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Protein fraction profile and physicochemical quality of *Blacksoyghurt* drink

Al-Baarri, A.N., Dwiloka, B., Mulyani, S., Pramono, Y.B., *Setiani, B.E., Rahmawati, A.A., Jordan, A. and Nababan, P.U.B.L.

Food Technology Study Programme, Faculty of Animal and Agricultural Science, Universitas Diponegoro, Semarang 50275, Indonesia

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Abstract

Blacksoyghurt is often processed using lactic acid bacteria to increase its nutritional content, but the growth of the microorganism affects the content and organoleptic analysis. Therefore, this study aimed to determine the effect of longer fermentation time on the chemical and organoleptic characteristics of *Blacksoyghurt*. The samples were divided into 4 treatment groups, which consisted of different durations, i.e., 0, 3, 6, 9, and 12 hrs. The result showed that variation in time significantly affected the protein content, fat content, pH, total acid, viscosity, total dissolved solids, and the organoleptic characteristics of the product, but no significant effect was observed on the total solids. Based on the chemical and organoleptic analysis, the best treatment was 12 hrs.

1. Introduction

Black soybeans are legumes in the Leguminosae group, which have a black color on the epidermis and are usually processed into raw materials for soy sauce production. They have a rich nutritional composition of 420 mg/g protein, 224 mg/g fat, 340 mg/g carbohydrates, 6 mg/g calcium, 5 mg/g phosphorus, and 0.1 mg/g iron (Kurniasih *et al*., 2013). Black soybeans are also classified as prebiotic sources because they contain oligosaccharides, such as raffinose and stachyose. These compounds can be processed by probiotics, such as lactic acid bacteria as a source of nutrients and carbon for their growth (Maryati *et al*., 2016). Due to the rich composition of the plant, it is often used for the production of various processed foods. *Blacksoyghurt* is one of its products, which has been fermented using lactic acid bacteria.

Lactic acid bacteria (LAB) are gram-positive bacteria that do not produce spores and are spherical or rod-shaped. They also produce lactic acid as the final product during their fermentation process (Okfrianti *et al*., 2018). Based on the type of fermentation, LAB can be divided into two categories: there are, homofermentative Embden Mayerhof Parnas (EMP) and heterofermentative/Hexose Monophosphate Pathway. The homofermentative process converts 95% of hexoses to lactic acid, while the heterofermentative converts carbohydrates into small amounts of the acid and other products, such as ethyl alcohol, acetic acid, formic acid and carbon dioxide (Hasanah, 2014). An example of homofermentative bacteria is *Lactobacillus acidophilus*, while heterofermentative bacteria are *Lactobacillus bulgaricus* and *Lactobacillus plantarum*.

Blacksoyghurt is obtained from the fermentation of soybean by lactic acid bacteria, which produce proteolytic enzymes. The catalyst hydrolyzes the main components of the substrate's protein into peptides and free amino acids through protein hydrolysis techniques (Lu *et al*., 2022). The growth of LAB is influenced by the length of fermentation. One of the factors that must be considered during the production of the product is variation in time because it has an effect on its nutritional content, such as protein and fat. Therefore, this study aimed to determine the best fermentation time for *Blacksoyghurt*.

2. Materials and methods

2.1 Preparation of raw materials

2.1.1 Soybean juice making

Black soybean samples were obtained randomly from Johar Baru Market, Semarang. They were then soaked for 8 hrs, washed, and placed in 0.5% $HaHCO_3$ for 30 mins. Subsequently, the samples were mashed

*Corresponding author.
Email: *bhaktietzasetiani@lecturer.undip.ac.id*

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with a water ratio of 1:8 and then filtered with gauze. A total of 10% sugar was added, and the mixture was homogenized at 4 rpm for 10 mins. The soybean juice was then pasteurized at 72°C for 15 s, and the temperature was lowered to 42°C.

2.1.2 Starter making

The starter was prepared based on the method described previously by Harjiyanti *et al*. (2013). Approximately 68.20 g of skim milk was dissolved in 500 mL distilled water, followed by pasteurization at 37°C for 15 s, and the temperature was lowered to 42°C. Subsequently, 10 g of freeze-dried LAB starter containing *Streptococcus thermophilus, L. bulgaricus* and *L. acidophilus* was incubated at 37°C for 4.5 hrs, and it served as the mother culture. A total of 125 g of skim milk was dissolved in 500 mL of distilled water and then pasteurized at 37°C for 15 s. A bulk starter culture was produced using approximately 25 g of the mother culture and then incubated at 37°C for 4.5 hrs.

