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The Effect of Addition of Wuluh Starfruit (*Averrhoa bilimbi* L.) Juice as a Coagulant in Cottage Cheese from Cow's Milk

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ABSTRACT

Lemon and lime have been widely used as coagulation media in the manufacture of soft cheese from cow's milk. However, the use of organic citric acid contained in wuluh starfruit (*Averrhoa bilimbi* L.) has still been rarely used. This study aimed at determining the effect of variations in the addition of starfruit juice in making cottage cheese from cow's milk. The experiment used a completely randomized design (CRD) with three repetitions and five treatments of Wuluh star fruit juice additions: SKA1 = 10%, SKA2 = 20%, SKA3 = 30%, SKA4 = 40% and SKA5 = 50%. From the results of the organoleptic assessment, it was concluded that cottage cheese from SKA3 treatment received the highest acceptance value of 6.07±0.69 with physico-chemical properties: yield (25.70±0.30%), moisture content (54.81±0.05%), ash content (2.30±0.02%), protein content (18.55±0.06%), fat content (24.19±0.04%), pH value (4.43±0.01), vitamin C content (127.58±0.00 mg/kg), antioxidant activity (47.49±0.46%) and salt content (135.66±0.58 ppm).

Key words: Antioxidants, citric acid, cottage cheese, cow's milk, organoleptic

INTRODUCTION

One of the foods that contain high nutrients is milk (Ali *et al.*, 2022). Milk is the result of milking from cow or other mammals, having white colour, and can be consumed or used as a safe and healthy food ingredient. Milk naturally contains vitamins, protein, calcium, magnesium, phosphorus, minerals and fat (Patahanny *et al.*, 2019). The composition of milk consists of water (87.20%), fat (3.70%), protein (3.50%), lactose (4.90%) and minerals (0.07%; Sanam *et al.*, 2014). Milk is a food product that is almost perfect in nutritional content and is highly recommended for consumption, especially by children in their infancy. While cow's milk is good food for humans, it is also suitable for bacteria. If milk is not immediately stored at a low temperature within 3 h, then milk spoilage will occur. Processing milk into various products is one way to overcome the spoilage of milk. Some products derived from fresh milk are yogurt, candy, ice cream, butter and cheese.

Cheese is produced by separating solid substances in milk through the coagulation process (Novianti *et al.*, 2018). Until now, the need for cheese in Indonesia has been obtained from imports, which makes the price of cheese, relatively expensive. The consumption of cheese is quite large, which is indicated by the continued increase in cheese imports. There are several types of cheese: soft, semi-hard and hard (Setiyorini *et al.*, 2014). Cottage cheese is a soft cheese with a non-sharp taste (Supriyanti and Fitria, 2014). Cottage cheese is usually used as a mixed ingredient in cakes or pairs well with bread. Most cottage cheese is produced with the addition of lemon or lime juice. The cheese contains all the essential nutrients from milk (*Ali et al.*, 2022). Cottage cheese is recommended for diabetic patients because of its low sugar content and high protein. The making process of cheese generally uses calf rennet or microbes (Li *et al.*, 2022). However, the price of rennet is relatively quite high. This problem can be overcome by making cheese with the acidification method.

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The principle of cheese acidification is to coagulate milk with the addition of acetic acid, citric acid, or other organic compounds, which can lower the pH of milk (Vergara-álvarez *et al.*, 2019). There is research about the making of cottage cheese with the addition of lemon with the best results by adding 5 % lemon with a protein value of 4.25 g (Supriyanti and Fitria, 2014). And also, there is research on the making of cream cheese from soybeans with acetic acid and produced the best cream cheese with the addition of 0.4% acetic acid with a protein value of 15.57% (Fauzia, 2016). Based on the research above, it is necessary to use other sources of natural organic acids in to manufacture of cottage cheese. One of the natural ingredients that can be utilized is wuluh starfruit.

Wuluh starfruit is a plant that is easy to grow naturally or by grafting. This fruit thrives in Indonesia, the Philippines, Sri Lanka, Myanmar and Malaysia (Aseptianova and Yuliany, 2020). Wuluh starfruit has a sour taste and is often used as a flavour enhancer (Putriana, 2018). Wuluh starfruit contains vitamin C, flavonoid compounds, and triterpenoids. According to research, the flavonoids and phenols contained in wuluh starfruit can be used as an antibacterial and anti-inflammatory agent. Moreover, this plant also contains amino acids, citric acid, cyanidin glucosides, potassium ion, sugar and vitamin A (Insan *et al.*, 2019). Considering the potential possessed by wuluh starfruit, the authors used it as a coagulant in the manufacture of cottage cheese from cow's milk.

