Physicochemical quality of fermented milk with additional red dragon fruit (*Hylocereus polyrhizus*) skin

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**Abstract.** Physicochemical qualities and total flavonoids qualities of fermented milk can be enhanced by the utilization of natural ingredients of red dragon fruit skin (*Hylocereus polyrhizus*). The purpose of this study was to analyze the effect of red dragon fruit skin concentration and length of incubation as well as their interaction toward physicochemical characteristics and total flavonoid qualities of fermented milk with the addition of red dragon fruit skin. The research method used was an experimental method by using a completely randomized design with 2 two factors. Factor A was the dragon fruit skin concentration 0%, 20%, 40%, and 60% and factor B was the incubation period ranging from 12 hours, 14 hours, until 16 hours. The treatments were repeated three times each. The data were processed statistically with analysis of variance followed by the least significant difference test. The result showed that a high concentration of red dragon fruit skin and long incubation could increase the activity of Lactic Acid Bacterial (LAB) during the fermentation process, so it increased the fat content but decreased carbohydrate and protein content of fermented milk. Therefore, it can be suggested that the concentration of dragon fruit skin used in producing fermented milk should be 60% and take 12 hours.

1. Introduction

Milk has been developed and processed by several researchers into several processed products such as fermented milk [1,2]. Fermented milk is one of the dairy products. The product has a distinctive taste and is nutritious for health. The final metabolism of the changes in milk nutrition by microorganisms determines the taste and aroma of this product [3,4]. Based on a research result, it is found out that the content of the fermented milk such as lactic acid, bacteriocin, and various other compounds can function as an antibacterial and antioxidant [3,5–9]. In addition, fermented milk can also prevent or treat diseases due to infection.

Due to the increase in public awareness about the relationship between diet and diseases, people become more selective in choosing food. The food currently being selected is food with high functional properties. The fermented milk developed as a product and that has been studied is the fermented milk with the addition of natural ingredients. The natural ingredients are easily obtained, and also have nutrients and other components needed by humans [6,7]. Dragon fruit skin as one of the agricultural wastes has the potentials to be a natural additive.
The red dragon fruit skin contains carbohydrates, fat, ash, protein, vitamin C, vitamin E, vitamin A, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and phytoalbumin. The advantage of red dragon fruit skin is that it is rich in polyphenols and is a source of antioxidants. Besides, the antioxidant activity in red dragon fruit skin is greater than the antioxidant activity in the flesh of the fruit, so it has the potential to be developed as a source of natural antioxidants [10,11]. Furthermore [12] states that dragon fruit peels still contain glucose, maltose, and fructose. The nutritional content found in dragon fruit peels has the potential to be processed into food processing ingredients. These components can be a source of nutrition for the microorganisms found in the fermented milk. The use of red dragon fruit skin in the production of fermented milk can increase the total nutrition of the fermented milk and can also affect its final quality both physically and chemically.

The production of fermented milk is very dependent on the long process of milk incubation that is taking place. The availability of nutrients and the diversity of microorganisms in the red dragon fruit skin may affect the performance of microbial decomposers during the process of making fermented milk. The incubation period could affect the fermentation process that occurs because it can affect the formation of lactic acid which is the primary metabolic process of fermentation [13,14]. Astawan [15] suggested that the longer the fermentation the more microorganisms are active, multiplying and the numbers are increasing. As more bacteria are formed and reproduce, the ability to break up the substrate is better, resulting in more lactic acid.

In relation to the theories above, a study on the addition of red dragon fruit skin with different incubation periods was carried out to investigate its potential in improving the physicochemical quality and total flavonoids of fermented milk.

2. Materials and methods

This research was carried out experimentally with a completely randomized design, factorial pattern (4×3) and threefold repetition. Factor A was the red dragon fruit skin with various concentration levels of 0%, 20%, 40% and 60%, and factor B was the incubation period ranging from 10 hours, 12 hours and 14 hours.

