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SUMMARY

The study of the dromedary milk casein micelle showed remarkable differences in comparison with that of the cow. With respect to the microstructure, scanning electron microscopy revealed a relatively large size with a maximum frequency situated around 0.4 - 0.5 μm and a distribution that could reach 0.6 μm . The study of the mineral repartition between the soluble and colloidal phases, showed that about 2/3 of the inorganic phosphorus and 2/3 of the magnesium were involved in the formation of the micelle. The latter was stable enough to bear the mechanical stress considering that the dromedary milk showed linear behaviour. Lowering of the pH had a delayed effect on the micelle. This effect was only observed at about pH 5.5. At pH 5.0 a state of transition characterized by deep biochemical modifications was observed. At lower pH values, some reverse tendencies were observed that resulted in a coagulum lacking firmness. The most significant structural stages were visualized by means of electron microscopy and the rheology in the permanent and harmonic modes.

RESUMO

O estudo da micela da caseína do leite do dromedário mostrou notáveis diferenças em comparação com aquela da vaca. Com respeito à microestrutura, a microscopia eletrônica de varredura revelou um tamanho relativamente grande com uma frequência máxima situada em torno de 0,4 - 0,5 μm e de uma distribuição que poderiam alcançar 0,6 μm . O estudo da repartição mineral entre as fases solúveis e coloidais mostrou que aproximadamente 2/3 do fósforo inorgânico e 2/3 do magnésio estiveram envolvidos na formação da micela. Este último era bastante estável para carregar o estresse mecânico visto que o leite do dromedário teve um comportamento linear. O abaixamento do pH teve um efeito demorado na micela. Este efeito foi observado somente em torno de pH 5.5. Em pH 5.0 um estado da transição caracterizado por modificações bioquímicas profundas foi observado. Em valores de pH mais baixos, algumas tendências reversas foram observadas, resultando num coágulo sem firmeza. Os estágios estruturais mais significativos foram visualizados por meio da microscopia eletrônica e da reologia em modos permanentes e harmônicos.

PALAVRAS-CHAVE
KEY WORDS

Dromedary milk; Casein micelle; Curd.
Leite do dromedário; Micela de caseína; Coalhada.

1. INTRODUCTION

Dromedary milk continues to be drunk fresh because it is not suitable for transformation into dairy products like other types of milk (FARAH; BACHMANN, 1987; MEHAIA *et al.* 1988; FARAH; ATKINS, 1992; WANGOH *et al.*, 1993; ABU-TARBOUSH, 1994). Difficulties are related concerning the control of the coagulation operation, which is a necessary stage in the development of dairy products. Thus, neither the enzymatic nor the mixed method forms a coagulum with the required qualities to undergo further technological treatment (FARAH, 1993).

The information currently available on dromedary milk concerns its general composition (SAWAYA *et al.*, 1984; MEHAIA, 1994), its behaviour during fermentation (ABU-TARBOUSH, 1996; ABU-TARBOUSH *et al.*, 1998), its aptitude for certain physical separations (MEHAIA, 1996) and its richness in molecules with antibacterial activity (ELAGAMY *et al.*, 1996).

Studies concerning its adaptation to technological transformations were limited to studying the feasibility of manufacturing certain products and mentioning the technological difficulties faced (ABU-LEHIA *et al.*, 1989; FARAH *et al.*, 1989; FARAH *et al.*, 1990; MOHAMED *et al.*, 1990; MEHAIA, 1993). However, the acidification and coagulation processes have not been extensively investigated. In these processes, the component playing a dominant role in the formation of the mechanical properties of the gel is the casein fraction. This has already been shown to be the case in cow's milk (BRULÉ *et al.*, 1997). Therefore, to understand dromedary milk curds, it may be necessary to study the casein structure and composition at the natural milk pH and its changes during pH lowering. This study attempted to investigate this from various approaches: electron microscopy, rheology and physico-chemistry. The results were compared with those of cow's milk.

