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Review

The minerals of milk

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Abstract – The salt of milk constitutes a small part of milk (8–9 g·L^{-1}); this fraction contains calcium, magnesium, sodium and potassium for the main cations and inorganic phosphate, citrate and chloride for the main anions. In milk, these ions are more or less associated between themselves and with proteins. Depending on the type of ion, they are diffusible (cases of sodium, potassium and chloride) or partially associated with casein molecules (cases of calcium, magnesium, phosphate and citrate), to form large colloidal particles called casein micelles. Today, our knowledge and understanding concerning this fraction is relatively complete. In this review, the different models explaining (i) the nature and distribution of these minerals (especially calcium phosphate) in both fractions of milk and (ii) their behaviour in different physico-chemical conditions, are discussed.

milk / minerals / salts / calcium / phosphate / casein micelle

1. INTRODUCTION

The milk components (proteins, fat, lactose, vitamins and minerals) are necessary to nurture the new-born and consequently they contribute to its growth. The mineral fraction, which is a small fraction of milk (about 8–9 g·L^{-1}), contains cations (calcium, magnesium, sodium and potassium) and anions (inorganic phosphate, citrate and chloride). In milk, these ions play an important role in the structure and stability of casein micelles [1–3]. A good understanding of the properties of milk minerals is important for fundamental research but also for the development of dairy products in which this fraction appears to be complex, dynamic, and in strong interaction with the protein fraction. In this review, the important characteristics of this salt fraction in different conditions are covered with an emphasis on recent findings. Thus, after indicating the milk mineral composition, we describe the distribution of ions between the different fractions of milk and the factors influencing this distribution. Then, particular attention will be paid to the structure and stability of casein micelles in relation with this mineral fraction.

2. MINERAL COMPOSITION OF COW MILKS

Concentration ranges of the different minerals in cow milk (expressed in massic and molar concentrations) are indicated in

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Table I. Mineral composition of cow milk.

Mineral	Concentration (mg·kg^{-1})	Concentration (mmol·kg^{-1})
Calcium	1043–1283	26–32
Magnesium	97–146	4–6
Inorganic phosphate	1805–2185	19–23
Total phosphore	930–992	30–32
Citrate	1323–2079	7–11
Sodium	391–644	17–28
Potassium	1212–1681	31–43
Chloride	772–1207	22–34

Table II. Total and soluble salt concentration (mM) in the skim milk of Ayrshire in early, middle and late lactation and from cows with subclinical mastitis [4].

	Bulk	Early	Middle	Late	Mastitic
Total calcium	30.1	33.2	29.4	32.1	29.4
Soluble calcium	9.5	12.8	9.4	8.0	9.1
Total magnesium	5.1	5.7	5.0	5.4	4.9
Soluble magnesium	3.3	3.9	3.2	3.3	3.2
Total inorganic phosphate	20.9	19.4	20.9	18.4	19.0
Soluble inorganic phosphate	11.2	10.5	11.7	7.0	9.2
Total citrate	9.8	9.8	9.1	8.5	8.8
Soluble citrate	9.2	9.1	8.9	7.8	8.3
Total sodium	25.5	29.7	24.8	48.8	34.5
Total potassium	36.8	41.8	40.3	26.9	36.1
Total chloride	30.3	36.7	29.7	46.5	40.5
pH	6.72	6.53	6.73	6.98	6.87

Table I. This composition is considered as relatively constant but slight variations can be observed in some cases. Thus, the calcium and phosphate contents are higher in milks rich in proteins. For example, the milk from Normandy cows has a higher mineral content than the milk from Friesian, Red Pied and Holstein cows. The concentration of minerals also varies with the time of the lactation period (Tab. II) and the most important changes in composition occur at around parturition; thus the calcium concentration in colostrum is much higher than that of normal milk and near the end of lactation. Mineral composition is also modified during mastitis and this specially concerns concentrations of sodium and chloride ions which are strongly increased (Tab. II, last column). Depending on the cause and severity of mastitis, these changes in composition can be more or less important. Concerning the effect of diet on the mineral composition of milk, there are few scientific studies. However, some differences in the milk salt concentration and especially in the citrate concentration are related during the change in season. In fact, there is a causal effect of diet on citrate concentration related to the de novo synthesis of fatty acids by the mammary gland [4].

