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The Fermentation Time Effect against the Isoflavones Profiles of Genistein and Daidzein of Blacksoyghurt as a Potential Functional-Probiotic Drink

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Abstract— **Blacksoyghurt is a fermented black soybean juice drink as a vegan probiotic product as an option as a substitute for dairy products. Black soybean glucoside isoflavone compounds are converted into aglycone compounds during the fermentation process which is antioxidant compounds. This study aims to determine the effect of fermentation time on the isoflavone profile of genistein and daidzein blacksoyghurt, as well as panelists' preferences as potential functional drinks. Blacksoyghurt was fermented with 5% lactic acid bacteria starter at 37°C for 0 hours, 3 hours, 6 hours, 9 hours, and 12 hours. The results of isoflavone, genistein, and daidzein profiles were analyzed by descriptive analysis using Microsoft Excel 19 for windows. LAB viability analysis was analyzed using one-way ANOVA followed by Duncan's analysis to determine whether there was a significant difference in treatment at the $p<0.05$ confidence interval. The hedonic test data were analyzed using the Kruskal Wallis test with a significance level ($p<0.05$), followed by the Mann-Whitney test. The longer blacksoyghurt fermentation time resulted in an increase in antioxidant levels of IC50 (1385.18 - 760.79), isoflavone profile (37.50 µg/g - 313.64 µg/g), and lactic acid bacteria (0 CFU/mL - 2.04 x 1010 CFU/mL), and decreased levels of genistein (28.22 µg/g - 22.97 µg/g) and daidzein (32.66 µg/g - 25.49 µg/g). Panelists prefer blacksoyghurt which has a sour taste, a distinctive yogurt aroma, and a thick texture. The best-recommended fermentation time is 12 hours for the isoflavone, genistein, and daidzein content, as well as the panelists' high preference.**

Keywords— **Antioxidant; blacksoyghurt; daidzein; genistein; hedonic; isoflavone.**

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I. INTRODUCTION

Yogurt is a functional food made from a mixture of milk and lactic acid bacteria *Streptococcus thermophilus* and Lactobacillus bulgaricus and the addition of *Lactobacillus thermophilus* bacteria as a source of probiotics [1]. As it is known that cow's milk contains lactose, some people are lactose intolerant. Therefore, an innovation of processed yogurt that does not contain lactose is carried out, namely by replacing the raw material of animal milk with vegetable milk, one of which is made from black soy milk.

Black soybean (*Glycine soja* (L) *Merrit*) is a native plant of Tropical Asia, such as Southeast Asia, including Indonesia which has been cultivated for a long time such as the Cikuray, Local Wonosari, Local Bantul, and Malika varieties. Black soybeans have the main content of 47% protein and 20% fat. Black soybeans also contain higher amounts of polyphenols, flavonoids, and antioxidants than yellow soybeans, each with 6.13 mg/g; 2.19 mg/g; and 0.65 mg/g [2].

The utilization of black soybeans as functional food processed products is still less attention and is not optimal compared to yellow soybeans. So far, in Indonesia, black soybean is only used as a raw material for making soy sauce. One alternative to black soy milk is processed into yogurt. Blacksoyghurt is one of the preparations made from black soybean juice which is fermented using lactic acid bacteria (LAB). Blacksoyghurt has the potential as a functional food because it has high antioxidants, has isoflavone, and plays a role as a probiotic due to lactic acid content.

Research that has been done is still limited to identifying that blacksoyghurt can reduce LDL in the blood [3]. The update carried out in this study was to optimize the main content of blacksoyghurt, namely isoflavones, daidzein, and genistein which act as antioxidants that increase along with the length of fermentation. Furthermore, in this research the addition of *L. plantarum* is needed to increase the probiotic content in blacksoyghurt since alfa-galactosidase enzyme as a soluble enzyme fermented oligosaccharides in black soybeans in the form of raffinose and stachyose. Therefore, further research is needed to determine the optimal fermentation time containing isoflavones, daidzein, and genistein with the addition of *L. plantarum* bacteria which are beneficial for body health and improve the immune system.

