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Effect of Temperature on the Substitution Equilibrium of Longan, Beetroot, and Coconut Juice on the Total Acid Content and Vitamin C Content of Cow's Milk Yogurt

Cindy Andriyani Siallagan, Chaira Adinda Rambe, Dinda Salsabillah, Kartika Tri Aullia Upara, Ribka Angeli Sinaga, Zuky Andus Rivaldi Situmorang*, Ahmad Nasir Pulungan, Moondra Zubir

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan 20221, Indonesia

**Corresponding author: zukysitumorang@gmail.com*

ABSTRACT

Variations in fermentation temperature and types of fruit juice added to yogurt affect the acid content, pH, and vitamin C content. This study analyzed yogurt with the addition of longan, beetroot, and coconut juice at fermentation temperatures of 35°C, 40°C, and 45°C. The results showed that beetroot juice had a stable acid content at all temperatures, while longan and coconut juices fluctuated. Increasing the fermentation temperature generally decreased the pH due to acid production by lactic acid bacteria, with pH stabilization occurring at a temperature of 40°C. Vitamin C content increased with increasing fruit juice concentration, but decreased during fermentation due to oxidation, with the greatest decrease in 12-hour fermentation. Beetroot juice was more stable in maintaining acid and vitamin C levels than longan and coconut juices. These results indicate that the selection of fruit juice types and fermentation temperatures is very important in maintaining the quality of yogurt.

Keywords: yogurt, fermentation, temperature, acid content, vitamin C

1. INTRODUCTION

Yogurt is a fermented milk product that has high nutritional value and is beneficial for health. The fermentation process by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* produces lactic acid which affects the taste, texture, and nutritional content of yogurt. One important factor in the quality of yogurt is the total acid content and vitamin C content, which can be affected by additional ingredients such as fruit juice.

The addition of fruit juice to yogurt is an interesting innovation because it can increase nutritional value, enrich the taste, and provide additional health benefits. Longan fruit (*Dimocarpus longan*) contains natural sugars, organic acids, and antioxidants that can support the fermentation process and increase total acid levels. Red beet (*Beta vulgaris*) is rich in betacyanin and vitamin C, which can strengthen antioxidant activity and affect the color stability of yogurt. Meanwhile, why, which refers to certain types of fruit, can

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have its own effect on the composition of acids and vitamins in yogurt.

This study aims to evaluate the effect of longan, red beet, and why fruit juice substitution on total acid levels and vitamin C content in cow's milk yogurt. The results of this study are expected to provide scientific insight for the development of more nutritious and innovative fruit juice-based yogurt, as well as support the improvement of the quality and health benefits of fermented milk products.

2. METHODOLOGY

2.1. Tools and materials

The raw materials used in this study were . While the chemicals used for analysis were fresh cow's milk, longan fruit, beetroot, coconut water, indicators, distilled water, plain biokult yoghurt bacterial starter, 0.01 N NaOH, 0.1 N standard iodine solution, and 1% starch.

The tools used are digital scales, ovens, water baths, incubators, glass jars, burettes, aluminum foil, filter paper, Erlenmeyar, beaker glasses, pH meter paper, watch glasses, glass funnels, dropper pipes, stirring rods, stoves, measuring flasks, knives, and spatulas.

Analysis of acid levels from the incubation process of cow's milk fermentation with added fruit juice, through a comparison of various temperature variations can be done using acid-base titration. Acid-base titration is a titration to determine the level or concentration of a base solution with an acid solution of known levels involving a standard NaOH solution. Acid-base titration is a neutralization titration method. The principle is that the sample or analyte is titrated drop by drop until the titration endpoint occurs, marked by the analyte turning pink (purple) in a basic atmosphere. In determining the sample level (vitamin C), the iodometric titration method can be used. Iodimetric titration is a redox titration involving standard iodine solutions (I2) and (I-). Basically, the oxidizing sample is reduced with excess potassium iodide, then the resulting iodine is titrated with a standard sodium thiosulfate solution. Iodimetric titration uses a standard iodine solution for direct titration. The principle is the redox reaction $I2 + 2e \rightarrow 2I^-$, where iodine functions as an oxidizing agent. Determination of metampirone levels under neutral conditions shows the end point of titration, with a persistent yellow color change under acidic conditions and a blue color change under acidic conditions.

