



ORIGINAL RESEARCH PAPER

Effect of purified *Calotropis gigantea* latex protease on the cheese quality

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Abstract

Cheesemaking has been relying solely on chymosin for so long. But factors like increased cheese production, diminishing supply of chymosin as well as religious and dietary limitations have been hindering the demand-production balance of cheese in the world. Hence, the primary objective of this study was to utilize the three-phase partitioning (TPP) purified *Calotropis gigantea* latex protease in cheesemaking. The cheese prepared using purified protease was compared with chymosin cheese for physicochemical, sensorial, textural, and microbiological evaluation. The optimum conditions for purified protease from response surface methodology (RSM) analysis were 6.25 milk pH and 45°C milk temperature. The physicochemical parameters (moisture content, protein, ash, calcium, salt content, and pH) of cheeses prepared from latex protease and chymosin were significantly different ($p < 0.05$). The yield of latex protease cheese was significantly higher ($p < 0.05$) than chymosin cheese. In terms of texture and aftertaste, the cheese made with latex protease had significantly lower ($p < 0.05$) mean scores than chymosin cheese. Compared to chymosin cheese, the latex protease cheese had a generally inferior textural character, with significantly ($p < 0.05$) lower values for hardness, chewiness, gumminess, cohesiveness, and resilience. The total viable count and Lactobacilli count of the cheeses produced with chymosin and latex protease showed a significant ($p < 0.05$) difference. Hence, this study highlighted that the TPP purified *C. gigantea* latex protease could be used as a plant coagulant for cheesemaking.

Keywords:

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Introduction

Cheese, a dairy product made from milk, comes in a variety of flavors, textures, and forms. The quality of cheese depends on factors such as the type of milk used in the cheesemaking process and the specific strains of bacteria and mold incorporated during preparation (Fox & McSweeney, 2017). Cheese production has been steadily increasing in most countries, with an annual growth rate of over 3%. It accounts for over 40% of global milk

production, and the worldwide annual production is about 22.17 million MT (Shahbandeh, 2023).

Milk has historically been coagulated using calf rennet. Traditional coagulants include chymosin and pepsin, derived from animal stomachs. But due to a rennet shortage brought on by a combination of rising cheese demand and cow diseases as well as dietary and/or religious restrictions, other milk clotting enzymes derived from plants and microorganisms are being researched and produced (Su et al., 2009). As an alternative, plant protease have good catalytic capabilities and can coagulate milk at a variety of pH and temperature conditions (Mazorra-Manzano et al., 2018). There is a growing interest in plant-based coagulants due to their potential sustainability and accessibility (Nicosia et al., 2022). Some plants shown to have proteases that provide good milk coagulating activity are *Cynara cardunculus* (Gomes et al., 2019), *Zingiber officinale* (Maskey et al., 2023), *Moringa oleifera* (Tajalsir et al., 2014), *Helianthus annuus* (Nasr et al., 2016), *Ananas comosus* (Bhagavathy et al., 2019; Gul et al., 2022), *Carica papaya* (Maskey & Shrestha, 2020), *Actinidia deliciosa* (Maskey & Karki, 2022), *Ficus carica* (Hachana et al., 2021), etc. The extract from *Cynara cardunculus* dried flowers is primarily utilized as a coagulant in the production of PDO cheeses, contributing to the unique characteristics. These cheeses include *Serpa* and *Serra da Estrela* (from Portugal) as well as *La Serena*, *Torta del Casar* and *Los Pedroches* (from Spain) (Roseiro et al., 2003).

Calotropis gigantea, found across tropical and 60 subtropical regions, is an important latex producing plant belonging to the Asclepiadaceae family (Rajagopalan et al., 2014). A characteristic feature of the Euphorbiaceae, Asclepiadaceae,

Moraceae, and Apocynaceae plant families is the presence of latex. The abundance and variety of proteases in plant latex make it a promising source of plant coagulants. *C. procera* and *C. gigantea*, members of the Asclepiadaceae family, have traditionally been used to treat various health conditions (Singh et al., 2010). Efforts have been made to purify and characterize the cysteine proteases in *C. gigantea* latex, such as Calotropin FI, FII, and Calotropin DI, DII (Abraham & Joshi, 1979; Pal & Sinha, 1980). Crude protease from its latex has promising milk clotting activity as well as protease activity and has the potential to replace calf rennet as milk coagulant in cheesemaking process (Rajagopalan & Sukumaran, 2018). Hence, the main objective of this study was to utilize purified *C. gigantea* latex protease in cheesemaking and compare the quality of prepared cheese with chymosin cheese.

