Physicochemical Properties of Black Soygurt Made from Black Soybeans (BS) and Black Sticky Rice (BR)

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Abstract. Back soygurt is a food product fermented by black soy milk using the bacteria Streptococcus thermophilus and Lactobacillus bulgaricus commonly used in the process of making yogurt. To improve the black color of the soyghurt produced, it was added black sticky rice extract. The objective of this study was to investigate the effect of the proportion of black soybean extract (BS) with black sticky rice extract (BR) and fermentation time, on physicochemical characteristics in black soygurt. This study uses a completely randomized factorial design consisting of two factors. The first factor was the proportion of black soybean extract and black sticky rice extract which consists of three levels (50:50, 60:40, and 70:30). Factor II was fermentation time which consists of 3 levels (10 hours, 12 hours and 14 hours). The data obtained were analyzed with ANOVA, and further tests with Duncan. The best treatment was the proportion of black soybean extract: black sticky rice extract (50:50) with 14 hours fermentation time. The characteristics of black soygurt from this treatment were total LAB 10.63 CFU/ml; acidity (pH) 4.23; intensity of color L* 58.28; soluble protein 1.58%; total anthocyanin 1.61 mg/100g; antioxidant activity 19.55%.

Keywords: blacksoygurt, soybean, stickyrice

1. Introduction
Soygurt is a food product fermented from soy milk using the bacteria Streptococcus thermophilus and Lactobacillus bulgaricus which are commonly used in the process of making yogurt [1]. Soygurt has several advantages, namely the price of raw materials that are relatively cheap than cow’s milk, the nutritional content is almost the same as cow's milk, does not contain lactose that can be consumed for patients with lactose intolerant [2]. Soybean is one of the beans that have very good nutritional content compared to other beans. Generally, soybeans used as raw material for soy milk are yellow soybeans. Alternative raw materials that can be used for making soy milk are black soybeans (Glycine soja Sieb). Black soybean is a local soybean that does not get much attention and is not as popular as yellow soybeans because the color is less attractive [3]. Bad taste in soy milk that is less liked by the community can be overcome by fermenting soy milk with lactic acid bacteria culture as well as in making yogurt. Yogurt on the market has a variety of flavors, colors and, aromas. To create new variations of yogurt products, soygurt will be made from black soybeans, and to increase the intensity...
Beneficial of black sticky rice is still not optimal so that it can be innovated as an additional ingredient in soygurt products.

Black sticky rice is easily available at an affordable price and has been known to contain anthocyanin compounds that have several pharmacological activities, one of which is antioxidant activity. The main anthocyanin components found in black sticky rice (Oryza sativa var. glutinosa) are cyanidine-3-glycosides and peonidine-3-glycosides [4]; [5] and [6]. The concentration of the addition of black sticky rice which is different and the time of fermentation affects the resulting soygurt. The fermentation time in making soygurt is one of the factors that need to be considered because it has various benefits, including preserving food products, giving flavor or flavor to food products. The existence of the fermentation process carried out by certain microbes is expected to increase the nutritional value of the fermented product. An improvement in the quality of fermented food products and innovation in the use of raw materials is expected to increase the consumer’s preference. Increased acceptance by consumers will increase demand for fermented products, especially fermented drinks. The objective of this study was to investigate the effect of the proportion of black soybean extract and black sticky rice extract in different fermentation time on the characteristics of black soygurt.

2. Material and Method

2.1. Material and Equipment

The materials used in this study were black soybean from mature Raya shop, skim milk, black sticky rice, granulated sugar, water, a culture of Lactobacillus bulgaricus and Streptococcus thermophilus from the UGM Center for Food and Nutrition Study which was reproduced by itself at the Micro Veterinary Laboratory of UPN "Veteran" Java East, while the materials for analysis include: aquades, Sodium carbonate, NaOH, copper sulfate, Buffer Na-K-tartar, folin ciocalteau reagents, BSA, KCL buffer pH 1.0, sodium acetate buffer pH 4.5, DPPH, methanol, MRS broth, MRS agar, indicators pp, NaCl, Anthrhone, glucose, arsenomolybdate, nelson reagent.

The tools used for the manufacture and analysis of synbiotic soygurt in this study include digital scales, blender and filter cloth, analytical scales, incubators, autoclaves, centrifuges, micropipets, water baths, thermometers, erlenmeyers, volume pipettes, test tubes, analytical scales, incubators, refrigerators, autoclaves, centrifuges, micropipets, water baths, thermometers, erlenmeyers, volume pipettes, test tubes, beaker glass, measuring cup, measuring flask, viscometer, magnetic stirrer, stirrer, petri dish, pH meter, vortex, in case, Colony counter, spectrophotometer.

