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Original article

Characterization of the dromedary milk casein micelle and study of its changes during acidification

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Abstract — Biochemical and physical properties of dromedary milk were studied at initial pH and during an acidification with glucono-δ-lactone. In comparison with bovine milk salt, citrate and nitrogen partition between soluble and micellar phases revealed several characteristics. Dromedary milk micelle presented a more important mineral plus citrate charge (~ 98 mg·g^{-1} casein). Micellar Mg, P and citrate proportions were higher, about 2/3, 2/3 and 1/3, respectively. During acidification, micellar mineral and citrate release started tardily (~ pH 5.8) and the maximum of casein and Pi solubilization took place at lower pH values, 4.9 and 4.8, respectively. Dromedary milk micelle seemed to maintain its integrity until about pH 5.5, below, it underwent extensive biochemical and structural modifications mainly at pH 5.0. At this point, dromedary milk was characterized by a loose microstructure, by a maximum of loss modulus and of micelle hydration and by a minimum of apparent viscosity. The pH 5.0 would be a transition pH between micellar structure and coagulum structure.

dromedary milk / micelle / acidification / microscopy / rheology

Résumé — Caractérisation de la micelle de caséine du lait de dromadaire et étude de son évolution au cours de l'acidification. Des propriétés biochimiques et physiques du lait de dromadaire ont été étudiées au pH initial et au cours d'une acidification par la glucono-δ-lactone. La détermination des répartitions de la fraction minérale, du citrate et de la fraction azotée du lait, entre les phases micellaire et soluble, a révélé de nombreuses particularités par rapport au lait bovin. La micelle du lait camelin présente une charge en minéraux plus citrate relativement plus importante (~ 98 mg·g^{-1} caséines). Les proportions en Mg, en P et en citrate micellaires sont plus élevées (respectivement 2/3, 2/3, 1/3). Au cours de l'acidification, la libération des minéraux et du citrate micellaires débute tardivement (~ 5,8) et la solubilisation maximale des caséines et celle du Pi est obtenue à des pH plus bas (respectivement 4,9 et 4,8). La micelle du lait de dromadaire paraît préserver son

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intégrité jusqu'aux environs du pH 5,5. En deçà, elle subit de profondes modifications biochimiques et structurales notamment vers le pH 5,0. En ce point, le lait de dromadaire est caractérisé par une microstructure ouverte, par un maximum de module de perte, par un minimum de viscosité apparente et par un maximum d'hydratation micellaire. Le pH 5,0 serait un pH de transition entre une structure de micelle et une structure de coagulum.

lait de dromadaire / micelle / acidification / microscopie / rhéologie

1. INTRODUCTION

Most of the dromedary milk is drunk fresh because, contrary to other types of milk, it does not easily lend itself to the transformation into dairy products [1, 17]. Difficulties are related to the control of the coagulation process which is a necessary stage in dairy product manufacture. Thus, whatever method is used, i.e. acid, enzymic or mixed, the formed coagulum does not present the requisite qualities to undergo further technological treatments [16].

Concerning cows' milk, it has already been established that the casein micelle has a dominant role in the edification of the physical properties of curd [10]. Therefore, to understand dromedary milk coagulum, it was necessary, beforehand, to study the composition and the structure of its colloidal phase at initial pH and during technological transformations.

The limited information available on this micellar fraction concerns the isolation of caseins and the study of micelle size. Compared to bovine milk caseins, caseins of dromedary milk show a different electrophoretic mobility [18], a poor rennetability [17] and partially similar amino acid sequences [24]. The dromedary milk is particularly marked by a relatively low content of κ-casein [24, 30] and by a relatively large average micelle diameter [11].

In the present paper, we tried to acquire some information about changes in dromedary milk micelles as a function of progressive pH lowering. First, we observed the solubilization of micellar components. Second, using microscopic, rheological and hydration studies, we attempted to investigate changes in micelle structure. The results were compared with those available on cows' milk.

2. MATERIALS AND METHODS

2.1. Milk samples

The milk came from the milking of an eighteen-dromedary herd (*Camelus dromedarius*) of Maghrabi breed belonging to the Institute of Arid Regions (Institut des Régions Arides, Medenine, Tunisia). The collected milk samples were pooled, kept refrigerated and transported to the laboratory about 6 h after milking. Upon its arrival, the pooled milk was then immediately skimmed with 3 successive centrifugations at 2 000 *g* and 10 °C for 15 min. Then, it was stabilized with sodium azide at 0.02% (w/v) and divided into portions of 50 mL. The acidification was carried out at 20 °C by addition of definite amounts of glucono-δ-lactone (GDL) so that all the desired pH values were attained after about 24 h. Thus, for pH 4.4, we added 1.05% (w/v) of GDL.

All assays were carried out on 6 different milk samples. Each sample was a pool of milk taken from 16 to 18 animals.

