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Dromedary milk lactic acid fermentation: microbiological and rheological characteristics

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The ability of dromedary skim milk to form an acid curd during a lactic acid starter fermentation was investigated. The activity of the starter in dromedary milk was characterized by a longer lag phase (~5 vs. ~1 h) and by an earlier decline phase. This suggests the presence of inhibiting factors. The maximum buffering capacity of dromedary milk as well as its minimum apparent viscosity were obtained at lower pH values. Similarly, its elastic modulus appeared later (pH 5.7 vs. 6.3). Because these rheological and biochemical events took place at lower pH values, dromedary skim milk seems to present a higher physical stability toward the increase of acidity. Determination of the rheological and microscopic characteristics of the dromedary milk coagulum (pH 4.4) did not reveal curd formation but indicated a fragile and heterogeneous structure. This coagulum, which is very different from that of cows' milk, seems to be made up of dispersed casein flakes. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 263–270.

Keywords: lactic fermentation; dromedary milk; curd; rheology; microstructure

Introduction

Lactic acid fermentation is the most widely used acidification process to coagulate bovine milk during the manufacture of cultured dairy products. Lactic acid bacteria are responsible for this bioprocess [3]. Lactic acid fermentation lowers pH, altering casein micelles that progressively lose their surface potential [13], minerals [30], caseins [11] and solvation [36]. The results of these modifications are the destruction of the micellar structure and the formation of a three-dimensional network or coagulum, which encloses all the aqueous phase [9]. Such a curd structure was not observed in dromedary milk [20,26]. In fact coagulation caused by the lactic fermentation did not produce a curd but simply flakes that lack firmness and that were unable to undergo further technological treatments [1].

Dromedary and bovine milk have a similar chemical composition [21]. However, dromedary milk is characterized by a larger average micelle diameter [6,10,21]. Data concerning dromedary milk fermentation are limited and refer only to the feasibility of certain processes. Such studies mention the technological difficulties encountered [22,33].

The aim of this study was to look for relationships between the physicochemical properties of fermented dromedary milk and factors inherent in this milk using microbiological, rheological and microscopic approaches. We traced the lactic fermentation of dromedary milk under conditions that were very similar to those used in cheese manufacture. The study compared dromedary milk with bovine milk in order to see differences between the two kinds of milk and, thus, to determine the reason why dromedary milk is not suitable for the manufacture of dairy products.

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Materials and methods

Milk samples

Dromedary milk (*Camelus dromedarius*) was supplied by the Institute of Arid Region herd (IRA, Médenine, Tunisie). Cows' milk (Holstein breed) was obtained from a private farm situated in the same area. Samples of dromedary milk and cows' milk were collected, kept refrigerated and transported to our laboratory within 6 h. Upon arrival, they were skimmed, at 2000×*g* and 10°C for 15 min. This operation was repeated three times for dromedary milk to achieve fat separation. The samples were then pasteurized at 63°C for 30 min.

All assays were carried out on six milk samples. Each sample was a pooled skim milk taken from 16 to 18 animals for dromedary milk and from 17 to 20 animals for bovine milk.

Milk fermentation

Acidification was carried out by addition of a mixed culture of *Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *diacetylactis* and *L. lactis* ssp. *cremoris*. This mother culture consists of a CH-normal O1 lactic acid starter (Chr. Hansen's Laboratory, Copenhagen, Denmark), revivified for 24 h at 25°C, in sterile reconstituted skim milk. The initial colony-forming unit (cfu) count of the culture was controlled before each experiment. After inoculation (2%, w/v), the milk was divided into 200-ml fractions. One was used to trace pH during the fermentation process. The incubation temperature was maintained at 20°C±0.1°C in a circulating water bath (Huber, Kältemashinenbau, Offenburg, Germany).

Titrable acidity

Titrable acidity, expressed in Dornic degrees (1°D=0.1 g lactic acid/l of milk), was determined by titration of 10 ml of sample with N/9 sodium hydroxide to a pink endpoint using phenolphthalein as indicator [2].

Table 1 Average chemical composition of dromedary and cows' skim milks

Component	Content (g/kg)			
	Dromedary		Cow	
	$\bar{X}$	SD	$\bar{X}$	SD
Total solids	96.07	2.00	98.20	1.82
Lactose	54.03	1.12	55.40	1.04
Total nitrogen	30.72	0.64	33.61	0.55
Caseins	20.60	0.42	27.00	0.32
Whey proteins	7.55	0.22	4.93	0.15
Nonprotein nitrogen	2.57	0.02	1.68	0.01
Fat	1.20	0.20	1.30	0.10
Ash	9.92	0.12	9.76	0.10
Calcium	1.22	0.03	1.35	0.03
Magnesium	0.10	0.01	0.11	0.01
Phosphorus	1.02	0.02	1.15	0.02
Citrate	1.85	0.04	1.72	0.03

$\bar{X}$, mean values of six pooled milks. SD, standard deviation.

