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PERFORMANCE IMPROVEMENT OF YOGURT THROUGH THE VARIATION OF ROSELLE EXTRACT ADDITION AND FERMENTATION TIME

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ABSTRACT

Yogurt was a functional drink that was high in nutritional value but had a relatively short shelf life. Roselle extract had a high content of bioactive compounds, namely anthocyanin, polypHenol, and vitamin C. The integration of yogurt with roselle extract was expected to improve product performance both from the properties of health and sensory. This study aimed to determine the effect of roselle extract addition and fermentation time on the characteristics of roselle-based yogurt products. The research method used was an experimental method with a completely randomized design with Duncan's further test with treatments (1) the addition of rosella extract consisted of 3 levels, namely 0.5, 1.5, and 2.5 %. (2) Fermentation time: 8, 12, and 16 hours. The observed variables included chemical (pH, total titrated acid/TTA, anthocyanins), microbiology (lactic acid bacteria/LAB), and sensory. The fermentation time affects the pH value and TAT. The 12 hours of fermentation time gave pH and TTA values, respectively: 3.77 and 1.23%. The variation in the addition of roselle extract significantly affected the anthocyanin, TTA, and LAB values. The addition of the extract as much as 1.5% gave the anthocyanins, TTA, and LAB value respectively 0.871 mg/mL, 1.31%, 561.11x 107CFU/mL. The best treatment was yogurt with a roselle extract concentration of 1.5% and 20 hours of fermentation. This yogurt had characteristic as follows: pH 3.7, TAT 1.33%, anthocyanins 0.8751 mg/mL, LAB 560 x 107 CFU/mL, reddish-yellow (4), distinctive aroma (4), Sour taste (4), fine texture (4), and liking (4).

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INTRODUCTION

Yogurt was one of the nutrient-rich beverages (Jannah et al., 2014). Yogurt drink was a fermented milk drink by Lactobacillus Bulgaricus and Streptococcus thermopHillus (Adolfson et al.,2004). Sunarlim (2007) stated that yogurt as milk fermentation had benefits: Increased nutritional value, increased food variation, as a natural preservative, and get the texture and flavor that was liked.

Recently, yogurt innovations that developed were the addition of yogurt efficacy and flavor improvement. The added efficacy of yogurt was often done with variations of types and amounts of probiotic bacteria. The flavor improvement was made with the addition of fruit, herbs, or other herbal plants.

Roselle is a herbal plant with efficacy as a colorant (Arueya and Akomolae, 2014; Mardiah, 2010), antioxidant, and antibacterial (Purbowati et al.,2018; Mourtzinos et al.,2008). These abilities of roselle were due to the content of bioactive compounds such as polypHenols, anthocyanins, flavonoids, vitamin C, and sterols.

The efficacy of roselle flower petals in diabetes prevention, was due to their high antioxidant content. The bioactive compounds in the cell plant were obtained by the extraction processed. According to Purbowati et al. (2016), MAE was an effective technique for extracting bioactive roselle compounds. The 4.91 minutes extraction time and extraction power of 250 W, extracted pHenolic compounds (19.45 ± 0.32 mg/g), anthocyanins (13.51 ± 0.03 mg/g) and vitamin C (20.47 ± 0.34 mg/g) efficiently.

Kharismawati et al. (2015) stated that the more intense red color in the roselle flower petals was resulted from the higher anthocyanin content. According to Purbowati et al. (2018), the antioxidant activity was positively correlated with a total pHenol, vitamin C, and anthocyanin content. These led to the addition of Rosela extract to add efficacy and as a colorant, limited by the acid flavor caused. Therefore, it was necessary to study the variation of the addition of Rosela extract with the content of bioactive components that still can be acceptable to consumers.

The addition of Roselle extract with a variety of concentrations in the making of yogurt drink also resulted in the adjustment of fermentation time done. The properties of the roselle extract that served as an antioxidant and antibacterial substance resulted in the fermentation time that must be done to produce yogurt products as prescribed standards.

This research sought to develop product innovations in yogurt by finding out the effect on the variation of the addition of roselle extract and fermentation time. The function of roselle extract as an antibacterial and antioxidant material until a certain concentration was thought to be able to control the number of prebiotic bacteria in yogurt while affecting the typical acid flavor and consumer-liked.

