Determination of anions of milk by ion chromatography

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Summary — The anions of milk have been successfully analysed by ion chromatography coupled with suppressed conductivity detection. With an appropriate sample preparation (ultrafiltration alone, or combined with dry mineralization), this method enabled simultaneous identification and quantification of chloride, phosphate and citrate ions with good repeatability (relative standard deviations of about 1%). Moreover, good sensibility (less than 20 µg/kg) and no interference between ions and other matrix components were determined. It was also shown that this method offers a very promising alternative for studying changes in the salt balance of milk during some technological treatments (acidification and increase of ionic strength of milk).

ion chromatography / milk / anion

Résumé — **Analyse des anions du lait par chromatographie ionique** - Les anions du lait ont été analysés par chromatographie ionique couplée à une détection par conductimétrie avec autosuppression. Avec une préparation d'échantillon appropriée (ultrafiltration seule ou combinée avec une minéralisation par voie sèche), cette méthode a permis l'identification et la quantification simultanée des ions chlorure, phosphate et citrate avec de bonnes répétabilités (déviations standard relatives d'environ 1 %). De plus, de bonnes sensibilités (inférieures à 20 µg/kg) et aucune interférence entre les ions à doser et les autres composés du milieu ont été déterminées. Par ailleurs, cette méthode constitue un excellent moyen pour étudier des changements d'équilibres salins durant certains traitements technologiques comme l'acidification ou l'augmentation de force ionique de laits.

chromatographie ionique / lait / anion

INTRODUCTION

The principal anions of skim milk are:

- phosphate (~ 2700–3000 mg/kg);
- citrate (~ 1500–2200 mg/kg);
- and chloride (~ 1000–1100 mg/kg).

Phosphate is present as: 1) inorganic phosphate (soluble phosphate and micellar calcium phosphate (MCP)); 2) organic

phosphate associated to small molecules (pentoses, hexoses, glycerol, serine and nucleotides); and 3) covalently bound to peptide chains of caseins; α_{s1} -B, α_{s2} -A, β -A², κ -B caseins have 8, 11, 5 and 1 phosphoserine residues per molecule respectively (Walstra and Jenness, 1984). In casein micelles, the MCP (about 50% of the inor-

ganic phosphate) plays an important role in maintaining the structure of casein micelles because these micelles are disaggregated when MCP is removed (Walstra and Jenness, 1984). Thus, modifications of physico-chemical parameters, such as pH, ionic strength and temperature, influence the dynamic mineral equilibrium of milk and some changes in mineral balance occurring during technological treatments are fundamental to a number of manufacturing processes. The phosphate concentration of milk affects almost all aspects of cheese manufacture (Lucey and Fox, 1993). The effects of these physico-chemical parameter modifications have been investigated by several authors (Brulé et al, 1974, 1977; Brulé and Fauquant, 1981; Walstra and Jenness, 1984; Grufferty and Fox, 1985; Visser et al, 1986; van Hooydonk et al, 1986; Dalglish and Law, 1988, 1989; Le Graet and Brulé, 1993). Moreover, chloride is totally in the aqueous phase and about 10% of citrate is electrostatically linked to casein micelles.

Different methods to determine the concentration of milk anions have been described in the literature. Usually, phosphate, chloride and citrate ions were determined by the following methods:

- phosphorus: colorimetric method (FIL, 1987), ^{31}P NMR (Visser et al, 1986), capillary ion electrophoresis (Schmitt et al, 1993);
- chloride: titrimetric (FIL, 1988a (Mohr method)), potentiometric (FIL, 1988b) and colorimetric (Herrero et al, 1992) methods, capillary ion electrophoresis (Schmitt et al, 1993);
- citrate: chromatographic (Marsili et al, 1981), spectrometric (Pierre and Brulé, 1983) and enzymatic (FIL, 1992a) methods, capillary ion electrophoresis (Schmitt et al, 1993).

Each method has its own merits, but they are time-consuming and/or require much material; occasionally they are also susceptible to interference. Moreover, with

each of these methods, except capillary ion electrophoresis (Schmitt et al, 1993), only one element can be determined at a time. For the determination of ions, an alternative method is the ion chromatography. The applications of this method are in the following areas (Weiss, 1995): environmental analysis (water, soil hygiene) (Stahl, 1994), detergent and household product industry (weakly basic cleansing agent, toothpaste, shampoo, etc), pharmaceutical industry, clinical chemistry (blood, saliva, urine) and food (vegetable, baby food) (Ruiz et al, 1995) and beverage industry (wine, apple juice, berry juice concentrate).

