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Research Paper

Chemical analysis of fermented milk during refrigerator storage with the addition of mangosteen rind extract (*Garcinia mangostana* L.)

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ABSTRACT: *This study aims to determine the effect of storage time of fermented milk with the addition of mangosteen rind extract stored at refrigerator temperature using starter Lactobacillus Plantarum strain SRCM 102737 and Lactobacillus brevis strain 200335 on moisture content, protein content, fat content, and pH. This study used fresh goat's milk and mangosteen rind extract. This study used a 3x4 Factorial Completely Randomized Design method with 3 replications. The treatment given was fermented milk stored at refrigerator temperature by storage time of 0 days (A1), 14 days (A2), 28 days (A3), and the difference in concentration of the mangosteen rind extract was 0% (B1), 1% (B2), 2% (B3) and 3% (B4). The variables measured were moisture content, protein content, fat content, and pH. The results showed that storage time at refrigerator temperature significantly affected storage time (P<0.05) on moisture content (83.16%), fat content (3.46%), and pH (4.44). The concentration of mangosteen rind extract had a significant effect (P<0.05) on protein content (4.30%) and fat content (3.44%). From this study, it can be concluded that storage of fermented milk with the addition of mangosteen rind extract as much as 3% at refrigerator temperature for up to 28 days is still able to maintain milk quality.*

KEYWORDS: *Fermented milk, Lactobacillus Plantarum strain SRCM 102737, Lactobacillus brevis strain 200335, storage, mangosteen rind extract*

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I. INTRODUCTION

Lactic acid bacteria are good bacteria that have many benefits and can be widely used in the processing of livestock products such as milk. The addition of lactic acid bacteria in the manufacture of fermented milk will add longer shelf life and offer better benefits if consumed by the body. [1] conducted a study on the manufacture of fermented milk using the bacterium *Pediococcus acidilactici* BK01 at storage for 28 days in the refrigerator. It was found that fermented milk with a storage of 28 days still had a good quality.

Fermented milk is a dairy product that is fermented using Lactic Acid bacteria. The raw materials used in the manufacture of fermented milk can be cow's milk, buffalo milk, sheep's milk, and goat's milk (Melia et al., 2020) [1]. One type of milk that is still not commonly consumed is goat's milk. Goat's milk has better advantages than cow's milk. According to [2], goat milk has a protein content of 4.3% and fat 2.8% is relatively better than cow's milk protein content with 3.8% protein and 5.0% fat. The mixture is more homogeneous and easy to digest because the fat granule molecules are smaller, namely, 3.49 mm while cow's milk is 4.55 mm and consists of short and medium-chain fatty acids that do not contain - lactoglobulin which is the cause of allergies [3]. Therefore, processing goat's milk into fermented milk, so that it will create a variety of fermented milk products that are not only derived from cow's milk but also goat's milk.

To improve the quality of fermented milk so that it can become functional food, probiotics and mangosteen rind extract are added which can improve the quality and nutritional value of fermented milk as a functional food, making it interesting to study. Mangosteen rind contains xanthone compounds, which are bioflavonoids with antioxidant, antibacterial, anti-allergic, antitumor, antihistamine, and anti-inflammatory properties [4]. [5] stated that the antioxidant ability of xanthones even exceeds vitamins A, C, and E which have been known as the most effective antioxidants in fighting free radicals in the body. Xanthones are very beneficial for the health of the body as an antioxidant, anti-histamine, anti-inflammatory, and anti-microbial.

II. MATERIALS AND METHODS

2.1. Research Materials

This study used lactic acid bacteria Lactobacillus Plantarum strain SRCM 102737 and Lactobacillus brevis starin 200335, Goat's milk was taken at Simpang Pelangi, Korong Gadang, Kuranji District, Padang City and mangosteen was taken at Jl. Puti Bungsu, Tunggang Incarnation, Lubuk Basung District, Agam Regency. The research method used a randomized block design (RAK) with 4 treatments with 3 replications. The treatments used were storage for A1 (0 days), A2 (14 days) and A3 (28 days) and the addition of mangosteen rind extract B1 (0%), B2 (1%), B3 (2%) and B4 (3 %). In this study, the measured variable are the moisture content, protein content, fat content and pH value of fermented milk.

