Impact of lactic acid bacteria on the coagulation of camel milk

CHETHOUNA Fatma

Laboratory of Research on Phoeniciculture, Faculty of Natural and Life Sciences, University of Kasdi Merbah, Ouargla, 30000, Algeria

abstract

If the low aptitude for enzymatic coagulation of camel milk has been the subject of numerous studies, this is far from being the case for its acid coagulation, where only a few studies have been devoted to it. The objective of this study is to evaluate the acid coagulation of camel milk.

Monitoring of pH, Dornic acidity and a count of lactic acid bacteria on MRS and M17 media was carried out. We determined the clotting time of camel milk treated with organic acids (citric, acetic and lactic acid produced by *Streprococcus salivarius subsp. thermophilus*, and *Lactobacillus delbrueckii subsp. bulgaricus* isolated from camel milk) in comparison with of **cow's milk**, and we also calculated the activity (UP) and clotting power (F) of these acids.

Monitoring of the evolution of pH and Dornic acidity showed a slow acidification of camel milk **compared** to cow's milk, the enumeration of lactic flora showed a lower rate in camel milk than that of cow. The clotting activity of organic acids (citric, acetic and lactic acid) recorded during this study is **(**18.76 s, 15.01 s and 15.79 s respectively**)**, was greater than that of enzymatic coagulants used by several researchers to coagulate camel milk, the clotting time of camel milk by organic acids is comparable (non-significant difference $p \geq 0.05$) to those of cow's milk and Berridge's standard solution.

This study showed that the acid coagulation of camel milk is inhibited by the growth rate of lactic acid bacteria, and the addition of organic acids to camel milk could give promising results to the food industries, particularly cheese manufacturing.

Keywords: cheese, clotting activity, clotting power; enzymatic coagulant, organic acid

Introduction

Camel milk has a physicochemical composition relatively similar to that of **cow's** milk, this milk is nevertheless distinguished by a high content of vitamin C and niacin and by the presence of a powerful protective system, linked to relatively high levels of Lysozyme, Lactoperoxidase (LP/SCN/H2O2 system), Lactoferrin and bacteriocins (Kanuspayeuva et *al*.,2004; Siboukeur, 2007, Chethouna, 2021, Boudjenah, 2012). Despite its richness, the transformation of this milk into derived products remains difficult, because of its slow coagulation.

Numerous scientific researches (Ramet,1992; Siboukeur, 2007; Ho et al., 2022.) relate the slow coagulation of camel milk to its reduced κ-casein (3.3% compared to 13% in cow's milk**)** , to resolve this research problem concerns the addition of coagulating enzymes of animal origin (calf or camel abomasum) (siboukeur 2007; boudjenah 2012), plant and microbial enzyme (*Rhizomucor miehei*) and plant extract (*Cynara cardunculus L.*) (El alia et al., 2023). These studies gives promising results and suggest the possibility of producing cheese from camel milk.

The isoelectric pH of camel casein is 4.3 compared to 4.6 for bovine casein, which requires a higher quantity of lactic acid (H+), the lactic acids produced by lactic acid bacteria can neutralize the electronegative charge of κ-caseins. Our hypothesis is that the reduced Kappa (κ) casein content is not the only cause of the delay in coagulation, but also the lactic acid bacteria content.

The objective of this study is to evaluate the acid coagulation of camel milk by enumerating the lactic acid bacteria in camel milk in compared to cow's milk and conducting coagulation tests with the addition of certain organic acid.

Material and method

Milk sampling

*Camel and cow’s milk samples were collected, from herds of dromedaries (*Camelus dromedarius*) of the Sahrawi population and from Guelmoise cattle in mid-lactation living in semi-extensive farming in the region of Touggourt south-eastern Algeria. Sampling was done monthly from September to May. Milk samples were stored in a cooler containing an ice pack

and immediately transported to the laboratory for analysis. all analysis were carried out in triplicate.

*The milk powder used was of the "law-heat" type, known for its ability to make cheese, and used as a standard substrate to compensate for a possible variation in coagulating ability of the milk.

In this study, cow's milk and low-heat milk powder were used as controls.

Physico-chemical analyzes

In order to have an idea of the lactic acid level in collected milk, Dornic acidity and pH measurements are carried out.

pH measurement

pH was measured at a temperature of (+ 20°C). The value was read directly on the pH meter after immersing its electrode in the sample.