2.2. Research methods

This study uses a completely randomized design factorial pattern with 2 factors that are repeated three times. The factor I: the proportion of black soybean juice and black sticky rice juice (50:50, 60:40, 70:30); factor II: Fermentation time (10 hours, 12 hours and 14 hours). The data obtained were analyzed using analysis of variance. If there are significant differences between treatments followed by the Duncan test (DMRT)

2.3. Making soygurt

Black soybean juice and black sticky rice juice were mixed according to the treatment, added 10% skim milk and 5% sucrose, pasteurized at 80°C, 15 minutes, cool to 40-42°C, 5% starter inoculation, 37°C incubation, time according to treatment. Analysis carried out includes Total LAB, pH, Total Acid, Dissolved Protein, Antioxidant Activity, Anthocyanin, Intensity of color (Brightness).
3. Results and Discussion

3.1. Total Lactic Acid Bacteria (LAB)

Figure 1 shows that the higher the proportion of black soybean extract and the lower the proportion of black sticky rice extract and the longer the fermentation time increase the total lactic acid bacteria in black soygurt. This is by following the results of the analysis of raw materials in black soybeans having a higher dissolved protein which is 18.37% compared to the black sticky rice which is 3.31%. Protein from black soybean can be used as a source of nitrogen for the growth of lactic acid bacteria. Black soybeans also have oligosaccharides in the form of stachyose and raffinose which can trigger the growth of LAB. [7], oligosaccharides used by lactic acid bacteria for cell growth and maintenance can also be used for product formation. During the fermentation process, sugar hydrolysis will occur by lactic acid bacteria. Oligosaccharides contained in black soybeans will be hydrolyzed into its constituent compounds. The results of sugar metabolism by lactic acid bacteria in the form of energy needed for bacterial cell growth and organic acids, especially lactic acid. According to [8], α-galactosidase and invertase enzymes will hydrolyze stachyose and raffinose into glucose, fructose and, galactose.

![Figure 1. Total lactic acid bacteria in black soygurt.](image1)

Total lactic acid bacteria will also increase with a longer fermentation time because lactic acid bacteria can utilize the nutrients optimally as a growth medium. According to [9], the length of fermentation time the total lactic acid bacteria increases, because lactic acid bacteria have a longer time to ferment or hydrolyze sugar into simpler components into lactic acid, CO₂, H₂O, and energy, then energy will be used for synthesis so that the amount cells become increased.

3.2. pH value

Figure 2 shows that the higher of black soybean and the lower the black sticky rice extract and, the length of fermentation time, the decreased the acidity (pH) in black soygurt. Caused by the higher proportion of black soybean extract, the more nitrogen sources in the form of protein so that lactic acid bacteria can grow optimally and produce the lactic acid, and reduce pH. According to [10], lactic acid bacteria will utilize these nutrients and produce organic acids during fermentation, as a result, these organic acids accumulate and the pH of the media decreases. Sucrose added to soygurt will also be broken down into glucose and fructose so that it can be used as a nutritional source of lactic acid bacteria to form lactic acid and the higher the lactic acid produced, the lower the acidity (pH) of the product produced. The longer the fermentation time, the lower the pH of soygurt produced. The decrease in pH is a result of the fermentation process that occurs due to the production of lactic acid derived from lactic acid bacteria. The lactic acid produced will dissociate to produce H⁺ and CH₃CHOHCOO⁻ so the higher lactic acid allows more H⁺ ions in soygurt, the lower the pH produced. The results of the pH analysis of soygurt conducted ranged from 4.13 to 4.59. These results indicate

![Figure 2. PH value in black soygurt.](image2)
that all treatments on soygurt products produced in this study have fulfilled SNI requirements (2981: 2009) which states that the pH quality for good yogurt ranges from 4-4.5.

3.3. Soluble Proteins

Figure 3. shows that the higher the proportion of black soybean extract and the lower the proportion of black sticky rice extract and the longer the fermentation time can increase the soluble protein in black soygurt. This was related to the amount of soluble protein found in raw materials before fermentation. Black soybean has 18.57% dissolved protein, while black sticky rice has 3.31% of soluble protein (Tabel 1). Increasing the growth of lactic acid bacteria can increase proteolytic enzymes so that it can improve the performance of the fermentation process followed by an increase in the breakdown of substrate components during the fermentation process such as the breakdown of proteins into simpler peptides, cause soluble protein also increases. According to [11], soluble protein levels are related to the proteolytic activity of lactic acid bacteria which converts protein to amino acids and peptides so that it will increase levels of soluble protein in yogurt.

![Figure 3. Soluble protein in black soygurt](image)

![Figure 4. Intencity of Color in black soygurt](image)

The longer the fermentation time will increase the level of dissolved protein soyghurt. This can be due to the more time spent by BAL in breaking down proteins into simpler peptides and the increase in organic acids produced as a result of BAL fermentation causes dissolved protein to also increase. According to [12], starter bacteria can produce protease enzymes which in turn cause hydrolyzed proteins to be the simplest component of proteins namely peptides and amino acids which are soluble proteins.

