Bipolar Membrane Electroacidification To Produce Bovine Milk Casein Isolate

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Bipolar membrane electroacidification (BMEA) has been developed previously (Bazinet et al., Report for the Canadian Electricity Association 9326 U 987, 1996; Bazinet et al., *J. Agric. Food Chem.* **1997**, *45*, 2419−2425, 3788−3794) and has been used for isoelectric precipitation of soybean proteins. The purpose of this study was to validate the feasibility of BMEA for the precipitation of milk casein and to investigate the effect of flow rate. High-purity isolates containing 1.23 and 2.00% ash and 85.4 and 91.6% total protein were obtained with flow rates of 0.2 and 1.2 gal/min. The molecular composition profiles of the isolates obtained by HPLC showed that only caseins were precipitated. However, except for protein precipitation curves, the flow rate did not influence the final composition and purity of the isolates. These results showed that BMEA is a new alternative process for the production of high-purity bovine milk casein isolate.

Keywords: *Electrochemical acidification; bipolar membrane; casein; milk; precipitation*

INTRODUCTION

Casein is extensively used in the manufacturing of food products for its nutritional quality and functional properties. However, highly pure casein can be obtained only by an insolubilization step, and centrifugation can then be used for a simple separation of the casein from whey (Varnam and Sutherland, 1994).

Two main types of casein usually produced in the industry are rennet and acid caseins. In the case of rennet casein, the underlying mechanism is identical to that of the production of cheese curd and depends on the unique sensitivity of the $Phe_{105}-Met_{106}$ bond of κ-casein to hydrolysis by acid proteinases, the active components of rennet. For acid casein production, the three main procedures used are based on isoelectric precipitation of casein by chemical, physicochemical, or fermentation acidification (Segalen, 1985; Southward, 1993; Varnam and Sutherland, 1994). Other techniques have been proposed for the production of acid casein: acidification of milk by ion-exchange plus acid (Salmon, 1983), electrodialysis of skim milk to pH 5.0 followed by acidification to pH 4.6 with acid (Laiteries Triballat, 1979), and acidification by water electrolysis at the surface of monopolar anion- or cation-exchange membranes stacked in an electrodialysis cell (Bolzer, 1985).

A procedure derived from electrodialysis and using bipolar membranes was developed to precipitate soybean protein (Bazinet et al., 1996, 1997a,b, 1998a). Bipolar membrane electroacidification (BMEA) uses a property of bipolar membranes to split water and the action of monopolar membranes for demineralization. When a current is passed across a bipolar membrane, electrical conduction is achieved by the transport of H^+ and OH^- ions generated by electrodissociation of water (Mani, 1991). The protons thus generated can come into contact with the proteins, bringing them to their isoelectric point and resulting in selective separation. Water dissociation may occur but with a lower efficiency, using monopolar membranes stacked in an electrodialysis cell (Bazinet et al., 1996; Korngold, 1984; Davis et al., 1997). This dissociation appears only when the current value exceeds the limiting current value (Brun, 1989; Korngold, 1984). Bipolar membranes are made to be used above limiting current conditions and need a lower current to reach the limiting current value than monopolar membranes (Bazinet et al., 1998c).

As the electrical water splitting in an electrodialysis cell at the surface of monopolar membrane is affected, among other factors, by the flow rate of the solution to be treated (Gardais, 1990; Klein et al., 1987), this study is a part of a broader research project aimed at precipitation of bovine milk protein, without the addition of acids, by decreasing the pH through electrodialysis. Its specific objectives were to validate the feasibility of BMEA for the precipitation of milk casein and to investigate the effect of flow rate. Both flow rates were compared in terms of electrodialysis cell parameters, percentage of proteins precipitated, protein composition profiles, and chemical composition of isolates produced.

MATERIALS AND METHODS

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

Methods. *Electroacidification Cell.* The electroacidification cell was the same as that used by Bazinet et al. (1997a,b) with four Neosepta CMX cationic membranes and three Neosepta BP-1 bipolar membranes (Tokuyama Soda Ltd., Tokyo, Japan).

