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Physicochemical Properties of Mozzarella Cheese using Coagulants Moringa Seed Extract (*Moringa oleifera*) with Microwave Assisted Extraction Method

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Abstract
Generally, cheese making uses the enzyme rennet, but this enzyme has critical point because its halal status has not been confirmed yet. Moringa seeds are alternative coagulants that have been used in water purification and cheese making and extracted using one of the methods developed, the Microwave Assisted Extraction (MAE). This study aims to obtain the best physicochemical quality of Mozzarella cheese using Moringa seed extract as a coagulant. The method in this study was completely randomized with 4 treatments and 4 replications consisting of the use of 0.025% commercial rennet enzyme as a control treatment (P0), the use of Moringa seed extract as much as 2% (P1), 2.5%, and 3% (P3). The parameters observed were moisture content, moisture content, pH, texture, yield and coagulation time. The data was analyzed by analysis of variance (ANOVA) and continued by Duncan's Multiple Range Test Method (DMRT) if there were differences. The results showed that the physicochemical of mozzarella cheese using Moringa seed extract as a coagulant gave a highly significant difference ($P<0.01$) on coagulation time, and a significant difference in texture ($P<0.05$) but did not give a significant difference ($P>0.05$) on moisture content, pH, and yield. The best concentration of Moringa seed extract is 2.5% and have moisture content 43.35%, pH 5.57, texture 2.33 N, yield of 12.74% and coagulation time of 15.5 minutes. Moringa seed extract can be used as an alternative coagulant in Mozzarella cheese making.

Keywords: Coagulant, Microwave Assisted Extraction (MAE), Moringa seed extract, Mozzarella cheese, Physicochemical

1. Introduction
Mozzarella cheese is a soft cheese whose manufacturing process does not use a fermentation process and includes fresh cheese. This cheese is used as a topping on food because of its soft texture, meltable and delicious taste [1]. Cheese is made by a coagulation process using bacteria or the enzyme rennet to separate casein from whey [2]. The process of coagulating casein to produce curd and whey until it is processed into cheese is the most important stage [3]. The addition of the enzyme rennet can change the part of k-casein so that casein micelles can combine and form a gel due to the loss of repulsion between casein micelles [4]. The functional properties of cheese are influenced by pH, calcium content, and the interaction between calcium and casein. [5] explains that calcium is an important mineral in the coagulation process of casein by rennet, especially at the stage of casein micellar aggregation in the cheese-making process. The isoelectric point occurs when the positive and negative charges of casein are balanced or have a zero value, which reaches a pH of 4.6 so that it can precipitate and separate curd and whey [6].

Generally, the enzyme rennet used is derived from calf abomasum, which is critical point because its halal status has not been confirmed yet, so an alternative coagulant is needed as a substitute for the rennet enzyme. Moringa seeds are an alternative coagulant that has been used in water purification and cheese making [8]. According to [7], Moringa is also a source of milk clotting enzymes. Moringa seeds can be extracted using one of the currently developed methods, like Microwave Assisted Extraction (MAE), which utilizes microwaves with a frequency of 300 MHz–300 GHz [9]. Moringa seed extract is used as a coagulant in the manufacture of a typical Southern Nigerian cheese called "wara". The results of the research by [10] showed that it has better coagulation and antioxidant properties than *Calotropis procera* extract, which is commonly used in conventional cheese making. This study also used *Moringa oleifera* seed extract as a coagulant, extracted by the MAE method, and aimed to obtain the best physicochemical quality of Mozzarella cheese.

2. Materials and methods

2.1 Materials

The materials used in this study were fresh cow's milk and Moringa seed extract, using MAE for 6 minutes. The equipment in this study used stainless steel pans, plastic containers, digital scales, drop pipettes, wooden spatulas, glass stirrers, Whatman No. 1, plastic filter, filter cloth, gloves, stove, thermometer, pH meter (WalkLAB Microprocessor pH Tester Trans Instruments Ti 9000), oven, desiccator, petri dish, stopwatch, 25ml measuring cup, beaker glass, TA. XT Plus Texture Analyzer, and freezer.

