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Effect of red rosella flower extract as an acidulant on yield, total dissolved solids, texture hardness, color, antioxidant activity, and pH value of Mozzarella cheese

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ABSTRACT

Mozzarella cheese is a highly sought-after dairy product, but the consumption of dairy-based products in Indonesia is still relatively low. The cheese-making process is highly dependent on the coagulation stage, which involves acidification using red rosella flower extract. This study aims to investigate the effect of using red rosella flower extract as an acidulant in Mozzarella cheese production on yield, total soluble solids, texture, color, antioxidant activity, and pH value of the resulting cheese. This study employed a completely randomized design (CRD) with four treatments, namely variations in the concentration of red rosella flower extract at T1 (4%), T2 (5%), and T3 (6%) compared to the control T0 (1,4% acetic acid). The data obtained were analyzed using Analysis of Variance (ANOVA) at a significance level of 5%, followed by Duncan's Multiple Range Test (DMRT). Data obtained from testing Mozzarella cheese with different concentrations of rosella flower extract treatment obtained yield values of 10.07-11.10%; total soluble solids 0.62-2.04 oBrix; texture 1025.10-2291.00 gf; total pH value 5.16-5.65; lightness value (L*) 53.78-87.22; redness value (a*) 2.49-7.31; yellowness value (b*) 6.97-16.67; antioxidant activity 18.42-37.11% inhibition. The higher the concentration of red rosella flower extract, the lower the yield of Mozzarella cheese, the higher the total soluble solids, the softer the texture of Mozzarella cheese, and the more acidic the pH value of Mozzarella cheese.

Introduction

Milk is a product produced from the process of milking a cow's udder and has characteristics such as a white liquid that naturally does not undergo reduction or addition of treatment except for the cooling process. The consumption level of processed milk-based products in Indonesia is still relatively low, which is around 8 liters per capita per year compared to other countries (Alfiah and Waluyo, 2015). Fresh milk that does not go through processing can only last for one day. The problem experienced can be overcome through the processing of cow's milk so that it can become a product that is more durable and not easily damaged (Hutagaol *et al*., 2024). One effective method to extend the shelf life of cow's milk is to process it into Mozzarella cheese.

The addition of rosella extract in the Mozzarella cheese making process aims to achieve a condition where protein molecules are in a neutral state or at the isoelectric point which will produce curd deposits. If the amount of curd formed is large, the yield value of the cheese formed is high (Putri *et al*., 2020). The average total soluble solids of fresh Mozzarella cheese from cow's milk is 11.34% (Elgaml *et al*., 2024). The more efficient the process of separating curd from whey in both the filtering and clumping stages, the higher the total solids contained in the cheese. Rosella petals contain organic acid ingredients that are used to improve the quality of Mozzarella cheese products, with the presence of anthocyanin pigments that form antioxidants. When the amount of anthocyanins increases, the antioxidant activity will also increase. Rosella petals contain effective natural acids that can lower the pH of milk. The addition of purple rosella flower extract results in a lower pH that plays a role in the coagulation process of casein protein, and antioxidants in Mozzarella cheese (Wardhani *et al*., 2018). However, it needs to be studied further whether the use of red rosella flower extract as an acidulant can be used for making Mozzarella cheese. Therefore, this study used red rosella flower extract as acidulant to determine the quality of Mozzarella cheese in terms of yield, total soluble solids, texture, color, antioxidant activity, and pH value. Developing a Mozzarella cheese formulation using a natural acidulant (red roselle extract) that not only substitutes acetic acid but also enriches the cheese with bioactive antioxidant compounds.

Materials and methods

Materials

The Materials used in this research are fresh cow milk from the Faculty of Animal Science and Agriculture, Diponegoro University, liquid rennet marketed by Cheese Journey, Vinegar (Belibis, Indonesian), distilled water, methanol, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) purchased from local chemical store in Semarang, ice water obtained from Food Chemistry and Nutrition Laboratorium, Diponegoro University, and rosella flowers obtained from Rasamala Traditional Market, Banyumanik, Semarang. The tools needed were pot, stove, stirrer (Memmert, Germany), analytical balance (Osuka, Japan), thermometer, beaker, measuring cup, erlenmeyer (Iwaki, Indonesian), filter cloth, spoon, plastic wrap, refrigerator (Modena, Indonesian), pH meter (IKAMaT, Indonesian), hand-held pocket refractometer (PAL-1, Japan), texture analyzer TA-TX Plus 100 (Lamy Rheology, France), centrifugation (OHAUS, USA), centrifuge tube (OHAUS, USA), drop pipette, color reader (Konica Minolta, Japan), and UV-VIS spectrophotometer (MRC, England).