Table III. Salts partition in cow milk [35].

Constituents	Concentration
Casein	26.1 g·L^{-1}
Total Ca	29.4 mM
Soluble Ca	9.2 mM
% of soluble Ca	31%
Micellar Ca	20.2 mM
Micellar calcium·g^{-1} of casein	0.77 mM
Total Pi	20.9 mM
Soluble Pi	11.2 mM
% of soluble Pi	54%
Micellar Pi	9.7 mM
Micellar Ca/micellar Pi	2.08
Total Mg	5.1 mM
Soluble Mg	3.3 mM
% of soluble Mg	65%
Micellar Mg	1.8 mM
Ester phosphate	3.5 mM
Total citrate	9.2 mM
Soluble citrate	8.2 mM
% soluble citrate	89%
Soluble Na	24.2 mM
Soluble K	34.7 mM
Soluble Cl	30.2 mM

3. DISTRIBUTION OF MACRO-ELEMENTS IN COW MILK

In milk, all of these macro-elements are distributed differently into diffusible and non diffusible fractions (essentially casein micelles). Potassium, sodium and chloride ions are essentially diffusible although calcium, inorganic phosphate and magnesium are partly bound to the casein micelles (Tab. III). About one-third of calcium, half the inorganic phosphate, two-thirds of magnesium and over 90% of citrate are in the aqueous phase of milk. A small proportion of calcium is also bound to α-lactalbumin (there is one atom of calcium per protein) [5]. No macro-element is bound in important amount to the fat globules and lactose.

3.1. Minerals in the diffusible fraction

3.1.1. Preparation of diffusible fraction

Experimentally, the determination of salt content in the aqueous phase is not problematic but implicates, before analysis, the obtention of this fraction. Four different methods to obtain this phase are used: dialysis, ultrafiltration, ultracentrifugation and rennet coagulation followed by recovery of whey. During the preparation of the sample by one of these techniques, it is imperative to work at a controlled pH and temperature since mineral equilibrium is very dependent on these physico-chemical parameters. For dialysis and ultrafiltration, it is recommended to use membranes with a molecular weight cut-off inferior to 10 000–15 000 Da in order to avoid a transfer of small molecules and proteins able to bind minerals in the dialysat or ultrafiltrate. For ultracentrifugation, different conditions of centrifugation can be used. Two typical conditions are 80 000 *g*, 2 h and 100 000 *g*, 1 h. However for those concentrations determined in the dialysat, rennet whey, ultrafiltrate or ultracentrifugal supernatant correspond rigorously to the real concentration in the milk; it is therefore necessary to use a correction factor. Thus, the mineral concentrations found in these samples must be converted into diffusible mineral concentrations by multiplying by a 0.96 correcting factor which takes into account the excluded volume effect. Moreover, some slight differences of results can exist depending on the type of sample preparation. Thus, the concentrations found in the ultracentrifugal supernatant are generally more important than those found in the ultrafiltrate. This difference is related to the presence of whey proteins and soluble caseins in the ultracentrifugal supernatant (and not in the ultrafiltrate) which are able to bind some ions such as calcium and magnesium.

Table IV. Concentrations (mM) of free and associated ions in the aqueous phase of milk [7].