Culture growth

Bacterial counts (colony-forming units per milliliter) on M.17 medium [8] were done using serial dilutions prepared with sterile peptone–water. Petri dishes were incubated for 48 h at 25°C.

Evaluation of proteolysis

Proteolysis of incubated milk was traced by the increase of nonprotein nitrogen (NPN) content during the lactic fermentation. Samples for analysis were prepared by adding 5 ml of trichloroacetic acid (24%, w/v) to 5 ml of milk. After 5 min of incubation at room temperature, the samples were filtered using Whatman No. 2 paper. The soluble nitrogen content was determined as mentioned below.

Measurement of buffering capacity

The buffering values (dB/dpH), corresponding to the relationship between increment of the added acid and the resultant increment in pH, were calculated according to Van Slyke [40] and plotted vs. pH. To simulate the lactic fermentation, titrations were performed using 0.5 N lactic acid (Sigma-Aldrich, Steinheim, Germany).

Composition analysis

Total nitrogen (TN), NPN and noncasein nitrogen (NCN) contents of the initial milks were determined by the Kjeldahl method [2] using a Büchi 425 and a Büchi 325 apparatus (Büchi, Flawil, Switzerland). Total casein content was calculated by difference between TN and NCN after separation according to Rowland [35].

Calcium and magnesium were measured using a Hitachi Z-6100 model atomic absorption spectrometer (Hitachi Instruments Engineering, Ibaraki, Japan) in the presence of lanthanum oxide (Sigma, St. Louis, MO, USA). The concentration of phosphorus (P) was determined by a colorimetric method with ammonium molybdate [34]. Citrate was determined enzymatically using a test kit (Bœhringer Mannheim, Germany; catalogue number 139076).

Soluble casein and soluble mineral contents of initial milks were determined in supernatants after separation of the soluble and micellar phases of milk by centrifugation (190 000×*g*, 60 min, 20°C).

Total solids, ash, lactose and fat contents were determined according to standard methods [2], respectively, by drying at 102–104°C, by incineration at 550°C, and by the Bertrand method followed by extraction with a soxhlet system.

Rheological measurements

All measurements were carried out using a StressTech Rheological rheometer (Rheological Instruments, Lund, Sweden) with a coaxial cylinder measuring system (diameters 25 and 27 mm) and at 20±0.1°C. The flow curves were determined at a gradient shear rate from 1 to 200 s^{-1} (gradient obtained at 20 min). The storage modulus (G') and the tangent of the loss angle ($\tan \delta$) were measured at a frequency of 1 Hz and in the linear viscoelastic regime, (strain <0.02). For the two modes, immediately after adding the lactic acid starter to the milk, 15.9 ml of inoculated milk was immediately transferred to the rheometer measuring system, which was protected with a cover to prevent evaporation. A separately thermostated sample, kept at the same temperature, was used to trace pH during lactic fermentation.

Scanning electron microscopy (SEM)

Samples of fermented milks were treated according to Attia *et al* [4] and were examined with a Philips XL 30 scanning electron microscope (Philips, Limeil Brevannes, France) after drying to CO_2 critical point on a Baltec CPD 030 apparatus and gold-coating on a Baltec MED 20 apparatus (Balzers Union, Balzers, Germany).

Results

Composition of milks

The two kinds of milk had a similar gross composition (Table 1). However, nitrogen compounds of dromedary milk were characterized by a lower casein content and higher whey protein and NPN contents. The study of mineral and casein partition between the dissolved phase and the micellar phase (Table 2) revealed that dromedary milk micelle was characterized by a higher mineral charge and a lower casein content.

Table 2 Partition of caseins and minerals of dromedary and cows' milks between soluble (SP) and micellar (MP) phases (mean values of six pooled milks)

Component	Content (%)			
	Dromedary		Cow	
	SP	MP	SP	MP
Calcium	34.90	65.10	34.16	65.84
Magnesium	36.45	63.55	42.32	57.68
Phosphorus	34.36	65.64	39.70	60.30
Citrate	67.90	32.10	89.00	11.00
Caseins	16.25	83.75	5.3	94.7

