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Properties of Soft Cheese Supplemented with Cinnamon Extract (*Cinnamomum burmannii*) During Storage

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ABSTRACT

An innovative milk-based functional product was developed to produce a quality and healthy animal product, such as antioxidant-rich cheese. The study was conducted to identify the chemical composition, lactic acid bacteria (LAB) viability, color characteristics, hardness, and stickiness of cheese with cinnamon extract during storage. The material used was cow milk from the Faculty of Animal and Agricultural Sciences Diponegoro University, cinnamon extract, animal rennet, and culture of *Lactobacillus plantarum* Kita-3 from Food and Nutrition Culture Collection (FNCC), Universitas Gadjah Mada. The research was conducted experimentally using a completely randomized design (CRD) with 4 treatments and 6 replications. The cheese was stored at 4-10°C and observed at 0, 10, and 20 d to determine titratable acidity (TA), moisture content (MC), free fatty acid (FFA), antioxidant activity, LAB viability, color characteristics, hardness, and stickiness. Treatments were addition of cinnamon extract 0% (P0), 3% (P1), 6% (P2), and 9% (P3). Data collected was analyzed by analysis of variance and continued with Duncan's multiple range test. The addition of cinnamon were not affected ($p>0.05$) to TA and FFA at 0 d observation, MC, b*, stickiness at 0 and 20 d, and hardness at 10 d. Meanwhile, it affected significantly ($p<0.01$) TA at 10 and 20 d, pH and LAB viability at all observations, MC at 10 d, FFA and hardness at 0 and 20, and stickiness at 10 d. It also affected significantly ($p<0.01$) increased antioxidant activity of cheese on 0 d, with the highest was 14.37% (6%). In summary, the addition of up to 9% cinnamon could produce a good quality of cheese during storage.

Keywords: A*ntioxidant, Cheese, Cinnamon, Lactobacillus plantarum kita-3*

Introduction

Society's awareness of consuming healthy food is currently increasing, not only to meet basic needs but also the health effects are also a consideration. One of the functional foods which has been widely developed is food containing probiotics, a substance that provides health effects by enhancing good microbiota in the digestive tract. The use of various food-grade additives in food could be applied to improve quality and food safety and prolong shelf-life. These additional ingredients usually include preservatives and antioxidants (Christaki *et al*., 2021). A recent study conducted by Ismiarti *et al*. (2023) showed that the addition of porang flour (*Amorphophallus oncophyllus*) and *Lactobacillus rhamnosus* was able to improve LAB total on fresh cheese, so that it could be qualified as probiotic cheese. Cheese is typically divided into hard and soft cheese. Soft cheese contains up to 80% water (Wulandari *et al*., 2021). Cheese would have a beneficial effect when combined with probiotics or prebiotics (Ismiarti *et al*., 2023). Apart from probiotics as functional foods, other important substances for health that could be applied into soft cheese were antioxidants.

Antioxidants are compounds which play an important role on inhibiting oxidation by reacting with free radicals. Free radicals are produced in the human body through physiological processes, contact with certain environments, and unhealthy eating habits (Stobiecka *et al*., 2022). Ingredients containing antioxidants have potency to delay or prevent damage due to oxidation of target molecules (Handito *et al*., 2022). Recent studies have been conducted to develop cheese with antioxidant-rich plant such catechins (Rashidinejad *et al*., 2016b), moringa, bidara, and bay leaves extract (Setyawardani *et al*., 2021), and orthodox black tea (Fadhlurrohman *et al*., 2023). Indonesia has rich-in-antioxidant herbal plants, such cinnamon (*Cinnamomum burmannii*). Cinnamon components consist of terpenes, phenylpropanoids, ligands, flavonoids, aromatic, and aliphatic components, coumarins, alcaloids, steroids, and other active substances (Chen *et al*., 2019). The great component on cinnamon are cinnamaldehyde, the essential oil from cinnamon has a cinnamaldehyde content of 92% (Pratiwi *et*

al., 2015). The high level of antioxidants in cinnamon is the reason for its use in food to develop herbs-based antioxidant-rich foods. Apart from that, a good sweet flavor could produce cheese which is more acceptable for sensory properties.