2.1.3 Blacksoyghurt

Blacksoyghurt was produced by mixing 1000 mL of black soybean extract with 5% bulk culture, followed by homogenization. Soybean juice was fermented with various treatments, i.e., 0 (T0), 3 (T1), 6 (T2), 9 (T3) and 12 hrs (T4) at 37°C. One hour before the end of the fermentation, *Lactobacillus plantarum* starter was added to the mixture.

2.2 Testing parameters

2.2.1 SDS Page

SDS Page was used to examine the protein content based on the method described previously by (Ghosh *et al*., 2013) using 12.5% separating and 3% stacking gels. They were then stained with 0.1% (m/V) Coomassie Brilliant Blue R-250 in methanol (y = 45%) and acetic acid (y = 10%). Subsequently, the gels were destained overnight in a solution containing methanol (y = 30%) and acetic acid (y = 45%). The protein fractions were then identified using a recombinant molecular mass standard mixture (TaKaRa Biotechnology Co, Ltd, Dalian, PR China).

2.2.2 Fat content

The fat content was examined based on the method described by Untoro *et al*. (2012). The samples were extracted with fat solvent liquid using the Soxhlet method.

2.2.3 Viscosity

The viscosity of the samples at 25°C was measured with an Ostwald U-tube, as described by Setianto *et al*. (2014).

2.2.4 pH

The pH of the sample at 25°C was measured with a pH meter based on the method described by Alfadila *et al*. (2020).

2.2.5 Total acid

The total acid content of the sample was examined, as described by Meirida *et al.* (2016) using the titration method. The percentage of lactic acid was used as an indicator of titratable acidity. Subsequently, the samples were titrated with 0.1 M sodium hydroxide until the final total acid was obtained.

2.2.6 Total dissolved solids

The total dissolved solids content was analyzed using a hand refractometer, as described by Ismawati (2016). Before the analysis, its prism was cleaned with alcohol to avoid errors. Afterwards, the sample dripped on the prism and the refractometer was directed to a light source. The scale that appeared shows the total solids content in °Bx.

2.2.7 Color content

The color content was measured using the L*a*b method, as described by Engelen (2017). The attributes of the samples were determined with a chromameter, which gave the L*, a*, and b* values directly. The color measurement was expressed in L*, indicating the lightness on a scale of 0 to 100 or from black to white. The a* (+,-) value shows the level of redness or greenness, while b* (+,-) indicates yellowness and blueness.

2.2.8 Organoleptic testing

The organoleptic testing was measured using the scoring method, as described by Adri *et al*. (2013). The sensory properties were evaluated by a trained panel consisting of 25 panelists. The samples in the plastic cups were then coded with three-digit numbers, and the order of presentation was randomized. Thereafter, a test form comprising four sensory attributes, viz., appearance, flavor, texture, and overall acceptability was given to each panelist.

2.2.9 Total solid content

The total solids content was measured using the method described by Budirahayu *et al*. (2020). The samples were dried at 100˚C to constant weight using an oven.

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2.3 Data analysis

Data from the SDS page, fat content, pH, viscosity, total acid, total dissolved solids, color content, organoleptic testing, and total solid content were analyzed using the one-way ANOVA and then followed by the Duncan analysis to identify the presence of significant differences in the treatments at a ($p < 0.05$) confidence interval. Furthermore, the color testing and protein fraction using SDS - Page were analyzed with descriptive analysis using Microsoft Excel 19 for Windows. The organoleptic data were analyzed using the Kruskal Wallis test with a significance level ($p < 0.05$), and then followed by the Mann-Whitney test.

