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ORIGINAL ARTICLE

Fresh cheese production using freeze-dried papain as a vegetable coagulant

Produção de queijo fresco usando papaína liofilizada como coagulante vegetal

Grégor Villacréz Chavez[1], Segundo Grimaldo Chávez[1], Ralph Rivera Botonares[2], Nuri Carito Vilca Valqui[3], Marileydi Zuta Puscan[1], Manuel Oliva[1], Daniel Tineo[1]*

[1]Universidad Nacional Toribio Rodríguez de Mendoza, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Chachapoyas, Amazonas, Perú
[2]Universidad Nacional de Jaén, Jaén, Cajamarca, Perú 3
[3]Instituto Nacional de Innovación Agraria (INIA), Estación Experimental Agraria Amazonas, Chachapoyas, Amazonas, Perú

***Corresponding Author:** Daniel Tineo, Universidad Nacional Toribio Rodríguez de Mendoza, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Chachapoyas, Amazonas - Perú, e-mail: dtineo@indes-ces.edu.pe

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Abstract

The study examined the efficacy of freeze-dried papain enzyme obtained from three *Vasconcellea* species (*V. pubescens, V. chachapoyensis*, *V. heilbornii*) as a natural coagulant in cheese making. Notably, the enzyme *V. pubescens* demonstrated the most promising results when concentrations of 2 g/L, 4 g/L, and 6 g/L were used to produce fresh cheese, while other enzyme species exhibited lower efficacy. The optimal yield of fresh cheese with minimal residual enzyme was achieved when a 2 g/L dose of papain enzyme was employed at a coagulation temperature of 30 °C, resulting in physicochemical and organoleptic characteristics comparable to those produced with commercial Hansen's rennet. Nevertheless, an increase in the coagulation temperature (42 °C) and a higher dose of papain enzyme (4 g/L) resulted in a reduction in the yield of fresh cheese and; consequently, the residual enzyme increased. Further studies are required to determine the purity of freeze-dried papain and the most effective dosage to increase profitability for producers and consumers. Such findings could facilitate the ecological application of this alternative in producing of fresh cheese.

Keywords: Enzyme; Freeze-dried papain; Fresh cheese; Vegetable coagulant; Cheese production; Papain.

Resumo

O estudo examinou a eficácia da enzima papaína liofilizada obtida de três espécies de *Vasconcellea* (*V. pubescens, V. chachapoyensis, V. heilbornii*) como coagulante natural na fabricação de queijo. Em particular, a enzima *V. pubescens* demonstrou os resultados mais promissores quando concentrações de 2 g/L, 4 g/L e 6 g/L foram usadas na produção de queijo fresco, enquanto outras espécies de enzimas apresentaram menor eficácia. O rendimento ideal do queijo fresco com o mínimo de enzima residual foi alcançado quando uma dose de 2 g/L da enzima papaína foi empregada

a uma temperatura de coagulação de 30 °C, resultando em características físico-químicas e organolépticas comparáveis às produzidas com o coalho Hansen comercial. No entanto, um aumento na temperatura de coagulação (42 °C) e uma dose maior de enzima papaína (4 g/L) resultaram em uma redução no rendimento do queijo fresco e, consequentemente, a enzima residual aumentou. São necessários mais estudos para determinar a pureza da papaína liofilizada e a dosagem mais eficaz para aumentar a lucratividade para produtores e consumidores. Essas descobertas poderiam facilitar a aplicação ecológica dessa alternativa na produção de queijo fresco.

Palavras-chave: Enzima; Papaína liofilizada; Queijo fresco; Coalho vegetal; Queijo produção; Papaína.

Highlights

• Studies on native species of the *Vasconcellea* genus are essential for their potential use as a source of the papain enzyme and its various applications

• The papain enzyme derived from *V. pubescens* has high proteolytic activity, making it suitable for coagulating milk and producing fresh bovine cheese

• Livestock producers have high expectations for using the papain enzyme as an ingredient in the production of fresh bovine cheese

1 Introduction

Cheese is an essential food for millions of families around the world due to its calcium, vitamin A, and riboflavin content (Mohapatra et al., 2019). As a result, global cheese production is around 19 million tonnes per year and is produced through a process of enzymatic coagulation of milk (Fox et al., 2017). The international market is estimated to be worth around US$77.6 billion in 2021 and is expected to reach US$113 billion by 2027 (Shahbandeh, 2022). On the other hand, there are more than 2 million cow's milk producers in Peru, representing approximately 452,218 families, whose national production of fresh milk in 2022 was 1 million 891 thousand tonnes, of which 43% is destined for cheese production (Instituto Nacional de Estadística e Informática, 2022).

Due to the increasing demand for cheese, numerous studies have identified several factors that contribute to the production of high quality cheese, such as milk composition, textural properties, enzyme concentration and coagulation time (Li et al., 2022). However, rennet concentration has a direct effect on coagulation rate and cheese quality (Radovanovic et al., 2021), as it is the key point for milk coagulation in the cheese-making process, the results of which are reflected in the quality of the final.

