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Probiotic drink based on black soybean enriched with double petals butterfly pea flower extract (*Clitoria ternatea* L.) on physical, chemical, and sensory

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Abstract

This study developed a probiotic black soyghurt drink by fermenting black soybean juice enriched with double petals butterfly pea flower extract (Clitoria ternatea L.) and yoghurt starter cultures. A factorial design (2x2) with Completely Randomized Design and three replications was applied to evaluate the effects of fermentation time (10 and 15 hours) and extract concentration (8% and 13%) on the physical, chemical, and sensory properties of black soyghurt. Results showed that fermentation time and extract concentration significantly influenced viscosity and a* color values, while L* and b* values remained unaffected. Chemically, water, ash, and protein contents were stable across treatments, whereas fat content, pH, total anthocyanin, and antioxidant activity varied significantly, with all parameters meeting SNI 2981:2009 yoghurt standards. Sensory evaluation revealed that taste was affected by fermentation time but not by extract concentration, while consistency influenced ranking but not hedonic tests. The addition of butterfly pea flower extract enhanced anthocyanin content and antioxidant activity, contributing to the functional quality of the probiotic drink. This formulation offers a promising functional beverage alternative based on black soybeans enriched with natural anthocyanin pigments.

Keywords: Black Soybeans; Black Soyghurt; Double Petals Butterfly Pea Flower; Anthocyanin Pigments

1. Introduction

Probiotics are living microorganisms that can stimulate health and can be added to food. The characteristics of probiotics are able to live in the human digestive tract, especially in the small intestine, and are able to survive in acidic conditions and contain bacteriocides derived from bile salts. The ability of probiotics against pathogenic bacteria is able to compete by producing lactic acid [1].

The use of probiotics is growing rapidly in various Asian countries, from the food industry to cosmetics. Probiotic food products based on fermented milk and food supplements in the South Korean market have been very successful, especially probiotic yoghurt [2]. Japan has also developed probiotic drinks containing lactic acid bacteria to improve gut health, reduce abdominal adiposity, and serum uric acid levels [3]. China continues to develop and introduce fermented food probiotic products and has been in the top 10 food trends there [4]. Research and development of new probiotic drinks based on non-dairy raw materials continues to be developed in Thailand because this functional drink causes lactose intolerance, cholesterol content, and allergies to milk protein [5]. Various food products, both food and drinks, that can potentially be probiotics contain live lactic acid bacteria. If these food products are consumed, they can be beneficial for humans because they will improve the properties of the intestinal microflora [6]. Probiotic products found on the market include kefir, sour milk, flavored fermented milk, and yogurt.

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Yoghurt is a product made from fermented or reconstituted milk by utilizing Streptococcus thermophillus and Lactobacillus bulgaricus bacteria or other suitable lactic acid bacteria, with or without the addition of other food ingredients, and permitted [7]. Development of yoghurt products as probiotic and functional drinks using black soybeans as an alternative to animal-based cow's milk in an effort to increase its consumption, which is still considered low.

Black soybeans (Glycine soja (L.) Merrit) contain various nutrients, including protein, carbohydrates, fat, minerals, essential amino acids, vitamin E, flavonoids, isoflavones, and anthocyanins [7]. Putri and Triandita [8] explained that black soybeans are a local type developed in Indonesia and contain high dietary fiber. Soybean skin contains 87% dietary fiber, consisting of cellulose 40-53%, hemicellulose 14-13%, and crude fiber 1-3%. The advantage of black soybeans is one of the food ingredients as a source of antioxidants, namely anthocyanins [9]. Black soybeans do not contain cholesterol but contain phytochemicals that are beneficial for the body, child growth, and lactose intolerance conditions. The use of black soybeans in addition to making soy sauce, also tauco, porridge, juice, black soyghurt, and so on.

Black soyghurt is the fermentation of black soybean juice into a functional drink with the help of lactic acid bacteria (LAB) types Lactobacillus bulgaricus and Streptococcus thermophillus. This drink contains high nutrients, especially protein, but is less popular with the public because it has a pungent aroma [10]. One way to increase its consumption is by enriching it with natural dyes containing anthocyanins, namely the double petals of the butterfly pea flower (Clitoria ternatea L.). Anthocyanin is a phenolic compound that influences the appearance of red to blue colors in some flowers, fruits, and leaves [11]. Butterfly pea flowers are compound flowers that are identical to the purple color of their petals [12]. This flower has a concentration five times greater than ordinary butterfly pea flowers, so it is very effective for use as a natural dye in food and drinks. To obtain natural dyes for butterfly pea flowers, one of the processes is done by extraction using citric acid, which is an organic acid.

Butterfly pea flower extract contains anthocyanin pigments that are purple to blue in color. Anthocyanin pigments extracted with the addition of citric acid are more stable than in neutral and alkaline solutions [13]. These natural dyes are quite stable when added to black soyghurt products that have a low pH.

Based on this description, the black soyghurt probiotic drink product with the addition of butterfly pea flower extract can be a solution and alternative for the development of new functional food products based on black soybeans.