2. MATERIAL AND METHODS

2.1 Milk samples

The milk was obtained from the milking of an eighteen-dromedary herd (*Camelus dromedarius*) of Maghrabi breed belonging to the institute of Arid Regions (Institut des Régions Arides, 4119 Medenine, Tunisie).

2.2 Acidification

The milk was acidified using slow hydrolysis with glucono- δ -lactone ([0.08 - 1.05%] w/v of GDL) (Merck, D-64293 Darmstadt, Germany). For this purpose the milk was divided into 16 fractions of 50 ml and GDL added to each fraction until the desired pH was reached.

2.3 Soluble solids and micellar phase separation

This was done by centrifugation at 190000g for 60min in a Beckman L7-55 ultracentrifuge (Beckman Instruments France S.A, 93220 Gagny, France) equipped with a SW41 rotor. After centrifugation, the supernatant was recovered for the measurement of soluble N and of soluble minerals while the residue was used for the determination of the casein solvation water.

2.4 Methods of analyses

pH was measured with a Metrohm 744 pH meter (Metrohm LTD, CH-9101, Herisau, Switzerland).

Nitrogen. Total nitrogen (TN), non-protein nitrogen (NPN) which is the TN fraction soluble in 12% trichloroacetic acid (w/v), non-casein nitrogen (NCN) and soluble nitrogen (SN), all extracted by the Rowland procedure (ROWLAND, 1938), were determined by the Kjeldahl method (AFNOR, 1993) after mineralization in a Büchi 425 unit, distillation in a Büchi 320 unit (Büchi Laboratoriums-Technik, CH-9230 Flawil, Switzerland) and titration with 0.1M HCl. The differences (TN-NPN), (TN-NCN) and (SN-NCN) were used in a series of calculations to obtain the contents of total protein, total casein and soluble casein (N X 6.38).

Minerals. Calcium, magnesium, potassium and sodium were measured in a Hitachi Z-model 6100 atomic absorption spectrometer (Hitachi Instruments Engineering Co., 882 Ichige, Katsuta-shi, Ibaraki-ken, 312 Japan), in the presence of lanthanum oxide (Sigma Chemical Co., P.O. Box 14508, St Louis, MO 63178 USA) for calcium and magnesium and caesium chloride (Merck) for potassium and sodium (AFNOR, 1993). Phosphorus was determined by a colorimetric method with ammonium molybdate (PIEN, 1969) using a Shimadzu UV - 160A spectrophotometer (Shimadzu Co., Kanda-Nishikicho 1-chome, Chiyoda, Tokyo, 101 Japan).

Casein solvation water. At each pH value, the residue after centrifugation was weighed, freeze dried for 48 h in a Usifroid SMH15 freeze dryer (Usifroid, 78310 Maurepas, France) and dried at 102-104°C for 24 h (GASTALDI *et al.*, 1996). The degree of hydration was expressed as g H₂O/g sedimented proteins, which were obtained by the difference between the protein content of the acidified milk and that of the corresponding supernatant.

Electrophoresis. Polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) was carried out using a Bio-Rad, Mini Protean II apparatus (Bio-Rad Laboratories, Hercules, California 94547, USA) according to a method based on that of LAEMMLI (1970).

The soluble and insoluble proteins were obtained according to 2.3.

Scanning electron microscopy (SEM). The samples underwent the treatments indicated by ATTIA *et al.* (1991a and b) and were subsequently observed using a Philips XL30 scanning electron microscope (Philips, BP 45, 94454 Limeil Brevannes, France) after drying to the CO₂ critical point in a

Baltec CPD 030 apparatus, and coating with gold in a Baltec MED20 apparatus (Balzers Union, FL-9496 Balzers, Germany). The distribution of micellar diameter was determined directly on the screen of the microscope, thanks to the precision of the measurements on a surface of $2 \times 10^{-8} \text{m}^2$.

Rheology. A StressTech Reologica rheometer (Reologica Instruments AB, Scheelevägen 30, 22363 Lund, Sweden) with coaxial cylinders was used at $20^\circ\text{C} \pm 0.1^\circ\text{C}$. The permanent mode used a constant shear rate equal to 200s^{-1} , or a variable one of 1 to 200s^{-1} . The Sinusoidal mode was followed at a frequency of 1 Hz in the linear viscoelastic region.

