

The Effect of Red Rosella Extract as an Acidulant on Water Content, Total Solid Ingredients, Total Acid, and Melasticity in Mozzarella Cheese

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Abstract - Mozzarella cheese is a highly popular dairy product; however, the consumption rate of dairy-based products in Indonesia remains relatively low. One way to extend the shelf life of milk is by processing it into mozzarella cheese. The production process of this cheese is highly dependent on the coagulation stage, which involves acidification using red roselle flower extract. This study aims to determine the effect of substituting acetic acid with red roselle flower extract as an acidulant on the moisture content, total solids, total acidity, and stretchability of mozzarella cheese. The research utilized a Completely Randomized Design (CRD) with four treatments, namely variations in the concentration of red roselle flower extract: P1 (4%), P2 (5%), and P3 (6%), compared to the control P0 (without roselle extract). The data obtained were analyzed using Analysis of Variance (ANOVA) at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT). The results showed that the use of different concentrations of red roselle flower extract as an acidulant in mozzarella cheese production caused changes in moisture content, total solids, total acidity, and stretchability. The best treatment based on all parameters was P3, which used 6% red roselle flower extract as the acidulant. The addition of varying concentrations of red roselle flower extract increased the moisture content, decreased the total solids value, increased total acidity, and enhanced the stretchability of the mozzarella cheese. The findings indicate that the addition of red roselle flower extract significantly affected the moisture content, total solids, total acidity, and stretchability. The P3 treatment with 6% red roselle flower extract produced mozzarella cheese with higher moisture content and stretchability. The total solids and total acidity values obtained were close to those of the control. This study demonstrates that red roselle flower extract can be used as an alternative acidulant in mozzarella cheese production to improve the final product quality.

Keywords: Mozzarella cheese, red rosella flower, water content, total solids, total acid, elongation.

I. INTRODUCTION

Milk is a food product with perfect nutritional value and is the best source of animal-derived amino acids[8]. The nutrients found in cow's milk include protein, calcium, vitamin A, vitamin B, vitamin D, amino acids, calories, fat, phosphorus, iodine, zinc, iron, copper, magnesium, vitamin E, and thiamine [12]. However, milk is also a perishable food item due to the growth and development of bacteria. To address milk spoilage, processing can be done to make the milk last longer. One way is to process it into cheese. Among the various types of cheese available, mozzarella cheese is one of the most popular variants. This cheese has a soft texture and savory taste that makes it favored as an addition to various food dishes.

Mozzarella cheese is a processed cow's milk product made through the process of acidification and the addition of the enzyme rennin to form curd. Mozzarella cheese production can be done through direct acidification, eliminating the need to wait for the action of bacterial starter cultures to produce lactic acid [18]. Cheese making through direct acidification is performed by adding acidic substances, such as citric acid, which will produce a mozzarella-type cheese that is usually white and consumed immediately without a ripening process. This step can be modified using natural acidulants as an alternative, while maintaining the essential process of curd formation. The use of roselle as a natural acidulant in the production of mozzarella cheese offers an effective direct acidification alternative because roselle's low pH content can replace the role of citric acid in lowering the milk's pH, thus keeping the rennin enzyme activity optimal for forming curd.

Roselle (*Hibiscus sabdariffa*) is a herbal plant from the Malvaceae family that originates from West Africa. This plant can grow up to 2-3 meters in height with palmate leaves and conspicuous flowers. Most roselle plants are used as ornamental plants, and some are believed to have medicinal properties[14]. Its flower calyx is rich in antioxidants that act as free radical scavengers and is often utilized as a natural colorant[6]. The roselle flower calyx contains various bioactive compounds beneficial for health, including organic

acids such as citric acid, malic acid, and tartaric acid. This acid content gives roselle its characteristic sour taste. Roselle is increasingly being cultivated due to its potential as a functional food ingredient.