2.2. Mangosteen rind extraction process

The procedure for making modified mangosteen rind extract [6], the flesh of the mangosteen rind was cut into small pieces, then dried in an oven at 60^{o}C until it dries. mash the dried mangosteen rind until crushed then do the sifting of the mangosteen rind. Maceration is done by soaking the mangosteen rind in ethanol 96% soak for 2 x 24 hours with the ratio between the mangosteen rind and ethanol 96% is 1:10 for the first day, and 1:4 for the second day. The maceration results are filtered using Whatman 41 paper, the filtered results are evaporated using rotary evaporation.

2.3. Fermented milk production

Goat milk was pasteurized at a temperature of 65^{o}C for 30 minutes. Starter *Lactobacillus Plantarum* strain SRCM 102737 and *Lactobacillus brevis* starin 200335 added as much as 5%, next incubated for 12 hours at 37^{o}C temperature.

2.4. Parameters

1. Moisture Content

The dishes are oven at 110ºC for one hour. The plates were cooled in a desiccator to remove moisture and weighed. The sample was weighed as much as 5 grams on a plate that had been dried and baked at 105ºC for 8 hours. The sample was then cooled in a desiccator for 30 minutes and weighed. This stage is repeated until a constant weight is achieved [7].

2. Protein Content

Destruction stage

The sample is weighed as much as 1 gram. The sample was put in a Kjeldahl flask. One grain of selenium is put into the tube and 1 gram of H2SO4 is added. The tube containing the solution is inserted into the heating device. The digestion process is carried out until the solution becomes clear.

Distillation stage

The clear solution was cooled and taken 25 ml of the sample and then added 150 ml of distilled water and 25 ml of 30% NaOH then distilled. The solution is heated (2/3 distilled) until all N from the liquid in the flask is captured by 0.05 N H2SO4 which has been mixed with methyl red indicator drops in Erlenmeyer

The titration stage

The distillate in erlenmeyer was titer with 0.01N NaOH. A total of 25 ml of 0.05 N H2SO4 plus 3 drops of the red methylated indicator was added to another Erlenmeyer and titrated with 0.1 NaOH, resulting in a color change from pink to yellow as blank [7].

3. Fat Content

The fat squash will be used in the oven for 30 minutes at a temperature of 100-105ºC. The fat flask is cooled in a desiccator to remove moisture and weighed. The sample is weighed as much as 1 g. then wrapped in filter paper, covered with fat-free cotton, and put in a soxhlet that has been connected to the fat flask. Samples were previously oversized and their weights were determined. The hexane solvent is poured until the sample is immersed and reflux or extraction is carried out for 5-6 hours or until the fat solvent that drops into the fat flask is clear. The fat solvent that has been used, refined and collected. The fat extract in the fat flask is dried in an oven at 100-105ºC for 1 hour. The fat flask is cooled in a desiccator and weighed. The drying stage of the fat flask was repeated until a constant weight was obtained [7].

4. pH

The degree of acidity of the sample was measured using a pH meter, and calibrated with a buffer solution with pH values of 4 and 7. The sample was prepared as much as 5 grams, then added with 10 mL of distilled water, after which the sample was stirred for five minutes. The sample is transferred to the measuring

cup, the pH meter is immersed in the sample approximately 2-4 cm. The pH value is obtained by reading the scale indicated by the needle [7].

