Bipolar Membrane Electroacidification of Demineralized Skim Milk

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The aim of this study was to evaluate the effect of decreasing the mineral content of skim milk by electrodialysis (ED) prior to electroacidification with bipolar membrane (BMEA) on the performance of the process, the chemical composition, and the physicochemical and functional properties of the isolates produced. ED used to demineralize the skim milk solution was very efficient. However, the electroacidification parameters were influenced by the demineralization level of the skim milk solution: the energy efficiency was decreased with an increase in demineralization, but it was still possible to perform BMEA at a very low conductivity level. Moreover, the isolates produced by BMEA after electrodialysis demineralization at different rates showed similar chemical composition, except on potassium and lactose contents for 75% demineralized isolate. These isolates, except on protein load for 75% demineralization rate, showed similar physicochemical and functional properties, whatever the demineralization rate.

Keywords: *Electrochemical acidification; electrodialysis demineralization; precipitation kinetic; functional properties; casein isolate*

INTRODUCTION

Hydrochloric acid is commonly used for casein production because the acid is available as a relatively inexpensive byproduct of the chemical industry (*1*). Other techniques have been proposed for the production of acid casein. Acidification of milk by ion exchange plus acid (*2*) or by ion exchange alone (*3*) has been developed in France. A proposed alternative involves electrodialysis (ED) of skim milk to pH 5.0 followed by acidification to pH 4.6 with acid (*4*). A specific advantage of these methods is the production of acid whey with reduced mineral content. This acid whey is more readily used than acid whey produced by the normal acidification process and may increase its value for further processing (*1*, *5*).

More recently, bipolar membrane electroacidification (BMEA) has been used for isoelectric precipitation of skim milk protein and production of isolates (*6*). BMEA uses the property of bipolar membranes to split water and the action of monopolar membranes for demineralization. According to results obtained by Bazinet et al. (*6*), the skim milk solution is demineralized at 30−40% of the initial level during the process. However, a higher demineralization rate of the whey would be of great interest for the dairy industry and for its use in such application as infant formula.

The aim of this study was therefore to evaluate the effect of decreasing the mineral content of skim milk by ED prior to electroacidification on the performance of the process and on the chemical composition and the physicochemical and functional properties of isolates produced by BMEA.

MATERIALS AND METHODS

Material. The raw material used in this study was reconstituted milk (10% w/v) from low-temperature spray-dried skim milk powder (Agropur, Granby, Canada). The averaged composition of the skim milk powder was the following (g/100 g): total protein, 33.9; whey protein, 7.4; fat, 0.6; carbohydrates, 53.5; ash, 8.2; moisture, 3.8.

Methods. *ED Cell.* The ED cell and stack system were the same that those used by Bazinet et al. (*7*) with 10 AR-103-QZL-388 anionic membranes (Ionics Inc., Watertown, MA), 9 CR-64-LMP-401, and 2 CR-61-AZL-389 cationic membranes (Ionics Inc.). This arrangement sets up three circuits: the skim milk (4 L); the concentrate, a 0.1 N KCl solution (6 L); and the electrolyte, 20 g/L Na_2SO_4 (6 L). The flow rate of the skim milk solution was controlled at 1.6 L/min. The anode of the module was made of platinum-plated niobium, and the cathode was a plate of stainless steel 316.

BMEA Cell. The module was an MP type cell (100 cm^2 of effective electrode surface) from ElectroCell Systems AB Co. (Täby, Sweden). This arrangement defines three closed loops, separated by cationic and bipolar membranes (Tokuyama Soda Ltd., Tokyo, Japan) containing the milk solution (3 L), a 0.25 N HCl solution (6 L), and a 20 g/L Na_2SO_4 solution (6 L). Each closed loop was connected to a separate external reservoir, allowing for continuous recirculation (*8*). The anode, a dimensionally stable electrode (DSA), and the cathode, a 316 stainless steel electrode, were supplied with the MP cell.