Soft cheese contains no more than eighty percent of water content, it is a food with a potential carrier of antioxidants from cinnamon because it can be stored for a relatively long time, has a solid consistency, and complete nutrients (Ismiarti *et al*., 2023) . A previous study that has been conducted proving that cheese was effective as an antioxidant carrier for green tea which contains catechins as the largest active substance (Rashidinejad *et al*., 2016b). Cheese is made from coagulating milk casein after acidification with renin enzyme. Acidification of cheese could be done directly using ingredients which has strong acidity or indirectly using acid-producing bacterial cultures. The LAB group has been proven to be effective in supplementing cheese, apart from the acidification process, it is also used to produce probiotic cheese (Sumarmono *et al*., 2020). *Lactobacillus plantarum* Kita-3 is a group of LAB isolated from Haloumi cheese developed by Rahayu *et al*. (2019). The use of cinnamon herbs is expected to maintain the viability of *Lactobacillus plantarum* Kita-3 culture, which is added as an acidifier, resulting in a milk-based functional food, a beneficial substance for a host. This study was conducted to investigate the chemical composition, LAB viability, color characteristics, hardness, and stickiness of cheese with cinnamon extract on 0, 10, and 20 d observations.

Materials and Methods

Material. Material used were cow milk from Faculty of Animal and Agricultural Science, Diponegoro University, cinnamon bark, commercial animal rennet, culture of *Lactobacillus plantarum Kita-3* from Food and Nutrition Culture Collection (FNCC) Universitas Gadjah Mada, phenolpthalein (Merck, 1%), absolute ethanol (Sigma Aldrich, 96%), de Mann Rogosa Sharpe Agar (MRSA) and broth (MRSB) (Merck, Germany), physiological NaCl 0.85%, 2,2 diphenyl 1 picrylhydrazyl (DPPH) (Sigma Aldrich, Germany). Equipment used were analytical scales, electric oven, burette, pH meter type HI1271 (Hanna Instrument, USA), desicator, Laminar Air Flow, spectrophotometer, micropipette 200µL (Socorex, Switzerland), colony counter, texture profile analyzer TA.XT plus C (Stable Micro System, UK).

Methods. Steps of this study were culture preparation, extraction of cinnamon, cheese production, observation, and determination of parameters. Treatments included the addition of cinnamon 0%, 3%, 6%, and 9% $^{v}/_{v}$, respectively. This experimental study was conducted with a completely randomized design with 4 treatments, with replication for 6 times. Observation was obtained at 0, 10, and 20 d on cold storage (4-10°C) and determining chemical composition (TA, MC, and FFA), antioxidant activity, LAB viability, color characteristics (L*, a*, and b*), hardness (g) and stickiness (g). Data was analyzed using analysis of variance and post hoc DMRT (Duncan's Multiple Range Test)

Culture starter preparation. Culture starter preparation was followed by Ouwehand et al. (2001). An 18 g of skim milk was added into 100 mL distilled water, sterilized in an autoclave (110°C) for 15 min, then cooled until 45°C. *Lactobacillus plantarum* Kita-3 isolate on MRSB was pipetted 100 µL into sterilized skim milk and incubated at 45°C for 18 h to produce a mother culture. The mother culture was then re-inoculated into sterile skim milk, incubated at 45°C then for 8 h, and ready to be used as an acidifier.

Cinnamon extraction. Cinnamon bark was rinsed in running water and then dried at 50°C for 3 d until dry, then ground using a blender and shifted to get cinnamon powder (BSN, 1995)The extraction method was followed by Kimestri *et al*. (2018) with a modification on the heating time. 100 g of cinnamon powder was dissolved in 1500 mL of distilled water and stirred until homogeneous, then filtered using a filter cloth to obtain cinnamon extract. The cinnamon extract was then heated at 100°C until boiling, then cooled and ready to be used.

