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Usefulness of *Moringa oleifera* seed extract as Coagulant for the production of fresh camel cheese

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ORIGINAL ARTICLE

Abstract

The current study aimed to enhance the coagulation properties of camel milk using enzymatic extracts derived from *Moringa oleifera* seeds. Experiments were conducted to evaluate the clotting activities of *Moringa* extract on camel milk and compare them with cow milk. Our results regarding the characterisation of the enzymatic extract showed an extraction yield of 54.3±1.8%. The optimum coagulation conditions were determined to be pH 5 and a temperature of 55°C. In addition, the enzymes exhibited substantial clotting activity (2.54 RU) on both camel and cow milk. The cheese samples showed significant oxidative and antibacterial activity.

Keywords: camel milk; cheese; clotting activity; extraction yield; MSE

Introduction

Camel milk is popular for its traditional anti-infective properties (Morrin *et al.,* 2021) as well as its anti-cancer properties (Krishnankutty *et al.,* 2018), anti-diabetic (Sboui *et al*., 2022; Ashraf *et al*., 2021). In addition, it is values more generally as a restorative agent for convalescent patients. These benefits are attributed to components such as antimicrobial factors such as Lactoferrin, Lysozyme, Lactoperoxidase, and Immunoglobulin (Konuspayeva *et al.,* 2004).

Camel milk is mainly consumed raw after milking or fermented, but it is rarely processed into cheese (Konuspayeva & Faye, 2016). Compared to cow milk, camel milk contains higher levels of whey protein, lower levels of αs1-casein, and a very low κ- to β-casein ratio. According to Bekele (2023), these factors affect the technological characteristics of milk's acidic or enzymatic coagulation process, resulting in final curds that are weak, brittle and have an open body and texture. Hence, several studies have focused on the functional and coagulation properties camel milk's proteins (Fguiri *et al.,* 2021; Mbye *et al.,* 2020). Despite the above difficulties, satisfactory cheese can be produced when cheese-making procedures are tailored to the unique characteristics of camel milk (Ramet, 2001). Much work was dedicated to improving the coagulum formation process using various proteolytic enzymes from animal, plant, and microbial sources.

Milk coagulation is the primary step in manufacturing most cheeses, and calf rennet has been the most widely used milk-clotting enzyme preparation (Mohamed *et al.,* 2009a). However, the last decades have witnessed a population explosion that increased cheese production and consumption demand. On top of this, the price of calf rennet significantly increased along with the reduced supply of natural calf rennet (Mohamed *et al.,* 2010). In addition, the use of animal rennet is limited by religious

and safety considerations, change in diet (vegetarianism), or fear of consumption of foods containing genetically engineered products (Roseiro *et al.,* 2003). All these factors have necessitated the search for new proteases with high specific milk clotting activities and low general proteolytic activities to be used as a rennet substitute. Accordingly, interest has been directed toward discovering a milk-clotting enzyme that would satisfactorily replace calf rennet in cheese manufacturing, and numerous enzyme preparations of animal, microbial, and plant origin have been studied (Jacob *et al.,* 2011). Microbial rennets produced by genetically engineered bacteria have proven to be suitable substitutes for animal rennet, but increasing attention has been directed towards natural rennet extracted from plants such as *Ananas comosus*, *Carica papaya*, *Calotropis procera, Ficus carica, Calm viscera*, *Cynara cardunculus* (Roseiro *et al*., 2003), Cynara scolymus (Sidrach *et al.,* 2005), and *Solanum dubium* (Mohamed *et al.,* 2009a,b) among others. Unfortunately, most of these plant rennets were found to be inappropriate because they possess high general proteolytic activity, which leads to the production of short peptides responsible for the defect in the flavour and texture of cheese (Anusha *et al.,* 2014). An exception to this general rule is represented by the aqueous extract of *Cynara cardunculus* flowers containing two aspartic acid-type proteases, named cardosin A and B (Verissimo *et al.,* 1995), which has been used for years for the manufacture of sheep milk cheese in several areas of Portugal and Spain. However, the flowers of *Cynara cardunculus* are not used to produce cow-milk cheeses as they tend to produce a bitter taste because of the formation of several peptides identifiable in the digests of isolated bovine β- and α-casein (Macedo *et al.,* 1996). Thus, searching for a rennet substitute from plant sources with a high ratio of milk clotting to general proteolytic activity was highly needed to overcome the abovementioned problems.

Moringa oleifera is grown in rural regions of Mexico, and its different parts, such as leaves, flowers, and seeds, are edible. It is a source of protein, calcium, iron, carotenoids, and phytochemicals, and it is employed for several applications in developing countries (Idris *et al.,* 2016). Previously, it has been reported that *M. oleifera* is an interesting source of milk clotting enzymes. Pontual *et al.* (2012) reported the caseinolytic and milk-clotting activities of *M. oleifera* flowers using azocasein and skim milk as substrates, respectively. *M.oleifera* seed extract was also used as a milk-clotting agent, and the resulting curd was white and firm (Tajalsir *et al.,* 2014). Despite the studies above on milk-clotting enzymes from *M. oleifera*, a deep evaluation of this potential source of a rennet substitute is still absent. Thus, this research aimed to determine the potential ability of *M. oleifera* seeds to coagulate camel milk and to investigate the use of *M. oleifera* in the production of soft cheese. In this study, we used cow milk as a control to assess the efficiency of the coagulation activity of *M. oleifera* seeds extract.

Materiel and Methods

Milk samples

Fresh camel milk was collected from female camels (*Camelus dromedarius*) belonging to the Arid Land Institute (IRA Medenine, Tunisia). Cow milk samples were collected from a private farm. Samples were brought to the laboratory in an isotherm container and were analysed and processed upon arrival.

Collection and preparation of Moringa Oleifera seed samples

Samples of *Moringa oleifera* seeds were obtained from a private farmer in the South of Tunisia. All samples were collected and dried at room temperature in the laboratory. Thereafter, each sample was ground using an all-purpose high-speed smashing machine and stored in closed containers in a freezer at -20°C until use (Amna *et al*., 2014).