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This arrangement defines three closed loops containing the milk solution, a 2 g·L^{-1} aqueous KCl solution, and a 20 g·L^{-1} Na_2SO_4 solution. Each closed loop was connected to a separate external 600 mL glass container (School, Duran, Germany), allowing for continuous recirculation. The electroacidification system did not allow for constant temperature control.

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 WMD-30RT-220 (Iwaki Walchem, Tokyo, Japan), and the flow rate was controlled using Model E-03248-58 flow meters (Cole-Parmer Instrument Co., Vernon Hills, IL). The anode, a dimensionally stable electrode (DSA), and the cathode, a 316 stainless steel electrode, were supplied with the MP cell.

Protocol. Electroacidification was performed in batch process using a current of 2.0 A, with electrolyte volumes of 1 L. The electroacidification was stopped after the pH reached 4.0. The initial pH varied between 6.5 and 6.7. Two flow rates for the milk solution during electroacidification were tested (1.2 and 0.2 gal/min), and three replicates of each condition were performed in this experiment.

During each treatment, 1.5 mL samples of the milk solution were taken, in the outlet line outside of the BMEA cell and just before the milk reservoir, at every 0.2 pH unit decrease from initial pH (∼6.6) to pH 4.0. The time required to reach pH 4.0, the anode/cathode voltage difference, the conductivity, and the temperature were recorded as the treatment progressed. The concentration of soluble protein was determined on freshly acidified 1.5 mL samples. At the end of each run, 500 mL of pH 4.0 milk was 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). The precipitate was washed twice with distilled water before being lyophilized for 48 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 composition profiles, and ash content measurements were performed.

Analysis Methods. (1) Ash Content. In accordance with Method 930-30 (AOAC International, 1995), ∼1.5 g of lyophilized sample was added to the cooled crucibles, and the mass was recorded. The samples were then ashed at 550 °C for 16 h and weighed again when they reached room temperature.

(2) Soluble Protein and Total Protein Determination. The protein concentration determination was done using an FP-428 LECO apparatus (LECO Corp., Saint Joseph, MI). The instrument was calibrated each time with ethylenediaminetetraacetic acid (EDTA) as a nitrogen standard. A preliminary comparison of the LECO and Kjeldahl methods has demonstrated a very good correlation between both methods (R^2 = 0.986 and 0.996 for the high and low ranges of protein, respectively).

For soluble protein determination the LECO conditions were the following:

sample size	75 mg
analysis constants	
oxidation furnace temp	900 °C
oxidation standby temp	650 °C
purge cycles	3
minimum timeout	30
comparator level	1.00
loop select	low range
flow constants	high, 30 s
	high, 30 s
	high, end
gases	O_2, 99.99%; He, 99.99%
calibration standard	75 mg of EDTA (no. 502-092, 9.56 ± 0.03% N, LECO

Conditions for total protein determination were the same as for soluble protein, except for loop select (high range), flow constant (high, 10 s; high, 30 s; high, end) and mass of EDTA (150 mg).

(3) Molecular Profiles. The chromatographic analysis of protein composition profile of the lyophilized protein isolate and skim milk was performed by reverse-phase HPLC according to the method of Jaubert and Martin (1992). Separation of α_{s1}-, α_{s2}-, β-, and κ-caseins was carried out on a 15-cm Vydac C4 column (Model 214 TP 5415, Vydac, Hesperia, CA) coupled with a Vydac Protein C4 guard column (Model 214 FSK 54, Vydac). Elution at 1 mL/min was achieved using a linear gradient from 37 to 57% solvent B [0.096% (v/v) trifluoroacetic acid in 80% (v/v) acetonitrile] and from 63 to 43% solvent A [0.1% (v/v) trifluoroacetic acid in water] for a total run time of 38 min at room temperature. The detection wavelength was 214 nm, and a 50 μL sample volume was injected.

