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Soy-yamgurt probiotic drink as a natural potential of antioxidant

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ABSTRACT. Yogurt is a popular healthy food, consumed by many people. Probiotic are used for better growth and survival of probiotic bacteria as well as to improve organoleptic, rheological and technological properties of soy-yamgurt probiotic. The aims of this study were to determine physicochemical changes and survival of probiotic bacteria due to the effect of ratio of yam-bean with soy-bean extract on the quality of soy-yamgurt probiotic drink. The quality examined were total bacteria, antioxidant activity, lactic acid content, and acceptability including color, texture, flavor, and overall acceptance. This research had been performed using Completely Randomized Design two factorials, consist of five levels i.e.: ratio of yam-bean with soy-bean extract (100:0; 75:25; 50:50; 25:75; 0:100) and the fermentation time (4,6 and 8 hours), the process was repeated three times. The results showed that ratio of yam-bean with soy-bean extract of 50:50 and fermentation for 6 hours incubation produced the best quality. Acceptable probiotic drink containing 1.44 x 10^9 CFU/mL lactic acid bacteria, IC_{50} of soy-yamgurt in the attenuation of free radical DPPH ranged from 58.718-18.112 mg/L in 5 minutes incubation and 39.7204-11.9925 mg/L in 60 minutes, and 0.48% lactic acid. This appearance of soy-yamgurt had yellow greenish color, desired texture and flavor, and received the highest score of overall acceptance.

1. Introduction

Among all the fermented dairy products, yogurt is one of the most popular and had more acceptability worldwide. This product is produced through lactic acid fermentation by *Lactobacillus delbruekii* subsp. *bulgaricus* and *Streptococcus salivarus* subsp. *thermophillus*. Probiotics have introduced as microorganisms which in sufficient amounts had health benefits for the host [2,4]. The most important health benefit of probiotics include reducing serum cholesterol level [21] , improving nutritional value, improving calcium absorption, proteins and vitamin synthesis, improving lactose digestion [4], promoting immune system function [1], preventing different type of cancer especially colon, preventing growth and activity of pathogenic microbes [20] and synthesizing bacteriocines [7].

Soy-yamgurt was a product of fermented milk produced from the activity of lactic acid bacteria and contain microorganisms alive, active and abundant (>10^7 CFU/mL). Raw material of soy-yamgurt not only from fresh milk, but its can also come from plant-based product diversification that mixed with skim milk powder as a source of lactose and lactic acid bacteria growth media. One of them is the raw material from soy-bean and yam-bean (*Pachyrhizus erosus L.*) extract.

It has been proven that the yam-bean had many benefits and a strong antioxidant activity, it also contains water-soluble polysaccharides (WSP) which proved to decrease diabetes mellitus [12]. Probiotic soy-yamgurt drink (a blend of soybean and yam-bean extract) turned out to have the high antioxidant activity and content of nutrients that are easy to digest due to the fermentation process.

Soy-yamgurt relatively more durable than fresh milk or milk powder dissolved in water or yam-bean extract, but its storage should be cold for a few days.

To enhance durability, increasing flavor and marketing development needed a further treatment. The treatment could also be expected to maintain or just reduce the nutritional value decline as little as possible, physico-chemical and organoleptic value. To create a more varian in its aroma and taste as well as increasing nutrition value, have been soy-yamgurt form. Among others: total bacteria 10^7 CFU/mL, total lactic acid 0.5-2% and minimum total solid 12%, good appearance, taste, and overall acceptance [14].

2. Material and Methods

2.1 Materials

Milk powder contains 1.0% fat was purchased from Supermarket in Medan, Indonesia. Microbial strains derived from plain yogurt commercial contaning *L. bulgaricus*, *S. thermophillus* and *L. acidophilus.* Yam bean *Pachyrhizus erosus* L. (PeL)) were identified as varieties "elephant" and collected from Binjai city, the province of North Sumatera, Indonesia.

Material analysis i.e.: NaOH 0, 1N, H_2SO_4 p.a, 96% alcohol, absolute alcohol, n-hexan, K_2SO_4, $CuSO_4$, phenolptalein, DPPH (2.2-3-diphenyl pikril hidrazil), aquadest, 0,9% physiological NaCl solution and MRS broth.