2.2. Research methods

The research method is the manufacture of yogurt from cow's milk by fermentation with the addition of longan, beet, and coconut juice which is incubated at various temperatures with testing the analysis of total acid content and vitamin C content in each fermented yogurt to which fruit juice is added.

2.3. Research Procedure Organoleptic Examination

Yogurt samples added with longan, beetroot and coconut juice based on the cooking process were checked for quality using the five senses with observations including shape, size, texture, color, taste and smell.

a. Acid Content Analysis

10 grams of sample was put into a 100 milliliter measuring flask and added with distilled water until the boundary mark. After that, the mixture was homogenized and filtered. 10 ml of filtrate was taken and put into an Erlenmeyer flask. 2 or 3 drops of pp indicator were added and titrated with 0.01N NaOH solution until the color of the solution turned pink and did not change for 30 seconds. At the end of the titration, how much NaOH was used at the end of the titration was calculated.

Total acid value of the product (%) = ((V x N x P x BE acid)/(sample weight x 1000)) x 100%.

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b. pH analysis

Approximately 30 ml of homogenized sample (fermentation medium) was taken and put into a 50 ml glass beaker. Before use, the pH meter was calibrated using pH 7 and 4 buffers, and then cleaned with distilled water and then the pH of the sample was measured. Before using distilled water to measure the pH of other samples, clean the pH meter.

c. Determination of Sample Content by Iodimetric Titration Method

10 grams of yogurt fermentation sample solution was put into an Erlenmeyer. Then, 1.2 ml of 10% H2SO4 solution and a few drops of 1% starch solution were added. The solution was then titrated with 0.01N standard iodine (I2) until the color became stable blue. Duplo measurements were carried out. The following formula is used to calculate the levels:

$$\text{Vitamin C Levels } = \frac{VI2\ X\ NI2\ X\ 8{,}808}{mg\ sample\ x\ 0{,}1} \text{ x } 100\%$$

Information:

VI_2	: Iodine Titration Volume
NI_2	: Iodine Normality
8,808	: Equivalence of Vitamin C to 0.1 N
Iodine Mg sample	: Sample Weight

3. RESULTS AND DISCUSSION

Tabel 1. Organoleptic Examination Results

Organoleptic Examination

Sample	T^0C	Smell	Flavor	Texture
Longan Fruit Juice	35	Normal	Sour	Thin
	40	Normal	Very sour	Thick
	45	Normal	Not too sour	More compact
Beetroot Juice	35	Yogurt	Very sour	Thick
	40	The smell of cow's milk	Sour	Thin
	45	Yogurt	Sour	More compact
Coconut Juice	35	Normal	Sour	Thin
	40	Normal	Not too sour	Normal
	45	The smell of cow's milk	Sour (no coconut taste)	Thick

From Table 1. Organoleptic examination aims to control product quality. Yogurt samples with the addition of several fruit juices were examined using the five senses with observations including shape, size, texture, color, taste, and smell. Organoleptic tests are based on human responses as subjective measures using the sensitivity of the five senses to measure the acceptance of product quality. The results of the organoleptic test can be seen in Table 1. Based on the results of the organoleptic examination, the addition of

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fruit juice in yogurt fermentation provides variations in smell, taste, and texture. Longan juice produces yogurt with a normal smell at all temperatures, a sour taste that decreases at high temperatures, and a texture that becomes denser as the fermentation temperature increases.

Beetroot juice showed a change in odor from yogurt to cow's milk at certain temperatures, with the most sour taste at low and high temperatures, and a texture that tended to be thinner at 40°C. Coconut juice had a normal odor at low temperatures, but a cow odor appeared at high temperatures. The sour taste remained, but the distinctive coconut flavor disappeared at 45°C, while the texture became thicker. From these data, longan juice provided increased aroma stability and density, beetroot juice produced a more sour but less stable yogurt, while coconut juice experienced a change in odor and lost its distinctive flavor at high temperatures.

