Production of Monascus Pigments on Durian Seed: Effect of Supplementation of Carbon Source

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Studies on the potential of durian seed as a substrate for monascus pigments production and the effect of carbon source supplementation on the monascus pigments production have been done. After pretreatment, durian seed was supplemented with carbon source (sorbitol, fructose, glucose and maltose) at 5% w/w, inoculated with *Monascus* sp. KJR2 culture, and then incubated for 14 days at room temperature (30°C). The fermented matter was dried and analyzed for the yellow, orange and red pigments. The results showed that durian seed has a good potential as a substrate for monascus pigment production. Supplementation of maltose, glucose and fructose are effective to increase monascus pigments production. Maltose is the most effective for carbon source supplementation, with water soluble yellow, orange and red pigments yield of 16.80 A/g, 11.49 A/g and 10.71 A/g, respectively as well as ethanol soluble pigments of 22.36 A/g, 6.93 A/g and 8.53 A/g.

Key words: durian seed, supplementation, carbon source, monascus pigments

Natural food colorants have manifold advantages over synthetic colorants in terms of both health and environment. It can be produced by animals, plants, and microorganisms. Among microbial sources of natural colorants, filamentous fungi are the most explored because it has higher potentiality to produce higher productivity with the available cultivation technology, than of plants or insects based natural colorants that are currently in use[9,12].

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Monascus has long been known that it produce yellow, orange and red pigments, which can be used for coloring foods. *Monascus* sp produce red, orange and yellow pigments during both solid state fermentation and submerged fermentation. Six major related pigments are divided into 3 groups : rubropunctatin and monascorubrin are orange pigments with different side chains on the ozo-lactone ring. The two corresponding red pigments, rubropunctamine and monascorubramine, are nitrogen analogues of the orange pigments. The yellow pigments, monascin (monascoflavin) and ankaflavin are the reduced forms of the two orange pigments. The pigments can easily react with amino group containing compounds in the medium such as proteins, amino acids or nucleic acids to form water-soluble pigments[7].

Traditionally, *Monascus* cultivates on rice substrate. Many studies showed that it grows in a wide variety of natural substrates besides rice i.e. corn, cassava, wheat, potato and adlay[6, 8, 13, 14]. Due to high cost of currently used technology of pigment production on an industrial scale, there is a need for developing low cost process for the production of pigments. From the literature it is evident that utilization of a cheaply available substrate through solid-state fermentation can attain the objective of pigment production in an economically feasible way [3]. Various agro-industrial residues such as rice bran, wheat bran, cassava bagasse, jackfruit seed have been exploited for pigment production[3, 7]. Durian seed is an agro-industrial residu and underutilized.

Durian (*Durio zibethinus* Murr) is one of high value tropical fruits in some Asian countries. In 2009, production of durian in Indonesia was 857.851 tons[4]. Durian seeds are usually discarded. Seeds make up around 5-15 % of the total fruit mass. Fresh durian seeds contain high moisture, carbohydrate and protein[2]. The objectives of this research were to studies the potential of durian seed as a substrate for monascus pigments production by solid-state fermentation process and the effect of carbon source supplementation on the monascus pigments production.

MATERIAL AND METHODS

Culture and Starter preparation

A culture of *Monascus* sp. KJR2 obtained from Center for Food and Nutrition Research, Widya Mandala Surabaya Catholic University. It was maintained on Saboraud's Dextrose Agar (SDA) slant, preserved at 4°C and sub cultured routinely in every four weeks. *Monascus sp.* KJR2 was grown on SDA slants at room temperature (30°C) under static conditions for 14 days, 10 mL of sterile distilled water was added and the spores were scraped under aseptic conditions. The spore suspension obtained was inoculated into Saboraud's Dextrose Broth (SDB) and then was incubated at room temperature (30°C) for 10 days. It was used as starter to produce monascus pigments on sterilized durian seed.

Solid-state fermentation (SSF)

Durian seeds (variety Manalagi) were obtained from local durian seller. Durian seeds were stored in a freezer (-4°C) until used. Proximate and starch content analysis of durian seed were conducted by standard methods of AOAC [1]. After boiling for 10 minutes in a $CaCO_3$ solution of 5% w/v to remove the mucus, then peeled the seed coat, cut into size of 0.5 cm x 0.3 cm x 0.3 cm. A 50 g of durian seed was transfer into 300 mL of flask, added with carbon sources (glucose, fructose, sucrose, maltose and sorbitol) at 5% by mass. The contents of the flasks were mixed thoroughly, autoclaved at 121°C for 15 minutes, then cooled to room temperature. It was inoculated with the spore suspension of *Monascus* sp. KJR2 and incubated at room temperature (30°C) for 14 days in static condition (with shaking daily). Red mold durian seed were dried in an oven at 45°C for 24 hours, and then ground. The product was then analyzed for the water soluble pigments and ethanol soluble pigments.

Pigment Analysis

Ethanol soluble pigments

Ethanol soluble pigments were analyzed according to Babitha *et al*.,[3] with little modification. A 1 g of fermented matter was taken in a 250-mL conical flask and mixed with 90% ethanol (adding 5 mL of distilled water per gram of fermented matter on dry basis). The content was mixed on a shaker at 200 rpm, allowed to stand for 15 minutes, and then filtered through Whatman No 1 filter paper. The filtrate was measured by using spectrophotometer (Shimadzu, UV 1601) at 400 nm for yellow pigment, 470 nm for orange pigment and 501 nm for red pigment. Pigment yield was expressed as Absorbance at corresponding wavelength per gram of dry substrate (A/g).