Rennet can be obtained from a range of animal, plant, and microbial sources. Hansen's rennet, known for its popularity in Peru, is one of the most sought-after rennet's. Its prime constituent is chymosin (CHY-MAXTM M, boasting 600 International Milk Clotting Units (IMCU) per mL; Chr-Hansen A/S, Hoersholm, Denmark) and is extracted from the abomasum of ruminants (Alihanoğlu et al., 2018). Vegetable rennets can be extracted from various plant species, including moringa (*Moringa oleifera* Lam.), papaya (*Carica papaya* L.), and pineapple (*Ananas comosus* (L.) Merr.), among others (Asrafuzzaman et al., 2011; Derso & Dagnew, 2019). The utilization of vegetable proteases is significant in producing cheeses intended for specific or organic markets (Ben-Amira et al., 2017). This is because animal-origin rennet can only meet 20% to 30% of the demand in the cheese industry (Alihanoğlu et al., 2018) and the high cost restricts its applicability in meeting the total demand (Li et al., 2022). Thus, vegetable-derived coagulants are gaining popularity in the cheese manufacturing industry and are known to improve the cheese's performance, colour, aroma, taste, and texture, which in turn, has led to increased consumer preference (Short et al., 2021).

Papain enzyme, derived from papaya latex (*C. papaya*), is a well-established source of vegetable rennet due to its resistance to acidic conditions and high temperatures (Ovando-Martínez & González-Aguilar, 2020). Nevertheless, *C. papaya* is not the only species suitable for the production of this enzyme; other sister species,

including some high-altitude papayas from the *Vasconcellea* genus, then they have been identified as possible alternatives (Tineo et al., 2020). The proteolytic property of this papain enzyme remains relatively unknown and consequently, it has not received much quantitative attention in the production of fresh cheeses that are similar to those made with commercial rennet. Cheese is known to contain food additives and other ingredients that are functionally necessary for processing, as reported by Norma Técnica Peruana (Peru, 2017). Therefore, we can conclude that when utilizing coagulants from enzymatic extracts of fruits of *Vasconcellea* species during the production of fresh cheese, we are adhering to the reference standard for Peru. Two species of *Vasconcellea*, *V. pubescens* and *V. x heilbornii*, have been extensively researched, with the latter being commercially recognized. Nevertheless, there are other species, including *V. chachapoyensis*, recently described in the northeast of Peru (Tineo et al., 2020), on the other hand, the proteolytic properties of which species are not yet understood. The genus shows adaptation to cold climates in the subtropical Andean region. Their agronomic potential in Andean communities is largely due to their disease resistance, tolerance to cold, high enzymatic activity in latex, and elevated protein and vitamin content (Scheldeman et al., 2011).

In this study, it could be analyzed the impact of freeze-dried papain concentration and coagulation temperature on yield, pH, texture, whey turbidity, residual enzyme, coagulating enzymes, and sensory and physicochemical characteristics of fresh cheeses. Three different concentration doses were used (2 g/L, 4 g/L, and 6 g/L) of freeze-dried papain enzyme from *V. pubescens*, *V. chachapoyensis*, and *V. x heilbornii* as a substitute for commercial Hansen's rennet.

2 Materials and methods

2.1 Conduct of the experiment

The study implemented a completely randomized design (CRD) with a 3Ax3Bx3C factorial arrangement. Factor A employed three *Vasconcellea* species (*V. chachapoyensis*, *V. heilbornii* and *V. pubescens*). Factor B included three concentrations of lyophilized papain enzyme (2, 4 and 6 g/L of milk), and Factor C involved coagulation temperatures (30 °C, 35 °C and 42 °C). The evaluation of 27 treatments (Table S1 in Supplementary Material), each with three replicates, resulted in 81 experimental units. A subsequent experiment was carried out to compare commercial Hansen's rennet (control treatment = T0) with the top 4 treatments (T19, T20, T24 and T27) from the previous experiment that achieved milk cutoff and coagulation.

2.2 Cheese production

The raw milk underwent quality control and was found to have an acidity of 0.14 g/100 g lactic acid, a density of 1.031 g/mL, fat content of 19.09 g/100 g fresh cheese, pH of 6.8 and a temperature of 19.9 °C. Membrane filtration was utilized to screen the milk and ultrafiltration membranes were implemented to fractionate the skimmed milk for protein concentration. This size separation is based on fractionation between different predominant milk proteins, such as α-lactoalbumin (ALA) and β-lactoglobulin (BLG), both of which have molecular masses of 14.4 kDa and 18.4 kDa, respectively. This is because BLG can exist as a mixture of monomers and dimers under milk processing conditions (Mercadante et al., 2012). In this study, ultrafiltration membranes with a molecular weight of 30 kDa were employed to fractionate ALA and BLG. Subsequently, pasteurization was performed at 65 °C for 30 minutes and cooled down to 48 °C. Afterwards, the milk was fermented and conditioned with the application of NaCl, $CaCl_2$ and 2% of *Lactobacillus lactis*. It was left to stand for 30 minutes. Then, 1 g of $CaCl_2$ was added per liter of milk, with lyophilized papain added at three concentrations (2, 4 and 6 g/L), and then set at three coagulation temperatures (30 °C, 35 °C, and 42 °C). Following homogenization, the mixture was allowed to stand for three hours in a water bath suspended in a flat Technopor float designed for this study. This was done to facilitate monitoring of temperature and coagulation time, to project this technology to the reality of local producers, who are located at different altitudinal levels with different economic and environmental conditions.