MATERIAL AND METHODE

Material

The materials used in this study were: fresh milk, Lactobacillus bulgaricus, and Streptococus thermophilus, roselle petals extract, cane sugar, skim milk, NaCl 0.85%, aquadest, nutrient agar, 0.1%, NaOH solution, 1% pHenolpHthalein indicator (PP), acidic ethanol.

Extraction

Ten grams of the dried roselle petals were ground for 1 minute using a grinder and sieved in 60 mesh. The extraction was done in 100 mL aquadest, microwave power extraction 250 W, and 5 minutes of extraction. The microwave used for extraction was Electrolux EMM 2007X. The slurry was radiated in a microwave oven at regular intervals (one-minute radiation and two minutes off) to keep the temperature did not rise above the boiling point (Purbowati et al., 2016). Roselle extract was filtered and concentrated with a vacuum evaporator at 70oC, 44 cmHg and blowing with N2 to ensure the solvent evaporate.

Making Yogurt

Fresh milk was pasteurized at a temperature of 72 ºC for 15 seconds to kill pathogenic bacteria in milk. Then the pasteurization milk was cooling until the temperature dropped to 37 ºC- 45 ºC and filtering. After screening, the inoculum bacteria starter as much as 5% of the total volume of milk gained. Fermentation was then carried out with a variation of the length of fermentation time, i.e., 12, 16,20 hours at 40 ºC. After the fermentation was completed and then added roselle extract with a variation of 0.5%, 1.5%, and 2.5%. Yogurt ready to be analyzed

PH Measurement (Widagdha and Nisa, 2015)

A homogenized sample was taken approximately 30 mL and placed in a glass beaker of 50 mL. Before used, the pH meter was calibrated using a pH buffer of 7 and 4 and was then cleaned with the Aquadest subsequently performed pH measurements of the sample. Each time it will measure the pH of another sample, the previous pH meter was cleaned with the aquadest.

Determination of Total Titrated Acid (TTA) levels (Widagdha and Nisa, 2015)

Determination of the total rate of acetic acid by titration using NaOH 0.1 N and PP indicator. A total of 10 grams of yogurt was inserted into the measuring flask 100 mL. Samples were then homogenized and filtered. A total of 10 mL of filtrate was taken and inserted into the Erlenmeyer, then added 2-3 drops of the PP indicator. The sample solution was then injected with a solution of 0.1 N NaOH until the color of the solution changes to pink and the color did not change back for 30 seconds. At the end of the titration was calculated the amount of NaOH used.

Total acid (%) = (V x N x P x BE lactic acid)/(sample weight x 1000) x 100%

Total Anthocyanin (Fuleki & Francis, 1968)

A total of 1 ml of the sample solution was added to 1 ml acidic ethanol (95% ethanol + N HCL (85:15 V/V)). Samples were stored at 40 °c for 24 hours. Then the sample solution was diluted up to 3 mL and homogenized using a vortex. The absorption of the sample solution was measured using a wavelength of 535 nm.

Total Plate Count (TPC) analysis (AOAC, 2000)

Determination of the number of lactic acid bacteria (LAB) in yogurt by TPC method, through the creation of growth media nutrient agar (NA), planting until calculating the number of colonies on the slab. The number of live microbes were achieved by multiplying the number of colonies by the dilution factor.

Sensory analysis (Rahayu and Nurosiyah, 2008)

Samples were served in a uniform size plastic cup. The panelist consisted of 25 semi-trained panelists with a range of 19-25 years of age, male or female student status. The five attributes assessed on the sensory analysis included color, flavor, texture, taste, and preference. The form of the color sensory Test questionnaire consisted of five scales of assessment, namely (1) yellowish-white, (2) light yellow, (3) yellow, (4) reddish-yellow, and (5) pink. On the form of the flavor sensory Test questionnaire consisted of five scales of assessment, namely (1) very non-typical, (2) not typical, (3) quite typical, (4) typical, (5) very typical. The form of the texture sensory Test questionnaire consisted of five scales of assessment, namely (1) not very smooth, (2) not smooth, (3) mild, (4) smooth, (5) very smooth. The form of the taste sensory Test questionnaire consisted of five scales of assessment, namely (1) not very acidic, (2) not acidic, (3) slightly acidic, (4) acidic, (5) very acidic. The form of the preference sensory Test questionnaire consisted of five scales of assessment, namely (1) very disliked, (2) dislikes, (3) somewhat fond, (4) Likes and (5) very fond.

