Effect of Cationic Membrane Permselectivity on the Efficiency of Skim Milk Electroacidification

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Bipolar membrane electroacidification (BMEA) uses the property of bipolar membranes to split water and the demineralization action of cation-exchange membranes (CEM). As milk mineral salt content is very sensitive to ionic strength and pH changes, the aim of this study was to better understand the effect of changes in mineral content during pH decrease and demineralization of skim milk. The objectives were to investigate the effect of different cationic permselective membranes (CSV and CMX membranes) on skim milk cation migration and protein precipitation during BMEA. The permselectivity of both membranes tested does not influence the final efficiency of BMEA. The purity of the bovine milk casein isolates produced was similar to or higher (97−98% versus 93.4−96.7) than those of commercial isolates, due to a reduced ash content (1.2 versus 2.0−3.8%) resulting from the CEM demineralizing phenomenon. For both membranes, the main ionic species to migrate was the potassium ions.

Keywords: *Electrochemical acidification; bipolar membrane; casein; milk; cationic permselectivity*

INTRODUCTION

Two main types of casein are usually produced in the dairy industry: rennet and acid casein (Segalen, 1985; Southward, 1993; Varnam and Sutherland, 1994). In the case of rennet casein, the underlying mechanism for purification is identical to that of production of cheese curd and depends on the unique sensitivity of the $Phe_{105}-Met_{106}$ bond in κ-casein to hydrolysis by acid proteinases, the active components of rennet. For acid casein production, three main procedures exist, all based on isoelectric precipitation of casein using acidification by chemical, physicochemical, or fermentation means (Segalen, 1985; Southward, 1993; Varnam and Sutherland, 1994).

A procedure developed for soybean protein precipitation (Bazinet et al., 1996, 1997a,b, 1998a), which was derived from electrodialysis, was tested for the production of acid casein (Bazinet et al., 1999a). This technology, generically termed bipolar membrane electrodialysis or more specifically bipolar membrane electroacidification (BMEA), uses the property of bipolar membranes to split water and the action of cation-exchange membranes (CEM) to demineralize.

A typical CEM for electrodialysis has a pore size of 10−20 Å with a capacity of 1.6−3.0 mequiv/g of dry weight of resin. The demineralization action of cation-exchange or cationic membranes (CEM) is closely linked to its internal structure. CEMs are made of a macromolecular material (skeleton) consisting of a hydrophobic polymer that carries ionizable groups. In a CEM, fixed anions are electronically neutralized by mobile cations in the interstices of the polymer. Under the influence of an electric field, cations move from one site to another in the network of anionic functional groups fixed on the skeleton and cross the membrane. Ions with the same charges as the ionized groups fixed on the polymer are rejected from the membrane core, as a result of an electrostatic repulsion called Donnan exclusion (Bazinet et al., 1998b). The concentration of fixed anionic charges, as well as the degree of cross-linking of the CEM, determines the ion permselectivity. The degree of cross-linking determines the tightness of the matrix structure and thus its porosity or its pore size (Streat and Cloete, 1987). The CEM used in electrodialysis is generally a monolayer membrane. Some special three-layer CEMs have been developed to obtain a monovalent cation permselectivity. On each side of the CEM layer, electrolyte polymers with different electric charges are applied to the surface of the membrane. The monovalent cation permselectivity thus obtained is referred to as a filtering effect, because of its resemblance to the trapping of multivalent ions with a filter.

In milk, casein interacts with ions and salt, particularly calcium phosphate, to form voluminous micellar complexes with diameters varying from 20 to 600 nm (Cheftel et al., 1985; Schmidt, 1982). About 7% of casein micelle dry matter is constituted by inorganic material, mainly calcium and phosphate (Walstra, 1990). A total of 68% of the calcium present in milk (117 mg of calcium/100 g of milk) is associated with the micelle, that is to say, ∼31 mg/g of dry micelle. The micelle also contains about 0.26 g of potassium, 0.11 g of magnesium, and 0.11 g of sodium per 100 g of dry micelle

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(Schmidt, 1982). By adjusting the pH of milk to the isoelectric point of casein, the intra- and interprotein electrostatic attractions are increased, which affect the stability of the mineral phase of the micelle.

As the mineral salt content is very sensitive to ionic strength and pH changes, the aim of this study was to better understand the effect of changes in mineral content during the pH decrease and demineralization of skim milk. Our objectives were to investigate the effect of cationic membrane permselectivity on skim milk cation migration and protein precipitation during BMEA and more generally on the global efficiency of BMEA. Two cationic membranes, with different permselectivities, were compared in terms of electrodialysis cell parameters, percentage of proteins precipitated, ash content, mineral content, protein molecular profiles, and chemical composition of isolates produced.