Before analysis, freeze-dried samples of isolates (55 mg) and lyophilized skim milk (LSM) (135 mg) were added to 4 mL of buffer solution (100 mM Tris-HCl, 8 M urea, 1.3% trisodium citrate, pH 7.0). The mixture was reduced in 10 mM 1,4-dithiothreitol (DTT) and maintained for 1 h at 37 °C. Before injection, the homogenate sample was diluted 10 times with solvent A and filtered on a 0.45 μm Millipore filter.

Statistical Analyses. The duration of the electroacidification, the voltage, the conductivity, and the percent soluble protein as a function of pH were subjected to a split-plot analysis of variance using SAS software (SAS, 1989). Regression equations and curve fitting were calculated for the voltage, duration, and percent soluble proteins as a function of pH using SigmaPlot (version 3.0 for Windows, Jandel Scientific, Corte Madera, CA). The ash content and the percent total protein data were analyzed by an analysis of variance and examined by Duncan tests to determine the significance of differences between the different samples.

RESULTS AND DISCUSSION

After electroacidification of the skim milk solution, the final product was still milky. However, after the solution was left to settle, the precipitate was composed of small and soft clumps of casein, and the supernatant was clear. In the BMEA cell, a slight fouling appeared due to the formation of a casein network in the spacer turbulence promoter formed by a double layer of wire mesh. The casein filled the 2 × 2 mm square of the turbulence promoter to form a soft white curd. However, the fouling produced by recirculation of these small particles did not alter the yield of casein recovered because the curd was formed only at the end of the process, when all of the casein was precipitated. An online centrifuge should allow the recovery of the casein particles and decreased fouling.

Electroacidification Parameters: Duration, Anode/Cathode Voltage Difference, and Conductivity. Results of the analysis of variance indicated that the flow rate of the milk solution had no significant effect on the duration of electroacidification ($P > 0.10$), on the anode/cathode voltage difference ($P > 0.92$), and on the conductivity ($P > 0.74$) during the electroacidification process. However, pH ($P < 0.001$) and the dual interaction of pH and flow rate ($P < 0.0009$) had a highly significant effect on the anode cathode voltage difference. The first-order linear regression calculated for the duration and the third-order regression curves calculated for the anode/cathode voltage difference as a function of pH produced coefficients of determination in the range of 0.950−0.999.

Duration of Electroacidification. To decrease the pH of skim milk from 6.6 to 4.0, the durations were the same with 17.8 and 18.1 min at 1.2 and 0.2 gal/min, respectively (Figure 1). The flow rate does not influence the duration of electroacidification. However, at the beginning of the process, the times to decrease the pH

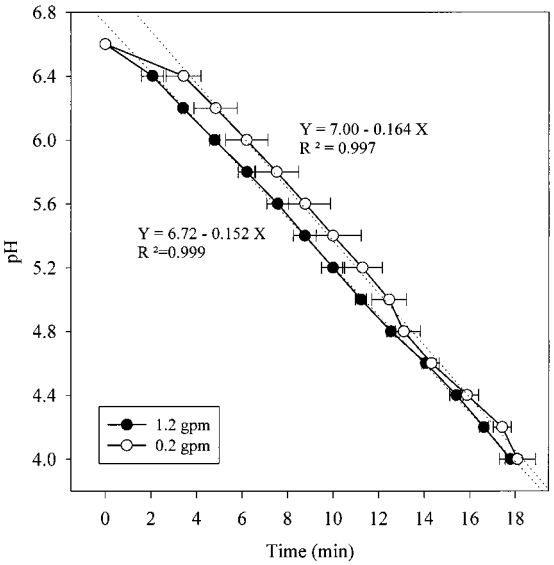

Figure 1. Effect of the flow rates, 1.2 and 0.2 gal/min, on the time required to decrease the pH by bipolar membrane electroacidification of a skim milk solution run at 2.0 A constant current.

