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Coagulation power comparison between fresh and powdered *biduri* (*Calotropis gigantea*) leaf extract in making *suspesi* soft cheese

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Abstract

Cheeses have different characteristics based on the coagulant used during their production. Making *suspesi* soft cheese by adding *biduri* (*Calotropis gigantea*) leaf extract is generally followed by heating. The present work focused only on the ability of *biduri* leaf extracts to coagulate milk protein, and not on heating of the extract. The present work aimed to analyse the coagulation power of fresh and powdered *biduri* leaf extracts, and to evaluate the physicochemical, chemical, and organoleptic characteristics of the produced *suspesi* soft cheeses. The present work used a completely randomised design with two treatments and six replications. The variables measured were physicochemical characteristics, including coagulation time, curd production, whey percentage, pH, and lactic acid titration. Physicochemical characteristic analyses of *suspesi* soft cheeses coagulated with fresh and powdered *biduri* leaf extracts showed significant differences ($p < 0.05$) in the coagulation time and curd production. On the other hand, it did not show significant differences ($p > 0.05$) in the percentage of whey, pH, and lactic acid titration. The chemical content of *suspesi* soft cheeses showed significant differences ($p < 0.05$) in the water, protein, and crude fat contents. The organoleptic characteristics of *suspesi* soft cheeses showed a significant difference ($p < 0.05$) in the colour, but did not show significant differences ($p > 0.05$) in the smell, taste, and texture. *Suspesi* soft cheese produced with fresh *biduri* leaf extract had better coagulation power, physicochemical characteristics, chemical content, and organoleptic characteristics.

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Introduction

Cheese is a typical food from a local area with its characteristics. The characteristics of the cheese produced depend on the type of milk and coagulant used. Several types of cheese come from a region's traditional food, such as *Dangke* from Enrekang in South Sulawesi, which is coagulated with protease enzymes found in papaya latex (Malaka *et al.*, 2017; Sulmiyati and Said, 2019). *Ewe bomubomu*, a cheese from Nigeria, is coagulated with calotropin from the leaves of *Calotropis procera* (Akinloye and Adewumi, 2014). *Örgü* cheese (braided cheese) from Jordan is coagulated with the rennet enzyme of animal origin (Çelebi and Şimşek, 2015). *Cimi tulum* cheese from Turkey is clotted with goat rennet (Karagozlu *et al.*, 2009). Soft white cheese from Sudan is coagulated with cassava powder (Dhuol and Hamid, 2013). A soft cheese called *wara* from West Africa is coagulated with leaf extract of *Calotropis procera* using cow's milk from White Fulani cows (Ojedapo *et al.*, 2014).

The abovementioned studies explain that milk coagulants come from different materials, although they all have similar property of coagulating the milk. The main raw material other than milk in cheese making is strongly influenced by the type of coagulant. The type of coagulant that is commonly used is animal-derived renin enzymes. There are limitations in this type of production as it is expensive, and very limited renin enzymes is produced (Sukainah *et al.*, 2021). According to Akinloye and Adewumi (2014), the use of renin enzymes is limited to people who consume foods of animal source, religious reasons, as well as limitations on the use of genetically animals. Several researches examined other coagulant ingredients as alternative which are obtained from protease, and other plant extracts that have functions as vegetable coagulant ingredients. According to Adetunji and

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Babalobi (2011), the use of plants extracts as coagulants in the production of soft cheese has been known for a long time. Previous research used coagulant ingredients derived from plants such as spinach leaf extract (El-Sayed, 2020), *Moringa oleifera* (Abdeen *et al*., 2021), *Cynara* L. (Roseiro *et al*., 2003), papaya latex (Malaka *et al.*, 2017; Sulmiyati and Said, 2019), cassava powder (Dhuol and Hamid, 2013), sunflower seeds (Nasr *et al*., 2016), *Calotropis procera* latex (Akinloye and Adewumi, 2014; Ojedapo *et al.*, 2014), and *Calotropis gigantea* latex (Margoutomo *et al*., 2019; Bulkaini *et al*., 2020; Witono *et al*., 2003). The present work's novelty lies in the sense that it focused on using *biduri* leaf extract obtained from the *biduri* plant (*Calotropis gigantea*).