MATERIALS AND METHODS

Material. The raw material used in this study was fresh homogenized skim milk (Quebon, Natrel, Longueil, PQ, Canada).

Methods. *(a) Electroacidification Cell.* The module used was an MP type cell (100 cm^2 of effective electrode surface) from ElectroCell Systems AB Co. (model MP cell, Täby, Sweden). The cell consists of eight compartments separated by four cationic membranes and three Neosepta BP-1 bipolar membranes from Tokuyama Soda Ltd. (Tokyo, Japan). This arrangement defines three closed loops containing the milk solution, a 2 g/L aqueous KCl solution, and a 20 g/L Na_2SO_4 solution. Each closed loop was connected to a separate external reservoir, allowing for continuous recirculation (Bazinet et al., 1997b).

The anode/cathode voltage difference was supplied by a variable 0−100 V power source, Powerstat model 236BU-2 (The Superior Electric Co., Bristol, CO). The electrolytes were circulated using three centrifugal pumps (model XVB56C34F2012b-W, Marathon Electric, Wausau, WI), and the flow rate was controlled using a model FC-FI-C-3/8 flow meter (Filter-Chem, Alhambra, CA). The temperature of the electrolytes was maintained at 20 °C by circulating water inside a stainless steel coil immersed in each of the reservoirs. The anode, a dimensionally stable electrode (DSA), and the cathode, a 316 stainless steel electrode, were supplied with the MP cell.

(b) Protocol. Electroacidification was carried out in batch process using a current of 2.0 A, and once a voltage of 60 V had been reached, it was maintained constant so as not to surpass the total power of the power supply. Electrolyte volumes of 4 L were used for the Na_2SO_4 and KCl solutions, whereas a 1.5 L volume was used for the milk solution. The electroacidification was stopped after the pH reached 4.2. The initial pH varied between 6.5 and 6.7.

Two membranes were tested during electroacidification: the Neosepta CMX membrane from Tokuyama Soda Ltd., which is permeable to monovalent and divalent cations, and the CSV membrane from Asahi Glass Co. Ltd. (Tokyo, Japan), which is permeable to monovalent cations only. Three replicates of each condition were performed in this experiment.

During each treatment, 1.5 and 30 mL samples of the milk solution were taken, respectively, every 0.4 and 0.8 pH unit decrease from initial pH (around 6.6) to pH 4.2. In this experiment, milk was diluted (1.1 L of milk and 0.4 L of distilled water) to minimize the risk of spacer fouling. The time required to reach pH 4.2, the system resistance, the conductivity, and the temperature were recorded as the treatment progressed. On freshly acidified 1.5 mL samples, the concentration of soluble protein was determined. The 30 mL samples were stored at −20 °C before ash content, sodium, potassium, and calcium concentrations, and protein profiles were performed. At the end of each run, 500 mL samples of the pH 4.2 milk solution were taken. These samples were centrifuged for 10 min at 4 °C, at 500*g* (centrifuge model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA), and the precipitate was washed twice with distilled water before being lyophilized for 24 h at room temperature (model Freezone 4.5, Labconco, Kansas City, MO). The lyophilized isolates were stored at 4 °C before total protein determination, protein profiles, and ash content were performed.

(c) Analysis Methods. (1) pH Measurement. The pH of the protein solution was measured with a pH meter model Φ11 (Beckman Instruments Inc., Fullerton, CA).

(2) System Resistance. The system resistance was calculated, using Ohm's law, from the voltage and the current intensity read directly from the indicators on the power supply.

(3) Conductivity. A YSI conductivity meter model 35 was used with a YSI immersion probe model 3418, cell constant $K = 0.1\ cm^{-1}$ (Yellow Springs Instrument Co., Yellow Springs, OH) to measure the conductivity of the protein solutions.

(4) Moisture. Moisture was measured according to AOAC Method 927-05 (AOAC International, 1995a).

(5) Ash Content. Ash content was determined according to AOAC Methods 930-30 and 945-46 (AOAC International, 1995b,c).

(6) Potassium, Sodium, and Calcium Concentration Determination. Sodium and potassium concentrations were determined by flame emission spectrophotometry (method 990-23, AOAC International, 1995d), whereas calcium concentration was measured by an atomic absorption spectrophotometric method (method 991-25, AOAC International, 1995e) with a Varian SpectrAA-100 (Malgruve, VIC, Australia). The ion concentration was measured on an ashed sample diluted in 20 mL of 2 N HCl. A specific hollow cathode lamp (model 3UNX-Ca, Cathodeon Limited, Cambridge, U.K.) at 422.7 nm wavelength was used to determine calcium concentration in the solution. For flame emission, sodium and potassium concentrations were measured at 589 and 766.5 nm wavelengths, respectively.