The research aimed to examine the interaction of differences in fermentation time and concentration of double petals butterfly pea flower extract on physical, chemical, and sensory properties of black soyghurt.

2. Material and methods

2.1. Materials and Equipment Research

Research materials are black soybeans (lingkarorganik.or.id), double petals butterfly pea flowers (tommyng gagitudech). Other ingredients are yoghurt starter (brand Biokul set plain yoghurt PT.Diamond Cold Storage, Bekasi), granulated sugar, skim milk (brand Indo Prima), citric acid (p.a. Merck), Ingredients for chemical analysis on black soyghurt are distilled water, H2SO4 (p.a), HgO (pa), NaOH-Na2S2O3 (p.a), K2SO4 (p.a), HBO3 (p.a), HBO3 (pa), n-hexane (p.a), indicators (methyl red and methylene blue), HCl 0.02 N (p.a), DPPH powder (2,2 - diphenyl - 1 pycrilhydrazine) 0.25 mM (p.a), methanol 70% (technical), KCI 0.025 M (pa), HCl 37% (technical), Na-acetate 0.4 M (p.a), buffer solution 4 and 7 (p.a), filter paper, thread, plastic wrap, aluminum foil.

The equipments used for chemical analysis are UV-Vis Spectrophotometer, color reader (TCR 200), viscotester model VT-04F, kjeldahl system, autoclave, petridish, vortex (LABINCO L46)), pH meter brand The pHep Family Hanna Instruments, waterbath, analytical balance (ABJ-NM / ABS-N), spatula (stainless 16 cm), beaker glass (Herma 250 ml), measuring cup 50 ml ± 0.5 ml (Herma), Erlenmeyer (Pyrex 250 ml), centrifuge (Hettich EBA 20), centrifuge tube, Erlenmeyer (Pyrex 250 ml), clamp (stainless 30 cm), dropper pipette, volume pipette, label paper, separating funnel, burette, test tube (Pyrex 13x100), electric heater (thru M.E.D), flask Kjeldahl (Pyrex 250 ml), oven binder, desiccator, mortar (10 cm), condenser tube, distillation apparatus, fat flask (Pyrex 250 ml), filter paper, Soxhlet (Pyrex 250 ml), porcelain cup (15 ml) and electric furnace (Neycraft). The tools used in the sensory test are sensory test form paper, stationery, and label paper.

2.2. Research Stages

2.2.1. Research Methods

Research on the process of making double petals butterfly pea flower extract, black soybean extract, black soybean yoghurt, and physical, chemical, and sensory analysis of black soyghurt products with the addition of double petals butterfly pea flower extract. Physical analysis includes viscosity (Ostwald), L*a*b* color (Chromameter), chemical analysis includes water content (thermogravimetry), ash, protein (Kjeldahl), fat (Soxhlet), pH (pH meter), total anthocyanin (pH difference), antioxidant activity (DPPH), and sensory in ranking and hedonic tests.

2.2.2. Making of Double Petals Butterfly Pea Flower Extract [13]

The process of extracting double-petalled butterfly pea flowers using the maceration method, using aqueous solvent added with 1% citric acid for 24 hours at a temperature of 25ºC. First, 100 g of double-petaled butterfly pea flowers were size-reduced, then 300 ml of water and 3 ml of citric acid were added. The extraction process was carried out for ± 24 hours at a temperature of 25ºC. The extract obtained was then filtered and stored in a glass bottle lined with aluminum foil.

2.2.3. Making Black Soybean Extract [14] modified

A total of 450 g (bk) of black soybeans were added with 1350 ml of water (1:3) and soaked for 8 hours. Furthermore, after draining, it was boiled by adding 1350 ml of water (1:3) at a temperature of 90ºC for 30 minutes. Before milling, the boiled black soybeans are drained, and then 1350 ml of water (1:3) is added, after which it is filtered, the result is black soybean extract.

2.2.4. Making Black Soyghurt

1100 ml of black soybean extract is pasteurized at 80ºC for 10 minutes, and 5% skim milk and 7.5% granulated sugar are added, then cooled until the temperature reaches 40ºC, and then inoculated with 5% yoghurt starter. Then the fermentation treatment is carried out for 10 and 15 hours at a temperature of 37ºC. After this process, the two fermentation treatments are added with 8 and 13% double petals butterfly pea flower extract and stored at a temperature of 4ºC.

2.3. Analysis Procedure

2.3.1. Analysis of Water Content (thermogravimetry)

Analysis of water content can be done by a heating method called thermogravimetry, which is a method carried out using an oven temperature of 100-105ºC for 3-5 hours, depending on the material. Heat the oven, then put the closed weighing bottle in the oven and dry it at a temperature of 102-105ºC for 30 minutes. After that, take the closed weighing bottle that has been dried, then cool it in a desiccator for 30 minutes, then weigh it. Do this three times to get a constant bottle weight. Weigh 2-4 g of black soyghurt, then place it in the closed weighing bottle that has been weighed earlier, and then put it in the oven for 6 hours. After 6 hours, take the closed weighing bottle, place it in a desiccator, and cool it for 30 minutes, then weigh it. Do 3 repetitions on each sample. Water content can be determined using the following formula:

$$\text{Water content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Sample weight}} \text{ x } 100\%$$

The reduction from this weight is the amount of water in the material.

