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Effects Of Sucrose Addition To Lactic Acid Concentrations And Lactic Acid Bacteria Population Of Butterfly Pea (*Clitoria Ternatea* L.) Yogurt

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Abstract. Butterfly pea yogurt (BPY) is a result of processed milk with the addition of the extract of the butterfly pea (*Clitoria ternatea* L.) Through the fermentation process of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as a lactic acid bacteria. The butterfly pea (*Clitoria ternatea* L.) is a source of the anthocyanin and potentially a natural blue dye. The study aims to determine sucrose's influence on lactic acid production and lactic acid bacteria of butterfly pea yogurt. The research design uses a completely random design. The study uses four treatments is a P1: 0% sucrose addition, P2: 4% sucrose, P3: 8% sucrose, P4: 12% sucrose, and yogurt control without sucrose addition as a comparison. Data is analyzed using variance analysis. Results showed that the concentration of lactic acid yogurt control 1.06 ± 0.79 , P1= 1.18 ± 0.22 , P2= 1.55 ± 0.39 , P3= 1.85 ± 0.24 and P4= 2.0 ± 0.31 . Whereas lactic acid bacteria population of yogurt control are $3,1 \times 10^6 \pm 0.79$, P1= $5.33 \times 10^6 \pm 0.97$, P2= $7.60 \times 10^6 \pm 0.26$, P3= $3.35 \times 10^7 \pm 0.98$ and P4= $2.86 \times 10^7 \pm 0.53$. The results indicate that the addition of sucrose 12% increase the concentration of lactic acid and lactic acid bacteria population significantly ($p < 0.05$) of butterfly pea (*Clitoria ternatea* L.) yogurt.

1. Introduction

Yogurt is the result of processed milk fermentation using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria or other suitable lactic acid bacteria. [1]. Yogurt processing with goat milk as an alternative to diversifying yogurt products. Therefore, it is necessary to develop goat milk processing methods with fermentation techniques aimed at diversification and to reduce the smell of goat milk prengus. As consumer interest in yogurt products increases, consumers are now switching from synthetic dyes to natural dyes because they are healthier or do not have negative side effects. Dyes derived from plants can be used as natural alternative dyes, one of which is the butterfly pea.

The addition of dyes to yogurt can increase consumer appeal to consume various color variants of yogurt. The butterfly pea (*Clitoria ternatea* L.) has a purplish-blue color pigment. Many research uses natural dyes such as pandan leaves, turmeric, and dragon fruit extract as natural coloring agents added to yogurt. The addition of butterfly pea extract as a natural dye for a popsicle and various foodstuffs has been done by [2]. The butterfly pea extract contains anthocyanin pigments [3] which can be used as an alternative to natural dyes that produce a purplish-blue color.

The quality of yogurt also depends on the bacterial cultures inoculated [4] and the resulting lactic acid [5]. The addition of sugar has been widely studied as one factor influencing the growth of lactic acid bacteria [6]. In this study, the addition of sugar to the yogurt with butterfly pea petal flower extract was investigated on lactic acid and the population of lactic acid bacteria.



2. Materials and Methods

2.1 Material

The main ingredient used to make yogurt in this study is commercial pasteurized milk circulating in the market. Dried butterfly pea (*Clitoria ternatea* L.) obtained from the producer “Kusuka Ubiku” under the trademark “Hasil Bumiku” in Bantul, Yogyakarta, Indonesia. Lactic acid bacteria starter obtained from the Biotechnology Laboratory at Faculty of Agriculture Technology Gadjah Mada University (FTP UGM). Other supporting materials were distilled water, 0.9% NaCl, and MRS media (de Man, Ragosa, Sharpe).