Biduri leaf extract has generally been used by the people of East Nusa Tenggara (NTT) in Indonesia as an ingredient to make a soft cheese called *suspesi*. *Biduri* leaves as a coagulant contain alkaloids, tannins, saponins, flavonoids, and glycosides (Singh *et al.*, 2014). According to Noviyanty *et al.* (2020), the tannin content in *biduri* leaves plays essential role as a coagulant that binds and precipitates milk proteins. *Biduri* leaf extract at a concentration of 100, 200, and 300 ppm had an average tannin contents of 1.16, 3.53, and 7.12 µg/mL, respectively. In addition, Witono *et al.* (2003) stated that *biduri* plant sap is used as a coagulant in cheese-making. Suresh and Ashok (2016) observed that the inside of *biduri* leaf contains flavonoids, coumarins, tannins, and phenolic compounds which play a role in health. Furthermore, Alafnan *et al*. (2021) reported that *biduri* plant contains flavonoids, thus can be used as a source of bioactive phytocompounds that have antioxidant and enzyme inhibitory potentials.

The people of NTT generally make cheese by adding *biduri* leaf juice to fresh milk, which is then heated. The present work used a method different from that used by the community: firstly pasteurisation; then, the temperature was lowered to 55°C; and *biduri* leaf extract was added to produce *suspesi* soft cheese. *Biduri* leaf extract was obtained by extraction from the leaves of the *biduri* plant in a liquid form, which was then dried. The present work focused on the coagulation power of *biduri* leaf extract in making soft cheese, and aimed to compare the coagulation power of *biduri* leaf extracts in fresh and powdered forms, and to evaluate the characteristics of the resulting *suspesi* soft cheeses.

Materials and methods

Research design

The present work was conducted at the Laboratory of Animal Products Technology, University of Nusa Cendana, Kupang, Indonesia from June to July 2022. The present work used a completely randomised design, with two treatments and six replications: P1 = fresh *biduri* leaf extract, and P2 = powdered *biduri* leaf extract. The present work had limitations in measuring the ability of *biduri* leaf extract as a coagulant, and comparing this ability in fresh and powdered forms. The physicochemical and organoleptic characteristics were tested at the Laboratory of Animal Products Technology, University of Nusa Cendana, Kupang, Indonesia. The chemical content analyses were carried out at the Laboratory of Animal Feed Chemistry, Faculty of Animal Husbandry, University of Hasanuddin, Makassar, Indonesia.

Biduri leaf extraction

Biduri leaf extract was obtained from the *biduri* plant. Extraction began by washing *biduri* leaves with clean water, and withering them at 40°C for 5 min to facilitate extraction. After that, leaves were mashed using a mortar, and squeezed to obtain fresh *biduri* leaf juice. To obtain the *biduri* leaf extract in powder form, the next step was to partially dry the fresh juice using a Memmert oven at 40°C. The physicochemical and organoleptic characteristics of fresh and powdered *biduri* leaf extracts are presented in Table 1.

Table 1. Physicochemical and organoleptic characteristics of fresh and powdered *biduri* leaf extracts.

Parameter	Fresh *biduri* leaf extract	Powdered *biduri* leaf extract
Colour	Dark green	Brownish green
Smell	Specifically *biduri* leaves	Specifically *biduri* leaves
Taste	Bitter	Bitter
Tannin*	0.5%	3.31%**
pH	6.4 - 6.9	6.3 - 6.4

*UV-visible spectrophotometric method (Noviyanty *et al.*, 2020). **Tannin content without dilution.

Suspesi soft cheese production

The cow's milk came from Sulu Labur Misi Course, Mandeu Village, Raimanuk District, Belu Regency, East Nusa Tenggara, Indonesia. The pH value of the milk was measured, and ranged from 6.5 to 6.7. The milk was then pasteurised using the high-temperature short time (HTST) method at 75°C for 15 s. The milk was then cooled to 55°C.