2.2 Methods

This study employed a completely randomized design (CRD) with 4 treatments: 0.025% rennet enzyme as a control treatment, Moringa seed extract 2% (P1), 2.5% (P2), and 3% (P3) with 4 replicates. Data was analyzed using Analysis of Variance (ANOVA), and if a significant difference was found, it was tested using Duncan's Multiple Range Test (DMRT).

2.2.1 The Manufacture of Moringa seed powder

The manufacture of Moringa seeds powder refers to [11] and has been modified. Dried Moringa seeds in the sun for 2 days. The moringa seeds were peeled from the skin and mashed with the moringa seeds using a blender. Then the moringa seeds were sieved using a 40-mesh sieve and then stored in a closed container at room temperature.

2.2.2 Moringa seed extraction preparation with Microwave Assisted Extraction (MAE) method

Moringa seed extraction preparation refers to [12] as follows: 100 grams of Moringa seed powder were weighed, then 300 ml of sodium phosphate pH 7 buffer solution was added, after 10 ml of EDTA solution was added and homogenized with a magnetic stirrer at 6 rpm for 30 minutes. Moringa seed extraction using the MAE method is based on [13] and has been modified as follows: Pour a homogeneous mixture of Moringa seeds into an Erlenmeyer flask, then extract in the microwave for 6 minutes at 100% power. Extraction was carried out with a minute flame system and a 2minute pause. After that, the extraction results were filtered using filter paper and allowed to stand at 4°C for 30 minutes. The filtrate was centrifuged for 30 minutes at a speed of 4,000 rpm. After that, the results of the centrifugation were filtered using filter paper and then 60% (w/v) ammonium sulfate were added. Then, centrifugation was carried out again for 30 minutes at a speed of 4,000 rpm, the precipitate was taken, and the sample was stored in a freezer.

2.2.3 The procedure for making Mozzarella Cheese

The procedure for making mozzarella cheese refers to [14] with the following modifications: The milk is pasteurized at 72ºC for 15 seconds, then the temperature is lowered to 35ºC, then 20 ml of starter is added and homogenized. Incubate at room temperature for one hour, then add 0.08% citric acid and homogenize. After a few minutes, 0.15% $CaCl_2$ was added and homogenized again. 0.025% rennet enzyme was added for the control treatment, 2%, 2.5%, and 3% Moringa seed extract were added for the other treatments, which were then allowed to stand for 1 hour to form curd. Cut the cord and let it rest for 15 minutes, then separate the curd and the curd using a filter cloth. The curd and whey were separated 3 times until there was no more flowing whey, then 1.5% salt and 1% sodium citrate were added, then stirred until smooth, worked and stretched at 75˚C then molded into a plastic container and frozen in the freezer.

2.2.4 Moisture content

The determination of moisture content was made using an oven based on [15]. The samples are weighed as much as 1-3 grams in a petri dish as the initial weight. The sample was dried in an oven at a temperature of 105ºC for 3 hours until the weight was constant. The sample was cooled in a desiccator and reweighed as a final weight until the weight was constant. The moisture content is calculated by the formula:

$$\text{Moisture content (\%)} = \frac{W0-W1}{W0} \times 100\%$$

Description: The initial sample weight (W0) and sample weight after drying (W1)

2.2.5 pH

The determination of pH value using a pH meter is based on [16]. Five grams of cheese were weighed, diluted with 25 ml of distilled water, added to 50 ml, and homogenized. Then, calibrated at pH 4 and 7, the pH value can be directly known by reading the number that has been shown (stable) on the pH meter.

