Arch Lebensmittelhyg 72,
185–191 (2021)
DOI 10.2376/0003-925X-72-185

ISSN 0003-925X

Korrespondenzadresse:
hachana@yahoo.fr

[1]) Animal Production Department, Higher Agronomic Institute of Chott-Meriam, University of Sousse, 4042 Susa Tunisia; [2]) Department of Agriculture, Forestry and Food Science, University of Torino, 10095 Grugliasco, Italy

Use of papaya leaf extract for the production of fresh goat's cheese as an alternative to animal rennet

Verwendung von Papayablattextrakt für die Herstellung von Ziegenfrischkäse als Alternative zu tierischem Lab

Yasser Hachana[1]), Iheb Frija[1]), Rabaa Maoual[1]),
Ibrahim El Akram Znaidi[1]), Riccardo Fortina[2])

Summary

Carica papaya leaf extract obtained by a simple and chemical-free method was first analyzed for levels of protein, polyphenols, proteolytic activity and milk clotting activity, and then evaluated in fresh goat's cheese production as an alternative to calf rennet. Milk clotting essays, cheese yields, protein fractions and sensory evaluations were determined considering animal rennet as reference. The highest cheese yield was obtained with 0.2% dose of fresh papaya leaf extract. Compared to calf rennet, papaya leaf extract remained active even at 95 °C. After 12 months of cold storage, the clotting ability of liquid extract decreased significantly, whereas the lyophilized extract maintained its full clotting potential. Cheese samples made with papaya leaf extract showed lower levels of casein but higher levels of pre-α-caseins and water-soluble nitrogen than those made with calf rennet. Sensory results proved that the use of papaya leaf extract in fresh goat's cheese production had no negative impact on color, consistency or taste, but on the contrary, it improved cheese's odor which was remarkably appreciated by panelists. This natural product could therefore be a good alternative to conventional animal rennet and could be used to attribute specificity to traditional fresh goat's cheese.

Keywords: Papaya leaves, calf rennet, goat milk, cheese yield, proteolysis, sensory quality

Introduction

Coagulation is a key step for successful cheese production, essentially ensured by two processes: acidification carried out using lactic ferments, and enzymatic by rennet. Animal clotting enzymes are often gastric proteases; the most commonly used is rennet, consisting mainly of chymosin or pepsin from cattle, pig or chicken (Jacob et al., 2011). Calf rennet is traditionally used as milk coagulant for cheese making. Animal rennet extracted from the abomasum of young ruminants has a proteolytic activity that specifically targets κ-caseins, increasing cheese yield and reducing protein losses during coagulation (Fox and Kelly, 2004). Only 20 to 30% of calf rennet requirements can be met (Jacob et al., 2011). In addition, several factors reported by Roseiro et al. (2003), namely high rennet prices, some religious concerns, emergence of vegetarian diets or prohibition of recombinant calf rennet use in some European countries, have encouraged the search for alternative sources of milk coagulation. Accordingly, several studies have investigated other novel sources of coagulating enzymes, including plant-based enzymes. Some vegetable coagulant, used as milk-clotting agent in cheese making, produced a curd with similar characteristics to those obtained using commercial chymosin (Liburdi et al., 2019). However, the use of plant proteases has not been developed on a large scale and it has been limited to a few types of artisanal cheese production due to their proteolytic action, disproportionate to their milk coagulation activity (Faccia et al., 2012). Papaya is a plant originating from tropical countries and belonging to the *Caricaceae* family, being able to grow in a wide range of climates (Amari and Mamboya, 2012). The main proteases isolated from the *Carica papaya* plant are papain, chymopapain, caricain and glycyl endopeptidase (Feijoo-Siota and Villa, 2001). Debuigne and Couplan (2009) reported that all papaya plant parts contain proteolytic enzyme called papain. According to Adetunji and Salawu (2008), crushed papaya leaves have demonstrated milk clotting abilities. This result was confirmed by Derso and Degnew (2019) who observed milk-clotting activity when using leaf extracts of *Carica papaya.* Attempts to use plant proteases in cheese making have often failed due to either weekly yield or poor organoleptic quality (Kumar et al., 2010).

The objective of this study was first to evaluate the protein and polyphenol contents, as well as the proteolytic properties of the papaya leaf extract, obtained by a simple and chemical-free method. In addition, the clotting power of this plant extract, stored for one year in different forms, was tested in fresh goat cheese production. Calf rennet was used as a reference coagulant. The second objective of the study was to determine the extract dose and milk heating temperature allowing the best cheese yield. The last objective was to test the effect of papaya leaf extract on the proteolytic activity and sensory quality of cheese.

