The potential of probiotic frozen yoghurt with the addition of fruits tamarillo to increase immunity

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Abstract. Frozen yoghurt is a food that has the potential to increase immunity in preventing Covid-19, which is known as a functional food. These functional foods contain nutritional components for the body, such as probiotics with the addition of active substances from fruit. The purpose of this study was to analyze the potential of frozen yoghurt and the effect of adding Fruits Tamarillo to increase immunity, seen from the test variables including pH value, TTA (Total Titrable Acid), water content, fat content, protein content, total lactic acid bacterial colonies, and antioxidant activity. The method used was a 3 x 3 factorial randomized block design experimental method with three replications. The treatment was the addition of the percentage of frozen yoghurt starter concentrations A1 (3%), A2 (5%), A3 (7%) and Fruits Tamarillo B1 (0%), B2 (5%), B3 (10%) concentrations. The results of this study obtained the best results with the addition of a 5% starter, and addition of 5% Fruits Tamarillo, namely a pH value of 4.66, Total Titratable Acidity (TTA) 0.67, total lactic acid bacteria colonies 95.7 x 10⁸ CFU / mL, and 44.25% antioxidant activity.

1. Introduction

Lactic acid bacteria is a type of good bacteria that has many benefits for human health. It is necessary to select the origin of the sample to be isolated as a potential lactic acid bacteria, such as traditional fermented food such as bekasam from South Sumatra. The results of the isolation of lactic acid bacteria as probiotics today are very much needed because they are related to public health and the surrounding environment. According to [1], lactic acid bacteria used to ferment milk also produce antimicrobial substances that act as natural antibiotics against pathogenic bacteria, help maintain digestive health, prevent various diseases, and increase digestibility in lactose sensitive individuals and increase the safe storage time of the product. Because of these benefits, much work to identify lactic acid bacteria and probiotics could potentially be developed to add value to food. As a result, lactic acid bacteria are classified as probiotics, mainly because they are antimicrobial, stomach-acid tolerant, and safe to use, but bacteria classified as probiotics should also have the ability to produce antimicrobial substances that can suppress the growth of pathogenic enteric bacteria. Such substances include organic acids, hydrogen peroxide, diacetyl, and bacteriocin [2].

Lactic acid bacteria were obtained from the isolation of traditional tapioca fermented food from South Sumatra. According to [3], the isolation of lactic acid bacteria from Bekasam South Sumatra has the potential for probiotics with lactic acid bacteria Pediococcus acidilactici. After obtaining the lactic
acid bacteria from Bekasam, it will be applied in making a frozen yoghurt starter. Yoghurt is fermented milk using a mixed microbe; generally, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, resulting in a pudding-like consistency. Previous research from [4] using substitution of lactic acid bacteria *Lactobacillus fermentum* L23 and *Streptococcus thermophilus*, resulted in the best concentration of starter giving at 5% concentration. Based on research results, [5] identified one lactic acid bacteria isolated from a sample of swamp buffalo milk harvested in the Agam district that showed high probiotic potential. Of all the isolates, BMA 3.3 had the best ability to survive at low pH, resist bile salts. It effectively inhibited the growth of pathogenic bacteria (*E. coli* O157: H7 and *S. aureus* ATCC 25923) and was resistant to three of the five most commonly used antibiotics and had medium hydrophobicity. This lactic acid bacterial strain was identified as *L. fermentum* strain L23. This isolate has potential as a natural addition in the production of fermented milk-based foods to enhance their health value. The use of Lactic Acid Bacteria, a combination of three bacteria in a yoghurt starter, where the process is started by *S. thermophilus* by producing organic acids which stimulate the growth of *L. fermentum* and *P. acidilactici*. Then *L. fermentum* plays a role in degrading glucose in products with the result of lactic acid. *P. acidilactici* plays a role in increasing, stimulating peptides at a temperature of 43°C.

To improve the final product's quality, after the yoghurt product is finished, it is then continued to the freezing stage known as frozen yoghurt. Frozen yoghurt is a dessert that combines the texture of ice cream with yoghurt's nutritional properties [6]. The process consists of mixing all the ingredients to make yoghurt, stirring naturally with a stabilizer/emulsifier and sugar, then freezing the mixture in a conventional ice cream freezer [7].