Methods

The research was conducted from February to April 2025 at the Food Chemistry and Nutrition Laboratory, Faculty of Animal and Agricultural Science, Diponegoro University. Semarang. The research included making red rosella flower extract, making Mozzarella cheese, and parameter testing. Parameter testing included yield produced, total soluble solids produced, texture formed, color, antioxidant activity, and pH value.

Procedure of red rosella flower extract

The preparation of red rosella flower extract refers to the research of Anggraeni (2017). The first stage of fresh rosella flowers was dried and

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then mashed until powdered, sieved using a 60 mesh sieve. Rosella flower powder was dissolved in water in a ratio of 100 g : 300 ml and pasteurized at 63-65°C for 30 minutes. The rosella solution was then filtered to extract the liquid.

Mozzarella Cheese sample making procedure

The procedure for making Mozzarella cheese follows Hakim's research (2020) The first stage is pasteurized milk at 70°C, then cooled until the milk temperature reaches 35°C. Then the milk was added with vinegar acid or red rosella flower extract according to the variation of concentration treatment, namely T0 (1.4% vinergar acid), T1 (4%), T2 (5%), and T3 (6%), rennet enzyme as much as 0.025% (v/v) of the milk volume after which it was allowed to stand for 10 minutes until curd was formed. Then the curd that has been formed is cut into the size of a dice, then separate the curd with whey by filtering it until it is completely separated. Stretching is done at 75-85°C and then the finished Mozzarella cheese is placed in ice water for 30 minutes.

Yield testing procedure

Yield is the ratio between the weight of cheese products produced and the amount of milk raw materials used. The measurement of yield aimed to determine the level of efficiency of the product produced (Fadhlurrohman *et al*., 2023). Mozzarella cheese yield testing was conducted by comparing the weight of curd separated from whey. The test is based on the research of Hamad (2015) by calculating the yield of Mozzarella cheese using the following formula.

Yield (%) = a/b x 100

Description:

a = weight of cheese produced (g)

b = weight of milk used (g)

Total dissolved solids testing procedure

The measurement of total dissolved solids is based on research of Alfarisi *et al*. (2023) which was modified, using a hand-refractometer. TDS measurement in Mozzarella cheese aims to evaluate the difference in the effects of pure acetic acid and rosella extract as coagulants. A 1 g sample of Mozzarella cheese was dissolved in distilled water until the volume reached 10 ml. The solution was then homogenized using a centrifuge at 2000 rpm for 10 minutes. Before use, the refractometer prism was cleaned with distilled water and dried with a soft cloth. A total of 2-3 drops of sample were dropped on the refractometer prism to measure the degree brix. The results of total dissolved solids (TPT) can be seen on the hand refractometer with units (°Brix).

Texture testing procedure

Texture measurement uses a method based on the research of Nugroho *et al*. (2018) related to indicators of hardness and softness. Cheese texture measurement can be done using the TA-TXPlus Texture Analyzer tool. This tool operates based on the principle of product durability measured through the applied compressive force, as well as the ability of food ingredients to return to their original condition after the pressure load is removed (Estiningtyas and Rustanti, 2014). The cheese texture test procedure starts with cutting the cheese sample into cubes with a side size of 3 cm. The probes were installed in the appropriate position with respect to the sample. The device was turned on and set so that the value on the monitor should show zero. The start test menu setting was selected so that the probe started piercing the sample. The test is considered complete when the probe returns to its initial position. The results of this test are displayed in the form of graphs and numerical values that provide information on the texture characteristics of the cheese being tested.

Testing procedure for pH value

Measurement of pH value was carried out based on AOAC (2005). A sample of 5 g was added with 5 ml of distilled water and homogenized. Next, the pH value was measured using a calibrated pH meter. Then the pH meter was dipped into the cheese solution.

Color and appearance testing procedure

Color measurement on Mozzarella cheese is done by using a tool called color reader in accordance with (Dwiokta, 2024). The sample that has been cut into equal pieces is then placed under the color reader lens, then if the tool is pressed, it will shoot a flash of light that will capture the L*, a* and b* values of the Mozzarella cheese sample and recorded by the computer. The L*, a* and b* values will indicate the bright color of the sample and the lightness or darkness of the material.