2.3.2. Ash Content Analysis

Ash content analysis is carried out to determine the mineral content in the test sample. The test method is to oven the porcelain cup in the oven for 30 minutes at a temperature of 105ºC, then remove the cup from the oven and cool it in a desiccator for 15 minutes then weigh the cup, weigh until 2 g of black soyghurt is then put in the cup, place the cup in the ashing furnace, ashing is carried out in an electric furnace at a temperature of 600ºC for 2 hours or until white ash is formed. After that, cool the cup in a desiccator for 30 minutes and weigh.The calculation formula for ash content analysis can be seen as follows:

$$\text{Ash content (\%)} = \frac{(\text{Cup weight} + \text{Ash}) - (\text{Cup weight})}{(\text{Sample weight (g)})} x\ 100\ \%$$

2.3.3. Protein Content Analysis (Kjeldahl)

The procedure for analyzing protein content in black soyghurt using the Kjeldahl flask method is to take a sample of 0.1-0.5 g of black soyghurt, put it in a Kjeldahl flask and add 40 mg of HgO, 1.9 mg of K2SO4 and 2 ml of H2SO4, place the flask containing several of these solutions on a heater with a temperature of 430ºC in an acid chamber. Destruction of the solution until clear for 1-1.5 hours. The results of the destruction are then cooled and diluted with 10-20 ml of distilled water slowly. The next step is the distillation process using the Kieltec system. The Kjeldahl flask from the destruction is then transferred to the distillation apparatus. Washing and rinsing the flask 5-6 times with 1-2 ml of distilled water, then transferring the washing and rinsing water to the distillation apparatus. Place a 125 ml Erlenmeyer flask containing 5 ml of HBO3 (boric acid) and 2-4 drops of indicator (a mixture of 2 parts of 0.2% methyl red in alcohol and 1 part of 0.2% methylene blue in alcohol) before distillation begins. The tip of the condenser must be submerged under the HBO3 solution. Add the transferred destruction sample with 8-10 ml of NaOH-Na2S2O3 (sodium triosulfate) solution. Then, carry out distillation until ± 15 ml of distillate is collected in the Erlenmeyer. Rinse the condenser tube with distilled water and collect the rinsing in the same Erlenmeyer. Dilute the contents of the Erlenmeyer flask to ± 50 ml, then titrate, drop 0.02 N HCl from the burette on the distilled sample. Titrate until the solution changes color to pink. Record the volume of HCl used. Calculate the total N (% protein) in the sample being tested. The calculation of total N can be seen below:

$$\mathrm{N} = \frac{(\mathrm{A} - \mathrm{B})\ \mathrm{x}\ \mathrm{N\ HCl}\ \mathrm{x}\ 14}{\mathrm{mg\ sample}}\ \mathrm{x}\ 100\%$$

$$\text{Protein content= N x Conversion Factor}$$

Note: A = ml of sample titration, B = ml of blank titration, Conversion factor = 6.25

2.3.4. Fat Content Analysis (Soxhlet)

Fat and oil content were tested using the Soxhlet method, first drying the fat flask in an oven at 105ºC for 30 minutes. Then cool in a desiccator for 15 minutes and weigh it (A). Weigh a sample of 5 g (S), then wrap it in filter paper and insert it into the fat sleeve. Cover the fat sheath with non-fat cotton and insert it into the Soxhlet tube extractor chamber, and then flush with fat solvent (hexane). The Soxhlet tube is installed on the distillation device. Installation of the fat flask that is ready on the distillation device with an electric heater at a temperature of 80 T Reflux, and carried out for at least 5 hours until the solvent that falls into the fat flask is clear. Distillation of the solvent in the fat flask, then the flask that has contained the extraction basil is then heated in an oven at a temperature of 105ºC for 60 minutes or until the weight is constant, then cooled in a desiccator for 20-30 minutes and weighed (B).

Calculation for fat content can be seen below:

$$\text{Fat content (\%)} = \frac{\text{Final weight (B)} - \text{Cup weight (A)}}{\text{Sample weight}}\ \mathrm{x}\ 100\%$$

2.3.5. Analysis of pH Levels (pH meter)

The pH test was carried out using a pH meter type The pHep Family Hanna Instruments. Before testing, first clean the cathode tip using distilled water and then dry it with a tissue. The cathode tip on the pH meter needs to be calibrated in buffer solutions 4 and 7 before use. After that, the cathode tip can be used for testing on black soyghurt samples. The cathode tip must always be cleaned with distilled water first for testing on each sample. The pH value can be known from the numbers listed on the pH meter.