Anion		Free ion		Complexed ion	
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
H_2Cit^-	+[1]	+	+	+	+
$HCit^{2-}$	0.04	0.01	+	+	+
Cit^{3-}	0.26	6.96	2.02	0.03	0.04
$H_2PO_4^-$	7.50	0.07	0.04	0.10	0.18
HPO_4^{2-}	2.65	0.59	0.34	0.39	0.52
PO_4^{3-}	+	0.01	+	+	+
Glc 1-PH^-	0.50	+	+	0.01	0.01
Glc 1-P^{2-}	1.59	0.17	0.07	0.10	0.14
H_2CO_3	0.11	–	–	–	–
HCO_3^-	0.32	0.01	+	+	+
CO_3^{2-}	+	+	+	+	+
Cl^-	30.9	0.26	0.07	0.39	0.68
HSO_4^-	+	+	+	+	+
SO_4^{2-}	0.96	0.07	0.03	0.04	0.10
RCOOH	0.02	–	–	–	–
$RCOO^-$	2.98	0.03	0.02	0.02	0.04
Free ion		2.00	0.81	20.92	36.29

3.1.2. Concentration and association of minerals in the diffusible fraction

In the diffusible fraction, ions are not totally "free" in solution and different associations of ions occur. Quantitatively, these associations depend on the association constants (or affinity) between cations and anions and the solubilities of the salts [1–3, 6, 7]. Analytically, the different diffusible salts can not be determined because they are inseparable in this fraction. However, Holt et al. [7, 8] have proposed a calculation for the ion equilibrium in milk diffusate. An example of calculation indicating these different associations is indicated in Table IV. Globally, calcium exists as ionic calcium (free ion) and a stable complex mainly with citrate (as the trivalent citrate), and to a lesser degree with inorganic phosphate (as a mixture of $H_2PO_4^-$ and HPO_4^{2-}) and with chloride. The low quantity of calcium phosphate in the diffusible fraction is due to the low solubilities of these salts. The other monovalent cations (sodium and potassium) exist mainly as free ions but a small part of these ions are associated with citrate, inorganic phosphate and chloride.

Taking into account these different associations, the diffusible fraction of milk at pH 6.6–6.7 appears to be supersaturated in calcium phosphate (Tab. V), and to have an ionic strength of about 80 mM.

3.2. Micellar calcium phosphate

3.2.1. Phosphoserine residues of casein molecules

All casein molecules have, in their sequences, phosphoserine residues in clusters (Fig. 1) (except for the κ-casein which has only one residue). The phosphate groups of these phosphoserine residues constitute the main binding sites of cations in the caseins. The cation binding capacity of the casein components decreases in the order $\alpha s_2 > \alpha s_1 > \beta >$ κ-casein, corresponding to their decreasing phosphoserine contents

Caséine β	GEIVE**S**L**SSS**EESITRINKKIEK
Caséine α_{s2}	KNTMEHV**SSS**EESII**S**QETYKQE
Caséine α_{s2}	EEEYSIG**SSS**EE**S**AEVA**T**EEVKI
Caséine α_{s1}	EL**S**KDIG**S**E**S**TEDQAMEDIKQMEAE**S**I**SSS**EEIVPN**S**VEQKHI
Caséine α_{s2}	TLNREQL**STS**EENSKKTVDMEST

Figure 1. Example of clusters of phosphoserine residues (S) present in casein molecules.

Table V. Chemical formulas and degrees of saturation (S) of different potential calcium and magnesium salts in a typical milk ultrafiltrate [2].

Compound	Abbreviation	Formula	Ca/P	S
Dicalcium phosphate	DCP	$CaHPO_4$	1.0	3
Dicalcium phosphate dihydrate	DCPD	$CaHPO_4.2H_2O$	1.0	6
Micellar calcium phosphate	MCP	$Ca(HPO_4)_{0.7}(PO_4)_{0.2}.xH_2O$	1.1	1
Octacalcium phosphate	OCP	$Ca_8H_2(PO_4)_6.5H_2O$	1.33	60
β-Tricalcium phosphate	β-TCP	$\beta\text{-}Ca_3(PO_4)_2$	1.5	200
Hydroxyapatite	HAP	$Ca_5OH(PO_4)_3$	1.67	8×10^8
Amorphous calcium phosphate	ACP	$Ca_3(HPO_4)_{0.2}(PO_4)_{1.87}.xH_2O$	1.45	0.2
Tricalcium citrate dihydrate	TCC	$Ca_3(Cit)_2.2H_2O$	–	0.4
Dimagnesium phosphate	–	$MgHPO_4$	–	0.3

(globally, the amount of calcium which is directly bound to the casein is equivalent to the number of ester groups present).