3. RESULTS AND DISCUSSION

3.1 Milk at the natural pH

Chemical composition. The mean composition of the dromedary skim milk used is shown in Table 1.

TABLE 1. Average composition of the dromedary skim milk used.

Composition	Content (g/kg d.b.)
Dry matter	97.8
Lactose	54.6
Total nitrogen components	32.2
Casein	22.2
Whey protein	8.1
Non-protein nitrogen	2.1
Fatty matter	1.4
Ash	8.6

Visualisation of micellar structure. Scanning electron microscopy (Figure 1) showed an organisation similar to that of cow's milk (ATTIA *et al.*, 1991a, b) with spherical and individualized shapes and variable sizes.

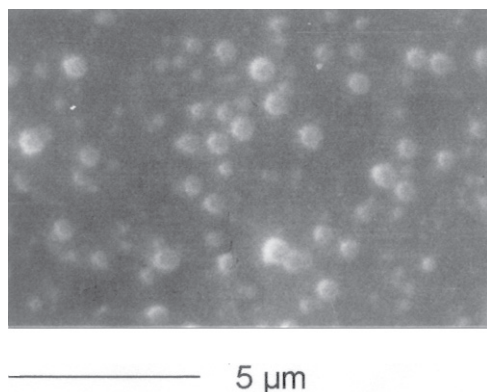


FIGURE 1. Scanning electron microscopy (SEM) micrograph of dromedary skim milk casein micelles.

However, the study of the micellar diameter (Figure 2) showed two important differences: (1) A greater size distribution that could reach approximately $0.6 \mu\text{m}$ vs. $0.3 \mu\text{m}$ for cow's milk (SCHMIDT, 1982); (2) Maximum frequency was located between $0.4 \mu\text{m}$ and $0.5 \mu\text{m}$ vs. 0.13 and $0.16 \mu\text{m}$ for the cow's milk (MCMAHON; BROWN, 1984).

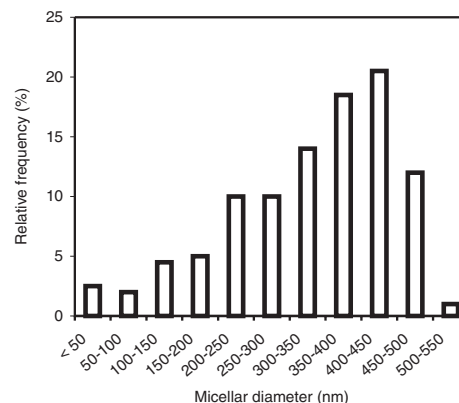


FIGURE 2. Size distribution of dromedary skim milk casein micelles.

Rheological behaviour. The flow curves, reproduced in Figure 3, gave a linear equation with constant coefficients. In the same way, the dynamic viscosity remained constant as related to the time of shearing, so the fluid does not have a thixotropic behaviour. Therefore, camel milk can be said to be able of bearing some mechanical strains without changing its microscopic structure. It means that, similar to cows' milk micelles, those of the dromedary milk were sufficiently stable to retain their integrity during pumping, agitation, skimming, homogenization etc.

The absolute viscosity of the camel milk ($1.72 \text{mPa}\cdot\text{s}$ at 20°C) was, however, lower than that of a cows' skim milk reconstituted at the same dry matter content and measured in the same conditions ($2.04 \text{mPa}\cdot\text{s}$). This difference was presumably the consequence of the relatively large size of dromedary milk micelle (BUSCALL *et al.*, 1988).

