

# Characteristic and Isoflavone Level of Soymilk Fermented by Single and Mixed Culture of *Lactobacillus plantarum* and Yoghurt Starter

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**Abstract** Fermented soymilk has better health benefits compared to unfermented soymilk. The health benefits of fermented soymilk are improving blood lipid profile, antioxidants and anti-inflammatory. Fermentation of lactic acid bacteria can increase the content of aglycon isoflavones, namely genistein and daidzein. The purpose of this study was to analyze the characteristics of soymilk and isoflavone level fermented with a single culture of *Lactobacillus plantarum* and mixed culture of *Lactobacillus plantarum* and yogurt starter. This research consisted of 4 treatments, namely unfermented, fermentation using *L. plantarum*, fermentation using *S. thermophilus* and *L. bulgaricus*, and fermentation using *L. plantarum*, *S. thermophilus*, *L. bulgaricus*. Samples analyzed proximate, titratable acid, pH, total lactic acid bacteria, total daidzein, genistein and sensory quality. The results showed that the total acid, pH, and total lactic acid bacteria for fermentation with mixed cultures were higher compared to *L. plantarum* single culture. Total daidzein and genistein for single culture and mixed culture were not significantly different between the two groups. Sensory analysis shows that soymilk with a single culture have the highest value for the parameters of taste, aroma and color.

**Keywords:** daidzein, genistein, mixed culture, soymilk, and single culture

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## 1. Introduction

Soy milk is a soy extraction product obtained from crushed or ground soybeans. The disadvantage of soy milk is the unpleasant smell caused by the enzyme lipoxygenase from soy which is active during the process of making soy milk. Lipoxygenase (EC. 1.13.11.12 linoleic: 13-oxidoreductase oxygen) catalyzes the oxygenation of long-chain fatty acids into hydroperoxides. An effort to reduce the unpleasant odor of soy milk is to ferment soymilk using lactic acid bacteria. Several studies have shown that fermented soy milk can provide better health benefits compared to unfermented soy milk. Some lactic acid bacteria that can be grown using soy milk carbohydrates that have been studied are *L. rhamnosus* [1], *L. paracasei* subsp. *paracasei* [2], *L. plantarum* [2,3], *Bifidobacteria bacterium* [4], and *Bifidobacteria lactis* [5].

Fermentation using lactic acid bacteria can increase isoflavones in the aglycon form of genistein and daidzein. Several studies have shown that lactic acid bacteria have the enzyme  $\beta$ -glucosidase which can hydrolyze glycosidic bonds between isoflavones and carbohydrates [6,7,8].

$\beta$ -glucosidases ( $\beta$ -D-glucoside glucohydrolase, EC 3.2.1.21) are enzymes that catalyze the hydrolysis of  $\beta$ -glycosidic bonds in oligosaccharides and glycosides to form glucose or oligosaccharides which are shorter or unbranched. According to [9], the  $\beta$ -glucosidase enzyme from *L. plantarum* can completely hydrolyze isoflavone glycosidic bonds during 48 hours fermentation at 37°C.

Fermented soy milk can reduce triglycerides, total cholesterol, and LDL in the blood [2,3,4,10,11,12]. Some studies also show that fermented soy milk can increase HDL levels in the blood [3,4]. Fermented soy milk can also be as an antioxidant and anti-inflammatory. Fermented soy milk can increase the activity of antioxidant enzymes in the body namely SOD, catalase and GSH-Px so that it can improve the body's antioxidant status [5,10,11]. The content of genistein fermented soymilk can improve antioxidant status through activation of nuclear respiratory factor 1 (Nrf 1). Nuclear respiratory factor 1 (Nrf 1) is a transcription factor that plays an important role in regulating antioxidant enzymes [13]. Fermented soy milk can also reduce oxidation products, namely malonaldehyde and free radicals [4,5,11]. Experiments using experimental animals have shown that fermented soymilk can reduce proinflammatory cytokines, namely TNF- $\alpha$ , IL-6 and IL-1B [4,14].