3.2.2. Micellar calcium phosphate

In a general point of view, the physical chemistry of the calcium phosphate salts is complex because these salts can have many compositions (different Ca/P ratio), exist in different forms (amorphous or differently crystallised) and the thermodynamical equilibrium is very slowly attained. Moreover, in casein micelles, there is an additionnal complication in defining this micellar calcium phosphate because calcium and inorganic phosphate are in strong interaction with phosphoserine residues of caseins. Indeed, micellar calcium is not totally associated to the colloidal inorganic phosphate and one part of it is directly bound to casein by the intermediate of phosphoserine residues which are organic phosphate. Thus, the colloidal calcium in milk can be defined as a mixture of calcium caseinate (containing organic phosphate) and calcium phosphate (which is an inorganic phosphate). However, experimentally, these two types of colloidal calcium are not separable, and consequently it is difficult to calculate the composition of calcium phosphate alone. In spite of these difficulties, different methodologies were used to determined its chemical composition and structure.

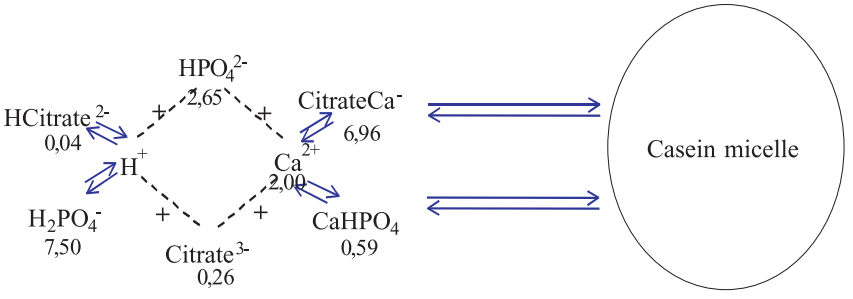

Figure 2. Mineral equilibrium between aqueous and micellar phases. The concentrations of different mineral associations are indicated in mM. The pH is 6.7.

After the analysis of micellar calcium phosphate composition, the Ca/P ratio was calculated by different authors. If the ester phosphate groups of the caseins together with their bound calcium are assumed not to be part of the calcium phosphate matrix, calcium would be of a tricalcium phosphate type. This is based on the Ca/P molar ratio of approximately 1.5. However, if one assumes that the casein ester phosphates are an integral part of the calcium phosphate matrix, resulting in a calcium to phosphate ratio close to 1.0, the micellar calcium phosphate would be of a brushite type [9]. This was confirmed by X-ray absorption and infrared spectroscopy since it was observed that the micellar calcium phosphate closely resembles brushite [9]. By calculation of the apparent solubility product, a structure more like that of dicalcium phosphate and a modified structure $Ca(HPO_4)_{0.7}(PO_4)_{0.2}$ was proposed by Holt [9, 10]. By microscopy, the micellar calcium phosphate appeared as several distinct regions of higher electron density (microgranules) with an estimated diameter of about 2.5 nm [11]. Using high-resolution transmission electron microscopy and diffraction methods, Mc Gann et al. [11] and Lyster et al. [12] indicated an amorphous nature of the micellar calcium phosphate. Van Dijk [13] proposed a specific formula showing the bond between micellar calcium phosphate and caseins. In this formula, a complex containing 4 inorganic phosphates, 2 phosphoserine residues and 8 cations (6 calcium) is described. However this formula was not in accordance with the presence of microgranules having the few nanometer diameter previously described.

