so that increased carrageenan concentration will produce more sticky sheet jam. Cohesiveness parameter shows how firm the product is. Increased carrageenan concentration will increased the cohesiveness value. Increasing of cohesiveness value proves that using higher carrageenan concentration will make the product firm enough so that is not easily to break. All of the texture parameters are related to moisture content. Increased carrageenan concentration will decrease the water content which create small gaps of matrix in the product so that the product become firmer and following by increasing of hardness value, decreasing of adhesiveness value, and increasing of cohesiveness value (Table 4.).

Table 4. Texture Analysis of Mulberry Sheet Jam

Carrageenan Concentration (%)	Hardness (g)	Adhesiveness (g.s)	Cohesiveness
0.50%	721.6500±354.00 ^a	-1397.0575±228.61 ^a	0.16±0.03 ^a
0.75%	815.0075±313.50 ^a	-1051.4525±64.71 ^b	0.16±0.01 ^a
1.00%	817.8850±288.78 ^a	-725.1125±130.39 ^c	0.17±0.02 ^a
1.25%	1037.6475±201.47 ^{ab}	-716.7600±190.95 ^c	0.18±0.03 ^{ab}
1.50%	1240.3650±235.75 ^b	-614.3650±327.93 ^c	0.19±0.04 ^{ab}
1.75%	1675.0925±490.99 ^c	-539.3550±569.51 ^c	0.22±0.02 ^b

Note: a. The average value ± deviation standard from 4 replications

b. Different *superscript* shows the significant different at $\alpha = 5\%$.

Syneresis Level

Syneresis level shows the amount of free water which is going out from the gel matrix of sheet jam after 4, 8, and 12 days storage. Lower syneresis level means longer storage period of the product. Syneresis level on 4 days storage is between (0.88-2.14%); Syneresis level on 8 days storage is between (1.17-2.48%); Syneresis level on 12 days storage is between (1.46-2.72%). From Tabel 5, it known that higher carrageenan concentration will decrease syneresis level. Carrageenan as hydrocolloid can help to hold the free water inside the matrix and lower the syneresis level. Longer storage period gives more chance to free water from the product matrix. Along storage period there could be changes in room temperature such as raising temperature which can cause more water freed from the matrix. Besides, longer storage period may result changes of gel matrix texture as we called relaxation. In this condition, water inside gel is going out if there is any hydrostatic tension (Nussinovitch, 1997).

Table 5. Syneresis Level of Mulberry Sheet Jam

Carrageenan Concentration	Syneresis Level of Days (%)		
	4	8	12
0.50%	2.14 ± 0.53	2.48 ± 0.66	2.72 ± 0.71
0.75%	1.90 ± 0.52	2.34 ± 0.65	2.37 ± 0.63
1.00%	1.71 ± 0.36	2.11 ± 0.59	2.22 ± 0.58
1.25%	1.54 ± 0.26	1.79 ± 0.36	2.06 ± 0.44
1.50%	1.27 ± 0.14	1.49 ± 0.22	1.81 ± 0.33
1.75%	0.88 ± 0.26	1.17 ± 0.39	1.46 ± 0.48

Note: a. The average ± deviation standard from 4 replications.

Antioxidant Activity

Antioxidant activity is comparing between mulberry sheet jam sample with sheet jam without mulberry puree. Carrageenan expectedly may coat the antioxidant of mulberry. From Table 6, it can be concluded that different carrageenan concentration giving no significant difference on DPPH scavenging activity. Increases of carrageenan concentration giving unstable effect for antioxidant activity of mulberry

sheet jam. The average of mulberry sheet jam antioxidant activity can be seen in Table 6.

Table 6. Antioxidant Activity of Mulberry Sheet Jam

Carrageenan Concentration	% Inhibition
0.50%	81.18
0.75%	81.39
1.00%	79.33
1.25%	80.34
1.50%	79.97
1.75%	79.98

Sensory Evaluation

Sensory evaluation includes three parameters which are taste, color, and texture. Sensory evaluation is using hedonic test which panelists are expected to give a score for each treatment based on their fondness. This temporary result used 82 untrained panelist of Widya Mandala Catholic University student. The result of sensory evaluation may seen at Table 7.

Table 7. Sensory Evaluation of Mulberry Sheet Jam

Carrageenan Concentration	Taste	Color	Texture
0.50%	5.3744±0.6496 ^b	4.7244±0.7329 ^a	4.5183±0.7198 ^a
0.75%	5.0683±0.7590 ^a	5.0976±0.7085 ^{bc}	4.7195±0.7679 ^{ab}
1.00%	5.1122±0.7459 ^a	5.0695±0.6415 ^{bc}	5.0878±0.8503 ^c
1.25%	5.0488±0.7357 ^a	5.0000±0.8648 ^b	4.7793±0.8234 ^{ab}
1.50%	4.9207±0.7973 ^a	5.1744±0.7468 ^{bc}	4.8159±0.8088 ^b
1.75%	4.8713±0.6886 ^a	5.2549±0.6547 ^c	4.7890±0.9242 ^{ab}

Note: a. The average value ± deviation standard from 4 replications

b. Different *superscript* shows the significant different at $\alpha = 5\%$.

Form the anova test, there is difference in the taste, color, and texture of mulberry sheet jam. The addition of 0.5% carrageenan shows the difference in taste and also being the best treatment in taste parameter. Carrageenan is tasteless but noticed may coated the mulberry flavour. So that, increased of carrageenan concentration will reduce sensory score of the taste. The acceptability of color shows the highest score at 1.75% carrageenan. The addition of 1.75% carrageenan shows the darkest product from lightness test. Decreased of product lightness may cause phsycological perception that in this treatment the amount of mulberry addition is higher. Panelist highest score of texture shows in mulberry sheet jam with 1% carrageenan. Based on objective texture test, increased carrageenan concentration gives harder texture of mulberry sheet jam. It proves that panellists choose the between treatment which has firm texture but not too hard.

The Best Treatment

The best treatment of mulberry sheet jam defined from sensory evaluation and antioxidant activity using spider web. The spider web were presented in Figure 1. Area score for the best treatment range from 50.0484-51.9071. Table 8 listed the area of each treatment.

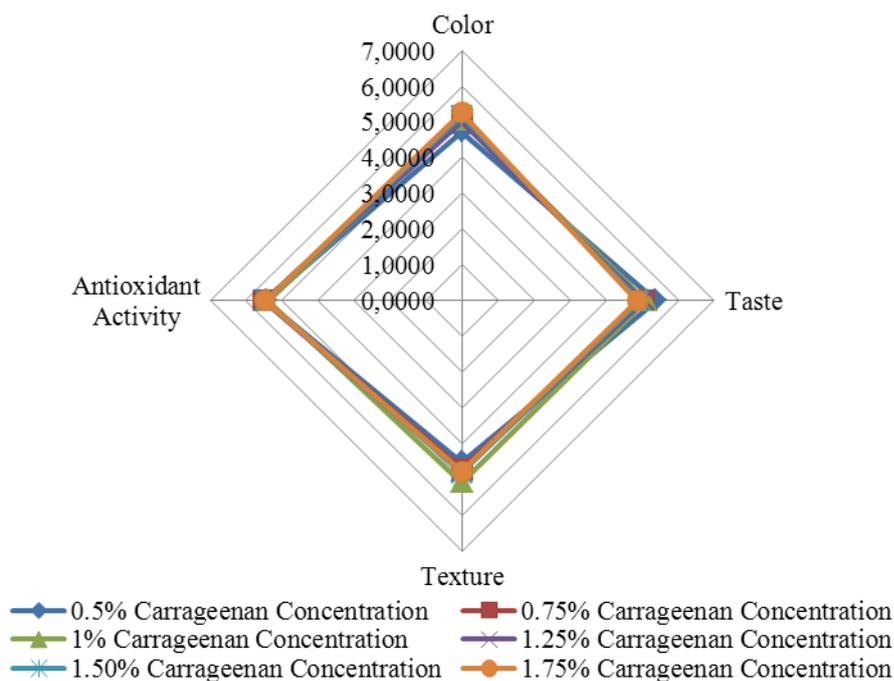


Figure 1. Spider Web of Mulberry Sheet Jam Best Treatment

Table 8. Area of Mulberry Sheet Jam Best Treatment

Carrageenan Concentration	Area
0.50%	50.0484
0.75%	51.9278
1.00%	53.3865
1.25%	51.5420
1.50%	51.7857
1.75%	51.9071

Based on the area of each treatment it can be concluded that mulberry sheet jam with 1% carrageenan as the best treatment. Addition of 1% carrageenan gives firm and also elastic texture of mulberry sheet jam. Mulberry sheet jam with 1% carrageenan has 42.59% water content; hardness score 817.88g; cohesiveness score 0.170; adhesiveness score -716.76g.s; and acceptability from taste parameter 5.1122; color 5.0695; texture 5.0878; and antioxidant activity 5.3998.

Conclusion

Different carrageenan concentration gives significant different in water content, texture (hardness, adhesiveness, and cohesiveness), also sensory evaluation (taste, color, and texture) of mulberry sheet jam. The best treatment choose from sensory evaluation and antioxidant activity which shows by the addition of 1% carrageenan which water content is 42.59%; hardness score 817.88g; cohesiveness score 0.170; adhesiveness score -716.76g.s; and acceptability from taste parameter 5.1122; color 5.0695; texture 5.0878; and antioxidant activity 5.3998.

References

[AOAC] Association of Official Analytical Chemist. 1995. *Official Methods of Analysis of The Association of Analytical Chemists*.

- USA: The Association of Official Analytical Chemists, Inc. 979. 12.
- Burey, P., B. R. Bhandari, T. Howes, and M. J. Gidley. 2008. *Critical Reviews in Food Science and Nutrition*. 48:361-377(2008).
- Bourne, M. C. 1982. *Food Texture and Viscosity: Concept and Measurement Second Edition*. New York: Academic Press.
- Hutchings, J.B. 1999. *Food Colour and Appearance 2nd edition*. Maryland: Aspen Pub.
- Imeson, A. 2010. *Food Stabilisers, Thickeners, and Gelling Agents*. Oxford: Blackwell Publishing Ltd. 39. 73-88.
- MacDougall, D.B. 2002. Colour Measurement of Food, (dalam *Colour in Food*, D.B. MacDougall, Ed.). Cambridge: Woodhead Publishing Limited and CRC Press, LLC. 40-45.
- Nussinovitch, A. 1997. *Hydrocolloid Application: Gum Technology in The Food and Other Industries*. London: Chapman and Hall Ltd. 5-12. 40-54.
- Phillips, G.O. and P.A. Williams. 2009. *Handbook of Hydrocolloids Second Edition*. UK: Woodhead Publishing Limited and CRC Press LLC. 83-94; 208-209.
- Sashikumar, J. M., Mahesu, and Jayadef. 2009. In Vitro Antioxidant Activity of Methanolic Extract of Berberis Tinctoria Lesch. *Journal of Herb Medicine and Toxicology* 3(2): 53-58.
- Wade, A dan P.J. Weller. 2006. *Handbook of Pharmaceutical Excipients 5th edition*. London: The Pharmaceutical Press. 122.
- Xrite. 2015. *A Guide to Understanding Color Communication*. https://www.xrite.com/documents/literature/en/L10-001_Understand_Color_en.pdf (June 10, 2016).
- Yenrina, R., Hamzah, N., dan Zilvia, R. 2009. Mutu Selai Lembaran Campuran Nenas (*Ananas Comusus*) dengan Jonjot Labu Kuning (*Cucurbita Moschata*). *Jurnal Pendidikan dan Keluarga UNP*. ISSN 2085-4285. Volume I (2):33-42.

Nanoencapsulation of *Andrographis paniculata* Extract as α -Glucosidase Inhibitors on the Formation of Hepatitis B Virus Envelope

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Abstract

Hepatitis B is a liver inflammation caused by hepatitis B virus (HBV). Currently, there is no specific handling for acute HBV infection. *Andrographis paniculata* is medicinal plant contain andrographolide that could inhibits α -glucosidase enzyme which may be involved on the formation of hepatitis B virus envelope. This research aims to do nanoencapsulation of *Andrographis paniculata* extract to increase its active compound bioavailability and see the ability of nanoparticle formed as α -glucosidase inhibitors through in vitro study. Nanoencapsulation of *Andrographis paniculata* extract use ionic gelation methods and chitosan as encapsulator. The optimum α -glucosidase inhibition of 37.17% was obtained at 16% concentration. Results showed that it takes 2.4 and 2 times of *Andrographis paniculata* extract nanocapsule to match the performance of Lamivudin and HP Pro as existing commercial hepatitis B drug.

Keywords : *Andrographis paniculata* extract ; α -glucosidase inhibitors ; nanoencapsulation

Introduction

Hepatitis is a disease characterized by an inflammation that occurs in the liver organ [1]. There are different types of hepatitis virus infection due to different viruses, namely hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV). Among the various types of hepatitis, hepatitis B is the largest and most important type to be discussed globally [2]. World Health Organization (WHO) estimates there are over 2 billion people in the world infected with hepatitis B virus, of which 350 millions of whom have been infected in chronic and become carrier. Indonesia is a country with a high additional hepatitis B that placed the second largest in Southeast Asian countries after Myanmar [3]. Currently, there is no specific handling for acute hepatitis B infection. Giving antibiotics does not have an impact on the handling of this infection. Because the disease is caused by a virus, the use of adrenocorticosteroids result no effect on the process of primary infection and will increase the rate of recurrence of the disease. Corticosteroid therapy used only in people with chronic active hepatitis with symptomatic, HBsAg negative, and have heavy injuries on liver biopsy. Effective treatment on interferon is not yet known. Haemodialysis, cross perfusion, transfusion, and immunoglobulin (IG) containing anti-HBs (HBIG) height has no effect on hepatitis, while specific antiviral drugs like Lamivudin (second generation of nucleoside analogue) is already available and others are in the development stage, but these medicines have not been evaluated for the treatment of hepatitis B acute [2].

Some researches result that the using of antiviral compounds that can reduce the level of hepatitis B virus circulating surface antigen (HBsAg) as the virus envelope give more beneficial immunological response [4]. Inhibition of hepatitis B virus envelope formation done by inhibits α -glucosidase enzyme activity which involved on the formation of glycan to bind with calnexin, a chaperon that plays role in protein folding to the formation of the hepatitis B virus [5][6]. Inhibition of α -glucosidase enzyme can reduce the circulation of the virus envelope so that virus secretion will not perfect and in the end the virus will die [4].

One of compounds that has the ability to inhibits α -glucosidase is andrographolide [7][8]. Andrographolide is the most active compounds found in plants *Andrographis paniculata*. *Andrographis paniculata* is one of 13 commodities of herbal plants that are assigned to the Government through Ditjen POM [9]. Plant part which contain a lot of active ingredient is leaf and root. Level of andrographolide and other diterpenoids at leaf is $2.39 \pm 0.008\%$ [10].

Based on the above issues and the facts of andrographolide activity, this research aims to do encapsulation of *Andrographis paniculata* leaf containing andrographolide and see the inhibiton activity against α -glucosidase enzyme. The encapsulation will use nanoparticles in order to make extract spread easily in blood and more accurate to achieve the cells target [11]. The reduced size of the particles will increase the surface area of the particles, solubility and thus for particles of the drug can increase its bioavailability in the human body.

Methods

Nanoencapsulation

One gram of chitosan dissolved in 50 mL of acetic acid 1% (b/v) then 0.15 g extract added and stirred with magnetic stirrer (Cole Parmer) until homogen. In other beaker glass (Pyrex Iwaki), 0.25 g of STPP dissolved in 25 mL of aquades, and 200 μ L Tween 80 0.1% (v/v) was added to the solution of STPP and stirred. Solution of chitosan-extract then added with syringe (Terumo) into STPP solution and stirred for 30 minutes. Then, the solution was sonicated using Ultrasonic Cleaner Elma S30H and centrifuged (Hanil) at 10,000 rpm speed for 15 minutes. The filtrat formed then are dried with freeze dryer. Calculation of loading capacity is done by dry nanocapsule formed, and calculation of encapsulation efficiency is done by the andrographolide level on nanocapsule using High Performance Liquid Chromatography (HPLC). Particle size and morphology are identified using Field Emission Scanning Electron Microscopy (FE-SEM).

Solution Prepare

Buffer phosphate pH 6.8 made by KH_2PO_4 (Merck) 0.1 M solution and NaOH (Merck) 0.1 N solution. Making substrate solution by dissolve 0.0315 g of p-nitrophenyl- α -D-glucopyranoside (Sigma Aldrich) in 20 mL buffer phosphate. Enzyme carrier solution made by 6 mg of BSA (Merck) dissolved in buffer phosphate until 6 mL, then enzyme solution made by dissolve α -glucosidase (Sigma Aldrich) in BSA solution until get 0,3 u/mL, 0,15 u/mL, 0,075 u/mL, and 0,0375 u/mL concentration. Na_2CO_3 (Merck) solution to end enzymatic reaction made by dissolve 1,06 g of Na_2CO_3 in 100 mL buffer phosphate. Sample of *Andrographis paniculata* crude extract [12], nanocapsule of *Andrographis paniculata* leaf extract, Lamivudin (Kimia Farma), HP Pro (Bio-Lab Medilab), and andrographolide

standard (Sigma Aldrich) dissolved by dimethyl sulfoxide (DMSO) (Merck) then buffer phosphate until get concentration of 0,5%, 1%, 2%, 4%, 8%, 12%, 16%, and 18%.

Optimization of α -Glucosidase Enzyme Concentration

Blank solution testing is done by adding 490 μ L of buffer phosphate and 250 μ L of substrate solution on 10 μ L DMSO. Solution then incubated for 5 minutes at 37°C, then into the solution 250 μ L of enzyme solution added with various concentrations (0.3 u/mL u/mL, 0.15, 0.075 u/mL, and 0.0375 u/mL), the solution incubated again for 15 minutes at 37°C. The reaction was stopped by adding 2000 μ L of Na₂CO₃. Its absorbance measured with UV-Vis spectrophotometer at 400 nm wavelength. Blank control testing is done by 490 μ L of buffer phosphate and 250 μ L of substrate solution on 10 μ L DMSO. Solution then incubated for 5 minutes at 37°C, then into the solution 2000 μ L of Na₂CO₃ was added, the solution incubated again for 15 minutes. Then, 250 μ L of enzyme solution with various concentrations (0.3 u/mL u/mL, 0.15, 0.075 u/mL, and 0.0375 u/mL) was added. Its absorbance measured with UV-Vis spectrophotometer at 400 nm wavelength.

α -Glucosidase Inhibition Assay[13]

Sample testing of crude extract, nanocapsule, Lamivudin (chemical medicine of hepatitis B), HP Pro (herbal medicine of hepatitis B), and andrographolide standard done by adding 10 μ L of sample with various concentrations into 490 μ L of buffer phosphate and 250 μ L of substrate solution. Solution then incubated for 5 minutes at 37°C. 250 μ L of enzyme solution 0.0375 u/mL was added then the solution was incubated for 15 minutes. Reaction was stopped by adding 2000 μ L of Na₂CO₃. Absorbance measured at 400 nm wavelength. Sample control testing done by adding 10 μ L of sample with various concentrations into 490 μ L of buffer phosphate and 250 μ L of substrate solution. Solution then incubated for 5 minutes at 37°C. 2000 μ L Na₂CO₃ was added then the solution was incubated for 15 minutes. Then 250 μ L of enzyme solution 0.0375 u/mL was added. Absorbance measured at wavelength 400 nm. Inhibiton calculated with the equation of:

$$\% \text{ Inhibition} = \frac{A_B - A_S}{A_B} \quad (1)$$

where A_B is blank absorbance minus blank control absorbance, and A_S is sample absorbance minus sample control absorbance.

Result and discussion

Nanoencapsulation

Dry nanocapsule extract produced is 1.88 g. Calculation result the loading capacity and the encapsulation efficiency that high enough of 46.29% and 73.47%. This is due to the magnitude of the chitosan concentration that cause chitosan molecules will be increasingly bound to each other which leads into the increasing of intermolecular crosslinking bonds [14]. The bonds formed at these conditions are strong and stable so produce a particle that hard and not easily broken. The resulting particle size ranged from 557.9 nm to 892.6 nm. This particle size meets the definition of nanoparticles between 10-1000 nm [15], but not yet to meet the range of nanoparticles for drug of <100 nm [11].

The morphology of the particles shown in Figure 1 are identified by FE-SEM 1,000x zoom in and 2,000x zoom in. Particles was not perfectly spheric with a smooth surface and agglomerated each other but still visible form of a circle. This is due to the use of high concentration of chitosan that cause increased

viscosity solution that produces a strong form of the particle surface with the interaction by TPP, so the particles result will form the more spherical [16].

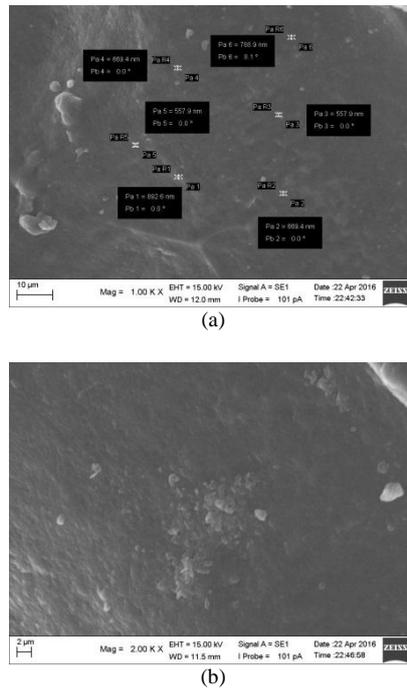


Figure 1 SEM result of *Andrographis paniculata* extract nanocapsule

α-Glucosidase Inhibition

α-glucosidase enzyme inhibition of sample shows in the Figure 2. The maximum inhibition and optimum concentration needed from each sample are different each other. Crude extract result 33.17% of maximum inhibition at 12% of concentration. Nanocapsule result higher maximum inhibition of 37.17%, however the concentration required to get that value is 16%. Maximum inhibition of Lamivudin is 90.85% and HP Pro is 77.42% which obtained by 12% concentration. Lamivudin is chemical compound which is a type of nucleoside analog drugs included in the treatment of hepatitis B, while the HP Pro is a drug containing lignin schisandrin and gomisin. Lamivudin inhibits 2.4 times bigger than nanocapsule, and HP Pro inhibits 2 times bigger.

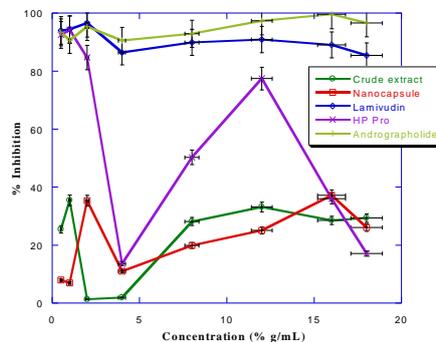


Figure 2 Inhibition of *α*-glucosidase by crude extract, nanocapsule, Lamivudin, HP Pro, and andrographolide standard

Andrographolide standard has better performance compared with Lamivudin and also alkaloids

and lignans on HP Pro that is visible from the resulting maximum inhibition percentage of 99.56% at 16% concentration. It is more proving that andrographolide and its derivative compounds contained in the *Andrographis paniculata* extract have the ability to inhibit α -glucosidase enzyme activity. Low inhibition by nanocapsule due to the very small levels of the active compounds (andrographolide) encapsulated (0.23%).

Concentration required by each sample to produce maximum inhibition of α -glucosidase that can inhibit the formation of N-glycan from glycoproteins MHBs and LHBs on hepatitis B virus envelope. The results obtained in this study indicate that the required concentration of *Andrographis paniculata* leaf extract nanocapsule leaf extract is 16% (g/mL) to inhibit protein folding on hepatitis B virus due to the inhibition of α -glucosidase.

Conclusion

Nanoencapsulation of *Andrographis paniculata* leaf extract with chitosan has 46.29% of *loading capacity* and 73.47% of encapsulation efficiency. Result of particle size from 557.9 nm until 892.6 nm. Maximum inhibition by nanocapsule is 37.17%.

Acknowledgement

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References

- Aini, Desna Quratul. 2015. Uji Pelepasan Terkendali Ekstrak Daun Keji Beling (*Strobilanthes crispus*) untuk Sediaan Obat Antihiperkolesterolemia. Depok: Universitas Indonesia
- Aisyah, Nurul. 2014. Ekstraksi Akar Ilalang (*Imperata cylindrica*) serta Aktivitas Inhibisinya terhadap α -Glukosidase yang Berfungsi dalam Pembentukan Virus Hepatitis B. Prosiding Seminar Nasional Rekayasa Kimia & Proses ISSN 1411-4216.
- Dai, Gui-Fu., Xu, Hai-Wei., Wang, Jun-Feng., Liu, Feng-Wu., & Liu, Hong-Min. 2006. Studies on the Novel α -Glucosidase Inhibitory Activity and Structure-Activity Relationships for Andrographolide Analogues. *Bioorganic & Medicinal Chemistry Letters* 16 : 2710-2713.
- Firdayani, dkk. 2012. Pengembangan Kandidat Senyawa Obat Turunan Naftokuinon sebagai Inhibitor Virus Hepatitis B. Pusat Teknologi Farmasi dan Medika BPPT.
- Herdiani, Elvina. 2012. *Potensi Tanaman Obat Indonesia*. [online] Available at <http://www.bbpp-lembang.info/index.php/arsip/artikel/artikel-pertanian/585-potensi-tanaman-obat-indonesia> [accessed 11 September 2015].
- Hidalgo, Maria A., Hancke, Juan L., Bertoglio, Juan C., & Burgos, Rafael A. 2013. Andrographolide a New Potential Drug for the Long Term Treatment of Rheumatoid Arthritis Disease. Intech.
- Kementrian Kesehatan Republik Indonesia. 2014. Situasi dan Analisis Hepatitis. Pusat Data dan Informasi.
- Ko, J.A., et al. 2002. Preparation and Characterization of Chitosan Microparticles Intended for Controlled Drug Delivery. *International Journal of Pharmaceutics* 249:165-174.
- Lazar, Catalin et al. 2007. Treatment of Hepatitis B Virus-Infected Cells with α -Glucosidase Inhibitors Results in Production of Virions with Altered Molecular Composition and Infectivity. *Antiviral Research* 76 (2007) 30-37.
- Misnadiarly, AS. 2008. Mengenal Penyakit Liver in "*Mediakom XII Edition*". Badan Litbangkes.
- Monharaj, VJ. & Chen, Y. 2006. Nanoparticles-A Review. *Tropical Journal of Pharmaceutical Research*; 5(1):561-573.
- Nanda, Rabindra K., Patil, Subodh S., and Navathar, Dipak A. 2012. Chitosan Nanoparticles Loaded with Thiocolchicoside. *Der Pharma Chemica* 4(4):1619-1625.
- World Health Organization. 2002. Hepatitis B. Departement of Communicable Diseases Surveillance and Response.
- Simsek, Ender et al. 2006. α -Glucosidase Inhibitors Have a Prolonged Antiviral Effect Against Hepatitis B Virus through the Sustained Inhibition of the Large and Middle Envelope Glycoproteins. *Antiviral Chemistry & Chemotherapy* 17:259-267.
- Xu, Hai-Wei., Dai, Gui-Fu., Liu, Gai-Zhi., Wang, Jun-Feng., dan Liu, Hong-Min. 2007. Synthesis of Andrographolide Derivatives: A New Family of α -Glucosidase Inhibitors. *Bioorganic & Medicinal Chemistry* 15 (2007) 4247-4255..

Zahrani, Khansa. 2016. Nanoenkapsulasi Ekstrak Daun Sambiloto (*Andrographis paniculata*) sebagai Inhibitor α -Glukosidase pada Pembentukan Amplop Virus Hepatitis B. Depok: Universitas Indonesia.

Comparative investigation of five chilled basic taste absolute thresholds on coastal and inland region : A case study at Tabanan Regency, Bali

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Abstract

Basic taste absolute threshold is an important factor on product development process because it can give information about people sensitivity to a taste. Current study involved 128 respondents ranging from 18 to 40 years old. Triangle method was used to determine the absolute threshold values of each panellist. The study was conducted in Kediri and Kerambitan representing coastal community as well as Baturiti and Penebel representing inland population. The individual threshold measured using Best Estimation Threshold (BET) Value. Analysis of Variance (ANOVA) shows that significant differences only found on the bitter taste (p -value < 0.05), whereas for the four basic tastes, there is no other significant differences. One of the factors that contribute to these differences is the habit of drinking alcoholic beverages; people who drink alcoholic beverages tended to have higher bitter threshold value than people who are not a regular drinker.

Keywords: Absolute threshold; chilled drink; Bali

Introduction

Product development is series of process to obtain and fulfil consumer needs and wants on particular food products. Taste is one of important factors in food product development stages as it can influence people perception on food and beverages. The sensitivity among people to taste can be different (Stone and Siedel, 2004), therefore the characteristic of market target is required to be well described to assure food products acceptability.

Taste sensitivity of respondents can be determined by measuring absolute threshold. By measuring absolute threshold, the minimum perceived tastant concentration can be detected (Meilgaard *et al.*, 2007). Absolute threshold value for each individual might be different and it depends on their daily food intake and activity (Pustiawati, 1999). Geographical location also have a role on different food habits, for example, people who lived in coastal area will be more easier to access seafood than people who lived in inland area.

Limited studies related to sensory threshold measurement are hardly found in the literature, particularly those focusing on Indonesian context. Unpublished-preliminary work conducted by our research group suggest that sensitivity difference on five basic tastes is partly contributed by different food habit and food choices among people who lived in West Java, Central Java and East Java. Refers to Baharrudin and Sharifudin (2015), geographical location allegedly have an influence on absolute

threshold value. This current study is focused on investigating possible differences on sensory threshold of conservative communities in Bali particularly at Tabanan Regency, whereas both types of coastal and inland communities can be observed

Materials and Methods

Respondents

Current study was conducted in Tabanan, Bali Indonesia. It involved 128 respondents ranging from 18 to 40 years old. All the respondents have to be healthy and have been living in each area minimum for 10 years. Current study also collect data about the dominant food taste for each region. Specific questions related to food habit, particularly related to alcoholic beverages consumption are also raised.

Materials

Five basic taste tested in current research was sweet, salty, sour, bitter and umami. Each basic taste use specific ingredients refers to Mojjet et al (2001); saccharose for sweet taste, natrium chloride (NaCl) for salty taste, citric acid for sour taste, caffeine for bitter taste, and monosodium glutamate (MSG) for umami taste. All those ingredients were diluted in mineral water. All the tastant concentrations can be seen on Table 1.

Table 1. Tastant concentrations

Sample Number	Concentration (g/L)				
	Citric Acid	Saccharose	NaCl	Caffeine	MSG
1	0.12	5	0.40	0.16	0.08
2	0.24	10	0.80	0.32	0.16
3	0.48	20	1.60	0.64	0.32
4	0.96	40	3.20	1.28	0.64
5	1.92	80	6.40	2.56	1.28

Adapted from Mojjet (2001)

Methods

Current research conducted by using home use testing, where the test was done in the respondent house or where they usually done their activities (Kemp et al., 2009). The method used in this research is *Triangle* method with two types of sample serving order; ascending concentration (ASC) and descending concentration (DSC). All sample served on 40 ml plastic cup at chilled temperature $7^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Temperature control was used as refers to Lipscomb (2015) that difference of serving temperature can influence panelist absolute threshold value. All the respondents got one of the samples serving order randomly. For each concentration in each taste, respondent got three cups of sample, a cup of *tastant* and two cups of plain water or two cups of *tastant* and a cup of plain water randomly. Each sample was given three digits code on the cup using sticker. For each concentration, respondent asked to choose one of the most different out of three served samples and they were required to provide an answer eventhough they failed recognising the different one. The absolute threshold value for each respondent was calculated by using Best Estimation Threshold (BET) method. The absolute threshold value for each group of the tests obtained by calculating the mean of the threshold value for each group.

Statistical analysis

The data was analyzed by Minitab 16 applying Nested-ANOVA to investigate the influence geographical location, gender and sample serving order. In addition, General Linear Model (GLM) was

also applied to evaluate the effect of alcoholic beverages consumption and age grouping to the absolute threshold value of respondents.

Result and discussion

Sweet-absolute threshold

Absolute threshold result for sweet taste can be seen on **Figure 1**. Absolute threshold concentration for people who lived in coastal area is 10.6 g/L (± 0.64) and for people who lived in inland area is 11.2 g/L (± 0.64). Nested ANOVA suggests that there is no significant difference ($p\text{-value} > 0.05$) between coastal and inland people on sweet taste absolute threshold.

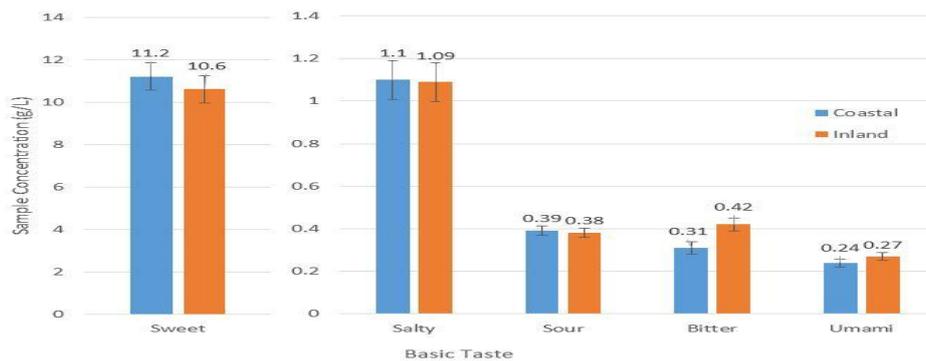


Figure 1. Five basic taste absolute threshold concentrations of coastal and inland people at Tabanan Bali

Salty-absolute threshold

Absolute threshold result for salty taste can be seen on **Figure 1**. Absolute threshold concentration for people who lived in coastal area is 1.1 g/L (± 0.091), while for people who lived in inland area is 1.09 g/L (± 0.091). Nested ANOVA suggests that there is no significant difference ($p\text{-value} > 0.05$) between coastal and inland people on salty taste absolute threshold.

Sour-absolute threshold

Absolute threshold result for sour taste can be seen on **Figure 1**. Absolute threshold value for people who lived in coastal area is 0.39 g/L (± 0.022), while for people who lived in inland area is 0.38 g/L (± 0.022). Nested ANOVA suggests that there is no significant difference ($p\text{-value} > 0.05$) between coastal and inland people on sour taste absolute

Bitter-absolute threshold

Absolute threshold result for bitter taste can be seen on **Figure 1**. People who lived in coastal area can detect caffeine concentration at 0.31 g/L (± 0.030), while people who lived in inland area can detect caffeine concentration at 0.42 g/L (± 0.030). This difference is confirmed by Nested-ANOVA ($p\text{-value} < 0.05$). The different on bitter taste may occur due to different habit between those two types of communities. The results of questionnaire showed that people who lived in the inland area drink more frequent alcoholic beverages than those who lived in coastal area. By using GLM analysis, people who frequently drink alcohol have higher absolute threshold value for bitter taste (0.44 g/L (± 0.037)) than those who are not alcohol drinker (0.33 g/L (± 0.026)).

Umami-absolute threshold

Figure 1. shows that people who lived in coastal area can detect umami taste on MSG concentration 0.24 g/L (± 0.019), while people who lived in inland area can detect umami taste on MSG concentration MSG 0.27 g/L (± 0.019). There is no significant difference ($p\text{-value} > 0.05$) in terms of umami threshold for both coastal and inland people.

Gender factor

Nested ANOVA result show that there is no significant difference ($p\text{-value} > 0.05$) in terms of gender factor on all five basic taste absolute thresholds. This result is in agreement with Hasanah *et al.* (2014) and Mojat *et al.* (2001) as they also reported that gender does not affect taste threshold values.

Sample serving order factor

ANOVA result suggests that sample serving order only contribute to provide sour taste absolute threshold significantly ($p\text{-value} < 0.05$). People lived in coastal area who provided with descending sample serving order have higher sour taste absolute threshold value (0.48 g/L ± 0.045) compare to people lived in inland area who provided with ascending sample serving order (0.25 g/L ± 0.045). Refer to Lawless and Heymann (2010), the difference may occur because respondent taste bud will be easier to get fatigue as they taste the highest concentration first. Thus respondent will be difficult to distinguish the rest of concentrations.

Age group factor

GLM analysis shows that there is no significant difference ($p\text{-value} > 0.05$) to absolute threshold value of five basic taste in terms of age grouping between 18-23 years old; 24-29 years old; 30-36 years old; and 37-40 years old. Refers to Guyton and John (2011), common people taste bud ability to taste will decrease when they aged more than 45 years old. Furthermore Mojat *et al.* (2001) reported that age does not provide any difference to the threshold value for 19 to 33 years old people. In addition, they suggest that the different perception will occur when the age range are huge, for example, group of people aged 65 to 75 years old compare to group of people aged 19 to 33 years old.

Alcohol consumption factor

Statistical analysis using GLM shows that alcoholic beverages consumption was not provide any significant difference ($p\text{-value} > 0.05$) to absolute threshold value of salty, sour and umami taste. The habit was only found to contribute for sweet taste absolute threshold. Group of people who drink alcoholic beverages required higher concentration of sweetness (9.6 g/L ± 0.79) compare to group of people who are not drinker (1.51 g/L ± 0.55). Refer to Silva *et al.* (2015), drinking alcoholic habit may affect people perception about food, people who drink alcoholic beverages will like sweet food more than other food. Refer to Lawless and Hymann (2010) the theory happen in this chase is simple contrast effect. This theory indicates that people who frequently consume sweet food will require lower concentration of sugar to detect sweetness.

Alcoholic beverages consumption tended to influence the bitter taste absolute threshold value. Group of people who drink alcohol have higher bitter taste absolute threshold value (0.4394 g/L ± 0.037) compare to group of people who are not drinker (0.3281 g/L ± 0.026). It may suggest that alcoholic beverages consumption may reduce taste bud sensitivity to bitter taste. Refers to Waterhouse *et al.* (2016), etanol compound as occurs in wine may provide direct bitter taste to someone who drink it.

Moreover, refers to Duffy (2004) people who drink more beer will be less sensitive to bitter taste compare to people who do not drink. Refer to Lawless and Heymann (2010), the theory applies in this case is sensory adaptation, where someone who consume more bitter food or beverages will need higher amount of bitter compound to detect bitterness.

Conclusion

Based on the current study conducted in Tabanan, Bali, it can be concluded that among 5 basic tastes, only bitter taste threshold was found to be different between coastal and inland people. One of the factors that can be attributed to the difference is the habit of people on alcoholic beverages consumption. It was observed that alcoholic beverages consumption tended to reduce the sensitivity of the taste bud to the bitter taste. Sample serving order was also found to be contributed to the difference of sourness threshold. This suggests that threshold experiments can be conducted by both ascending and descending order, except for sourness.

Acknowledgement

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References

- Baharuddin, A.R., Sharifudin, M.S., 2015. The Impact of Geographical Location on Taste Sensitivity and Preference. *Int. Food Res. J.* 22, 731–738.
- BPS, 2015. Perkembangan Pariwisata Bali September 2015. Accessed on 29 of March 2016. <http://bali.bps.go.id/webbeta/website/brs_ind/brsInd20151102132512.pdf>.
- Duffy, V.B., Peterson, J.M., Bartoshuk, L.M., 2004. Associations Between Taste Genetics, Oral Sensation and Alcohol Intake. *J. Physiology & Behavior* 82 (2004), 435-445.
- Guyton, Arthur C. and Hall, John E., 2011. Textbook of Medical Physiology: Twelfth Edition. 2014. Saunders Elsevier. Philadelphia.
- Hasanah, U., Adawiyah, D. R., Nurtama B., 2014. Preferensi dan Ambang Deteksi Manis dan Pahit: Pendekatan Multikultural dan Gender. *J. Mutu Pangan* vol 1(1): 1-8, 2014
- Lawless, H.T., Heymann, H., 2010. Sensory Evaluation of Food 2nd Ed., Springer Science & Business Media. New York.
- Lipscomb, K., 2015. Effect of Temperature on Sensing Intensity of Basic Tastes: Sweet, Salty and Sour. Clemson University. South Carolina
- Meilgaard, M.C., Carr, B.T., Civille, G.V., 2007. Sensory Evaluation Techniques, Fourth Edition. CRC Press. Boca Raton.
- Mojet, J., Christ-Hazelhof, E., Heidema, J., 2001. Taste Perception with Age: Generic or specific losses in threshold sensitivity to the five basic tastes?. *Chem. Senses* 26: 845-860, 2001.
- Puspitawati, I., 1999. Psikologi Faal. Universitas Gunadarma, Jakarta.
- Silva, C.S., Dias, V.R., Almeida, J.A.R., Brazil, J.M., Santos, R.A., Milagres, M.P., 2015. Effect of Heavy Consumption of Alcoholic Beverages on the Perception of Sweet and Salty Taste. *Alcohol and Alcoholism J.* 51(3): 235-241.
- Stone, H., Sidel, J.L., 2004. Sensory Evaluation Practices, 3rd ed. Elsevier Academic Press. San Diego.
- Waterhouse, A.L., Sacks, G.L., Jeffery, D.W. 2016. Understanding Wine Chemistry. John Wiley & Sons, Inc. Chicester.

Gelatine from Cricket (*Gryllus assimilis*) through Acid Method, Characterization, and Application as Clarifier in Apple Juice Industry

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ABSTRACT

Gelatine is a derived compound produced from collagen fibers of connective tissue, skin, and bone through hydrolysis by acid or alkaline solution. It is very widely used in industry, e.g. in the food industry as a stabilizer and/or clarifier. Moreover, gelatine is also used as a raw material for capsule production and film processing. Currently, gelatine in Indonesia is mainly imported from China which are generally made from pig bones or skins. Therefore, mostly the imported gelatine is not halal for populations who are Moslems. As a result, it is necessarily conducting research to produce gelatine using raw materials which is comply with kosher/halal law and safe, e.g. crickets. The aim of this research is to study the effect of extraction temperature and time on the yield of gelatine obtained. The method implemented in this research was acid extraction by using various extraction temperatures (30, 50, 60 and 70 °C) and extraction times (1, 2, 3 and 4 h). The result showed that the increasing of yield occurred as the increasing of extraction temperatures and times. The characterization using FTIR analysis showed that the gelatine produced from cricket has the functional groups which are similar to those of commercial cow skin gelatine and could significantly reduce the turbidity of apple juice. It is concluded that the cricket gelatine can be used as clarifier for apple juice.

Keywords: Gelatine, cricket, acid method, clarifier, apple juice

Introduction

Gelatine is a protein derivative of collagen fibers existing in the skin, bone, and cartilage. Its amino acid composition is slightly similar to collagen, where 2/3 of all the amino acids is glycine, while the rest are proline and hydroxyproline. Gelatine does not contain tryptophan, therefore it can not be classified as a complete protein (Chaplin, 2005). The process of gelatine production is based on collagen chain termination which is carried out under acidic or alkaline condition. Termination of the collagen chain can also be assisted by conducting the extraction at high temperature. Acid process is more favor to change the triple helix collagen fibers into a single chain, while the alkaline solution can only produce double chain (Ward & Court, 1977). Thus, it results in, higher amount of hydrolyzed collagen than the alkaline solution.

Gelatine has many uses, especially in pharmaceuticals and food industries, i.e. as a forming agent, thickener, emulsifier as well as clarifier. Currently, most gelatine products consumed in Indonesia are imported from China which are mostly made from pig skins and bones, meanwhile such gelatine is not halal for *kosher and safe* moslems. This condition reflects the need of a study to use gelatine alternative gelatine sources, such as cricket (*Gryllus assimilis*).

A number of researchers have studied on the use of mammals, poultries as well as fishes as the raw materials to produce gelatines. Aryanti (1998) used sheep bone, Kartika and Nursyabani (2003) used chicken bone, while Karlina *et al.* (2009) used stingray (*Himantura gerardi*) bone, and Fatimah and Dewi (2008) used milkfish (*Chanos-Chanos Forskal*). The other researches were Hao *et al.* (2009) who used sturgeon skin. In addition, Nofri (2014) have succeeded in using tuna fish (*Thunnus Sp*), while Permata (2014) used cat fish bone. Moreover, Mariod *et al.* (2011) have prepared gelatine from melon bugs (*Aspongubus Viduatus*) and sorgum bugs (*Agonoscelis pubescens*). As the growing interest in developing alternative raw materials to make gelatine, one of them is the cricket. Producing gelatine from cricket is expected (a) to increase the economic value of crickets that has not been widely utilized and (b) to produce halal gelatine.

Materials and Method

Materials and Apparatus

The cricket used as raw material was obtained from local seller in the region of Kalijudan, Surabaya. The chemicals used in this research were 37% hydrochloric acid, 98% sulphuric acid, oxalic acid, sodium borax, sodium hydroxide, Kjeldahl tablet, phenolphthalein, methyl red and potassium bromide. The apparatus used consisted of Fourier Transform Infrared Spectrophotometer (Shimadzu), analytical balance (Sartorius Basic), furnace (Thermolyne Furnace), desiccator (Glaswerk), freezer (Panasonic), centrifuge, hot plate, oven, Kjeldhal apparatus, water bath, glasswares, penetrometer, and turbidimeter.