2.3 Milk cutoff time

The onset of coagulation, also known as the milk cutoff time, was determined by observing the time of application of lyophilized papain from the three *Vasconcellea* species. This led to the colloidal concentration, which marks the beginning of the separation of protein content from whey. All experimental units that coagulated were assigned a value of 1, while those that did not were assigned a value of 0.

2.4 Coagulation and yield analysis

The method assessed milk coagulation by measuring the duration of the reaction between milk protein (casein) and the enzyme papain until curd-cutting clots formed. The yield was calculated as a percentage (%) by dividing the weight of freshly made cheese by the volume used and then multiplying by one hundred (100) (Equation 1).

$$\% \text{ Performance} = \frac{\text{Final weight of fresh cheese obtained (g)}}{\text{Initial volume of fresh cow's milk (L)}} \text{ x } 100 \quad (1)$$

2.5 Evaluation of serum turbidity and residual enzyme

The turbidity of the whey was measured using a PF-12 Plus turbidimeter and reported in Nephelometric Turbidity Unit and the Formazin Nephelometric Unit (NTU/FNU) scales. Regarding the other enzyme, a 20 mL experimental substrate consisting of skimmed milk with acidity of 0.14 g/100 g lactic acid, density of 1.031 g/mL, fat content of 19.09 g/100 g fresh cheese, pH of 6.8, and temperature of 19.9 °C, combined with sodium cacodylate buffer (0%) was used. Thus, 05 M hydrochloric acid (pH = 6.0) containing 0.1 M calcium chloride, 0.86 M sodium chloride, and purified water was placed in twelve Falcon tubes. The tubes were centrifuged for thirty minutes at 30 °C using the Hettich Universal 360 R centrifuge from Germany. Likewise, 1 mL of fresh cheese extract was added to the substrate, and it was incubated for five, ten, and fifteen minutes in an IC 111 ECO INCUCELL incubator from the United States, set to 30 °C. At the end of each incubation period, a predetermined amount of freeze-dried papain enzyme and commercial Hansen's rennet, used in producing fresh cheese, were added to evaluate coagulation duration. The time elapsed from adding fresh cheese extract to the experiment substrate until the predetermined amount of freeze-dried papain and commercial rennet were added is referred to as the “incubation time.” The time from adding freeze-dried papain and commercial Hansen’s rennet until visible curd formation on the experiment tube wall was named “coagulation time”. The slope was calculated using the aforementioned times via a regression line. Coagulant units (CU/mL of fresh cheese) were used to determine coagulant retention in the fresh cheese (Equation 2).

$$\text{CU/mL} = \frac{-S}{1+S} * \frac{\text{Weight of supernatant}}{\text{Fresh cheese weight}} * \frac{100}{FD} * 1000 \quad (2)$$

S: The slope is calculated from a regression line between incubation time and clotting time.

2.6 Extraction of coagulating enzymes

To extract the coagulation enzymes, 40 g of freshly grated cheese was mixed with 60 g sodium cacodylate buffer containing 0.1 M $CaCl_2$, 0.86 M NaCl, 0.05 M HCl, and pH 6.0. The mixture was homogenized, weighed, and incubated at 30 °C for 4 hours before being centrifuged at 9000 x g and 4 °C for 1 hour. The supernatant was obtained by filtration through Whatman N° 42 paper. The amount retained in these fractions was determined by weighing the supernatant.

2.7 Evaluation of sensory analysis

The assessments were conducted objectively, with no subjective evaluations made. The hedonic scale test sheet, administered to the panelists, evaluated the cheese's acceptability based on its colour, smell, taste, and

texture. The sources, proposed by experts, were scored on a scale where a rating of 1 signified low or no acceptance, and a score of 7 represented high acceptance levels (Faccia et al., 2013).

2.8 Physic-chemical analysis of cheese

The experiments were conducted on the most successful treatments for producing the cheese. The pH was measured at a temperature of 15 °C by direct measurement at four different points using a Hanna HI-2213 penetration pH meter from Romania. The fat content was evaluated using the Gerber-Van Gulik method, while the ash (method 935.42), titratable acidity (method 920.124), protein (method 935), and moisture (method 926.08) were also measured in triplicate using the same methodology (Association of Official Analytical Chemists, 1995).