Determination of Dornic Acidity

According to the Dornic method, titration was carried out using an N/9 sodium hydroxide solution (NaOH) (0.111 mol/l) and phenolphthalein -2% alcoholic solution- as an indicator (NF V04-305, 1985). 1°D corresponds to 0.1 g of lactic acid per liter of milk.

Microbiological analysis

Lactic acid bacteria count

The culture media used were:

- M17medium used for the enumeration of lactic acid bacteria (mesophilic lactococci), with incubation at 37°C for 48 hours (guiraud, 2012);
- M.R.S (Man Rogosa and Sharp) medium is used for the enumeration of Lactobacillus, with incubation at 37 °C for 48hours (Larpent et al., 1997); (NF: 15787. 2009) .

The inoculations were carried out in petri dishes. Counts were performed using a colony counter. Countable petri plates contained between 30 and 300 colonies per plate (Guiraud 2012). For this purpose, serial dilutions of milk sample were performed (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}).

Coagulant

The coagulants used in this study were

organic acids (citric acid, acetic acid and lactic acid produced by lactic acid bacteria isolated from camel milk).

The lactic acid bacteria isolated from camel milk were:

- *Streprococcus salivarius subsp. thermophilus,*
- *Lactobacillus delbrueckii subsp. Bulgaricus*

Lactic acid production

During this study, we purified the lactic acid produced by *St. thermophilus* and *Lb. delbrueckii* **a**ccording to European patent EP 1 094 054 A1, as follows:

1. **Fermentation:** MRS broth was inoculated with lactic ferments (*St. thermophilus* and *Lb. delbrueckii*) at a rate of 10%. The pH wa**s** adjusted to 5.5–6.5 during fermentation by adding ammonium hydroxide.
2. **Filtration:** Microfiltration was performed using a TECHSEP membrane with a porosity of 0.1 µm to eliminate cells.
3. **Clarification:** The clarified medium was concentrated in a vacuum evaporator at 50°C until its ammonium lactate concentration reached 40%. The concentrated aqueous solution **was** then acidified to a pH of approximately 2.0 by the addition of pure sulfuric acid.
4. **Chromatography:** This concentrated and acidified solution underwent chromatographic separation on a strong cation exchange resin composed of sulfonic acid polystyrene crosslinked with 7% divinylbenzene..

Clotting activity

Coagulant activity was measured according to the method of Berridge (1945), modified by Collin et al. (1977). It was carried out on a standard substrate prepared by dissolving "low-heat" type milk powder at 10% (W/V) in a $CaCl_2$ solution (0.01 M) and adjusting the pH to 6.5 using a 0.1 N NaOH solution.

The technique consisted of adding 1 mL of citric, acetic, or lactic acid to 10 mL of substrate, then recording the coagulation time at 30°C. A unit of coagulant activity (U.A.C.*)* was calculated using Berridge's formula (1945):

$$U.A.C. = \frac{10 \times V}{Tc \times Q}$$

U.A.C. = Unit of coagulant activity;

V = volume of standard substrate used;

Q = volume of coagulant extract;

Tc = coagulation or flocculation time. (sec)

The clotting activity was also expressed as "SOXHLET clotting force" (F), using the following formula:

$$F = \frac{U.A.C.}{0.0045}$$

Clotting time

According to Ramet (1993), the optimal coagulation temperature for camel milk ranges from 40-42°C. Thus coagulation or flocculation time of camel milk was measured directly at 42°C and compared with cow's milk and standard substrate at the same temperature (positive control).

The coagulation time measured during this study was defined as the time between the addition of acid to milk and the appearance of flakes or curds on the inner wall of the test tube after inversion.

Result and discussion

Physico-chemical analysis

Evolution of pH and Dornic Acidity of Camel and Cow Milk Stored at 30°C

During storage, the camel milk sample acidified more slowly than the cow milk sample (Figure 1 and 2). On Day 4 (D0+3), the camel milk sample had pH values higher than the isoelectric pH of camel caseins (pH = 4.3). The recorded Dornic acidity values confirmed this relatively slow trend, which did not exceed 40°D on Day 4. This slow acidification of camel milk during fermentation has been widely documented (Farah et al., 1990; Ramet, 1992, 1994; Kamoun, 1995; Abou-Tarbousch et al., 1998; Siboukeur, 2007).

Camel milk has a high buffering capacity, which slows down the drop in pH. It also contains an antimicrobial system that inhibits microbial proliferation more effectively than the milk of other domestic species. This system **is** likely responsible for the renowned fortifying and therapeutic properties of camel milk, widely acknowledged by nomadic populations (Yagil, 1982; Ramet, 1987). Notably, camel milk contains high levels of lysozyme (Barbour et al., 1984) and vitamin C (Siboukeur, 2007; Boudjenah, 2012; Chethouna, 2022).