The extract of red roselle flowers substituted as an acidulant will affect the properties of mozzarella cheese, such as total acid, water content, stretchability, and total solids. Water content influences the cheese's texture, where a higher water content results in a softer texture. Total solids affect the shelf life and flavor intensity of the cheese. The organic acids in roselle, such as citric, malic, and ascorbic acid, can increase the acidity of dairy products[10]. Stretchability is an important parameter that determines consumer acceptance.

II. MATERIALS AND METHODS

2.1 Materials

The materials and tools to be used in this research are fresh cow's milk milked in the morning from the Farm of the Faculty of Animal and Agricultural Sciences, Diponegoro University, liquid rennet marketed by Cheese Journey by Zaidaneam, Belibis brand vinegar acid, aquadest, ice water, and roselle flowers obtained from the Rasamala Traditional Market, Banyumanik, Semarang. The tools used in this research were a pot, stove, stirrer, Osuka-5002 analytical balance, thermometer, beaker glass, measuring cup, Erlenmeyer flask, filter cloth, spoon, plastic wrap, refrigerator, moisture content oven, and a ruler.

2.2 Methods

The research was conducted during the period of February - April. The research included the making of red roselle flower extract, the making of mozzarella cheese, and parameter testing. The tested parameters included the resulting moisture content, the total resulting solid materials, the total acid formed, and the product's stretchability.

2.3 Making Red Rosella Flower Extract

The making of red roselle flower extract refers to the research by[10]. The first stage was to dry the fresh roselle flowers and then grind them into a powder, sieved using a 60-mesh sieve. The roselle flower powder was dissolved in water with a ratio of 100 g : 300 ml and pasteurized at a temperature of 63-65°C for 30 minutes. The roselle solution was then filtered to extract the liquid extract. The process of making roselle flower extract can be seen in Figure 1.

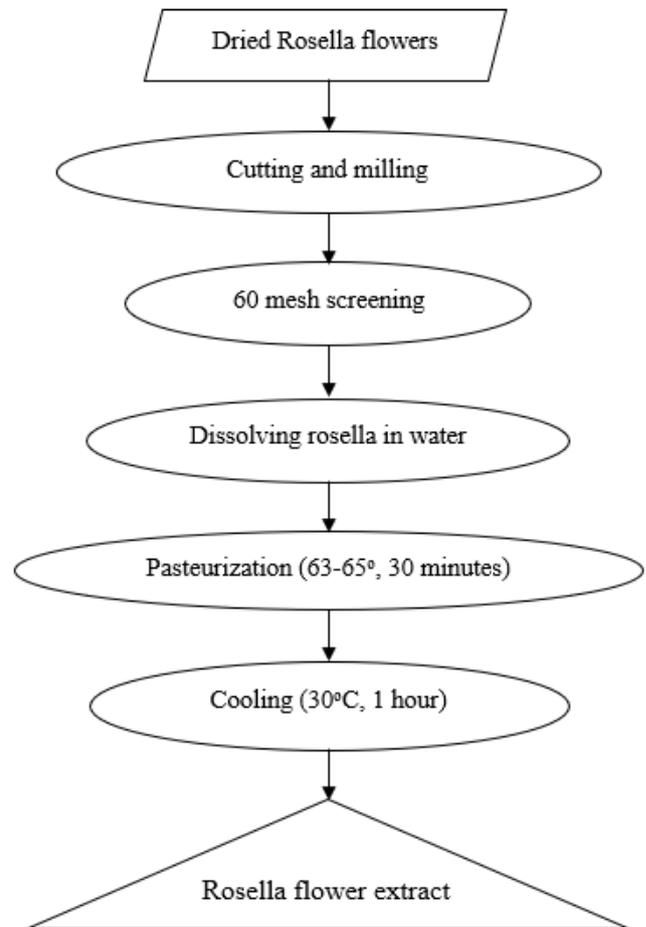


Figure 1: Flowchart of the Process of Making Red Rosella Flower Extract [10]

2.4 Making Mozzarella Cheese

The procedure for making mozzarella cheese follows the research by [11]. The first stage is to pasteurize the milk at a temperature of 70°C, then cool it down until the milk temperature reaches 35°C. Then, vinegar acid and red roselle flower extract are added to the milk according to the treatment concentration variations, namely P0 (0%), P1 (4%), P2 (5%), and P3 (6%), and 0.025% (v/v) of rennet enzyme from the milk volume is added, after which it is left to stand for 10 minutes until the curd is formed. Then the curd that has been formed is cut into dice-sized pieces, then the curd is separated from the whey by filtering until it is completely separated. Stretching is carried out at a temperature of 75 – 85°C then the finished mozzarella cheese is placed in ice water for 30 minutes. Figure 2 presents the flow chart for making mozzarella cheese.