Cheese making. Cheesemaking followed Ismiarti *et al*. (2023) starting with pasteurization of 5 l milk for each treatment using the LTLT method (63°C for 30 min), then adding cinnamon extract. The temperature was decreased to 37°C. *Lactobacillus plantarum* Kita-3 starter was added at 5% ($^{v}/_{v}$) and incubated to lower the pH to 6.1. Rennet was added at 0.06 mL/L milk and incubated at 37°C for 30 min until curd formed. The curd was filtered using a filter cloth to separate from the whey and then pressed for 1 h. The cheese formed was then soaked using NaCl solution 5%($^{w}/_{v}$) for 30 min and the cheese was stored in the refrigerator at 4-10°C.

Determination of raw material composition. Protein, fat, and lactose content of milk were measured following Sudarmadji *et al*. (2007), protein using semi-micro Kjeldahl method, fat using Babcock method, and lactose using Luff Schoorl. Antioxidant activity was measured following a method developed by Fadhlurrohman *et al*. (2023), samples were measured 1 g, then dissolved with 5 mL of methanol. The sample was centrifuged for 10 min at 4000 rpm. The supernatant produced was pipetted 1 mL and 1 mL of 0.20 M DPPH was added. Then, the sample was incubated in a dark room for 30 min. Finally, the absorbance of the sample was tested on a spectrophotometer with a 517 nm wavelength. For a blank, 1 mL of methanol was added to 1 mL of DPPH.

Determination of chemical composition. The chemical characteristics of cheese measured were TA, MC, and FFA, according to Sudarmadji *et al*. (2007). The TA test

was carried out by weighing a 10 g sample and grinding it, adding 2 drops of 1% phenolphthalein, and then titrating it using 0.1 N NaOH until the color was pink. Determination for MC was done by the thermogravimetric method by weighing a 1-2 g sample and then heating at a temperature of 105°C for 18 h until constant weight. FFA was carried out by weighing 10 g samples and grinding them. The sample was then added with 50 mL of heated 96% ethanol absolute. The mixture was then added with 2 mL of 1% phenolphthalein and titrated until pink.

Determination of color and texture characteristics. A colorimeter (Konica Minolta CR-10) was used to measure the color characteristics (L*, a*, and b*) following the International Commission on Illumination (CIE) color scale (Setyawardani *et al*., 2021). L* meant light to dark, a* was green to red b was yellow to bule. Then, texture (hardness and stickiness) was analyzed using a texture profile analyzer (Stable Micro System, United Kingdom). A cubical sample (1x1x1) was tested by compression mod with load cell 10 kg.

Results and Discussion

Composition of raw materials

The cheese in the study was made from cow's milk, cinnamon extract, and Lactobacillus plantarum Kita 3 starter. The composition of the raw materials is as follows: protein 2.80%, fat 3.62%, lactose 4.21%, cinnamon extract's antioxidant activity (DPPH test) 65.82%, and Lactobacillus plantarum Kita-3 total 8.34 log CFU/g.

The composition of raw materials greatly influenced the characteristics of the cheese produced. The composition of milk was based on the Indonesian National Standard of raw milk, has a minimum protein content of 2.80%; 3% fat, and dry matter without fat 7.8% (BSN, 2011). The milk used in making cheese has protein and fat levels that meet SNI, while the dry ingredients without fat were slightly below standard. Juniawati et al. (2017) confirmed that milk protein and fat levels affect cheese yield.

Effect of cinnamon extract on chemical composition of cheese

The chemical composition of cheese with the addition of cinnamon extract and *Lactobacillus plantarum* Kita-3 are presented in Table 1. The addition of cinnamon extract have no significant effect ($p>0.05$) on TA and FFA of cheese at 0 d of observation and MC at 0 and 20 d, however, it has significant effect ($p<0.01$) on TA at 10 and 20 d observation, pH on all d of observation, MC on 10 d, and FFA on 0 and 20 d. The addition of cinnamon extract also has significant effect ($p<0.01$) on antioxidant activity on 0 d of product. The average of antioxidant activity of each treatments were 6.12±0.08; 12.51±1.66; 14.37±0.86; 14.09±1.09%, respectively.

