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Study of Making Black Soybean Soyghurt (*Glycine soja* L Merrit) Using *Lactobacillus acidophilus* to Inhibit Pathogenic Bacteria

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ABSTRACT

Black soybean soyghurt is a very beneficial health drink because it contains good nutrition and as an antibacterial. This study used a factorial 3 x 3 x 3 Completely Randomized Design (CRD). The collected data were analyzed using Analysis of Variance (ANOVA). If there is a significant difference with the control then it is continued with Duncan's multiple distance test. The results obtained are the best fat content of 1.978%, ash content of 0.241 and pH 4.055. All research results were in accordance with the 1992 Indonesian National Standard (SNI). Meanwhile, the inhibition zone of *Lactobacillus acidophilus* against *Escherichia coli* was 15.7 mm.

KEY WORDS: Study, Soyghurt, Black soybean, *Lactobacillus acidophilus*, Zone of inhibition, pathogenic bacteria

INTRODUCTION

Black soybean is one of the varieties of soybean (*Glycine max* (L) Merrit). Botanically and nutritionally, black soybeans have many similarities with yellow soybeans, but the black color makes this soybean has specific uses, one of which is for making soy sauce. Along with the development of soybean processing time, its utilization has been developed, including making soyghurt.

Soyghurt is a fermented product of soy milk. The manufacture of soyghurt needs to be introduced in Indonesia because soyghurt products have high nutritional value and are still difficult to obtain in the domestic market. In addition, the use of soy milk for yogurt will also help diversify processed soybean products as a source of quality protein. The addition of probiotic bacteria to soyghurt, such as *Lactobacillus acidophilus*, has the potential to improve product quality and health status. Soyghurt can provide ideal conditions for the growth of probiotic bacteria and for the human digestive system. In making soyghurt, it is possible to diversify the culture of Lactic Acid Bacteria (LAB) which are probiotics such as *L acidophilus*, with the aim of improving the balance of microflora in the intestine. Soyghurt has several advantages compared to other fermented milk products, namely soyghurt contains protein ranging from 3-3.5% with lower production costs. Soyghurt can also provide many health benefits, including preventing degenerative diseases such as cancer, osteoporosis and coronary heart disease. In addition, it can also help prevent diarrhea, prevent an increase in blood cholesterol levels that are too high and help diversify processed soybean seeds as a source of quality protein (LPM, 2019).

Soyghurt products are expected to be an alternative drink with probiotic content and high isoflavones as components that can reduce free radical activity in the body. Therefore, it is necessary to develop yoghurt products made from vegetable milk such as soybeans (Naila, 2020). The problem of food safety is a global problem so that attention needs to be paid in setting public health policies. Foodborne diseases often occur from food contamination, especially in developing countries with poor sanitation and hygiene conditions but also in developed countries (Nurul, 2022), therefore it is necessary to study pathogenic diseases, one of which is *Escherichia coli*. These bacteria are normally (commensal) found in the large/small intestinal tract of healthy children and adults and the number can reach 109 CFU/g. *Escherichia coli* is known as a cause of diarrheal disease. However, there are only a few E. coli that are pathogenic (Zulva, 2020).

MATERIALS AND METHODS

L acidophilus bacteria were obtained from the Microbiology Laboratory of the Bandung Institute of Technology, Indonesia, and Malika black soybeans were obtained from online stores. Before using *L acidophilus*, a growth curve was made, to find out at what age the bacteria were used.

The research method used a 3x3 factorial Completely Randomized Design (CRD) with 3 replications. The first factor consisted of the inoculum dose (D) with the following treatment levels: The first factor was the type of bacteria,

namely *Lactobacillus acidophilus*. The second factor is the inoculum dose (D) consisting of d1 = 2 %, d2 = 3 %, d3 = 4 %. The third factor is the fermentation time (W) with the following levels: w1 = 4 hours, w2 = 8 hours and w3 = 12 hours.

Parameter

Parameters measured were nutrient content, namely the zone of inhibition, fat content, ash content and pH.

Data analysis

Data from *Lactobacillus acidophilus* fermentation were analyzed using Anova (Analysis of Variance). If there is a significant effect of the treatment, it is continued with Duncan's multiple distance test.