Protocol. Electrodialysis was performed in batch process using a current of 2.0 A. After reaching 30 V, the voltage was maintained constant at 30 V to limit water splitting (*7*). The

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initial pH varied between 6.5 and 6.7. Electroacidification was carried out in batch process using a current of 2.0 A; after reaching 90 V, the voltage was maintained constant at 90 V in order to not surpass the total power of the power supply. The electroacidification was stopped when all of the caseins were precipitated (pH_c). As the pH_c values vary with the demineralization level, the respective values of pH_c were determined in a preliminary study. Four demineralization rates were tested during electroacidification: 0, 25, 50, and 75%. Three replicates of each condition were performed in this experiment.

Samples (1.5 mL) of the milk solution were taken at the beginning and at the end of the ED process, at the beginning of the BMEA process, and at every 0.2 pH unit decrease during electroacidification from pH 5.8 to the pH_c value. The time required to demineralize at the desired rate by ED and to reach the pH_c value by BMEA were recorded, as was the conductivity of the skim milk solution as the treatments progressed. The concentration of soluble protein was determined on freshly acidified 1.5 mL samples. At the end of each BMEA, samples of ~2.5 L of the electroacidified milk solution were recovered. 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 double-distilled water, and the pH was adjusted to 6.6 with 1 N NaOH. The sodium caseinates (CAS) produced were lyophilized for 24 h at room temperature (model Freezone 4.5, Labconco, Kansas City, MO). The lyophilized CAS were stored at 4 °C before chemical composition and physicochemical and functional properties were performed.

Analysis Methods. (a) 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.

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

(c) Energy and Relative Energy Consumption. The voltage as a function of time multiplied by the current was integrated to determine the energy consumption (*7*, *9*, *10*).

(d) Protein Content. The protein content of 1.5 mL samples of freshly acidified milk and of CAS isolate powder was determined by an FP-428 LECO apparatus (LECO Corp., St. Joseph, MI), according to the conditions and parameters used by Bazinet et al. (*6*).

(e) Lactose Concentration. Protein solutions (5% w/v) were acidified with 1.0 N hydrochloric acid to a pH below the isoelectric point. Samples were then centrifuged for 15 min at 2000*g* and 20 °C in a Beckman GS-6 centrifuge (Beckman Instruments Inc., Mississauga, ON, Canada). Fifteen microliters of the 0.45 μm filtrated supernatant was injected on an Ion-300 column (Mandel Scientific Co., Rockwood, ON, Canada) connected to an HPLC (Waters Associates, Milford, MA) having a UV detector (210 nm) (model 490, Waters) and a refractive index detector (model R410, Waters) according to the method of Doyon et al. (*11*). A 0.0054 N H_2SO_4 solution was used as mobile phase at a flow rate of 0.4 mL/min. The concentration of lactose was determined using a commercial D-lactose solution (Sigma Chemical Co., St. Louis, MO) of known concentration.

(f) Ash Content. Ash content was determined according to AOAC methods no.930-30 (*12*) and 945-46 (*13*).

(g) Potassium, Sodium, Magnesium, and Calcium Concentration Measurements. Sodium, potassium, magnesium, and calcium concentrations were determined by inductively coupled plasma (ICP, Optima 3300, dual view, Perkin-Elmer, Norwalk, CT). The wavelengths used to determine sodium, calcium, magnesium, and potassium concentrations were 589.59, 422.67, 285.21, and 766.49 nm, respectively (*14*). The analyses were carried out in radial view. Samples were prepared from known weight skim milk solution ash dissolved in 10 mL of HCl (2 N) and diluted with HCl (2 N) to be within the calibration ranges for each cation.

(h) Protein Profile. The chromatographic analysis of the protein profile of the lyophilized protein isolate and skim milk samples was performed by reversed-phase HPLC according to the method of Jaubert and Martin (*15*), in the conditions used by Bazinet et al. (*6*).

(i) Specific Viscosity. Ten milliliters of a 4% (w/v) protein solution was introduced into a calibrated viscometer size 100 (Cannon-Fenske RoutineViscosimeter, Cannon Instrument, VWR, Ville-Mont-Royal, PQ, Canada) placed at 25 °C in a thermostatic water bath. The time needed for the solution to flow through the thin capillary was measured precisely and divided by the time needed for double-distilled water to flow in the same conditions in order to give the relative viscosity (η_r) of the protein solution. The analysis was repeated five times for each solution. Specific viscosity was calculated from relative viscosity and protein concentration according to the following equation:

$$\eta_{sp} = (\eta_r - 1)/[\text{protein}] \quad (1)$$

η_{sp} is the specific viscosity (mL/g), η_r the relative viscosity of the protein solution, and [protein] the protein concentration of the solution (g/mL).