Raw Material Analysis

Tabel 2. Characteristics of Raw Materials for Longan, Beetroot and Coconut Juice

Parameter Longan Juice	Cow's Milk			Beetroot Juice			Coconut Juice			
	35⁰C	40⁰C	45⁰C	35⁰C	40⁰C	45⁰C	35⁰C	40⁰C	45⁰C	
Total Acid %	-	0,045	0,036	0,054	0,045	0,045	0,045	0,045	0,018	0,036
Ph	4,0	5,0	5,2	5	4,8	5,1	4,5	5,0	4,0	5,4

Based on Table 2. the addition of fruit juice to yogurt shows different variations in acid and vitamin C levels depending on the type of fruit juice and processing temperature. Longan juice has a varying acid content, namely 0.045% at 35°C, decreasing to 0.036% at 40°C, and increasing to 0.054% at 45°C. Beetroot juice shows a stable acid content of 0.045% at all temperatures. Meanwhile, coconut juice has a lower and more fluctuating acid content, namely 0.045% at 35°C, decreasing drastically to 0.018% at 40°C, and increasing again to 0.036% at 45°C. From these data, it can be concluded that beetroot juice has the most stable acid content, while coconut juice tends to have a lower and unstable acid content.

Table 3. Results of measuring Vitamin C levels using Iodimetric Titration

Sample	Titration volume (ml)	Sample Volume (ml)	% Vitamin C	Average % Vitamin C
Longan Fruit Juice	**0,25**	**10**	**0,0220**	
	0,2	**10**	**0,0176**	**0,0190**
	0,2	**10**	**0,0176**	
Beetroot Juice	**0,5**	**10**	**0,0440**	
	0,5	**10**	**0,0440**	**0,0381**
	0,3	**10**	**0,0264**	
Coconut Juice	**0,4**	**10**	**0,0352**	
	0,2	**10**	**0,0176**	**0,0322**
	0,5	**10**	**0,0440**	

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Based on the data obtained, the levels of vitamin C in various types of fruit juices showed different variations. Beetroot juice had the most stable vitamin C levels, which was 0.045% in all samples tested. This shows that beetroot juice is the best choice if the consistency of vitamin C levels in the product is prioritized. Longan juice has vitamin C levels that vary between 0.036% and 0.054%, with an average of 0.045%. Although there is a slight fluctuation, the vitamin C levels in longan juice are still relatively high and stable.

Meanwhile, coconut juice shows the lowest levels of vitamin C, with values ranging from 0.018% to 0.045%, and an average of only 0.033%. The variation in vitamin C levels in coconut juice is quite significant, which shows the instability of the vitamin C content in this sample. From the data obtained, it can be concluded that beetroot juice is the best choice to maintain vitamin C levels in the product. Longan juice can still be used because it has fairly stable levels, while coconut juice is less than optimal in increasing the vitamin C content in processed products such as yogurt.

Analysis of Acid Levels and Vitamin C Levels in Yogurt After Fermentation

1. pH analysis

The results of the pH analysis of longan, beetroot, and coconut fruit juice yogurt with the treatment of adding fruit juice and fermentation time obtained an average pH ranging from 43.2 - 50.1. The effect of the treatment of adding fruit juice and fermentation time on the pH of longan, beetroot, and coconut fruit juice yogurt can be seen in Figure 1.