MATERIALS AND METHODS

The tools used in the cheese-making process were: mixer, oven, digital scale, filter cloth, plastic utensils, knife, pH meter and glassware. This study used chemical instruments in the form of: UV-vis spectrophotometer (Thermoscientific Genesys 150), Kjeldahl testing device, Soxhletation extraction device, laboratory oven (Memmert UN 110), furnace (Carbolite AAF 1100), pH meter (Hanna Instrument HI 2211) and saltmeter (Lutron YK-31SA) and other instruments.

The raw material for the manufacture of cottage cheese is fresh cow's milk obtained from Padang Panjang Dairy House, West Sumatra, Indonesia. Other materials used were 100% wuluh starfruit juice, 3% sodium chloride and 0.01% calcium chloride.

The chemicals used in the parameter testing consisted of: aqua DM (Bratachem), selenium mix (Merck), boric acid (Pudak Scientific), sodium hydroxide (Merck), tashiro indicator (Merck), hydrochloric acid (Merck), hexane (Bratachem), sulfuric acid (Smart Lab), DPPH (Sigma-Aldrich) and methanol (Merck).

One kg Wuluh starfruit was weighed and washed with running water and mashed using a blender. Separation of pulp and juice was done using a cloth filter. Wuluh starfruit juice was directly added to milk according to the treatment (v/v). Preparation of 0.4% citric acid solution was done by dissolving 1 g of synthetic citric acid in 100 ml of distilled water (w/v) and then thoroughly stirred. 0.4% citric acid solution was added to milk as a control.

Fresh cow's milk was pasteurized at 72°C for 20-30 sec while stirring. The pasteurized milk was then cooled to a temperature of 35°C. Wuluh starfruit juice with five treatments was added gradually so that the pH level did not drop too low. High milk temperature can increase the pH which will affect coagulation process. As a control, 0.4% citric acid solution was used as a coagulant. The curd formation occurred in ±30 min after the addition of coagulant (Ali *et al.,* 2022).

The research design used was a one-level completely randomized design (CRD) with five levels of treatment and three replications. The data were analyzed using analysis of variance (ANOVA) with F test and Duncan's New Multiple Ranger Test (DNMRT) at 1% significance level.

Curd yield values expressed in per cent were determined prior to storage by comparing the weight of curd produced with the weight of fresh cow's milk used as raw material.

The proximate analysis consisted of moisture content using the air oven method, ash content using the dry ashing method, fat content using the soxhletation extraction method, protein content using the kjeldahl method and carbohydrate content using the by difference method (Syukri *et al.,* 2014).

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical was used to determine the antioxidant activity of the samples by DPPH method (Salihat & Putra, 2021). pH measurement was carried out using a pH meter. Measurement for

vitamin C was carried out by titration method with starch indicator (Sembara *et al.,* 2021). Salt content was measured using a salt meter. Organoleptic tests were carried out with four parameters: color, aroma, texture, and taste because the panelist preference for a product was influenced by taste, aroma, texture, and color.

RESULTS AND DISCUSSION

Acidification was used as a method in the process of making cottage cheese from cow's milk in this study. Belimbing wuluh (*Averrhoa bilimbi* L.) extract was used as a coagulant. As a control, 0.4% synthetic citric acid was used because citric acid was the dominant organic acid contained in wuluh starfruit (Wiradimadja *et al.*, 2015). The concentration of wuluh starfruit juice and synthetic citric acid used was based on the volume of milk which was the raw material. Cottage cheese products from five treatments and control are seen in Fig. 1.