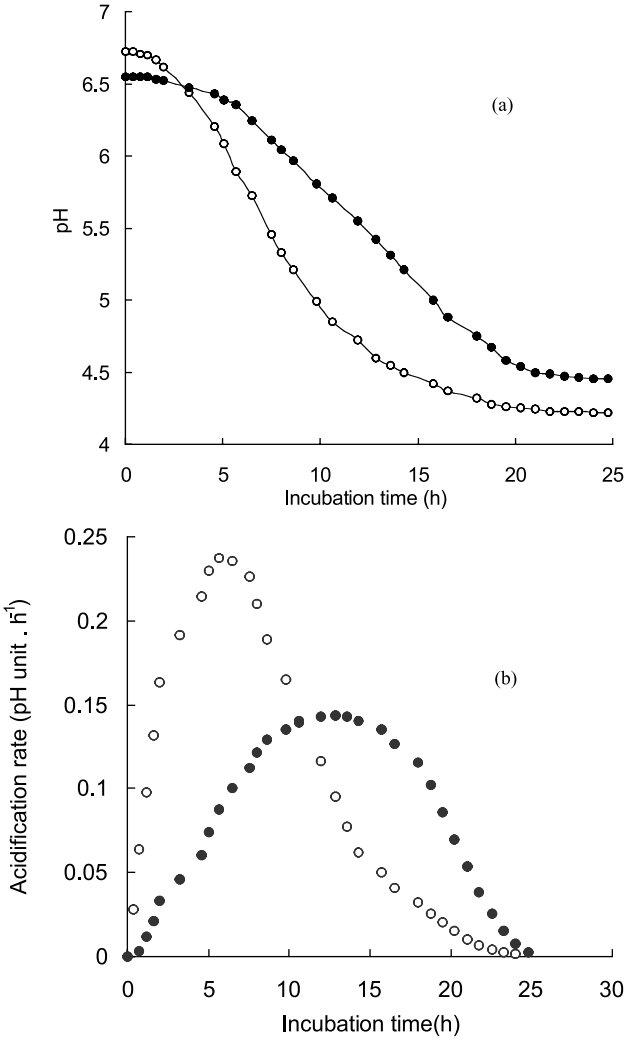

Figure 1 Kinetics of acidification of dromedary (•) and cows' (○) skim milks during a fermentation at 20°C, with a lactic acid starter (CH-normal O1; 2%). (a) pH changes. (b) Acidification rate. Values are means of six pooled milks.

Lactic fermentation ability

Kinetics of acidification: At the onset, the dromedary milk fermentation exhibited a slow acidification rate (Figure 1). Thus, after 7.5 h postinoculation, the pH of this milk had a 0.44 pH unit (SD=0.01) decrease; however, the pH of the bovine milk had a 1.27 pH unit (SD=0.01) decrease (Figure 1a). The maximum of the acidification rate (Figure 1b) was observed later for dromedary milk (′12 vs. ′6 h for cows' milk).

Development of lactic acid starter: In both milks, the growth curves of the inoculated culture showed the usual sequence of phases (Figure 2b). However, a longer lag phase was observed in dromedary milk (′5 vs. ′1 h for cows' milk). Besides, in this milk, the kinetics of the acid production (Figure 2a) was slow because between 5 and 15 h postinoculation the cows' milk acidity had a 55.5°D change (SD=2.08). However, that of dromedary milk had only a 36.1°D change (SD=1.46). At the end of the log phase, the lactic bacterial count in the dromedary milk was approximately four times lower than that in the bovine milk (4.7×10^4 cfu/ml; SD=0.32×10^4 against 17.62×10^4 cfu/ml; SD=0.28×10^4). Finally, in both milks, the pH corresponding to the maximum number of cells presented a deviation in comparison with that corresponding to the maximal acidity. This deviation was pronounced in dromedary milk (′9 h against ′3 h for bovine milk). Colonies of the organisms plated from fermented dromedary milk were characterized by markedly reduced sizes in comparison with those plated from fermented cows' milk (Figure 3).

Proteolytic activity: The increase in NPN content was faster in dromedary milk than in bovine milk, and the deviation increased as incubation time increased (Figure 4). A change in slope was observed for both curves (at ′7 h postinoculation for bovine milk and at ′11 h postinoculation for dromedary milk). After 24 h incubation, NPN content increased from 2.57 (SD=0.02) to 5.94 g