2.2 Primary culture preparation

To prepare the primary culture , 250 mL of crude milk heated 80-85^{o}C for 20 min, it is transferred to 500 mL erlenmeyer flasks, then culture solid (plain yogurt commercial) containing yogurt starter added to Erlenmeyer flasks (3%). It is incubated at 40-42^{o}C for 6 hours. The process was repeated 3 times, to form a solid structure and used for better growth and survival of probiotic bacteria. At the end they were refrigerated.

2.3 Sample preparation.

The making of yam-bean extract from yam-bean tubers (5 months harvesting time) was selected, trimmed, and shelled. Yam-bean were sliced and washed, then blanched for 7-10 minutes, blended and added water (yam-bean: water = 1:1), filtered.

The soybeans used were first sorted, then soaked in a solution of $NaHCO_3$ (baking soda) of 0.5% for 30 minutes and boiled for 30 minutes. The soybean shell was separated by squeezing and washed with water until the shell easy to separate. Soybean was milled with hot water as much as 6 parts. Soybeans was filtered by filter cloth that has been blanched and soybean extract was heated to boiling. After boiling, it is left for 20 minutes at 80^{o}C [5].

2.4 Symbiotic yogurt production

The materials used to make soy-yamgurt were 250 mL sterile containers containing the pasteurized of soybean extract and yam-bean extract (80^{o}C for 20 minutes), 10% dried skim milk (1% fat) were inoculated simultaneously with 3% of the starter (*L. bulgaricus, S. thermophillus,* and *L. acidophillus*) derived from the commercial culture, 1% sugar, and 0,6% gum Arabic and then incubated at 40-42^{o}C for 4, 6 and 8 hours.

2.5 Observation

The observations were analyzed by total lactic acid [10]), determination of water content [18], sensory evaluation [15], activity antioxidant by DPPH method [8], determination of LAB colonies [3]. Soy-yamgurt were evaluated using 4 score Hedonic test. The samples were evaluated by 15 panelists regarding the organoleptic attributes including, color, flavor, taste and texture.

2.6 Microbial test

Microbial test consisted of sample culture in MRS broth using pour plate method according to the standard. In order to provide this, proper dilution of the samples were made in sterile ringer solution and the plates were incubated at 37^{o}C following the culture preparation. Colony counts were measured following 72h incubation period [3].

2.7 Test of antioxidant activity by DPPH method

The antioxidant activity was determined by the free radical method of DPPH [16]. 0.004% DPPH solution was prepared by dissolving 100% of the DPPH crystals in 100 mL ethanol p.a. A total of 200 µL of ethanol (pro analyzed) was transferred into the cuvette and then added 3 mL DPPH solution, stir well and immediately made visible light spectra (400-600 nm). For test of sample, 200 µL sample was transferred into the cuvette, then added 3 mL DPPH solution, subsequently made visible light spectra (400-600 nm). At the 5th minute, read absorbance at 497-517-537 nm and done in 60th minute. The calculation of DPPH absorbance and solution sampling determined using Equation 1, whereas non antiradical activity determined using equation 2.

The Absorbance value of DPPH and sample solution = A517 - ((A497+A537)/2) ……..(1)

The % of DPPH Attenuation = (1-(A value of test sample/A value of the DPPH)) x 100% …..(2).

3. Results and Discussion

3.1 Probiotic bacterium counts (PBC)

Figure 1 shows the higher of yam-bean extract of the sample had the higher PBC, it means sample solution containing water soluble polysaccharide (WSP) from yam-bean [, there was significantly different between the sample that contains soy-bean extract. The addition of various amount of yambean and soybean extract of the soy-yamghurt probiotic drink gave significantly different effect to the total bacteria ($p<0.01$). Figure 1 shows that the higher soybean extract, the total bacteria of soy-yamgurt were declined, but it was still in a standardized Standard National Indonesia.