Fermented cow's milk and soy milk usually use starter yogurt, namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. To increase aglycon isoflavone levels in soy milk, it is necessary to study fermented soymilk using lactic acid bacteria that have a high  $\beta$ -glucosidase enzyme. One of the lactic acid bacteria that has a high  $\beta$ -glucosidase enzyme is *L.plantarum*. Research on fermentation using mixed cultures between the yogurt starter and *L.plantarum* does not yet exist. Result of mixed fermentation cannot be predicted based on what happens in pure culture. This study aims to examine the characteristics of fermented soymilk using a single culture *L.plantarum* and a mixture of yogurt starter

## 2. Material and Methods

### 2.1. Bacteria

Lactic acid bacteria strain *Lactobacillus plantarum* FNCC 0027, *Streptococcus thermophilus* FNCC 0040, and *Lactobacillus bulgaricus* FNCC 0041 obtained from the Universitas Gadjah Mada culture collection.

### 2.2. Fermentation of Soymilk

Soybeans were soaked in water for 8 hours, washed and removed from the skin. Then the soybean was ground with a ratio of soy and water of 1:5. After the waste was separated, soymilk was sterilized at 115°C for 10 minutes. Sterilized soymilk was divided into two parts, fermented and unfermented soymilk. Fermented soymilk is inoculated with *Lactobacillus plantarum* (LP) or mixed cultures *L.bulgaricus* + *S.thermophilus* (LB+ST) and *L.plantarum* + *S.thermophilus* + *L.bulgaricus* (LB+ST+LP) by 3% (v / v). Bacterial concentration was  $10^7$  CFU / ml. Then the soy milk is incubated for 18 hours at 37°C.

### 2.3. Growth and Acid Production

Total lactic acid bacteria use the pour plate method. 1 gr of soy milk diluted with sterile 0,85% saline (w/v). The last dilution level was planted in MRS media and incubated for 48 hours. The growing colonies are then counted using a colony counter. Plates containing 25-250 colonies were counted and recorded as CFU per gram of fermented soy milk. The Titrable acidity was measured by titration with 0,1 N NaOH solution and expressed as percentage lactic acid (AOAC).

### 2.4. Sensory Analysis

Sensory analysis carried out using 25 panelists. Twenty-five panelist consisting of student and staff of the Department Agroindustrial Technology (Trunojoyo University, Indonesia). Panelists were given a questionnaire to evaluate sensory differences among the product presented by giving a score between 1-5 (1-extremely bad, 5-extremely good). Assessment include color, taste, flavor and texture . sample were presented in white plastic cups and marked with 3-digit codes. Water was provided for rinsing between samples. The result as a quantitative descriptive analysis (QDA) profile [15]

### 2.5. Isoflavon Analysis by HPLC

The product was extracted first using the enzyme sulfatase for 45 minutes at 37°C. The extraction results are then separated using high performance liquid chromatography (HPLC Agilent 1200 series). The solvent used was acetonitrile from a concentration of 25% to 50% (v / v) in 50 Mm buffered sodium acetate (pH 4.8) for 10 minutes then followed by 50% acetonitrile for 10 minutes [16]

### 2.6. Statistical Analysis

Hypothesis testing for variables with ratio data scale uses parametric statistical tests. Data was analyzed using the Analysis of Variance (ANNOVA) test with a 95% confidence level and the difference was declared significant if  $p < 0.05$ . If there are significant differences, then proceed with the Least Significant Difference (LSD) test with  $p < 0.05$ . As for the organoleptic test variable with ordinal data scale, it was analyzed using a non parametric statistical test, the Kruskal-Wallis test and continued with the Mann-Whitney test.

## 3. Result and Discussion

The nutritional content analyzed in this study is moisture content, carbohydrate content, protein content, fat content, and ash content. The results showed that the values of moisture content, carbohydrate content, and ash content were not significantly different for all treatments. This shows that fermentation does not affect the moisture, carbohydrate and ash of the ingredients. The Proximate analysis was shown in Table 1.

Different protein levels for the unfermented soybean milk treatment group with fermented soymilk. Unfermented soymilk has the lowest protein content compared to other treatments. Fermented soy milk with LB + ST has the highest protein content, followed by LB + ST + LP and LP. Fermentation can increase protein levels of soy milk due to the presence of protein from these bacterial cells [17]. Several studies have shown increased levels of protein in fermented soymilk. The free amino nitrogen of soymilk fermented with the four probiotic strain *L. casei*, *B. Animalis* V9, *L. acidophilus* NCFM dan *L.rhamnosus* GG meningkat [18]. Research by [19] shows that protein content was increased as fermentation time increased. The improvement of protein content might be due to some anabolic processes leading to polymer build-up or microbial cell proliferation.

