The Effect Of Adding Butterfly Pea Flower Extract (Clitoria ternatea L.) and the Ratio of Starter Lactobacillus bulgaricus And Streptococcus thermophilus onYoghurt Quality

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Abstract

This research was conducted to determine the effect of adding the extract of butterfly pea flower (Clitoria ternatea L.) and the ratio of starter Lactobacillus bulgaricus and Streptococcus thermophilus on the quality of yogurt. This study used a factorial Completely Randomized Design with two factors, i.e the addition of butterfly pea extract (I = 4% ; 8%; 12%) and the ratio of starter Lactobacillus bulgaricus and Streptococcus thermophilus (A = 1:1 ; 1:2; 2:1). The results obtained showed that the addition of butterfly pea flower extract had a highly significant effect on the pH value, total acid, antioxidant activity, total lactic acid bacteria, viscosity, total soluble solid , color value, color hedonic and general acceptance hedonic. Ratio of starter Lactobacillus bulgaricus and Streptococcus thermophilus had a highly significant effect on the pH value and total acid, had a significant effect on antioxidant activity,viscosity and total soluble solid. The interaction between the two factors had a significant effect on the total acid value of yogurt. Yoghurt with the best quality was addition of butterfly pea extract 12% (I_3) and the ratio of starter Lactobacillus bulgaricus and Streptococcus thermophilu 2:1 (A_3). It was based on the parameters of antioxidant, total lactic acid bacteria, color value, and organoleptic.

Keywords : Butterfly Pea Flower, Lactobacillus bulgaricus, Streptococcus thermophilus, and Yoghurt

1. Introduction

Yogurt is processed milk fermented by bacteria. Yogurt product fermentation uses lactic acid bacteria. The minimum standard for the number of colonies in yogurt is 10^7 CFU/mL or 7 log CFU/mL [1]. The activity of LAB (Lactic Acid Bacteria) causes the milk to taste sour due to lactic acid bacteria fermenting lactose in milk [2]. Yogurt has the benefit of maintaining stomach health and preventing digestive cancer because the probiotic content can balance the microflora of the digestive tract [3]. Yogurt can also be consumed by lactose intolerant sufferers, fights the growth of pathogenic bacteria, and can stimulate the nervous system, especially the digestive tract [2]. The working principle of the bacteria found in yogurt is to create a balance in the intestinal microflora because it produces lactic acid. The acidity of the fermentation can slow down the disease bacteria that cannot stand the acidic environment. The results of the fermentation form organic acids to produce a yogurt flavor [4].

Butterfly pea flower (Clitoria ternatea L.) is a plant from Ternate, Maluku. The main content of butterfly pea flower as a coloring agent is anthocyanin pigment. Anthocyanins are polar and stable in acidic conditions, the solvents for anthocyanins are distilled water and tartaric acid [5]. Butterfly pea flowers contain chemical compounds that are useful for antioxidants, antidiabetics, immunomodulators, anti-obesity, anti-inflammatory, anti-microorganism, and anti-cancer [6]. The main content of the butterfly pea flower is flavonoids with a concentration of 20.07 ± 0.55 (mmol/mg flower). Flavonoids are the main group of polyphenolic chemical compounds and are secondary metabolites in plants (Saleem, et al., 2017). Flavonoids give color, aroma, taste to fruits, flowers, and seeds in plants [7]. Flavonoids act as natural antioxidants in fruits, cereals and vegetables [8]. Anthocyanin is a flavonoid pigment that is an antioxidant. The color intensity and stability of anthocyanins depend on factors such as pH, light quality, temperature, metal ions, oxygen, enzymes, oxygen, ascorbic acid, and sugar [9].