2.9 Texture assessment

The texture characteristics of the fresh cheese samples were evaluated in four replicates utilizing a Brookfield CT3 Texture Analyzer from the United States. Double compression testing was performed using a TA4/1000 cylindrical probe with a diameter of 25 mm, following the methodology recommended by Mazorra-Manzano et al. (2013). The pretest, test, and posttest were conducted under controlled conditions at velocities of 2 and 3 mm/s, and a distance of 61 mm. The fresh cheese samples used for testing were 5 × 5 × 1.5 cm in size (height × width × length). Texture parameters assessed comprised fracturability, hardness, cohesiveness, adhesiveness, gumminess and resilience. The TexturePro Software CT V1.8 Build 31 by Brookfield Engineering Labs, Inc. was used to collect the data.

2.10 Statistical analysis

The mean ± standard deviation (SD) expressed the results of each experiment. Statistical analyses were carried out using Infostat statistical software v.2017.

3 Results and discussion

3.1 Milk cutting and coagulation time

The study examined the impact of different enzyme sources (*V. chachapoyensis, V. heilbornii,* and *V. pubescens*), doses (2, 4, and 6 g/L), and temperatures (30 °C, 35 °C, and 42 °C) on milk coagulation. Figure 1A displays the milk clotting time using the specified enzyme, dose, and temperature sources. First, the results showed that the *V. chachapoyensis* enzyme caused an immediate clotting effect at all three temperatures (30 °C, 35 °C, and 42 °C) and enzyme doses (2, 4, and 6 g/L) (Figure 1A). Second, the enzyme from *V. heilbornii* caused milk shearing at 30 °C with a dose of 2 g/L, but not at 35 °C. However, increasing the dose (6 g/L at 35 °C) did cause milk shearing. At 42 °C, milk shearing occurred after more than 19 minutes with a dose of 2 g/L (Figure 1A). Third, the enzyme from *V. pubescens* caused milk shearing at all three temperatures (30 °C, 35 °C, and 42 °C) with doses of 2, 4, and 6 g/L. Using 2 g/L of enzyme, the milk shearing after 10 minutes at 30 °C, 5 minutes at 35 °C, and 1 minute at 42 °C (Figure 1A). Milk shearing occurs at different rates depending on the temperature and enzyme dosage. At 30 °C and 35 °C, milk shearing occurred after 15 minutes with 4 g/L of enzyme and after 15.8 minutes with 6 g/L of enzyme. At 42 °C, milk shearing occurred after 6.5 minutes with 4 g/L of enzyme and after 8.7 minutes with 6 g/L of enzyme (Figure 1A).

On the other hand, a grouping analysis was performed of coagulation time, revealing the formation of three treatment groups. The first group consists of treatments T14, T11, and T1, which involve the use of enzymes from *V. chachapoyensis* and *V. heilbornii* that produce milk cutting but with low coagulation power (Figure 1B). A second group consisting of four treatments (T19, T20, T24, and T27) used *V. pubescens* as the enzyme

source. This proved to be the most suitable option, as it produced adequate coagulation at both the three enzyme doses and the three coagulation temperatures (see Figure 1B). Finally, a third group included the remaining treatments whose enzyme source was from the three *Vasconcellea* species that did not produce adequate coagulation (see Figure 1B). Therefore, this suggests that natural coagulants offer several benefits in cheese production, particularly their ability to produce unique aromas and flavours when they come into contact with milk due to the variety of enzymes, bioactive compounds and microbial activity involved in the diverse stages of cheese making. However, plant rennet's effectiveness as coagulants in the cheese industry is hampered by its catalytic instability at milk coagulation pH values and temperatures (Ahmadi et al., 2021). In the case of papain, stability is conferred within a milk pH range of 3 to 9; however, optimal efficiency is achieved at acidic pH (Ahmadi et al., 2021). Notwithstanding this result, papain derived from *C. papaya* leaves exhibited higher protease activity at pH 7.5 (Derso & Dagnew, 2019). For this study, milk was observed to have a pH of 6.8, whereby the enzymes from *V. heilbornii* and *V. pubescens* induced cleavage and coagulation of milk at varying doses. However, the clots produced by *V. heilbornii* indicated inconsistencies, conceivably owing to a weak interaction between the different constituents of milk and the enzyme. Other factors, including enzyme source, collection time, and purity level, have also been identified as affecting the stability of the enzyme. This has been demonstrated in the use of other types of kiwis, ginger, and melon proteases (Mazorra-Manzano et al., 2013).