To improve the quality of frozen yoghurt produced so that it can be a functional food with active compounds that are good for the body. One of the ingredients that can be added is the Fruits Tamarillo (*Solanum betaceum* Cav.). The addition of Fruits Tamarillo to yoghurt aims to take advantage of the active compound content in the form of antioxidants and can reduce cholesterol in the body. Fruits Tamarillo contains phytochemicals, including β-carotene anthocyanins, flavonols, phenolic acids, and antioxidant activity.

1.1. The purpose of the research

In this regard, the potential for improving yoghurt products' quality is by modifying the work to be frozen yoghurt and adding Tamarillo juice. With the use of lactic acid bacteria from Bekasam from South Sumatra as a frozen yoghurt starter with the addition of Fruits Tamarillo, it is hoped that it can increase the body immunity. Based on these considerations, this study aims to analyze The Potential Of Probiotic Frozen Yoghurt With The Addition Of Fruits Tamarillo (*Solanum Betaceum* Cav.) To Increase Immunity.

2. Materials and methods

2.1. The material of the research

This study was conducted at the Laboratory of Animal Product Technology, Faculty of Animal Science, Andalas University, Padang, in November 2019. The materials used in this study for the isolation of lactic acid bacteria were taken from Bekasam in Banyuasin district, South Sumatra and in the manufacture of products using fresh cow's milk obtained from existing farms in the city of Padang and Fruits Tamarillo from a fruit plantation in Alahan Panjang, Solok district.

2.2. Methods of the research

The method used in this study is an experimental method with a randomized block design with a factorial pattern of 3x3 treatment and three replications. The treatments were A1 (rate of yoghurt starter concentration 3%), A2 (rate of yoghurt starter concentration 5%) and A3 (rate of yoghurt starter concentration 7%); B1 (increase in the rate of Fruits Tamarillo to 0%), B2 (increase in the rate of Fruits Tamarillo to 5%) and B3 (increase in the rate of Fruits Tamarillo to 10%).
2.3. Procedure starter yoghurt
In this study, a starter was made based on the modification of the [8] method patent no. SID201804980.

2.4. Procedure frozen yoghurt
Making yoghurt according to [9] with the following modifications: Pasteurize milk at a temperature of 65 °C for 30 minutes; Reducing the temperature of pasteurized milk to 43 °C; Inoculating starter yoghurt (a combination of Lactobacillus fermentum, Streptococcus termophilus and Pediococcus acidilactici) based on starter percentage treatment A1 (3%), A2 (5%), and A3 (7%); Continue the incubation process at 43 °C for 12 hours; After the yoghurt product was finished, Fruits Tamarillo was added based on the percentage treatment of B1 (0%), B2 (5%), and B3 (10%) and incubated for 24 hours; Then proceed to the frozen yoghurt stage and stored in the freezer; After that, the samples were tested on the observed variables.

2.5. Parameter testing
Some of the variables measured in this study include pH value [10], and Total Titratable Acidity (TTA) [11], total lactic acid bacteria Colonies [12], and Antioxidant Activity [13].

2.6. Data analysis
Analyses used in this study were carried out according to [14] procedure. Processing the test data using IBM SPSS statistics 23 and if there is an influence from the treatment, it continues with the Duncan Multiple Range Test (DMRT).

3. Results and discussion

3.1. pH value
The pH value of frozen yoghurt in the combination of starter concentration and concentration of Fruits Tamarillo to frozen yoghurt showed no interaction with the pH value of frozen yoghurt (analyzed using SPSS version 22 for windows software). Table 1 below shows the results of further data testing using the DMRT follow-up test at the 5% level for each factor.

| Factor A (Starter) | Factor B (Fruits Tamarillo) | Average  |
|--------------------|-----------------------------|----------|
|                    | B1  | B2  | B3   |
| A1                 | 4.80 | 4.67 | 4.70 | 4.72<sup>a</sup> |
| A2                 | 4.78 | 4.66 | 4.64 | 4.69<sup>ab</sup> |
| A3                 | 4.76 | 4.64 | 4.64 | 4.68<sup>b</sup> |
| Average            | 4.78<sup>a</sup> | 4.66<sup>b</sup> | 4.66<sup>b</sup> |

Note: Numbers in the same row followed by the same lowercase letter and numbers in the same column followed by the same capital letter are not significantly different according to the 5% level of DMRT.

