FULL FACTORIAL EXPERIMENTAL DESIGN TO DETERMINE FACTORS INFLUENCED TOTAL BACTERIAL CONTENT IN MAKING VEGETABLE SAUSAGE

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ABSTRACT

Sausage has became one of the favorite food of Indonesian society but people are less aware of the adverse health impact because of the content of sausages are consumed. Vegetable sausage can be an alternative food to minimize the negative impact on health. In making vegetable sausage, it should contain the appropriate nutrition. Vegetable sausage must have the appropriate nutritional content of Indonesian National Standard (SNI 01-3820-1995), min.13% protein, fat max. 25%, and microbial contamination (ISO 7388-2009) 1 x 104 colonies / gram. In this paper, will be discussed design of making process of vegetable sausage using full factorial experimental design. Response of experiment is total bacterial content and the factors are boiling time and and the water composition.

Keywords: vegetable sausage, full factorial experimental design

INTRODUCTION

Sausage has been a favorite food consumed by the people of Indonesia since the 1980s. Everyone already knows sausages as delicious food made from basic ingredients are chopped meat, mashed, and given flavorings and preservatives then wrapped with animal gut or artificial wrapper but people doesn't aware of the impact on health. The consumption of 50 grams of sausage a day can increase bowel cancer by 20 percent associated with processed meat. Moreover, in some types of sausages contain high levels of fat, cholesterol and sodium that potentially lead to obesity, heart disease, stroke, and hypertension. Hence the need for an alternative to reduce the negative impact on health.

Vegetable sausage is a sausage that only contain vegetable protein and fat and also materials of manufacture only derived from plant materials without any additional animal ingredients. Nutrient content in the sausage has been set according to the Indonesian National Standard (SNI 01-3820-1995); sausage good must contain a minimum of 13% protein, a maximum of 25% fat, and carbohydrates at least 8%. In addition, a good sausage must also comply with the maximum limit of microbial contamination in the sausage, which is produced sausages should not exceed the maximum limit contamination bacteria 1 x 10^5 colonies / gram [1]. According Susiwi [2], the number of bacteria influenced by water, pH and temperature.

Design of experiments is a procedure that needs to be taken before the experiment should be done so that the necessary data can be obtained, so that an objective analysis and conclusions can be done (Montgomery, 2001)[3]. Full Factorial Design is the method used in this experiment in which all levels of a particular factor combined with all levels of each of the other factors in the experiment. The aim of this research is to find optimal parameter in vegetable processing.

Vegetable sausage

Vegetable sausageis sausage that made from mixture of basic ingredients. The main raw material is the manufacture of sausages vegetable oyster mushrooms, red beans, wheat flour and tapioca flour. Oyster mushrooms have a springy nature, rich in fiber and protein so it can be used as a mixture of vegetable sausage while red beans contain 17% protein. Protein content of both material is high enough as a source of vegetable protein diet and vegetarians (Adaninggar, 2013). [4]

RESEARCH METHODOLOGY

The aim of this research is to determine factors of the optimal level of boiling time and water composition in making vegetable sausage. The response is total bacterial content. The objective is to determine the level of boiling time and water composition so the bacterial content minimum.

1. Statistical analysis

The analysis of variances and design of experimental (DOE) 3^2 factorial were used to investigate the influence of experimental factors such as boiling time and water composition. The level of these factors can be seen on Table-1.

Table-1. Factors and level.

Factor	Level	Value
Boiling Time (A)	1(low)	28 minutes
	2 (center)	30 minutes
	3 (high)	32 minutes
Water Composition (B)	1(low)	500 mL
	2 (center)	550 mL
	3 (high)	600 mL

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Randomized design of experiment with 2 replication shown in Table-2.

Table-2. Design matrix for design of experimental (DOE) 3^2 factorial.

Exp	A (level)	B (level)	Exp	A (level)	B (level)
1	1	2	10	3	2
2	2	3	11	1	1
3	1	1	12	1	3
4	3	1	13	3	2
5	1	2	14	2	2
6	3	1	15	2	1
7	2	2	16	1	3
8	2	1	17	3	3
9	2	3	18	3	3

2. AEROBIC PLATE COUNT (APC)

The aerobic plate count (APC) is intended to indicate the level of microorganism in a product. Detailed procedures for determining the APC of foods have been developed by the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Calculation of microrganism using equation 1).

$$N = \frac{\sum C}{[(n_1 \times n2) + (0.1 \times n_2)+(\dots)] \times d} \qquad (1)$$

where:

N   = Number of colonies per ml or g of product

$\sum C$ = Sum of all colonies on all plates counted

n_1 = Number of plates in first dilution counted

n_2 = Number of plates in second dilutioncounted

d   = Dilution from which the first counts were obtained

3. PROTEIN TEST AND FAT TEST

This test is used to examine whether the sausages meets the requirements of SNI namely a maximum fat content of 25% and minimum 13% of protein

4. RESULT AND DISCUSSIONS

Result of experiment are display at Table-3.

Table-3. Result of experiment.