The equipments used in this study were sample bottles, measuring cups, micropipettes, tips, analytical scales, incubators, refrigerators, autoclaves, sonicators, spectrophotometers, bunsen, blenders, knives, pans, Petri dishes, ovens, Kjehdal flasks, measuring flask, fume hood, Erlenmeyer, dropper, distillation flask, and test tube. Furthermore, the ingredients used were full cream milk, jelly, commercial bacteria (Lactobacillus bulgaricus, Streptococcus thermophilus, L. acidophillus and Bifidobacterium longum), aquadest, alcohol, spiritus, red dragon fruit skin (Hylocereus polyrhizus), tissue, Tropicana sugar, buffer solution, selenium, H₂SO₄, H₃BO₃ 2%, HCl 0.01%, NaOH 30%, chloroform, phenolphthalein, AlCl₃, NaCOOCH₃, methanol.

2.1. Research procedure

2.1.1. Preparation of the dragon fruit skin solution (Hylocereus polyrhizus). The preparation process of the red dragon fruit skin solution consisted of some stages. First, the red dragon fruit skin was peeled. Second, the skin was cleaned by using sterile aquadest. Third, the clean skin was then blended with distilled water with a ratio of 1: 1 which was equal to 100 g of red dragon fruit skin to 100 mL distilled water. Then, the skin of the red dragon fruit was blended until smooth and fluffy [16].

2.1.2. Making a starter. The process of making a starter was begun by mixing distilled water with 0.15% jelly (w/v), and then it was heated. After that, the distilled water and jelly were added with 10% (w/v) full cream milk, and 20%, 40%, and 60% (w/v) red dragon fruit skin extract. The mixture was placed in a sample bottle and was then heated again at the temperature level of 105ºC for 5 minutes with an autoclave. The equipment that was already in extreme cold was then inoculated with 3% (v/v) commercial product culture. The mixture was homogenized and incubated at 42ºC for 12 hours.
Activation was undertaken twice before being used as a starter for the fermented milk and storage of culture in frozen conditions [17].

2.1.3. Making fermented milk products. The process of making fermented milk was done by mixing distilled water with 0.15% jelly (w/v) and then heated with 10% (w/v) full cream milk, 20%, 40%, and 60% (w/v) red dragon fruit skin extract, and 3% (w/v) Tropicana sugar. The mixture was then heated at 105 degrees Celsius for 5 minutes. After that, it was inoculated with 3% (v/v) of the starter and then incubated at 42º C for 10, 12, and 14 hours. The incubated samples were ready to be tested [18].

2.2. Sample testing

2.2.1. Carbohydrate content. Carbohydrate (lactose) content was calculated based on the differences between the amount of water, fat and protein content and ash by 100. The carbohydrate (lactose) content was calculated using the following formula:

\[
\text{% carbohydrate content} = 100 - (\text{water} + \text{protein} + \text{fat} + \text{ash}) \times 100\%
\]

2.2.2. Measurement of hydrogen potential (pH). Hydrogen potential (pH) was measured at room temperature by using a pH meter. The pH meter was turned on and calibrated with a pH buffer of 4 and 7. After calibrating, the electrode was dipped in the sample solution of fermented milk, and the pH measurement was set. Furthermore, the electrode was allowed to immerse for a while. The read value was recorded as the pH of the sample. After measuring, the pH meter was then rinsed with distilled water and dried with a tissue.

2.2.3. Protein content. There was 0.1–0.5 g of the samples put into a 100 mL Khjehdal flask and homogenized with 1 gram selenium mixture and 10 mL H₂SO₄. The samples were destructed until clean in a fume hood and then poured into a measuring flask while rinsing it with distilled water. The samples were mixed with 10 mL H₃BO₃ 2% + 4 drops of indicator solution in Erlenmeyer. Five mL of the solution was pipetted into the distillation flask. The solution was titrated with 0.01% HCl and added 5 mL 30% NaOH + 100 mL distilled water. The protein content was calculated using the following formula:

\[
\text{% Protein content} = \frac{V_1 \times N \times HCl \times 6.38 \times p \times 14 \times 100\%}{\text{Sample weights}}
\]