On the other hand, Berhe et al. (2018) suggested that the limited growth rate of lactic ferments in camel milk is due to the rate of proteolysis rather than its antimicrobial system. This particular behavior also explains the difficulties in transforming camel milk into cheese.

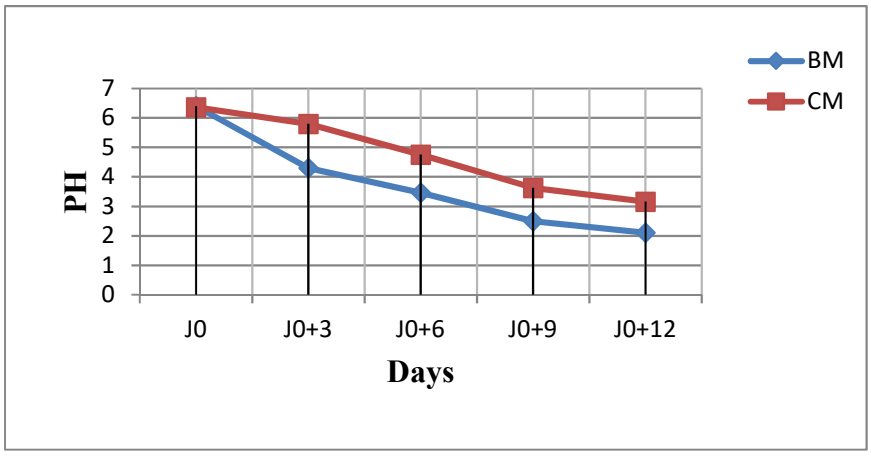

Figure 01: evolution of pH of camel and bovine milk during storage at 30°C

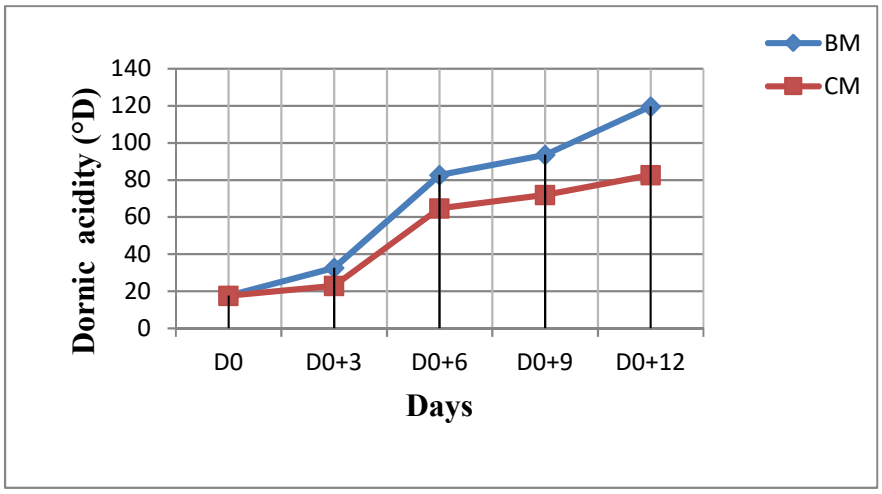

Figure 02: évolution of dornic acidity of camel and bovine milk during storage at 30°C

Enumeration of lactic acid bacteria

The slow acidification of camel milk compared to cow's milk, as previously observed, could be linked to the growth rate of lactic acid bacteria (LAB) in the two types of milk. Therefore, we enumerated LAB in samples of camel and bovine milk over ***a*** nine-month period (Table 1). The LAB count in camel milk was consistently lower than in cow's milk, regardless of the sampling month. Statistical analysis revealed a significant difference ($p \leq 0.05$) between the two samples.

We also observed an increase in LAB counts for both types of milk from April to November, followed by a decrease from December to March. This trend can be explained by temperature variations, as our study was conducted ***in*** an arid and semi-arid climate, where temperatures increase from April to mid-November. It is noteworthy that LAB are mesophilic bacteria (optimal growth temperature: 20–40°C).