II. Material and Method

A. Research Procedure

The research procedure was carried out with the first stage, namely purchasing samples of Malika variety black soybeans purchased from Johar Baru Market, Semarang, and stored in tightly closed packaging to maintain humidity and prevent mold growth. The next stage in this research is processing black soybeans into black soybean juice, which is then processed into yogurt with various treatments using a starter bulk culture and starter L. plantarum. Further testing is carried out on the viability of LAB, isoflavone profile, genistein, daidzein, antioxidant activity, and hedonic tests, which were then descriptive data analysis was carried out for the profile parameters of isoflavones, genistein, and daidzein, as well as antioxidant activity, as well as statistical data analysis for LAB viability parameters and hedonic tests.

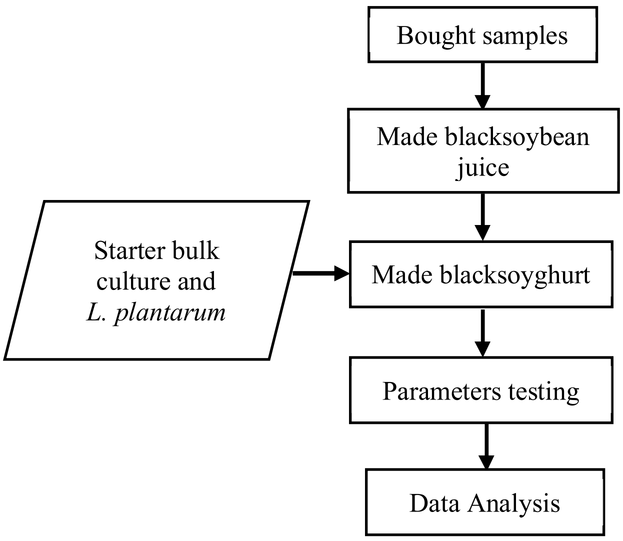

Fig. 1 Research Procedure Flowchart

B. Preparation Samples

Black soybean samples were taken randomly from Johar Baru Market, Semarang. Black soybeans were soaked for 8 hours, washed, and then soaked in 0.5% $NaHCO_3$ for 30 minutes. Black soybeans are mashed with a water ratio of 1: 8, then filtered with gauze. About 10% sugar was added and then homogenized at 4 rpm for 10 minutes. The blacksoymilk was pasteurized at 37 °C for 15 seconds and lowered to 42 °C.

The starter was prepared according to Harjiyanti et al. [4]. Mother starter culture made from 68.20 g of skim milk was dissolved into 500 ml of distilled water and pasteurized at 37 °C for 15 seconds. The temperature was lowered to 42 °C. Prepared a set freeze-dried LAB starter (*S. thermophilus*, *L. bulgaricus*, and *L. acidophilus*) about 10 g as a mother culture. The mother starter culture was then incubated at 37 °C for 4.5 hours. The bulk culture starter was made of 125 g of skim milk dissolved in 500 mL of distilled water. Then pasteurized at 37°C for 15 seconds. The addition of 25 grams of mother culture is needed, then incubate at 37 °C for 4.5 hours.

Blacksoyghurt drink made with 1000 mL of black soybean juice was added with 5% bulk culture and then homogenized. Soybean juice was fermented with various treatments, as followed 0 hours (T0), 3 hours (T1), 6 hours (T2), 9 hours (T3), and 12 hours (T4) at 37°C. One hour before the end of fermentation, *L. plantarum* starter is added.

C. Profile Isoflavone Testing

Determination of isoflavone profile with total flavonoid according to Elshafei et al. [5] UV-Vis Spectrophotometer method. Profile testing isoflavones consists of standard solution preparation and flavonoid assay. Standard solution preparation was carried out by using 5 mg of quercetin standard dissolved in 5 ml of methanol. Made standard concentrations are 0 ppm, 20 ppm, 40 ppm, 60 ppm, and 80 ppm. The wavelength was determined using the spectrophotometric method UV-Vis with maximum absorbance is 510 nm.

