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Effects of acidification on physico-chemical characteristics of buffalo milk: A comparison with cow's milk

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Abstract

Composition and physico-chemical properties of buffalo and cow milks were compared at their initial pH and during acidification. As compared to cow milk, buffalo milk was richer in fat, lactose, protein (especially caseins) and minerals such as calcium, magnesium and inorganic phosphate. Along with these differences of major components, the capacity of milk to be acidified (named buffering capacity) was higher for buffalo milk than for cow milk. The precipitation/aggregation of caseins at their isoelectric pH, solubilization of calcium and inorganic phosphate and decrease in hydration of casein as a function of decrease in pH were significant for both milks. For both species, these molecular changes were qualitatively similar but quantitatively different. These quantitative differences during acidification were related to the differences of composition between the milks.
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Keywords: Milk; Buffalo; Cow; Composition; Physico-chemical characteristics; Acidification

1. Introduction

Buffalo's milk is ranked second in the world after cow's milk, being more than 12% of the world's milk production (CNIEL, 2002). In India and Pakistan (both producing about 80% of the world's production of buffalo milk), this milk is used for making different dairy products, such as butter, butter oil (ghee), soft and hard cheeses, condensed and evaporated milk, ice cream and yoghurt. Parts of these products are acidified using traditional methods, without any scientific evidence and without having knowledge of the molecular distribution of the major milk components as a function of pH.

The molecular changes induced by acidification in cow milk are relatively known and many physico-chemical modifications of casein micelles have been described. Among the molecular changes occurring during acidification, protonation of acid groups, including demineralisation of casein and decrease in solubility, hydration and zeta potential of caseins, are the most described (Banon & Hardy, 1992; Brulé & Fauquant, 1981; Gaucheron, 2004; Le Graët & Brulé, 1993; Snoeren, Klok, Van Hooydonk, & Damman, 1984). On the other hand, the scientific literature concerning the description and understanding of the effects of acidification of buffalo milk is poor (Ganguli, 1992).

In the present paper, some physico-chemical characteristics of buffalo milk at natural pH (different protein fractions, fat, lactose, total and diffusible minerals) and during acidification (precipitation/aggregation of protein, mineral solubilization and micellar hydration) are described and the results compared with those obtained for cow milk.

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2. Materials and methods

2.1. Milk samples

Fresh raw bulk whole buffalo milk (Murrah breed of *Bubalus bubalis*) and cow milk (Holstein breed of *Bos taurus*) were obtained from the Cantal region (Coopérative de Bufflonnes, Zone Artisanale, 15600 Maurs, France) and from Société Laitière (35590, l'Hermitage, France), respectively. 0.3 g/l of thimerosal (Sigma–Aldrich, St. Louis, USA) was added as a preservative.

2.2. Acidification of buffalo and cow milks

Milk was acidified with 1 M HNO_3 under vigorous stirring at 20 °C. The dilution caused by the HNO_3 addition was kept constant by adding an appropriate volume of Milli Q-water, so the fixed volumes were 7 and 5 ml for buffalo and cow milk, respectively. Before analysis, the samples were left overnight at room temperature. All the experiments were carried out in duplicate.

2.3. Preparation of ultrafiltrates and pellets of casein micelles

Acidified milk samples at different pH were ultracentifuged at 20 °C for 1 h at 100,000*g* (Sorvall, Discovery 90SE, Hitachi, USA) with a T-865 rotor. Supernatants were carefully removed and micellar pellets were drained. Ultrafiltration of supernatants was carried out at 20 °C to eliminate whey proteins with an ultra free membrane Vivaspin 20 (Vivascience, Sartorius group, Germany, molecular mass cut-off: 10 kDa) for 2 h at 1800*g* in a SV 11 TH centrifuge (Firlabo, Lyon, France). Ultrafiltrate (UF) was collected for further analyses.

2.4. Analyses

2.4.1. General composition

Fat was determined according to IDF (1997). Lactose was determined by using a lactoscope (Delta instruments, Laboratoire Humeau, France). Ash was determined after mineralisation of milk at 550 °C for 7 h according to IDF (1964a), and pH was measured using a H1 9024 Microcomputer pH meter (Hanna Instruments, Portugal).

