Enhancement of the lactic acid bacteria population

by Ignatius Srianta

Enhancement of the lactic acid bacteria population, chemical and organoleptic properties of soygurt with nutraceuticals from purple sweet potato (*Ipomoea batatas* L) paste

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Abstract. Soygurt is a type of probiotic beverage that is made from soy milk fermented by lactic acid bacteria (LAB). Purple sweet potato-PSP (*Ipomoea batatas* L) contains various nutraceutical compounds which may amplify the health benefits of soygurt once added at optimum level. The aim of this experiment was to evaluate the impact of PSP at various concentrations on LAB population, chemical and organoleptic properties of soygurt. The purple sweet potato paste (PSPP) was added at six different concentrations i.e. 0%, 3%, 6%, 9%, 12%, and 15% (w/v) of the total mixture. Statistical analysis with ANOVA at α of 5% showed that the addition of PSPP caused an increase in the total plate count of LAB cells (from 7.9900 to 9.7459 log cfu/g), pH (from 4.735 to 4.298), and total lactic acid (from 0.267 to 0.466%). Based on the organoleptic evaluation preferences, soygurt at 3% and 6% PSPP have been accepted by the panelists in terms of its taste preference at 5.35 (sufficient) and its mouthfeel at 5.88 (like). Notwithstanding, soygurt with 12% PSPP was the optimum concentration with respect to its color at 5.40 (sufficient). In conclusion, PSPP concentrations affect significantly the LAB population, chemical, and organoleptic properties of soygurt. **Keywords**: soygurt, soymilk, purple sweet potato paste

1 Introduction

In the midst of the COVID-19 pandemic, all aspects of life lead to the new lifestyle or '*new norm*', such as working online from home, keeping social distancing, using masks, washing hands consistently with soap etc.,[1]. Such an era where public awareness and understanding of healthy food and beverages are getting higher, that lead to selective action towards food products [2]. The preference of functional food is the best option because aside from obtaining selected nutrients, we could consume its active compounds indirectly [3]. One of these

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functional foods that is quite attractive is probiotic that has been proven to provide health benefits over the time. A typical example of probiotic products is yogurt [4].

Yogurt is a semi-solid fermented milk product which made with or without the addition of dairy products such as skimmed milk powder and whey concentrates. Lactic acid bacteria (LAB) are common bacteria that used in yogurt manufacture such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* [5]. In general, yogurt is made from mammalian milk such as cow's milk, but with the development of food technology and diversification, plant-based milk such as soymilk has been introduced as an alternative product, thus still edible by the consumers who unable to digest cow's milk protein (casein) or cannot consume cow's milk. Nonetheless, soymilk is infamous of its beany flavor. Such unpleasant odor is originated from the formation of volatile carbonyl compounds during the catalysis of unsaturated fatty acids by lipoxygenase enzyme [6]. Hence, it is necessary to fermented soymilk into soygurt.

Soygurt that made from pure soymilk has different nutritional components than conventional cow's milk in terms of its carbohydrates content, proteins and soluble solids. Soymilk contains oligosaccharides that have not been utilized perfectly in lactic acid fermentation. With reference to Mital and Steinkrus [7], carbohydrates in soy milk are raffinose (0.07%), stachyose (0.39%) and sucrose (0.48%), however, only sucrose that can be utilized completely by LAB for cells growth. Therefore, it is necessary to add other source of carbohydrates to produce soygurt that meets the standard of conventional yogurt [8].

Purple Sweet Potato (PSP) is a one of potential local food from Indonesia that is readily available and could be used as a source of carbohydrates in soygurt manufactured. PSP contains antioxidant, anti-cancer substances and dietary fiber that provide nutraceuticals benefit [9]. For instance, anthocyanins in PSP range from 14.68-210 mg/100 g and have potential as natural dyes [10]. Carbohydrates in PSP consist of starch, oligosaccharides (raffinose and stachyose), sucrose, glucose and fructose which could be used as nutrients source for LAB growth. Retnati et al [11] reported that addition of 10% purple sweet potato filtrate into yogurt resulted in the highest LAB cells after 8 hours fermentation (3.95×10^9), compared to white and orange sweet potato filtrate (1.54×10^9 and 3.17×10^9). Such an increase in number of LAB cells growth would affect significantly the chemical and organoleptic properties of soygurt. Hence, the aim of this experiment was to evaluate the impact of PSP at various concentrations on LAB population, chemical and organoleptic properties of soygurt.