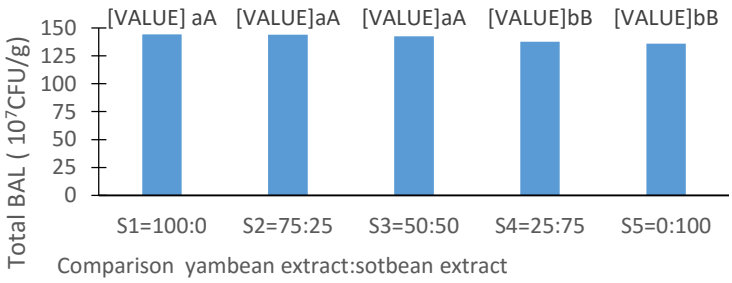


Figure 1. Total plate count of lactic acid bacteria in each treatment.

The total of bacteria on soy-yamgurt products is *L. bulgaricus*, *S. thermophillus* and *L. acidophilus* without any other microbial impurities, it can be seen from the growth of bacterial colonies in MRSA medium. The high number of total LAB soy-yamgurt is caused by the relatively higher sugar content of yam-bean extract, so it is suspected that all the glucose is fermented as a source of energy to form lactic acid. This indicates that the presence of FOS (fructo-olygosaccharide) deposits in yam bean and soy bean extract in the form of GOS (galactooligosaccharide) were not entirely used by microbes for growth. In this research using a banana yogurt products for the growth of *Lactobacillus acidophillus* CCRC 10695, pointed out that the source of FOS on bananas did not change significantly [6]

The starter bacteria growth is influenced by various factors, among them are nutrients that are closely related to the composition of the raw materials, such as lactose for growth and amino acids, vitamins and other supporting factors such as environmental conditions (pH, oxygen, and water activity), and the type of bacteria [13]. The addition of skim milk powder on this research serves as a

source of lactose and protein for the growth and activity of the starter bacteria, because both soy-bean and yam-bean extract did not contain lactose or casein. This is supported by the opinion of [13] which states that the availability of the nutrients that are appropriate to the needs of the bacteria produce a high increasing number of bacterial cells. At the beginning of fermentation, the pH of the initial raw material is relatively high around 5.6-6.8 which is.

At that point, the *S. thermophilus* grew earlier and caused a decrease in pH to 5.0-5.5, then the *L. bulgaricus* will multiply and changed the lactose into lactic acid and caused the pH decreased to 4.4-3.8 [9]. When *L. bulgaricus* began to grow, it will release amino acids, such as valin, histidin and glycine that are needed for the growth of *S. thermophilus*. In contrast, S. *thermophilus* will help lower the pH (increasing levels of acid), synthesis the formic acid and folic acid that stimulate the growth of *L. bulgaricus* [9]. The *L. bulgaricus* growth at the beginning of fermentation was slow, but the growing activity of proteolytic *L. bulgaricus* released a number of peptides and amino acids that can stimulate the growth of *L. acidophilus*. In mutual, *L. acidophilus* produced folic acid that can stimulate the growth of *L. bulgaricus* [17].

3.2 Total dry solids

Total dry solids of soy-yamgurt probiotic drink have a very important role, beside as a source of nutrition for the activity and bacterial starter culture developments, as well as to form a texture and flavor components in the produced fermented product. Total dry solids is influenced by the composition of the raw material used. Skim milk powder has a role as the starter bacteria growth media, because the powdered milk contains a high dry ingredients consisting of relatively high protein, fat and lactose as the main energy source to speed up the bacteria starter activities and to form lactic acid and flavor components. The bacteria are able to use the nutrients in the ingredients to form secondary metabolites and a mass of cells, so the total dry solids has increased with the increasing amount of soy-bean extract and the increasing of fermentation temperature [16], it is presented in Figure 2.