Fresh *biduri* leaf extract was added to some of the pasteurised milk, and powdered *biduri* leaf extract (diluted to 10:1 dilution; 1 g of *biduri* leaf extract + 9 mL of distilled water (Waterone)) at 3% (v/v) was added to the rest. After the curd formed, it was allowed to stand for 5 min; then, the curd and whey were separated using a sieve until the water was no longer dripping (~1 h).

Curd production, whey percentage, pH, and lactic acid percentage

The curd produced (w/v), which was separated from whey, was weighed using a digital scale [Kern (PBS/PBJ); maximum weight: 620 g, and minimum weight: 0.1 g]. Then, the percentage of cheese produced was calculated using Eq. 1. The percentage of whey (v/v) was measured by using a measuring cup, and the results were calculated using Eq. 2. For lactic acid titration, Mann's acid test method was used (Hadiwiyoto, 1994). For pH measurements, a pH meter (Ezodo PH5011) was used, which was previously calibrated using buffer solutions of pH 4.01, 7.01, and 10.01 (Hanna Instruments) (Hadiwiyoto, 1994).

Cheese production (%) =
(Curd mass / Initial milk volume) × 100 (Eq. 1)

Whey percentage (%) =
(Whey volume / Initial milk volume) × 100 (Eq. 2)

Chemical content

Protein content was determined using the Kjeldahl method. Fat content was determined using the Soxhlet method. Water content was determined using the heating method. Carbohydrate content was determined using the Luff Schoorl method (AOAC, 2005). Tannin content was determined using the UV-visible spectrophotometry method (Noviyanty *et al.*, 2020).

Organoleptic quality

The organoleptic quality of *suspesi* soft cheeses was tested by 15 semi-trained panellists on the parameters of colour, smell, taste, and texture. The organoleptic quality assessment parameters and scores are tabulated in Table 2.

Statistical analysis

Physicochemical characteristics, protein content, and organoleptic data of *suspesi* soft cheeses produced were analysed by independent *t*-tests using SPSS software version 16.0.

Results

Physicochemical characteristics of suspesi soft cheeses

The physicochemical characteristics of *suspesi* soft cheeses coagulated with fresh and powdered *biduri* leaf extracts were different regarding the coagulation time and curd production. The effects (means ± standard deviations) of the type of coagulant on the physicochemical characteristics of the *suspesi* soft cheeses are displayed in Table 3.

Suspesi soft cheeses coagulated with fresh *biduri* leaf extract had faster coagulation time, higher curd production, lower whey percentage, higher pH value, and lower lactic acid titration than *suspesi* soft

Table 2. *Suspesi* soft cheeses' organoleptic quality scale assessment.

Parameter	Organoleptic quality score				
	1	**2**	**3**	**4**	**5**
Colour	Dark green/ dark brown	Green/ brown	Quite green/ quite brown	A bit green/ a bit brown	White
Smell	Not *suspesi* scented	Slightly *suspesi*/ unscented	Quite *suspesi* scented	Slightly *suspesi* scented	*Suspesi* scented
Taste	Very bitter	Bitter	Quite bitter	Slightly bitter	Not bitter
Texture	Too soft	Soft	Quite soft	A bit dense	Dense

Table 3. Means ± standard deviations of the effects of coagulant extract on physicochemical characteristics of *suspesi* soft cheeses.

Parameter	P1	P2	*p*-value
Coagulation time (s)	146.72 ± 6.05[a]	1,585.22 ± 497.18[b]	0.000
Curd production (%)	26.58 ± 1.41[a]	17.29 ± 1.73[b]	0.000
Whey percentage (%)	71.90 ± 4.08[a]	75.83 ± 4.40[a]	0.139[ns]
pH	6.85 ± 0.05[a]	6.83 ± 0.08[a]	0.687[ns]
Lactic acid titration (%)	0.25 ± 0.05[a]	0.27 ± 0.06[a]	0.528[ns]

P1: *biduri* leaf extract (v/v); P2: powdered *biduri* leaf extract (v/v). Different lowercase superscripts in the same row indicate significant differences ($p < 0.05$). [ns]non-significant.

cheese coagulated with the powdered *biduri* leaf extract (Table 3). Based on the statistical analyses, coagulation time and curd production showed significant differences ($p < 0.05$), but the percentage of whey, pH, and lactic acid titration did not show significant differences ($p > 0.05$). The present work demonstrated that the coagulation ability of fresh *biduri* leaf extract was better than the powdered form. The coagulation ability was measured by the faster coagulation time and higher curd production, although the tannin content (0.5%) in the fresh form was lower than that in the powdered form (3.31%; without dilution) (Table 1).