(j) Interfacial Area (IA) of the Emulsions. Oil emulsions (33% v/v) were produced by mixing commercial corn oil (Mazola) and 4% (w/v) protein solution with a Polytron (model PT 10-35, probe PTA 10S, Kinematica AG, Littau, Switzerland) for 30 s at 9000 rpm and homogenized at a pressure of 10000 psi with an Emulsiflex-C5 homogenizer (Avestin, Ottawa, ON, Canada). IA of the emulsions was calculated from the turbidity of diluted emulsions (*16*). Emulsions were diluted to a final oil volume fraction of 6×10^{-5} in sodium phosphate buffer (0.01 M, pH 7.0) containing 0.5% sodium dodecyl sulfate (SDS, Bio-Rad Laboratories Canada Ltd., Mississauga, ON, Canada) according to the method of Britten and Giroux (*17*). Optical density was measured in duplicate at 500 nm with a Beckman DU-640 spectrophotometer (Beckman Instruments Inc., Mississauga, ON, Canada). IA was calculated according to the method of Cameron et al. (*18*). Emulsion stability was measured by determining the IA of the emulsion stored for 6 weeks at 4 °C.

(k) Protein Load of the Emulsions. Protein load was calculated from protein depletion in the serum phase after emulsion formation according to the method of Britten and Giroux (*17*). Serum phase was separated from the emulsion by centrifugation (25000*g* for 1 h at 4 °C) using a Beckman centrifuge (model J2-21, rotor type JA 20-1). Protein was determined in the aqueous phase before and after emulsion formation using Bradford's method (*19*) calibrated with a bovine serum albumin (BSA) standard (Bio-Rad Laboratories Canada Ltd., Mississauga, ON, Canada). Protein load results were expressed as milligrams per square meter. For that purpose, protein concentration depletion in the aqueous phase was divided by the IA of the emulsion.

(l) Foaming Properties. Foaming properties were measured according to the method of Waniska and Kinsella (*20*). Fifteen milliliters of 0.5% (w/v) protein solution was used. The solution in the column was sparged with nitrogen gas at a constant flow rate of 19 mL/min until foam volume reached 70 mL. Protein solution was added as required to maintain the volume constant at 15 mL. Time required to reach 55 mL of foam and the volume of protein solution added were recorded. At the end of the sparging, the volume of liquid drained from the foam after 2 min was measured. The analyses were done at room temperature and repeated five times for each solution.

(m) Solubility Profile. Hydrochloric acid (0.2 N) was added gradually to 250 mL of 2% (w/v) protein solution; 1.5 mL aliquots were taken at pH 6.6, 5.8, 5.4, 5.2, 5.0, 4.8, 4.6, 4.4, 4.2, and 4.0 and centrifuged at 500*g* for 10 min at 4 °C. Protein concentration was measured in the supernatant using Bradford's method (*19*). Nonlinear regression equations were

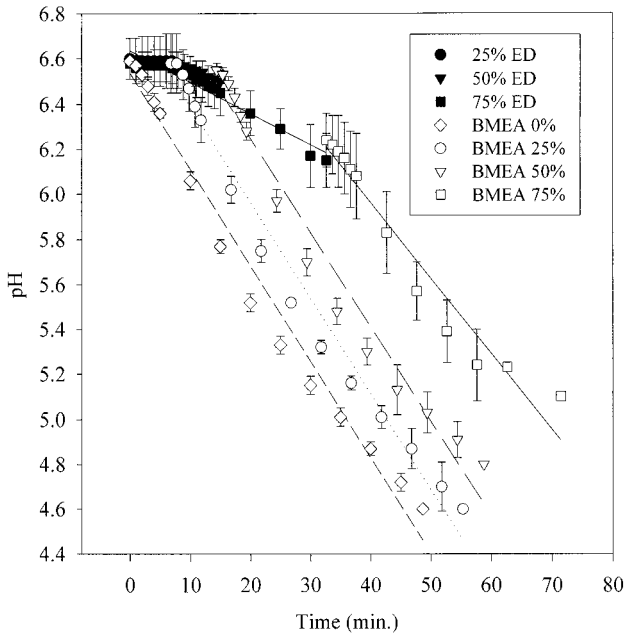

Figure 1. Effect of demineralization rate on the duration of ED phase, BMEA phase, and global process (ED plus BMEA).