3. Results and discussion

3.1 Soy protein fraction through SDS page

The SDS page revealed that all treatments showed almost the same line, but they had different thickness levels. The thick line observed implies that there was an increase in protein components. The type of protein was indicated by the blue line, where molecular weights of 19 – 20, 37 - 49, 53 - 42, 76 - 57, and 83 - 57 kDa revealed the presence of basic glycinin (B), acid glycinin (A) (Nielsen, 1985), β-Conglutinin, α-Conglutinin, and α'-Conglutinin, respectively (Aresse *et al*., 1991). Figure 1 shows that the length of treatment affected the protein components of *Blacksoyghurt*. The band became thinner due to denaturation, which occurred during fermentation by LAB. Zufahmi *et al*. (2015) reported that the difference in the thickness of the band formed was caused by the difference in the number of molecules that migrated. Soybean proteins were hydrolyzed into simpler molecules during the fermentation by *L. acidophilus* and *Streptococcus thermophilus*. The results showed that the peptide profile produced was unchanged (Shirotani *et al*., 2021). The presence or absence of bands at a certain migration distance indicates the presence or absence of proteins that migrated and stopped at that distance during the electrophoresis process. A previous study revealed that the non-fermented and fermented products of *Blacksoyghurt* have a pH of 6.51 and 4.27-4.75, respectively. This decrease caused a denaturation process, where the proteins in the solution reduced their solubility, settled down and agglomerated (Osundahunsi *et al*., 2007). The fermentation of the product led to changes in the soy protein fraction components, such as β-Conglutinin and α-Conglutinin.

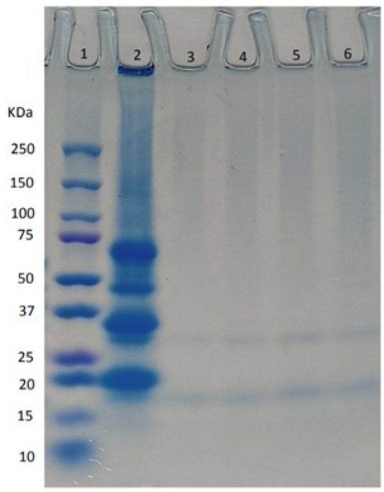

Figure 1. Protein fraction results using the SDS page method. The SDS-PAGE pattern of the *Blacksoyghurt* with various fermented hours on 12.5% separating and 3% stacking gels; 1. Molecular weight standards 2. T0, 3. T1, 4. T2, 5. T3, 6. T4.

3.2 Fat content

Figure 2 shows that there was a decrease in the fat content of *Blacksoyghurt* from 47.06% to 40.46% along with the length of fermentation. This was due to various fat conversion reactions by LAB, such as isomerization, hydration and saturation (Wang, 2022). The longer the fermentation time, the lower the fat content of the product due to protein synthesis from fat (Ye *et al.*, 2013). Lipolytic and similar enzymes can contribute to the breakdown and extraction of lipid constituents. They also have selective reductive activity, which involves the use of lipid-associated components as a carbon source (Obadina *et al*., 2013; Adebo *et al*., 2022).

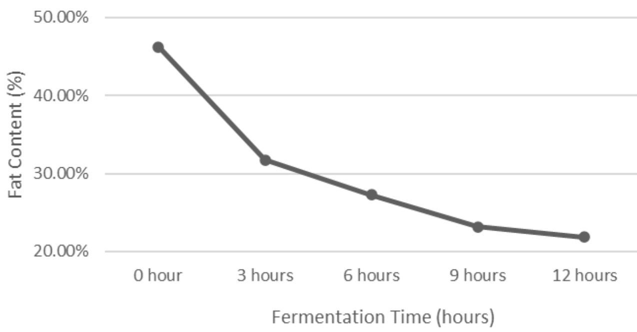

Figure 2. Fat content of the *Blacksoyghurt*.

3.3 Protein content

Figure 3 shows that the difference in black soybean fermentation times has a significant effect ($p < 0.05$) on the protein content, which ranged from 2.13% to 3.69% and increased with the duration. LAB produces

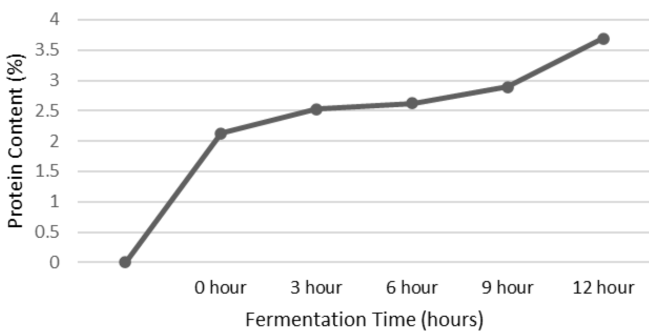

Figure 3. Protein content of the *Blacksoyghurt*.