2.2. Soluble and micellar phase separation

Separation of the soluble and micellar phases of the initial milk (pH 6.55) and of the acidified milks was achieved by ultracentrifugation at 190 000 *g* for 60 min at 20 °C using a Beckman L 7-55 apparatus (Beckman Instruments France S.A., Gagny, France) equipped with a rotor SW 41. After centrifugation, the supernatants were carefully removed and kept at –20 °C until soluble nitrogen, soluble mineral and soluble citrate measurements. The concentrations of these components were determined taking into consideration the pellet volume. Study of the solvation water was done on the micellar pellets, just after the ultracentrifugation.

2.3. Mineral and citrate analyses

Calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) were measured by a model Hitachi Z-6100 atomic absorption spectrometer (Hitachi Instruments Engineering Co., Ibaraki-ken, Japan) in the presence of lanthanum chloride for Ca and Mg, and in the presence of cesium chloride for K and Na. The concentration of total phosphorus (P) was determined by a colorimetric method with ammonium molybdate [31]. Micellar organic phosphorus (Po) content was deduced by the difference between the milk's total P at native pH and soluble P at pH 4.8 (i.e. maximum of P solubilization). For each pH, soluble inorganic phosphorus (Pi) was estimated by the difference between soluble P and Po linked to the dissociated micellar caseins. Citrate content was determined enzymatically using a commercially available test kit (Bœhringer, Mannheim, Germany, catalog number 139076). Each measurement was carried out in duplicate. The repeatability was estimated to be 2.5%.

2.4. Nitrogen

The total nitrogen (TN) content of the milk and of the supernatants of acidified milks were determined by the Kjeldhal method [2] after mineralization on a Büchi 425 unit, distillation on a Büchi 320 unit (Büchi Laboratoriums-Technik, Flawil, Switzerland) and titration with HCl (0.1 mol·L^{-1}). The corresponding protein contents were calculated using 6.38 as the conversion factor. Milk casein content was calculated by the difference between (TN) and non-casein nitrogen (NCN) after separation according to Rowland [33]. Casein contents of supernatants were calculated by the difference between TN content of these supernatants and NCN content of the corresponding milk. Each measurement was carried out in duplicate. The repeatability was estimated to be 3.5%.

2.5. Solvation water

The solvation of the casein micelles was determined according to Thompson et al. [38]. For each sample, the corresponding pellet remaining after ultracentrifugation was weighed, lyophilized during 48 h on a Usifroid SMH 15 apparatus (Usifroid, Maurepas, France) and dried at 102–104 °C during 24 h. After drying, the loss in weight was expressed as g H_20·g^{-1} dry pellet. The protein content of each pellet was determined by the difference between total protein of the milk and total protein of the corresponding supernatant (i.e. soluble protein of milk). Each measurement was carried out in duplicate. The repeatability was estimated to be 2%.

2.6. Scanning electron microscopy (SEM)

Samples were treated according to Attia et al. [6]. They 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).

2.7. Rheological measurements

All measurements were carried out using a StressTech Reologica rheometer (Reologica Instruments AB, Lund, Sweden) with a coaxial cylinders measuring system (diameters 25 and 27 mm) and at 20 ± 0.1 °C. In the permanent mode, samples were subjected to a constant shear rate 200 s^{-1}). Dynamic measurements were followed at a frequency of 1 Hz and in the linear viscoelastic region (strain < 2%). For the two modes, after addition of the definite amount of GDL to the milk, the mixture was stirred manually for 1 min and then 15.9 mL of the mixture was immediately transferred to the rheometer measuring system which was covered to prevent evaporation. A separately thermostated sample, kept at the same temperature (20 °C), permitted the pH values to be followed. Each analysis was done in duplicate. Repeatability was estimated to be 6%.

3. RESULTS

3.1. Solubilization of micellar components as a function of pH

3.1.1. Mineral and citrate partition in milk

The average mineral and citrate charges of dromedary milk and its partition between micellar and serum phases, at initial pH (pH 6.55) are presented in Table I. Almost all K and all Na was soluble whereas the Ca, Mg, P and citrate were divided up between the 2 phases of milk with micellar parts close to 2/3, 2/3, 2/3 and 1/3 respectively.

Figure 1 illustrates the pH lowering effect on the soluble mineral and citrate contents. Soluble Ca, Mg, P and citrate concentrations increased during acidification and presented sigmoidal curves. The solubilization of these minerals started toward pH 5.8 and their corresponding curves presented slope changes at about pH 5.2–5.0. The maximum solubilization of P, 0.7 $g\cdot kg^{-1}$ (SD = 0.01), and that of citrate, 1.72 $g\cdot kg^{-1}$ (SD = 0.04), were obtained at about pH 4.8. For lower pH values, soluble P and citrate contents decreased.

