Journal of Animal & Veterinary Advances
MEDWELL PUBLICATIONS

Camel Milk-Clotting Properties of Latex Protease from *Ficus carica*

[1]Imen Fguiri, [1]Amel Sboui, [1, 3]Naziha Ayeb, [2]Manel Ziadi, [1]Meriem Guemri, [1]Samira Arroum, [1]Mohamed Dbara and [1]Touhami Khorchani
[1]*Laboratory of Livestock and Wild Life Institute of Arid lands (IRA Medenine), 4119 Medenine, Tunisia*
[2]*Laboratory of Microbial Ecology and Technology (LETMi), National Institute of Applied Sciences and Technology (INSAT), BP 876, 1080 Tunis, Tunisia.*
[3]*Regional Center for Agricultural Research (CRRA) Sidi Bouzid, Tunisia*

Key words: Camel milk, *ficus carica*, latex, cheese, proteolytic activity, extracted

Corresponding Author:
Naziha Ayeb
Regional Center for Agricultural Research (CRRA), Sidi Bouzid, Tunisia

Page No.: 99-106
Volume: 19, Issue 7, 2020
ISSN: 1680-5593
Journal of Animal and Veterinary Advances

Abstract: This research aimed to prospect latex fractions from *Ficus carica* for new plant peptidases with milk-clotting activities of camel milk, for use as rennet alternatives. Latex fractions, extracted from the fig tree (*Ficus carica*), show proteolytic and milk-clotting activity. The enzymatic preparation was obtained by fractionation of latex from fig tree by FPLC; having a proteolytic activity of 23491.24 IU L^{-1}. After manufacturing process, *Ficus carica* latex protease with the ability to coagulate milk can be used as alternatives to commercial animal chymosin in the cheese manufacturing process. The cheese yield is determined at different enzymes doses and it was found that 1mL of the enzyme extract in 100 mL of camel milk gives a yield of 15%. The physicochemical and microbiological characterization of camel milk cheese compared to cow milk cheese showed that camel milk cheese was more acidic, richer in protein (50.04 g L^{-1}) but less loaded with total mesophilic flora.

INTRODUCTION

Plant proteases play crucial roles against unfavorable conditions including water and environmental stress[1]. These proteases were used in different industries including pharmaceutical and food industry for bioactive peptides production and meat tenderization[2, 3].

At the moment several examples of the use of enzymes and of specifically proteases in different areas from the industry can be mentioned: modified proteins for the food industry, baking, beer elaboration, cheese production, detergent dust preparation, treatment of industrial effluents, textile industry, manufacture of leather, pharmaceutical industry, cleaning of surgical supplies and biomedicals[4]. Species belonging to the Moracées family usually contain proteolytic enzymes in latex. Latex is a milky fluid with a complex mixture of constituents, like proteins, vitamins, carbohydrates, lipids, terpenes, alkaloids and free amino acids. The presence of some enzymes like chitinases and proteases in latex vacuoles suggest that they may help plants for defense against pathogens, parasites and herbivores by attacking the invader once the plant cell is lysed[5].

Camel milk has been used fresh or fermented form in different regions of the world. In the world, camel milk is better known for its fermented products: shubat in

Kazakhstan; chal in Turkmenistan; khoormog in Mongolia; gariss in Sudan; suusac in Kenya, zrig-in Mauritania, rather than for its types of cheeses: chuku in Niger or caravan in Mauritania, fresh camel cheese in Morocco[6].

Camel milk is technically more difficult to process than milk from other animals. However, satisfactory cheese can be made when cheese-making procedures are adapted to camel milk's particular characteristics[7].

Cheese making technology aims to preserve milk, so that, consumption can be postponed for periods from few days to several months. The preservation of the product is obtained mainly through lactic acidification and limited dehydration. However, the processing of camel milk into cheese is technically more difficult than milk from other domestic dairy animals. This is mainly due to its low total solids content, unique composition and casein properties. Its suitability for cheese making decreases significantly in the hot season when camel milk production is influenced by water and feed availability, as under water shortage conditions camel milk contains abnormally low milk solids and its cheese processing ability is poor[8]. In spite of the above difficulties, efforts have been made for cheese preparation from camel milk. Thus, the objective of this research is the preparation of fresh cheese from camel milk using enzymatic extract of fig tree latex (*Ficus carica*) as coagulation enzymes.