The mathematical model that describes the influence of the two factors is formulated as follows:

$$Y_{ij} = \mu + D_i + W_j + D_iW_j$$

Information :

i = 1,2,3.....

j = 1,2,3.....

Yij = Variables of the i-th and j-th responses.

= Average response variable

Di = Effect of treatment dose i of the inoculum dose factor

Wj = Effect of dose treatment to j time effect factor

DiWj = Influence factor interaction inokucum dose and fermentation time.

Research Procedure

Making *Lactobacillus acidophilus* starter

Making MRSA media then put in a petri dish.Pour the bacteria into 1 ml petri dish that has been given MRSA media. Store in the incubator for 12 hours. The harvested bacteria were put into 10 ml physiological NaCl then centrifuged at 600 rpm for 20 minutes, the precipitate + physiological NaCl was separated and equated with Mc Farlan 2 standard.

Making Mc Farlan Solution 2 : To make a standard Mc Farlan 2, weigh 0.2 grams of barium chloride plus 9.8 ml of H2SO4.

Antibacterial activity test (Ernawati et al, 2016)

Bacterial testing was carried out using the disc diffusion method (Kirby-Bauer Test). A test tube containing pathogenic bacteria *(E coli*) was taken 1 ose and then grown on a petri dish containing NA, then placed a paper disc that had been soaked with

L acidophilus for 10 minutes and slightly pressed to stick. Incubate for 24 hours, the inhibition zone formed will appear clear color around the paper disc.

Making Black Soy Milk (Pricilia et al, 2015)

Black soybeans are good soaked in water for 12-24 hours, boiled for 15 minutes, then remove the husk. After that, 8000 ml of hot water with a temperature of 60-90^{o}C was added, then blended and then filtered. The filtered result obtained is soy milk.

Make Black Soy Soyghurt

Soy milk that has been made is then fermented by *Lactobacillus acidophilus* (the zone of inhibition against *E coli* is known) with each dose of inoculum and then fermented according to the time of the research treatment.

Fat Analysis (Asmariani et al, 2017)

The sample was weighed and put into a paper sleeve which was corked and lined with cotton. The sample was put into a Souxlet which was connected to a fat flask containing boiling stones that had been dried and the weight was known. The sample was extracted using hexane for 6 hours, then the hexane was distilled and the fat extract was dried in a drying oven at 105^{0}C. The fat extract was cooled and weighed. Fat content is calculated using.

$$\text{Fat Level \%} = \frac{W2}{W1} \times 100\ \%$$

W1 = Weight of flask before extraction (grams)

W2 = Weight of empty fat pumpkin (grams)

Ash Content Analysis (Amelia et al, 2015)

The ash content test is a petri dish dried in an oven at 105^{0}C for 1 hour then cooled in a desiccator for 15 minutes then weighed 2 grams of the sample in a petri dish and then put into an ashing furnace (Furnace) with a temperature of 600^{0}C for 3 hours and then cooled in a desiccator until 120^{0}C temperature. The cup and ash were weighed so that a constant weight was obtained, then the ash content was calculated using the formula:

$$\text{Ash Content \%} = \frac{W2}{W1} \times 100\ \%$$

pH testing (AOAC, 1995)

pH measurement is done using a pH meter. The pH meter was standardized first according to the buffer for pH 4 and 7 according to the pH range of the yughurt. Measurements were made by dipping the pH meter electrode into 10 ml of the sample.

RESULTS

Pathogenic Bacteria

E coli bacteria are normally (commensal) found in the large/small intestinal tract of healthy children and adults and the number can reach 10^9 CFU/g. The name of this bacteria may already be familiar to our ears. This bacterium, which is one of its natural habitats is the human intestine, is often found in an environment with poor sanitation. *E coli* can be found in agricultural products that are polluted from the environment around agricultural land.