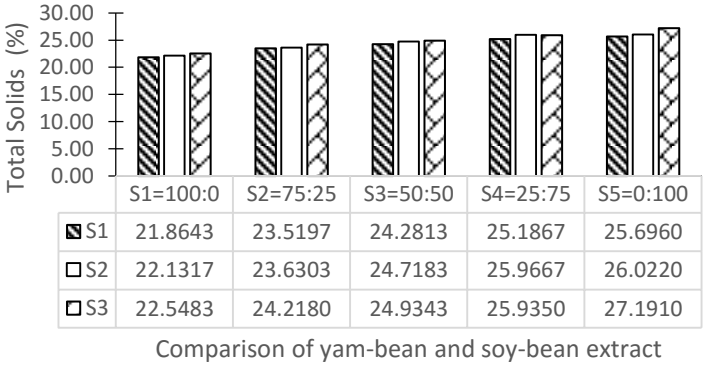


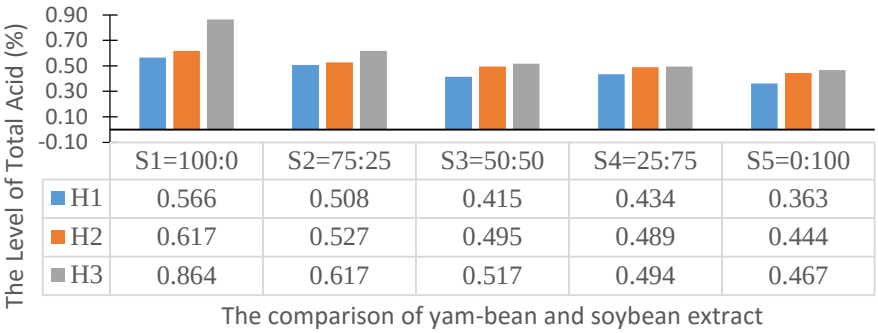
Figure 2. The chart of average total solids in each treatment.

3.3 Lactic acid levels

The lactic acid content are obtained by titration which was assumed as the levels of acid dominant formed during fermentation. The addition of yam-bean extract was significantly effect from 0.682 to 0.424%, the higher the amount of yam-bean extract the higher lactic acid value. It means that all the treatments of the addition of soy-bean and yam-bean extract produced beverage with the lactic acid levels were 0.5-2.0% [13], except for the addition of 100% soy-bean extract, to be more clear it can be seen in Figure 3.

Lactic acid is the main metabolite (85%) that are produced from lactose by the LAB starter. The process of lactose into lactic acid are influenced by several factors i.e.: the number and type of starter,

starter condition, temperature, incubation time and nutritional content of lactose in milk [13]. Soy-yamgurt had a specific sour taste derived from lactic acid bacteria activity. Lactose as a source of energy utilized by the bacteria starter to support its growth through the glycolysis; lactose is hydrolyzed by the β-galactosidase into glucose and galactose.



	S1=100:0	S2=75:25	S3=50:50	S4=25:75	S5=0:100
H1	0.566	0.508	0.415	0.434	0.363
H2	0.617	0.527	0.495	0.489	0.444
H3	0.864	0.617	0.517	0.494	0.467

Figure 3. The chart of the average total acid in each treatment.

Furthermore, glucose is converted into pyruvate and then pyruvic acid is converted to lactate acid by the lactate dehydrogenase enzyme [19]. The overhaul process of lactose into lactic acid is influenced by several factors were the number and type of starter, starter conditions, temperature, incubation time and nutritional content of lactose in milk, the added initial raw material [13]. In the fermentation process, the growth of *S. thermophillus* is faster than *L. bulgaricus* and *L. acidophilus*. The temperature and incubation time effected the levels of lactic acid, as it caused the difference in the growth and composition of microbes contained in the starter, so it will produce lactic acid which varies on the final fermented product [9]

3.4 Influence of the substrate variation and fermentation time on the antioxidant activity

The antioxidant activity is calculated based on the DPPH free radical attenuation by soy-yamgurt antioxidant components. The presence of antioxidant activity in the raw materials is expected to add functional value on the soy-yamgurt, beside the probiotic bacteria content. The antioxidant activity of soy-yamgurt can be seen in Figure 4 and 5.

IC_{50} value is the concentration of sample that gives 50% DPPH attenuation [17]. The result showed that the IC_{50} value were obtained on the 5^{th} minutes or 60^{th} minutes, where the value ranged between middle to low, except in the treatment of the S1H1 and S2H1 on the 5^{th} minutes, the value of IC_{50} is still above 100 mg/L. According to [8] that the compound had to be a powerful antioxidant activity if the IC_{50} value is less than 50 ppm. This indicates that soy-yamgurt has a powerful to strong activity to reduce free radicals.