Another aspect of micellar calcium phosphate is its exchangeability with the diffusible fraction. Indeed, calcium is more or less exchangeable between these fractions and Pierre et al. [14] using ^{45}Ca, showed that the time required to exchange all the calcium directly bound to phosphoserine residues was less than one minute. Calcium engaged in colloidal phosphate is only partly exchangeable. Calcium bound to the first acidity of phosphate is exchanged in less than one minute, the one bound to the second acidity is slowly exchanged (70% in 24 h) and the one bound to the third is not exchanged. Figure 2 summarises the mineral equilibrium existing between the diffusible and non diffusible fractions (micellar casein) in the milk.

3.2.3. Role of micellar calcium phosphate in the casein micelle

As previously described, there are significant amounts of calcium phosphate and calcium citrate in casein micelles. This micellar calcium phosphate, which is mainly associated to αs_1-, αs_2-, and β-caseins, is

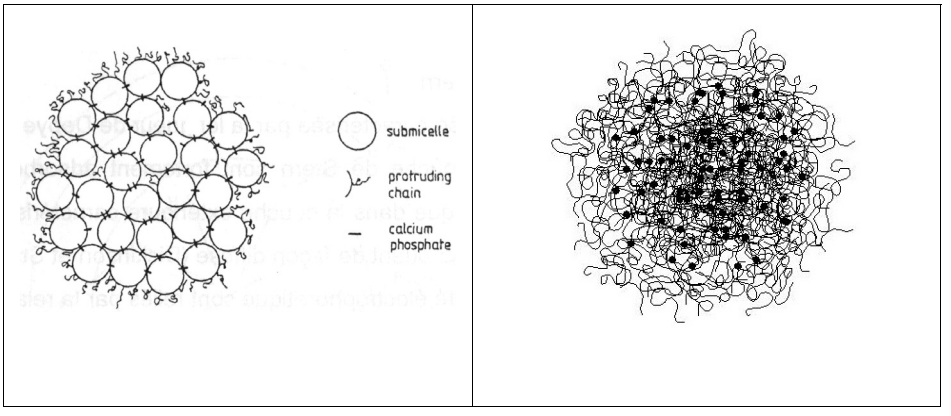

Figure 3. Models of casein micelles. Left: Model of Schmidt [15]; Right: Model f Holt and Horne [16].

responsible for the structure and stability of casein micelles. It is, at the same time, a cross-linking and also a neutralising agent of phosphoserine residues because it is positively charged and binds the negative sites of casein molecules. In a typical casein micelle, there are about 10^4 polypeptide chains of casein molecules associated with about 3.10^3 microgranules of an amorphous calcium phosphate. In all models of the casein micelle structure proposed, micellar calcium phosphate is an integral part of the casein micelle. Today, models of Schmidt [15] and the one of Holt and Horne [16] are essentially used (Fig. 3). Schmidt [15] proposed a subunit structure linked by micellar calcium phosphate although the submicelles are not considered in the model of Holt and Horne [16]. These latter authors consider caseins as rheomorphic proteins and the micellar calcium phosphate named calcium phosphate nanoclusters are mainly bound to the phosphoserine residues of casein and probably glutamate and aspartate residues. In the last model, the size of the calcium phosphate nanoclusters is to the order of the nanometer and nanoclusters are constituted of a core of calcium phosphate and a shell of casein phosphopeptide.

4. INFLUENCE OF PHYSICO-CHEMICAL CONDITIONS ON MINERAL DISTRIBUTION AND STABILITY OF CASEIN MICELLES

As described previously in this review, micellar calcium phosphate plays a key role in the maintenance of the structure of the casein micelle. However, depending on the physico-chemical conditions, the structure of casein micelles can be more or less destroyed by dissociation of caseins and salts [17]. On the one hand, it is useful to modify the physico-chemical conditions because the induced-changes allow to have a better understanding of the properties of milk minerals and especially the interactions between the salts of calcium phosphate and the casein molecules. On the other hand, these properties of dissociation are commonly exploited for the manufacture of dairy products. Figure 4 summarises some physico-chemical events occurring during technological operations such as acidification, heat treatment, cooling, addition of chelatants, NaCl, or divalent cations.