(7) Soluble Protein and Total Protein Determination. The protein concentration was determined using an FP-428 LECO apparatus (LECO Corp., St. Joseph, MI) following the same conditions and parameters used by Bazinet et al. (1999a).

(8) Protein Profiles. The chromatographic analysis of the molecular profile of the lyophilized protein isolate and skim milk samples was performed by reverse-phase HPLC according to the method of Jaubert and Martin (1992), in the conditions used by Bazinet et al. (1999a).

(d) Statistical Analyses. The time required for pH decrease measures of conductivity and system resistance as a function of pH were subjected to an analysis of variance using SAS software (SAS, 1989), and linear regression was calculated using SigmaPlot (version 3.0 for Windows, Jandel Scientific, Corte Madera, CA). The concentration of soluble protein, the ash content, the concentration of Ca, Na, and K ions in ash, and the percentage of κ-, α_{s1}-, α_{s2}-, and β-caseins and whey protein in milk solution as the pH decreased were analyzed with a split-plot analysis of variance, since the Huynh−Feldt condition was met (Huynh and Feldt, 1970). Regression contrasts were calculated for each univariate analysis of variance, using SAS software, to examine the effect of interaction between the variables. Duncan tests were used to determine the significance of the difference between both membranes. The total protein, ash content, concentrations of Ca, Na, and K in ash, and composition in κ-, α_{s1}-, α_{s2}-, and β-caseins and whey protein in the isolates produced with different membranes by BMEA were subjected to an analysis of variance and as well as Duncan tests to determine the significance of difference between isolates.

RESULTS AND DISCUSSION

Electroacidification Parameters: Duration, System Resistance, and Conductivity. Results of the analysis of variance indicated that the pH has a highly significant effect on the duration of BMEA ($P < 0.0001$) and on the system resistance ($P < 0.0004$), and the dual

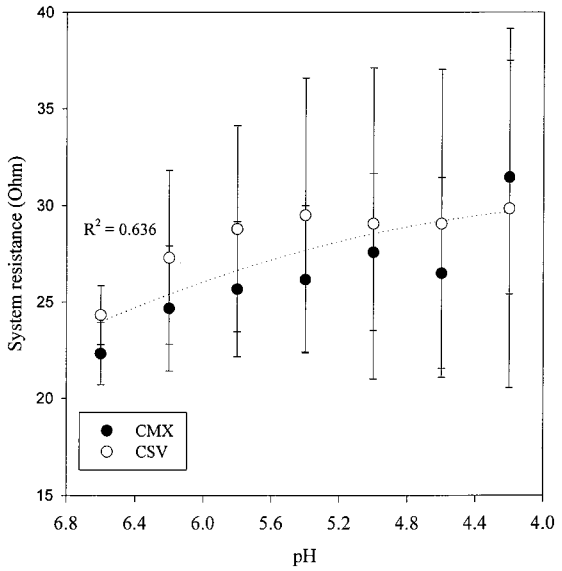

Figure 1. Effect of membrane permselectivity, CMX and CSV membranes, on the system resistance during bipolar membrane electroacidification of a skim milk solution run at 20 °C.

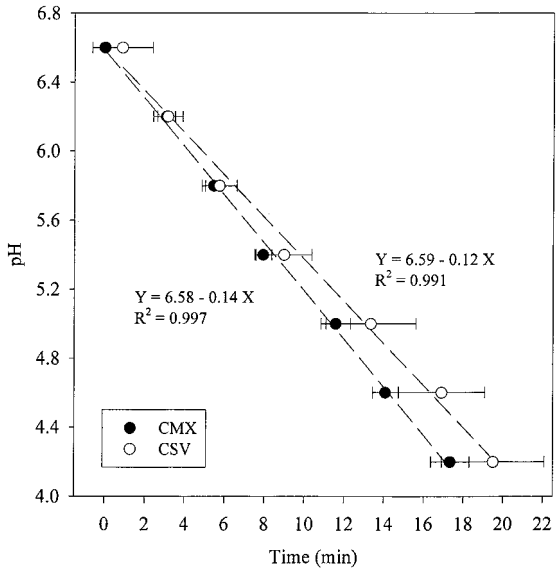

Figure 2. Effect of membrane permselectivity, CMX and CSV membranes, on the time required to decrease the pH by bipolar membrane electroacidification of a skim milk solution run at 20 °C.

interaction of the pH and the membrane has a significant effect on the duration ($P < 0.032$) of BMEA and on the conductivity ($P < 0.0009$) of the milk solution. The regression calculated for the variables as a function of pH produced coefficients of determination ranging between 0.636 and 0.997.