Chemical content of suspesi soft cheeses

The chemical contents differed between *suspesi* soft cheeses coagulated with fresh *biduri* leaf extract and powdered *biduri* leaf extract. The effects (means ± standard deviations) of the type of coagulant on the chemical contents of suspesi soft cheeses are shown in Table 4.

The chemical content of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract exhibited higher crude protein, carbohydrate, and fat contents than that coagulated with the powdered *biduri* leaf extract. The water content of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract was lower than that coagulated with the powdered *biduri* leaf extract (Table 4). The results of statistical analyses showed significant differences ($p < 0.05$) in the water content, crude protein, and crude fat, but no significant difference ($p > 0.05$) in the carbohydrate content. This indicated that fresh and powdered extracts affected the chemical content of the cheeses produced. The ability of coagulation in fresh form was better than that in powdered form, which was in line with our results of chemical analyses.

Organoleptic characteristics of suspesi soft cheeses

Differences were noticed in the organoleptic characteristics of *suspesi* soft cheese coagulated with *biduri* leaf extracts in the fresh and powdered forms, in terms of colour and consistency of the cheeses. The effects (means ± standard deviations) of the type of coagulant on the organoleptic characteristics of *suspesi* soft cheeses are shown in Table 5.

The organoleptic characteristics of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract were different from that coagulated with powdered *biduri* leaf extract. Table 4 compares the characteristics of colour, smell, taste, and texture of the *suspesi* soft cheeses coagulated with fresh and powdered *biduri* leaf extracts. The statistical analyses showed a significant difference ($p < 0.05$) in colour, but no differences in the smell, taste, and texture of the cheeses. These results indicated that the organoleptic characteristic that differed between the *suspesi* soft cheeses produced by using fresh and powdered *biduri* leaf extracts was the colour.

Table 4. Means ± standard deviations of the effects of coagulant type on the chemical content of *suspesi* soft cheeses.

Parameter	P1	P2	*p*-value
Water (%)	53.17 ± 2.39[a]	61.94 ± 2.59[b]	0.000
Crude protein (%)	14.26 ± 0.69[a]	12.28 ± 0.84[b]	0.001
Carbohydrates (%)	13.82 ± 2.79[a]	10.94 ± 2.80[a]	0.104[ns]
Crude fat (%)	16.73 ± 2.43[a]	13.52 ± 2.09[b]	0.034

P1: fresh *biduri* leaf extract (v/v); P2: powdered *biduri* leaf extract (v/v). Different lowercase superscripts in the same row indicate significant differences ($p < 0.05$). [ns]non-significant.

Table 5. Means ± standard deviations of effects of coagulant type on organoleptic characteristics of *suspesi* soft cheeses.

Parameter	P1	P2	*p*-value
Colour	3.05 ± 0.05[a] (quite green)	3.30 ± 0.20[b] (quite brown)	0.013
Smell	3.29 ± 0.38[a] (quite suspesi scented)	3.12 ± 0.14[a] (quite suspesi scented)	0.337[ns]
Taste	4.91 ± 0.05[a] (not bitter)	4.79 ± 0.18[a] (not bitter)	0.148[ns]
Texture	3.15 ± 0.12[a] (quite soft)	3.07 ± 0.06[a] (quite soft)	0.191[ns]

P1: *biduri* leaf extract (3%) (v/v); P2: powdered *biduri* leaf extract (v/v). Colour scale: (1) dark green/dark brown; (2) green/brown; (3) quite green/quite brown; (4) slightly green/slightly brown; and (5) white. Smell scale: (1) not suspesi scented; (2) not slightly suspesi scented; (3) quite suspesi scented; (4) slightly suspesi scented; and (5) suspesi scented. Taste scale: (1) very bitter; (2) bitter; (3) quite; (4) slightly bitter; and (5) not bitter. Texture scale: (1) very soft; (2) quite soft; (3) quite soft; (4) a bit dense; and (5) dense. Different lowercase superscripts in the same row indicate significant differences ($p < 0.05$). [ns]non-significant.