Where
- \(V_1\): sample titration volume
- \(N\): Normality of 0.02 N HCl or H₂SO₄ solution
- \(P\): 100/5 dilution factor

2.2.4. Fat content. The fat content was determined by the Soxhlet method. Three grams of the samples put into a 10 mL scale test tube and chloroform was added until approaching the scale. The test tube was tightly closed, shaken and then left overnight. The tube was placed near the 10 mL scale mark with the same fat solvent, shaken until homogeneous and then filtered with filter paper into the test tube. Five milliliters of the sample was pipetted into a cup whose weight had been known (a) and then dried at 100ºC for 3 hours. The cup containing the sample was put into the desiccator for 30 minutes, then weighed (b). The fat content was then calculated by using the following formula:

\[
\text{% fat content} = \frac{p \times (b - a) \times 100\%}{\text{Sample weights}}
\]

Where
- \(p\): Dilution = 10/5 = 2
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Remodel the nutrients. Nutrition was revamped in the form of carbohydrates into lactic acid due to the long incubation period, meaning the longer the incubation period, the longer the time used by lactic acid bacteria to remodel the nutrients. Nutrition was revamped in the form of carbohydrates into lactic acid. This is consistent with the opinion of [19] who states that during the fermentation process, LAB will utilize sugar or carbohydrates found in the media to form lactic acid. During the fermentation process, LAB activity in fermented milk lactose, carbohydrates consisting of glucose, maltose, and fructose. Besides milk lactose, LAB also used carbohydrates in the dragon fruit skin as energy sources in the fermentation process. During the fermentation process, the LAB carried out a metabolic process that produced lactic acid. This is consistent with the opinion of [12] that the red dragon fruit skin contains carbohydrates consisting of glucose, maltose, and fructose. Furthermore, [20] also states that during the fermentation process, the LAB will utilize sugar or carbohydrates found in the media to form lactic acid. The LAB activity in the fermentation process can produce lactase enzymes that can break down lactose into glucose and galactose and produce lactic acid [21].

Duncan’s test results on the treatment of the incubation period show that the carbohydrate content of fermented milk at the incubation period of 10 hours was not significantly different (p>0.05) to the incubation period of 12 hours. However, the carbohydrate levels of fermented milk in the incubation period of 10 and 12 hours were significantly different (p<0.01) to the incubation time of 14 hours. It happened because the longer the incubation period, the longer the time used by lactic acid bacteria to remodel the nutrients. Nutrition was revamped in the form of carbohydrates into a simple sugar that

**Table 1. Characteristics of the fermented milk carbohydrates at different concentrations of red dragon fruit skin and incubation time.**

| Red dragon fruit skin concentration (%) | Incubation period | Mean values |
|-----------------------------------------|-------------------|-------------|
| 0                                       | 10 hours          | 12 hours    | 14 hours   | Mean values     |
| 0                                       | 9.58±0.03<sup>e</sup> | 9.89±0.02<sup>g</sup> | 10.17±0.18<sup>b</sup> | 9.88±0.27<sup>10</sup> |
| 20                                      | 9.72±0.05<sup>f</sup> | 9.37±0.13<sup>d</sup> | 9.14±0.05<sup>bc</sup> | 9.41±0.26<sup>c</sup> |
| 40                                      | 9.27±0.01<sup>cd</sup> | 9.09±0.01<sup>b</sup> | 9.13±0.07<sup>bc</sup> | 9.16±0.09<sup>b</sup> |
| 60                                      | 9.06±0.05<sup>b</sup> | 9.24±0.01<sup>cd</sup> | 8.67±0.09<sup>a</sup> | 8.89±0.25<sup>h</sup> |

Mean values 9.41±0.27<sup>10</sup> 9.39±0.32<sup>h</sup> 9.28±0.58<sup>a</sup>

Note: <sup>ABCDE</sup>Superscripts that follow the mean values in the same row and column showed highly significant differences (p<0.01).
<sup>abcdef</sup>Superscripts that follow the mean values at different treatment interactions showed highly significant differences (p<0.01).