The fat content of fermented soymilk includes low-fat yogurt because it ranges between 0.6 - 2.9%. Soymilk raw material has lower fat content when compared to cow's milk. The fat content for LB + ST + LP treatment has the highest value and is different for all other treatments. Fat content increases with the type of lactic acid bacteria that are added.

Fermented soymilk will increase titrable acidity from 0 in unfermented soymilk to 1.089. Changes in titrable acidity because carbohydrate was changed in to lactic acid by lactic acid bacteria. This also causes a decrease in the pH value during the fermentation of soy milk. The pH

value of soy milk dropped from 6.5 to 3.66 in fermented soymilk. The results of measurements of pH and titrable acidity values are shown in Table 2. The highest titrable acidity being LB + ST treatment (1.089%), followed by LB + ST + LP (1.035%), LP (0.741%), and unfermented (0). Instead the highest pH value was found in the unfermented treatment (6.5), followed by the treatment of LP (4,033), LB + ST + LP (3.75), and LB + ST (3,667). From the results of titrable acidity and pH values it can be concluded that the production of lactic acid by *L.plantarum* during fermented soymilk is not as much as the production of lactic acid by *L.bulgaricus* and *S.thermophilus* which is a yogurt starter. Fermented soymilk using a mixed culture of 3 strains of bacteria gives the results of pH and titrable acidity values that are not different from the pH value and the titrable acidity from fermentation using 2 strains bacteria

The pH value and the production of lactic acid are affected by the strains of lactic acid bacteria involved. Lactic acid production is influenced by the ability of lactic acid bacteria to use available carbon sources. According to [20] and [21], *S. thermophilus*, *L. acidophilus*, *L. cellobiosis* and *L. plantarum* can use the main carbohydrates in soybeans (sucrose) so that they show significant growth and produce lactic acid during fermentation soy milk. In contrast, *L. bulgaricus* cannot use carbohydrates in soybeans so that their growth is inhibited and the production of acid is small.

The pH and titrable acidity values are also influenced by single culture or mixed culture. The results of this study indicate that fermentation with a single culture of *L.plantarum* produces pH and titrable acidity of 4 and 0.741. Fermentation using mixed cultures results in lower pH values and higher total acidity. Fermented soymilk with a mixture of *Saccharomyces boulardii* and *L.plantarum* cultures produced a pH value of 5.87. Whereas fermented soymilk with a mixture of *Saccharomyces boulardii* and *L.bulgaricus* cultures gave a lower pH value of 5, 16 [17]. Compared with other studies by [18], fermented soymilk using 6 probiotics reached a pH value of 4.5 at 9.5 hours fermentation time for *L. rhamnosus* GG and 21 hours for fermentation by *L.acidophilus*. Research by [22] showed that fermented soymilk using mixed cultures of *S. infantarius* and *Weissella* sp.4 gave lower pH values than fermentation using single culture *Weissella* sp.4, but was higher than *S.infantarius* single culture fermentation.

During the fermentation of soymilk there is an increase in total lactic acid bacteria. Fermentation with LB + ST + LP mixed cultures produced the highest total lactic acid bacteria which was  $9,20833 \pm 0.582801$  log CFU/g. Fermentation with single culture LP produced the lowest total lactic acid bacteria that was  $8.3396767 \pm 0.158829$  log cfu/g.

The highest total lactic acid bacteria were obtained from treatment using mixed cultures. This shows that there is a symbiosis between the lactic acid bacteria used. The growth of *L.plantarum* bacteria as a single culture was lower than 1 log CFU/g compared to fermentation using mixed cultures. LB and ST mixed culture is a starter for yogurt so it does have a symbiosis in growth in cow's milk or soymilk

Several studies on the use of mixed cultures show that the bacteria *S.thermophilus* and *L.bulgaricus* are the dominant bacteria in fermentation of cow's milk and soy milk. Research by [23] showed that *S.thermophilus* had better growth in cow's milk and soy milk compared to 4 other probiotic strains. Research by [24] showed that *L. helveticus* and *B.longum* growth decreased in fermented soymilk using mixed cultures with *S.thermophilus*. The *L. helveticus* population decreased by 0.6 log CFU / ml, while the *B.longum* population declined more than 10-fold when compared between single culture fermentation and mixed culture. So it can be concluded that *S.thermophilus* is too dominant so that it inhibits the growth of other lactic acid bacteria. Fermentation using mixed cultures between *S.infantarius* 12 and *Weissella* sp. 4 gives better results compared to fermentation using a single culture [22].