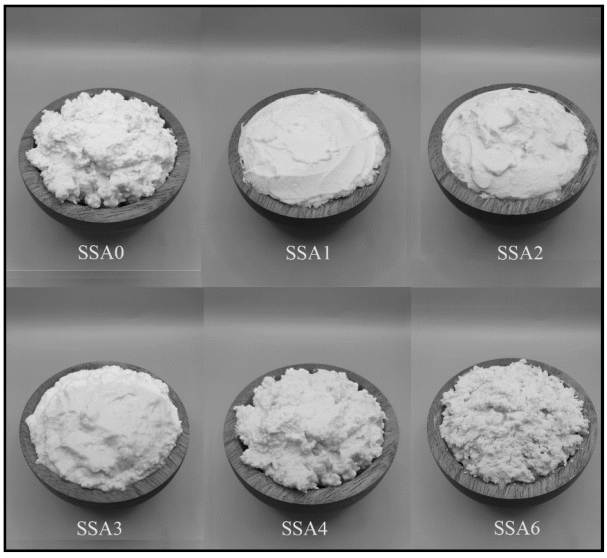

Fig. 1. Cheese from cow's milk with several treatments by the addition of belimbing wuluh.

The addition of wuluh starfruit juice to cow's milk caused the moisture content contained in the resulting cottage cheese to increase. In the SSA1 treatment, the water content value was 53.24% which increased to 68.25% in the SSA5 treatment (Table 1). The water content value of the SSA1 treatment was lower than the control treatment (SSA0), which used pure citric acid (0.4%), namely, 54.81%. The increase in moisture content in each treatment was affected by the addition of the amount of wuluh starfruit juice. The value of the moisture content began to increase in the SSA2 treatment compared to the control (SSA0). Increasing the concentration of wuluh starfruit juice caused coagulation of casein in cow's milk to occur more optimally, which impacted more curd being formed so that the moisture content contained in the cheese was also higher.

Moisture content was the amount of water contained in the material expressed in per cent (Syukri *et al.*, 2018; Syukri *et al.*, 2022). Moisture content was an essential characteristic that significantly affected food products. The moisture content also determined the freshness and durability of these food products. High moisture content made it easy for bacteria, mold and yeast to multiply, which caused changes in food products. In addition, the water content also determined the texture of the product. Fresh cheese products such as cottage cheese have a more significant percentage of moisture content, so they have a relatively short shelf life and a soft texture (Kumari *et al.,* 2022; Nair *et al.,* 2022).

Table 1. Proximate composition of cottage cheese from cow's milk with variations in the addition of wuluh starfruit juice

Parameters	Treatments					
	SSA0	SSA1	SSA2	SSA3	SSA4	SSA5
Moisture (%)	$54.81^{b}\pm0.05$	$53.24^{a}\pm0.03$	$56.80^{c}\pm0.01$	$64.60^{d}\pm0.02$	$67.80^{e}\pm0.01$	$68.25^{f}\pm0.02$
Ash (%)	$2.30^{b}\pm0.02$	$2.12^{a}\pm0.01$	$2.41^{c}\pm0.02$	$2.69^{d}\pm0.04$	$2.73^{d}\pm0.02$	$2.96^{e}\pm0.02$
Protein (%)	$18.55^{f}\pm0.06$	$17.72^{e}\pm0.03$	$16.23^{d}\pm0.03$	$15.83^{c}\pm0.02$	$12.54^{b}\pm0.12$	$11.24^{a}\pm0.09$
Fat (%)	$24.19^{f}\pm0.04$	$23.76^{e}\pm0.03$	$21.68^{d}\pm0.17$	$19.24^{c}\pm0.08$	$17.22^{b}\pm0.07$	$16.26^{a}\pm0.06$

Different superscripts are significantly different between treatments.

An increase in the amount of wuluh starfruit juice used in the process of making cottage cheese caused increase in ash content. The SSA1 treatment had the ash content of 2.12 % and continued to increase to 2.96% in the SSA5 treatment. The ash content of the control treatment (SSA0) was 2.30%. The average ash content of the five treatments using wuluh starfruit juice was not too far from the ash content value of the control treatment.

The percentage of ash content began to increase in the SSA2 treatment compared to the control (SSA0). The SSA3 and SSA4 treatments had not many different heights. Wuluh starfruit juice had an ash content of 0.30%. This percentage caused an increase in the ash content of cottage cheese to be higher, along with the higher concentration of wuluh starfruit juice used.

Ash content was an indicator in determining a food product's purity and cleanliness level. An ash content value that was too high can be a sign that a food product was unhygienic and had the possibility of being contaminated with dangerous minerals such as heavy metals. Ash content can indicate the mineral content contained in a material. Food ingredients consisted of organic matter and water 96%, while the rest was inorganic material, namely ash containing minerals (Ali *et al.,* 2022). It can be said that these cottage cheese products contained minerals that were not too high.