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proteolytic enzymes, which hydrolyze the substrate's protein into peptides and free amino acids (Hou *et al*., 2017). Hydrolysis increases the content of the hydrolyzate product through reactions with protease enzymes (Zhao *et al*., 2012). These biological catalysts are produced around the cell wall, cytoplasmic membrane, or inside the cell, where they are also used by *Lactobacillus acidophilus* for growth. The reshuffling of protein complex compounds into simpler compounds, such as amino acids, is important in soybean fermentation (Sharma *et al*., 2021).

3.4 Viscosity

Table 1 shows that the longer the fermentation time, the higher the viscosity of *Blacksoyghurt*. The high value obtained was caused by the increase in the amount of lactic acid produced. Li *et al*. (2014) revealed that organic acids are produced from the breakdown of sugar, and they cause acid accumulation as well as an increase in viscosity. Diantoro *et al*. (2015) also reported that the lactic acid formed during the process was removed from the cells. Therefore, the longer the duration of the fermentation, the higher the total acid and texture of the product. *Blacksoyghurt* fermentation with LAB makes the product thicker due to coagulation caused by the microbe (Devi *et al*., 2014). Viscosity has a negative correlation with the level of acidity because an isoelectric point occurs at low pH (Diasari *et al*., 2021).

Table 1. Viscosity of the *Blacksoyghurt*.

Fermentation Time	Viscosity (Cp)
0 hr	1.36 ± 0.05^{d}
3 hrs	1.83 ± 0.06^{c}
6 hrs	1.94 ± 0.06^{b}
9 hrs	2.04 ± 0.12^{ab}
12 hrs	2.08 ± 0.33^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05).

3.5 pH

The level of microbial activity as well as the number of microbes increased along with the fermentation time. This led to a decrease in the pH of the medium where the chemical change of sugar into acidic components occurred (Mohammadi *et al*., 2017). The acidity level of *Blacksoyghurt* is presented in Table 2. The decrease in pH causes the taste of the product to become sour due to the formation of lactic acid as the main product of the metabolism. Yusmarini *et al*. (2012) reported that the higher the amount of sugar available, the higher the level of organic acids produced. Gawad *et al*. (2014) also reported that LAB converts complex compounds, such as protein, fat, and carbohydrate into simple components, including lactic acid, free fatty acids, and amino acids.

Table 2. pH of the *Blacksoyghurt*

Fermentation Time	pH
0 hr	6.51 ± 0.37^{a}
3 hrs	4.75 ± 0.68^{b}
6 hrs	4.52 ± 0.59^{b}
9 hrs	4.28 ± 0.52^{b}
12 hrs	4.27 ± 0.50^{b}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05).

3.6 Total acid

The increase in total acid was caused by the increased number of LAB, which produced more lactic acid. This is in agreement with the results reported by Septiani *et al*. (2013), that the condition was caused by an increase in microbe population, and this was indicated by a decrease in pH because more sugar was hydrolyzed to acids. Table 3 shows the total acid content of *Blacksoyghurt*. Teguh *et al*. (2015) reported that an increase in lactic acid levels occurred due to the activity and growth of LAB, which breaks down simple sugars through the glycolysis process. This is because several strains can now remodel the sugar component to the maximum level (Setiarto *et al*., 2017).

Table 3. Total acid of the *Blacksoyghurt*.

Fermentation Time	Total Acid (%)
0 hr	0.16 ± 0.33^{d}
3 hrs	0.34 ± 0.82^{b}
6 hrs	0.40 ± 0.54^{bc}
9 hrs	0.47 ± 0.62^{b}
12 hrs	0.62 ± 0.17^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05).

3.7 Total dissolved solids

Table 4 shows that the different fermentation times have a significant effect ($p < 0.05$) on *Blacksoyghurt* TDS. The total soluble solids level ranged from 11.95 – 10.13°Bx and decreased with the treatment duration. Putri, Budiharjo and Kusdiyantini (2014) reported that bacterium breaks down sugar into simpler forms during fermentation. Yulianto and Widyaningsih (2013) also revealed that the total dissolved solids can be used to determine the sugar content of the material. Black soybeans contain oligosaccharides in the form of raffinose and stachyose, but they need to be hydrolyzed to increase digestibility. One of the ways that is often used to reduce their level is through fermentation into yogurt using *Lactobacillus plantarum* (Salahudin and

Utomo, 2012).

Table 4. Total dissolved solids of the *Blacksoyghurt*.