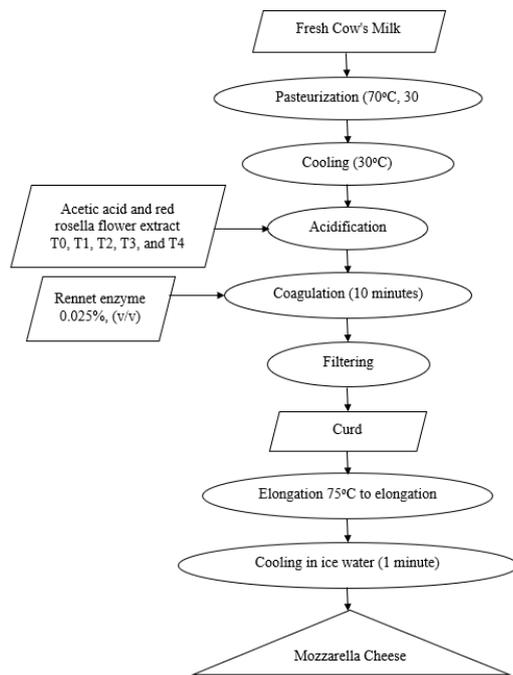


Figure 2: Flowchart of Mozzarella Cheese Making Process [11]

III. PARAMETER TESTING

3.1 Moisture Content

Analysis of moisture content was carried out following the oven method based on the research by[4]. This method applies the principle of reducing the sample weight after heating in an oven at 105°C. The procedure begins by drying an empty dish in the oven for about 15 minutes. The heated dish is then cooled in a desiccator before being weighed to obtain the empty dish weight. In the next step, a 2 gram sample is placed in the dish and heated in the oven at a temperature of 105°C for 6 hours. After heating, the sample is cooled in a desiccator before weighing is performed.

The calculation of moisture content can be done using the following formula:

$$\text{Moisture Content (\%)} = \frac{\text{weight B} - (\text{weight C} - \text{weight A})}{\text{weight B}} \times 100$$

Description:

Weight A = Weight of the porcelain dish

Weight B = Initial weight of the sample

Weight C = Final weight of the sample

3.2 Total Solids

The total solids content test in mozzarella cheese refers to research by[3]. This procedure begins with an analysis of the sample's water content. After obtaining the water content, the total solids are then calculated.

The calculation of total solid materials can be done using the following formula:

$$\text{Total Solids (\%)} = 100\% - \text{Moisture Content}$$

3.3 Total Acid

The analysis of total acid content was conducted by adopting the titration method according to the procedure by[16]. The process begins by grinding a 2.5-gram cheese sample using a mortar. The ground sample is then transferred into a 25 ml volumetric flask, and distilled water is added up to the mark. Afterward, the sample undergoes a filtration process using filter paper and is collected in a beaker. From the resulting filtrate, a 25 ml aliquot is taken, and 3 drops of phenolphthalein (PP) indicator are added. The titration is performed using a 0.1 N NaOH solution until a stable pink color change is observed.

The total acid can be calculated using the following formula:

$$\text{Total Acid (\%)} = \frac{(A \times B \times N \times (\text{dilution factor}))}{C \times 1000} \times 100$$

Description:

A = Volume of NaOH

B = Molecular Weight (Acetic Acid)

C = Sample Weight (g)

N = Normality of NaOH (N)

3.4 Stretchability

The stretchability of the mozzarella cheese was tested using a measurement method with a ruler, as described in the research by [5]. The testing process begins by heating a sample of mozzarella cheese in an oven at a temperature of 185°C for 5 minutes. After heating, the sample is removed from the oven and tested by placing a fork in the center of the cheese. Then, the cheese is pulled vertically, and the length of the stretch is measured in centimeters using a ruler.