This article describes the use of ion chromatography to determine the principal anions of milk (chloride, phosphate and citrate). Modifications of pH and ionic strength on the salt balance of milk were also studied.

MATERIALS AND METHODS

Milk

Reconstituted milk was prepared at room temperature from INRA low heat skim milk powder at a concentration of 10% (w/w) in water. 0.01% thiomersal (Sigma Chemical Co, Saint Louis, MO, USA) was added to prevent bacterial growth.

Acidification of milk

Two hours after the reconstitution of the milk, acidification was realised by addition of 1 mol/L HCl. In order to obtain the same final volumes, the dilution effects were corrected by water addition. After overnight storage at room temperature, the pH values were measured. A range of pH values between 6.73 and 3.93 was obtained.

Increase of ionic strength of milk

0.5, 1 and 2% (w/w) of dry NaCl was added to milk. After overnight storage at room temperature, the pH values were measured.

Conventional methods for mineral content determination

Chloride concentration was determined by potentiometry (chloride analyser 926-Ciba Corning Diagnostics, Halstead, UK) (FIL, 1988a).

Phosphorus concentration was determined according to FIL method (1987) by molecular absorption spectrometry of phosphomolybdate compounds. A conversion factor of 95/31 was used to convert the phosphorus concentration into phosphate concentration.

Citric acid concentration was determined out by enzymatic method (FIL, 1992a) using citrate lyase, malate dehydrogenase, lactate dehydrogenase and NADH, H⁺ (cat No 139076, Boehringer, Mannheim, Germany). After enzymatic reactions, the absorbance decrease of NADH, H⁺ which is proportional to citric acid concentration, was determined at a wavelength of 340 nm.

For each sample, two independent measurements were carried out.

Ion chromatography for mineral content determination

A Dionex DX-500 high-performance liquid chromatographic system (Jouy-en-Josas, France) was used.

Lactate, acetate, propionate, butyrate, chloride, nitrate, succinate, carbonate, sulfate, phosphate and citrate were separated on an

anion exchange column (AS11 IonPac column, 4 × 250 mm) fitted with a AG11 guard column. The AS11 IonPac analytical column stationary phase was composed of a 13 µm highly cross-linked polyethylvinylbenzene/divinylbenzene substrate agglomerated with anion exchange latex that had been completely aminated. The anion trap column (ATC-1) was also used. This system was positioned between the gradient pump pressure transducer and the injection valve. Its application was to strip anionic contaminants such as carbonate from the hydroxide eluent and thus improve the signal to noise ratio of the analysis. Separation under linear gradient elution conditions used NaOH (from a fresh bottle of 50% NaOH solution that was low in carbonate concentration, JT Baker, Deventer, the Netherlands). The gradient elution program is reported in table I. Solutions A, B and C were 2 mmol/L NaOH, 200 mmol/L NaOH and 18-MΩ water respectively. Separations were carried out at 20 °C and at a flow-rate of 2 mL/min. After elution and before detection by the ED40 conductivity detector maintained at 35 °C, an auto-suppression external water mode was used by an anion self-regenerating suppressor (ASRS-I, 4 mm). This system provided high capacity suppression of traditional eluents and simplified the detection of ion chromatography because it maximised signal to noise ratio for high sensitivity analysis (Weiss, 1995).

Standard solutions of all the investigated anions were prepared from 1000 mg/L commercial

Table I. Gradient elution program used for analysis of anions by ion chromatography. Eluents A, B and C were 2 mmol/L NaOH, 200 mmol/L NaOH and 18-MΩ water, respectively. The complete chromatographic conditions are described in the legend to figure 1.

Programme du gradient d'élution utilisé pour l'analyse des anions par chromatographie ionique.

Les éluants A, B et C sont respectivement une solution de NaOH à 2 mmol/L, une solution de NaOH à 200 mmol/L et de l'eau à 18 MΩ. Les conditions chromatographiques complètes sont décrites dans la légende de la figure 1.