Determination of flavonoid content, namely by 10 ml of blacksoyghurt sample added 5 ml of KOH and 5 ml of propanol, then heated in a water bath for 15 minutes until left to cool. Then filtered with Whatman filter paper 04. The filtrate results were taken 1 ml, then 1.5 ml of aquademine and 0.2 ml of 5% sodium nitrite were added. The sample was allowed to stand for 6 minutes and added AlCl3 1: 1 methanol 0.3 ml and then allowed to stand for 6 minutes. Then, added sodium hydroxide 1 N 2 ml, and allow to stand for 15 minutes. The absorbance sample was determined by UV-Vis spectrophotometry at a length of 510 nm.

$$Consentrate = \frac{\text{Absorbance}}{3{,}96} \quad (1)$$

$$\text{Total Flavonoids} = \frac{\text{Concentrate} \times \text{Dilution}}{\text{Sample volume (mL)}} \quad (2)$$

D. Deidzein and Genistein Testing

Determination of genistein and daidzein levels according to Bettaiah and Prabhushankar [6] with High-Performance Liquid Chromatography method. There are 2 stages, namely sample preparation and determination of aglycone content. Sample preparation was carried out using extraction, 500 ml sample of blacksoyghurt is put into the Erlenmeyer added with 500 ml of ethyl acetate. The solution was macerated for 1 hour with stirrer, the ethyl acetate part is then taken by evaporation using a rotary evaporator to produce a thick extract. Sample extract 3 mg and dissolved in 10 ml of methanol: water (8:2) which is then fed into the HPLC tool for analysis.

Determination of the amount of genistein and daidzein was carried out with column C18 reverse phased. Standard genistein and daidzein are reconstituted respectively in methanol: water (8:2) with a concentration of 25 ppm, 20 ppm, 15 ppm, 10 ppm, and 5 ppm. The solution is put into each tool as much as 2 ml. The sample was injected with as much as 10 l. The mobile phase used is acetonitrile: water (8:2), flow rate 0.5 ml/minute at 40 °C, at length 255 nm wave. The data obtained is in the form of an area which is then determined the values of a, b, and r by comparing the concentration of the sample (ppm) with the area. Created a linear regression equation y = a + bx.

E. Lactic Acid Bacteria (LAB) Viability Testing

LAB viability according to Mangalisu [7] total plate count (TPC) method. Petri dishes were sterilized using oven at 170 ºC for 1 hour. Then the media is prepared by MRS Agar as much as 34.1 grams dissolved in 500ml aquadest. Then the medium is heated and dissolved using Magnetic Hot Plate Stirer. Then, wet sterilization was carried out on the media and 0.85% physiological NaCl diluent by autoclaving at a temperature of 121 ºC and a pressure of 1 atm for 15 minutes. The pour plate method was used to test for lactic acid bacteria. The tested sample was diluted with a dilution of 10^{-1} – 10^{-8}. One mL of the sample from the 10^{-6}, 10^{-7}, 10^{-8} dilutions was put into a sterile petri dish. Each sample was made in duplicate in a petri dish. The petri dish containing the diluted sample was filled with MRS agar (Merck) to a volume of 15 mL, and the petri dish was shaken in a circle to spread the MRS agar the bacteria uniformly. The petri dish which had been filled with agar was allowed to stand/ After the medium solidified, the petri dish was incubated at 37 ºC for 24 hours in an inverted position. Colony growth in each plate was calculated by TPC number in 1 ml, multiplying the mean number of colonies by dilution factor used for Colony Forming Unit/mL or Colony/mL.

F. Antioxidant Activity IC50

According to Toledo et al. [8], Antioxidant activity is *2,2-diphenyl-1-picrylhydrazyl* (DPPH) IC 50 method. The sample was prepared with 10 grams of TCA added 50 ml of aquademin. 5 ml of the sample was put into a test tube and added 5 ml of TCA solution (1:1) then vortexed for 30 seconds. After that the sample was centrifuged at 5000 rpm for 6 minutes. DPPH solution was made with 2 mg of DPPH dissolved in 100 ml methanol. After that the DPPH solution was vortexed for 30 seconds. Then 5 samples of blacksoyghurt (0 hours, 3 hours, 6 hours, 9 hours, and 12 hours) were diluted with 50, 100, 150, and 200 levels for each treatment. The first dilution is done by mixing 50 l of sample and 150 l of methanol, etc. At each level of dilution, 4.8 ml of DPPH solution was added.

G. Hedonic Testing

Hedonic testing (preferred level) according to Fadly et al. [9]. Product preference testing will be carried out on products that have been dehydrated and carried out on 30 semi-trained panelists. Samples were presented to the panelists with a size of ±20 ml. The selected product was carried out using the hedonic quality method based on the organoleptic response. Each treatment was sampled by 30 panelists for organoleptic testing and given a score with a range of 1-4. The highest score indicates the most preferred treatment by the panelists, while the lowest score indicates the most disliked treatment.