2 Materials and Methods

2.1 Materials

These are the employed materials: Soymilk "CIP", commercial *freeze-dried yogurt starter* "Yogourmet" that consists of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*, sugar "Gulaku", gelatin powder "Gelnex", and purple sweet potato (*Ipomoea batatas* L) "Gluduk Putra Mas".

2.2 Preparation of PSPP

Purple sweet potato was peeled to separate the flesh from its peel, rotten or damaged part. Then it was washed with clean water and cut into several pieces. Purple sweet potato was steamed at 98°C±2°C for 10 minutes and mashed with sterile mortar and pestle. Mashing process carried out under aseptic condition inside enkas. Subsequently, the PSPP was

weighed based on each treatment, then kept inside a sterile container and stored in frozen temperature -15°C±1°C for later use.

2.3 Preparation of soygurt with different concentrations of PSPP

Soymilk was heated until the temperature reached 50°C then 5% of sugar (w/v) was added gradually. The soymilk was then pasteurized for 15 minutes at 80-90°C with constant stirring to avoid the sugar clumped or burnt at the bottom of the pan. Consequently, all process carried out in the laminar air flow to minimize contamination. 0.5% gelatin powder was added at temperature 70°C±1°C, and it was stirred evenly. The mixture was then divided into six parts as follows: 0%, 3%, 6%, 9%, 12%, and 15% PSPP (w/v), and cooled until the temperature reached 42°C±1°C. Thereafter, freeze dried yogurt starter (0.5%) was inoculated, stirred and incubated in 43°C±2°C for 5 hours. Manufactured soygurt was stored in 4°C±1°C for 17 hours.

2.4 Analysis of Soygurt

The LAB population, pH and total acidity of manufactured soygurt were measured in triplicates to avoid error during the 17 hours storage at 4°C±1°C.

2.5 Microbiological Analysis of Soygurt

The LAB population in the manufactured soygurt determined by the total plate count method that refers to Fardiaz [12]. It was determined by making a serial dilution to 10 of one gram of each sample of soygurt. Thereafter, 1 mL of each sample of soygurt was placed on MRS agar plates and incubated at 37°C for 48 hours. Colony counts were converted to log CFU/g.

2.6 pH of Soygurt

The pH of soygurt was measured with an electronic digital-type pH meter "SI Analytics Lab-885". pH value was determined based on the methods described by Muchtadi et al [13]. Firstly, the electrode of pH meter was adjusted and calibrated at room temperature using buffer solutions of pH 4 and 7. Then, the electrode of pH meter was immersed in a sterile cup containing 25 mL of soygurt and readings were recordly directly.

2.7 Acidity of Soygurt

Acidity of soygurt was calculated based on the method described by Widagdha and Nisa [14]. The acidity value estimated as the amount of 0.1 N NaOH solution (mL) used to neutralize 10 g of soygurt samples. Phenolphtalein was used as an indicator to achieve a pink color. Acidity was expressed as percentage (%) lactic acid and was computed using the following formula:

$$\% \text{ Lactic Acid} = \frac{Volume\ of\ NaOH\ used\ (mL)\ x\ N\ x\ BM\ lactic\ acid}{Volume\ sample\ (mL)\ x\ 1000} \ x\ 100\% \tag{1}$$

2.8 Sensory Evaluation

The preference test of the panelists using the Hedonic Scale Scoring method for color, taste and mouthfeel. The scale used is a sensory scale with value conversion as follows 1 = very dislike; 2 = dislike; 3 = imperfect; 4 = neutral; 5 = sufficient; 6 = like; 7 = really like. The

organoleptic test performed by 40 untrained panelists. The test were carried out on a RAL basis as much as 25 mL of sample per concentration. Each of the panelists tested each sample and filled in a questionnaire that has been provided.

## 2.9 Statistical Analysis

The data were analyzed by "IBM SPSS statistics 19". Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) with α = 5% were used to differentiate between the mean values.