2.3 Data analysis

The data on the physicochemical testing and flavonoid total were processed statistically with an analysis of variance based on a completely randomized design with a factorial pattern of 2 factors (4×3) with three repetitions. The treatment that significantly affected was further tested with the Duncan test [19].

3. Results and discussion

3.1 Carbohydrate content

Carbohydrate is an organic substance consisting of carbon, hydrogen, and oxygen. Carbohydrates can be classified based on the number of simple sugar molecules in the carbohydrate. Monosaccharides, disaccharides, and polysaccharides are some carbohydrate groups. The carbohydrate content of fermented milk with the addition of red dragon fruit skin and different incubation times were presented in Table 1.

**Table 1. Characteristics of the fermented milk carbohydrates at different concentrations of red dragon fruit skin and incubation time.**

| Red dragon fruit skin concentration (%) | Incubation period | Mean values |
|-----------------------------------------|-------------------|-------------|
| 0                                       | 10 hours          | 12 hours    | 14 hours   | Mean values     |
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| 20                                      | 9.72±0.05<sup>f</sup> | 9.37±0.13<sup>d</sup> | 9.14±0.05<sup>bc</sup> | 9.41±0.26<sup>c</sup> |
| 40                                      | 9.27±0.01<sup>cd</sup> | 9.09±0.01<sup>b</sup> | 9.13±0.07<sup>bc</sup> | 9.16±0.09<sup>b</sup> |
| 60                                      | 9.06±0.05<sup>b</sup> | 9.24±0.01<sup>cd</sup> | 8.67±0.09<sup>a</sup> | 8.89±0.25<sup>h</sup> |

Mean values 9.41±0.27<sup>10</sup> 9.39±0.32<sup>h</sup> 9.28±0.58<sup>a</sup>

Note: <sup>ABCDE</sup>Superscripts that follow the mean values in the same row and column showed highly significant differences (p<0.01).
<sup>abcdef</sup>Superscripts that follow the mean values at different treatment interactions showed highly significant differences (p<0.01).

Table 1 showed that the higher the concentration of the red dragon fruit skin and the longer the incubation period, the lower the carbohydrate content of the fermented milk. Analysis of variance showed that the concentration of red dragon fruit skin, incubation time and the interaction between had a very significant effect (P<0.01) on fermented milk. Duncan's test results on the treatment of red dragon fruit skin concentration showed that the carbohydrate content of fermented milk had a very significant difference (p<0.01) between each red dragon fruit skin concentration. It happened because the red dragon fruit skin contains carbohydrates consisting of glucose, maltose, and fructose. Besides milk lactose, LAB also used carbohydrates in the dragon fruit skin as energy sources in the fermentation process. During the fermentation process, the LAB carried out a metabolic process that produced lactic acid. This is consistent with the opinion of [12] that the red dragon fruit skin contains glucose, maltose, and fructose. Furthermore, [20] also states that during the fermentation process, the LAB will utilize sugar or carbohydrates found in the media to form lactic acid. The LAB activity in the fermentation process can produce lactase enzymes that can break down lactose into glucose and galactose and produce lactic acid [21].
produced lactic acid. One of the bacteria used in this study was *L. bulgaricus*. This type of LAB can convert lactose or other types of sugar into lactic acid. This is in accordance with the opinion of [22] that the *L. bulgaricus* bacteria were classified as homofermentative because it is only able to produce lactic acid in the main product of glucose fermentation. The fermentation of pentose sugar by *L. bulgaricus* will produce lactic acid and acetic acid. The formation of lactic acid from lactose is used as a source of energy and carbon during the growth of bacteria in the fermentation process so that the pH value will decrease.