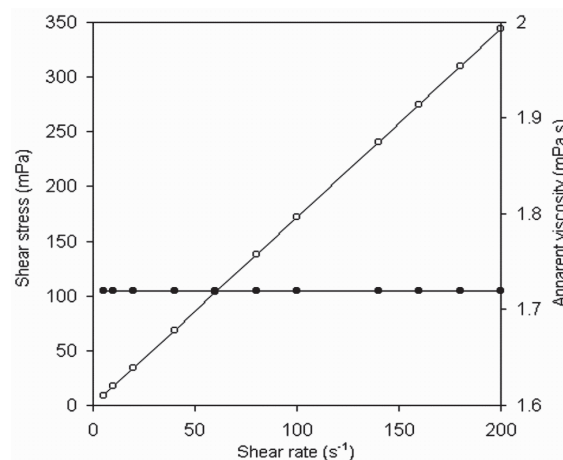


FIGURE 3. Change of shear stress and apparent viscosity with shear rate for dromedary skim milk: at native pH and at 20°C .

Mineral distribution between soluble and colloidal phases. Table 2 showed that about 2/3 of the Ca, P and Mg were present in the micellar phase. However, almost all the K and the Na were free. It seemed that, as in the case of the bovine milk micelle, the Na and K were not much involved in the micellar structure of camel milk while Mg, Ca and P participates in the formation of this micelle.

However, among the Ca, Mg and P, only the Ca presented an identical distribution in the two types of milk. Indeed, Mg and P were involved to a more important extent in the formation of the dromedary milk micelle than in that of the cow: 2/3 vs. 2/5 for Mg and 2/3 vs. 1/2 for P. This relatively large presence of P and Mg in the camel milk micelle was certainly another consequence of the relatively large micellar size. Indeed, it is an established fact that large micelles are richer in saline bridges binding submicelles than small micelles (BRULÉ *et al.*, 1997).

TABLE 2. Average contents of the main minerals of dromedary skim milk and their distribution between the soluble and colloidal phases.

Mineral element	Total content (g/kg)	Soluble fraction (%)	Colloidal fraction (%)
Potassium	1.72	98.0	2.0
Sodium	0.66	96.0	4.0
Calcium	1.23	35.3	64.7
Magnesium	0.09	36.2	63.8
Inorganic phosphorus	0.70	37.7	62.3

Molecular weight of the soluble and colloidal proteins. Figure 4, shows the electrophoregrams for the two phases revealing proteins that are specific to dromedary milk. The insoluble phase gave no band that could be identified with that of cow's milk casein. Therefore, all the insoluble proteins (25 kDa, 28 kDa, 31 kDa and 35 kDa) were specific to dromedary milk.

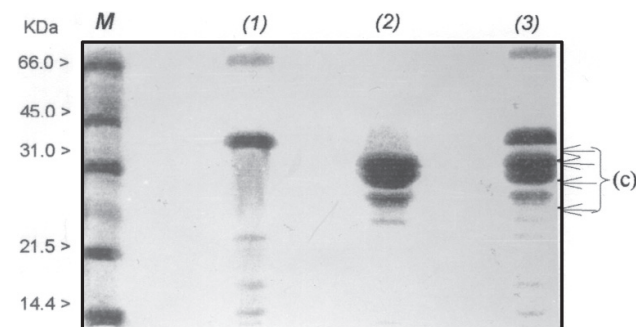


FIGURE 4. Separation of dromedary skim milk proteins by SDS-PAGE: (M) molecular mass standards; (1) soluble phase; (2) insoluble phase; (3) skim milk; (c) caseins.

These results were in partial agreement with those of FARAH; FARAH-RIESEN (1985) who isolated only two bands,

of 32 and 35 kDa, with those of LARSSON-RAZNIKIEWICZ; MOHAMMED (1986), who isolated three proteins of 25, 27 and 31 kDa, and with those of OCHIRKHUYAG *et al.* (1997), who obtained three casein fractions with molecular weights of 27.5, 35.3, and 26.3 kDa, identified as being homologous with the b, a₅₁ and a₅₂ casein of bovine milk.