In table 1, it shows that frozen yoghurt with the difference in the addition of starter concentration (Factor A) has a significant effect (P <0.05) on the pH value of frozen yoghurt. The mean pH value of frozen yoghurt with the difference in the addition of starter concentration (Factor A) ranged from 4.68-4.72. The highest value in the starter treatment is 3% (A1) with an average pH value of 4.72 frozen yoghurt and the lowest pH value in 7% (A3) with an average pH value of 4.68 frozen yoghurt. As for the effect of the addition of starter (factor A) on decreasing the pH of yoghurt due to the results of the metabolism of Lactic Acid Bacteria, namely L. fermentum, S. thermophilus and Pediococcus acidilactici, the largest is lactic acid. However, A2 and A3 treatments were not significantly different (P> 0.05). This happens because the addition of a starter to 5% and 7% has an optimal effect on reducing pH so that the addition of a higher starter does not affect the pH value. The
decrease in the pH value is due to the increasing accumulation of the starter because the higher the
starter's addition, the higher the production of lactic acid and other organic acids, which affects the
decrease in the pH value. As seen in the research results, the highest addition of starter was 7% (A3),
resulting in the lowest yoghurt pH of 4.68. This is supported by the opinion of [15] that lactose is
hydrolyzed by the beta-galactosidase enzyme to produce glucose and galactose, the result of which is
lactic acid.

In frozen yoghurt with the addition of the concentration of Fruits Tamarillo (Factor B), it showed
an effect (P <0.05) on the pH value, which ranged from 4.66 - 4.78. It is known that the addition of
Fruits Tamarillo can affect reducing the pH value of frozen yoghurt. The decrease in the yoghurt's pH
was due to the increasing accumulation of Fruits Tamarillo, which was influenced by the nature of
tamarillo, which was in the pH range of 4.6, so that the higher the Fruits Tamarillo, the higher the
acidity of the yoghurt. As seen in the research results, the highest addition of Fruits Tamarillo at 10%
resulted in the lowest pH value of 4.66. This is in line with the opinion of [16] which states that phenol
components can donate hydrogen atoms and can stop free radical reactions by turning them into stable
compounds. Similar to the research. This research results in the same pH range as the pH of yoghurt in
the [17] study, the pH of buffalo milk yoghurt is 4.6, and the pH of cow's milk yoghurt is the result of
research by [18] which is 4.6.

3.2. Total Titratable Acidity (TTA)
Total Titratable Acidity (TTA) of frozen yoghurt in the combination of addition of starter
concentration and concentration of Fruits Tamarillo to frozen yoghurt showed no
interaction with the TTA value of frozen yoghurt (analyzed using SPSS software version 22 for
windows). In table 2 below shows the results of further data testing using the DMRT follow-up test at
the 5% level for each factor.

| Factor A (Starter) | Factor B (Fruits Tamarillo) | Average |
|--------------------|----------------------------|---------|
|                    | B1  | B2  | B3  |         |
| A1                 | 0.60| 0.66| 0.75| 0.67b   |
| A2                 | 0.61| 0.67| 0.84| 0.70a   |
| A3                 | 0.65| 0.77| 0.83| 0.75a   |
| Average            | 0.62c| 0.70b| 0.80a|

Note: Numbers in the same row followed by the same lowercase letter and numbers in the same
column followed by the same capital letter are not significantly different according to the 5%
level of DMRT.

