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Characteristics of Suspesi with Different Level of Biduri Leaf Extract at a Temperature of 75°C, Traditional Cheese from Nusa Tenggara Timur

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ABSTRACT

The characteristics of the cheese produced are influenced by many factors, including the characteristics of the coagulant used. The characteristics of biduri leaf extract influence the characteristics of the suspesi. The research aimed to analyze the physicochemical, microstructure, and organoleptic characteristics of suspesi using different levels of biduri leaf extract given at a temperature of 75°C. This experimental study used a Completely Randomized Design with five treatments and three replications. Treatment is provided in making suspesi by adding biduri leaf extract, L1 = 2%, L2= 2.5%, L3 = 3%, L4 = 3.5%, L5 = 4%. Each level of bidi leaf extract was added at a temperature of 75°C. The variables measured were coagulation time, curd production, whey percentage, pH of curd, lactic acid percentage, water content, protein, fat, carbohydrates, L*, a*, b* color measurements, hardness, tannin content, microstructure, and organoleptic characteristics. The results obtained were that the significant effect ($P<0.05$), where there is an increase in coagulation time, whey percentage, lactic acid percentage, and organoleptic characteristics at the hedonic quality aspect of color, taste, texture, hedonic/preference aspect of taste and texture. and there has been a decrease in curd production. Furthermore, did not show a significant effect ($P>0.05$) on the pH of curd, water, protein, fat, carbohydrates content, hedonic quality aspects of smell, hedonic aspect of color, smell, the color of L*, a*, b*, Wi, Yi, hardness, tannin content. The microstructure there was to bond loose between protein and fat molecules. The conclusion given biduri leaf extract at different levels added at a temperature of 75°C, the characteristics of suspesi in physicochemical characteristics, organoleptic, and the microstructure of suspesi had decreased in line with the increase in the level of biduri leaf extract. The best suspesi was at a level of giving biduri leaf extract at 2%.

Keywords: Calotropis, Cheese, Level, Nusa Tenggara Timur, Suspesi

Introduction

The characteristics of the cheese produced are influenced by many factors, namely the composition of the milk (Mbye *et al.*, 2022), the coagulant used (Malaka and Sulmiyati, 2010; Sulmiyati and Said, 2019; Sulmiyati and Malelak, 2022; Sulmiyati and Malelak, 2023), addition of salt/NaCl (Elisabeta *et al.*, 2018; Elisabeta *et al.*, 2018; Ayyash and Shah, 2011; Møller *et al.*, 2013; Hayashida *et al.*, 2023), cheese-making process (Mbye *et al.*, 2022; Malaka and Sulmiyati, 2010; Sulmiyati and Malelak, 2022; Sulmiyati and Malelak, 2023), temperature (Mbye *et al.*, 2021), pressure (Mbye *et al.*, 2021), storage (Lazárková *et al.*, 2021; Kreutz *et al.*, 2020), packaging (Gorrasi *et al.*, 2016; Malaka *et al.*, 2017a).

Suspesi is a cheese product originating from Timor Tengah Selatan, Nusa Tenggara Timur, Indonesia (Sulmiyati and Malelak, 2022; Sulmiyati and Malelak, 2023). Suspesi have two types, wet suspesi and dry suspesi. There is no definite standard for the characteristics of suspesi. Standardization of suspesi is limited to water content and organoleptic characteristics to differentiate types of suspesi, whether wet suspesi or dry suspesi. Wet suspesi have a water content of around 50-55% and have a greenish-white color, while dry suspesi have a water content of around <20% and have a brownish color. Research conducted by Sulmiyati and Malelak (2022), to produce suspesi, there are characteristics differences in hardness, color of L*, and tannin content using fresh biduri leaf extract at levels of 3% and 5% using an application temperature of 55°C. Further research by Sulmiyati and Malelak (2023) showed that the characteristics of the cheese produced using fresh biduri leaf extract have better physicochemical, chemical content and organoleptic characteristics compared to using dried biduri leaf extract as a coagulant in making suspesi cheese. Milk casein coagulation is influenced by tannins in biduri leaf extract. According to (Noviyanty *et al.*, 2020), tannin is a

type of polyphenol found in plants that functions to bind and precipitate protein. Welin *et al.* (2023), Adding 2% biduri leaf extract at a temperature of 55°C provides better physicochemical and organoleptic characteristics.

Based on the result of previous research, temperature is one factor that influences cheese characteristics. Therefore, the research was carried out using different levels of biduri leaf extract and biduri leaf extract was added at temperature 75°C. This research aimed to analyze the effect of a temperature of 75°C with the level of biduri leaf extract on the characteristics of physicochemical, chemical, the color characteristics of L*, a*, b*, hardness, tannin content, microstructure, and organoleptic of suspesi.

Materials and Methods

Materials

The research materials used were cow's milk from Misi Farms Atambua, Nusa Tenggara Timur, biduri leaf from biduri plants taken around Kupang City, Nusa Tenggara Timur, NaOH 0.1 N, Indicator phenolphthalein.

Research design

This experimental study uses a Completely Randomized Mono Factor Design with five treatments and three replications. Biduri leaf extract is given at a temperature 75°C with different level, Treatment is given in making suspesi L1 = Biduri leaf extract level 2% temperature 75°C, L2= Biduri leaf extract level 2.5%, L3 = Biduri leaf extract level 3%, L4 = Biduri leaf extract level 3.5%, L5 = Biduri leaf extract level 4%.

Methods

The procedure of making biduri leaf extract. This research began with making biduri leaf extract. The method refers to the method (Sulmiyati and Malelak, 2023). The biduri leaf is washed and cleaned, then the biduri leaf is pounded using a mortar. Once smooth squeezed, they are pressed to obtain the extract of the biduri leaf. The resulting biduri leaf extract can be seen in Figure 1.

The procedure of making suspension. The procedure for making suspesi is a modification of the method (Sulmiyati and Malelak, 2023), the milk is pasteurized *High-Temperature Short Times* method, at 75°C for 15 s, then at a temperature of 75°C biduri leaf extract is added to the milk at a level of 2%, 2.5%, 3%, 3.5%, and 4% (v/v), left to stand until perfect coagulation, after the curd is formed the separate the curd from the whey while pressing to obtain a solid curd (draining time ~4-6 h).

