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Effect of temperature on the salt balance of milk studied by capillary ion electrophoresis

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ABSTRACT

Many inorganic species, such as calcium, phosphate and magnesium, are in equilibrium between the liquid and colloidal phases of milk and hence are of importance with respect to the coagulation properties of milk. Capillary ion electrophoresis makes possible the determination of anions and cations in less than 6 min. The soluble phase of milk was obtained by ultrafiltration and samples had to be diluted 250-fold before analysis. Cold storage increased soluble calcium and phosphate concentrations, and warm-up of the milk restored the initial ionic equilibria. More drastic heat treatments (80–90°C) caused precipitation of tricalcium phosphate and calcium citrate.

INTRODUCTION

The saline components of milk (5–9.5 g/l) consist of many different species which are present as ions, salts or undissociated complexes [1]. The inorganic species are mainly calcium, potassium, magnesium, sodium, chloride and phosphate; citrate is the most important of the organic anions.

Many components, such as chloride, sodium and potassium, are completely soluble whereas others, such as calcium, magnesium, inorganic phosphorus and citrate, are in equilibrium between the liquid and colloidal phases. They take part in the formation of casein micelles and hence can be considered as part of the proteinic structure of milk. Studies on the distribution of mineral species between the liquid and colloidal phases have already been published [2]. Physico-chemical parameters (ionic strength, pH and temperature) influence the equilibrium of milk [3,4].

The saline equilibrium has a direct impact on the coagulation aptitude and cheese-making from milk. For this reason, it is important to study and to control the concentrations of these ions. The dairy industries needs to know the saline equilibria of milk in order to determine the optimum time for starting the production of cheese.

Conventional methods of ion analysis are not convenient; they suffer from many limitations and are materials and/or time consuming. Moreover, milks are now standardized in proteins, calcium and fats.

The recently introduced technique of capillary ion electrophoresis (CIE; Waters trade name Capillary Ion Analysis, CIA) is advantageous for ion analyses. Speed of analysis, reliability and the low cost of use of this method are especially attractive. It allows improvements in the determination of inorganic ions.

The development and evaluation of an effective sample preparation procedure are the first step in the study of the effect of temperature on milk equilibrium, and were the main aim of this work.

EXPERIMENTAL

Materials and reagents

The CIE system used was a Waters–Millipore (Milford, MA, USA) Quanta 4000 with a fused-silica capillary (60 cm × 75 μm) I.D.

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A Model M14011 centrifuge was obtained from Jouan (St. Herblin, France). Ultrafiltration was performed with Millipore (Milford, MA USA) Ultrafee-MC filters with a molecular mass cut-off (MMCO) of 10 000.

CIA-PAK UV Cat-1 reagent and OFM Anion-BT were obtained from Waters-Millipore and sodium chromate tetrahydrate from Aldrich (Milwaukee, WI, USA).

Milk samples

Milk was obtained from a farm near Nancy (France) from the morning milking of a Holstein breed herd. Samples were stored in a cold room at 4°C and heated quickly to the appropriate temperature when required.

Sample preparation

The soluble phase was obtained as follows: 0.5 ml of raw milk was placed in an Ultrafree-MC filter with an MMCO of 10 000 and centrifuged at 3000 *g* for 15 min. The ultrafiltrates obtained were diluted 250-fold with 18-MΩ water.

Ion determination

Ion determinations were performed according to Jandik *et al.* [5]. Injection was hydrostatic (injection volume 10 nl for 30 s) for both anions and cations. For cations, indirect UV detection at 214 nm was used, the electrolyte was 5 m*M* UV-Cat-1–6.5 m*M* α-hydroxyisobutyric acid (HIBA) (a metal ion complexant which improves the separation of cations) (pH 4.4) and the applied voltage was 20 kV. For anions, indirect UV detection at 254 nm was used, the electrolyte was chromate containing OFM Anion-BT and the applied voltage was 20 kV.

RESULTS AND DISCUSSION

Evaluation of the technique

The sample preparation method was optimized to make it simple, rapid and reliable. Three different methods to obtain the soluble phase of milk were tested: dialysis, ultracentrifugation and ultrafiltration [2]. We selected ultrafiltration as it is faster and requires only a centrifuge.

Several commercial filtration systems were tested (Sartorius Centrisart I with MMCO 5000 and 10 000, Millipore Ultrafree-MC with MMCO 5000 and 10 000 and Filtron Microsep with MMCO 3000 and 5000). Ease of use, cost and repeatability of results were the criteria for selection. The Millipore Ultrafree-MC with MMCO 10 000 appeared to be the best compromise for our purposes.