Quantitatively, Mg and Ca were dissolved with different rates (Fig. 2). The first cation was released more rapidly than the second one. Their solubilization continued up to pH 4.7 and pH 4.5, respectively. The comparative study of micellar concentration of Pi and Ca during acidification (Fig. 2) revealed a slow solubilization at the onset, and a rapid one from pH 5.5 with a real inflection point at about pH 5.0. The micellar Ca / micellar Pi ratio was practically constant at the onset. But starting from pH 5.5, this ratio increased until about pH 4.8. At this point, all micellar Pi was

Table I. Average contents of main minerals and of citrate in dromedary skim milk and their partition between soluble and micellar phases ($\bar{x}$: mean values of 6 pooled milks, SD: standard deviation).

Tableau I. Teneurs moyennes en principaux minéraux et en citrate du lait écrémé de dromadaire et leurs répartitions entre phases soluble et micellaire ($\bar{x}$: moyenne des valeurs obtenues sur 6 laits de mélange, SD : écart type).

Mineral	Total content ($g\cdot kg^{-1}$)		Soluble fraction ($g\cdot kg^{-1}$)		Micellar fraction (%)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
K	1.72	0.06	1.69	0.04	1.7	0.1
Na	0.66	0.02	0.64	0.01	3.0	0.1
Ca	1.23	0.04	0.43	0.02	65.0	1.0
Mg	0.090	0.003	0.033	0.002	63.3	1.2
P	1.02	0.02	0.35	0.02	65.6	0.9
Citrate	1.82	0.04	1.24	0.03	31.8	1.5

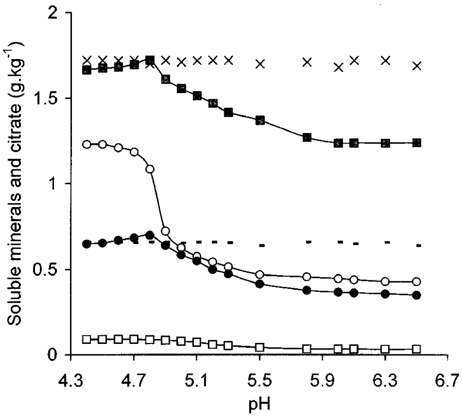

Figure 1. Solubility of minerals and of citrate in dromedary skim milk during acidification by GDL: (Mg), □; (P), ●; (Ca), ○; (Na), –; (Citrate), ■; (K), ×. Values are the means of results of 6 pooled milks. (SD of some values are indicated in the text.)

Figure 1. Évolution de la concentration en minéraux solubles et en citrate soluble au cours de l'acidification, par la GDL, du lait écrémé de dromadaire : (Mg), □ ; (P), ● ; (Ca), ○ ; (Na), – ; (Citrate), ■ ; (K), ×. Les valeurs sont les moyennes des résultats obtenus pour 6 laits de mélange. (Les écarts types de quelques valeurs sont indiqués dans le texte.)

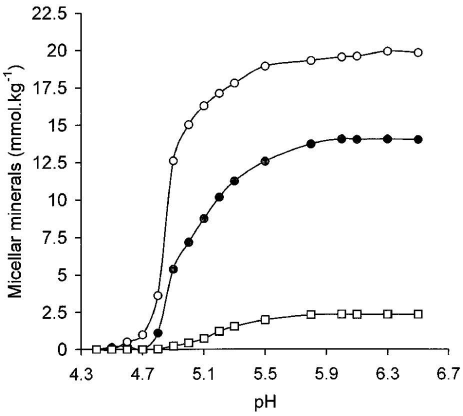

Figure 2. (Ca), ○; (Mg), □; (Pi), ● contents in the micellar phase of dromedary skim milk during acidification by GDL. Values are the means of results of 6 pooled milks. (SD of some values are indicated in the text.)

Figure 2. Évolution de la teneur des micelles en (Ca), ○ ; (Mg), □ ; (Pi), ● au cours de l'acidification, par la GDL, du lait écrémé de dromadaire. Les valeurs sont les moyennes des résultats obtenus pour 6 laits de mélange. (Les écarts types de quelques valeurs sont indiqués dans le texte.)

solubilized while a certain amount of Ca remained in the micelle.

The micellar change of [Ca + Mg] concentration as a function of micellar [Pi + Citrate] concentration (Fig. 3) was practically linear between pH 5.8 (the beginning of demineralization) and pH 4.9. In that interval, cation release seemed to be related to that of anions by the following equation: [micellar cations] = *a* [micellar anions] + *b*. The value of the constant *b* gave an estimation of micellar cation charge of the dromedary milk when all micellar anions were released.

3.1.2. Nitrogen partition in the milk

Table II presents the main nitrogen fractions of dromedary milk at initial pH. The determination of the amount and of the partition of caseins permitted an estimation of micelle mineralization of dromedary milk (~ 98 mg of salts plus citrate·g^{-1} of caseins) (Tab. III).

Figure 4 illustrates the micellar casein solubilization of dromedary milk during acidification. The change in soluble casein content as a function of pH presented a bell shaped curve and showed a maximum (37.2%; SD = 1.6) at about pH 4.85. Just below this point, soluble casein content dropped rapidly.