Preparation of extract

Moringa seed extract (MSE) was prepared according to the methods described by Tajalsir *et al.* (2014) and Mohamed Ahmed *et al.* (2010). Briefly, 5 g of the obtained powder was macerated in 50 ml NaCl solution (5%) by stirring at 37°C for 4 h. The mixture was filtered (filtration tissue) and then centrifuged for 10 minutes at 3500 rpm and 4°C. The sediment was neglected, and the filtrate was taken and used to estimate the enzymatic activity according to the method.

Moringa seed extract characterisation

Extraction yield

The extraction yield R constitutes the ratio of the volume of the extract obtained and the total volume of the solution prepared for extraction. This yield is given as a percentage according to the following formula:

$$R = \frac{\text{Extracted volume}}{\text{Total volume}} \times 100$$

Physicochemical characterisation

The pH and dry matter of the enzymatic extract were determined using International standard methods (Afnor, 1993). The protein content was determined

according to the Bradford method (1976), using bovine serum albumin (BSA) as the standard.

Determination of optimal temperature, pH and $CaCl_2$

The effect of temperature on the milk clotting activity (MCA) was determined using Berridge substrate solution (milk powder in $CaCl_2$ solution 0.01M, pH 6.5) according to the International Dairy Federation (IDF) (1976). Berridge solution was incubated with the appropriate extract by varying the temperature from 30 to 60°C with an interval of 5°C. In test tubes containing 10 ml of the milk, 1 ml of the enzyme extract was added, and the flocculation time was determined.

The effect of pH on MCA was tested at 30°C using Berridge substrate solutions prepared at different pH: from 5 to 8 with an interval of 0.5.

The optimal $CaCl_2$ concentration is checked by Berridge solution prepared with a calcium ion concentration range of 0.01 to 0.09 M. In the tubes containing 10 ml of $CaCl_2$ milk at pH=6.4 and T=30°C, 1 ml of extract is added, and the flocculation time is determined.

Polyphenol content

The polyphenol contents of the extracts were determined by the method described by Shetty *et al.* (2005). 1 ml of extract was transferred into a test tube and followed by the addition of 1 ml of 95% ethanol and 5 ml of H_2O. Folin-Ciocalteu reagent (diluted 1:1 with distilled water) was added to each sample, and the mixture was vortexed. Na_2CO_3 (5%, 1 mL) was added to the reaction mixture and then incubated for 60 min at room temperature. Absorbance values were determined at 725 nm. Standard curves were established simultaneously for each assay using different concentrations of gallic acid (5–60 µg/mL) in methanol. Polyphenol levels in the extracts were expressed as micrograms of gallic acid equivalents (µgGAE) per ml of the sample (Amirdivani *et al.,* 2011).

Milk-Clotting Activity

Flocculation time (FT)

10 ml of skimmed milk was put in a test tube maintained in a water bath at 30°C, and then 1 ml of the extract was added. The flocculation time is noted as soon as the first flakes appear on the tube's wall (Bergere *et al.,* 1997). The flocculation time was used to calculate coagulant activity.

Milk-clotting activity (MCA)

The coagulant activity is determined according to Berridge's method (1945), which was modified by Collin *et al.* (1977). A unit of enzymatic activity or rennet unit corresponds to the number of units of weight or volume of milk, which can be coagulated by 1 ml of coagulant preparation in 100 seconds and at 30°C (Bengana, 2001). The formula is as follows:

$$MCA = \frac{10 \times V}{Tf \times Q}$$

MCA: unit of coagulant activity
V: volume of milk used
Q: volume of coagulant extract
Tf: flocculation time

Cheese-making process

Fresh camel and cow milk were pasteurised at 65°C for 30 minutes and then cooled to 40°C. The extract was then added to 10% milk and mixed thoroughly. The mixture was incubated at 55°C until coagulation. After coagulation, the whey was drained, and the obtained camel (CAMSE) and cow (CMSE) cheeses were kept at 4°C for further analysis.

Cheeses characterization

The cheese yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{W cheese}}{\text{W milk}} \times 100$$

With:

W (Weight) milk = volume of milk (ml) × 1.03

The physicochemical characteristics were determined using International standard methods (Afnor, 1993).

As microbiological analysis, the total viable counts were determined on a plate count agar (Oxoid *et al*., UK) at 30°C for 72 h; total coliforms on violet red bile agar (Oxoid) at 30°C for 24 h; Staphylococcus on Bair Parker (Oxoid) at 37°C for 24 h; Lactic acid bacteria on MRS agar (Oxoid) at 30°C for 48h under anaerobiosis; and yeasts and moulds on Sabouraud chloromphenicol agar (Oxoid) at 30°C for 72h. Results were expressed as log colony-forming units per ml of milk or gram of cheese.

The fat-soluble vitamins and sugar content were determined by LC-MS chromatography, according to Albala Hurtado *et al.* (1997). GC-MS determined the fatty acids profile. The milk fat was extracted by centrifugation of camel milk (3500 rpm, 20 min, 4°C). Then, the milk fat was subjected to methylation using a methanol KOH (2 N) solution. The solution was mixed with hexane to extract the fatty acids methyl ester (FAME), and the supernatant was analysed using gas chromatography QP2010 Shimadzu (Tokyo, Japan) coupled with mass

spectrometry. The Fatty acids were quantified and identified using FAME internal standards (2013).

Antioxidant activity of cheese

Ferric-reducing power test

The FRAP test is a direct test of total antioxidant power. An antioxidant can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), according to the reaction described by (Oyaizu *et al.,* 1986).

One ml of the extract was mixed with 2.5 ml of 1% potassium ferrocyanide (K_3Fe) solution and 2.5 ml of 0.2 M phosphate buffer pH 6.6. The mixture was incubated at 50°C for 20 min in a water bath. The reaction was stopped by adding 2.5% trichloroacetic acid (TCA). After centrifugation at 3000 rpm for 10 min, 2.5 ml of supernatant was collected and mixed with 3 ml of distilled water and 0.5 ml of 0.1% iron chloride ($FeCl_3$) solution. The positive control is represented by a solution of a standard antioxidant: ascorbic acid. The absorbance was measured for all samples at 700 nm. An increase in absorbance corresponds to an increase in the reducing power of the sample (Wang, 2012).