Time (min)	% A	% B	% C
0.00	10.0	0	90.0
0.10 (injection)	10.0	0	90.0
5.00	50.0	0	50.0
20.00	12.5	11.0	76.5
22.00	0.0	15.0	85.0
23.00	10.0	0	90.0
30.00	10.0	0	90.0

solutions (Merck, Darmstadt, Germany) except for citric acid which was prepared from citric acid monohydrate commercial salt (Merck). Before injection with an auto-sampling device (25 µL volume), suitable concentrations (0, 1, 2, 5, 10 and 20 mg/kg for chloride and citrate, and 0, 2, 5, 10, 20 and 40 mg/kg for phosphate) were obtained by dilution with 18-MΩ water.

The detection limits were found by preparing dilute solutions of ions and by identifying the concentrations of ions that gave a signal calculated as twice the baseline noise.

In order to estimate the repeatability (or precision) of this method for each ion, relative standard deviations (RSD) were calculated as follows: $RSD\% = (\sigma / m) \times 100$; m and σ being the mean and the standard deviation of series of determination respectively.

Sample preparation

The milk aqueous phase was obtained as follows: 7 mL of milk was placed in an ultrafiltration membrane cone (molecular mass cut-off = 25 000 Da, CF 25, Amicon, Epemont, France) and centrifuged at room temperature (1000 g for 30 min). Before ion chromatography analysis, the ultrafiltrate was diluted 200-fold (0.1 g in 20 mL) with 18-MΩ water and not in solution containing NaOH to avoid carbonation of the sample.

For the determination of total phosphate (organic and inorganic) content in ultrafiltrate or in milk, a preparation of ashes by dry mineralization was carried out. A dry ashing followed by a dissolving of ashes by HNO₃ rather than a wet mineralization by acid such as H₂SO₄ was chosen because, in the presence of concentrated acid, the concentration of anions (SO₄²⁻ in this case) would be too high and could induce an overloading of the chromatographic column. Therefore, 1 g of sample was mineralised at 550 °C for 4 hours. A previous experiment had shown that a dry ashing at a temperature higher than 550 °C induced a loss of chloride ions probably by volatilisation (FIL, 1992b). The ashes obtained were dissolved in 1 mL of 1 N HNO₃. Then, the volume was adjusted to 200 mL with 18-MΩ water before analysis by ion chromatography. It is noteworthy that the HNO₃ solution used does not contain residual anions as chloride, sulfate, phosphate and citrate. For sample storage, polyethylene vessels were used.

RESULTS AND DISCUSSION

General aspects

A typical chromatogram of standard anions (lactate, acetate, propionate, butyrate, chloride, nitrate, succinate, carbonate, sulfate, phosphate and citrate) is presented in figure 1. The chromatographic peaks were

Fig 1. Chromatogram of anions (lactate (Lac), acetate (Ace), propionate (Pro), butyrate (But), chloride (Chl), nitrate (Nit), succinate (Suc), carbonate (Car), sulfate (Sul), phosphate (Pho) and citrate (Cit)) of a standard mixture containing 5 mg/kg of each ion except for carbonate and phosphate ions which were at concentrations of 10 mg/kg. Chromatographic conditions for anions were as follows: eluent, NaOH under linear gradient conditions which are indicated in table; flow rate, 2.0 mL/min; AS11 column with an AG11 as a guard column; injection volume, 25 µL; suppressed conductivity detection.

Chromatogramme des anions lactate (Lac), acétate (Ace), propionate (Pro), butyrate (But), chlorure (Chl), nitrate (Nit), succinate (Suc), carbonate (Car), sulfate (Sul), phosphate (Pho) et citrate (Cit) d'une solution étalon contenant 5 mg/kg de chaque ion, sauf pour les ions carbonate et phosphate, qui étaient à une concentration de 10 mg/kg. Les conditions chromatographiques étaient les suivantes : éluant, NaOH utilisé sous les conditions de gradient linéaire qui sont indiquées dans le tableau 1 ; débit, 2,0 mL/min ; colonne AS11 avec une précolonne AG11 ; volume d'injection : 25 µL ; détection conductimétrique avec autosuppression.

identified by injecting each ion separately. The organic acids (acetate, lactate, propionate and butyrate) were partially separated. The other peaks were well resolved and consequently the quantification steps were easy. The difference between chromatographic peak areas with equal molar concentrations of different ions is related to their equivalent ionic conductivity which are characteristic for each ion (CRC Handbook of chemistry and physics, 1991). Moreover, as the pH value of eluent (containing NaOH) was about 12, all the anions were eluted in their fully dissociated forms. It is to be noted that under these chromatographic conditions, the citrate molecules may be separated from their structural isomer isocitrate (in milk, 1% of citrate is isocitrate; Walstra and Jenness, 1984), because both molecules have different retention times: 20.2 and 21.0 min respectively.