Analysis Data

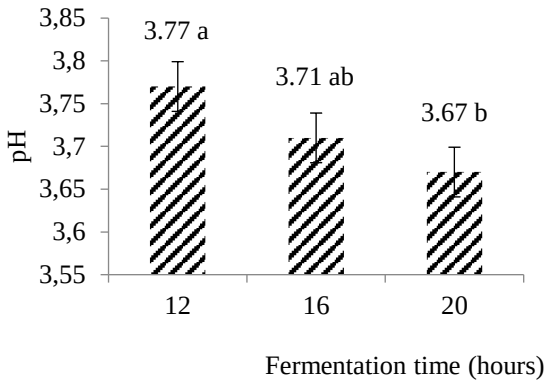
Chemical variables (pH, TTA, reducing sugars), and microbiology (LAB) data were analyzed by ANOVA at the level of 5%. If there was a real influence, continue with Duncan's multiple area tests. Data on the organoleptic test were analyzed by a descriptive statistical method and to determine the best treatment using the exponential comparison method (MPE).

RESULT AND DISCUSSION

The results showed that the duration of fermentation had a significant effect on the pH value, total titrated acid and had no significant impact on anthocyanins and total lactic acid bacteria. Variations in the addition of roselle extract significantly affected anthocyanins, total titrated acid, and total lactic acid bacteria and had no significant effect on pH. The interaction between the two treatments did not affect substantially to all variables.

pH

The pH value was one of the crucial characteristics in the evaluation of dairy products such as yogurt. The results of measuring the pH value of yogurt at different fermentation times were presented in Figure 1



Note: Same letters above the bars indicate no significant difference.

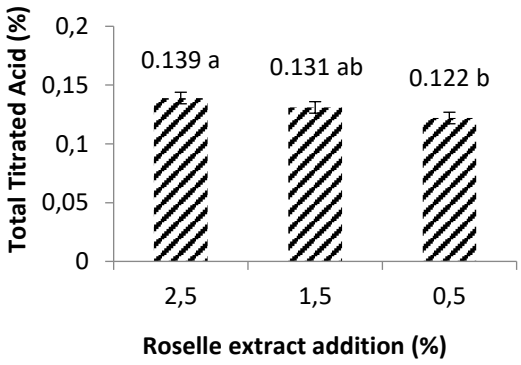
Figure 1 The effect of fermentation time to yogurt pH value

Figure 1 showed the average pH of yogurt with 12 hours, 16 hours, and 20 hours fermentation time treatment, respectively, 3.8; 3,7; 3,6. It can be seen that longer fermentation time had decreased the pH value. Jannah et al.(2014) stated the low pH value was generally related to the increase number of organic acids produced by lactic acid bacteria. Winarno and Fernandez (2007) stated that the pH of fermented milk was influenced by the concentration of lactic acid bacteria and the fermentation time. Organic acids contained in yogurt increased due to the increasing number of microbes that remodel lactose into lactic acid. The more sugar sources that can be metabolized, the more organic acids were produced so that the pH value automatically dropped.

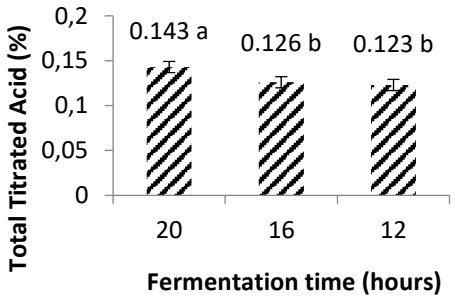
In the treatment of variation, roselle extract addition did not significantly affect the pH value, but there was still a pH decrease. The average pH of yogurt with the addition of roselle calyx extract treatment at concentrations of 0.5%, 1.5%, and 2.5% respectively 3.7; 3,7; 3,6. With the addition of roselle calyx extract, yogurt became more acidic. The more concentration of rosella calyx extract was added the lower pH even though the difference was not so significant. According to Purbowati et al. (2018), roselle petals extract had a pH value of around 2. As a result of adding this extract would make yogurt pH value lower than before

Total Titrated Acid (TTA)

Figure 2 showed that the addition of roselle petals extract significantly affected the total titrated acid. The average value of total titrated acid in yogurt due to the addition of roselle petal extract ranged from 1.22 to 1.39%. Compared with quality standards ranging from 0.5-2.0% (as lactic acid), then the yogurt produced in this study has met the standard. The highest total titrated acid value of 1.39% was obtained by adding yogurt with roselle flower extract at a concentration of 1.5% (B3).