III. RESULTS AND DISCUSSION

3.1. Moisture Content

Table 1. The average value of moisture content

Factor A (Storage Time)	Factor B (Concentration of KBM Extract)				Average
	B1	B2	B3	B4	
A1	84,29	84,88	84,18	83,29	84,16[a]
A2	83,80	83,82	83,46	83,11	83,55[ab]
A3	83,42	83,17	82,98	83,04	83,16[b]
Average	83,84	83,96	83,54	83,15	

Description: The mean of superscripts with lowercase letters ([ab]) shows a significant difference (P<0.05)

Based on the results of the study, it can be seen that there is no interaction from the addition of mangosteen rind extract concentration on the storage time of fermented milk. Meanwhile, the storage time of fermented milk for 0 days (A1), 14 days (A2), and 28 days (A3) had a significant effect (P<0.05) on the moisture content of fermented milk. The average moisture content of fermented milk during storage ranged from 83.16% - 84.16%. The length of storage will affect the quality of dairy products, moisture content is one that greatly determines the quality of fermented milk products. [8] stated that the factor that greatly affects product quality is moisture content. Changes in moisture content can be affected by the storage temperature of the product. [9] stated that the length of storage will affect the moisture content of foodstuffs, where the longer the storage time, the amount of moisture content in food products decreases along with the length of storage. Based on the results of the study, the average moisture content of fermented milk ranged from 83.16% - 84.16%. This is by the research of [1] the average moisture content of fermented milk with the addition of starter *Pediococcus acidilactici* BK01 with a storage time of up to 28 days ranged from 84.92% - 85.57%. [10] stated that the moisture content of yogurt using *Lactobacillus fermentum* L23 and *Streptococcus thermophilus* bacteria with the addition of dragon fruit extract ranged from 81.22% - 83.91%.

3.2. Protein Content

Table 2. Average protein content

Factor A (Storage Time)	Factor B (Concentration of KBM Extract)				Average
	B1	B2	B3	B4	
A1	3,63	3,76	3,80	4,15	3,84
A2	3,64	3,79	4,37	4,38	4,05
A3	3,65	3,95	4,38	4,36	4,09
Average	3,64[c]	3,83[bc]	4,18[ab]	4,30[a]	

Description: The mean of superscripts with lowercase letters ([abc]) shows a significant difference (P<0.05)

Table 2 shows the average protein content of fermented milk with the concentration of the addition of mangosteen rind extract (Factor B) 0%, 1%, 2%, and 3% ranging from 3.64% - 4.30%. The treatment of mangosteen rind extract with different concentrations (Factor B) had a significant effect (P<0.05) on the protein content of fermented milk. The higher the concentration of mangosteen rind extract, the higher the protein content of fermented milk. This could be because the protein content in the mangosteen rind extract affects the protein content of fermented milk. According to [11], the protein content in the mangosteen rind is around 3.02%. Therefore, the greater the addition of mangosteen rind extract will increase the protein content of fermented milk. In addition, mangosteen peel extract contains phenolic compounds that can affect in protein content of fermented milk. [12] stated that antioxidants contained in mangosteen rind extract can prevent damage caused by free radicals to normal cells and proteins.

Based on the research conducted, the range of fermented milk protein content ranged from 3.64% - 4.30%. Based on the research of [10] that yogurt with the addition of dragon fruit extract has protein levels in the range of 2.66% - 4.25%. The research of [1] stated that fermented milk using a bacterial culture of *Pediococcus acidilactici* BK01 at storage for up to 28 days had protein levels in the range of 3.53% - 3.58%. When compared with Indonesian national standards [13] the minimum protein content for yogurt fermented

milk products is 2.7%. The results of the study with a storage period of 28 days with the addition of mangosteen rind extract up to 3% meet Indonesian national standards.

3.3. Fat Content

Table 3. Average fat content

Factor A (Storage Time)	Factor B (Concentration of KBM Extract)				Average
	B1	B2	B3	B4	
A1	3,70	3,52	3,50	3,46	$3,55^{b}$
A2	3,52	3,46	3,45	3,42	$3,47^{a}$
A3	3,48	3,46	3,45	3,42	$3,46^{a}$
Average	$3,57^{c}$	$3,48^{b}$	$3,47^{a}$	$3,44^{a}$	

Description: The mean of superscripts with lowercase letters (^{abc}) shows a significant difference (P<0.05)

Table 3 shows the average fat content of fermented milk with storage time (Factor A) 0, 14, and 28 days ranging from 3.46% - 3.55%. The decrease in fat content of fermented milk can be caused by the activity of lipase enzymes produced by microorganisms found in fermented milk. This is by the opinion of [14] which states that the decrease in kefir fat content can be caused by the production of lipase enzymes by microorganisms found in kefir. The lipase enzyme produced will hydrolyze fat which will cause a decrease in fat content in milk. [15] stated that during the fermentation process bacteria will undergo three main reactions in decomposing milk components, namely the decomposition of lactose into lactic acid, hydrolyzing casein into peptides, degrading protein into amino acids, and breaking down milk fat into free fatty acids. The decrease in fat content is related to the degradation of fat in milk into free fatty acids and volatile components. This is by [16] statement that lipolysis of fat components in milk will form free fatty acids.