MATERIALS AND METHODS

Sampling: Milk samples were collected from camels (*Camelus dromadarius*) belonging to the herd of the Arid lands Institute (IRA Medenine) and from cows of the southern region of Tunisia. The samples were brought to the laboratory in an isotherm container and were analyzed upon arrival.

The latex sample of *Ficus carica* was collected from different varieties of southern Tunisian fig (Bayoudhi, Ragoubi, Magouli Abiadh), early in the morning by superficial incision of stem or leaves of healthy plants and allowing the milky latex to drain in clean glass vials separately, brought to the laboratory and kept in refrigerator till the experiment started.

Preparation of crude enzyme: Latex was homogenized and filtered. Filtrate latex sample was centrifuged at 7000 rpm for 30 min at 4°C. The resulting supernatant of latex enzyme called "Crude enzyme" or "Centrifugal fraction" was used for further investigation of protease enzyme assay[9].

Solvent precipitation: The crude extract was subjected to protein precipitation by adding 3 volumes of chilled acetone slowly to 1 volume of crude extract and the precipitate was separated by centrifugation at 7000 rpm for 30 min at 4°C. The precipitate was dissolved in a minimum volume of water and the protein was precipitated again by adding chilled acetone. After a centrifugation (7000 rpm, 30 min, 4°C), the precipitate was collected and dried at room temperature. The fraction obtained was used for proteins estimation and enzyme assay.

Protein determination: The content of proteins was determined following the method of Lowry *et al*.[10] using Bovine Serum Albumin (BSA) as standard. The protein content of the column eluent was also monitored spectrophotometrically at 280 nm.

Proteolytic activity: The proteolytic activity was performed using casein as substrate as described by Kunitz[11]. The enzyme solution (1 mL) was mixed with 1 mL of casein solution and kept at 50°C for 10 min. The reaction was stopped by adding 5.0 mL of 5.0% w/v TCA solution. The absorbance of the clear filtrate was measured at 280 nm against blank solution.

The unit of proteolytic activity was defined as the amount of protease which caused an increase of one unit of absorbance per minute of enzymatic reaction.

Purification of enzymatic extract: The enzymatic extract was purified using a Fast Protein Liquid Chromatography system (AKTA purifier GE (Healthcares, Sweden)). The enzymatic extract was injected into the mono Q 5/50 GL anion exchange column at a flow rate of 1 mL min for 30 min. Elution of the protein fractions were ensured using two solvents (A) (20 mM Tris pH 7.7) and (B) (20 mM Tris pH 7.7+1M NaCl). After collection, the absorbance at 280 nm and protease content of each fraction were determined.

Optimum pH and temperature of the enzyme activity: Optimum pH and temperature were determined according to the method of Kunitz[12]. To study the effect of pH on enzyme activity, the purified enzyme solution was incubated with casein solution of various pH values (4.5-8.0) at 50°C for 10 min and activities were measured. In order to determine the optimum temperature, the purified enzyme solution prepared in 0.02 M phosphate buffered saline PBS (8 g L^{-1} NaCl, 0.2 g L^{-1} KCl, 1.44 g L^{-1} Na_2HPO_4, 0.24 g L^{-1} KH_2PO_4, at pH 7.2) was incubated with casein solution at various temperatures ranging from 40-70°C for 10 min in a controlled temperature water bath and their activities were tested.