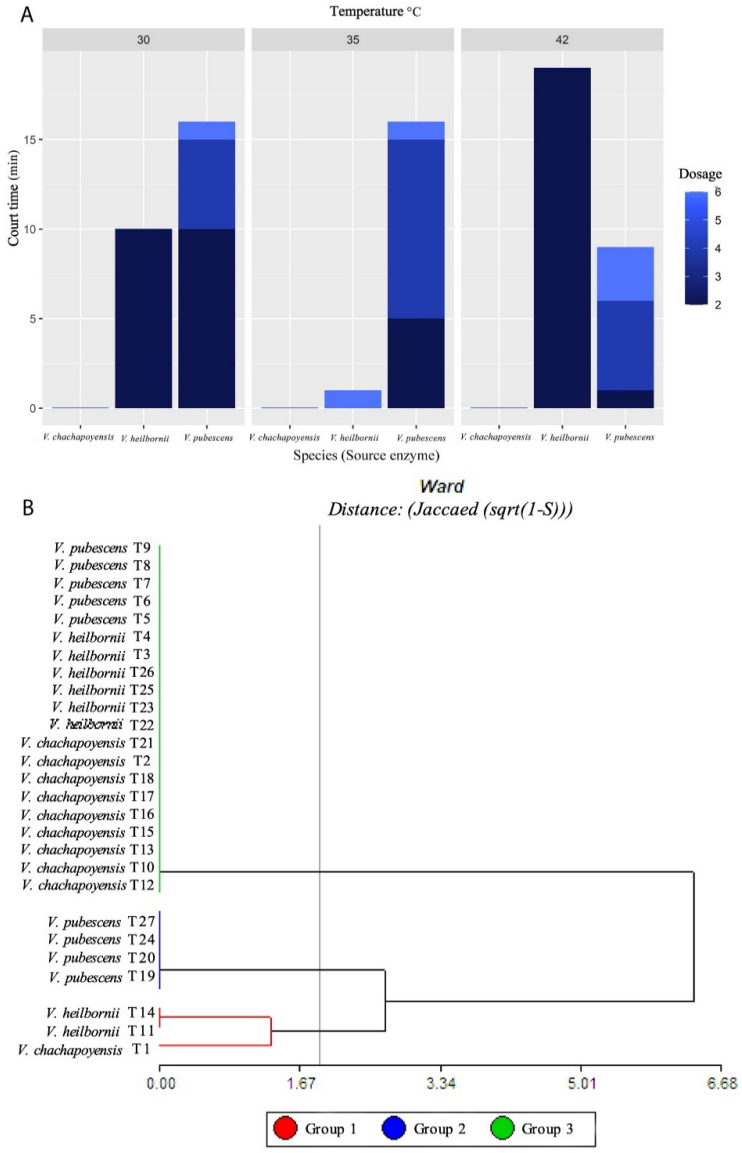

Figure 1. (A) Effect of enzyme source, dose and coagulation temperature on milk cutting time (CT) and coagulation (B).

Different enzymes display varied coagulation temperatures dependent on their composition and length of incubation, as demonstrated by Derso & Dagnew (2019). According to Anusha et al. (2014), plant-based enzymes exhibited exceptional stability within the temperature range of 30 ºC to 60 ºC. In our study, we assessed the enzyme reactions at temperatures of 30 °C, 35 °C, and 42 °C, all of which proved positive. However, the yield, organoleptic properties, and texture of the final product exhibited variation. The yield decreased alongside raised papain levels and the coagulation temperature. The decrease in cheese yield is attributed to the stronger bonds formed between water-retaining proteins caused by higher levels of coagulant concentration and temperature. This results in increased syneresis, as noted by Nazish et al. (2022). Longer coagulation durations generally lead to higher cheese production, indicating that the concentration of papain and temperature are both critical factors that impact the production of fresh cheese. For example, using 2 g/L of papain and decreasing the temperature to 30 °C can result in an increased yield, which is directly linked to higher moisture content (Li et al., 2022). Additionally, this study measured turbidity, which is the clarity of a substance. Any increase in turbidity indicates a darker sample. In serum, higher turbidity suggests a higher concentration of suspended fats, lactose, proteins, and other compounds (Muvdi-Nova et al., 2021). The study revealed that whey samples treated with natural coagulants displayed a lower mean turbidity compared to those treated with a commercial coagulant, as shown in Table 1. This could be attributed to the diverse chemical reactions initiated by the natural coagulants when mixed with the sample (Asrafuzzaman et al., 2011).

Table 1. Cheese yield and whey turbidity.

Parameters		Cheese yield (%)	*Whey turbidity* (NTU)
Sources enzyme	Commercial Hansen's rennet (control)	20.40 ± 0.00 [a]	617.00 ± 0.00 [a]
	V. pubescens	17.89 ± 3.29 [b]	614.75 ± 3.60 [b]
Dose	0.02 g/L	20.40 ± 0.00 [a]	617.00 ± 0.00 [a]
	2 g/L	19.58 ± 1.58 [a]	612.67 ± 4.23 [b]
	4 g/L	19.40 ± 2.17 [a]	617.00 ± 0.00 [a]
	6 g/L	13.13 ± 1.78 [b]	616.67 ± 0.58 [a]
Temperature	30 °C	20.23 ± 1.06 [a]	616.00 ± 1.00 [a]
	35 °C	18.80 ± 1.90 [a]	609.33 ± 3.21 [b]
	42 °C	17.64 ± 3.69 [a]	616.89 ± 0.33 [a]
Treatments	T0 (control)	20.40 ± 0.00 [a]	617.00 ± 0.00 [a]
	T19	20.23 ± 1.06 [a]	616.00 ± 1.00 [a]
	T20	18.80 ± 1.90 [a]	609.33 ± 3.21 [b]
	T24	19.40 ± 2.17 [a]	617.00 ± 0.00 [a]
	T27	13.13 ± 1.78 [b]	616.67 ± 0.58 [a]

The means represented by the same letter (a or b) in the column are not significantly different from one another (Tukey, $p \leq 0.05$). Similarly, the means represented by different letters (a and b) in the column are significantly different from one another (Tukey, $p \leq 0.05$).