Cations in the ultrafiltrates obtained by centrifugation at different speeds (1000 and 3000 *g*) and for various times (5, 7, 10 and 15 min) were determined by CIE (results not presented). The quality of the ultrafiltrate was not affected by time or speed; its composition remains constant (for a concentration factor lower than fivefold) according to the work of Brulé *et al.* [6]. A sufficient volume of ultrafiltrate (50 μl) was obtained by centrifugation at 3000 *g* for 15 min. Before injection, a 250-fold dilution with 18-mΩ water was made for both anions and cations.

CIE results for calcium (nine samples) were confirmed by atomic absorption spectrometry. The correlation between the two techniques was excellent ($y = -0.1077 + 0.9761x$ with $r = 0.99$; where y is the calcium concentration given by AAS and x by CIE).

Complete sample preparation requires about 20 min. The determination of cations analysis is not affected by anions, and *vice versa.* Moreover, a complete analysis for anions or cations is achieved with just one injection. Conventional methods of ion analysis do not have this advantage.

The electropherograms of milk ultrafiltrates show peaks for potassium, sodium, calcium and magnesium for cations and chloride, sulphate, phosphate and citrate for anions (Fig. 1). Both were obtained in less than 6 min. The flexibility, speed of analysis and efficiency of CIA make it the best method for the determination of ions in milk.

A repeatability study of the CIE method gave relative standard deviations (R.S.D.s) of 5.2%, 6% and 6.9% for sodium, calcium and magnesium respectively. The relatively high values could be improved by a larger dilution of the samples.

The 250-fold dilution leads to slight asymmetry of the peaks owing to an overloading of the capillary. This asymmetry results in a decrease in precision. R.S.D.s better than 3% can be obtained with a larger dilution (results not presented). Concerning anions, the R.S.D.s are good for chlorides (2.7%), owing to the good symmetry of the peak, but the determination of citrate (R.S.D. = 5.7%) and phosphate (R.S.D. = 4.5%) is less precise owing to

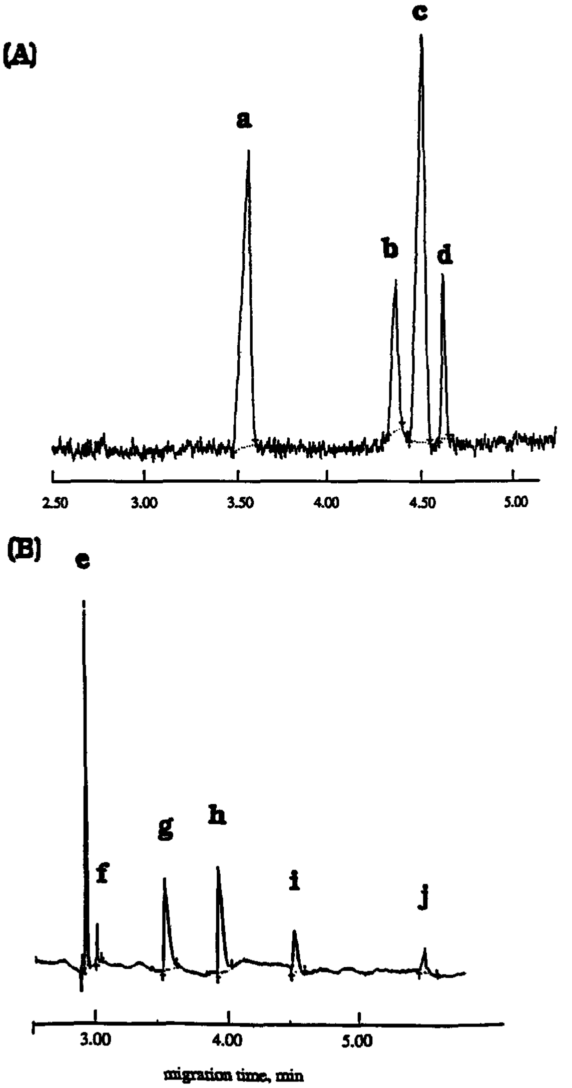

Fig. 1. CIE of (A) cations and (B) anions in the soluble phase of milk. The ultrafiltrate was diluted 250-fold with 18-mΩ water. The applied voltage was 20 kV. Peaks: a = potassium; b = calcium; c = sodium; d = magnesium; e = chloride; f = sulphate; g = citrate; h = phosphate; i = carbonate; j = lactate.

the high mobility of the electrolyte and the too large dilution. The use of an electrolyte optimized for phosphate and citrate (*i.e.*, *p*-hydroxybenzoate) would certainly improve the precision.

The optimum dilution was found to be 1:500 for cations and 1:1000 for anions. Sufficient precision was obtained with a common dilution of 1:250 for both anions and cations, hence we chose this simplified sample preparation procedure.

Effect of temperature on ionic equilibria

Among the different factors having an influence on the saline equilibria of milk, temperature is certainly the most important. The cooling of milk at the farm and storage at 2–4°C for 48–72 h are common practice in the dairy industry.