Duncan's test results on the concentration interaction of the red dragon fruit skin and incubation time showed that the levels of fermented milk carbohydrates were significantly different (p<0.01) between each treatment. The higher the concentration of red dragon fruit skin and the longer the incubation process, the lower the carbohydrate content. This is due to the increase in the concentration of red dragon fruit skin so that it will increase the substrate for the metabolism of bacteria. Furthermore, the longer the incubation, the more the lactic acid bacteria will actively utilize the carbohydrates to grow and form lactic acid. This is consistent with the opinion of [23] that the length of fermentation greatly affected the activity of lactic acid bacteria. The LAB growth is triggered by an adequate source of carbon for its life. The red dragon fruit skin contains a complete nutritional component in that the carbohydrate content is around 73.2 g / 100 g. Thus, besides cow milk, carbon source also comes from dragon fruit skin which can stimulate microbial growth in fermented milk. Furthermore, [24] also states that the total sugar content of probiotic drinks decreases along with the increase in sucrose concentration. This study was comparing total sugar before and after adding sucrose. The results showed that at the concentration of 5% sucrose, the total sugar obtained before was 9.8% and after was 7%, while at the addition of 7% sucrose, the total sugar obtained before was 11% and after was 8%.

The mixed culture used is a mixture of *Streptococcus* and *Lactobacillus*. Both of these bacteria work in symbiosis in remodeling sugar into a simple sugar that was converted to lactic acid. This is consistent with the opinion of [25] that the reducing sugar resulted is closely related to the activity of enzymes released by microbes, especially lactic acid bacteria that dominate during fermentation. The higher the enzyme activity, the higher the reducing sugar produced. Furthermore, the reducing sugar resulted is reused by lactic acid bacteria as a carbon source for its activities, so the longer the fermentation, the more the reducing sugar will decrease.

### 3.2. pH value

Potential hydrogen (pH) is used to express the level of acidity or base possessed by food. A low pH is a suitable environment for the growth of fermenting bacteria. The results of the analysis of the pH value of fermented milk with the addition of red dragon fruit skin and incubation time were presented in table 3.

This research result shows that the pH value of the fermented milk with the addition of red dragon fruit skin and incubation time is around 4.03-4.49. Table 3 showed that the average pH decreased along with the increase in the concentration of red dragon fruit skin but the increase in pH value with a longer incubation period. The analysis of variance showed that the concentration factor of dragon fruit peel had a very significant effect (p<0.01) on the pH value of the fermented milk. However, the treatment of the incubation period and the interaction between the concentration of red dragon fruit skin and incubation time did not significantly affect (p>0.05) on the pH value of the fermented milk. The nutritional content found in the skin of dragon fruit, especially carbohydrates in the form of fructose sugar can be a source of energy for LAB. The increase in the energy sources accompanied by the longer incubation time can stimulate the growth of LAB in producing lactic acid so that it affects the pH value.
The 3. of 100% extract of red dragon fruit skin is the best treatment in making fermented milk. This is in line with the research of the production of lactic acid by fermentation and in the next phase to 4.6 at the incubation period. Furthermore carbohydrate in milk used by starter cultures with raw milk, the most important component of milk is lactose and casein. Lactose is the main fermented milk. This is consistent with the opinion of more active to produce lactic acid.

Fructose and maltose content compared to other concentration treatments. fermented milk with the addition of red dragon fruit skin at the concentration of 60%, has the highest pH value of fermented milk. This is presumably due to the short period of the incubation interval, to the decrease in pH. The treatment of adding red dragon fruit skin concentration. However, the pH value of the fermented milk was not significantly different (<0.05) between the control treatment and the concentration of 20%, 40%, and 60%. The fermented milk without the addition of red dragon fruit skin (control) which has the highest pH value of fermented milk can be caused because the bacteria in the fermented milk only breaks down the lactose into glucose and galactose as the main energy source. Otherwise, the fermented milk with the addition of red dragon fruit skin at the concentration of 60%, has higher fructose and maltose content compared to other concentration treatments.

Duncan's further test results on the treatment of the red dragon fruit skin concentration show that the pH value of the fermented milk control treatment was significantly different (<0.01) to the treatment of adding 60% red dragon fruit skin concentration. However, the pH value of the fermented milk was not significantly different (<0.05) between the control treatment and the concentration of 20%, 40%, and 60%. The fermented milk without the addition of red dragon fruit skin (control) which has the highest pH value of fermented milk can be caused because the bacteria in the fermented milk only breaks down the lactose into glucose and galactose as the main energy source. Otherwise, the fermented milk with the addition of red dragon fruit skin at the concentration of 60%, has higher fructose and maltose content compared to other concentration treatments.