Escherichia coli is known as a cause of diarrheal disease. However, there are only a few *E coli* that are pathogenic. One of the pathogenic *E coli* strains is *E coli* 0157:H7 (Nurul, 2022). This strain of *E coli* produces a toxin called Shiga

toxin. *Lactobacillus acidophilus* is a Gram positive bacteria, has the ability to remodel simple carbohydrates into lactic acid, with increasing lactic acid, the pH of the environment becomes low which causes other bacteria not to grow. When colonization occurs on the surface of the gastrointestinal tract, lactobacilli prevent fungal growth and suppress *E coli* and other Gram-negative pathogens. In this study, *L acidophilus* 600 x 10^6 CFU/ml or 60 x 10^7 CFU/ml was used, the inhibitory power was 15.7 mm. In line with the opinion of Suriawiria, 1986 which states that Lactobacillus can inhibit pathogenic microbes in 10^7.

Fat

Vegetable fats are good fats that come from plants. There are various benefits of vegetable fats to maintain a healthy body, including lowering cholesterol levels and preventing heart disease. Vegetable fat is different from saturated fat and trans fat which can actually contribute to increasing the amount of bad cholesterol (low density lipoprotein / LDL) in the blood and trigger the emergence of various diseases in the body. The results of the fat study on black soybean soyghur are presented in Table 1.

Table 1: Effect of inoculum dose on fat content

Inoculum dose	Average (%)	Signification
d1(2%)	1.283	a
d2(3%)	2.153	b
d3(4%)	2.252	c

Note: Lowercase letters indicate a significant difference according to Duncan's distance test at the 5% level.

Table 1 shows that there is a very significant difference, the difference is in d3 the highest fat content is 2.252%, then followed by d2 at 2.153% and the lowest is in d1 at 1.283%. The higher the microbial dose, the more lactic acid bacteria (LAB), the more nutrients needed, one of which is fat. According to Weerathilake (2014) the fat content in soyghurt is strongly influenced by the mixed ingredients at the time of making soyghurt and the fat content contained in the soyghurt product is contained from the raw materials used.

Table 2 : Effect of fermentation time on fat content

Fermentation time	Average(%)	Signification
w2 (8 hours)	1.845	a
w3 (12 hours)	1.865	b
w1(4 hours)	1.978	c

Note: Lowercase letters indicate a significant difference according to Duncan's distance test at the 5% level.

Based on Table 2, the best fat content at w1 fermentation time is 4 hours, the fat content is 1.978% and the lowest is at 12-hour fermentation time is 1.865%. The longer the fermentation time, the more LAB, the more fat needed for nutrition so that less fat is produced. According to the results in Table 1, the more inoculum doses, the shorter the fermentation time to produce a lot of fat. The fat content of this study is in accordance with the 1992 Indonesian National Standard (SNI), that the fat content is not more than 3.8% (Yuliasni, 2007).

Ash Level

Ash content is a mixture of inorganic or mineral components contained in a food. Foodstuffs consist of 96% inorganic materials and water while the rest are mineral elements. The element is also known as organic matter or ash content. The ash content can indicate the total minerals in a food ingredient (Zahro, 2013).

Table 3: Effect of inoculum dose on ash content

Inoculum dose	Average(%)	Signification
d3 (4%)	0.216	a
d1 (2%)	0.221	a
d2 (3%)	0.253	b

Note: Lowercase letters indicate a significant difference according to Duncan's distance test at the 5% level.

Based on Table 3 the effect of dose on ash content, the highest ash content was found at a dose of d2 of 0.253%, followed by d1 of 0.221% and d3 of 0.216%. d3 and d1 were not significantly different, so the optimal dose to get high ash content was 3% dose. Ash content is an organic substance left over from the combustion of an organic material. According to SNI, the ash content in yogurt is not more than 1% (Inmas, 2019). The high and low ash content of a material is related to minerals (Askar, 2005).

Table 4: Effect of fermentation time on ash content

Fermentation time	Average (%)	Signification
w1 (4 hours)	0.217	a
w3 (12 hours)	0.233	b
w2 (8 hours)	0.241	b

Note: Lowercase letters indicate a significant difference according to Duncan's distance test at the 5% level

The results in Table 4, show that the fermentation time of the ash content, w2 gave the highest yield of 0.241 %, followed by w3 which was 0.233% and finally w1 of 0.217%. W2 and w3 did not give a significant difference but with w1 significantly different. The optimal time to ferment soyghurt by *L acidophilus* is 8 hours. Fermentation can give a new taste and texture to the product so that the product quality is better. The acids formed during fermentation also give soyghurt its distinctive taste. This is in line with the results of research by Dewi, 2011 which states that the addition of different sugars to soyghurt will produce different organic acids which in turn will get good quality soyghur.

pH (Power of Hydrogen or Hydrogen Potential)

pH is a measure of the concentration of hydrogen ions in water. In chemistry, pH is a measurement of the hydrogen ion concentration in a water-based solution, which is a measure of the acidity or alkalinity of an aqueous solution or other liquid. The pH scale from 0-14 is usually measured with a pH meter. A low pH means more hydrogen ions in the liquid while a high pH indicates less hydrogen ions (Anugrah, 2022).