Antioxidants from butterfly pea flowers combined with yogurt can produce various benefits. LAB has antioxidant activity because it can produce hydroxyl radical absorbers in the form of metabolites produced by bacteria. Milk fermented by lactic acid bacteria has higher free radical scavenging activity than milk fermented by single bacteria [10]. Yogurt fermentation by

LAB produces a soft and semi-solid texture, and produces a sour taste (Rahman, et al., 2019). The use of a starter with different percentages and different raw materials gives a different final quality and can change the nutrition and characteristics of yogurt [11].

Lactobacillus bulgaricus is a homofermentative bacterium with a bacillus shape and a purplish color. With a size of 0.5-0.8 x 2-9 μm , it can grow optimally at 37°C and is able to break down sugar into lactic acid. While Streptococcus thermophilus is a coccus shaped bacterium, with a bluish color that grows optimally at 45°C with a diameter of < 1 μm and can produce lactic acid optimally under anaerobic conditions. The composition ratio of the two bacteria is 1:1, this composition can obtain a distinctive yogurt taste [12].

Milk is a liquid produced from cows that have not been given any treatment and its content is not reduced or added to anything. High nutrition in milk is used by bacteria to feed its growth which reduces the function of milk and results in damage [13]. Granulated sugar is a class of sucrose ($C_{12}H_{22}O_{11}$) which is made from sugarcane and undergoes a crystallization process. Sugar acts as a source of nutrients that are used for microbial growth [14]. The addition of sugar can bind water to the product so that the resulting texture becomes thicker and more homogeneous. Skimmed milk is the product of milk that has had some or all of its fat removed when it is allowed to stand or is separated from its centrifugal separator. This type of milk contains lactose which is capable of metabolizing lactic acid so that it can be utilized by Lactobacillus bulgaricus and Streptococcus thermophilus as energy for their development [15]. The addition of skim has the same goal as sugar, which is to improve the texture of yogurt because it can bind water to the product.

2. Material and Methodology

2.1. Materials

The material of this research is pure milk, ried butterfly pea flowers, Lactobacillus bulgaricus, Streptococcus thermophiles, skimmed milk and sugar.

2.2. Making Telang Flower Sari

Brewed 5 g of dried telang using 200 mL of water with a temperature of 60 º C for 30 minutes, then filtered and then discarded the dregs.

2.3. Single Starter Manufacturing

Pasteurized 100 mL of pure milk plus 10 g of skim milk at 85 º C for 30 minutes, poured into a glass jar that has been sterilized 50 mL each. Allow to stand until the temperature is 40oC then put LB bacteria and ST bacteria as much as 4% of the milk into the jar. Incubate at 40º C for 12 hours, then add 100 mL of pure milk and 10 g of pasteurized skimmed milk at 85º C for 30 minutes. Incubated at 40oC for 12 hours, then put in the refrigerator at 4º C so that the fermentation does not continue.

2.4. Mix Strater Making

Pasteurized 150 mL of pure milk plus 15 g of skim milk at 85 º C for 30 minutes, poured into a jar and allowed to stand until the temperature was 40 º C. Enter the starter LB and ST as much as 4% of the weight of the material in a ratio of 1:1 (3 ml : 3 ml), 1:2 (2 ml : 4 ml) and 2:1 (4 ml : 2 ml) respectively, stir gently. Incubate at 40 ˚ C for 12 hours, then put in the refrigerator at 4 ˚ C so that the fermentation does not continue.

2.5. Yogurt Making

Put 20 g of skimmed milk and 20 g of sugar into 200 mL of cow's milk, then pasteurized for 30 minutes at 85 º C. Put in a jar that has been sterilized and allowed to stand until a temperature of 40 º C, then added the essence of the butterfly pea flower with a concentration of 4%, 8%, and 12% of the volume of milk then stirred until well blended. Added starters Lactobacillus bulgaricus and Streptococcus thermophilus as much as 4% of the volume of milk with treatment ratios of 1:1, 1:2, and 2:1. Incubate at a temperature of 45 º C for 6 hours, then put in the refrigerator at a temperature of 4 º C so that the fermentation does not continue repeated 3 times.