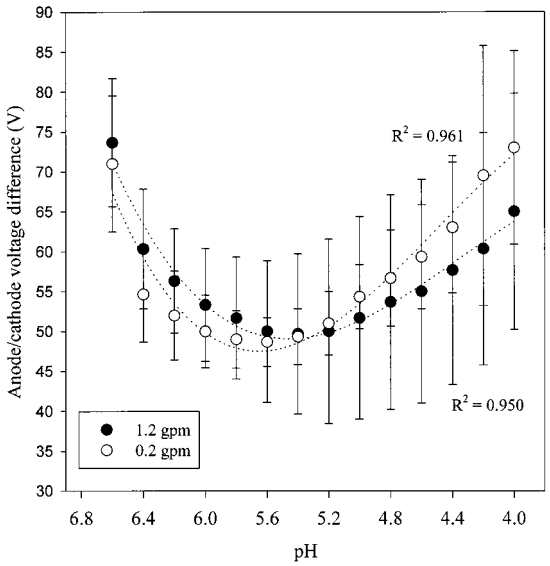

Figure 2. Effect of the flow rates, 1.2 and 0.2 gal/min, on the anode/cathode voltage difference during bipolar membrane electroacidification of a skim milk solution run at 2.0 A constant current.

from 6.6 to 6.4 were different: 2.1 and 3.4 min for 1.2 and 0.2 gal/min, respectively. This delay in acidification between 1.2 and 0.2 gal/min was found from pH 6.4 to 5.0 and disappeared at pH 4.8. Considering that the system was at solution equilibration at pH 6.6, the acidification process was found to be linear in time, which was confirmed by the regression coefficients calculated in Figure 1. From pH 6.4 to 4.0, delay in acidification was progressively diminished to disappear at the end of the process. This difference in time should be due to a faster circulation of the H^+ produced at the bipolar membrane at high flow rate, resulting in a better mixing of H^+ continuously produced with the milk protein solution in the bulk container. Moreover, the disappearance of delay in acidification between 1.2 and 0.2 gal/min could be explained by a strong precipitation of milk protein between pH 5.0 and 4.8. These results are confirmed by the fact that the production of H^+ and,

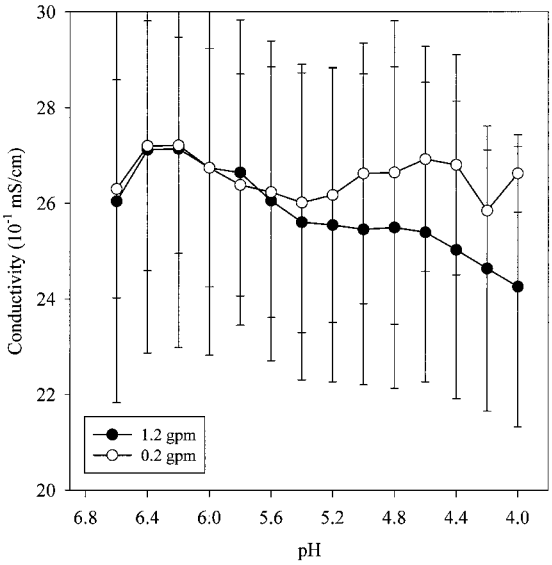

Figure 3. Effect of the flow rates, 1.2 and 0.2 gal/min, on the conductivity of skim milk solution during bipolar membrane electroacidification run at 2.0 A constant current.

consequently, the time of acidification were previously shown to mainly depend on protein concentration and current density (Bazinet et al., 1997a,b, 1998b; Mani, 1991). When protein concentration and current density were maintained constant, the time of acidification was the same whatever the other conditions, except in the case of complete fouling of the electrodialysis cell spacers.

Anode/Cathode Voltage Difference. At both flow rates, the evolution of anode/cathode voltage difference was the same: a drop followed by an increase (Figure 2). During the acidification of skim milk, at 1.2 and 0.2 gal/min, respectively, the voltage dropped from 73.7 to 49.7 V and from 71.0 to 48.7 V and then increased from 49.7 to 65.0 V and from 48.7 to 73 V. This phenomenon was previously observed by Bazinet et al. (1997a,b) on soybean protein. The decrease in voltage would be the result of the higher conductivity of H^+ generated at the bipolar membrane in the replacement of ions migrating across the cationic membrane, to maintain the electrical neutrality of the solution (Bazinet et al., 1998a): The molar conductivity of H^+ is much higher than that of all the other ions (Brett and Oliveira-Brett, 1994). This replacement in part of the milk cations by H^+ in the protein solution and the migration of these cations from the protein compartment to the KCl and Na_2SO_4 compartments across the cationic membrane, coupled in the KCl compartment with generation of OH^- at the anionic exchange layer of the bipolar membrane, induced a decrease in the overall resistance of the system and, consequently, a decrease in anode/cathode voltage difference (Bazinet et al., 1998a). This phenomenon progressed during the entire process, but when the protein began to precipitate, the overall resistance of the system increased because of a slight fouling in the spacers of the cell.