3.2 Cheese yield and whey turbidity

The study exclusively employed papain enzyme extracted from *V. pubescens* due to its exceptional capacity to enhance milk coagulation and subsequent cheese production. Experimental findings demonstrated that T19 had the highest cheese production (20.23% ± 1.06%) compared to other analyzed enzymes, with similar performance to commercially available Hansen's rennet (20.40 ± 0.00%) (Table 1). In contrast, T27 presented the lowest cheese yield (13.13% ± 1.78%) (Table 1). Besides, Table 1 shows that an increase in both enzyme dosage and temperature led to a decrease in cheese yield. However, samples with a papain concentration of 2 g/L obtained favorable results, which aligned with those obtained at high temperature levels of 30 °C.

On the other hand, the mean serum turbidity levels varied based on the different doses of papain and temperatures applied. Increased turbidity levels were noted in the control treatment (T0) and T24, with a recorded value of 617.00 ± 0.00 NTU (Table 1). The T27 treatment exhibited a turbidity reading of 616.67 ± 0.00 NTU, which was similar to the previously mentioned higher values. On one hand, treatment T19 showed a turbidity measurement of 616.00 ± 0.00 NTU, and treatment T20 recorded a lower turbidity of 609.33 ± 3.2 NTU (Table 1).

3.3 Residual coagulant activity

For this analysis, we opted for the treatments that generated the highest yields of fresh cheese. The control group selected was Treatment T0 and Table 2 displays significant levels of residual coagulant activity at 101.00 ± 0.00 UC/mL. T20 has been identified as the treatment exhibiting the lowest residual coagulation activity (84.67 ± 2.52 coagulation units per milliliter) when applied at a temperature of 35 °C (Table 2). A clear correlation has been established between the amount of enzymes incorporated into the milk and the residual enzyme concentrations within the cheese. This correlation signifies that elevated doses of papain enzyme plus increased coagulation temperatures are responsible for higher levels of residual enzyme, as Gumus & Hayaloglu (2019) have also pointed out. These findings are significant, as even small quantities of coagulant in cheese can have a notable impact on blood clotting in humans (Lin et al., 2023), while elevated levels of papain in food may pose health risks, the acceptable daily intake limit has not yet been established by the scientific community. Our research suggests that the use of papain enzyme results in lower residual enzyme levels compared to Hansen's rennet, which is available commercially.

Table 2. Effect of enzyme source, dose and coagulation temperature on the residual enzyme.

Parameters		Residual enzyme (CU/mL)
		F = 14.77
		p = 0.005
Sources enzyme	Commercial Hansen's rennet (Control)	101.00 ± 0.00 [a]
	V. pubescens	90.78 ± 6.36 [b]
Dose	0.02 g/L	101.00 ± 0.00 [a]
	2 g/L	90.33 ± 6.62 [b]
	4 g/L	91.67 ± 7.09 [b]
Temperature	30 °C	96.00 ± 2.65 [a]
	35 °C	84.67 ± 2.52 [b]
	42 °C	96.33 ± 6.80 [a]
Treatments	T0 (control)	101.00 ± 0.00 [a]
	T19	96.00 ± 2.65 [a]
	T20	84.67 ± 2.52 [b]
	T24	97.67 ± 7.09 [ab]

The means represented by the same letter (a or b) in the column are not significantly different from one another (Tukey, $p \leq 0.05$). Similarly, the means represented by different letters (a and b) in the column are significantly different from one another (Tukey, $p \leq 0.05$).

3.4 Sensory evaluation

The study examined the acceptance of fresh cheese using the enzyme papain as a natural coagulant. Only those treatments that achieved adequate coagulation and cheese production were evaluated (T0, T19, T20, T24, and T27). Figure 2 indicates that there were no significant differences in the aroma or hue attributes of cheese samples. However, using higher quantities of papain in the milk (4 g/L and 6 g/L) resulted in

significant variations in the texture and taste of the cheese. Treatment T27 was found to decrease the acceptability ratings for both texture and flavour, specifically at a papain concentration of 6 g/L. There was a noticeable difference in scores, with 3.02 ± 1.53 and 3.95 ± 1.53, respectively (Figure 2). The outcomes suggest that the higher papain levels in the cheese impacted its overall appeal. The judges deemed samples T24 and T27 to be less agreeable in both texture and taste. Conversely, panelists favoured treatments T19 and T20 the most (Figure 2).