Cheese manufacture: The milk was pasteurized at 65°C for 30 min. The temperature of milk was brought down to 40°C. The starter culture (*Lactococcus lactis*) isolated from artisanal fermented milk was then added to decrease pH at 5.5. After about 1 h, the enzymatic preparation was then added at the rate of 1 mL L^{-1} of milk and mixed

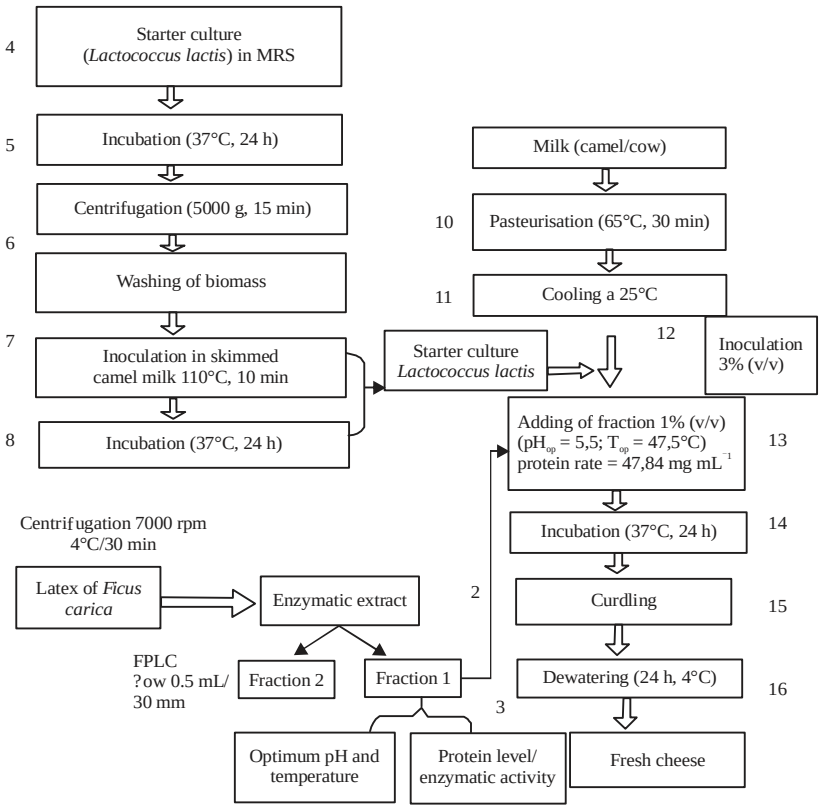

Fig. 1: Diagram of production of fresh cheese with enzymatic extract of fig tree latex

thoroughly. The mixture was incubated for 24 h at 37°C after coagulation, the whey was drained to obtain a fresh cheese (Fig. 1).

Physiochemical and microbiological analysis: The physicochemical characteristics of milk were determined using International standard methods. The protein content was determined according to the Bradford method using Bovine Serum Albumin (BSA) as standard.

As to microbiological analyses, the total viable counts were determined on plate count agar (Oxoid Ltd., Basingstoke, UK) at 30°C for 72 h, total coliforms on violet red bile agar (Oxoid) at 30°C for 24 h, mesophilic and thermophilic lactobacilli on MRS agar (Oxoid) at 30 and 45°C for 48 h under anaerobiosis, respectively, lactococci on M17 agar (Oxoid) at 30°C for 48 h, yeasts and molds on Worth agar (Oxoid) at 30°C for 72 h. All determinations were made in duplicate and expressed as log colony-forming units per gram of cheese.

Lipid extraction, fatty acid analysis and vitamins composition: Fat were extracted with chloroform and methanol as described by Bligh and Dyer[12] and the fat content was determined from dried lipid weights. Fatty acids were identified by using FAME standards and expressed as mg/g of fat.

The fat-soluble and water-soluble vitamins were determined by LC-MS chromatography according to Albala-Hurtado[13].

Statistical analysis: Statistical analyses were performed using SPSS 14.0 Software (SPSS Inc.; Chicago, IL, USA). Significant differences among treatments were tested by ANOVA followed by Duncan test with a level of significance at $p = 0.05$. Data were expressed as mean values ±Standard Deviation (SD). All experiments were performed in duplicate and repeated three times.

RESULTS AND DISCUSSION

Milk composition

Physicochemical composition: The physicochemical characteristics of camel and cow's milk are given in Table 1. A significant difference between cow and camel milk composition was shown only in dry matter content.