3 Results and Discussion

3.1 Lactic Acid Bacteria Population

Figure one (1) demonstrated the changes of lactic acid bacteria (LAB) population in all soygurt samples. Total plate count in all soygurt samples increased throughout the addition of PSPP. The higher PSPP concentration the greater total plate count in the soygurt samples. The LAB population of soygurt ranged from 7,9900-9.7459 log cfu/g. Based on the microbial population requirements of SNI 2981-2009 [15], the minimum amount of living probiotic cells are 7 log cfu/g, so all the samples had already met the criteria of fermented milk/yogurt.
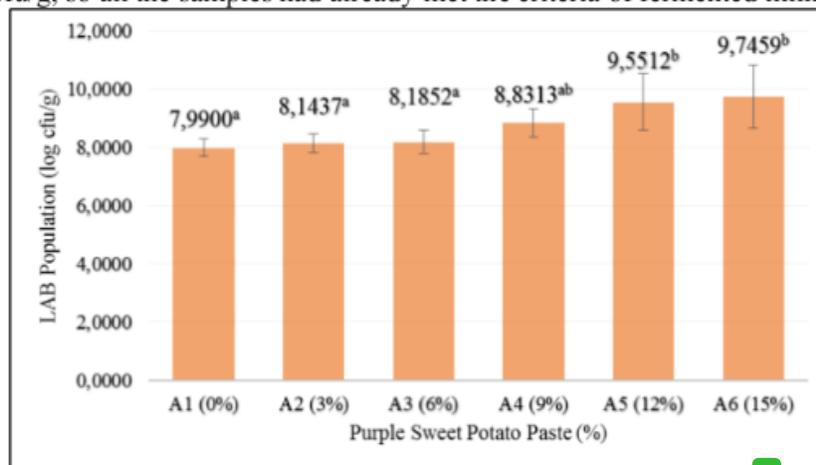
Fig. 1. The total LAB of soygurt at different concentrations of PSPP. Means with the same superscript letter are not significantly different (P >0.05) based on DMRT.

This study showed that concentration of PSPP in samples A1, A2, A3, and A4 did not affect significantly (p>0.05) LAB population in soygurt. These findings were similar to those reported by Retnati et al [11], who demonstrated that yogurt with the addition of sweet potato filtrate had higher amount of LAB population compared to those of the control (i.e. without addition of sweet potato filtrate), nonetheless with insignificant difference. This occurrence could happen due to lack of essential nutrients to support the LAB growth, hence the formation of mass cells is limited. Furthermore, A1 had the lowest LAB population that resulted by the soymilk as the only source of nutrients. LAB could utilize the nitrogen and some of the oligosaccharides (e.g., raffinose, stachyose, and sucrose) in the soymilk, however a very limited amount to growth [16]. Moreover, the results pointed out that A5 and A6 were significantly (p<0.05) higher than A1, A2, and A3, which were not significant (p>0.05) compared to A4. The significant increase of LAB population was due to the utilization of carbohydrates (starch, sucrose, maltose, glucose, and fructose), proteins, fibers, minerals, and

vitamins (B$_1$, B$_2$, C, and E) in the PSPP by LAB as source of nutrients for the cells growth [11].