2.6. Data analysis

This study used a factorial Completely Randomized Design (CRD) consisting of two factors, namely: factor I: addition of butterfly pea extract (I): I_1 (4%), I_2 (8%), and I_3 (12%), factor II: comparison of starter Lactobacillus bulgaricus and Steptococcus thermophilus (A): A_1 (1:1), A_2 (1:2), and A_3 (2:1). The number of treatment combinations (Tc) is 3 x 3 = 9, the accuracy in this study was repeated 3 times. The treatment which had a significant and very significantly different effect was continued with further tests using the Duncan Table by comparing the LSR (Least Significant Range) values. Parameters tested were pH value, total acid, antioxidant activity, total lactic acid bacteria (LAB), viscosity, total soluble solids, color (^{o} Hue), organoleptic, and anthocyanin content in raw materials.

3. Results

Characteristics of yogurt with the addition of butterfly pea extract and comparison of Lactobacillus bulgaricus and Streptococcus thermophilus straters at several treatment levels can be seen in Table 1 and Table 2.

3.1. pH value

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). Comparison of starter Lactobacillus bulgaricus and Streptococcus thermopilus (Table 2) gave a highly significant different effect (P<0.01).

The addition of more and more butterfly pea flower extract resulted in a decrease in the pH value, where the pH obtained ranged from 4.4011 – 4.4667. This is influenced by the vitamin C compound contained in the butterfly pea flower. According to Dianatasya's analysis [16], the value of vitamin C infused butterfly pea flower water with 1:10 water and butterfly pea flower immersion treatment was 3.66%. The pH value in treatment I_1 was the highest pH value with a value of 4.4667, this was due to the small amount of butterfly pea flower extract added, namely 4%, so the addition of ascorbic acid to the yogurt was also small.

The pH value obtained from the starter comparison ranges from 4.178 to 4.4533. The use of more and more Lactobacillus bulgaricus causes the pH to decrease. According to Oberman [17], during Streptococcus thermopilus fermentation grows first and produce formic acid, acetic acid, lactic acid, and acetaldehyde. The presence of formic acid produced by Streptococcus thermopilus can cause a decrease in pH and stimulate the growth of Lactobacillus bulgaricus and produce lactic acid, amino acid valine, histidine and glycine. This is also in accordance with Widyanto [18] which states that Lactobacillus bacteria metabolize lactose into lactic acid and Lactobacillus is also the highest lactic acid producing bacteria.

3.2. Total Acid

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). Comparison of starter Lactobacillus bulgaricus and Streptococcus thermopilus (Table 2) gave a highly significant different effect (P<0.01). The interaction between the addition of butterfly pea flower extract and the comparison of starter Lactobacillus bulgaricus and Streptococcus thermophilus had a highly significant different effect (P<0.01).

The addition of more and more butterfly pea flower extract resulted in an increase in total acid. This is due to the presence of vitamin C contained in the butterfly pea flower. According to Dianatasya's research [16], the value of vitamin C (ascorbic acid) infused water of butterfly pea flowers by immersing the flowers of sea cucumbers in water with a ratio of 1:10 is 3.66%.

Lactobacillus bulgaricus bacteria in the starter ratio causes an increase in total acid, the more Lactobacillus bulgaricus, the higher the total acid. The Lactobacillus bulgaricus bacteria converting lactose into lactic acid, Lactobacillus bulgarigus is also the highest lactic acid producing bacteria [18]. The interaction relationship between the addition of butterfly pea flower extract and the ratio of starter to total acid is shown in Fig 1.

3.3. Antioxidant Activity (IC_{50})

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). A comparison of starter Lactobacillus bulgaricus and Streptococcus thermopilus (Table 2) gave a significantly different effect (P<0.05).