2.2.6 Texture

Texture testing was carried out with the TA-XT Plus Texture Analyzer according to [17]. The sample is cut into cubes with a side size of 3 cm, then a probe is inserted and adjusted to its position. Turn on the tool and make sure that the value on the monitor is zero, then press the start test menu so that the probe will move to pierce the sample and return to its original position when the test is complete. The test results are observed, which are seen in the form of numbers.

2.2.7 Yield

The percentage yield test was carried out by weighing the cheese with a digital scale according to the research of [18]. The mass / weight of the cheese is then recorded and calculated based on the calculation formula as below:

$$\text{Cheese Yield (\%)} = \frac{\text{Weight of formed cheese (gram)}}{\text{The volume of milk used (gram)}} \times 100\%$$

2.2.8 Coagulation time

Coagulation time or Rennet Coagulation Time (RCT) is performed by measuring the elapsed time between the addition of a known amount of renin to the volume of milk added at a certain temperature until coagulation (clumping) occurs and is assessed visually [19]. Analysis of coagulation time is measured in minutes. The following are the RCT determination steps:

1. The enyme rennet was dissolved in water in a 1:100 (v/v) ratio.
2. The time was measured with a stopwatch until the milk flocculation started at 35˚C.
3. The samples were observed and the coagulation assessment was divided into four groups, namely:
4. Fast coagulation, if the coagulation time lasts for 10 minutes.

5. Moderate coagulation, if the coagulation time lasts for 15 minutes.
6. Coagulation is slow, if the coagulation time is more than 15 minutes.
7. Non-coagulation, if the sample does not coagulate.

3. Results and discussion

The average values of moisture content, pH, texture, yield, and coagulation time of mozzarella cheese with the use of Moringa seed extract as a coagulant are presented in Table1.

Table 1: Average Value of Physicochemical Properties of Mozzarella Cheese Using Moringa Seed Extract as Coagulant with MAE Method

Parameter	Treatment			
	P0	P1	P2	P3
Moisture content (%)	42.00 ± 2.37	40.40 ± 4.04	43.35 ± 1.55	40.52 ± 3.36
pH	5.60 ± 0.11	5.64 ± 0.15	5.57 ± 0.19	5.77 ± 0.07
Texture (N)	2.53 ± 0.20^{k}	2.66 ± 0.40^{k}	2.33 ± 0.55^{k}	3.41 ± 0.35^{l}
Yield (%)	12.65 ± 0.45	12.38 ± 0.40	12.74 ± 0.33	12.69 ± 0.22
Coagulation time (minutes)	12.45 ± 0.11^{q}	15.68 ± 0.45^{t}	15.15 ± 0.43^{s}	14.98 ± 0.94^{r}

3.1 Moisture content

The results of the analysis of variance showed that the use of different Moringa seed extracts as coagulants did not provide a significant difference (P>0.05) in the moisture content of Mozzarella cheese. The highest average value of moisture content in Table 1 is in P2, which is 43.35% ± 1.55 with a concentration of using Moringa seed extract 2.5% and the lowest value is in P1, which is 40.40% ± 4.04 with a concentration of using Moringa seed extract 2%. Moisture content indicates the amount of water contained in a product formed. High moisture content affects the texture of Mozzarella cheese. Mozzarella cheese moisture content in the results of this study was not much different from the study of [20]. Processed cheese with the addition of 4% Moringa seed extract produces 42.4% water content, an increase in water content is followed by an increase in the concentration of Moringa seed extract. According to the [21], the moisture content of Mozzarella cheese ranges from 40–50%.