Fermentation soy milk will increase aglycon isoflavones level, namely genistein dan daidzein. The result of measurements of genistein and daidzein level are shown in Table 3. The results showed that daidzein increased more than genistein during fermented soymilk. Genistein increased 82.8%, from 33, 16292  $\mu\text{g} / \text{g}$  to 193.7317  $\mu\text{g} / \text{g}$ . Daidzein increased by 93.56%, from 9,854  $\mu\text{g} / \text{g}$  to 153, 01  $\mu\text{g} / \text{g}$ .

Fermentation using *L.plantarum* both for single culture and mixed culture can increase aglycon isoflavones levels. The LB + ST treatment group had lower levels of genistein and daidzein when compared to the LP and LB + ST + LP groups. According to [9], the  $\beta$ -glucosidase enzyme from *L. plantarum* can completely hydrolyze isoflavone glycosidic bonds during 48 hours fermentation at 37°C. The  $\beta$ -glucosidase enzyme from lactic acid bacteria is an intracellular, extracellular, and membrane bound enzyme. Several studies have shown that lactic acid bacteria have high  $\beta$ -glucosidase activity. Research by [25] showed that *L. casei* (116.06 mU / mL) had the highest  $\beta$ -glucosidase activity. Research by [10] which showed that the lactic acid bacteria which had the highest activity was *L.rhamnosus*. While research by [26] shows that the lactic acid bacteria that have the highest  $\beta$ -glucosidase activity are *L.casei* and the lactic acid bacteria that has the lowest activity is *L. rhamnosus*. Several studies have shown *L.plantarum* to have a high  $\beta$ -glucosidase activity [9,24,27]. The enzyme activity of  $\beta$ -glucosidase from *L.plantarum* is 1.7 - 1.8 U / g 19. Research by [28] which showed that the lactic acid bacteria which had the highest activity was *B.subtilliss* HJ18-9.

Research by [27] gave the same result that the aglycon isoflavones which increased the most during fermented soymilk was daidzein. Research by [18] showed that the aglycon isoflavone compound which most increased during fermentation was genistein, which increased from 2.54  $\mu\text{g} / \text{g}$  to 26.63  $\mu\text{g} / \text{g}$ . After that followed by daidzein compound which increased from 3.33  $\mu\text{g} / \text{g}$  to 16,41  $\mu\text{g} / \text{g}$ . Research by [29] shows that fermentation soymilk by single culture of two strain of *Lactobacillus paracasei*, two strain of *Lactobacillus acidophilus*, and one strain of *Bifidobacterium* significantly increased bioactive isoflavone aglycones at the range of 62-96%.

Aglycon isoflavone levels (genistein and daidzein) produced from single culture fermentation using

*L.plantarum* were not significantly different from mixed culture groups of *L.plantarum* + *S.thermophilus* + *L.bulgaricus*. This shows the symbiosis between the three strains of lactic acid bacteria in converting glycosidic isoflavones to aglicon isoflavones. Fermented soymilk using a mixture of *S. infantarius* and *Weissella* sp.4 cultures in a ratio of 1: 3, 1: 5 and 1:10 resulted in high aglicon isoflavone levels [22]. Different results were shown by the research of [24]. The results showed that the bioconversion of isoflavones was bound to the mixed

fermentation culture of *S.thermophilus* and *L. helveticus* 50% lower when compared to single culture fermentation of *L. helveticus*. Research by [17] shows that fermented soymilk with a mixed culture of a mixture of *Saccharomyces boulardii* and *L.plantarum* produces daidzein and genistein levels of 3.71 mg /100 mL and 8.95 mg/mL. Whereas fermentation with a mixture of *Saccharomyces boulardii* and *L.bulgaricus* cultures resulted in lower daidzein and genistein levels of 3.33 mg / 100 mL and 8.09 mg/100 mL.