Total nitrogen content (TN) of milk, non-casein nitrogen (NCN) and non-protein nitrogen (NPN) fractions were prepared according to IDF (1964b). For NCN, milk was acidified to pH 4.6 with a mixture of 10% (v/v) acetic acid and 1 M acetate buffer. For NPN, about 40 ml of 15% (v/v) trichloroacetic acid were added to 10 ml of milk. NCN and NPN samples were filtered through Whatman papers (Whatman Int. Ltd., Maidstone, UK) No. 42 and 40, respectively. TN, total soluble nitrogen content (TN_s), NCN and NPN were determined by the Kjeldhal method (IDF standard 20B, 1993). Nitrogen content was converted into equivalent protein content using 6.38, 6.25 and 3.60 as converting factors for TN, NCN and NPN contents, respectively (Karman & Van Boekel, 1986). Casein nitrogen (CN) was calculated as [CN] = [TN] − [NCN].

Dry matter was determined by drying 5 g of milk sample at 103 °C for 7 h in a capsule containing sand according to IDF (1987).

2.4.2. Major mineral contents

Cation concentrations (calcium, magnesium, sodium and potassium) were determined by atomic absorption spectrometer (Varian 220FS Spectr AA, Les Ulis, France) (Le Graët & Brulé, 1993). Anion concentrations (chloride, inorganic phosphate and citrate) were determined by ionic chromatography coupled with suppressed conductivity detection (Dionex DX 500, Dionex, Voisin-le-Bretonneux, France) (Gaucheron, Le Graët, & Piot, 1996). The total concentration was determined in the diffusible phase of milk acidified to about 3.5 and 4.6 for cations and anions, respectively. Ionic concentration determined in the UF was converted into diffusible concentration by multiplying by the 0.96 correcting factor, as described by Pierre and Brulé (1981). This correction takes into account the excluded volume effect.

2.4.3. Size of particles and aggregates

The size distribution of particles and aggregates was determined by laser light scattering, using a Mastersizer 2000 (Malvern Instruments Ltd., Worcestershine, UK) at two different wavelengths (He/Ne laser: 633 nm and electroluminiscent diode: 466 nm). A 500 µl sample was dispersed into the apparatus circulating cell containing 100 ml of Milli Q-water at 20 °C. The parameters of size distribution were calculated by the Mastersizer software, and the results of modal diameter (diameter at maximum peak of the main population) were expressed.

2.4.4. Water content of ultracentrifuged pellets

Drained pellets obtained by ultracentrifugation were weighed and then dried after thorough mixing with sand in capsules at 103 °C for 7 h. The difference between the weight before and after drying, expressed as solvation (g of water/g of dry pellet) was taken as the amount of tightly bound water of ultracentrifuged pellets.

3. Results and discussion

3.1. Composition

3.1.1. General composition

Comparison of the overall compositions of buffalo and cow milk showed that large differences existed between them (Table 1). Thus, contents of TN, fat, lactose, ash and dry matter were higher for buffalo milk than for cow milk whereas normal pH values were similar for milk from both species. These results are in agreement with the findings of various authors (Ganguli, 1992; Patino, 2004; Roy, Nagpal, Sadana, & Sharma, 1972; Spanghero & Sus-