Conductivity. Results of the statistical analysis indicate that the flow rate did not affect the conductivity (Figure 3). The conductivity changed in the same way during electroacidification whatever the flow rate, as shown in Figure 3. This demonstrates that conductivity at a level of 2.6−2.4 mS/cm does not appear to be a limiting factor in the electroacidification of milk protein.

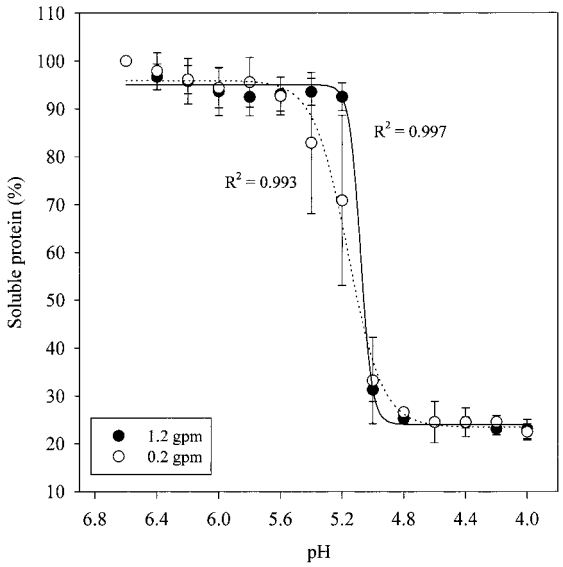

Figure 4. Effect of the flow rates, 1.2 and 0.2 gal/min, on the percentage of soluble proteins in the skim milk solution during bipolar membrane electroacidification run at 2.0 A constant current.

The apparent lower decrease in conductivity, ~0.1−0.2 mS/cm during electroacidification, was similar to the results obtained by Bazinet et al. (1997b) on soybean protein.

Soluble Protein. The analysis of variance of the data shows that the flow rate ($P > 0.50$) had no significant effect, whereas pH ($P < 0.0001$) and the dual interaction of pH and flow rate ($P < 0.0009$) had highly significant effects on the amount of soluble proteins. The equations of the curves representing percentage of soluble proteins as a function of pH were calculated and modeled; R^2 ranged between 0.993 and 0.997.

The flow rate had no effect on the amount of soluble protein, which decreased from ~100% to 21.5−24.4% (Figure 4). However, the rates of decrease of the soluble protein from pH 6.6 to 4.0 were different between the two flow rates. Thus, at the beginning of the procedure, from pH 6.6 to 5.6, soluble protein amounts were the same for both flow rates, ranging between 93 and 100%. When the pH dropped from 5.6 to 5.2, at 0.2 gal/min, soluble protein decreased to 82.9% at pH 5.4 and to 70.9% at pH 5.2, whereas at 1.2 gal/min, soluble protein was constant at 93−100%. At pH 5.0, soluble protein was comparable for both flow rates at 31.3 and 33.2%. As the electroacidification continued from pH 4.8 to 4.0, soluble protein was constant at ~24% whatever the flow rate.

