

# Molecular changes during chemical acidification of the buffalo and cow milks

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**ABSTRACT** - Composition and physico-chemical properties of buffalo and cow milks were compared at their initial pH and during chemical acidification. As compare to cow milk, buffalo milk was richer in caseins and minerals such as Ca, Mg and Pi. Along with these differences, the capacity of buffalo milk to be acidified named buffering capacity was also higher than cow milk. The pH decrease induced aggregation of caseins at their isoelectric pH and solubilisations of Ca and Pi were significant for both milks. For both species, these molecular changes were qualitatively similar but quantitatively different. These differences suggested that the acidification process in dairy technology which is well established for cow milk can not be directly extrapolated to buffalo milk and some adaptations are necessary.

**Key words:** Buffalo milk, Cow milk, Acidification, Molecular changes.

**INTRODUCTION** - Buffalo's milk is ranked second in the world after cow's milk, being more than 12 % of the world's milk production. This milk is used for making different dairy products. Parts of these products (cheese, yogurts) 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. In the present paper, some physico-chemical characteristics of buffalo milk at natural pH and during acidification have been described and the results have been compared with those obtained for cow milk.

**MATERIALS AND METHODS** - 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 (Mauris, France) and from Société Laitière (Hermitage, France), respectively. 0.3g/L thimerosal was added as a preservative. Acidification of milks was carried out with 1 M HNO<sub>3</sub>. Total nitrogen (TN), non casein nitrogen (NCN) and non protein nitrogen (NPN) were determined by Kjeldhal method. Nitrogen contents were converted into equivalent protein contents using 6.38, 6.25 and 3.60 as converting factors for TN, NCN and NPN contents, respectively. Casein nitrogen (CN) was calculated as  $[CN] = [TN] - [NCN]$ . Total and diffusible cation and anion concentrations were determined by atomic absorption spectrometer and ion chromatography, respectively.

**RESULTS AND CONCLUSIONS** - Contents of TN were higher for buffalo milk than for cow milk whereas normal pH was similar for milks from both species. The CN content

was also significantly higher for buffalo milk than for cow milk. NCN content corresponding to whey proteins, proteose-peptone and NPN, was almost similar in both milks. NPN content, which contains creatin, urea and free amino acids, was also similar. The total calcium (Ca) and inorganic phosphate (Pi) concentrations were higher in buffalo milk than in cow milk. The concentrations of diffusible Ca and Pi were similar in both milks. From these total and diffusible concentrations, it could be deduced that the quantities of Ca and Pi associated with casein micelles were also higher in buffalo milk than in cow milk. Thus, in our study, 82 % and 72 % of Ca and 66 % and 48 % of Pi were in the micellar phase of buffalo and cow milk, respectively. Assuming that all the casein molecules existed in a micellar form, the amounts of Ca associated to casein were 1.12 and 0.84 mM and Pi 0.53 and 0.36 mM per gram of casein for buffalo and cow milk, respectively. The molar ratio of micellar Ca/micellar Pi was 2.10 and 2.35 for buffalo and cow milk, respectively. Total and diffusible concentrations of magnesium (Mg) and sodium (Na) ions were higher in buffalo milk than in cow milk whereas total and diffusible concentrations of potassium (K) and chloride (Cl) were higher in cow milk than buffalo milk. Total citrate concentration was similar in both milks whereas the diffusible content was lower in buffalo milk than in cow milk. Concerning composition, these results (Table 1) agree with what found in previous studies (Ganguli, 1973; Spanghero & Susmel, 1996).

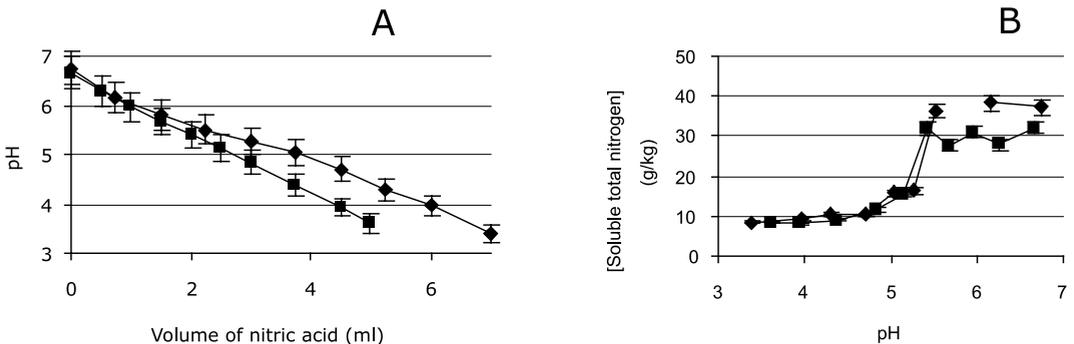
Table 1. Proteins and minerals composition of buffalo and cow milks.