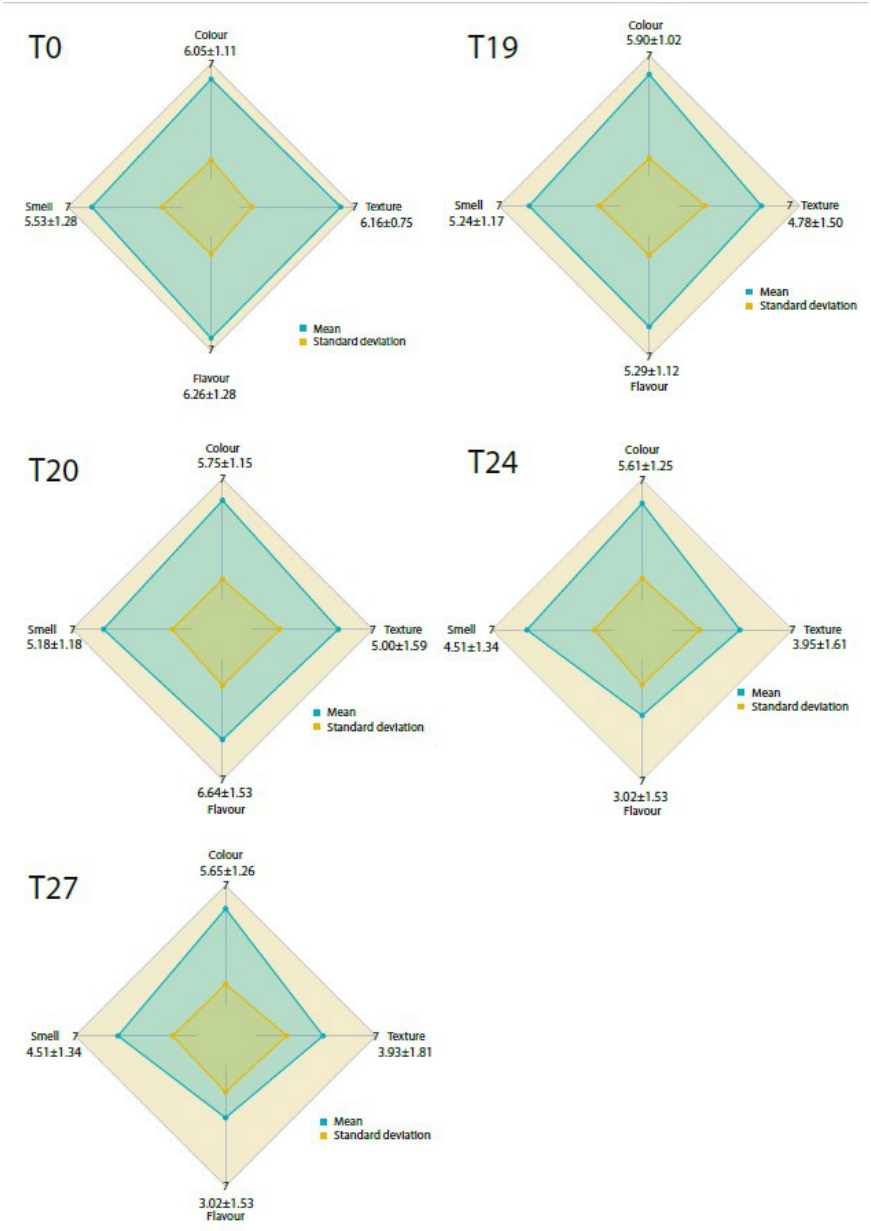

Figure 2. Radar graph of the evaluation of the sensory quality of the cheese.

3.5 Physic-chemical analysis of cheese

Only the cheeses that received the highest sensory scores (T19 and T20) were analyzed in this study. Table S2 (Supplementary Material) presents the physical and chemical characteristics of the cheese, such as moisture, protein, ash content, and pH levels. In this study, only the cheeses that received the highest sensory scores (T19 and T20) were analyzed. In treatment T19 (2 g/L; 30 °C) cheese with the best protein, moisture,

ash and pH content was obtained. Furthermore, it has been observed that an increase in temperature (35 °C) produces a more acidic cheese since the flavour and quality of the cheese are always affected by the production environment as well as by the processing techniques (Radovanovic et al., 2021). These effects are important for the production of fresh cheeses, where flavour, aroma, texture and colour properties are important for marketing and sales purposes (Short et al., 2021). It is also important to mention that the inclusion of 3-methyl-butanoic acid and 1-pentane has an unfavorable effect on the flavour of the cheese, causing an increase in papain concentration and an inappropriate bitter taste (Ali et al., 2022; Li et al., 2022; Nadzri et al., 2021). As a result, cheese products containing these compounds are rejected by customers and considered unsuitable for the market. The physic-chemical properties of cheese show corresponding trends. An increase in pH leads to a reduction in fat, protein, and acidity content. This alteration is attributed to the existence of water-soluble minerals, including ionic calcium, sodium, and potassium (Deshwal et al., 2020). As a result, cheese products containing these compounds are turned down by customers and regarded as inappropriate for the market. The physic-chemical properties of cheese exhibit corresponding trends. An increase in pH leads to a reduction in fat, protein, and acidity content. This alteration is assigned to the existence of water-soluble minerals, including ionic calcium, sodium, and potassium (Deshwal et al., 2020).