Table 1
Overall composition of buffalo and cow milks

	Buffalo	Cow
pH	6.81 ± 0.06	6.76 ± 0.04
Fat (g/kg)	70 ± 6	41 ± 1
Lactose (g/kg)	52.1 ± 1.1	48.0 ± 0.1
Ash (g/kg)	8.4 ± 0.2	7.7 ± 0.1
TN (g/kg)	43.5 ± 3.4	33.5 ± 0.3
NCN (g/kg)	8.9 ± 1.6	7.4 ± 0.5
NPN (g/kg)	1.0 ± 0.4	0.9 ± 0.02
CN (g/kg)	34.6 ± 1.1	26.1 ± 0.8
Total Ca (mM)	47.1 ± 1.2	30.5 ± 0.8
Diffusible Ca (mM)	8.2 ± 0.2	8.6 ± 0.2
Total P_i (mM)	27.7 ± 1.4	19.2 ± 1.0
Diffusible P_i (mM)	9.2 ± 0.5	9.9 ± 0.5
Total Mg (mM)	7.3 ± 0.2	4.6 ± 0.1
Diffusible Mg (mM)	3.5 ± 0.1	3.0 ± 0.1
Total Na (mM)	20.3 ± 0.5	17.5 ± 0.4
Diffusible Na (mM)	18.4 ± 0.5	15.9 ± 0.4
Total K (mM)	28.7 ± 0.7	42.0 ± 1.0
Diffusible K (mM)	26.0 ± 0.7	37.3 ± 0.9
Total Cl (mM)	16.6 ± 0.8	21.8 ± 1.0
Diffusible Cl (mM)	16.3 ± 0.8	22.8 ± 1.0
Total citrate (mM)	8.3 ± 0.4	8.8 ± 0.4
Diffusible citrate (mM)	7.1 ± 0.4	8.2 ± 0.4
Dry matter (g/kg)	174.5 ± 8.2	136.7 ± 10.8

Results correspond to the averages of three independent determinations, 15 days apart. TN, NCN, NPN and CN correspond to the contents of total nitrogen, non-casein nitrogen, non-protein nitrogen and casein nitrogen.

mel, 1996). The CN content ([TN] − [NCN]) was also significantly higher for buffalo milk than for cow milk. However, CN content, as compared to TN content, corresponded to the same percentage (about 80%) in both milks. NCN contents (about 20% of TN in both milks), which corresponds to whey proteins (especially α-lactalbumin and β-lactoglobulin), proteose–peptone and NPN, were almost similar in both milks. NPN contents (creatin, urea and free amino acids) were also similar. These results were in accordance with those described by Pandey, Katpatal, Bisht, and Mahesh (1986) and Taha (1989).

Dry matter was about 40 g/kg higher in buffalo milk than in cow milk. These results were similar to the findings of Ganguli (1992) and Spanghero and Susmel (1996). In both milks, when arithmetic additions of all the major component concentrations (protein, fat, lactose and ash) responsible for total solids content of both milks were done, they gave 174 g/kg and 130 g/kg for buffalo and cow milk, respectively, similar to the experimentally observed values (Table 1).

3.1.2. Mineral contents

The total calcium and inorganic phosphate concentrations were higher in buffalo milk than in cow milk (Table 1). Ranjan et al. (2005) also observed this difference of total calcium concentration between milks of these species. The concentrations of diffusible calcium and inorganic phosphate were similar in both milks. From these total and diffusible concentrations, it could be deduced that the quantities of calcium and inorganic phosphate associated with casein micelles were also higher in buffalo milk than in cow milk. Thus, in our study, 82% and 72% of calcium and 66% and 48% of inorganic phosphate were in the micellar phases of buffalo and cow milk, respectively. Assuming that the casein molecules existed in a micellar form, the amounts of calcium and inorganic phosphate associated with casein were 1.12 and 0.84 mM calcium per gram of casein and 0.53 and 0.36 mM inorganic phosphate per gram of casein for buffalo and cow milk, respectively. The molar ratios of micellar calcium/micellar inorganic phosphate were 2.10 and 2.35 for buffalo and cow milk, respectively. These differences in micellar mineralisation could be attributed to the higher phosphorylation of casein molecules in the case of buffalo milk or to a difference in the quantity of micellar calcium phosphate. Total and diffusible concentrations of magnesium and sodium ions were higher in buffalo milk than in cow milk, whereas total and diffusible concentrations of potassium and chloride were higher in cow milk than in buffalo milk. Total citrate concentrations were similar in the two milks, whereas the diffusible content was lower in buffalo milk than in cow milk.

The physiological differences of the animal, stage of lactation and some common factors such as season, feed, breed, time and sequence of milking could be responsible for the differences in concentration of protein, fat, lactose, ash and consequently dry matter, which were found to be higher in buffalo milk than in cow milk.