2.3.6. Total antioxidant analysis (pH difference)

The following are the steps for total anthocyanin analysis using the pH difference method according to AOAC (2005):

Making pH buffer solution 1. KCI 0.025 M solution (1.86 g in 980 ml distilled water) is added with 6.3 ml of 37% HCI. Preparation of pH 4.5 buffer solution. 0.4 M Na-acetate solution (54.43 g in 960 ml of distilled water) was added with 20 ml of 37% HCI.

2.3.7. Determination of Total Anthocyanin:

Dissolve the sample with acidic methanol solvent in a ratio of (1:1) in a glass. The sample solution was stirred until homogeneous, then the beaker was covered with aluminum foil. Macerate the sample at a temperature of -23ºC for 1 hour. Put the sample into two test tubes, each containing as much as 1 ml. The first tube was added with a pH 1 buffer solution as much as 9 ml. The second tube was added with pH 4.5 buffer solution as much as 9 ml, scanning anthocyanin with λ = 400-550 nm in both test tubes, to determine the maximum wavelength of anthocyanidin owned by each sample. Calculation of absorbance at maximum λ and wavelength of 700 nm in each sample is as follows:

$$A = (A_{vis\text{-}max} - A_{700nm})_{value\ pH\ 1} - (A_{vis\text{-}max} - A_{700nm})_{value\ pH\ 4,5}$$

$$\text{Antocyanin concentration (mg/L)} = \frac{\text{A x MW x DF x 1000}}{\varepsilon \text{ x l}}$$

Description:
A = Absorbance
MW = Molecular Weight (Molecular Weight of cyanidin glucoside = 449.2)
DF = Dilution factor (dilution factor = 10 ml/ 0.1 ml)
ε = molar absorptivity/ molar extinction coefficient (29,600 L cm - 1)
l = Cuvette width (1 cm)

2.3.8. Analysis of Antioxidant Activity (DPPH)

The method used in the analysis is the DPPH method, with a wavelength (λ) of 517 nm with a spectrophotometer. First, make a 0.25 mM DPPH solution. The formula for calculating the need for DPPH powder:

$$\text{Concentration} = \frac{\text{mass (mg)}}{\text{Mr x Volume (L)}}$$

DPPH powder is dissolved with 70% methanol in a 50 ml measuring flask to the limit and homogenized. DPPH storage in dark, closed, and cold conditions, and must be used immediately. Extraction of active ingredients: a sample of 1 g is weighed and put into a centrifuge tube. A total of 9 ml of 70% methanol solution is added. Centrifuge at a speed of 4000 rpm for 10 minutes. Separation of the supernatant for the antioxidant activity test. Analysis of antioxidant activity: 1 ml of supernatant is taken and put into a test tube. DPPH 0.25 mM solution, as much as 2 ml, was added and homogenized. Close the mouth of the test tube with plastic wrap and the body of the tube with aluminum foil tightly so that light does not enter. Storing the sample for 30 minutes in the dark. Using a UV-Vis spectrophotometer with a wavelength of 517 nm to read its absorbance. The inhibition formula in % is as follows:

$$\text{inhibition} = \frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} \text{ x } 100\%$$

2.3.9. Sensory Analysis (ranking and hedonic tests)

Sensory properties /tests are carried out using a scalar test, namely a ranking test, an affective test method (acceptance test), and a hedonic test. Filling out a form containing questions and responses from panelists regarding black soyghurt products covering aspects of taste, aroma, color, and consistency. A total of 25 semi-trained panelists tested the black soyghurt products given. The ranking test scale for taste includes (1) very sour, (2) rather sour, (3) not sour, while for consistency, (1) very viscous, (2) rather viscous, (3) not viscous. The scale (score) used in the hedonic test is (1) very dislike, (2) dislike, (3) rather dislike, (4) neutral, (5) rather like, (6) like, (7) very like

2.4. Experimental Design

The experimental design used was Factorial with a Completely Randomized Design (CRD) consisting of two factors (2 x 2) with 3 replications. Data obtained from the analysis results will be tested using Analysis of Variance (ANOVA) with a confidence level of 95%. If there is a significant difference between treatments, further testing is carried out using Duncan's Multiple Range Test (DMRT). Data is processed using the SPSS version 26 program.

3. Results and discussion

3.1. The physical properties of black soyghurt

The results of the analysis of the physical properties of black soyghurt enriched with double petals butterfly pea flower extract can be seen in Table 1.