Discussion

Physicochemical characteristics of suspesi soft cheeses

Coagulation time

The coagulation time of fresh *biduri* leaf extract was faster than that of the powdered *biduri* leaf extract at 146.72 ± 6.05 and 1,585.22 ± 497.18 s, respectively (Table 3). Statistical analyses showed a significant difference ($p < 0.05$) in the coagulation time of milk protein using *biduri* leaf extract in fresh form as compared to powdered form, although the tannin content in fresh *biduri* leaf extract was lower than that in powdered *biduri* leaf extract (not diluted with water). Several factors can be attributed to this such as the tannin content in powdered form was analysed without being dissolved in water; in the present work, *biduri* leaf extract powder which had been dissolved in water was used, thus the tannin content in the solution would be lower as compared to the powdered form. Several other factors were also observed such as there was dissolved latex which could likely have important role in milk protein coagulation, and the heating factor during the drying of *biduri* leaf extract influenced the activity of other potential enzymes in milk protein coagulation (Witono, 2013). Previous research used *biduri* plant as a coagulant in cheese-making by utilising the sap which contains protease that could function as coagulant (Witono *et al.*, 2003). Many factors influence milk casein coagulation, namely the type of the enzyme, water content, cutting time, coagulation temperature, and milk composition (Britten and Giroux, 2022).

The coagulation time using fresh *biduri* leaf extract in the present work was much faster than that reported by Ojedapo *et al.* (2014), in which the coagulation time of soft cheese using *C. procera* leaf extract was 16.75 ± 0.70 min for unsalted cheese, and 14.75 ± 2.90 min for salted cheese. Abebe and Emire (2020) also observed lower coagulation time using coagulant of *C. procera* (14.30 ± 1.2 to 16.36 ± 0.9 min). Nasr *et al*. (2016) showed that the coagulation time using rennet (0.91 ± 0.07 h) as a coagulant was faster as compared to coagulant material from sunflower seed enzyme (3.60 ± 0.27 h). Therefore, the use of *biduri* leaf extract has the potential to be used as a milk coagulant in cheese-making.

Curd production

Suspesi soft cheese coagulated with fresh *biduri* leaf extract had 9.29% higher curd production (26.58 ± 1.41%) than that coagulated with powdered *biduri* leaf extract (17.29 ± 1.73%; Table 3). Statistical analyses showed significant difference for this parameter ($p < 0.05$), thus indicating that the ability of fresh *biduri* leaf extract to coagulate milk casein was better than that of the powdered *biduri* leaf extract. According to Mona *et al.* (2011), the factors that influence the production of cheese are milk composition, casein content, milk quality, milk somatic cell number, type of pasteurisation process used, type of coagulant, hardness of the curd at the time of cutting, and the manufacturing process.

Noviyanty *et al*. (2020) stated that tannins play a role in coagulating proteins. The average tannin content in *biduri* leaf extract at a concentration of 100, 200, and 300 ppm was 1.16, 3.53, and 7.12 g/mL, respectively.