Table 01: Comparison of lactic acid bacteria levels in camel and cow's milk over 9 months

Month	Lactic acid bacteria	Camel's milk		Cow's milk	
		Mean	Standard deviation	Mean	Standard deviation
September	*Lactococcus*	1.73×10^4	1.49×10^3	2.85×10^5	4.02×10^4
	Lactobacillus	1.77×10^4	9.72×10^2	5.07×10^5	1.39×10^5
October	*Lactococcus*	1.75×10^4	1.02×10^3	2.7×10^5	1.23×10^4
	Lactobacillus	2.75×10^4	2.01×10^3	2.53×10^5	2.30×10^4
November	*Lactococcus*	2.41×10^4	2.85×10^3	3.66×10^5	3.26×10^4
	Lactobacillus	2.95×10^4	3.62×10^3	3.39×10^5	2.98×10^4
December	*Lactococcus*	4.28×10^3	7×10	5.1×10^4	9.58×10^2
	Lactobacillus	5.49×10^3	2.86×10^2	4.94×10^4	9.04×10^2
Janury	*Lactococcus*	9.02×10^2	5.1×10	2.59×10^3	3.1×10
	Lactobacillus	8.09×10^2	7.4×10	1.49×10^3	2.02×10^2
February	*Lactococcus*	9.05×10^2	5.5×10	5.09×10^3	2.32×10^3
	Lactobacillus	9.26×10^2	7.2×10	2.89×10^3	1.45×10^3
March	*Lactococcus*	1.79×10^3	6.62×10^2	2.33×10^4	7.62×10^3
	Lactobacillus	1.6×10^3	2.6×10^2	2.08×10^4	7.22×10^3
April	*Lactococcus*	5.61×10^3	6.17×10^2	6.87×10^4	3.44×10^3
	Lactobacillus	6.29×10^3	8.6×10	5.64×10^4	3.74×10^3
May	*Lactococcus*	1.81×10^4	1.13×10^3	5.96×10^5	3.32×10^4
	Lactobacillus	3.75×10^4	7.51×10^3	5.66×10^5	3.31×10^5

P value≤0.05, the difference is significant

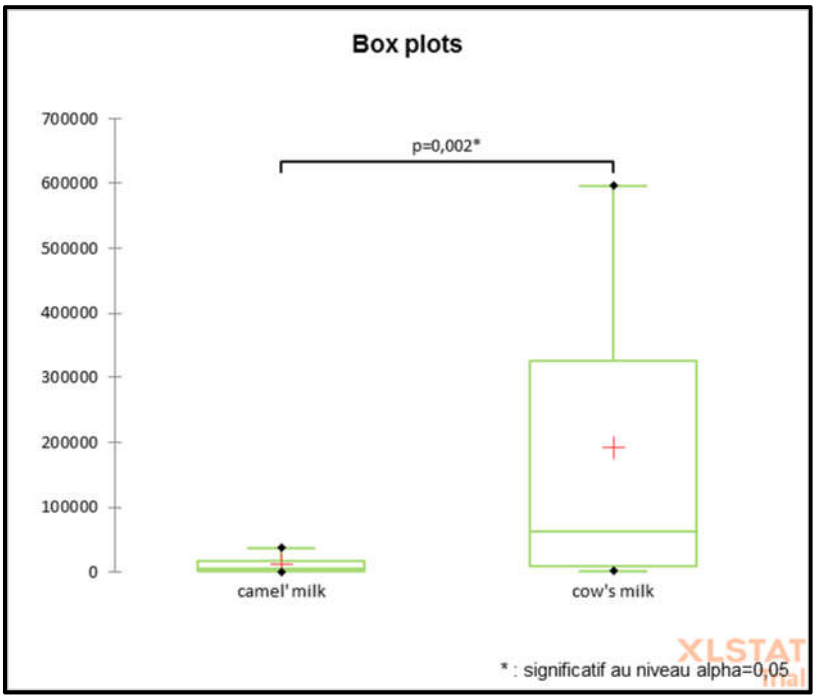

Figure 03: Box plots representing the difference in lactic acid bacteria content in cow and camel milk

Clotting time of camel milk treated with organic acids

Based on the results of statistical analyses (using XLSTAT software, version 2022, Kruskal-Wallis test), we can conclude that the flocculation or coagulation time of camel milk treated with organic acids (citric, acetic, and lactic acid) does not show a significant difference ($p \geq 0.05$). The recorded coagulation times were 5.33 s, 6.33 s, and 5.33 s, respectively (Table 02).

Additionally, we compared the coagulation time of camel milk with that of cow's milk and Berridge's standard solution, as illustrated by the boxplot (Figure 04). The results also indicate that the difference is not statistically significant ($p \geq 0.05$). Thus, it can be concluded that the coagulation time of camel milk treated with organic acids is comparable to that of cow's milk and Berridge's standard solution.