Table 1 The physical properties of black soyghurt

Analysis	Factor A Fermentation Time (hours)	Factor B The enrichment with Double Petals Butterfly Pea Flower Extract (%)	
		8	13
Viscosity	10	475.00 ± 0.50^{Aa}	200.00 ± 0.50 ^{Ab}
	15	360.50 ± 0.50 ^{Ba}	262.50 ± 0.50^{Bb}
L* (brightness)	10	52.65 ± 1.75 ^{Aa}	51.65 ± 2.59^{Aa}
	15	50.81 ± 1.21^{Aa}	51.16 ± 1.31^{Aa}
a* (green-red)	10	16.06 ± 0.93^{Aa}	13.68 ± 2.95 ^{Ab}
	15	14.09 ± 2.74 ^{Ba}	10.87 ± 1.34^{Bb}
b*(blue-yellow)	10	-1.67 ± 2.21^{Ba}	-1.60 ± 0.15^{Ba}
	15	1.12 ± 2.12^{Aa}	0.49 ± 0.26 ^{Aa}

Description: The average is obtained from three replications, numbers followed by different capital letters (A and B) indicate a significant difference in fermentation time, and numbers with different lowercase letters (a and b) indicate a significant difference in the concentration of double petals butterfly pea flowers extract (P<0.05)

3.1.1. Viscosity

Viscosity is the thickness of a food product. Viscosity is influenced by the total solids content in milk in making yogurt [15]. Other factors that can affect the viscosity of a fermented product are protein content, pH, type of starter culture, and incubation time [16].

The research that has been conducted shows the highest viscosity results in the treatment of 10 hours of fermentation and the addition of 8% double petals butterfly pea flower extract. This is in contrast to the condition where the longer the fermentation, the higher the viscosity. The results of this analysis are influenced by changes in the fermentation process, causing the viscosity to decrease. This research is supported by [17], who stated that increasing dissolved solids in soyghurt can affect the increase in viscosity value. During fermentation, lactic acid bacteria convert lactose into lactic acid, which causes the pH to decrease and the protein content in the soyghurt to coagulate so that the viscosity increases [18]. The treatment of fermentation time and the addition of double petals butterfly pea flower extract affect the physical properties, namely the viscosity of black soyghurt.

*3.1.2. Color L * (black-white)*

The degree of black or white is indicated if the L * value = 0, indicating perfect black, and if the L * value = 100 indicates perfect white (Suliasih et al., 2018). The L * value in the 10-hour fermentation treatment decreased with the increasing addition of double petals butterfly pea flower extract. These results are supported by research conducted by Suliasih et al. [15] that the addition of red dragon fruit to making yoghurt has a significant effect on brightness. The decrease in color intensity is influenced by the addition of dragon fruit, which contains anthocyanin pigments that cause a red color in yoghurt. This color pigment causes a decrease in the brightness value of yoghurt. However, in the 15-hour fermentation treatment, the L* value increased; this is thought to be because the anthocyanin pigment in the double petals of the butterfly pea flower extract was damaged during the mixing process and during fermentation. The same opinion was also expressed by Amperawati et al. [19], stating that the higher the L* value, the lower the anthocyanin content and total dissolved solids. The fermentation time treatment did not have a significant effect, but the addition of pea flower extract had a significant effect on the L* color in black soyghurt.

3.1.3. Color a (green-red)*

The a* value can be known if the -a* value (negative) indicates a greenish color, which is between 0 and -80, while if the +a* value (positive) indicates a reddish color, which is between 0 and +80 [20].

The results showed that the higher the addition of double-petalled butterfly pea flower extract, the a* value decreased in the 10 and 15-hour fermentation treatments. This condition is thought to be due to damage to the anthocyanin pigment of the double petals of the butterfly pea flower during the extraction process and the high water content. The results of this analysis are supported by research by Nasrullah et al. (2020), which states that high water content cannot preserve anthocyanin pigments. Meanwhile, according to research conducted by Wibawanti and Rinawidiastuti [20], it was stated that the more mangosteen peel extract was added, the higher the a* value in yogurt. Another opinion about the addition of red dragon fruit in making yogurt has a significant effect on the a* value. The high intensity of the color is influenced by the addition of dragon fruit, which contains anthocyanin pigments that tend to be red-purple, causing the yogurt to turn red. This color pigment causes an increase in the a* value (tending to be red) in the resulting yogurt (15]. The treatment of fermentation time and the addition of the concentration of double petals butterfly pea flowers affect the a* color in black soyghurt.

3.1.4. Color b (blue-yellow)*

The b* value can be known if the -b* value (negative) indicates a blue color, which is between 0 and -70, while if the +b* value (positive) indicates a yellow color, which is between 0 and +70 [20].

The b* value in the 10 and 15 hour fermentation treatment decreased along with the increasing addition of double petals butterfly pea flower extract. According to research by Suliasih [15], the addition of red dragon fruit in making yogurt had a significant effect on the b* value. The treatment of fermentation time and the addition of pea flower concentration affected the b* value in black soyghurt.

3.2. The chemical properties of black soyghurt

The results of the analysis of the chemical properties of black soyghurt enriched with double petals pea flower extract can be seen in Table 2.