The yield of curd coagulated by *biduri* leaves was much lower than that obtained using protease. Sulmiyati and Said (2019) stated that the curd coagulated by crude papain at 0.5 - 2% using buffalo milk was 37.04 - 52.88%. Witono *et al.* (2003) reported that the enzyme level extracted from *biduri* was 0.3%, with an incubation period of 120 min, which resulted in a cheese yield of 4.44%. Al-Zoreky and Almathen (2021) showed that using camel chymosin as a coagulant with cultured milk yielded 8.75 ± 1.68% of soft cheese, and with non-cultured milk yielded 3.34 ± 1.14% of soft cheese. Cow's milk coagulated with *C. procera* had a soft cheese yield value of 25.60% (Akinloye and Adewumi, 2014). The yield of soft cheese coagulated with *C. procera* leaf extract was 19.26 ± 3.14% for unsalted cheese, and 16.63 ± 2.86% for salted cheese (Ojedapo *et al.* 2014). Abebe and Emire (2020) produced lower yield curd by using coagulant from *C. procera* (14.13 ± 0.43 to 17.89 ± 1.10%). Nasr *et al*. (2016) observed that the curd yield using rennet as coagulant (18.73 ± 1.10%) resulted in lower curd yield as compared to coagulant of seed sunflower enzyme (20.78 ± 5.37%). Based on these results, curd could be more produced as compared to other coagulant ingredients besides the coagulant material from papaya enzymes (papain). Therefore, *biduri* has the potential as a coagulant in cheese-making as compared to other *Calotropis* sp. namely *C. procera*, which has been used in traditional cheeses from South Africa.

Whey percentage

The whey percentage of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract was lower than powdered *biduri* leaf extract. The percentages of whey coagulated with fresh and powdered biduri leaf extracts were 71.90 ± 4.08 and 75.83 ± 4.40%, respectively. The results of statistical analyses showed no significant difference ($p > 0.05$) for this parameter. The percentage of whey has a relationship with the production of curd, where the higher the amount of curd produced, the lower the percentage of whey. These results showed that the percentage of whey was higher using powdered *biduri* leaf extract as compared to fresh *biduri* leaf extract, in line with the resulting curd production which was much higher using fresh *biduri* leaf extract as compared to using powdered *biduri* leaf extract. Among the factors that can affect this is temperature when drying *biduri* leaves, which impact on the activity of enzymes contained in *biduri* leaf extract. According to Witono (2013), drying has an effect on enzyme activity, where drying using a freeze dryer minimises damage to the components contained in the material, including enzymes and proteins, as compared to regular dryers. The whey percentage in the present work was much higher than that obtained by Sulmiyati and Said (2019) which was 41.38 - 52.32% from buffalo's milk *Dangke* coagulated with crude papain at 0.5 - 2%. Meanwhile, soft cheese made from cow's milk coagulated with *C. procera* had a whey yield of 72.47% (Akinloye and Adewumi, 2014). Abebe and Emire (2020) produced whey volume of 601.90 ± 5.69 to 858.40 ± 6.40 mL using coagulant from *C. procera*.

pH and lactic acid titration

The pH value of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract (6.85 ± 0.05) was higher than that coagulated with powdered *biduri* leaf extract (6.83 ± 0.08; Table 3). Statistical analyses did not show significant differences ($p > 0.05$) in the pH value and lactic acid percentage. Therefore, the pH value of *biduri* leaf extract coagulant in fresh and powdered form did not affect the pH content of the *suspesi* soft cheeses produced. pH value and lactic acid titration obtained are influenced by the pH value of the coagulant used. Based on previous studies, the pH value of cheese was higher than soft cheese of cow's milk coagulated with *C. procera*, which had a pH value of 6.47 (Akinloye and Adewumi, 2014). The *suspesi* soft cheese coagulated with fresh *biduri* leaf extract had a lactic acid titration of 0.25 ± 0.05%, whereas the *suspesi* soft cheese coagulated with powdered *biduri* leaf extract had 0.27 ± 0.06% (Table 3). The titration value of lactic acid is inversely proportional to the pH value obtained. The higher the pH value of the curd, the lower the titration value of lactic acid. The titration of lactic acid in the present work was higher than that obtained by Sulmiyati and Said (2019), where the percentage of lactic acid in buffalo's milk in *Dangke* was 0.15 - 0.17%. Abebe and Emire (2020) observed that pH value of cheese using a coagulant from *C. procera* was 6.12 ± 0.01 to

6.35 ± 0.03, and titrable acidity of 0.106 ± 0.001 to 0.110 ± 0.003.