Materials and Methods

Materials

The latex of *C. gigantea* plant was collected in a sterilized tube by breaking upper tender stems from areas around Dharan (26.8406°N, 87.2914°E). Fresh cow milk was purchased from the local market of Dharan. The freeze-dried DVS culture (TCC-20) (Chr. Hansen, Denmark) was procured from Trishul Trade Links, Kathmandu, Nepal.

Ammonium sulfate, skimmed milk powder (SMP), dialysis membrane, L-cysteine were procured from the HiMedia Laboratories Pvt. Ltd., India while tert-butanol was purchased from Thermo Fisher Scientific Pvt. Ltd., India. All of the chemicals used were of the analytical grade.

Isolation of purified latex protease

Extraction of crude protease from latex of *C. gigantea* plant was performed according to Mazorra-Manzano et al. (2013) with minor modifications. The latex and sodium phosphate buffer (50mM, pH 7) were blended in the ratio of 1:1 (w/v). The slurry was stirred for 45 min at 4°C and filtered through cheesecloth. The filtrate was centrifuged at 5000 rpm (for 10 min at 4°C) to separate contaminants. The supernatant was precipitated with ammonium sulfate up to 80% saturation, followed by centrifugation at 15000 rpm (for 5 min at 4°C). The precipitated protein fraction was dissolved in phosphate buffer (50 mM, pH 7) and dialyzed overnight with three changes of pH 7 buffer (4°C) using a dialysis membrane with a molecular weight cut-off (MWCO) of 12 kDa.

The purification of crude dialyzed latex extract was carried out by TPP as described by Maskey et al. (2024). The crude dialyzed extract was saturated with 52% ammonium sulfate at 25°C and the pH was maintained at 6.0. Then tert-butanol was added 1.5 times to the volume of crude extract. The mixture was vortexed and allowed to stand for 30 min at room temperature (25°C), then centrifuged at 5000 rpm for 10 min at 4°C to separate the upper butanol phase, middle interfacial phase (IP), and lower aqueous phase (AP). The upper butanol phase and AP were discarded, while the IP was dissolved in the phosphate buffer (pH 7.0)

containing 10 mM L-cysteine and 2.5 mM EDTA. The dissolved IP was dialyzed overnight (12 h) and stored at -20°C for further analysis.

Milk clotting activity determination

Milk clotting activity (MCA) of purified protease was assessed through the procedure derived by the IDF (2007). Reconstituted milk was prepared by combining 5.5 g SMP with 50 ml CaCl₂ solution (0.5%, w/v), maintaining pH at 6.5, and incubated at 37°C for 5 min. A vial containing 2 ml of milk and 200 µl of enzyme was incubated, with the vial regularly checked for any signs of clot at intervals of 10 s. One unit of MCA was defined as the amount of enzyme required to coagulate 10 ml of reconstituted skim milk at 37°C in 40 min (Berridge, 1952). MCA was determined by using equation 1.

$$MCA \left(\frac{U}{ml} \right) = \frac{2400 \times V_S}{T \times V_E} \quad (1)$$

where T = time required for clotting (s); V_S = milk volume (ml); V_E = enzyme volume (ml).

RSM optimization

The impact of milk pH and temperature on cheesemaking process was analyzed by using response surface methodology (RSM). The two-factor central composite rotatable design (CCRD) comprising 13 experimental runs was employed through Design Expert software (Version 13.0.5, Stat-Ease Inc., Minneapolis). The three coded levels utilized in the optimization study were -1, 0 and +1 as presented in Table 1. The fixed factor was the concentration of latex protease i.e., 0.25%. It was selected on the basis of coagulation time and curd firmness after clotting. Also, the use of low amount of plant coagulants may avoid the negative impact of excessive proteolysis of caseins (Esteves et al., 2003).