Research Procedure

The first step is the preparation of raw materials by discarding the entrails of the cricket, then washing the rest using warm water at 50°C to remove the remaining fat and it was dried until the moisture content less than 10%. Prior to be used, it was crushed to powder form and stored in desiccator. The following step is the demineralization. In this step, the cricket powder was soaked into 6% HCl solution for 18 h with three times replacement of the HCl solution every 6 h. Then, the cricket powder was washed with distilled water until the pH of 4-5. The next step was the extraction process using distilled water at various extraction temperatures and times. The solution was filtered and dried to reach constant weights. The last step was to apply the cricket gelatine as a clarifier for juice manufacturing. The cricket gelatine was added into the apple juice at various ratio (m/v), and turbidity of the juice was measured in comparison the values before and after treatment with the cricket gelatine. The reduction of turbidity percentages were expressed in %.

Application of Cricket Gelatine as a Clarifier

The cricket gelatin, from the treatment producing the highest yield, was added into the apple juice by varying the ratios (m/v) and stirred for 5 minutes at 360 Rpm, then stirred for 30 minutes at 155 Rpm. During the stirring, the coagulation and flocculation occur and producing sediment after settling for 2.5 hours. The turbidity before and after addition of gelatine were measured.

Result and discussion

The Effects of Various Extraction Temperatures and Times on the Yield

The effect of extraction temperature on the yield is shown in Figure 1, while the effect of extraction time is shown in Figure 2.

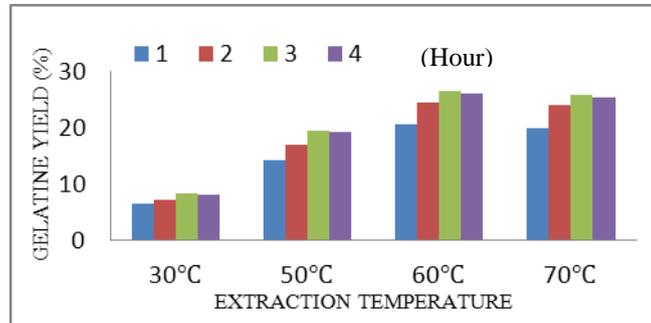


Figure 1. The effect of extraction temperature to the yield of gelatine (n=2)

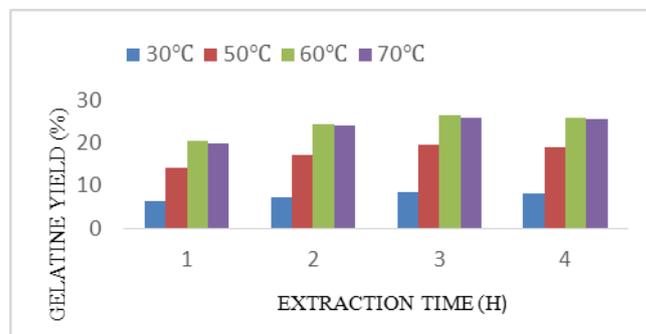


Figure 2. The effect of extraction time to the yield of gelatine (n=2)

As shown on Figure 1 and 2, at the same extraction times, the increasing yields are in line with the increasing extraction temperatures at the range of 30°C - 60°C, but the yield decreases at a temperature of 70°C. The diffusion process of gelatine in water at heating of 30°C - 60°C occurs was more effective, so that the yields increased. Ockerman and Hansen (2000) states that the high extraction temperatures increase the yield of gelatine due to the open collagen structure because multiple bonds in protein molecules had been broken. It helps break the hydrogen bonds in the hydrolyzed gel. The more gel of hydrogen bonds are broken, the more collagen has dissolved in hot water so that the yield of gelatine increase. However, the extraction temperature which is closed to denaturations temperature i.e.80 °C can lead to proteins denaturation. It makes gelatine be insoluble in water and will be removed from extracts during filtration process, so reduces the yield of extraction.

Similarly, it also happens for the variation of extraction time. At the same extraction temperature, the increasing yield occurs as the increasing of extraction time. This is presumably because the amount of H⁺ ion has hydrolyzed more collagens, so that more collagens decomposed into gelatine. But, the extraction time which is to high such as 4 hours can makes decreasing of gelatine yield due to the increasing excess of heat energy that goes into the extraction system which can increase the breakdown of proteins. And also occurs the advanced hydrolysis in collagen that has been converted into gelatine. From

the research, the conditions that produce the highest yield is extraction temperature of 60°C for 3 hours.

FTIR analysis

The aim of FTIR analysis is to confirm the presence of gelatine in the final product by comparing the functional groups of the cricket gelatine and commercial gelatine. The results are as follows:

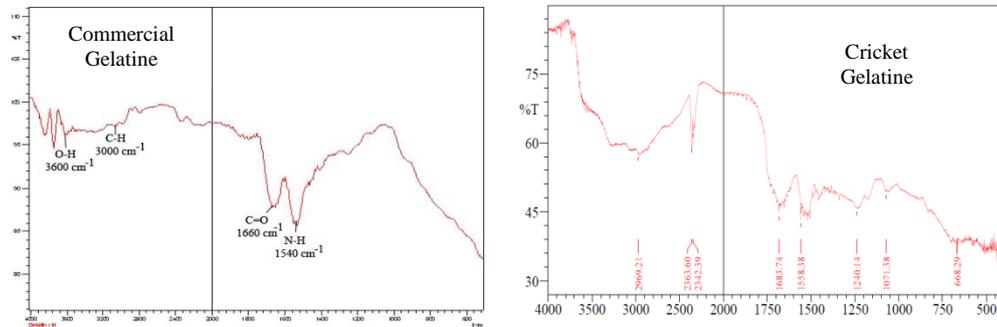


Figure 3. FTIR analysis of cricket gelatine and commercial gelatine

Figure 3 shows the results of FTIR analysis to identify the functional groups of the cricket gelatine and commercial gelatine. Interpretation of the FTIR spectra indicates that both of them having the same functional groups of O-H, C-H, C=O and N-H. At cricket gelatine, the peak of O-H appears at wave number of 3295 cm⁻¹ whereas in commercial gelatine at 3600 cm⁻¹. It is in agreement with the FTIR spectra of general knowledge of gelatine that the functional groups O-H in the range of wave number 3600-3200 cm⁻¹ (Chaplin, 2005). Peak of C=O of cricket gelatine appears at wave number of 1683.74 cm⁻¹ whereas in commercial gelatine at wave number 1660 cm⁻¹. It is in accordance with the FTIR spectra wherein the functional group C = O contained in the range of wave number 1800-1650 cm⁻¹. Peak of N-H cricket gelatine at wave number 1558.38 cm⁻¹, whereas commercial gelatine at wave number 1540 cm⁻¹. It accordance with the FTIR spectra literature wherein the functional group N-H secondary as a marker cluster chains of amino acids contained in the range of wave number 1600-1460 cm⁻¹. C-H peak of cricket gelatine is at the wave number 2969.21 cm⁻¹, whereas C-H peak of commercial gelatine at wave number 3000 cm⁻¹. This is consistent with the literature in which the FTIR spectra of secondary C-H group function in the range of wave number 3000-2840 cm⁻¹ (Pretsch, 2000). The FTIR spectra, there are also impurities indication in the form of calcium carbonate located at the absorption peak of 1470-1440 and 880-710 cm⁻¹ (Larkin, 2011). In the experimental results of cricket gelatine, calcium carbonate peak lies in wave numbers of 1455 and 714 cm⁻¹.

Gelatine Characteristis

The cricket gelatine characterization was carried out only for the product with the highest yield, i.e. gelatine which was made at 60°C for 3 hours. The characterization consisted of protein content, moisture content, ash content, and Bloom gel strength. The cricket gelatine characteristics are presented in Table 1.

Table 1. Gelatine Characteristics

Characteristics	Cricket Gelatine	SNI No. 3735-1995
Colour	Brown-Red	colourless
Odor	Normal	-
Water content	3.9 %	max. 16%
Protein content	74.25 %	-
Ash content	0.59 %	Max. 3.25%
<i>Bloom gel</i>	155 g Bloom	-

Water Content

Water can be either intracellular or extracellular components of a product (Fever, 1989). It determines the freshness, appearance and durability of foodstuffs. As shown on Table 1, the water content of the cricket gelatine (3.9%) is lower than the requirement of Indonesian National Standard (16%) and commercial gelatine (8-15%).

Ash Content

The aim of ash content analysis is to measure the mineral content of cricket gelatine. The result shows that the ash content of cricket gelatine is 0.59%, which is lower than commercial gelatine (0.77%), even much lower than SNI (3.25%). This is due to the three times replacement of HCl solution during the demineralisation. In the demineralization step, the reaction between HCl and calcium phosphate as the composer of bone occurs, producing CaCl₂ which is soluble in the HCl solution and easily separated through filtering. The reactions is $\text{Ca}_3(\text{PO}_4)_2 (\text{s}) + 6 \text{HCl} (\text{aq}) \rightarrow \text{CaCl}_2(\text{aq}) + 2 \text{H}_3\text{PO}_4 (\text{aq})$.

The ash content depends on the mineral components that are bound to collagen and has not been released during the demineralization and washing, so it is still contained in the cricket gelatine (Aviana, 2002).

Bloom Gel Strength

Bloom gel strength or gelatine gel strength is one of the parameters to determine the texture and the force to produce a certain deformation (Suptijah, 2013). The bloom strength of the cricket gelatine is presented in Table 1, i.e. 155 g Bloom, it has already met with SNI (75-250 gBloom).

Odor and Taste

Odor and taste is one of the parameters that determine the quality of the cricket gelatine. Based on the results of characterization in Table 1, it can be concluded that the cricket gelatine comply with SNI, i.e. having normal odor and taste as well as acceptable for consumers.

Application of Cricket Gelatine as a Clarifier

The initial turbidity of apple juice was 450 NTU, while the turbidities after the addition of gelatine are presented in Table 2.

Table 2 shows that the higher ratio of the cricket gelatine to the apple juice volume (m/v) produces the higher % reduction of turbidity. This is because the more amount of gelatine added to the apple juice, the more suspension of solid bound to the colloid of the apple juice. As a result, after 2.5 settling time, the turbidity decreases. However, the addition of gelatine at high ratio did not significantly affect to the increasing of % reduction of turbidity, because the gelatine used as a clarifier has reached the maximum

ability to bind to colloid content of apple juice.

Table 2. The effects of cricket gelatine addition to the reduction of apple juice turbidity

Ratio of Cricket Gelatine to Apple Juice (m/v)	Turbidity of Apple Juice		Reduction of Turbidity (%)
	Initial Turbidity (NTU)	Final Turbidity (NTU)	
0.1 : 200	450	350	22.22
0.2 : 200		100	77.78
0.3 : 200		50	88.89
0.4 : 200		40	91.11
0.5 : 200		35	92.22

Conclusion

The higher the extraction temperature, the higher the yield of cricket gelatine, but the yield decreases if the temperature is too high. The longer the extraction time, the higher the yield of cricket gelatine, but the yield decreases if the extraction time is too long. FTIR analysis shows that there are similarity between the functional groups of the cricket gelatine and commercial gelatine. While the characteristics of cricket gelatine results include ash content of 0.59%, 74.25% protein content, water content of 3.9%, gel strength of 155 gBloom, normal odor and taste as well as acceptable by consumers. The higher ratio of the gelatine to the apple juice volume (m/v) produces the higher % reduction of turbidity. However, the addition of gelatine at high ratio does not significantly affect the increasing reduction of turbidity, because the gelatine used as clarifier has reached the maximum ability to bind to colloid content of apple juice.

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References

- Aryanti, R., 1998. *Kajian proses produksi gelatin dari tulang domba menggunakan proses asam*. Skripsi. Fakultas Teknologi Pertanian, Institut Pertanian Bogor. Bogor
- Aviana, T., 2002. Pengaruh Jenis dan Konsentrasi Larutan Perendaman serta Metode Pengeringan terhadap Sifat Fisik, Kimia, dan Fungsional Gelatin dari Kulit dan Tulang Cucut. Skripsi. Fakultas Teknik Pertanian – IPB, Bogor.
- Chaplin, M. *Gelatine*. 2005 [cited 2010 17 September]; www/isbuc.ac.uk.
- Badan Standarisasi Nasional, 1995. *Syarat dan Uji Mutu Gelatin*. Jakarta: Dewan Standarisasi Nasional.
- deMan, John M., 1989. *Principles of Food Chemistry*. USA: Aspen Publishers.
- Fatimah, Dewi, 2008. *Efektivitas Penggunaan Asam Sitrat dalam Pembuatan Gelatin Tulang Ikan Bandeng (Chanos-Chanos Forskal)*, Chemical Departemen. Universitas Islam Negeri Malang: Malang.
- Hao, Shuxian, Laihao Li, Xianqing Yang, Jianwei Cen, Hong Shi, Qi Boa, and Junyan He, *The Characteristics of Gelatine Extracted from Sturgeon (Acipenser baeri) Skin Using Various Pretreatments*. Food Chemistry, 2009. 115: p. 124-128.
- Karlina, Intan Riezky and Lukman Atmaja. 2009. *Ekstrak Gelatin dari Tulang Rawan Ikan Pari (Himantura gerardi) pada Variasi Larutan Asam untuk Perendaman*. Jurusan Kimia FMIPA., Institut Teknologi Sepuluh Nopember.
- Kartika, R.D dan Nursyabani, A.A. 2003. *Pembuatan Gelatin dari Tulang Ayam*, Universitas Islam Indonesia., Yogyakarta.
- Larkin, Peter J., *Infrared and Raman Spectroscopy*. 2011, USA: Elsevier.
- Nofri Sandria, Desmelati, Merry Sukmiwati. 2014. *Studi Ekstraksi Gelatin dari Mata Ikan Tuna (Thunnus Sp)*.
- Permata, W., Yenita, Faradhita Widiastri. 2014. *Gelatin dari Tulang Ikan Lele (Chaliasbatrachus) : Pembuatan dengan Metode Asam, Karakterisasi dan Aplikasinya Sebagai Thickener pada Industri Sirup*.
- Pretsch, Ernoe *Structure Determination of Organic Compounds*. 2000, Germany: Springer-Verlag Berlin Heidelberg.
- SNI 06-3735. 1995. *Mutu dan Cara Uji Gelatin*. Badan Standarisasi Nasional, Jakarta.
- Suptijah, P., Suseno, H.S., dan Anwar, Cholil. 2013. *Analisis Kekuatan Gel (Gel Strength) Produk Permen Jelly dari Gelatin Kulit Ikan Cucut dengan Penambahan Karaginan dan Rumput Laut*. Vol. 16 No. 2. Institut Pertanian Bogor: Bogor

Characteristics of Baby Food Composed of Pre-Gelatinized Canna and Rice Flour

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Abstract

Rice is commonly used in baby food, but *Canna* (*Canna edulis* Ker.) flour is rarely used although some research showed that *Canna* flour was easily digested. Previous study showed that *Canna* flour had unstable pasting because of unique starch granule properties in gelatinization, so that it has to be pre-gelatinized before processed as flour. Whereas variance in rice was significantly influenced the flour characteristics, especially water absorption, viscosity, and pasting profile. This research used pre-gelatinized of white *Canna* tuber (PGC) and *Mentik Wangi* rice flour (MWR). Two ratio of water: flour (9:1 and 11:1, % w/w) that nested in three proportion of PGC: MWR flour (40:60, 50:50, 60:40, % w/w) were used as factor in baby porridge formula. The flour proportion gave significantly effect on starch digestion value, but no effect on viscosity and dextrin content of the porridge. The highest proportion of PGC produced the highest of digestion value (183,8 mg/g sample). Ratio of water: flour as nested factor significantly influenced to all characteristics of the porridge. For each proportion of the flour, the higher ratio of water was the higher of dextrin content and the digestion value, but lower of viscosity of the porridge. Pre-gelatinized *Canna* flour improved the pasting profile of MWR flour then whole characteristics of the porridge.

Keywords: Pre-gelatinized Canna Flour, *Mentik Wangi* Rice Flour, Baby Food, Baby Porridge, starch digestion value

Introduction

Weaning or complementary food for baby generally includes formula milk and milk-based solid food. Those food have to be nutritious (high energy and protein, completed with vitamin and mineral), good sensory characteristics, low cost and made from local ingredients (Manulang, 1994). Infants require additional energy by 24-30% due to the nutritional content of breast milk is not sufficient. Indonesia has many varieties of flours that still are inferior and have not been used optimally, including Canna flour. Research done by Suprijono and Sutedja (2008) showed that Canna flour could substitute wheat flour in cookies for children. Canna flour substituted in cookies increased the swelling power of the cookies. This swelling power was higher than the ones in 100% wheat flour-cookies. This phenomenon was also found by Muzaifa *et al.* (2014) in *Kwetiau*, a type of noodle, which used 100% Canna flour. Water absorption characteristic was higher in Canna flour but lower in the cookies, even though Canna starch had higher water absorption than wheat flour.

Baby food for 7-10 months generally is porridge that varied based on the consistency and the main ingredient. Instant baby porridge generally circulating in Indonesia are made with the main ingredient of white rice and brown rice. This phenomenon can be explained because rice is the staple food consumed by the Indonesian people. Traditionally and commercially baby porridges were made from high-starch flour, like banana and mung bean, or by mix of flour from cereals, beans or peas (cowpea, soybean, jack

bean) and tubers, like sweet potato and cassava, but Canna flour hasn't been used. In fact, the aim of those mixture is to improve the nutrition quality of the baby porridge.

The Canna (*Canna edulis* Kerr.; Indonesia: Ganyong) flour is high in carbohydrate (85,16-96,53% *by difference*), but low in protein (0,45-1,44%) with lysine as limited amino acid, and total dietary fibre (8,59%) (Hill, 1952; Carolina and Ilmi, 2016). It has high solubility and low gel consistency, then it is easier to be digested (Flach and Rumawas, 1996; Widowati *et al.*, 2001). Microscopic observation of Canna flour indicated that gelatinization made shape of the starch granule similar a sack. This inconvenient was improved when used pre-gelatinized Canna flour. This characteristic will influence the viscosity and consistency of baby food. Because the viscosity and consistency are the most important characteristics for baby porridge, it need the research focus in baby porridge made from rice flour and modification of Canna flour.

Materials and Methods

Materials

White Canna tuber flour used in this research was bought from "Harapan Mulya Corp"-Ciamis, West Java, Indonesia, then was pre-gelatinized. The rice was *Mentik Wangi* cultivar, which bought from UD Usaha Bersama at Surabaya rice market, East Java, Indonesia.

Chemicals

All reagent and solvent used for chemical characteristics analysis were pro analysis grade. Chemicals for analysis are Iodine, HCl 0.1 N, NaOH, hexane, acetone, H₂SO₄, K₂S₂O₄, HgO, Zn powder, cellite, CH₃COONa, ethanol 96%, dinitrosalicylic, and buffer Na-phosphate 0.1M; pancreatic enzyme from porcine pancreas (Sigma, P7545) and amyloglucosidase from *Aspergillus niger* (Fluka/Sigma-Aldrich, A9913), glucose (Merck 1.08342), and maltose.

The Rice and Canna Flour Preparation

Rice flour was prepared using wet-milling method. *Mentik Wangi* rice was soaked into water (rice: water = 1:2, w/v, kg/L) in room temperature for two hours. This process aims to make the seeds wet enough before milling. After the milling process, then dried in cabinet dryer at 60°C until the water content reached 8-10%. The dried rice sieved to pass 80 mesh of size.

The suspension of Canna flour was prepared by mixing Canna flour and water in ratio 8:100, g/g. The suspension was heated until 70°C. It was spread on baking tray, then dried in cabinet dryer at 60°C until the water content reached 8-10%. The dried Canna sol was grinded using cereal miller repeatedly until has been finely ground and got size 80 mesh. The product was called as Pre-gelatinized Canna Flour.

The Rice and Canna Flour Analysis

The physicochemical properties of the flour that should be analyzed include yield, starch granule structure (microscopic), starch content (AOAC, 1996), and water absorption (Ganjyal *et al.*, 2006; Shimelis *et al.*, 2006). To evaluate the effect of pre-gelatinization, the Canna flour then was analysed total dietary fibre content, viscosity, and digestibility value (Parada and Aguilera, 2009). The mix of pre-gelatinized Canna and rice flour was analyzed further, included the water absorption (Ganjyal *et al.*, 2006; Shimelis *et al.*, 2006), total and insoluble dietary fibre content (Asp *et al.*, 1983).

The Baby Porridge Formula, Processing, and Analysis

It is a nested factorial research; using water-the flour ratio, which nested in Pre-gelatinized Canna: rice flour ratio. The water: flour ratio consists of two levels, those were 9:1 (A1) and 11:1 (A2), ml/g from total weight of ingredient. The Canna: rice flour ratio consists of three levels, those were 40:60 (P1), 50:50 (P2), and 60:40 (P3), g/g from total of flour weight. Unit of research experiment was 450 g. Composition of each ingredient was shown at Table 1. All treatments were replicated three times respectively. Research parameters were viscosity, digestibility value, and dextrin content of the baby porridge; and supported by water absorption and dietary Fibre content data.

Table 1. The Baby Porridges Formula

No	Ingredient	Proportion (%)		The Composition					
		A1	A2	P1A1	P1A2	P2A1	P2A2	P3A1	P3A2
1.	Pregelatinized-Canna flour (g)	9.2	7.7	16.56	13.80	20.70	17.25	24.84	20.70
2.	Rice flour (g)			24.84	20.70	20.70	17.25	16.56	13.80
3.	Water (ml)	82.9	84.4	373	380	373	380	373	380
4.	Skim Milk (g)		6.5	29.25	29.25	29.25	29.25	29.25	29.25
5.	Sugar (g)		1.4	6.35	6.35	6.35	6.35	6.35	6.35
Unit Experiment Total		100		450	450	450	450	450	450

All ingredients were prepared as shown in Table 1. The ingredients were mixed using spoon, then water was added slowly until all ingredient mixed well. The suspension was heated until 95°C that was preserved for 15 minutes; it has to be stirred during heating to avoid the clumping. The porridge had to be 45°C at room temperature, before analysis.

The baby porridge then is a sample for analysis of viscosity using viscometer, starch digestibility *in vitro* (Parada and Aguilera, 2009), dextrin content using titrimetric/iodometric method (Member Companies of The Corn Refiners Association, 2001).

Statistical Analysis

Data was analysis statistically using ANOVA at $\alpha=0.05$ to evaluate the effect of pre-gelatinized Canna: Rice ratio and the nested: water: flour ratio on physicochemical characteristic and digestibility of the flour and the baby porridge. Analysis further was done using LSD at $\alpha=0.05$

Result and Discussion*The Effect of Rice-Pre-gelatinized Canna Flour Ratio on Physicochemical Characteristics of Flour*

Pre-gelatinization changed the characteristics of Canna flour. It increased the size of Canna starch granule more than ten times (Table 2.), although the size varies. It also resulted in increasing of the yield, starch content, water absorption, and digestibility value. The increase of size starch granule and starch content enhanced hydrophilicity, which was proved by the increase of water absorption. The moisture content of the material would affect the solubility of a substance in water. Pre-gelatinized canna flour gave lower water content. High water content within the material causing the material becomes difficult to disperse in water because the material tends to closely, not to form pores, resulting materials which not be able to absorb large amounts of water. The higher the moisture content of a substance has the lower

solubility, otherwise low water levels cause high solubility for flour easily disperse in water. The similar result obtained by Listyoningrum and Harijono (2015), the water content in instant porridge feeding from green beans higher than commercially baby porridge, so the solubility was lower. Interestingly, pre-gelatinization slightly influenced the viscosity and total dietary fibre of Canna flour.

Table 2. Physicochemical Properties of Canna and Rice Flour

No	Characteristics	Canna Flour		Rice Flour
		Non-pre-gelatinized	Pre-gelatinized (8%)	
1.	Yield (%)	0,00	3,05	69,3
2.	Starch granule size ($\mu\text{m}^2 \pm \text{SD}$)	2.966,07 \pm 748,12	25.854,70 \pm 24.118,34	57,76 \pm 9,67
3.	Water content (%)	20,73	8,35	10,42
4.	Starch Content (mg/g, db)	85,26	138,16	85,77
5.	Total Dietary Fibre Content (mg/g, db)	14,57	15,92	-
6.	Water absorption (%)	69,3	525,5	114,84
7.	Viscosity (dPa.s)	13,70	15,00	-
8.	Digestibility value (mg/g sample)	12,18	124,27	-

Table 3 below showed that pre-gelatinized Canna flour significantly influenced the physicochemical characteristics of the mix flour. The higher ratio of pre-gelatinized Canna flour the higher water absorption, total dietary fibre and insoluble dietary fibre content of the mix flour. Pre-gelatinized Canna flour in ratio 60% (P3) gave the highest characteristics. It is consistent with data in Table 2. Pre-gelatinization that increased significantly the water absorption of Canna flour, improved this characteristic in the mix flour. Total dietary fibre of pre-gelatinized Canna flour was 15,92% and rice flour was 4,67-7,57% (Widowati dkk., 2009). It indicated that pre-gelatinization in Canna flour that more significantly influenced the total and insoluble dietary fibre content of mix flour than rice flour.

Table 3. Physicochemical Characteristics of Rice-Pre-gelatinized Canna Flour

NO	Characteristics	Pre-gelatinized Canna: Rice Flour Ratio*		
		P1	P2	P3
1.	Water absorption (%)	336.21 ^a	356.98 ^a	370.33 ^a
2.	Total Dietary Fibre content (%)	16.19 ^a	20.08 ^b	20.55 ^c
3.	Insoluble Dietary Fibre content (%)	14.18 ^a	17.06 ^b	18.43 ^c

* Pre-gelatinized Canna: Rice ratio : P1= 40:60, P2= 50:50, P3= 60:40

Values with different letters in the same line were differ significantly, $p < 0.05$.

The Effect of Rice-Pre-gelatinized Canna Flour Ratio on Physicochemical Characteristics of Baby Porridge

Table 4 showed that pre-gelatinized Canna: rice ratio did not affect significantly the viscosity and dextrin content of the baby porridge, but significantly affect the digestibility value. The higher ratio of pre-gelatinized Canna, the higher digestibility value of the baby porridge. The increase of starch granule shape, water absorption and digestibility of pre-gelatinized Canna flour (Table 2) significantly increased the digestibility of baby porridge. It had to be higher than rice flour, to make the increase significantly.

Comparing data of viscosity in Table 4 and 5, viscosity of baby porridge more significantly influenced by water: flour ratio than the flour ratio. The viscosity decreased with the increase of water ratio. It may influence by the increase of dextrin content of baby porridge. Dextrin is more soluble than starch thereby decreasing the viscosity. Cooking temperature (95°C) during baby porridge making seems gave different influences for pre-gelatinized Canna and rice flour to make a viscosity and consistency need by the baby porridge. It was higher than gelatinization temperature of rice starch (72-93°C) and

Canna starch (60-70°C (Suprijono and Widyastuti, 2010) or 70,5-72°C (Richana and Sunarti, 2004)). Pre-gelatinized *Canna* flour improved the pasting profile of rice flour then whole characteristics of the porridge.

Table 4. Physicochemical Characteristics of Baby Porridge Based on Pre-gelatinized Canna: Rice Flour Ratio

NO	Characteristics	Pre-gelatinized Canna: Rice Flour Ratio*		
		P1	P2	P3
1.	Viscosity (dPa.s)	30.29 ^a	28.09 ^a	27.92 ^a
2.	Dextrin content (%)	14.14 ^a	15.11 ^a	14.14 ^a
3.	Digestibility value (mg/g sample)	109.10 ^a	108.47 ^a	183.80 ^b

* Pre-gelatinized Canna: Rice ratio : P1= 40:60, P2= 50:50,P3= 60:40

Values with different letters in the same line were differ significantly, p<0.05.

The Effect of Water: Flour Ratio on Physicochemical Characteristics of Baby Porridge

Table 5. Physicochemical Characteristics of Baby Porridge Based on Different Water: Flour Ratio

NO	Characteristics	P1*		P2*		P3*	
		A1**	A2**	A1**	A2**	A1**	A2**
1.	Viscosity (dPa.s)	35.57 ^b	25.01 ^a	30.84 ^y	25.33 ^x	40.57 ⁿ	15.27 ^m
2.	Dextrin content (%)	13.96 ^a	14.31 ^b	14.04 ^x	16.19 ^y	13.47 ^m	14.81 ⁿ

* Pre-gelatinized Canna: Rice ratio : P1= 40:60, P2= 50:50,P3= 60:40

** Water: flour ratio : A1= 9:1, A2= 11:1

Values with different letters in the same line were differ significantly, p<0.05.

Conclusion

Pre-gelatinized Canna: rice flour ratio significantly influenced digestibility of the baby porridge. The viscosity and dextrin content of baby porridge were more significantly influenced by water: flour ratio than the flour ratio.

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References

- AOAC (1996), "Official Methods of Analysis", Association of Official Analysis Chemistry, Washington DC.
- Asp, N.G, Johansson, C.G., Hallmer, H., and Siljestrom, M. (1983), "Rapid Enzymatic Assay of Insoluble and Soluble Dietary Fiber", *J. Agric. Food Chem.* Vol. 31, pp. 476-482.
- Flach, M. and Rumawas, F. (1996), "Plant Resources of South East Asia", Backhuys Pub., London
- Ganjyal, G., Hanna, M.A., Supprung, P., Noomhorm, A., and Jones. D. (2006), "Modeling Selected Properties of Extruded Rice Flour and Rice Starch by Neural Networks and Statistics", *Cereal Chemistry*, Vol. 83 No. 3, pp. 223-227.
- Hill, A.F. (1952), "Economic Botany, A Textbook of Useful Plants and Plant Product", Mc. Graw-Hill Book Co. Inc., Newyork
- Listyoningrum, H. dan Harijono (2015), "Optimasi Susu Bubuk dalam MPASI", *Jurnal Pangan dan Agroindustri* Vol. 3 No 4 pp.1302-1312,
- Manulang, M., Endang, S., and Eveline, S. (1994), "Pengolahan Makanan Bayi". Buletin Teknologi dan Industri Pangan pp. 48-55
- Member Companies of the Corn Refiners Association (2001), "Dextrin Analysis Reducing Sugar", <http://www.corn.org/methods/D-52.pdf>
- Muzaifa, M., Sulaiman, M.I., and Liyuza (2014), "Evaluasi Sifat Fisik Pati Ganyong (*Canna edulis* Kerr.) sebagai Bahan Baku Pembuatan *Kwetiauw* pada Tingkat Substitusi yang Berbeda", *Agricultural Science and Technology Journal (SAGU)*, Vol.13 No. 2, pp. 35-40

- Parada, J. and Aguilera, J. M. (2009), *In Vitro* Digestibility and Glicemic Response of Potato Starch is Related to Granule Size and Degree of Gelatinization, *Journal of Food Science*, Vol. 74 No. 1 pp. E34-E38.
- Richana, N. and Sunarti, T.C. (2004), "Karakterisasi Sifat Fisikokimia Tepung Umbi dan Tepung Pati dari Umbi Ganyong, Suweg, Ubi Kelapa dan Gembili", *Jurnal Pascapanen*, Vol. 1 No.1, pp. 29-37
- Shimelis, E.A., Meaza, M. dan Rakshit, S.K. 2006. Physicochemical Properties, Pasting Behaviour and Functional Characteristics of Flours and Starches from Improved Bean (*Phaseolus vulgaris L.*) Varieties Grown in East Africa, *Agricultural Engineering International, the CIGR Ejournal*, Vol.VIII, pp. 623-629.
- Sudarmadji, S, Bambang, H., and Suhardi (1997), "Prosedur Analisa untuk Bahan Makanan dan Pertanian", 4th edition. Liberty, Yogyakarta.
- Suprijono, M.M. and Sutedja, A.M. (2008), "Efek Metode *Blanching* Uap dalam Pembuatan Biskuit Tepung Tempe terhadap Penerimaan Konsumen", Prosiding Seminar Nasional Teknologi Pangan, PATPI. Yogyakarta: Book C pp. TP-148 - TP-155.
- Suprijono, M.M., and Widyastuti, T.E.W. (2010), "Optimasi Penggunaan Tepung Ganyong dalam Pembuatan Produk Biskuit Balita", Research Report. Surabaya: Widya Mandala Catholic University Surabaya
- Widowati, S., Hadiatmi, Soewarno T., Soekarto dan Damayanti, N. (2001), "Karakterisasi Sifat Fisikokimia Tepung Ganyong (*Canna Edulis, Kerr*) dan Kesesuaiannya untuk Produk Pangan" Himpunan Makalah Seminar Nasional Teknologi Pangan, PATPI, Semarang, pp. AO18-109 - AO18-120.

Modeling and Optimising Tensile Strength of Biodegradable Film as the Effect of Fungal Fermented Cassava (“Gathotan”) Flour

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Abstract

A biodegradable film was made from mixed of tapioca starch and “gathotan” (a traditional fungal fermented cassava) flour to increase efficiency of raw material. The effect of all ingredients on tensile strength of film was examined. Experiment design was central composite design for response surface methodology, using three factors namely proportion of gathotan flour (6, 7, 8, 9, 10 g/10 g mixed flour and starch), glycerol (0.5, 1.0, 1.5, 2.0, 2.5 mL), and citric acid concentration (0.3, 0.4, 0.5, 0.6, 0.7 mL). All ingredients were mixed thoroughly with distilled water to make 100 mL suspension, and then cooked in a hot plate at 90°C while stirred continuously until all part of the suspension was gelled. Gel was then spread in a petri dish, and dried in an oven at 70°C until dry. Tensile strength was measured using a miniature tensile grips TA 96-B on a texture analyser (TAXT Plus, Stable Micro System, UK). Results showed that proportion of gathotan, its linear and quadratic function, had significant effects. Quadratic function of glycerol concentrations also showed significant effects. Equation for tensile strength was expressed as: $Y = 1095.50 - 218.17 x_1 - 17.10 x_2 - 6.78 x_3 + 9.98 x_1^2 - 636.07 x_2^2 - 74.83 x_3^2 + 50.21 x_1 x_2 + 10.38 x_1 x_3 + 168.81 x_2 x_3$, where x_1 : gathotan proportion, x_2 : citric acid concentration, and x_3 : glycerol concentration. Optimization model showed that maximum tensile strength of film was 211.548 N/mm², which was made with 6 g gathotan, 0.320 mL citric acid, and 0.722 mL glycerol. Further Biodegradability, permeability and other analysis of film are yet to be done.

Keywords: gathotan, biodegradable film, cassava, tensile strength

Introduction

Indonesia’s need of plastic bag and container has been increasing steadily to reach 200 tonnes annually (Surono, 2013). It is highly preferred material for packaging, since it is strong, durable, heat resistant, and water proof. This leads to enormous waste and creates environmental problem due to inability to deal with plastic waste. Reduce in soil fertility and soil water quality istwo critical consequences. Therefore, there is a strong need to make degradable plastic. Recently biodegradable plastic has been studied. Raw material used is starch or protein. Several important qualities of plastic are non-adhesive, shiny, and transparent; and they become important parameters for successful biodegradable film, a part from degradability (EzeohaandEzenwanne, 2013).

In this work, we use cassava fermented by *Botryodiplodiatheobromae* (called ‘gathotan’) (Purwandari, 2014) as raw material for the making of biodegradable plastic film. We studied the biodegradability and tensile strength of the film as the effect of ingredients.

Materials and Methods

Cassava tubers fermented by *Botryodiplodiatheobromae* (“gathotan”) was dried in a cabinet dryer, and ground, then sieved using 100 mesh sift. The flour (6, 7, 8, 9, 10 g/10 g flour blends containing gathotan flour and tapioca) was then mixed with glycerol (0.5, 1.0, 1.5, 2.0, 2.5 mL), citric acid (0.3, 0.4, 0.5, 0.6, 0.7 mL), and tapioca. Distilled water was then added to make a 100 mL suspension. The

suspension was stirred continuously at 90°C in a water bath, until gelled. The mixture was then degassed using vacuum pump for 10 minutes. It was then spread in a petridish to make 3 cm layer, and then dried in a drying oven at 70°C until dry. The experimental design followed a central composite design with three factors, on response surface methodology (Table 1 and 2).

Table 1. Coding of factors

Factors	Code				
	-1.68179	-1	0	1	1.68179
	Tak Code				
X1	6	7	8	9	10
X2	0.3	0.4	0.5	0.6	0.7
X3	0.5	1	1.5	2	2.5

Table 2. Central composite design for the experiment

Run	X1	X2	X3
1	9(-1)	0.4(-1)	1(-1)
2	7(1)	0.4(-1)	1(-1)
3	9(-1)	0.6(1)	1(-1)
4	7(1)	0.6(1)	1(-1)
5	9(-1)	0.4(-1)	2(1)
6	7(1)	0.4(-1)	2(1)
7	9(-1)	0.6(1)	2(1)
8	7(1)	0.6(1)	2(1)
9	10(-1.68179)	0.5(0)	1.5(0)
10	6(1.68179)	0.5(0)	1.5(0)
11	8(0)	0.3(-1.68179)	1.5(0)
12	8(0)	0.7(1.68179)	1.5(0)
13	8(0)	0.5(0)	0.5(-1.68179)
14	8(0)	0.5(0)	2.5(1.68179)
15	8(0)	0.5(0)	1.5(0)
16	8(0)	0.5(0)	1.5(0)
17	8(0)	0.5(0)	1.5(0)
18	8(0)	0.5(0)	1.5(0)
19	8(0)	0.5(0)	1.5(0)
20	8(0)	0.5(0)	1.5(0)

X1 : gathotan flour (in gram)
 X2 : citric acid (mL)
 X3 : glycerol (mL)

Tensile strength was examined using tensile grips TA 96-B in a texture analyser (TA-XT Stable Micro System). A 40 mm x 5 mm x 5 mm sample, 0.03 mm thick was mounted, and every measurement were repeated five times. Data were processed using Minitab 15 statistical package.

Results and Discussion

Tensile strength of the film samples are presented in Table 3.

Table 3. Texture analyzer readings of biodegradable film made from gathotan flour

Run sampel	X1 (gram)	X2 (mL)	X3 (mL)	peak positive force (kg force)	N	tensile strength (N/mm ²)
1	9	0.4	1	2.556	25.0658	167.1053
2	7	0.4	1	2.28	22.35916	111.7958
3	9	0.6	1	2.009	19.70156	131.3437
4	7	0.6	1	2.664	26.12492	174.1661
5	9	0.4	2	2.799	27.44881	137.2441
6	7	0.4	2	2.53	24.81082	165.4055
7	9	0.6	2	3.849	37.7458	251.6386
8	7	0.6	2	2.323	22.78085	151.8723
9	10	0.5	1.5	4.192	41.10948	274.0632
10	6	0.5	1.5	2.724	26.71331	178.0888
11	8	0.3	1.5	1.87	18.33844	122.2562
12	8	0.7	1.5	1.705	16.72034	111.4689
13	8	0.5	0.5	1.91	18.7307	124.8713
14	8	0.5	2.5	2.051	20.11344	134.0896
15	8	0.5	1.5	2.091	20.50571	117.1755
16	8	0.5	1.5	2.942	28.85116	192.3411
17	8	0.5	1.5	3.363	32.97976	219.8651
18	8	0.5	1.5	2.78	27.26249	181.7499
19	8	0.5	1.5	2.602	25.5169	170.1127
20	8	0.5	1.5	2.239	21.95709	146.3806
21	9	0.4	1	0	0	0
22	7	0.4	1	0	0	0
23	9	0.6	1	0	0	0
24	7	0.6	1	0	0	0
25	9	0.4	2	0	0	0
26	7	0.4	2	0.898	8.806372	58.70914
27	9	0.6	2	1.08	10.59118	70.60788
28	7	0.6	2	0.791	7.75706	51.71373
29	10	0.5	1.5	0.939	9.208444	61.38963
30	6	0.5	1.5	0.838	8.217973	54.78648
31	8	0.3	1.5	0.606	5.94283	39.61887
32	8	0.7	1.5	0.714	7.001948	46.67965
33	8	0.5	0.5	0.559	5.481917	36.54612
34	8	0.5	2.5	0.279	2.736055	15.6346
35	8	0.5	1.5	0.308	3.020448	20.13632
36	8	0.5	1.5	0.812	7.963	53.08667
37	8	0.5	1.5	0.819	8.031646	45.89512
38	8	0.5	1.5	0.773	7.58054	43.31737
39	8	0.5	1.5	0.759	7.443247	42.53284
40	8	0.5	1.5	1.055	10.34602	68.97344
41	9	0.4	1	1.572	15.41605	102.7737
42	7	0.4	1	1.531	15.01398	75.06991
43	9	0.6	1	1.091	10.69906	85.59244
44	7	0.6	1	2.231	21.87864	109.3932
45	9	0.4	2	1.876	18.39728	91.98638
46	7	0.4	2	1.4224	13.94898	92.99319
47	9	0.6	2	1.756	17.22048	114.8032
48	7	0.6	2	3.325	32.60711	217.3807

49	10	0.5	1.5	2.267	22.23168	148.2112
50	6	0.5	1.5	1.671	16.38691	109.2461
51	8	0.3	1.5	0.614	6.021283	34.40733
52	8	0.7	1.5	0.884	8.669079	49.53759
53	8	0.5	0.5	0.752	7.374601	42.14058
54	8	0.5	2.5	0.879	8.620045	49.2574
55	8	0.5	1.5	0.738	7.237308	41.35604
56	8	0.5	1.5	1.217	11.93469	59.67347
57	8	0.5	1.5	1.407	13.79796	68.98978
58	8	0.5	1.5	0.9	8.825985	44.12993
59	8	0.5	1.5	0.692	6.786202	45.24135
60	8	0.5	1.5	1.207	11.83663	59.18313
61	9	0.4	1	0	0	0
62	7	0.4	1	0	0	0
63	9	0.6	1	0	0	0
64	7	0.6	1	0	0	0
65	9	0.4	2	0	0	0
66	7	0.4	2	0	0	0
67	9	0.6	2	0	0	0
68	7	0.6	2	0	0	0
69	10	0.5	1.5	0	0	0
70	6	0.5	1.5	0	0	0
71	8	0.3	1.5	1.095	10.73828	71.58855
72	8	0.7	1.5	1.48	14.51384	96.75895
73	8	0.5	0.5	2.357	23.11427	154.0952
74	8	0.5	2.5	2.837	27.82147	111.2859
75	8	0.5	1.5	2.842	27.8705	185.8033
76	8	0.5	1.5	1.598	15.67103	41.7894
77	8	0.5	1.5	1.603	15.72006	39.30015
78	8	0.5	1.5	1.93	18.92683	63.08945
79	8	0.5	1.5	1.425	13.97448	46.58159
80	8	0.5	1.5	1.354	13.2782	37.93773
81	9	0.4	1	1.213	11.89547	67.97409
82	7	0.4	1	1.309	12.8369	73.35374
83	9	0.6	1	1.126	11.04229	73.61525
84	7	0.6	1	1.698	16.65169	111.0113
85	9	0.4	2	1.324	12.984	74.19431
86	7	0.4	2	1.161	11.38552	75.90347
87	9	0.6	2	1.696	16.63208	83.16039
88	7	0.6	2	2.018	19.78982	131.9321
89	10	0.5	1.5	2.114	20.73126	138.2084
90	6	0.5	1.5	1.398	13.7097	91.39798
91	8	0.3	1.5	1.683	16.50459	110.0306
92	8	0.7	1.5	1.382	13.55279	90.35194
93	8	0.5	0.5	1.15	11.27765	75.18432
94	8	0.5	2.5	1.91	18.7307	124.8713
95	8	0.5	1.5	1.549	15.1905	101.27
96	8	0.5	1.5	0.895	8.776952	58.51301
97	8	0.5	1.5	0.915	8.973085	59.82057
98	8	0.5	1.5	0.927	9.090765	60.6051
99	8	0.5	1.5	1.017	9.973363	66.48909
100	8	0.5	1.5	0.864	8.472946	56.4863

Optimization of tensile strength

Tensile strength of the biodegradable film as the effect of ingredient is expressed as the equation below. Y: tensile strength, x_1 : proportion of gathotan flour, x_2 : concentration of citric acid, x_3 : concentration of glycerol. Tensile strength was affected significantly by proportion of gathotan, quadratic function of gathotan proportion, and quadratic function of glycerol.

$$Y = 1095.50 - 218.17 x_1 - 17.10 x_2 - 6.78 x_3 + 9.98 x_1^2 - 636.07 x_2^2 - 74.83 x_3^2 + 50.21 x_1x_2 + 10.38 x_1x_3 + 168.81 x_2x_3$$

Maximum tensile strength was 211.548 N/mm², made from 6 g of gathotan, 0.320 mL citric acid, 0.722 mL glycerol (Figure 1a and b).