Inorganic species in raw milk cooled and stored at 4°C for several days were determined by CIE (Fig. 2). The soluble calcium concentration increased during the first day of cooling and remained constant thereafter. This demonstrates, in accordance with the works of Brulé and Fauquant [7], that part of the colloidal calcium migrates to the liquid phase. We have observed the same effect for phosphate, while sodium and chloride are not affected

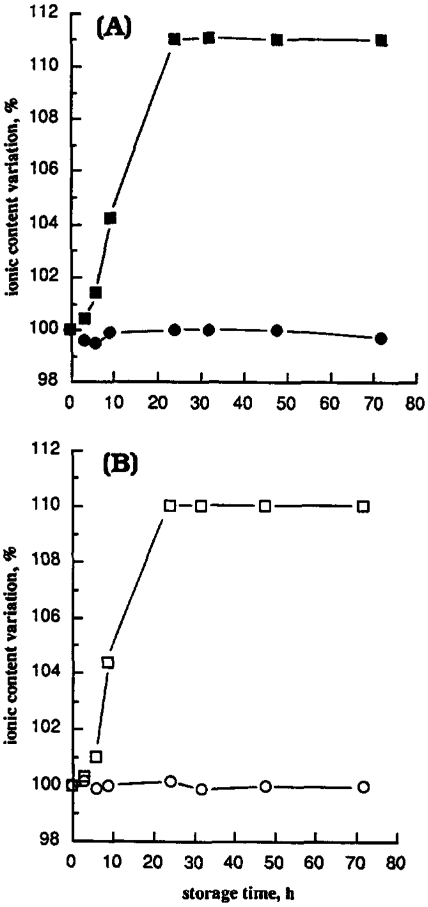

Fig. 2. Effect of cooling of milk on the concentrations of (A) (■) calcium and (●) sodium and (B) (□) phosphate and (○) chloride in the soluble phase of milk.

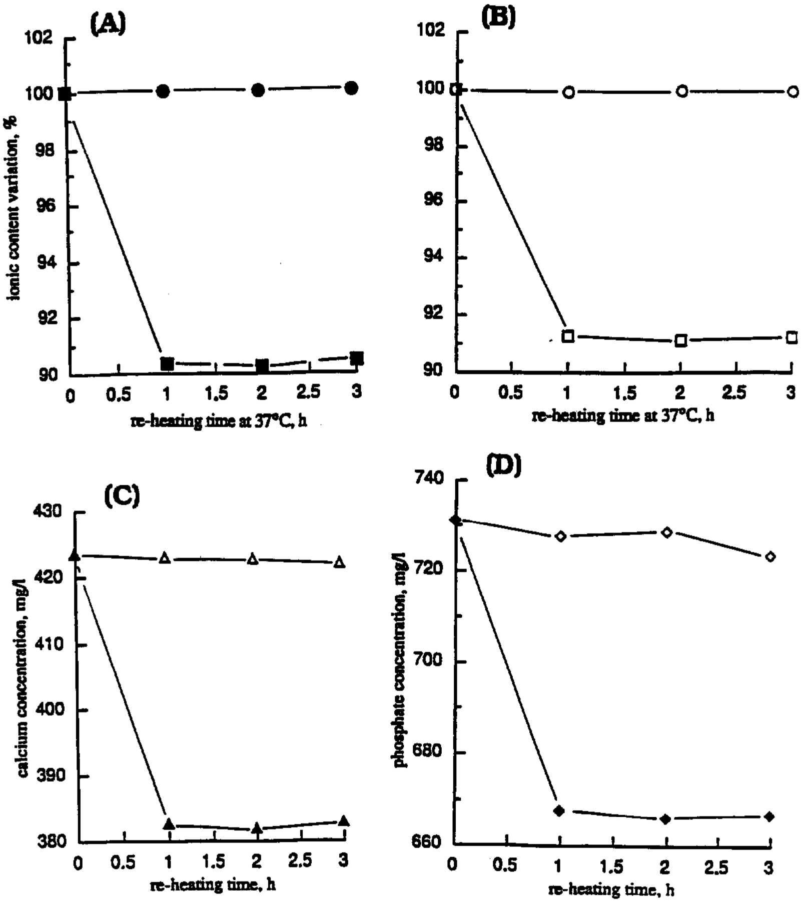

Fig. 3. (A,B) Influence of warming of milk after cold storage on the minerals in the soluble phase: ■ = calcium; ● = sodium; □ = phosphate; ○ = chloride. (C,D) Influence of warming temperature on the concentrations of (C) soluble calcium and (D) soluble phosphate. Measurements were performed at (△,◇) and (▲,◆) 37°C.

by cooling. These results are in good agreement with those of other workers [8–10]. The concentration of calcium phosphate in the liquid phase was increased by cooling [11], which induced a decrease in the mineral charge of the casein micelles.