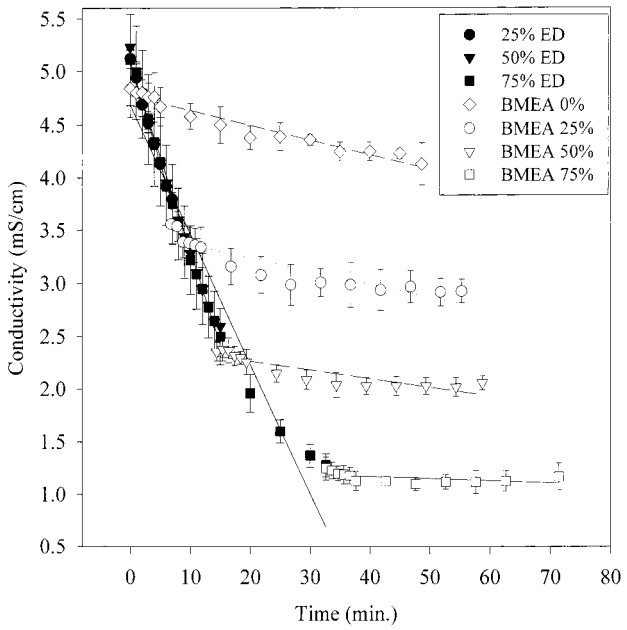

Figure 2. Evolution of skim milk conductivity during ED and BMEA phases carried out at four demineralization rates.

calculated according to the procedure of Bazinet et al. (*14*):

$$S_p = b + \frac{a}{1 + \exp\left[-\left(\frac{pH_x - c}{w}\right)\right]} \quad (2)$$

S_p is the percentage of soluble protein (%), pH_x the pH value ranging from pH 6.6 to 4.0, a the amplitude of the curve (% unit), b the percentage of soluble protein at the isoelectric point (%), c the center or point of inflection, and w the width of the transition region of the sigmoidal curve (pH unit).

Statistical Analyses. Using SAS software (*21*) the data from the compositional, physicochemical, and functional analyses of CAS produced by BMEA were submitted to an analysis of variance with regression contrasts to examine the effect of the demineralization treatment prior to BMEA. Repeated measure analyses of variance were performed for soluble protein and protein fractions as treatment progressed and for isolate solubility profile. Linear regression equations for the duration and the conductivity of the skim milk solution as the ED and BMEA progressed, and nonlinear regression equations for the solubility as a function of pH, were calculated using Sigmaplot (version 2.01 for Windows, Jandel Scientific, Corte Madera, CA).

RESULTS AND DISCUSSION

ED Parameters: Duration, Conductivity, Resistance, and Energy Consumption. *Duration.* The duration of the global process (BMEA plus ED) differed according to the demineralization level ($P < 0.0187$) (Figure 1). The time to reach pH_c increased in a linear fashion with an increase in demineralization: 48.7, 55.3, 58.8, and 71.5 min for 0, 25, 50, and 75% demineralization level, respectively. Moreover, according to the linear coefficients calculated for the BMEA phase, the BMEA has been carried out in similar way whatever the demineralization level.

Conductivity of the Skim Milk Solution. As expected, the variation in conductivity of the skim milk solution from the beginning to the end of the global process was influenced by the demineralization level ($P < 0.0001$) (Figure 2): the variation in conductivity increased in a linear fashion from 0.7 to 3.9 mS/cm with an increase in demineralization from 0 to 75%. During the ED phase, the conductivity of the milk solution decreased in a similar way whatever the demineralization level with an averaged linear coefficient of −0.134 ($R^2 = 0.937$), whereas during the BMEA phase the conductivity decrease was lower due to an increase in demineralization and disappeared at 75%. Hence, during BMEA, the conductivity decreased by 13×10^{-3} and 2×10^{-3} mS/cm·min for 0 and 75% demineralized milk, respectively.