Decreasing the flow rate accelerated the precipitation of protein by increasing the residence time of the protein in the electrodialysis cell. Consequently, at 0.2 gal/min, as the generation of H^+ was constant and as a lower volume of protein solution was in contact with more H^+, the pH decreased more rapidly and more protein precipitation occurred. Also, as protein was longer in contact with the electrogenerated H^+, a pH gradient occurred between the inlet of the electrodialysis cell, corresponding to the bulk container solution, and the outlet of the cell. At high flow rate, however, the shorter residence time was not sufficient to create a measurable pH gradient between the inlet and the outlet of the cell. Then, as the mixing between the outlet solution and the bulk solution was high, supplementary passes through the cell were required to obtain a pH close to the isoelectric point and to allow protein precipitation. The different evolution of soluble protein is confirmed by the calculations of the inflection points and the width of transition of the solubility curves: pH 5.08 and 0.04 pH unit and pH 5.16 and 0.11 pH unit at 1.2 and 0.2 gal/min, respectively. The 24% soluble protein remaining would correspond to the whey proteins, which are soluble in the pH range from 4.8 to 4.0. This is confirmed by percentages of whey protein in milk composition cited in the literature ranging from 14 to 24% (Cheftel et al., 1985; Brunner, 1981; Swaisgood, 1982; Lorient, 1991).

Chemical Composition. The chemical composition, in terms of ash content and total protein, of isolates obtained at pH 4.0 after electroacidification was compared to that of an LSM.

The ash content of isolates was lower in comparison with LSM: 1.23 ± 0.01 and 2.00 ± 0.60% for 0.2 and 1.2 gal/min electrochemical isolates in comparison with 8.05 ± 0.01% for LSM. The ash content of LSM is in accordance with data found in the literature, 8.05 versus 7.9% (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Renner et al., 1996), whereas the ash content of the electroacidified isolate was lower than data cited in the literature, 2.0−3.8 and 8.0−10.5% for commercial casein and coprecipitated casein, respectively (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Alais, 1984; Walstra and Jenness, 1984). This difference in ash content indicates a demineralization phenomenon acting during electroacidification. Indeed, to maintain the milk solution electrically neutral, one cationic charge must cross the cationic membrane for each H^+ produced at the bipolar membrane (Bazinet et al., 1997a,b, 1999).

The percentage of total protein is 2.3−2.5 times higher for electroacidified isolate in comparison with LSM. This result confirms the efficiency of BMEA for the precipitation and the separation of milk casein. Total protein in LSM is in accordance with the literature, 36.9 versus 36.2% for regular nonfat dried milk (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Renner et al., 1996), whereas the percent total protein of electroacidified isolates (91.6 ± 4.6 and 85.4 ± 4.1% for 0.2 and 1.2 gal/min isolates, respectively) is similar to or slightly higher than data found in the literature, 83.0−88.5 and 83.0−85.0% for commercial casein and coprecipitated casein, respectively (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Alais, 1984). The difference in ash content could explain the difference in percent total protein observed for the isolates obtained by BMEA versus commercial isolates.

Molecular Profile Analysis. The comparison of the molecular profiles obtained by HPLC showed that BMEA allows the separation of high-purity bovine milk casein (Figure 5). Whey protein peaks, mainly α-lactalbumin and β-lactoglobulin, with retention times ranging from about 35 to 39 min, do not appear or appear in a very low quantity on the isolate profiles (Figure 5). BMEA allows a good separation of casein from raw milk. Moreover, the flow rate does not influence the purity and the molecular profile of the final isolate obtained by BMEA.

CONCLUSION

Results obtained in this study show that BMEA is a new alternative process for the production of high-purity casein bovine milk isolate. Moreover, except for protein precipitation curves, flow rate does not influence the final composition and purity of the isolate.

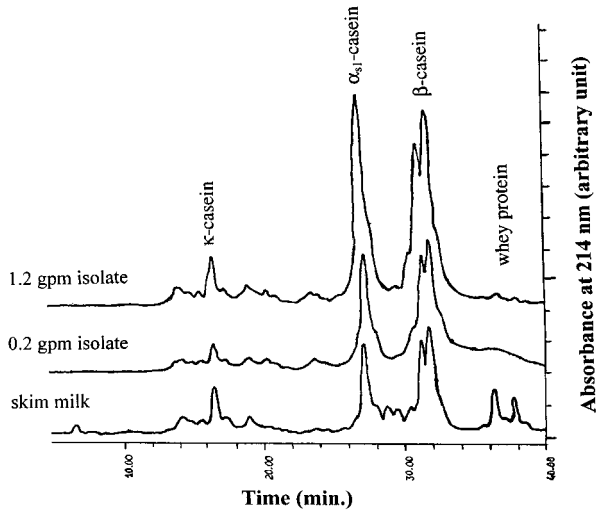

Figure 5. Reverse-phase HPLC chromatograms of skim milk and of isolates produced by bipolar membrane electroacidification of skim milk run at two different flow rates and at 2.0 A constant current.