4.1. Acidification

The different acido-basic groups of milk constituents (organic and inorganic phosphate,

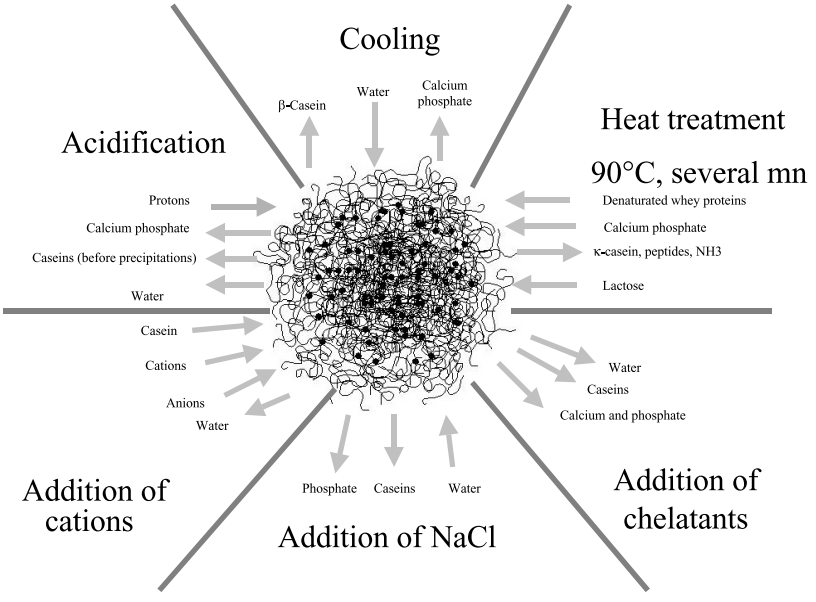

Figure 4. Modification of salt equilibrium in different physico-chemical conditions.

citrate, carboxylic residues, etc.) become more and more protonated when the pH of milk is reduced. Consequently, micellar calcium phosphate and the small amounts of magnesium and citrate associated to casein micelles are dissolved and the caseins are liberated into the diffusible fraction of milk. The extent of dissociation depends on both pH and temperature [9, 18–20]. It is generally admitted that, in milk at pH 5.2 one part of calcium but the totality of inorganic phosphate are solubilised suggesting that the micellar calcium (inorganic) phosphate is completely "destroyed" at this pH value. Between the native pH of milk and pH 5.2, a correlation between solubilised calcium and solubilised inorganic phosphate is observed. Calcium is totally solubilised only at pH 3.5 [9]. The compilation of these results of pH-induced solubilization suggests that the calcium ion is implicated in two types of association : the first is an association with inorganic phosphate and the second is with organic phosphate (phosphoserine residues of casein molecules). These modifications of repartition induced by acidification are irreversible and it is impossible to reconstruct the micellar calcium phosphate and the micellar edifice by an increase in pH.

4.2. Heat treatment

Casein micelles are considered as remarkably stable against heat treatments. However, several changes depending on the intensity of the heat treatment were determined. Among these changes, it is described that calcium phosphate present in the diffusible fraction is less soluble during heat treatment and consequently some decreases in calcium and inorganic phosphate in this fraction were determined after this type of treatment [21]. Wahlgren et al. [22] showed, using NMR of ^{43}Ca and ^{31}P, an association of these constituents to the casein micelles during heat treatment (from 30 to 64 °C). If the heat treatment is less than 95 °C during few minutes, the modifications of salt equilibrium are considered as

reversible. In contrast, severe heatings (for example, 120 °C during 20 min) cause irreversible changes in casein micelles and in the salt partition. Phosphoserine residues of caseins can be partially hydrolysed and the decrease of calcium and phosphate contents in the diffusible fraction does not correspond to the simple precipitation of diffusible calcium phosphate. Moreover, changes in the structure and composition of the original micellar calcium phosphate into a more insoluble form are described [21]. The new phases formed are not clearly identified although some authors relate the formation of crystalline β-tricalcium phosphate [23] or hydroxyapatite [20].