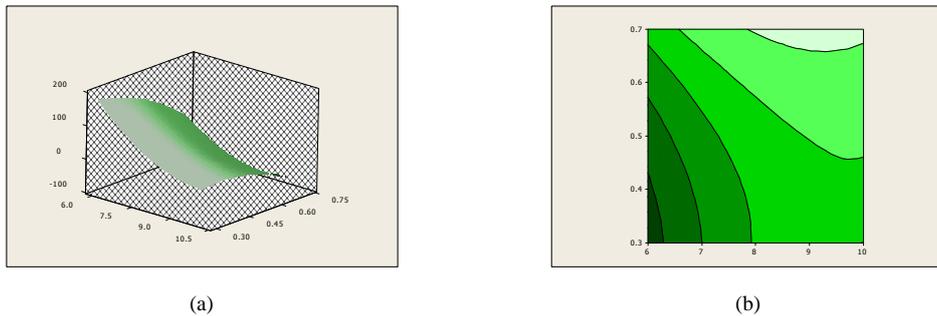


Figure 1. Tensile strength of biodegradable film as the effect of gathotan and tapioca concentration (a) surface plot (b) contour plot.

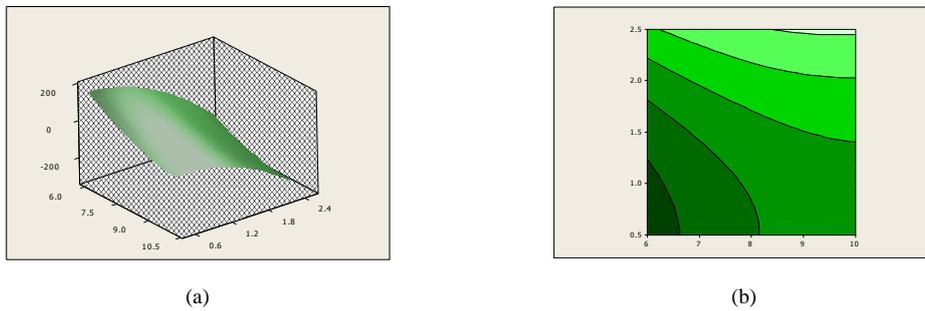


Figure 2. Tensile strength of film as the effect of glycerol and gathotan flour concentration (a) surface plot, (b) contour plot

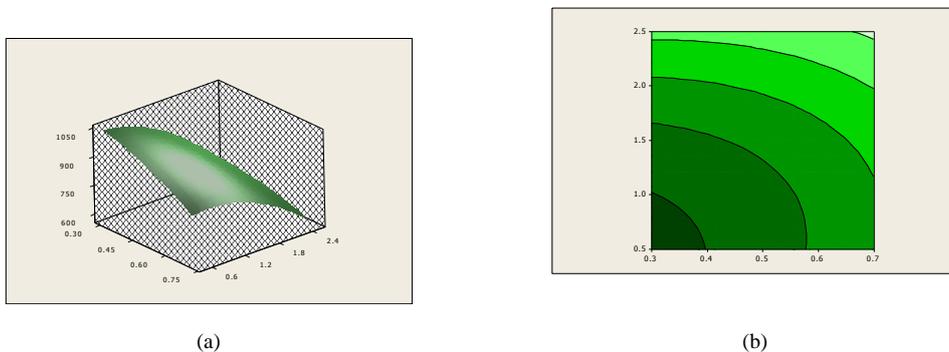


Figure 3. Tensile strength of biodegradable film as the effect of glycerol and citric acid concentration (a) surface plot (b) contour plot.

Conclusion

Gathotan flour proportion, its linear and quadratic function, significantly affected tensile strength of biodegradable film. Maximum tensile strength was 211.548 N/mm².

References

- Anonim. Unit Conversion Tables. [online] https://www.isa.org/uploadedFiles/Content/Training_and_Certifications/ISA_Certification/CCST-Conversions-document.pdf. Accessed 15 September 2016.
- M.P. Cereda, A.C. Bertolini, A.P. Silva, M.A. Oliveira, and R.M. Evangelista. 1995. Películas de almidón para la preservación de frutas. In: Congreso Polimeros Biodegradables: Avances y Perspectivas. Buenos Aire. 42. dalam Ezeoha.S. L and J. N. Ezenwanne. 2013. Production of Biodegradable Plastic Packaging Film from Cassava Starch. IOSR Journal of Engineering (IOSRJEN). Vol. 3. e-ISSN: 2250-3021, p-ISSN: 2278-8719.
- Purwandari, U., Tristiana, G.R. dan D, Hidayati. 2014. Gluten-free noodle made from gathotan flour: antioxidant activity and effect of consumption on blood glucose level. International Food Research Journal. Vol. 21. No. 4:1629-1634.
- Surono, U. B., 2013, Berbagai Metode Konversi Sampah Plastik Menjadi Bahan Bakar Minyak. JURNAL TEKNIK VOL.3 NO.1 ISSN 2088 –3676.

Sensory Properties and Antioxidant Activity of Steamed Rice with Various of Black and White Rice Ratio

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Abstract

Black rice (BR) has higher content of fiber than white rice (WR). Furthermore, black rice contain anthocyanin which has numerous beneficial for health, including anti hyperglycemic effect and protection from cardiovascular disease. Unfortunately, it has firmer texture and distinguished flavor, which makes it not as preferable as WR. Adding white rice increased the acceptance of steamed black rice. The percentages of white rice which added in this study were 0% (S100); 25% (S75); 50% (S50); 75% (S25); and 100% (S0). Scoring preference test was used for sensory analysis, followed by proximate analysis for fiber and water content, total starch using Nelson-Somogyi method, total anthocyanin using pH differential method, total phenolic using Folin-Ciocalteu method, and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). S50 had the highest sensory preference while S25 was the lowest due to its pale color. S50 had higher fiber, anthocyanin, and phenolic content and higher antioxidant activity than S0, while it had higher total starch, water content, and tenderness than S100. Cooking process had reduced antioxidant activity on steamed BR, while the adding of WR had reduced it more. Based on the results, S50 was the best possible mix because it had the highest preference index and still had anthocyanin and phenolic content and antioxidant activity.

Keywords: Black rice; sensory preference; anthocyanin; phenolic; antioxidant.

Introduction

Rice (*Oryza sativa*) is consumed as staple for nearly half of the world's population, especially in Asia. Even before the 1960s, Indonesia has become the largest rice consuming country per capita, followed by China and India (Mohanty, 2013). While Asia is provenas rice's center of species origin, Indonesia is believed to become the secondary of species origin for it has a great diversity of rice genetics (Sitaresmi *et al.*, 2013).

Two main subspecies of rice are *japonica* and *indica*, while Chang (1984) separated the third subspecies i.e. *javanica* or *tropical japonica*. Morphologically, it is closer to *indica*, thus some rice workers tend to classify it as its variant. But genetically, it is closer to *japonica* (Chakraborty, 2001; Chang, 1984; Haryanti *et al.*, 2013). While Garris *et al.* (2005) further divided *indica* as *indica* and *aus*, and *japonica* as *aromatic*, *temperate japonica*, and *tropical japonica*; by genetic evidence they suggested that *temperate japonica* was derived from *tropical japonica*.

Historically, the evidence of rice cultivation in Bali and Java was only recorded from the ninth century AD which stated that rice had become a major subsistence crop at the time (Christie, 2007). Some rice cultivars which are widely known today, such as *Mentik Wangi*, was already recorded on Javanese literature from the 19th century, i.e. *Serat Centhini* (Ranggasutrasna *et al.*, 2008). White rice is the cultivar

which is commonly consumed, while brown rice is usually consumed for health reasons. Black rice is unknown by most Javanese population up to the last decade, but still is often mistaken as black glutinous (waxy or sticky) rice.

Black rice cultivar is mainly cultivated in Asia especially China, followed by Sri Lanka, Indonesia, India, etc. Some internationally well-known cultivars of black rice are Forbidden rice, Chinese black rice, and Indonesian black rice (Ujjawal, 2016) such as *Cempo Ireng* from Yogyakarta, *Melik*, *Cibeusi*, *Toraja*, and *Jlitheng* (Kristamtini, 2009; Pramitasari, 2012).

The dark purple color of black rice is derived from anthocyanin pigments (Indradewa, 2012). It contains high level of nutrients such as protein, minerals, and dietary fiber which are higher than brown and white rice (Ujjawal, 2016). Since ancient time, black rice has been believed as healthy food (Guo *et al.*, 2007) and now is considered as functional food for its anthocyanin component (Indrasari *et al.*, 2008). The anthocyanin of black rice is proven to have antihyperglycemic effect, increase insulin sensitivity, alleviate pancreatic and hepatic inflammation (Krisbianto *et al.*, 2016; Pramitasari, 2014), and has higher antioxidant activity than α -tocopherol (Swasti, 2007).

Unlike white rice, black rice is not considered as a common staple for Indonesian population (Indradewa, 2012). Taste, aroma, texture, and health aspects, among others, are taken into consideration in selecting varieties of rice for consumption (Indrasari *et al.*, 2008). Black rice has a harder texture than white rice (Pramitasari, 2012). Although it has stronger fragrant aroma than white rice (Kristamtini, 2009; Ujjawal, 2016), its aroma is resembles but milder than black glutinous rice and different from those of white rice. It may lead Indonesian people, especially in Java and Bali, to think that black rice is better to eat as snack just like black glutinous rice because its aroma is not suitable to go with most of Indonesian side dishes. The modification of steamed black rice as staple food is necessary to deal with its less desirable organoleptic properties.

This study was stressed on the organoleptic and nutritive changing on steamed black rice which was mixed with different ratio of white rice. It was expected that the mixing of black rice with white rice would increase panelists' preference. On the other hand, it would reduce its nutritive value, as well as the capacity of black rice as functional food. All the rice varieties were from widely known local cultivars, i.e. *Cempo Ireng* for black rice and *Mentik Wangi* for white rice.

Materials and Methods

Materials

Polished white rice cultivar *Mentik Wangi* was purchased from farmland at Salam, Magelang, while black rice cultivar *Cempo Ireng* was from Lumpang Community, Yogyakarta.

Ethanol 96%, citric acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-(+)-ascorbic acid (vitamin C) was purchased from J.T. Baker (Selangor, Malaysia). Other chemicals were from Merck Millipore and kindly provided by Food Chemical and Biochemical Laboratory, Faculty of Agriculture Technology, Gadjah Mada University (UGM).

Sample preparation

Black rice and white rice were mixed with the concentration of black rice: 0% (S0), 25% (S25), 50% (S50), 75% (S75), and 100% (S100) w/w. Mixed rices were cooked in rice cooker (National SR-WO6N) with the addition of water at a ratio of 1:3 w/v for 30 minutes. Mixed steamed rice samples were used for sensory evaluation, crude fiber, water content, and total starch analyses.

For other analyses, mixed steamed rice samples were dried in cabinet dryer with a temperature of 50°C for 16 hours. Dried mixed rices, black rice grain (R100), and white rice grain (R0) were grounded by using dry grinder (BL-301 GS G/Y) to be less than 60 meshes. A total of 25 grams of each powder were macerated in 250 ml ethanol-citric 3% for 1 hour. It was then filtered with filter paper to separate its extract from the cake. The extracts were used as samples for total anthocyanin, total phenolic content, and antioxidant activity using DPPH analyses.

Sensory evaluation

A focus group consist of eight semi-trained panelists were asked to perform organoleptic test with a hedonic scale of 1 to 5. Each member did a closed assessment and followed by an open discussion (Meilgaard *et al.*, 2000; Wong, 2008). Descriptive test was used for hardness assessment of the samples, while scoring preference test was for overall preference of the samples, i.e.: color, texture, and flavor.

Water content, crude fiber, and total starch

Water content of mixed steamed rice samples was analyzed by thermogravimetric method which was described on AOAC Official Method 934.01. Ceramic fiber filter method for crude fiber analysis was based on AOAC Official Method 962.09 by using 1.25% H₂SO₄ and 1.25% NaOH solution to digest the dried samples.

Nelson-Somogyi method was used to determine the total starch of mixed steamed rice samples (Nelson, 1944). Before the analysis, the starch component of the samples was hydrolyzed with HCl 30% and then neutralized with NaOH 40% (Poedjiadi, 1994).

Total anthocyanin, total phenolic content, and DPPH analyses

Total anthocyanin was determined by pH-differential method (Giusti and Wrolstad, 2001). Each 0.9 ml potassium chloride 0.025 M (pH 1) and sodium acetate 0.4 M (pH 4.5) buffers were mixed with 0.1 ml sample extract respectively. After 15 min incubation, absorbance was measured at 530 nm and 700 nm using spectrophotometer (Spectronic 200). Molecular weight (M_w = 449.2 g/mol) and molar absorptivity (ϵ = 26900 L/mol.cm) were regarded as cyanidin-3-glycoside.

The method for total phenolic content was based on Shui and Leong (2006) with gallic acid (0-1.0 mg/ml) as standard and the absorbance was measured at 765 nm. The data was served as mg Gallic Acid Equivalent (GAE)/g. Antioxidant activity by DPPH method was based on Krisbianto *et al.* (2016) with vitamin C (0-40 mg/L) was used as standard and the absorbance was measured at 515 nm. The data was served as mg Ascorbic acid Equivalent Antioxidant Capacity (AEAC)/g.

Statistical analysis

Completely Randomized Design was used for chemical analysis and organoleptic testing. The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) with SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) at the 95% confidence level.

Result and discussion

Steamed rice specification

Water content, crude fiber, and total starch of mixed steamed rices are shown in Table 1. S0 (100% of white rice) had the highest water content and total starch while S100 (100% black rice) was the lowest. Higher white rice ratio lead to higher water content and total starch of mixed steamed rices. The water content and total starch of S25 (75% white rice) was not significantly different with S0, while S50 and S75 were not significantly different to S100. This result was similar to Ayabe *et al.* (2014) who compared the physicochemical properties of nonsticky (*japonica*) Okunomurasaki black rice and polished Koshihikari white rice upon cooking. On the other hand, although the fiber contents were reduced along with the higher ratio of white rice, the results were not significantly different between the samples.

Table 1. Steamed rice specification

	Water content (%wb), mean (SD)	Crude fiber (%db), mean (SD)	Total starch (%db), mean (SD)
S0	63.68 (1.84) ^a	0.15 (0.11) ^a	43.16 (0.44) ^a
S25	61.72 (0.47) ^a	0.17 (0.19) ^a	35.80 (0.21) ^{ab}
S50	52.45 (0.13) ^b	0.19 (0.00) ^a	32.29 (6.69) ^b
S75	52.34 (0.46) ^b	0.21 (0.25) ^a	28.71 (1.17) ^b
S100	52.08 (1.03) ^b	0.22 (0.14) ^a	28.40 (5.15) ^b

Description: different superscript on the same column show a significant different ($p > 0.05$). SD = standard deviation; wb = wet basis; db = dry basis.

Mizuma (2014) proposed that polishing process would increase the starch content of polished rice by remove the outer layers which were high in protein and fat compared to the endosperm. While Haryadi (2006) stated that fat and protein inhibit water absorption of rice during cooking, Mizuma (2014) explained that starch absorbed more water than protein but fat inhibit water absorption.

Functional properties

Anthocyanin has a strong antioxidant activity, thus makes black rice as a functional food. Table 2 shows anthocyanin (TA) and phenolic contents (TPC) of mixed steamed black rices, black rice grain (R100), and white rice grain (R0), along with the antioxidant activity. The results show that both cooking process and the addition of white rice had significantly reduced TA, TPC, and antioxidant activity of the samples. The cooking process of black rice had reduced its TA around 68.55%, similar to Hiemori *et al.* (2009) that found the cooking process of black reduced its TA around 65-80%. The addition of white rice by 25% (S75), 50% (S50), and 75% (25%) lowered the TA by 7.69%, 20.50%, and 74.34% respectively. On the other hand, Kurilich *et al.* (2005) stated that cooking process might increase the bioavailability of anthocyanin because the destruction of cell walls had made anthocyanin more accessible by our digestive system.

Some researchers found that anthocyanin had a very low bioavailability and most of the anthocyanin that we consume would be excreted via urine and feces (Aguilar, dkk, 2013; Kurilich, dkk, 2005). However, it still showed a high systemic activity and gave an oxidative protection to the mucosa of our digestive system (Stintzing dan Carle, 2004).

Table 2. Functional properties of rice

	Total anthocyanin (mg/g), mean (SD)	Total phenolic content (mgGAE/g), mean (SD)	DPPH (mgAEAC/g), mean (SD)
R0	-	0.19 (0.11) ^{ab}	0.44 (0.17) ^{ab}
R100	69.02 (0.96) ^d	3.58 (0.23) ^c	2.41 (0.04) ^d
S0	-	0.01 (0.06) ^a	0.34 (0.11) ^a
S25	5.57 (0.96) ^a	0.16 (0.02) ^{ab}	0.53 (0.14) ^{ab}
S50	17.26 (0.96) ^b	0.38 (0.00) ^b	0.58 (0.10) ^{ab}
S75	20.04 (0.00) ^c	0.67 (0.02) ^c	0.63 (0.01) ^b
S100	21.71 (1.67) ^c	1.22 (0.13) ^d	0.97 (0.11) ^c

Description: different superscript on the same column show a significant different (p >0.05). SD = standard deviation; GAE = gallic acid equivalent; AEAC = ascorbic acid equivalent antioxidant capacity.

Based on the data of this research, for adults about 60 kg who eat 100 gram of mixed rices three times a day, we can estimate the anthocyanin intake for S100, S75, S50, and S25 are 109 ppm, 100 ppm, 86 ppm, and 28 ppm respectively. These calculations are higher than Krisbianto *et al.* (2016) who used 40 ppm and 80 ppm anthocyanin on rats. The results showed that 40 ppm and 80 ppm of anthocyanin had antihyperglycemic effect, increased insulin sensitivity, and alleviate pancreatic and hepatic inflammation on hyperglycemic rats. But a further research on human needs to be conducted to prove the functional properties of mixed steamed rices.

The antioxidant activity was also found on S0 and R0. These results might be due to the phenolic component of white rice cultivar *Mentik Wangi*. Pearson correlation test (data not shown) showed that TA and TPC had very strong and positive correlation to DPPH scavenging activity of mixed black rice extracts.

Sensory evaluation

Singh *et al.* (2013) found a significant linear correlation between the hardness of date fruits with water content and crude fiber. Figure 1 shows the hardness of mixed steamed rices on the scale of 1 to 5. Compared to Table 1, the hardness level of mixed steamed rices is positive correlated to the water content, while crude fiber content might not have much distribution.

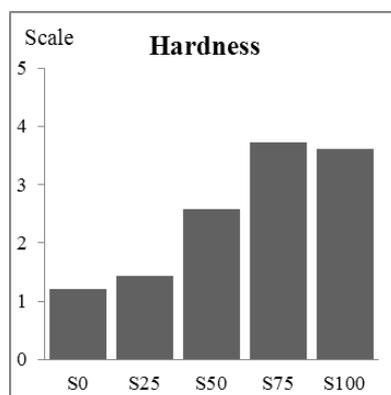


Figure 1. Hardness of mixed steamed rices on the scale of 1 (very tender) to 5 (very hard).

The hardness of steamed rice is highly affected with its amylose-amylopectin contents. High amylose rices (25-33%) are dry and hard upon cooling, low amylose rices (2-9%) are moist and sticky, and waxy rices contain about 1-2% of amylose (Mutters and Thompson, 2009). Kristamtini *et al.* (2011) found that *Mentik Wangi* contained about 15-16% amylose and 35-42% amylopectin so that this cultivar belong to the low amylose rices (10-20%). Amylose has a high rate of retrogradation thus the steamed rices with high content of amylose will have a hard texture. On the other hand, amylopectin is better to holding water content which result in a tender texture of steamed rice (Pramitasari, 2012).

Juliano and Perez (1983) found that the hardness of steamed rice was positive correlated with its amylose content. By the rice cooker method, the same water: rice ratio for cooking led to a higher degree of hardness to high amylose rices and lower degree of hardness to low amylose rices.

Figure 2 shows the preference index of mixed steamed rices, comprised the texture, color, and flavor, on the scale of 1 to 5. The mean (SD) preference index of S0 was 3.38 (1.31) and was not significantly different to S75 and S100. Although the three of it were in neutral-like category, after the discussion it appeared only S0 was considered as neutral by the panelists because it had a tender texture and a delicate, pandan-like aroma. White rice cultivar *Mentik Wangi* is a local cultivar from Yogyakarta and for centuries has been known for its fragrance aroma (Kristamtini *et al.*, 2011; Ranggasutrasna *et al.*, 2008). The fragrant aroma of aromatic rice like *Mentik Wangi* is derived from active component 2-acetyl-1-pyrroline (Indrasari *et al.*, 2008).

On the other hand, black rice cultivar *Cempo Ireng* was considered to have a soft, caramel-like aroma. After a further discussion, it appeared that S75 and S100 were considered to have similar properties, i.e. the hardest texture just like half-cooked white rice, unappealing darkest color (Figure 3), and detected bitterness. Bett-Garber *et al.* (2012) identified the taste of black rice as oily, meaty, medicinal, sweet aromatic, smoky, astringency, and bitterness. The bitterness and astringency were caused by the high phenolic content of pigmented rice grains. Table 2 shows that black rice had a higher concentration of phenolic components than white rice.

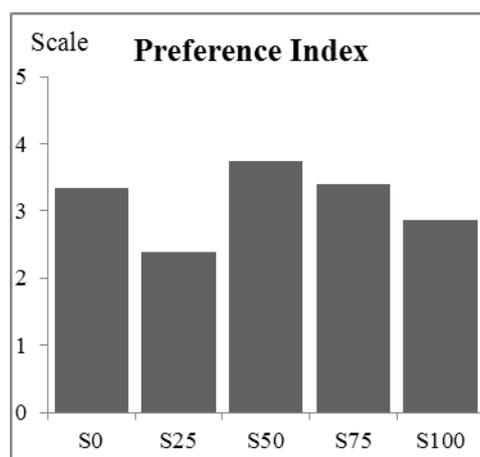


Figure 2. Preference index of mixed steamed rices on the scale of 1 (very dislike) to 5 (very like).



Figure 3. Colors of mixed steamed rices.

S25 had the lowest preference index, i.e. 2.38 (0.74), mostly because of its uniformity and faded purple color with dark spots (Fig. 3). The concentration of black rice that was only 25% unable to dye the rest of white steamed rice and the texture was also harder than the white part, led to an unpleasant gritty texture of mixed steamed rice.

The highest preference index, i.e. 3.75 (1.04), was S50. It had a uniform dark purple color and texture, while the aroma of black rice was not very dominant.

Conclusion

The most preferred ratio of black rice: white rice was 1:1 (S50). The addition of 50% white rice cultivar *Mentik Wangi* enabled to alleviate the less desirable organoleptic properties of black rice cultivar *Cempo Ireng* comprised the texture, color, and flavor. On this ratio, the mixed steamed rice still had antioxidant activity.

References

- Aguilar, F., Crebelli, R., Dusemund, B., Galtier, P., Gott, D., Gundert-Remy, U., König, J., Lambré, C., Leblanc, J-C., Mortensen, A., Mosesso, P., Oskarsson, A., Parent-Massin, D., Rose, M., Stankovic, I., Tobback, P., Waalkens-Berendsen, I., Woutersen, R.A., and Wright, M. (2013), "Scientific opinion on the re-evaluation of anthocyanins (E 163) as a food additive", *European Food Safety Authority Journal*, Vol. 11, No. 4, pp. 3145.
- Ayabe, S., Wada, N., Oishi, K., and Kasai, M. (2014), "Changes in the physicochemical properties of black rice upon cooking", *日本調理科学会誌*, Vol. 47, No. 2, pp. 76-83.
- Bett-Garber, K.L., Lea, J.M., Champagne, E.T., and McClung, A.M. (2012), "Whole-grain rice flavor associated with assorted bran colors", *Journal of Sensory Study*, Vol. 27, pp. 78-86.
- Chakraborty, S. (2001), *Rice Breeding and Genetics*, Concept Publishing Company, New Delhi, pp. 38-47.
- Chang, T. (1984), "The ethnobotany of rice in island Southeast Asia", *Asian Perspective*, Vol. XXVI, No. 1, pp. 69-76.
- Christie, J.W. (2007), "Water and rice in early Java and Bali", in Boomgard, P. (Ed.), *A World Water: Rain, Rivers and Seas in Southern Asian Histories*, NUS Press, Singapore, pp. 235-237.
- Garris, A.J., Tai, T.H., Coburn, J., Kresovich, S., and McCouch, S. (2005), "Genetic structure and diversity in *Oryza sativa* L.", *Genetics*, Vol. 169, No. 3, pp. 1631-1638.
- Guo, H., Ling, W., Wang, Q., Liu, C., Hu, Y., Xia, M., Feng, X., and Xia, X. (2007), "Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. Indica) on hyperlipidaemia and insulin resistance in fructose-fed rats", *Plant Foods for Human Nutrition*, Vol.62, No.1, pp. 1-6.
- Haryadi (2006), *Teknologi Pengolahan Beras*, UGM Press, Yogyakarta.
- Haryanti, W.D.U., Kusumaningrum, H.P., and Budiharjo, A. (2013), "Deteksi jenis padi *Indica* dan *Japonica* padi gogo rancak beras merah varietas Slegreng dan Mandel berbasis fragmen ORF100 dan ORF29", *Jurnal Sains dan Matematika*, Vol. 21, No. 4, pp. 98-102.
- Hiemori, M., Koh, E., and Mitchell, A.E. (2009), "Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. *Japonica* var. SBR)", *Journal of Agricultural and Food Chemistry*, Vol. 57, No. 5, pp. 1908-1914.
- Inradewa, D. (2012), "Kesiapan varietas padi dalam mendukung ketahanan pangan di Indonesia", in Sunarminto, B.H. (Ed.),

- Pertanian Terpadu untuk Mendukung Kedaulatan Pangan Nasional*, BPFE, Yogyakarta, pp. 131-157.
- Indrasari, S.D., Purwani, E.Y., Widowati, S., and Damaradjati, D.S. (2008), "Peningkatan nilai tambah beras melalui mutu fisik, cita rasa, dan gizi", in Daradjat et al (Ed.), *Padi: Inovasi Teknologi Produksi*, Puslitbangtan, Bogor, pp. 565-590.
- Juliano, B.O. and Perez, C.M. (1983), "Major factors affecting cooked milled rice hardness and cooking time", *Journal of Texture Studies*, Vol. 14, pp. 235-243.
- Krisbianto, O., Astuti, M., and Marsono, Y. (2016), "Antihyperglycemic effect and antioxidants properties of black rice (*Oryza sativa* L. *Indica*) cereal and anthocyanin extract on health and histopathology of hyperglycemic rats", *Pakistan Journal of Nutrition*, Vol. 15, No. 7, pp. 702-707.
- Kristantini (2009), "Mengenal beras hitam dari Bantul", *Tabloid Sinar Tani*, 13 May.
- Kristantini, Widyayanti, S., and Rahayu, S. (2011), "Respon padi local Mentik Wangi terhadap pendekatan teknologi SRI (*System of Rice Intensification*) dan PTT (Pengelolaan Tanaman Terpadu)", *Widyariset*, Vol. 14, No. 3, pp. 565-570.
- Kurilich, A.C., Clevidence, B.A., Britz, S.J., Simon, P.W., dan Novotny, J.A. (2005), "Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots", *Journal of Agricultural and Food Chemistry*, Vol. 53, No. 15, pp. 6537-6542.
- Meilgaard, M., Civille, G.V., and Carr, B.T. (2000), *Sensory Evaluation Techniques*, Third Edition, CRC Press, Boca Raton.
- Mizuma, T. (2014), "Effects of the rice polishing ration on water absorption of rice", *関西福祉科学大学紀要*, No. 18, pp. 33-39.
- Mohanty, S. (2013), "Trends in Global Rice Consumption", *Rice Today*, Vol. 12, No. 1, pp. 44-45.
- Mutters, R.G. and Thompson, J.F. (2009), *Rice Quality Handbook*, UCANR Publications, California, pp. 12-13.
- Nelson, N. (1944), "A photometric adaptation of the Somogyi method for the determination of glucose", *Journal of Biological Chemistry*, Vol. 153, pp. 375-380.
- Poedjiadi, A. (1994), *Dasar-Dasar Biokimia*, UI Press, Jakarta.
- Pramitasari, R. (2012), "Evaluasi sensoris, nilai gizi, dan sifat fisik minuman berbasis beras (*Oryza sativa* L.) untuk orang lanjut usia", Essay, Agriculture Technology Faculty, Gadjah Mada University, Yogyakarta.
- Pramitasari, R. (2014), "Potensi antioksidan minuman fungsional berbasis beras hitam (*Oryza sativa* L. *Indica*) dan kedelai hitam (*Glycine max* L. Merr) untuk lansia penyandang diabetes mellitus tipe 2", Thesis, Agriculture Technology Faculty, Gadjah Mada University, Yogyakarta.
- Ranggasutrasna, Yasadipura II, and Sastradipura (2008), *Centhini Tambangraras-Amongraga*, Vol. 3, pp. 314.
- Singh, V., Guizani, N., Al-Alawi, A., Claereboudt, M., and Rahman, M.S. (2013), "Instrumental texture profile analysis (TPA) of date fruits as a function of its physico-chemical properties", *Industrial Crops and Products*, Vol. 50, pp. 866-873.
- Sitairesmi, T., Wening, R.H., Rakhmi, A.T., Yunani, N., and Susanto, U. (2013), "Pemanfaatan plasma nutfah padi varietas local dalam perakitan varietas unggul", *IPTEK Tanaman Pangan*, Vol. 8, No. 1, pp. 22-30.
- Stintzing, F.C. and Carle, R. (2004), "Functional properties of anthocyanins and betalains in plants, food, and in human nutrition", *Trends in Food Science and Technology*, Vol. 15, No. 1, pp. 19-38.
- Swasti, Y.R. (2007), "Aktivitas antioksidan antosianin beras hitam dalam Low Density Lipoprotein (LDL) plasma darah manusia secara in vitro", Thesis, Agriculture Technology Faculty, Gadjah Mada University, Yogyakarta.
- Ujjawal, K. (2016), *Black Rice: Research, History and Development*, Springer International Publishing, Switzerland.
- Wong, L.P. (2008), "Focus group discussion: a tool for health and medical research", *Singapore Medical Journal*, Vol. 49, No. 3, pp. 256-261.

Mold Contamination and Aflatoxin B₁ Levels in Salted Fish Commodities

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Abstract

Salted fish is subjected to a process of salting and drying as a method of inhibiting fish spoilage. Nevertheless, salted fish can be contaminated by mold and aflatoxin B₁ which is produced by mold. The aim of this study was to determine which types of mold could contaminate salted fish, which was obtained from the markets of Pasar Kenjeran, Surabaya and Pasar Beringharjo Yogyakarta, and also to assess the levels of aflatoxin B₁ contamination in the salted fish. The samples were cultivated directly in DRBC and DG-18 media and then mold enumeration carried out based on the colonies formed. For identification purposes, the mold which grew on the surface of the sample was isolated on MEA media using the three point method, then the isolates obtained were identified using macromorphology and micromorphology. The measurement of aflatoxin B₁ levels was done using an ELISA (Enzymatic Linked Immuno Sorbent Assay) test. Research results showed that the molds identified as contaminants on the salted fish were *Aspergillus tamaris*, *Aspergillus flavus* both of which are aflatoxin producing molds, *Aspergillus sydowii*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium citrinum*, and *Penicillium chrysogenum*. *Rhizopus sp.* contamination was also found. As for the level of aflatoxin B₁, all samples were found to be positively contaminated with aflatoxin B₁, the highest contamination of a sample occurring in Lidah salted fish (75.81 ppb) and the lowest in Rese salted fish (4.38 ppb).

Keywords: salted fish, mold, Aflatoxin B1

Introduction

Indonesia is an archipelago country consisting of 13,667 islands, with most of its territory covered by water. With Indonesia's vast extent of coastline, fish as a commodity is easily obtained. Based on data from buku Kelautan dan Perikanan dalam Angka (Book of Marine and Fisheries Statistic) in 2014, figures show that production of caught fish in 2013 reached 5,707,013 tons, while availability for consumption amounted to 11,822 tons in that year. Numbers for fish consumption in 2013 amounted to 35.21 kg / capita / year. The protein content in fish is potentially high, ranging from 15 to 20%. Not only is fish a considerable source of animal protein, it is also rich in calcium and phosphorus.

However, fish is perishable and needs to be preserved. One way of doing this is by a process of salting. Salted fish is high in protein at about 42 grams per 100 grams (Ahmad, 2014). During the salting process, salt penetration into the body of the fish occurs due to varying salt concentrations (Sutarni, 2011). Salted fish has a lower water content, which can increase shelf life. In Indonesia, the technique of salting fish is often used because the process is easy and cheap.

The low water content will inhibit the growth of bacteria spoilage. However, salted fish can be contaminated by mold. The mold can be active and grow at temperatures of 20-30°C, with a relatively low a_w at 0.85 (Rahayu, 2014). Mold contamination of food negatively affects the quality of the product, thereby decreasing the product's value.

Cases of mold contamination have been reported in dried salted fish, dried fish and smoked fish. Smoked fish from tropical areas has the potential for contamination by toxigenic molds, such as *Aspergillus flavus* (Adebayo-Tayo et al., 2008). *Aspergillus flavus* is also the dominant species that contaminates smoked fish from the Sierra Leone area, followed by contamination from *Aspergillus ochraceus*, *Aspergillus niger* and *Aspergillus tamarii* (Jonsyn, 1992). *Aspergillus flavus* was also isolated from dried fish from Sri Lanka and Indonesia (Atapattu, 1990). *Aspergillus tamarii* was also found to be the predominant contaminant in Maldives fish (Mohamed, 2013). *Aspergillus sydowii* is a species isolated from dried fish sold in traditional markets in Jakarta (Santoso et al, 1999).

Some mold can produce mycotoxins, such as aflatoxin. Aflatoxin is a mycotoxin produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and is hepatotoxic, mutagenic, teratogenic, and carcinogenic to both humans and animals (Alberts et al, 2006). An outbreak of aflatoxin was first noticed in the early 1960s in the UK (turkey X disease) (Do, 2007). Epidemiological studies believe that aflatoxin can cause liver cancer in humans (Samson and Hoekstra, 2010). Distribution of cases of aflatoxin is commonly found in developing countries (Samuel, 2009). Aflatoxins are very stable at high temperatures, therefore they are not easily destroyed by a treatment process which uses high temperatures. Of the several types of aflatoxin, aflatoxin B1 is the most dangerous.

Detection and identification of mold contamination on salted fish in Indonesia is required as food safety information. The aim of this study is to determine the type of mold that can contaminate salted fish, including aflatoxin, and determine the amount of contamination by alfatoksin B1 in salted fish.

Materials and Methods

Sample preparation

A total of 20 kinds of salted fish used in this study were purchased in February 2015. The types of salted fish and the source can be seen in Table 1.

Table 1. Fish Species Source

Type of fish	Species	Source
Bulu bebek	<i>Thryssa mystax</i>	
Campur	-	
Glomo	<i>Johnius coitor</i>	
Janjan	<i>Anguilla rostrata</i>	Pasar
Keteng	<i>Mystus planiceps</i>	Kenjeran, Surabaya
Kindo	Unknown	
Lidah	<i>Cynoglossus lingua</i>	
Talang	<i>Chorinemus tala</i>	
Banyar peda	<i>Rastrelliger kanagurta</i>	
Belek	Unknown	
Ebi	<i>Litopenaeus vannamei</i>	
Kacangan	<i>Charangidae sp</i>	
Layur	<i>Trichurus savala</i>	Pasar
Pedho alit	<i>Rastrelliger scombridae</i>	Beringharjo, Yogyakarta
Pethek	<i>Leiognathus equulus</i>	
Rese	<i>Acetes indicus</i>	
Sero	Unknown	
Teri	<i>Stolephorus tri</i>	
Teri Nasi	<i>Stolephorus commersonii</i>	

Each sample was stored in polyethylene plastic using a salted fish code type. Samples were stored in a cool room (4°C) in the microbiology PSPG laboratory (Center for the Study of Food and Nutrition). Before performing the tests, the salted fish was allowed to thaw at ambient temperature (±28°C, for 1 hour).

Mold Enumeration

All of the different kinds of salted fish were inoculated on DRBC media (Oxoid) and DG-18 (Oxoid) directly (Rahayu *et al.*, 2014), and large-sized samples were cut with sterile scissors. They were then incubated at room temperature for 5 days. Colonies formed were differentiated by appearance and color. Enumeration shows percentage of samples contaminated. With the following formula:

$$\frac{\text{number of samples contaminated by certain mold}}{\text{total sample cultivated}} \times 100\%$$

Determination of Aflatoxin B1 Contamination

A total of 25 grams of previously crushed samples was extracted using 70% methanol by shaking for around 3 minutes. The extract was then separated from the pulp using Whatmann paper No.1. AflatoxinB1 content was tested in accordance with the manual on RIDASCREEN Aflatoxin B1 ELISA kit (Bio Scientific).

Results and Discussion

Mold Enumeration and Identification

From observation of the incubated samples, it became clear that all of the samples on DRBC and DG 18 media were contaminated by mold. Colonies formed were not very diverse on both media. Colonies formed were coloured as follows: green colony, brown colony, yellow colony, bluish grey colony, bluish green colony, black colony and white colony. Suspected mold contamination from the colonies formed on the salted fish was from the *Aspergillus*, *Penicillium* and *Rhizopus* genera, Yeast was also found to be present, but was not identified.

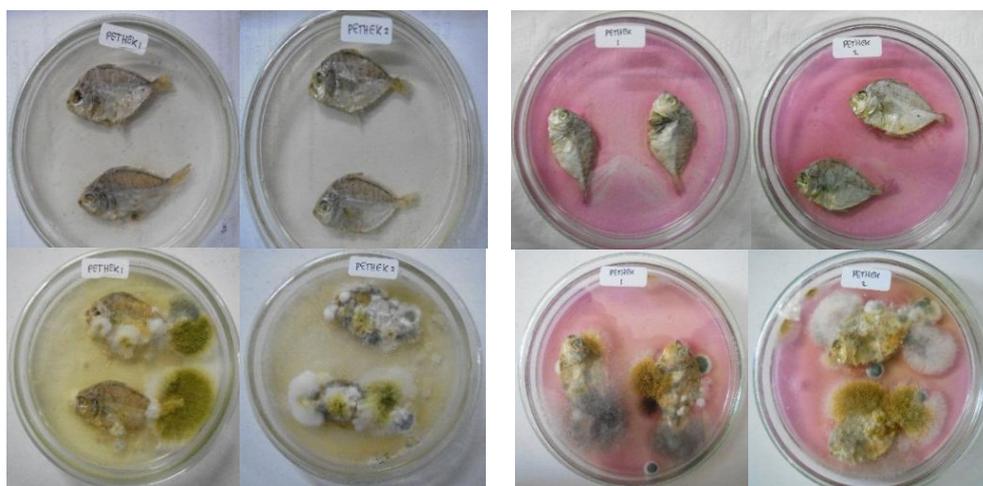


Figure 1. Mold Diversity contaminating on salted fish

Seven isolates in this study were identified based on micromorphology and macromorphology characteristics. Mold identification is very important, especially when testing for the presence of an aflatoxin producer, which is very dangerous to human health. From all of the samples, seven species of mold were identified, these being: *Aspergillus tamarii*, *Aspergillus flavus*, *Aspergillus sydowii*, *Aspergillus niger*, *Penicillium citrinum*, *Penicillium chrysogenum* and *Rhizopus sp.* Samples taken from Kenjeran market, Surabaya were dominated by green colonies. On the other hand, samples from Beringharjo market, Yogyakarta were dominated by black and white colonies. This was due to differences in the supply chain and the handling. Samples from Kenjeran market, Surabaya were being sold in coastal and open roadside stalls, in addition to which, the production sites and the dried fish stalls were close to each other. While the samples from Beringharjo market, Yogyakarta consisted of salted fish supplied from Pati, Central Java and the stalls were all in one room, which meant that a large proportion of the mold found in the salted fish samples was indoor fungi. The growths of *Aspergillus flavus* and *Aspergillus tamarii* on the identification medium bore similar characteristics. The frequency of mold contamination on salted fish is shown in Figure 2. Samples from Kenjeran market, Surabaya showed that all samples were found to have mold contamination. The dominant mold was 100% *Aspergillus tamarii* (9/9 samples) and 89% *Aspergillus flavus* (8/9 samples). Lidah salted fish had the highest frequency of *Aspergillus flavus* contamination which was about 92%. Spores of *Aspergillus flavus* were found in the tropical air. In Indonesia itself, which is a tropical country, the drying of salted fish still takes advantage of the use of sunshine, thus increasing the likelihood of contamination by fungal spores. The samples from Beringharjo market, Yogyakarta were also contaminated by mold. The dominant molds were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus tamarii*, and *Rhizopus sp* with the frequency of each contamination being approximately 45% (5/11 samples). The highest *Aspergillus flavus* contamination was found on Pethek salted fish with 50%. By the presence of *Aspergillus flavus* on salted fish, it is possible to find aflatoxin contamination. Contamination on examined salted fish was dominated by *Aspergillus*, followed by *Penicillium*, it's supported by Hassan *et al.* (2011) who showed that the most dominant contamination of salted fish which is randomly collected from different markets at Giza Governorate was by *Aspergillus spp* members, at about 83.3% with *Aspergillus flavus* contamination being at 66.6%. According to Pitt and Hocking (2009), *Aspergillus* and *Eurotium* were the most dominant contamination species found on dried food in tropical and subtropical regions. *Aspergillus* and *Eurotium* were also dominant on smoked dried fish from warm water regions (Adebayo-Tayo *et al.*, 2008). This was because *Aspergillus* usually grows faster than *Penicillium* but takes longer for sporulation and is generally able to produce spores that are resistant to light, dryness and chemicals thus having a longer life (Rahayu, 2014).

Another important genus which was identified is *Penicillium*, although it had a lower frequency than the *Aspergillus* genus. *Penicillium citrinum* and *Penicillium chrysogenum* were also identified on some of the samples of salted fish. The highest *Penicillium citrinum* contamination was found on Ebi salted fish, at about 50% and the highest *Penicillium chrysogenum* contamination was found on Lidah salted fish, at about 33%. *Penicillium citrinum* produces citrinin toxin which is nephrotic and carcinogenic for animals (Flajs *et al.*, 2009).

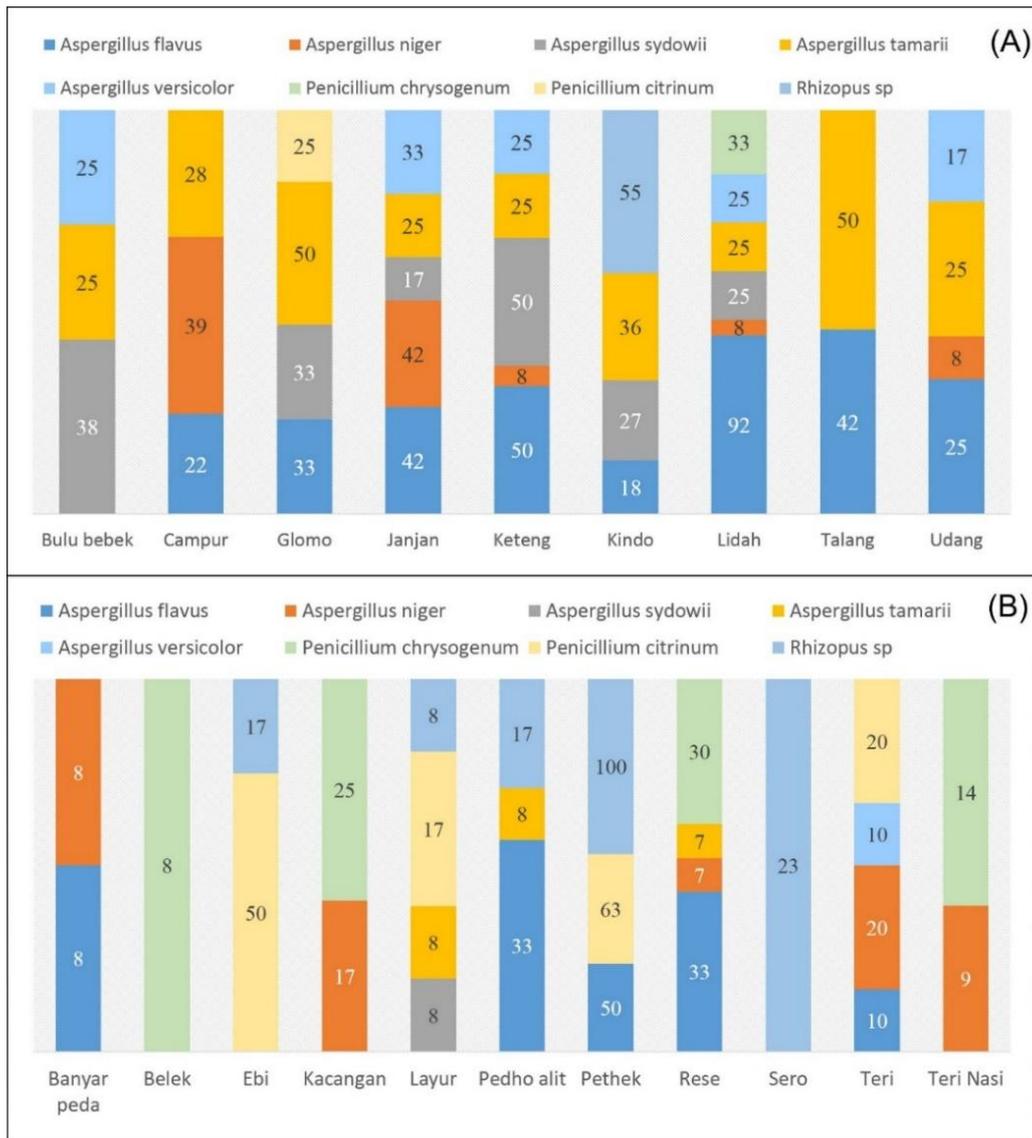


Figure 2. Frequences of Mold Contamination on Salted Fish
 (A).Frequency of Mold on Salted Fish from Kenjeran Market, Surabaya
 (B).Frequency of Mold on Salted Fish from Beringharjo Market, Yogyakarta

Seven of the isolates identified had halotolerant and xerophilic properties. *Aspergillus sydowii* is a mold mostly found on marine organisms (Samson, 2010). According to Prakash *et al.* (2011) *Aspergillus flavus* and *Aspergillus niger*, which originated from dried seafood products in India showed salt resistance of up to 18%. In Indonesia, which is a tropical country, the drying of salted fish is done conventionally using sunlight, thus increasing the possibility of mold spore contamination. *Aspergillus flavus* was also found to be a dominant contaminant on smoked dried fish (Adebayo-Tayo *et al.*, 2008). In the case of Maldives fish, which is a salted fish from the Maldives region, *Aspergillus tamarii* contamination was higher than *Aspergillus flavus* contamination, because Maldives fish has a low salinity at an average 2.08% (Mohamed, 2013). It is similar to the total mold contaminant in samples from Kenjeran market, Surabaya.