Research variables

The physicochemical analysis of suspesi. Physicochemical analysis measures curd production, whey percentage, curd pH, and lactic acid percentage. Curd production is measured by weighing the curd formed, and the result of the curd weight is entered in the formula equation 1. Measurement of the percentage of whey by weighing the whey produced from separating the curd is then entered in the formula equation 2. The pH of the curd is measured by weighing the curd and then adding distilled water with a ratio of 1:1 using a pH meter [Ezdo pH5011] (Hadiwiyoto, 1994). Measurement of the percentage of lactic acid using the Mann's Acid Test method (Hadiwiyoto, 1994), then the titration results are included in equation 3.

$$\text{Curd production (\%)} = \frac{\text{curd mass (g)}}{\text{Milk volume (ml)}} \times 100\% \quad (1)$$

$$\text{Whey percentage (\%)} = \frac{\text{whey mass (g)}}{\text{Milk volume (ml)}} \times 100\% \quad (2)$$

$$\text{Lactic acid percentage (\%)} = \frac{\text{ml NaOH (ml) x 0.009 x 100\%}}{\text{milk mass (g)}} \quad (3)$$

The chemical content analysis of suspesi. Analysis of the chemical content of suspesi, including measurements of water content, protein content, fat content, and carbohydrate content, was carried out at the Feed Chemistry Laboratory of Hasanuddin University. Analysis of water content refers to the heating method, analysis of protein content uses the Kjeldahl method, analysis of fat content uses the Soxhlet method, and analysis of carbohydrate content uses the Luff Schoorl method (AOAC, 2005).

Color analysis of suspesi. The color of suspesi is observed L*, a*, b* color. Color of L*, a*, b* analysis was conducted at the Animal Products Technology Laboratory, Hasanuddin University, using a color meter [Color Meter TES 135A]. Color standard L* = 100 (Brightness), 0 = darkness, color a*= Positive: redness, negative: greenness, color b*: positive: yellowness, negative: blueness. The measurements of the Whiteness Index (Wi) Hunter and the Yellowness Index (Yi) FC using equations 4 and 5 (Hirschler, 2012; Miloradovic *et al.*, 2018).

$$\text{Whiteness Index (Hunter)} = L^* - 3 \times b^* \quad (4)$$

$$\text{Yellowness Index (FC)} = 142.86 \times b^*/L^* \quad (5)$$

Tannin content analysis of suspesi. Tannin content measurements were carried out at the Feed Chemistry Laboratory of Hasanuddin University using the UV-VIS Spectrophotometer method with standard absorbance concentrations of 0=0; 1=0.038; 2=0.113; 4=0.216; 8=0.422; 10=0.546 (AOAC, 2005).

The hardness analysis of suspesi. Suspesi hardness was conducted at the agricultural technology Laboratory Politeknik Negeri Pertanian Kupang. Hardness measurement tool using a texture analyzer [Brookfield CT3 4500] (Sulmiyati and Malelak, 2022).

Microstructure analysis of suspesi. Observations of the Suspesi microstructure began with slide preparation at the Balai Besar Veteriner-Maros Laboratory, South Sulawesi, then continued with observations of Suspesi the microstructure at the Maros Pet Care Laboratory using an electron microscope [Olympus CX 21 LED], and advance camera [Optic Plus] (Malaka *et al.*, 2017a). The Suspesi microstructure standard used is a score of 0 – 4, where a score of 0 = no binding occurs; a score of 1 = weak ties uneven distribution; a score

a  b  c 

Figure 1. Biduri leaf and biduri leaf extract from *Calotropis gigantea* plant (The characteristics of biduri leaf extract are dark green, have a distinctive smell of biduri leaf, and a bitter taste), Informant: a = biduri leaf the front, b = biduri leaf the back, c = biduri leaf extract.

Table 1. Organoleptic hedonic quality of suspesi scale instrument

Variables	Hedonic quality scale				
	1	2	3	4	5
Color	dark green	green	quite green	rather green	white
Smell	not smell *suspesi*	rather not smell *suspesi*	quite smell *suspesi*	rather smell *suspesi*	smell *suspesi*
Taste	very bitter	bitter	quite bitter	rather bitter	not bitter
Texture	very soft	soft	quite soft	rather solid	solid

Table 2. Organoleptic Hedonic/preference of suspesi scale instrument

Variables	Hedonic scale/preference				
	1	2	3	4	5
Color	dislike	rather dislike	rather like	like	very like
Smell	dislike	rather dislike	rather like	like	very like
Taste	dislike	rather dislike	rather like	like	very like
Texture	dislike	rather dislike	rather like	like	very like

of 2 = moderate ties, less even distribution; a score of 3 = strong and even bond; and score 4 = very strong and even ties.

Organoleptic analysis of suspesi. The organoleptic test used 15 semi-trained panelists on the quality of Suspesi using two organoleptic scales, namely the hedonic quality scale and the hedonic scale from the aspects of color, smell, taste, and texture, with a scale of 1-5. The characteristics of the panelists used were students aged 19-20 years old and females. The instrument for assessing the hedonic quality scale can be seen in Table 1, and the hedonic scale can be seen in Table 2.

Data analysis

Quantitative data obtained were analyzed using One Way analysis of variance. Data analysis with a significant effect ($p<0.05$) will be followed by the Duncan Test using SPSS Version 16 software. Qualitative data is analyzed descriptively. Quantitative data are illustrated with mean values ± Standard Deviation. Descriptive data is shown in the form of images.