Microbiological quality: The microbiological quality of camel and cow's milk was showed in Table 2. The

Table 1: Physicochemical characteristics of 2 types of milk (cow and camel)

Variables	Cow milk	Camel milk	p-values
pH	6.533^{a}±0.20	6.445^{a}±0.240	0.594
Acidity (°D)	14.800^{a}±1.374	16.970^{a}±1.773	0.286
Density	1.029^{a}±0.001	1.027^{a}±0.001	0.366
Viscosity (Cp)	2.886^{a}±0.176	3.320^{a}±0.190	0.694
Fat (g L^{-1})	26.443^{a}±5.317	23.886^{a}±1.109	0.425
MD(g L^{-1})	112.543^{a}±3.910	111.930^{b}±18.858	0.032
Ash(g L^{-1})	7.774^{a}±0.585	9.160^{a}±0.087	0.220
Protein(g L^{-1})	31.363^{a}±4.484	36.323^{a}±0.890	0.220

F: Fat; MD: Dry Matter; ^{a,b}means in the same line followed by the same letter are not statistically different p>0.05; NS: Not significant

Table 2: Microbiological quality of cow and camel milk

Variables	TAPC (CFU mL^{-1})	Coliforms (CFU mL^{-1})	Yeast and molds (CFU mL^{-1})	LAB (CFU mL^{-1})
Cow milk	9.166^{a} 10^{5}±0,101	9.33^{a}±0.251	2.83^{a} 10^{4}±0.473	2.54^{a} 10^{4}±0.221
Camel milk	1.75^{a} 10^{4}±0,266	4.66^{a}±0.463	7.33^{a} 10^{3}±0.64	1.25^{a} 10^{5}±0.107
p	0.140	0.218	0.212	0.186

TAPC: Total Aerobic Plat Count; LAB: Lactic Acid Bacteria; ^{a,b}means in the same line followed by the same letter are not statistically different p>0.05; NS: Not significant

Table 3: Protein content, optimum pH and temperature and proteolytic activity of crude enzyme

Protein content	Proteolytic activity	Optimum pH	Optimum temperature
47.84 mg mL^{-1}	2231,9 UI L^{-1}	[5-6]	[45-50°C]

Table 4: Cheese yield (%) according to the fraction dose

Milk	Fraction volume (mL fraction/100 mL milk)	Yield (%)
Camel	1	15.6
	2	13.5
	3	11.4
	4	11.2
Cow	1	12.1
	2	11.4
	3	11.2
	4	11.1

bacterial load is lower in camel milk than in cow's milk. It is noted that the TAPC in camel milk (1.75.10 CFU mL^{-1}) is lower than that in bovine milk (9.166.10 CFU mL^{-1}). The analysis revealed the existence of total coliforms and yeasts and molds with average values, which remain much lower in camel milk (an average value of 7.33 CFU mL^{-1} for yeasts and molds and 4.66 CFU mL^{-1} for coliforms) compared to cow's milk (respectively 2.83×10 and 9.33 CFU mL^{-1}). Camel milk was richer in lactic acid bacteria than cow milk.

Characterization of crude enzyme: The protein content was 47.84 mg mL^{-1}. The optimum activity of crude enzymatic extract of fig tree latex was characterized with an optimum temperature range (45-50°C) an optimum pH range[5-6] and an enzymatic activity of 2231.9 IU L^{-1} (Table 3).

Purification of enzyme: The elution profile of the Fast Protein Liquid Chromatography system (FPLC) was shown in Fig. 2 Tow fractions were shown after purification of the enzymatic extract by FPLC.

Only the fraction 1 showed considerable proteins content (9.15 mg mL^{-1}) which represents almost 1/5 of that found in the crude extract (47.84 mg mL^{-1}) and an enzymatic activity 5 times higher than that of the crude extract (23491.24 IU L^{-1}). As a result, only fraction1 was used for cheese making.

The optimal enzymatic activity of Fraction 1 was at pH = 6.5 and temperature = 42°C. These conditions are slightly different from those found for the crude enzymatic extract.

Cheese manufacturing

Cheese yield: The results of cheese yield were summarized in Table 4. For camel milk, the cheese yield is not proportional to the dose of the fraction used for coagulation with a maximum at the lowest dose (1 mL of extract/100 mL of milk). For cow's milk the cheese yield is proportional to the dose used with an optimum at 1.5 mL of fraction/100 mL of milk.