Table 2. Mold Identified on Salted Fish

Salted Fish	Origin	Identified mold
Bulu bebek		<i>Aspergillus sydowii, Aspergillus tamarıi, Aspergillus versicolor</i>
Campur		<i>Aspergillus flavus, Aspergillus tamarıi, Aspergillus niger</i>
Glomo		<i>Aspergillus flavus, Aspergillus, tamarıi, Aspergillus sydowii, Penicillium citrinum</i>
Janjan		<i>Aspergillus flavus, Aspergillus, tamarıi, Aspergillus niger, Aspergillus sydowii, Aspergillus versicolor</i>
Keteng	Kenjeran market, Surabaya	<i>Aspergillus sydowii, Aspergillus flavus, Aspergillus tamarıi, Aspergillus versicolor, Aspergillus niger</i>
Kindo		<i>Aspergillus flavus, Aspergillus tamarıi, Aspergillus sydowii, Rhizopus sp</i>
Lidah		<i>Aspergillus flavus, Aspergillus, tamarıi, Aspergillus niger, Aspergillus sydowii, Aspergillus versicolor, Penicillium chrysogenum</i>
Talang		<i>Aspergillus flavus, Aspergillus tamarıi</i>
Udang		<i>Aspergillus flavus, Aspergillus niger, Aspergillus tamarıi, Aspergillus versicolor</i>
Banyar peda		<i>Aspergillus niger, Aspergillus flavus</i>
Belek		<i>Penicillium chrysogenum</i>
Ebi		<i>Penicillium citrinum, Rhizopus sp</i>
Kacangan		<i>Aspergillus niger, Penicillium chrysogenum</i>
Layur	Beringharjo market, Yogyakarta	<i>Aspergillus tamarıi, Penicillium citrinum, Aspergillus sydowii, Rhizopus sp</i>
Pedho alit		<i>Aspergillus flavus, Aspergillus tamarıi, Rhizopus sp</i>
Pethek		<i>Aspergillus flavus, Aspergillus tamarıi, Penicillium citrinum Rhizopus sp</i>
Rese		<i>Aspergillus flavus, Aspergillus tamarıi, Aspergillus niger, Penicillium chrysogenum</i>
Sero		<i>Rhizopus sp</i>
Teri		<i>Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Penicillium citrinum</i>
Teri Nasi		<i>Aspergillus niger, Penicillium chrysogenum</i>

Aflatoxin B₁ contamination in Salted Fish

The presence of *Aspergillus flavus* indicates the possibility of contamination by aflatoxin B₁ in salted fish. Of the samples analyzed, all were positively contaminated by aflatoxin B₁. The highest aflatoxin B₁ contamination was on the Lidah salted fish taken from Kenjeran market, Surabaya which had about 75.81 ppb. And the lowest aflatoxin B₁ contamination was on Rese salted fish taken from Beringharjo market, Yogyakarta at about 4.38 ppb. The Aflatoxin B₁ levels of the salted fish are shown in Figure 3.

If the results using the ELISA method are correlated with the frequency of *Aspergillus flavus* contamination on the samples (Figure 4), it can be fully understood that the entire amount of aflatoxin B₁ in the samples was not governed by the amount of *Aspergillus flavus* contamination. If correlated with the mold species that had contaminated each of the samples with aflatoxin, not all samples were contaminated with *Aspergillus flavus* which is an aflatoxin B₁ producer. Furthermore, the results of aflatoxin contamination using the ELISA method showed that each of the samples was contaminated in different amounts.

Aflatoxin contamination has no correlation to the frequency of the appearance of *Aspergillus flavus* on samples because *Aspergillus flavus* does not grow well. The growth of *Aspergillus flavus* is hampered by other more dominant molds, so there were some samples that were not contaminated with *Aspergillus flavus*. According to Goto *et al.* (1996) *Aspergillus tamarıi* also can produce aflatoxin B₁ and B₂, probably the presence of aflatoxin produced by *Aspergillus tamarıi* instead *Aspergillus flavus*. Moreover, the ELISA method has low specificity to detect other components which have a similar

structure to aflatoxin B₁, such as AFB₂, AFG₁ and AFG₂ (Leszczynska *et al.*, 2001). The salt content in the samples also provides a matrix effect that can affect absorbance readings, but it is also possible to reach a cross-linking component of the sample thus affecting the selectivity and specificity of ELISA absorbance readings that have an impact on other components, such as aflatoxin B₁ (Rachmawati *et al.*, 2004).

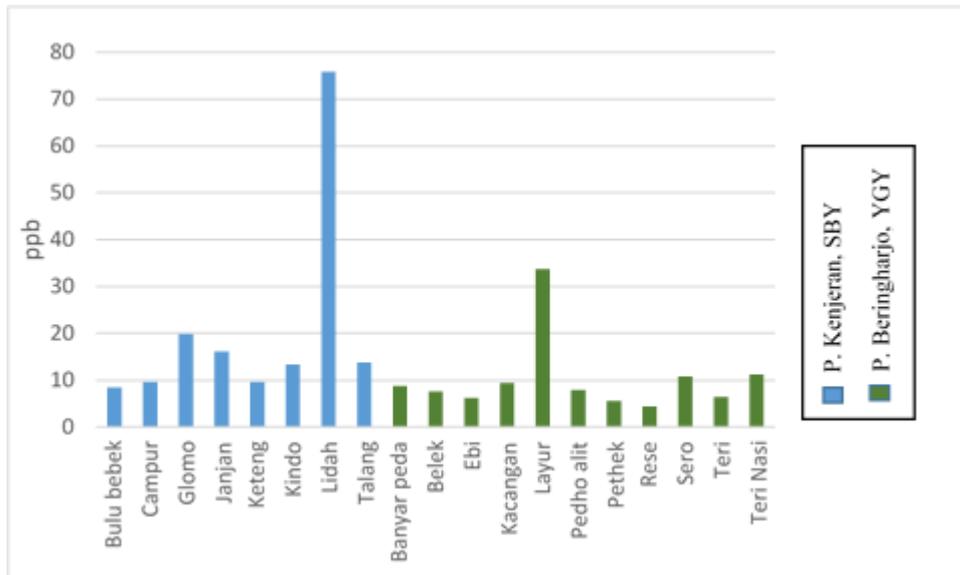


Figure 3. Aflatoxin B1 Level in Salted Fish

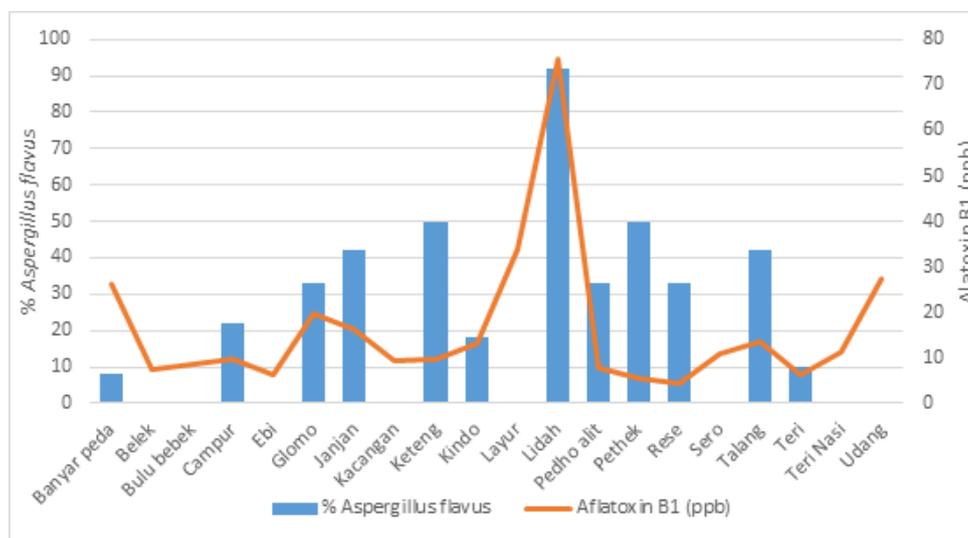


Figure 4. Corellation Frequency of Aspergillus flavus and Aflatoxin B1 level

Conclusions

Mold identification is very important. Salted fish commodities have been identified as having mold contamination, one of which was an aflatoxin B₁ producer. Aflatoxin B₁ detection using ELISA showed that all samples had been positively contaminated with Aflatoxin B₁. This was caused by poor handling of the salted fish commodities.

References

- Achmad. 2014. Seputar Kandungan Gizi Pada Ikan Asin Kering Dan Manfaat Kandungan Didalamnya. <http://bkpd.jabarprov.go.id/>. Diakses pada tanggal 2 Januari 2015 pukul 15.27.
- Adebayo- Tayo, B. C., Onilude, A. A., and Patrick, U. G. 2008. Mycoflora of Smoked Dried Fishes Sold in Uyo Eastern Nigeria. *World Journal of Agricultural Science*, 4, 346-350
- Atapattu, R., and Samarajeewa, U. 1990. Fungi Associated with Dried Fish in Sri Lanka. *Mycopathologia*, 111, 55-59
- Alberts, J. F., Engelbrecht, Y., Steyn, P. S., Holzappel, W. H., and Van, Zyl. W. H. 2006. Biological Degradation of Aflatoxin B1 by *Rhodococcus erythropolis* Cultures. *International Journal of Food Microbiology*. 189 : 121-126
- Do, J. H., Choi, Dong-Kug. 2007. Aflatoxin: Detection, Toxicity, and Biosynthesis. Department of Biotechnology, Konkuk University. Korea. 12 : 585-593
- Flajs, D., and Peraica, M. 2009. Toxicological Properties of Citrinin. *Archives of Industrial Hygiene and Toxicology*, 60: 457-464
- Goto, Tetsuhisa., Wicklow, Donald T., and Ito, Yoko. 1996. Aflatoxin and Cyclopiazonic Acid Production by a *Sclerotium*-Producing *Aspergillus tamarii* Strain. *Applied and Environmental Microbiology*. 4036-4038
- Hassan, Atef. A., Hassan, Manal. A., Howayda., El-Shefei, M., Rasha, M. H. S. El Ahl., and El-Dayem, R. H. Abd. 2011. Detection of Aflatoxigenic Mould Isolated from Fish and Their Products and Its Public Health Significance. Dept of Mycology & Mycotoxin and Food Hygiene Animal Health Research Institute. Egypt. 9 :106-114
- Jonsyn, F. E., and Lahai, G. P. 1992. Mycotoxigenic flora and Mycotoxins in Smoke Dried Fish from Sierra-Leone. *Nahrung (Food)*, 36, 485-489
- Leszczynska, Joana., Maslowska Joana., Owczarek Alina., and Kucharska Urszula. 2011 Determination of Aflatoxin in Food Products by the ELISA Method. *Bioinorganic and Analytical Chemistry Group. Institute of General Food Chemistry, Lodz Poland*. 19. (1) : 8-12
- Mohamed, S. 2013. Isolation and Identification of Mycoflora and Toxigenic Fungi from Maldive Fish. Thesis. Toxigenic Fungi and Mycotoxin Productin in Maldive Fish (Smoked Dried Tuna Fish). Chapter 3, 42-66
- Pitt, J. I., and Hocking, A. D. 2009. *Fungi and Food Spoilage* 3rd edition. Springer Science + Business Media : New York
- Prakash, S., Jeyasanta, I., Carol, R., and Patterson, J. 2004. Microbial Quality of Salted and Sundried Sea Foods of Tuticor in Dry Fish Market Southeast Coast of India. *International Journal of Microbiological Research*, 2, 188-195
- Rachmawati, Sri., Lee, A., Murdiati, T.B., Kennedy, I. 2004. Pengembangan Enzyme Linked Immunosorbent Assay (ELISA) Teknik untuk Analisis Aflatoksin B₁ pada Pakan Ternak. Balai Penelitian Veteriner. Bogor
- Rahayu, Endang. S., Sardjono., Samson, Robert. A. 2014. Jamur Benang (mold) pada Bahan Pangan. PT. Kanisius : Yogyakarta
- Santosa, I., Gandjar, I., Sari, R. D., and Sembiring, N. D. 1999. Xerophilic Moulds Isolated from Salted and Unsalted Dried Fish from Traditional Markets in Jakarta. Faculty of Mathematic and Science University of Indonesia
- Samson, R. A., and Hoekstra, E. S. 2010. *Introduction to Food Airborne Fungi* 7th edition . CBS utrecht : Netherland
- Samuel, Nir., Ezri Yosef., Farah Raymond., Igor Vackzman., Hussein Amer., Rubinshtein Orit., Assy Nimer. 2009. Acute Aflatoxicosis Resulting in Fulminant Hepatic Failure and Rhabdomyolysis. *Department of Internal Medicine. Israel*. (2). 48-50
- Sutarni. 2011. Faktor-Faktor yang Mempengaruhi Produksi Pengawetan Ikan Asin Teri di Kecamatan Labuhan Maringgai Kabupaten Lampung Timur. Program Studi Agribisnis Jurusan Ekonomi dan Bisnis Politeknik Negeri Lampung . Vol 7

Physico-chemical Characteristics and Sensorial Attributes of Steamed Orange-Fleshed Sweet Potato Genotypes

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ABSTRACT

The orange-fleshed sweet potato is rich in nutrients, particularly betacarotene as a pro-vitamin A. However, texture of the steamed root tends to be tender/soft and moist along with the darkness of the orange flesh. This would limit the uses of sweet potato for food with respect to their sensorial acceptances. In this study, eleven genotypes (MSU 06039-21, MSU 06044-03, MSU 05036-23, MSU 06039-07, MSU 06042-18, MSU 06043-42, MIS 0651-15, MIS 0651-09, MIS 0651-09, MSU 06071-82, and MSU 05036-17) of orange-fleshed sweet potato and one control variety (Beta2) were studied for physico-chemical characteristics and sensorial attributes in the form of steamed tubers. The results showed that the moisture content of the steamed tubers positively correlated with the moisture content of the fresh ones ($R^2 = 0.76$). The steamed tubers had higher reducing sugars compared to their counter parts. Amylose content of the fresh tubers had no correlation with the steamed tubers' hardness. The MIS0651-15 clone gave the steamed tubers with the highest hardness (the firmest texture) whereas Beta 2 was the most tender. The hardness of steamed tubers positively correlated with texture profiles judged by panelists through organoleptic test ($R^2 = 0.49$). MSU 05036-17 clone showed the highest scores of colour, aroma, and texture preferences, followed by MSU06071-82, MSU 06039-07, MSU05036-11, and MSU06042-18. This suggests that the steamed orange-fleshed sweet potato genotypes are tailored for cake and another food products ingredients with tender texture.

Keywords: Orange-fleshed sweet potato, physico-chemical properties, sensorial attributes.

Introduction

Most of sweetpotato production (89%) in Indonesia is used as an ingredient in food consumption to the level of 7.1 kg/capita/year (FAOSTAT, 2011). The natural compounds of betacarotene in orange and yellow fleshed sweet potato can function as an antioxidant and provitamin A that is the advantages which needs to be promoted to enhance sweet potato image which is considered inferior food. The provitamin A, betacarotene, can be converted into vitamin A in the intestinal mucosa in humans. It is the highest vitamin A activity (100%) when compared with the other carotenoids (Woolfe, 1992). Vitamin A deficiency can lead to disorders of the vision, as nyctalopia, xerophthalmia/keratomalacia until permanent blindness as well as disruption of the growth and immune to disease (Gopalan, 1992).

The betacarotene content of sweet potato positively correlates with the intensity of the yellow/orange colour (Purcell and Walter, 1968 in Woolfe, 1992; Simonne *et al.*, 1993; Ginting *et al.*, 2008). The results of the eight clones of orange fleshed sweet potato of a hybrid and local varieties show the range of betacarotene between 292 to 12.032 $\mu\text{g}/100\text{ g}$ with the yellow to orange shifting colours (Ginting *et al.*, 2008). The two sweet potato varieties, namely Beta 1 and Beta 2 had been released in 2009 with considerably high betacarotene content (12.031 μg and 4.629 $\mu\text{g}/100\text{ g}$, respectively) (ILETRI, 2012).

Problems encountered in the establishment of sweet potato varieties containing high betacarotene is the high moisture content and low dry matter content (< 30%) (Yamakawa 1998) as well as sweetness and soft texture in the mouth aftersteaming (Woolfe, 1992). These properties are less

favoured, either by the breeder as well as industry and consumer of sweet potato. Therefore, this research was conducted on the physico-chemical observation of fresh and steamed tubers toward the consumer acceptance.

Materials and Methods

The experiment was conducted in the Chemical and Food Processing Laboratory, ILETRI in October 2010. Material experiment is eleven sweet potato clones as a food rich in betacarotene based on the colour of the fleshed sweet potato (orange) with high yield potential (> 25 ton/ha) and one variety (Beta-2) as a comparison. Clones/varieties planted on the Dry Season II 2010 at Tumpang, Malang and harvested at the age of 4.5 months. The fresh samples were cut section of the base and the tip, peeled and then shredded for moisture content analysis with the gravimetry method according to SNI 01-2891-1992 (National Standardization Agency of Indonesia, 1992). Observations for Hunter colours (L^* , a^* , b^*) of the flesh root were done using a colour reader Minolta CR-200b. In addition, fresh tubers were also cut into small cubes, and then dried in an oven for 24 hours at 60 °C. The dry matters were milled for reducing sugar analysis with Somogyi Nelson methods (Sudarmadji *et al.*, 1997) and amylose content (Juliano, 1971). Analysis of the chemical properties of steamed sweet potato included moisture and sugar reduction contents, while analysis of physical properties such as hardness, viscosity and their behaviour when chewed (*chewiness*), used steamed tubers from each sweet potato clone using a texture analyzer for 5 replications. The acceptance levels of sensorial attributes (colour, texture, and taste) of the steamed tubers are analyzed using Hedonic test with 20 panelists, score ranged from 1 (strongly dislike) to 5 (really like). Data were obtained from three replications for each sweet potato clone.

Results and Discussions

Physical and chemical properties of fresh and steamed tubers

The colour of fresh sweet potato

Nine sweet potato clones have red skin colour and three other has slightly yellow skin colour (Table 1). Visually, 12 clones have various flesh colours from slightly orange (MSU 06039-07) to dark orange (MSU 05036-23). Measurement of colour/brightness (L^*) with colour reader also showed that MSU06039-07 clone with the slightly orange flesh had the highest brightness level (L^*) but the MSU 05036-23 clone with dark orange colour the lowest (Table 1).

Moisture content

Moisture content of fresh roots ranged from 70.22% (MIS 0651-09) to 82.62% (MSU 05036-23) as shown in Table 2. The difference in moisture content is mainly caused by the difference of the sweet potato clones because all of the clones are planted on the same season and location as well as technique. The ranges, obtained in the research is slightly wider range compared with observation on the eight yellow/orange sweet potato clone 72.41 to 80.97% studied before (Ginting *et al.*, 2008). The sweet potato has a high moisture content when the value is greater than 73.5% and is lower when the value is less than 65.5% (Antarlina, 1997). Among the 12 clones/varieties observed, the seven clones (MSU 06039-21, MSU 06044-03, MSU 05036-11, MSU 05036-23, MSU 06043-42, MIS 0651-15, and Beta 2) have higher moisture content compared with five other clones (MSU 06039-07, MSU 06042-18, MIS 0651-09, MIS 06071-12, and MSU 05036-17). According to Yamakawa (1998), orange fleshed sweet

potato has high water content and low levels of dry matter content (< 30%). The results showed that moisture content of the steamed roots positively correlated with the moisture content of the fresh roots ($R^2 = 0.76$) (Figure 1).

Table 1. Physical characteristics of 12 sweetpotato clones/varieties

Sweetpotato Clones	Peel colours	The Colour of Flesh			
		Visual	L *	a *	b *
MSU 06039-21	Red	Orange +	76.1 b	19.3ef	45.0 e
MSU 06044-03	Pink	Orange +++	71.4 cf	34.0 cd	54.7 c
MSU 05036-11	Red	Orange ++	73.1 cd	31.9 d	53.3 cd
MSU 05036-23	Red	Orange ++++	68.0 i	39.1 b	58.9 ab
MSU 06039-07	Red	Orange	79.3 à	21.2 e	51.5 d
MSU 06042-18	Red	Orange +++	70.2 gh	34.2 cd	57.8 ab
MSU 06043-42	Slightly Yellow	Orange ++	73.4 c	33.5 cd	53.7 c
MIS 0651-15	Slightly Yellow	Orange ++	72.1 de	34.4 cd	57.1 b
MIS 0651-09	Red	Orange +++	74.2 c	34.8 c	57.4 b
Beta 2	Red	Orange +	78.2 à	16.6 f	46.9 e
MSU 06071-82	Slightly Yellow	Orange ++	70.8 fg	37.5 b	58.5 ab
MSU 05036-17	Red	Orange +++	69.5 h	42.0 à	59.6 à
LSD 5%	-	-	1.2	2.8	2.0
CV (%)	-	-	1.0	5.2	2.1

Values followed by different letters are significantly different at $P < 0.05$

L* : lightness level that ranges from 0 (dark/black) to 100 (bright/white)

a* : green (-100) up to red (+100)

b* : blue (-100) up to yellow (+100)

Table 2. Moisture content of fresh and steamed orange fleshed sweetpotato

Clones/Varieties	Moisture content (%)		Reducing sugar (% dw)		Amylose (% dw)
	Fresh tubers	Steamed tubers	Fresh tubers	Steamed tubers	
MSU 06039-21	80.08 b	78.54 b	3.76 fg	11.91 e	23.56 bcde
MSU 06044-03	76.32 c	74.31 e	1.94 i	13.06 de	23.71 bcd
MSU 05036-11	77.13 d	75.17 d	8.11 a	13.07 de	25.47 a
MSU 05036-23	82.63 a	79.80 a	8.07 a	26.53 a	23.73 bcd
MSU 06039-07	73.07 gh	72.03 gh	3.90 f	12.35 e	21.36 f
MSU 06042-18	72.50 h	75.78 c	6.29 c	24.29 a	22.49cdef
MSU 06043-42	75.24 f	72.43 fg	6.35 c	15.43 c	21.90 ef
MIS 0651-15	75.30 f	71.60 h	7.00 b	15.88 c	23.72 bcd
MIS 0651-09	70.22 i	68.85 i	2.50 h	15.37 c	24.89 ab
Beta 2	79.43 c	78.62 b	4.63 e	11.50 e	23.92 abc
MIS 06071-82	73.21 g	72.60 f	5.32 d	21.64 b	22.08def
MSU 05036-17	72.83 gh	74.09 e	3.58 g	14.61 cd	23.31 bcde
LSD5%	0.58	0.49	3.76	2.24	1.69
CV (%)	0.45	0.39	3.63	8.13	4.27

LSD: Least Significant Difference

CV: Coefficient Variation

dw: dry weight

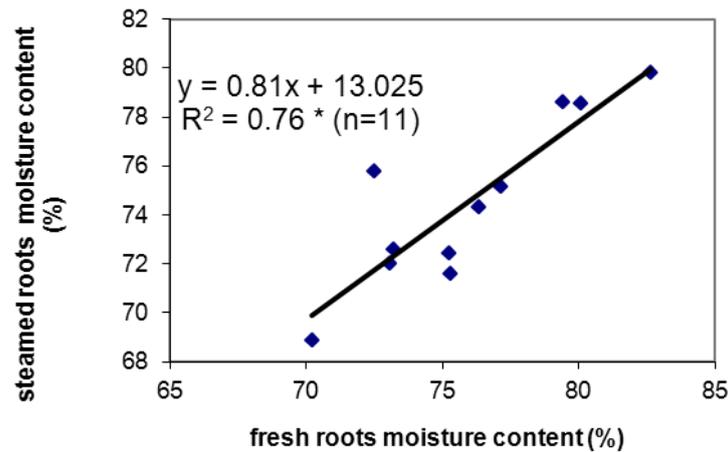


Figure 1. Correlation between moisture content of fresh and steamed sweetpotato of 12 genotypes.

Reducing sugar and amylose contents

The reducing sugar content ranged between 1.94 to 8.11% dw with the highest value was the MSU 05036-11 clone and MSU 05036-23 clone (Table 2). According to Woolfe (1992), orange fleshed sweet potato has sweet and tender/moist texture in the mouth. Sweet potato reducing sugar levels are categorized as high when the value is > 6.83% dw and is low is < 3.91% dw (Antarlina, 1997). Based on these criteria, there were three clones high in reducing sugar content, four clones moderate, and five clones low. The results of this study showed a trend of increasing reducing sugar levels along with the increase intensity of sweet potato flesh colour, such as in the MSU 05036-23 clone, even though some of them have a reducing sugar levels sufficiently low, as MSU 06044-03 and MIS 0651-09 clones (Table 2). The reducing sugar levels of sweet potato this research slightly wider than the research results obtained by Ginting *et al.* (2008) on the eight clones/varieties (MSU 01015-7, MSU 01035-5, MIS 559-3, MSU 01035-2, AC Merah, MSU 01015-6, MIS 943-1, and MSU 99062-3) of yellow/orange fleshed sweet potato which value 3.42 to 8.18% dw. Beta 1 variety (formerly MSU 0105-07 clone) which contains dark orange has reducing sugar amounting to 8.18% dw (Ginting *et al.*, 2008). Reducing sugar of steamed sweet potato increased when compared with that of fresh one, the highest value was for the MSU 05036-23 and MSU 06042-18 clone (Table 2). This increase is closely related with the activity of the enzyme α -amylase which is naturally found in the sweet potato and its activities at the optimum temperature of 70-75 °C (Walker *et al.*, 1976 in Losh *et al.*, 1981). These enzymes hydrolyze starch into maltose and dekstrin, depending on the enzyme activity on each sweet potato variety. This resulted in the number of reducing sugar increases when steaming is done at a temperature of $\pm 90^{\circ}\text{C}$ (S-101 Technical Committee 1980 in Woolfe, 1992).

Sweet potato genotype showed significant effects on the amylose content ($P < 0.05$) with the highest value (25,47% dw) seen in MSU 05036-11 clone (Table 2). Groups of sweet potato genotype having high amylose content when its value is greater than 25% dw and belongs to low group when it is less than 19% dw (Anonymous, 2008). The role of amylose is in absorbing water, especially in the process of gelatinization. In addition, it also determines texture of cooked sweet potato because the higher

levels of amylose, the texture of the flesh tend to be the harder/denser because it is able to absorb a lot of water and stay intact in the high temperature (Damardjati *et al.*, 1989).

Texture profile of steamed root

Hardness observed on the steamed sweet potato showed a moderate wide range with the highest value on MIS 0651-15 clone, while the lowest value obtained for the Beta 2 variety (Table 3). This can be related to moisture content of steamed sweet potato Beta 2 variety which was also moderate (Table 2).

Table 3. Texture profile of 12 genotypes orange fleshed sweetpotato steamed

Clones/Varieties	Hardness (N)	Adhesiveness (mm)	Cohesiveness	Springiness (%)	Chewiness (N.mm)
MSU 06039-21	2.14	0.02	0.48	84.67	86.31
MSU 06044-03	5.16	0.02	0.16	84.92	74.04
MSU 05036-11	3.94	0.13	0.18	82.36	59.11
MSU 05036-23	2.87	0.20	0.16	69.95	33.00
MSU 06039-07	7.01	0.30	0.14	72.01	70.97
MSU 06042-18	8.63	0.02	0.16	94.26	127.07
MSU 06043-42	8.48	0.03	0.13	74.14	79.22
MIS 0651-15	14.52	0.96	0.11	76.97	126.70
MIS 0651-09	8.04	0.73	0.14	94.21	106.06
Beta 2	1.67	0.35	0.21	46.02	16.24
MSU 06071-82	7.38	0.24	0.11	84.18	70.58
MSU 05036-17	5.34	0.15	0.13	84.26	59.90
Mean \pm standard deviation	3.58 \pm 6.27	0.26 \pm 0.30	0.18 \pm 0.10	79.00 \pm 12.95	75.77 \pm 33.33

Based on the average values (Table 3), the six clones/varieties belongs to the hard group and other six clones belong to tend. The level of crispness flesh (springiness) also varied with the range was not so wide with the highest value markedly was observed for the MSU 06042-18 and MIS 0651-09 clones and lowest for Beta 2 variety. The level of plasticity of the flesh when it was chewed (chewiness) as a result of the multiplication of violence with a cohesiveness and springiness also seem highest on the MSU 06042-18 and MIS 0651-15 clones. The texture profile varied between clones/varieties related to levels of amylose/ amylopectin, moisture, and reducing sugar of tubers (Utomo, 2009).

The sensorial attributes of steamed root

Two genotypes (MSU 14007-13 and MSU14009-39) with high beta-carotene or the dark orange of flesh sweet potato colour (Table 1) showed low scores of colour acceptance. Betacarotene is susceptible to heat treatment, thus steaming may cause degradation due to oxidation and/or trans-cis-isomerization (Wu *et al.*, 2008). Finally, this study obtained that the taste of five clones (MSU 05036-11, MSU 05036-17, MSU 06039-07, MSU 0651-09 and MSU06071-82) were scored moderately liked by the panelists (Table 4).

Steamed sweet potato's texture was firm: less preferred compared with that which was elastic texture (Table 4). This is evident from clones MSU 05036-17 and MSU 06071-82 which were moderately preferred because it had slightly elastic texture. The MSU 05036-17 clone had the highest score for the level of preference for colour, flavour, and texture, followed by the clone of MSU 06071-82, MSU 06039-07, 05036-11 MSU, and MSU 06042-18 (Table 4). The hardness of steamed sweet potato positively correlated with texture profiles judged by panelists through organoleptic test ($R^2= 0.49$) (Figure 2).

Table 4. Sensorial attributes of 12 genotypes of sweet potato steamed

Clones/Varieties	Acceptance score			Texture
	Colour	Taste	Texture	
MSU 06039-21	2.3	2.5	2.4	1.9
MSU 06044-03	3.5	3.2	3.0	2.9
MSU 05036-11	3.5	3.9	2.9	2.9
MSU 05036-23	3.0	2.3	2.0	2.0
MSU 06039-07	3.5	3.5	3.4	3.3
MSU 06042-18	3.5	3.2	3.3	3.5
MSU 06043-42	3.6	2.5	3.4	3.8
MIS 0651-15	2.8	2.8	3.2	3.2
MIS 0651-09	2.6	3.8	3.1	3.3
Beta 2	2.2	2.5	2.3	2.4
MSU 06071-82	3.6	3.6	3.5	3.4
MSU 05036-17	4.0	4.2	3.7	3.3

Acceptance score: 1 = disliked very much, 2 = moderately disliked, 3 = slightly liked, 4 = moderately liked, 5 = liked very much
 Texture: 1 = very firm, 2 = firm, 3 = slightly elastic, 4 = elastic, 5 = very elastic

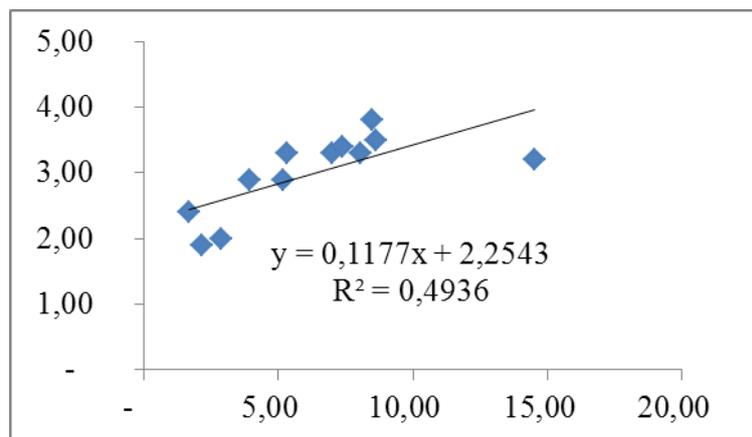


Figure 2. Correlation between texture measured using a texture analyzer and organoleptic test

Conclusions

1. This study confirms the darker of colour of sweet potato flesh, the higher of moisture content and the tender texture of sweet potato flesh.
2. The MSU 05036-17 clone had the highest score for the level of preference for colour, flavour and texture. These genotypes suitable for food products prepared from sweet potato steamed.

References

Antarlina, S. S., 1997. Karakteristik ubijalar sebagai bahan tepung dalam pembuatan kue *cake*. In Budijanto, S., F. Zakaria, R. Dewanti-Hariyadi, B. Satiawiharja (Eds). Prosiding Seminar Nasional Teknologi Pangan Denpasar 16-17 Juli 1997. PATPI-Menpangan RI. p. 188-204.

ILETRI. 2012. Deskripsi varietas unggul kacang-kacangan dan umbi-umbian. Balai Penelitian Tanaman Kacang-kacangan dan Umbi-umbian. Malang. 185 pp.

Damardjati, D. S., S.D. Indrasari, Santosa B.A.S. 1989. Pengembangan Teknologi Pasca Panen Sekunder Tanaman Pangan di Pedesaan. BALITTAN Sukamandi.

FAOSTAT, 2011. Statistical database of food balance sheet. www.fao.org. (accessed on 6th September, 2016).

Ginting, E., Jusuf M, Rahayuningsih, St. A. 2008. Sifat fisik, kimia dan sensoris delapan klon ubijalar kuning/orange kaya beta karoten. In Saleh, N, Rahmianna, A. A, Pardono, Samanhudi, Anam, C, and Yulianto (Ed). Prosiding Seminar Nasional Pengembangan Kacang-kacangan dan Umbi-umbian: Prospek Pengembangan Agro Industri Berbasis Kacang-kacangan dan Umbi-umbian. p. 392-405.

Gopalan, C., 1992. Nutrition in development transition in South-East Asia. WHO Regional Office for South-East Asia. New Delhi.

Juliano, B.O., 1971. A Simplified Assumy For Milled Rice Amylose. Cereal Sci. Today 16:334-340.

Simonne, A.H., Kays, S.J, Koehler, P.E, Eitenmiller, R.R. 1993. Assessment of β -carotene content in sweet potato breeding lines in relation to dietary requirements. J. Food Comp. Anal. 6, 336-345.

Sudarmadji, S., Haryono, B., Suhardi., 1997. Prosedur Analisa untuk Bahan Makanan dan Pertanian. Liberty. Yogyakarta.

- Utomo, J. S., 2009. Development of Restructured Sweetpotato French-Fry-Type Product. Thesis Doctor of Philosophy. Universiti Putra Malaysia. 2009. Kuala Lumpur.
- Yamakawa, O., 1998. Development of new cultivation and utilization system for sweetpotato toward the 21st century. In D. R. La Bonte, M. Yamashita and H. Mochida (Eds). Proceedings of International Workshop on Sweet Potato System toward the 21st Century. Miyakonojo, Japan, December 9-10, 1997. Kyushu National Agricultural Experiment Station. p. 1-8.
- Woolfe, J. A., 1992. Sweet potato an untapped food resource. Cambridge University Press. Cambridge. p. 60-61; 71-79; 146-158.
- Wu, X., Sun, C., Yang, L., Zeng, G., Liu, Z., Li, Y. 2008. β -carotene content in sweet potato varieties from China and the effect of preparation on β -carotene retention in the Yunshu No. 5. *Innov. Food Sci. Emerg. Tech.* 9, 581-586.

Characterization of Modified Corn Flour and Coarse Grits Using several soaking Treatments

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Abstract

Corn grains should be processed into intermediate products to extend the shelf life and to improve its added-value, such as flour and grits. The objective of this research was to study the effect of soaking treatment on the properties of corn flour and grits. Soaking treatment led to a slight increase on the moisture (4.1 – 5.0%) and protein content (8.8 – 9.7%), while ash (0.2 – 0.5%), fats (4.2 – 4.7%) and carbohydrate content (80.7 – 82.0%) tended to decrease on both flour and grits. The color of corn flour and coarse grits ranged between 93.4 and 94.4 (L), -1.2 to -2.7 (a), and 28.5 to 39.7 (b). Starch content of corn flour (73.8%) and grits (72.8%) tended to decline in the range of 66.1-70.2% for corn flour due to soaking treatments and of 66.3-69.1% for grits. The amylose content ranged from 22.3 to 30.3%. Lactic acid bacteria activities during soaking influenced the ratio of amylose – amylopectin contents, both in flour and grits.

Keywords : corn flour, corn coarse grits, soaking treatment.

Introduction

Corn is one of the strategic commodities in Indonesia, which is potentially able to be developed into staple food similar to rice. In addition, it also could be used as industrial raw materials, feed and biomass production. Corn demands are from food and non-food industries, such as cracker/keropok, sweetener including sorbitol, instant noodles, cookies, paper, textiles, and plywood industries.

Based on the last five years data, corn production in Indonesia increased approximately 2.5% a year, from 17.6 million tones in 2011 to 18.5 million tones in 2013, and increased to 19.6 million tones in 2015 (BPS, 2015). However, the peak corn harvest period coincides with rainy season and simple post-harvest handling at the farm level causes yield losses. Yield/mass losses in corn occur because of poor post-harvest handling and during storage that can reach 3-5% per month at the farm level (Dorosh, 1987). Beside mass losses, the mycotoxin contamination is also a serious problem due to quality deterioration. According to Sauer *et al.* (1992), mycotoxin contamination causes decreasing germination power, discoloration, musty odor, mass losses, chemical and nutritional changes in grain stored.

Extending the shelf life and improving the product added-value, corn grains should be processed into intermediate products, such as: flour, starch, and grits. The objective of this research was to study the influences of several soaking treatments on corn flour and grits properties.

Methods

This research was conducted at laboratory of Indonesian Center for Agricultural Postharvest Research and Development – Bogor, from July until October, 2015. The yellow corn grains used were from Bogor. Dried corn grains were processed according to procedures shown in the Fig. 1. The resulting samples were analysed for color (chromameter method), proximate (moisture, ash, protein, fat and carbohydrate content) (AOAC, 2006), the amylose – amylopectin content (IRRI method), and starch. Moisture content was determined using gravimetric method at 105°C until it reach constant weights. The ash content was determined by AAS, heating in 500 – 600°C for 6 hours, determination of fat content used Soxhlet method with hexan solvent. Protein content was determined by micro-Kjeldahl method. Carbohydrate was determined by different method. Amylose content was determined by spectrophotometry using amylose derived from pure potato amylase as standart solution. Starch content was determined by washing with distilled water, and then using HCl. Data analysis was ANOVA at 5% level of significance.

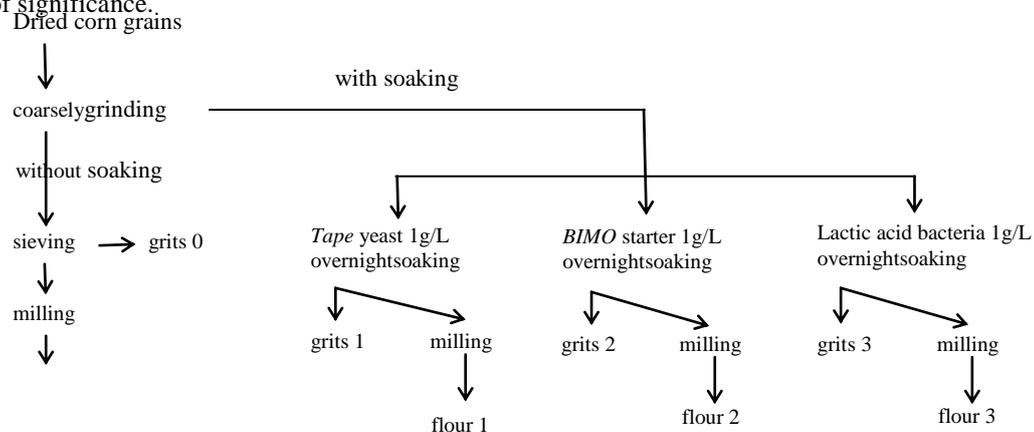


Figure 1. The processing procedure of corn flour and coarse grits

Result and Discussion

Richana (2010) reports that corn grains contain starch 54.1-71.7%, protein 11.1-26.6%, fat 5.3-19.6%, and fibre 2.6-9.5%. In this study, corn flour and grits without soaking had 3.8% moisture, 1.4% ash, 7.0% protein, 5.1% fat, and 82.8% carbohydrate. The soaking treatment led to a slight increase of moisture (4.1– 5.0%) and protein content (8.8 – 9.7%) whereas ash (0.2 – 0.5%), fats (4.2 – 4.7%) and carbohydrate content (80.7 – 82.0%) tended to decrease. Moisture content of agricultural products affect its shelf life, likewise corn flour and grits. It is affected by drying process as well. Optimum drying process would influence the corn flour and grits shelf life, because it reduced the moisture content without damaging the chemical structure such that the growth of microbes and enzyme activities could be inhibited. Corn flour and grits with and without soaking treatment still has low moisture content. According to the SNI (Indonesian National Standard) of flour, the moisture contents of the grits and corn flour still fell within safe moisture content for storage (< 14%). Soaking treatment slightly increased the protein content from 7.0% without soaking treatment, to 8.8 – 9.7% with soaking treatment. The corn grain after coarsely grinding would undergo the fermentation process during soaking that would improve

the protein content, because it used fermentation starter like *Tape* yeast, *BIMO* starter, and lactic acid bacteria.

The color of corn flour and coarse grits ranged between 93.4 and 94.4 (L), -1.2 and -2.7 (a), and 28.5 and 39.7 (b). Corn flour without soaking treatment contained 73.8% starch, and soaking treatment tended to decline the starch (66.1-70.2%) content. While coarse grits without soaking treatment contained 72.8% starch, and it decreased with soaking treatment (66.3-69.1%). Starch content is an important component in determining the quality of food. Natural starch is a mixture of amylose and amylopectin. Amylose and amylopectin ratio influences the processing of corn product, related to its energy for gelatinization or boiling the maize starch.

The corn flour and coarse grits contained 22.3-30.3% of amylose (with and without soaking). It was in line with Richana et al. (2011, 2013) that reported the amylose content of corn grains (15.7-29.0%) and fermented corn flour (19.7-23.9%). Amylose has an important role in gelatinization process; it affects the character of starch paste. Amylose is a polymer of glucoses that are not branched, while amylopectin is a branched polymer of glucoses and have larger molecular size. Because of that, amylose can be digested more slowly than amylopectin. Lactic acid bacteria soaking treatment tended to maintain the amylopectin and decreasing the amylose for both corn flour and grits.

Table 1. Characteristics of corn flour and coarse grits.

Treatment	Color			Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Starch (%)	Amylose (%)	Amylopectin (%)
	L	a	b								
Corn flour											
Control	93.41	-2.72	28.52	3.79 a	1.38	6.99	5.02	82.82	73.80	28.63	45.17 c
soaking:											
▪ <i>Tape</i> yeast	93.54	-2.63	29.33	4.15 b	0.18	9.07	4.72	81.88	66.13	28.27	37.86 a
▪ <i>BIMO</i> starter	94.03	-2.52	28.92	4.48 c	0.21	8.75	4.74	81.82	70.16	30.27	39.89 ab
▪ Lactic acid bacteria	93.82	-1.22	39.73	4.96 d	0.48	9.69	4.19	80.68	67.38	23.25	44.13 c
Corn grits											
Control	93.42	-2.72	28.52	3.83 a	1.37	6.99	5.07	82.74	72.84	27.80	45.04 c
soaking :											
▪ <i>Tape</i> yeast	94.04	-2.62	29.33	4.12 b	0.17	9.05	4.67	81.99	68.83	28.87	39.96 ab
▪ <i>BIMO</i> starter	94.22	-2.53	28.92	4.46 c	0.19	8.78	4.74	81.83	69.06	29.27	39.79 ab
▪ Lactic acid bacteria	94.43	-1.24	39.73	4.91 d	0.49	9.68	4.18	80.76	66.28	22.25	44.03 c

*Means in the same column followed by the same letter are not significantly different at 0,05%.