Results and Discussion

Physicochemical characteristics of suspesi
Coagulation time

The effect of different levels of biduri leaf extract added at a temperature of 75°C has a coagulation time of range 0.193±0.031-0.833±0.369 m (Table 3). The coagulation time was the fastest in suspesi given biduri leaf extract at a level of 4% (v/v) and the longest coagulation time at 2% (v/v). Statistical analysis results showed a significant effect ($p<0.05$) on coagulation time. The results of the Duncan test for L1 treatment (2% v/v) showed differences with L2 treatment (2.5% v/v); L3 (3% v/v); L4 (3.5% v/v); and L5 (4% v/v). This indicates that the higher the level of biduri leaf extract added, the faster coagulation occurs. The same thing happened in the research Hachana *et al.* (2021), that the higher the temperature used, the faster the clotting time. Where a temperature of 40°C with a dose of 2% fresh caprifig tree extract produces a milk clotting time of 20 minutes, and successively, the milk clotting time gets faster at this temperature 60°C, 80°C, 90°C, 100°C, and 110°C, respectively 8 min, 3 min, 1 min, and 1 min. Many factors influence the coagulation time, including the type of coagulant used. Sulmiyati and Malelak (2023) the coagulation time was faster using fresh biduri leaf extract than fresh biduri leaf extract powder.

The type of coagulant material greatly influences coagulation. Biduri leaf extract (*Calotropis gigantea*) contains enzymes that play a role in coagulation of milk casein. The coagulation mechanism can be enzymatic because biduri leaf extract contains protease enzymes. According to Rajagopalan *et al.* (2018), *C. gigantea* sap contains the enzymes Calotropain FI, FII, Calotropin DI and DII. Apart from that, the occurrence of coagulation can also be influenced by tannin. According to Noviyanty *et al.* (2020), biduri leaf extract also contains tannin, which plays on important role in the coagulation of milk protein (casein).

Curd production

The effect of different levels of biduri leaf extract added at a temperature of 75°C has a curd production of 31.340% ± 2.270 – 44.453% ± 4.018 (Table 3). The highest curd production was in suspesi given biduri leaf extract at a level of 2% (v/v) and the lowest curd production was at a level of 4% (v/v). Statistical analysis results showed a significant effect ($p<0.05$) on the curd production produced. The results of the Duncan test for L1 treatment (2% v/v) showed differences with of L2 treatment (2.5% v/v); L3 (3% v/v); L4 (3.5% v/v); dan L5 (4% v/v). Treatment of L2 (2.5% v/v) showed differences with of L1 treatment (2% v/v), L4 (3.5% v/v), and L5 (4% v/v). Treatment of L4

Table 3. Characteristics of physicochemical cheese with biduri leaf extract levels at temperature 75°C

Variables	Treatments					P-Value
	L1	L2	L3	L4	L5	
Coagulation time (minutes)	0.833±0.369[b]	0.493±0.090[a]	0.407±0.133[a]	0.397±0.040[a]	0.193±0.031[a]	0.018
Curd production (%)	44.453±4.018[c]	39.307±0.752[b]	37.700±0.703[b]	32.453±3.560[a]	31.340±2.270[a]	0.001
Percentage of whey (%)	50.833±3.182[a]	54.953±6.456[ab]	60.820±2.543[bc]	60.940±3.312 [bc]	64.267±3.884[c]	0.016
pH of curd	6.900±0.100	6.900±0.000	6.867±0.058	6.933±0.058	6.900±0.000	0.737[ns]
Lactic acid percentage (%)	0.143±0.021[a]	0.160±0.036[a]	0.253±0.078[ab]	0.310±0.101[b]	0.307±0.084[b]	0.041

showed differences with L1 (2% v/v), L2 (2.5% v/v), and L3 (3% v/v). This indicates that the higher the biduri leaf extract added, the decreased curd production. This is due to the activity of the protease enzyme contained in biduri leaf extract, although the previous hypothesis was that the coagulation of milk casein was influenced by the tannin content (Noviyanty *et al.*, 2020). In several other research results, biduri leaf extract also contains protease enzymes. According to Anusha *et al.* (2014); Rajagopalan *et al.* (2018) there are protease enzymes. According to Anusha *et al.* (2014); Rajagopalan *et al.* (2018) there are Calotropin FI, FII, Calotropin DI, and DII enzymes in *Calotropis gigantea*. Further research conducted by Abebe and Emire (2020) utilization of the calotropin enzyme taken from the leaves of the Calotropis procera plant which is used as a coagulant in making traditional East African cheese. According to Silva *et al.* (2020), that the protease enzyme found in Calotropis procera sap is a type of cysteine protease. Therefore, the enzyme activity that occurs is characterized by the amount of curd produced being influenced by temperature, where in this study, the temperature of giving biduri leaf extract was 75°C, so the enzyme activity did not work optimally to coagulate milk casein. According to Witono *et al.* (2007) the protease enzyme found in the biduri plant (*Calotropis gigantea*) has an optimum temperature of 55°C and thermostability at 90°C.

The research Hachana *et al.* (2021) that the yield of cheese decreased at doses of 1%, 4%, and 5%, and there was a decrease in the yield of cheese at a temperature of 80°C at a dose of 2% fresh coagulant (caprifig tree extract). The curd production produced is different from the research Welin *et al.* (2023), that the higher the level of biduri leaf extract results in increased curd production in cheese coagulated with biduri leaf extract 1% – 2.5% (v/v) at a temperature of 55°C. Same thing, research Li *et al.* (2022), that the higher the papain concentration, the yield increases with an average increase of 1-1.8%. This shows that using a temperature of 75°C has an influence on the curd production produced. In general, according to Sales *et al.* (2020), the increase in cheese yield is influenced by high rennet concentrations and long coagulation times. Further, according to research Arlene *et al.* (2015), the type of milk and enzyme used have an influence on the yield of cheese. According to Mona *et al.* (2011), the cheese yield is influenced by variations in cheese and the composition of milk and cheese, the composition of the milk (fat and casein/protein), the composition of the cheese (moisture and salt), fat loss, and the length of the cheese-making process. Furthermore, in detail, the factors that influence cheese yield, milk composition, genetic variants of milk, type of milk, physiological factors of livestock, conditions of the cheese-making process, milk storage, milk standardization, type of starter culture used, milk pasteurization, milk homogenization, type of coagulant used.