Table 2 The chemical properties of Black Soyghurt

Analysis	Factor A Fermentation Time (hours)	Factor B The enrichment with Double Petals Butterfly Pea Flower Extract (%)	
		8	13
Water Content (%)	10	84.52 ± 0.53Ab	85.67 ± 0.28Aa
	15	84.50 ± 0.00Ab	85.33 ± 0.28Aa
Ash Content (%)	10	0.17 ± 0.28 Aa	0.17 ± 0.28 Aa
	15	0.33 ± 0.28 Aa	0.17 ± 0.28 Aa
Protein Content(%)	10	3.24 ± 0.01 Aa	3.26 ± 0.08Aa
	15	3.20 ± 0.00Aa	3.18 ± 0.11Aa
Fat Content(%)	10	0.92 ± 0.00Bb	0.99 ± 0.00 Ba
	15	1.03 ± 0.01 Ab	1.11 ± 0.01Aa
pH (degree of acidity)	10	4.71 ± 0.02Aa	4.68 ± 0.01 Ba
	15	4.46 ± 0.01 Ab	4.45 ± 0.01Bb
Total anthocyanin (mg/L)	10	4.93 ± 0.00Bb	5.81 ± 0.00 Ba
	15	5.01 ± 0.00 Ab	6.11 ± 0.00Aa
Antioxidant Activity (% inhibition)	10	30.84 ± 0.28Bb	35.05 ± 0.38 Ba
	15	34.19 ± 0.43 Ab	37.32 ± 0.40Aa

Description: The average is obtained from three replications, numbers followed by different capital letters (A and B) indicate a significant difference in fermentation time, and numbers with different lowercase letters (a and b) indicate a significant difference in the concentration of double petals butterfly pea flowers extract (P<0.05)

3.2.1. Water Content (%)

Water content is the amount of water contained in a product or food ingredient. The concentration of the addition of double petals butterfly pea flower extract to black soyghurt has a significant effect on the water content. The higher the concentration of double-petalled butterfly pea flower added, the higher the water content. This is supported by research by Nizori et al. [22], who argue that the results of the analysis of the water content of a sample can be influenced by the raw materials used. The analysis method for the water content test used is the gravimetric method, which has the disadvantage of also evaporating non-water components in the sample, so that it is counted as the total water content. According to research by Labiba et al. [23], the water content produced ranges from 81.74-84.84%. The decrease in water content in the soy yogurt is influenced by the addition of soybeans, which causes the protein content to be high, so that it has the ability to bind water. The length of fermentation has no effect, but the addition of double petals butterfly pea flower concentration affects the water content in black soyghurt.

3.2.2. Ash Content (%)

The results of statistical tests that have been carried out show that black soyghurt with fermentation time treatment and the addition of double petals butterfly pea flower extract is not significantly different between treatments and meets SNI 2981:2009, which is a maximum of 1% with an average ash content ranging from 0.17-0.33%. According to research by Husni et al. [24], the ash content in yogurt ranges from 0.84-1.17%, in this study, several treatments did not comply with the SNI yogurt, which is a maximum of 1%. Meanwhile, in the study by Labiba et al. [23], the ash content ranged from 0.39-0.49%. The treatment of fermentation time and the addition of double petals butterfly pea flower concentration did not affect the ash content of the black soyghurt produced, but met the SNI standard quality.

3.2.3. Protein Content (%)

The results of the study showed that the protein content in the 10-hour fermentation treatment increased with the increasing addition of double petals butterfly pea flower extract. These results are supported by research by Handayani and Wulandari [17], which states that the large number of lactic acid bacteria in soyghurt causes high protein content, because protein is a component of microbes. However, in the 15-hour fermentation time treatment, the protein content decreased; it is suspected that the protein was damaged due to the acid content in the double petals of the butterfly pea flower extract. The cause of protein damage is influenced by several factors, including heating, high acid or base conditions, heavy metal cations, and saturated salts [25]. The treatment of fermentation time and concentration of double petals butterfly pea flower did not affect protein content, but met the standard quality of SNI 2981: 2009 from protein content (3.18-3.41%) in black soyghurt. Another opinion, states that the soybean fermentation process produces proteolytic enzymes produced by lactic acid bacteria that can hydrolyze the main components of soybean protein into peptides and free amino acids through protein hydrolysis techniques [26]. Proteolytic enzymes are produced around the cell wall, cytoplasmic membrane, or inside the cell, which are then used by Lactobacillus acidophilus for its growth. The process of breaking down proteins, which are complex compounds, then becomes simpler in the form of amino acids, which are important in soybean fermentation [27].

3.2.4. Fat Content (%)

The results of the study showed that the highest fat content was found in the treatment of 15 hours of fermentation and the addition of 13% double petals butterfly pea flower extract of 1.1%, meeting SNI 2981:2009 with a maximum of 3.3%. The fat content in this study was a combination of black soybeans and skim milk. The fermentation process carried out caused the fat content not to decrease in quantity but rather to increase [17]. This statement is also supported by the research of Labiba [23], which explains the composition of fat in soybeans, namely linoleic acid, where lactic acid bacteria have difficulty breaking down fat in soyghurt so that it cannot be hydrolyzed. The treatment of fermentation time and concentration of double petals butterfly pea flower extract affects the fat content in black soyghurt.