	Buffalo	Cow
pH	6.81 ± 0.06	6.76 ± 0.04
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 / diffusible Ca (mM)	47.1 ± 1.2 / 8.2 ± 0.2	30.5 ± 0.8 / 8.6 ± 0.2
Total / diffusible Pi (mM)	27.7 ± 1.4 / 9.2 ± 0.5	19.2 ± 1.0 / 9.9 ± 0.5
Total / diffusible Mg (mM)	7.3 ± 0.2 / 3.5 ± 0.1	4.6 ± 0.1 / 3.0 ± 0.1
Total / diffusible Na (mM)	20.3 ± 0.5 / 18.4 ± 0.5	17.5 ± 0.4 / 15.9 ± 0.4
Total / diffusible K (mM)	28.7 ± 0.7 / 26.0 ± 0.7	42.0 ± 1.0 / 37.3 ± 0.9
Total / diffusible Cl (mM)	16.6 ± 0.8 / 16.3 ± 0.8	21.8 ± 1.0 / 22.8 ± 1.0
Total / diffusible citrate (mM)	8.3 ± 0.4 / 7.1 ± 0.4	8.8 ± 0.4 / 8.2 ± 0.4

The pH of buffalo milk decreased slower than pH of cow milk during acidification (Figure 1A). For example to obtain pH 4.0 in buffalo and cow milk, 6.0 and 4.5 mL of acid was needed, respectively, which indicated that the buffering capacity of buffalo milk was higher than that of cow milk. The buffering capacity of milk is related to its composition in acidobasic compounds (Salaün et al., 2005); in our case, the difference observed between the milk of these species was probably related to the higher casein and Pi contents in buffalo milk as compared to cow milk (Table 1). Acidification process of milk induced precipitation of casein (Figure 1B). For both milks, the observed decrease in total soluble nitrogen corresponded to a progressive neutralization of casein as a function of pH with the consequence of their

precipitation around their isoelectric pH. For casein from cow milk, it is admitted that the value of this characteristic is 4.6 (Walstra & Jenness, 1984). The comparison of amino acids contained in casein molecules of these species indicated a good homology suggesting that the isoelectric pH might be similar for both species.

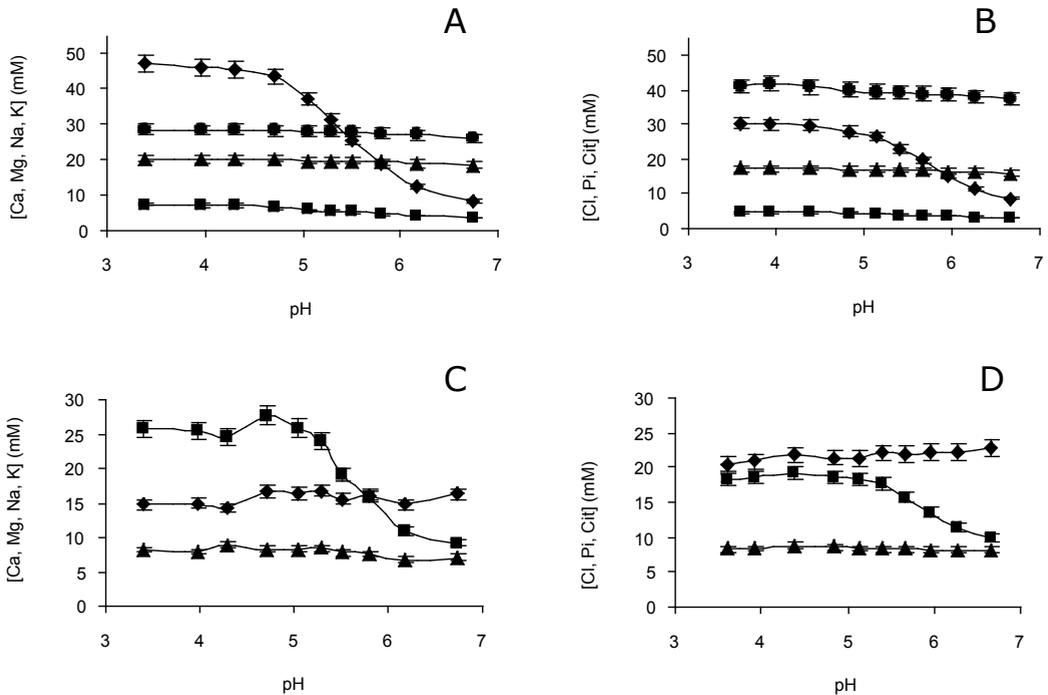
Figure 1. (A) pH of milks (60 mL) as a function of added volume of HNO<sub>3</sub> (1 M) (■: cow ; ◆: buffalo). (B) Effect of pH on soluble nitrogen content (■: cow; ◆: buffalo).



The diffusible concentration of Ca and Pi increased as a function of acidification (Figure 2). The solubilization of Ca was considered for both species as total at pH 3.5 and of Pi 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 Pi was totally solubilized, we could consider that the solubilization concerned essentially the Ca directly associated to phosphoserine residues of casein molecules. Dalgleish & Law (1989) and Le Graët & Brulé (1993) also found the same results for cow milk. Slight and gradual solubilizations of Mg, Na, K, Cl and citrate were also determined as a function of pH decrease. These solubilization trends were very similar for both milks.

This work showed that the proteins and minerals composition of buffalo and cow milks were different. The casein micelles from buffalo milk were more mineralised than cow milk. During acidification, some molecular changes such as precipitation / aggregation of caseins, solubilizations of Ca and Pi have been occurred. These changes were qualitatively similar for both species. It was noteworthy that the buffering capacity was higher for buffalo milk than cow milk. This difference suggested that the acidification process in dairy technology, which is well established for cow milk, can not be directly extrapolated to buffalo milk and some adaptations are necessary.

Figure 2. Concentrations of diffusible ions as a function of pH. A and B correspond to the diffusible concentrations of Ca (◆), Mg (■), Na (▲) and K (●) of buffalo and cow milk, respectively. C and D correspond the diffusible concentrations of Cl (◆), Pi (■) and Cit (▲) for buffalo and cow milk, respectively.



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