This is consistent with the research results found by [26] that the availability of carbohydrates as an energy source causes an increase in the metabolic activity by L. acidophilus bacteria which contributes to the decrease in pH. The treatment of incubation time did not show any significant effect (<0.05) on the pH value of fermented milk. This is presumably due to the short period of the incubation interval, but there is an increase in pH along with the longer incubation period.

According to [28], pH 4.5 is the point of stopping fermentation and in the next phase, there is post acidification which occurs due to the natural production of lactic acid by L. bulgaricus as a natural activity of proteolytic bacteria. The increase in the levels of lactic acid will further reduce the pH value of the treatment.

Based on the analysis conducted, the concentration of 60% is the best treatment for the pH value of fermented milk. This is in line with the research of [30] who obtained a pH value of 4.03. The addition of 100% extract of red dragon fruit skin is the best treatment in making probiotic drinks.

### 3.3. Protein content

The protein content of fermented milk is the total amount of protein from the raw material used and the LAB protein inside. The protein content of the fermented milk with the addition of red dragon fruit skin and different incubation times were presented in table 4.
The optimal incubation temperature and incubation time will directly increase the most optimal for the bacteria used. The optimal incubation temperature and incubation time will vary depending on the type of bacteria being used. For example, some bacteria may grow best at lower temperatures, while others may require higher temperatures. Similarly, the optimal incubation time may vary depending on the bacteria and the desired outcome.

The 12 hour incubation treatment show the highest protein content. It proves that the treatment affects the protein molecule will form positive and negative ions with the same amount. In the acidic state, the protein will be positively charged while at high pH (alkaline atmosphere), it will be negatively charged. At pH 4.8–6.3 (isoelectric pH), the amino acids would be in a dipolar or zwitterion state. In this situation, the least solubility of protein is found in water, so that the protein will clot and settle.

The low protein content of red dragon fruit skin may also be caused by the low pH value of the fermented milk products produced. Each amino acid has a different isoelectric point. At the low pH (acidic atmosphere), the amino acids will be positively charged while at the high pH (alkaline atmosphere), it will be negatively charged. At pH 4.8–6.3 (isoelectric pH), the amino acids would be in a dipolar or zwitterion state. In this situation, the least solubility of protein is found in water, so that the protein will clot and settle.

Table 3. Characteristics of fermented milk protein in red dragon fruit skin concentration and different incubation times.

| Red dragon fruit skin concentration (%) | Incubation time | Mean values |
|----------------------------------------|----------------|-------------|
|                                        | 10 hours       | 12 hours    | 14 hours    |
| 0                                      | 2.65±0.03\(^a\) | 2.64±0.05\(^c\) | 2.73±0.04\(^e\) | 2.67±0.05\(^c\) |
| 20                                     | 2.54±0.02\(^d\) | 2.85±0.05\(^d\) | 2.62±0.01\(^f\) | 2.67±0.14\(^d\) |
| 40                                     | 2.44±0.05\(^c\) | 2.75±0.02\(^f\) | 2.41±0.02\(^c\) | 2.53±0.16\(^b\) |
| 60                                     | 2.27±0.02\(^b\) | 2.55±0.02\(^d\) | 2.20±0.02\(^a\) | 2.34±0.16\(^a\) |

Mean values: 2.48±0.15\(^A\), 2.70±0.12\(^b\), 2.49±0.21\(^A\)

Abc superscripts that follow the mean values in the same row and column showed highly significant differences (p<0.01).