3.2.5. pH (degree of acidity)

The treatment of fermentation time and the addition of double petals butterfly pea flower extract affect the pH of black soyghurt. The lowest pH was 4.45 at a fermentation time of 15 hours, and the addition of 13% double petals butterfly pea flower extract. The results of the study have met SNI 2981:2009 yoghurt, namely pH 4-5. According to Sembiring [19] fermentation time affects the lactic acid produced; the longer the fermentation, the higher the lactic acid produced, and the pH decreases. In a study conducted by Nirmagustina and Wirawati, the treatment of 15-hour fermentation time produced a low pH of around 4.05 [28]. The bacteria used in making yogurt are also called lactic acid bacteria (LAB) and are able to produce lactic acid. During the fermentation process, probiotic bacteria are able to break down lactose in milk raw materials into glucose and galactose by the lactase enzyme, and then form lactic acid. This causes the pH of the

milk to drop and changes the taste of the milk to a distinctive sourness. This acidic atmosphere can coagulate proteins so that the viscosity of the yogurt will increase or produce a thick or solid yogurt appearance.

3.2.6. Total Anthocyanin (mg/L)

Anthocyanin is a water-soluble flavonoid compound that can produce red to blue pigments and is stable at low pH. The results of this study showed that the longer the fermentation and the addition of double petals butterfly pea flower extract, the total anthocyanin in black soyghurt increased. The highest total anthocyanin was in the 15-hour fermentation treatment and the addition of 13% double petals butterfly pea flower extract of 6.11 mg/L. Anthocyanin is a compound that is easily damaged by several factors, including exposure to light, hot temperatures, and alkaline conditions [29]. The higher the concentration of double-petaled butterfly pea flowers added, the higher the total anthocyanin produced. Butterfly pea flowers contain anthocyanins because their petals are blue [30]. The fermentation process causes the pH of black soyghurt to decrease. These conditions are very suitable for anthocyanin pigments because they are stable in acidic conditions. The treatment of fermentation time and concentration of double petals butterfly pea flowers affects the total anthocyanin in black soyghurt.The materials and methods should be typed in Cambria with font size 10 and justify alignment. Author can select Normal style setting from Styles of this template. The simplest way is to replace (copy-paste) the content with your own material. Method and analysis which is performed in your research work should be written in this section. A simple strategy to follow is to use keywords from your title in first few sentences.

3.3. The sensory properties of black soyghurt

The results of the sensory properties analysis of black soyghurt enriched with double petals butterfly pea flower extract can be seen in Table 3

Table 3 The sensory properties of Black Soyghurt

Analysis	Factor A Fermentation Time (hours)	Factor B The enrichment with Double Petals Butterfly Pea Flower Extract (%)	
		8	13
The sensory properties of black soyghurt (ranking)			
Taste	10	1.86 ± 0.50^{Aa}	1.80 ± 0.40 ^{Aa}
	15	1.60 ± 0.56^{Aa}	1.73 ± 0.69^{Aa}
Consistency	10	2.33 ± 0.60 ^{Aa}	2.03 ± 0.61 ^{Ab}
	15	2.00 ± 0.64 ^{Ba}	1.73 ± 0.69 ^{Bb}
The sensory properties of black soyghurt (hedonic)			
Color	10	4.66 ± 1.34^{Aa}	5.00 ± 1.05^{Aa}
	15	4.90 ± 1.47^{Aa}	5.03 ± 1.44 ^{Aa}
Flavor	10	4.30 ± 1.31^{Aa}	4.43 ± 1.30 ^{Aa}
	15	4.73 ± 1.20^{Aa}	4.63 ± 1.15 ^{Aa}
Taste	10	3.86 ± 1.16^{Ba}	4.60 ± 1.16^{Ba}
	15	4.80 ± 1.51 ^{Aa}	4.66 ± 1.47^{Aa}
Consistency	10	4.63 ± 1.21^{Aa}	4.75 ± 1.10^{Aa}
	15	4.83 ± 1.11^{Aa}	4.90 ± 0.95 ^{Aa}

Description: The average is obtained from three replications, numbers followed by different capital letters (A and B) indicate a significant difference in fermentation time, and numbers with different lowercase letters (a and b) indicate a significant difference in the concentration of double petals butterfly pea flowers extract ($P<0.05$)

3.3.1. The sensory properties of black soyghurt (ranking)

Taste

The results of the statistical analysis showed that the sensory analysis of taste was not significantly different between treatments. The average panelist assessment of the acidity level of black soyghurt was 1.60-2.33 (slightly sour). The lowest average was found in the treatment of 15 hours of fermentation time and the addition of 8% double petals

butterfly pea flower extract. This condition is thought to be because the longer the fermentation, the higher the lactic acid content, so that the taste becomes sour. This is supported by research by Dianasaril et al. [31] stating that total lactic acid increases with longer incubation, causing the pH value to decrease so that the taste becomes sour. The treatment of fermentation time and the addition of double petals butterfly pea flower extract did not affect the taste with the ranking test on black soyghurt.