The concentration of addition of mangosteen rind extract (Factor B) 0%, 1%, 2% and 3% ranged from 3.44% - 4.57%. The addition of mangosteen rind extract in the manufacture of fermented milk (Factor B) 0%, 1%, 2%, and 3% had a significant effect (P<0.05) on the fat content of fermented milk. Fat content decreased slightly with increasing concentration of mangosteen rind extract. This can be caused because the mangosteen rind contains low-fat content so that the more addition of the mangosteen rind there will be a decrease in the fat content of fermented milk. [17] stated that the mangosteen rind extract contains a fat content of 0.32%. The small amount of fat in the mangosteen rind extract will cause a decrease in the fat content of fermented milk as the concentration of the addition of the mangosteen rind extract increases. In this study, the fat content of fermented milk during 28 days of storage ranged from 3.46% - 3.55%. When compared with the Indonesian national standard [13] the fat content for yogurt fermented milk products is 3%. Based on Codex [18] states that the fat content for fermented milk is less than 10%. The results of the study with a storage period of 28 days with the addition of mangosteen rind extract up to 3% met Indonesian national standards and Codex standards.

3.4. **pH Value**

Tabel 4. pH Value

Factor A (Storage Time)	Factor B (Concentration of KBM Extract)				Average
	B1	B2	B3	B4	
A1	4,73	4,70	4,67	4,67	$4,69^{a}$
A2	4,53	4,50	4,50	4,50	$4,51^{b}$
A3	4,47	4,43	4,43	4,43	$4,44^{c}$
Average	4,58	4,54	4,53	4,53	

Description: The mean of superscripts with lowercase letters (^{abc}) shows a significant difference (P<0.05)

Based on the table above, it can be seen that the storage time of fermented milk was 0 days (A1), 14 days (A2) and 28 days (A3) had a significant effect (P<0.05) on the average pH value of fermented milk. The effect of decreasing the pH of fermented milk can be caused by the activity of lactic acid bacteria which break down lactose in milk into lactic acid, causing a decrease in the pH value of fermented milk. [19] stated that lactic acid bacteria will utilize existing carbohydrates to produce lactic acid, this will lower the pH and increase the acidity of yogurt. The decrease in pH of fermented goat's milk during storage was due to an increase in the total acid production of fermented milk. [20] stated that the pH value of fermented milk will decrease along with the length of storage in the refrigerator. [21] stated that the decrease in pH during storage was due to the metabolic activity of the culture, the longer the product was stored the more lactic acid was formed which caused the pH to tend to decrease. The pH value of fermented milk during storage ranges from 4.44-4.69. This is

by the statement of [1] added that fermented milk with a storage period of 28 days using Pediococcus acidilactici BK01 culture had a pH range of 4.28-4.48. According to Adriani [22], the quality of fermented milk is good based on the pH value, which is in the range of 3.8 to 4.6. So it can be concluded that the pH value of fermented milk with the addition of mangosteen rind extract stored in the refrigerator for 28 days still has good quality.

IV. CONCLUSION

The results concluded that the storage time of fermented milk (Lactobacillus plantarum strain SRCM 102737 and Lactobacillus brevis strain 200335) with the addition of mangosteen rind extract had a significant effect ($P<0.05$) on moisture content, fat content, and pH value. The concentration of mangosteen peel extract had a significant effect ($P<0.05$) on protein and fat levels. Fermented milk with the addition of 3% mangosteen rind extract still able to maintain its quality as a food product suitable for consumption up to 28 days of storage.

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