3.5 Data Processing and Analysis

The data obtained from all tests were statistically analyzed using the ANOVA (Analysis of Variance) method at a 5% significance level. If the analysis results indicated a significant treatment effect, the analysis was continued with the DMRT (Duncan's Multiple Range Test) to identify differences among the treatments. This statistical analysis was performed using SPSS software, version 26.0 for the Windows operating system.

IV. RESULTS AND DISCUSSION

4.1 Moisture Content

One of the factors that influence the ability of mozzarella cheese to bind water is the level of acidity. Acid increases the amount of nonmicellar calcium that dissolves in the whey, while micellar calcium remains trapped within the cheese. The more nonmicellar calcium that is formed, the less calcium remains in the cheese, which makes the cheese softer and its water content higher. This decrease in calcium level increases protein hydration, causing more whey to be trapped during the syneresis process. Syneresis is the contraction of the curd to expel whey, which is a key factor in determining the cheese's moisture content. The breaking and formation of new bonds between curd particles trigger syneresis. The lower the level of acid coagulation, the greater the curd's ability to retain water. Conversely, the moisture content decreases because the cheese's water binding capacity is reduced as the rennet concentration and coagulation duration increase. This decrease in moisture content makes the cheese more resistant to spoilage. This is consistent with the opinion of [2], who state that high moisture content will cause cheese products to spoil faster.

Red roselle flower extract contains organic acids such as acetic acid, malic acid, and ascorbic acid, which are capable of lowering the milk's pH, thus helping to optimize the coagulation process in mozzarella cheese production. The coagulation process under increasingly lower pH conditions can increase the curd's ability to retain water, consequently increasing the moisture content in the mozzarella cheese. This aligns with the statement of [1], who state that a lower pH condition during coagulation can increase the curd's water retention, resulting in higher moisture content in mozzarella cheese. Curd formed at a low pH has stronger bonds between protein molecules and water. This occurs because of the interaction between casein protein and water. Curd formation at a high acidity level will increase the water-binding capacity.

Table 1: Results of Mozzarella Cheese Moisture Content Testing with red rosella flower extract acidifier

Treatment	Moisture Content (%)
P0	54,63±0,64 ^b
P1	52,79±0,44 ^a
P2	53,69±1,17 ^{ab}
P3	55,80±0,78 ^c

4.2 Total Solids

The addition of roselle flower extract can affect the total solids content of mozzarella cheese. The higher the

concentration of roselle extract added, the lower the amount of total solids formed. The acid content in the roselle extract plays an important role in this process because different levels of acidity will affect the coagulation of casein in the milk. This coagulation process determines how effectively the curd can be separated from the whey. If the curd is easily separated, the cheese's total solids will increase. This is consistent with the opinion of [17], who state that the easier the curd is separated from the whey, the higher the cheese's total solids. Adding acid during cheese making can also increase the effectiveness of the rennet enzyme, resulting in a denser curd. This prevents casein and fat from dissolving extensively in the whey, which leads to an increase in the total solids of the mozzarella cheese.

The total solids measured in mozzarella cheese include organic acids, protein, and other components. The organic acids found in roselle have a high concentration because they come from the roselle flower extract, which contains natural acids. This is consistent with the opinion of [7], who state that roselle flower extract has a relatively high content of organic acids. Protein in cheese is formed through the coagulation process of milk when the pH reaches the isoelectric point, causing the protein to clump and turn into a solid. Roselle flowers also contain various solid ingredients such as protein, fat, fiber, and vitamin C. These contents contribute to increasing the total solids in mozzarella cheese to which roselle flower extract is added.