Table 1

Experimental factors and coded levels in RSM design for purified latex protease

Factors	Symbol	Coded units		
		-1	0	+1
Milk pH	A	6.00	6.25	6.50
Milk temperature (°C)	B	40	45	50

The response MCA) for different experimental combinations were related to the coded variables (X_i, i=1 and 2) by a second-degree polynomial equation 2.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (2)$$

The coefficients of the polynomial were represented by β₀ (constant), β₁, β₂, (linear effects); β₁₂ (interaction effects); β₁₁, β₂₂ (quadratic effects); and ε (random error). Data were modelled by multiple regression analysis and the statistical significance of the terms was examined by analysis of variance (ANOVA).

Production of cheese

The cheeses were produced following the procedure given by Walstra et al. (2006) with slight modifications. The cheese prepared using latex protease was marked as cheese A, while cheese prepared using chymosin was marked as cheese B. Fresh cow milk was heated to 80°C and then cooled to 45°C. Then, freeze dried thermophilic culture (50U) was added and allowed to incubate at 42°C. This was followed by the addition of CaCl₂ (0.02%). For cheese A, purified protease was added at the rate of 0.25% of the milk (v/v) while for cheese B, chymosin was added at the rate of 2.5 g/100 L milk. The incubation temperatures for cheeses A and B were 45°C and 40°C, respectively. The time of incubation was 40 min. The curd was cut vertically and horizontally into 1 cm³ and then cooked up to 50°C until the curd was firm. After drainage of whey, the curd grains were molded and pressed using 10 kg weight for 12 h. The pressed curds were then immersed into 15% brine solution for 6 h. After brining, the cheese blocks were vacuum packaged in LDPE (low density polyethylene) and stored at refrigerated condition (4°C).

Physicochemical analysis

Physicochemical analysis including pH, acidity, fat content, protein content, ash content, moisture, and salt content of cheese was determined following the procedures given by AOAC (2005). The yield of cheese was determined by using equation 3 as per Nasr et al. (2016).

$$\text{Yield (\%)} = \frac{\text{Weight of cheese (kg)}}{\text{Weight of milk (kg)}} \times 100 \quad (3)$$

Sensory evaluation

Sensory analysis was conducted using hedonic tests according to Hosiana et al. (2020) and descriptive tests according to Rajagopalan & Sukumaran (2020), to determine the cheese flavor, appearance, texture and taste quality response. Cheeses were analyzed organoleptically by a panel of 10 semi-trained panelists (25-45 years old; seven males and three females) from Central Department of Food Technology, Dharan. Refrigerated cheese samples were thawed to room temperature before serving and 5g of sample portion was given to each panelist. The panelists evaluated the cheese samples for sensory attributes (texture, appearance, flavor, odor, aftertaste, and overall acceptance) by using a five-point hedonic scale, with 1 being poor and 5 being excellent quality.

Texture profile analysis

The texture profile analysis (TPA) of the cheese samples were carried out using TA.XT plus texture analyzer (Stable Micro Systems Ltd., England) according to Sharma & Vaidya (2018). The double compression test of cheese sample was performed by using a probe with a diameter of 75 mm (P/75). The cheese sample having a temperature of 20°C was cut into a rectangular shape with the dimensions of 14 mm in width, 22 mm in length, and 14 mm in height. After adjusting the distance of 5 mm, the cheese sample was compressed by a strain rate of 70%, with two

sequential cycles at a test speed of 2 mm/s for 5 s. The texture parameters of cheese (hardness, adhesiveness, springiness, cohesiveness, chewiness, gumminess, and resilience) were computed as per Gunasekaran & Ak (2002).

Microbiological analysis

The microbiological analysis of cheese samples was performed by following the methods described by Freitas et al. (1996). Total viable counts were determined using plate count agar (PCA), coliform bacteria using violet red bile agar (VRBA), yeast and molds using potato dextrose agar (PDA), and Lactobacilli count using de Man-Rogosa-Sharpe (MRS) agar. All the microbiological tests were carried out in triplicate, and the results were expressed as log CFU/g of cheese.

Statistical analysis

The data were analyzed using IBM SPSS Statistics (Version 27) using independent samples t-test at 5% significance level. All of the graphs were created with Microsoft Excel (2019).