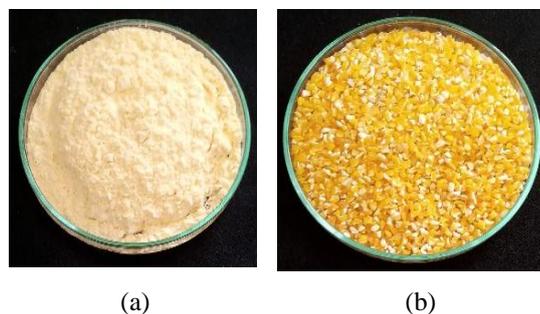


Figure 2. (a) Corn flour and (b) Corn grits with *Tape* yeast soaking treatment

Conclusion

Soaking treatment tended to increase the moisture and protein content but reduced ash, fat, carbohydrate, and starch contents. Lactic acid bacteria soaking treatment affected the ratio of amylose – amylopectin both on corn flour and grits.

References

- AOAC. 2006. Official methods of analytical of the association of official analytical chemist. Washington DC: AOAC.
- Dorosh, P.A. 1987. International Trade in Corn. *In* Timmer, C.P. (ed) The Corn Economy of Indonesia. Cornell University Press, Ithaca.
- Richana N. 2010. Modified corn starch to substitute wheat flour using. *Warta Penelitian & Pengembangan Pertanian* 32(6): 5-7. (*in Bahasa Indonesia*)
- Richana N., Budiyanto A., Mulyawanti I. 2011. Lactic acid bacteria for fermentation of the modified corn flour production. *Proceeding of The 3rd International Conference of Indonesian Society for Lactic Acid Bacteria*, January 21-22, 2011. Yogyakarta: Indonesian Society for Lactic Acid Bacteria. (11 p.)
- Richana N., Ratnaningsih, Arif A.B., Hayuningtyas M. 2013. Characterization of eight maize varieties with a low glycemic index to support food security. *Proceeding of International Maize Conference*. Gorontalo, November 21-23, 2012. Jakarta: Ministry of Agriculture Republic of Indonesia. p. 178-183.
- Sauer, D.B., Meronuck, R.A. and Christensen, C.M. 1992. Microflora. *In* Sauer. D.B. (ed). *Storage of Cereal Grains and Their Products*. Americans Association of Cereal Chemists, Inc., St. Paul. Pp . 313 – 340.

Ethanol Producing Yeast Isolation from Indonesian Local Fruits for Ethanol Production

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Abstract

Ethanol is an alternative fuel and has more advantages than fossil fuel that we use nowadays, such as more eco friendly, can be produced over and over again, and come from abundant material. Yeast has important role in the efficiency of ethanol fermentation from sugar. The objective of this research is to obtain potential yeast from local fruits and used them from ethanol production. The samples are flesh of mature kweni mango (*Mangifera odorata* Griffith) and Jackfruit (*Artocarpus heterophyllus*). The samples were inoculated in Malt Extract Broth (MEB) with additional of glucose 20% and ethanol 7% for screening. Yeast growth indicating sample then isolated in Malt Extract Agar (MEA) with additional of glucose 20% and ethanol 7% by streak plate. After that, the isolated yeast were tested for its capability in producing ethanol from high concentration of glucose media by inoculating in Pepton Glucose Yeast (PGY) Broth with additional glucose 20%. The result is, obtained most potential yeast for ethanol production from kweni mango (Isolate MKP-12) that could produce ethanol up to 7,79% (w/v) in 2 days, has 0,47 $Y_{p/s}$ point or 93,1% theoretical.

Keywords: Isolation; Ethanol Producing Yeast; Indonesian Fruit; Ethanol Fermentation

Introduction

The using of ethanol as alternative fuel to replace fossil has been observed from early 1980 (Jacques *et al.*, 2003). The most significant advantages of ethanol compared to fossil fuel is it can be produced over and over again in short period time. Ethanol can be produced by fermenting sugary material using yeast through anaerobic condition. To produce ethanol, yeast needs to consume sugar as carbon sources. Only simple sugar such as monosaccharide or disaccharide that can be consumed and almost all yeast species can consume glucose as their carbon sources. However, the ability of each yeast to consume glucose and produce ethanol is different. Therefore, using the most potential yeast is important to improving the efficiency of ethanol production.

Theoretically, yeast can convert each gram of glucose into 0,51 gram ethanol and 0,49 gram CO₂. However, not all of the glucose are converted into ethanol and CO₂. Under anaerobic condition, glucose will be consumed by yeast and will going through glycolysis metabolic pathway (Embden-Meyerhof-Parnas or EMP pathway). In EMP pathway, two molecules of ATP will be produced and used to drive the biosynthesis of yeast cells. Various by product are also produced during ethanol production. Glycerol, producing at a level of about 1% (w/v), is the main one. (Bai *et al.*, 2007). These metabolites production is undesirable due to loss of ethanol yield.

Although ethanol is produced by yeast itself, the increasing concentration of ethanol will initially inhibitory and latterly lethal to yeast, making them have resistency limit for ethanol concentration (Walker, 1998). Concentration of glucose affect the physiology of yeast. Low concentration of glucose will lead yeast to convert glucose into yeast cell producing mode, instead of producing ethanol. It happens because, when the carbon source supply is limited, yeast must maximize the efficiency of converting glucose into yeast cell to sustains its colony. This is the so-called Crabtree Effect (Bamfort, 2005). However, too high glucose concentration can increase the osmotic pressure and inhibit the yeast itself.

There are several criterias to select yeast for ethanol production starter to make the production more efficient. Some of the criteria areit can produce less metabolite other than ethanol, has resistency against high concentration of ethanol and glucose, and can reach the highest concentration of ethanol in less time. Therefore, this research was aimed to obtain yeast with those following criterias from Indonesian local fruit for ethanol producing.

Materials and Methods

Yeast sources

In this study, the yeast were isolated from the flesh of kweni mango and jackfruit fruit. Only fruit which had ripened and no defect that were selected. The kweni mango were obtained from Jombang and Ciputat Market, Tangerang, Banten. The jackfruit were obtained from Kota Baru, Yogyakarta. These fruit were choosen because they contain high concentration of sugar and naturally contain ethanol. it is indicated from their strong alcoholic smell. Also as control, another yeast were isolated from ethanol fermentation media of ethanol distillery in Bekonang, Sukoharjo, Central Java.

Media and chemicals

Media for isolation and screening consisted of malt extract, glucose, ethanol, and agar. For fermentation, mediaconsisted of pepton, glucose, and yeast extract. Malt extract, agar, pepton and yeast extract were purchased from Oxoid. Glucose and ethanol were purchased from Merck.

Chemicals for glucose concentration analysist were dinitrosalicylic acid (DNS), Rochelle salt (potassium sodium tartrate tetrahydrate) and NaOH. Chemicals for ethanol concentration analysist were potassium dichromate ($K_2Cr_2O_7$), potassium carbonate (K_2CO_3), and sulfuric acid (H_2SO_4).

Yeast isolation

A little part of inner fruit flesh were put in to5 mL of broth media containing malt extract 2% (Malt Extract Broth or MEB); glucose 20%; and ethanol 7%in sterillized tube. Afterthat, the samples were incubated in ambient room for ± 4 days. The addition of high concentration glucose and ethanol was to make the selection media, so only yeast with high ethanol and osmotic resistency that could grow in media. Medium that were possitively contained selected yeast, indicated by increasing of media turbidity and appearance of carbon dioxide bubble in the surface of media, then were plated in agar media containing malt extract 2%; glucose 20%; ethanol 7% and agar 2% (Malt Extract Agar or MEA). The

plating were done by streak plate method and then incubated in ambient room for ± 4 days. Plating were redone until pure colony were gained. Isolated yeast then were stored in 2 mL of sterilized skim milk 10% and glicerol 20% (1:1) in -40°C .

Starter preparation

Starters were prepared by culturing each isolate in 25 mL MEB with addition of glucose 20% after the isolates were thawed. The medium then were incubated in ambient room for 4 days.

Ethanol fermentation analysis

Ethanol fermentation were performed anaerobically by inoculating 2,5 mL screened yeast isolates starter into 22,5 mL media containing pepton 0,75%; yeast extract 0,45%; and glucose 20% (PGY). Glucose content analysis was done by DNS method (Toledo *et al.*, 2012). Ethanol content analysis was done using micro diffusion method (Macleod, 1949). Biomass content was done by measure absorbance of its medium.

Ethanol yield ($Y_{p/s}$) was calculated using :

$$Y_{p/s} = \frac{p - p_0}{s_0 - s}$$

P = final product (ethanol) concentration;

S_0 = initial substrate concentration.

P_0 = initial ethanol concentration;

(Riadi, 2007)

S = final substrate (glucose) concentration;

While % theoretical was calculated by:

$$\% \text{ theoretical} = \frac{Y_{p/s}}{0.51} \times 100\%$$

Result and Discussion

Yeast isolation

By using MEB medium, 30 samples from kweni mango are positively contain yeast. 20 samples from jackfruit samples and 6 samples from Bekonang distillery sample also indicated that it contain yeast. Growth of yeast will make the turbidity of medium changes because yeast cells are produced over times. CO_2 is by product of ethanol fermentation. The appearances of CO_2 indicates that yeast are able to grow and convert sugar into ethanol. High concentration of ethanol and glucose in these media make sure that those yeast has high resistency into ethanol and osmotic pressure. There is possibilities that those yeast could produce high yield and maximum concentration of ethanol.

Positive samples were plated into MEA by streak plate until pure isolate were obtained. After that, each different morphology of yeast colony were picked for further assay. 2 isolates were recovered from kweni mango sample, 3 isolates from jackfruit sample and 2 isolates from Bekonang distillery sample.

Ethanol fermentation analysis

The result of ethanol fermentation analysis are shown in Table 1. That table show the ability of each yeast to produce highest concentration of ethanol and time that needed to reach that concentration. Table 1 also show their yield of ethanol from glucose and how close that yield value to theoretical yield of ethanol from glucose.

Table 1. Ethanol fermentation analysis result

Isolate	Origin	Max ethanol concentration (%) (w/v)	Time (day)	$Y_{p/s}$	% theoretical
MKP12	Kweni mango	7.79	3	0.47	93.13
MKP42		6.87	3	0.42	81.45
FINK1	Jackfruit	4.90	4	0.35	68.57
FINK2		5.98	4	0.36	71.20
FINK3		7.05	4	0.37	72.99
BR131	Bekonang distillery	6.92	4	0.37	71.78
BR232		7.61	3	0.44	85.63

Isolate MKP-12, from kweni mango, could reach highest maximum ethanol concentration among the other isolates. It could reach higher max concentration of ethanol than control isolates (isolates from Bekonang distillery). Isolate FINK-3 could reach higher max concentration of ethanol than jackfruit isolates and control isolates, but its max concentration was not higher than isolate MKP-12 could reach. Also isolate MKP-12 could reach its max ethanol concentration in just 3 days while isolate FINK-3 needs 4 days. While isolate BR-232 could reach its max ethanol concentration faster than the other Bekonang distillery isolate and jackfruit isolates. High max ethanol concentration can be useful for ethanol production. The following process after fermentation is ethanol separation. Usually, distillation method is used to separate ethanol due to alcohol volatility. Distillation process sometimes repeated to reach several concentration of ethanol. High concentration of ethanol after fermentation process, make distillation process easier reach to ethanol targeted concentration.

The highest value of $Y_{p/s}$ is obtained by MKP-12 isolate from kweni mango sample. It had 0.47 value of $Y_{p/s}$ or 93.13% theoretical. It means that, isolate MKP-12 could convert every gram of glucose into 0.47 gram of ethanol. While from jackfruit sample, isolate FINK-3 had the highest $Y_{p/s}$ value. However, it was not higher than BR-232 isolate's $Y_{p/s}$ value. $Y_{p/s}$ value represent how efficient a microorganism consumed substrate and produce its product. Isolate with high $Y_{p/s}$ value is desirable in ethanol production because it can produce more ethanol from less glucose. Figure 1 shows that the glucose concentration will decrease over time, while ethanol and biomass concentration is increase. It indicates that those isolate are able to consume glucose and produce ethanol and yeast cell. From the ethanol curve of isolate MKP-12 and BR-232, both had reach their peak in 3 days and slightly decrease afterthat. While isolate FINK-3 had reach their peak in 4 days. There is a possibility that isolate FINK-3 could reach higher ethanol content after 4 days. However, more than 4 days for fermentation process is less economic beneficial.

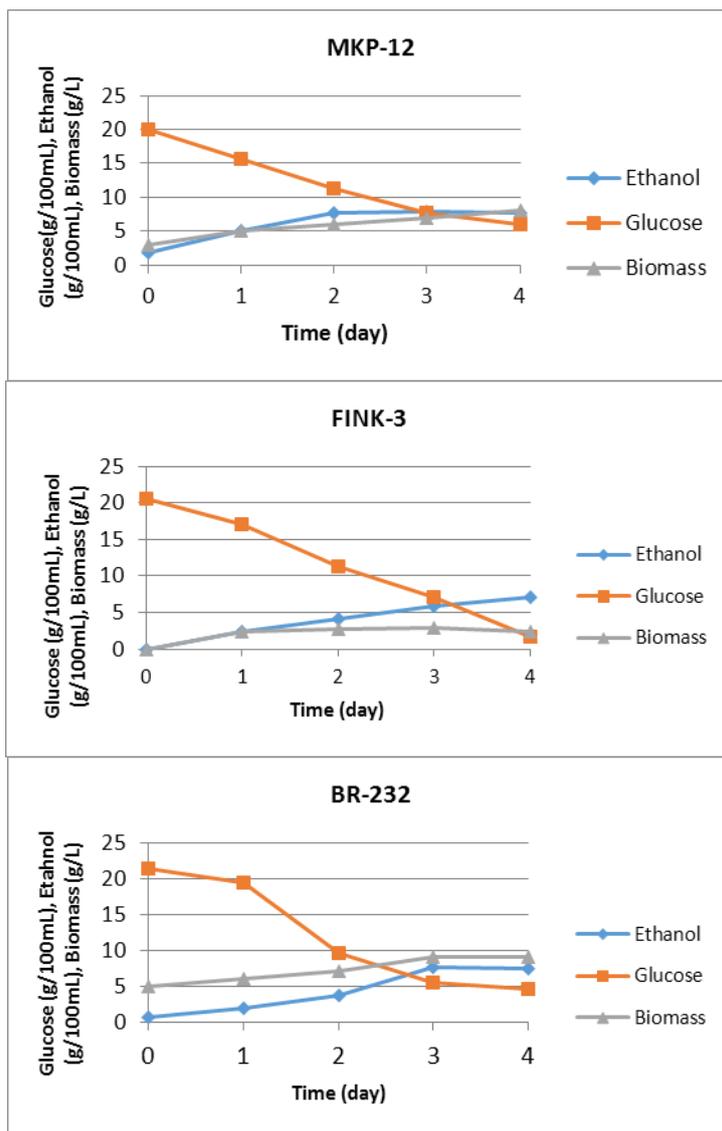


Figure 1. Fermentation curve isolate MKP12, FINK3, and BR232

Conclusion

From this research, obtained isolate yeast that most potential for ethanol production. Isolate MKP-12, which is isolated from kweni mango, could produce max ethanol concentration up to 7.79% in 3 days. Isolate MKP-12 also has 0.47 value of $Y_{p/s}$ or 93.13% theoretical which is higher than control isolates.

Acknowledgement

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Reference

- Bai, F., Anderson, W., & Moo-Young, M. (2007). Ethanol Fermentation Technologies from Sugar and Starch Feedstocks. *Elsevier*, 89-95.
- Bamfort, C. W. (2005). *Food, Fermentation and Micro-organisms*. Blackwell: Science Ltd.
- Jacques, K., Lyons, T., & Kelsall, D. R. (2003). *The Alcohol Textbook 4th Edition*. Nottingham: Nottingham University Press.
- Macleod, L. D. (1949). Determination of Alcohol by Microdiffusion. *J. Biol. Chem*, 181 : 323 - 331.
- Riadi, L. (2007). *Teknologi Fermentasi*. Yogyakarta: Graha Ilmu.
- Vagner de Alencar Arnaut de Toledo, M. C.-T. (2012). Spectrophotometry as a Tool for Dosage Sugars. Dalam J. Uddin, *Macro to Nano Spectroscopy* (hal. 269 - 290). Intech.
- Walker, G. M. (1998). *Yeast Physiology and Biotechnology*. London: John Wiley & Son.

Conditions of Hygiene and Sanitation “Otak-Otak Bandeng” Production in Distric of Gresik

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Abstract

The purpose of this research is to know hygiene and sanitation conditions of production otak-otak bandeng in Gresik, covering of food handler's hygienic, sanitation of processing, food, tools, and sales. This study was descriptive quantitative research. The data undertaken by observation to determine conditions of hygiene and sanitation of otak-otak bandeng in district of Gresik. The subject of research was five of otak-otak bandeng business in Subdistrict of Duduk Sampeyan, Manyar and Gresik in District of Gresik. Technical analyst data was done by the percentage of continued with a descriptive analysis. The results of this research indicated: 1) Personal hygiene food handelrs otak-otak bandeng was good enough (76%), 2) In general, sanitary processing (dapur) in fairly good condition (62%), 3) Sanitary conditions in sales was good enough (76%), 4) Food Sanitation in General was good enough (75%), 5) Sanitary equipment in the condition quite well (66%). Based on it, hygiene and sanitation of food handlers, kitchen processing, food, equipment, and sales quality needs to be improved.

Keywords: Hygiene, Sanitation, Otak-Otak Bandeng

Introduction

Otak-otak bandeng is one food of East Java especially in district of Gresik made from milk fish. More detail in accordance with Saparianto (2007), that otak-otak bandeng processed from milk fish, foemerly of separated from spines and the skin, then spines fish dumped and his skin used as wrapping meat fish already seasoned. Compared with other food of Gresik, otak-otak bandeng is more attractive to visitor or travellers who had visited Gresik.

As food typical of Gresik, many people interest otak-otak bandeng not in accordance with durability their products that is short, because elementary substance which used, technique processing and packaging and sales less supportive so endurance their products low. One of the factor in impervious to being low is processing otak-otak bandeng still simple or traditional. Processing a still the traditional allow the presence of food contamination that cousesotak-otak bandeng can't last long.

Food contamination that occur during the process food processing can cause disease due to food as: food poisoning caused by bacteria contamination that produce toxic contaminated and other material is poisonous like streptococcus bacteria; disease infection is a disease caused by pathogen bacteria like streptococcus. It is influenced by cooking less perfect, preservation and conservation manner that is less well as storage methods, and the existence of parasitic infection as taeniasaginata and Taeniasolium cause of anemia and disorder on the eyes and brain.

In Indonesia, cases of a disease due to food especially processed fish are not much or rarely

reported. According to BPOM Indonesia (2015), the cause of the remarkable disease due to food are microbes (confirm) as one incident (1,64%), microbes (suspect) as 26 events (42,62%), chemistry (suspect) as 7 events (11,48%) and that known causes 27 events (44,26%). A kind of food cause of food poisoning 2015 is a native of home cooking households 25 events (40,98%), street food 14 events (22,95%), food from food service as many as 13 events (21,31%) and processed food as many as 9 events (14,75%).

Considering from the data, then disease due to food from processed a fish can come from food produced by households and food industry processing. Based on it, it is important to note of factors that causes of a low processed especially otak-otak bandeng in origin production. The purpose of this study is to observe condition of hygiene and sanitation of otak-otak bandeng production in district of Gresik, covering food handler's hygiene, sanitation of food, food processing place (kitchen), equipment and sales food market.

Materials and Methods

This study was descriptive quantitative research. The data undertaken by observation to determine conditions of hygiene and sanitation of otak-otak bandeng in district of Gresik. The subject of research was five of otak-otak bandeng business in Subdistrict of Duduk Sampayan, Manyar and Gresik in District of Gresik. Technical analyst data was done by the percentage of continued with a descriptive analysis.

Result and discussion

1. Characteristics of Respondents

Table 1. Characteristic Respondent Distribution

No	Group	Respondent	%
A. Gender			
1	Male	1	6,7
2	Female	14	93,3
Total		15	100
B. Age			
1	30-39	5	33,33
2	40-49	8	53,33
3	≥50	2	13,33
Total		15	100
C. Last Education			
1	Elementary School	1	6,67
2	Junior High School	1	6,67
3	High School	12	90
4	University	1	6,67
Total		15	100
D. Selling way			
1	At a special space/Store	14	93,3
2	Around	1	6,7
Total		15	100
E. Long working			
1	0-15 years	7	46,7
2	>15-30 years	6	40
3	>30-45 years	1	6,7
4	>45 years	1	6,7
Total		15	100
F. Sanitation training			
1	Ever	1	6,7
2	Never	14	93,3
Total		15	100

Most food handler makers and sellers of otak-otak bandeng are women. In terms of the perspective of

age, food handler classified as adults who has the experience more along with long or experience in producing the otak-otak bandeng, although only a small minority had once followed training sanitation. The more experience so can trigger they to think positively of the way handling good food and hygienic. It's same with Marsaulina research (2004) in Jakarta that concludes the connection between cleanliness individuals with food handler's age.

Higher experience of food handler have the better hygiene condition. A number of studies linking various single age category of food handlers with their manners and knowledge. Survey of schools in England show that 81 % of the people of the age of 55 years always ensure that the food served in a state of heat and eat it as soon as served, meanwhile the people that are less than 24 years who performs the correct procedure only 54 % (WHO, 2006) .The level of education of food handler is extremely diverse, ranging from a graduate of a primary school to college.

A number of studies connect the level of food handler education with cleanliness. Education could not influence food handlers knowledge in hygienic behavior. A number of studies connect the level of food handler education with cleanliness. According to Notoatmojo (2003), knowledge and attitudes about health would affect to the attitudes behavior as a result over a long period of health education.

2. Food handler Hygiene

a. Health

Food handlers health covering health conditions and things associated with attempts to maintain the health of each food handler, as are presented in Table 2.

Table 2. Health of Food Handler

No	Group	Respondent	Prosentase (%)
A. Disease is on suffered now			
1	Spread	1	6,67
2	uncontaminated	1	6,67
3	None/healthy	13	86,67
Total		15	100
B. Health check routine			
1	Routine	3	20
2	Not routine	12	80
Total		15	100
C. Health check in 1 year latest			
1	Never	0	0
2	Once	12	80
3	Twice	2	13,3
4	More than twice	1	6,67
Total		15	100
D. Health check place			
1	Community health center (Puskesmas)	14	93,3
2	Hospital	0	0
3	Health clinic	1	6,67
Total		15	100

Food handler is a person who handles food and play important roles in displacement and prevention of disease due to food (Isara and Isah, 2009) so, his health must be protected. Most of food borned diseases are caused by microorganisms which grow during the preparation, process, and storage of food. As reported by BPOM Indonesia (2015), that the cause of extraordinary occurrence disease due to food are microbes (44,26 %) the rest is because chemicals and other are unknown. At this time, only a small part of food handlers were suffering of flu or infectious diseases, the rest suffer from a disease that is less

risky against contamination of food, like rheumatic fever. Because of seemingly good conditions, medical examination are not always routines. According to Fatonah (2005), medical examination done for everyone especially who's directly connected with food processing, a food handler to be free from infectious disease. If the food handler is sick, especially a contagious disease, he should go to the doctor thus the disease will not affect the food. The unhealthy of food handler can spread a virus into food.

b. Food Handler Cleanliness

Table 3. Food Handler Cleanliness

No	Aspect Observed	Evaluation		Conclusion
		Yes	No	
1	Short nail and clean	15	0	Less good(50%)
2	Neat hair	10	5	
3	Using clean clothes when process food	8	7	
4	Using clean clothes when selling	5	10	
5	Process food on the table	0	15	
Total		38	37	

In general, cleanliness of food handler is less especially the use of work clothes at the time of the process and selling food as well as undertaking the production process. The habit of washing the hands had been done on a regular basis. Moreover, their nails are good. Food handler should take into account of washing hands properly to help avoid the contamination of microorganisms. Washing hands in advance before handle the food can minimize the risk of bacteria contamination.

Nails are part of a hand which are often be a source of contamination or resulting in cross contamination. Most common source of contamination is coming from men (Green and Selman, 2005), especially by direct contact of food by hand. If food handler ia not clean, so food can be polluted (McSwane *et al.*, 2003). Food handlers are able to send pathogenic bacteria to food with the hand contaminated by organisms of their digestive tract. Hence, contact hand directly with ready to eat food are potentially admit pathogenic bacteria in foods (Guzewich and Ross, 1999).

c. Habits / Food Handler Behavior

Table 4. Food Handler Behavior

No	Aspect Observed	Evaluation		Conclusion
		Yes	No	
1	Performs activities processing with good manners	9	6	Less good(51%)
2	Not use jewelry when process food	5	10	
3	Process food by using a spoon / claw	8	7	
4	Washing the hands	9	6	
Total		31	29	

To keep food to stay safe, food handler be barred from using jewelry when processing the food, for example ring, because the skin below the ring is a good place to bacteria for growth, so fallen bacteria of the ring will contaminating the food. In the process of making otak-otak bandeng, there are stage of kneading batter, where at this stage is carried out using hand, when the food handler use rings, contact between ring and dough will transfer the bacteria from skin under the ring to the dough.

In general, the individual hygiene consists of cleanliness, health, habits and behavior are quite good (76%). The central government of Indonesia by statute No.18 year 2012 on Food, has set standards and requirements that food and drink worthy and safe to eat in the community, where the article 71 paragraph

1 and 2 explained that everyone involved in a food chains have to control the risk of hazard on food and everyone who perform the process of the preparation of food production obliged to the requirements sanitation and food safety.

3. Observation

Table 5. The condition of the kitchen

No	Aspect Observed	Evaluation		Conclusion
		Yes	No	
1	The kitchen clean	2	3	Good enough (75%)
2	Tiles in the floor	4	1	
3	Air ventilation	2	3	
4	The closed trash and clean	0	5	
5	The trash in a condition closed	0	5	
6	The trash which is already full immediately disposed	4	1	
7	Place wash proper equipment in the kitchen	3	2	
Total		15	20	

The kitchen has an important role in the process of food preparation, therefore cleanliness of the kitchen and its environment should always maintained well. Not all the kitchens for food processing are clean although almost all facilities has already been good, especially for the garbage storage which usually are inadequate. Good facilities and clean condition can reduce the contamination. The parameters of good condition of kitchen are for example have the floor height above the water surface level, smooth, not slippery and easily cleaned. Kitchen should have a good ventilation system that air can easily swap with outside fresh air. In addition, the ventilation also provides a tunnel for smoke from food processing to leave the kitchen.

Hot air and humid are the conditions need for bacteria to grow, so the kitchen must be kept clean to keep cool and not fetid. Food processing will also yield garbage. Garbage bin should be provided in the kitchen while it should be closed and the garbage disposed periodically. Therefore could avoid bad smell and attract insects. However, from this research, 5 food handlers do not have clean garbage bin in the kitchen. Meanwhile, 5 food handlers garbage were placed in an open condition..

Table 6. The condition of food

No	Aspect Observed	Evaluation		Conclusion
		Yes	No	
1	Fresh Raw Materials (fish)	5	0	Good enough(73%)
2	Dry substances kept in a container and orderly	1	4	
3	Available freezers for storing fresh material (fish , meat , eggs)	5	0	
Total		11	4	

Sanitation is very important for food handler. Sanitation can reduce the possibility of pollution to food, so that sickness caused by food can be minimized even terminate. Most of food handlers have applying sanitation although were not well implemented. Material used to make otak-otak should be a good material, fresh, and dry. According to Anonymous (2009), food is all of their food and drink, including additives.

Ingredients of packaged food for street food must be from material listed on the department of health, not expired, not defective or not damaged, including the use of basic material of milkfish. Fish is a source of

animal protein that easily damaged, so that should be treated and stored properly. Fish used in the condition of fresh and no foul, besides no visible signs of damage fish because microbes like the presence of a foul odor because ammonia gas, sulphide or foul other compounds, the establishment of mucus on the surface of fish, the change in color, namely the skin and flesh of fish become dull or pale and the change in the flesh of fish not springy be again.

From 5 places, one place keep the material in dry and neat condition. Meanwhile, 4 other place keep all materials not in closed container. Every food placed separately according to its kind, in each container. Food produced in a large scale usually are not immediately consumed, hence should be stored. Storage methods depend on the variety and number of foodstuffs. Foodstuffs which dry wrapped on sack or plastic can be stored in the open space, while food derived from animal should be kept at the cold or freezing temperatures.

Table 7. The equipment condition

No	Aspect Observed	Evaluation		Conclusion
		Yes	No	
1	Equipment always clean condition	4	1	Good enough(60%)
2	Washing equipment correctly	4	1	
3	Equipment washed every finished use	1	4	
4	Processing equipment feasible	3	2	
5	Equipment made of material safe	3	2	
6	Available store shelves special equipment	3	2	
7	Equipment in the in face down position	3	2	
Total		21	14	

From the result of research of equipment it can be concluded that to 4 place of respondents, the condition of equipment were clean. However, in the remaining one place, the equipment condition of respondent was lacking or not clean. There is regulation by Ministry of health Indonesia Republic No. 942 / Menkes /SK/2003 which specify on how to maintain the cleanliness of equipment. Based on observations during the survey, only 4 place respondents do laundering equipment properly namely by removing dirt with water, cleanse with soap, then rinse with water. But not all places are able to wash crockery. This does not suit report that was delivered by Purnawijayanti (2001:44) that dirty equipment must be washed after use and disinfected or drained with the help of the rays of the sun.

Equipment used should not be broken, dented scratched or crack because it will be suitable for dirt or bacteria. Moreover, equipment may not be able to be washed perfectly so can be a source of contamination. Equipment used should not have crack and rupture, so they do not cause accident (hands injury). After washing, equipment should be stored properly, by putting on a shelf in reversed.

Equipments are kept on a special rack to avoid dust, insect, and rodent. The result of this research in accordance with research conducted by Hartono and Susanna (2003) which reported that the methods of washing and storing of the equipment shall based on regulation that equipment will always in a clean state before used because contaminant are no longer exist by washing and appropriate storage.

Table 8. The stall condition

No	Aspect Observed	Evaluation		Conclusion
		Yes	No	
1	The clean container	5	0	Good enough(76%)
2	Stalls clean condition	4	1	
3	Case display in good condition	2	3	
4	Available instrument responsible for food	4	1	
5	The condition of case display clean	4	1	
Total		19	6	

Sanitation of stall or place to sell the product is mainly to avoid pollution caused by dust or smoke, and also flies. There should also garbage can with perfect condition available, so could not be penetrated by flies and insects. Based on the results, in all 5 places, clean container were already used. Container for storage such as basin, pot, were all clean.

Following the process of procurement, acceptance of foodstuffs, washing, compounding, making, editing shapes, and packaging will be followed. Food and beverage must be served with clean container therefore safe for health (Depkes RI, 2003). While from 4 place, food handler have a clean stall. The clean and healthy stall will be a hygienic and enjoyable place to work. Cleanliness of the stall will greatly determine the quality and security food produced. On the contrary, microorganisms are growing well on a moist and warm environment, as in food placed in dirty compartment.

Based on the results, 4 place having a display case for storing foods in the condition of being open even if there is one that is closed only occasionally while it has no buyers. Open display of the food can increase the risk of contamination of food by the environment, either through the air, dust, vehicles smoke even insects, while in one place, a display case for storing foods in the condition of closed. The results of the study stated that in one place tools are available (a brace, a spoon, or forks) to take food and 4 other place, food handler not wear the tools or brace when taking or holding food. These microorganisms which attached to the hand will drift off and multiplied in food.

Conclusion

The hygiene sanitation condition of otak-otak bandeng production that includes: Personal hygiene and sanitation is quite good (76%). Meanwhile, sanitary conditions of processing in a kitchen is quite good (75%), however, cleanliness and facilities environment needs to be improved. Food sanitary conditions quite good (73%) but still require more improvement. Moreover, equipment sanitary conditions quite good (60%) it is necessary to improve hygiene and storage of equipment. Sales facility sanitary conditions quite good (76%), nevertheless, cleanliness and facilities of stall still needs to be improved.

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References

- Anonym. 2009. *Keputusan Menteri Kesehatan RI Nomor 942/Menkes/SK/VII/2005 tentang Persyaratan Higiene Sanitasi Makanan Jajanan*, www.blogspot.com. Diakses 20 Maret 2013
- B POM RI. 2015. Laporan Tahunan BPOM RI. http://www.pom.go.id/new/index.php/browse/laporan_tahunan/21-09-2006/21-09-2016/1. Diunduh 10 Pebruari 2016.
- Departemen Kesehatan RI, 2003. "*Peraturan Menteri Kesehatan No. 715 tahun 2003 tentang Persyaratan Hygiene Sanitasi Jasaboga*". Jakarta :Departemen Perburuhan RI.
- Fathonah, Siti. 2005, *Higiene dan Sanitasi Makanan*, Fakultas Teknik Universitas Negeri Semarang, Semarang
- Green, L.R., and Selman, C. 2005. *Factors Impacting Food Workers' and Managers' Safe Food Preparation Practices: A Qualitative Study*. *Food Protection Trends*, 25 (12), 981-990. <http://www.ophp.oregon.gov/DHS/ph/foodsafety/snet/snetworkerarticle.pdf> Diakses 15 Agustus 2015
- Guzewich, J., and Ross, M.P. 1999. *Evaluation of Risks Related to Microbiological Contamination of Ready-To-Eat Food by Food Preparation Workers and The Effectiveness of Interventions to Minimize Those Risks*. Retrieved from <http://www.fda.gov/Food/FoodSafety/RetailFoodProtection/ucm210138.htm> Diakses 12 Agustus 2014
- Kepmenkes RI No. 942/MENKES/SK/VII/2003 *tentang Pedoman Persyaratan Hygiene Sanitasi Makanan Jajanan*.

- <http://www.depkes.go.id/download/SK942.03.pdf>, diakses pada 20 Maret 2012.
- Marsaulina, Irmawati, 2004. *Study Tentang Pengetahuan Perilaku Dan Kebersihan Penjamah Makanan Pada Tempat Umum Pariwisata di DKI Jakarta (TMII, TIJA, TMR)*. Sumatra :Universitas Sumatra Utara
- McSwane, D., Rue., N., and Linton, R. 2003. *Essentials of Food Safety and Sanitation* (3rd ed.). Chicago, IL: National Restaurant Association Educational Foundation
- Purnawijayanti, H. 2001. *Sanitasi Higiene dan Keselamatan Kerja Dalam Pengolahan Makanan*. Kanisius. Yogyakarta.
- Saparinto, C. 2007. *Membuat Aneka Olahan Bandeng*. Jakarta: Penebar Swadaya.
- Notoatmodjo S. 2003. *Promosi Kesehatan dan Ilmu Perilaku*. Jakarta :Rineka Cipta.
- Susanna, Dewi dan Hartono Budi, 2003. *Pemantauan Kualitas Makanan Ketoprak dan Gado-Gado di Lingkungan Kampus UI Depok Melalui Pemeriksaan Bakteriologis*. Makara Seri Kesehatan 7(1): 21-29. Diakses 4 Januari 2013.
- WHO. 2006. *Penyakit Bawaan Makanan :Fokus Pendidikan Kesehatan*, Penerbit Buku Kedokteran EGC, Jakarta

The Physical Properties and Acceptability of Duck Meat Cured in Curcumin Extract with Added STPP

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Abstract

Duck meat was taken from laying ducks which had become unproductive and also from older male ducks. The duck meat texture was tough and the high fat content of the duck meat caused it to oxidize easily during storage and processing. It is known that curing duck meat in curcumin extract and freezing for storage can inhibit oxidation of the fat, but the meat turns yellowish in colour and the texture of the frozen meat when cooked is tougher. The purpose of this study was to evaluate the effect of curing of duck meat in curcumin extract with the addition of STPP (sodium tripolyphosphate) on the physical properties (colour, texture and water holding capacity) of the duck meat and on its acceptability. The research used Randomized Complete Design, and the duck meat was cured in curcumin extract at concentrations of 0.3% and 0.4% (w/w), then STPP was added at various concentrations of: 0.0; 0.10; 0.15; 0.20 and 0.25% (w/w). The duck meat texture was tested using a Texture Analyzer and the colour tested with a Colour Reader. Acceptability of the cured duck meat with added STPP was determined by Hedonic Test based on smell, colour, texture and flavour. The results of this study concluded that raw duck meat texture was based on resistance to force and deformation, which were not significantly different. Resistance to force was between 545,91g and 597,76g and the deformation was between 70,47% and 73,03%. Water holding capacity of cured meat was between 40,05% and 46,77%, which is higher than fresh meat at 26,81%. However, the color of the cured duck meat wasn't as bright as fresh duck meat, as shown by the lightness (L) of between 34,04 and 36,46 against 34,43 for the fresh duck meat, and yellowness (b) of between 21,43 and 22,98 for cured meat and 22,49 for fresh meat. The research showed that the duck meat cured with curcumin extract at 0.3% and 0.4% concentrations and added with 0:10 to 0:20% STPP resulted in the most acceptable product, based on texture, color and water holding capacity, especially the brighter color and softer texture.

Keywords: duck meat, curcumin, STPP, texture.

Introduction

Duck (*Anas platyrhynchos*) is a type of poultry farmed for meat and eggs. The quantities of duck meat on the market are still very limited as supply mostly comes from culled females (54.35%), but as much as 35.41% can come from male salvage and up to 18% from young females (Hardjosworo, 2001). Duck meat is the meat of culled non-productive female layers and older males. Reject duck meat has a clay-like texture and a fat content reaching 1.84%, in contrast to chicken meat at only 1.05% fat (Ali *et al.*, 2007). Unsaturated fatty acids (AL TJ) make up more than 60% of the total fatty acids, which results in the duck meat being easily oxidized, thereby degrading flavour, destroying nutrients and leading to a build-up of toxic substances. According to Baggio and Bragagnolo (2006) meat, during processing and storage, can be the subject of oxidation induced by the presence of heat, light, metal and oxygen which will produce ROS (Reactive Oxygen Species) such as aldehydes, peroxide and cholesterol oxides that can lead to degenerative diseases such as cardiovascular disease and early aging.

Attempts to inhibit oxidation of the fat in duck meat were made by Dewi and Astuti (2013) by adding 0.3% turmeric extract as a natural source of antioxidants and then curing for 10 minutes. Storage was undertaken for 8 weeks in a freezer. Turmeric is known to contain curcumin which can inhibit lipid peroxidation (Jayaprakasha *et al.*, 2006). The results show that extract of turmeric can inhibit an increase in the numbers of peroxides and TBARS of duck meat, and the texture of duck meat after storage becomes more tender. But curcumin is yellow in colour and has a distinctive turmeric flavour,

which can affect the acceptability of the product. In addition, the research is still limited to storage only. Yet according to Sampaio *et al.* (2012), lipid oxidation will continue during cooking.

The problem is that the texture of duck meat becomes more tender after storage in a freezer (Dewiand Astuti, 2013), but according to Fernandes *et al.* (2013) storing lamb and mutton at freezing temperatures (-18° C) causes low water retention or Water Holding Capacity (WHC), so that the texture of the meat after cooking is hard. Abdel *et al.* (-) states that the addition of sodium tripolyphosphate (STPP) to lamb meat which is to be frozen can inhibit the decrease in WHC compared to the control, so the texture of the cooked meat is softer and is preferred by the consumer. And according to Marsha *et al.* (2013) the use of STPP in turkey meat, in addition to controlling the WHC, can also inhibit the oxidation of fat by slowing down the penetration of heat into the material. Hence this objective study to evaluate the effects of curing duck meat in curcumin extract, and the addition of STPP on the physical properties and acceptability of duck meat, both raw and after cooking.

Materials and Methods

This study was undertaken with the aim of producing duck meat with a soft texture after frozen storage, to measure the effects of antioxidants, provide high acceptability and stability during processing, and to be safe for human consumption.

Materials

The materials used for the study of duck meat were derived from duck breeders in the village of Argomulyo, Sedayu, Bantul, Yogyakarta. Turmeric, as a natural source of antioxidants, was purchased from a local market in the Yogyakarta area. Analysis of the base material (duck meat) included water content (AOAC, 1990). Water holding capacity (Hamm, 1960 in Soeparno, 2009) was tested using a Texture Analyzer and colour was tested using a Colour Reader. Chemicals used in all the pro qualifying analyses were obtained from Merck.

Methods

The research method consists of five steps. These are : 1) Preparation of turmeric curcumin extract by sorting tubers, then peeling and washing. Curcumin extraction using maceration method (Marsono *et al.*, 2005), 2) Curing fresh duck meat with turmeric curcumin extract (with a variation of 0.3% and 0.4%) and variation of the addition of STPP at 0.00; 0.10; 0.15; 0.20; and 0.25%, 3) Storage of duck meat (phase 2) at freezing temperatures (-10°C) for 8 weeks, 4) Testing the physical properties (texture, color, WHC), 5) Determining the organoleptic acceptability of (phase 3) raw duck meat by sensory test.

Statistical analysis

The experimental design used was completely randomized design, factorial pattern with factors such as variation of the ratio of fresh meat to the amount of curcumin extract and STPP to determine the differences between treatments used by the F test, then the real difference between the samples was determined by Duncan's Multiple Range Test (DMRT) (Gacula and Singh, 1984)

Result and discussion

Texture of duck meat

The results showed that there was no interaction effect from each curcumin and STPP treatment on the texture of the cured duck meat (Table 1). The addition of 0.3% and 0.4% curcumin did not affect the texture of the cured raw duck meat. This is consistent with the results of research by Dewi and Astuti (2014) which states that adding curcumin only increases antioxidants, which does not affect the texture of duck meat. The texture of the cured raw duck meat is also not influenced by the addition of STPP because STPP, as well as increasing the water retention capabilities of meat during cooking, can also help maintain meat texture and tenderness (Petracci *et al.* 2013). Tender meat has a much higher market value than tough meat. This is likely due to the meat used being a sample of different muscles, as in this study by using only a few ducks but utilizing all the meat from each carcass. Soeparno (2009) states that muscle difference affects the texture and tenderness of meat.

Table 1. Texture of cured duck meat (g)

Curcumin (%)	STPP (%)					Average (ns)
	0	0,1	0,15	0,2	0,25	
0,3	559,25	542,50	686,50	599,88	578,38	593,30
0,4	565,75	650,50	509,01	491,94	554,88	554,42
Average (ns)	562,50	596,50	597,76	545,91	566,63	

Deformation of duck meat

Deformation is a change in the meat texture caused by cutting the meat to measure texture. Raw cured duck meat has relatively similar deformation and is not affected by curcumin treatment, STPP and interaction (Table 2). This may be because the texture is not significant so deformation is insignificant as well.

Table 2. Deformation of cured duck meat (%)

Curcumin (%)	STPP (%)					Average (ns)
	0	0,1	0,15	0,2	0,25	
0,3	55,44	77,63	64,51	72,65	72,22	68,49
0,4	69,57	68,43	76,42	69,32	72,00	71,15
Average (ns)	62,51	73,03	70,47	70,99	72,11	

If the cured duck meat was overcooked, the deformation was affected by the addition of STPP. Deformation of cured cooked duck meat was greatest with the addition of STPP by 0.1%. Higher additions of STPP showed no more significant deformation. This is because in meat products, STPP is generally used to maintain texture or tenderness after cooking.

Brightness (L) of cured duck meat

The brightness of cured duck meat was influenced significantly by the addition of STPP, but there was no significant effect from the addition of curcumin as well as its interaction (Table 3). The addition of 0.4%

curcumin gave the same relative brightness as the addition of 0.3%. Curcumin only serves to add antioxidants, which are substances that can inhibit an oxidation reaction in materials susceptible to oxidation (Fennema, 1996). It is likely that it has no real effect on the brightness of the flesh. The brightness of cured duck meat was significantly enhanced by the STPP treatment with the lowest brightness coming from the addition of 0.2% STPP and the highest brightness of 36.46 from 0.1% addition of STPP. Petracci *et al.* (2013), stated that some of the functional ingredients used in meat, STPP being one of them, are also able to increase the water retention of meat during cooking.

Table 3. Lightness of cured duck meat

Curcumin (%)	STPP (%)					Average(ns)
	0	0.1	0.15	0.2	0.25	
0,3	35,08	36,17	35,82	30,35	33,73	34,20
0,4	33,78	36,74	36,45	32,99	34,34	34,85
Average	34,43 ^a	36,46 ^a	36,14 ^a	31,67 ^b	34,04 ^a	

Redness of cured duck meat

The colour of cured duck meat is not affected by the addition of curcumin, STPP or its interaction (Table 4). Redness value which is positive showed that duck has a reddish colour, while if the value is negative then the meat will be a greenish colour. In this study the positive value means that the cured duck meat in this research is reddish in colour. Red cured duck meat indicates normal meat.