(a) System Resistance. The evolution of the system resistance is the same whatever the permselectivity of the membrane, and it is characterized by a slight increase (Figure 1). The system resistance increased from 24.3 to 29.8 Ω and from 22.3 to 31.5 Ω for the CSV and CMX membranes, respectively. This increase should be mainly due to a slight fouling of the spacers in the cell by the precipitated protein.

Indeed, as the mesh wire design of the spacer is fine, precipitated protein forms a slight deposit on the mesh, which increases the global system resistance. This result is in agreement with those Bazinet et al. (1998a) obtained on soybean protein.

(b) Duration. The time required to decrease the pH of a skim milk solution from 6.6 to 4.2 was 17.3 and 19.5 min for electroacidifications carried out with CMX and CSV membranes, respectively (Figure 2). This is confirmed by the calculated slopes of −0.12 and −0.14 for CSV and CMX membranes, respectively. The acidification of skim milk solution, during BMEA, whatever the membrane was carried out in a linear fashion, as confirmed by coefficients of determination of 0.991 and 0.997 calculated for CSV and CMX membranes, respectively.

However, the fact that the BMEA was not run at a constant current explains the difference observed in duration between the membranes. The CSV membrane is a cationic monovalent permselective membrane, and by its intrinsic selectivity, it slows the crossing of divalent cations. As the BMEA was not carried out at constant current, the impact of membrane selectivity was even higher; the CSV membrane increases the global resistance of the system by allowing mainly the migration of monovalent cations. This is confirmed by the different system resistance values discussed above (24.3 and 22.3 Ω for CSV and CMX membranes,

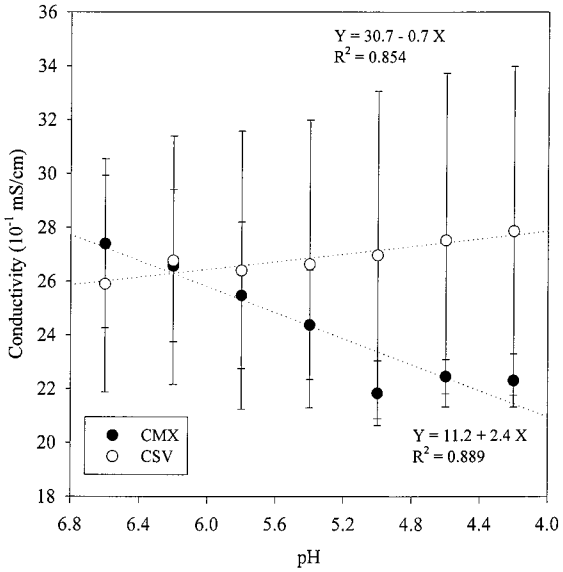

Figure 3. Effect of membrane permselectivity, CMX and CSV membranes, on the conductivity of the skim milk solution during bipolar membrane electroacidification run at 20 °C.

respectively). Due to a higher resistance of the system, and at a constant voltage, the current intensity is lowered with CSV stacking and, consequently, the number of H^+ electrogenerated is lowered. As the duration is directly linked to the quantity of H^+ electrogenerated (Bazinet et al., 1998a, 1999b), the duration of BMEA with CSV membrane is longer.

(c) Conductivity. Results of the statistical analysis indicate that the evolution of the conductivity as pH decreases is different for both membranes (Figure 3). The conductivity changes differently during BMEA according to the membrane used: for the CSV membrane, the conductivity was characterized by a slight increase (or stabilization) from 2.6 to 2.7 mS/cm, whereas for the CMX membrane a decrease in conductivity was noted from 2.7 to 2.2 mS/cm. A decrease in conductivity obtained with the CMX membrane is in accordance with previous results obtained by Bazinet et al. (1999a). This