The curd production produced was higher than in the research (Sulmiyati and Malelak, 2023), the curd production produced in suspesi using biduri leaf extract level of 3% at a temperature of 55°C resulted in curd production of 26.58±1.41%. The same thing, the research Welin *et al.* (2023) produces curd production produced from cheese by providing biduri leaf extract at a level of 1.5-2.5% range 19.50±1.53 - 29.56±4.16%.

Whey percentage

The effect of different levels of bidi leaf extract added at a temperature of 75°C produces a whey percentage of range 50.833±3.182-64.267±3.884% (Table 3). The highest whey percentage was in suspesi given biduri leaf extract at a level of 4% (v/v) and the lowest whey percentage was at 2% (v/v). Statistical analysis results showed a significant effect ($p<0.05$) on the percentage of whey produced. The results of the Duncan test for treatment L1 (2% v/v) showed a difference from treatment L3 (3% v/v); L4 (3.5% v/v); and L5 (4% v/v). L2 treatment did not differ from L1 (2% v/v), L3 (3% v/v), and L4 (3.5% v/v). Treatment L3 (3% v/v) did not difference from treatment L4 (3.5% v/v) and L5 (4% v/v). This shows that the higher the level of biduri leaf extract added, the whey percentage increases and is inversely proportional to the curd production produced, where the more curd is formed, the whey percentage decreases. The process of coagulating milk casein is influenced by the characteristics of the coagulant material used, in making suspesi using biduri leaf juice, where the enzyme activity contained in the coagulant extract does not optimally coagulation if given at a temperature of 75°C. Resulting in a decrease in curd to form as indicated by the high percentage of whey produced in line with the increase in the level of biduri leaf extract added.

This result is different from Welin *et al.* (2023), using the temperature of giving biduri leaf juice at a temperature of 55°C with a level of 1.5%-2.5% (v/v), that the higher the level of biduri leaf extract added, the lower the percentage of whey produced, however, this is in line with the curd production produced, that the higher the curd obtained, the lower the whey produced. Produces percentage of whey produced from cheese by added biduri leaf extract at a level of 1.5%-2.5% at

a temperature of 55°C range 62.40%±4.20-67.00%±10.20.The percentage of whey produced is lower than in research Sulmiyati and Malelak (2023), that the percentage of whey produced in the suspesi using biduri leaf extract level of 3% at a temperature of 55°C produces a percentage of whey 71.90%± 4.08.

pH and lactic acid percentage of curd

The effect different level of biduri leaf extract added at temperature of 75°C has the pH of the curd 6.867 ± 0.058 – 6.900 ± 0.000 (Table 3). The results of statistical analysis showed no significant effect (p>0.05) on the pH of the curd produced. This shows that the pH of the suspesi curd is in the same range. This shows that giving biduri leaf extract at different levels did not have an effect on increasing or decreasing the pH of the curd. This is because the pH of the curd produced has the same pH as biduri leaf extract. The results were the same in the study Sulmiyati and Malelak (2023) the pH of suspesi curd at the biduri leaf extract level of 3% at a temperature of 55°C produces a curd pH 6.85± 0.05. The pH value of the curd produced is also influenced by the pH of the coagulant material, where the pH of biduri leaf extract is in the range 6.4-6.9.

Percentage of lactic acid range 0.143±0.021-0.310±0.101 (Table 1). The highest percentage of lactic acid was found in suspesi given biduri leaf extract at a level of 3.5% (v/v) and the lowest percentage of lactic acid was at a level of 2% (v/v). Statistical analysis results showed a significant effect (p<0.05) on the percentage of lactic acid. This shows an increase in acidity, as indicated by the percentage of lactic acid produced in line with the level of biduri leaf extract added. Even though the pH values obtained were almost the same, it was seen that the acidity of the milk increased in line with the level of biduri leaf extract added. However, it can be seen that the percentage of lactic acid produced in treatment L1 is no different between L2 and L3. Treatment L4 has no differences with treatments L3 and L4, Treatment L3 has no differences with L1, L2, L4, and L5. Research result Sulmiyati and Malelak (2023), the percentage of lactic acid suspesi in biduri leaf extract level is 3% at temperature 55°C 0.25%± 0.05. However, the percentage of lactic acid in this study was lower than the research results Welin *et al.* (2023) range 0.43±0.07-0.67± 0.010% on levels 1.5%-2% at temperature 55°C.

Chemical content of suspesi

The effect different level of biduri leaf extract added at temperature of 75°C has Water content range 53.380±2.843-58.290±0.721%, protein content range 14.205±1.421-15.445±0.318%, fat content range 18.220±0.283-20.030±2.630%, carbohydrate Content range 4.980±1.344–10.735±4.971% (Table 4). The statistical analysis results showed no significant effect (P>0.05) on water, protein, fat, and carbohydrate content. This shows that the levels of biduri leaf extract given at different levels at a temperature of 75°C produce the same chemical content. Research result Arlene *et al.* (2015), that the type of milk used has an influence on the protein and fat content, while the type of enzyme used in making cheese has no influence.

Based on the water content, the suspesi in this study are included in the semi-soft - soft cheese category based on According to Heller (2008); (Bulkaini *et al.*, 2020), that the water content in the semi-soft cheese category is 45% - 55% and soft cheese if it has a water content of >55%. The resulting water content of the suspesi is following SNI 01-2980-1992. However, the study's protein and fat content were below standard SNI 01-2980-1992. According to protein content minimum 19.5%, minimum fat content 25%. Research of Sulmiyati and Malelak (2023), chemical content of suspesi cheese using fresh biduri leaf extract at a level of 2% (v/v) at a temperature of 55°C, water content 53.17%, protein content 14.26%, fat content 16.73%, carbohydrate content 13.82%, fat content 16.73%.