Table 4. Redness of cured duck meat

Curcumin (%)	STPP (%)					Average (ns)
	0	0,1	0,15	0,2	0,25	
0,3	7,58	6,25	7,00	7,67	6,22	6,94
0,4	7,20	8,07	7,38	6,69	7,02	7,27
Average (ns)	7,39	7,16	7,19	7,18	7,52	

Yellowish color of cured duck meat

The result showed that the value of yellowness of cured duck meat was significantly influenced by the addition of curcumin, but was not influenced by the addition of STPP and there was no interaction (Table 5). In addition, curcumin added at 0.4% had a higher yellowness value, i.e. had a more yellow coloured flesh than the 0.3%.

Table 5. Yellowness of cured duck meat

Curcumin (%)	STPP (%)					Average
	0	0,1	0,15	0,2	02525	
0,3	20,17	21,40	24,05	21,26	21,76	21,73 ^a
0,4	24,80	24,10	20,40	21,60	24,20	23,02 ^b
Average (ns)	22,49	22,75	22,23	21,43	22,98	

Ali *et al.* (2007) stated that the color of the duck meat has a very high redness value, but it has a low brightness value. Dewi and Astuti (2014) stated that the addition of curcumin gave an improved flesh color. It is further mentioned that there is a relationship between the amount of curcumin and the length of time curing the duck meat on the colour. i.e. if lower levels of curcumin are used to get the desired yellow color, then curing time has to be longer.

Water Holding Capacity (WHC) Value of cured duck meat

The results of this study indicate that the WHC value of duck meat was significantly influenced by the addition of STPP, but that it was not affected by the addition of curcumin and its interaction (Table 6).

The addition of STPP increased the WHC value, but at an addition of 0.25% (Table 6).

Table 6. WHC Value of cured duck meat

Curcumin (%)	STPP (%)					Average (ns)
	0	0,1	0,15	0,2	0,25	
0,3	30,28	41,01	43,00	43,22	38,82	39,27
0,4	23,33	41,25	50,13	50,32	41,28	41,26
Average	26,81 ^a	41,13 ^b	46,57 ^b	46,77 ^b	40,05 ^b	

This is consistent with the statement of Thomas (1997) cit. Dewanti (2009) that STPP can absorb, bind and hold water, increase water holding capacity (WHC) and tenderness. Yuanita (2008) states that the STPP FG is able to break the bonds of the water in the meat, thus causing a decrease in the levels of WA (water activity) which is an essential component in inhibiting the growth of microbes. A high WHC value for meat shows that the meat can hold water, so the water level is higher. This is consistent with the research results of Pratiwi (2016), which state that higher the levels of STPP FG used, up to a level of 4% in broiler meat soaking, the lower the water content. An improvement in the FG STPP treatment levels is also accompanied by an increase in the elasticity of the meat and its water holding capacity. This means that a decrease in water content does not occur in the water-bound or immobilized free water, but is due to the evaporation of free water (Yuanita *et al.* 1997).

Sensory Test of Cured Duck Meat

The results of the study of sensory testing of cured raw duck meat show that the smell, texture are affected on the whole by the addition of curcumin and STPP (Table 7). The smell of the raw meat with curcumin added at 0.3% is less preferred than that at 0.4%;, with the highest score of unlikeability being 0.3% curcumin and 0.2% STPP giving a score of 4.60 or the most disliked. The colour test by sensory testing was not affected by either the curcumin treatment or the STPP. The texture of the raw duck meat was tested by pressing with a finger. The results of the tests of sensory texture of the meat turned out to be significantly different, with the least preferred texture coming from the addition of 0.4% curcumin and 0.25% STPP. But the most preferred overall by the panelists in a sensory test came from the addition of curcumin at 0.3% and 0.4% and STPP 0.25%.

In the sensory test of cured cooked duck meat the smell was not affected by treatment with curcumin and STPP. While colour, texture and taste were on the whole affected by treatment with curcumin and STPP,

the addition of curcumin made no significant difference to the smell of the cooked meat, possibly because 0.3% and 0.4% additions of curcumin after cooking had a relatively similar smell.

Table 7. Sensory Test of Cured Duck Meat

		Smell	Color (ns)	Texture	Overall
Basic Material		3,53 ^{ab}	3,47	4,27 ^b	3,73 ^{ab}
Curcumin (%)	STPP (%)				
0,30	0,00	4,53 ^{ab}	3,73	4,20 ^{ab}	4,33 ^b
	0,10	4,27 ^{ab}	3,21	3,33 ^{ab}	3,73 ^b
	0,15	3,80 ^{ab}	3,33	3,27 ^{ab}	3,47 ^{ab}
	0,20	4,60 ^b	4,00	3,87 ^{ab}	4,13 ^{ab}
	0,25	4,47 ^{ab}	3,40	3,33 ^{ab}	3,25 ^{ab}
0,4	0,00	3,30 ^a	3,67	3,47 ^{ab}	3,53 ^{ab}
	0,10	3,73 ^{ab}	3,40	3,93 ^{ab}	3,40 ^a
	0,15	3,33 ^a	3,47	3,93 ^{ab}	3,47 ^{ab}
	0,20	4,13 ^{ab}	3,60	3,67 ^{ab}	3,87 ^{ab}
	0,25	3,40 ^{ab}	3,53	3,07 ^a	3,20 ^a

Table 8. Sensory Test of Cured Duck Meat

		Smell (ns)	Color	Texture	Flavore	Whole
Basic Material		3,67	4,40 ^b	3,13 ^{ab}	3,33 ^{ab}	3,53 ^{ab}
Curcumin (%)	STPP (%)					
0,30	0,00	3,20	3,80 ^{ab}	3,80 ^{ab}	4,33 ^{bcdef}	4,20 ^{abc}
	0,10	3,33	3,33 ^{ab}	3,19 ^{ab}	3,13 ^a	3,47 ^a
	0,15	3,93	3,60 ^{ab}	2,73 ^a	3,93 ^{abcde}	3,73 ^{ab}
	0,20	3,27	3,13 ^a	2,80 ^a	3,47 ^{abc}	3,30 ^a
	0,25	3,67	3,44 ^{ab}	3,47 ^{ab}	3,80 ^{abcd}	3,93 ^{abc}
0,4	0,00	3,13	3,27 ^a	4,20 ^{ab}	4,67 ^{def}	4,13 ^{abc}
	0,10	4,20	3,13 ^a	4,27 ^b	5,00 ^{ef}	5,00 ^c
	0,15	4,04	3,47 ^{ab}	4,33 ^b	5,13 ^f	4,67 ^{bc}
	0,20	4,07	3,07 ^a	3,53 ^{ab}	4,60 ^{cdef}	4,40 ^{abc}
	0,25	4,13	3,40 ^{ab}	3,20 ^{ab}	4,60 ^{cdef}	4,40 ^{abc}

While the most preferred colour of cooked cured meat was achieved by adding STPP at 0.2% and curcumin at 0.3% to 0.4%. Preferred texture of the cooked cured meat is achieved by addition of curcumin 0.3% and STPP at 0.1 to 0.2%, and curcumin at 0.4% with STPP at 0.2% to 0.25%. While the preferred taste of cooked cured meat is with curcumin added at 0.3% and STPP from 0.1% to 0.25%, and curcumin at 0.4% with STPP from 0.2% to 0.25%. On the whole the most preferred cooked cured duck meat is with an addition of curcumin at 0.3% with STPP from 0.1% to 0.2%, and curcumin added at 0.3% with STPP at 0.20% to 0.25%.

Conclusion

The results of this study concluded that raw duck meat texture was based on resistance to force and deformation, which were not significantly different. Resistance to force was between 545,91g and 597,76g and the deformation was between 70,47% and 73,03%. Water holding capacity of cured meat was between 40,05% and 46,77%, which is higher than fresh meat at 26,81%. However, the color of the

cured duck meat was not as bright as fresh duck meat, as shown by the lightness (L) of between 34,04 and 36,46 against 34,43 for the fresh duck meat, and yellowness (b) of between 21,43 and 22,98 for cured meat and 22,49 for fresh meat. The research showed that the duck meat cured with curcumin extract at 0.3% and 0.4% concentrations and added with 0:10 to 0:20% STPP resulted in the most acceptable product, based on texture, color and water holding capacity, especially the brighter color and softer texture.

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References

- Abdel-Ad Aal, H.A. and H.M.A. Mohamed. -. Change in the ultrastructure of lamb muscles as influenced by freezing and sodium tripolyphosphate (STPP). Department of Food Science, Faculty of Agriculture, Minia University, El-Minia, Egypt. *Lucraristititific*. Vol. 56. SeriaZootehnie.
- Ali. M.S., G.H. Kang, H.S.Yang, J.Y. Jeong, Y.H. Hwang, G.B. Park dan S.T. Joo. 2007. A Comparison of meat characteristics between duck and chicken breast. *Asian-Aust. J. Anim. Sci.* 20 : 1002-1006.
- AOAC, 1990. *Official Methods of Analysis Association Official Agricultural Chemistry*. Washington D.C.
- Baggio,S.R. dan N. Bragagnolo, 2006. Cholesterol oxide, cholesterol, total lipid and fatty acid content in processed meat products during storage. *LWT*. 39 : 513-520.
- Dewanti, T. 2009. STPP PenggantiBoraks (Bleng) pada Krupuk Puli danBakso, <http://terminalcurhat.blogspot.com/2009/03/stpppenganti-boraks-bleng-pada-krupuk.html> diakses 6 Januari 2016
- Dewi, S.H.C. 2010. Sifat Fisik Daging Sapi pada Lama Penyimpanan yang Berbeda. Laporan Penelitian. Universitas Mercu Buana. Yogyakarta.
- Dewi, S.H.C. 2011. Populasi Mikroba dan Sifat Fisik Daging Sapi Beku pada Lama Penyimpanan yang Berbeda. Laporan Penelitian. Universitas Mercu Buana. Yogyakarta.
- Dewi, S.H.C. dan Astuti,N. 2013. Ekstrak Kurkumin Kunyit Untuk Menghambat *Off-Flavor* Daging Itik Selama Pangolahan. Laporan Penelitian. LPPM. Universitas Mercu Buana Yogyakarta.
- Dewi, S.H.C. dan Astuti,N. 2014. Akseptabilitas dan Sifat Daging Itik Afkir yang Dilakukan Curing Menggunakan Ekstrak Kurkumin Kunyit untuk Menghambat Oksidasi lemak Selama Penyimpanan. *Jurnal Agritech*. Fakultas Teknologi Pertanian. Universitas Gadjah Mada. Yogyakarta. Vo. 34 (4) : 415-421.
- Fennema, O.R., 1996. *Principles of Food Science*. Marcell Dekker Inc. New York.
- Fujiwara,H., M. Hosokawa, X. Zhou, S. Fujimoto, K. Fukuda, K. Toyoda, Y. Nishi, Y. Fujito, K. Yamada, Y. Yamada., Y. Seino and N. Inagaki. 2008. Curcumin Inhibits Glucose Production in Isolated Mice Hepatocytes. *Diabetes Research and Clinical Practice*. 80 : 188-191.
- Gacula, M.C. dan J. Singh, 1984. *Statistical Methods in Food and Consumer Research*. Academic Press, Inc. Orlando. San Diego. New York. London.
- Hardjosworo. 2001. *Ternak Itik*. Penebar Swadaya. Jakarta.
- Jayaprakasha, G.K, L. Jaganmohan Rao , K.K. Sakariah. 2006. Antioxidant activities of curcumin, demethoxy curcumin and bisdemethoxy curcumin *Food Chemistry* 98 : 720–724.
- Marsha, L.S., J.R. Clause, N.G. Marriots, W.N. Eigel, H. Wang. 2013. Reduction in Lipid Oxidation by Incorporation of Encapsulated Sodium Tripolyphosphate in Ground Turkey. *Meat Science*. 95: 376-380.
- Marsono, Y., Safitri, R. dan Noor, Z., 2005. Antioksidan dalam Kacang-kacangan : Aktivitas dan Potensi serta Kemampuannya Menginduksi Pertahanan Antioksidan pada Model Hewan percobaan. Laporan Penelitian Hibah Bersaing XII.
- Petracci,M., M. Bianchi, S. Mudalal, C. Cavani. 2013. *Functional Ingredients for Poultry Meat Products*. Trend in Food Science and Technology. 33 : 27-39.
- Pratiwi, Y.D. 2016. Pengaruh kadar *sodium tripolyphosphate* food grade (STPP FG) terhadap kualitas daging broiler. Skripsi. Universitas Mercu Buana Yogyakarta. Yogyakarta.
- Sampaio, G.r., T. Saldanna, R.a.M. Soares, E.A.F.S. Torres. 2012. Effect of natural Antioxidant Combinations on Lipid oxidation in Cooked Chicken Meat During Refrigerated Storage. *Food Chemistry*. 135 : 1383-1390.
- Soeparno. 2009. *Ilmu dan Teknologi Daging*. 4th edition. GadjahMada University Press. Yogyakarta.
- Yuanita L., Surodjo S., dan Wikandari P. 1997. *Pengaruh Penggunaan Alkali Fosfat Sebagai Pengganti Boraks Terhadap Kualitas Daging Olahan*. Laporan Penelitian. Lembaga Penelitian IKIP Surabaya.
- Yuanita, L. 2008. Penentuan Kadar STPP *Food Grade* Untuk Meningkatkan Masa Simpan Ikan Nila Tilapia (*Oreochromis niloticus* L.). *Jurnal Berkala Penelitian Hayati: 13 (179–186)*.

The Improvement of Nutritional Value of Analog Rice from Lesser Yam Tubers (*Dioscorea esculenta* L.) Enriched with Soybean Flour

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Abstract

The research to produce of analog rice had been conducted in the previous year with extrusion techniques. The most preferred analog rice by consumers was made from the proportion of lesser yam : mocaf flour 90%:10% using 2% of carrageenan. In addition to the availability of rice as staple food, problem in Indonesian population is lack of protein intake, which lead to malnutrition especially in toddlers. One solution to fight malnutrition is adding important nutrients into food, which commonly called fortification. The fortification can be made by coating and premix/mixing. The fortifications by coating on the surface of the rice grains were judged ineffective because it would be loss when washed and soaked. Therefore, this research was done by fortification on analog rice by mixing with soybean flour. The aims of fortification is to improve the nutritional value of protein in analog rice produced. The addition of soybean flour on this research were 0%, 1%, 2%, 3%, 4% and 5%. The data obtained were analysis by ANOVA. If there was a noticeable difference between the treatments, further analysis with LSD (Least Significant Different Test) were carried out. The results in this research showed that increasing the addition of soybean flour can increase the nutritional value of protein in analog rice from 6.704% to 8.098%. The addition of soybean flour cause a decrease starch levels in analog rice from 85.582% to 84.108%, while increased amylose from 19.514% to 20.05% and declined of amylopectin from 66.069% to 64.057%; an increase of the rehydration power on analogue rice were found from 34.44% to 53.33%; an increase of expansion of volume were measured from 165.56% to 177.78%. The highest sensory score of analog rice was the treatment F3 wicth the score of color is 3.35, taste is 2.95 and texture is 2.40.

Keywords: analog rice, fortification, lesser yam, soybean

Introduction

Lesser yam (*Dioscorea esculenta* L.) is one of the many local species of plants grow in various regions of Indonesia, and usually found as wild plant in the garden and in the forests. The tuber of this plant is usually utilized as a source of carbohydrate as an alternative of rice, but the utilization is still very limited. The superiority of lesser yam compared to other crops are easily grown on non-irrigated land and the critical land, without intensive farming and also can be grown as intercrops (Gsianturi, 2003).

Tubers are generally contain high levels of carbohydrates, but low protein content compare with cereals. Because of the high content of carbohydrates, these tubers known simply as a cheap source of calories. In addition to the primary function as a source of carbohydrate and calories, tubers are also contain bioactive compound and micronutrients.

Development of analog rice from lesser yam tubers is one effort to create an innovation based on

local food source available, and providing alternative food that is cheap and nutritious, so it can be beneficial to strengthen food self-sufficiency. Previous research that has been done were development of analog rice from lesser yam tubers using seaweed and carrageenan as ingredients (Winarti, et al., 2016a), as well as the development of analog rice using mocaf flour (Winarti, et al., 2016b). In this research, the analog rice was made from lesser yam tuber and were fortified with soybean flour to increase nutritional value of the product.

In addition to the availability of rice as staple food, problem which arise in Indonesian population is the lack of protein intake, leads to malnutrition especially in children. Solution to the problems is by adding nutrients into food, which commonly called as the fortification. The fortification can be made by coating and premix/mixing. The fortifications by coating on the surface of the rice grains were known as ineffective because it would be loss when washed and soaked. Thus, the fortification with the premix method becomes one of effective solutions.

The premix method can be applied in the processing of analog rice from lesser yam tubers which then the shape was recreated to be similar with rice. The substance used as fortification agents are trapped in the matrix of analog rice so that it is not lost on the process of leaching. In the process of production of analog rice, binding agent such as alginat, agar and carrageenan can be added for binding the nutrients substance.

The addition of carrageenan is expected to improve the texture of cooked analog rice to become more soft and sticky. The texture, appearance, flavor and color are important parameters and must be observed in the production of analog rice. Some of the ingredients that are added could affect the consumer preferences. The quality of the cooked rice also largely determined by the nature of the physico-chemical of rice such as temperature of starch gelatinization, the volume expansion, viscosity, water absorption and consistency of starch gelling (Purwani, 2001).

The research objective were to determine the effect of enrichment with soybean flour on nutritional quality and consumer preference of analog rice from lesser yam tubers.

Material and Methods

The main material used for this research was lesser yam tubers (*Dioscorea esculenta* L.) which was obtained from the Sawahan, Nganjuk region, East Java. Supporting materials were mocaf, corn starch, soybean flour and carragennan. Meanwhile, the equipment used in this research includes cabinet dryers, single screw extruder, disk mill, plastic strapping, vacuum sealer, soaking tub, strainers, cans and plastic tools.

The procedure of analog rice was as follows. In brief, lesser yam flour was mixed with other ingredients such as corn starch, mocaf flour, skim milk, vegetable oil and GMS. Then, different proportion of soybean flour were added at concentrations of 0%, 1%, 2%, 3%, 4% and 5% from the total weight of the dough and mixed thoroughly until homogeneous. The flour mixture/dough was then formed to granules of rice shaped analog with a single screw extruder (length 1.8 m, with diameter of 2mm) at a temperature of 100°C. Analog rice were then dried at 60°C, for 24 hours.

Hedonic scale scoring test was used to evaluate the sensory quality of cooked analog rice with 20 consumers as panelists. Range value in this test was 1 until 5, in which 1 is dislike it very much to 5

which is like it very much.

Results and Discussion

Protein Content

The results in this research showed that the enrichment of soybean flour gave significant difference effects on protein levels of analog rice ($p \leq 0.05$). The increase of the amount of soybean flour added leads to increase the protein levels on analog rice. The results of the protein content on analog rice is presented in Table 4.1.

Table 4.1. The average of protein levels on analog rice which fortified with soybean flour.

The addition of soybean flour (%)	Protein Content (%)
0	6.704±0.06 ^a
1	6.809±0.02 ^b
2	7.456±0.01 ^c
3	7.906±0.02 ^d
4	8.051±0.02 ^e
5	8.098±0.03 ^e

Note: different letters following the values in each column indicates significantly different ($p \leq 0.05$)

From Table 4.1, it can be seen that the addition of soybean flour can significantly increase the levels of protein on analog rice. This is because soybean contains high levels of protein that is an average of 35% (Koswara, 2009). Moreover, for superior varieties of soybean, can contain protein levels of 40-50%.

Starch, Amylose and Amylopectin content

The result in this study show that levels of starch, amylose and amylopectin on analog rice from lesser yam tubers enriched with soybean flour are different. Starch, amylose and amylopectin content on analog rice is presented in Table 4.2.

Table 4.2. The average content of starch, amylose and amylopectin on analog rice fortified with soybean flour

The addition of soybean flour (%)	Starch content (%)	Amylose content (%)	Amylopectin content (%)
0	85.582±0.48 ^b	19.514±0.6 ^a	66.069±0.93 ^c
1	84.847±0.46 ^{ab}	19.549±0.31 ^a	64.798±0.87 ^{abc}
2	85.330±0.08 ^{ab}	19.581±0.25 ^a	65.749±0.17 ^{cb}
3	84.432±0.34 ^{ab}	19.436±0.26 ^a	64.996±0.08 ^{abc}
4	84.627±0.04 ^{ab}	20.080±0.24 ^a	64.547±0.27 ^{ab}
5	84.108±0.28 ^a	20.051±0.07 ^a	64.057±0.21 ^a

Note: different letters following the values in each column indicates significantly different ($p \leq 0.05$)

The results showed that the addition of soybean flour can effect on the levels of starch, amylose and

amylopectin of analog rice ($p \leq 0.05$). Increasing the levels of soybean flour could significantly decrease the starch and amylopectin contents, though it was not significant for amylose levels (Table 4.2). This is because the material balance of the nutritional value in the ingredients of analog rice. The higher the percentage levels of proteins and other components in a material, leads to the lower the percentage levels of other materials, including the levels of starch. This is in accordance with the opinion of Winarno (2002), which states that the proportion of components in foodstuffs is in balance condition, if one of the components in a material increase then it will cause a decrease in the other components, and vice versa.

Composition of the starch are amylopectin and amylose, both are mutually inversely proportional, that is in food formulation, if the amylose content is increase, the amylopectin levels will decrease, and vice versa. This is in line with the results obtained in this research, which the levels of amylopectin in analog rice decreased by the increase of amylose content.

Rehydration Power

Rehydration power/water absorption of analog rice was determined to know the ability of the rice to absorb water when rice is cooked. Water absorption can also be used to predict the shelf life of analog rice and appropriate packaging methods. Water absorption is influenced by several factors, among others, long-chain of starch, the number of polar groups or hydroxyl groups, the surface area of powder and water content (Hariyadi, 2011).

The results in this study showed that the increase of soybean flour levels were responsible for the increase of the rehydration power, but not significantly different (Table 4.3.). This is due to the fact that the increase of soybean flour cause to the increase of amylose levels on analog rice. Amylose has ability to bind the water easily and also to release again, so analog rice with high amylose levels will easily absorbs water so that increased the rehydration power.

Tabel 4.3. The average of rehydration power on analog rice fortified with soybean flour

The addition of soybean flour (%)	Rehydration Power (%)
0	34.44±0.33 ^a
1	33.33±0.67 ^a
2	34.44±0.67 ^a
3	35.56±0.33 ^a
4	42.22±0.33 ^a
5	50.33±0.67 ^a

Note: different letters following the values in each column indicates significantly different ($p \leq 0.05$)

Expansion Volume

The expansion volume of analog rice is the ability of rice to swell after steaming. Expansion volume has an important role to the quality of analog rice. The mechanism of expansion of analog rice is when rice is soaked in water and heated, the rice will absorb the water and the process of gelatinization of the starch is occurred. Starch properties can trap water to form a three-dimensional network. Therefore leads to the expansion of the volume.

The results from this research show that addition of soybean flour can increase the expansion volume on analog rice (Table 4.4), although do not significantly different. The increase of expansion volume could be caused by the denaturation process of protein from soybean flour when heated. When protein is denatured, it will form a three-dimensional gel which is capable of trapping the water. The protein also has the ability to spontaneously absorb water from the environment containing liquid/water absorption (Joon, 2011).

Table 4.4. The average of expansion volume on analog rice fortified with soybean flour

The addition of soybean flour (%)	Expansion Volume (%)
0	165.56±0.67 ^a
1	167.78±0.67 ^a
2	168.89±0.33 ^a
3	170.11±0.33 ^a
4	171.11±0.67 ^a
5	177.78±0.33 ^a

Note: different letters following the values in each column indicates significantly different ($p \leq 0.05$)

Sensory Quality

The quality of food can be measured by three parameters which are chemical, physical and sensory. The acceptance of food products by consumers is determined by many factors, especially sensory quality. Sensory properties are the properties of the materials scoring by human senses, namely the senses of sight, smell and taste.

The results showed that the highest preference of taste, texture and color of the cooked analog rice by consumer is the analog rice with 3% of soybean flour (Table 4.5). The addition of 3% of soybean flour can enhance the taste due to the protein in soybean flour. The preference on texture could be due to the chewy texture of analog rice enriched with 3% of soybean flour. Heating of soybean protein caused the denaturation of proteins results in the formation of chewy texture. The relationship between the additions of soybean flour with the consumers preference are presented in Figure 4.1.

Table 4.5. Sensory quality on analog rice fortified with soybean flour

The addition of soybean flour (%)	Average of Color	Average of Taste	Average of Texture
0	2.75 ^{ab}	2.35 ^a	2.65 ^{bc}
1	2.45 ^a	2.65 ^a	2.30 ^{ab}
2	2.55 ^a	2.60 ^a	2.50 ^{ab}
3	3.35 ^b	2.95 ^a	3.40 ^c
4	2.65 ^{ab}	2.80 ^a	2.85 ^{bc}
5	2.95 ^{ab}	2.40 ^a	2.10 ^a

Note: different letters following the values in each column indicates significantly different ($p \leq 0.05$)

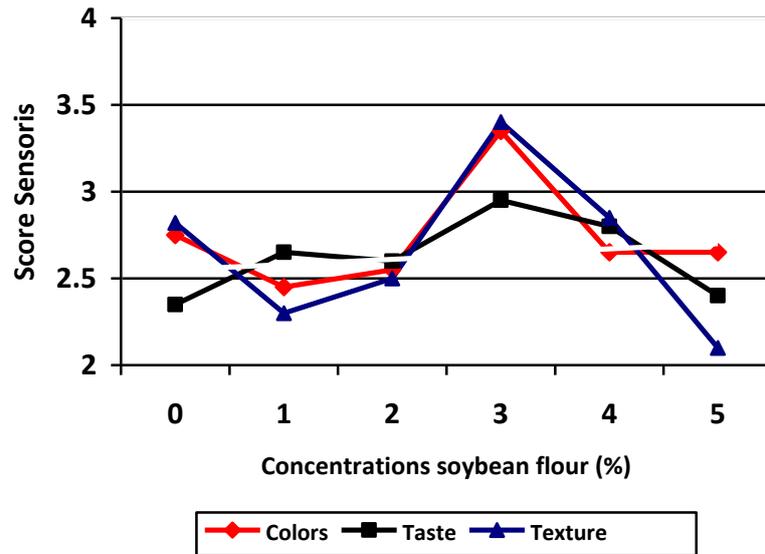


Figure 4.1 The quality of colors, taste and texture on analog rice fortified with soybean flour

Conclusions

The results showed that the increase of soybean flour levels can increase the levels of protein, amylose, rehydration power and expansion volume of analog rice. Starch and amylopectin levels on analog rice from lesser yam tubers werw decreased. The addition of 3% of soybean flour on analog rice is the most preferred by consumers.

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References

- Budi, F.S., Hariyadi, P., Budijanto, S. and Syah, D. 2013. Extrusion Process Technology of Analog Rice. PANGAN, Vol. 22 No. 3: 263-274.
- Gaggia, F., Mattarelli, P. and Biavati, B. 2010. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology 141:515-528.
- Gibson, G.R., 2004. Prebiotics. Best Practice & Research Clinical Gastroenterology. Vol.18, No.2, pp.287-298. (Chapter in book).
- Gsianturi, 2003. Strengthening Food Security With Tubers. Article presented at National Food Industry, Technology Expert Association Food Indonesia.
- Haryadi, 2001. Modified Cassava Flour Technology. AGRITECH, Vol. 31:2;86-92.
- Marsono, Y. 2002. The glycemic index some tubers. Article presented at National Food Industry, Technology Expert Association Food Indonesia, Surabaya 10-11 October 2002.
- Roberfroid, M.B., 2005. Introducing inulin type fructans. British Journal of Nutrition, 93, Suppl.1, S13-S25.
- Setiawati, N.P., Santoso, J., Purwaningsih, S. 2014. THE CHARACTERISTICS OF ARTIFICIAL RICE WITH SEAWEED *Eucheuma cottonii* ADDITION AS A DIETARY FIBER SOURCE. Journal of Tropical Marine Science and Technology, Vol. 6, No.1:197-208.
- Subagio, A. 2007. Industrialisasi Modified Cassava Flour (MOCAF) as raw material to Food Industry to support the diversification

of the national food staple. Food Technology Faculty, Jember University.

- Umar, M. 2013. MOCAF Flour as a substitute for wheat flour. <http://allinonenewfree.blogspot.com/2013>.
- Winarti, S., E. Harmayani and R. Nurismanto. 2011. Ekstraksi of Inulin From Various Yam Tuber (*Dioscorea* spp.), article presented in AFC (Asian Food Conference), Bangkok 15-19 June 2011.
- Winarti, S., dan E.A. Saputro. 2013. Characteristics of prebiotic flour from yam tubers (*Dioscorea* spp.). Journal of Chemical Engineering, Vol.8, No.1: 17-21.
- Winarti, S.; Harmayani, E and Nurismanto, R. 2011. Characteristics and Profile of Inulin from Various Yam Tubers (*Dioscoreas* pp.). AGRITECH, Vol.31, No.4: 378-383
- Winarti, S., E.Harmayani and R.Nurismanto. 2011. Ekstraksi of inulin from various yam tubers (*Dioscorea* spp.), article was presented in AFC (Asian Food Conference), Bangkok 15-19 June 2011.
- Winarti, S., Harmayani, E., Marsono, Y., and Pranoto, Y. 2013. Effect Foaming on Drying Inulin from Lesser Yam Tubers (*Dioscorea esculenta*) to Phisichochemical Characteristics Prebiotics Activity. AGRITECH, 33(4):311-319.
- Winarti, S., Harmayani, E., Marsono, Y., and Pranoto, Y. 2013. Effect of inulin isolated from lesser yam (*Dioscorea esculenta*) on the growth of probiotics bacteria and SCFA formation during fermentation. International Research Journal of Microbiology (IRJM), Vol 4(2): 53-63.
- Winarti, S., Harmayani, E., Marsono, Y., Pranoto, Y., Nishi, K., and Sugahara, T. 2014. Immunostimulatory and Prebiotic Activities of Inulin Extracted from Lesser Yam Tuber (*Dioscorea esculenta*). Bali International Seminar on Science and Technology (BISSTECH 2). UPNV Jatim and STIKOM Bali. Denpasar, 2-4 September 2014.
- Winarti, S., Harmayani, E., Marsono, Y., and Pranoto, Y. 2014. Quantification of Colonic Microbiota Sprague Dawley Rats with Diet Containing Lesser Yam Inulin by Florescent in Situ Hybridization (FISH) Method. International Convergence Food for a Quality Life. SEAFast CENTER and PATPI. Jakarta, 15-16 Oktober 2014.
- Winarti, S., Susiloningsih, E.K.B., and Hidayat, R. 2016. Substitution Effect of Mocaf (Modified Cassava Flour), Carrageenan and Seaweed on Properties of Analog Rice from Lesser Yam Tubers (*Dioscorea esculenta* L.). article was presented in FIAC (Food Innovation Asia Conference), Bangkok 16-18 June 2016.

Do Indonesian Teenage and Young Adult Females Consume Foods Adequately?

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Abstract

Teenage and young adult females (TYAF) are currently one of the main target groups of nutrition and health programs in developing countries. Iron tablet supplementation is one of the popular nutrition intervention program for TYAF while a food based education intervention is rarely studied since no adequate information on which food groups are inadequately consumed by TYAF. The aim of this study is to analyze the mean actual intake of the major food groups and the prevalence of TYAF inadequately consumed each major food group as recommended by the new Indonesia dietary guideline. This study was a cross sectional study using secondary data from the Total Diet Study 2014 of Ministry of Health Indonesia. The subjects included 15583 healthy women aged 15-29 years old. The foods were categorized into five major food groups: cereals and tubers, animal protein foods, plant protein foods, vegetables, and fruits. The adequacy level of each major food group intake was categorized into – excessive (>110%), adequate (90-110%), inadequate (80-90%), and severe inadequate (<80%). The results show that the mean actual intake of animal protein foods, plant protein foods, vegetables, and fruits among TYAF were below the recommendation, among teenage females group were 112.3±102.9 g; 35.3±68.6 g; 41.4±48.6 g; 22±61.9 g, respectively, and among young adults females group were 120.3±111 g; 40.6±75 g; 53.1±56.2 g; 26.5±66.1 g, respectively. The prevalence of TYAF who had inadequate and severe inadequate level intake of animal protein foods, vegetables, and fruits among teenage females were 55.2%; 80.2%; 99.4%; and 90.0%, respectively, while among adult females were 50.6%; 76.5%; 98.9%; and 92.0%, respectively. Teenage and young adult females in Indonesia were inadequately consumed animal protein foods, plants protein foods, vegetables, and fruits. In conclusion, this implies that they are prone to quality protein and micronutrient deficiency.

Keywords : teenage, young adult, female, adequate food

Introduction

The most important period for women before pre-pregnancy and pregnancy are in teenage and young adult age. During this period, dietary intake should be balanced and adequate to achieve optimum status of the nutrition and health for giving birth (Martorell *et al.*, 1992; Merchant and Kurz 1993; Bartley *et al.*, 2005; Koletzko *et al.*, 2011). Poor dietary intake can cause teenage and young adult females (TYAF) undernourished then if this continuously happened during pregnancies, it could lead to low birth weight infants. Pregnant teenage were more likely have about twofold increased risk of preterm births and low birth weight infants compared with pregnant women (Cordeiro *et al.*, 2012; Sharma *et al.*, 2003; Gillespie *et al.*, 2001).

TYAF in Indonesia are the priority target of nutrition and health programs. Iron supplementation programs have been initiated by the Indonesia's government since 1970 to combat anemia, however the prevalence of anemia among TYAF still remains the same (Briawan 2008). The prevalence of anemia among teenage and young adults aged 15-24 years old were rising from 6.9% in 2007 to 18.4% in 2013, and the trend of the proportion of anemic women were higher than men (Riskesdas 2013; Riskesdas

2007). TYAF are susceptible to anemia so that iron supplementation becomes the common nutrition intervention program for these groups. However, the anemia is not only consequence of iron deficiency, but also causes of a wide variety micronutrient deficiency such as folic acid, vitamin B₁₂, and vitamin A or other nutrients (Nelms *et al.*, 2010; Lynch 2007). Most of the coexisting micronutrient deficiencies in developing countries are affected by the disappointing responses often observed with single micronutrient supplements. Consequently, there is increasing emphasis on food-based approaches (Gibson 2011).

Dietary diversification was one of natural food-based approach, since the malnutrition due to lack not only consumption of nutritious food but also a variety of food. The food based education program in a promoted variety of food often disregarded and become unfinished agenda. The domination of one food groups could affect inadequacy intake of other food groups (Sari and Rosa 2015; Rahmawati 2015; Achadi 2014; Marwati 2013; Hardinsyah 2007). Indonesian Dietary Guidelines were updated in 2014 and contains of food groups intake recommendation which could fulfil daily nutrition adequacy for the specific age and gender. The guideline also suggests consuming a variety of foods to achieve balance nutrition. There are no latest studies on the inadequacy of food intake among TYAF. Therefore, this study aims to analyze the daily average intake of the major food groups and the prevalence of inadequacy of the major food groups intake in TYAF.

Methods

Design and subjects

The design of this study was a cross-sectional study, using secondary data conducted by the National Institute of Health Research and Development (NIHRD), Ministry of Health Indonesia. The data were collected in 2014 from all provinces in Indonesia. The subjects of this study were taken from individual samples of Individual Food Consumption Survey (IFCS) which was part of the Total Diet Study (TDS) 2014. The subject's criteria of this study were 15-29 years old females in a good health condition while the exclusion criteria was a subject who pregnant and breastfeed condition at the same time. The total of the subjects after exclusion process were 15583.

Food group intake analysis

Individual food consumption data were collected by 24 hours recall. After that, all the food serving list was converted into their raw form (uncooked) and assigned those ingredients into 5 five major food groups consist of: cereals and tubers; animal protein foods, plant protein foods, vegetables, and fruits. For example, if they consumed *Gado-gado* (Indonesian salad), tempeh and peanut sauce considered as plant protein foods while kale and spinach considered as vegetables. Foods that were not consumed as mixtures were assigned directly to their appropriate their food group. Each food group was described based on the proportion of subjects who were consumed (%), mean (g), standard deviation (g), and the median (g) daily intake. Then the average intake of each food group compared to the recommendation. The recommended servings of each food group number were converted into edible gram weights based on method of Indonesia Ministry of Health 2014 (Table 1).

The adequacy level of each food group was determined as the percentage of the food intake over its recommended quantity for the specific age based on the dietary guideline for Indonesian 2014, then it was classified into 4 levels : excessive intake (>110%), adequate (90-110%), inadequate (80-90%), and

severe inadequate (<80%). Based on age, all subjects were divided into two groups: teenage (15-18 years old) and young adult (19-29 years old). The teenage group consisted of 5406 subjects and 10177 subjects for young adult. The proportion subject of adequacy level of each major food group was statistically differenced with Chi-Square and Mann Whitney Test compared between both groups. All statistical analysis was performed using Microsoft Excel 2007 and IBM-SPSS 22 software.

Table 1. Food group recommended daily intake from Indonesia Dietary Guideline, Ministry of Health 2014

Food Group	Food Item	Serving	Weight (g)
Cereals and tubers	Rice or sweet potato	5	200
Animal protein foods	Fish or poultry	3	135
Plant protein foods	Tempeh or tofu	3	150
Vegetables	Spinach	3	300
Fruits	Orange or banana	4-5	200-250

Results and discussion

The food consumption described by the proportion of subjects who were consumed food groups and the mean actual intake of food groups in Table 2. Generally, the results show that only cereals and tubers had the average actual daily intake relatively higher than the recommendation among subjects while four other major food groups were below the recommendation. Actual daily intake of animal protein foods was slightly lower than recommendation, whereas plant protein foods were sharply lower than recommendation. Then, the daily mean actual of vegetables and fruits intakes have the highest difference than recommendation compared with others.

The subjects 15 to 18 years of age had a lower actual mean intake of source of protein foods, vegetables and fruits than subjects 19 to 29 years of age. Then, most of subjects who had single and student status were less consumed animal protein foods, plants protein foods, vegetables, and fruits. Furthermore, subjects with large family had the highest intake of cereals and tubers whereas they had the lowest intake of animal and plant protein foods. Compared to urban areas, subjects who lived in rural area consumed higher cereals and tubers however, they had a low intake of source of protein foods, vegetables and fruits. And regarding education and socioeconomic level the average intake of cereals and tubers declined to their level, whereas animal protein foods and fruits increased. Nevertheless, the mean actual intake of animal protein foods, plant protein foods, vegetables were lower than recommendation in all levels of education and SES. Subjects who had the lowest education and SES level were consumed higher cereals and tubers while they were fewer of consuming sources of protein foods, vegetables and fruits.

Cereal and tubers remained the first choice of food groups in majority among teenage and young adult females (TYAF) while vegetables and fruits became the last choice to consume. The common cereals were consumed among TYAF consist of rice, bread, wheat, and their products which had been the majority staple food. The most of this kind of staple foods was categorized as simple carbohydrates. Despite they were the source of carbohydrate which can contribute the main energy and necessarily support daily activity, the long-term over-intakes of simple-carbohydrates rich food and poor-intakes vegetables and fruits could lead to non-communicable diseases in the future (Lock *et al.*, 2005; Liu *et al.*, 2000).

Table 2. Mean of actual daily food groups intake of TYAF compared to recommended intake

Characteristics	n(%)	Cereal & tubers %(mean±SD)median	Animal protein foods %(mean±SD)median	Plant protein foods %(mean±SD)median	Vegetables %(mean±SD)median	Fruits %(mean±SD)median
Recommendation		200	135	150	300	200-250
Age						
15-18 yo	5406(34.7)	99.9(269.3±152.2)246	85(112.3±102.9)108.8	40.3(35.3±68.6)63.2	79.4(41.4±48.6)40.2	27.6(22±61.9)52.5
19-29 yo	10177(65.3)	99.9(280.8±161.6)250.5	84.6(120.3±111.1)119.4	42.6(40.6±75)68.8	85.4(53.1±56.2)47.4	30.3(26.5±66.1)63
Marital status						
Single	8906(57.2)	99.9(265.1±125.2)240.5	85.8(116.2±82.5)112.8	40.8(36.1±37.4)64.1	81.3(43.6±29.8)41.1	29.2(24.2±35)57
Married	6499(41.7)	99.9(293.1±171.8)264.9	83.3(119.3±113.2)119.8	43.2(42.2±78.2)72	85.8(56.4±60)51	29.5(26.1±66.4)62.3
Widow	178(1.1)	99.4(265.8±157.1)230	84.8(119.4±105.3)114.8	44.9(42.1±71.4)60.5	90.5(52.7±47.7)42.8	28.7(22.8±56.5)52.5
Family size						
Small	7199(46.2)	99.9(275±162.9)249.5	84.9(119.7±110.2)117.3	44(42.7±78.3)71.1	84.1(51.3±54.8)46.8	29.3(25.3±63.3)62
Middle	5830(37.4)	99.9(275.7±152.9)250	85(117±107.7)115.5	42.2(38±70.4)65	82.4(47.2±53.8)43.5	29.2(23.9±61.3)58.4
Large	2554(16.4)	99.8(284.2±157.8)252.5	83.8(112.8±104.2)109.7	35(29.3±60.9)60.5	83(47.3±51.6)43.5	29.6(26.5±75.4)54
Education						
No school	1116(7.1)	99.6(354.7±290.7)280	69(96±116.4)108	24.2(23.6±62.6)67.6	79.1(50.4±59.4)46.4	22.5(26.9±90.1)75
Elementary school	3119(20)	99.9(285.7±163.2)255	78.9(99.6±103.1)100.6	40.6(37±73.7)64.4	82.1(50.1±54.5)46.8	25.6(20.1±61.6)49.4
Junior high school	5193(33.3)	99.9(270.3±139.4)250	84.6(112.1±106)108.9	43.6(41.2±73.3)70	82.2(46.6±52)43.2	27.8(22.1±58.4)56
High school	4995(32.1)	100(264.1±130.9)245.4	89.5(129.5±107)124.6	43.6(40.4±73.6)68	84.8(49.5±53.8)44.4	31.4(26.9±62.9)63.5
Diploma/university	1160(7.5)	100(261.3±128.3)245.7	95.6(159±112.6)145	46.6(39.5±73)56	88.8(54.4±55.7)47.5	44.4(40.4±75.6)69
Job status						
Studying	4367(28)	100(264.8±137.4)242.5	86.9(116.4±101.7)112	42(36.9±69.5)63.1	80.2(42.1±47.7)39.7	29.2(24.4±66.3)57.6
Not working	6835(43.9)	99.9(278.9±145)255	85.1(119.3±111.8)116.6	40.3(38.6±74.6)69.4	83.6(50.3±55.2)46	29.1(23.5±59.6)56.3
Working	4381(28.1)	99.8(285.5±193.5)250	82(116±109.3)118.2	44.1(40.8±73.5)67	85.9(54±57.1)48	29.9(27.7±70.6)66.6
Living area						
Urban	7169(46)	100(263.1±126.9)243	91.1(130±105)120.1	50.5(46.5±76.2)67.1	83.1(45.2±47.1)42	31.4(26.4±62.4)62.2
Rural	8414(54)	99.8(288.4±180.2)255	79.3(106.9±110)109.2	34.4(32.1±69.3)66	83.4(52.4±59)47.4	27.6(23.7±66.7)57
Socio-economic status (SES)						
Low	5745(36.9)	99.8(300.5±197.5)260.5	73.9(94.6±106.2)101.6	33.1(32±71.6)66.8	82.6(52.6±60)47.1	25.3(23.6±72.3)58.7
Middle	6651(42.6)	100(264.1±128)246.5	89.2(120.5±103.6)113.6	47.7(44.9±75.3)70.6	84(47.1±49.2)44	29.6(22.8±54.6)56.3
High	3187(20.5)	100(260.7±129)242.1	95.1(152.9±111.3)141	45.3(38.1±68.8)59	83.1(46.7±51.6)42.3	36(32±69.4)67.5
Total	15583(100)	99.9(276.8±156.9)250	84.7(117.6±108.4)115.4	41.9(38.7±72.9)72.6	83.3(49.1±54)50	29.4(25±64.7)62.3

The intakes of both sources of protein foods among TYAF were below recommendation. It was important to consume both animal and plant protein food adequately because they completed each other. Neumann *et al.* (2002) found that the maternal intake of animal source foods during pregnancy was positively associated with the beginning of infant growth and was the major factors that predicted pregnancy weight gain, birth weight, and birth length. Animal source food supply not only high-quality and readily digested protein and energy, but also a compact and efficient source of readily available micronutrients. In terms of quality protein, plant protein foods were lower than the animal protein food, but it contained a lower proportion of saturated fat than animal food source. In addition, in Indonesia plant protein foods were the complement foods usually consumed with animal protein foods, they certainly were not consumed in large portion. Tempeh is the most common example, it contained isoflavones, fiber, antioxidants, and anti-cholesterol, which can prevent degenerative diseases (Ministry of Health 2014; Karyadi and Lukito 1996).