Physicochemical composition: The physicochemical composition of the cheese produced from camel and cow milk after enzymes coagulation (the enzymatic extract of fig tree latex and chymosin) was illustrated in Table 5. Camel milk cheese was more acidic and richer in protein (50.04 g L^{-1}) than cow milk cheese. The camel milk cheese with latex was more acidic, richer in fat, dry matter, ash and proteins content than that with chymosin.

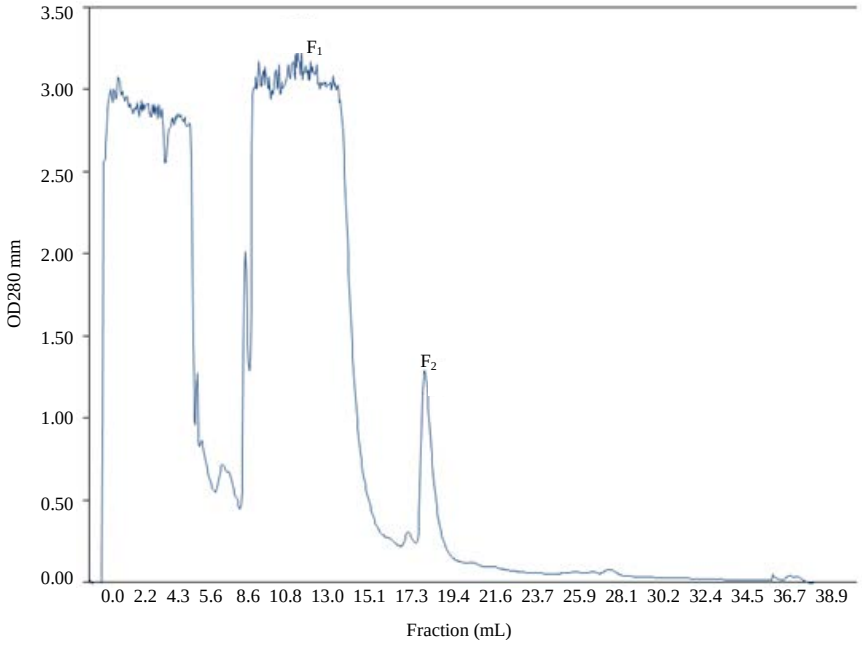

Fig. 2: Purification of acetone-precipitated fraction by FPLC column chromatography

Table 5: Physicochemical parameters of Camel and Cow Cheese with Latex (and Camel with Chymosin

Variables	CaChL	CoChL	CaCC	p-values
pH	4.41^{b}±0.026	4.53^{a}±0.62	5.00±0.12	0.038
Acidity (°D)	133.960^{a} ±1.32	113.70^{b} ±2.89	70.00±1.02	0.000
F (g L^{-1})	119.23^{a}±14.33	212.23^{a}±5.10	67.35±0 .003	0.262
DM (g L^{-1})	268.1^{a}±5.401	268.06^{a}±8.4	33.1±1.55	0.730
Ash (g L^{-1})	7.47^{b}±4.25	11.3^{a}±0.2	2.71±0.47	0.000
Proteins (g L^{-1})	50.04^{a}±12.91	39.21^{b}±6.28	42.50± 0.71	0.025
Phosphorus (g L^{-1})	0.199^{a}±0.077	0.175^{a}±0.081	0.43±0.02	0.215

F: fat; DM: Dry Matter; CaChL: Camel Cheese with Latex; CoChL: Cow Cheese with latex; CaCC: Camel Cheese with chymosin; ^{a,b}means in the same line followed by the same letter are not statistically different p>0.05; NS: Not significant

Microbiological quality: The microbiological quality of camel and cow milk cheese manufacturing with the enzymatic extract of fig tree latex compared with camel milk cheese with chymosin was showed in Table 6. Concerning TAPC, coliforms and yeast and molds, they are more abundant in cow cheese (1.466105, 3.33 10^2 and 7.43 10^7 CFU mL^{-1}) than in camel cheese produced by the two types of enzymes (the purified enzymatic extract of fig tree latex and chymosin).