Water soluble pigments

Water soluble pigments were analyzed according to Carvalho *et al*.,[6] with little modification. Water soluble pigments analysis was conducted with extraction of the pigments with distilled water. A 1 g of fermented matter was taken in a 250-mL conical flask and mixed with distilled water (adding 5 mL of distilled water per gram of fermented matter on dry basis). The content was mixed on a shaker at 200 rpm, allowed to stand for 15 minutes, centrifuged at 5000 rpm 30°C for 15 minutes, and then filtered through Whatman No 1 filter paper. The filtrate was measured by using spectrophotometer (Shimadzu, UV 1601) at 400 nm for yellow pigment, 470 nm for orange pigment and

501 nm for red pigment. Pigment yield was expressed as Absorbance at corresponding wavelength per gram of dry substrate (A/g).

Data analysis

The obtained data will be analyzed using analysis of variance (ANOVA) at α = 5%. If the ANOVA test results indicate a significant effect, followed by Duncan's Multiple Range Test (DMRT) at α= 5% to determine the level of treatment that gives a significan difference.

RESULTS AND DISCUSSION

Chemical composition of durian seed and its potential as *Monascus* substrate

Table 1 show the chemical composition of durian seed. The protein content of durian seed is comparable to the jackfruit seed of 4.2% [3]. The starch content of durian seed was lower than that of rice of 77% [8] and jackfruit seed of 36.7% [3]. The chemical composition reflects that durian seed has a potential as monascus substrate. Durian seed need a pretreatment prior to be used as a substrate for monascus pigments production because it contains sticky mucus. The mucus was removed by boiling it in calcium carbonate solution at 5% w/v for 10 minutes. Figure 1 is the photographs of fermented matter without carbon source supplementation, after 3; 8 and 13 days fermentation. This figure showed that durian seed is suitable for *Monascus* growth and pigments production.

Effect of carbon source supplementation

Table 2 and 3 showed the water and ethanol soluble monascus pigments, respectively. Generally, *Monascus* sp KJR2 grew well on durian seed and produced considerable amount of yellow, orange and red pigments. Water soluble yellow, orange and red pigments yield of control (no sugar addition) were 10.30 A/g; 7.82 A/g; and 6.76 A/g,

Table 1. Chemical composition of durian seed

Component	Content (g/100g)
Moisture	54.09 ± 0.71
Ash	1.58 ± 0.12
Crude fat	1.32 ± 0.10
Crude protein	3.40 ± 0.29
Starch	18.92 ± 0.59

Table 2. Water soluble of monascus pigments

Carbon source	Yellow (A/g)	Orange (A/g)	Red(A/g)
Control*)	10.30 ^{a}	7.82 ^{ab}	6.76 ^{a}
Sorbitol	9.54 ^{a}	6.30 ^{a}	5.99 ^{a}
Fructose	14.32 ^{b}	10.04 ^{bc}	9.52 ^{b}
Glucose	14.51 ^{b}	10.06 ^{bc}	9.58 ^{b}
Maltose	16.80 ^{b}	11.49 ^{c}	10.71 ^{b}

Note: *)control is no carbon source supplementation
Different character in the same column indicated a significantly difference at α = 5%

Table 3. Ethanol soluble of monascus pigments

Carbon source	Yellow (A/g)	Orange (A/g)	Red (A/g)
Control*)	7,28 ^{a}	2,40 ^{a}	3,29 ^{a}
Sorbitol	13,12 ^{b}	4,36 ^{ab}	5,63 ^{ab}
Fructose	20,55 ^{cd}	8,13 ^{c}	10,08 ^{c}
Glucose	17,15 ^{bc}	7,11 ^{bc}	8,89 ^{bc}
Maltose	22,36 ^{d}	6,93 ^{bc}	8,53 ^{bc}

Note: *)control is no carbon source supplementation
Different character in the same column indicated a significantly difference at α = 5%

Fig. 1. Photographs of fermented matter without carbon source supplementation on the day 3 (a); day 8 (b); and day 13 (c) of fermentation

Fig. 2. Photographs of fermented matter supplemented with maltose on the day 3 (a); day 8 (b); and day 13 (c) of fermentation

respectively. Supplementation of fructose, glucose and maltose resulted significant increasing of pigments production. Addition of sorbitol have no effect on the growth and pigments production significantly even tend to inhibit the growth. Babitha et al [3] found the similar result that monascus pigments yield was poor when jackfruit powder supplemented by sorbitol. Data obtained showed that maltose gave the highest both water and ethanol soluble pigments yield, followed by glucose and fructose. Figure 2 showed the photographs of fermented matter supplemented with maltose on the day 3 (a); day 8 (b); and day 13 (c) of fermentation. In the case of glucose supplementation, other researchers also found the similar results that glucose as good substrate for Monascus growth and pigment production [10, 11, 15]. However, Babitha et al [3] found the extremely different result which was glucose supplementation into jackfruit powder gave poorest monascus pigments production. Utilization of carbon sources for growth appears to be strain specific.

The durian seed is suitable as substrate for *Monascus* growth and pigments production. Maltose was the most effective carbon source to be supplemented into the durian seed for Monascus growth and pigments production, followed by glucose and fructose.

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