**Table 1. Proximate analysis of unfermented soymilk, fermented soymilk by single and mixed culture**

Sample	Moisture (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)
Unfermented	92.41 ± 2.04 <sup>a</sup>	1.36 ± 0.22 <sup>a</sup>	3.42 ± 0.59 <sup>a</sup>	2.35 ± 0.57 <sup>a</sup>	0.45 ± 0.02 <sup>a</sup>
LP	91.85 ± 0.82 <sup>a</sup>	1.37 ± 0.09 <sup>a</sup>	3.63 ± 0.24 <sup>b</sup>	2.67 ± 0.69 <sup>a</sup>	0.46 ± 0.02 <sup>a</sup>
LB+ST	91.73 ± 1.33 <sup>a</sup>	1.42 ± 0.14 <sup>a</sup>	4.11 ± 0.19 <sup>c</sup>	2.26 ± 0.68 <sup>a</sup>	0.46 ± 0.02 <sup>a</sup>
LB+ST+LP	91.81 ± 1.00 <sup>a</sup>	1.64 ± 0.11 <sup>b</sup>	3.87 ± 0.19 <sup>bc</sup>	2.2 ± 0.32 <sup>a</sup>	0.46 ± 0.03 <sup>a</sup>
P	0.250	0.015	0.015	0.520	0.901

Values are mean±SD from 6 replicates

The same letter in the same row are not significantly different (P < 0,05)

LP: Fermented using *L.plantarum*, LB+ST: Fermented using *L. bulgaricus* and *S. thermophilus*, LB+ST+LP : Fermented using *L. bulgaricus*, *S. thermophilus*, and *L.plantarum*.

**Table 2. Titratable Acid,pH value and population lactic acid bacteria of unfermented soymilk, fermented soymilk by single and mixed culture**

Sample	Titratable Acid (%)	pH value	Population (Log CFU/mL)
Unfermented	0 ± 0 <sup>a</sup>	6.5 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
LP	0.74 ± 0.16 <sup>b</sup>	4.03 ± 0.81 <sup>b</sup>	8.39 ± 0.15 <sup>b</sup>
LB+ST	1.09 ± 0.17 <sup>c</sup>	3.66 ± 0.10 <sup>c</sup>	9.19 ± 0.32 <sup>c</sup>
LB+ST+LP	1.03 ± 0.26 <sup>c</sup>	3.75 ± 0.23 <sup>c</sup>	9.20 ± 0.58 <sup>c</sup>
P	0.000	0.000	0.000

Values are mean±SD from 6 replicates

The same letter in the same row are not significantly different (P < 0,05).

LP: Fermented using *L.plantarum*, LB+ST: Fermented using *L. bulgaricus* and *S. thermophilus*, LB+ST+LP: Fermented using *L. bulgaricus*, *S. thermophilus*, and *L.plantarum*.

**Table 3. Genistein dan Daidzein level of unfermented soymilk, fermented soymilk by single and mixed culture**

Sample	Genistein (µg/g)	Daidzein (µg/g)
Unfermented	33.16 ± 4.62 <sup>a</sup>	9.85 ± 3.49 <sup>a</sup>
LP	183.47 ± 22.47 <sup>b</sup>	146.13 ± 14.86 <sup>b</sup>
LB+ST	174.52 ± 12.86 <sup>b</sup>	143.55 ± 22.44 <sup>b</sup>
LB+ST+LP	193.73 ± 16.36 <sup>b</sup>	153.01 ± 27.35 <sup>b</sup>
P	0.003	0.000

Values are mean±SD from 6 replicates

The same letter in the same row are not significantly different (P < 0,05)

LP: Fermented using *L.plantarum*, LB+ST: Fermented using *L. bulgaricus* and *S. thermophilus*, LB+ST+LP: Fermented using *L. bulgaricus*, *S. thermophilus*, and *L.plantarum*.

**Table 4. Factors Affecting Genistein and Daidzein Level**

Correlation	p-value	β
Titratable acid → genistein	0.000	-0.669
Total LAB → genistein	0.000	1.577
pH → genistein	0.623	-0.371
Titratable acid → daidzein	0.007	0.612
Total LAB → daidzein	0.528	0.218
pH → daidzein	0.735	0.886

**Table 5. Sensory analysis fermented soymilk**

Sample	Colour	Aroma	Taste	Texture
LP	4.08 ± 0.74 <sup>b</sup>	3.38 ± 0.75 <sup>a</sup>	3.27 ± 0.96 <sup>a</sup>	3.96 ± 0.87 <sup>b</sup>
LB+ST	3.04 ± 0.92 <sup>a</sup>	2.96 ± 0.72 <sup>a</sup>	3.08 ± 0.89 <sup>a</sup>	2.77 ± 1.03
LB+ST+LP	3.19 ± 0.85 <sup>b</sup>	3.08 ± 0.93 <sup>a</sup>	2.88 ± 0.91 <sup>a</sup>	2.88 ± 0.82 <sup>b</sup>
P	0.000	0.106	0.191	0.000

Values are mean±SD from 6 replicates

The same letter in the same row are not significantly different (P < 0,05)

LP: Fermented using *L.plantarum*, LB+ST: Fermented using *L. bulgaricus* and *S. thermophilus*, LB+ST+LP : Fermented using *L. bulgaricus*, *S. thermophilus*, and *L.plantarum*.