Results and Discussion

Optimization of milk pH and temperature for cheesemaking

The experimental results were fitted with the quadratic second order polynomial equation. The equation of MCA (in terms of coded values) is presented in equation 4. The results of the experiment are shown in Table 2. The statistical significance of the equation was analyzed by ANOVA for the quadratic model of responses (Table 3).

$$\text{MCA (U/ml)} = 1254.59 - 639.03A + 662.41B - 143.36AB + 262.52A^2 + 23.38B^2 \quad (4)$$

Where A and B are coded values of pH of milk and temperature of milk respectively. A², B² and AB are model terms.

From analysis of MCA (Table 3), A and B had significant (p<0.05) negative and positive effect on clotting power at 95% confidence level. Similarly, AB had non-significant (p>0.05) negative effect. But A² and B² had non-significant (p>0.05) positive effect on MCA. The model p-value less than 0.0001 indicated significant regression model, while the insignificant (p>0.05) lack of fit revealed well fitted model. The value of predicted R² was 0.9366, which is in reasonable agreement with adjusted R² 0.9763. Therefore, the MCA could be analysed and predicted by using this regression model.

Figure 1 illustrates the interactive impact of each of the two factors employed to determine the optimal level for maximum MCA in the response surface plots. With decreasing milk pH and increasing milk temperature, the MCA of latex protease enhanced. Similar response was observed by Maskey & Shrestha (2020) for crude papaya latex protease and Maskey & Karki (2022) for partially purified kiwifruit protease.

Hence the optimum operating conditions for maximum MCA of purified *C. gigantea* latex protease were 6.25 milk pH and 45°C milk temperature. The response predicted for these optimum conditions was MCA of 1254.59 U/ml.

Table 2

Central composite design with experimental values for optimization

Run	A: pH	B: Temperature (°C)	MCA (U/ml)
1	6.25	55	1846.15
2	6.5	35	406.78
3	6.25	35	631.58
4	6.25	45	1333.33
5	6.0	55	3000
6	6.25	45	1200
7	6.5	55	1500
8	6.5	45	774.19
9	6.0	45	2181.82
10	6.25	45	1411.77
11	6.25	45	1142.86
12	6.0	35	1333.33
13	6.25	45	1263.16

Table 3

ANOVA for quadratic model of MCA

Source	Sum of Squares	df	Mean Square	F-value	P-value
Model	5405000	5	1081000	99.83	<0.0001
A	2450000	1	2450000	226.29	<0.0001
B	2633000	1	2633000	243.15	<0.0001
AB	82211.23	1	82211.23	7.59	0.0283
A²	190300	1	190300	17.58	0.0041
B²	1509.32	1	1509.32	0.1394	0.7199
Residual	75793.89	7	10827.70		
Lack of Fit	30573.35	3	10191.12	0.9015	0.5145
Pure Error	45220.54	4	11305.13		
Cor Total	5480000	12			

Verification of model

To validate the adequacy of regression model equations, 3 experimental runs were performed. Table 4 summarizes the outcomes of the confirmatory tests.

The experimental value was near to the predicted value, indicating that this model was accurate enough to predict the optimal value. Hence, milk pH 6.25 and milk temperature 45°C were selected cheesemaking.