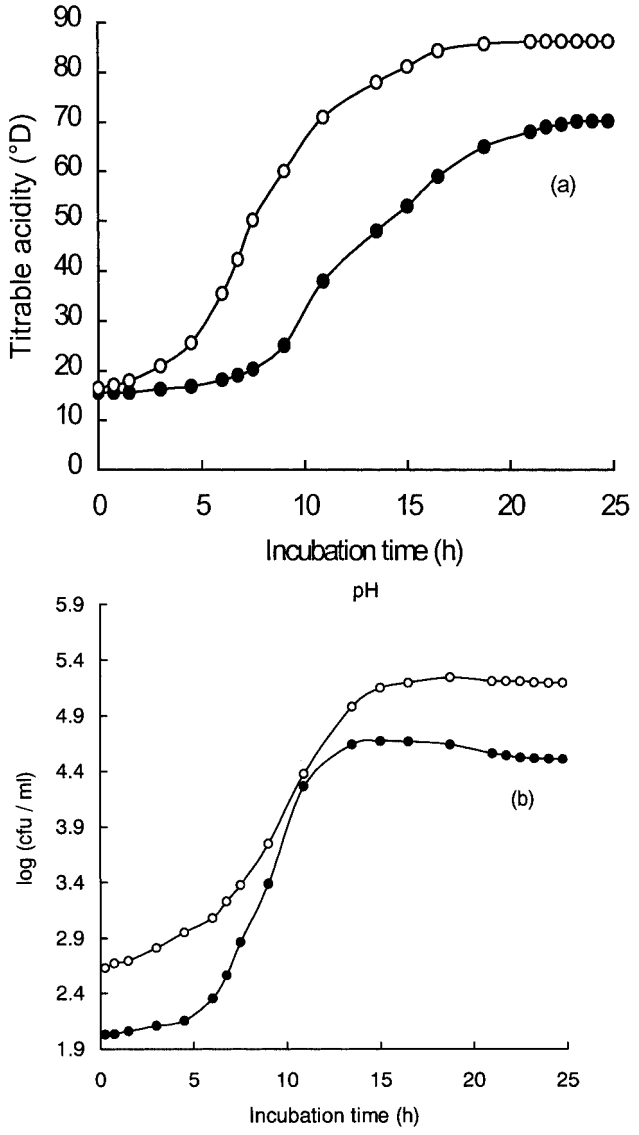

Figure 2 Activity, at 20°C, of a lactic acid starter (CH-normal O1; 2%), in dromedary skim milk (•) and in cows' skim milk (○). (a) Lactic acid production (1°D = 0.01% of lactic acid). (b) Bacterial growth (the first colony-forming unit measure corresponds to 0.25 h postinoculation). Values are means of six pooled milks.

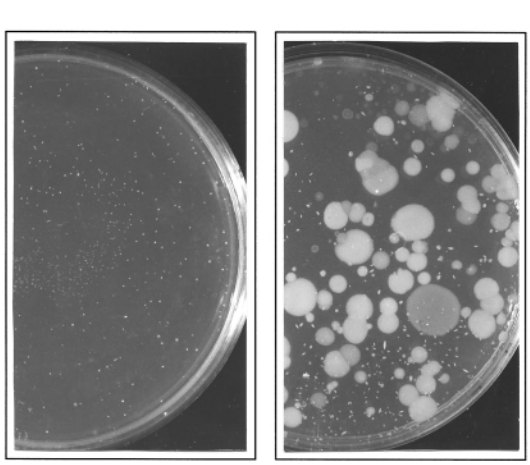

Figure 3 Aspect of colonies grown from fermented dromedary (a) and cows' (b) skim milks on medium M17; incubation 25°C for 48 h.

kg^{-1} (SD=0.01) and from 1.68 to 2.87 g kg^{-1} (SD=0.03) for dromedary milk and for cows' milk, respectively.

Buffering capacity

In both kinds of milk, the buffering curve vs. pH showed a bell shape (Figure 5). The buffering capacity of dromedary milk was higher than that of bovine milk, except in the pH interval between wn>/5.20 and 5.80. The maximum buffering capacity took place at pH 4.75 (SD=0.04) and pH 5.15 (SD=0.03) for dromedary milk and bovine milk, respectively.

Rheological study

Apparent viscosity changes (Figure 6): After a slight and progressive decrease, the apparent viscosity increased until the last measured point (Figure 6). The minimum of viscosity for dromedary milk was observed at a lower pH value (′5.0 vs. ′5.5 for bovine milk). Finally, whatever the postinoculation time, dromedary milk seemed less viscous. Thus, at the onset of the fermentation process, the apparent viscosity at 200 s^{-1} was 1.7 mPa s (SD=0.01) for dromedary milk against 2.08 mPa s (SD=0.02) for bovine milk. At pH 4.32 it was, respectively, 2.16 mPa s (SD=0.01) against 3.4 mPa s (SD=0.05).

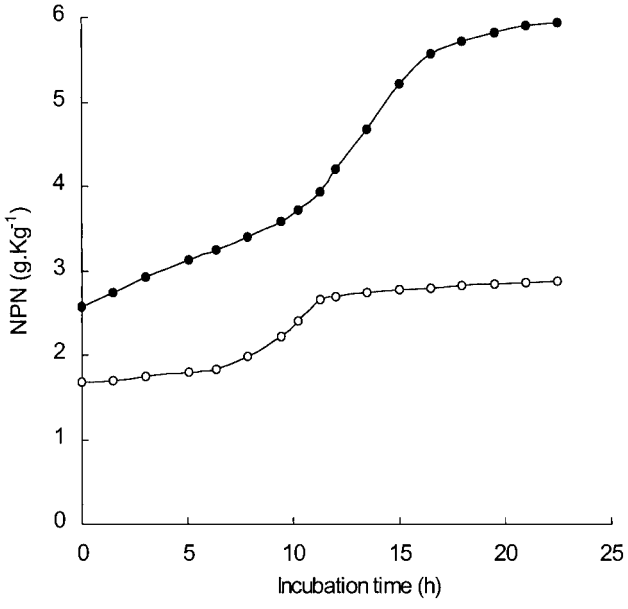

Figure 4 Changes in NPN of dromedary milk (•) and of cows' milk (○), during a fermentation, at 20°C, with a lactic acid starter (CH-normal O1; 2%). Values are means of six pooled milks.