3.6 Cheese texture analysis

Table 3 presents the textural features of cheeses produced using various coagulating agents, such as commercial and vegetable sources. Technical term abbreviations are fully explained upon first use. The application of the commercial coagulating agent Hansen (T0) resulted in cheeses with similar degrees of firmness, gumminess, fracturability, cohesiveness, and resilience when compared to cheeses coagulated with papain (2 g/L, at 42 °C) (Table 3). This indicates that several factors affect the acceptance of dairy products among consumers, namely texture, adhesiveness, resilience, and consistency (Kaczyński et al., 2023). In addition to the stress placed upon the interdependence of product composition and processing methods (Fox et al., 2017). Therefore, to enhance the appeal of dairy products, manufacturers need to recognize the significant impact of texture, as stated by Li et al. (2022).

Table 3. Physical characteristics of fresh cheese parameters.

Parameters		Fracturability (N)	Hardness (N)	Cohesiveness (-)	Adhesiveness (g/s)	Gumminess (N)	Resilience (mj)
Source of enzyme	Hansen's rennet	23.93 ± 0.41 [a]	23.87 ± 0.16 [a]	4.04 ± 0.24 [a]	2.75 ± 2.78 [b]	96.70 ± 4.65 [a]	1.51 ± 0.19 [b]
	V. pubescens	22.82 ± 1.19 [b]	22.04 ± 0.58 [b]	1.85 ± 1.05 [b]	4.40 ± 0.88 [a]	41.29 ± 12.24 [b]	0.03 ± 0.01 [a]
Dose	0.02 g/L	23.93 ± 0.41 [a]	23.87 ± 0.16 [a]	4.04 ± 0.24 [a]	2.75 ± 0.78 [b]	96.70 ± 4.65 [a]	1.51 ± 0.19 [a]
	2 g/L	22.82 ± 1.19 [b]	22.04 ± 0.58 b	1.85 ± 1.05 [b]	4.40 ± 0.88 [a]	41.29 ± 22.24 [b]	0.03 ± 0.01 [b]
Temperature	30 °C	23.75 ± 0.38 [a]	21.76 ± 0.32 [b]	0.92 ± 0.15 [c]	3.98 ± 0.71 [a]	21.84 ± 3.57 [c]	0.02 ± 0.00 [b]
	35 °C	21.89 ± 0.93 [b]	22.32 ± 0.69 [b]	2.77 ± 0.52 [b]	4.83 ± 0.91 [a]	60.74 ± 11.51 [b]	0.05 ± 0.02 [b]
	42 °C	23.93 ± 0.41 [a]	23.87 ± 0.16 [a]	4.04 ± 0.24 [a]	2.75 ± 0.78 [b]	96.70 ± 4.65 [a]	1.51 ± 0.19 [a]
Treatments	T0	23.93 ± 0.41 [a]	23.87 ± 0.16 [a]	4.04 ± 0.24 [a]	2.75 ± 0.78 [b]	96.70 ± 4.65 [a]	1.51 ± 0.19 [a]
	T19	23.75 ± 0.38 [a]	21.76 ± 0.32 [b]	0.92 ± 0.15 [c]	3.98 ± 0.71 [a]	21.84 ± 3.57 [c]	0.02 ± 0.00 [b]
	T20	21.89 ± 0.93 [b]	22.32 ± 0.69 [b]	2.77 ± 0.52 [b]	4.83 ± 0.91 [a]	60.74 ± 11.51 [b]	0.05 ± 0.02 [b]

The means represented by the same letter (a or b) in the column are not significantly different from one another (Tukey, $p \leq 0.05$). Similarly, the means represented by different letters (a and b) in the column are significantly different from one another (Tukey, $p \leq 0.05$).

4 Conclusion

This study demonstrates that lyophilized papain enzyme from *Vasconcellea* species is a viable option for the production of fresh bovine cheese. Of the three species studied as a source of the enzyme, *V. chachapoyensis*

and *V. heilbornii* did not yield promising results for the production of fresh cheese. Nevertheless, the enzyme extracted from *V. pubescens* exhibited the greatest potential, as it resulted in a higher yield of fresh cheese with low residual enzyme. The optimal dose and temperature for preparation were determined to be 2 g/L of lyophilized papain per liter of milk, incubated at 30 °C for 10 minutes. The yield and physic-chemical characteristics were similar to those of the cheese produced with commercial Hansen's rennet as a coagulant. However, when a higher concentration of lyophilized enzyme (4 g/L) and an incubation temperature of 42 °C are employed, the yield is diminished, increasing the residual enzyme concentration. Consequently, further studies are required to ascertain the purity and dosage of these enzymes, to enhance profitability and facilitate their ecological application in the production of fresh cheese.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Evaluation of the cutting and coagulation times of the combination of the 27, using three different species, doses and temperatures.

Table S2. Physic-chemical analysis of cheese

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