Materials and methods

Coagulant extracts preparation

Fresh and mature leaves and stems of *Carica papaya* (650 g), collected from the Higher Agronomic Institute of Chott-Meriam Tunisia Farm, were carefully rinsed under running water, cut into small pieces using pruning shears, and then mechanically crushed using a Moulinex (JU610D10) centrifugal extractor without using any chemicals. The resulting green-colored liquid was quickly transferred into 45 mL vials and centrifuged at 5000 rpm at 4 °C for 35 minutes, in order to separate the grinding residues and reduced the pigment content, preventing undesirable greenish coloring of cheese. The coagulant extract yield (%) was calculated as the difference between the initial weight of fresh leaves and stems and the final weight of the supernatant. The extract was divided into three batches, each of 200 mL. The first batch was used fresh, the second one was stored for 12 months in a dark bottle at 4 °C, and the third was lyophilized using a freeze dryer (FreeZone Dry 4.5 USA) and stored at 4 °C for 12 months. The amount of lyophilized extract from the initial batch was 6.2 g (yield: 3.1%).

Coagulant extract characterization

Total protein content of papaya leaf extract was determined in triplicate according to the Bradford method (1976), using Coomassie brilliant blue G-250 dye.

Polyphenols content of papaya leaf extract was measured in triplicate by the Folin-Ciocalteu's method (Fu et al., 2011). Polyphenols content was expressed as milligrams gallic acid equivalents per gram of dry papaya leaf extract (mg GAE /g DE).

Milk clotting activity of papaya leaf extract was assessed in triplicate by the method of Anusha et al. (2014), and expressed in terms of Soxhlet units (SU).

The proteolytic activity of papaya leaf extract was determined in triplicate by the method of Chopra and Mathur (1983), in which enzyme activity was determined by the casein digestion assay.

Raw milk composition and clotting assays

Raw milk was collected in 2019 and 2020 from the alpine goat herd of the professional agricultural training center of Monastir Tunisia. Bulk milk was controlled for pH (Hanna HI 5522 pH meter) and titrable acidity with a 0.1 N NaOH solution and phenolphthalein (Dornic acidimeter, Gerber Instruments, Germany). Chemical composition was assessed in triplicate using Milko-Scan FT 120 (Foss-Electric, DK). Milk was also analyzed for total aerobic mesophilic flora according to AFNOR (2013). In 2019, the clotting assay was carried out in triplicate on 80 samples of milk using variable doses of fresh Papaya leaf extract (0.1, 0.2, 0.3%) and temperatures (40, 60, 80 and 95 °C) in order to obtain the best cheese yield with the minimum amount of extract.

Cheese production and yield

Cheese production using fresh Papaya leaf extract or calf rennet was performed in 2019; the refrigerated and lyophilized Papaya leaf extracts were tested after 12 months of storage, in 2020. The extract dose was 0.2 mL/L of milk; the lyophilized extract was first dissolved into 200 mL of distilled water. Calf rennet dose (Soxhlet Unit = 1/30.000 ± 5%; pepsin/chymosin ratio = 1/6.9 ± 5%; WalcoRen 95L300 Quebec, Canada) was 0.2 mL/L of milk. In both years, common cheese-making procedure was used. Bulk milk was heated to 80 °C for 2 minutes, then cooled to 40 °C and added with a coagulant. After clotting (15 minutes), the resulting curd was cut and carefully stirred, then poured into small 100 g molds and gently pressed by hand to remove whey. All cheese samples were stored at 4 °C for further essays. Cheese yield (g/L of milk) was calculated as the ratio between the mass of the curd (g) after 2 hours of draining and the amount of milk (L). For each type of Papaya leaf extract coagulant (fresh, refrigerated at 4 °C and lyophilized), the yield was calculated as the average of 3 replicates.