3.2. Changes of physical properties as a function of pH

3.2.1. Micellar hydration changes (Fig. 5)

At initial pH (pH 6.55), the solvation water of casein micelles was 3.32 g H_2O·g^{-1} dry micelles (SD = 0.06). The hydration curve showed, first, a decrease until pH 5.5 followed by an increase with a maximum at about pH 4.95, 3.32 g $H_2O \cdot g^{-1}$ dry micelles (SD = 0.09). Below this point, micellar hydration decreased again.

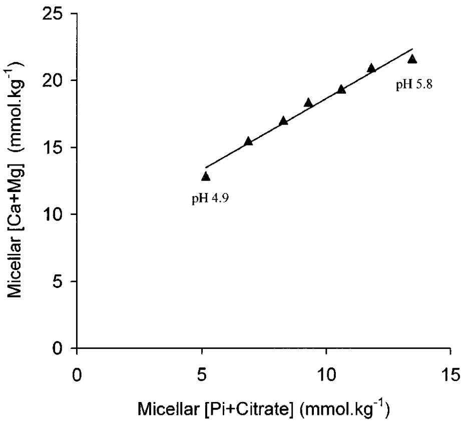

Figure 3. Micellar [Ca + Mg] content as a function of micellar [Pi + Citrate] content during dromedary skim milk acidification by GDL between pH 5.8 and pH 4.9 ($y = 1.0697\,x + 7.9518$, $R^2 = 0.9764$). Values are the means of results of 6 pooled milks. (SD of some values are indicated in the text.)

Figure 3. Évolution de la teneur en [Ca + Mg] micellaire en fonction du [Pi + Citrate] micellaire au cours de l'acidification par la GDL, du lait écrémé de dromadaire, dans l'intervalle de pH [5,8–4,9] ($y = 1{,}0697\,x + 7{,}9518$, $R^2 = 0{,}9764$). Les valeurs sont les moyennes des résultats obtenus pour 6 laits de mélange. (Les écarts types de quelques valeurs sont indiqués dans le texte.)

Table II. Content of nitrogen compounds in dromedary skim milk ($\bar{x}$: mean values of 6 pooled milks, SD: standard deviation, cas.: caseins).

Tableau II. Teneurs en fractions azotées du lait écrémé de dromadaire ($\bar{x}$: moyenne des valeurs obtenues sur 6 laits de mélange, SD : écart type, cas. : caséines).

Type of analysis	Content	
	$\bar{x}$	SD
TN ($g \cdot kg^{-1}$)	32.4	0.7
N C N ($g \cdot kg^{-1}$)	10.2	0.4
Total cas. ($g \cdot kg^{-1}$)	22.2	0.5
Soluble cas. (%)	15.4	1.1
$\frac{\text{Total cas.}}{\text{TN}}$ (%)	68.5	1.2

Table III. Estimation of the mineral and citrate contents in dromedary skim milk micelles (mean values from 6 pooled milks).

Tableau III. Estimation de la charge en minéraux et en citrate de la micelle de caséine du lait écrémé de dromadaire (les valeurs correspondent à la moyenne des résultats obtenus sur 6 laits de mélange).

Mineral	Content ($mg \cdot g^{-1}$ caseins)
Ca	42.6
Mg	3.0
Pi	18.7
K	1.6
Na	1.1
Citrate	30.9
Total	97.9

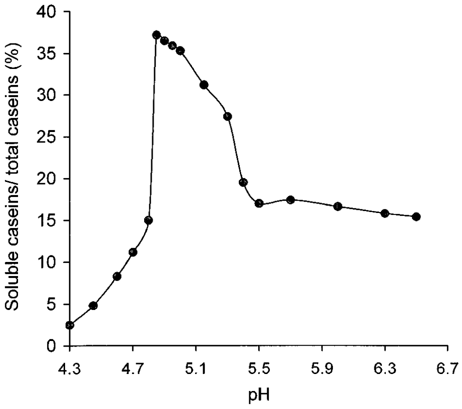

Figure 4. Solubility of casein in dromedary skim milk during acidification by GDL. Values are the means of results of 6 pooled milks. (SD of some values are indicated in the text.)

Figure 4. Évolution du pourcentage des caséines solubles au cours de l'acidification, par la GDL, du lait écrémé de dromadaire. Les valeurs sont les moyennes des résultats obtenus pour 6 laits de mélange. (Les écarts types de quelques valeurs sont indiqués dans le texte.)

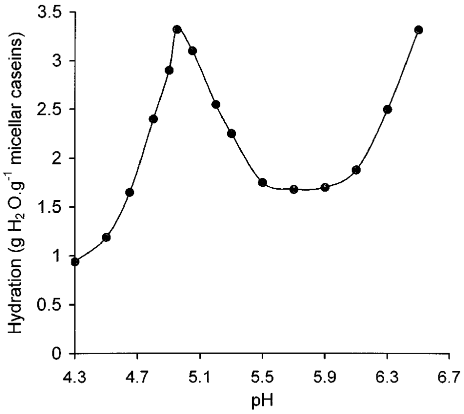

Figure 5. Changes in micellar casein hydration in dromedary skim milk during acidification by GDL. Values are the means of results of 6 pooled milks. (SD of some values are indicated in the text.)