H. Data Analysis

The data of isoflavone, genistein, and daidzein profiles were analyzed by descriptive analysis using Microsoft Excel 19 for windows. The viability of lactic acid bacteria was analyzed using one-way ANOVA followed by Duncan analysis to identify the presence of significant differences in treatment at an interval confidence of $p<0.05$. Hedonic test data were analyzed using the Kruskal Wallis test with a significance level ($p<0.05$), followed by the Mann-Whitney test.

III. Results and Discussion

A. Isoflavone Profile

The isoflavone profile in blacksoyghurt was highest at 12 hours of treatment. The results can be seen in Table 1. This phenomenon is due to a change in the phenolic component through the activity of the tannase enzyme made the phenolic group loses glucose (aglycone) and has more hydroxyl groups (OH) which cause high antioxidants resulting in the degradation of LAB [10]. The decreasing isoflavone profile at 3 hours to 9 hours of fermentation because of fermentation can significantly reduce the total isoflavone content [11]. The decreasing isoflavones are caused by the incomplete hydrolysis of β-glucosidase by *L. plantarum*. The β-glucosidase enzyme *L. plantarum* can hydrolyze glycosidic bonds completely at 48 hours of fermentation at 37°C and the activity of β-glucosidase in *L. plantarum* is high at around 0.91 U/mL obtained at pH 5 and pH 6 [12].

TABLE I
ANALYSIS OF ISOFLAVONE PROFILES IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Isoflavone Profile (μg/g)
0 Hour	37.50
3 Hours	190.91
6 Hours	170.45
9 Hours	165.18
12 Hours	313.64

The isoflavone profile is directly proportional to the high protein content of soybeans. The soybean fermentation process produces proteolytic enzymes produced by LAB, which can hydrolyze the main components of soybean protein into peptides and free amino acids through protein hydrolysis techniques [13]. The hydrolysis of soybean protein by lactic acid bacteria causes the protein to become short peptides with antioxidant activity [14]. The structure of soybean protein during the hydrolysis process will be modified, and the R groups of the more active amino acids will be exposed because soy peptides have higher antioxidant activity than protein before the hydrolysis process [15].

The results of the research that has been carried out show that there is an increase in the isoflavone content of non-fermented soybean extract (control) with fermented soybeans. The fermentation process in black soybeans can increase the isoflavone profile in the product due to changes in β-glucosidase from yogurt culture in soy milk which converts isoflavones from glucosides to aglycones [16]. With the fermentation process, soybeans will degrade soy protein into simpler forms such as oligopeptides and di, tri-peptides, thereby eliminating the problem of protein allergy and serving as a good source of bioactive peptides [17].

B. Genistein and Daidzein

Based on the research, the daidzein amount is higher than the genistein. These data deal with the research conducted by Lee et al. [18], which said the amount of daidzein was 436,1 μg/g while genistein was 258.2 μg/g. The research data can be seen in Table 2. The dominant amount of daidzein in blacksoyghurt is due to the activity of the bacteria *L. acidophilus* and *L. plantarum* which have a high activity to convert daidzein compared to genistein [19]. The difference in the amount of daidzein and genistein is also due to the greater amount of daidzein than the amount of genistein contained in soybean juice before fermentation.

TABLE II
GENISTEIN AND DAIDZEIN CONTENT IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Genistein (μg/g)	Daidzein (μg/g)
0 Hour	28.22	32.66
3 Hours	27.04	29.55
6 Hours	25.48	29.86
9 Hours	22.97	25.49
12 Hours	25.69	28.66

Genistein and daidzein in soybeans should increase with the length of the fermentation process, but in this study, there was a decrease in aglycones during the fermentation process. The decrease in aglycones in blacksoyghurt is due to microbial activity that can stop the aglycone formation process so that the aglycone content in blacksoyghurt decreases. Microbial activity can be influenced by LAB which is added in the process of making yogurt. The decrease in the number of blacksoyghurt aglycones is due to microbial activity that can stop the aglycone formation process. Not all lactic acid bacteria can secrete β-glucosidase in the medium and cannot degrade the isoflavones contained in soybean juice [19].