(a)



(b)

Note: Same letters above the bars indicate no significant difference

Figure 2 The effect of concentration of roselle extract (a) and fermentation time (b) on total titrated acid yogurt.

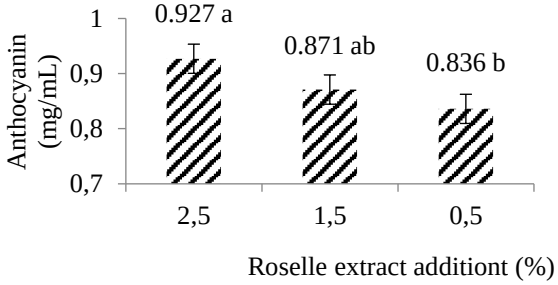
The value of total titrated acid in rosella probiotic yogurt showed a high percentage of dissociated and undissociated acids produced by the starter of lactic acid bacteria and roselle flower petal extract. According to Purbowati et al. (2018) roselle calyx extract contained anthocyanin (13.51 ± 0.03 mg / g), vitamin C (20.47 ± 0.34 mg / g) and had a pH value of 2

Fermentation time significantly affected the total value of titrated acid. The average value of TTA on the fermentation period of 12 hours (A1), 16 hours (A2), and 20 hours (A3) respectively, were 1.23%, 1.26%, and 1.43%.

This was consistent with the research of Chen et al. (2017), which stated that the longer yogurt time fermentation, the total value of acid increased. This was due to the increasing number of lactic acid from the hydrolysis of lactose (milk sugar) and sucrose by lactic acid bacteria. This hydrolysis resulted in lower lactose concentration in yogurt than in milk, which in part explains why yogurt is more tolerant than fresh milk by a person with lactose maldigestion. Jannah et al. (2014) also stated this condition occurred because the lactic acid bacterias found in yogurt have a longer time to utilize the nutrients contained in the medium (yogurt) to be converted to lactic acid.

Anthocyanin

Figure 2 showed that the addition of roselle petals extract significantly affected anthocyanin content. The average value of anthocyanin at additions of 0.5% (B1), 1.5% (B2), 2.5% (B3) respectively were 0.836, 0.871 and 0.927 mg/mL. From these results, it can be seen that an increase in the amount of anthocyanin contained despite the slight difference. This was because the amount of rosella flower petal extract added was different. According to Kharismawati et al.(2018), the higher addition of roselle extract, the higher the anthocyanin content in the functional jelly drink. The more acidic the solution, the red anthocyanin will be stronger. This was due to the fact that anthocyanin was degraded by an increasing pH.

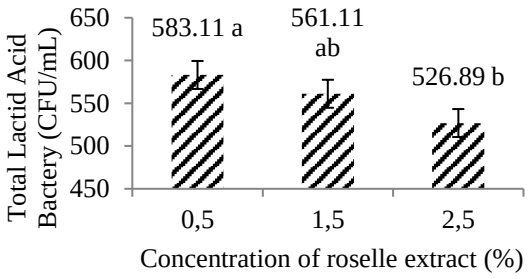


Note: Same letters above the bars indicate no significant difference

Figure 3 The effect of roselle extract addition on anthocyanin .

These were also in line with Purbowati et al.(2016) that stated anthocyanin content in roselle petals was 13.51 ± 0.03 mg/g

Lactic Acid Bacteria



Note: Same letters above the bars indicate no significant difference

Figure 4 The effect of roselle extract addition on total lactic acid bacteria yogurt.

Variance analysis results showed that the variation in roselle extract addition affected the number of lactic acid bacteria in yogurt. The higher the roselle extract addition, the amount of LAB contained in yogurt drinks decreased. The average value of LAB in the treatment of addition of roselle calyx extract at 0.5% (A1), 1.5% (A2), and 2.5% (A3) respectively 5.82 x 107, 4.97 x 10 7, and 4.61 x 107. These results indicate a decrease in the number of microbes contained. This was because of the antibacterial properties contained in anthocyanin that affect the presence of microbes in yogurt.