3.2 Acidification of the dromedary skim milk

Changes in the composition of the milk fractions. The effects of lowering the pH of the dromedary milk on the demineralisation of the micelles and on the solubilisation and hydration of the casein fractions are illustrated in Figures 5, 6 and 7. The change in mineral content of the soluble phase (Figure 5) was similar to that of cow's milk (ATTIA, 1987) with, on the one hand, straight lines indicating values close to 100% for Na and K and, on the other hand, sigmoid curves for Ca, P and Mg. However these latter minerals distinguished themselves, in the dromedary milk, by three remarkable differences: (1) The demineralisation occurred later, at approximately pH 5.5 vs. pH 6.0 for cow's milk (ATTIA, 1987); (2) The point of inflection reflecting an increase in the solubilization of the micellar minerals was located at approximately pH 5.0 vs. pH 5.5 for cow's milk (ATTIA, 1987); (3) The casein fractions maintained a certain mineral charge, notably Ca, even at very low pH. In cow's milk, the casein fractions seemed to be totally demineralised at a pH value close to pH 5.0 (HEERTJE *et al.*, 1985; VAN HOOYDONK *et al.*, 1986; ATTIA, 1987).

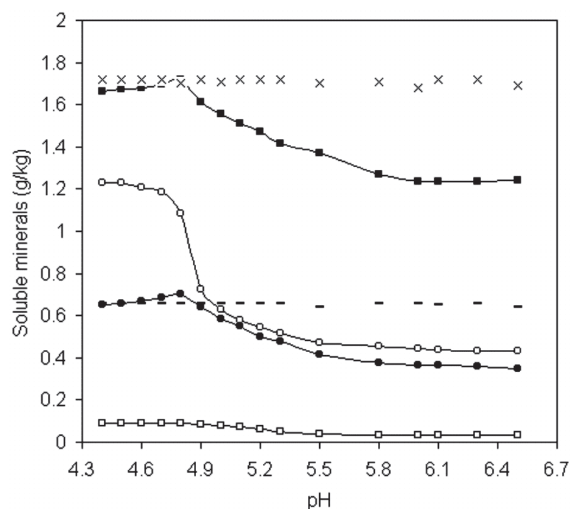


FIGURE 5. Evolution of percent soluble minerals during acidification of dromedary skim milk with GDL. Mg (□); P (●); Ca (○); Na (-); Citrate (■); K (×).

In addition to changes in the solubilization of the casein fractions and in their hydration (Figures 6, 7), they showed trends similar to those observed in cow's milk (SNCEREN *et al.*, 1984; DALGLEISH; LAW, 1988) but with the maximum at a lower pH (pH 5.0 vs. pH 5.4 and 5.5 respectively). These two properties seemed closely related to the phenomenon of

demineralisation. Hence a cause and effect relation could be conceived between the relative richness of dromedary milk micelles in insoluble salts (Table 2) and the greater resistance of the micelles to acid. Below pH 5.0 there was an inversion of trends with considerable soluble casein reduction and a drop of hydration. The dromedary milk acidified at this pH was therefore at the threshold of extensive biochemical changes, which would correspond to a real point of transition between two different protein structures. Thus, one could put forward the following hypotheses: (1) dissociation of the micellar material of the dromedary milk was efficient at approximately pH 5.0. The separation of the casein monomers resulted in the micellar skeleton losing its form; (2) solubilization of the colloidal phase minerals continued below pH 5.0, especially with respect to minerals directly bound to micellar and submicellar caseins; (3) A spatial rearrangement occurred between submicellar units and/or certain caseins of the dromedary, leading to some different types of aggregation.

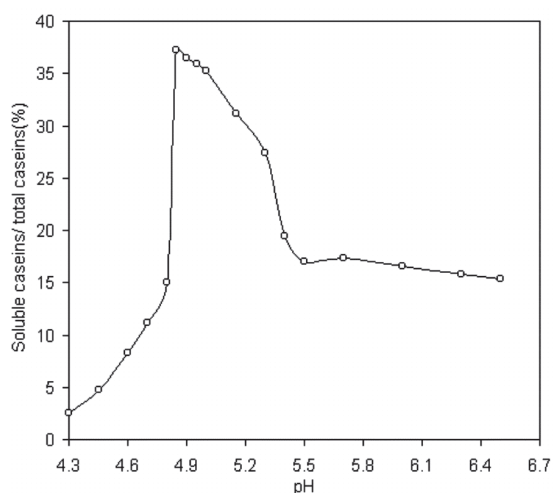


FIGURE 6. Evolution of soluble casein during acidification of dromedary milk with GDL.