Experiment	Bacterial content (x 10^4 colony/grams)	Protein content	Fat content
1	0,8123	16.13%	3.49%
2	0,9786	16.33%	3.56%
3	0,7926	16.36%	3.73%
4	0,8213	16.50%	3.71%
5	0,8130	16.55%	3.49%
6	0,8240	16.56%	3.67%
7	0,8324	16.71%	3.64%
8	0,7932	16.79%	3.73%
9	0,9790	16.97%	3.57%
10	0,8234	15.94%	3.75%
11	0,7926	16.71%	3.77%
12	0,9756	15.77%	3.61%
13	0,8230	15.75%	3.62%
14	0,8320	15.91%	3.70%
15	0,7930	16.97%	3.75%
16	0,9810	16.79%	3.64%
17	0,9980	15.65%	3.78%
18	0,9960	16.79%	3.67%

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The results of the above experiments then conducted analysis of variances (ANOVA) to determine the influence of factors on the bacterial content on Table-3.

Table-4. Analysis of variances (α= 0, 05).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Boiling Time	2	0,0012	0.0012	0.0006	262,44	0.13
Water	2	0.1194	0.1194	0.0397	25935,93	0.00
Water*Boiling Time	4	0.0008	0.0008	0.0002	88,18	0.00
Error	9	0.0000	0.0000	0.0000		
Total	17	0.1215				
S=0.00151767		R-Sq=99.98%		R-Sq (adj) = 99,97%		

1. Hypothesis testing

Testing of each source of variation is done by using p-value. Hypothesis in this ANOVA are:

H_0 :$\mu_1=\mu_2=\mu_3$ there is no difference in mean of bacterial content between the level

H_1 :$\mu_{1\neq} \mu_{2\neq}\mu_3$ at least one difference in mean of bacterial content between the level

The test criteria are as follows:

If the p-value $< \alpha$, then reject H_0 that means there are at least one level significantly different.

If the p-value $\geq \alpha$, then failed to reject H_0 that means no significantly different.

Boiling time factor

The p-value was 0.13. which means there is no significant difference between the level of the boiling time factor. This suggests that the boiling time no significant effect on total bacterial content in vegetable sausage products

Water composition factor

For the composition of the water, the value of p was 0.000. Thus, the conclusion is reject H_0, which means there are significant differences between the levels in the composition of the water. This shows that the composition of the water significant effect on total bacterial content in vegetable sausage products.

Boiling time and composition boiling water interaction

For interaction with the boling time and water composition, the value of p was 0.000. So the conclusion is reject H_0, which means there are significant differences between the levels in time factor boiling with water composition. This suggests that the interaction with the time factor boiling water composition effect on total bacterial content in vegetable sausage products.The differences of bacterial content betwen level of each factor as shown in Figure-1 and mean of bacterial content of each level shown in Table-4.
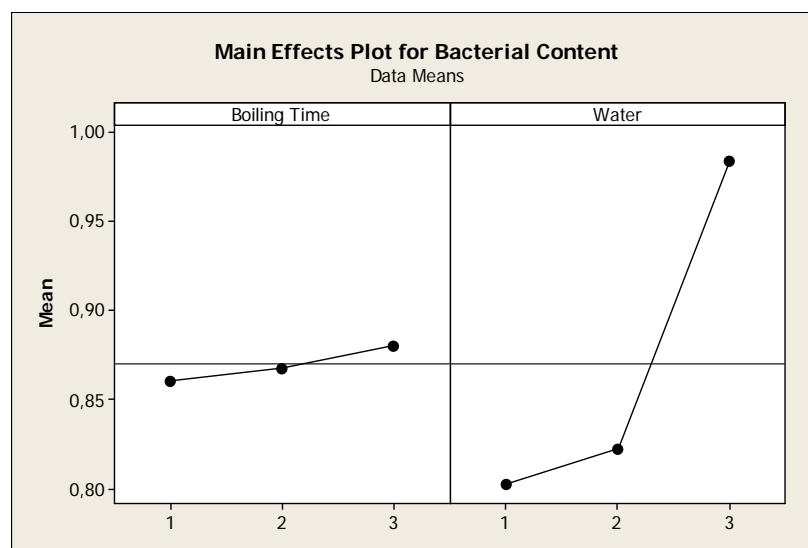

Figure-1. Main effects plot for total bacterial content.

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Table-5. Mean of bacterial content.

Factor	Bacterial content (x 10^4 colony/grams)		
	Level 1	**Level 2**	**Level 3**
Boiling Time	0,8611	0,8730	0,8810
Water	0,8028	0,8227	0,9847

Figure-1 and Table-4 show that optimal level of water composition is 500 mL while boiling time be on every level in range. Table 3 shows that protein content and fat content meet the requirement of Indonesian National Standard (SNI 01-3820-1995).

CONCLUSIONS
The experimental results show that the boiling time did not have a significant effect on the bacterial content. The water composition has a significant impact on bacterial content. The optimal level of boiling time and water composition is 28 minutes and 500 mL respectively. Producing vegetables sausage at this level will meet the requirement of Indonesian National Standard (SNI 01-3820-1995).

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