The level of efficiency of LAB in converting isoflavones depends on the variety of strains and varieties of soybean used for the optimum activity of the β-glucosidase enzyme at the pH 4.5 and temperature 60°C [20]. The β-glucosidase enzyme hydrolyzed the sugar bonds on the glucosides and released the phenolic aglycone bonds.

C. The Viability of LAB

During the fermentation process, LAB experienced an increase in the number of cells. This proves that an adequate amount of nutrients causes an increase in the total number of lactic acid bacteria. The availability of abundant nutrients increases the number of LAB because of the large amount of energy produced for the growth process. The increase in the number of lactic acid bacteria during the fermentation process occurs under the fermentation process to break down the sugar by LAB for metabolism. The longer the fermentation time, the more sugar is broken down by LAB for growth. During fermentation, lactic acid bacteria can break down glucose into lactic acid and other sugars such as fructose, sucrose, and maltose [21].

L. plantarum bacteria is added as a part of starter bacteria, which can break down starch and soybean oligosaccharides into simpler compounds, resulting in increasing the level of reducing sugar. *L. plantarum* is able to break down starch. In addition, in the opinion of [22], *L. plantarum* has the enzyme α-galactosidase as an enzyme dissolved where it can ferment raffinose and stachyose thereby increasing the sugar in blacksoyghurt. Black soybean oligosaccharides are prebiotics which is able to increase the growth of LAB while it is expected to increase the effectiveness of LAB as a probiotic [23].

The amount of LAB in fermented beverage products is important because it is beneficial for health. According to [11], the condition of the probiotic drink contained more than 10^8 CFU/mL of viable LAB. The ability of fermented drinks to inhibit pathogenic bacteria is closely related to the amount of LAB contained in fermented drinks. Lactic acid bacteria will produce compounds that can inhibit the growth of enteric pathogenic bacteria [24]. Based on the data in Table 3. it can be seen that there was an increase in total LAB from10^8 - 10^{10} CFU/mL, it is indicated that the blacksoyghurt product is a probiotic drink product.

TABLE III
LAB BLACKSOYGHURT VIABILITY IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	LAB Viability (CFU/mL)
0 Hour	0.00^{b}
3 Hours	1.96×10^{8b}
6 Hours	1.04×10^{9b}
9 Hours	9.19×10^{9ab}
12 Hours	2.04×10^{10a}

Information:
$^{a\text{-}b}$ Values with different lowercase superscripts indicate a significant difference ($p<0.05$).

D. Antioxidant Value

The data in Table 4. revealed that the antioxidant activity as IC50 blacksoyghurt ranged from 760.79 – 1385.18 and decreased along with the prolonged fermentation time. It was indicated that the IC_{50} value decreased with the prolong of the fermentation period. The highest IC_{50} value was in the control treatment of 1385.18. While the lowest IC_{50} value is at 12 hours of fermentation at 760.79. According to Darikvand et al. [25] the lower the antioxidant IC_{50} value indicates that a food product has a higher antioxidant activity because using lower concentrations can inhibit DPPH by 50%.

TABLE IV
ANTIOXIDANT IC_{50} IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Antioxidant IC_{50}
0 Hour	1385.18
3 Hours	830.72
6 Hours	830.72
9 Hours	785.74
12 Hours	760.79

Antioxidants are often associated with free radicals. According to Krishnaswamy et al. [26], free radicals are often associated with several pathological events such as inflammation, aging, and the cause of cancer. According to Mahmood [27], free radicals are atoms or molecules that have unpaired electrons, where free radicals are formed as an intermediate in an organic reaction that occurs through a homolysis process of covalent bonds. According to Hamid et al. [28], the reactivity of free radical compounds will quickly attack surrounding cellular components such as proteins, carbohydrates, lipids, lipoproteins, RNA, and DNA compounds. As a result, the reactivity of free radicals will cause damage to the function and structure of cells. Fermentation by LAB can change the antioxidant activity of blacksoyghurt.

In this study, the production of blacksoyghurt products was based on the low pH of yogurt, which was around 4.5 so that it was compatible with anthocyanins which were more stable at low pH. This is following the opinion of Khumkarjorn et al [29] which states that anthocyanins have the most optimal stability in the range of pH 5. According to Adebo and Medina-Meza [30] , the value of antioxidant activity increases during the fermentation process related to the available sugar content, where the hydrolysis of sugar by lactic acid bacteria will cause more phenol compounds to be liberated so that it can increase antioxidant activity.