In table 2, it shows that frozen yoghurt with the difference in the addition of starter concentration
(Factor A) has a significant effect (P <0.05) on the TTA value of frozen yoghurt. The average cost of
TTA for frozen yoghurt with the difference in the addition of starter concentration (Factor A) ranged
from 0.67- 0.75%. The highest value of the starter treatment is 7% (A3) with an average TTA value of
frozen yoghurt is 0.75%, and the lowest TTA value is in the starter treatment of 5% (A1) with an
average TTA value of frozen yoghurt is 0.67%. It is known that the addition of starter concentration
has little effect in increasing the TTA value of frozen yoghurt. The increase in TTA of frozen yoghurt,
and the addition of a starter is because the higher the starter concentration, the higher the activity of
lactic acid bacteria in producing organic acids during fermentation. As a result, it increases frozen
yoghurt TTA. As seen in the study results, the highest addition of starter, 7% (A3), resulted in the
highest TTA of 0.75%. According to [15], sugar, namely lactose, is the primary basis for the formation
of yoghurt, which is actively transported across the S. thermophillus membrane through the mediation
of the enzyme galactose permease. Pgalactosidase enzyme hydrolyzes lactose to glucose and
galactose. Furthermore, glucose is metabolized to pyruvate via the Emden-Meyerhof-Parnas (EMP)
pathway, and lactate dehydrogenase converts pyruvate into lactic acid.
In frozen yoghurt with the addition of Fruits Tamarillo concentration (factor B), it showed a very significant effect (P < 0.01) on the TTA value ranging from 0.62–0.80%. The results of the analysis of variance showed that frozen yoghurt with the addition of Fruits Tamarillo (Factor B) had a very significant effect (P < 0.01) on the TTA value of frozen yoghurt, which was the highest in the treatment of adding Fruits Tamarillo 10% (B3) with an average The TTA value was 0.80%. The lowest was Fruits Tamarillo 0% (B1) addition with an average TTA value of 0.62%. It is known that the addition of Fruits Tamarillo can affect increasing the amount of frozen yoghurt TTA. The increase in TTA for frozen yoghurt and the increasing accumulation of Fruits Tamarillo (factor B) was caused by the high TTA content in Tamarillo, which could affect the TTA value of frozen yoghurt. This is supported by the opinion of [19] that Tamarillo Fruits have a titrated acidity of 1.0-2.6% (g / 100 g). As seen in the study results, the highest addition of Fruits Tamarillo was 10% (B3), resulting in the highest TTA of 0.80%. TTA of frozen yoghurt in this study was higher than that of [18], cow and sheep milk yoghurt, with a TTA of 0.7% and 0.9%.

3.3. Total lactic acid bacteria colonies

| Factor A (Starter) | Factor B (Fruits Tamarillo) | Average |
|--------------------|-----------------------------|---------|
|                    | B1  | B2  | B3  |       |
| A1                 | 51.3| 74.7| 64.0| 63.3b|
| A2                 | 74.7| 95.7| 90.0| 86.8a|
| A3                 | 64.3| 86.3| 81.0| 77.2ab|
| Average            | 63.4b| 85.6a| 78.3ab|

Note: Numbers in the same row followed by the same lowercase letter and numbers in the same column followed by the same capital letter are not significantly different according to the 5% level of DMRT.

In table 3, it shows that frozen yoghurt with the difference in the addition of starter concentration (Factor A) has a significant effect (P < 0.05) on the total lactic acid bacteria colonies frozen yoghurt. The total average of lactic acid bacteria colonies frozen yoghurt with the difference in the addition of starter concentration (Factor A) ranged from 63.3 - 86.8 x 10⁸ CFU / mL. Where the highest value in the starter treatment is 5% (A2) with a total of lactic acid bacteria colonies frozen yoghurt on average 86.8 x 10⁸ CFU / mL and the lowest is in the starter treatment 3% (A1) with a total Lactic Acid Bacteria colonies frozen yoghurt an average of 63.3 x 10⁸ CFU / mL. The results showed that using a combination of the bacteria Lactobacillus fermentum L23, Pedicoccus acidilactici, and Streptococcus thermophilus produced quite a lot of total lactic acid bacteria colonies up to 86.8 x10⁸ CFU / ml. This is because in the milk fermentation process in the form of yoghurt a starter is needed, which usually consists of two or more mixtures of Lactic Acid Bacteria. Mixed culture for starters is necessary so that interactions between bacteria occur. Still, there will be a decrease when the growth of lactic acid bacteria is faster, and nutrients are decreasing, where there is a decrease in the provision of starter concentrations of 7% with a total of 77.2 x 10⁸ CFU / mL lactic acid bacteria colonies. This is supported by the opinion of [20] that bacterial growth will be optimal when the amount of nutrients available in the media or products helps, when the amount of nutrients is in the reduced media, there will be competing with other microbes, resulting in decreased microbial numbers. The decrease in total lactic acid bacteria colonies was also influenced by the increasingly acidic condition of the product due to lactic acid production by lactic acid bacteria.