Color of suspesi

The effect of different biduri leaf extract added at temperature of 75°C resulted in L* color in the range of 77.65 ± 3.41 – 82.96 ± 4.52. Color a* is 6.60 ± 5.02-10.95 ± 3.23. Color b* is in the range 24.33±5.40-30.16±2.40. Wi (hunter) value is in the range -10.37±2.99-7.96± 20.28. Yi (FC) value is in the range 43.34±11.61-53.73±1.46 (Table 5). The results of statistical analysis showed that the level of biduri leaf extract added at a temperature of 75°C showed no significant effect (p>0.05) on color L*, color a*, and color b*, Wi (hunter), and Yi (FC) value. The L* color result for suspesi describes cheese with a bright color. The color result of a* suspesi describes a redness. The color results of b* suspesi describes a yellowness. This shows that the colors L*, a*, and b* produced based on the level of biduri leaf extract do not have a significant effect. Even though statistically, it is described as having no effect, the results obtained show a decrease in lightness, which can be seen in the L* value. The Wi (hunter) values showed no significant effect (p>0.05), but it can be seen that there is a decrease in the lightness level of the cheese in line with the increase in the level of biduri leaf extract added and the Yi (FC) values increase

Table 4. Characteristics of chemical content cheese with biduri leaf extract levels at temperature 75°C

Variabels	Treatments					P-Value
	L1	L2	L3	L4	L5	
Water content (%)	54.275±1.336	56.225±4.447	58.190±0.622	58.290±0.721	53.380±2.843	0.300^{ns}
Protein content (%)	15.015±0.474	15.395±0.898	15.445±0.318	15.145±0.700	14.205±1.421	0.632^{ns}
Fat content (%)	19.075±0.983	20.030±2.630	19.800±1.117	18.220±0.283	20.310±0.481	0.597^{ns}
Carbohydrate content (%)	10.150±0.156	6.565±2.524	4.980±1.344	6.800±1.103	10.735±4.971	0.266^{ns}

in line with the increase in the level of biduri leaf extract.

The results of this research have the same results as the results of research conducted by Sulmiyati and Malelak (2022), which shows that the L* color produces a lower brightness level in line with increasing the level of biduri leaf extract at the level of 5% (v/v) compared to the addition of biduri leaf extract at 3%. Meanwhile, the a* color results produced do not match the results obtained visually (Figure 2), visually the suspesi is greenish in color with the level of green color increasing in line with the level of biduri leaf extract added. Further according to Miloradovic *et al.* (2018), heating treatment using a temperature of 80°C for 5 minutes and heating treatment using a temperature of 90°C for 5 minutes using cow's milk and goat's milk has the same L*, a*, b* color values in Quark cheese. Factors that determine the influence of the cheese's color are the chemical composition of the milk used. Research Cunha *et al.* (2010), that color variations in *requeijão cremoso* cheese products using vegetable fat as a substitute for milk cream tend to increase as the proportion of substitutes increases. The factor of ripening time, research conducted by Diezhandino *et al.* (2016), that there was a decrease in the brightness level of the L* color produced in *valdeón cheese* up to 120 days of ripening.

The hardness of suspesi

The effect different level of biduri leaf extract added at temperature of 75°C, which was added to the level of hardness of the specialty cheese, was 31.00±2.65-39.00±7.57 g (Figure 3). The statistical analysis results showed that the level of biduri leaf extract added at a temperature of 75°C showed no significant effect ($p>0.05$, $P = 0.417$) on the level of hardness of suspesi. The level of hardness of the cheese shows that the level of biduri leaf extract at the level of 2% and 4% has a higher cheese hardness compared to the level of biduri leaf extract at the level of 2.5% – 3.5%, but it can be seen that the hardness of the cheese increases in line with the level of biduri leaf extract. The same of research Sulmiyati and Malelak (2022), there was an increase in the hardness of the suspesi cheese produced in line with the increasing level of biduri leaf extract added, the suspesi cheese using an application temperature of 55°C showed a high level of hardness at the 5% level compared to the 3% level of biduri leaf extract. When compared with giving biduri leaf extract at a temperature of 55°C Sulmiyati and Malelak (2022) with the research results obtained using the temperature of giving biduri leaf extract at 75°C showing a higher level of hardness, based on these results it is clear that temperature has an influence on texture and hardness. According to Mbye *et al.* (2022), high pasteurization can have an influence on the quality of the cheese produced, especially on cheese yield and texture Further, according to Malaka *et al.* (2017a), the hardness of the Dangke cheese produced is correlated with the microstructure According to Issa Ado *et al.* (2018), It can be seen

Table 5. Characteristics of color suspesi cheese with biduri leaf extract levels at temperature 75°C

Variables	Treatments					P-Value
	L1	L2	L3	L4	L5	
Color L*	80.940±4.416	82.957±4.520	81.720±2.733	77.650±3.406	80.123±4.210	0.567^{ns}
Color a*	9.093±5.158	6.597±5.016	9.533±5.284	7.197±5.686	10.953±3.229	0.814 ^{ns}
Color b*	24.327±5.400	25.780±1.668	28.103±1.746	27.340±0.404	30.163±2.397	0.201 ^{ns}
Wi (Hunter)	7.963±20.278	5.617±2.665	-2.583±7.670	-4.363±4.490	-10.367±2.985	0.233^{ns}
YI (FC)	43.337±11.613	44.393±1.515	49.220±4.521	50.377±2.813	53.730±1.464	0.233^{ns}

L1 = Biduri leaf extract level 2% temperature 75°C, L2= Biduri leaf extract level 2.5% temperature 75°C, L3 = Biduri leaf extract level 3% temperature 75°C, L4 = Biduri leaf extract level 3.5% temperature 75°C, L5 = Biduri leaf extract level 4% temperature 75°C. Wi = whiteness Index, YI = Yellowness Index.
ns = non significant.

Figure 2. Suspesi cheese with biduri leaf extract levels at temperature 75°C (L1 = Biduri leaf extract level 2% temperature 75°C, L2= Biduri leaf extract level 2.5% temperature 75°C, L3 = Biduri leaf extract level 3% temperature 75°C, L4 = Biduri leaf extract level 3.5% temperature 75°C, L5 = Biduri leaf extract level 4% temperature 75°C).