There were differences in the physicochemical characteristics of the *suspesi* soft cheese coagulated with fresh *biduri* leaf extract as compared to powdered *biduri* leaf extract. Based on the coagulation time and resulting curd production, the physicochemical characteristics of the *suspesi* soft cheese coagulated with *biduri* leaf extract had faster coagulation ability than powdered *biduri* leaf extract. According to Mona *et al.* (2011), the characteristics of different types of cheese depends on the variations during cheese-making, such as unique composition of the chemical content of milk (especially fat and protein), fat loss, and curd produced during cheese-making.

Chemical characteristics of suspesi soft cheeses

Water content

The water content of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract was 53.17 ± 2.39%, whereas that coagulated with powdered *biduri* leaf extract was 61.94 ± 2.59%. This showed that the water content using coagulant material from powdered *biduri* leaf extract had higher water content as compared to fresh *biduri* leaf extract. These results were lower when using coagulants from *C. procera* (61.70%) and papaya (62.50%) as reported by Adetunji and Babalobi (2011). The water content of *suspesi* soft cheese coagulated with *biduri* leaf extract was within the prescribed standard SNI 01-2980-1992 (National Standardization Agency of Indonesia, 1992). Based on SNI 01-2980-1992, the water content of processed cheese is 46 - 56% (FAO, 2006), and the standard water content is 18 - 20%.

Crude protein content

The crude protein content of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract was 1.98 (14.26 ± 0.69%), higher than that coagulated with powdered *biduri* leaf extract (12.28 ± 0.84%). Previous research by Ojedapo *et al.* (2014) revealed that the protein content of White Fulani unsalted cheese was 14.74 ± 1.02%, and salted cheese was 20.13 ± 1.02%, using cow's milk coagulated with *C. procera* leaf extract. The protein content of spinach powder-supplemented UF-soft cheese was 9.29 - 10.03% (El-Sayed, 2020). The obtained protein content was also lower than reported by Adetunji and Babalobi (2011), where the protein content of the cheese produced using coagulants from *C. procera* was 33.84%, and papaya was 31.60%.

Carbohydrate content

Suspesi soft cheeses coagulated with fresh and powdered *biduri* leaf extracts had carbohydrate contents of 13.82 ± 2.79 and 10.94 ± 2.80%, respectively. Statistical analyses results did not show significant difference ($p > 0.05$) in the type of coagulant used. The carbohydrate contents in the present work were higher than that in El-Sayed's (2020) study which stated that spinach powder-supplemented UF-soft cheese had a carbohydrate content of 6.20 - 7.28%. The content of carbohydrates (lactose) is higher than in research by Adetunji and Babalobi (2011), where the lactose content of the cheese produced using coagulants from *C. procera* was 8.10%, and papaya was 2.05%.

Fat content

The crude fat contents of *suspesi* soft cheese coagulated with fresh and powdered *biduri* leaf extracts were 16.73 ± 2.43 and 13.52 ± 2.09%, respectively. The fat content of our *suspesi* soft cheese coagulated with fresh *biduri* leaf extract was higher than that of White Fulani unsalted cheese (12.68 ± 2.72%) and salted cheese (15.30 ± 1.97%), made from cow's milk coagulated with *C. procera* leaf extract (Ojedapo *et al.*, 2014). El-Sayed's (2020) study showed that the fat content in spinach powder-supplemented UF-soft cheese was 11.50 - 13.00%. The fat contents of the *suspesi* soft cheese made with the two types of coagulants were not within the standard SNI 01-2980-1992, 25%. The fat content recorded in the present work was also lower than reported by Adetunji and Babalobi (2011) of 31.45% using coagulant from *C. procera*, and 22.33% using papaya.

The *suspesi* soft cheese coagulated with fresh *biduri* leaf extract had better chemical characteristics in terms of water, crude protein, carbohydrate, and crude fat contents than the *suspesi* soft cheese coagulated with powdered *biduri* leaf extract.

Organoleptic characteristics of suspesi soft cheeses

The type of coagulant used had an influence on the colour of the produced *suspesi* soft cheeses. The main quality indicator of the produced *suspesi* soft cheese was also not to have bitter taste. Pereira *et al.* (2009) found that the organoleptic characteristics of

cheese are strongly influenced by the level of appearance, taste, and texture. The organoleptic characteristics of the cheese produced did not affect or increase the organoleptic quality, since the cheese produced in the present work did not undergo ripening. According to Vítová *et al*. (2011), ripening can improve organoleptic quality.