Nitrogen fractions and urea polyacrylamide gel electrophoresis (urea-PAGE)

The nitrogen fractions and urea-polyacrylamide gel electrophoresis analysis of cheese samples made with fresh Papaya leaf extract and calf rennet were performed to assess the differences in the proteolytic activity. Nitrogen fractions were separated according to the procedure described by Kuchroo and Fox (1982). Extracts were used to determine Non-Protein Nitrogen (NPN) in 12% trichloroacetic acid and Water Soluble Nitrogen (WSN) at pH 4.6. The Kjeldahl method (AFNOR, 2014) was used to determine the Total Nitrogen content (TN), WSN and NPN. Ammonia Nitrogen (NH_3N) was determined using an ammonia selective electrode (Orion high-performance, Thermo Scientific 9512HPBNWP) according to the procedure described by Molimard et al. (1994). Amino Acid Nitrogen (AAN) was determined in triplicate according to the method described by Folkertsma and Fox (1992).

Urea-polyacrylamide gel electrophoresis (urea-PAGE) was performed using a SE 400 Vertical Electrophoresis Unit (GE Healthcare SE400-15-1.5). Analyses were carried out according to Veloso et al. (2002). Protein band density was determined using CD60 densitometer (Desaga Sarstedt). Caseins were quantitatively determined by peak area integration of the densitometer traces, using digital image processing of the stained gel by ImageQuant TL 7.0 software, combined with the CD60 densitometer.

Sensory evaluation

In order to assess the impact of Papaya leaf extract on cheese flavor, sensory attributes of cheese samples (color, taste, aftertaste, consistency and overall appreciation) were evaluated on a 0 (lowest) to 20 (highest) points scale by a volunteer group of 80 unqualified tasters, including professors, students, administrative staff and workers. Two coded cheese samples were presented to each taster for evaluation: one produced from calf rennet and the other from Papaya leaf extract. During evaluation, candidates were required to rinse their mouths with mineral water after each cheese tasting. Spontaneous attributes were used to describe the perceived differences between cheeses. Scores for each sample were obtained by averaging scores of 80 panelists.

Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model procedure of XLSTAT 2016-0.2, to determine the effect of Papaya leaf extract on fresh goat cheese quality. Significantly different means were identified using Duncan test. All statements of significance were based on 5 % probability. All analyses were carried out in triplicate.

Results and discussion

Extract characterization

Papaya leaf extract characteristics are presented in Table 1. Papaya leaf extract is characterized by protein and polyphenol levels of 1.3 ± 0.2 mg/mL and 89.4 ± 1.1 mg GAE/g DE respectively, and a proteolytic activity and milk clotting activity of 7.5 ± 0.7 U/mL and 197.3 ± 12.4 SU/mL respectively. Waheed et al. (2017) reported similar results for protein content (1.2 mg/mL) and proteolytic activity (7 U/mL), but lower milk clotting activity (170 SU/mL), for the same type of plant extract. However, Derso and Dagnew (2019), using papaya leaf extract solution obtained by adding 5% NaCl in sodium acetate buffer, observed similar milk clotting activity (195 SU/mL).

TABLE 1: *Characteristics of papaya leaf extract.*

Determination	Value
Protein (mg/mL)	1.3 ± 0.2
Polyphenols (mg GAE/g DE)	89.4 ± 1.1
Proteolytic activity (U/mL)	7.5 ± 0.7
Milk clotting activity (SU/mL)	197.3 ± 12.4

GAE = gallic acid equivalent per gram of Dry Extract

TABLE 2: *Physico-chemical and bacteriological composition of raw goat's milk.*

Parameters	Milk[1]	Milk[2]
Acidity (°D)	14.22 ± 0.12	15.1 ± 0.18
pH	6.73 ± 0.02	6.59 ± 0.01
Fat (g/L)	3.70 ± 0.03	3.58 ± 0.01
Protein (g/L)	3.31 ± 0.01	3.38 ± 0.02
Lactose (g/L)	4.96 ± 0.03	4.89 ± 0.01
TAMF (cfu/mL)	93 x 10^{-4} ± 5.4	76 x 10^{-4} ± 8.1

[1]Milk = goat milk used with fresh extract (2019); [2]Milk = goat milk used with refrigerated and lyophilized milk (2020); TAMF = total aerobic mesophilic flora.

TABLE 3: *Goat milk clotting assay: Cheese yield (g/L) at different doses of fresh Papaya leaf extract and milk heating temperatures.*

Dose (%)	Temperature (°C)	Cheese Yield (g/L)	Mean ± SD
0.1	40	0	187.6[c] ± 6.7
0.1	60	180.1 ± 0.9	
0.1	80	193.2 ± 1.1	
0.1	95	189.5 ± 0.7	
0.2	40	0	301.27[a] ± 1.4
0.2	60	301.8 ± 1.0	
0.2	80	299.7 ± 0.5	
0.2	95	302.3 ± 0.6	
0.3	40	0	277.2[b] ± 7.7
0.3	60	278.6 ± 1.3	
0.3	80	284.1 ± 1.6	
0.3	95	268.9 ± 1.1	

[abc] Values with different superscript letters within the same column have significant differences (p <0.05).