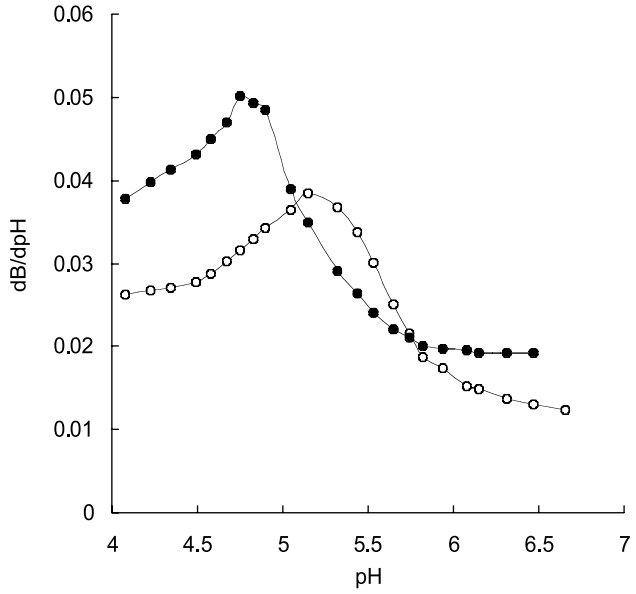

Figure 5 Changes in the buffering values of dromedary (•) and of cows' (○) skim milks [(volume of the added acid×normality)/pH change]. This capacity was determined on 100-ml milk volumes. Values are means of six pooled milks.

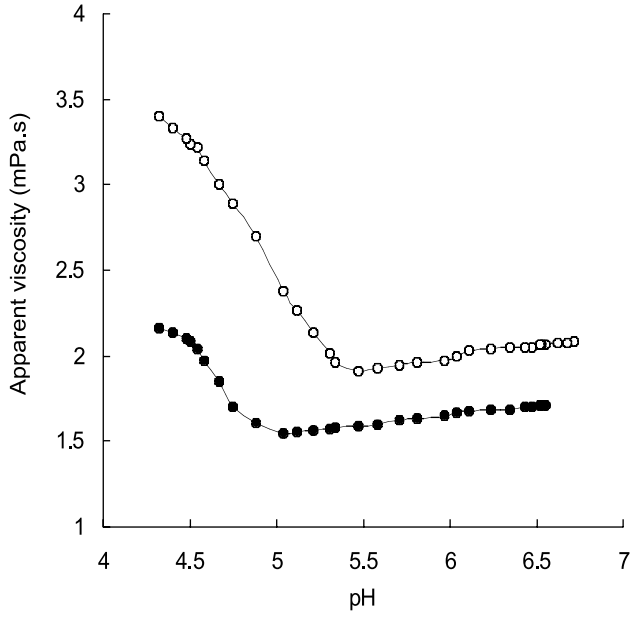

Figure 6 Changes in the apparent viscosity (200 s^{-1}; 20°C) of dromedary (•) and of cows' (○) skim milks during a fermentation by a lactic acid starter (CH-normal O1; 2%). Values are means of six pooled milks.

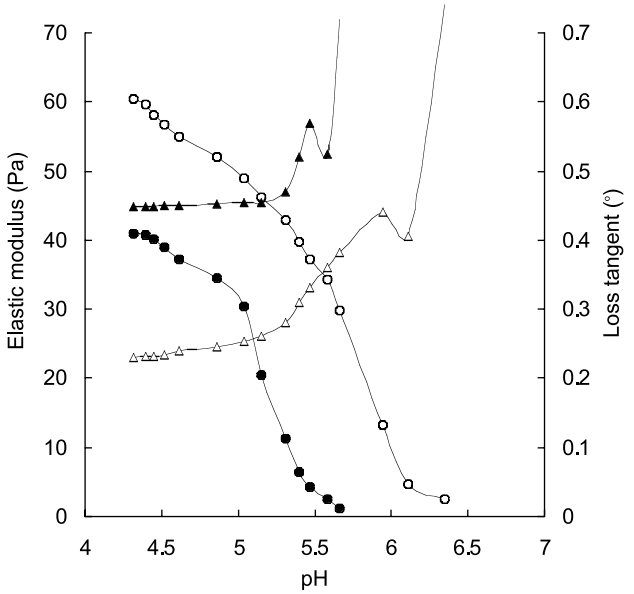

Figure 7 Changes in the elastic modulus (G') and in the loss tangent (tan δ) of dromedary skim milk (filled symbols) and of cows' skim milk (open symbols), during a fermentation, at 20°C, by a lactic acid starter (CH-normal O1; 2%) (●, ○): G' modulus; (▲, △): tan δ.