The increase in the addition of wuluh starfruit juice caused the value of protein content to decrease. The SKA1 treatment had protein content of 17.72%, lower than the control (SKA0) with a value of 18.55%. In the SKA5 treatment, the protein content decreased to 11.24% as more wuluh starfruit juice was added. In each treatment, the protein content decreased along with the increase in wuluh starfruit juice. The values of protein content decreased compared to the control (SKA0).

The protein produced in the cheese was influenced by the processing, namely the curd coagulation method with the addition of acid (Komansilan *et al.,* 2021). Increasing the addition of wuluh starfruit juice caused the protein in the cheese to become more damaged (protein denaturation). This statement was in line with research that states that a decrease in pH can cause the denaturation of proteins formed through coagulation (Setiani *et al.,* 2021). Citric acid contained in wuluh starfruit is a weak acid, has coagulation power, and produces low protein deposits, which range from 54.73-61.94%. So that it can only cause protein denaturation in smaller amounts with its low electronegativity.

The decrease in the fat content of cottage cheese was associated with an increase in the moisture content. Wuluh starfruit juice had a moisture content of 94.78 %. Wuluh starfruit juice with high moisture content decreased the fat content of the cheese along with the increase in the use of wuluh starfruit juice as a coagulant. This statement was in accordance with the statement which says that several factors can accelerate the breakdown of fat, such as air, light, temperature, and moisture content (Pastukh & Zhukova, 2021).

Low-fat cheese was good for health. Reducing saturated fat consumption also means reducing bad cholesterol (LDL) and the risk of coronary heart disease. Several factors can affect the quantity of fat in cheese, including processing factors, such as errors in the manufacturing process and storage factors (Novianti *et al.*, 2018).

Yield was the quotient of the dry weight of a product by the weight of raw material (Senduk *et al.*, 2020). The yield of cheese was obtained by weighing the cheese product and comparing it to the weight of fresh milk; the final result was in per cent units. The highest percentage of yield was obtained by cheese with SSA4 treatment (addition of 40% wuluh starfruit juice), namely, 35.64% (Table 2). At the same time, the lowest yield was in the cheese with SSA1 treatment (addition of 10% wuluh starfruit juice), namely, 14.37%. It can be concluded that the greater the addition of wuluh starfruit juice, the greater the yield of cheese produced from fresh cow's milk, but the yield value decreased in the SSA5 treatment. This decrease was due to the pH, which had passed the optimum value for the coagulation process of casein in milk to become curd, namely, 4.6-5.5 (Troch *et al.*, 2017). A pH value that was too low could cause the size of the formed curd particles to become too smooth, making it challenging to filter when separating the curd from the whey.

Curd was a cheese that was produced from the precipitation or coagulation of casein contained in milk. The curd coagulating process became more optimal as the pH decreased which was

Table 2. Physico-chemical properties of cottage cheese from cow's milk with variations in the addition of wuluh starfruit juice

Parameters	Treatments					
	SSA0	SSA1	SSA2	SSA3	SSA4	SSA5
Yield (%)	25.70±0.30	14.37±0.08	19.15±0.11	24.88±0.04	35.64±0.05	28.92±0.04
pH	4.43^{d}±0.01	5.70^{f}±0.00	5.40^{e}±0.00	4.02^{c}±0.00	2.64^{b}±0.00	2.05^{a}±0.01
Vitamin C (mg/kg)	127.58^{a}±0.00	139.60^{b}±0.09	152.22^{c}±0.00	189.17^{d}±0.00	241.96^{e}±0.01	272.77^{f}±0.01
Antioxidant activity (%)	47.49^{b}±0.46	40.47^{a}±0.13	48.81^{c}±0.08	61.58^{d}±0.07	70.39^{e}±0.35	82.34^{f}±0.17
Salt (ppm)	135.66^{c}±0.58	115.33^{a}±0.58	117.66^{b}±0.58	117.66^{b}±0.58	116.00^{a}±0.01	116.00^{a}±0.01

Different superscripts are significantly different between treatments.

proportional to the increased concentration of wuluh starfruit juice used. Wuluh strarfruit juice containd several organic acids (citric acid, oxalic acid, acetic acid, lactic acid and formic acid) with a low pH value so that it could precipitate casein in cow's milk (Wiradimadja *et al.*, 2015).