Effect of coagulant dose and milk heating temperatures on cheese yield

Chemical composition and characteristics of goats bulk milk used in 2019 and 2020 for clotting tests and cheese making trials is reported in Table 2.

Cheese yield values (average of three replicates) at varying doses of fresh Papaya leaf extract and milk temperatures, and the average of each dose at different temperatures are shown in Table 3.

The highest cheese yield was obtained with 0.2% dose (301.27 ± 1.4 g/L), while the lowest at 0.1% dose (187.6 ± 6.7 g/L). Difference between the highest and lowest yield was significant, 113.67 g/L. At 40 °C goat's milk has not coagulated whatever the extract dose. According to literature, the most effective coagulant dose of vegetal extract depends on the type of plant as well as the type of cheese. Akinloye and Adewumi (2014) reported adequate yields in the production of cow's milk cheese using a 3.3% dose of fresh extract of papaya leaves. In our trial, the 0.2% dose of Papaya leaf extract coagulant allowed the production of the highest cheese yield. This dose was used for measuring the effects on cheese yield and clotting time of goat milk samples at increasing temperatures (from 60 to 95 °C). Results (expressed as mean of three determinations) are shown in Table 4.

The increase of milk temperature didn't have a significant impact on cheese yield *(p >0.05)*, but it affected the coagulation time. A significant reduction of clotting time *(p < 0.05)* was observed as the milk heating temperature increased. At 60 °C, the clotting time was 17.1 ± 0.01 minutes, significantly longer than other temperatures. At 80 °C, the clotting time was 8.9 minutes faster than at 60 °C. Whereas at 90 °C, the clotting time was 2.3 ± 0.01 minutes, significantly faster than at 60 °C and 80 °C (Table 4). In line with our findings, Hafid et al. (2020) showed that more the temperature of milk added with papain increases, more its coagulation is fast. This profile is similar to that generally reported for plant proteases. Compared to conventional proteases of animal origin, papaya leaf extract was more heat stable and remained active even at 95 °C, thus potentially allowing the production of cheese from pasteurized milk without adding lactic ferments. Derso and Dagnew (2019) reported that milk coagulation activity increased with incubation temperature and reached its maximum level at 70 °C for *Carica papaya* leaf extract.

Effect of shelf life and storage method on cheese yield

Table 5 shows the effect of shelf life and storage method of papaya leaf extract on cheese yield compared to calf rennet.

Shelf life as well as storage method of papaya leaf extract significantly affected cheese yield *(p < 0.05)*. Cheese yield obtained by calf rennet (329.6 ± 24.4 g/L milk) was significantly higher (P < 0.05) than those obtained with fresh, chilled and freeze-dried papaya leaf extract (297.9 ± 20.2 g/ /L, 171.1 ± 17.8 g/L and 295.2 ± 11.2 g/L of milk respectively). Refrigerated extract resulted in significant decrease in cheese yield compared to fresh extract and calf rennet (-126.8 and -158.5 g/L milk, *p < 0.05*). With the lyophilized extract, cheese yield was similar to that obtained with fresh extract (295.2 vs 297.9 g/L milk respectively). These results clearly indicate that the clotting ability of papaya leaf extract decreased significantly *(p < 0.05)* after 12 months of refrigerated storage, but still remained active allowing a yield of 171.1 g/l. On the contrary, the lyophilized extract maintained the same clotting ability of the fresh extract even after one year of storage (295.2 vs 297.9 g/L). According to Tejada et al. (2008b), refrigerated storage is not appropriate for long conservation of aqueous plant extract. They reported that lyophilization and freezing have proven to be ideal for the long-term storage of plant coagulant. Similarly, Hafid et al. (2020) proved that latex extracted papain remained more stable at –20 °C than at 4 °C or 25 °C after more than 5 weeks of storage.