The calcium content retained in cheese can also affect the moisture content of Mozzarella cheese. The combination of the addition of citric acid and the use of a starter that breaks down lactose into lactic acid can lower the pH and optimize the work of enzymes in Moringa seed extract to form curd. The addition of protease enzymes can cause calcium to dissolve and diffuse faster in water than in the gel, so that calcium will be removed by the cheese curd along with the whey. The difference in the average value of moisture content between other treatments is almost the same. This is thought to be caused by the absence of differences in the calcium content in the cheese produced by citric acid so that it has the same ability to bind water in the curd. [22] stated that the increase in whey fluid (calcium nonmcellar) causes the calcium retained in the cheese to decrease (calcium micellar) so that the cheese becomes softer and the water content is higher. [23] also added that the difference in moisture content in cheese is also caused by the presence of water in the cheese, which is at three levels: bound in the structure of the curd component, retained on hygroscopic curd particles, and as free water. In addition, the degree of suppression when expelling the whey results in the presence of free water in the curd.

3.2 pH

The results of the analysis of variance showed that the use of different Moringa seed extracts as coagulants did not give a significant difference (P>0.05) to the pH value of Mozzarella cheese. The average value in Table 1 of the results of the study increased along with the concentration of the addition of Moringa seed extract. The average value of pH levels of Mozzarella cheese is the lowest in P2, which is 5.57 ± 0.19 with the use of 2.5% Moringa seed extract, and the highest value is found in P3, which is 5.77 ± 0.07 with the concentration of Moringa seed extract used at 3.0%. According to [24], the pH level of Mozzarella cheese ranges from 5.1 to 5.4.

The addition of Moringa seed extract with concentrations of 2% and 3% in fresh cheese resulted in pH values of 6.51 and 6.55 [20]. In line with the results of [25], the addition of moringa seed extract as much as 1% and 2% reached pH values of 6.54 and 6.56, respectively. This shows that the more concentration of Moringa seed extract added, the more the pH value increases because Moringa seed extract can inhibit bacterial growth and reduce the amount of lactic acid that has been fermented by lactose. The concentration of citric acid used in this study was relatively the same, namely 0.08%, so that it did not significantly affect the curd coagulated by the protease enzyme of Moringa seed extract. In addition, the increase in the pH value was caused by the increasing heating temperature during the stretching process.

3.3 Texture

The results of the analysis of variance showed that the use of different Moringa seed extracts as coagulants gave a significant difference (P<0.05) to the texture of Mozzarella cheese. The lowest average value of Mozzarella cheese texture in Table 1 is in P2 with a concentration of 2.5% Moringa seed extract, which is 2.33 N ± 0.55, and the highest value is in P3, which is 3.41 N ± 0.35 with a concentration of 3% Moringa seed extract. [14] stated that the good texture of Mozzarella cheese is soft and stretchy. The hardness value of the cheese can affect the quality of the cheese and it does not stretch well.

Texture, which describes the structure or cohesiveness of cheese, is one of the factors that influence consumer acceptance of food products. The amount of water in mozzarella cheese can affect its texture. Increasing water content will cause the texture to become soft. This is in accordance with the opinion of [26] that the interaction between cheese components such as fat, protein, water and pH affect the texture of the cheese. The water content of the P3 treatment was 40.52%, so that it affected the texture of the Mozzarella cheese by 3.14 N. The high calcium content in the curd also gave the P3 a harder texture. This was due to the calcium retained in the cheese being cross-linked with casein. The cross-links make the protein network stronger, thereby reducing the melting power of the cheese and increasing the hardness of the cheese.

3.4 Yield

The results of the analysis of variance showed that the use of different Moringa seed extracts as coagulants did not give a

significant difference (P>0.05) to the yield of Mozzarella cheese. The highest average value of Mozzarella cheese yield in Table 1 is in P2 which is 12.74% ± 0.33 with a concentration of 2.5% Moringa seed extract, and the lowest value is in P1, which is 12.38% ± 0.40 with a concentration of 2% Moringa seed extract. The results of this study are not much different from the research of [27], which found 12.24% with the use of tangerine juice on Mozzarella cheese with a concentration of 4%. Research conducted by [28] also produced a yield of 12.93% with the addition of 0.5% acetic acid.