Antioxidant activity testing procedure

Antioxidant activity testing was carried out using the DPPH method in accordance with (Subagio and Morita, 2001). Samples as much as 0.1 g were suspended with 20 ml of methanol in Erlenmeyer and stirred for 10 minutes. Then centrifuged at 5000 rpm for 5 minutes. Then 1 ml of filtrate was taken plus 0.5 ml of DPPH reagent (4x10-4) and allowed to stand for 20 minutes after adding methanol to 5 ml. The absorbance was immediately measured at 517 nm, then the absorbance was read. The data generated will be converted into tables using Microsoft Excel.

Data analysis

Data from the yield, total soluble solids, and texture tests were analyzed using Analysis of Variance (ANOVA) with a statistical significance level of 5%. The ANOVA test was conducted to determine the effect of the treatment, if there is an effect on the treatment then a further test will be carried out using Duncan's Multiple Range Test (DMRT) test. The DMRT test aims to determine the differences between the treatments given.

Results

Yield and total dissolved solids

Based on the results shown in Table 1, the addition of red rosella flower extract at different concentrations had a significant effect on Mozzarella cheese yield ($p<0.05$). The highest yield value was obtained in treatment T1 (4% rosella extract) at 11.10±0.46%, but statistically it was not significantly different from T0 (1.4% acetic acid control) at 10.93±0.54%. Meanwhile, treatments T2 (5% rosella extract) and T3 (6% rosella extract) showed lower yields, namely 10.45±0.60% and 10.07±0.62%. This difference is thought to be related to the composition of organic compounds in rosella extract, which contains organic acids (mainly citric acid and malic acid) and other bioactive compounds that can affect the milk protein coagulation process. Excessively high extract concentrations tend to increase acidity, resulting in less than optimal casein coagulation and lower yields compared to medium concentration treatments.

Table 1. Yield and total dissolved solids by acidification of red rosella flower extract test results.

Treatment	Yield (%)	Total Dissolved Solids (°Brix)
T0	10.93±0.54^{a}	0.62±0.08^{a}
T1	11.10±0.46^{a}	0.88±0.15^{b}
T2	10.45±0.60^{ab}	1.46±0.11^{c}
T3	10.07±0.62^{b}	2.04±0,19^{d}

Data are shown as mean values of 5 replicates±standard deviation. T0 = 1.4% acetic acid acidulantr; T1 = 4% red rosella flower extract acidulant; T2 = 5% red rosella flower extract acidulant; T3 = 6% red rosella flower extract acidulant.

Total dissolved solids (TDS) in Mozzarella cheese were also significantly affected by treatment ($p<0.05$). The TDS value increased with increasing rosella extract concentration, starting from 0.62±0.08°Brix (T0), 0.88±0.15°Brix (T1), 1.46±0.11 °Brix (T2), to the highest at T3 of 2.04±0.19°Brix. This increase indicates that the addition of rosella extract can enrich the dissolved solids content in cheese. This can be explained by the high content of organic acids, anthocyanins, and phenolics in rosella extract that are soluble in the cheese serum phase. These compounds increase the total soluble solids when compared to the use of pure acetic acid as a coagulant. These results are consistent with the literature reporting that the presence of natural organic acids and phytochemical compounds from plants can increase the soluble solids content in fermented milk products.

Texture hardness and pH Value

The addition of red rosella flower extract had a significant effect ($p<0.05$) on the texture of Mozzarella cheese. The highest cheese texture hardness value was obtained in treatment T1 (4% red rosella extract) at 2291.00±211.82 gf, followed by T2 (1.4% acetic acid) at 1705.20±163.92 gf. A decrease in texture value occurred with increasing acidity using rosella extract in T3 (5% red rosella) at 1207.70±106.22 gf, and the lowest texture value was shown by treatment T0 (6% red rosella extract) with a result of 1025.10±101.18 gf. Changes in texture caused by excessive proteolytic activity, resulting in the breakdown of many peptide bonds in casein proteins. The higher the concentration of acid added, the less stable the curd structure will be, resulting in a softer cheese texture.

Differences in acid concentration had a significant effect ($p<0.05$) on the pH value of Mozzarella cheese. Mozzarella cheese made using 1.4% acetic acid (T0) as the acidifier had the highest pH value of 5.16. Mozzarella cheese production using rosella extract as the acidifier showed a decrease in pH value as the rosella extract concentration increased, with recorded values of 5.65 at T1 (4%), 5.56 at T2 (5%), and 5.25 at T3 (6%). All pH values are acceptable, as pH values between 5.0 and 6.0 are in line with the quality standards set by SNI 01-3816-1995.