DPPH radical scavenging activity

The determination of antioxidant activity by the DPPH test was carried out using the method described by (Brand-Williams *et al.,* 1995), slightly modified by (Pyo *et al.,* 2006). A DPPH (0.1 mM) solution was prepared by dissolving 3.94 mg of this product in 100 ml of methanol. Then, add 1 ml of extract or 1g of cheese and 1.5 ml of methanol. After incubation for 24 h, the extracts were centrifuged at 3500 rpm for 20 min at 4°C, then 1 ml of the DPPH solution and 1.5 ml of methanol were added to 0.5 ml of supernatant. The control tube contains 1.5 ml of the DPPH solution and 1.5 ml of methanol. The absorbance was measured at 517 nm after 30 minutes of incubation in the dark. The antioxidant activity linked to the trapping effect of the $DPPH^{\cdot}$ radical was expressed as inhibition percentage (IP) using the following formula:

$$IP = A_a - \frac{A_b - A_c}{A_a} \times 100$$

A_a: absorbance of DPPH
A_b: Sample absorbance
A_c: absorbance of the white tube

Antibacterial activity

The antibacterial activity of the selected isolates was determined by agar diffusion assay. The indicator bacteria used in this study were *E. coli, Klebsiella pneumonia: Kp, S. aureus, Salmonella tipi, Listeria inocula, Pseudomonas aeruginosa, and Enterococcus feacalis* (Lab collection).

The BHI (Brain Heart Broth) was inoculated with the pathogenic strains and then incubated for 24 hours at 37°C.

Barefoot *et al.* (1983) recommended methods include bringing extract and cheese into contact with the pathogenic indicator strain. Wells (4.5mm) were dug under sterile conditions on Muller-Hinton agar inoculated with the inducing (pathogenic) strain. The wells are filled with 80 µl of extract and cheese. The Petri dishes were put at a temperature of +4°C/4 h to allow good diffusion of the antimicrobial substance (Doumadji *et al.,* 2010). The plates were incubated at 37°C. The inhibition zone formed around the wells was examined after 24 to 48 h of incubation (Hwanhlem *et al.,* 2011).

The reading was done by measuring the diameter in mm of the inhibition zones (Zi). Inhibition was considered positive if the diameter was greater than 2 mm (Thompson *et al.,* 1996). The measurement of the inhibition diameter Zi was carried out according to the following formula:

Zi (mm) = inhibition zone diameter (mm) – Wells diameter (4,5 mm)

Statistical study

All tests were replicated three times, and the data are expressed as the mean ± standard deviation (SD). The present work's statistical analysis was carried out using XLSTAT software (2014.5.03, Addinsoft, Pearson edition, Waltham, MA, USA). Differences are considered significant at $p < 0.05$.

Result and Discussion

Characterisation of enzymatic extract

The physicochemical characteristics of MSE are presented in Table 1.

The extraction yield can be considered very interesting. The yield of a vegetal extract is influenced not only by the type of initial material but also by the extraction

Table 1. Physicochemical characteristics of enzymatic extract.

	MSE
Yield (%)	54.3±1.8
pH	4.2±0.15
Dry Matter (g/l)	58.3±2.3
Protein Content (g/l)	15.42±0.9

conditions (extraction solution, extraction duration, pH, temperature, etc.) (Benyahia, 2013).

MSE presents a high total protein content (15.42 g/l), which seems higher than that found by Muñoz *et al*. (2017), who showed a protein content in the same type of extract equal to 10.3±0.45mg/ml.

Polyphenol contents

MSE presented a polyphenol content of 148.5 µgGAE/ml. Furthermore, the leaves of *M. oleifera* were known for their richness in phenolic compounds more than Seeds (Kasolo *et al.,* 2010; Moyo *et al.,* 2011; Baba *et al.,* 2015). Indeed, it has been reported that the phytochemical composition of plants depends on several variables, such as the age of the plant, the harvest season, the harvested part and the climatic conditions (Miliauskas *et al.,* 2004; Tomas-Menor *et al.,* 2013).

Optimum conditions of the enzymatic extract

As shown in Figure 1, the temperature for the optimal clotting activity of the enzymatic extract of Moringa Seeds was 55°C. The effect of temperature proceeds mainly on the secondary phase of coagulation, specifically the aggregation step. This is due to the importance of hydrophobic interactions in the aggregation of hydrolysed micelles (Boudjneh, 2012)

The optimal clotting activity of MSE was at pH 5. Ramet (1989) reported that all enzymes used in cheese manufacture are acidic proteases, and their activity is generally optimal at pH values close to 5.5. Few studies on milk-clotting substitutes for calf rennet applied to camel milk are available. This agrees with Yonas *et al.* (2014), who reported that the highest clotting activity was observed at pH 5.0.

$CaCl_2$ concentration has no significant effect on clotting activity. Similar results were mentioned by Castillo *et al.* (2002), who considered that the main effect of $CaCl_2$ is important in aggregation and firming. Thus, it is usually added as a texturing agent. Indeed, the presence of ionised calcium is essential for the achievement of the secondary phase of milk coagulation since Ca^{2+} ions neutralise negative casein micelle residues to form a firm curd in the second phase of the coagulation process (Barrett *et al.,* 1998).

Milk clotting activity (MCA)

The obtained milk clotting activity for MSE was 2.54 RU, which was higher than that reported by Boudjinah *et al.* (2011) and Siboukeur *et al.* (2005). Their studies found