Elastic modulus and loss tangent changes (Figure 7): During fermentation, the elastic modulus ($\acute{G}$) of dromedary milk appeared at a lower pH value (pH ′5.7 vs. ′6.3 for bovine milk) and showed lower values at any fermentation stage (Figure 7). At the manifestation of $\acute{G}$, the loss angle tangent (tan δ) started dropping. Then it had a sudden but slow increase close to pH 6.0 for bovine milk and close to pH 5.5 for dromedary milk. After that, it decreased notably to reach approximately the values 0.23 for bovine milk and 0.45 for dromedary milk. This decrease phase was much shorter for dromedary milk because it corresponded to a drop of ′0.35 vs. ′1.5 pH unit for cows' milk, i.e., a fermentation time interval of 3 and 11 h, respectively.

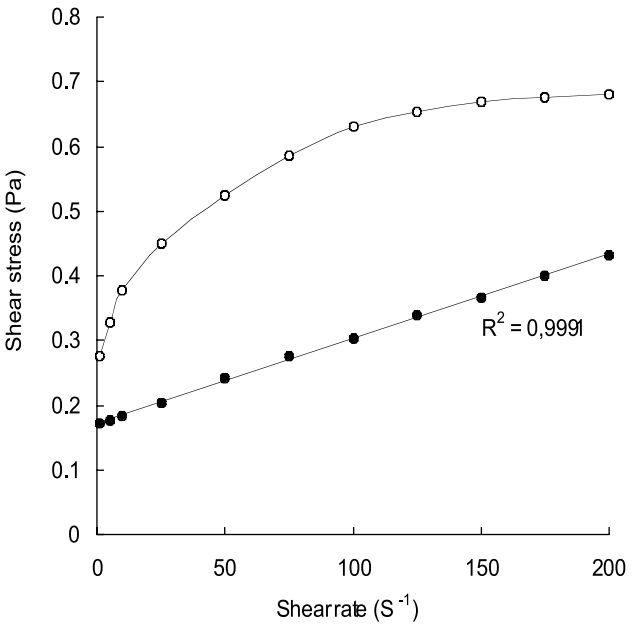

Figure 8 Flow curves, at 20°C, of dromedary skim milk (●) and of cows' skim milk (○) curds (pH 4.4) obtained by a fermentation with a lactic acid starter (CH-normal O1; 2%).

Coagulum flow curves (Figure 8): The rheograms shear stress (τ) – shear rate (D) indicated that the coagulum of both milks (pH 4.4) behaved as a plastic body (Figure 8). However, dromedary milk coagulum displayed a Bingham fluid behavior ($\tau=\tau_s+\alpha D$; α=constant), whereas the bovine milk coagulum displayed a nonideal plastic body behavior. The yield stress (τ_s) of dromedary milk coagulum was lower than that of bovine milk coagulum (0.17 Pa, SD=0.01 vs. 0.27 Pa, SD=0.01).

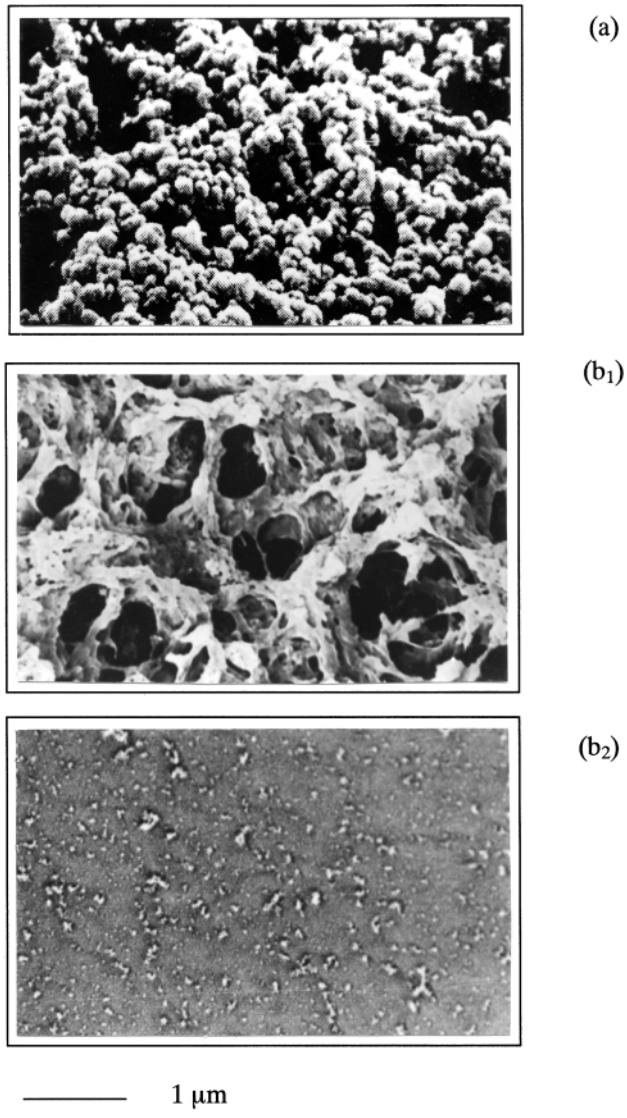

Figure 9 SEM micrographs of cows' (a) and of dromedary (b) skim milk curds (pH 4.4) obtained by a fermentation, at 20°C, with a lactic acid starter (CH-normal O1; 2%), (b_1) Flakes sample. (b_2) Soluble phase sample.