The addition of more and more butterfly pea flower essence caused the resulting IC_{50} value to decrease significantly. This is due to the polyphenol content in the butterfly pea flower. Yogurt fermentation process causes the polyphenol content to increase, this is because lactic acid bacteria produce β -glucosidase enzymes which can break down phenolic glucosides thereby increasing the concentration of polyphenols. According to Pratiwi [19], polyphenols can regulate enzymes and help reduce and inhibit the formation of free radicals.

The use of Lactobacillus bulgaricus which is more than Streptococcus thermophilus causes IC_{50} to decrease. The results of previous studies said that Lactobacillus bulgaricus has high antioxidant activity, and the increase in LAB helps degrade proteins into small peptides with antioxidant activity so that the antioxidant activity of yogurt increases [20].

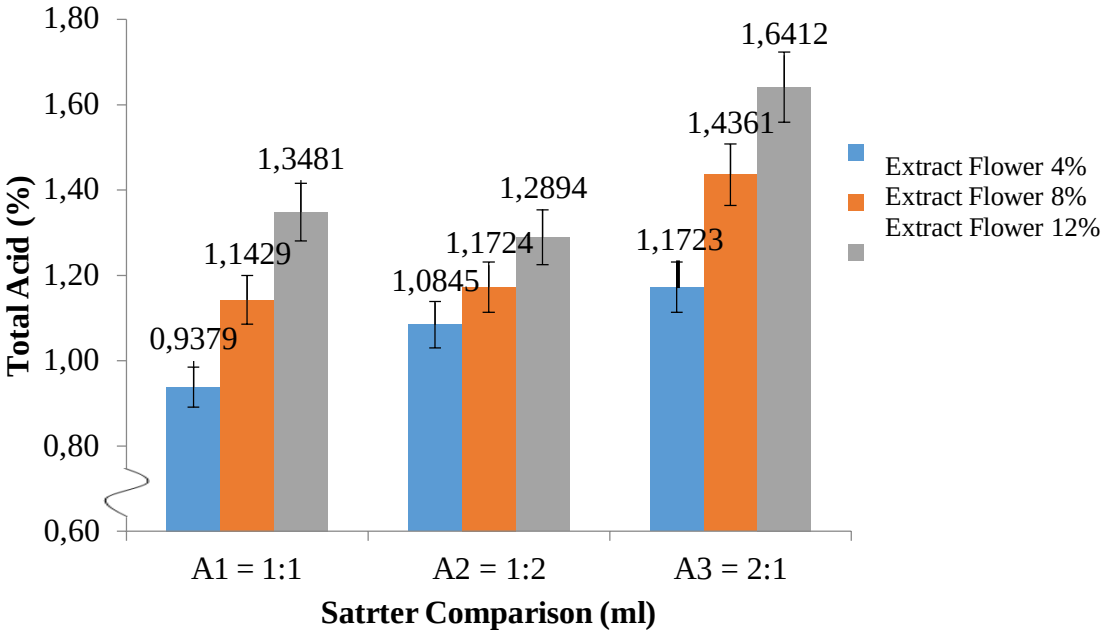

Figure 1 . Interaction of addition of butterfly pea flower extract and ratio of starter to total yogurt acid

Table 1. Effect of the addition of butterfly pea flower essence on the parameters observed