The acidity level of a food product can be determined by measuring the pH value. The results of the analysis of diversity showed that differences in the treatment of wuluh starfruit juice in the making process of cottage cheese from cow's milk had a highly significant effect on the pH value. The more wuluh starfruit juice was added, the pH value of cow's milk cottage cheese decreased. The pH value in SSA0 (control) was 4.43, which was lower in the SSA1 (5.70) and SSA2 (5.40) treatments (Table 2). The pH value of the cottage cheese produced was classified as acidic because in this acidic environment, the casein in milk precipitated completely into curd.

The pH value decreased with the addition of wuluh starfruit juice in each treatment. The acidity of starfruit juice was inseparable from the organic acid content that was found in starfruit (Wijayanti *et al.*, 2019). The pH value of unripe wuluh starfruit was 1.99. The low pH value of wuluh starfruit juice also caused a decrease in the pH value of the cheese produced, along with the increasing concentration of wuluh starfruit juice used as a coagulant. Increasing acidity was one of the main factors in cheese making because curd formation required a certain amount of acid.

The more the addition of wuluh starfruit juice, the vitamin C content of the cheese products increased. In the control treatment (SSA0), which used 0.4 % citric acid, the vitamin C content was 127.58 mg/kg, lower than the SSA1 to SSA5 treatments, which used wuluh starfruit juice as a coagulant. Along with the addition of wuluh starfruit juice in the manufacture of cottage cheese, the vitamin C content in cottage cheese increased to 272.77 mg/kg in the SSA5 treatment (Table 2). The increase in vitamin C content in cow's milk cottage cheese was in line with the increase in the concentration of wuluh starfruit juice. Unripe wuluh starfruit had a vitamin C content of 141.29 mg/kg and ripe wuluh starfruit of 106.65 mg/kg (Sari, 2018). More the addition of wuluh starfruit juice, the higher the vitamin C. A decreasing pH value and a stronger sour taste characterized the increase in vitamin C in the resulting cottage cheese product.

The antioxidant activity ranged from 40.47-82.34%. The increased antioxidant activity was in line with the increase in vitamin C. Wuluh starfruit was a fruit with antioxidant activity that was beneficial to human health because it contained high vitamin C. Antioxidant compounds can donate one or more electrons to free radicals that cause cancer (Budaraga & Salihat, 2020; Gaglio *et al.*, 2021; Pereira *et al.*, 2020).

The increase in the addition of wuluh starfruit juice in the making process of cottage cheese caused a higher salt content. The control treatment (SSA0) using 0.4% citric acid had a higher salt content than the treatment using wuluh starfruit juice (SSA1-5). This higher salt content was due to the lower the pH value, the more acidic the taste of the cheese produced, so the salt content of the cheese was also lower. At the same time, the SKA3-5 treatment had a higher salt content than the control treatment. The more the addition of wuluh starfruit juice, the salt content increased but slightly decreased in the SSA4 and SSA5 treatments. The increase in salt content in the SSA1 to SSA3 treatment was in line with the decrease in the pH value. Salt would be increasingly difficult to dissolve, causing

precipitation to occur, which caused the reading on the saltmeter to be high. In comparison, a slight decrease in the SSA4 and SSA5 treatments could occur due to an error in reading the tool. Salt has a function as a food additive and a source of sodium and serves for preservation in the manufacture of cottage cheese from cow's milk.

Highest taste evaluation for cow's milk cottage cheese was in the SSA3 treatment, namely, 6.64 (like extremely), while the lowest score was in the SSA5 treatment with a value of 4.16 (neither like nor dislike). As a comparison, the SSA0 treatment had a taste value of 4.72 (like slightly). The panelists most accepted cottage cheese taste in the SSA3 treatment with the most suitable taste of salty and typical milk (Table 3). In addition, the typical sour taste of wuluh starfruit juice contained in the cheese produced was also not too dominant. The more added wuluh starfruit juice, the sourer the cheese would be so that the milky and salty taste would disappear. This resulted in the panelist acceptance of the cheese in the SSA4 and SSA5 treatments decreasing compared to the other treatments.