After several days of cold storage, the milk must be re-equilibrated to allow processing; the removal of the soluble phase modifies the rennet coagulation properties of milk [12,13].

Cooled samples were warmed to 20 or 37°C (Fig. 3). Treatment at 20°C for 3 h did not restore the initial ionic equilibria. At this temperature, re-equilibration takes a very long time [2,9]. At 37°C, the soluble calcium content decreased quickly during the first hour, nearly reaching its initial concentration in raw milk. The same results could be obtained for soluble phosphate, according to other workers [3,4].

These results demonstrate the reversibility of the ionic equilibria and the need for a re-equilibration time before the beginning of cheese manufacture.

More drastic heat treatments were also evaluated. Cooled milk was warmed at 50, 60 or 70°C for 2 min (Table I). This treatment restored the concentration of soluble calcium and phosphate to the levels observed in fresh milk before cooling. Hence the re-equilibration could be performed in 1 hour at 37°C or in 2 min at 60°C.

TABLE I

EFFECT OF HEATING MILK AT SEVERAL TEMPERATURES ON THE CONCENTRATIONS OF SOLUBLE PHOSPHATE AND CALCIUM.

Control: milk stored at 4°C (= 100%) Conditions: applied voltage, 20 kV; injection, 10 nl. For phosphate: electrolyte, chromate with OFM Anion-BT; indirect UV detection at 254 nm. For calcium: electrolyte, 5 m*M* UV Cat-1–6.5 m*M* HIBA (pH 4.4); indirect UV detection at 214 nm.

Ion	Thermal treatment			
	Control	50°C, 2 min	60°C, 2 min	70°C, 2 min
Soluble phosphate	100	91.0	89.6	89.3
Soluble calcium	100	88.7	86	86.2

TABLE III

EFFECT OF INDUSTRIAL UHT TREATMENT ON THE SOLUBLE PHASE OF MILK

Control: raw sample (= 100%). Conditions: applied voltage, 20 kV; injection, 10 nl. For calcium: electrolyte, 5 mM UV Cat-1–6.5 mM HIBA (pH 4.4); indirect UV detection at 214 nm. For anions: electrolyte, chromate with OFM Anion-BT; indirect UV detection at 254 nm.

Ion	Thermal treatment		
	Raw	Pasteurized	Sterilized
Calcium	100	99	92.0
Phosphate	100	94.5	90.1
Citrate	100	97.9	89.7

Drastic heat treatments of milk are frequently used in the dairy industry. Thus, for the study of an industrial UHT treatment, several experiments on the laboratory scale were performed at 80 and 90°C (Table II). We observed an important decrease in soluble calcium and phosphate contents, whereas the contents of soluble sodium and chloride were unchanged. The former decrease was greater than that observed during the re-equilibration after cold storage. The soluble citrate also decreased, but less significantly. This can be explained by the fact that the solubility of calcium phosphate decreases when a drastic thermal treatment is used; the colloidal calcium phosphate reaches saturation and precipitates as tricalcium phosphate [1]. Precipitation of calcium citrate could be the reason for the decrease in soluble citrate [14].

This study was completed by collection of samples in an industrial UHT installation at three different stages: raw milk (stored at 4°C), milk after pasteurization at 88°C for 20 s and UHT milk (indirect sterilization for 2 s at 143°C) (Table III). As expected, the soluble calcium, phosphate and citrate contents decreased slowly after pasteurization, and slightly more after sterilization, but the decrease was smaller than expected. The short dura-

TABLE II

EFFECT OF THERMAL TREATMENT ON IONS IN THE SOLUBLE PHASE OF MILK

Control: milk stored at 4°C (= 100%). Conditions: applied voltage, 20 kV; injection, 10 nl. For cations: electrolyte, 5 m*M* UV Cat-1–6.5 m*M* HIBA (pH 4.4); indirect UV detection at 214 nm. For anions: electrolyte, chromate with OFM Anion-BT; indirect UV detection at 254 nm.

Ion	Thermal treatment					
	Control	80°C, 2 min	90°C, 2 min	90°C, 5 min	90°C, 10 min	90°C, 15 min
Calcium	100	82.9	77.2	75.9	74.5	73.2
Sodium	100	100.5	100	100.8	100	100
Chloride	100	100	100	99.9	99.7	99.8
Phosphate	100	86.5	84.4	83.5	81.2	78.7
Citrate	100	94.8	92.6	90.7	89.8	88.3

tion of the thermal treatment could explain this. It appears that not only temperature, but also its duration, are of importance for the study of these ionic equilibria.

CONCLUSIONS

CIE is a very convenient technique for estabilishing the modifications of ionic equilibria in milk under the influence of temperature changes. Sample preparation is minimal and the run time is less than 6 min. CIE is a very powerful tool and can be used for analysis in control laboratories in the dairy industry and in other food and beverage industries.

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