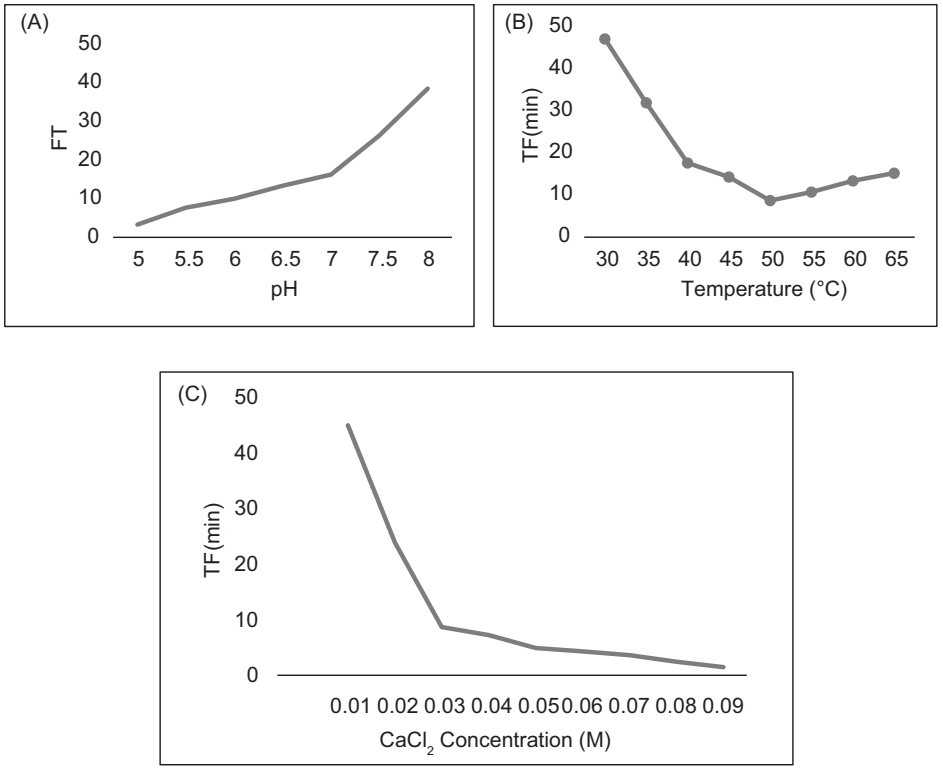

Figure 1. Optimal coagulation conditions of MSE. (A) optimum temperature; (B) optimum pH; (C) calcium chloride concentration.

Table 2. Physicochemical characteristics of camel and cow cheese coagulated with MSE.

Cheese	pH	Acidity (°D)	Dry Matter (%)	Ash (%)	Fat (%)	Fat-soluble vitamins (ppm)	
						Vit A	Vit K
CMSE	5.65±0.02	38.5±0.75	24.1±0.05	2.52±0.17	3.15±0.1	2.986±0.665	1.502±0.364
CAMSE	5.38±0.01	55±1.25	28.86±0.7	2.9±0.05	3.82±0.1	–	0.974±0.482

CMSE: Cow milk cheese made using Moringa seed extract.
CAMSE: Camel milk cheese made using Moringa seed extract.

maximum coagulant activities of 0.360 RU and 0.174 RU while working on the camel abomasum and 0.149 RU using commercial cow rennet, respectively.

Physicochemical characteristics of curd cheese

The physicochemical characteristics of camel and cow cheese with enzymatic extracts of Moringa Seeds are shown in Table 2.

Table 2 showed that CAMSE was more acidic and richer in dry, ash and fat matter than CMSE. Fresh cheeses of both types of milk were characterised by low and minimal mineral content due to the significant losses that occur during coagulation at the whey level. This is still in the range of 60–70% reported for fresh cheese (Lobato-Calleros *et al.,* 2006; Torres-Llanez *et al.,* 2006)

The major fat-soluble vitamin in prepared cow cheeses is vitamin A, which is described as an indicator of good cheese quality. In addition, the variation in the fat-soluble vitamin content of cheeses depends on the fat content of the milk used as raw materials, the addition of cream and the dry matter concentration achieved during draining (Bouraoui, 2014). Vitamin K is found in cow and camel cheeses.

Fatty acid content

The relative proportions of the different fatty acids obtained by gas chromatography are indicated in the following table (Table 3).

The composition of CAMSE revealed a low content of short and medium-chain fatty acids (from C4 to C12) and a relatively high content of long-chain fatty acids. These results support the good digestibility of this fat and a relatively important nutritional criterion (Gnan & Sheriha, 1986; Farah & Ruegg, 1989; Gorban & Izzeldin, 2001). The level of palmitic acid (C16:0) in CAMSE (32.393%) was similar to that reported in camel milk (31.45 %, according to (Attia *et al.,* 2000).

Table 3. Composition of cow and camel cheeses in fatty acids.

	Cow cheese	Camel cheese
C4:0	3.7±0.86	2.61±1.549
C6:0	0.754±0.696	0.702±0.595
C8:0	0.452±0.344	0.398±0.308
C10:0	0.961±0.871	0.92±0.777
C12:0	1.429±0.875	1.365±0.832
C13:0	0.232±0.206	0.033±00
C14:0	10.852±0.834	10.576±0.845
C14:1	1.146±0.011	0.999±0.108
C15:0	1.042±0.006	0.98±0.086
C15:1	0.093±00	*
C16:0	31.995±0.012	32.393±0.641
C16:1	6.462±4.576	6.246±4.467
C17:0	0.506±0.053	0.467±0.076
C17:1	0.315±0.092	0.262±0.049
C18:0	12.188±0.971	11.222±00
C18:1	25.966±1.305	25.563±1.306
C18:2	1.015±0.836	2.77±0.731
C18:3	0.206±0.011	0.167±0.004
C20:0	0.35±0.096	0.222±00
C20:1	0.103±0.002	0.084±0.008
C20:3	0.099±0.015	*
C20:4	0.142±0.025	0.136±0.014
∑SFA	67.009	61.868
∑UFA	32.991	38.132

CAMSE contained 61.868% of saturated fatty acids and 38.132% of unsaturated fatty acids.

Sugar composition

The sugar contents of the obtained cheeses are mentioned in Table 4.

CMSE are richer in lactose than camel cheeses. This richness can be attributed to cow milk's higher lactose content than camel milk (Konuspayeva *et al.,* 2008).

Microbiological quality

The microbiological quality of cow and camel cheeses is shown in Table 5.

The microbiological quality of the cheeses shows that cow cheese is richer in total aerobic mesophilic flora than camel cheese. This may be due to antibacterial proteins in camel milk (Jrad *et al.,* 2013). Indeed, the total flora is a usual criterion for the hygiene of processes in factories. According to Martin (2012), their evidence in foods means inadequate or too careful conservation of raw materials. Despite the high load of total mesophilic flora, the number of germs found remains below the thresholds indicated by the Tunisian standards relating to the microbiological specifications of milk and derived products (NT 16.40. 1988) (10^4) CFU/ml)).