Table 1. Chemical composition of cheese with cinnamon extract

Variable	Treatment	Storage (d)		
		0	10	20
Titratable Acidity	0%	0,68±0,00	0,76±0,00^{c}	0,97±0,00^{b}
	3%	0,60±0,00	0,87±0,00^{c}	1,00±0,00^{b}
	6%	0,66±0,00	1,01±0,00^{b}	1,12±0,04^{a,b}
	9%	0,68±0,01	1,19±2,25^{a}	1,23±0,04^{a}
	Average	0,65±0,00	0,96±0,00	1,08±0,02
Moisture Content	0%	55,18±1,78	53,56±1,47^{b}	52,74±6,23
	3%	55,32±0,40	53,73±0,14^{d}	55,19±7,11
	6%	56,47±1,86	55,71±0,41^{c}	54,59±2,48
	9%	55,52±5,05	52,91±2,45^{a}	55,78±0,21
	Average	55,62±2,27	53,98±1,12	54,57±4,91
Free Fatty Acid	0%	3,54±0,06^{c}	1,27±0,89	2,82±0,00^{c}
	3%	4,34±0,25^{b}	1,52±1,75	3,20±0,02^{b}
	6%	4,36±0,14^{b}	1,90±3,60	3,65±0,02^{a}
	9%	4,75±0,33^{a}	2,14±5,18	3,21±0,01^{b}
	Average	4,25±0,20	1,70±2,85	3,22±0,02

Notes: ^{a,b,c} different subscripts on the same row mean significantly different ($p<0.01$)

Fortification of dairy products, one of which was cheese with herbal plants, was developed widely because of its fairly wide market segment and was in demand by society. The addition of herbs to cheese had an important role, such as improving product quality with the presence of bioactive components to prevent several diseases (Al-Hamdani *et al*., 2021). TA values of cheese could interpret a trend in probiotic growth in cheese (Ismiarti, 2023). During the fermentation process, probiotics are used as a starter, doing metabolism processes that produce lactic acid, and then pH decreases. In line with a study conducted by Setyawardani *et al*. (2017), during the fermentation process, the pH of kefir decreased due to lactic acid bacteria (LAB) changing lactose into lactic acid. The addition of cinnamon extract did not change the acidity of the cheese on 0 d observation. It indicated that there was no metabolic activity from the added starter, so it did not produce metabolites that affected the acidity level. The higher level of cinnamon extract added could produce a more acidic cheese. One of the factors causing it was the pH of the extract, which was lower than milk; it was 5.7. Another factor explained by Rashidinejad *et al*. (2016) was due to lactose metabolism which produced lactic acid. In addition, herbal extracts contained phenol components which allowed them to degrade into phenolic acids with different acid strengths due to oxidative and hydrolysis

processes. Cinnamon is a herb that has antimicrobial properties (Ramayana *et al*., 2018); however, at certain levels, it did not affect it (Rashidinejad *et al*., 2016a). Therefore, the use of appropriate levels needed to be considered especially in cheeses acidified by lactic acid bacteria.

The addition of cinnamon extract has no effect ($p>0.05$) on MC of the cheese. This was in accordance with research by Wasliyah *et al*. (2022) that cheese with the addition of bay leaf powder has the same MC. During observation, the MC of the cheese had a tendency to decrease. The addition of 9% cinnamon extract produced cheese with the lowest MC on the 10 d of observation, followed by the addition of 0%, 6%, and the highest 3% on the same day. Wasliyah *et al*. (2022) stated that the MC easily decreased in cheese, whereas during the draining process, the curd and whey were easily separated. Research by Setyawardani *et al*. (2021) showed that the use of herbs caused cheese solids to decrease, meaning the MC of the cheese is higher than the control. The MC of cheese which tends to decrease during storage was not in line with research Al-Hamdani *et al*. (2021) which stated that during storage for up to 21 d there was a decrease in total solids, so the MC increases. The decrease in MC during the observation was due to the reduction in whey during storage, which dripped through the surface of the cheese so the MC decreased. The higher level of extract increased the water of the milk before curd was formed; this caused cheese with the addition of cinnamon extract to have higher MC compared to the control. In addition, cinnamon, with its bioactive component and lower pH than milk, caused instability of milk casein before acidification. Setyawardani *et al*. (2021) emphasized that the use of herbs would initiate the acidification of milk in the presence of lactic acid bacteria, thus affecting the aggregation of cheese casein.