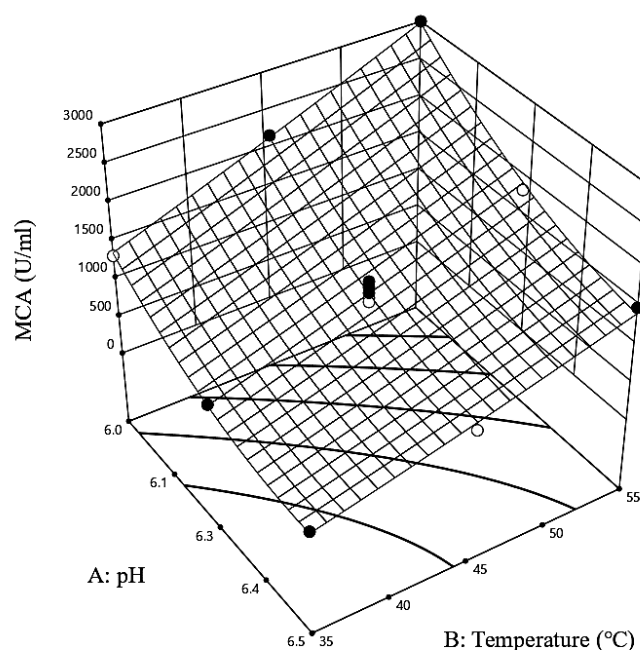


Figure 1

Response surface plot for MCA of latex protease

Table 4

Response predicted and experimental values

Treatments	Optimum conditions		Predicted mean value	Experimental mean value	Deviation (%)
	pH	Temp. (°C)			
MCA (U/ml)	6.25	45	1254.59	1242.11	1.01

Chemical composition of cheese

The chemical composition of the cheeses made from purified latex protease (A) and chymosin (B) has been shown in Table 5.

According to the study by Fox & McSweeney (2017), the moisture content of fresh cheese should be around 40-50%. The moisture percentage is in line with the findings of Mahajan & Chaudhari (2014) but lower than the findings of Tossou et al. (2018) and higher than that of Rana et al. (2017). The moisture content of cheese A was significantly ($p < 0.05$) greater than that of cheese B. Sanjuán et al. (2002) also observed more moisture content in vegetable rennet cheese compared to animal rennet cheese.

Table 5
Chemical composition of cheeses

Parameters	Cheese A	Cheese B
Moisture (%)	49.59 ^b ±0.31	48.61 ^a ±0.19
Protein % (wb)	22.23 ^b ±0.27	21.41 ^a ±0.86
Fat % (wb)	22.50 ^a ±0.57	23.00 ^a ±0.56
Ash % (wb)	3.82 ^b ±0.14	3.62 ^a ±0.14
Salt content % (wb)	2.65 ^b ±0.06	2.45 ^a ±0.11
Calcium (mg/100 g)	549.89 ^b ±4.06	419.54 ^a ±2.04
pH	5.85 ^b ±0.06	5.65 ^a ±0.06

Note. Values are the means ± standard deviations (SD) of three determinations. Values in the row bearing similar superscript are not significantly different at 5% level of significance.

Table 6
Yield of cheeses prepared by plant coagulants

Coagulant	Yield (%)	Reference
<i>Wrightia tinctoria</i> stem	13.44	Rajagopalan & Sukumaran (2020)
<i>Ginger (Zingiber officinale)</i>	15.4	Mazorra-Manzano et al. (2013)
<i>Melon (Cucumis melo)</i>	15.1	Mazorra-Manzano et al. (2013)
<i>Kiwifruit (Actinidia deliciosa</i> cv. Hayward)	20.27	Nicosia et al. (2022)
<i>Sunflower (Helianthus annuus)</i> seeds	20.78	Nasr et al. (2016)
<i>Cardoon (Cynara cardunculus</i> L.) flowers	22.8	Gomes et al. (2019)
<i>S29534-Enzeco® (Commercial plant rennet)</i>	11.43	Rajagopalan & Sukumaran (2020)
<i>Calotropis procera</i>	14.47	Aworh & Muller (1987)

The protein content in cheese A was significantly ($p < 0.05$) higher than that in cheese B. Omueti & Jaiyeola (2006) reported that retention of whey in final cheese might increase the protein content as well. This correlates with the higher moisture content in cheese A. The lower value of fat content recorded in cheese A might be due to processing variables. Due to strong proteolytic activity of plant protease, the casein network breakdown in cheese results in higher fat losses in whey (Nuñez et al., 1991). In contrast to the results of this study, lower fat contents were observed in cheeses prepared by using *C. procera* leaves (Aworh

& Muller, 1987), ash gourd proteinase (Gupta & Eskin, 1977), papaya latex (Maskey & Shrestha, 2020), and kiwifruit protease (Maskey & Karki, 2022). Aworh & Muller (1987) even found more moisture and protein in those cheeses produced with vegetable rennet.