The bacterial flora present in cheeses can originate from the starting milk, the equipment, and the production environment or from the addition of lactic ferments during manufacturing (Ozturkoglu-Budak *et al.,* 2017; Pyz-Lukasik *et al.,* 2018).

Table 4. Composition of cheeses obtained in sugars (g/L).

	Camel cheese	Cow cheese
Fructose	0.14±0.03	0.17±00
Glucose	0.14±00	0.15±00
Sucrose	0.17±0.01	0.15±0.05
Lactose	7.04±0.70	9.33±3.10

Antioxidant activity

The results of the antioxidant activity of MSE and the camel and cow cheeses are represented in Figure 2. The MSE presents a high antioxidant activity (33.64%), which agrees with the results of Dalei *et al.* (2016). With different methods (DPPH and Frap test), camel cheeses' anti-radical activity is higher than cow cheeses. This can be attributed to the richness of camel milk in vitamin C.

Antibacterial activity

Figure 3 represents the results of the antibacterial activity of an enzymatic extract of Moringa Seeds and camel and cow cheeses.

The MSE shows an inhibitory effect on all strains tested with an inhibition diameter varying between 11 mm ±1 and 15.5 mm ±0.5.

Indeed, in plants, contamination by pathogenic microorganisms leads to a sharp increase in the levels of phenolic compounds, which corresponds to establishing the plant's defence mechanism (Macheix *et al.,* 2006; Meziani

Table 5. Microbiological quality of cow and camel cheeses with Moringa seeds.

	FMAT (UFC/g)	CT (UFC/g)	MY (UFC/g)	Staphylococcus (UFC/g)	LAB (UFC/g)
Camel cheese	2.38±0.11 × 10^3	6.18±0.04 × 10^2	0	3.54 ±0.06 × 10^2	2.58±0.15 × 10^3
Cow cheese	6.81±0.26 × 10^3	9.54±0.18 × 10^2	0	2.50±0.16 × 10^2	1.23±0.23 ×10^3

FMAT: total aerobic mesophilic flora; CT: Total coliforms MY: Molds and Yeast; LAB: Lactic Acid Bacteria.

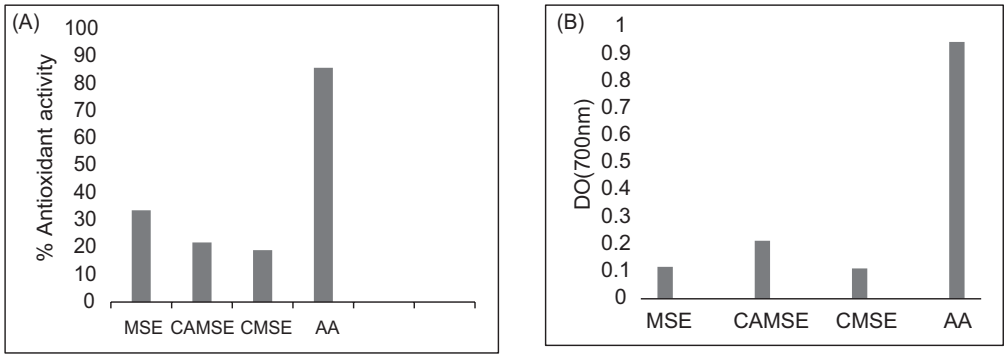

Figure 2. Antioxidant activity of MSE and obtained cheese. (A) DPPH test. (B) Frap test with MSE: enzymatic extract of Moringa seeds; CAMSE: Camel cheese with Moringa seeds extract; CMSE: Cow cheese with MSE; AA: ascorbic acid.

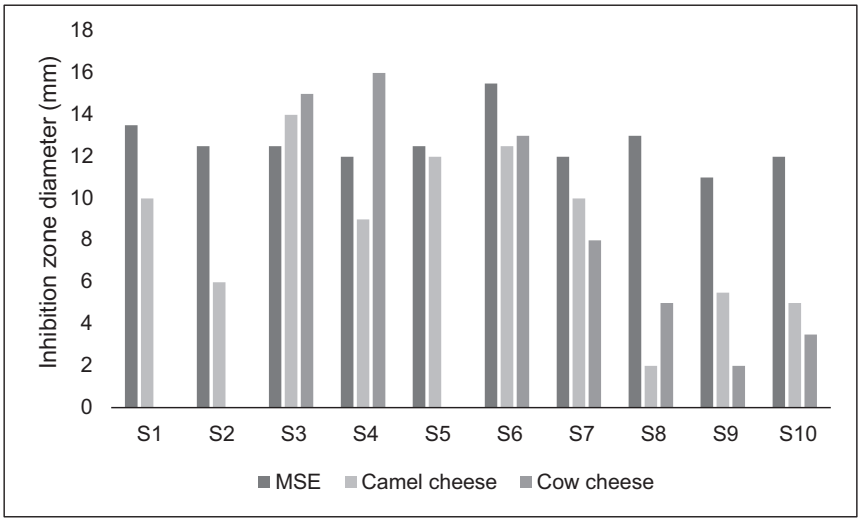

Figure 3. Antibacterial activity of MSE and camel and cow cheese against 10 pathogenic strains. S1: *Streptococcus pyogenes*. S2: *Listeria inocua*. S3: *Pseudomonas aeruginosa*. S4: *Escherichia coli*. S5: *Candida Albicans*. S6: *Micrococcus Luteus*. S7: *Staphylococcus aureus*. S8: *Klebsiella pneumoniae*. S9: *Salmonella typhi*. S10: *Enterococcus faecalis*.; MSE: Moringa Seeds extract; CAMSE: Camel Cheese with enzymatic seeds. CMSE: Cow cheese with enzymatic seeds.

et al., 2015). Some authors have noted that the antibacterial activity of phenolic compounds is probably due to their ability to combine with extracellular soluble proteins and, thus, with bacterial cell walls (Tsuchiya *et al.,* 1996).