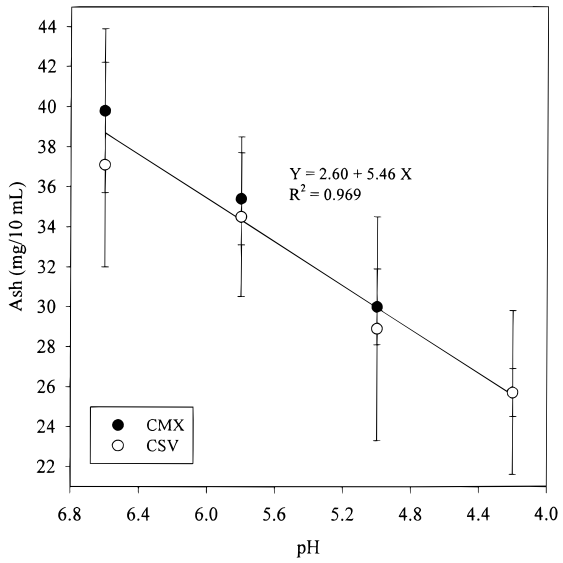

Figure 4. Effect of membrane permselectivity, CMX and CSV membranes, on the evolution of the ash content in the skim milk solution during bipolar membrane electroacidification run at 20 °C.

demonstrates that the BMEA was carried out under excellent conductivity conditions and that conductivity at a level of 2.2−2.7 mS/cm does not appear to be a limiting factor in BMEA of skim milk solutions.

Ash Content. The analysis of variance of the data shows that the pH ($P < 0.0001$) has a highly significant effect on ash content. The regression calculated for the ash content as a function of pH produced a coefficient of determination of 0.969.

As the pH decreased, the ash content (milligrams per 10 mL) also decreased for both membranes tested (Figure 4). Thus, the average initial ash content was ~38.4 mg/10 mL at pH 6.6 and decreased linearly to 34.9, 29.4, and 25.7 at pH 5.8, 5.0, and 4.2, respectively, a de-ashing rate of 33.1%. This result agrees with the data in the literature (Bazinet et al., 1997a, 1998a).

Bazinet et al. (1998a, 1999b) observed that the quantity of H^+ necessary for electroprecipitation is related to the concentration of protein present in the solution. As the protein content was the same for both membranes, the same number of H^+ to be electrogenerated is consequently necessary. Hence, to obtain electrical neutrality of the skim milk solution, one cationic charge must cross the cationic membrane for each H^+ produced at the BPM interface. This decreases the quantity of minerals, and therefore the overall ash content, by desalinization of the protein compartment (Bazinet et al., 1997b; Houldsworth, 1980; Chaput, 1979).

Concentrations of Calcium, Sodium, and Potassium. The analyses of variance of the data show that the pH has a significant effect on calcium ($P < 0.0001$) and potassium ($P < 0.0001$) concentrations, as well as the dual interaction of pH and type of membrane on calcium ($P < 0.0482$) concentration.

As the BMEA proceeded, the concentrations of potassium, sodium, and calcium decreased as a function of pH but in different ways according to the membrane permselectivity (Figure 5). The potassium ion concentration decreased very rapidly, from 579.8 to 308.0 mg/L (−46.8% variation) from pH 6.6 to 4.2, respectively. The sodium ion decreased ($P = 0.0598$) very slowly from 360.5 to 300.2 mg/L (−16.7% variation) from the beginning to the end of the BMEA run. The evolution of the calcium concentration was different according to the permselectivity of the membrane: with the CMX membrane, the calcium concentration in the milk solution decreased from 779.7 to 675.6 mg/L (−13.3% variation) from pH 6.6 to 4.2, respectively, whereas with the CSV membrane, the decrease was from 766.1 to 569.8 mg/L (−25.6% variation). Therefore, the calcium ion seems to migrate in a higher quantity with the CSV membrane than with the CMX membrane.

Furthermore, from pH 5.0 to 4.2, according to the membrane permselectivity, the three ions have a different evolution: the sodium migrates in a linear fashion during all of the BMEA treatments with the CSV membrane, whereas with the CMX membrane, its concentration drops drastically in this particular interval. In the same way, the calcium migrates linearly during BMEA with CMX membranes, whereas with CSV membranes its concentration dropped. Contrary to both previous ions, the migration of potassium seems to be reduced between pH 5.0 and 4.2: with the CSV and CMX membranes the concentration of potassium was stable; there was no change to the rate of migration of potassium ions. The significant decreases noted for the calcium and sodium ions for the CSV and CMX membranes, respectively, seem to compensate for the reduced migration rate of the potassium ions. In addition, calcium, a divalent ion, migrated across the CSV membrane commercially sold as a monovalent permselective membrane.

These results confirm previous results indicating that monovalent ions seem to be much more mobile than divalent salts and that they can be easily removed from milk. Lower mobilities of the divalent ions and their abilities to form complexes with proteins would be involved (Young, 1974; Hiraoka et al., 1979; Higgins and Short, 1980; Perez et al., 1994).