The resulting calibration functions of chloride, phosphate and citrate ions (table II) showed that the methods gave excellent linearity between injected concentrations (up to 20 mg/kg for chloride and citrate, and up to 40 mg/kg for phosphate) and chromatographic peak areas (conductivity signals). Higher concentrations were not tested in order to avoid overloading of the chromatographic column and because the concentration range of the standard solutions used in this study corresponded to possible concentrations of these ions in milk or in dairy products. For all ions tested, the detection limits were lower than 20 µg/kg (table II). In this study, these values were not restrictive because the ion concentrations in the aqueous phase of milk are very high. Each ion contributes differently to the current transport and the differences in detection limits between each ion were due to the differences in their equivalent ionic conductivity (CRC Handbook of chemistry and physics, 1991). These detection limits were approximately the same as those obtained by standard methods.

Anion determination in the aqueous phase of milk

Unmodified milk

Figure 2 shows anionic chromatographic profile of diluted milk ultrafiltrate. Additions of chloride, phosphate and citrate standards (+ 50 and 100% of the initial concentration value) to the analysed samples indicated that for these samples, there was no substantial matrix interference because the added concentrations were totally recovered (results not shown).

Chloride and citrate concentrations

In milk ultrafiltrate, concentrations of chloride and citrate were 1060 and 1500 mg/kg respectively. These values obtained by ion chromatography are normal for an unmodified milk and were confirmed by potentiometry for chloride and by enzymatic method for citric acid (results not shown). The RSD were 0.8% and 1.1% respectively ($n = 6$).

Fig 2. Chromatogram of anions in the aqueous phase of milk. The ultrafiltrate was 200-fold diluted with 18-M Ω water. The complete chromatographic conditions are described in the legends to figure 1 and table I.

Chromatogramme des anions de la phase aqueuse du lait. L'ultrafiltrat était dilué 200 fois avec de l'eau à 18-M Ω . Les conditions chromatographiques complètes sont décrites dans les légendes de la figure 1 et du tableau I.

Table II. Calibration parameters of chloride, phosphate and citrate ions by ion chromatography. Regression equations ($y = ax + b$) were calculated from injected concentration (y) as a function of the conductimetric signal (x) obtained. The detection limit was calculated as being the concentration which gave a signal corresponding to twice the baseline noise. r^2 corresponds to the correlation coefficient obtained by comparison of injected concentrations and conductivity signals obtained. The chromatographic conditions are described in the legends to figure 1 and table I.

Caractéristiques des droites de calibration des ions chlorure, phosphate et citrate par chromatographie ionique. Les équations de régression ($y = ax + b$) ont été calculées à partir de la concentration injectée (y) en fonction du signal conductimétrique obtenu (x). La limite de détection fut calculée comme étant la concentration qui donna un signal correspondant à deux fois le bruit de fond. r^2 correspond au coefficient de corrélation obtenu par comparaison des concentrations injectées et des signaux conductimétriques obtenus. Les conditions chromatographiques complètes sont décrites dans les légendes de la figure 1 et du tableau I.

	Time rétention (min)	a	b	r^2	Détection limit ($\mu\text{g/kg}$)
Chloride	7.2	$1.01 \cdot 10^{-5}$	- 0.067	0.999	~ 10
Phosphate	17.6	$3.06 \cdot 10^{-5}$	0.582	0.999	~ 10
Citrate	20.2	$4.23 \cdot 10^{-5}$	- 0.316	0.999	~ 20

Phosphate concentration

In milk ultrafiltrate, the phosphate concentration was 930 mg/kg and the RSD was 0.8% ($n = 6$). The phosphate concentration of the same ultrafiltrate determined by colorimetric method (FIL, 1987) was 1190 mg/kg. In fact, in all tested samples (results not shown), differences of about 250 mg/kg were always observed. An ultrafiltrate of milk contains phosphorus in many forms. All phosphorus is present as orthophosphate, but part of it is bound to small organic components which are able to pass through the ultrafiltration membrane. These phosphate groups are either esterified to serine and threonine residues of peptides or esterified to several small molecules like pentoses, hexoses, glycerol, serine and nucleotides (Walstra and Jenness, 1984). The phosphate concentration in milk ultrafiltrate as phosphate esters ranges between 150–450 mg/kg (Walstra and Jenness, 1984). This range was in accordance with the differences found in this study.