3.4. The Black Colour Intensity

Figure 4, shows that the higher the proportion of black soybean extract and the lower the proportion of black sticky rice extract and the length the fermentation time can increase the value of (brightness). This is related to the total anthocyanin in the raw material. The total anthocyanin in black sticky rice extract was 4.61 mg/100g while the total anthocyanin in black soybean extract was 0.68 mg/100g. The black sticky rice extract has a higher total anthocyanin than black soybean extract, therefore, soygurt with the proportion of black soybean juice and black sticky rice juice (50:50) will reduce the value of L (brightness) in soygurt or it can be said that the product is getting darker. [13], the chromophore group itself is a color-carrying group in a pigment where the higher the concentration of pigment, the higher the number of chromophore groups, which causes the color to become darker. The length of the fermentation time, the value of L (brightness) in soygurt was an increase, this can be due to acid conditions anthocyanin brightness will increase because it will be red. According to [7], that anthocyanin is a water-soluble pigment, in acidic conditions anthocyanin brightness will increase because it will be red. Fermentation will cause the brightness level of the product to increase.
3.5. Anthocyanine Content

Figure 5 shows that the higher the proportion of black soybean extract and the lower the proportion of black sticky rice extract and the length of fermentation time will reduce the total anthocyanin in soygurt. This relates to the total anthocyanin raw material used. The total anthocyanin in black sticky rice is 105.21 mg/100g while the total anthocyanin in black soybean is 40.11 mg/100g so that the lower the black sticky extract used, the lower the total anthocyanin in soygurt. The longer the fermentation time it will increase the total anthocyanin in soygurt. This can be caused because, during the fermentation of BAL, the enzyme α-galactosidase is needed to hydrolyze the sugars in black soybean extract and black sticky rice extract. The results of sugar metabolism by BAL in the form of organic acids, especially lactic acid, along with an increase in total acid during fermentation, a decrease in pH occurs. At pH anthocyanin, acid is in the most stable condition and highest color intensity. According to [14], the lower the pH value, the more concentrated the color of red and stable. According to [15], in acidic conditions will cause more and more anthocyanin pigments in the form of colored cavities flavilium and absorbance measurements will show an increasing amount of anthocyanin and cause vacuole cell walls to rupture so that more anthocyanins are extracted.

3.6. Antioxidant Activity

Figure 6 shows that the higher the proportion of black soybean extract and the lower the proportion of black sticky extract can reduce the antioxidant activity in black soygurt, but the longer the fermentation time can increase the antioxidant activity. This is related to the antioxidant activity of the raw materials used. The antioxidant activity of black sticky rice is 29.49% while the antioxidant activity of black soybean is 13.09% so that the higher the proportion of black sticky juice used, the higher the antioxidant activity. High antioxidant activity in black sticky rice is also caused by the presence of anthocyanin which can act as an antioxidant. Based on the analysis of raw materials, the total anthocyanin black sticky rice is higher at 105.21 mg/100g compared to black soybean at 40.11 mg/100g. According to [16], anthocyanin is a phenolic compound of the flavonoid group which has the mechanism of capturing free radicals by releasing hydrogen atoms from its hydroxyl group. The mixing of black soybean juice and black sticky rice in various proportions has decreased slightly after being fermented into soyghurt products, this can be caused due to the influence of temperature on the pasteurization process, the presence of oxidation and anthocyanin degradation during fermentation which causes antioxidants in the product has decreased slightly. [17], the natural antioxidants are generally in the form of concentrated liquids and sensitive to heating. According to [18], during the fermentation process anthocyanin content will be degraded by the lactic acid bacteria to produce its derivatives.

![Figure 5. Anthocyanine Content in black soygurt.](image1)

![Figure 6. Antioxidant Activity in black soygurt.](image2)
The length of fermentation time, the higher the antioxidant activity in black soyghurt. This is because the longer the fermentation time, the total lactic acid that accumulates in soyghurt is also higher so that it can result in antioxidant activity also increasing. According to [9], the presence of lactic acid which can remodel lactose into lactic acid which is synergistic that is giving H+ ions to free radicals that have unpaired electrons thereby increasing antioxidant activity. [19], lactic acid contains α-hydroxy acids (AHA) which function as antioxidants. Therefore, antioxidant activity is influenced by lactic acid (CH3CHOHCOOH) produced by probiotic bacteria as donors of hydrogen atoms for molecules or toms that have unpaired electrons in their outermost orbits (free radicals).

4. Conclusion
The best treatment of this research was the proportion of black soybean extract: black sticky rice extract (50:50) and 14 hours fermentation time resulted in black soygurt with 10.63 CFU/ml total LAB, 4.23 pH, 1.58% soluble protein, L* 58.28 intensity of color; 19.55% antioxidant activity, and 1.61 mg/100g total anthocyanins.

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