Test Parameters	Addition of Butterfly Pea Flower Extract (%)		
	4	8	12
pH	4.4667 ± 0.0376 ^{aA}	4.4533 ± 0.0115 ^{aA}	4.4011 ± 0.0102 ^{bB}
Total Acid (%)	1.0649 ± 0.1184 ^{cC}	1.2505 ± 0.1614 ^{bB}	1.4165 ± 0.1884 ^{aA}
Antioxidant Activity (ppm)	115.7131 ± 0.2470 ^{aA}	112.9690 ± 0.1913 ^{bB}	109.7150 ± 0.4598 ^{cC}
Total Lactic Acid Bacteria (CFU/mL)	1.1051 x 10 ^{9} ±0.0040 ^{aA}	1.3834 x 10 ^{9} ±0.0179 ^{bB}	1.6376 x 10 ^{9} ±0.0126 ^{cC}
Viscosity (Pa.s)	1.5484 ± 0.0181 ^{aA}	1.5025 ± 0.0059 ^{bB}	1.4544 ± 0.0154 ^{cC}
Total soluble solids (^{O}Brix)	14.6228 ± 0.1324 ^{aA}	13.4900 ± 0.0725 ^{bB}	12.5906 ± 0.3993 ^{cC}
Color Value (^{O}Hue)	226.5544 ± 0.2402 ^{cC}	238.8848 ± 0.3775 ^{bB}	266.2362 ± 0.2360 ^{cC}
Color Hedonic	4.1056 ± 0.0051 ^{cC}	4.2078 ± 0.0051 ^{bB}	4.4489 ± 0.0102 ^{aA}
General Acceptance Hedonic	4.2556 ± 0.0051 ^{cC}	4.2822 ± 0.0038 ^{bB}	4.3300 ± 0.0067 ^{aA}

Note: The test was carried out with 3 repetitions. Differences in lowercase letters indicate significant differences in 5% and capital letters are very real at 1%.

3.4. Total Acid Bacteria

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect ($P<0.01$). This is due to the content of polyphenols such as flavonoids, anthocyanins, and flavonols. Fermentation by LAB produces β-glucosidase enzymes which can break down phenolic glucosides which increase the concentration of polyphenols [19]. These polyphenolic compounds can stimulate the growth of Lactobacillus but inhibit the growth of pathogenic bacteria.

Table 2. The effect of starter ratio on was observed

Test Parameters	Starter Comparison (mL)		
	1:1	1:2	2:1
pH	4.4533 ± 0.0404 ^{aA}	4.4500 ± 0.0426 ^{aA}	4.4178 ± 0.0255 ^{bB}
Total Acid (%)	1.1430 ± 0.2051 ^{bB}	1.1821 ± 0.1028 ^{bB}	1.4165 ± 0.2350 ^{aA}
Antioxidant Activity (ppm)	113.0939 ± 2.9022 ^{aA}	112.8067 ± 2.9848 ^{bB}	112.4969 ± 3.1236 ^{cC}
Total Lactic Acid Bacteria (CFU/mL)	1.3824 x 10 ^{9} ±0.2714 ^{aA}	1.3714 x 10 ^{9} ±0.2610 ^{aA}	1.3724 x 10 ^{9} ±0.2674 ^{aA}
Viscosity (Pa.s)	1.4922 ± 0.0435 ^{bB}	1.4983 ± 0.0524 ^{aA}	1.5149 ± 0.0467 ^{aA}
Total soluble solids (^{O}Brix)	13.3945 ± 1.1225 ^{cB}	13.5269 ± 1.0695 ^{bA}	13.7819 ± 0.8794 ^{aA}
Color Value (^{O}Hue)	243.7158 ±20.3784 ^{aA}	243.9371 ± 20.5179 ^{aA}	244.0224 ± 20.0327 ^{aA}
Color Hedonic	4.2489 ± 0.1739 ^{aA}	4.2567 ± 0.1826 ^{aA}	4.2567 ± 0.1725 ^{aA}
General Acceptance Hedonic	4.2844 ± 0.0369 ^{aA}	4.2933 ± 0.0321 ^{aA}	4.2889 ± 0.0467 ^{aA}

Note: The test was carried out with 3 repetitions. Differences in lowercase letters indicate significant differences in 5% and capital letters are very real at 1%.

3.5. Viscosity

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). Comparison of starter Lactobacillus bulgaricus and Streptococcus thermopilus (Table 2) gave a highly significant different effect (P<0.05).

The decrease in viscosity occurred due to the addition of more butterfly pea flower extract. This happens because the butterfly pea flower essence is made from dried butterfly pea flowers which are brewed with water. The addition of butterfly pea flower extract reduces the total solids in the product which causes the product to become thinner. The amount of water in the product can reduce the ability of proteins to bind water.