3.2. Acidification of milk

3.2.1. Buffer capacity

The pH of buffalo milk decreased more slowly than did the pH of cow milk during acidification (Fig. 1). For example, to obtain pH 4.0 in buffalo and cow milk, 6.0 and 4.5 ml of acid were needed, respectively, which indicated that the buffer capacity of buffalo milk was higher than that

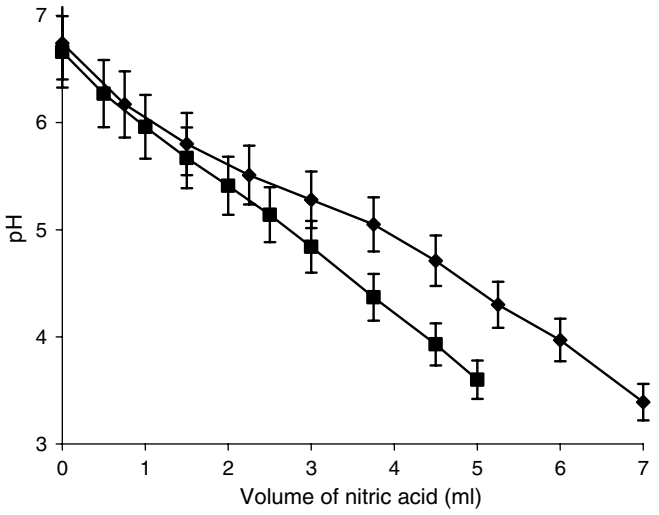

Fig. 1. pH of milks (60 ml) as a function of added volume of HNO_3 (1 M). ■: cow and ♦: buffalo.

of cow milk. The buffering property of milk is related to its composition in acido-basic compounds (Salaün, Mietton, & Gaucheron, 2005); in our case, the difference observed between the milks of these species was probably related to the higher casein content in buffalo milk than in cow milk (Table 1). Moreover, inorganic phosphate, which also contributes to the buffering capacity, was also higher in buffalo milk than in cow milk (Table 1). Ismail, El Deeb, and El Difrawi (1973) described that both milks exhibited the same buffering intensity, which was not in accordance with the findings of this study. This discrepancy could be attributed to the difference in the protocols used for the determination of the buffering capacity. These results showed that the acidification process, which is well established for cow milk, cannot be directly extrapolated to buffalo milk and some adaptations are necessary.

3.2.2. pH-induced protein aggregation

Acidification of milk induced different molecular changes causing precipitation (Fig. 2) and aggregation (Fig. 3) of casein. Fig. 2 shows similar decrease of TN_s content from normal pH to about 3.5 for both milks. TN_s content remained constant and corresponded to the NCN content below pH 4.6 for both milks, as indicated in Table 1. In parallel with this decrease in TN_s content, the formation of large aggregates in whole milks was observed (Fig. 3a and b for buffalo milk and cow milk, respectively). At normal pH for both milks, two major populations were determined, corresponding to casein micelles (modal diameter of about 180 nm for both milks) and fat globules (modal diameter of about 5.0 and 4.5 μm for buffalo milk and cow milk, respectively). However, during acidification, the percent of these populations decreased and the formation of large aggregates was observed. At pH 5.28, the size distribution profile of buffalo milk showed the presence of large aggregates although this was not the case at pH 5.41 for the cow milk. At pH 4.71 and 4.84 (for buffalo and cow milk, respectively), individual populations of casein micelles and fat globules were strongly decreased, confirming the precipitation/aggregation of casein, as shown previously in Fig. 2. At pH below 4.0, only one population, with a modal diameter of about 60 μm, was observed and no difference between the two species was determined. To better understand the protein precipitation/aggregation, a similar determination was carried out on skim milk (Fig. 3c and d for buffalo and cow milk, respectively). Similar results were observed, suggesting that the role of fat on the size of the aggregates was negligible.