The analysis revealed that the load of lactic acid bacteria in camel cheese produced with the purified enzymatic extract of fig tree latex is the most important (8.750.10 CFU mL^{-1}), comparing it with that found in camel cheese produced with chymosin (111.3.10² CFU mL^{-1}) and in cow cheese with latex (8.100 10^7 CFU mL^{-1}).

Vitamins and fatty acids compositions of cheese

Fat and water soluble Vitamins compositions: The fat-soluble vitamins analyzed were: Vitamin, A, D, E, K and the water-soluble vitamins were: Vitamin C, B2, B5, B7 and B12. Camel cheese with latex showed higher levels of fat-soluble vitamins (Vit A (135,079 mg kg^{-1}), K2 and Vit E) compared to cheese made from cow's milk. Camel cheese has a high content of ascorbic acid (6.189 mg kg^{-1}) compared to cheese made from cow's milk (Table 7 and 8).

Fatty acid: The fatty acid compositions of the two types of cheese were determined by GC-MS was shown in the Table 9. The major fatty acid in camel milk cheese was oleic acid (28.4%) followed by palmitic acid (27.1%) and stearic acid (23.6%).

The percentage of unsaturated fatty acids known by their nutritional interests in this type of cheese is 34.36% which was higher than that found in cow milk cheese (29.1%). Fatty acids are detected in camel milk cheese.

The physicochemical composition of camel and cow milk was analyzed before starting the experimentation. The pH and acidity of camel milk were in the range of the normal values reported in the literature[16]. The acidic value of camel milk was due to the presence of vitamin C

Table 6: Microbiological quality of Camel and Cow Cheese with Latex (CaChL and CoChL) and Camel cheese with Chymosin (CaCC)

Variables	TAPC CFU mL^{-1})	Coliforms (CFU mL^{-1})	Yeast and molds (CFU mL^{-1})	Lactic acid bacteria (CFU mL^{-1})
CoChL	1.466^{a} 10^{5}±0.219	3.330^{a} 10^{2}±0.71	7.430^{a} 10^{7}±0.296	8.100^{a} 10^{7}±0.101
CaChL	4.160^{a} 10^{4}±0.626	10.500^{b}±0.707	1.330^{b} 10^{5}±0.424	8.750^{a} 10^{8}±0.707
CaCC	136.10^{2}	0	12	$111,3.10^{2}$
p	0.281	0.024	0.072	0.947

TAPC: Aerobic Total Plate Count; CoChL: Cow cheese with latex; CaChL: Camel Cheese with latex; CaCC: Camel cheese with chymosin; a,b means in the same line followed by the same letter are not statistically different p>0.05; NS: Not significant

Table 7: Water- soluble vitamins of Camel and Cow Cheese with Latex (CaChL and CoChL) and Camel cheese with Chymosin (CaCC)

Water soluble vitamins (mg kg^{-1})	CachL	CochL	CachC
Ascorbic acid	6.36	5.43	6.23
B5	0.15	0.51	1.23
B7	3.25	0.08	3.35
B2	0.05	0.21	0.00
B12	0.12	0.00	0.25

Table 8: Fat-soluble vitamins Camel and Cow Cheese with Latex (CaChL and CoChL) and Camel cheese with Chymosin (CaCC)

Fat soluble vitamins (mg kg^{-1})	CachL	CochL	CachC
Retinol	135.08	39.49	0.956
K2	0.789	0.506	0.123
Tocopherol	1.968	0.37	0.548

Table 9: Fatty acid composition of camel and cow milk cheese (%)