Fermentation Time	Total Dissolved Solids (°Bx)
0 hr	11.95±0.45^{c}
3 hrs	11.18±0.33^{b}
6 hrs	11.00±0.44^{b}
9 hrs	10.43±0.74^{ab}
12 hrs	10.13±0.29^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05).

3.8 Color

Table 5 shows that the brightness level of L* on *Blacksoyghurt* ranges from 35.918 – 53.242, and it increased along with the length of fermentation. Armanzah and Hedrawati (2016) reported that when anthocyanins are degraded, the black pigment fades and the product becomes brighter. The a* value of the product ranged from -4.074 to -1.64. The color became redder along with the treatment duration length, and this was indicated by the increasing a* value. Hermawati *et al*. (2015), reported that the brightness of anthocyanins increases in acidic conditions because they turn red. The b* value ranged from 5.698 to 9.766. Rohman and Maharani (2020) reported that the yellow color was caused by the presence of two yellow pigments in skim milk raw materials, there are carotene and riboflavin, which are also widely contained in milk whey.

Table 5. Color L*a*b* of the *Blacksoyghurt*.

Fermentation Time	L*	a*	b*
0 hr	35.918	-4.074	5.698
3 hrs	47.052	-2.316	9.766
6 hrs	49.406	-2.316	9.766
9 hrs	53.242	-1.64	9.384
12 hrs	5.43	-2.148	9.618

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05).

3.9 Organoleptic

Table 6 shows that the panelists preferred 12 hrs for the fermentation of *Blacksoyghurt*. This was indicated by the aroma and taste parameters, which were higher compared to other treatment durations. Meanwhile, for the color parameter, the highest score was obtained at 3 hrs because a longer fermentation time gave the product a distinctive aroma and increased its sourness (Das *et al*., 2019). The sour taste of yogurt is influenced by the ability of LAB to ferment sugars into organic acids (Hidayat *et al*., 2013).

Table 6. Organoleptic *Blacksoyghurt*.

Sensory Attributes	Fermentation time				
	0 hr	3 hrs	6 hrs	9 hrs	12 hrs
Color	3.12±0.66^{a}	3.80±0.50^{bc}	3.80±0.41^{c}	3.36±0.70^{ad}	3.52±0.51^{d}
Aroma	1.60±0.76	1.80±0.76	1.72±0.61	1.72±0.61	1.88±0.83^{s}
Taste	1.32±0.63^{a}	2.60±0.86^{b}	3.13±0.68^{c}	3.00±0.91^{bc}	3.24±0.78^{c}
Overall	3.00±0.65	2.67±0.70	2.41±0.65	2.56±0.71	2.64±0.70

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05).

3.10 Total solid

Table 7 shows that the different fermentation times did not have a significant effect (p > 0.05) on the total solids of *Blacksoyghurt* in the T0, T1, T2, T3, and T4 treatments. The content obtained from the product ranged from 11.59 – 12.65% and increased along with the length of the process. Although no significant effect was recorded in the test, its value in the product increased. This was because the number of lactic acid bacteria increased due to growth, and this led to the addition of mass (Nizori *et al*., 2008). The increasing value was also influenced by the addition of skim milk to the yogurt starter. This is in agreement with Serlahwaty *et al*. (2015), who reported that the addition of skim milk can trigger LAB growth as well as increase the nonfat solids content of *Blacksoyghurt*. Naibaho *et al*. (2020) reported that total solids are a combination of carbohydrates, protein, fat, vitamins, and minerals found in soy milk, as well as other added ingredients. The content obtained in the product caused a 100% reduction in its moisture level. This is in accordance with the opinion of Putri *et al*. (2014) that there was a change in glucose into lactic acid, which led to the production of water, carbon dioxide, and other metabolites during fermentation. Therefore, an increase in the water content of *Blacksoyghurt* caused a decrease in the total solids

Table 7. Total solid of *Blacksoyghurt*.

Fermentation Time	Total Solid (%)
0 hr	11.59±1.10
3 hrs	12.54±0.85
6 hrs	12.65±1.16
9 hrs	11.71±0.45
12 hrs	12.28±0.36

Values are presented as mean±SD.

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produced.

4. Conclusion

Based on the chemical and organoleptic analysis, the best time for the fermentation of *Blacksoyghurt* production was 12 hrs. It gave the product a good protein fraction and aroma and the taste was also preferred by the panelist.

Conflict of interest

The authors declare no conflict of interest.

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