2.2 Extraction of butterfly pea powder

The butterfly pea (*Clitoria ternatea* L.) extract (BPE) is based on the research of [7], [3] and [6] with simple modification. The powdered butterfly pea flower petal was extracted using maceration technique at a temperature of 60°C for 37 minutes. Furthermore, the sample was filtered using a 70 mm Whatman filter paper. The extraction process was carried out with a ratio of butterfly pea:distilled water at 3:1 (w/v). The solution was the result of the extraction then added in the process of making yogurt.

2.3 Preparation of yogurt starter

The starter of *Lactobacillus bulgaricus* (LB) and *Streptococcus thermophilus* (ST) obtained from FTP UGM. The bacterial starter was then grown on MRS media with a temperature treatment of 40°C for 24 hours before use, according to the method used by [4], with minor modifications.

2.4 Yogurt production (without BPE addition)

Yogurt production with lactic acid bacteria (LB and ST) follows a method done by [8] and [6] with minor modification. The culture bacteria added to pasteurized milk with a ratio of 1:9 and incubated at a temperature of 37°C for 24 hours.

2.5 Butterfly pea yogurt (BPY) with sucrose addition

The butterfly pea yogurt (BPY) fermentation procedure followed the research by [9] with BPE addition by 10% and sucrose addition by 0%, 4%, 8%, and 12% (v/v). The butterfly pea powder extract was added to the pasteurized milk and then stirred until it is thoroughly mixed. LB and ST starter with a population of 1×10^8 CFU/ml added to the milk solution. Inoculation or fermentation is done in the incubator with a temperature of 45°C for 24 hours until formed coagulation. Furthermore, yogurt was stored at a temperature of 4°C. All these experiments performed in triplicate, the lactic acid content and total lactic acid bacteria were determined after the incubation time was 48 hours.

2.6 Analytical methods

Total lactic acid bacteria determined according to standard plate count (SPC) method of [8]. The lactic acid concentration determined with total titrated acid method by [10]. All sample analyses were carried out in triplicate. Experimental data were subjected to one-way analysis of variance (ANOVA) using the Duncan Multiple Range Test (DMRT) test ($p < 0.05$). The IBM SPSS software 22.0 was used.

3. Results and Discussion

3.1 Lactic Acid Content

According to National Standard Indonesia (SNI 2981: 2009), a good level of yogurt lactic acid is 0.5-2.0%. The lactic acid content of this research result are shown in Figure 1:

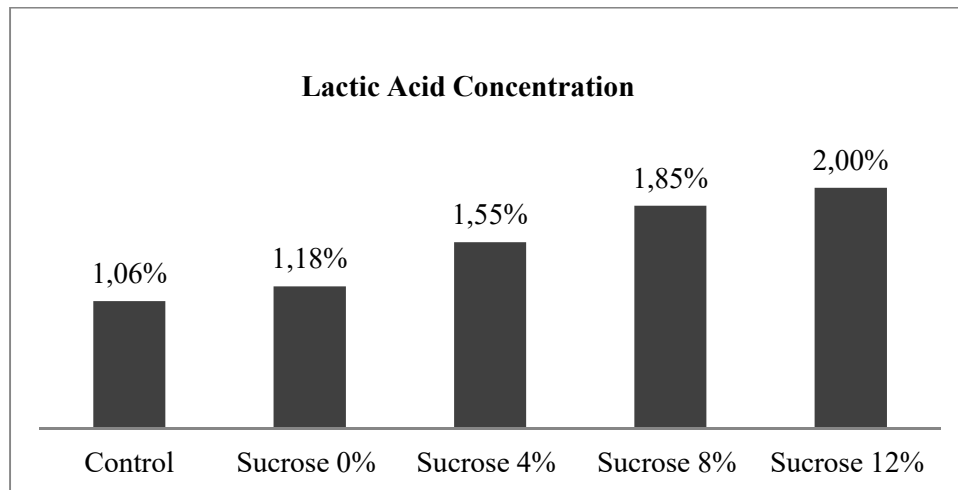


Figure 1. Levels of lactic acid yogurt butterfly pea

Based on the results of the research data, the concentration of lactic acid in control yogurt was $1.06\% \pm 0.79$, 0% sucrose $1.18\% \pm 0.22$, 4% sucrose $1.55\% \pm 0.3$, 8% sucrose $1.85\% \pm 0.24$ and 12% sucrose $2.00\% \pm 0.31$. Lactic acid concentration data without 0% and 4% sucrose compared with control yogurt was not significantly different, while with the addition of 8% sucrose and 12% sucrose compared to control, it was significantly different ($p < 0.05$).