Bioconversion of isoflavones bound to aglycon isoflavones is influenced by total lactic acid bacteria and fermentation time. Some studies show that bioconversion is no more than 50% if the total bacteria is less than  $10^8$  CFU/mL and the fermentation time is less than 8 hours [24]. Research by [27] showed bioconversion of isoflavones bound to aglycon isoflavones of more than 50% under fermented soybean milk using *L.plantarum* with total bacterial finish reaching  $9.9 \pm 0.3$  log CFU/mL and 48 hours fermentation time. Research by [30] shows that fermented soymilk for 24 hours can hydrolyze bound isoflavones by more than 50%. However, different studies are shown by [22], fermented soymilk using *Weissela* sp.4 can hydrolyze 99% daidzin and genistin in soy milk during 6 hour fermentation.

The pH value also affects the activity of the  $\beta$ -glucosidase enzyme. According to research by [26], the most isoflavone bioconversion occurs when the pH drops from 6.7 - 4.55, while the isoflavones bioconversion only occurs by 6-14% when the pH drops from 4.15 - 3.85. Other studies show pH values ranging from 4.3 to 4.5 can cause the bioconversion of isoflavones by more than 50% [22,24,25,27]. The production of the  $\beta$ -glucosidase enzyme occurs during the logarithmic phase of lactic acid bacteria which is about the first 6 hours. Therefore, many bioconversions occur when pH decreases from 6.7 to 4.55 to 16.21. A pH value that is too low will also cause the inactive  $\beta$ -glucosidase enzyme because the  $\beta$ -glucosidase enzyme is sensitive to low pH.

Factors affecting aglycon isoflavones level were analyzed using regression. The regression results between variables are presented in Table 4. The table presents that aglycon isoflavone levels are influenced by titratable acid and total lactic acid bacteria. The relationship between titratable acid and aglycon isoflavones levels is negative, meaning that the higher the titratable acid, the free isoflavones levels are lower. The relationship between total lactic acid bacteria and free isoflavones is positive, meaning that the higher the total lactic acid bacteria, the higher the aglycon isoflavones levels.

The sensory analysis is a test to measure the panelists' impression of the good or bad of a product. The range of sensory analysis scales used in this study ranged from extreme good to extreme bad. The scale used is 1 to 5, with a value of 1 being very bad and a value of 5 being very good. The sensory analysis scale used is a general quality scale that is good or bad. Quality parameters measured in this study are color, aroma, taste, and texture.

The sensory analysis results showed that the LP treatment had the highest value for all parameters, namely color, texture, aroma and taste (Table 5). The sensory analysis for texture and color parameters showed that the LP treatment was different from the other two treatments. As for the taste parameters, the LP treatment has the highest value, followed by the LB + ST treatment, and the LB + ST + LP treatment. The LP treatment also has the highest value for the aroma parameter followed by LB + ST + LP, and LB + ST.

The result of all parameters showed that the LP treatment had the best sensory value compared to the other two treatments. If it is related to the pH value, the LP treatment which has a pH value above 4 so that it is more acceptable to consumers in terms of taste and aroma. The

reported optimum pH of fermented soymilk is 4.2 to 4.3. While the texture of the LP treatment which is still liquid is more acceptable when compared to the texture of the other treatment. Previous research has shown that a common problem associated with fermented soymilk is low acidity and flavor intensity [15].

## 4. Conclusion

Fermented soymilk with single culture and mixed culture will give different pH values, titratable acid, and total lactic acid bacteria. However genistein and daidzein levels did not differ between single culture and mixed culture treatments. Fermentation for 18 hours with mixed culture can increase the levels of aglycon isoflavones but produce pH values below 4. This affects consumer acceptance of the fermented soymilk produced. The optimum fermentation time needs to be further investigated to obtain fermented soymilk products which are high in aglycon isoflavones and preferred by consumers.

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