Fatty acid	Camel cheese (%)	Cow cheese (%)
Hexanoic Acid	0.048	1.646
Octanoïc acid	0.037	1.057
Decanoïc acid	0.098	2.606
Dodecanoïc acid	0.589	3.250
Tridecanoïc acid	0.054	-
Tetradecanoïc acid	6.212	12.265
(Cis-9) methyl myristoleate	0.668	1.317
Pentadecanoïc acid	1.361	1.498
(Cis-10) Pentadecanoïc acid	0.433	-
Hexadecanoïc acid	27.416	34.132
9- hexadecenoïc acid	4.555	1.739
Heptadecanoïc acid	0.827	0.816
Cis-10 Heptadecanoïc acid	0.346	0.435
Methyl stearate	23.645	10.166
9- octadecenoïc acid	0.804	-
9- octadecenoïc acid (Z)	28.413	22.348
9,12- octadecenoïc acid (Z, Z)	2.901	2.932
9, 12,15- octadecatrienoïc acid (Z, Z, Z)	0.530	0.438
eicosanoïc acid	0.705	-
5, 8, 11,14- eicosatetraenoïc acid	0.360	-
Butanoic acid	-	2.992

(ascorbic acid)[14, 15] which gives the milk a slightly acidic taste[16]. This acidity could also be attributed to the richness of this milk in various organic acids (citric acid, orotic acid and butyric acid)[15].

The proteins content is higher in camel milk (36.323 g L^{-1}) than in cow's milk (31.363 g L^{-1}). These values are similar to those reported by Sboui *et al*.[16] which are 34.15 g L^{-1} for camel milk and 30.5g L^{-1} for cow's milk. The proteins content varies according to the stage of lactation and the species[17].

The bacterial load (TAPC, coliforms, yeast and molds) was lower in camel milk than in cow's milk. According to El Hatmi *et al*.[18], this is due to its high content of soluble proteins that have an antimicrobial effect and its high ascorbic acid content which lowers the pH. Indeed, the presence in camel raw milk of factors limiting bacterial proliferation has been demonstrated: high content of lysozyme[19] and vitamin C[20].

The proteins content in the crude extract (47.84 mg mL^{-1}) is very high compared to that mentioned by Nouani *et al*.[21] (22 mg mL^{-1}). This parameter, combined with the apparent characteristics of crude sap, affects the sensory properties of fresh cheeses as also reported by Garg *et al*.[22] and Walstra *et al*.[23].

For camel milk, the cheese yield is not proportional to the dose of fraction 1. In fact, the cheese yield is influenced by the richness of the milk in protein, fat, calcium. Camel milk cheese made with enzymatic extract was more acidic, richer in protein and phosphorus than cow milk cheese and camel milk cheese made with

chymosin. Benkerroum *et al*.[6] revealed the effect of the type and the effect of concentration of rennet on camel milk coagulation.

These results indicated that the cheese obtained has an acceptable hygienic quality as suggested by the absence of faecal coliforms. This could be explained by the good hygienic quality of the pasteurised milk used as raw material to manufacture the cheese in addition to the proper sanitary conditions in which the cheese samples were handled and prepared. Furthermore, the inherent antimicrobial activity of camel milk could concur with the highly competitive nature of the lactic acid bacteria of the starter culture to limit the growth of undesirable micro-organisms during the fermentation[6]. Such an assumption could be supported by the relatively low total aerobic count in camel cheese compared to that obtained from cow milk which normally exceeds 7 log units[24]. No other data to our knowledge are available in the literature regarding the hygienic quality of cheese produced from camel milk to be compared to our results.

CONCLUSION

In this study, it was demonstrated that a cheese with acceptable physicochemical, microbiological and nutritional quality could be obtained from camel milk using an enzymatic extract of fig. Camel milk coagulation with this enzymatic extract was optimized at 45-50°C and 5-6 as pH range. However, more research are needed to study the mechanism of enzymatic coagulation of camel milk to improve the quality and the yield of camel milk cheese and to use the nutritious whey that is produced from cheese making with camel milk.