E. Hedonic Quality

Sensory attributes presented in the hedonic quality test or blacksoyghurt preference include color, aroma, taste, texture, and after taste. The following are the results of the hedonic quality test or blacksoyghurt preference.

TABLE V
COLOR HEDONIC QUALITY IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Color	Scoring Criteria
0 Hour	3.13 ± 0.78^{a}	Like
3 Hours	2.53 ± 0.73^{b}	Dislike
6 Hours	2.50 ± 0.73^{b}	Dislike
9 Hours	2.73 ± 0.64^{b}	Dislike
12 Hours	2.83 ± 0.75^{a}	Dislike

Information:
$^{a\text{-}b}$ Values with different lowercase superscripts indicate a significant difference ($p<0.05$).

Based on the data in Table 5, it can be seen that the panelists' preference for blacksoyghurt color ranged from 2.50 to 3.13 and decreased in the control treatment (P0) to 6 hours (P2) but increased at 9 hours of fermentation (P3) and 12 hours (P4). The panelist's level of preference for blacksoyghurt was the highest for color, namely in the control treatment of 3.13 which was included in the "like" assessment criteria. Meanwhile, the panelists' lowest preference level for blacksoyghurt color was in the 6-hour fermentation time treatment (P2) of 2.50 which was included in the "dislike" assessment criteria.

Color is the appearance that is first seen by consumers and color often determines a food product in society. The color acceptance of a food product varies depending on geographical, natural, and social aspects of the receiving community. The anthocyanin content influences the color of black soybeans in the skin of black soybeans. According to Han et al. [31] anthocyanins are water-soluble pigments naturally found in several plants and fruits, one of which is black soybeans. This pigment gives black soybeans their black color. The more the ratio of the content of black soybeans in blacksoyghurt will produce a darker product color. The color of yoghurt can also be affected by the homogenization process.

Based on the data in Table 6, it can be seen that the panelists' preference level for the aroma of blacksoyghurt ranged from 1.97 to 3.13 with the highest level of preference being the control treatment (P0) of 3.13 which was included in the "like" assessment criteria. While the 3-hour fermentation time (P1) has the lowest level of preference of 1.97, which is included in the "very dislike" assessment criteria. The level of blacksoyghurt preference decreased from treatment P0 to P1, increased from P1 to P2, decreased from P2 to P3, and increased again from P3 to P4.

TABLE VI
AROMA HEDONIC QUALITY IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Aroma	Scoring Criteria
0 Hour	3.13 ± 0.73^{a}	Like
3 Hours	1.97 ± 0.80^{b}	Very Dislike
6 Hours	2.13 ± 0.78^{b}	Dislike
9 Hours	2.03 ± 1.03^{b}	Dislike
12 Hours	2.30 ± 0.95^{b}	Dislike

Information:
$^{a\text{-}b}$ Values with different lowercase superscripts indicate a significant difference ($p<0.05$).

Aroma is an odor received by the nose and brain which is a mixture of four main odors: fragrant, sour, rancid, and charred. Aroma is a parameter that can affect the quality of a food product. Aroma can determine the delicacy of the food product. Fermentation time can affect the smell and sour taste of yogurt. The longer the fermentation, the stronger the sour taste and smell. The aroma of blacksoyghurt can come from the activity of LAB that produces acid, producing a sour yogurt aroma. The sour aroma is obtained through fermentation, which can cause oligosaccharides' breakdown in black soybeans. In addition, the longer the fermentation can increase the LAB, which can cause the blacksoyghurt aroma to become sourer. According to Zhang et al. [32], the aroma of blacksoyghurt can be influenced by the high-fat content of black soybeans where soy fat can react with lipoxygenase enzymes which can cause unpleasant aromas. In this study, grinding was carried out with hot water for approximately 5 minutes to inactivate the lipoxygenase enzyme to reduce unpleasant odors. This is in accordance with the opinion [33], which states that the unpleasant odor in soybeans can be removed by soaking and can be done by destroying the soybean enzyme system by heat treatment. However, based on research, soybeans' unpleasant odor is difficult to completely remove.