Table 2: Test Results for Total Solid Mozzarella Cheese Ingredients with red rosella flower extract acidifier

Treatment	Total Solids (%)
P0	45,37±0,64 ^b
P1	47,21±0,44 ^c
P2	46,31±1,17 ^{bc}
P3	44,20±0,78 ^a

4.3 Total Acid

Acidification is an important stage in the coagulation of milk protein during mozzarella cheese production. This process helps the enzyme work optimally in curd formation and whey separation. The level of acidity plays a major role in all stages of milk coagulation. The addition of roselle flower extract concentration can affect the total acidity in mozzarella cheese because roselle contains various organic acids with a pH ranging from 1.5 - 2.5. This is in accordance with the statement of [13] which states that roselle flower extract has a pH of 2.24. The higher the concentration of roselle extract used, the higher the resulting acidity level of the mozzarella cheese.

An increase in the percentage of roselle extract causes a higher acidity value to be maintained in the coagulum during the coagulation process with the rennet enzyme, which results in a greater acid titration value. Cheesemaking with the rennet enzyme still requires a combination of acid to assist the coagulation process [19]. A high amount of acid and optimal rennet action will maximize the coagulation process because the acid component is also coagulated. The total acid in mozzarella cheese is usually calculated based on the product's acidity equivalent to lactic acid, so roselle extract as a source of organic acids directly affects the level of acidity and the success of the coagulation process.

Table 3: Total Acid Test Results for Mozzarella Cheese with red rosella flower extract acidifier

Treatment	Total Acid (%)
P0	6,44±0,77 ^c
P1	2,58±0,29 ^a
P2	4,52±0,50 ^b
P3	5,82±1,60 ^c

4.4 Stretchability

The addition of red roselle flower extract can affect the physical properties of mozzarella cheese, especially in increasing the cheese's stretchability. Roselle flowers contain organic acids that play a role in the acidification process during curd formation. Optimal acidification helps the rennet enzyme work better, so the formed curd becomes denser and the resulting mozzarella cheese has good stretchability. The addition of acid can increase the amount of micellar calcium retained in the cheese, making the cheese softer and easier to stretch. A softer cheese texture usually also indicates higher moisture content. Stretchability in mozzarella cheese is also influenced by the process of heating the curd which is then gradually pulled until cheese with a stretchy texture is formed. This aligns with the opinion of [9], who state that the level of stretchability can occur due to the stretching process on the curd that has been separated from the whey. The stretchability ability of mozzarella cheese comes from the strong bonds of casein molecules, giving the cheese its stretchy property.

Excessively high addition of roselle extract in cheesemaking can increase the protein hydrolysis process due to a greater acid content. This hydrolysis process cuts long protein chains into shorter chains, which can occur through enzymes or by acid. Excessive use of acid can accelerate and strengthen protein hydrolysis, causing mozzarella cheese to lose its ability to form strong and dense elastic fibers when heated. This is consistent with the opinion of [15], who state that increasing acid concentration will shorten the protein chains, thus decreasing the cheese's elasticity.

Table 4: Test Results for the Stretchability of Mozzarella Cheese with red rosella flower extract as acidifier

Treatment	Stretchability (cm)
P0	152,40±4,39 ^c
P1	84,00±4,95 ^a
P2	116,20±5,89 ^b
P3	180,60±4,83 ^d

V. CONCLUSION

Based on the research that has been conducted, it can be concluded that the use of red roselle flower extract with different concentrations as an acidulant in the production of mozzarella cheese caused changes in the activities of moisture content, total solids, total acidity, and stretchability. The variation of added red roselle flower extract increased the moisture content, decreased the total solids value, increased total acidity, and enhanced the stretchability of the mozzarella cheese product.

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Citation of this Article:

Heni Rizqiati, Anang Mohamad Legowo, Fariz Nurmita Aziz, & Sadhewa Mahardika Hardian Syahputra. (2025). The Effect of Red Rosella Extract as an Acidian an Water Content, Total Solid Ingredients, Total Acid, and Melasticity in Mozzarella Cheese. *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 9(11), 238-243. Article DOI <https://doi.org/10.47001/IRJIET/2025.911029>