Figure 4 shows camel cheese has strong activity against Pseudomonas aeruginosa, Candida albicans, and Micrococcus luteus, with an inhibition zone ranging from 12±0.5 to 14±2mm. It also has middling activity against Streptococcus pyogenes, Escherichia coli, and Staphylococcus aureus (inhibition diameter between 9±1 and 10±0.5mm) and low activity against Listeria inocula, Salmonella typhi, and *Enterococcus faecalis*.

The antibacterial activity of camel cheese was higher than that of cow cheese, which can be due to the combination of MSE seeds and the antibacterial activity of camel milk (Jrad *et al.,* 2013).

Conclusion

The extract of Moringa *oleifera seeds* revealed a high milk-clotting activity on camel milk and high antioxidant and antibacterial effects.

Moringa oleifera seed extract can be used as an alternative to rennet to produce camel milk cheese. However, further studies are requested to complete the purification and characterisation of this promising extract.

References

AFNOR. (1993). Contrôle de la qualité des produits alimentaires: lait et produits laitiers : analyses physicochimiques (4th ed.). AFNOR. Paris. France. p. 581.

Albala-Hurtado S., Veciana-Nogues M.T., IzquierdoPulido M., & Mariné-Font A. (1997). Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. Journal of Chromatography A, *778*, 247–253. https://doi.org/10.1016/s0021-9673(97)00387-7

Amirdivani Sh., Hj Baba A.S. (2011). Changes in yogurt fermentation characteristics. Moreover, antioxidant potential and in vitro inhibition of angiotensin-1 converting enzyme upon the inclusion of peppermint. dill and basil. LWT, 44(6), 1458–1464. https://doi.org/10.1016/j.lwt.2011.01.019

Amna E.T., Ebraheem A.S.A., Abdelmoez M.A., Fatima J. Khider. F.J,. Mohamed O.E., et al. (2014). Partial purification of milk clotting enzyme from the seeds of *Moringa oleifera*. Journal of Microbiology Biotechnology and Food Sciences, 4(1), 58–62. https://doi.org/10.15414/jmbfs.2014.4.1.58-62

Anusha R. Singh M.K., & Bindhu O.S. (2014). Characterization of potential milk coagulants from *Calotropis gigantean* plant parts and their hydrolytic pattern of bovine casein. European Food Research and Technology, 238, 997–1006. https://doi.org/10.1007/s00217-014-2177-0

Ashraf A., Mudgil P., Palakkott A., Iratni R., Gan C.Y., Maqsood S., et al. (2021). Molecular basis of the anti-diabetic properties of camel milk through profiling of its bioactive peptides on dipeptidyl peptidase IV (DPP-IV) and insulin receptor activity. Journal of Dairy Science, 104(2021), 61–77. https://doi.org/10.3168/jds.2020-18627.

Attia H., Kherouatou N., Nasri M., & Khorchani T. (2000). Characterization of the dromedary milk casein micelle and study of its changes during acidification. Le Lait, 80, 503–515. https://doi.org/10.1051/lait:2000141

Baba M.D., Yakubu G., Yelwa J.M., & Haruna L. (2015). Costs and Returns of Moringa (*Moringa oleifera*) Production in Zuru Local Government Area of Kebbi State. Nigeria. New York Science Journal, 8(1), 36–40.

Barefoot S.F., & Klaenhammer T.R. (1983). Detection and activity of lacticin B. a bacteriocin produced by *Lactobacillus acidophilus*. Applied and Environmental Microbiology, 45, 1808–1815. https://doi.org/10.1128/aem.45.6.1808-1815.1983

Barrett A.J., Rawlings N.D., & Woessner J.F. (1998). Handbook of proteolytic enzymes (pp. 843–846). Academic Press: SanDiego. CA. USA.

Bekele B. (2023). Innovative approach of cheese making from camel milk: a review. IntechOpen, https://doi.org/10.5772/intechopen.108700

Bengana M. (2001). Isolement, purification et caractérisation des enzymes protéolytiques (pepsine. chymosine) issues de caillettes de bovins adultes; incorporation de ces préparations dans la fabrication du fromage à pâte molle type camembert à la laiterie de Draa Ben Khada (Thèse de Magister). Institut National Agronomique.El-Harrach.Alger.

Benyahia F.A. (2013). Thèse. Extraction de la pepsine et utilisation dans l coagulation du lait en vue d'une valorisation des proventricules de volailles au profit de la filière lait en Algérie (pp. 173). Université de Constantine.

Berridge N.J. (1945). The purification and crystallization of rennin. The Biochemical Journal, 39, 179–186. https://doi.org/10.1042/bj0390179

Boudjenah H.S. (2012). Aptitudes à la transformation du lait de chamelle en produits dérivés: effet des enzymes coagulantes extraites de caillettes de dromadaires (PhD. Thesis). Universite Mouloud Mammeri de TiziOuzou (Algérie). Tizi Ouzou. Algeria.

Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999

Brand-Williams W., Cuvelier M.E., & Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Castillo A.R., Meehl J.B., Morgan G., Schutz-Geschwender A., & Winey M. (2002). The yeast protein kinase Mps1p is required for assembly of the integral spindle pole body component Spc42p." Journal of Cell Biology, 156(3), 453–465. https://doi.org/10.1083/jcb.200111025

Collin J.C. Grappin R., & Legraet Y. (1977). Etude de la méthode de mesure selon BERRIDGE. du temps de coagulation du lait additionné d'une solution enzymatique. Revue Laitière Française, 355, 389–394.

Dalei J., Rao V., Sahoo D., Rukmini M., & Ray R. (2016). Review on nutritional and pharmacological potencies of Moringa oleifera. European Journal Of Pharmaceutical and Medical Research 3(1):150–155

De Lima C.J., Coelho L.F. & Contiero J. (2010). The use of response surface methodology in optimization of lactic acid production: focus on medium supplementation. temperature and pH control. Food Technology and Biotechnology, 48(2), 175–181.

Doumandji A., Hellal A., & Saidi N. (2010). Purification de la bactériocine à partir de Lactobacillus acidophilus 11. Revue de Microbiologie Industrielle, Sanitaire, et Environnementale, 4(2), 25–47.