The high yield of Mozzarella cheese treatments P0, P1, P2, and P3 can be influenced by the composition of fat and protein content of the cow's milk raw materials used, namely 2.39% and 4.05%, respectively. Milk with high protein and fat content can produce an optimal yield. The protein content in Moringa seed powder is also high, at around 35.95%. The increase in concentration used in Moringa seed extract also affects the yield. The average yield of P1 treatment was around 12.38%, increasing to 12.74% with a concentration of 2.5% Moringa seed extract, while P2 treatment was not much different from P3 treatment, which was 12.69% with a concentration of 3% Moringa seed extract. The yield that did not differ much was thought to be caused by the use of Moringa seed extract, which was not much different in concentration, so that it had the same ability to bind curd. The use of excessive or insufficient coagulant causes the curd to become brittle and softer so that a lot of casein and fat are dissolved with the whey during the cutting process.

3.5 Coagulation time

The results of the analysis of variance showed that the use of different Moringa seed extracts as coagulants gave a very significant difference (P<0.01) to the coagulation time of Mozzarella cheese. The average value in Table 1 decreased along with the concentration of the use of Moringa seed extract. The average value of the coagulation time of the highest Mozzarella cheese was found at P1, which was 15.68 ± 0.45 with a concentration of 2% Moringa seed extract, and the lowest value was found at P0 as a control treatment, which was 12.45 ± 0.11 with the use of 0.025 % rennet enzyme.

The time that elapses after the addition of the enzyme rennet to milk at a certain temperature is called the coagulation time. Milk coagulation is an important thing to observe because it can affect the yield and quality of the resulting cheese. This is in accordance with the opinion of [29] that rennet coagulation time (RCT), the time required for curd formation, firmness, and curd syneresis, were used to evaluate the gelation potential index of milk by protease enzymes. Moringa seeds contain positively charged proteins which are important as biocoagulant agents [30]. This positive charge makes casein micelles unstable and reduces electrostatic repulsion. Moringa seed extract also contains a protease enzyme that can break the peptide bond of -casein when approaching the isoelectric pH of 4.6. The coagulation activity of the moringa seed protease enzyme is optimum when the pH reaches 5 and the temperature is 55–65˚C. The use of other ingredients such as citric acid, starter, and $CaCl_2$ helps increase the enzyme to form curd and shorten the coagulation time. [31] argue that the factors that affect milk coagulation are enzyme concentration, temperature, and protein (casein fraction) κ-casein and β-casein affect the stability of casein micelles and curd activity, which results in a shorter coagulation time so that the resulting curd is more consistent.

3.6 Best Treatment

Determination of the best treatment is used as a consideration for decision making by comparing the variables and treatments used. The best treatment was the use of Moringa seed extract with 0.025% P0 renet enzyme (control treatment). The effectiveness index method developed by [32] was used to determine the use of Moringa oleifera seed extract as a coagulant as much as 2% (P1), 2.5% (P2), and P3 (3%) on Mozzarella cheese using quantitative data parameters such as water content, pH, texture, yield, and coagulation time The best treatment at P2 with the use of Moringa seed extract as much as 2.5%, the physicochemical properties of Mozzarella cheese resulted in a water content of 43.354%, pH 5.57, texture 2.33 N, yield of 12.38%, and a coagulation time of 15.5 minutes.

Table 2: The Best Treatment of Physicochemical Mozzarella Cheese Using Coagulant Moringa Seed Extracts

Parameter	Value
Moisture content (%)	43.35
pH	5.57
Texture (N)	2.33
Yield (%)	12.74
Coagulation time (minutes)	15.55

4. Conclusion

The best use of Moringa seed extract as a coagulant in the manufacture of Mozzarella cheese is 2.5% (P2) and has good physicochemical qualities, moisture content of 43.35%, pH 5.57, texture 2.33 N, yield 12.74%, and the coagulation time was 15.55 minutes. Moringa seed extract can be used as an alternative coagulant in the manufacture of Mozzarella cheese.

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