Thus, the organic phosphate concentration of ultrafiltrate was not evaluated by

direct ion chromatography analysis. On the contrary, the concentration of total phosphate (inorganic + organic) obtained by dry ashing of milk ultrafiltrate and ion chromatography (1180 mg/kg) (fig 3) was not significantly different from the concentration obtained by FIL method (1987), ie, mineralization in the presence of H_2SO_4 and molecular absorption spectrometry (1190 mg/kg). Chloride concentration was the same as that found before ashing (1060 mg/kg). On the other hand, in the case of dry mineralization, citrate was destroyed because no chromatographic peak corresponding to this ion was detected (fig 3). It is noteworthy that, in the case of this mineralization, the small chromatographic peaks eluted at 3.8 and 4.6 min were not identified but can not correspond to the acetate and butyrate ions because these ions are also destroyed during the mineralization. The high concentration of nitrate (about 65 000 mg/kg) (fig 3) corresponded to the presence of 1 mL of HNO_3 (1 mol/L) in 200 mL which was necessary to dissolve the ashes obtained after dry ashing and does not affect the chromatographic separation.

Other ions

In milk ultrafiltrate, other small chromatographic peaks eluted at 3.8 and 4.6 min (fig 2) could correspond to acetate and butyrate respectively. Traces of these ions (less than 1 mg/kg) were qualitatively determined by injection of ultrafiltrate added with acetic and butyric acid solutions. Other anionic species such as lactate, propionate, nitrate and succinate were not detected in these ultrafiltrate samples.

Chromatographic peak eluted at 12.0 min (fig 2) could correspond to carbonate ion. It was identified by injection of ultrafiltrate added with carbonate solution. It should be noted that residual carbonate was also

Fig 3. Chromatogram of anions in the ashes of milk ultrafiltrate. The ashes of ultrafiltrate were obtained by dry mineralization at 550 °C for 4 hours. Then, they were dissolved in 0.5 mL of 1 mol/L HNO_3 and the volume adjusted to 200 mL, with 18-M Ω water. The complete chromatographic conditions are described in the legends to figure 1 and table 1.

Chromatogramme des anions de cendres d'un ultrafiltrat de lait. Les cendres de l'ultrafiltrat ont été obtenues par minéralisation sèche à 550 °C pendant 4 heures. Puis elles ont été dissoutes dans 0,5 mL d' HNO_3 à 1 mol/L et le volume ajusté à 200 mL, avec de l'eau à 18 M Ω .

Les conditions chromatographiques complètes sont décrites dans les légendes de la figure 1 et du tableau 1.

detected in fresh 18-M Ω water used for sample dilution and that the chromatographic area of this peak was approximately the same as those found with the ultrafiltrate. So, we can conclude that carbonate was absent in ultrafiltrate.

The chromatographic peak eluted at 13.1 min corresponded to sulfate ion (fig 2). It was identified by injection of ultrafiltrate added with sulfate solution. The concentration value of sulfate ions was 140 mg/kg in the ultrafiltrate and 200 mg/kg after dry mineralization followed by analysis by ion chromatography of the same ultrafiltrate (fig 3). This difference in concentration could correspond to the presence of sulphur-containing compounds able to pass through ultrafiltration membrane (thiocyanate, amino acids such as methionine and cysteine) but which are not directly detectable by ion chromatography as sulfate ion. One part of these compounds are probably transformed in sulfate ion during dry ashing. These values of sulfate concentrations are in accordance with the literature (Walstra and Jenness, 1984).

Acidification of milk

Inorganic species in the aqueous phases of the milk acidified at different pH values (between 6.73 and 3.93) by HCl were determined by ion chromatography. For all samples and in spite of the high number of protons and chloride ions, no change in retention times and in chromatographic peak resolutions was observed.