The ash content of the cheese depends on minerals present in cheese, and amount of salt used in brining of cheese (Maskey & Karki, 2022). The ash content as well as calcium content in cheese A were significantly more ($p < 0.05$) than cheese B. Similar results were found by Sanjuán et al. (2002), Khan & Masud (2013), Maskey & Shrestha (2020) as well as Maskey & Karki (2022). The overall salt content of the cheeses was found to be lower than the findings of Sanjuán et al. (2002), and Sousa and Malcata (1997). The cheese A had more ($p < 0.05$) salt content and pH than cheese B. The variation in pH values may be attributed to the difference in initial pH of coagulants used, and their protease activity. Similar results were observed by Sousa & Malcata (1997), Khan & Masud (2013), Maskey & Shrestha (2020), and Maskey & Karki (2022).

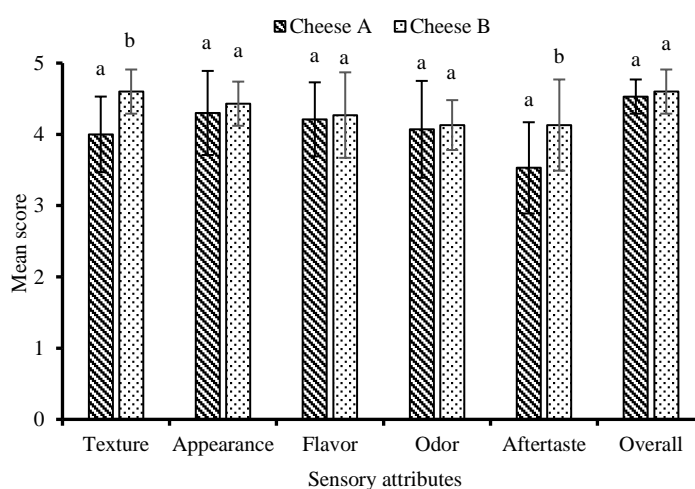


Figure 2
Graphical representation of mean sensory scores of cheeses

Note. Values on top bars bearing similar superscript are not significantly different at 5% level of significance.

Yield

Cheese yield is one of the critical things to look at in cheese making because this affects the profit of the cheesemaker (Mohsin et al., 2024). The yield of cheese A was observed to be 13.68% which was significantly more ($p < 0.05$) than cheese B (13.04%). The yield obtained falls in the range of the yields shown in other studies conducted for various plant coagulants as shown in Table 6. The cheeses made by *Wrightia tinctoria* (Rajagopalan & Sukumaran, 2020) and *C. procera* (Aworh & Muller, 1987) had similar yields to this study. These observations revealed that the type of coagulant used can be a significant contributor to the cheese yield (Rajagopalan & Sukumaran, 2020). The moisture content may also have attributed to the higher ($p < 0.05$) yield of cheese A. Factors such as milk quality, milk type, pretreatments, gel firmness, curd particle size, and cooking rate influence milk gel composition,

moisture expulsion, and fat loss in cheesemaking (Fox & McSweeney, 2017).

Sensory evaluation

Graphical representation of sensory scores of the cheese samples is given in Figure 2. The representation of organoleptic analysis for texture, color, flavor, and aroma are shown in Figure 3(a-d).

The average texture scores were found significantly ($p < 0.05$) higher for cheese B than A. This may be due to the variation in moisture content (Karki & Ojha, 2018). The organoleptic analysis of both cheeses revealed that 60% of the panelists found cheese B to have hard texture whereas only 53% of them found cheese A to be of hard texture. Cheese A was found chewier and softer as compared to cheese B. However, less than 10% found creaminess only in cheese A. Similar findings have been reported for ewe's milk cheeses made using vegetable coagulant from *Cynara cardunculus*, which were softer and creamier than those made using rennet. The intense level of proteolysis which occurred in cheeses made using vegetable protease hydrolyze the casein network, creating a more homogeneous structure, thus prompting greater creaminess and softening of the cheese (Tejada et al., 2007).