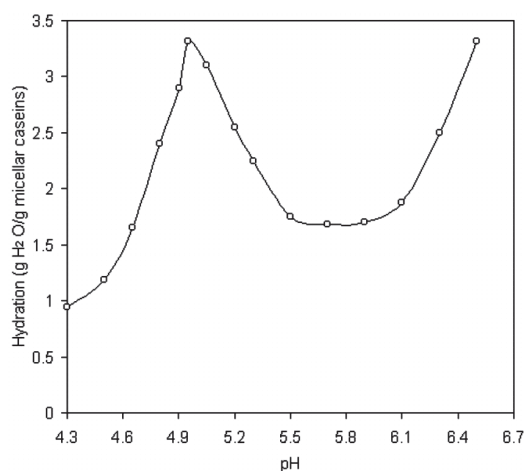


FIGURE 7. Evolution of casein hydration during acidification of dromedary milk with GDL.

Changes in the rheological parameters. The rheological study (Figures 8 and 9) seemed to reinforce the hypotheses presented above. Indeed, the apparent viscosity (Figure 8), practically constant at the onset of acidification, decreased slightly as from pH 5.5 to reach a minimum close to pH 5. This minimum seemed to correspond to a transitory organisation state, which permitted below the edification of an insoluble structure below pH 5.0. This new structure was characterized by a remarkable increase in the apparent viscosity.

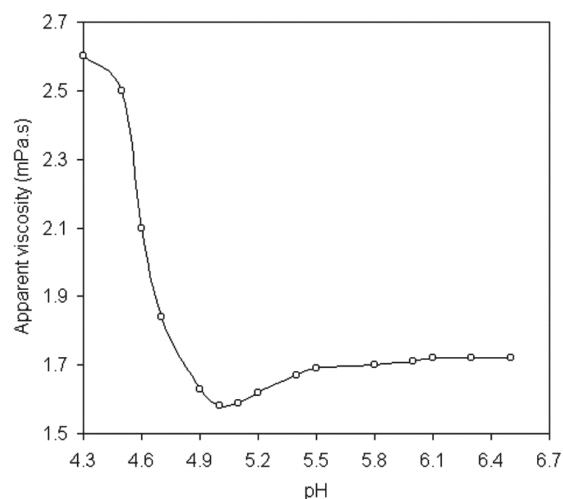


FIGURE 8. Change in apparent viscosity (at 200 s^{-1}) of dromedary skim milk during acidification.

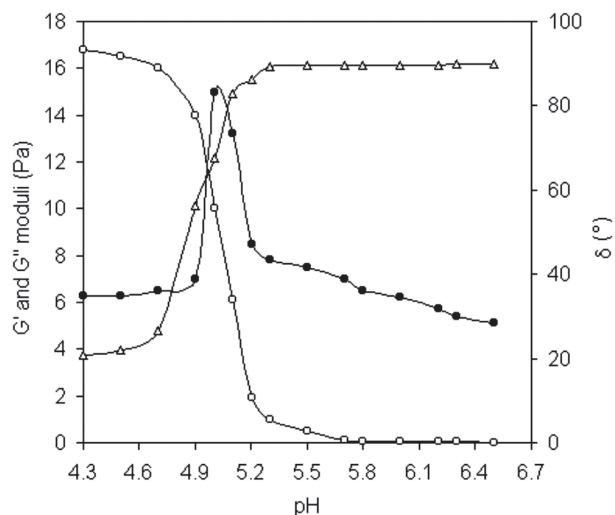


FIGURE 9. Change in storage (G') and loss (G'') moduli and in the phase angle (δ) during dromedary skim milk acidification. G' (○); G'' (●); δ (Δ).