However, in this experiment, sodium migrated more slowly than calcium. The migration rates of the different ions, in skim milk, are different according to the de-ashing rate and their respective absolute conductivity and mobility values of 73.5 S·cm^2/mol and 7.6 cm^2/s·V for potassium, 50.1 S·cm^2/mol and 5.2 cm^2/s·V for sodium, and 119.0 S·cm^2/mol and 6.2 cm^2/s·V for calcium (Hiraoka et al., 1979; Brett and Oliveira-Brett, 1994). According to these conductivity and mobility values, calcium has the higher conductivity but a medium mobility due to its structure, the potassium has a medium conductivity and the higher mobility, whereas the sodium has the lower conductivity and mobility. Moreover, the mobility of calcium can be changed by the formation of a complex with protein. Hiraoka et al. (1979) studied the evolution of the sodium, potassium, and calcium concentrations during demineralization of skim milk by electrodialysis. Potassium, sodium, and calcium were shown to migrate at different rates from 0 to 90% de-ashing rates. All of the sodium and potassium were removed, whereas about one-fourth of calcium was not yet removed. At low de-ashing rates, from 0 to ~30%, sodium and calcium de-ashing kinetics are inverted. Therefore, our experiment was carried out in the de-ashing rate range where the sodium and calcium kinetics are inverted because the de-ashing rate calculated from ash data was ~33%.

Soluble Protein. The analysis of variance of the data shows that the pH ($P < 0.0001$) has a significant effect on the percent soluble protein. A nonlinear regression

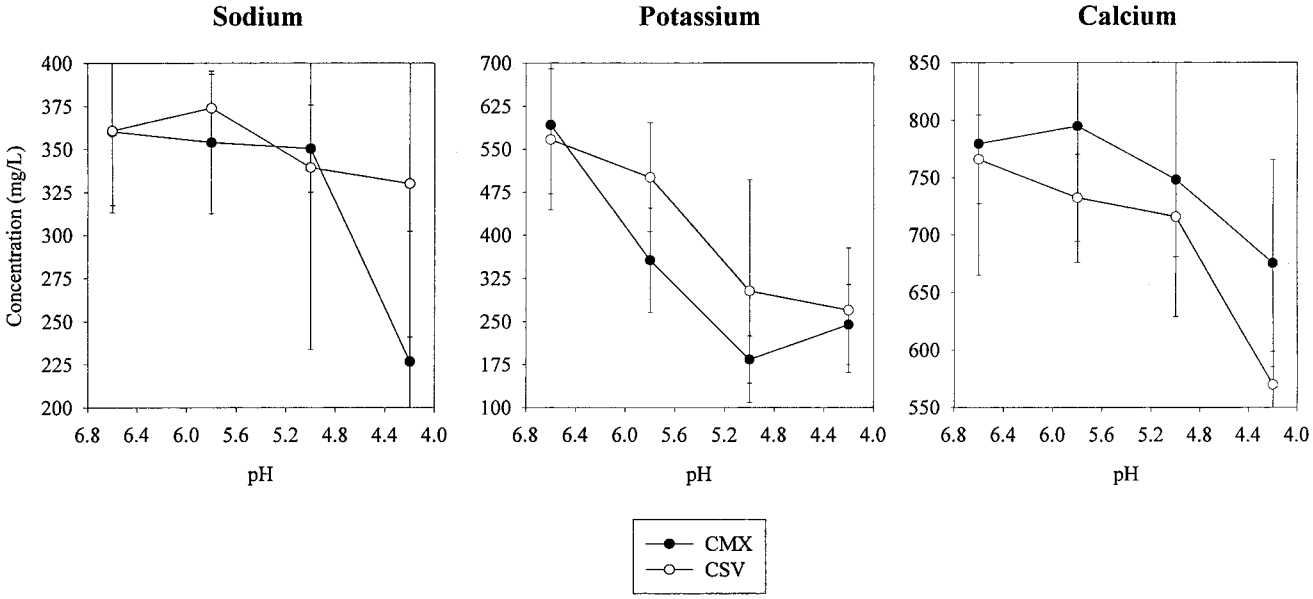

Figure 5. Effect of membrane permselectivity, CMX and CSV membranes, on the evolution of the sodium, potassium, and calcium concentrations in the skim milk solution during bipolar membrane electroacidification run at 20 °C.