Table 2. Texture and pH value by acidification of red rosella flower extract test results.

Treatment	Texture (gf)	pH Value
T0	1025.10±101.18[a]	5.16±0.016[a]
T1	2291.00±211.82[b]	5.65±0.018[b]
T2	1705.20±163.92[c]	5.56±0.016[c]
T3	1207.70±106.22[d]	5.25±0.019[d]

Data are shown as mean values of 5 replicates±standard deviation. T0 = 1.4% acetic acid acidulantr; T1 = 4% red rosella flower extract acidulant; T2 = 5% red rosella flower extract acidulant; T3 = 6% red rosella flower extract acidulant.

Color

The color values of Mozzarella cheese in each treatment had a significant effect ($p<0.05$) on lightness (L*), redness (a*), and yellowness (b*). Mozzarella cheese made using 1.4% acetic acid (T0) as an acidifier had the highest lightness (L*) value of 87.22. Mozzarella cheese production using rosella extract as an acidifier showed a decrease in lightness (L*) with increasing rosella extract, with values of 64.45 at T1 (4%), 61.07 at T2 (5%), and 53.78 at T3 (6%). The brightness value in Mozzarella cheese decreases due to the addition of anthocyanins in the dark red rosella extract.

The production of Mozzarella cheese using 6% rosella extract (T3) as an acidifier has the highest redness (a*) value of 7.31. The production of Mozzarella cheese using rosella extract as an acidifier showed an increase in redness value (a*) with increasing rosella extract, with values recorded at 5.76 in T2 (5%), 4.45 in T1 (4%), and 2.49 in 1.4% citric acid acidifier (T0). The addition of rosella extract to Mozzarella cheese increases anthocyanin content, making the Mozzarella cheese redder in color.

The production of Mozzarella cheese using 1.4% acetic acid (T0) as an acidifier resulted in the highest yellowness (b*) value of 16.67. Mozzarella cheese production using rosella extract as an acidifier showed a decrease in yellowness (b*) with increasing rosella extract, with values of 8.87 at T1 (4%), 7.75 at T2 (5%), and 6.97 at T3 (6%). When rosella extract is mixed with milk with a neutral pH, anthocyanins turn purple and lose their yellow color in the milk.

Table 3. Color of Mozzarella cheese by acidification of red rosella flower extract test results.

Treatment	*Lightness* (L*)	*Redness* (a*)	*Yellowness* (b*)
T0	87.22±0.44[a]	2.49±0.13[a]	16.67±0.41[a]
T1	64.45±0.45[b]	4.45±0.32[b]	8.87±0.30[b]
T2	61.07±0.40[c]	5.76±0.28[c]	7.75±0.28[c]
T3	53.78±0.48[d]	7.31±0.20[d]	6.97±0.32[d]

Data are shown as mean values of 5 replicates±standard deviation. T0 = 1.4% acetic acid acidulantr; T1 = 4% red rosella flower extract acidulant; T2 = 5% red rosella flower extract acidulant; T3 = 6% red rosella flower extract acidulant.

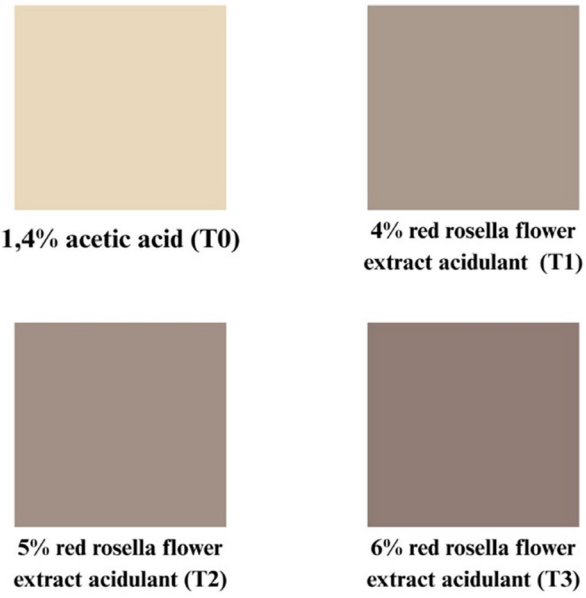

Fig. 1. Mozzarella cheese coloring with red rosella flower extract acidulant.