TABLE VII
TASTE HEDONIC QUALITY IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Taste	Scoring Criteria
0 Hour	3.33 ± 0.71^{a}	Like
3 Hours	2.20 ± 0.96^{bc}	Dislike
6 Hours	2.30 ± 0.99^{bc}	Dislike
9 Hours	1.90 ± 0.92^{b}	Very Dislike
12 Hours	2.47 ± 1.00^{c}	Dislike

Information:
$^{a\text{-}b}$ Values with different lowercase superscripts indicate a significant difference ($p<0.05$).

Based on the data in Table 7, it appears that the panelists' preference level for the taste of blacksoyghurt ranges from 1.90 to 3.33, where the highest level of preference is the control treatment (P0) which is 3.33 with the "like" assessment criteria. Meanwhile, the 9-hour fermentation time (P3) had the lowest level of preference, namely 1.90 which was included in the "very dislike" assessment criteria. The panelists' preference for the taste of blacksoyghurt decreased with the length of the fermentation period but increased during the 12-hour fermentation period (P4).

The sour taste in yogurt is influenced by LAB activity which gives a sour taste that comes from the breakdown of carbohydrates, namely oligosaccharides in black soybeans. In addition, the sour taste can also be caused by the content of polyphenol compounds in black soybeans. According to [34] polyphenol compounds can act as antibacterial that can change cell proteins and can damage plasma membranes in bacteria. The plasma membrane in bacteria works to identify the substrate in which bacteria grow. According to [35] damage to the plasma membrane in bacteria can interfere with the fermentation process, which causes less lactic acid to be produced.

The taste of blacksoyghurt can also be affected by additional ingredients during the manufacturing process, such as the addition of sugar to black soybean juice. This is in accordance with the opinion of Kim and Han [21], which states that the addition of granulated sugar in soybean juice before fermentation can help produce yogurt products with a balanced sweet and sour taste so that the panelists prefer the product.

TABLE VIII
TEXTURE HEDONIC QUALITY IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Texture	Scoring Criteria
0 Hour	2.97 ± 0.85^{a}	Dislike
3 Hours	2.50 ± 0.86^{b}	Dislike
6 Hours	2.50 ± 0.82^{b}	Dislike
9 Hours	2.37 ± 0.85^{b}	Dislike
12 Hours	2.53 ± 0.78^{b}	Dislike

Information:
$^{a-b}$ Values with different lowercase superscripts indicate a significant difference ($p<0.05$).

Based on the data in Table 8, it can be seen that the panelists' level of preference for the blacksoyghurt texture ranged from 2.37 to 2.97, with the highest level of preference being the control treatment (P0) which was 2.97 with the assessment criteria of "dislike". While the 9-hour fermentation time (P3) has the lowest level of preference, which is 2.37 with the "dislike" assessment criteria. The panelists' preference for blacksoyghurt texture decreased with the length of fermentation. However, there was an increase in the 12 hours of fermentation time treatment (P4).

The texture of yogurt is influenced by the viscosity formed from the activity of LAB that occurs during the fermentation process due to the presence of a substrate in the form of oligosaccharides derived from black soybeans. The carbohydrates in black soybeans consisting of oligosaccharides can replace lactose contained in cow's milk to become prebiotics for LAB. Quality yogurt is one that has a smooth and good texture, does not foam, has a distinctive taste and aroma, does not have a bitter taste, and has an acidity level that does not exceed the limit. According to Li et al. [36], The texture of yogurt is formed because the protein in soy juice coagulates and forms a gel-like structure caused by bacterial activity, wherein the gel's process will change the texture, and a flavor is formed. The texture of blacksoyghurt is also influenced by the number of bacteria included in the fermentation process and the length of fermentation. The longer the fermentation increases, the viscosity of the blacksoyghurt. The texture of yogurt can be influenced by the viscosity and pH contained in yogurt.

TABLE IX
AFTER TASTING HEDONIC QUALITY IN BLACKSOYGHURT WITH DIFFERENT FERMENTATION TIMES

Fermentation Time	Texture	Scoring Criteria
0 Hour	3.10 ± 0.92^{a}	Like
3 Hours	2.37 ± 1.03^{b}	Dislike
6 Hours	2.47 ± 1.01^{b}	Dislike
9 Hours	2.10 ± 0.99^{b}	Dislike
12 Hours	2.57 ± 0.97^{b}	Dislike

Information:
$^{a-b}$ Values with different lowercase superscripts indicate a significant difference ($p<0.05$).