The use of Lactobacillus bulgaricus in the manufacture of yogurt increased viscosity. This is due to the large number of Lactobacillus bulgaricus used in yogurt. The statement of Lestari [21], that Lactobacillus bulgaricus can increase viscosity. The occurrence of casein (milk protein) fermentation by LAB in yogurt processing can reduce the pH value. Viscosity increased at pH 5.3 due to decreased solubility of casein and at pH 4,8 – 4,7 casein precipitated perfectly. This situation causes the viscosity to increase because casein has hydrophilic properties [22].

3.6. Total Soluble Solids

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). Comparison of starter Lactobacillus bulgaricus and Streptococcus thermopilus (Table 2) gave a highly significant different effect (P<0.05).

The addition of more and more butterfly pea flower essence causes the total soluble solids to decrease. This is due to the water content contained in the butterfly pea flower essence. This is in line with the viscosity of the yogurt, the addition of water used for making butterfly pea flower extract causes the total solids in the product to become less [23]. The addition of butterfly pea flower extract which contains water makes the yogurt also contain more water.

Starter comparison gives a significantly different effect. . The fermentation carried out by LAB in making yoghurt produces lactic acid which can lower the pH value. A decrease in the pH value of and an increase in the content of acid can increase the viscosity of the product which also increases the total solids of the product. According to Ismawati [24] protein, lactic acid, total sugar, and organic acids contained in the product are dissolved solids.

3.7. Color Value (^{o}Hue)

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). The decrease in L value and increase in ^{o}Hue was caused by the addition of butterfly pea flower extract, this is because the anthocyanin content of butterfly pea flowers contributes color to the plant. Anthocyanins are used as natural dyes in food [25]. Previous studies stated that the L value decreased as the concentration of the butterfly pea flower increased and the ^{o}Hue value increased as the concentration of the butterfly pea flower used for product production increased [26].

3.8. Color Hedonic

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). The addition of more butterfly pea flower essence causes the hedonic value of the color to increase. This is because the hedonic color in treatment I_1 yogurt looks blue-green and in treatment I_3 yogurt looks dark blue. The difference in color in the yogurt is caused by the addition of butterfly pea flower extract causing the brightness level of the yogurt to decrease so that the color looks darker. This was tested from the results of the yogurt color value on I_1 obtaining the L value of 73.9811 (high) and on I_3 obtaining the L value of 66.7344 (low).

3.9. General Acceptance Hedonic

The addition of butterfly pea flower extract (Table 1) had a highly significant different effect (P<0.01). The large use of butterfly pea flower essence in yogurt increased the hedonic value of general acceptance. This is based on other hedonic acceptance, panelists like treatment I_3 with the addition of 12% butterfly pea flower extract, at this concentration the color of the yogurt is not too thick, the taste is not too sour and the sour aroma is not too concentrated so the panelists prefer yogurt with the addition of 12% butterfly pea flower extract.

4. Conclusion

The addition of butterfly pea flower extract had a very significant effect (P<0.01) on pH, total acid, antioxidant activity, total LAB, viscosity, total soluble solid, color value, color hedonic, and general acceptance hedonic. Comparison of Lactobacillus bulgaricus starters and Streptococcus thermophilus with a very significant effect of (P<0.01) on the pH value of total acid, a significant effect of P<0.05) on antioxidant activity, viscosity, and total soluble solids.

The interaction between the addition of butterfly pea flower extract and the comparison of starter LB and ST had a significantly different effect (P< 0.05) on the total acid value of yogurt. From research on yogurt with the addition of butterfly pea flower extract and the comparison of the starter with the best quality is the treatment with the addition of 12% (I_3) and starter ratio Lactobacillus bulgaricus and Streptococcus thermophilus 2:1 (A_3). This is based on antioxidant parameters, total LAB, color value, and organoleptic.

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