The inorganic phosphate concentrations in the milk aqueous phases increased during acidification (fig 4). At the pH value of 6.73, the inorganic phosphate concentration was 930 mg/kg. Then, at the pH value of about 5, 1870 mg/kg of inorganic phosphate were determined and showed a supplementary solubilization of 50% of total inorganic phosphate. The increase in inorganic phosphate concentration in the aqueous phase was related to a protonation of inorganic phosphate and of acidic

groups of caseins (carboxyls, phosphates of phosphoseryl residues) and consequently corresponded to a direct solubilization of MCP. At the pH value of 3.94, electrostatic binding of inorganic phosphate to caseins is possible because at this pH value, caseins are positively charged and can bind negative ions such as phosphate. Thus, to determine the concentration of total inorganic phosphate, it was necessary to carry out ultrafiltration of acidified milk at a pH value close to 5. Moreover, at this pH value, the ultrafiltration was easier than at other pH values because caseins are precipitated.

Fig 4. Effect of acidification on the anion concentrations: chloride (■), phosphate (✚) and citrate (*), in the aqueous phase of milk. A range of pH values between 6.73 and 3.93 was obtained by HCl addition. These ion concentrations were determined by ion chromatography. The chromatographic conditions are described in the legends to figure 1 and of table I.

Effet de l'acidification sur la concentration des anions chlorure (■), phosphate (✚) et citrate () contenue dans la phase aqueuse de lait. Une gamme de pH entre 6,73 et 3,93 a été obtenue par addition d'HCl. Les concentrations des ions ont été déterminées par chromatographie ionique. Les conditions chromatographiques complètes sont décrites dans les légendes de la figure 1 et du tableau I.*

At the same time, in the pH range 6.73–3.93, the citrate concentration increased slightly. At the pH value of 6.73, the citrate concentration was 1490 mg/kg (100%). At the pH value of 4.71, 1650 mg/kg of citrate were present in the aqueous phase of milk. This value corresponded to a 10% solubilization. The significant decrease in the citrate concentration between pH 4.41 (1650 mg/kg) and pH 3.93 (1590 mg/kg) probably corresponded to the binding of negatively charged citrate molecules to positive charges of caseins. Thus, to determine the concentration of total citrate, it was necessary to carry out ultrafiltration of acidified milk at a pH value close to 5.

The high and linear increase in chloride concentration (from 1052 to 3270 mg/kg) corresponded to the HCl addition necessary for the acidification.

These results obtained by ion chromatography were in accordance with those obtained by conventional methods used in this work (results not shown) and agreed well with the literature (Walstra and Jenness, 1984; van Hooydonk et al, 1986; Dalgleish and Law, 1988, 1989; Le Graet and Brulé, 1993).

Increase of ionic strength of milk

Anionic species in aqueous phase NaCl added milk were determined by ion chromatography (fig 5). For all samples and in spite of the high concentration of sodium and chloride ions, no change in retention times and in chromatographic peak resolutions was observed.

The inorganic phosphate concentrations of the aqueous phase were slightly increased (from 930 to 1000 mg/kg) after NaCl additions (up to 2%). At the same time, increases of soluble calcium concentrations were described and may be due to an exchange of sodium for calcium which was attached directly to the phosphoseryl residues of caseins (Brulé et al, 1974; Grufferty and Fox, 1985; Le Graet and Brulé, 1993). These slight increases in in-

organic phosphate concentrations were probably due to slight pH decreases which occurred after additions of NaCl. pH values were 6.73, 6.68, 6.66 and 6.62 for NaCl addition of 0, 0.5, 1 and 2% (w/w) respectively.

The citrate concentration of the aqueous phase was not affected by the addition of NaCl.

The high and linear increase of chloride concentration (from 1060 to 12 830 mg/kg) corresponds to the NaCl addition (0, 0.5, 1 and 2%, w/w).

These results obtained by ion chromatography were in accordance with those

obtained by conventional methods used in this work. Moreover, these results were similar to those obtained by other authors (Grufferty and Fox, 1985; Le Graet and Brulé, 1993).