REFERENCES

01. Garcia-Lorenzo, M., 2007. The role of proteases in plant development. Ph.D. Thesis, Umea University, Umea, Sweden.
02. Sullivan, G.A. and C.R. Calkins, 2010. Application of exogenous enzymes to beef muscle of high and low-connective tissue. Meat Sci., 85: 730-734.
03. Salami, M., A.A. Moosavi-Movahedi, F. Moosavi-Movahedi, M.R. Ehsani and R. Yousefi *et al*., 2011. Biological activity of camel milk casein following enzymatic digestion. J. Dairy Res., 78: 471-478.
04. Walsh, G., 2004. Proteins Biochemistry and Biotechnology. Wiley & Sons, New York, USA.,.
05. Vierstra, R.D., 1996. Proteolysis in Plants: Mechanisms and Functions. In: Post-Transcriptional Control of Gene Expression in Plants, Filipowicz, W. and T. Hohn (Eds.). Springer, Berlin, Germany, pp: 275-302.
06. Benkerroum, N., M. Dehhaoui and R. Tlaiha, 2011. The effect of concentration of chymosin on the yield and sensory properties of camel cheese and on its microbiological quality. Int. J. Dairy Technol., 64: 232-239.
07. Ramet, J.P., 2001. The Technology of Making Cheese from Camel Milk (Camelus Dromedaries). FAO., Rome, Italy.
08. Khan, H., I.H. Athar and M. Aslam, 2004. Evaluation of cheese prepared by processing camel milk. Pak. J. Zool., 36: 323-326.
09. Chanda, I., S.K. Basu, S.K. Dutta and S.R.C. Das, 2011. A protease isolated from the latex of Plumeria rubra Linn (Apocynaceae) 1: Purification and characterization. Trop. J. Pharmaceutical Res., 10: 705-711.
10. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
11. Kunitz, M., 1947. Crystalline soybean trypsin inhibitor: II. General properties. J. Gen. Physiol., 30: 291-310.
12. Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.
13. Albala-Hurtado, S., M.T. Veciana-Nogues, M. Izquierdo-Pulido and A. Marine-Font, 1997. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. J. Chromatogr. A, 778: 247-253.
14. Farah, Z., 1993. Composition and characteristics of camel milk. J. Dairy Sci., 60: 603-626.
15. Haddadin, M.S., S.I. Gammoh and R.K. Robinson, 2007. Seasonal variation in the chemical composition of camel milk in Jordan. J. Dairy Res., 10: 1-5.
16. Yagil, R., O. Zagorski and C.V. Creveld, 1994. Science and Camel's Milk Production (Some Keys for Nutrition and Marketing). In: Camels and Dromedaries, Dairy Animals, Marin, S.G. (Ed.). L'Ecume des Pages, Paris, France, pp: 75-89.
17. Guliye, A.Y., C. van Creveld and R. Yagil, 2002. Detection of subclinical mastitis in dromedary camels (*Camelus dromedarius*) using somatic cell counts and the N-acetyl-ß- D-glucosaminidase test. Trop. Anim. Health Prod., 34: 95-104.
18. El-Htami, H., K. Touhami and A. Hamadi, 2006. Characterization and composition of camel's (Camelus dromedaries) colostrum and milk. MHA (Sousse), 18: 13-17.
19. Barbour, E.K., N.H. Nabbut, W.M. Frerichs and H.M. Al-Nakhli, 1984. Inhibition of pathogenic bacteria by camel's milk: Relation to whey lysozyme and stage of lactation. J. Food Prot., 47: 838-840.
20. Yagil, R., A. Saran and Z. Etzion, 1984. Camel milk for drinking only. Comp. Biochem. Physiol., 78: 263-266.

21. Nouani, A., N. Belhamiche, R. Slamani, S. Belbraouet and M.M. Bellal, 2009. Characterization of the purified coagulant extracts derived from Artichoke flowers (*Cynara scolymus*) and from the fig tree latex (*Ficus carica*) in light of their use in the manufacture of traditional cheeses in Algeria. Int. J. Dairy Technol., 62: 112-117.
22. Garg, S.K. and B.N. Johri, 1994. Rennet: Current trends and future research. Food Rev. Int., 10: 313-355.
23. Walstra, P., T.J. Geurts, A. Jellema and M.A.J.S. Van Boekel, 1999. Dairy Technology: Principles of Milk Properties and Processes. 3rd Edn., Marcel Dekker Inc., New York, ISBN: 978-0-8247-0228-1 pp: 4.
24. Marino, M., M. Maifreni, S. Moret and G. Rondinini, 2000. The capacity of Enterobacteriaceae species to produce biogenic amines in cheese. Lett. Applied Microbiol., 31: 169-173.