Figure 5. Évolution de l’hydratation des caséines micellaires au cours de l’acidification, par la GDL, du lait écrémé de dromadaire. Les valeurs sont les moyennes des résultats obtenus pour 6 laits de mélange. (Les écarts types de quelques valeurs sont indiqués dans le texte.)

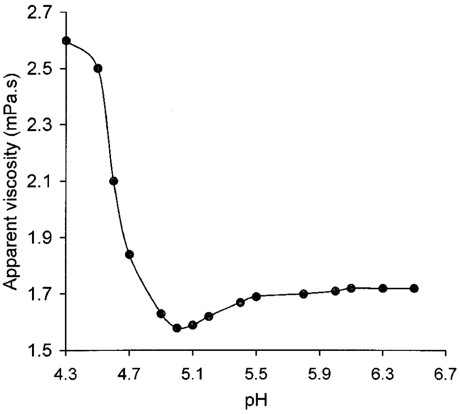

Figure 6. Changes in apparent viscosity of dromedary skim milk during acidification by GDL. Values are the means of results of 6 pooled milks. (SD of some values are indicated in the text.)

Figure 6. Évolution de la viscosité apparente du lait écrémé de dromadaire au cours de l’acidification par la GDL. Les valeurs sont les moyennes des résultats obtenus pour 6 laits de mélange. (Les écarts types de quelques valeurs sont indiqués dans le texte.)

3.2.2. Apparent viscosity changes in milk (Fig. 6)

Dromedary milk’s apparent viscosity underwent three stages of change. First, it remained practically constant until about pH 5.5, 1.69 mPa·s (SD = 0.01). Second, it decreased slightly to reach a minimum close to pH 5.0, 1.58 mPa·s (SD = 0.01). Third, it increased gradually until pH 4.3 which was our last measured pH value, 2.6 mPa·s (SD = 0.16).

3.2.3. Storage and loss moduli changes (Fig. 7)

The study in oscillatory mode showed, near pH 5.0, a maximum value of loss modulus (G’’), 15.00 Pa (SD = 1.05), and an inflection point on the elastic modulus (G’) curve. Just below this point, towards pH 4.95, the two moduli crossed each other,

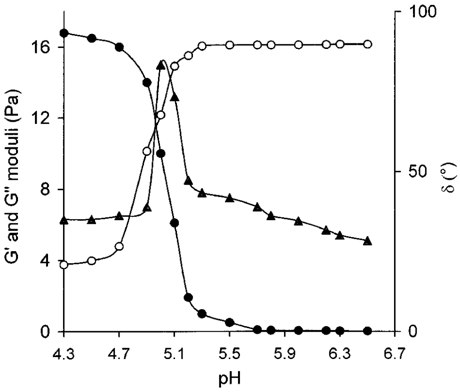

Figure 7. Changes in storage (G’) and loss (G’) moduli, and in phase angle (δ) during acidification of dromedary skim milk by GDL (G’), ●; (G”), ▲; (δ), ○. Values are the means of results of 6 pooled milks. (SD of some values are indicated in the text.)

Figure 7. Évolution du module de conservation (G’), du module de perte (G’) et de l’angle de déphasage (δ) au cours de l’acidification, par la GDL, du lait écrémé de dromadaire. (G’), ● ; (G”), ▲ ; (δ), ○. Les valeurs sont les moyennes des résultats obtenus pour 6 laits de mélange. (Les écarts types de quelques valeurs sont indiqués dans le texte.)

one rising (G’) and the other dropping (G’’). The phase angle (δ) did not change much at the onset but showed an abrupt drop from pH 5.5, 89.5° (SD = 0.4), to pH 4.7, 26.6° (SD = 0.9).

3.2.4. Micellar microstructure changes (Fig. 8)

At the initial pH, the individualized micelles appeared in a spherical shape with different sizes (Fig. 8a). Then, they aggregated toward pH 5.5 in globular or linear shapes while maintaining their integrity (Fig. 8b). Toward pH 5.0 (Fig. 8c), the micelles fused leading to a real three-dimensional structure. Finally, at pH 4.4 (Fig. 8d), the milk showed a very loose network covered with clearly visible casein aggregates.