Fresh extract of papaya leaves resulted in lower cheese yield than rennet (297.9 ± 20.2 g/ /L vs 329.6 ± 24.4 g/L milk), but remained satisfactory compared to what has been reported in other studies. The effect of plant coagulants on cheese yield was always controversial. Some authors affirm that these products significantly reduce cheese yield, while others report better yields than those obtained with chymosin. Liburdi et al. (2019) reported higher proteolytic activity and lower milk clotting capacity of papain compared to other plant coagulants. On the contrary, Rana et al. (2017) observed the same cheese yield by using rennet or papaya latex (220 g kg^{-1}), which was lower than that achieved in this work. Boshra and Tajul (2013) reported that papain protease has similar milk clotting capacity as rennet and allows the same yield in cheese making. Cheese produced with papaya protease showed the best milk clotting ability and physicochemical composition as compared to moringa extract (Waheed et al., 2017).

Protein fractions and Urea-PAGE results

The Urea-PAGE electrophoretograms of the pH 4.6 insoluble fractions of fresh goat cheeses, produced with calf rennet (CR) and fresh Papaya leaf extract (FPLE) are illustrated in Figure 1. Four zones were delimited representing casein fractions ranking from those with the highest molecular weight to the lowest molecular weight as follows; γ-caseins (γ-CN), β-caseins (β-CN), αs-caseins (αs-CN) and pre-αs-caseins (pre-αs-CN) (Figure 1).

Variations in casein fractions and their degradation products expressed as a percentage of total optical density of produced farm cheeses are shown in Table 6.

The coagulant type used in cheese manufacturing significantly *(p < 0.05)* affected the concentrations of α-casein and β-casein, as well as their degradation products, γ-casein and pre-α-casein. Levels of β-casein, α-casein and γ-casein fractions were significantly *(p < 0.05)* higher in cheese samples made with calf rennet than in those produced

TABLE 4: *Effect of milk temperature on cheese yield and clotting time (dose of fresh papaya leaf extract: 0.2%).*

Milk temperature (°C)	Cheese Yield (g/L)	Clotting time (Min)
60	303.1 ± 2.8	17.1[a] ± 0.01
80	294.2 ± 3.1	8.2[b] ± 0.04
95	298.9 ± 2.2	2.3[c] ± 0.01

[abc] Values with different superscript letters within the same column have significant differences (*p* <0.05).

TABLE 5: *Effect of shelf life and storage method of Papaya leaf extract on cheese yield compared to calf rennet.*

Coagulant type	Time storage	Cheese yield (g/L)
Liquid PLE	Fresh	297.9 ± 20.2[b]
Refrigerated PLE (4 °C)	12 months	171.1 ± 17.8[c]
Lyophilized PLE	12 months	295.2 ± 11.2[b]
Calf rennet	Fresh	329.6 ± 24.4[a]

[abc] Values with different superscript letters within the same column have significant differences (*p* <0.05). PLE = Papaya leaf extract.

TABLE 6: *Variations in casein fractions of fresh goat cheeses made with fresh papaya leaf extract and calf rennet.*

Casein fractions (%)	Papaya leaf extract	Calf rennet
α-Casein	31.6 ± 3.21[b]	39.1 ± 1.97[a]
β-Casein	37.8 ± 1.29[b]	41.7 ±1.15[a]
γ-Casein	7.9 ± 1.67[b]	10.3 ± 2.78[a]
Preα-Casein	12.4 ± 0.74[b]	2.6 ± 1.01[a]

[ab] Values with different superscript letters within the same column have significant differences (*p* <0.05). PLE = Papaya leaf extract.

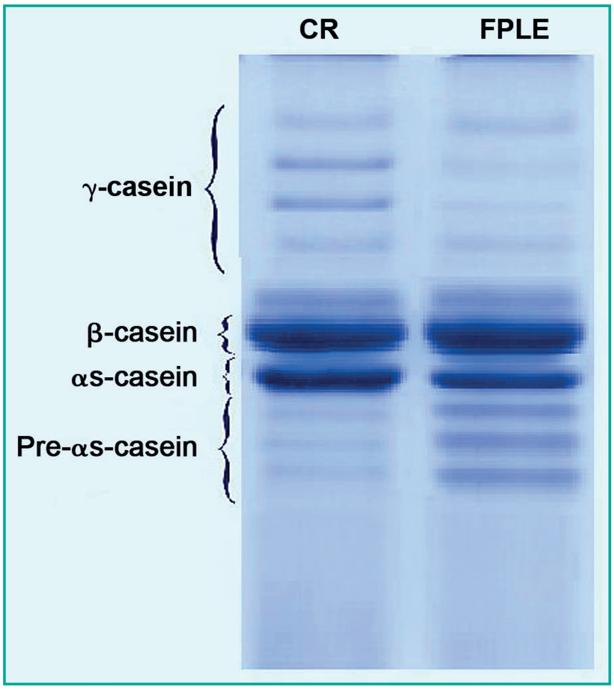

FIGURE 1: *Urea-polyacrylamide gel electrophoresis (urea-PAGE) of the water-insoluble extract of fresh goat's cheeses produced with calf rennet (CR) and fresh Papaya leaf extract (FPLE).*