4.3. Cooling

On the contrary to the heat treatments, the solubility of micellar calcium phosphate increases when the temperature decreases and consequently a cooling of milk induces a dissolution of micellar calcium phosphate [24]. Ichilczyk-Leone et al. [25] cooled skim milk and showed that one part of micellar calcium phosphate is transferred in the diffusible fraction within 24 h. Quantitatively, they observed increases of about 10% of calcium and inorganic phosphate in the diffusible fraction. These changes are reversible and the previous partition may be re-established on re-warming.

4.4. Addition of chelatants

Chelatants have high affinities for cations and are able to displace them, especially calcium. Thus, the addition to milk of calcium-chelating agents (citrate, EDTA, oxalate, etc.) or cation exchange resins induces an increase of calcium and inorganic phosphate contents in the diffusible fraction of milk [26–30]. Consequently, the casein micelles are more or less destroyed by the disruption of micellar calcium phosphate. For the industrial processes, these chelatant additions can resolve some important problems because they improve the heat stability and storage life of concentrated milk products and prevent deposit formation on heat exchangers and various membrane surfaces.

4.5. Addition of NaCl

The addition of NaCl to milk leads to a slight decrease in pH and increases in Ca^{2+} concentrations in the diffusible phase. These changes would correspond to exchanges of divalent cations or protons which were attached directly to phosphoseryl residues of casein molecules by Na^{+} [9, 28, 30, 31]. Moreover, it is well known that the increase in ionic strength induces decreases in activity coefficients of the diffusible ions and consequently increases in the dissociation of the ion pairs [3]. As a consequence of these exchanges, the hydration of casein micelles is increased [28, 32] although their size and charge stay constant.

4.6. Addition of divalent cations (especially calcium)

The addition of divalent cations induces important modifications in the salt distribution between aqueous and micellar phases. These modifications depend on the type of cation (calcium, magnesium, iron, zinc, copper), the concentration at which they are added, the eventual modification and regulation of pH consequently to cation addition. Theoretically, the modifications of salt balance are controlled by the solubilities of cation phosphate and cation citrate salts in the diffusible fraction [33]. In the literature, the modifications of salt balance of milk induced by calcium addition are the most described [34]. Thus, after the addition of 10 mM calcium chloride to milk, about 80% of this ion was associated to casein micelles. In parallel, displacements of inorganic phosphate and citrate ions from the diffusible fraction to the micellar edifice were observed. Consequently, casein micelles were strongly modified: for example, their zeta potentials and hydration decreased with as a consequence a decrease in their heat stability [34].

5. CONCLUSION

The mineral fraction has been subjected to extensive investigations for over 60 years. Most of these research works can be separated into two categories:

(1) The applied research studying the role of minerals during different industrial processes or their influence on the functionality of dairy products;

(2) The basic research studying the physical chemistry (concentration, repartition, solubility, nature of associations) of these minerals and especially calcium phosphate.

From these results, it appears that these minerals represent a small fraction of milk (compared to the lipid or protein fractions) but they play important roles in the structure and stability of the casein micelles. Small changes of the physico-chemical conditions can induce some variations in their composition or repartition and consequently can have important consequences on the casein micelles. However and in spite of the existence of different models, several questions have no response today. Currently the questions which seem essential are the following: what is the detailed structure of the micellar calcium phosphate? What are the existing interactions between this complexed salt and casein molecules to form casein micelles?

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