Consistency

The results of statistical analysis showed that the average panelist assessment of the consistency of black soyghurt was 1.73-2.33 (slightly thick). The lowest average was found in the treatment of 15 hours fermentation time and the addition of 13% double petals butterfly pea flower extract. This condition is thought to be because black soyghurt, with the treatment of long fermentation and the addition of double petals butterfly pea flower extract, affects the consistency. The longer the fermentation time causes the pH decrease and the consistency of the yogurt increases. The treatment of long fermentation and the addition of double petals butterfly pea flower extract affect the consistency of the ranking test on black soyghurt.

3.3.2. The sensory properties of black soyghurt (hedonic)

Color

The quality of a food ingredient can be seen from its color. In addition, color is also an attraction for consumers in accepting a food product. The results of the statistical analysis showed that the color of black soyghurt with the treatment of long fermentation and the addition of double petals butterfly pea flower extract did not differ significantly between treatments. The average panelist assessment of the color of black soyghurt was 4.33-5.03 (neutral-rather like). These results were obtained through a hedonic test, where the highest average showed that the panelist's response to the color in the 15-hour fermentation time treatment and the addition of 13% double petals butterfly pea flower extract was quite similar. It is suspected that the appearance of black soyghurt is influenced by the addition of double petals butterfly pea flower extract, causing the color of the black soyghurt to become darker. Double petals butterfly pea flower extract contains anthocyanin pigments that are stable in acidic conditions; different pH levels also affect the differences in color obtained [12]. The treatment of fermentation time and the addition of double petals butterfly pea flower extract did not affect the panelists' preference for the color of black soyghurt.

Flavor

The results of the statistical analysis showed that the sensory analysis of aroma in black soyghurt with the treatment of fermentation time and the addition of double petals butterfly pea flower extract did not differ significantly between treatments. The average panelist assessment of the aroma of black soyghurt was 3.66-4.73 (neutral-quite like). This condition is thought to be because the aroma produced is unpleasant (still has a distinctive soybean aroma), so that the panelists are less interested. This statement is supported by Cahyani [10], which states that soyghurt drinks have high nutritional value but are less popular with the public because they have an unpleasant aroma. The highest average aroma value was in the 15-hour fermentation treatment and the addition of 8% double petals butterfly pea flower extract. This is thought to be because the aroma of black soyghurt is influenced by the fermentation time and the addition of butterfly pea flower extract. This statement is supported by Handayani and Wulandari [17], which states that fermentation in soybeans produces organic acids that can help reduce unpleasant aromas and improve taste. In this study, the use of 4 types of bacteria was also thought to help reduce unpleasant aromas in black soyghurt. The treatment of fermentation time and the addition of double petals butterfly pea flower extract did not affect the aroma preference of black soyghurt.

Taste

The results of the statistical analysis showed that the average panelist assessment of the taste of black soyghurt was 3.46-4.86 (neutral-quite like). This result was obtained through a hedonic test; from this study, the effect of fermentation time was significantly different, but the addition of double petals butterfly pea flower extract was not significantly different. Meanwhile, in the study of Husni et al. [24], it was explained that the addition of S. polycystum extract gave significantly different results. The highest average showed that the panelist response to the taste in the treatment of 10-hour fermentation time and the addition of 8% double petals butterfly pea flower extract was somewhat similar. The treatment of fermentation time had a significant effect, but the addition of double petals butterfly pea flower extract did not affect the panelists' preference for the color of black soyghurt.

Consistency

The results of statistical analysis showed that the sensory analysis of consistency in black soyghurt with fermentation time treatment and the addition of double petals butterfly pea flower extract did not differ significantly between treatments. The average panelist assessment of the texture of black soyghurt was 4.56-4.90. These results were obtained through hedonic tests, where the highest averages showed that the panelist response to consistency in a fermentation time treatment of 15 hours and the addition of 13% double petals butterfly pea flower extract was neutral. This condition is thought to be because the texture produced in this treatment is not thick enough (not in accordance with yoghurt in general), so that the panelists are less interested. The concentration of added skim milk must be increased as a filler so that it acts as a total solid that helps form the consistency of black soyghurt. This statement is supported by research by Handayani and Wulandari [17], which states that increasing milk concentration can help increase the consistency of soyghurt. The treatment of fermentation time and the addition of double petals butterfly pea flower extract did not affect the panelists' preference for the consistency of black soyghurt.

4. Conclusion

Fermentation time and addition of double petals butterfly pea flower extract to black soyghurt did not affect the physical properties of L* brightness (white-black) and b* (blue-yellow), but affected the viscosity and a* (green-reddish). The interaction of treatments did not affect the chemical properties of water, ash, and protein content, but affected fat, pH, total anthocyanin, and antioxidant activity. In all treatments, the ash, protein, fat, and pH content met the SNI 2981:2009 yoghurt quality standards. Furthermore, the treatment did not affect the ranking test on taste, but affected consistency. While in the hedonic test, it did not affect color, aroma, and consistency, but it did affect taste.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no known financial, personal, or professional conflicts of interest that could have influenced the work reported in this manuscript.

Statement of ethical approval

This work complies with all applicable ethical standards, including research integrity, proper acknowledgment of sources, and adherence to relevant institutional and publication guidelines. All procedures were conducted responsibly with respect for academic and professional norms.

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