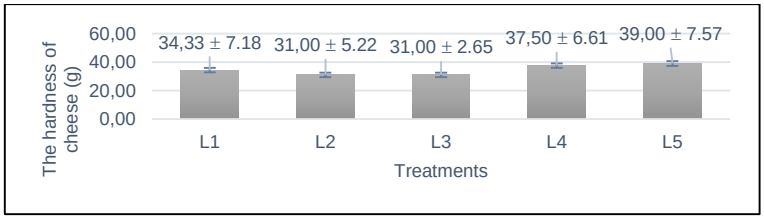

Figure 3. The hardness of suspesi cheese (g) with biduri leaf extract levels at temperature 75°C (L1 = Biduri leaf extract level 2% temperature 75°C, L2= Biduri leaf extract level 2.5% temperature 75°C, L3 = Biduri leaf extract level 3% temperature 75°C, L4 = Biduri leaf extract level 3.5% temperature 75°C, L5 = Biduri leaf extract level 4% temperature 75°C).

that curd with *homogenized anhydrous milk fat* (HAMF), and the curd produced by *homogenized cream* (HC) has a higher level of hardness than *curd of non-homogenized cream* (NHC). This difference is influenced by the protein and fat content, the large size of the fat globules, and the absence of connectivity. The same research Wang *et al.* (2023), that the decrease in hardness is influenced by the low-fat content, which can stabilize the gel network in the filer component. This can be compared to the decrease in hardness in cheese that added *Lycium barbarum polysaccharide* (LBP) isolate compared to the hardness of cheese that added *Jujube polysaccharide* (JP), and control treatments.

Apart from that, there are many factors that influence the hardness of the cheese produced, namely, the chemical components of the milk; according to Abdalla *et al.* (2022), the hardness of Mozzarella cheese that uses camel milk is lower than that of cheese that uses cow's milk. Furthermore, Cunha *et al.* (2010) states that the use of vegetable fat has an effect on increasing the size of fat globules, which correlates with increasing the hardness of the cheese. Salt level factor, according to Kaya (2002), There was an increase in the hardness of *Gaziantep cheese* in line with the salt concentration given at a concentration of 5-25%. Furthermore, *Telemea cheese* has a higher hardness using NaCl than KCl, respectively 3.55±0.27 N and 2.32±0.19 N. Factors of use of variations in bacteria used, according to Hussein and Shalaby (2014), the use of bacteria *Kareish cheese* made with starter culture containing *Lb. delbrueckii ssp. bulgaricus* and *Str. Thermophilus* has a higher cheese hardness than other bacterial variations in *Kareish cheese,* producing a hardness of 5.5±0.51 N. The ripening time factor, according to Vásquez *et al.* (2018), the hardness of the cheese produced is correlated with the ripening time of the Swiss-type cheese, and according to Malaka *et al.* (2017b), there was an increase in cheese hardness with the addition of passion fruit juice at the 10% level until ripening on day 28, respectively 1.63-3.70 kg/cm^2.

Tannin content of suspesi

The effect different level of biduri leaf extract added at temperature of 75°C had a suspesi tannin content of 0.28±0.02-0.33±0.04% (Figure 4). The results of statistical analysis showed that the level of biduri leaf extract added at a temperature of 75°C showed no significant effect (p>0.05, p= 0.658) on the tannin content of suspesi. The tannin content produced shows that the tannin content increases in line with the level of biduri leaf extract produced. The results obtained were the same as the research Sulmiyati and Malelak (2022), that there was an increase in the tannin content in suspesi in line with the level of biduri leaf extract added, where the tannin content obtained from suspesi with the addition of biduri leaf extract at a temperature of 55°C with levels of 3% and 5% was in the range of 0.25±0.04-0.33±0.04%. Furthermore Sulmiyati and Malelak (2023), that the tannin content contained in fresh biduri leaf extract is 0.5% and dried biduri leaf extract is 3.31%.

The tannin content plays a role in the coagulation of milk casein, but apart from the tannin content produced, which participates in the coagulation of milk casein, there are enzymes in the *Calotropis* plant. According to Anusha *et al.* (2014); Rajagopalan *et al.* (2018) there are *Calotropain FI, FII, Calotropin DI* and *DII* enzymes found in *Calotropis gigantea*. Furthermore, research was conducted by Abebe and Emire (2020) utilizing *calotropin enzyme* taken from the leaf of the *Calotropis procera* plant, which is used as a coagulant in making traditional cheese from East Africa. According to Silva *et al.* (2020), the protease enzyme found in *Calotropis procera latex is a cysteine protease*. The research of Anusha *et al.* (2014), shows *C. gigantea* plants in the latex have higher *Caseinolytic activity* (CA) continued in the leaf, flowers, and stems, however *Milk-Clotting Activity* (MCA), which is highest in the latex than in the stem, leaf, and flower, then for the *Milk-Cloting Index* (MCI) is highest in the latex, then stems, flowers, and the lowest is the leaf. According to Mohsin *et al.* (2024), there are types of protease enzymes derived from plants that have the potential to be used as coagulants in making cheese, *Cynara cardunculus, Cynara scolymus, Salpichroa origanifolia, Withania coagulans, Helianthus annus, Ficus religiosa, Solanum dubium, Streblus asper, Cucumis melo L., Euphorbia prunifolia, Solanum elaeagnifolium, Ficus carica, Calotropis gigantea, Calotropis procera, Actinidia chinensis, Carica papaya, Ananas comosus, and Zingiber officinal.*

Microstructure of suspesi

The effect different level of biduri leaf extract added at temperature of 75°C, the microstructure of the suspesi shows that the bonds between protein and fat molecules appear loose in line with

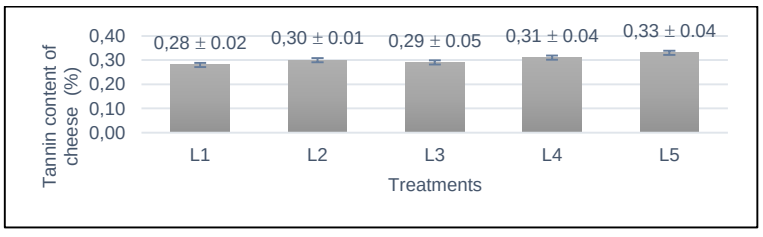

Figure 4. Tannin content of suspesi cheese (%) with biduri leaf extract levels at temperature 75°C (L1 = Biduri leaf extract level 2% temperature 75°C, L2= Biduri leaf extract level 2.5% temperature 75°C, L3 = Biduri leaf extract level 3% temperature 75°C, L4 = Biduri leaf extract level 3.5% temperature 75°C, L5 = Biduri leaf extract level 4% temperature 75°C).