3.5 The Characteristics of Soy-yamgurt Sensory Evaluation Quality of Substrate Variation with Fermentation Time.

The characteristics of soy-yamgurt sensory evaluation were including the hedonic values of color, flavor, taste and texture. The quality of sensory evaluation is an important parameter associated with the panelist acceptance of a product. For more details can be seen in Tables 1 and 2. The hedonic value of soy-yamgurt on taste gave highly significant interactions ($p<0.01$). In general the acceptance of panelists on taste and texture qualities tended to increase in longer fermentation time, but all the statistical results showed that 6 hours fermentation gave the best results. It is showed that the substrate variation with higher soy-bean extract, the consistency and firmness of the soy-yamgurt tended to be more viscous.

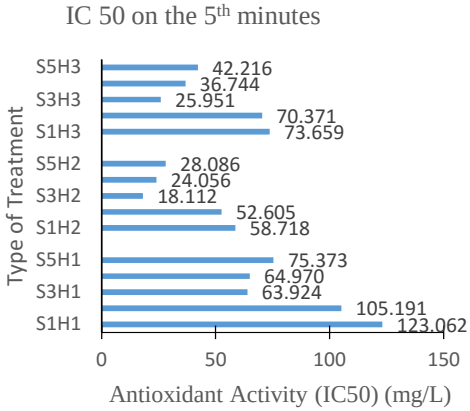


Figure 4. IC 50 antioxidant activity in various treatment on the 5th minute.

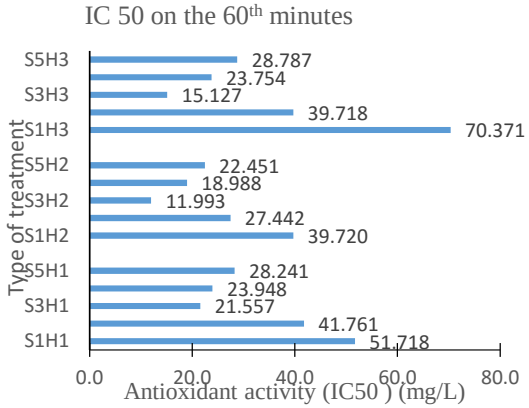


Figure 5. IC 50 antioxidant activity in various treatment on the 60th minute.

The increasing of soy-bean extract, tend to increase the organoleptic value, more acceptable taste. The best quality of sensory was S4 (yam-bean extract with soy-bean extract = 25:75).

Table 1. The effect of the substrate variation for the sensory evaluation quality characteristic of soy-yamgurt.

The Compari-son of Yam-bean extract with soy-bean extract	The hedonic value (numeric)			
	Color	Flavor	Taste	Texture
S1=100:0	3,682bB	3,061cC	2,991cC	2,782dC
S2=75:25	3,790aA	3,417aA	3,373bB	3,447bcB
S3=50:50	3,380dD	3,101cC	3,399bB	3,581aA
S4=25:75	3,600cC	3,282bB	3,380bB	3,383cB
S5=0:100	3,412dD	3,449aA	3,561aA	3,474bB

Description: the data were composed of 3 replication, the value that are followed by different small letters in one column showed significant ($p < 0.05$) and highly significant effect ($p < 0.01$) (Capital letters).

Table 2. The effect of fermentation time on the sensory quality characteristic of soy-yamgurt

Fermentation time	Hedonic value (numeric)			
	Color	Flavor	Taste	Texture
H1= 4 hours	3,485aA	3,383bB	3,432bB	3,289cB
H2= 6 hours	3,521aA	3,449aA	3,711aA	3,453aA
H3= 8 hours	3,340bB	3,361bB	3,317cC	3,387bA

Description: The data were composed of 3 replication, the value that are followed by different small letters in one column showed significant ($p < 0.05$) and highly significant effect ($p < 0.01$) (capital letters)

4. Conclusions and Suggestions

The soy-yamgurt from yam-bean and soy-bean extract was analyzed in the quality characteristic including total solids, total acid titration, organoleptic test and antioxidant activity with the DPPH free radical attenuation method.

It can be concluded that the using of yam-bean and soy-bean had resulted in improving sensory and rheological attributes of soy-yamgurt. The best treatment in this study was the S3H2 (the comparison of yam-bean and soy-bean extract 50:50) and 6 hours fermentation time.

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