3.2 Chemical Properties

Fig. 2. highlights that the addition of PSPP had a significant effect on the mean value of pH of soygurt sample. The statistical analysis implied that A6 presented the maximum decrease in pH values and was significantly ($p<0.05$) less than A1, A2, A3, and A4, which were not significant ($p>0.05$) compared to A5. The mean values of acidity (pH) ranged from 4.298-4.735. PSP contains some potential macronutrients such as carbohydrates (e.g. starch, sucrose, maltose, and glucose) that is used as substrates for the growth and maintenance of cells. These saccharides able to produce lactic acid. The greater the amout of lactic acid formed during fermentation, the lower pH of the soygurt [17, 8]. These results are in accordance with Nizori et al [18], who reported that synbiotic soygurt that inoculated with *L. bulgaricus, S. thermophiles*, and *L. acidophilus* could actively cooperate to produce lactic acid and other organic acids that lead to the increase of soygurt acidity.
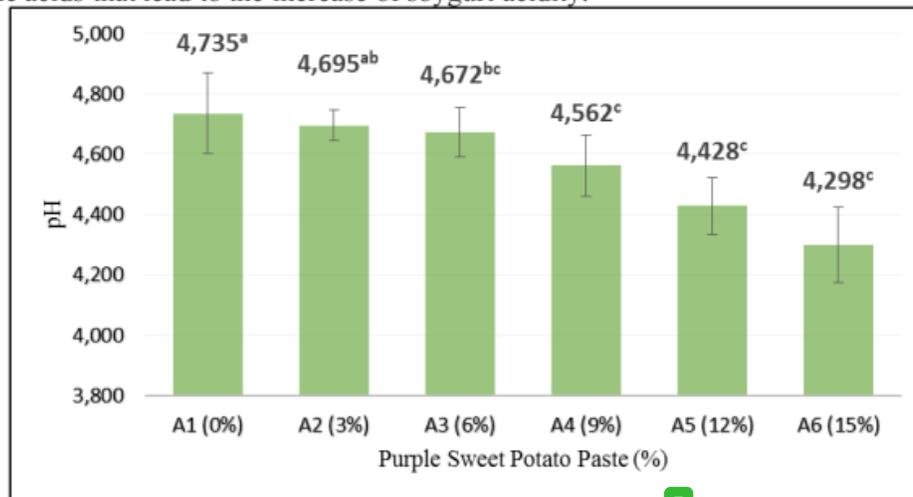

Fig. 2. The pH of soygurt at different concentration of PSPP. Means with the same superscript letter are not significantly different ($P>0.05$) based on DMRT.

Despite the increase in acidity of manufactured soygurt displayed by A5 and A6, the statistical analysis revealed the absence of significant differences. These results are in agreement with Sintasari et al [19], who declared that during fermentation process, there is a maximum amount of substrate that could be utilized as carbon source for energy production, thus not all components in PSPP metabolized to produce lactic acid or the other organic acids.

LAB used in this study were homofermentative bacteria that produce 85% lactic acid and acetic acid, butyric acid, pyruvic acid and formic acid in small quantities from glucose conversion [20, 21]. Soygurt acidity was affected significantly ($p<0.05$) due to different concentrations of PSPP (**Fig. 3.**). The mean values of total acidity ranged from 0.2670% to 0.4660%. The higher the concentration of PSPP, the higher lactic acid production and subsequently the lower pH value. Value of total acidity was inversely proportional to the pH value.
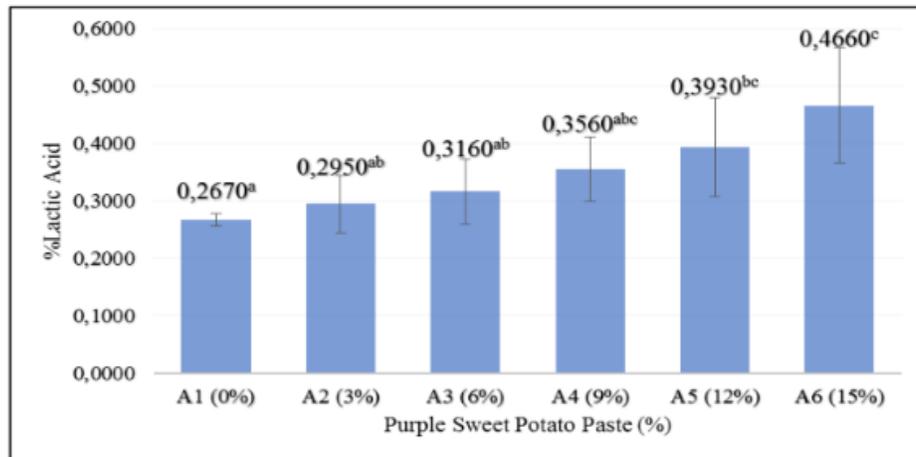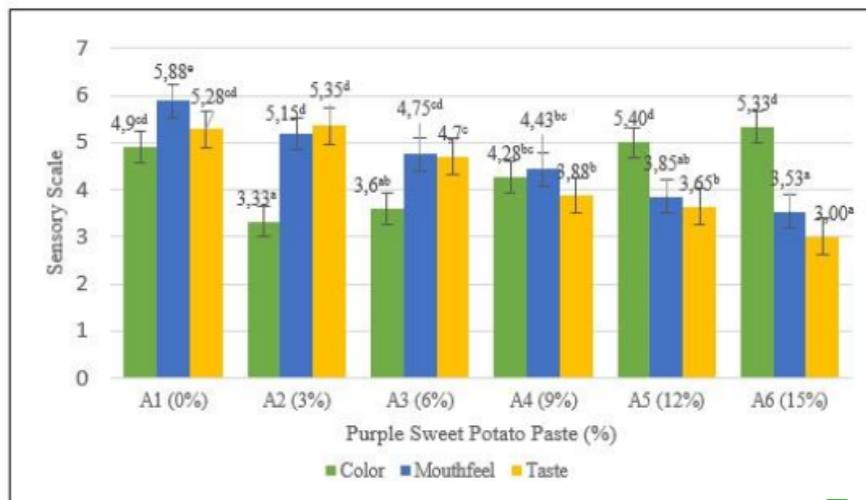
Fig. 3. The % lactic acid of soygurt at different concentrations of PSPP. Means with the same superscript letter are not significantly different (P >0.05) based on DMRT.