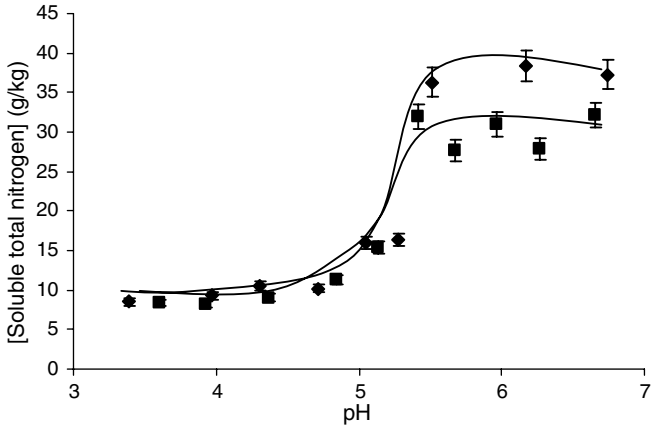

Fig. 2. Effect of pH on soluble nitrogen content (■: cow and ♦: buffalo). Soluble nitrogen content was determined after filtration of acidified milks on Whatman filter No. 41.

For both milks, the observed decrease in TN_s corresponded to a progressive neutralization of casein as a function of pH with the consequence of their precipitation/aggregation around their isoelectric pH. For casein from cow milk, it is admitted that the value of this characteristic is 4.6 (Walstra & Jenness, 1984) whereas, for casein from buffalo milk, no information on this characteristic was found in the literature. However, the comparison of amino acids contained in casein molecules of these species indicated a good homology (Abd El-Salam, 1975; Addeo, Mercier, & Ribadeau-Dumas, 1977; Ganguli, Prabhakaran, & Iya, 1964; Nagasawa, Kiyosawa, Kuwahara, & Ganguli, 1973; personal results) suggesting that the isoelectric pH might be similar for milk from both species. This similarity in amino-acid composition could also explain an identical process of protein precipitation/aggregation during acidification.

3.2.3. pH-induced solubilization of minerals

In parallel with the precipitation/aggregation of casein, the pH-induced solubilization of minerals was also determined (concentrations of diffusible cations in Fig. 4a and b for buffalo and cow milk, respectively and diffusible anions in Fig. 4c and d for buffalo and cow milk, respectively).

At the normal pH, the differences in the diffusible concentration of each of the ions between the two species were the same as those reported in Table 1. Then, the diffusible concentrations of calcium and inorganic phosphate increased as a function of acidification. For calcium, the solubilization was considered for both species as total at pH 3.5. For inorganic phosphate, total solubilizations were observed at pH 4.7 and 4.9 for buffalo and cow milk, respectively. Between normal pH and pH 4.9–4.7, this solubilization corresponded to the neutralization of the casein molecules and to the dissociation of micellar calcium phosphate. At pH lower than 4.9–4.7, as inorganic phosphate was totally solubilized, we could consider that the solubilization concerned essentially the calcium directly associated with phosphoseryl residues of casein molecules. Similar results were obtained previously for cow milk (Dalgleish & Law, 1989; Gastaldi, Lagaude, & Tarodo de La Fuente, 1996; Le Graët & Brulé, 1993; Van Hooydonck, Hagedoorn, & Boerrigter, 1986; Visser, Minthan, Smits, Tjan, & Heertje, 1986). The relationships between micellar calcium and inorganic phosphate concentrations were plotted at different pH values (Fig. 5). On the one hand, good corre-