Farah Z. & Rüegg M.W. (1989). The size distribution of casein micelles in camel milk. Food Microstructure, 8, 211–216. https://doi.org/10.1017/S0022029900024183

Fguiri I., Atigui M., Sboui A., Arroum S., Marzougui Ch., Dbara M., et al. (2021). Camel Milk-Clotting Using Plant Extracts as a Substitute to Commercial Rennet. Journal of Chemistry, 2021, 6680246. https://doi.org/10.1155/2021/6680246

Gnan S. O., & Sheriha A. M. (1986). Composition of Libyan camel milk. Australian Journal of Dairy Technology, 41, 33–35.

Gorban A.M.S., & Izzeldin O.M. (2001). Fatty acids and lipids of camel milk and colostrum. International Journal of Food Sciences and Nutrition, 52(3), 283–287. https://doi.org/10.1080/713671778

Hwanhlem N., Buradaleng S., Wattanachant S., Benjakul S., Tani A., & Maneerat S. (2011). Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (Plasom) and production of Plasom from selected strains. Food Control, 22(3–4), 401–407. https://doi.org/10.1016/j.foodcont.2010.09.010

Idris M.A., Jami M.S., Hammed A.M., & Jamal P. (2016). Moringa oleifera seed extract: A review on its environmental applications. International Journal of Applied Environmental Sciences, 11, 1469–1486.

Jacob M., Jaros D., & Rohm H. (2011). Recent advances in milk clotting enzymes. International Journal of Dairy Technology, 64(1), 14–33. https://doi.org/10.1111/j.1471-0307.2010.00633.x

Jrad Z., El Hatmi H., Fguiri I., Arroum S., Assadi M., & Khorchani T. (2013). Antibacterial activity of Lactic acid bacteria isolated from Tunisian camel milk. New Microbes and New Infections, 7(12), 1002–1008. https://doi.org/10.5897/AJMR12.488

Kasolo J.N., Bimenya G.S., Ojok L., Ochieng J., & Ogwal-Okeng J.W. (2010). Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities. Journal of Medicinal Plants Research, 4(9), 753–757. https://doi.org/10.5897/JMPR10.492

Konuspayeva G., Loiseau G., & Faye B. (2004). La plus-value «santé» du lait de chamelle cru et fermenté : l'expérience du Kazakhstan. Rencontres autour des Recherches sur les Ruminants, 11.

Krishnankutty R., Iskandarani A., Therachiyil L., Uddin S., Azizi F., Kulinski M., Bhat A.A., & Mohammad R.M. (2018). Anti-cancer activity of camel milk via induction of autophagic death in human colorectal and breast cancer cells. Asian Pacific Journal of Cancer Prevention, 19. 3501. https://doi.org/10.31557/APJCP.2018.19.12.3501

Lobato-Calleros C., Ramos-Solis L., Santos-Moreno A., & Rodriguez-Huezo M. (2006). Microstructure and texture of panela type cheese-like products: use of low methoxyl pectin and canola oil as milk-fat substitutes. Revista Mexicana de Ingeniería Química, 5, 71–79. 2006.

Macedo I.Q., Faro C.J., & Pires E.M. (1996). Caseinolytic Specificity of Cardosin. An Aspartic Protease from the Cardoon *Cynara*

cardunculus L. Action on Bovine β- and α-Casein and Comparison with Chymosin. Journal of Agricultural and Food Chemistry, 44, 42–47. https://doi.org/10.1021/jf9406929

Macheix J.J. Fleuriet A., & Jay-Allemand C. (2005). Les composés phénoliques des végétaux. Un exemple de métabolites secondaires d'importance économique. Presses Polytechniques et Universitaires Romandes, CH-1015, 192.

Martin Rosset W. (2012). Nutrition et alimentation des chevaux. Nouvelles recommandations alimentaires de l'Inra. Ouvrage collectif. Éditions Quæ. pp. 1952–1251.

Mbye M., Sobti B., & Al Nuami K.M. (2020). Physicochemical properties. sensory quality. and coagulation behaviour of camel versus bovine milk soft unripened cheeses. NFS Journal, 20, 28–36. https://doi.org/10.1016/j.nfs.2020.06.003

Meziani S., Oomah B.D., Zaidi F., Simon-Levert A., Bertrand C., & Zaidi-Yahiaoui R. (2015). Antibacterial activity of carob (*Ceratonia siliqua L.*) extracts against phytopathogenic bacteria *Pectobacterium atrosepticum*. Microbial Pathogenesis, 78, 95–102. https://doi.org/10.1016/j.micpath.2014.12.001

Miliauskas G., Venskutonis P.R. & Van Beek T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry, 85, 231–237. https://doi.org/10.1016/j.foodchem.2003.05.007

Mohamed Ahmed I.A., Morishima I., Babiker E.E. & Mori N. (2009a). Characterization of partially purified milk-clotting enzyme from *Solanum dubium Fresen* seeds. Food Chemistry, 116 (2), 395–400. https://doi.org/10.1016/j.foodchem.2008.11.072

Mohamed Ahmed I.A., Morishima I., Babiker E.E. & Mori N. (2009b). Dubiumin. a chymotrypsin like serine protease from the seeds of *Solanum dubium Fresen*. Phytochemistry, 70(4), 483–491. https://doi.org/10.1016/j.phytochem.2009.01.016

Mohamed Ahmed I.A., Babiker E.E., & Mori N. (2010). pH stability and influence of salts on activity of a milk-clotting enzyme from *Solanum dubium* seeds and its enzymatic action on bovine caseins. LWT-Food Science and Technology, 43, 759–764. https://doi.org/10.1016/j.lwt.2009.12.011

Morrin S.T., Buck R.H., Farrow M., Hickey R.M. (2021). Milk-derived anti-infectives and their potential to combat bacterial and viral infection. Journal of Functional Foods, 81, 104442. https://doi.org/10.1016/j.jff.2021.104442

Moyo B., Masika P.J., Hugo A., & Muchenje V. (2011). Nutritional characterization of Moringa (*Moringa oleifera Lam.*) leaves. African Journal of Biotechnology, 10(60), 12925–12933. https://doi.org/10.5897/AJB10.1599

Ozturkoglu-Budak S., & De Vries R.P. (2017). Mold-ripened and raw milk cheeses: Production. risks. and benefits to human health. Dairy in Human Health and Disease across the Lifespan. Elsevier, 353–361. https://doi.org/10.1016/B978-0-12-809868-4.00027-3

Paquot, C. (2013). Standard Methods for the Analysis of Oils, Fats and Derivatives. Elsevier.