Colour

The green colour of the cheese came from the *biduri* leaf extract used, which was dark green (Figure 1c). The powdered *biduri* leaf extract was brownish green in colour (Figure 1d). Therefore, the resulting *suspesi* soft cheeses coagulated with both types of leaf extract imparted the green and brown colours of their extracts (Figures 2a, 2b).

Smell

The smell of *suspesi* soft cheeses was not significantly different. The *suspesi* soft cheeses coagulated with fresh and powdered *biduri* leaf extract were quite *suspesi* scented.

Figure 1. *Biduri* (*Calotropis gigantean*): **(a)** plant; **(b)** leaf pollen; **(c)** leaf extract; **(d)** leaf extract dilution 10^{-1}.

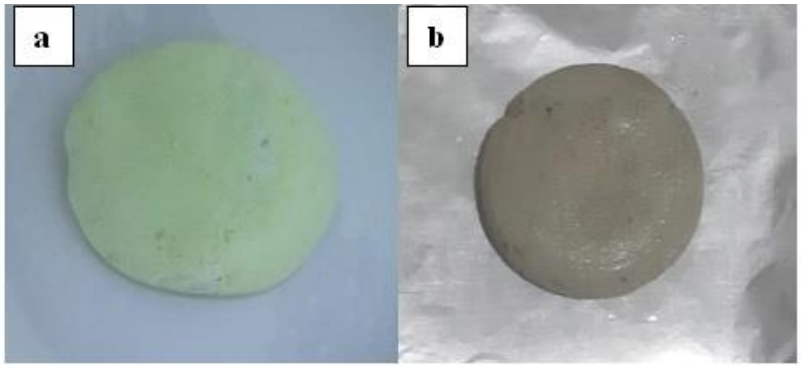

Figure 2. *Suspesi* soft cheeses coagulated with **(a)** *biduri* leaf extract, and **(b)** powdered *biduri* leaf extract.

Taste

The taste of *suspesi* soft cheeses made using both fresh *biduri* leaf extract and powdered *biduri* leaf extract was described as not bitter. The bitter taste is due to tannin. Noviyanty *et al.* (2020) stated that organoleptic results of cheese made using fresh *biduri* leaf extract were blackish green colour and bitter taste. These results showed that the coagulant used could affect the colour of the resulting *suspesi* soft cheese. A study by Pereira *et al.* (2009) showed that the level of consumer acceptance was strongly influenced by the organoleptic characteristics of cheese such as appearance, taste, and texture.

Texture

The texture of *suspesi* soft cheeses coagulated with both fresh and powdered *biduri* leaf extracts was fairly soft. According to Pereira *et al.* (2009), the cheese texture is indirectly influenced by chemical components, such as protein content and fat fraction at the micro- and macrostructure levels, thus affecting the cheese characteristics. The organoleptic characteristics of *suspesi* soft cheese coagulated with fresh *biduri* leaf extract were better than the powdered *biduri* leaf extract. Therefore, 3% fresh or powdered *biduri* leaf extract is the reference standard to make *suspesi* soft cheese.

Conclusion

Suspesi soft cheese that used fresh *biduri* leaf extract had better coagulation power than powdered extract. The characteristics of the *suspesi* soft cheese coagulated with fresh *biduri* leaf extract were: coagulation time, 146.72 s; curd production, 26.58%; whey percentage, 71.90%; pH, 6.85; lactic acid titration, 0.25%; water content, 53.17%; crude protein, 14.26%; carbohydrate, 13.82%; crude fat, 16.73%; colour, 3.04 (relatively green); smell, 3.29 (sufficiently flavoured with *suspesi*); taste, 4.91 (not bitter); and texture, 3.14 (relatively soft). Research should be conducted on the standardisation of the amount of *biduri* leaf extract and temperature used in the process to produce the best *suspesi* soft cheese.

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