Purbowati et al. (2016) stated that in roselle, almost 70% of the polyphenol compound was anthocyanin. According to Purbowati et al. (2018), Roselle extract had antibacterial activity against Staphylococcus aureus and E colii, based on clear zone formed. The antibacterial activity was expressed by the long diameter of the clear zone generated around the disc. Diameter <6 mm indicated extracts were not active, while diameter> 6 mm, extracts were classified as having an antibacterial activity (Mudi and Ibrahim, 2008). The essential component of the cell wall of gram-positive bacteria was peptidoglycan, one of which was the amino acid alanine, hydrophobic (nonpolar). Antibacterial compounds reacted with phospholipid compounds from the cell membrane resulting in cell lysis. According to the study (Rahayu, 2009), a synergistic effect between antimicrobial compounds and acidic pH can strengthen antibacterial activity. This was consistent with the research (Purbowati et al., 2018) that high

phenolic compounds and high acidity levels can increase the antibacterial activity.

The decrease in LAB population in both types of yogurt during the storage period still fulfilled the SNI 01-2981-2009 value of Yogurt Quality (Badan Standarisasi Nasional, 2009) was a minimum of 107 CFU mL-1. The number of probiotics required by the Swiss Food Regulation and International standard was> 106 CFU/g (Jay et al. 2005).

Variable Sensory

The popularity of yogurt was not only due to various therapeutic effects and health claims but also for its sensory properties. The primary sensory attributes of yogurt included color, flavor, taste, and texture. These had played an essential role in determining the acceptability and preference of yogurt to the customers.

Color

The results of organoleptic color in yogurt with the variation roselle extract addition and different fermentation time can be seen in Figure 5.

In Figure 5 the results showed that the addition of roselle petals extract and different fermentation times in the control treatment, A1B1, A1B2, A1B3, A2B1, and A3B1 had modus value 1, which means it had a yellowish-white color. Whereas the treatment of A2B2, A2B3, A3B2, and A3B3 have a modus value of 4, which means it had a reddish-yellow color. The reddish color was produced from the addition of roselle calyx extract. The red color of the roselle was derived from the content of anthocyanin. The more extracts were added, the color of the resulting yogurt will be reddish. Fermentation time also affected the color. These were because the fermentation time affects the total titrated acid produced. The chemical characteristics show that the fermentation times of A1 and A2 were not different, whereas A3 was different from A1 and A2. The total acid resulted in the pH value produced. The acidity conditions affected the color stability of the anthocyanin produced. The longer the fermentation was done, the red anthocyanin will be more stable.

According to Choirul et al.(2014), anthocyanin was a water-soluble pigment that was naturally present in various types of plants and fruits. The pigment will give pink, blue and purple colors to the fruits, flowers, and leaves included in the flavonoid class. Anthocyanins were very stable at low pH (2-4), and they were red, at pH 4-6 anthocyanins were purple, at pH 7-8 were blue, then yellow at pH> 8 (Brannen et al., 2002).

Flavor

The flavor was a sensory impression of food and other substances and was determined primarily by the chemical senses of taste and odor. The significant component commonly reported responsible for yogurt flavor were lactic acid, acetaldehyde, diacetyl, acetoin and 2-butonan (Cheng et al., 2010). Well flavored yogurt resulted when proper levels of these compounds were produced. Yogurt itself generally had a distinctive aroma characteristic, such as sour aroma. This aroma raised because during fermentation. There was a process of converting lactose into lactic acid. Lactic acid made yogurt having a distinctive sour aroma (Chen et al., 2017).