with papaya leaf extract; the latter showed higher levels *($p < 0.05$)* of pre-α-caseins. These results are consistent with findings of Tejada et al. (2008a), who reported higher levels *($p < 0.05$)* in α- β- and γ-casein fractions of cheeses made with animal rennet than those made with vegetable coagulants. Pino et al. (2009) observed an increase in γ-caseins fractions in cheese batches made with calf rennet compared to those made with vegetable coagulant. They also reported greater breakdown of αs-casein in cheeses made with plant-based coagulant than in cheeses made with calf rennet.

Differences between soluble nitrogen fractions (WSN, water-soluble nitrogen at pH 4.6; NPN, non-protein nitrogen; AAN, amino acid nitrogen; NH_3N, ammonia nitrogen) in fresh goat cheese samples produced with fresh papaya leaf extract and calf rennet are presented in Table 7. Significant differences *($p < 0.05$)* were observed for water-soluble

TABLE 7: *Variations in nitrogen fractions (mean of three determinations: g /100 g of total nitrogen, TN) of fresh goat cheese samples made with fresh papaya leaf extract and calf rennet.*

N fractions (g/100 g TN)	Fresh Papaya leaf extract	Calf rennet
WSN	$15.8^a \pm 3.51$	$10.1^b \pm 2.25$
NPN	4.2 ± 0.58	3.7 ± 0.95
AAN	0.3 ± 0.01	0.2 ± 0.03
NH_3N	0.4 ± 0.04	0.3 ± 0.02
Protein N	93.1 ± 0.29	96.2 ± 1.41
Casein N	$81.2^b \pm 2.47$	$87.8^a \pm 1.98$
Polypeptide N	$13.1^a \pm 2.03$	$6.3^b \pm 1.67$
Peptide N	4.0 ± 0.33	3.5 ± 0.15

[ab] Values with different superscript letters within the same row have significant differences ($p < 0.05$). PLE = Papaya leaf extract. WSN = water-soluble nitrogen; NPN = non-protein nitrogen; AAN = amino acid nitrogen; NH_3N = ammonia nitrogen.

nitrogen (WSN), casein-N and polypeptide-N components, while non-protein nitrogen (NPN), amino acid nitrogen (AAN), ammonia nitrogen (NH_3N) and protein-N were similar between the two types of cheese. Cheese samples made with papaya leaf extract showed lower levels of casein N (81.2 vs 87.8 g 100 /g TN) but higher levels of WSN (15.8 vs 10.1 g 100 /g TN) than those made with calf rennet, indicating a more intense proteolytic action of papaya leaf enzymes. Pino et al. (2009) observed higher levels of WSN in cheeses produced with vegetable coagulants compared to those made with calf rennet. Tejada et al. (2008a) attributed the increase of WSN, NH_3N and AAN in ewe's cheese to the more intense proteolytic action of plant coagulants as compared to that of calf rennet.

Sensory analysis

Based on sensory analysis results, the color, taste, aftertaste and consistency parameters of fresh goat cheese samples produced with papaya leaf extract were not different from those produced with calf rennet (Figure 2).

Significant differences *($p < 0.05$)* were observed between the two cheeses for odors and global appreciation parameters. Papaya leaf extract cheese was much more appreciated *($p < 0.05$)* by panelists. It obtained the highest scores for odor and global appreciation compared to rennet cheese. No differences were observed in color, taste, aftertaste and consistency (Figure 2). Similar results were reported by Rana et al. (2017) who found that papaya la-