4. DISCUSSION

The total mineral and citrate concentrations of dromedary milk (Tab. I) were found to be similar to the mineral composition of cow’s milk [3, 8, 22, 39]. Besides, almost all K and all Na were soluble, whereas Mg, Ca, P, and citrate were divided up into micellar

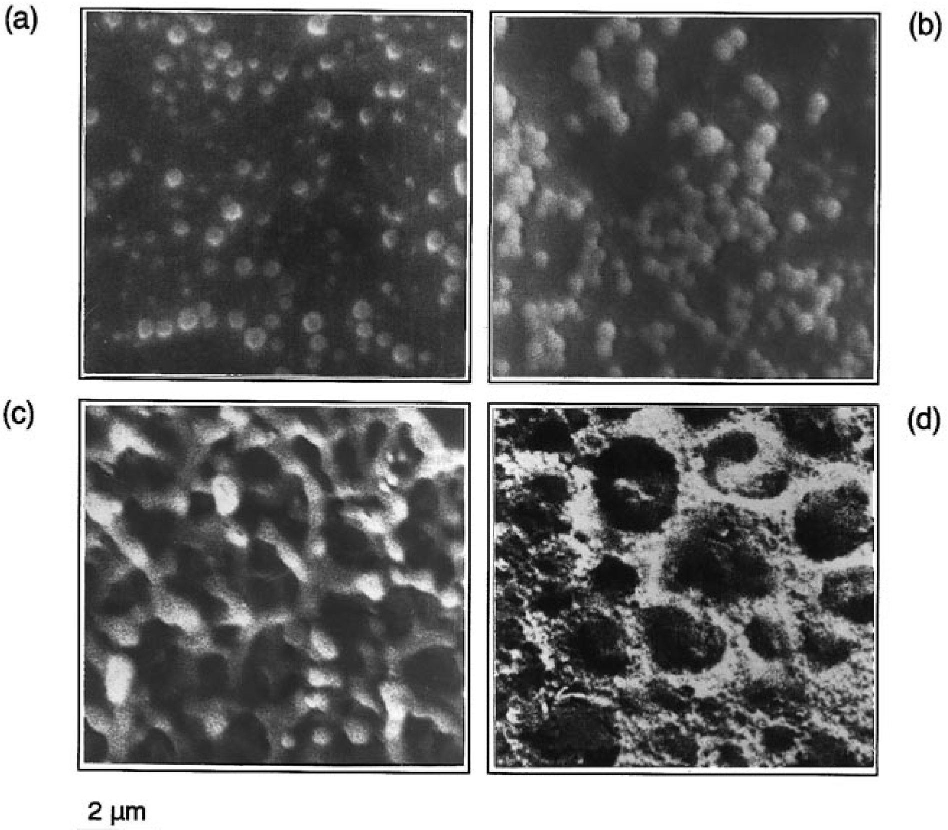

Figure 8. SEM photographs of dromedary skim milk acidified at different pH by GDL. (**a**) pH 6.55; (**b**) pH 5.5; (**c**) pH 5.0; (**d**) pH 4.4.

Figure 8. Observation en microscopie électronique à balayage du lait écrémé de dromadaire acidifié par la GDL, à différents pH. (**a**) pH 6,55 ; (**b**) pH 5,5 ; (**c**) pH 5,0 ; (**d**) pH 4,4.

and soluble phases. However, in dromedary milk only Ca had a partition identical to that of cows' milk since Mg, P and citrate were involved to a more important extent in the micellar phase than in that of the cow, about 2/3, 2/3 and 1/3 respectively versus about 2/5, 3/5 and 1/10 for bovine milk micelles.

Dromedary milk micelles had a salt plus citrate charge of about 98 $mg \cdot g^{-1}$ caseins (Tab. III). It was therefore more mineralized than its homologous in bovine milk (67 $mg \cdot g^{-1}$ caseins) [34]. The most pronounced difference was found for citrate (30 vs. 4 $mg \cdot g^{-1}$ caseins for cows' milk micelle). This higher mineral content would be related to the larger micelle size of dromedary milk [11] since it is an established fact that large micelles contain more saline bridges than small ones [10, 13].

The acidification of dromedary milk led to a progressive solubilization of micellar Ca, Mg, P and citrate (Fig. 1). This phenomenon is identical to that observed in cows' milk and it was illustrated by similar sigmoid curves [14, 19, 26, 39]. However, the characteristic points of these curves were different. Firstly, the demineralization of dromedary milk micelles started at around pH 5.8 whereas, that of cows' milk begins at the onset of acidification. Secondly, the inflection points of the different curves took place around pH 5.2–5.0 compared to pH 5.5 for cows' milk. Thirdly, maximum solubilization of P and of citrate were obtained at a lower pH, 4.8 versus 5.2 for cows' milk.

In comparison with bovine milk, the demineralization of dromedary milk micelles was, therefore, translated towards a lower pH. This result could be attributed to significant differences between cow and dromedary milk in their primary structures and composition as recently reported by Kappeler et al. [24] and by Ochirkhuyag et al. [30].

Between pH 5.8 and 4.9, the pH lowering seemed to concern mainly the mineral solubilization of the colloidal complex phosphate-citrate of Ca and Mg as justified by the proportional release of micellar cations and of micellar anions (Fig. 3). In the case of cows' milk, the solubilization of this complex, related to the ionization regression of casein acid functions, started from the onset of acidification and took place over a larger interval [26, 39, 40].

The comparison of changes in Ca and Pi solubilization (Figs. 1 and 2) also suggested that the calcium directly associated with caseins started solubilization only at around pH 4.8 when all the colloidal phosphate-citrate of the Ca and Mg edifice was released.