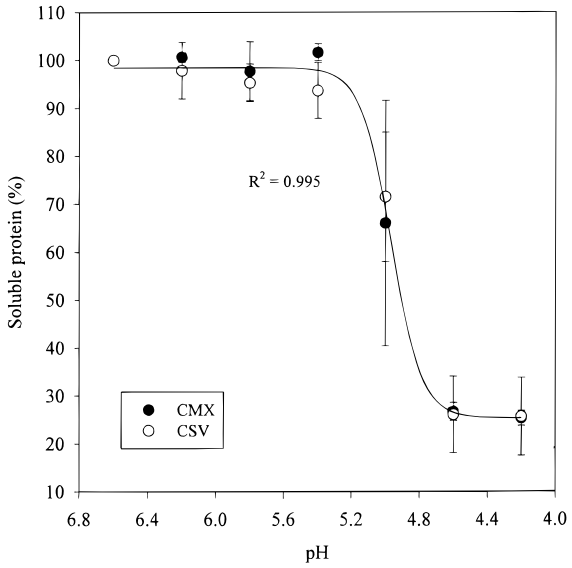

Figure 6. Effect of membrane permselectivity, CMX and CSV membranes, on the evolution of the percent soluble protein in the skim milk solution during bipolar membrane electroacidification run at 20 °C.

model of the percent soluble protein as a function of pH (all membrane types averaged) was calculated and produced a good coefficient of determination (R^2 = 0.995).

The membrane permselectivity had no effect on the final percent soluble protein, which decreased from ~100% to 25.3−25.6% soluble protein. Soluble protein decrease during the pH decrease from 6.6 to 4.2 was the same (Figure 6) and could be modeled as a sigmoidal curve. From pH 6.6 to 5.4, soluble protein was unchanged. When the pH dropped to 5.0, the percent soluble protein decreased to ~69%. As the BMEA continued from pH 4.6 to 4.2, the percent soluble protein was constant at ~25%.

The difference of cationic permselectivity of the membrane does not appear to have an effect on the percent soluble protein during BMEA of skim milk. The percent soluble protein of 25% obtained at the end of the run is in accordance with the literature (Brunner, 1981; Swaisgood, 1982; Cheftel et al., 1985; Lorient, 1991). In this experiment, the percent soluble protein obtained at pH 5.0 is higher than that obtained in the previous experiment. In this experiment the skim milk was diluted with water (1.1 L of skim milk and 0.4 L of water). This dilution, in addition to the dead volume of the electrodialytic system (tubing, reservoir, flow meter, and pump), has decreased the ionic strength and the protein concentration of milk, which are important factors in protein precipitation (Cheftel et al., 1985; Kinsella et al., 1985). Bazinet et al. (1997a, 1998a) have demonstrated on soybean protein that the protein−solvent interactions are increased as is the solubility of the protein when ionic strength and protein concentration are lowered.

Table 1. Supernatant Composition in Each Protein Fraction during BMEA[a]

pH	κ-casein	α_{s2}-casein	α_{s1}-casein	β-casein	whey protein (α-la and β-lg)
6.6	12.0 a	6.3 a	32.6 a	35.7 a	13.4 a
5.8	12.3 a	4.6 b	28.9 ab	30.8 ab	13.5 a
5.0	8.5 b	4.3 b	25.9 b	27.9 b	13.6 a
4.2	1.7 c	0.6 c	0.3 c	1.4 c	13.3 a

[a] Data from both permselective membranes are averaged. Means within a column marked with different letters are significantly different ($P < 0.05$).

Table 2. Percent Total Protein, Ash Content, and Moisture of the Isolates Produced by BMEA with Different Permselective Membranes[a]

membrane	total protein (% dry basis)	ash content (% dry basis)	moisture (%)
CMX	97.8 ± 0.3 a	0.98 ± 0.05 a	9.8 ± 4.2 a
CSV	98.2 ± 1.6 a	1.49 ± 0.01 b	4.9 ± 0.5 a

[a] Means within a column marked with different letters are significantly different ($P < 0.05$).

Molecular Profiles. The ANOVA showed a highly significant effect of pH on κ-casein ($P < 0.0001$), α_{s2}-casein ($P < 0.0001$), α_{s1}-casein ($P < 0.0001$), and β-casein ($P < 0.0001$), which were confirmed by Duncan tests. The pH was demonstrated to have no effect on

Table 3. Percentage of Each Protein Fraction in the Isolates Produced by BMEA with Different Permselective Membranes[a]

membrane	κ-casein	α_{s2}-casein	α_{s1}-casein	β-casein	whey protein (α-la and β-lg)
CMX	11.3 ± 2.6 a	6.5 ± 0.5 a	33.6 ± 1.5 a	47.3 ± 1.5 a	1.3 ± 0.1 a
CSV	13.1 ± 1.2 a	5.9 ± 0.2 a	34.8 ± 1.4 a	45.2 ± 2.6 a	1.1 ± 0.2 a

[a] Means within a column marked with different letters are significantly different ($P < 0.05$).

whey proteins (α-la and β-lg) by both ANOVA results ($P > 0.97$) and Duncan tests (Table 1).