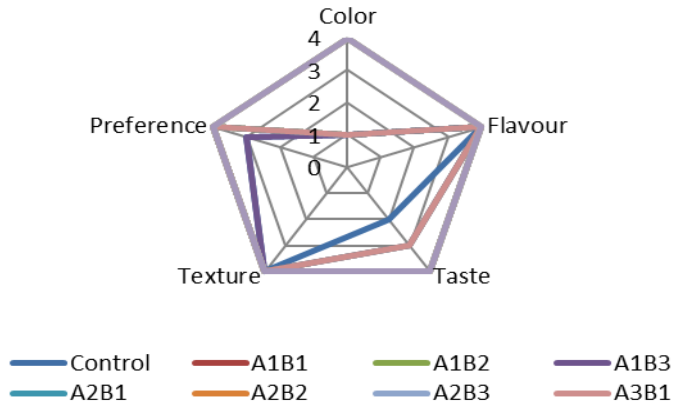


Figure 5 Result of organoleptic test on yogurt

Figure 5 showed that all yogurt produced from 8 combinations had a modus value of 4, which meant it had a typical flavor. These were influenced by the microbial content present in yogurt. According to Smit et al. (2005), the flavor in yogurt was produced by L. bulgaricus bacteria during the fermentation. Total lactic acid bacteria based on the combination of the addition of roselle extract and the fermentation time carried out were not significantly different, so that the flavor of yogurt produced was the same. The initial stage of incubation in yogurt was dominated by S. thermophilus, which decreased the pH from 6.5 to 5.5 by the redox reaction. At pH below 5 L. bulgaricus optimally produced acetaldehyde and lactic acid, and fermentation stopped when a pH of 4.5 or acidity of 0.9% to 0.95% or 1% was reached (Tabatabaie and Mortazavi, 2008). According to Irkin and Eren (2008) that L. bulgaricus had more role in the formation of aroma, while S. thermophilus had more role in the formation of yogurt flavor.

Taste

The taste was the sensation produced when a substance in the mouth reacted chemically with taste receptor cells located on taste buds in the oral cavity and can be expressed as sweet, sour, salty, bitter, and umami (Chen, 2017). Taste parameters were determining the level of acceptance of a food by consumers . According to SNI (01-2981-1992) yogurt had a distinctive taste like acid. In fresh milk, lactose was also the major energy and carbon source for the growth of LAB. LAB converted lactose into lactic acid, which gave yogurt the characteristic acidic taste.

Figure 5 showed the control yogurt had a modus value of 2, where the taste of yogurt was not acidic. For treatment A1B1, A2B1, A3B1 had a modus value of 3, which meant it had a slightly acidic taste, whereas, for the treatment A1B2, A1B3, A2B2, A2B3, A3B2, A3B3 had a modus value four, which meant it had an acidic taste. It can be seen that the addition of roselle calyx extract and the longer fermentation time will increase the sour taste of yogurt. The sour taste in yogurt came from the lactic acid contained in yogurt. The high content of lactic acid will make the yogurt taste more acidic. According to Chen et al. (2017), lactic acid produced during the fermentation process can increase flavor and increase acidity or decrease its pH. The formation of lactic acid and the results of lactic acid bacterial metabolites in the fermentation will affect the taste of yogurt.

Texture

The starter culture type affected the qualities of yogurt samples. Analysis of the texture indicated that the addition of roselle petals extract and different fermentation times did not have an effect on the texture. It had been reported that EPS produced by yogurt starter cultures could affect the texture of yogurt and improved sensory characteristics such as mouthful, shininess, clean-cut, ropiness, and creaminess (Behare et al., 2010). This was possible because of the equality of the coagulum formed at each treatment. This assumption was based on the equivalency of the pH value produced in each treatment that was between 3.6-3.7. According to Bylund (1995), the consistency of the coagulum determined the texture of yogurt. This consistency was influenced by pH when the pH was outside the normal pH range of milk because in this range, casein can form a network with whey protein, which has been denatured during pasteurization as an integral part of the process of making yogurt (Azizah et al., 2013). It suggested the strains producing higher exopolysaccharides might contribute to the higher viscosity of fermented milk. Guzel-Seydim et al. (2005) reported that exopolysaccharide filaments attached mucous bacteria to the protein matrix and thus caused more viscous-like behavior.

Based on Figure 5, it can be seen that the yogurt with the addition of roselle flower extract treatment and different fermentation time did not affect the preference because the modus value was almost 4, which meant that the panelists liked the roselle yogurt product.

CONCLUSION

The variation in fermentation time affected the pH value and TTA. The 12- hour fermentation time gave pH and TTA values, respectively: 3.77 and 1.23%. The variation in the addition of roselle extract significantly affected the anthocyanin, TTA, and LAB values. The addition of the extract as much as 1.5% gave the anthocyanin value, TTA, and LAB, respectively 0.871 mg /mL, 1.31%, and 561.11x107CFU/mL. The best treatment was yogurt with roselle extract addition 1.5% with 20 hours of fermentation. This yogurt had characteristic as follows: pH 3.7, Total titrated acid (TTA) 1.33%, anthocyanins 0.8751 mg/mL, TPC 560 x 10^7 CFU/mL, reddish-yellow (4),

distinctive aroma (4), Sour taste (4), fine texture (4), and liking (4).

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