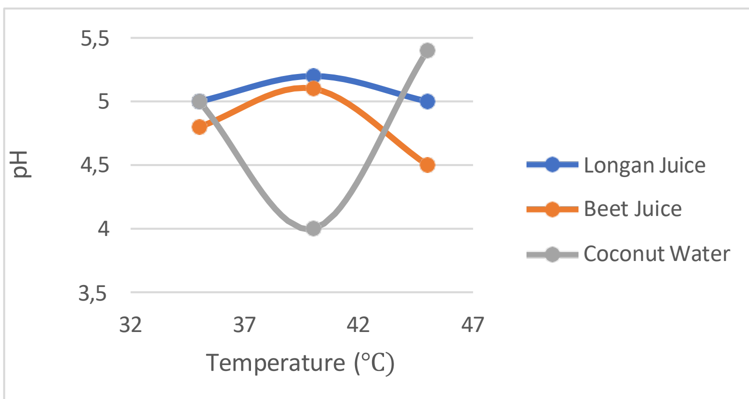

Figure 1. Yogurt pH graph with added fruit juice and fermentation time

From the graph Figure 1. Shows the change in pH value. The decrease in pH value is caused by an increase in total acid in the ingredients. The high acid content is likely caused by the addition of fruit juice, which can stimulate the activity of lactic acid bacteria. A suitable environment for these bacteria allows them to break down nutrients in the substrate, thus producing more acid. Interestingly, the highest pH value was found at a temperature of 400C with a pH of 4. This is thought to occur because the acidity level that is too high at this concentration can inhibit the adaptation phase of lactic acid bacteria, so that lactic acid production becomes more limited and the pH value does not experience a significant decrease. In general, the more acid that is formed, the lower the pH value produced. In addition, the longer the fermentation process takes place, the lower the pH formed in yogurt. This decrease is related to the activity of lactic acid bacteria that convert lactose in milk into lactic acid. This increase in lactic acid levels will increase the concentration of hydrogen ions, which ultimately causes the pH of yogurt to decrease. The fermentation that occurs is characterized by

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the growth of the lactic acid bacteria population in yogurt, which contributes to the accumulation of organic acids and causes a decrease in pH.

2. Analysis of the Effect of Variations in Yogurt Fermentation Incubation on Acid Content

The results of the acid content analysis based on the data in Table 2, the addition of fruit juice to yogurt showed different variations in acid content depending on the type of fruit juice and the processing temperature used. This indicates that temperature can affect chemical reactions and the activity of microorganisms during the fermentation process as seen in Figure 2.

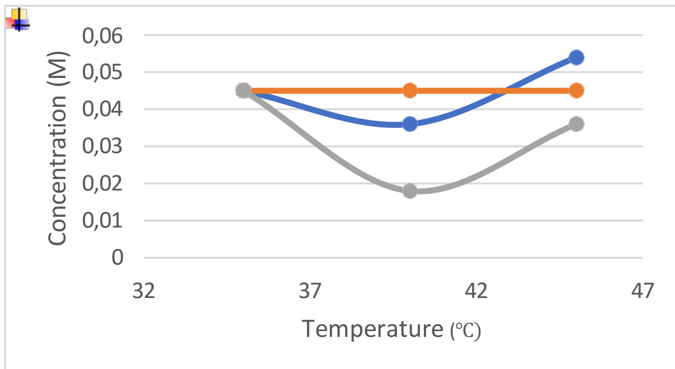

Figure 2. Graph of total yogurt acid with the addition of fruit juice due to the treatment of adding longan, beetroot and coconut fruit juice with fermentation time

Based on the data obtained from Figure 2. shows the pH balance in each fruit juice. In yogurt added with longan juice, the acid content experienced quite dynamic changes. At a temperature of 35°C, the acid content was recorded at 0.045%, then decreased to 0.036% at a temperature of 40°C, and increased again to 0.054% at a temperature of 45°C. This pattern shows that at a temperature of 35°C, the acid content has formed in a certain amount. However, at a temperature of 40°C, there was a decrease in the acid content which can be interpreted as a condition where the temperature is at a point that is not optimal for the activity of enzymes or microorganisms that produce acid, so that acid production is inhibited. The increase in acid content again at a temperature of 45°C indicates that higher temperatures can reactivate the reaction mechanism that produces acid, so that there is an increase in acid content in yogurt. Meanwhile, yogurt with the addition of beetroot juice showed stable acid levels at all temperatures tested, which remained at 0.045% at 35°C, 40°C, and 45°C. This shows that the components of beetroot juice have high chemical stability against temperature variations, so that the acid formation reaction does not experience significant changes. This stability indicates that both microbial activity and chemical reactions that produce acid are not significantly affected by increasing temperatures within the range studied.