Sucrose is a nutrient for lactic acid bacteria that will affect lactic acid bacteria's activity in the fermentation process. [11] stated that yogurt's viscosity would increase with the higher concentration of sucrose and the addition of skim milk. According to [12], the addition of sucrose and skim milk, either separately or together, was proven to affect the production of lactic acid in green bean yogurt. The more significant dissolved substantial component in a solution will increase the viscosity. [11] also stated that the most dissolved substantial component is sucrose. During the fermentation process, sucrose would be broken down into lactic acid. The yogurt's pH has decreased, and there is a coagulation of milk protein, resulting in clotting, which causes the yogurt's viscosity to increase. [13] stated that lactic acid production in the fermentation process resulted in a decrease in the pH value, which led to the coagulation of vegetable protein and the formation of a thick texture of yogurt.

3.2 Total Amount of Lactic Acid Bacteria (LAB)

Table 1. Bacterial population using the SPC method (CFU / ml)

Yogurt	LAB population (CFU / ml)
Yogurt Control	$3.10 \times 10^6 \pm 0.79$
(P1) Sucrose 0%	$5.33 \times 10^6 \pm 0.97$
(P2) Sucrose 4 %	$7.60 \times 10^6 \pm 0.26$
(P3) Sucrose 8 %	$3.35 \times 10^7 \pm 0.98$
(P4) Sucrose 12 %	$2.86 \times 10^7 \pm 0.53$

Description: Yogurt (UHT Milk)

The population of LAB based on the SPC data showed that the total LAB in yogurt control are 3.1×10^6 CFU/ml, (P1) sucrose 0% are 5.33×10^6 CFU/ml, (P2) 4% sucrose are 7.60×10^6 CFU/ml, (P3) 8% sucrose are 3.35×10^7 CFU/ml and (P4) 12% sucrose is 2.86×10^7 CFU/ml.

The addition of sucrose 0% and 4% to BPY showed no significant difference in the LAB population than the control yogurt. While the BPY with the sucrose addition of 8% and 12% showed significant differences ($p < 0.05$) with the control yogurt. This result indicates a higher sucrose concentration in the BPY, which contains carbohydrates as a nutrition source for LAB growth [14]. However, all these treatments are under the [1], namely the minimum total LAB in yogurt without heat treatment after fermentation are 10^7 CFU/ml. The number of bacterial colonies can grow up to the maximum number in the media, which is influenced by the media's availability of nutrients. The availability of nutrients dramatically influences the population of lactic acid bacteria. Skim milk rich in carbohydrates (lactose) and protein, and sucrose is a growing medium for LAB [15].

Lactic acid bacteria can degrade various types of sugar into lactic acid. The sugar contained in milk and fruit can stimulate growth and increase LAB activity in yogurt. Good yogurt contains a minimum standard population of LAB, which is 10^7 CFU/ml for each product [7].

Yogurt has long been known as a source of probiotics. The primary role of probiotics, in general, is to optimize digestion metabolism through the mechanism of repair of microbiota populations in the digestive tract. At least 15 types of health problems that are generally studied have proven the health effects of probiotics, [16], [19] – [20]. Over time, yogurt product innovation has evolved, producing a variety of yogurt products. Various changes and product differentiations continue to be made to add value to the health and appearance of yogurt, one of which is by adding blue pea flower extracts [17], [18], [21]–[22]. A similar study was also conducted by [12]–[13], who examined probiotic drinks from young coconut water with lactic acid bacteria starter *Lactobacillus casei*. The total results of the LAB after fermentation ranged from 7.3×10^{13} CFU/ml to 1.9×10^{14} CFU/ml. These results indicate that the higher the sucrose concentration, the higher the LAB activity. The treatment 15% and 3% sucrose concentrations gave the best results in producing lactic acid bacteria.