Anion determination in milk

To determine the total phosphate (inorganic + organic) concentration by ion chromatography, a chromatographic injection of milk without prior preparation of sample was not suitable. So, one solution was to carry out a dry ashing of the milk, as carried out with the ultrafiltrates, before analysis by ion chromatography. Results obtained with this method (2770 mg/kg) indicated that it was possible to prepare sample in this way because the total phosphate concentration thus obtained was similar to those obtained by the FIL method (1987) (2720 mg/kg). The chloride concentration of 1060 mg/kg was the same as those found in ultrafiltrate. The sulfate concentration was 390 mg/kg (against 140 and 200 mg/kg in an ultrafiltrate and in ashes of ultrafiltrate respectively). These differences were probably related to a transformation of sulphur compounds into sulfate ions during dry ashing. However, as observed in the case of dry mineralization of milk ultrafiltrate, no chromatographic peak of citrate was detected because it was destroyed during the dry mineralization.

Fig 5. Effect of ionic strength on the anion concentrations (chloride (■), phosphate (◆) and citrate (✱)) in the aqueous phase of milk. A range of added NaCl between 0 to 2% (w/w) was added. The ionic concentrations were determined by ion chromatography. The complete chromatographic conditions are described in the legends to figure 1 and table I.

Effet de la force ionique sur les concentrations des anions de la phase aqueuse de lait chlorure (■), phosphate (◆) et citrate (✱). Une gamme de NaCl allant de 0 à 2% (p/p) a été ajoutée au lait. Les concentrations des ions ont été déterminées par chromatographie ionique. Les conditions chromatographiques complètes sont décrites dans les légendes de la figure 1 et du tableau I.

CONCLUSION

Several methods have been developed for determination of anions of milk (Marsili et al, 1981; Pierre and Brulé, 1983; Walstra and Jenness, 1984; Visser et al, 1986; FIL, 1987, 1988a, 1988b, 1992a; Herrero et al, 1992; Schmitt et al, 1993). In this study, ion chromatography in combination with an adequate sample preparation (table III) has been successfully used for the qualitative and quantitative determinations of anions (chloride, phosphate and citrate) of milk.

Table III. Sample preparations before ion chromatography of milk anions.
Préparations d'échantillon avant chromatographie ionique des anions du lait.

<i>Anion determinations</i>	<i>Sample preparations</i>
Chloride, inorganic phosphate ^a , and citrate ^a concentrations in the aqueous phase	Ultrafiltration of milk
Chloride and total (inorganic and organic) phosphate concentrations in the aqueous phase	Dry mineralization of milk ultrafiltrate ^b
Chloride and total (inorganic and organic) phosphate concentrations of milk	Dry mineralization of milk sample ^b

^a The concentrations of total inorganic phosphate and of total citrate can be determined after ultrafiltration of acidified milk at a pH value close to 5. ^b Citrate determination can not be determined.

^a Les concentrations du phosphate inorganique total et du citrate total peuvent être déterminées après ultrafiltration de lait acidifié à une valeur de pH d'environ 5. ^b La concentration de citrate ne peut être déterminée.

Also, beside rapid quantification of these ions, ion chromatography provides a powerful method for analysing the mineral transformations undergone by milk during technological treatments such as acidification or increase in ionic strength. Even after 4000 analyses, no change in retention times or peak resolutions was observed.

One of the most obvious advantages of this technique is that multiple elements can be determined in one sample with no serial dilutions and the complete analysis can be performed by using only one instrument. The total time for each analysis was less than 32 min per sample.

Moreover, with the auto-sampling device, about 45 samples can be analysed in a single day. Conventional methods such as potentiometric, colorimetric and enzymatic methods do not have these advantages. This method can be used for analysis in control laboratories in the dairy industry and in other food and drink sectors as described by Weiss (1995).

The potential of this method for quantitative applications appears to be extraordinary because it seems possible to characterise:

- 1) the mineral composition of different dairy products (milks, caseinates, whey products, purified milk proteins, yoghurts, cheeses);
- 2) the concentrations of the main anions in the aqueous phase of milk during technological processes such as thermal treatments, acidification, membrane separation;
- 3) the progress of biochemical reactions by microorganisms during cheese ripening by measurement of the organic acid contents such as lactate, acetate, propionate, butyrate and succinate. However, further work is necessary to improve the chromatographic resolution of lactate, acetate, propionate and butyrate peaks.

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