In contrast to longan juice and beetroot juice, yogurt added with coconut juice showed greater fluctuations in acid levels. At a temperature of 35°C, the acid content was recorded at 0.045%, then decreased drastically to 0.018% at a temperature of 40°C, before increasing again to 0.036% at a temperature of 45°C. This fluctuation indicates that the components of coconut juice are more sensitive to temperature changes. The drastic decrease in acid levels at a temperature of 40°C is most likely caused by enzyme inactivation or degradation of acid compounds that occurs at that temperature, thus inhibiting acid formation. However, the increase in acid levels again at a temperature of 45°C indicates that although degradation occurs at a certain temperature point, at a higher temperature the acid formation reaction is active again,

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although it does not reach the initial level as at a temperature of 35°C. The results of the data analysis obtained indicate high stability of acid levels to temperature changes, while longan juice and coconut juice show more dynamic variations in acid levels. These differences are most likely due to the chemical characteristics and composition of each fruit juice, which influence the enzymatic response and microbial activity during fermentation.

3. Analysis of the Effect of Yogurt Fermentation Incubation Variations on Vitamin C Levels

From the data results show that the average value of vitamin C levels in yogurt due to the addition of longan, beetroot, and coconut juice and the fermentation time of the average levels of each fruit juice is 0.0190% longan fruit, 0.0381 beetroot, and 0.0322 coconut fruit. Changes in the average levels of vitamin C in various treatments can be seen in Figure 3.

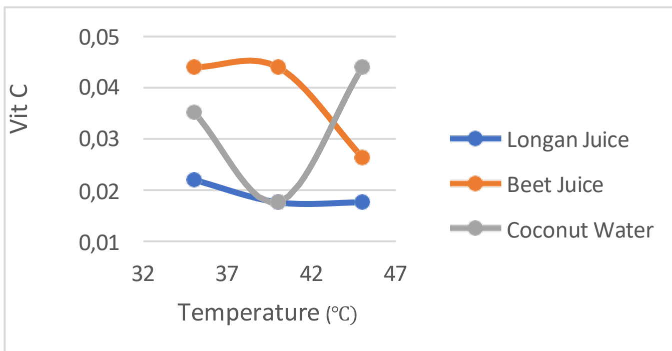

Figure 3. Graph of vitamin C levels in yogurt with the addition of longan, beetroot, and coconut juice due to the treatment of adding fruit juice and fermentation time.

From Figure 3. The increase in the concentration of fruit juice added to yogurt is directly proportional to the amount of vitamin C detected. The more fruit juice used, the higher the vitamin C content in yogurt. This causes the amount of remaining vitamin C to remain high even though oxidation occurs during the fermentation process. However, as the fermentation time increases, the levels of vitamin C in yogurt decrease. The most significant change in vitamin C levels occurred in fermentation for 12 hours, while the smallest change in vitamin C levels was recorded in fermentation for 6 hours. This phenomenon is likely caused by the fermentation temperature approaching the temperature threshold that accelerates the oxidation of vitamin C to dehydroascorbic acid. This decrease in vitamin C levels occurs due to the oxidation process, where ascorbic acid changes to L-dehydroascorbic acid due to heat exposure. Furthermore, L-dehydroascorbic acid can undergo further degradation to L- diketogulonic acid, which no longer has activity as vitamin C.

CONCLUSION

The results showed that fermentation temperature and type of fruit juice significantly affected the acid content, pH, and vitamin C content in yogurt. Longan and coconut juices experienced fluctuations in acid levels at various temperatures, while beet juice showed stable acid levels at all fermentation temperatures. In general, the higher the fermentation temperature, the lower the pH value due to increased acid production by lactic acid bacteria, although at 40°C pH stabilization occurred due to less than optimal environmental

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conditions for bacteria. The vitamin C content in yogurt increased with the addition of fruit juice, but decreased during fermentation due to oxidation accelerated by temperature. The most significant decrease in vitamin C levels occurred in fermentation for 12 hours. From these results, it can be concluded that the selection of the type of fruit juice and fermentation temperature is very important to maintain the stability of acid levels, pH, and vitamin C in yogurt. Beetroot juice is the best choice in maintaining stable acid and vitamin C levels, while longan juice and coconut juice show more dynamic variations. The results of this study can be a reference in the development of fruit juice-based yogurt that is more nutritious and stable.

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