4. Conclusion

The content of lactic acid and total lactic acid bacteria of butterfly pea (*Clitoria ternatea* L.) yogurt is significantly affected by sucrose addition. The higher sucrose addition resulted in higher lactic acid content and total lactic acid bacteria of butterfly pea yogurt.

5. References

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Effects of Sucrose Addition to Lactic Acid Concentrations and Lactic Acid Bacteria Population of Butterfly Pea (*Clitoria ternatea* L.) Yogurt

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Abstract. Butterfly pea yogurt (BPY) is a result of processed milk with the addition of the extract of the butterfly pea (*Clitoria ternatea* L.) Through the fermentation process of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as a lactic acid bacteria. The butterfly pea (*Clitoria ternatea* L.) is a source of the anthocyanin and potentially a natural blue dye. The study aims to determine sucrose's influence on lactic acid production and lactic acid bacteria of butterfly pea yogurt. The research design uses a completely random design. The study uses four treatments is a P1: 0% sucrose addition, P2: 4% sucrose, P3: 8% sucrose, P4: 12% sucrose, and yogurt control without sucrose addition as a comparison. Data is analyzed using variance analysis. Results showed that the concentration of lactic acid yogurt control 1.06 ± 0.79 , P1= 1.18 ± 0.22 , P2= 1.55 ± 0.39 , P3= 1.85 ± 0.24 and P4= 2.0 ± 0.31 . Whereas lactic acid bacteria population of yogurt control are $3,1 \times 10^6 \pm 0.79$, P1= $5.33 \times 10^6 \pm 0.97$, P2= $7.60 \times 10^6 \pm 0.26$, P3= $3.35 \times 10^7 \pm 0.98$ and P4= $2.86 \times 10^7 \pm 0.53$. The results indicate that the addition of sucrose 12% increase the concentration of lactic acid and lactic acid bacteria population significantly ($p < 0.05$) of butterfly pea (*Clitoria ternatea* L.) yogurt.

2

1. Introduction

Yogurt is the result of processed milk fermentation using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria or other suitable lactic acid bacteria, with or without the addition of other food ingredients and permitted food additives [1]. Yogurt processing with goat milk as an alternative to diversifying yogurt products. Therefore, it is necessary to develop goat milk processing methods with fermentation techniques aimed at diversification and to reduce the smell of goat milk prengus. As consumer interest in yogurt products increases, consumers are now switching from synthetic dyes to natural dyes because they are healthier or

2

do not have negative side effects. Dyes derived from plants can be used as natural alternative dyes, one of which is the butterfly pea.

The addition of dyes to yogurt can increase consumer appeal to consume various color variants of yogurt. The butterfly pea (*Clitoria ternatea* L.) has a purplish-blue color pigment. Many research uses natural dyes such as pandan leaves, turmeric, and dragon fruit extract as natural coloring agents added to yogurt. The addition of butterfly pea extract as a natural dye for a popsicle and various foodstuffs has been done by [2]. The butterfly pea extract contains anthocyanin pigments [3] which can be used as an alternative to natural dyes that produce a purplish-blue color.

The quality of yogurt also depends on the bacterial cultures inoculated [4] and the resulting lactic acid [5]. The addition of sugar has been widely studied as one factor influencing the growth of lactic acid bacteria [6]. In this study, the addition of sugar to the yogurt with butterfly pea petal flower extract was investigated on lactic acid and the population of lactic acid bacteria.