Coagulum structure

At the end of the incubation (pH 4.4), there was a real curd in cows' milk. It was a homogeneous and smooth mass, which entirely occupied the initial volume of milk. On the contrary, the fermented dromedary milk sample did not produce a curd structure. Indeed, we observed few dispersed small casein fragments at the surface and a film of firm gel at the bottom of the vessel.

SEM micrographs (Figure 9) showed that the structure of the fermented bovine milk consisted of individualized particles that were coalesced in chains leading to a relatively homogeneous sieve. However, for fermented dromedary milk, two structures were observed according to whether the analysis concerned dispersed flakes or the aqueous phase. In the first case (Figure $9b_1$), we observed a loose network presenting a tangle of chains. In the second case (Figure $9b_2$), we observed very small protein aggregates that had different shapes.

Discussion

During the lactic fermentation process, dromedary milk showed a behavior different from that of bovine milk at the microbiological level (Figures 1–4), at the biochemical level (Tables 1 and 2; Figure 5) and at the structural level (Figures 6–9). This difference is certainly due to intrinsic characteristics because in similar fermentation conditions, cow's milk formed a firm curd whereas dromedary milk did not (Section 3.5, Coagulum structure).

Dromedary milk appeared less favorable for the lactic fermentation because the activity of the inoculated lactic starter was lower in this milk than in bovine milk. Thus, in the dromedary milk fermentation, the acidification rate was lower (Figure 1), the lactic acid production (Figure 2a) was less marked and the final bacterial counts (Figure 2b) were much lower (3.2×10^4 vs. 15.7×10^4 cfu/ml).

The abrupt loss of colony-forming units just at the beginning of dromedary milk fermentation, ′15 min (Figure 2b), was rather surprising. It could only be explained by a rapid and strong inhibition of the lactic starter by some inhibiting agents. The surviving lactic bacteria grew rapidly (Figure 2b), suggesting that they are not affected by the inhibition phenomenon.

These observations would be related to the possible presence of a high content of natural protective proteins, some of which are present in this milk, such as lysozyme, lactoferrin, lactoperoxidase, immunoglobulin G and A, etc. [7,15,17,41].

Moreover, the colonies grown from the fermented dromedary milk were small compared to those of fermented cows' milk (Figure 3), which suggests the existence of inhibition factors. These results confirmed those previously reported by Gnan *et al* [24,25] who noted a drastic drop of the initial concentration of *Lactobacillus acidophilus* starter. The bacterial counts dropped from 3×10^2 cfu/ml to less than 10^2 cfu/ml in a few minutes [24]. These authors also noted a large difference in size between the colonies grown from camel milk and those grown from bovine milk. The small colonies grown from dromedary milk were the size of a pinhead [25].

The proteolytic activity of the lactic starter was much more marked for the dromedary milk as shown by the relatively large increase in the NPN in this milk (Figure 4). Proteinases have been found in the strains of lactic starters [27]. These activities increased greatly below pH 5.5 [38], i.e., in the zone where the solubilization of micellar caseins of bovine milk is high [11,36]. These proteinases were necessary to provide sufficient available nitrogenous compounds for lactococci, which preferentially attacked caseins in general, and the β fraction, in particular [14,18]. These facts would explain the relatively higher NPN content in dromedary milk during the fermentation process (Figure 4). Dromedary milk was characterized by a relatively high initial soluble casein content (Table 2) and by a relatively high β casein content [29]. This intense proteolytic activity of the lactic acid starter may also explain the earlier decline phase of lactic acid bacteria in dromedary milk (Figure 2). Indeed, the high proteolytic activity could induce an inhibiting effect on this growth [28]. This phenomenon would explain, for dromedary milk, the large deviation between the end of the log phase and the maximum acid production (Figure 2).