Free fatty acids play an important role in cheese flavor because mono and diglycerides could be hydrolyzed by carboxy-esterase or mono and diglyceride lipase (Amar *et al*., 2017). The higher level of cinnamon extract produced cheese with a higher FFA. This indicated that cinnamon extract caused lipolysis in cheese. During the 10 d of observation, FFA tended to decrease and increased again on 20 d of observation. Dopieralska *et al*. (2020) stated that during storage, the FFA content changed because fat, especially unsaturated fat, was oxidized. Setyawardani et al. (2019) emphasized that LAB culture added to cheese would develop in the presence of the substrate contained in the cheese. This produced metabolites in the form of lactic acid and acid numbers which were closely related to FFA.

Based on the result, the addition of cinnamon could increase the antioxidant activity of cheese on 0 d. As a statement by *Rashidinejad et al*. (2016) that cheese was an excellent carrier for antioxidants because it has a long shelf-life compared to other dairy products and is also nutritionally complete. The addition of 6% cinnamon produced the highest antioxidant content of cheese (14.37%). Handito *et al*. (2022) stated that herbs could be used as a coloring agent. Besides, it has side functions, such as antioxidant, anticancer, or anti inflammation. Antioxidant properties were trusted to delay or prevent damage caused by oxidation on molecule targets. Fadhlurrohman *et al*. (2023) confirmed that the use of herbs could improve the antioxidant activity of cheese, the addition of 2% orthodox black tea produced 33.49% antioxidant activity in the final product. It could was potential to produce functional food with highly antioxidant content.

Effect of cinnamon extract on LAB viability of cheese

The requirements for probiotic cheese must contain LAB 5.00-7.00 log CFU/g (FAO & WHO, 2002). Cheese with the addition of cinnamon extract and the acidifier *Lactobacillus rhamnosus* Kita-3 has LAB viability ranging from 7.68-8.67 log CFU/g, so it could be categorized as a probiotic cheese. The viability of cheese LAB during observation is presented in Figure 1. The addition of cinnamon extract affects ($p<0.01$) LAB viability on cheese at each observation. The addition of 9% cinnamon extract produced cheese with the lowest LAB viability on observation day 0. However, during the observation, there was increasing viability; the addition of 9% extract produced the highest viability in observation 20 d. The improvement of LAB viability during observation indicated metabolic activity, resulting in the breakdown of lactose into lactic acid. Setyawardani *et al*. (2019) emphasized that during ripening, LAB still undergoes metabolism but is hampered by low temperatures. This process still produced cheese substrate and lactic acid which caused a decrease in pH.

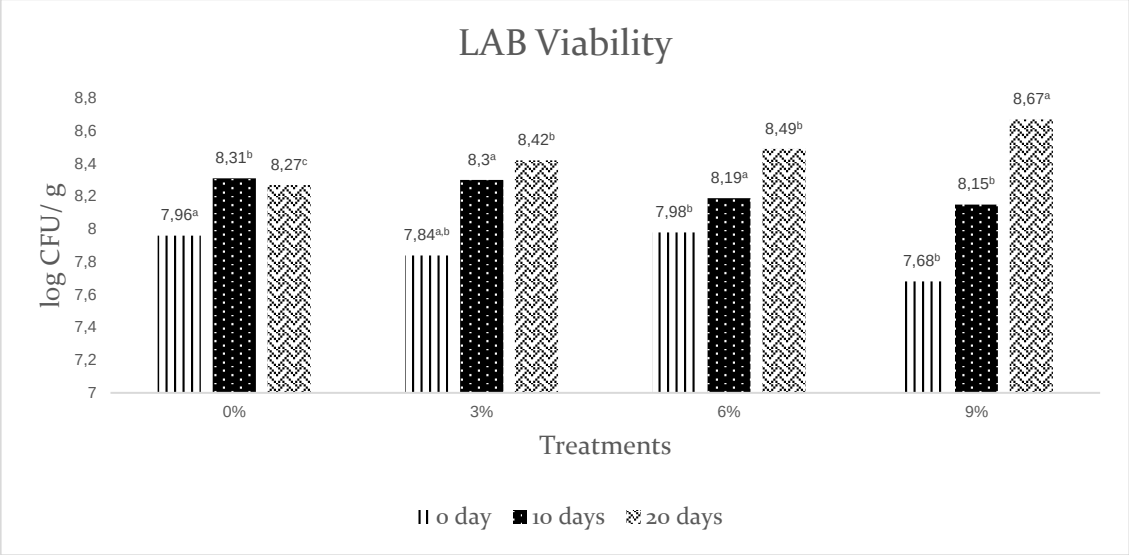

Figure 1. LAB viability of cheese with cinnamon extract during storage
[a,b,c]different subscripts at diagram mean significantly different (p<0.01)