The low protein content of red dragon fruit skin may also be caused by the low pH value of the fermented milk products produced. Each amino acid has a different isoelectric point. At the low pH (acidic atmosphere), the amino acids will be positively charged while at the high pH (alkaline atmosphere), it will be negatively charged. At pH 4.8–6.3 (isoelectric pH), the amino acids would be in a dipolar or zwitterion state. In this situation, the least solubility of protein is found in water, so that the protein will clot and settle. This is in line with the opinion of [31] stating that the proteins are used to make LAB cells. Protein will be converted into amino acids. Protein is an organic source that will be used in the fermentation process. Microorganisms will be able to grow quickly in the presence of organic nitrogen.

Table 4 showed the protein content of fermented milk produced in general, which is in the range of 2.20–2.85. The protein content of the fermented milk has decreased along with the increase in the concentration of red dragon fruit skin and the longer the incubation process. Based on the variance analysis results, it was shown that the concentration factor of dragon fruit peel and incubation time, as well as the two interactions, had a very significant effect (p<0.01) on the protein content of the fermented milk.

Duncan test results showed that the concentration of fermented milk skin without the addition of red dragon fruit skin (control) was not significantly different (p>0.05) with the concentration level of 20% but was very significantly different (p<0.01) to the concentration 40% and 60%. The decrease in the protein of this study was due to LAB which was proteolytic so that it could break down the proteins into peptides and amino acids. The LAB utilized the amino acids in the form of nitrogen for cell synthesis so that they could survive. This is consistent with the opinion of [31] states that the proteins are used to make LAB cells. Protein will be converted into amino acids. Protein is an organic source that will be used in the fermentation process. Microorganisms will be able to grow quickly in the presence of organic nitrogen.

The low protein content of the fermented milk with the addition of red dragon fruit skin may also be caused by the low pH value of the fermented milk products produced (table 4). Each amino acid has a different isoelectric point. At the low pH (acidic atmosphere), the amino acids will be positively charged while at the high pH (alkaline atmosphere), it will be negatively charged. At pH 4.8–6.3 (isoelectric pH), the amino acids would be in a dipolar or zwitterion state. In this situation, the least solubility of protein is found in water, so that the protein will clot and settle. This is in line with the opinion of [33] suggesting that the principle of changing the composition of milk in fermented milk is denaturation of protein due to acid or low pH during the fermentation process. The most dominant protein in milk is the casein protein (alpha, beta and k). In the acidic state, this protein will be positively charged, while in the alkaline state it will be negatively charged, and at the isoelectric pH, the protein molecule will form positive and negative ions with the same amount. In the circumstances, in accordance with the results of the study, it is known that the pH obtained is below the casein isolate pH point, so that the protein denaturation that occurs is irreversible.

Duncan's test results on the treatment of the incubation time show that the protein content of the fermented milk for a 10-hour incubation process was not significantly different (p>0.05) from the 14 hours incubation period but was very significantly different (p<0.01) to the 12 hours incubation time. The 12 hours of incubation treatment show the highest protein content. It proves that the treatment is the most optimal for the bacteria used. The optimal incubation temperature and incubation time will increase the microbial’s activity and its number. The many more bacteria are, the protein in the media will also increase because the protein is a constituent of microbes. This is in line with the research conducted by [34]. The study also used a combination of 4 types of bacteria. The results show that the...
addition of soursop amounting to 10% with a 12-hour incubation period can improve the physicochemical and organoleptic quality of yogurt.

Duncan's test results on the 14 hours of incubation treatment show a decrease in the protein content. This is due to the increase in LAB that utilized protein. The LAB utilized protein to stimulate its growth, so that the longer the incubation period was, the lower the protein content got. This is consistent with [35] research results concluding that the protein content of goat's milk curd decreases along with the length of incubation. It is because the LAB fermentation process requires nutrients to survive. These nutrients are derived from milk, so that the protein content that initially gets high decreases along with the duration of the fermentation.

Duncan's test results on the interaction between the concentration of red dragon fruit skin and incubation time show that the protein levels of the fermented milk were significantly different (p<0.01) between each treatment. There was a decrease in the protein content with an increase in the concentration of red dragon fruit skin and the longer the incubation time was. This is due to the increase in the concentration levels of dragon fruit peel causing the increase in the microorganism's activity in fermented milk. The increase in the activity of microorganisms was proved by the high content of lactic acid as shown in table 2. The increase in the activity was accompanied by the microorganism's need for amino acids. This condition decreased the protein content. The protein content of the fermented milk also continued to decrease because of the longer incubation process. This happened because the microorganisms remained alive, grew and required amino acids.