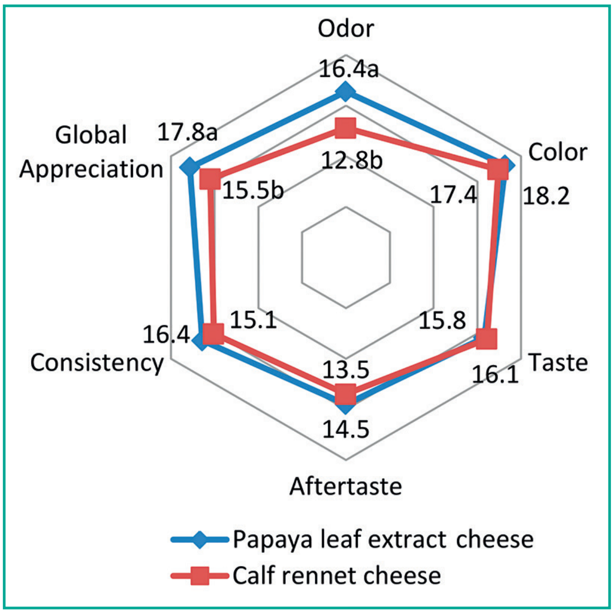

FIGURE 2: *Cheese sensory profile. [ab] Values with different superscript letters have significant differences ($p < 0.05$).*

tex cheese looked better with a smoother surface, free of cracks and mold, and obtained the highest score compared to rennet cheese. Vegetable coagulants have even been used in producing some of the most prestigious cheeses, such as Roquefort or Serra da Estrela, without affecting their sensory quality (Roseiro et al., 2003). Contradictory results have been reported by some authors who have pointed out negative effects when using vegetable coagulant for cheese production. These defects have generally been attributed to intense proteolysis caused by plant enzymes (Low et al., 2006; Bruno et al., 2010).

Journal of Food Safety and Food Quality 72, Heft 6 (2021), Seiten 177–214

Conclusions

Papaya leaf extract has the advantage of preserving its coagulation ability even when used at high temperatures and allowed a suitable cheese yield compared to calf rennet. It can be stored in freeze-dried form for 12 months without losing its clotting potential. Furthermore, the use of papaya leaf extract in fresh goat's cheese production has no negative impact on color, consistency or taste, but on the contrary, it has improved cheese odor that was remarkably appreciated by panelists. This natural product, simply extracted by chemical-free method, could be a good alternative to conventional animal rennet and could be used to attribute specificity to traditional fresh goat's cheese of controlled origin.

Acknowledgement

The authors would like to thank the technical staff of the Animal Production Laboratory of the Higher Agronomic Institute of Chott-Meriam Tunisia for their valuable contribution.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID IDS

Yasser Hachana:
https://orcid.org/0000-0002-0700-8246
Iheb Frija:
https://orcid.org/0000-0002-7610-3431
Rabaa Maoual:
https://orcid.org/0000-0002-5757-0707
Ibrahim EL Akram Znaidi:
https://orcid.org/0000-0003-2259-2484
Riccardo Fortina:
https://orcid.org/0000-0002-3949-4402

References

Adetunji VO, Salawu OT (2008): West African soft cheese 'wara' processed with Calotropis procera and Carica papaya: A comparative assessment of nutritional values. Int J Dairy Sci 6: 58–65.

AFNOR (2013): Food microbiology. Horizontal method for the enumeration of microorganisms. Part 1: Colony counting at 30 °C by the deep plating technique. ISO 4833-1.

AFNOR (2014): Milk and milk products. Nitrogen content determination. NF EN ISO 8968-1. V04-221-1

Akinloye AM, Adewumi OO (2014): Effects of local coagulants on the yield of cheese using cow and sheep milk. Int J Sustain Dev 3: 150-161.

Amari E, Mboya F (2012): Papain, a Plant Enzyme of Biological Importance: A Review. Am J Biochem Biotechnol 8: 99–104.

Anusha R, Singh MK, Bindhu OS (2014): Characterisation of potential milk coagulants from Calotropis gigantea plant parts and their hydrolytic pattern of bovine casein. Eur Food Res Technol 238: 997–1006.

Boshra V, Tajul AY (2013): Papaya an innovative raw material for food and pharmaceutical processing industry. Health Environ J 4: 68–73.

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

Bruno MA, Lazza CM, Errasti ME, López LM, Caffini NO, Pardo MF (2010): Milk Clotting and Proteolytic Activity of an Enzyme Preparation from Bromelia hieronymi Fruits. LWT-Food Sci Technol 43: 695–701.

Chopra, AK, Mathur DK (1983): Factors affecting protease production by Bacillus stearothermophilus RM-67. J Food Prot 46: 1020–1025.