Table 5: Effect of inoculum dose on pH

Inoculum dose	Average (%)	Signification
d3 (4%)	4.08	a
d2 (3%)	4.37	b
d1(2%)	4.4	c

Note: Lowercase letters indicate a significant difference according to Duncan's distance test at the 5% level

Based on Table 5, all of them show significant differences, the highest pH is at d1 which is 4.4, followed by d2 at 4.37 and the lowest is d3 at 4.08. The higher the dose added to the soyghurt, the more lactic acid formed, resulting in a decrease in pH. This study is in line with the results of research by Yulian Candra S, 2014 which states that lactic acid bacteria (LAB) utilize monosaccharides during fermentation so that the lactic acid formed produces metabolites, the more lactic acid causes the pH to drop and the decrease in pH causes the formation of casein coagulant so that the texture becomes thick. . Indratiningsih, et al., (2004) added that the starter inoculation is possible for lactose degradation and lactic acid production which results in a decrease in pH and the formation of yogurt clots. The decrease in pH is also thought to be due to the pH of salak pondoh which has a pH of 4.46 so that it can accelerate the acidic conditions in the yogurt drink.

Table 6: Effect of fermentation time on pH

Fermentation time	Average (%)	Signification
w1 (4 hours)	4.055	a
w3 (12 hours)	4.248	b
w2 (8 hours)	4.565	c

Note: Lowercase letters indicate a significant difference according to Duncan's distance test at the 5% level

Based on Table 6, it shows that w1 and w3 and w2 show significant differences, where the highest pH value is found in w2 at 4,565, followed by w3 at 4.248 and the lowest at w1 at 4055. In accordance with the results in Table 5, the more the dose of inoculum given, the lower the pH, so in a short time of 4 hours there has been a decrease in pH. The pH value of the research results is in accordance with SNI 1992 which states that quality yogurt has a pH of 4.1 – 4.5 (Yuliasni, 2007). In research, Zubaedah et al., 2010 stated that the pH of salak fruit was 4.46 which was added to yughurt containing sugar which could be used by LAB for fermentation, resulting in a decrease in pH. In accordance with the opinion of Supriyadi, et al., (2002) that salak fruit contains sucrose, fructose, and glucose. The results of research by Djaafar and Rahayu, (2006) stated that during the fermentation process LAB will utilize existing carbohydrates to form lactic acid, resulting in a decrease in the pH value.

DISCUSSION

The fat content in black soybean soyghurt is 2.52%. When compared to the requirement for youghut according to SNI (1992) in Yuliasni 2007. which is the highest 3.8%, this result can still be considered. The fat content in yogurt is strongly influenced by the fat content in the mixture when making yogurt and the fat content contained in yoghurt products (Weerathilake 2014). Amir, 2015 states that the fat content in yoghurt depends on the basic ingredients, namely skim milk and soy milk which contain quite high fat. The decrease in the value of fat contained in soyghurt is influenced by the amount of LAB. The ash content of the research results is 0.241 in accordance with SNI, which is not more than 1% (Yuliasni, 2007). The ash content indicates the presence of minerals contained in the food product. The pH of the research results is in accordance with SNI, which is 4.055 according to SNI, namely 4.1 - 4.5. (Yuliasni, 2007).

CONCLUSION

From the results of the study, it can be concluded as follows: The highest fat was produced at an inoculum dose of d3 with a fermentation time of w1 of 1.978%, the best ash content was produced at d2 of a fermentation time of w1 of 0.241% while the best pH was found in d3 fermentation time of w1 of 4.055 while the *Lactobacillus acidophilus* inhibition zone against *Echechia coli* at 15.7 mm.

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