The prevalence of food groups intake adequacy among teenage and young adult females described by Table 3, it shows that more than 50%, teenage and young adult females were categorized as excessive cereals and tubers intake. Comparing the percentage of adequacy level in cereals and tubers intake between teenage group and young adult group, the statistical result shows that there were no significant differences between both groups in subjects who were categorized as excessive, adequate, and inadequate. However, there were significant differences in subjects who were categorized as severe inadequate.

Table 3. Prevalence (%) of adequacy level of food group intake between teenage (15-18 yrs old) and young adult (19-29 yrs old) females

Food groups	Excessive >110		Adequate 90-110		Inadequate 80-89		Severe inadequate <80	
	15-18 yo	19-29 yo	15-18 yo	19-29 yo	15-18 yo	19-29 yo	15-18 yo	19-29 yo
Cereals and tubers	57.0	60.0	15.8	15.2	5.5	5.6	21.7 ^a	19.1 ^b
Animal protein foods	34.6 ^a	38.5 ^b	10.2	10.9	7.0	6.3	48.2	44.3
Plant protein foods	12.9	15.7	6.9	7.8	4.5	4.4	75.7	72.1
Vegetables	0.3	0.4	0.3	0.6	0.1	0.5	99.3 ^a	98.4 ^b
Fruits	7.0	4.3	3.0	3.7	3.3	2.5	86.7	89.5

^{a,b} *P*-value < 0.05; teenage (15-18 yrs old) versus young adult (19-29 yrs old) females were significantly different

Contrary to cereals and tubers, majority of subject was categorized as inadequate and severe inadequate in consuming animal protein foods, plant protein foods, vegetables, and fruits. Most of teenage females and young adult females were categorized as inadequate and severe inadequate intake of animal protein. There were no significant differences between teenage and young adult females who were categorized as adequate, inadequate, and severe inadequate comparing the percentage of adequacy level

in animal protein food intake between both groups whereas there were significant differences in both groups who were categorized as excessive level. Similar to animal protein foods, more than a half of teenage and young adult females were categorized as inadequate and severe inadequate intake of plant protein foods although there were no significant differences between two groups in all adequacy levels.

Besides that, nearly 100% of teenage and young adult females were categorized as inadequate and severe inadequate intake of vegetables and fruits. They were lack of consuming vegetables, and there were significant differences between these two groups who were categorized as severe inadequate level. Similar to vegetables, there were almost all of teenage and young adult females were insufficiency of daily fruit intake whilst there were no significant differences between two groups in all adequacy levels.

Based on Table 2 and Table 3, this study presents that TYAF were the vulnerable group of inadequate intake of sources protein foods, vegetables, and fruits. TYAF who were not married yet, as a student, living in rural and large family, and had low education and SES level should be priority group of food based education intervention. The previous study based on *the Basic Health Research (Riskesmas) 2010* showed that teenage females (16-18 years old) and young adult females (19-29 years old) were lower daily intake of sources of protein foods, vegetables, and fruits (Rahmawati 2015; Marwati 2010). National study regard anemia in Indonesia showed that teenage female who live in rural with a low educational background and often skip breakfast had a higher risk of anemia than others. When adolescence, teenage faced the menstruation period, which need more intake source foods of iron. Moreover, teenage who lived in rural with a low educational background had limited access to quality of foods and less information regard nutritious foods (Permaesih and Herman 2005).

Poor dietary intake, both in terms of the total quantities of food consumed and the contribution made by micronutrient-rich foods to the diet was the main cause of micronutrient malnutrition. Regarding this study, it was remained a major challenge of meeting recommendation from a diversified micronutrient-rich diet among TYAF. Indonesia, where had majority cases in the micronutrient, were predominantly exist in the context of food insecurity driven by poverty and agricultural underdevelopment. The last national health survey report from Riskesdas (2013) presented that the prevalence of anemia in teenage and young adult group (15-24 years old) was 18.4%. In addition, the Total Diet Study (2014) also reported that majority of the teenage (13-18 years old) and adult women (19-55 years old) had a low energy sufficiency level (<70%) and protein sufficiency level (<80%).

The qualitative study in East Nusa Tenggara found that teenage females (10-19 years old) had an unhealthy habit which caused poor of nutritious and variety foods. They were eating only rice without any vegetables or side dish, eating less desirable food, reducing portion size, skipping meals, saving

pocket money and earning money to buy food, and this had been happening in the long time so that were put them at risk of an insecure food situation (Fatmaningrum *et al.*, 2015). Another study in rural districts of Bangladesh showed that the women of reproductive age (15-49 years old) who were in the middle and high SES level and ability in literacy significantly improved food security and dietary diversity. Women in the highest SES were significantly less likely to experience food shortages; they tend to prioritize expenditure of household resources on food. Moreover, literacy ability could increase nutrition knowledge; they gained more information than women who had not literacy ability (Harris-fry *et al.*, 2015).

Thompson (2007) and Tontisirin (2002) reviewed that in developing countries the food-based interventions tend to be neglected in favor of singular fortification and supplementation programs as they are considered attractive in their apparent simplicity and cost-effectiveness. In practice, however, many such programs are proving difficult to manage, more costly than expected to implement, and less effective than promised. Consequently, for combating iron and other micronutrient deficiencies, it is important to ensure which the comprehensive approach is taken and which dietary diversification is recognized as essential.

The teenager could be as key the priority target of food-based intervention through nutrition education approach. When teenage in adolescence period, they were in the risk taking period, which can have dire consequences on adult health (Lassi *et al.*, 2015). Teenage females should be at the heart of a life course approach because soon they will become a mother. It is also necessary for young adult females; they need improvement of quality dietary intake to raise health and nutritional status. Finally, enhancing in the health and nutritional status among TYAF, means provide good health and nutrition status for their children and families in the future (Branca *et al.*, 2015). Investing in food-based intervention for TYAF would optimize the chances of improving pregnancy outcome, growth, and cognitive development of the next generation. Therefore, actions need to be taken to prevent and control quality protein and micronutrient deficiencies among TYAF.

Conclusions

TYAF in Indonesia were inadequately consumed animal protein foods, plants protein foods, fruits, and vegetables. Improving their intake through greater consumption of quality protein foods, fruits and vegetables as the preferred intervention must be placed high on the food development policy programs. Furthermore, using these data might explore the food based recommendation formulation with Linear

Programming tools to achieve specific major dietary goals such as choosing foods which giving more contribution to nutrient deficiency among both the target groups. This findings could also inspire innovation for food product development through fortification as well as bio-fortification to increase food quality.

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References

- Martorell R., Rivera J., Kaplowits H., Pollitt E., 1992. Long-Term Consequences of Growth Retardation During Early Childhood, Hernandez M., Argente J, Editor. Excerpta Medica, New York, p. 143-149.
- Merchant KM.and Kurz, KM., 2014. Women's Nutrition Through The Life Cycle: Social and Biological Vulnerabilities: The Health Of Women: a Global Perspective, Koblinsky MA., Timyan J., Gay J., 1993, Editor. Westview Press, Boulder, Colo, p. 63-90.
- Bartley K., Underwood BA.,Deckelbaum JR., 2005. A Life Cycle Micronutrient Perspective for Women's Health. Am J ClinNutr Vol. 81 (suppl), 1188s-93s.
- Koletzko B., Brands B., Demmelmair H., 2011. The Early Nutrition Programming Project (Earnest): 5 Y Of Successful Multidisciplinary Collaborative Research. Am J Clin Nutr. 94 (suppl), 1749s-53s.
- Cordeiro LS., Wilde PE., Semu H., 2012. Household Food Security is Inversely Associated with Undernutrition among Adolescent in Kilosa, Tanzania. J Nutr, p. 1 - 7.
- Sharma AK.,Chabra P., Gupta P., 2003. Pregnancy in Adolescents: A Community Based Study, Indian. Journal Preventive and Social Medicine, Vol 34, p. 24 - 32.
- Gillespie S., 2001. Empowering Women to Achieve Food Security: Health and Nutrition. Policy brief 8 of 12, available at: <http://ageconsearch.umn.edu/bitstream/16032/1/vf010006.pdf> (accessed 10 August 2016)
- Nelms M., SucherKP., Lacey K., Roth SL, 2010. Nutrition Therapy & Pathophysiology. Yolanda, Cossio, p 1-1080.
- Lynch S., 2007. Nutritional Anaemia: Iron Metabolism, Kraemer K., and Zimmermann MB Editor. SIGHT AND LIFE : Switzerland, p. 59-76.
- Gibson RS., 2011. Combating Micronutrient Deficiencies, Food-based Approaches: Strategies for Preventing Multi-micronutrient Deficiencies - a Review of Experiences with Food-based Approaches in Developing Countries, Thompson B. and Amoroso L., Editor. CAB International and Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
- Sari K., and Roshia BC., 2015. Several Dominants Risk Factor Related to Obesity in Urban Childbearing Age Women In Indonesia. *Health Science Journal of Indonesia*, Vol 6 (1): p. 1-68, ISSN 2087-7021, E ISSN 2338-3437.
- Achadi EL., 2014. Critical Period 1000 First Day of Life and The Long-Term Impact for Health, National Seminar PDGMI themed "Nutrition for Sustainable Development Life Course Approach to Address Current Nutritional Issues. Surakarta, Indonesia.
- Hardinsyah, 2007. Reviewed of Food Consumption Variances Determinant Factors. *Jurnal Gizi dan Pangan* Vol 2(2). PERGIZI PANGAN Indonesia, Jakarta.
- Lock K., Pomerleau J., Altmann DR., McKee M., 2005. The Global Burden of Disease Attributable to Low Consumption of Fruit and Vegetables: Implication for The Global Strategy on Diet. *Bulletin of the World Health Organization* Februari, Vol. 83(2).
- Liu S., Willett WC., Stampfer MJ., Hu FB., Franz M., Sampson L., HennekensCH., and Manson JE., 2000. A Prospective Study of Dietary Glycemic Load, Carbohydrate Intake, and Risk of Coronary Heart Disease in US Women. *Am J ClinNutr* 2000, 71: p. 1455-61.

- Ministry of Health Republic of Indonesia, 2014. Indonesia Dietary Guideline 2014. Ministry of Health : Jakarta
- Karyadi D., and Lukito W., 1996. Beneficial Effects of Tempeh in Disease Prevention Treatment,. Nutrition Reviews, Vol 54, No. 11: p. S94-S98.
- [NIHRD] National Institute of Health Research and Development, Ministry of Health Indonesia, 2007 Riset Kesehatan Dasar (Riskesdas) 2007, NIHRD: Jakarta.
- [NIHRD] National Institute of Health Research and Development, Ministry of Health Indonesia, 2013, Riset Kesehatan Dasar (Riskesdas) 2013,NIHRD: Jakarta.
- [NIHRD] National Institute of Health Research and Development, Ministry of Health Indonesia, 2015, Report of Total Diet Study : Individual Food Consumption Survey (IFCS). NIHRD: Jakarta.
- Mawarti SP., 2013. Development of Balance Nutrition Index among Adult Women in Indonesia, Dissertation. IPB : Bogor.
- Rahmawati, 2015. Development of Balance Nutrition Index among Teenage Females in Indonesia, Dissertation. IPB : Bogor.
- Permaesih D., and Herman S., 2005. Factors Influencing Anemia among Adolescent. Bul. Penel. Kesehatan, Vol. 33, No. 4: p. 162-171.
- Fatmaningrum D., Roshita A., and Februhartanty J., 2015. Coping Strategies for Food Insecurity Among Adolescent Girls during the Lean Season in East Nusa Tenggara, Indonesia: A Qualitative Study. British Journal of Nutrition (2016), 116(S1), p. S42–S4, doi:10.1017/S0007114515004092.
- Harris-Fry H., Azad K., Kuddus A., Shaha S., Nahar B., Hossen M., and Younes L., 2015. Socio-Economic Determinants of Household Food Security and Women's Dietary Diversity in Rural Bangladesh: A Cross-Sectional Study. Journal of Health, Population and Nutrition Vol.33(2), doi: 10.1186/s41043-015-0022-0.
- Thompson B., 2007. Nutritional Anaemia: Food Based Approaches for Combating Iron Deficiency, Kraemer K., and Zimmermann MB, Editor. SIGHT AND LIFE: Switzerland.
- Tontisirin K., Nantel G., and Bhattacharjee L., 2002. Food-Based Strategies to Meet the Challenges of Micronutrient Malnutrition in The Developing World. Proceedings of the Nutrition Society (2002) Vol. 61, p. 243-250, doi : 10.1079/PNS2002155.
- [FAO] Food and Agriculture Organization of the United Nations, 2006. The State of Food Insecurity. FAO: Washington, DC.
- [WHO] World Health Organization, 2003. Diet, Nutrition, and The Prevention of Chronic Diseases, WHO: Geneva.
- Lassi ZS., Salam RA., Das J., Wazny K., Bhutta ZA, 2015. An Unfinished Agenda on Adolescent Health: Opportunities for Interventions., Seminar Perinatology. Elsevier Inc., doi: 10.1053/j.semperi.2015.06.005.
- Branca F., Piwoz E., Schultink W., Sullivan M., 2015. Nutrition and Health in Women, Children, and Adolescent Girls. BMJ, Vol. 351 doi: <http://dx.doi.org/10.1136/bmj.h4173>.

The Effects of Cooking Methods on the Chemical (β -Carotene and Water Content), Physical (pH and Color) and Organoleptic (Color, Flavor, Texture) Characteristics of Papaya Leaf (*Carica papaya*, *Linn*)

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Abstract

Food as an essential commodity of human life needs some pre-processing to fulfil its role. Papaya leaves contain high vitamin A and β -carotene but it had bitter taste and hard texture. The aim of this study was to determine the effects of cooking methods on β -carotene and water content, pH, color and organoleptic characteristics of papaya leaf (*Carica papaya*, *Linn*). This experimental study used 2 factors of Randomized Group Design, boiling methods (water and clay water boiling) and types of cooking (urap-urap, sauted, bumbu kuning, buntil, and boiled) with 3 replications. The cooking types and boiling methods significantly changed the water content ($p=0.000$ and $p=0.044$), redness color ($p=0.000$), and organoleptic characteristics (color, flavor and texture) ($p=0.000$) but pHs were not different ($p=0.240$ and $p=0.432$). The best treatment was bumbu kuning boiled with clay water. **Keywords** : cooking, papaya leaves, β -carotene, water content, pH, color, organoleptic

Introduction

Food as an essential commodity of human life needs several pre-processings to fulfil its role. In order to produce safe foods and the benefit from its nutrition could be maximized. Besides, processing was carried out so that the foodstuffs were acceptable, especially in appearance and texture (Apriyantono, 2002). Processing could produce several foods with desirable properties, but on the other hand, processing could also generate the toxic compounds, loss of nutrients, and the changing of organoleptic properties towards less favoured (Apriyantono, 2002).

A review about the treatment caused changes in nutrient contents in food on the effect of home processing and storage against the levels of ascorbic acid and beta carotene on *Bathua* (*Chenopodium album*) and *Fenugreek* (*Trigonella foenum graecum*) leaves shows the nutrients declined significantly due to drying, blanching, and cooking (Yadav and Seghal, 1997). Another study (Moscha *et al.*, 19967) reports that blanching and traditional cooking significantly increase carotenoid levels of *Cowpea* leaves, beans, and pumpkin although in *Amaranth* and sweet potatoes, processing at high temperature could significantly lower carotenoid levels. A similar study shows that beta carotene in red clover leaves damage very quickly on acid medium with aerobic condition, and this loss was comparable to the

increasing of processing temperature (Kalaç *et al.*, 2007). But, another studies state that food processing increase gastric and intestinal of food absorption of 50-95% compared with raw foods (Sisson, 2010).

Papaya leaves contained 18.250 SI of vitamin A (IPTEKnet, 2005) and 11.565 µg equivalent (Duke, 1947) per 100 g higher than carrot, however these vegetables are less attractive because its bitter taste and hard texture. Based on those phenomena, there need a proper handling in the processing of papaya leaves in order to produce acceptable physical and organoleptic quality as well as to minimize the loss of beta carotene content. The aim of this study was to determine the effect of cooking methods on beta carotene and water content, pH, color and organoleptic characteristic of papaya leaves (*Carica papaya*, Linn).

Materials and Methods

Materials

This study using local variety of papaya leaves (*Carica papaya*, Linn) from papaya plantation in Jeru Village, Turen District, Malang and the ingredients to papaya leave processing include oils, herb and spices and water.

Chemicals

Chemicals used to analyze the papaya leaves were providable from Laboratory of Biochemistry and Nutrition Agriculture, Brawijaya University. Chemicals with pro analyzed purity (p.a) include petroleum ether, acetone, alumina, Na₂SO₄ anhidrat, except aquadest with thechnical purity.

Preparation of Processed Papaya Leaves

Papaya leaves were boiled prior to processing. Boiling methods were grouped into two types, i.e. boiling with water and with clay water then papaya leaves were processed into bumbu kuning, buntill, sauted, and urap-urap (all are Javanese traditional cooking methods).

Physicochemical and Organoleptic Properties

This study using experiment methods with two factors Randomized Group Design (RGD) include type of processings and boiling methods. Boiling methods were consisted of boiling with water (P) and clay water (TN). Type of processing were consisted of urap-urap (1); sauted (2); bumbu kuning (3); buntill (4); dan boiled (5). Each treatment were done 3 (three) replicates. Physicochemical properties were analyzed including beta carotene, water content, pH, color (L*,a*,b*) and organoleptic properties include color, flavor, and texture acceptance, so that the best treatment to deliver good characteristics of papaya leaves could be achieved. The organoleptic properties were analyzed using 5 points of hedonic scale (Lawless, 1998). That scales were transformed become numeric scale 1-5 (1= very dislike and 5= very like) (Soekarto, 1985). The assessment of organoleptic properties were done by 45 semi-trained panelists aged 20-28 years old, consist of 7 male and 38 female panelists.

Statistical analysis

The results were analyzed using SPSS 16.0 for windows with two-way Anova with 95% level of

confidence. Interaction of two factors were analyzed using *Duncan Multiple Range Test* (DMRT) then were analyzed the best treatment of processed papaya leaves using De Garmo effectiveness method.

Result and discussion

Characteristics of raw papaya leaves which were analyzed include color (L^*, a^*, b^*) and pH (physical properties), beta carotene, and water content (chemical properties) as well as organoleptic properties (color, flavor, and texture). The results for each treatment on papaya leaves can be seen in Table 1 and Figure 1, 2, and 3.

Table 1. Physicochemical Characteristics of Processed Papaya Leaves

Treatment	Beta Carotene ($\mu\text{g/g}$)	Water Content (%)	pH	Color		
				(L^*)	(a^*)	(b^*)
TN1	42.20 \pm 10.046	69.04 \pm 1.321 ^b	5.96 \pm 0.650	37.40 \pm 1.100	7.27 \pm 0.603 ^c	23.23 \pm 0.416
TN2	58.67 \pm 6.486	83.13 \pm 3.441 ^b	6.41 \pm 0.096	28.63 \pm 0.513	2.77 \pm 0.208 ^e	15.30 \pm 1.253
TN3	64.83 \pm 8.756	66.26 \pm 1.302 ^{ab}	6.32 \pm 0.118	31.60 \pm 1.258	5.47 \pm 1.387 ^d	13.37 \pm 1.250
TN4	40.07 \pm 6.929	69.20 \pm 0.887 ^d	6.44 \pm 0.197	45.87 \pm 2.107	6.70 \pm 1.200 ^a	22.60 \pm 0.755
TN5	52.72 \pm 6.429	88.27 \pm 0.101 ^c	6.57 \pm 0.847	27.70 \pm 1.473	3.20 \pm 0.693 ^a	12.77 \pm 1.361
P1	43.80 \pm 9.291	67.81 \pm 1.383 ^{ab}	5.88 \pm 0.472	37.40 \pm 0.833	4.33 \pm 0.252 ^{cd}	22.67 \pm 1.250
P2	56.29 \pm 3.067	81.72 \pm 3.183 ^b	6.25 \pm 0.571	28.60 \pm 1.153	3.23 \pm 0.416 ^{bc}	14.53 \pm 3.707
P3	62.80 \pm 3.462	62.77 \pm 5.579 ^a	6.38 \pm 0.156	33.17 \pm 1.761	3.63 \pm 0.153 ^{ab}	18.00 \pm 4.073
P4	43.86 \pm 8.942	66.97 \pm 2.007 ^d	6.43 \pm 0.295	38.70 \pm 3.011	4.90 \pm 0.173 ^{ab}	20.47 \pm 1.557
P5	62.62 \pm 6.003	87.23 \pm 0.923 ^c	6.16 \pm 0.720	27.73 \pm 0.551	3.53 \pm 0.839 ^a	13.40 \pm 1.212

All values are means \pm SD
 Boiling methods includes TN = boiled with clay water; P = boiled with water
 Type of processing includes 1=urap-urap; 2=sauted; 3=bumbu kuning; 4=buntil; 5=boiled
 Analyzed using two-way Anova and Post Hoc DMRT (Duncan Multiple Range Test)
^{abcd} Means for groups in homogenous subsets are displayed as Post Hoc DMRT result

Beta Carotene Contents

Beta carotene contents of papaya leaves are significantly influenced with type of processing ($p < 0.05$) while boiling methods ($p < 0.05$) and interaction of both type of processing and boiling methods ($p > 0.05$) does not affect its contents. Boiling methods of papaya leaves did not affect beta carotene content associated with pHs of water clay which were higher than water. Morris *et al.* (2006) report that loss of labile nutrients (include vitamin A and carotenoids) due to processing depends on the presence of heat and other treatment conditions such as pH (Morris *et al.*, 2006). Type of processing of papaya leaves affects beta carotene content associated with heat processing duration that leads to oxidation so that its content declined. Rodriguez *et al.* (2004) state that deep frying for a long time in combination with many preparation and cooking methods, such as baking and pickling, resulting in loss of carotenoids (Purcell *et al.*, 1969). Eskin (1979) reports that carotenoids damage at high temperature due to thermal degradation that result in decomposition when involve an oxidative condition (Eskin, 1979).

Post hoc test showed that there were differences of beta carotene contents in papaya leaves processed into urap-urap and sauted dish compared to boiled dish, buntil, or bumbu kuning. This is associated with the presence of oil in papaya leaves processed into urap-urap and sauted dish from shredded coconut and coconut oil so that beta carotene could be dissolved and declined. Bauernfeind and

Klaul (1981) mention that the main characterization of beta carotene is insoluble in water, ethanol, and methanol but soluble in vegetable oils and its melting point range between 178-184°C. Beta carotene contents of papaya leaves processed into boiled and buntill dish were higher than sauted and urap-urap; this related to boiling and steaming temperatures which was lower than sauteing. Azizah *et al.* (2009) report that during the boiling process, food would be exposed to heat (100°C) lower than stir-frying (180°C) (Azizah *et al.*, 2009). Wasantwisut *et al.* (1995) state that blanching, boiling, and stir-frying in many vegetables produce beta carotene contents of 89-95%, 80-96%, and 58-82%, respectively (Wasantwisut *et al.*, 1995). Beta carotene content in papaya leaves processed into bumbu kuning dish were related to cooking method using coconut milk that can cause beta carotene of papaya leaves to dissolve into coconut milk's lipid; then, it would remain soak the papaya leaves. The presence of beta carotene in coconut milk are also related with the higher of beta carotene content in papaya leaves. Coconut oil contains 10 SI vitamin A (IPTEKnet, 2005).

Water Contents

Water contents of papaya leaves were significantly influenced with both type of processing ($p < 0.05$) and boiling methods ($p < 0.05$) although its interaction did not affect its contents ($p > 0.05$). This are related to the higher concentration of minerals in clay water than those in water due to the higher of clay's mineral contents. Fanning *et al.* (1989) report that the primary mineral in Indonesian farm land are Magnesium, Iron, Calcium, Pottasium, and Sodium (Fanning *et al.*, 1989). Post hoc test using Duncan Multiple Range Test showed that papaya leaves dishes processed into bumbu kuning either boiled in water and clay water and urap-urap boiled in water only were significantly different from the dishes boiled either in water and clay water, aswell as buntill, urap-urap, and sauted underwent boiling in with clay water only. Water contents of sauted dish of papaya leaves boiled in water only were significantly different from those boiled and buntill either in water or clay water. The water contents of boiled papaya leaves either boiled in water or clay water were significantly different from those in buntill either boiled in water or clay water.

The results indicate that papaya leaves processed into bumbu kuning dishes either boiled in water or clay water have the lowest water content. This related to the duration of stewing process resulting in long periode of evapoartion so that its water content decline significantly. Muchtadi and Ayustaningwarno (2010) write that most water molecules in food would be evaporated and they left the empty spaces due to heat treatment. The empty spaces would be filled with lipids so that the papaya leaves are apparently soaked (Muchtadi and Ayustaningwarno, 2010). The results also sugest that the highest water content was obtained from papaya leaves processed into buntill dishes either boiled in water or clay water). This related to the evaporate water that condensed later on due to the cooking of buntill used banana leaves to cover it so that the water moecules were kept trapped inside increasing its water content.

pH Value

The pHs of papaya leaves were not significantly affected by the type of processings ($p > 0.05$), boiling methods ($p > 0.05$) or its interactions ($p > 0.05$). These associated with the facts that were no

differences between water's and water clay's pHs. Besides, there were also no differences between cooking environment (using coconut milk or scraped coconut) so that the pHs were no different either those of processed papaya leaves. pH of coconut milk is 6.10-7.00 whereas pH of fresh coconut is 5.50-7.80 (Center Food Safety and Applied Nutrition FDA-US, 2003).

Brightness (L)*

The brightness values (L*) of papaya leaves were significantly affected by the types of processing ($p < 0.05$) but not boiling methods ($p < 0.05$). This associated with similarity of pHs between water and clay water. The stability of plant pigments is affected by pH, light, and heat (British Nutrition Foundation, 2004). Interaction of both types of processing and boiling methods significantly affected the brightness of papaya leaves ($p < 0.05$).

Post hoc test using Duncan Multiple Range Test showed that the brightness of papaya leaves processed into buntil and boiled dishes were significantly different from those of bumbu kuning, sauted, and urap-urap dishes. The brightness values of papaya leaves processed into bumbu kuning dishes were significantly different from those of sauted and urap-urap dishes and similarly for sauted papaya leaves compared to those of urap-urap dishes. The brightness values of papaya leaves processed into buntil and boiled dishes were the lowest. During the boiling process, water were left to boil first and it covered up the whole surface of papaya leaves. The boiling of vegetable should be done after the water boiled to reduce cooking time, covering it with a pot lid, and make sure that water covering up the surface of papaya leaves to retaining color and flavor (Drummond *et al.*, 1993). The brightness values of papaya leaves processed into bumbu kuning dishes are higher than those of buntil and boiled dishes. These related with acidity of coconut milk that affected color changing. Acidic environment could cause the alteration of color from green to yellow (Vaclavik *et al.*, 2008).

Redness (a)*

The redness values (a*) were significantly affected by the types of processing, boiling methods, and their interaction ($p < 0.05$). This related to mineral composition of clay that the mineraks can oxidize beta carotene so changing *trans* isomer into *cis*. During heating process, cromoplast would dissappear and its caretenoid pigments would be more appearing on the cell wall (Purcell *et al.*, 1969). Post hoc test using Duncan Multiple Range Test showed that the redness of papaya leaves processed into boiled and buntil dishes undergoing both boiled in water and clay water as well as bumbu kuning dishes making from boiled papaya leasves in water only were significantly different from those of urap-urap dishes both they were boiled with water and clay water, bumbu kuning and boiled dishes made from papaya laves boiled in water clay only. The redness of papaya leaves processed into urap-urap dishes from papaya leaves boiled in water only and bumbu kuning dishes using papaya leaves boiled in water clay only were significantly different from those of urap-urap and sauted dishes boiled in water clay only. Herbs and spices could give color and flavor changes to the processed food (Vaclavik *et al.*, 2008).

Yellowness (b)*

The yellowness values (b*) of papaya leaves were significantly affected by the types of

processing ($p < 0.05$). Boiling methods and the interaction of both type of processing and boiling methods insignificantly affected the yellowness. This associated with beta carotene contents of processed papaya leaves, by which the cooking environments might affect the changing of yellowness. The longer the heating process, the more cell would be damaged and severely and most debris would appear as spreaded yellowness color (Purcell *et al.*, 1969). Post hoc test using Duncan Multiple Range Test showed that yellowness of papaya leaves processed into buntil and bumbu kuning dishes were significantly different from those of urap-urap and sauted dishes. Covering food up with a pot lid during brief cooking like stir-frying is strongly recommended for cooking vegetables containing beta carotene to prevent pigment oxidation (Vaclavik *et al.*, 2008).

Color Acceptance

The color acceptance of processed papaya leaves were affected by types of processing ($p < 0.05$) and boiling methods ($p < 0.05$), but their interactions were not ($p > 0.05$). Post hoc test using Duncan Multiple Range Test shows that color acceptance of papaya leaves processed into bumbu kuning and buntil dishes boiled in water only were significantly different from those of urap-urap, sauted and boiled dishes either boiled in water or clay water, as well as bumbu kuning and buntil dishes boiled in water clay only.

The color acceptance of papaya leaves processed into bumbu kuning and buntil dishes boiled in water clay only were different from those of urap-urap, sauted, boiled dishes undergoing boiling in water or clay water, bumbu kuning and buntil dishes boiled in water only. The color acceptance of papaya leaves processed into urap-urap (boiled in water only) and bumbu kuning dishes (boiled in water clay only) were different from those of urap-urap, sauted, and boiled dishes (boiled in water clay only). The color acceptance of papaya leaves processed into sauted, boiled (boiled in water only), buntil and boiled dishes (boiled in water clay only) were significantly different from those of urap-urap and sauted dishes (boiled in water clay only).

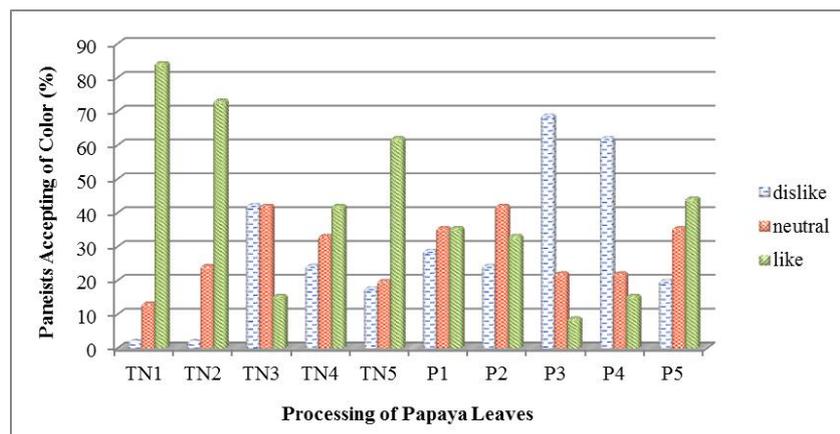


Figure 1. Panelists acceptance on papaya leaves color

This associated with the differences of cooking methods which can produce the different food color so that affect panelist responses. Urap-urap and sauted dishes boiled in clay water only showed the highest color acceptance. It related to the addition of herbs and spices like red chili, galingale, onion, and garlic in urap-urap dishes could cause a redness color of coconut and papaya leaves that the dish would be

more colorful. Besides, in sauted dishes, the presence of oil in papaya leaves processed into sauted dishes can cause the color of papaya leaves surface glossier that increase panelist acceptance of color. The panelists prefer buntill dish boiled in clay water only associated with the alkali pH of water clay although it slightly higher than water pH, so that the green color is more stable when steamed compared with buntill dish boiled in water. Besides, papaya leaves processed into bumbu kuning dish have the lowest panelist acceptance of color due to long time of cooking and the acidity of coconut pH so that the color become fade and resulted in panelists' dislikeness.

Flavor Acceptance

Flavor acceptance of papaya leaves were significantly not only affected with type of processing and boiling methods but also both interaction ($p < 0.05$). Post hoc test using Duncan Multiple Range Test shows that flavor acceptance of all papaya leaves boiled with water were different compared with boiled in clay water. Flavor acceptance of papaya leaves processed into boiled, buntill, and sauted dishes boiled in clay water were different compared with bumbu kuning and urap-urap dishes boiled in clay water.

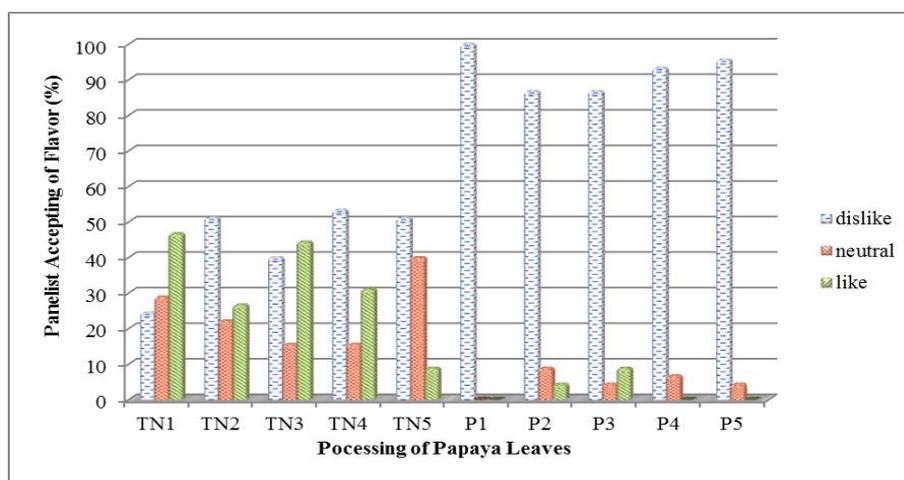


Figure 2. Panelists Acceptance on Papaya Leaves Flavor

The consumer like almost *Solanum* plant, except *Solanum scabrum* because of its bitter taste (Muthoni *et al.*, 2010). Urap-urap and bumbu kuning (boiled with clay water only) showed the highest flavor acceptance of panelists were associated with the presence of coconut milk and spices so that preferably to panelist. The lower of flavor acceptance of buntill, sauted, and boiled are associated with steaming process that cause flavor from spices and coconut will be evaporated and condensated so that papaya leaves become tasteless. Many flavor component would disappear through evaporation or dilute into cooking liquid, besides the longer the cooking process, the more flavor dissolved in water or evaporated in air (Mizer *et al.*, 1978).

Texture Acceptance

Texture acceptance of papaya leaves are significantly not only affected with with type of processing ($p < 0.05$) and boiling methods ($p < 0.05$) but also both interaction ($p < 0.05$). Post hoc test using DMRT shows that texture acceptance of papaya leaves processed into urap-urap, sauted, buntill and boiled (boiled in water only) were different with bumbu kuning (boiled in water only) and all papaya leaves

boiled with clay water. The texture acceptance of papaya leaves boiled in clay water are different with urap-urap, sauted, bumbu kuning, and boiled (boiled in clay water only). The texture acceptance of papaya leaves processed into bumbu kuning (boiled in water only) were different with bumbu kuning (boiled in clay water only).

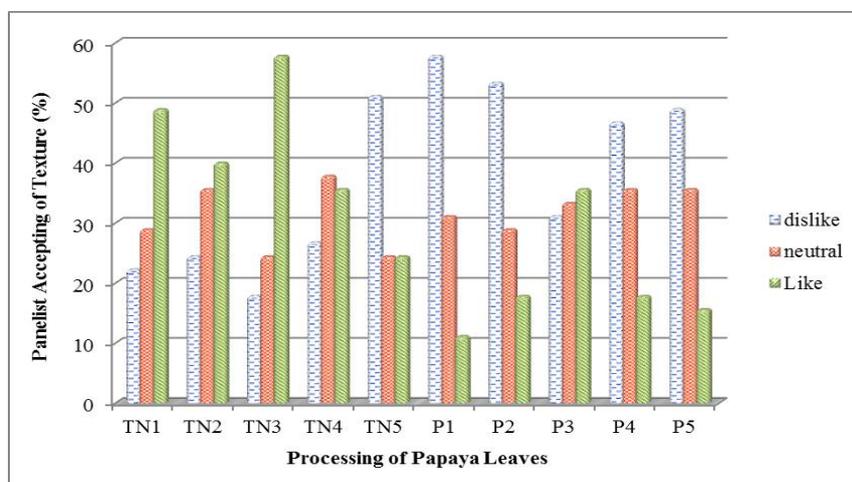


Figure 3. Panelists Acceptance on Papaya Leaves Texture

The texture acceptance of papaya leaves processed into bumbu kuning (boiled in water only) were related to the presence of texture softening due to the length of cooking. On the contrary, texture of bumbu kuning dishes (boiled in clay water only) showed the lowest of panelists acceptance, related to its harder texture compared with bumbu kuning dishes (boiled in water only) so that panelists consider it as an inappropriate combination with the sticky of coconut milk broth. Papaya leaves processed into urap-urap were associated with the difference of coconut and papaya leaves texture which can be combined to increase panelist acceptance.

The Best Treatment of Papaya Leaves

According to analysis of all characteristic of processed papaya leaves, the best treatment were determined using effectiveness method then the result were compared with characteristics of raw papaya leaves. The result could be seen in Table 2.

Table 2. Comparison of the best papaya leaves processed characteristics (bumbu kuning boiled in clay water vs. raw papaya leaves)

Parameters	Bumbu Kuning (TN3)	Raw Papaya Leaves	Δ (%)
Beta Carotene (µg/g)	64.83	104.15	37.75%**
Water Content (%)	66.26	79.15	16.28%**
pH	6.32	6.75	6.37%**
Brightness (L*)	31.63	19.2	64.73%*
Redness (a*)	5.47	4.6	18.91%*
Yellowness (b*)	13.37	13.7	2.41%**
Color Acceptance	2.73	-	-
Flavor Acceptance	3.20	-	-
Texture Acceptance	3.51	-	-

* = increase
 ** = decline

The best papaya leaves processed were bumbu kuning dishes (boiled in clay water) which declined of its beta carotene content, water content, pH and yellowness color, although its brightness and redness color were increase. The decline of beta carotene of bumbu kuning dishes (boiled in clay water) related to the cooking process were done twice (boiling followed by stewing) in a long time. This is mentioned that carotene very easily damaged by heating process as well as oxidation and isomerization can also occur when it exposed to heat and light (Morris *et al.*, 2006). Previous study about processing in broccoli showed that any types of processing in fresh broccoli pruned to show a decline in beta carotene structure, either cis or trans (Bernhardt *et al.*, 2006).

Papaya leaves processed into bumbu kuning dishes (boiled in clay water) also showed a decline in water content due to the heating from cooking process that can cause the permeability alteration of cell's membrane of papaya leaves. It can be associated with the loss of water and nutrients in papaya leaves then it dissolve into cooking liquid. Another study found heat treatment in vegetables can cause the cell wall become permeable, thus favorable for loss of water and nutrient, lectin and other components (Arthey *et al.*, 2991). The decline of pH can be associated with the alteration of cooking environment due to the addition of coconut milk. The acid pH of both papaya leaves and coconut milk can lower the pH of bumbu kuning dishes (boiled in clay water). pH of coconut milk was 6.10-7.00 (Center Food Safety and Applied Nutrition FDA-US, 2003). Besides, most of vegetables have acid pH, ranging from 5.0 to 5.6 (Brown, 2008).

The decline of yellowness color of papaya leaves processed into bumbu kuning (boiled in clay water) were related with the presence of oxygen component on yellow pigment (xantophyl) resulted the easier of this pigment to oxidize and its color become brighter. Xantophyl was a yellow-orange pigment constitute derivate from carotene, consist of carbon, hydrogen, and oxygen. This pigment could be oxidized due to its higher content of double bounds so that could become flavorless and colorless (Vaclavic *et al.*, 2008). According to Table 2, the brightness and redness values increase. It can be related with the length of cooking process that can change its color become brighter, besides stewing using coconut milk can dissolve the chlorophyl, the green color into coconut oil. The brighter green color would be appeared a few minutes after boiling due to the released of trapped air between cell space (McGee, 2004). Another study said that chlorophyl was a fat soluble green pigment that found in chloroplast cell. This pigment would be appeared in fatty cooking liquid of vegetables (Vaclavic *et al.*, 2008).

In Indonesia there is no daily recommendation of beta-carotene, but in Canada there is a daily recommendation intake of beta carotene (conversion factor 1 IU of beta carotene = 0.15 µg Retinoic Acid Equivalent (RAE); 1 µg of beta carotene = 0.50 µg RAE) so that can be determined a specific recommendation to consume processed papaya leaves to fulfil daily requirement (Canada Health, 2007). Therefore, to fulfil their daily requirement someone has to consume at least 130 µg of beta carotene or 65 µg RAE/day, equivalent with 2 g papaya leaves processed into bumbu kuning dish undergoing boiling in clay water). The maximum consumption per day should not exceed to 6,000 µg of beta carotene or equivalent with 92 g of papaya leaves processed in clay water.

Conclusion

There are differences of beta carotene contents of papaya leaves processed into bumbu kuning, buntill, boiled, sauted, and urap-urap dishes. There are differences of water contents and organoleptic properties (color, flavor, and texture) for each type of processing and boiling method but not for pHs. There are differences of brightness, and redness, and yellowness for each type of papaya leaves processing. Interaction of types of processing and boiling methods not only affect the brightness and redness values but also flavor and texture acceptances by panelist. The best treatment of processing papaya leaves based on this research is bumbu kuning dish boiled in clay water as a pretreatment and it is suggested to consume 2 – 92 g/day of it to fulfil daily requirement of beta carotene referring to Canadian recommendation intake.

References

- Apriyantono, A. 2002. Pengaruh Pengolahan Terhadap Nilai Gizi dan Keamanan Pangan. Disampaikan pada Seminar Online Kharisma ke-2. *Menjadi Ratu Dapur Profesional: Mengawal Kesehatan Keluarga Melalui Pemilihan dan Pengolahan Pangan yang Tepat*. Dunia Maya. 16-22 Desember 2002.
- Arthey, D. and Dennis, C. 1991. Vegetable Processing. Great Britain: Chapman & Hall.
- Azizah, A.H., et al. 2009. Effect of Boiling and Stir Frying on Total Phenolics, Carotenoids and Radical Scavenging Activity of Pumpkin (*Cucurbita moschato*). International Food Research Journal Vol 16: 45-51.
- Bauernfeind, J.C., Klaul, H. 1981. Carotenes as Colorants and Vitamin A Precursors. Florida : Academic Press.
- Bernhardt, S. and Schlich, E.. 2006. Impact of Different Cooking Methods on Food Quality: Retention of Lipophilic Vitamins in Fresh and Frozen Vegetables. <http://www.asianfood.info>. Accessed on December, 13, 2010.
- British Nutrition Foundation. 2004. Fruit and Vegetables. <http://www.britishnutrition.org.uk>. Accessed on December, 13, 2010.
- Brown, C. A. 2008. Understanding Food: Principles and Preparation, 3rd Edition. USA: Thomson Wadsworth, Thomson Corporation.
- Canada Health. 2007. Beta-Carotene. <http://www.hc-sc.gc.ca>. Accessed on December, 20, 2010.
- Center Food Safety and Applied Nutrition FDA-US. 2003. Approximate pH of Foods and Food Products. <http://www.vf.cfsan.fda.gov>. Accessed on December, 13, 2010.
- Drummond, E.K et al. 1993. Cook's Healthy Handbook: Good Nutrition and Safety in Your Kitchen. Canada: John Wiley & Sons, Inc.
- Duke A. J.. 1947. Handbook of Energy Crops: Carica papaya. <http://www.hort.purdue.edu>. Diakses tanggal 14 Oktober 2009.
- Eskin. 1979. Plant Pigment, Flavor and Texture. New York : Academic Press.
- Fanning, D. S. and M. C. B. Fanning. 1989. Soil: Morphology, Genesis and Classification. New York: John Wiley & Son.
- IPTEKnet. 2005. Kelapa. <http://www.ipitek.net.id>. Accessed on Agustus, 29, 2009.
- Kalaç, P et al. 2003. Losses of Beta-Carotene in Red Clover in Acid Medium During Ensiling. Animal Feed Science and Technology Volume 4(2):81-89.
- McGee, H. 2004. On Food and Cooking: The Science and Lore of the Kitchen. New York: Macmillan Library Reference USA, Inc.
- Mizer A., David et al. 1978. Food Preparation for The Professional. New York: Harper & Row Publishers, Inc.
- Morris, A et al. 2006. Effect of Processing on Nutrient Content of Foods. *Cajarticleso3c2004.qxp* 9/21/2006 Vol 37 (3).
- Moscha, T.C et al. 1997. Effect of traditional processing practices on the content of total carotenoid, β -carotene, α -carotene and vitamin A activity of selected Tanzanian vegetables. Plant Foods for Human Nutrition Journal Volume 50(3): 189-201.
- Muchtadi, R. T. dan Ayustaningwarno, F.. 2010. Teknologi Proses Pengolahan Pangan Cetakan Keempat. Bandung: Alfabeta.
- Muthoni, J et al. 2010. Participatory Characterization and Evaluation of Some African Leafy Vegetables in Lari, Kiambu West District, Central Kenya. Journal of Horticulture and Forestry Vol 2 (2): 012-016.
- Purcell, E. et al. 1969. Relationship of Vegetable Color to Physical State of The Carotenes. Agricultural and Food Chemistry Vol 17 (1): 41.
- Rodriguez, B et al. 2004. HarvestPlus Handbook for Carotenoid Analysis. Washington DC and Cali: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT).
- Sisson, M. 2010 The Importance of Cooking in the Evolution of the Human Brain. <http://www.marksdailyapple.com/about-2/mark-sisson/>. Diakses tanggal 2 Januari 2010.
- Yadav, S.K and Sehgal, S. 1997. Effect of home processing and storage on ascorbic acid and beta-carotene content of Bathua (*Chenopodium album*) and fenugreek (*Trigonella foenum graecum*) leaves. Plant Foods for Human Nutrition Journal Volume 50(3): 239-47.
- Vaclavik, A et al. 2008. Essentials of Food Science. New York: Springer Science+Business Media, LLC.
- Wasantwisut, E., P. Sungpuag, V. Chavasit, U. Chittchang, S. Jittinandana and T. Viriyapanich. 1995. Identifying and Recommending Vitamin A Rich Foods in Northeast Thailand. In *Empowering Vitamin A Foods*. Eds. E. Wasantwisut and

- G. A. Attig. Bangkok: Institute of Nutrition, 69-90.
- Lawless, T et al. 1998. Sensory Evaluation of Food Principles and Practices. USA: Chapman & Hall.
- Soekarto, T. S. 1985. Penilaian Organoleptik. Jakarta: Bhatara Karya Aksara.