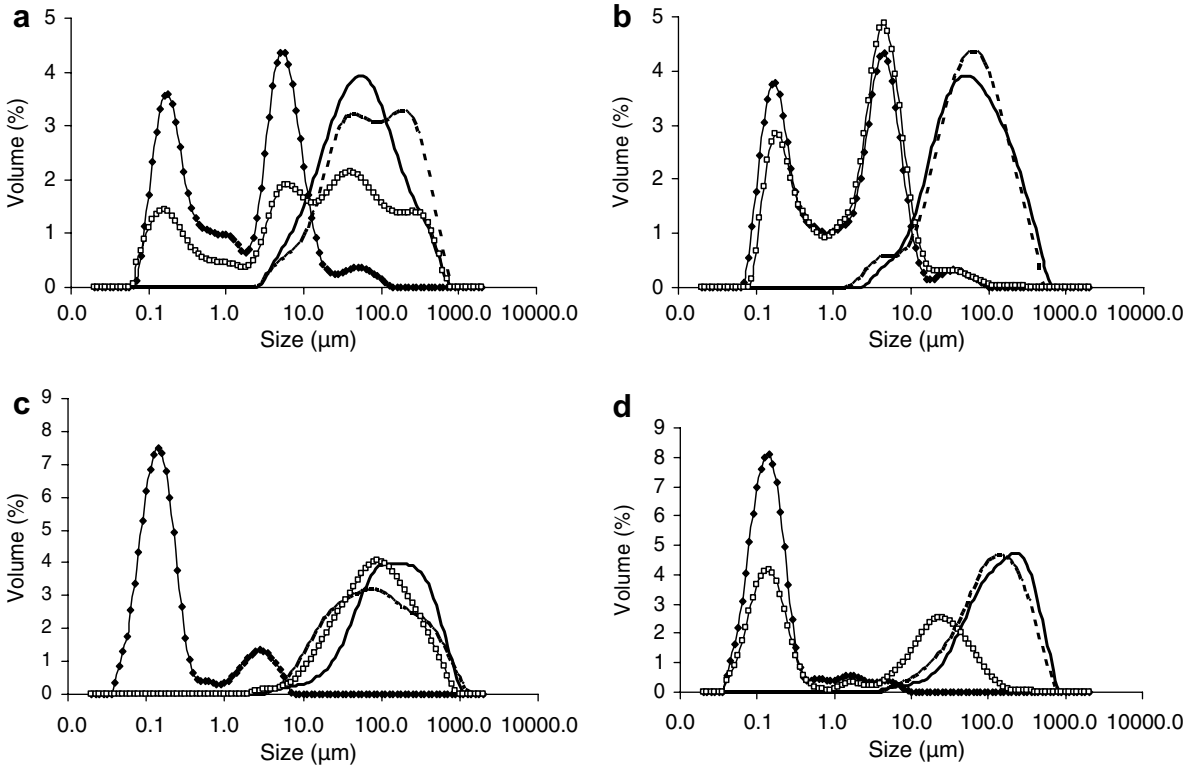

Fig. 3. Size of particles and aggregates as a function of pH. For whole milks (graphs a and b for buffalo and cow milk, respectively), symbols ♦, □, --- and — correspond to 6.74, 5.28, 4.71, 3.39 and 6.66, 5.41, 4.84, 3.60 for buffalo and cow milk, respectively. For skim milk (graphs c and d for buffalo and cow milk, respectively), symbols ♦, □, --- and — correspond to 6.76, 4.89, 4.60, 3.31 and 6.74, 5.38, 4.77, 3.67 for buffalo and cow milk, respectively.