Antioxidant activity

The results of the study indicate that the production of Mozzarella cheese using red rosella flower extract showed a linear increase in antioxidant activity with the addition of treatment. The production of Mozzarella cheese using 1.4% acetic acid as the acidifier does not contain antioxidants, then there is an increase in antioxidant activity with a concentration of red rosella flower extract acidifier of 4% to 18.42%, followed by an increase in antioxidant activity with a 5% concentration of red rosella flower extract acidifier to 27.28%, and further increased with a 6% concentration of red rosella flower extract acidifier to 37.11%. The increase in antioxidant activity is consistent with the increasing use of red rosella flower extract.

Discussion

The average yield obtained was 10.07%–11.10%. The yield results in this study were higher than those reported by Rosyidi *et al*. (2007), which had an average yield of 9.41%–10.15%. The mixture of organic acids, citric acid, and malic acid has an acidity closer to the isoelectric point of milk casein at pH 4.7 compared to acetic acid in vinegar. The cheese yield produced using citric acid is higher than that produced using acetic acid (Mbye *et al*., 2020). The isoelectric point is the state where casein pro-

tein molecules have no charge, causing denaturation and precipitation of proteins to occur optimally. Milk in an acidic state causes casein proteins to become unstable and form solid clumps called curds. The coagulation process involving protease enzymes is greatly influenced by the acidity level of the milk, so the quality and consistency of the curds depend heavily on the pH during acidification. Rennet enzymes work by cutting κ-casein at positions Phe_{105} and Met_{106} to produce homogeneous curd and ensure that the water content trapped in the curd has a relatively even distribution (Fox *et al*., 2017). The decrease in yield for treatments T2 and T3 after the optimum point in treatment T1 is due to changes in milk acidity during the coagulation process. This aligns with the findings of Lanur *et al*. (2025), who stated that excessively high acid levels can cause excessive damage to milk proteins, particularly casein, due to intensive proteolytic activity, resulting in more casein dissolving into the whey and producing less curd. Excessive acid concentration in milk can trigger enzyme activity during the coagulation process and affect curd strength. Adding acid to cheese at the appropriate pH affects whey release from the cheese and can increase casein molecule binding, leading to curd formation (Bansal and Veena, 2024).

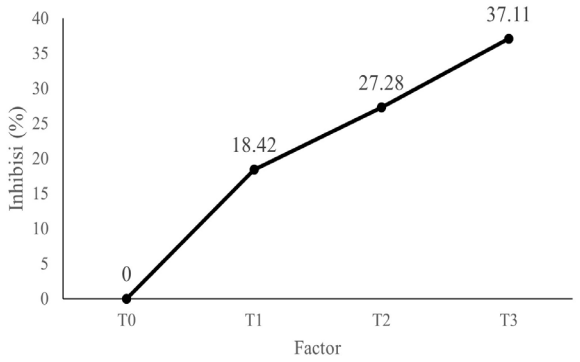

Fig. 2. Graph of antioxidant activity of Mozzarella cheese acidified with red rosella flower extract.
T0: 1.4% acetic acid acidulant; T1: 4% red rosella flower extract acidulant; T2: 5% red rosella flower extract acidulant; T3: 6% red rosella flower extract acidulant.

The treatment that produced the highest total dissolved solids value was T3, while the treatment that produced the lowest total dissolved solids value was T0 because the components measured as total dissolved solids include sucrose, reducing sugars, organic acids, and proteins, so that the higher the concentration of acid added, the higher the total dissolved solids contained in the cheese. Because the components measured as total dissolved solids include sucrose, reducing sugars, organic acids, and proteins, the higher the concentration of acid added, the higher the total dissolved solids contained in the cheese. The average total dissolved solids value obtained was 0.62– 2.04 oBrix. Organic acids such as citric acid and malic acid are the main components in rosella extract that contribute to total dissolved solids. The composition of total dissolved solids formed consists of organic acids, pigments, total sugars, and proteins (Diasari *et al*., 2021). An increase in the concentration of red rosella extract proportionally shows a significant increase in organic acid content and total dissolved solids. Anthocyanin content, which is a natural pigment in the flavonoid compound group in red rosella petals, is also soluble in water and gives cheese its natural red color (Winarti and Firdaus, 2010). The presence of anthocyanins not only indicates a change in color but also a change in pH value. The lower the pH value, the higher the content of organic acids present (Nugroho *et al*., 2018).