Pyo Y.H., Lee T.C., & Lee Y.C. (2006). Effect of lactic acid fermentation on enrichment of antioxidant properties and bioactive isoflavones in soybean. Journal of Food Science, 70(3), S215–S220. https://doi.org/10.1111/j.1365-2621.2005.tb07160.x

Pyz-Łukasik R., Knysz P. & Gondek M. (2018). Hygiene quality and consumer safety of traditional short- and long-ripened cheeses from Poland. Journal of Food Quality, 2018, 1–7. https://doi.org/10.1155/2018/8732412

Ramet J.P. (1989). Production de Fromages à Partir de Lait de Chamelle en Tunisie (pp. 1–33). FAO. Rome. Italy.

Ramet J.P. (1997). Technologie comparée des differents types de caillés. in Le Fromage. A. Eck & J. C. Gillis. (Eds.). Technique & Documentation (pp. 333–364). Lavoisier. Paris. France. 1997.

Ramet J.P. (2001). The Technology of Making Cheese from Camel Milk (Camelus dromedarius). Food Agriculture Organisation. Rome. Italy.

Roseiro L.B., Barbosa M.M., Ames J. & Wilbey R. (2003). Cheese making with vegetable coagulants; the use of *Cynara cardunculus* L. for the production of ovine milk cheeses. International Journal of Dairy Technology, 56, 76–85. https://doi.org/10.1046/j.1471-0307.2003.00080.x

Sánchez-Muñoz M.A., Valdez-Solana M.A., Avitia-Domínguez C., Ramírez-Baca P., Candelas-Cadillo M.G., Aguilera-Ortíz M., et al. (2017). Utility of milk coagulant enzyme of *Moringa oleifera* seed in cheese production from soy and skim milks. Foods, 6(8), 62. https://doi.org/10.3390/foods6080062

Sboui A., Atig C., Khabir A., Hammadi M., & Khorchani T. (2022). Camel milk used as an adjuvant therapy to treat type 2 diabetic patients: effects on blood glucose. HbA1c. cholesterol. and TG levels. Journal of Chemistry, 5860162(2022), 1–6. https://doi.org/10.1155/2022/5860162

Shetty Sh., Secnik K., & Oglesby A.K. (2005). Relationship of glycemic control to total diabetes-related costs for managed care health plan members with type 2 diabetes. Journal of Managed Care Pharmacy, 11(7), 559–64. https://doi.org/10.18553/jmcp.2005.11.7.559

Siboukeur O., Mati A., & Hessas B. (2005). Amélioration de l'aptitude à la coagulation du lait cameline (*Camelus dromedarius*): utilisation d'extraits enzymatiques coagulants gastriques de dromadaires. Cahiers Agricultures, 14(5). 473–478.

Sidrach L., García-Cánovas F., Tudela J., & Rodríguez-López J.N. (2005). Purification of cynarases from artichoke (*Cynara scolymus* L.): Enzymatic properties of cynarase A. Phytochemistry, 66(2005), 41–49. https://doi.org/10.1016/j.phytochem.2004.10.005

Silvestre M.P.C.. Carreira R.L., Silva M.R., Corgosinho F.C., Monteiro M.R.P., & Morais H.A. (2012). Effect of pH and temperature on the activity of enzymatic extracts from pineapple peel. Food and Bioprocess Technology, 5(5), 1824–1831. https://doi.org/10.1007/s11947-011-0616-5

Tajalsir A.E., Ebraheem A.S., Abdallah A.M., Khider F.J., Elsamani M.O., & Ahmed I.A.M. (2014). Partial purification of milk-clotting enzyme from the seeds of *Moringa oleifera*. Journal of Microbiology, Biotechnology and Food Sciences. 4, 58. https://doi.org/10.15414/jmbfs.2014.4.1.58-62

Thompson J.K., Collins M.A., & Mercer W.D. (1996). Characterization of a proteinaceous antimicrobial produced by *Lactobacillus helveticus* CNRZ 450. Journal of Applied Bacteriology, 80, 338–348. https://doi.org/10.1111/j.1365-2672.1996.tb03229.x

Tomás-Menor L., Morales-Soto A., Barrajón-Catalán E., Roldán-Segura C., Segura-Carretero A., et al. (2013). Correlation

between the antibacterial activity and the composition of extracts derived from various Spanish *Cistus* species. Food and Chemical Toxicology, 55, 313–322. https://doi.org/10.1016/j.fct.2013.01.006

Torres-Llanez M.J., Vallejo-Cordoba B., Dıaz-Cinco M.E., Mazorra-Manzano M.A., & Gonzalez-Cordova A.F. (2006). Characterization of the natural microflora of artisanal Mexican Fresco cheese. Food Control, 17(9), 683–690. https://doi.org/10.1016/j.foodcont.2005.04.004

Tsuchiya H., Sato M., Miyazaki T., Fujiwara S., Tanigaki S., Ohyama M., Tanaka T., & Iinuma M. (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. Journal of Ethnopharmacology, 50(1), 27–34. https://doi.org/10.1016/0378-8741(96)85514-0

Veisseyre R. (1979). Technologie du Lait: Constitution. R´ecolte. Traitement et Transformation du Lait (pp. 9–33). La Maison Rustique. Paris. France.

Verissimo P., Esteves C., Faro C., & Pires E. (1995). The vegetable rennet of *Cynara cardunculus* L. contains two proteinases with chymosin and pepsin-like specificities. Biotechnology Letters, 17, 614–645. https://doi.org/10.1007/BF00129389

Wang H., Ullah M.M., Klaser A., Laptev I. & Schmid C. (2009). Evaluation of local spatio-temporal features for action recognition. Presented at BMVC 2009-British Machine Vision Conference. https://doi.org/10.5244/C.23.124

Yonas H., Eyassu S., & Zelalem Y. (2014). Clotting activity of camel milk using crude extracts of ginger (*Zingiber officinale*) rhizome. African Journal of Food Science and Technology, 5(3), 90–95. https://doi.org/10.14303/ajfst.2013.047