Effect of cinnamon extract on physical properties of cheese

The color of cheese with cinnamon extract during storage was shown in Table 2, while TPA was in Figure 2. The addition of cinnamon significantly affected (p<0.01) lightness (L*) and a*. Color was an essential parameter of cheese according to consumer preferences. Pinho et al. (2004) described L*, a*, and b* were parameters to describe color characteristic. L* meant light/dark (0% meant dark, 100% light), a* was green/red (value from -60% meant greenish, then 60% reddish). Then, b* was yellow/blue (a value from -60% meant bluish, and 60% was yellowish). Texture was used to assess cheese's texture that simulates the mouth feel or compression of teeth when the chewing process (Ozcan *et al*., 2017)The highest L* was cheese with no cinnamon addition, while the highest level of cinnamon was the lowest L*. This means the increased level of cinnamon produced cheese with a darker color. During cold storage, the color tends to change. Setyawardani *et al*. (2018) stated that the lightness of the cheese was not influenced by the temperature of storage; it was affected by the duration of storage. Contrary to a*, the addition of cinnamon increased a*, which meant the higher level of cinnamon caused the cheese to be reddish. It was because the color of the cinnamon extract was reddish brown, which affected the cheese color. The addition of cinnamon has no effect on b* of cheese at 0 and 20 d storage but is significantly affected (p<0.01) at 15 d storage. The color of the cheese with cinnamon was more yellow compared with the control (higher value of b*). From Table 2, it could be interpreted that during storage, the cheese tended to be more yellow. The factor affected was the decreasing MC during cold storage. Study conducted by Setyawardani et al. (2018) showed that during storage, the total solid and fat ratio of cheese increased, and it was affected by the yellow color of cheese at the end of storage. At 15 d of storage, the level of cinnamon caused the cheese to yellower because of the cinnamon color that combined into the cheese. In line with Aydın & Tarakçı (2021)The use of herbs on cheese could increase the yellow color because the herbs' pigments are dehydrated, and water is reduced during the drying process.

Table 2. Color characteristic of cheese with cinnamon during storage

Variable	Treatments	Storage (d)		
		0	10	20
L*	0%	46.11±1,96[a]	46.94±0.07[a]	44.52±0.73[a]
	3%	44.10±0.89[b]	45.14±0.41[b]	43.82±0.78[b]
	6%	43.98±1.45[b,c]	44.14±0.37[c]	42.80±0.43[c]
	9%	42.75±2.34[c]	43.91±0.29[d]	42.34±0.10[c]
	Average	44.24±1.66	45.01±2.28	43.37±0.51
a*	0%	-1.14±130[d]	0.27±0.04[d]	0.63±0.11[d]
	3%	2.41±0.27[c]	2.78±0.29[c]	2.64±0.47[c]
	6%	3.19±0.40[b]	3.60±0.27[b]	4.61±0.18[b]
	9%	4.07±0.91[a]	4.19±0.06[a]	4.86±0.37[a]
	Average	2.13±0.72	2.71±0.17	3.18±0.28
b*	0%	18.97±1.45	19.64±0.07[c]	21.53±0.72
	3%	19.39±0.68	20.20±0.29[b]	21.99±0.52
	6%	19.51±0.33	20.42±0.04[a,b]	22.43±0.09
	9%	20.29±0.50	20.38±0.04[a]	21.94±0.48
	Average	19.54±0.74	20.16±0.11	21.97±0.45

Notes: [a,b,c] different subscripts on the same row mean significantly different (p<0.01)