Table 02: coagulation time of camel milk treated with organic acids in comparison with cow milk and Berridge solution

		Camel's milk		Cow's milk		Standard berridge solution		
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	P value
Clotting time on (s)	citric acid	5.33	0.57	5.33	0.57	5.33	0.57	0.721
	acetic acid	6.33	1.52	6.66	1.52	6.66	0.57	
	lactic acid	5.33	0.57	6.33	0.57	6.33	0.57	

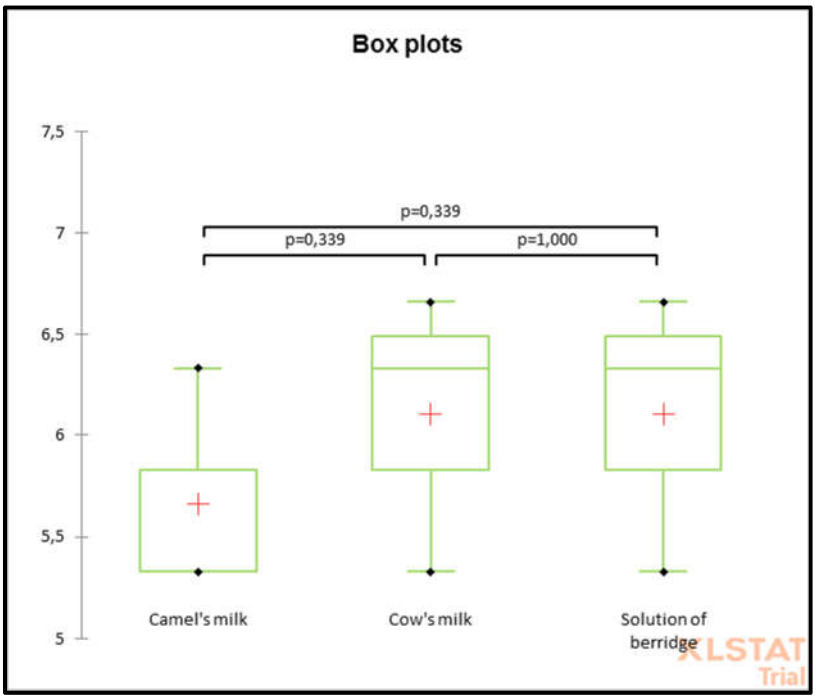

Figure 04 : boxplot shows the difference in clotting times of the three types of milk

.

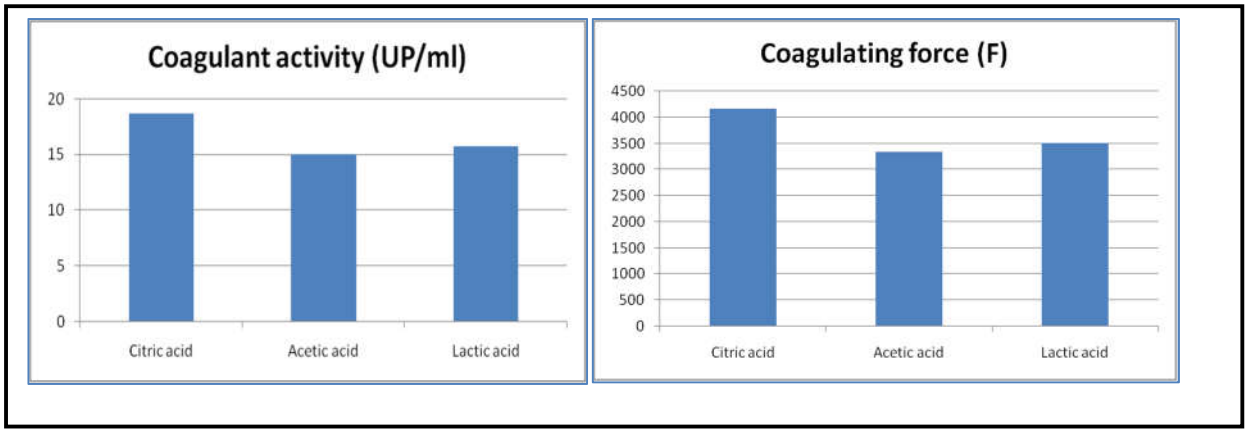

Figure 05: Activity and clotting power of organic acids tested on standard Berridge solution

Conclusion

The coagulation of camel milk, whether enzymatic or acidic, is known to be slow. While enzymatic coagulation can be improved through the addition of coagulating enzymes, acid coagulation remains less understood, despite its importance in certain industries that require a combination of both coagulation methods.