Among the panelists, more than 70% of the panelists found cheese A to be pale yellow in color while almost 50% of them found cheese B to be white. However, this difference in organoleptic viewpoint did not affect mean sensory scores for appearance of cheeses A and B as no significant difference ($p > 0.05$) was found. In terms of flavor, the cheese B had slightly ($p > 0.05$) higher score than cheese A. This was also evident in higher panelist acceptance % for both sour and salty flavor of cheese B (>45%) in comparison to cheese A (<35%). Flavor of cheese can be influenced by innate properties of the product along with flavor profile of the enzyme (Mijan et al., 2010). There was no significant difference ($p > 0.05$) found in the sensory scores for odor of both cheeses. About 45% of the panelists found acidic aroma in cheese B while 60% found milky aroma in cheese A. The cheese B had significantly ($p < 0.05$) higher score for aftertaste than cheese A. More than 35% of the panelists found bitter aftertaste in cheese A which might be the reason for low aftertaste score. Similar bitter aftertaste was seen in the soft cheeses made with *C. gigantea* by Noviyanty et al. (2020) and Sulmiyati & Malelak (2023). Most plant-derived milk clotting enzymes are suspected to have high proteolytic activity, resulting in the formation of bitter peptides (Liu et al., 2021).

Finally, the overall acceptance of the cheese B was slightly ($p > 0.05$) higher than that of the cheese A. Since the overall acceptance accounts for all sensory parameters of the cheeses and does not overlook other parameters for one, it can be said that cheese B was marginally better than cheese A.

Texture profile analysis

The results of texture profile analysis (TPA) of cheese samples are presented in Table 7.

The hardness of cheese A was significantly ($p < 0.05$) greater than cheese B. Cheese prepared from plant proteases is usually soft as α - and β - caseins are targeted by these proteases due to their high caseinolytic activity (Abd El-Salam et al., 2017). Significantly

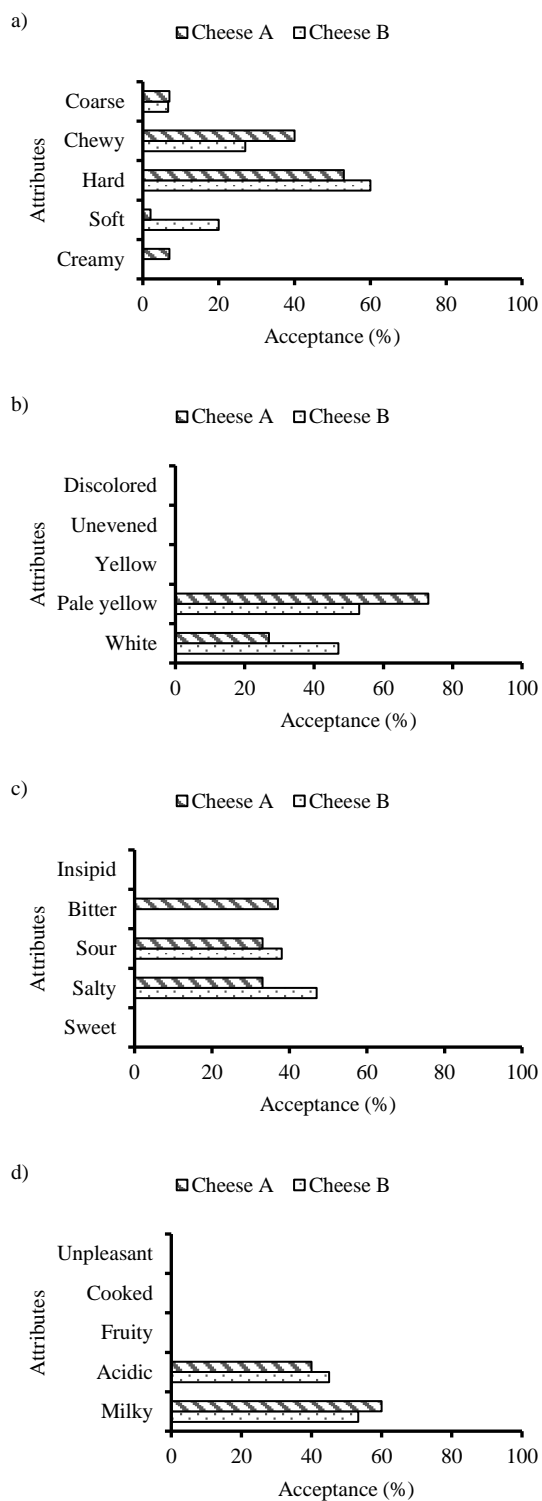


Figure 3 Organoleptic analysis of cheeses for a) texture, b) color, c) flavor and d) aroma