the addition of biduri leaf extract. The structure of suspesi at the level of providing biduri leaf extract at the level of 2% – 3.5% shows a suspesi structure with fat bonds that are dominant compared to the protein bonds of the suspesi and the protein and fat bonds appear to be loose and uneven (score of 2) but at the level of providing biduri leaf extract at the level of 4% shows that the bonds look very loose and uneven with a more dominant fat bond structure (score of 1). These results indicate that there is a decrease in the bond structure between protein and fat in the suspesi produced in line with the increase in the level of biduri leaf extract added (Figure 5). According to Mbye *et al.* (2022), high pasteurization can have an influence on the quality of the cheese produced, especially on cheese yield and texture.

Research result Pavia *et al.* (1999), that the brine vacuum impregnation method applied to *Manchego-type cheese* affects the cheese's microstructure, thus affecting the homogenization of fat distribution and compacting the protein network. Furthermore, Türkmen and Güler (2022), that the cheese microstructure is influenced by the type of rennet, salt concentration (Brine salt), and curing time. Meanwhile, according to Buffa *et al.* (2001), based on the results of research comparing cheese produced using goat's milk with different methods, namely using fresh milk, pasteurized milk (75°C for 15 s), and pressure treated (500 MPa, 15 minutes 20°C), that the cheese Pasteurized shows an open structure with many cavities and irregularities, while pressure treated cheese produces the most regular and closed structure of the protein matrix and the cheese fat globules are homogeneously distributed in the protein matrix compared to cheese from fresh and pasteurized milk.

Organoleptic characteristics of suspesi

The effect of different levels of biduri leaf extract added at a temperature of 75°C has an organoleptic hedonic quality scale in the color aspect range 2.244±0.435-3.733±0.447, smell range of 4.689±0.633-4.822±0.387, taste was range 1.933±0.252-5.000±0.000, texture was range 2.689±0.468-2.978±0.336 (Figure 6). The results of statistical analysis showed a significant effect ($p<0.05$) on the hedonic quality scale of the suspesi in aspect of color, taste and texture but showed no significant effect ($p>0.05$) on the smell of the suspesi. This shows that the hedonic quality decreases in organoleptic quality, which is in line with the increase in biduri leaf extract levels. The organoleptic characteristics of suspesi can be seen visually in Figure 2. In line with the hedonic quality, it impacts the organoleptic characteristics in terms of hedonic suspesi.

The effect of the temperature of 75°C on the level of added biduri leaf extract has organoleptic characteristics in terms of the level of hedonic scale/preference in the color aspect 4.022±0.543-4.267±0.654, smell 4.222±0.765= 4.378±0.684, taste 1.000±0.000-5.000±0.000, texture 2.600±0.495-2.956±0.367 (Figure 7). The results of the statistical analysis showed significant effect ($p<0.05$) on the hedonic scale of the suspesi in terms of taste and texture but showed no significant effect ($p>0.05$) on the color and smell of the suspesi. This shows that the level of biduri leaf extract added at a temperature of 75°C influences in terms of taste and texture. According to Sulmiyati and Malelak (2023), the indicator of the quality of the suspesi does not give a bitter taste. The bitter taste produced is influenced by the added biduri leaf extract According to Noviyanty *et al.* (2020), that biduri leaf extract has a dark green color and has a bitter taste. The same result is according to Sulmiyati and Malelak, (2023), the characteristic of biduri leaf extract is that it is dark green and has a bitter taste. Furthermore Li *et al.* (2022), organoleptic characteristics are not influenced by temperature and coagulation time, but the differences in cheese produced are based on differences in papain concentration, especially those related to saltiness and sweetness.

The conclusion from an organoleptic perspective is that the level of biduri leaf extract added at a temperature of 75°C has an influence on the organoleptic quality scale of the color, taste and texture of the suspesi. The descriptive characteristics of the suspesi produced by the panelists have an influence on the level of preferences, especially in terms of taste and texture. This indicates that the panellists level of preference is influenced by whether there is a bitter taste in the suspesi as well as whether the resulting texture is soft. Meanwhile, color did not have a significant influence on the panellists level of preference, nor did the smell of suspesi. The texture of the suspesi produced shows the level of preference for the texture of the suspesi produced, although statistically, it has a significant effect, the texture preference value has a description of somewhat liking it for all levels of giving biduri leaf extract and all panellists prefer the texture at the level of giving biduri leaf extract of 2.5% - 3%. According to (Noviyanty *et al.*, 2020), that biduri leaf extract has a dark green color and has a bitter taste. The same results according to (Sulmiyati and Malelak, 2023), that the characteristics of biduri leaf extract are dark green and have a bitter taste.

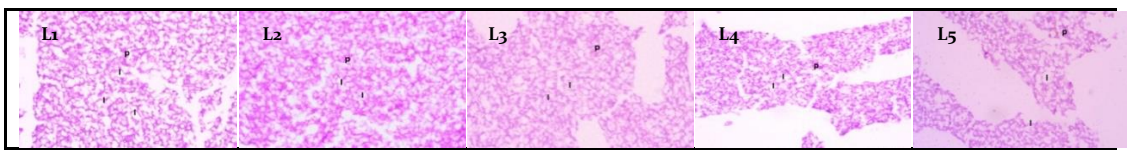

Figure 5. Microstructure of suspesi cheese with biduri leaf extract levels at temperature 75°C, magnification 40x (L1 = Biduri leaf extract level 2% temperature 75°C, L2= Biduri leaf extract level 2.5% temperature 75°C, L3 = Biduri leaf extract level 3% temperature 75°C, L4 = Biduri leaf extract level 3.5% temperature 75°C, L5 = Biduri leaf extract level 4% temperature 75°C).