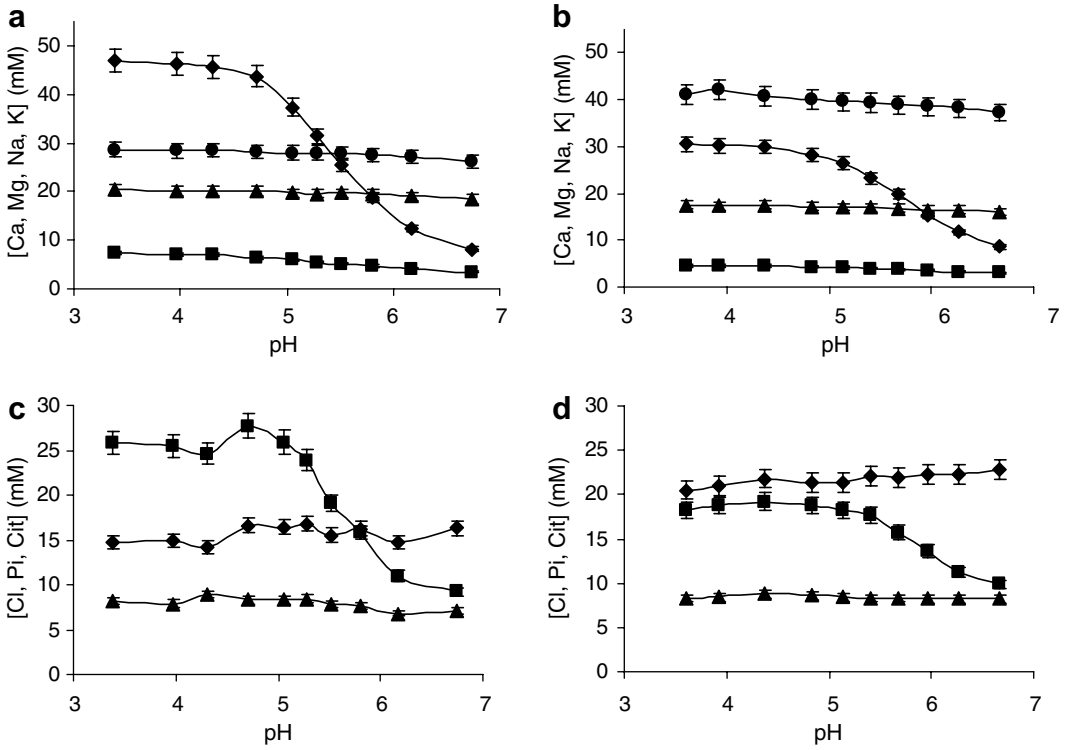

Fig. 4. Concentrations of diffusible ions as a function of pH. Figures a and b correspond to the diffusible concentrations of Ca (♦), Mg (■), Na (▲) and K (●) of buffalo and cow milk, respectively. Figures c and d correspond the diffusible concentrations of Cl (♦), P_i (■) and Cit (▲) for buffalo and cow milk, respectively.

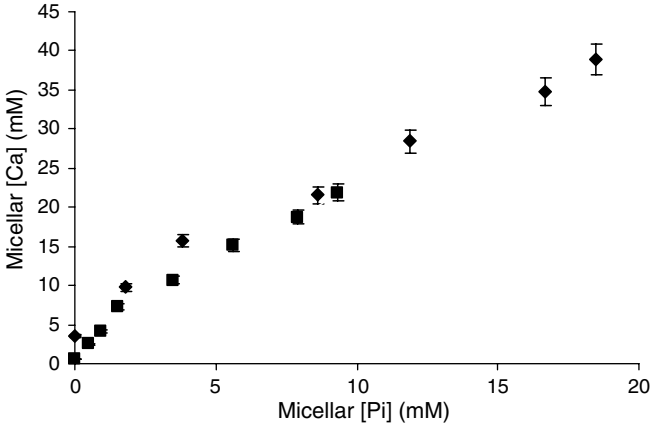

Fig. 5. Content of micellar calcium as a function of content of micellar inorganic phosphate during milk acidification (from pH 6.74 to pH 4.71 for buffalo milk and from pH 6.66 to pH 4.84 for cow milk). ■: cow and ♦: buffalo.

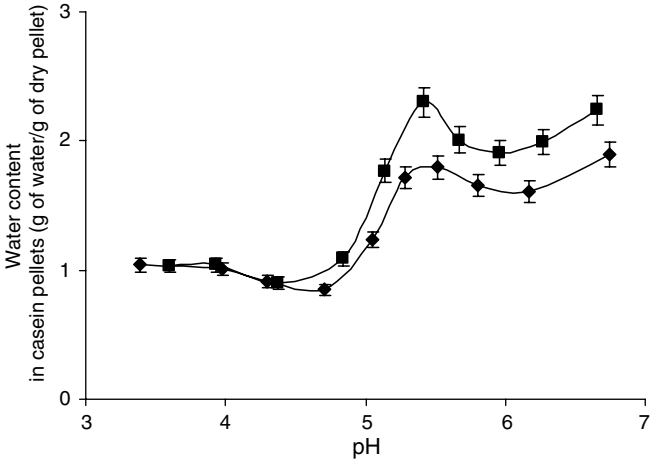

Fig. 6. Effect of pH on water content of casein pellets obtained by ultracentrifugation (■: cow; ♦: buffalo).