The study in the oscillatory mode (Figure 9) permitted the visualization of this transition point. In fact, it was characterized by a maximum value of viscous modulus (G'') and by an inflection point on the elastic modulus (G') curve. Below this point, pH 5.0, the two moduli crossed each other, one rising (G') and the other dropping (G''), and the milk became a real viscoelastic body.

The dromedary milk microstructure seemed to maintain its integrity up to pH 5.5 as indicated by the practically constant values of the phase angle of the (Figure 9). The angle then showed a fast and sharp drop with a tangent toward pH 5.0. At this point, the elastic character prevailed over the viscous character and permitted a new spatial redistribution, creating new links between the casein fractions. These bonds were permanent since they were capable of preserving a part of the energy that they were provided with during the strain.

Observations during scanning electron microscopy

The main structural stages could be visualized by scanning electron microscopy (Figure 10). Micelles that were separate at the onset pH (Figure 1), began to gather together in globular or linear shapes as the pH approached 5.5, while keeping their integrity. Near pH 5.2, some micelles merged to form different sized clusters. At pH 5.0, a complete fusion of these clusters was observed. This fusion led to a real three-dimensional network, having some linear and continuous nodes. This structure tightened to give a very reticulate network near to pH 4.7. Finally, at pH 4.4, a loose network was formed. It presented partially broken up nodes covered with aggregates. These were the completely demineralised casein flakes.

To obtain a macroscopic idea of the gel cohesion formed, we followed its flow curve (Figure 11). The corresponding rheogram showed a remarkable reduction of the apparent viscosity and the presence of a yield stress beyond which perfect proportionality was observed between the stress and the rate of shearing. It was an ideal plastic non-Newtonian fluid.

This result confirmed the crumbly and fragile appearance of the structure observed at pH 4.4 (Figure 10). Indeed, once the flow was induced, bonds assuring the cohesion were broken and the casein particles moved completely and instantaneously in the same direction as the flow.

The coagulum of cow's milk also behaved like a non-Newtonian body but it was pseudoplastic (ATTIA *et al.*, 1993). Its flow curve was not linear and therefore its microscopic structure changed with the shearing. This indicated an important difference in nature and/or in the number of bonds involved in the development of the acidic curds of the two types of milk.

It was thought that the reorganisation of the micelle and of its sub-units below pH 5.0, would determine the rheological properties of the acid gel of dromedary milk. However, this spatial reorganisation was in fact determined by the possibilities of interaction between the various types of casein. At this level, two meaningful differences were considered in relation to the bovine milk micelle: (1) A relatively low percentage of k-casein was expected in dromedary milk since an inversely proportional relation was found between micellar size and the k-casein content (MCGANN *et al.*, 1980; BRULÉ *et al.*, 1997). In the cow's milk, the k-casein participates, with the other types of casein, in the formation of the acid coagulum, due to hydrophobic and electrostatic interactions (BRULÉ *et al.*, 1997). LARSSON-RAZNIKIEWICZ; MOHAMED (1986) and recently OCHIRKHUYAG *et al.*, (1997) found the presence of an analogue of k casein in dromedary milk, but could not quantify it because of its very low content, as compared to that of cow's milk; (2) All the casein fractions of the dromedary milk were certainly different at the level of their