Derso AG, Dagnew GG (2019): Isolate and Extract for Milk Clotting Enzymes from the leaves of Moringa Oleifera, Carica Papaya and Mangifera Indica and Use in Cheese Making: The Case of Western Hararage Region, Ethiopia. J Food Nutr Res 7: 244–254.

Faccia M, Picariello G, Trani A, Loizzo P, Gambacorta G, Lamacchia C, Di Luccia A (2012): Proteolysis of Cacioricotta cheese made from goat milk coagulated with caprifig *(Ficus carica sylvestris)* or calf rennet. Eur Food Res Technol 234: 527–533.

Folkertsma B, Fox PF (1992): Use of Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. J Dairy Res 59: 217–224.

Fox PF, Kelly AL (2004): The caseins In Proteins in Food Processing, pp 29–71. Yada R Y, ed. Boca Raton. FL: CRC Press.

Fu L, Xu BT, Xu XR, Gan RY, Zhang Y, Xia EQ, Li HB (2011): Antioxidant capacities and total phenolic contents of 62 fruits. Food Chem 129: 345–50.

Hafid K, John j, Mansour Sayah T, Domínguez R, Becila S, Lamri M, Dib AL, Lorenzo JM, Gagaoua M (2020): One-step recovery of latex papain from Carica papaya using three phase partitioning and its use as milk-clotting and meat-tenderizing agent. Int J Biol Macromol 146: 798–810.

Jacob M, Jaros D, Rohm H. (2011): Recent advances in milk clotting enzymes. Int J Dairy Technol 64: 14–33.

Kuchroo CN, Fox PF (1982): Soluble nitrogen in Cheddar cheese: Comparison of extraction procedures. Milchwissenschaft 37: 331–335.

Kumar A, Grover S, Sharma J, Batish VK (2010): Chymosin and other milk coagulants: sources and biotechnological interventions. Crit Rev Biotechnol 30: 243–258.

Liburdi K, Boselli C, Giangolini G, Amatiste S, Esti M (2019): An evaluation of the clotting properties of three plant rennets in the milks of different animal species. Foods 8: 1–13.

Low YH, Agboola GS, Zhao J, Lim MY (2006): Clotting and proteolytic properties of plant coagulants in regular and ultrafiltered bovine skim milk. Int Dairy J 16: 335–343.

Molimard P, Lesschaeve I, Bouvier I, Vassal L, Schlich P, Issanchou S, Spinnler H (1994): Amertume et fractions azotées de fromages à pâte molle de type camembert: rôle de l'association de Penicillium camemberti avec Geotrichum candidum. Lait 74: 361–374.

Pino A, Prados F, Galán E, McSweeney PLH, Fernández-Salguero J (2009): Proteolysis during the ripening of goats'milk cheese made with plant coagulant or calf rennet. Food Res Int 42: 324–330.

Rana MS, Hoque MR, Deb GK, Nahar TN, Habib R, Siddiki MSR (2017): Preparation of cottage cheese from buffalo milk with different levels of papaya latex. Bangladesh J Anim Sci 46: 128–133.

Roseiro LB, Barbosa M, Ames JM, Wilbey RA (2003): Cheese making with vegetable coagulants the use of Cynara L for the production of ovine milk cheeses. Int J Dairy Technol 56: 76–85.

Tejada L, Abellan A, Cayuela J, Martınez-Cacha A, Fernandez-Salguero J (2008a): Proteolysis in goats' milk cheese made with calf rennet and plant coagulant. Int Dairy J 18: 139–146.

Tejada L, Vioque M, Gomez R, Salguero JF (2008b): Effect of lyophilisation, refrigerated storage and frozen storage on the coagulant activity and microbiological quality of Cynara cardunculus L. extracts. J Sci Food Agric 88: 1301–1306.

Veloso A, Teixeira N, Ferreira I (2002): Separation and quantification of the major casein fractions by reverse-phase high-performance liquid chromatography and urea–polyacrylamide gel electrophoresis Detection of milk adulterations. J Chromatogr A 967: 209–218.

Waheed M, Hussain MB, Majeed M, Rehman TU, Khan MU, Shariati MA, Plygun SA, Glinushkin AP (2017): Quality evaluation of cheese prepared by plant coagulants: carica papaya and moringa oleifera leaves. Russ J Agric Socio-Econ Sci 4: 232–239.

Present address of corresponding author:
Yasser Hachana
Animal Production Department
Higher Agronomic Institute of Chott-Meriam
University of Sousse
4042 Susa
Tunisia
hachana@yahoo.fr