lation coefficients ($R^2 = 0.98$) between the solubilization of calcium and inorganic phosphate were observed for both species. On the other hand, the comparison of slopes of the regression lines suggested similar processes of mineral solubilization for buffalo and cow milks. For both species, there were about 2 mM of solubilized calcium for 1 mM of solubilized inorganic phosphate. This ratio was in accordance with that indicated by Law (1996). Slight and gradual solubilization of magnesium, sodium, potassium (Fig. 4a and b for buffalo and cow milk, respectively) and chloride and citrate (Fig. 4c and d for buffalo and cow milk, respectively) was also determined as a function of pH decrease. Solubilization phenomena were very similar for both milks.

3.2.4. Water content of pellets of casein micelles

At normal pH, the water content of ultracentrifuged pellet from buffalo milk was lower than that from cow milk (1.90 and 2.24 g of water/g of dry pellet for buffalo milk and cow milk, respectively) (Fig. 6). Kuchroo and Malik (1976) have found similar differences between buffalo and cow milks. The most influential factors which can contribute to these significant differences are structure and mineralisation of casein micelles and glycosylation of κ-casein. In this work, the higher mineralisation of casein micelles from buffalo milk (Table 1 and Fig. 4) could be an important factor but no information concerning the structure of casein micelles was available to test this hypothesis. Concerning glycosylation, Sabarwal and Ganguli (1977) indicated that the κ-casein from cow milk had a higher sialic acid value than had κ-casein from buffalo milk. Moreover, the same authors indicated that the glycopeptide released from cow κ-casein had a higher molecular weight than had those released from buffalo κ-casein.

The water content of casein micelles pellets changed during acidification (Fig. 6). The different changes of water associated with casein are relatively well known for cow milk and are helpful for the discussion of the results observed in buffalo milk, knowing that the observed variations were qualitatively similar for these two species (Fig. 6). First, from initial pH to about 6.0, the water content of casein micelles pellets decreased. In this pH range, protonation of the negatively charged organic and inorganic phosphate groups and sugar residues of κ-casein occurred. Consequently, a demineralisation of casein was observed, as shown in Fig. 4. In parallel, a reduction of repulsive forces between adjacent chains, with a progressive collapse of the outer hairy layer, was described by Roefs, Walstra, Dalgleish, and Horne (1985) and Banon and Hardy (1992). All these events contributed to the decrease in voluminosity. Second, from pH 6.0 to 5.4 micellar hydration increased. This phenomenon, relatively well known, correlated with the micellar casein dissociation, with an expansion of the micelle structure and an increase of water–casein interactions, leading to a maximum gain in voluminosity at about pH 5.4 (Creamer, 1985; Snoeren et al., 1984). Third, from pH 5.4 to 4.5, charge neutralization with precipitation/aggregation of casein (Fig. 2) and demineralisation (Fig. 4) continued, so the decrease in the water content associated with casein was observed at pH close to the isoelectric pH. Below this value, casein started to exert positive charge effects and was able to bind water.

4. Conclusion

This work showed that the overall compositions of buffalo and cow milks were different. Buffalo milk had higher concentrations of protein, fat, ash and lactose than cow milk. The casein micelles from buffalo milk were more mineralised and less hydrated than their counterparts cow milk. During acidification, some molecular changes, such as precipitation/aggregation of casein, solubilizations of calcium and inorganic phosphate and decrease in hydration of casein, occurred. These molecular changes were qualitatively similar for both species. It was noteworthy that the buffering capacity was higher for buffalo milk than for cow milk. This difference suggested that the acidificat-

ion process in dairy technology, which is well established for cow milk, cannot be directly extrapolated to buffalo milk and some adaptations are necessary. On the other hand, it will be interesting, for the future, to test the influence of different factors such as type of milk (with and without fat, differently heated) and type of acidification (chemical or biological) on the molecular changes described in this work.

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