The solubilization of Ca and that of Mg (Fig. 1) continued until close to the pH observed for cows' milk (pH ~ 4.6) [9, 14]. However, contrary to what had been reported for cows' milk [9], the compared changes in solubilization of these two cations (Fig. 2) showed an important difference in behaviour. The relatively fast release of Mg would be related to a higher affinity degree between Ca and micellar caseins. So, contrary to cows' milk, these two minerals would not occupy identical sites within the dromedary milk micelle.

The decrease of P and citrate solubility, observed below the pH of maximum solubilization (Fig. 1) is a phenomenon which was shown for cows' milk [20] and also for micelle suspensions of bovine caseins [27] below pH 4.6. It must be due to interactions between P and citrate acid groups with weak pKa (2.10 and 3.13 respectively) and some positive charges of dromedary milk caseins.

Another separation process, the ultrafiltration, should be used in order to consolidate the original results concerning the mineral fraction partition and composition of dromedary milk.

The total nitrogen content (TN) of dromedary milk ~ 32 $g \cdot kg^{-1}$ was similar to that reported for cows' milk [3, 28]. However, this milk was characterized by a relatively low content of total caseins (~ 22 $g \cdot kg^{-1}$ vs. 28 $g \cdot kg^{-1}$ on average for cows' milk) and by a relatively low total casein / TN ratio (68.5% against 79% on average for cows' milk). The partition of caseins in milk at initial pH (Tab. II), has notably revealed a higher content of soluble caseins (15.4% of total caseins) than that observed on the cows' milk which was approximately 7% [12]. It is probable that the possibilities of interactions between casein molecules within the micelle were different in the two kinds of milk. This difference would essentially concern the hydrophobic bonds whose importance depends on the number and polarity of the lateral chains of some amino acid residues [21].

The casein micelle fraction presented a relatively low content of caseins (Tab. II) and a relatively high content of minerals plus citrate (Tab. III) which may give to the dromedary micelle an important mineral plus citrate / casein ratio (~ 98 $mg \cdot g^{-1}$ of caseins). As for cows' milk [4, 23, 34], the minerals of the colloidal phase would assure the intra- and intersubmicellar bonds. In fact, during acidification, these salts moved towards the aqueous phase leading to a destabilization of the dromedary milk micelle and to a partial solubilization of its caseins. The curves reflecting this dissociation were similar to those reported for cows' milk [12, 35, 39]. However, the maximum of solubilization of dromedary milk caseins was obtained at a lower pH (4.9 vs. 5.4 for bovine milk). A relation of cause and effect could be considered on the one hand between the relatively low casein content and the relatively high mineral charge of dromedary milk micelles (Tabs. II and III) and on the other hand its late disintegration (Fig. 4). Thus, the maximum casein dissociation obtained close to pH 4.9 coincided with the important release of minerals constituting the micellar saline bridges (Figs. 1 and 2). Below this maximum, the decrease of soluble casein content could express the beginning of casein precipitation. Dromedary milk caseins must present isoelectric pH values similar to those of bovine milk [24].

The hydration curve profile of the casein micelle appeared (Fig. 5) similar to that observed for cows' milk [19, 35, 36]. However, the limits of the three characteristic stages of the curve were not the same. Thus, the initial stage of decrease continued until pH 5.5 against pH 6.0 for the bovine milk. The increase phase was limited by the pH values 5.5 and 5.0 against 6.0 and 5.4 for cows' milk. The third phase did not present a minimum as indicated by Snœren et al. [35] since the hydration drop continued until the last measured point (~ pH 4.3). As for their homologous in bovine milk, the hydration of dromedary milk micelles seemed to be closely related to their mineral charges and to their protein composition. In fact, we noticed that the maximums of micelle hydration (Fig. 5), mineral solubilization (Fig. 1) and casein dissociation (Fig. 4) took place at relatively lower pH values. The maximum hydration of the dromedary milk micelle observed at about pH 5.0 could, thus, be correlated to the phenomenon of micellar casein dissociation which reached a maximum close to pH 4.9 (Fig. 4). The release of the caseins that increased from pH 5.5 would induce an expansion of the micelle structure leading to a maximum gain in voluminosity at about pH 5.0 (Fig. 5). This loosening of the colloidal phase was illustrated by numerous events. Thus, this pH 5.0 was characterized by a minimum of apparent viscosity (Fig. 6) which is an indication of the decrease of the micellar particles' number after their grouping. Similarly, the loss modulus (G'') which expressed the importance of the bonds susceptible to breaking, was at its maximum (Fig. 7). Finally, at this point the micellar microstructure (Fig. 8c) seemed quite loose. These three remarks would reflect the transitory character of this structure close to pH 5.0.