This study demonstrated that camel milk coagulates more efficiently when organic acids are added, particularly lactic acid produced by lactic acid bacteria isolated from camel milk. Our findings suggest that the delay in the acid coagulation of camel milk is primarily due to its low lactic acid bacteria content**.**

As a perspective, we recommend further investigation into the factors limiting the growth and development of lactic acid bacteria in camel milk, as addressing these limitations could enhance its coagulation properties and improve its industrial applications.

References

Alia, OAE., Zine-Eddine, Y., Ajbli, N., Kzaiber, F., Oussama, A., Boutoial, K. (2023). Optimization of camel milk coagulation: the use of coagulants of microbiological and plant origin. Acta Scientiarum Polonorum Technologia Alimentaria, 22(1), 81-91.

Abu-Tarboush, HM., Al-Dagal MM., Al-Royli MA., (1998):Growth, viability and proteolytic activity of Bifidobacteria in whole camel milk. J. Dairy Sci., 81, 354-361.

Barbour EK., Nabbut NH., Frerichs WN., Al Nakhli HM. (1984).Inhibition of pathogenic bacteria by camel's milk ; relation to whey lysozyme and stage of lactation. J. Food Protect., 47, 838-840.

Belhamiche N. (2005). *Extraction, purification et caractérisation de la coagulase de Mucor pusillus* (Doctoral dissertation, INA).

Berhe, T., Ipsen, R., Seifu, E., Kurtu, MY., Eshetu, M., Hansen, E.B. (2018). Comparison of the acidification activities of commercial starter cultures in camel and bovine milk. *LWT*, *89*, 123-127. https://doi.org/10.1016/j.lwt.2017.10.041

Boudjenah, S. (2012). Aptitudes à la transformation du lait de chamelle en produit dérivés: effet des enzumes coagulantes extraites de caillettes de dromadaires. Thèse de doctorat. Universite Mouloud Mameri.

Chethouna, F. (2021).*Contribution à l'étude des substances antibactériennes produites par la flore lactique isolée à partir du lait camelin (Camelus dromedarius) de la région d'Ouargla*. Thèse de doctorat. Université Kasdi Merbah Ouargla.

Chethouna, F., Boudjenah, S, Beldi, N., Siboukeur, O. (2022). Comparative study of the physico-chemical and microbiological characteristics of raw and pasteurized camel milk. *Emirates Journal of Food and Agriculture*, *34*(10), 850-858.

Farah, Z., Streiff, T., Bachmann, M. R. (1990). Preparation and consumer acceptability tests of fermented camel milk in Kenya. *Journal of Dairy Research*, *57*(2), 281-283.

Guiraud, JP. (2012). Microbiologie alimentaire. Dunod, Paris, 651p.

Ho, TM., Zou, Z., Bansal, N. (2022). Camel milk: A review of its nutritional value, heat stability, and potential food products. Food Res. Int., 153, 110870. https://doi.org/10.1016/j.foodres.2021.110870

Kamoun, M. (1995). Le lait de dromadaire : production, aspects qualitatifs et aptitude à la transformation. Option Médit., 13, 81-103.

Konuspayeva, G., Loiseau, G., Faye, B. (2004). La plus-value santé du lait de chamelle cru et fermenté: l'expérience du Kazakhstan. Institut de l'élevage.

Larpent JP., Copin MP., Germonville A., Jacquet M. , Thetas JL. (1997). Microbiologie du lait et des produits laitiers ; in : « Microbiologie alimentaire ». ed. larpent, Tec. Doc., 1ère Ed., Lavoisier, Paris

Outaleb T., 2006. Aptitude à la coagulation du lait par la pepsine ovine. Mém.Ing.Agr., Institut National Agronomique, El Harrach (Alger), 54p

Ramet JP., (1993). La technologie des fromages au lait de dromadaire (Camelus dromedarius). Etude F.A.O., Production et santé animales, 113

Ramet, J P. (2001). The technology of making cheese from camel milk (Camelus dromedarius) No.113. Food and Agriculture Org.

Siboukeur O K., (2007). Etude du lait camelin collecté localement : caractéristiques physico-chimiques et microbiologiques ; aptitudes à la coagulation.thèse de doctorat en Sciences Agronomiques université INA ELHarrach-Alger

Yagil, R. (1982). Camels and Camel Milk. FAO, Animal Production and Health, Paper N°

26, 1-69.