Membrane permselectivity had no effect on the evolution of each milk protein in the supernatant during BMEA of skim milk (Table 1). However, the evolution of the percentage of protein during pH decrease is different for each protein.

These results agree with previous data obtained for soluble protein and give more information on the differential precipitation evolution of each protein. Moreover, the initial casein percentages, transformed as percentage of caseinic fraction, are in accordance with values in the literature: κ-casein, 13.8 versus 13.9%; α_{s2}-casein, 7.3 versus 10.7%; α_{s1}-casein, 37.6 versus 38.7%; and β-casein, 41.2 versus 36.6% (Lorient, 1991; Swaisgood, 1982; Cheftel et al., 1985; Whitney, 1988). In the same way, the percentage of whey protein (mainly α-la and β-lg) of 13.5 is also in accordance with the percentage of whey protein in milk composition cited in the literature, ranging from 14 to 24% of milk protein, BSA, proteose peptone and Ig included (Cheftel et al., 1985; Brunner, 1981; Swaisgood, 1982; Lorient, 1991).

Chemical Composition of Isolates Produced. The chemical composition of isolates obtained at pH 4.2 after BMEA with both membranes was compared in terms of ash content, total protein, and percentage of each protein fraction (Table 2).

Ash contents of isolates were different for both membranes ($P < 0.001$): the CSV membrane reduced the migration of cations by electrodialysis phenomena in comparison with CMX membrane. The ash content of electroacidified isolate was lower than that of commercial casein, 2.0−3.8%, and coprecipitated casein, 8.0−10.5% (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Alais, 1984; Walstra and Jenness, 1984). Demineralization phenomenon acting during electroacidification would therefore produce isolates with lower salt contents (Bazinet et al., 1997a,b, 1999b).

The total protein content was shown to be the same for the permselectivity of both membranes ($P > 0.69$): 98% (both membranes averaged). The protein content, on a dry basis, of electroacidified isolates is slightly higher than that of commercial casein, 93.4−96.7%, and coprecipitated casein, 86.0−87.0% (Bassette and Acosta, 1988; Renner et al., 1996; Alais, 1984; Walstra and Jenness, 1984). The difference in ash content may explain the difference in percent total protein observed for the isolates produced by BMEA versus commercial isolates.

The comparison of the molecular profiles obtained by HPLC and the statistical analysis results of each protein fraction showed that there was no difference between the two membranes (Table 3 and Figure 7). BMEA allows the separation of high-purity bovine milk casein, and the permselectivity of both membranes tested does not influence the purity of the isolates produced.

Conclusion. We can conclude, from the data presented in this study, that the permselectivity of both membranes tested does not influence the final efficiency of BMEA. Moreover, these results confirm that BMEA allows the production of bovine milk casein isolates with a higher purity than those of commercial isolates, due to a lower ash content resulting from the CEM demineralizing phenomenon. However, the stacking of CSV membranes as CEM seems to stabilize the skim milk solution conductivity by differential mineral kinetics: for both membranes, the main ionic species to migrate was potassium, whereas the secondary species would be sodium and calcium for CMX and CSV membranes, respectively.

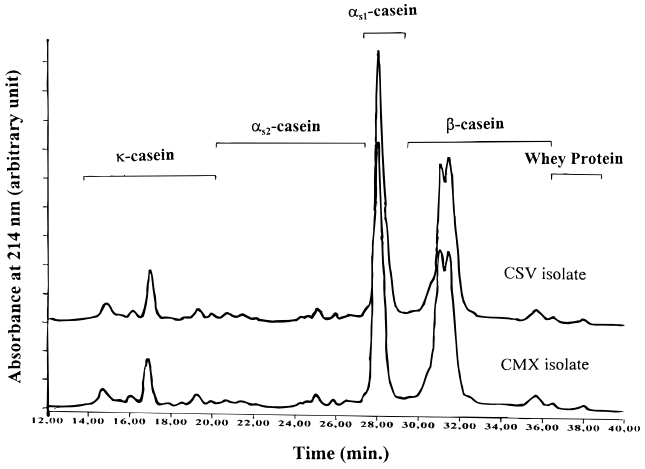

Figure 7. Reverse-phase HPLC chromatograms of isolates produced by bipolar membrane electroacidification of skim milk run with CMX and CSV membranes, at 20 °C.

An important point noted in this study was that the CSV membrane, commercially sold as a monovalent permselective membrane, was demonstrated to be, during skim milk BMEA, permselective to calcium, which is a divalent ion.

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