The panelist's highest assessment of the aroma of cow's milk cottage cheese was in the SSA3 treatment with a value of 5.92 (like moderately), while the panelist's lowest assessment of the aroma was in the SSA5 treatment with a value of 4.32 (neither like nor dislike). For the control treatment (SSA0), the aroma value was 5.36 (like slightly). The cottage cheese from the SSA3 treatment had a distinctive aroma of cow's milk combined with a slightly sour aroma typical of wuluh starfruit. However, the more wuluh starfruit juice added to cow's milk, the more sour the smell of the cheese, which caused the smell of milk to disappear.

The highest assessment of texture was found in the SSA5 treatment, which was 6.72 (like extremely) with the smoothest and softest texture compared to other treatments, including the control. The cheese produced in the SSA5 treatment had the softest and smoothest texture, which was characteristic of cottage cheese. This was due to the highest concentration of wuluh starfruit juice used so that the coagulated casein particles became smaller (Adrianto *et al.,* 2020). In addition, the higher moisture content also influenced the texture of the cheese, which was softer and smoother. This statement is supported by the average value of the moisture content of all the treatments listed in Table 1. Based on panelist assessment, the lowest texture assessment value was found in SSA1 treatment with a value of 4.36 (neither like nor dislike), where the texture of the cottage cheese was the coarsest. As a comparison, cheese produced from pure citric acid coagulant 0.4% (SSA0) had a texture value of 5.08 (like slightly).

The organoleptic test parameter that was measured using the sense of sight and was one of the parameters that indicated whether a processed product was attractive or not was colour. The highest colour assessment for cow's milk cottage cheese was in the SSA2 treatment with a value of 6.28 (like moderately), while the lowest rating was in the SSA5 treatment with a value of 4.04 (neither like nor dislike). The more the addition of wuluh starfruit juice, the colour of the cheese became more yellowish white. The colour of the SSA2 treatment was closest to the colour of cottage cheese in general, which was white. In the SSA0 and SSA1 treatments, the cheese was milky white. While in the SSA3, SSA4 and SSA5 treatments, the white colour of the

Table 3. Organoleptic properties of cottage cheese from cow's milk with variations in the addition of wuluh starfruit juice

Parameters	Treatments					
	SSA0	SSA1	SSA2	SSA3	SSA4	SSA5
Taste	4.72±0.79	5.44±1.12	5.52±1.12	6.64±0.57	4.24±1.27	4.16±0.85
Aroma	5.36±1.35	5.76±1.33	5.68±1.18	5.92±1.50	4.48±1.45	4.32±0.99
Texture	5.08±1.19	4.36±1.04	4.80±0.87	5.76±1.16	6.16±1.18	6.72±0.46
Color	5.32±1.55	5.44±1.47	6.28±0.94	5.24±1.45	5.08±0.47	4.04±0.98
Acceptability	5.12±0.29	5.32±0.70	5.48±0.48	6.07±0.69	4.99±0.86	4.81±1.28

The evaluation is identified using a 7-point hedonic scale (1–dislike extremely, 2–dislike moderately, 3–dislike slightly, 4–neither like nor dislike, 5–like slightly, 6–like moderately and 7–like extremely).

cheese became greenish white as the concentration of wuluh starfruit juice was increased. This greenish colour was caused by the chlorophyll pigment contained in starfruit (Sitompul *et al.*, 2022).

Recapitulation of organoleptic acceptance is a combination of taste, aroma, texture and colour assessments. The highest acceptance was obtained in the SSA3 treatment with a value of 6.07 (like moderately), while the lowest acceptance was obtained in SSA5 treatment with a value of 4.81 (like slightly). The SSA0 treatment used as a control had an acceptance value of 5.12 (like slightly).

CONCLUSION

Wuluh starfruit juice that was used as a coagulant, had an influence on the physico-chemical properties and organoleptic properties of cottage cheese products from cow's milk. Based on the results of the organoleptic evaluation, the cottage cheese product from cow's milk that the panelists most accepted was cheese from the SSA3 treatment (addition of 30% wuluh starfruit juice) with an average acceptance value of 6.07±0.69. The SSA3 treatment had the following physico-chemical properties: yield (25.70±0.30%), moisture content (54.81±0.05%), ash content (2.30±0.02%), protein content (18.55±0.06%), fat content (24.19±0.04%), pH value (4.43±0.01), vitamin C content (127.58±0.00 mg/kg), antioxidant activity (47.49±0.46%) and salt content (135.66±0.58 ppm). Microbiological properties, heavy metal content and shelf life of cottage cheese products can be carried out to complement this research further.

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