The high purity of the isolate is explained by the demineralization process coupled with the action of bipolar membranes. Bipolar membrane decreases the pH, without the addition of acid, and cations migrate accross the cation-exchange membrane (CEM) to decrease the ash content of the final product in order to maintain the electroneutrality of the milk solution.

However, a deposit, probably calcium hydroxide, appears on the CEM side in contact with the base. The calcium ion migrating from the milk solution could precipitate with OH^- produced on the anionic side of the bipolar membrane to form a fouling complex on the CEM. Hence, further research is needed to study the wide possibilities of BMEA and to understand the membrane phenomena associated with the migration of cations.

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LITERATURE CITED

Alais, C. Produits Laitiers Divers. In *Science du Lait: Principe des techniques laitières*, 4th ed.; SEPAIC: Paris, France, 1984; pp 723−764.

AOAC International. Method 930-30: Ash of dried Milk. In *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC: Arlington, VA, 1995; Vol. 2.

Bassette, R.; Acosta, J. S. Composition of milk Products. In *Fundamentals of Dairy Chemistry*, 3rd ed.; Wong, N. P., Jenness, R., Keeney, M., Marth, E. H., Eds.; Van Nostand Reinhold: New York, 1988; pp 39−79.

Bazinet, L.; Lamarche, F.; Labrecque, R.; Toupin, R.; Boulet, M.; Ippersiel, D. *Systematic Study on the Preparation of a Food Grade Soyabean Protein*; Report for the Canadian Electricity Association 9326 U 987; Research and Development: Montréal, May 1996.

Bazinet, L.; Lamarche, F.; Labrecque, R.; Ippersiel, D. Effect of KCl and Soy Protein Concentrations on the Performance of Bipolar Membrane Electro-acidification. *J. Agric. Food Chem*. **1997a**, *45*, 2419−2425.

Bazinet, L.; Lamarche, F.; Labrecque, R.; Ippersiel, D. Effect of Number of Bipolar Membrane and Temperature on the Performance of Bipolar Membrane Electro-acidification. *J. Agric. Food Chem*. **1997b**, *45*, 3788−3794.

Bazinet, L.; Lamarche, F.; Ippersiel, D. Comparison of Chemical and Bipolar-membrane Electrochemical Acidification for Precipitation of Soybean Proteins. *J. Agric. Food Chem*. **1998a**, *46*, 2013−2019.

Bazinet, L.; Lamarche, F.; Ippersiel, D. Bipolar-Membrane Electrodialysis: Applications in the Food Industry. *Trends Food Sci. Technol*. **1998b**, *9* (3), 107−113.

Bazinet, L.; Lamarche, F.; Ippersiel, D. L'Électroacidification avec Membranes Bipolaires: Une Électrotechnologies Environnementale pour la Production d'Isolats de Protéines de Soya. *Ind. Aliment. Agric.* **1998c**, *11*, 24−29.

Bazinet, L.; Lamarche, F.; Ippersiel, D. Ionic Balance: A closer Look at the K^+ migrated and H^+ generated during Bipolar Membrane Electro-Acidification of Soybean Proteins. *J. Membr. Sci.* **1999**, *154*, 61−71.

Bolzer, R. Installation and process for the preparation of acid caseinates. U.S. Patent 4,559,119, 1985.

Brett, C. M. A.; Oliveira-Brett, A. M. Fundamentals of Kinetics and Mechanism of Electrode Reactions. In *Electrochemistry: Principles, Methods, and Applications*; Oxford University Press: New York, 1994.