At the biochemical level, dromedary milk fermentation also differed from bovine milk as shown by changes in buffering capacity (Figure 5). This property controlled pH during fermentation and therefore reflected the release rate of micellar minerals to the milk-soluble phase. The general shape of the buffering capacity curves was identical for both milks and was comparable to that reported for cows' milk by Lucey *et al* [31]. The maximum buffering capacity observed at about pH 5.1 has been related to that corresponding to total solubilization of the micellar complex inorganic phosphate–calcium that took place at around the same pH [12,23,39]. Nevertheless, the same correlation could be assumed for dromedary milk with differences: (1) The buffering capacity of this milk started to increase only near pH 5.7 (vs. pH 6.7 for bovine milk). Consequently, the solubilization of micellar buffers, mainly the phosphate buffer (HPO_4^-) and the citrate buffer (H-citrate$^-$) would start at around this pH (5.7). (2) The maximum buffering capacity was at a relatively lower pH (pH 4.7 vs. pH 5.2 for bovine milk). Therefore, the total release of the micellar mineral complex took place only at about pH 5.7. (3) The maximal capacity was higher than that of the cows' milk. This suggests a rapid production of lactate ions due to significant demineralization of dromedary milk micelles at about pH 4.8. This result confirmed the higher mineral charge of the dromedary milk micelle mainly in citrate buffer (Table 2).

At the physical level, the rheological study also revealed several specificities of fermented dromedary milk. Thus the general shape of the curves representing the apparent viscosity changes (Figure 6) was in agreement with the results reported on cows' milk [23]. However, the minimum viscosity related to a reduction in the number of suspended particles was observed at a lower pH for dromedary milk. This confirms the hypothesis of a late release of micelle minerals and thus explains the higher stability of dromedary milk toward increased acidity. Below this minimum, the apparent viscosity of bovine milk was much higher than that of dromedary milk (Figure 6) indicating that the protein aggregates of fermented dromedary milk were very fragile. The increase of the apparent viscosity observed in this second phase was due to the destruction by shearing of bonds between the casein particles.

Contrary to the permanent mode, which was destructive, the sinusoidal mode was nondestructive and permitted the acid milk fermentation (Figure 7). The viscoelastic properties of the fermented dromedary milk appeared at a relatively low pH. This milk, therefore, remains a Newtonian liquid up to an advanced stage of fermentation, which confirms its relatively higher physical stability toward the lactic acidification process (Figure 6).

Changes in loss tangent (tan δ) of milk as related to pH presented similar curves for both milks (Figure 7). However, tan δ

values of dromedary milk were higher suggesting that during lactic fermentation the nonpermanent protein–protein bonds were more frequent in dromedary milk. In fact, the loss angle (δ) of a product presents an estimation of its Newtonian character (nonpermanent bonds) in comparison with its elastic character (permanent bonds). The three phases of the tan δ vs. pH curve (Figure 7) seemed to correspond to three different physical states of the fermented milk. Thus, the initial drop of tan δ was related to a decrease of the Newtonian character. The increase observed in the second phase would indicate, for both milks, that structural reorganization is necessary to acquire the viscoelastic properties. Finally, the third phase corresponded to elaboration of lactic curd. This latter phase was very short in the case of dromedary milk and, therefore, would not be enough to permit elaboration of a curd structure. In fact, hydrophobic, hydrogenous and nonmineral electrostatic bonds, which characterize the lactic curd of bovine milk, take time to be formed [37]. As a result, it is very probable that the rapid transition of dromedary milk from the liquid state to the gel state would be one cause of the absence, in the fermented milk, of a real curd and the presence of protein flakes. These flakes would be the beginnings of a curd structure or simple physical contacts between caseins as shown by the flow curve of this dromedary milk pseudocoagulum (Figure 8). Indeed, once the cohesive forces were destroyed, the particles forming the clusters instantaneously moved toward the same direction of the flow. However, the bovine milk curd behaved as a nonideal plastic body (Figure 8). This is in agreement with the results reported by Fangary *et al* [19]. In fact, below the yield stress, the breaking of the interprotein particle bonds was progressive and the state of its microstructure varied during shearing [5]. This difference in flow behavior between dromedary and bovine fermented milks indicates an important difference in the nature, in the number and in the strength of bonds involved. Therefore, the possibilities of aggregation between the casein particles would be different in both fermented milks as visualized by SEM (Figure 9). Thus, the protein particles of cows' milk coagulum interact to form a homogeneous open structure (Figure 9a). However, the particles of dromedary milk could either fuse into small coagulum fragments (Figure $9b_1$) or disperse into microscopic particles of casein (Figure $9b_2$). From a physical point of view, the two types of milk were not similar. Their differences may be attributed partly to micellar size. In fact, an inversely proportional relation exists between the micellar size and the κ-casein content [16,32]. Similarly, large micelles contain more saline bridges than small ones [9,12].

Conclusion

The present study permitted the visualization, both macroscopically and microscopically, of the inability of dromedary milk to produce an acid lactic curd. This was perceived under two aspects: a mechanical aspect and a microbiological aspect. The former concerned the total absence of a firm curd in dromedary milk whereas the latter was related to a relatively low rate of the lactic fermentation process in dromedary milk compared to that of bovine milk. The difference could be attributed to inherent factors in dromedary milk. One of these may be the mineral and/or the casein composition of its colloidal phase. Another is the probable presence of inhibition factors, which could be either natural or elaborated during fermentation.

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