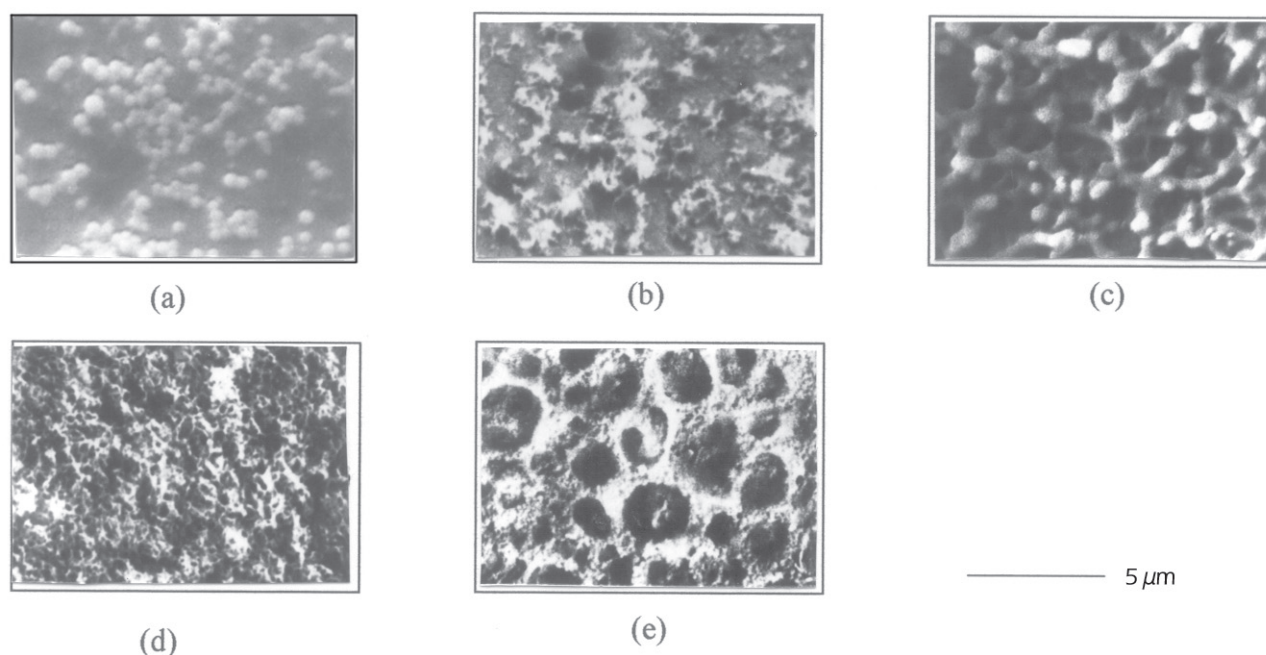


FIGURE 10. Scanning electron microscopy (SEM) photographs of dromedary skim milk acidified using GDL. (a) pH 5.5; (b) pH 5.2; (c) pH 5.0; (d) pH 4.7; (e) pH 4.4.

chemical structures; since they had different molecular weights. For example, the variations that could affect the rate and the localization of carbohydrate and phosphoryl fragments, would give rise to the micelles and caseins of the dromedary milk in a physico-chemical environment different from that of the micelles and caseins of acidified cow's milk.

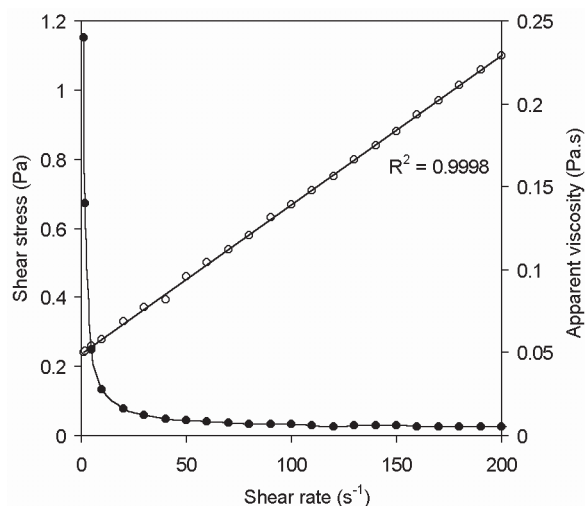


FIGURE 11. Rheogram at 20°C (shear stress – shear rate), ○; and (apparent viscosity – shear rate), ● of acid coagulum of dromedary skim milk acidified using GDL, at pH 4.4.

4. CONCLUSIONS

The physico-chemical, rheological and microscopic approaches used permitted the presentation of evidence of certain biochemical, structural and physical particularities of the dromedary milk micelles. During the lowering of the pH, these particularities marked the progress of the coagulation process that led to a pseudo curd. To look for ways of improving the technological possibilities of this curd, it seems imperative to increase the understanding of the effects associated with the specific properties of the dromedary milk micelle.

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