Brun, J. P. Électrodialyse. In *Procédés de Séparation par Membrane: Transport, Techniques membranaires et Applications*; Masson: Paris, France, 1989.

Brunner, J. Cow Milk Proteins: Twenty Five Years of Progress. *J. Dairy Sci*. **1981**, *64*, 1038−1054.

Cheftel, J. C.; Cuq, J. L.; Lorient, D. In *Protéines Alimentaires: Biochimie, Propriétés Fonctionnelles, Valeur Nutritionnelle, Modifications Chimiques*; Technique et Documentation Lavoisier: Paris, France, 1985.

Davis, T. A.; Genders, J. D.; Pletcher, D. A *First Course in Ion Permeable Membranes*; The Electrochemical Consultancy: Romsey, 1997; 255 pp.

Gardais, D. Les Procédés Électriques de Traitement des Rejets Industriels. In *Environnement et Electricité*; Electra; Doppee difffusion: Avon, France, 1980.

Hargrove, R. E.; Alford, J. A. Composition of Milk Products. In *Fundamentals of Dairy Chemistry*, 2nd ed.; Webb, B. H., Johnson, A. H., Alford, J. A., Eds.; AVI Publishing: Westport, CT, 1974; pp 58−86.

Jaubert, A.; Martin, P. Reverse-Phase HPLC Analysis of Goat Caseins. Identification of α_{s1} and α_{s2} Genetic Variants. *Lait* **1992**, *72*, 235−247.

Klein, E.; Ward, R. A.; Lacey, R. E. Membrane Processes—Dialysis and Electrodialysis. In *Handbook of Separation Process Technology*; Rousseau, R. W., Ed.; Wiley-Interscience: New York, 1987; pp 954−981.

Korngold, E. Electrodialysis-Membranes and Mass Transport. In *Synthetic Membrane Process*; Bedford, G., Ed.; Academic Press: New York, 1984; pp 191−220.

Laiteries Triballat. French Patent Appl. 2 418 627, 1979.

Lorient, D. Données récentes sur les protéines laitières. Thème 2: État physico-chimique de la matière protéique. *Actes du Colloque "Protéines et rendements en industries laitières"*; ENSAIA Nancy: Oct 2−3, 1991; pp 43−59.

Mani, K. N. Electrodialysis Water Splitting Technology. *J. Membr. Sci.* **1991**, *58*, 117−138.

Renner, E.; Renz-Schauen, A.; Drathen, M. *Nutrition Composition Tables of Milk and Dairy Protducts*; Drathen, M., Ed.; Justus-Liebig-Universität Giessen Verlag: Giessen, Germany, 1996.

Salmon, M. U.S. Patent 4,423,081, 1983.

SAS Institute Inc. In *SAS/Stat User's Guide*, Vol. 2, version 6, 4th ed.; SAS Institute Inc.: Cary, NC, 1989; 846 pp.

Segalen, P. Description des Différentes Techniques de Fabrication des caséines et caséinates. In *Lait et Produits Laitiers,- Vaches, Brebis, Chèvres- Vol.2, Les Produits laitiers—Transformation et Technologies*; Techniques et Documentation Lavoisier; APRIA: Paris, France, 1985; pp 395−405.

Southward, C. R. Utilization of Milk Components: Casein. In *Modern Dairy Technology, Advances in Milk Processing,* 2nd ed.; Robinson, R. K., Ed.; Chapman and Hall: London, U.K., 1993; Vol.1, Chapter 8, pp 375−432.

Swaisgood, H. E. Chemistry of milk protein. In *Developments in Dairy chemistry—1*; Fox, P. F., Ed.; Elsevier Applied Sciences Publishers: London, U.K., 1982; pp 1−59.

Varnam, H. A.; Sutherland, J. P. Milk and Milk Products—technology, chemistry and microbiology*;* In *Dairy Protein Products*; Chapman and Hall: London, U.K., 1994; Chapter 4, pp 159−182.

Walstra, P.; Jenness, R. Properties of Milk Concentrates. In *Dairy Chemistry and Physics*; Wiley-Interscience: New York, 1984; pp 301−335.

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