2. Materials and Methods

2.1 Material

The main ingredient used to make yogurt in this study is commercial pasteurized milk circulating in the market. Dried butterfly pea (*Clitoria ternatea* L.) obtained from the producer “Kusuka Ubiku” under the trademark “Hasil Bumiku” in Bantul, Yogyakarta, Indonesia. Lactic acid bacteria starter obtained from the Biotechnology Laboratory at Faculty of Agriculture Technology Gadjah Mada University (FTP UGM). Other supporting materials were distilled water, 0.9% NaCl, and MRS media (de Man, Ragosa, Sharpe).

2.2 Extraction of butterfly pea powder

The butterfly pea (*Clitoria ternatea* L.) extract (BPE) is based on the research of [7], [3] and [6] with simple modification. The powdered butterfly pea flower petal was extracted using maceration technique at a temperature of 60°C for 45 minutes. Furthermore, the sample was filtered using a 70 mm Whatman filter paper. The extraction process was carried out with a ratio of butterfly pea:distilled water at

3:1 (w/v). The solution was the result of the extraction then added in the process of making yogurt.

2.3 Preparation of yogurt starter

The starter of *Lactobacillus bulgaricus* (LB) and *Streptococcus thermophilus* (ST) obtained from FTP UGM. The bacterial starter was then grown on MRS media with a temperature treatment of 30°C for 24 hours before use, according to the method used by [4], with minor modifications.

2.4 Yogurt production (without BPE addition)

Yogurt production with lactic acid bacteria (LB and ST) follows a method done by [8] and [6] with minor modification. The culture bacteria added to pasteurized milk with a ratio of 1:9 and incubated at a temperature of 37°C for 24 hours.

2.5 Butterfly pea yogurt (BPY) with sucrose addition

The butterfly pea yogurt (BPY) fermentation procedure followed the research by [9] with BPE addition by 10% and sucrose addition by 0%, 4%, 8%, and 12% (v/v). The butterfly pea powder extract was added to the pasteurized milk and then stirred until it is thoroughly mixed. LB and ST starter with a population of 1×10^8 CFU/ml added to the milk solution. Inoculation or fermentation is done in the incubator with a temperature of 45°C for 24 hours until formed coagulation. Furthermore, yogurt was stored at a temperature of 4°C. All these experiments performed in triplicate, the lactic acid content and total lactic acid bacteria were determined after the incubation time was 48 hours.

2.6 Analytical methods

Total lactic acid bacteria determined according to standard plate count (SPC) method of [8]. The lactic acid concentration determined with total titrated acid method by [10]. All sample analyses were carried out in triplicate. Experimental data were subjected to one-way analysis of variance (ANOVA) using the Duncan

Multiple Range Test (DMRT) test ($p < 0.05$). The IBM SPSS software 22.0 was used.

3. Results and Discussion

3.1 Lactic Acid Content

According to National Standard Indonesia (SNI 2981: 2009), a good level of yogurt lactic acid is 0.5-2.0%. The lactic acid content of this research result are shown in Figure 1:

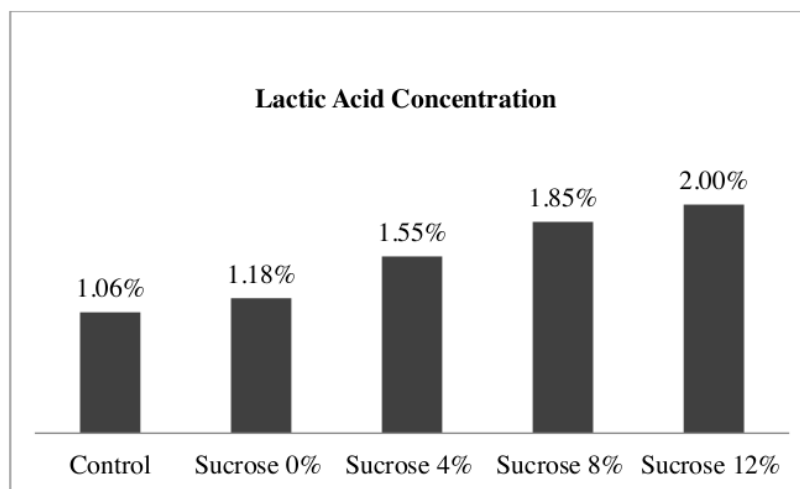


Figure 1. Levels of lactic acid yogurt butterfly pea

Based on the results of the research data, the concentration of lactic acid in control yogurt was $1.06\% \pm 0.79$, 0% sucrose $1.18\% \pm 0.22$, 4% sucrose $1.55\% \pm 0.3$, 8% sucrose $1.85\% \pm 0.24$ and 12% sucrose $2.00\% \pm 0.31$. Lactic acid concentration data without 0% and 4% sucrose compared with control yogurt was not significantly different, while with the addition of 8% sucrose and 12% sucrose compared to control, it was significantly different ($p < 0.05$).

Sucrose is a nutrient for lactic acid bacteria that will affect lactic acid bacteria's activity in the fermentation process. [11] stated that yogurt's viscosity would increase with the higher concentration of sucrose and the addition of skim milk. According to [12], the addition of sucrose and skim milk, either separately or together, was proven to affect the production of lactic acid in green bean yogurt.

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3.2 Total Amount of Lactic Acid Bacteria (LAB)

Table 1. Bacterial population using the SPC method (CFU / ml)

Yogurt	LAB population (CFU / ml)
Yogurt Control	$3.10 \times 10^6 \pm 0.79$
(P1) Sucrose 0%	$5.33 \times 10^6 \pm 0.97$
(P2) Sucrose 4 %	$7.60 \times 10^6 \pm 0.26$
(P3) Sucrose 8 %	$3.35 \times 10^7 \pm 0.98$
(P4) Sucrose 12 %	$2.86 \times 10^7 \pm 0.53$

Description: Yogurt (UHT Milk)

The population of LAB based on the SPC data showed that the total LAB in yogurt control are 3.1×10^6 CFU/ml, (P1) sucrose 0% are 5.33×10^6 CFU/ml, (P2) 4% sucrose are 7.60×10^6 CFU/ml, (P3) 8% sucrose are 3.35×10^7 CFU/ml and (P4) 12% sucrose is 2.86×10^7 CFU/ml.

The addition of sucrose 0% and 4% to BPY showed no significant difference in the LAB population than the control yogurt. While the BPY with the sucrose addition of 8% and 12% showed significant differences ($p < 0.05$) with the control yogurt. This result indicates a higher sucrose concentration in the BPY, which contains carbohydrates as a nutrition source for LAB growth [14]. However, all these treatments are under the [1], namely the minimum total LAB in yogurt without heat treatment after fermentation are 10^7 CFU/ml. The number of bacterial colonies can grow up to the maximum number in the media, which is influenced by the media's availability of nutrients.

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Lactic acid bacteria can degrade various types of sugar into lactic acid. The sugar contained in milk and fruit can stimulate growth and increase LAB activity in yogurt. Good yogurt contains a minimum standard population of LAB, which is 10^7 CFU/ml for each product [7].

A similar study was also conducted by [12], who examined probiotic drinks from young coconut water with lactic acid bacteria starter *Lactobacillus casei*. The total results of the LAB after fermentation ranged from 7.3×10^{13} CFU/ml to 1.9×10^{14} CFU/ml. These results indicate that the higher the sucrose concentration, the higher the LAB activity. The treatment 15% and 3% sucrose concentrations gave the best results in producing lactic acid bacteria.

4. Conclusion

The content of lactic acid and total lactic acid bacteria of butterfly pea (*Clitoria ternatea* L.) yogurt is significantly affected by sucrose addition. The higher sucrose addition resulted in higher lactic acid content and total lactic acid bacteria of butterfly pea yogurt.

References

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