The highest Mozzarella cheese texture value was obtained in treatment T1 using 4% rosella, while the lowest texture value was shown in treatment T3 using 6% rosella because lower pH levels can cause excessive protein denaturation and cause more protein to dissolve into the whey, causing the curd structure to become less stable and resulting in a softer cheese texture. The average texture value produced was 1207.70 – 2291.00 gf. The acidic properties of rosella flower extract, namely citric acid and malic acid, can coagulate protein networks (Hartanti and Lestari, 2018). Changes in texture values caused by excessive proteolytic activity result in the breakdown of many peptide bonds in casein protein (Nugroho *et al*., 2018). Higher acid concentrations result in less stable curd structure, leading to softer cheese texture. Lower pH levels can cause excessive protein denaturation, resulting in more soluble protein in the whey (Wardhani *et al*., 2018). An imperfect curd due to a pH that is too low causes the curd to be insufficiently dense and unable to bind water stably. Milk coagulation caused by a pH that is too low results in a curd that is not compact, leading to a very high water content (Anggraini *et al*., 2013). Very high water content can make the final cheese product have a soft texture, be easily broken, and lack elasticity. The water content in cheese can affect the texture and density of the cheese (Arifiansyah *et al*., 2015).

The production of Mozzarella cheese using 6% rosella extract (T3) as an acidifier resulted in the highest redness (a*) value of 7.31. The addition of rosella extract to Mozzarella cheese increased the anthocyanin content, making the Mozzarella cheese redder in color. The higher the rosella flower extract content, the redder the color becomes, accompanied by a decrease in brightness (L*) value, but the redness value increases. (Gregory *et al*., 2024). Mozzarella cheese production using 1.4% acetic acid (T0) as an acidifier has the highest yellowness (b*) value of 16.67. When rosella extract is mixed with milk at a neutral pH, anthocyanins will turn violet as the pH increases (Anggistia *et al*., 2016). Anthocyanins undergo color degradation depending on pH. Anthocyanins exist in five equilibrium forms depending on pH conditions. Anthocyanins appear red at pH < 4, gray at pH 4–5, violet at pH 6–7, blue at pH 7–8, and yellow at pH > 8 (Ifadah *et al*., 2022).

The increase in antioxidant activity is consistent with the increase in red rosella flower extract used. This is in line with the opinion (Diba'Izzati *et al*., 2024) that the higher the amount of rosella added, the greater the antioxidant activity in the product. Red rosella flowers contain anthocyanins as antioxidants. This antioxidant activity also supports the health such as protection against oxidative stress, premature aging, and prevention of various degenerative diseases, thereby helping to prevent conditions like kidney disease, coronary heart disease, hypertension, cancer, and diabetes (Pratiwi, 2018). The high vitamin C content in red rosella contributes to significant antioxidant activity. Vitamin C is a water-soluble compound that functions as a strong antioxidant, capable of neutralizing free radicals by donating electrons. This activity is crucial in preventing oxidative stress, which can damage cells and body tissues (Nurnasari and Khuluq, 2017).

In general, the lower the pH value of milk, the softer the curd will be, while a high pH will produce hard Mozzarella cheese. This is in line with the opinion of Nugroho *et al*. (2018), who state that a low pH will produce curd with a brittle texture that is easily broken. Vinegar acid plays a role in lowering the pH, aiming to optimize the activity of rennet during the curd formation process. Optimizing rennet activity results in curd that can hold more protein at the isoelectric point. In Mozzarella cheese production, acidifiers can be substituted with red rosella flower extract to break down casein. This aligns with the findings of Wahab *et al*. (2021), who state that red rosella flowers contain malic acid and citric acid. The natural acids in red rosella are not as strong as acetic acid, thus requiring a different formulation.

Conclusion

Based on the research conducted, it can be concluded that red rosella flower extract can act as an acidulant in making Mozzarella cheese by affecting yield, total soluble solids, texture, color, and pH value. The higher the concentration of red rosella flower extract, the lower the yield of Mozzarella cheese, the higher the total soluble solids, the softer the texture of Mozzarella cheese, and the more acidic the pH value of Mozzarella cheese.

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Conflict of interest

The authors declare that in this study, there is a potential conflict of interest because the researcher has a personal interest related to the services of the company that uses students as research subjects. The researcher is committed to ensuring that all data obtained and analysis conducted are not affected by personal interests and maintained the integrity and objectivity of this research.

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