Effect of Gelatin and Sugar Concentration on the Characteristic of Panna Cotta

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Abstract

Panna cotta is an Italian dessert made from heavy whipping cream, milk, gelatin and sugar. The formula of Italian panna cotta is less suitable to be applied in Indonesia because heavy whipping cream is unavailable in Indonesia and people don't like thick cream. Replacement of heavy whipping cream with light whipping cream and change in light whipping cream-milk ratio into 1:9 were done to adjust panna cotta with the preference of panelist. This affected on the concentration of gelatin and sugar should be added. The purpose of this study was to determine the effect of gelatin and sugar concentration and their interaction on the characteristic of panna cotta and to determine gelatin and sugar concentration that could produce the most prefer of panna cotta. The research design was a factorial randomized block design that consisted of two factors: the concentration of gelatin (1%; 1,25%; and 1,5%) and the concentration of sugar (2,5%; 5%; and 7,5%) to obtain nine combination treatment and replicated three times. Data were analyzed using ANOVA test at $\alpha = 5\%$, if the results showed a significant effect, data were analyzed by Duncan's Multiple Range Test at $\alpha = 5\%$ to determine the combination treatment that gave a significant difference. The results indicated interactions between gelatin and sugar concentration provided significant effect on texture (hardness) of panna cotta. Increasing gelatin concentration caused an increased in hardness, but when sugar concentration was increased, the increasing of hardness were declined. Increasing concentration of gelatin and sugar caused a decreased in syneresis. Hedonic score of gel firmness was increased as increasing of gelatin and sugar concentration up to 1,25% gelatin and 5% sugar. Hedonic score of ease to melt and ease to swallow were increased as decreasing in gelatin concentration and increasing in sugar concentration. The recommended treatment was 1% of gelatin and 7,5% of sugar.

Keywords: Panna Cotta; Gelatin; Sugar

Introduction

Milk is one source of animal protein that is very important for health because it contains complete nutrition. According to USDA Foreign Agricultural Service (2014), the average milk consumption in Indonesia is still low. Badan Pusat Statistik (2013) showed that the low of milk consumption was caused by people that don't like the taste of milk and prefer soft drinks and other beverage. This indicate that milk consumption has to be improved by processing milk into a product, such as dessert.

Panna cotta is an Italian dessert made from heavy whipping cream, milk, gelatin and sugar then served cold. Panna cotta has the character resembles pudding with more soft gel, but it can maintain its shape when it was cut. One thing that make panna cotta different from the other dessert is it will melt in the mouth.

Panna cotta in Italia is made by heavy whipping cream and milk with the same ratio (1:1). This type of cream and the formula is less suitable to be applied in Indonesia because heavy whipping cream is

unavailable in Indonesia and people don't like thick cream. Replacement of heavy whipping cream with light whipping cream and change in light whipping cream-milk ratio into 1:9 were done to adjust panna cotta with the preference of panelist. This affected to the concentration of gelatin and sugar should be added. This change caused a decreased in fat content of 69,10% of the original formula thereby affected to the texture of panna cotta and give impact on the concentration of gelatin should be added.

Gelatin is protein extracted and processed by partial hydrolyzing from collagen tissue in bone, cartilage and skin of animals (Karim, 2009). Gelatin in panna cotta play a role as gelling agent. Gelatin form thermoreversible gel at temperature below 35°C (Mariod dan Adam, 2013). Gelatin gel will melt when heated above the melting temperature, 27-34°C, thus gelatin gels tend to melt in the mouth (deMan, 1999).

According to deMan (1999), gel formation occurs in two stages process. The first stage is denaturation of the native protein into unfolded polypeptides. The second stage is association of polypeptides to form gel matrix. Gel formation is the phenomenon involving the association or cross-linking of the polymer chains to form a three dimensional network that traps or immobilises the water within it to form a rigid structure that is resistant to flow. The associated regions known as junction zone, may be formed by two or more polymer chains. Junction zones are bound by weak hydrogen bonds (Saha and Bhattacharya, 2010).

The characteristic of gelatin gel is affected by concentration of gelatin. As the concentration of gelatin increases, the rate of gelation also increases, thereby increasing the firmness (Mariod and Adam, 2013). If the concentration of the gelatin is too low, it will form a soft gel. This research used three levels of gelatin concentration, that are 1%; 1,25%; and 1,5%.

According to Weaver and Daniel (2003), gelatin gel is also affected by presence of another compound, such as sugar (sucrose). Sucrose is hygroscopic because thier polyhydroxyl group capable to form hydrogen bonds with water. This can interfere gelatin to trap water and affect to the characteristic of gelatin gel, so it should be determined the most appropriate concentration of sucrose for the best characteristic of panna cotta. This research used three levels of sucrose concentration, that are 2,5%; 5%; and 7,5%.

Various level in concentration of gelatin and sugar affect to characteristic of panna cotta. This become an underlying suspicion that there is interaction effect between concentration of gelatin and sugar to the characteristic of gelatin gel, therefore reasearch about effect of gelatin and sugar concentration on the characteristic of panna cotta should be done.

Materials and Methods

Materials

Light whipping cream, pasteurized milk and sugar are comercial products obtained from supermarket in Surabaya. Gelatin used for making panna cotta is bovine gelatin. Filter paper was used for syneresis analysis and mineral water for sensory analysis.

Instrument

Gas stove (*Rinnai*), refrigerator (*Electrolux*), weigher (*Acculab*), cylinder cup (volume 22 mL), spoon, stirrer, pan, thermometer 0-100°C, measuring cup 200 mL (*Iwaki Pyrex*) and beaker glass 250 mL (*Iwaki Pyrex*) were used for producing panna cotta. Cylinder cup (height: 4 cm, radius: 2 cm), analytical weigher (*Mettler Toledo*), spoon, weighing bottle, exicator, silica gel, Texture Analyzer (*TA-Xt Plus*), thermometer infrared (IRtek IR 60), plastic container (10x15 cm), oven (*Binder*), refrigerator (*Electrolux*) and questionnaire were used for physic and sensory analysis.

Experimental Design

The research design was a factorial randomized block design that consisted of two factors: the concentration of gelatin (1%; 1,25%; and 1,5%) and the concentration of sugar (2,5%; 5%; and 7,5%) to obtain nine combination treatment and replicated three times. Data were analyzed using ANOVA test at $\alpha = 5\%$, if the results showed a significant effect, data were analyzed by Duncan's Multiple Range Test at $\alpha = 5\%$ to determine the combination treatment that gave a significant difference. Concentration of gelatin and sugar for the best panna cotta were determined by the highest score in sensory analysis.

Panna Cotta Making

Some of pasteurized milk were set aside to soak with gelatin in ratio 1:15 (gelatin:milk) for 15 minutes (mixture 1). The rest of pasteurized milk were cooked with light whipping cream and sugar until 70°C (mixture 2). Mixture 1 were blended to mixture 2 and were cooked until 70°C then stand for 1 minute. This mixture were pour into several cup size adjusted to the requirement for analysis. This mixture was cooling down until reached the room temperature to prevent condensation at the lid of the cup, then was setting in refrigerator at $\pm 5^{\circ}\text{C}$ for overnight.

Texture Analysis

Textural parameter (hardness) was measured by Texture Analyzer (Pang *et al.*, 2014) at $15 \pm 1^{\circ}\text{C}$. A 0,5" (12,7mm) radius cylindrical probe was used to penetrate the sample. Sample in the cylinder cup was put on the container filled by ice cube in order to maintain the temperature. This container were put under the probe, then probe will penetrate the sample and back to the initial position. Hardness was defined by the maximum peak force during the first compression cycle.

Syneresis with modification

Syneresis was measured by measuring the water expelled from the sample panna cotta which has been stored overnight in refrigerator at 5°C (Anggraini, 2008). Water expelled was measured by tilting the cylinder cup filled by sample, then the water was absorbed by the filter paper, then the weight of the water was measured. Syneresis was measured every day until seven days storage in refrigerator.

Sensory Characteristic

Sensory analysis were done using 9-point hedonic scale which score 1 for dislike extremely and score 9 for like extremely. This research was done by 80 untrained panelists to record their opinion about gel

firmness, ease to melt and ease to swallow. The test consisted of two steps, the first step for gel firmness and ease to melt, then the second step for ease to swallow. A new sample come from refrigerator was prepared for every steps.

Result and discussion

Hardness

Hardness is defined as the force that should be given to deform an object. Hardness is measured as the maximum peak force during the first compression cycle (Bourne, 2002). The higher hardness, the more force needed to deform panna cotta, so it indicated the gel more firm. Hardness of panna cotta ranged from 8,684 g to 26,732 g as shown in Figure 1. The result indicated that interactions between gelatin and sugar concentration provided significant effect on hardness of panna cotta.

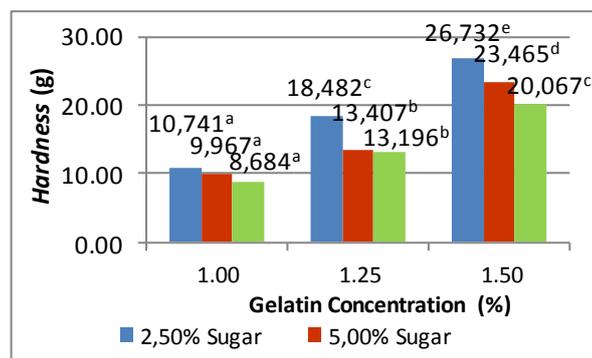


Figure. 1. Hardness of panna cotta as the function of gelatin and sugar concentration. Different superscripts letter indicate significant differences between samples ($\alpha = 5\%$).

The difference effect of gelatin concentration on hardness in every concentration of sugar indicated interaction between gelatin and sugar. Increasing concentration of sugar interfered gelatin gel formation because of competition of water available between gelatin and sugar. Increasing gelatin concentration caused increased in hardness, but when sugar concentration was increased, the increasing of hardness were declined. The higher concentration of sugar, the more water were bound therefore the less water available for gelatin. This caused gelatin gel weaken due to a lack of water available to maintain gel integrity. According to Choi (2004), sugar retarded gelatin gelation. The presence of sugar reduce gelation rate and prevent of gelatin chains from approaching each other kinetically during gelation because of increased viscosity. The movement of the protein aggregates were slow down in sugar system because increasing the viscosity. This may resulting in increasing distance between entangled points of the chain thereby decreasing the gel firmness.

Syneresis

The result showed that concentration of gelatin and sugar provided significant effects on syneresis of panna cotta respectively. Increasing concentration of gelatin caused decreased in syneresis because of the ability of gel to trap water in matrix system. The higher concentration of gelatin, the more matrix that can trap water therefore the less water expelled from gel during storage and impact on decreasing syneresis.

Increasing concentration of sugar caused more water will be bound by hydrogen bond. The higher concentration of sugar, the more water bound. This resulting decreased in water that has to be trapped by gelatin therefore the ability of gel matrix to retain water in it became higher therefore reduced the level of syneresis. Decreasing syneresis were also evidenced in the research done by Sugiarto (2011) which showed that the higher concentration of sugar in reeds jelly drink with the addition of carrageenan caused the amount of water expelled was reduced so that the syneresis.

Percentage syneresis of panna cotta also increased during storage. Syneresis occur due to the instability of gel matrix in trapping water. According to Glicksman (1983), the instability were caused by the formation of cross-linking between gelatin chains continuously during storage. This process caused gel shrank and loss of elasticity. As the result, the gel becomes brittle and loss the ability of trapping water. The percentage of syneresis was also increased due to the weakening ability of sugar in binding water in gel system during storage. Percentage syneresis of panna cotta can be seen in Table 1.

Table 1. Syneresis of Panna Cotta for 7 Days

Percentage of Syneresis on Day -	Gelatin (%)				Sugar (%)	
	1,00	1,25	1,50	2,50	5,00	7,50
1	0,077 ^c	0,070 ^b	0,059 ^a	0,076 ^c	0,069 ^b	0,062 ^a
2	0,097 ^c	0,080 ^b	0,058 ^a	0,087 ^c	0,079 ^b	0,069 ^a
3	0,324 ^c	0,240 ^b	0,215 ^a	0,291 ^c	0,264 ^b	0,232 ^a
4	0,673 ^c	0,609 ^b	0,357 ^a	0,592 ^c	0,544 ^b	0,503 ^a
5	1,411 ^c	0,994 ^b	0,634 ^a	1,109 ^c	1,006 ^b	0,875 ^a
6	1,952 ^c	1,336 ^b	0,769 ^a	1,467 ^c	1,332 ^b	1,259 ^a
7	2,542 ^c	1,729 ^b	1,209 ^a	1,968 ^c	1,812 ^b	1,700 ^a

Different manuscript letter in a row indicate significant differences between samples ($\alpha = 5\%$)

Sensory Characteristic

Hedonic score of panna cotta can be seen in Table 2. The results showed that increasing concentration of gelatin and sugar caused increasing in preference of panelists to gel firmness up to gelatin concentration levels of 1,25% and 5% sugar. Increasing concentration of gelatin above 1,25% with various concentration of sugar caused decrease in preference of gel firmness. The most preferred treatment was concentration of gelatin 1,25% and 5% sugar. This produce gel that were firm enough. Gelatin concentration above 1,25% in various concentration of sugar has a lower score. This treatment produced a rigid gel because the higher concentration of gelatin gave more intense intermolecular contacts and stronger protein-protein interaction. The use of gelatin concentration below 1,25% at various concentration of sugar also gave a lower score because it produced a very soft gel.

Score of ease to melt and ease to swallow were increased due to decrease in concentration of gelatin and increase in concentration of sugar. The highest score was obtained in 1% gelatin and 7,5% sugar. This treatment produced panna cotta that was melt easily inside the mouth. Cross-link formation of gelatin chains were not intense in low concentration of gelatin. It only formed a little entangled points of polypeptide chains, so the hydrogen bonds binding it were more easy to be break and gelatin gel were melt easily by heat in the mouth. High concentration of sugar also caused gel more easy to melt because sugar increased viscosity of the system. Cross-link formation in viscous system occurred slowly as

described before, therefore the entangled points were not intense and it caused gel more easy to melt.

Table 2. Hedonic Score of Panna Cotta

Attributes	Sample								
	A ₁ B ₁	A ₂ B ₁	A ₃ B ₁	A ₁ B ₂	A ₂ B ₂	A ₃ B ₂	A ₁ B ₃	A ₂ B ₃	A ₃ B ₃
Gel Firmness	4,34 ^a	6,23 ^{cd}	5,69 ^b	5,65 ^b	6,69 ^e	5,63 ^b	5,81 ^{bc}	6,55 ^{de}	6,08 ^{bc}
Ease to Melt	6,29 ^d	4,89 ^b	4,40 ^a	6,86 ^e	6,14 ^{cd}	5,08 ^b	7,43 ^f	6,49 ^{de}	5,78 ^c
Ease to Swallow	6,65 ^d	5,50 ^c	4,55 ^a	7,13 ^e	5,75 ^c	4,99 ^b	7,59 ^f	6,69 ^d	5,76 ^c
Total Score	17,28	16,62	14,64	19,64	18,58	15,70	20,83	19,73	17,62

A₁B₁ = 1% gelatin, 2,5% sugar; A₂B₁ = 1,25% gelatin, 2,5% sugar; A₃B₁ = 1,5% gelatin, 2,5% sugar; A₁B₂ = 1% gelatin, 5% sugar; A₂B₂ = 1,25% gelatin, 5% sugar; A₃B₂ = 1,5% gelatin, 5% sugar; A₁B₃ = 1% gelatin, 7,5% sugar; A₂B₃ = 1,25% gelatin, 7,5% sugar; A₃B₃ = 1,5% gelatin; 7,5% sugar. Different manuscript letter in a row indicate significant differences between samples ($\alpha = 5\%$)

Ease to swallow was related to gel firmness and ease to melt. Treatment of 1% gelatin and 7,5% sugar produced the softest gel therefore it was the most easy to swallow. Panna cotta with properties of the most easy to melt would undergo rapid change from solid to liquid so that it would have a tendency to flow and swallow easily in esophagus. Based on the highest score, the recommended treatment was 1% of gelatin and 7,5% of sugar.

Conclusion

Interactions between gelatin and sugar concentration provided significant effect on hardness of panna cotta. Increasing gelatin concentration caused an increased in hardness, but when sugar concentration was increased, the increasing of hardness were declined. Increasing concentration of gelatin and sugar caused a decreased in syneresis. Hedonic score of gel firmness was increased as increasing of gelatin and sugar concentration up to 1,25% gelatin and 5% sugar. Hedonic score of ease to melt and ease to swallow were increased as decreasing in gelatin concentration and increasing in sugar concentration. The treatment recommended was 1% gelatin and 7,5% sugar.

References

- Angraini, D. S. (2008), "Pengaruh Konsentrasi Karagenan dan Tripotassium Citrate Terhadap Sifat Fisikokimia dan Organoleptik Jelly Drink", *Skripsi*, Fakultas Teknologi Pertanian, Universitas Katolik Widya Mandala Surabaya, Surabaya.
- Badan Pusat Statistik. (2013), *Ringkasan Eksekutif Pengeluaran dan Konsumsi Penduduk Indonesia*, Badan Pusat Statistik, Jakarta.
- Bourne, M. C. (2002), *Food Texture and Viscosity: Concept and Measurement 2nd Edition*, Academic Press, New York.
- Choi, Y. H., Lim, S. T., Yoo, B. (2004), "Measurement of Dynamic Rheology During Ageing of Gelatine-Sugar Composites", *International Journal of Food Science and Technology*, Vol 39, pp. 935-945.
- deMan, J. M. (1999), *Principles of Food Chemistry Third Edition*. Aspen Publishers, Inc, USA.
- Glicksman, M. (1983), *Food Hydrocolloids*, Vol. II, CRC Press, Boca Raton.
- Karim, A. A., Bhat, R. (2009), "Fish Gelatin: Properties, Challenges, and Prospects As an Alternative to Mammalian Gelatins", *Food Hydrocolloids*, Vol. 23, pp. 563-576.
- Kartika, B., Hastuti, P., Supartono, W. (1988), *Pedoman Uji Inderawi Pangan*, Universitas Gadjah Mada, Yogyakarta.
- Kusnandar, F. (2010), *Kimia Pangan: Komponen Makro*, PT. Dian Rakyat, Jakarta.
- Mariod, A. A., Adam, H. F. (2013), "Review: Gelatin, Source, Extraction And Industrial Applications", *Acta Sci. Pol., Technol. Aliment.* Vol. 12 No.2, pp. 135-147.
- Pang, Z., H. Deeth, P. Sopade, R. Sharma, N. Bansal. (2014), "Rheology, Texture and Microstructure of Gelatin Gels With and Without Milk Proteins", *Food Hydrocolloids*, Vol. 35, pp. 484-493.
- Saha, D., Bhattacharya, S. (2010), "Hydrocolloids as thickening and gelling agents in food: a critical review", *Journal of Food Science and Technology*, Vol. 47 No. 6, pp. 587-597.
- Sugiarto, A. W. (2011), "Pengaruh Konsentrasi Karagenan dan Gula Pasir terhadap Sifat Fisikokimia dan Organoleptik Jelly Drink Alang-Alang (*Imperata cylindrica* (L.) P. Beauv.)", *Skripsi*, Fakultas Teknologi Pertanian, Universitas Katolik Widya Mandala Surabaya, Surabaya.
- USDA FAS. (2014), *Indonesia Dairy and Products Annual Report 2014*. Global Agricultural Information Network Report.
- Weaver, C. M., Daniel, J. R. (2003), *The Food Chemistry Laboratory: A Manual for Experimental Foods, Dietetics, and Food Scientists*, CRC Press, USA.

Mineral Composition and Antioxidant Reducing Power of Green Algae *Caulerparacemosa* from Talango Islands, Indonesia

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Abstract

Green algae *Caulerpa racemosa* was obtain from Talango Islands, Indonesia. Fresh, semi-dried, and dried *C. racemosa* were assayed for mineral composition, phytochemistry screening, and antioxidant reducing power. The analysis shows that the mineral composition varies. The highest mineral content results are calcium> potassium> sodium. Phytochemical analysis results show the components of alkaloids, phenolics, glycosides and saponins detected from the three treatments *C. racemosa*. Dried *C. racemosa* has reduced the activity of the nicest.

Keyword: Fresh; semi-dried; dried; antioxidant; phytochemical

Introduction

Seaweed is a functional food which consumed by Asian people, mainly in the form of fresh, dried, or easy to served. Seaweed also has potential as medicine, several compounds from seaweed has been widely reported to have antibacterial activity, anticoagulant, laxative, anti-ulcer and agents of suspending the preparation radiology (Ratana-arporn and Chirapart, 2006; Chanda *et al.*, 2010). Seaweed contains including an extensive nutrients such as protein, fat, carbohydrates, vitamins, minerals and bioactive compounds (Holdt and Kraan, 2011). In contrast to terrestrial plants, the nutrient content of seaweed is strongly influenced by their environment, such as temperature, salinity, light and nutrients (Mwalugha *et al.*, 2015).

Green seaweed *Caulerpa racemosa* widely spread especially in the tropics to temperate regions (Verlaque *et al.*, 2003). Many found in shallow to deep. *C. racemosa* has a core, colonize and modular, and not much has cellulose (Ukabi *et al.*, 2012). *C. racemosa* has mineral content that is essential for the body, such as Na, K, Ca, and Mg (Kumar *et al.*, 2011).

Drying seaweed with heating has a variety of purposes, one of which was to extend the shelf life. Gupta, et al. (2010) stated that heating seaweed at various temperatures and times reduced the total number of microbial cells. However, it had been widely known that the drying of fruits and vegetables can alter the nutritional content including phytochemical content material and little information about preparation seaweed extract by drying the green seaweed, especially *C. racemosa*. This study aimed to

provide information on the effect of the drying process to the phytochemical content and reducing power activity of *C. racemosa* from methanol extract.

Material and Method

Material

C. racemosa was collected from Sumenep district, Madura, Indonesia (Talango Island S 07° 05', 21.5" and 113° 56', 307"). The collection samples were washed with seawater thereafter with tap water to removed salt and other unwanted materials. The sample was divided into three portions. First, fresh seaweed was obtained by draining a sample. Second, the seaweed was dried at a temperature of 40-50°C for 6 hours in cabinet drying to obtain a semi-dry sample. Third, the seaweed was dried at at the same temperature for 24 hours to obtain a dry sample. Mineral contents were observed from fresh, semi-dry and dry samples.

Chemicals

Chemicals used were potassium hexacyanoferrate, trichloroacetic acid, FeCl₃, Million's reagent, Fehling A, Fehling B, NaOH 2%, acetic acid, H₂SO₄, Mayer's and Wagner's reagents, methanol.

Minerals Analysis

Three sample was digested by dry ashing the dissolved in HCL 1 M. The finnal diluted solution for calcium contained 1% lanthanum to overcome interferences. The element s in *C. racemosa* were quantified with atomic absorption spectrophotometry. Concentration of elements were determined from calibration curve of standart (Norziah and Cing, 2000).

Extraction Samples

Each sample was extracted with 300 ml methanol per day for 3 days at room temperature. Macerate was then collected and filtered with filter paper. Filtrate evaporated with rotary evaporation at 40° C under vacuum to obtain dark green concentrated extract. Each samples tested preliminary phytochemical screening of nine different chemical compound (alkaloids, phenolic groups ,terpenoids, flavonoids, carbohydrates, proteins, glicoside, saponins, and steroids) and reducing power activity.

Phytochemical Screening

Phytochemical screening was done base on Yadav and Agarwala (2011) method. The following is methods for screening phytochemical for *C. racemosa*

Protein

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Carbohydrate

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Phenolic

Crude extract was mixed with 2ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

Flavonoid

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Saponin

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Glycoside

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Steroid

Crude extract was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Terpenoid

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H₂SO₄ was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Alkaloid

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Reducing Power Activity

The reducing power of all samples was determined as described by Li *et al.* (2012) with some modifications. 2.0 mL of each sample at few concentrations was dissolved in distilled water and mixed with 2.5-mL aqueous potassium hexacyanoferrate solution (1%). Sample was incubated at 50°C for 30 min, then added 1.5 mL trichloroacetic acid (10%). Finally, 2.0 mL of the upper layer was mixed with 2.0 mL distilled water and 0.5 mL aqueous FeCl₃ (0.1%), and the absorbance recorded at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical Analysis

Each data showed mean of standard error of three replications. Data analysis was performed using Minitab 16 for Windows. Significance was considered by Analysis of variance (ANOVA) between each treatment when $p < 0.05$.

Result and Discussion

Mineral Analysis

Some of the minerals contained in *C. racemosa*. The macro mineral of *C. racemosa* was Potassium, Calcium and Sodium (Kumar *et al.*, 2011). The results of the analysis of *C. racemosa* fresh mineral, semi-dried, and dried presented on Table 1.

Table 1. Mineral Analysis of Green Seaweed *C. racemosa*

Minerals (mg/100g)	Fresh	Semi-dried	Dried
Na	13,05 ± 0,08 ^a	13,85 ± 0,32 ^b	14,00 ± 0,08 ^b
K	77,59 ± 0,74 ^a	86,77 ± 2,58 ^b	92,50 ± 1,73 ^c
Ca	149,66 ± 6,61 ^a	163,99 ± 1,73 ^b	168,64 ± 2,12 ^b
Mg	2,21 ± 0,02 ^a	2,56 ± 0,07 ^b	2,90 ± 0,06 ^c
Fe	9,52 ± 0,30 ^a	9,95 ± 0,06 ^a	10,13 ± 0,07 ^a
Zn	-	-	-

(a-c) representative significantly difference (p <0.05)

Mineral analysis of *C. racemosa* showed that the calcium content was the highest of minerals, followed by Potassium and Sodium. Khairi and El-Sheikh (2015), explains that potassium, calcium and sodium is a mineral found in many marine algae. In chlorophycean species, Ca was a most affluent among these elements during summer season. The results showed that the content of each mineral increased with the drying treatment. This is due to the drying may be due to loss of moisture can cause more concentrated inorganic compounds in seaweed *C. racemosa*. Minerals were not damaged by heat treatment and have low volatility, resulting in increased levels of minerals due to the loss of moisture content of the material (Agoreyo *et al.*, 2011). Mineral content influenced by the wave exposure, species, seasonal, annual, environmental, physiological factor and the type of processing and method of mineralization (Komalavalli and Lalitha, 2015).

Phytochemical Analysis

Phytochemicals screening was aimed for preliminary study of the extract. Phytochemical compounds analyzed were alkaloids, phenolics, terpenoids, flavonoids, carbohydrates, proteins, glycosides, saponins, and steroid. Phytochemical analysis results are presented in Table 2.

Table 2. Phytochemical Analysis of Green Seaweed *C. racemosa*

Phytochemicals	Fresh	Semi-dried	Dried
Alkaloids	+++	+++	+++
Phenolics	+	+	+
Terpenoids	+++	-	-
Flavonoids	+	+	+
Carbohydrates	+++	++	++
Proteins	-	-	-
Glycosides	+++	++	++
Saponins	++	+	-
Steroids	+++	-	-

The analysis showed that fresh *C. racemosa* positive for alkaloids, phenolics, terpenoids, flavonoids, carbohydrates, glycosides, saponins and steroids. Semi-dried detected alkaloids, phenolics, flavonoids, carbohydrates, glycosides, and saponins. Dried showed alkaloids, phenolics, flavonoids, carbohydrates, gikosida, and saponins. Karthick *et al.* (2014) showed the methanol extract of *C. scalpelliformis* positive for steroids, tannins, flavonoids, saponins and terpenoids. *C. racemosa* positive for alkaloid, phenolic, tannin, steroid and triterpenoid in methanol extract, while water extract positive for alkaloid, carbohydrate, glycoside and saponins (Rahul *et al.*, 2014).

Several different treatments showed differences positive phytochemical content. This suggests that the drying treatment can affect the content of phytochemistry. According to Gupta *et al.* (2011),

drying at different temperatures cause some changes phytochemical content which can also affect the activity.

Alkaloids, steroids, terpenoids were a class of compounds which is essential from *C. racemosa* (Ornano *et al.* 2014). Alkaloid is an organic compound that has the amine group and nitrogen atom (s) in a cyclic ring (Kuramoto *et al.*, 2004; Guven *et al.*, 2010). Some alkaloids have been found from *C. racemosa*. Caulerpin which is bisindole alkaloid compounds have been used as anti-inflammatory and antinociceptive. Racemosin a and b is also a compound bisindole alkaloid from *C. racemosa* (Liu *et al.*, 2013).

Terpenoid or isoprenoid the main classes of natural compounds that are similar to terpenes, derived from five-carbon isoprene units and various modifications (Yermakov *et al.*, 2010). Caulerpyne is sesquiterpenoid were first identified by Amico *et al.* (1978). Caulerpenyne of *C. racemosa* have phytotoxic activity (Raniello *et al.*, 2007).

Steroids are organic compounds that taste of plants and animals. The fundamental structure of steroid is four carbon rings called the steroid nucleus (Patel and Savjani, 2014). 28-oxostigmastane steroid is a steroid that has been identified from *C. racemosa* (Yang *et al.*, 2015).

Reducing Power Activity

Reducing power *C. racemosa* obtained by measuring the changes of Fe^{3+} to Fe^{2+} which is done by measuring the absorbance of the sample after treated with a solution of $FeCl_3$. The higher the value of the absorbance at 700 nm showed that the higher the activity of the reducing sample. Absorbance of reducing power activity is presented in Figure 1 .

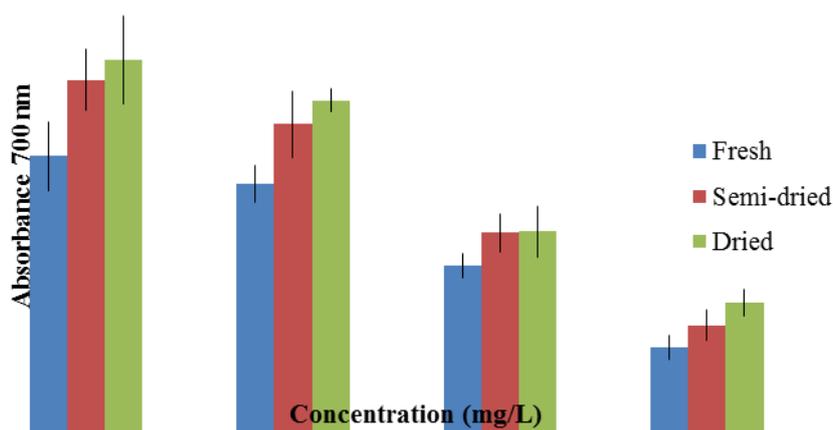


Figure 1. Absorbance of Reducing Power Activity of *C. racemosa* at 700 nm

Reducing power analysis showed that *C. racemosa* dry has the highest antioxidant activity which is shown by the high value of the absorbance. Antioxidant activity is usually associated with phytochemical content material. Although some phytochemical components more found in fresh *C. racemosa*, but the highest antioxidant activity obtained from dried *C. racemosa*. It may have caused the

drying process by heating can result in unnecessary downtime for endogenous oxidative enzymes that contribute to damage the antioxidant compounds (Rajauria *et al.*, 2010).

The ability of this reduction only saw antioxidant compounds capable of reducing ion Fe [III], which is a compound that can give an electron donor and has a lower reduction potential of Fe [II] (Fidrianny *et al.*, 2014). Reducing power analysis was used to evaluate the ability of antioxidants to donate electrons (Kumar and Jain 2012). All samples showed reducing power activity corresponding concentrations of each and potentially as an electron donor in radical compounds, transforming it into a more stable and terminate the chain reaction of free radicals.

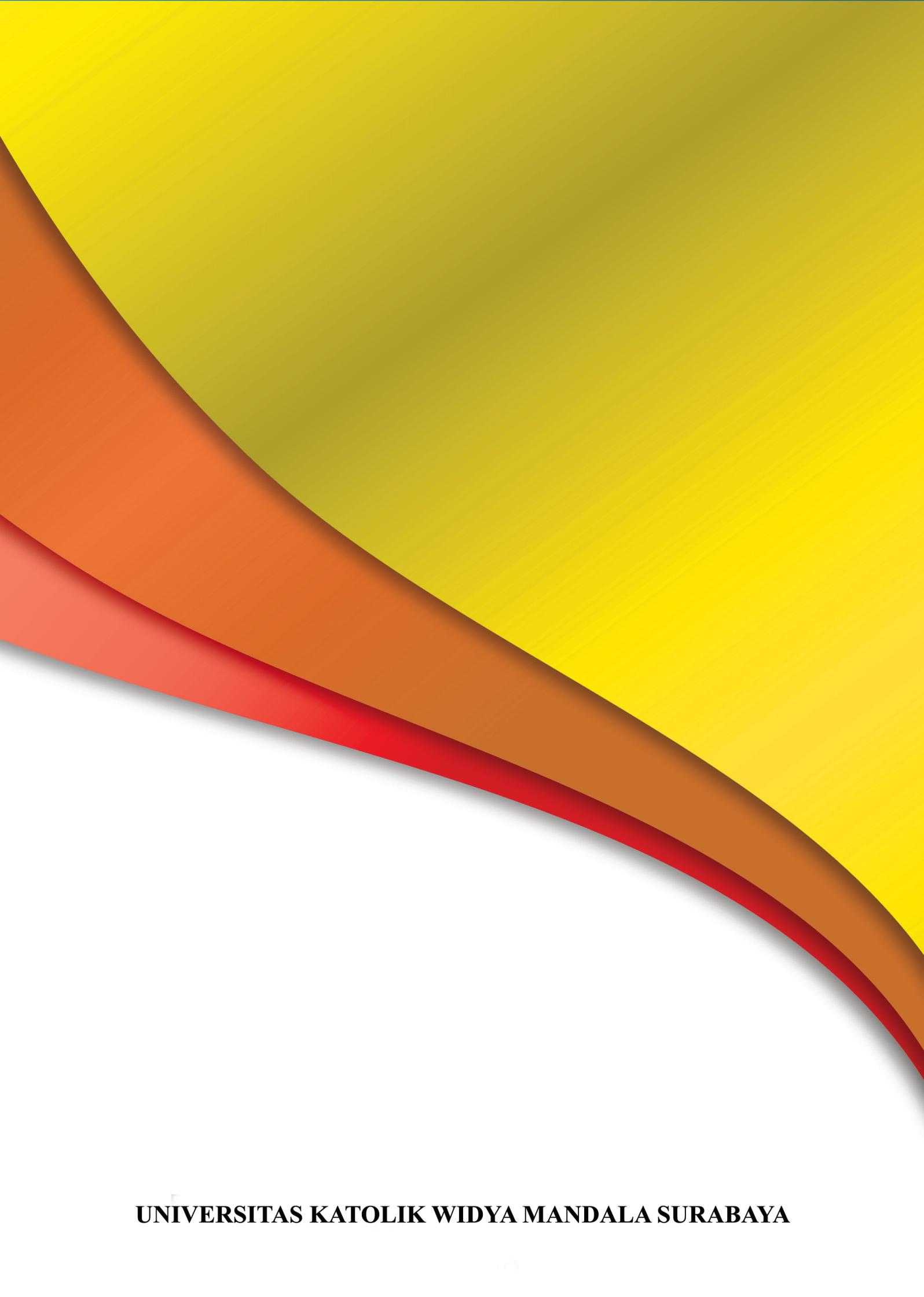
Conclusion

Seaweed *C. racemosa* was a source of minerals. The highest mineral content in *C. racemosa* from Madura was Calcium. Phytochemical compounds may contribute to its antioxidant activity, but to note a few things, including the presence of endogenous oxidative enzymes.

Reference

- Agoreyo, B.O., Akpiroroh, O., Orukpe, O.A., Osaweren O.R. and Owabor, C.N. The Effect of Various Drying Method on the Nutritional Composition of *Musa Paradisiaca*, *Diocorearotundata*, and *Colocasia esculenta*. *Asian Journal of Biochemistry*. DOI:10.3923/ajb.2011, p. 5.
- Amico, A., Oriente, G., Piattelli, M. and Tringali, C. 1978. Caulerpenyne, an Unusual Sequiterpenoid from the Green Alga *Caulerpa prolifera*. *Tetrahedron Letter*, 38, p. 3593.
- Chanda, S., Dave, R. Kaneria, M. and Nagani, K. 2010. Seaweeds: A Novel, Untapped Source of Drugs from Sea to Combat Infectious Disease. *Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. Formatex, p. 473.
- Fidrianny, I., Puspitasari, N., and Singgih, W.M. 2014. Antioxidant Activities, Total Flavonoid, Phenolic, Carotenoid of Various Shells Extract from Four Species of Legumes. *Asian Journal of Pharmaceutical and Clinical Research*, 7 (4), p. 45.
- Gupta, S., Cox S. and Abu-Ghannam N. 2011. Effect of Different Drying Temperatures on The Moisture and Phytochemical Constituents of Edible Irish Brown Seaweed. *LWT-Food Science and Technology*. doi:10.1016/j.lwt.2010.12.022, p. 6.
- Gupta, S., Rajauria, G., and Abu-Ghannam, N. 2010. Study of the Microbial Diversity and Antimicrobial Properties of Irish Edible Brown Seaweeds. *International Journal of Food Science and Technology*, 45: 485-489.
- Güven, K.C., Percot A. and Sezik, E. 2010. Alkaloid in Marine Algae. *Marine Drug*, 8, p. 269.
- Holdt, S.L., and Kraan, S. 2011. Bioactive Compounds in Seaweed: Functional Food Applications and Legislation. *Journal Application Phycology*. 23, p. 544.
- Karthick, N., Fathimal, M.A. Ramesh, K.H. Sridhar, Natrajan M., Divya V.V, Umavanitha M., and S. Umamaheswari. 2014. Screening of phytochemicals and antimicrobial activity of *Caulerpa pascuella* collected from Manapad Coast, Tuticorin District, Tamilnadu, South India. *Journal of Coastal Life Medicine*, 2(2), p. 109.
- Khairy, H.M. and El-Sheikh M.A.. 2015. Antioxidant Activity and Mineral Composition of Three Mediterranean Common Seaweeds from Abu-Qir Bay, Egypt. *Saudi Journal of Biogeological Science*, 22, p. 627.
- Komalavalli, N and Lalitha N. 2015. Trace Metals and Mineral Contents of Some Green Algal Seaweed from Vadakadu (Rameswaram) Coastal Region, Tamilnadu, India, *International Journal of Advance in Interdisciplinary Research*, (2) 1, p. 27.
- Kumar, M., Gupta V., Kumari, P., K. Reddy, CR, and Jha, B. 2011. Assessment of Nutrient Composition and Antioxidant Potential of *Caulerpaceae* Seaweeds. *Journal of Food Composition and Analysis*, 24: 270-278.
- Kumar, T. and Jain, V. 2015. Appraisal of Total Phenol, Flavonoid Contents, and Antioxidant Potential of Folkloric *Lansea coromandelica* using *In Vitro* and *In Vivo* Assays. *Scientifica*, <http://dx.doi.org/10.1155/2015/203679>, p. 7
- Kuramoto, M., Arimoto H. and Uemura, D. 2004. Bioactive Alkaloid from the Sea: A Review. *Marine Drugs*, 2, p. 40.
- Li, Z., Wang B., Zhang Q., Qu Y, Xu H., and Li, G. 2012. Preparation and Antioxidant Property of Extract and Semipurified Fraction of *Caulerpa racemosa*. *Journal Appl Phycol*, 24, p. 1530.
- Liu, D.Q., Mao, L.S., Zhang, H.Y., Yu, X.Q., Feng, M.T., Wang, B., Feng, L.H. and Guo, Y.W. 2013. Racemosin A and B, Two Novel Bisindole Alkaloids from the Green Alga *Caulerpa racemosa*. *Fitoterapia*, 91, p. 1.
- Mwalugha, H.M., Wakibia J.G, Kenji G.M. and Mwasaru, M.A. 2015. Chemical Composition of Common Seaweeds from the Kenya Coast. *Journal of Food Research*, (4) 6, p.28.
- Norziah, M.H. and Ching, C.H. 2000. Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chemistry*, p. 71.
- Ornano, L., Donno Y, Sanna C., Ballero M., Serafini M., and Bianco A. 2014. Phytochemical Study of *Caulerpa racemosa* (Forsk.) J. Agarth, an Invading Alga in the Habitat of La Maddalena Archipelago. *Natural Product Research*, <http://dx.doi.org/10.1080/14786419.2014.945928>, p. 1.
- Patel, S.S. and Savjani, J.K. 2015. Systematic Review of Plant Steroids as Potential Anti-Inflammatory Agents: Current Status and Future Perspective. *The Journal of Phytopharmacology*, 4 (2), p. 1.
- Rahul, M., Suresh N., and Anil T. 2014. Evaluation of Physicochemical Properties of Seaweed, *Caulerpa racemosa*. *International Journal of Research Ayurveda Pharmacy*, 5 (4), p. 543.
- Rajauria, G., Jaiswal, N., Abu-Ghannam A.K. and S. Gupta. 2010. Effect Hydrothermal Processing on Colour, Antioxidant and Free Radical Scavenging Capacities of Edible Irish Brown Seaweeds. *International Journal of Food Science and Technology*, 45, p. 2489.
- Raniello, R., Mollo, E., Lorenti, M., Gavagnin, M., and Buia M.C. 2007. Phytotoxic Activity of Caulerpenyne from the Mediterranean Invasive Variety of *Caulerpa racemosa*: a Potential Allelochemical. *Biol Invasion*, 9, p. 1.

- Ratana-Arporn and Chirapat. 2006. Nutritional Evaluation of Tropical Green Seaweeds *Caulera lentillifera* and *Ulva reticulata*. Kasesart Joournal (Natural Science): 40, p. 75.
- Ratana-Arporn, P. and Chirapat A. 2006. Nutritional Evaluation of Tropical Green Seaweeds *Caulerpalentillifera* and *Ulva reticulata*. Kasesart Journal (Natural Science), 40 (Suppl): 75-83.
- Ukabi, S., Dubinsky Z., Steinberger Y, and Israel A. 2012. Surveying *Caulerpa* (Chlorophyta) Species Along the Shores of the Eastern Mediterranean. Mediteranian Marine Science, (13) 1, p. 5.
- Verlaque, M., Durand C. Huisman J.M., Boudouresque C.M, and Y.L. Parco. 2003. On the identity and origin of the Mediterranean invasive *Caulerparacemosa* (Caulerpales, Chlorophyta). European Journal of Phycocloid, 38, p. 325
- Yadav, R.N.S. and Agarwala M. 2011. Phytochemical Analysis of Some Medicinal Plants. Journal of Phytology, (3) 12, p.11.
- Yang, P., Liu, D.Q., Liang, T.J., Li, J. Zhang, H.Y., Liu, A.H., Guo, Y.W. and Mao, S.C. 2015. Bioactive Constituens from the Green Alga *Caulerpa racemosa*. Bioorganic and Medical Chemistry, 23, p. 1.
- Yermakov, A.I., Khlaifat, A.L., Qutob, H., Abramov, R.A., Khomyakov, Y.Y. 2010. Characteristic of the GC-MS Mass Spectra of Terpenoids (C₁₀H₁₆). Chemical Sciences Journal, 7, p. 1.



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