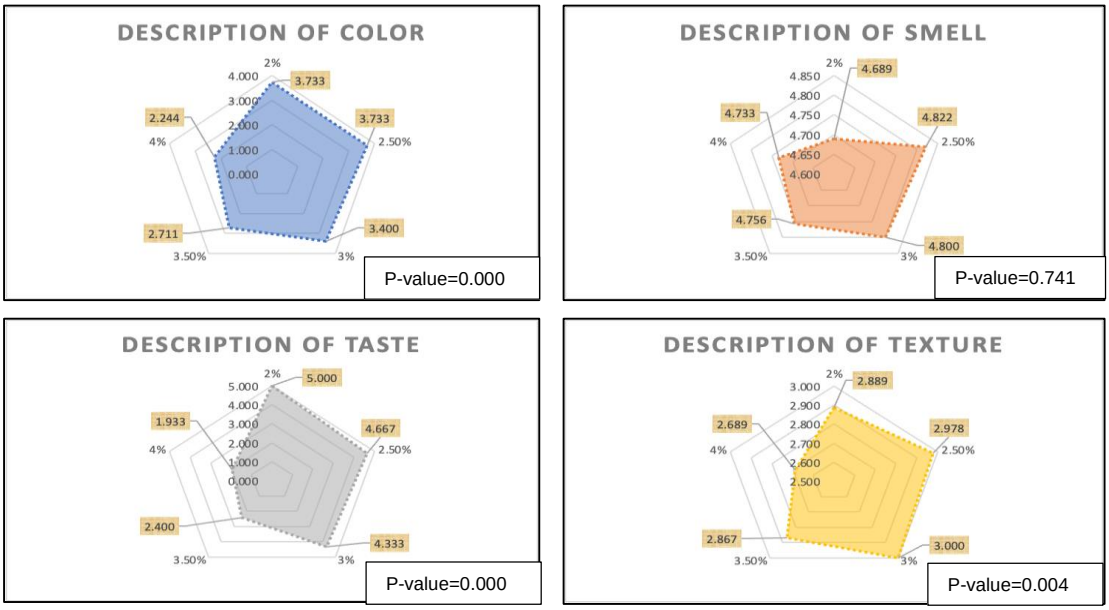

Figure 6. Organoleptic hedonic quality of suspesi cheese with biduri leaf extract levels at temperature 75°C (Information: color scale, (1) dark green, (2) green, (3) quite green, (4) rather green, (5) white Smell scale, (1) not smell suspesi, (2) rather not smell suspesi, (3) quite smell suspesi, (4) rather smell suspesi, (5) smell suspesi, Taste scale, (1) very bitter, (2) bitter, (3) quite bitter, (4) rather bitter, (5) not bitter Texture scale, (1) very soft, (2) soft, (3) quite soft, (4) rather solid, (5) solid

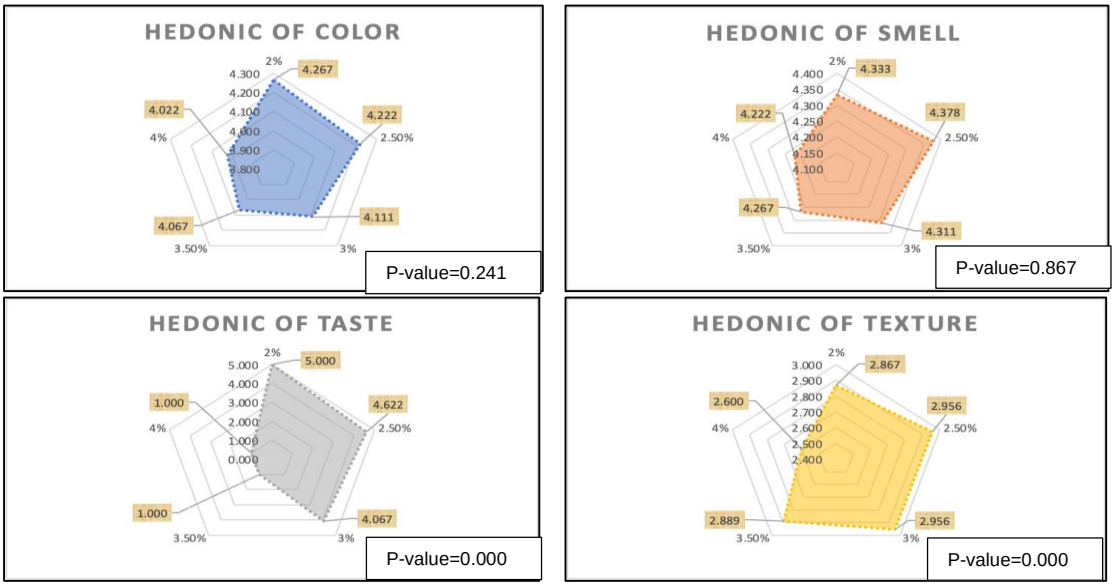

Figure 7. Organoleptic Hedonic/preference of suspesi cheese with biduri leaf extract levels at temperature 75°C (Information scale of hedonic: (1) dislike, (2) rather dislike, (3) rather like, (4) like, (5) very like.

Conclusion

Given biduri leaf extract at different levels added at a temperature of 75°C, the characteristics of suspesi in coagulation time, curd production, whey percentage, lactic acid percentage, organoleptic quality in terms of organoleptic and hedonic descriptions in the aspects of color, smell, taste, and the texture and microstructure of the suspesi had decreased, which showed that the bond was very strong, loosening of protein and fat bonds at 4% biduri leaf extract level. The best suspesi was at a level of giving biduri leaf extract at the level of 2% at a temperature of 75°C. The suggestion that giving biduri leaf extract can be recommended is giving biduri leaf extract should not >3% using a temperature of giving biduri leaf extract of 75°C.

Conflict of interest

No conflict of interest.

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Author's contribution

S.S conceived and designed the experiments; S.S and G.E.M.M performed the experiments; G.E.M.M analyzed the data with analysis tools; S.S and G.E.M.M wrote the paper, reviewed, and edited the article until the final article.

Ethics approval

The conducted research is not related to animals use.

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