The relatively high dromedary milk micelle hydration at initial pH (3.32 $g\ H_2O \cdot g^{-1}$ proteins) seemed to be surprising, knowing its high mineralization (Tab. III). This, suggested that fixation sites of water molecules did not present the same partition as that on the bovine milk micelle. This hypothesis seems plausible since the first stage of the hydration curve showed a drop in micelle solvation of about 50% (Fig. 5) against only 10 to 20% for bovine milk micelles [19, 35]. Furthermore, this initial reduction of dromedary micelle hydration is certainly very slightly related to the solubilization of colloidal minerals since the micelle demineralization phenomenon started only toward pH 5.5 (Fig. 1). We could therefore suppose that the main part of the hydration of the dromedary milk micelle is related to caseins, the charge of which was rapidly neutralized by the lowering of pH. Thus, the release of these caseins would be concomitant to that of an important decrease of the micelle solvation water.

Microscopic observation of dromedary milk micelles at initial pH (pH 6.55) revealed a structure similar to that of bovine milk micelles [6]. However, dromedary milk micelle diameters seemed bigger. Such a difference of micellar size between the two kinds of milks was reported by Buchheim et al. [11] who used the cryofracture technique to evaluate the average micellar diameter. By using the drying critical point technique and by carrying out direct measurements on the screen of the electron microscope on 800 particles, we have estimated to be 2/3 the proportion of micelles presenting a size between 0.35 µm and 0.5 µm (data not shown).

Several particularities of dromedary milk micelle seemed to contribute to its relatively large size. First, it has a relatively important mineral charge (Tab. III). Second, it has a relatively low content of caseins (Tab. II). Such a reverse correlation between the size of the micelles and casein content was reported for caprine micelles [32]. Third, it has a relatively high hydration (Fig. 5) which is synonymous to important voluminosity. Fourth, it has a relatively low content of κ-casein as reported by Larsson-Raznikiewicz and Mohamed [25], Ochirkhuyag et al. [30] and Kappeler et al. [24]. In fact, an inversely proportional relation was found between the micellar size and κ-casein content [15, 29].

During the acidification process, the dromedary milk micelles seemed to pass through three different stages. From initial pH (6.55) to pH 5.5, the only marked event was the drop of hydration (Fig. 5). In fact, the solubilization of minerals and consequently the charge regression were very reduced (Fig. 1). The micelle seemed to keep its integrity as indicated by the practically constant values of the milk's apparent viscosity (Fig. 6) and of the milk's phase angle (Fig. 7). This result suggested a very limited presence if not an absence of micellar links affecting the Newtonian behaviour of initial milk. Besides, the microstructure of the micelles seemed to be individualized (Figs. 8a and 8b), despite a certain closeness probably induced by the important hydration decrease (Fig. 5).

In the second stage, between pH 5.5 and pH 5.0, the milk began to lose its Newtonian behaviour to acquire progressively a viscoelastic behaviour. In fact, from pH 5.5, G' was > 0, and the phase angle was < 90°. This stage was marked by an important dissociation of micellar caseins (Fig. 4) and of the colloidal complex phosphate-citrate of Ca and Mg (Figs. 2 and 3). These phenomena led to the fusion of the micellar material and to the elaboration of an open and porous structure close to pH 5.0 (Fig. 8c). This latter must correspond to a transitory organization state since the curve, expressing biochemical and structural changes in micelles, indicated abrupt alterations in slope at this level. A similar transitory state, observed at a higher pH, was reported by Attia et al. during the ultrafiltration of acidified bovine milk. At pH 5.5, these authors signaled an abrupt amelioration of the permeate flux [5] and an abrupt aeration of the membrane deposit formed by a micellar network [7].

The third stage starts below pH 5.0 and involves the collapse of the micellar structure and the edification of an insoluble structure. This collapse seemed to be sharp as shown by the very fast solubilization of what remains from the micellar minerals (Fig. 2), by the important increase of the milk's apparent viscosity (Fig. 6), and by the spectacular drop of the phase angle (Fig. 7). Consequently, the destruction of the micelles is concomitant to a predominance of new types of bonds having a permanent character. In fact, below pH 4.8, the storage modulus (G') prevailed over the loss modulus (G'') (Fig. 7). The ultimate term of this stage was a network of completely demineralized casein aggregates which had smaller sizes than those of micelles (Fig. 8d). By vision or by touch, the structure of the edified curd seemed to be very different from that of a bovine acid coagulum. It is rather a pseudo-curd made up of groups of casein flakes totally devoid of firmness.

We think that the sudden disappearance of the micellar state is among the possible causes of the extreme fragility of that curd. In fact, the very progressive establishment of hydrophobic, hydrogenous and electrostatic non-mineral bonds, which characterize the network of the acid bovine milk coagulum [37], takes place only slightly in the dromedary milk coagulum. In fact, the pH interval between the points corresponding to a maximum of biochemical modifications, 4.8–4.9 (Figs. 1 and 4–6) and those corresponding to the elaboration of that pseudo-curd, 4.8–4.7, justified by the phase angle stabilization (Fig. 7), are not enough to establish these characteristic bonds in dromedary milk.

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