3.4. Fat content
Milk fat is a chemical compound that is included in the ester group which is composed of various fat and glycerol. Ninety percent of the milk fat components are fatty acids which are divided into unsaturated fatty acids and saturated fatty acids. The fat content of the fermented milk with the addition of red dragon fruit skin and different incubation time are presented in table 5.

**Table 4.** Characteristics of fat in the fermented milk with different concentrations of red dragon fruit skin and incubation time.

| Red dragon fruit skin concentration (%) | Incubation time | Mean values |
|-----------------------------------------|-----------------|-------------|
|                                         | 10 hours | 12 hours | 14 hours |                |
| 0                                       | 0.91±0.03 | 0.82±0.04 | 0.69±0.58 | 0.80±0.30<sup>A</sup> |
| 20                                      | 0.44±0.02 | 0.62±0.02 | 0.86±0.06 | 0.64±0.18<sup>B</sup> |
| 40                                      | 1.45±0.03 | 1.28±0.01 | 1.36±0.45 | 1.36±0.23<sup>C</sup> |
| 60                                      | 1.93±0.05 | 1.55±0.02 | 1.64±0.02 | 1.70±0.17<sup>C</sup> |
| Mean values                             | 1.18±0.58 | 1.07±0.38 | 1.14±0.50 |                |

<sup>A-B-C</sup> superscripts that follow the mean values in the same row and column showed highly significant differences (p<0.01).

Table 5 showed the fat content of the fermented milk produced in general is in the range of 0.44–1.93. The fat content of the fermented milk has increased along with the increase in the concentration of red dragon fruit skin and the longer the incubation period was. Based on the variance analysis results, it is showed that the treatment of the dragon fruit peel concentration had a very significant effect (p<0.01), while the treatment toward the incubation period and its interaction with the red dragon fruit skin concentration had no significant effect (p>0.05) on the fat content of the fermented milk.

Duncan's test results on the treatment of the dragon fruit peel concentration show that the fat content of the fermented milk control was not significantly different (p>0.05) to the concentration of 20% but was significantly different (p<0.01) to the concentration of 40% and 60%. It is caused by the fat content of the raw materials used, namely full cream milk and dragon fruit skin. According to [14], the fat content of full cream milk powder is not below 26% which is about 30 g, while the fat content of dragon fruit peels is 0.8 g / 100 g.
The increase in fat content was also due to the change from some carbohydrates to fat. The amylose in starch was converted to maltose and a little glucose. It is in accordance with the opinion of [36] citing that the remaining excess of glucose in small amounts will then be converted to fat. In addition, the fermentation bacteria have strong reducing properties, in that under the conditions of active fermentation, the media containing sugar and other compounds added will convert aldehyde to alcohol. After that, glycerol is made to affect the increase in fat content.

The treatment of the incubation period also show an increase in the fat content of the fermented milk, but the 12 hours of incubation was the lowest fat content. The decrease in the fat content was probably due to the 12 hours of incubation and became the optimum incubation time for the growth of the mixed culture used. The optimum incubation time for bacteria was more active and increasingly required energy for the fermentation process. Hence, besides the use of carbohydrates, fat was also used as an energy source. The findings are in accordance with the opinion of [37] stating that the first component attacked by bacteria is a carbohydrate (lactose). The second one is protein and the last one is fat. The fat content in the fermented milk will be remodeled into fatty acids. This simple compound will be absorbed by the body as nutrition. The more active bacteria in the fermented milk will accelerate the breakdown of fats by bacteria as an energy supply for the growth of these bacteria.

4. Conclusions
The higher the concentration and the longer the incubation, the higher the fat content but the lower the carbohydrate and protein content. Therefore, fermented milk with the addition of red dragon fruit skin should use 60% dragon fruit skin concentration and 12 hours incubation period.

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