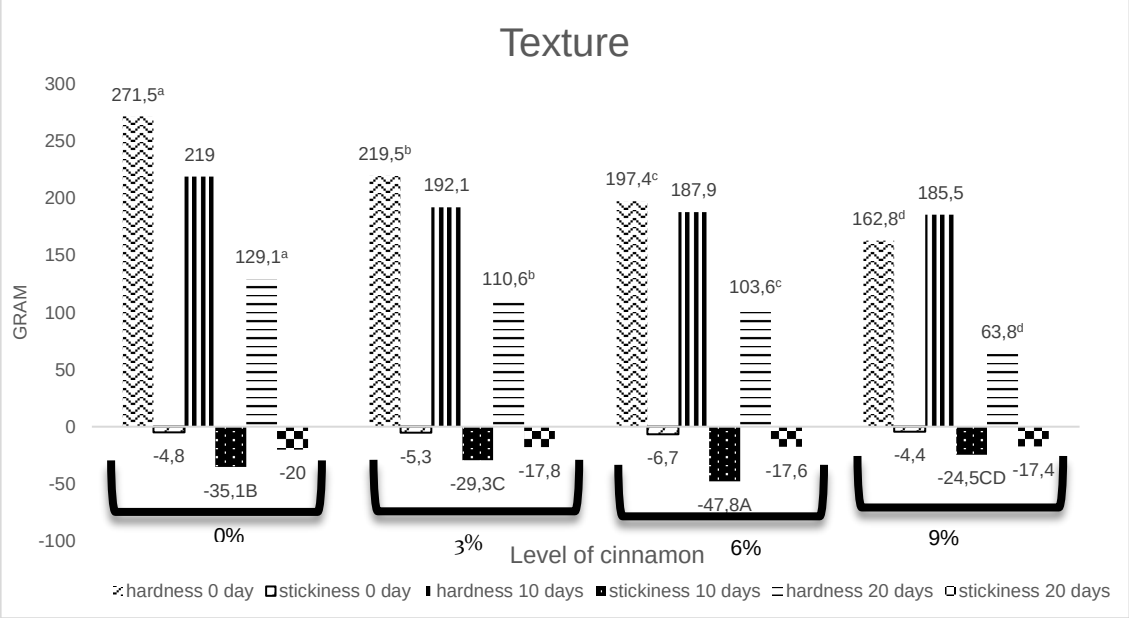

Figure 2. hardness and stickiness of cheese with cinnamon extract during storage
[a,b,c,d] different subscripts at diagram mean significantly different (p<0.01)

The hardness was lower on cheese with cinnamon, while increasing level of cinnamon made the lowest hardness (p<0.01). The hardness of cheese tends to be lower when cool and stored until 20 d, but at 10 d storage, all treatments have the same hardness (p>0.05). It was not in line with El-Batawy & Soliman (2017) that hardness of cheese would increase during storage, indicated that during storage cheese could maintain acceptability of texture properties. The addition of cinnamon has not affected (p>0.05) cheese at 0 and 20 d storage but significantly affected it at 10 d storage. Agree with Fadhlurrohman *et al*. (2023), adding orthodox black tea could decrease cheese hardness but not affect stickiness. Factors affecting cheese texture were defined by (Ozcan *et al*., 2017) included interaction between casein and water, MC, pH, calcium, temperature, sodium chloride, and proteolysis process.

Conclusion

The addition of cinnamon extract to soft cheese could maintain chemical composition during storage until 20 d. It also increased the antioxidant activity of cheese on 0 d, with the highest being 14.37%. LAB viability improved during storage until 20 d in the range of 7.68-8.67 log CFU/g; then it met the requirement of probiotics cheese. While cinnamon extract affected to color characteristic of cheese and decreased hardness and stickiness during storage. Cinnamon extract affected to cheese color to be darker and reddish.

Conflict of interest

Authors declared that there is no potential conflict of interest regarding this manuscript.

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Author's contribution

This study was carried out by five authors, with each their own contribution. Ismiarti was the research leader, designing methods and writing the manuscript. Nadlirotun Luthfi as the data analyst, Beta Novia Putri, Septi Setyas Tuty, and Sava Talia Rahmah (students) were doing laboratory analysis and collecting data.

Ethics approval

The conducted research was not related to either human or animal use.

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