3.3 Sensory Evaluation

3.3.1 Color

Based on ANOVA at $\alpha = 5\%$, different concentrations of PSPP had a significant effect on color preference in soygurt. Increasing the concentration of PSPP caused the color more intense. Opportunately, Soygurt with a deeper purple color was considered more attractive by the panelists.

Fig. 4. The organoleptic value of soygurt at different concentrations of PSPP. Means with the same superscript letter are not significantly different (P >0.05) based on DMRT.

The purple color was formed because PSP contains an antiocyanin type cyanidin 3-rhamnoside [22]. The 3%; 6%; and 9% concentrations had lower preference values than the 0% concentration. Panelists preferred 0% concentration as at this concentration, the purple color is less visible and causes a dull color-like effect.

3.3.2 Taste preference

Based on ANOVA at α = 5%, different concentrations of PSPP had a significant effect on taste preference in soygurt. **Fig. 4** depicted that there was a decrease as the concentration of PSPP increases in organoleptic testing of taste. The addition of PSPP could be an additional source of energy for the metabolic process of LAB that led to increasing production of lactic acid, lower the pH, then resulted in sour taste [17]. At concentrations of 0% and 3%, soygurt still has a sour taste that resembles the sour taste of commercial yogurt.

3.3.3 Mouthfeel

Mouthfeel is the rheological property of food during the first step in the mouth. Based on ANOVA at α = 5%, different concentrations of PSPP had a significant effect on mouthfeel preference in soygurt. As demonstrated in **Fig. 4**, there is a decrease in mouthfeel preference for soygurt as the concentration of PSPP increases. This is due to the carbohydrate and dietary fiber content of PSPP. According to Nisviaty [23], PSP has a high dietary fiber content of 4.72% per 100 grams. In addition to high dietary fiber, PSP also has a high starch content of 22.64% [24]. Soygurt with the addition of PSPP could increase the dissolved solids thus the texture of the manufactured soygurt feels more fibrous when entered the mouth and causes a rougher taste.

4 Conclusion

The influence of PSPP concentrations on the LAB population, chemical and organoleptic of soygurt was successfully evaluated. PSPP increased LAB population, acidity scale (pH) and the production of lactic acid significantly. In addition, total lactic acid bacteria ranged from 7.9900 to 9.7459 log CFU/g, pH ranged from 4.298 to 4.735 and total lactic acid ranged from 0.267% to 0.466%. Based on the panelists score on sensory evaluation, the average color preference ranged from 3.33 to 5.40 (imperfect to sufficient), taste preference ranged from 3.00 to 5.35 (neutral to sufficient), and mouthfeel preferences ranged from between 3.53 to 5.88 (imperfect to like). These reported findings will be useful to manufacturers producing enhanced quality soygurt.

5 Acknowledgements

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