Based on the data in Table 9, it can be seen that the level of preference of the panelists to the after taste of blacksoyghurt ranged from 2.10 to 3.10, with the highest preference value being the control treatment (P0) which was 3.10 with the assessment criteria "like". While the 9-hour fermentation time (P3) has the lowest level of preference, which is 2.10 with the "dislike" assessment criteria. The panelists' preference for after taste blacksoyghurt decreased with the length of fermentation. However, there was an increase in the 12-hour fermentation time treatment (P4).

Some blacksoyghurt panelists commented on the presence of a bitter after taste, thus affecting the assessment score. The bitter after taste of blacksoyghurt can be caused by the content of saponins in black soybeans. According to Mikołajczyk-bator [37], this bitter taste can be caused by the saponin compounds found in soybean seeds, which cannot be completely removed while cooking soybean juice. However, reducing the bitter aftertaste can be done by soaking and heating. This is in accordance with the opinion of Acquah et al. [33], which states that the combination of boiling and soaking can reduce saponin compounds in black soybeans.

In addition, according to Chitisankul et al. [38], the bitter and chalky taste as an aftertaste can also be caused by the content of isoflavones, the dominant form of phenolic compounds in soybean seeds. Daidzein and genistein are the main components of isoflavones produced by the activity of the beta-glucosidase enzyme, especially during yogurt fermentation [39]. Processing by soaking and/or heating can improve the taste of soybean juice but does not completely eliminate the unpleasant taste.

Based on the spider web graph in Fig. 2. the panelists' preference level on blacksoyghurt products based on the parameters of color, aroma, taste, texture. After taste, the outermost line in Figure 2, is the P0 (control) treatment. The outermost line shows that the treatment on these parameters has the highest value compared to other treatments. And the line that is close to the control treatment is P4, so it can be concluded that the panelists' preference for blacksoyghurt is blacksoyghurt with 12 hours of fermentation.

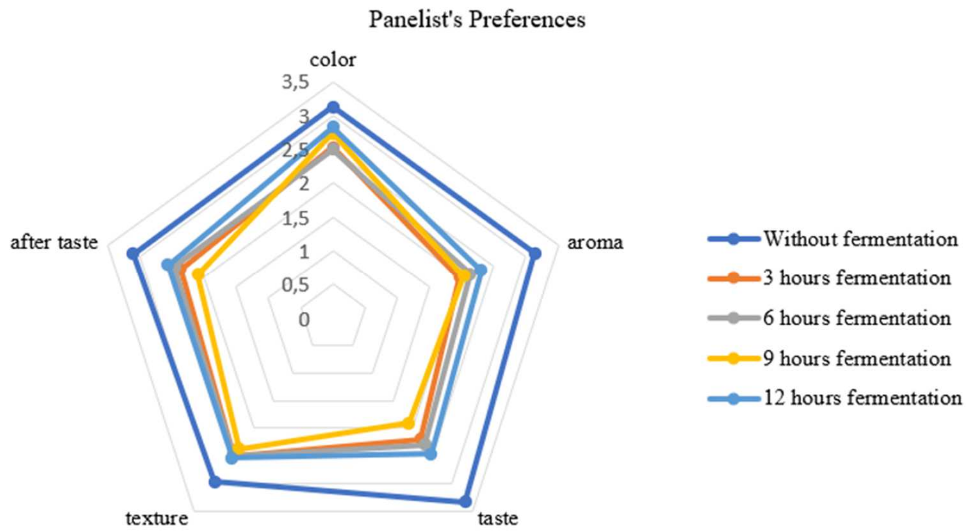

Fig. 2 Spider Web Panelist's Preferences

IV. CONCLUSION

The best *blacksoyghurt* fermentation time is 12 hours which relied on total isoflavone content of 313.64 µg/g; genistein content 25.69 µg/g; daidzein content 28.66 µg/g; antioxidant value of 760.79 and LAB viability about 2.04 x 10^{10} mg/mL. Panelists prefer *blacksoyghurt* which has a sour taste, a distinctive aroma of yogurt, and a thick texture. Therefore, the duration of fermentation significantly affected and proved to claim that *blacksoyghurt* is potentially functional and probiotic drink.

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