($p < 0.05$) lower gumminess and chewiness of cheese A in comparison to cheese B was observed. Decreased hardness is known to cause a reduction in gumminess and chewiness of the cheese prepared (Sant'Ana et al., 2013). Similar results for gumminess and chewiness were observed by Abd El-Salam et al. (2017), Sant'Ana et al. (2013), Mazorra-Manzano et al. (2013), and Rajagopalan & Sukumaran (2020). The result for cohesiveness showed significantly lesser ($p < 0.05$) value for cheese A than cheese B. But the springiness of both cheeses was found to be similar ($p < 0.05$). Cheese made with kiwifruit, melon or chymosin protease had similar cohesiveness, but ginger cheese had lower springiness (Mazorra-Manzano et al., 2013). Significantly higher ($p < 0.05$) adhesiveness was obtained in cheese A compared to cheese B. The resilience was significantly higher ($p < 0.05$) in cheese B. Identical resilience values were reported for mozzarella cheese prepared using *Dregea sinensis* and rennin (Wang et al., 2017). The overall textural performance of cheese A in this study indicated that *C. gigantea* latex protease might be more suitable for the production of semi hard cheese varieties like blue cheese, brie, etc. (Fox & McSweeney, 2017).

Table 7

Texture profile parameters of cheeses

TPA parameters	Cheese A	Cheese B
Hardness (g)	7163.475 ^a ± 11.564	7922.867 ^b ± 10.302
Gumminess	5454.886 ^a ± 5.444	7050.209 ^b ± 1.552
Chewiness	5458.46 ^a ± 8.402	7050.135 ^b ± 8.398
Cohesiveness (%)	76.202 ^a ± 0.045	89.193 ^b ± 0.238
Springiness (%)	99.952 ^a ± 0.053	99.994 ^a ± 0.005
Adhesiveness (g.s)	0.852 ^b ± 0.002	0.729 ^a ± 0.008
Resilience (%)	35.329 ^a ± 0.384	49.331 ^b ± 0.219

Note. Values represent mean of the three determinations ± SD. Values in the same row with different superscripts differ significantly at 5% level of significance.

Microbiological analysis

The results of microbiological analysis of cheeses A and B are presented in Table 8.

Coliform was not detected in either of the cheese samples. It might be due to heat treatment and maintenance of hygiene during cheesemaking. Heat-treated milk significantly improves the microbiological safety of cheese, with pasteurization being the most common method to prevent pathogen contamination (Johnson & Lucey, 2006). Significant difference ($p < 0.05$) was found for both total viable count and Lactobacilli count of cheeses A and B. Similar results were obtained by Galán et al. (2012), and Sousa & Malcata (1997) in cheeses prepared with animal rennet and aqueous extracts of *C. cardunculus* flowers. The microbial count was found to be lesser in comparisons to the

findings of Nuñez et al. (1991), Vioque et al. (2000), and Galán et al. (2012). The yeasts and molds count of both cheeses were lesser than the findings of Vioque et al. (2000) and Mohsin et al. (2024). Freshly made cheese with low pH is very susceptible to the growth of yeast and mold, and these microorganisms can proliferate to around 6-7 log CFU/g during the early ripening stage (Hayaloglu, 2016).

Table 8

Microbiological analysis of cheeses (log CFU/g)

Parameters	Cheese A	Cheese B
Total viable count	6.149 ^a ± 2.760	6.322 ^b ± 2.858
Yeasts and molds	2.295 ^a ± 0.419	2.236 ^a ± 0.393
Lactobacilli	5.759 ^b ± 0.263	5.415 ^a ± 0.255

Note. Values are the means of three determinations ± SD.

Conclusions

On the basis of this study, purified *C. gigantea* latex protease can be used as an alternative to chymosin in cheesemaking. There were differences observed in the physicochemical characteristics, yield, and microbiological profile of cheeses made using latex protease and chymosin. The panelists' overall acceptance of the purified protease proved that it is suitable for use in cheesemaking, even though the latex protease cheese's overall textural performance and sensory scores were lower than those of chymosin cheese. However, extended studies on cheese ripening may be beneficial for further conformance on the potential use of the TPP purified *C. gigantea* latex protease as a milk clotting agent.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare no conflict of interest.

Ethical approval

This work did not involve any animal study.

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