In frozen yoghurt with the addition of the concentration of Fruits Tamarillo (Factor B), there was a significant effect (P <0.05) on the total lactic acid bacteria colonies, which ranged from 63.4 - 85.86 x 10⁸ CFU / mL. Where the highest was in the addition of 5% (B2) Fruits Tamarillo with an average total of lactic acid bacteria colonies 85.86 x 10⁸ CFU / mL and the lowest was in the expansion of 0% (B1) Fruits Tamarillo treatment with an average total of lactic acid bacteria colonies of 63.4 x 10⁸ CFU / mL. The effect of adding Fruits Tamarillo to frozen yoghurt is due to the addition of Fruits Tamarillo...
(Factor B), which can act as a prebiotic for nutrition for lactic acid bacteria so that it can affect the total lactic acid bacteria found in frozen yoghurt. According to research by [21], Fruits Tamarillo contains hydrocolloid prebiotics found in the seeds and pulp. Hydrocolloid extract in tamarillo was proven to increase the number of \textit{Lactobacillus} and \textit{Pediococcus} during 24 hours fermentation. So with the addition of Fruits Tamarillo, it allows the growth of the total amount of lactic acid bacteria colonies frozen yoghurt. According to [22], the total population of bacteria in yoghurt is $10^7$ CFU/mL.

### 3.4. Antioxidant activity

The antioxidant activity of frozen yoghurt in the combination of the addition of starter concentration and the concentration of the expansion of Fruits Tamarillo to frozen yoghurt showed no interaction with the antioxidant activity of frozen yoghurt (analyzed using SPSS software version 22 for windows). In table 4 below shows the results of further data testing using the DMRT follow-up test at the 5% level for each factor.

| Factor A (Starter) | Factor B (Fruits Tamarillo) | Average |
|--------------------|-----------------------------|---------|
|                    | B1  | B2  | B3  |       |
| A1                 | 8.78| 44.45| 48.38| 33.87 |
| A2                 | 10.38| 44.25| 48.75| 34.46 |
| A3                 | 11.84| 45.16| 48.37| 35.13 |
| Average            | 10.33$^c$| 44.62$^b$| 48.50$^a$|        |

Note: Numbers in the same row followed by the same lowercase letter and numbers in the same column followed by the same capital letter are not significantly different according to the 5% level of DMRT.

In table 4, it shows that frozen yoghurt with the difference in the addition of starter concentration (Factor A) has no significant effect ($P < 0.05$) on the antioxidant activity of frozen yoghurt. The mean of frozen yoghurt antioxidant activity with the difference in the addition of starter concentration (Factor A) ranged from 33.87% -55.13%. The absence of an increase with the addition of a starter of frozen yoghurt was due to the \textit{Lactobacillus} starter's low activity. This is following the opinion of [23], which states that sugar's ability to be converted into lactic acid by lactic acid bacteria is synergistic by providing H$^+$ ions to free radicals, thereby increasing primary antioxidant activity.

In frozen yoghurt with the addition of the concentration of fruits Tamarillo (factor b), there was a significant effect ($p < 0.05$) on the antioxidant activity ranging from 10.33% -48.50%. An increase in antioxidant activity and the addition of fruits Tamarillo (factor b) is due to supplements from fruits tamarillo, which contain phenolic compounds that function as antioxidants. according to the research results, the antioxidant activity in fruits Tamarillo is 64.99%. So that the higher the fruit Tamarillo added, the higher the antioxidant activity of the yoghurt. As seen in the research results, the highest addition of fruits Tamarillo was 10%, resulting in the most increased antioxidant activity, namely 48.50%.

### 4. Conclusion

The results of this study showed a significant effect ($P < 0.05$) on each factor along with the best value where the starter concentration (factor A) affected the pH value (4.68), TTA (0.75%) and total lactic acid bacteria colonies ($86.8 \times 10^8$ CFU / mL). Then the concentration of Fruits Tamarillo (factor B) affects the pH value (4.66), TTA (0.80%), total lactic acid bacteria colonies ($85.6 \times 10^8$ CFU / mL), and antioxidant activity (48.50%). This study's results are expected to be a reference in the development of frozen yoghurt products that can increase immunity.
Acknowledgment
This research was supported by research cluster publications to professors (Project No: T/8/UN.16.17/PT.01.03/Pangan-PTU-KRP2GB/2020) LPPM Andalas University, Padang. Chairman: Dr Sri Melia, STP, MP.

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