Global Resistance of the Cells. As expected, the variation of the cell resistance during the BMEA phase was influenced by the demineralization level ($P < 0.0172$). The resistance variation increased from 14 to 23.3 V with an increase in demineralization from 0 to 25% and stabilized at 24 V thereafter. In addition, during the ED phase, resistance of the ED cell increased in a similar way whatever the demineralization level, with an averaged linear coefficient of 0.754 ($R^2 = 0.985$). During BMEA, the resistance increase was higher by an increase in demineralization to stabilize at 50 and 75% demineralization. Hence, during BMEA, the resistance increased by $(256-525) \times 10^{-3}$ Ω/min from 0 to 50% demineralization to stabilize at 0.568 Ω/min for 75% demineralized milk.

Energy and Relative Energy Consumption. During the first 18.7 min of the ED phase, the energy needed at one time was the same whatever the demineralization level ($R^2 = 0.985$) (Figure 3). After 18.7 min of ED, particularly at 75% demineralization, the energy needed at one time decreased constantly. In fact, after 18.7 min of demineralization the maximum voltage of the power supply was reached, due to an increase in resistance, and the voltage was fixed at 90 V. Consequently, because the current intensity decreased, the energy needed at one time decreased also.

During BMEA, the increase in energy needed at one time varied by 1.03−1.5 J/min with an increase in demineralization from 0 to 25% and stabilized at 1.4 J/min for 50% demineralization; afterward, for 75% demineralization BMEA, the energy needed at one time

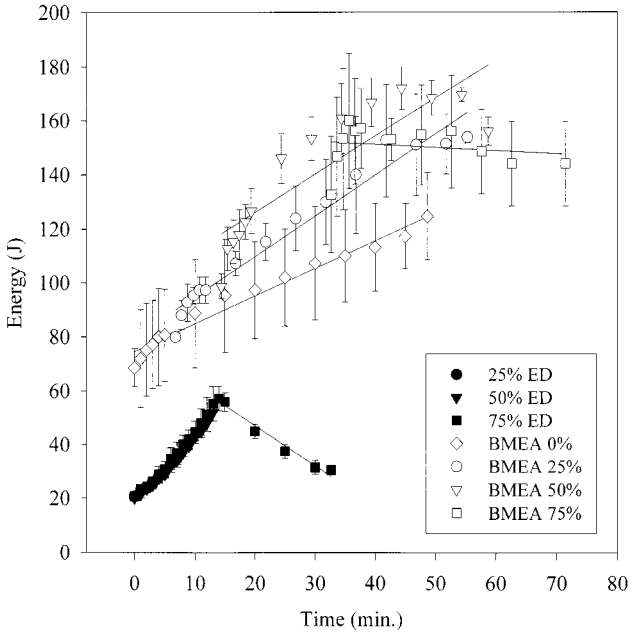

Figure 3. Evolution of energy needed at one time during ED and BMEA phases carried out at four demineralization rates.

could be considered as stable with a linear coefficient of −0.11 J/min.

As expected, the relative energy consumption expressed in kilowatt hours per kilogram of isolate produced increased in a linear fashion by 200% with an increase in demineralization from 0 to 75%.

ED to demineralize the skim milk solution was very efficient. However, the electroacidification parameters were strongly modified and related to the demineralization level of the skim milk solution. These results were in accordance with data in the litterature: as the demineralization progresses, the resistance of the system increased; a high demineralization level was related to a higher energy consumption and a higher electrical efficiency factor (*7*, *10*, *22*). Moreover, a pH decrease was observed during the ED phase when the demineralization level was >25%. Delbeke (*23*) observed the same phenomenon during different demineralization levels of cheese whey: decreases of 0.39 and 1.38 pH units were obtained with 70 and 90% demineralization, respectively. Perez et al. (*10*), with demineralized whey permeates and retentates obtained by ultrafiltration using ED, observed similar decreases in pH ranging from 0.13 to 0.55 pH unit for 40−65% demineralization rates. This change in pH during demineralization can be explained as follows: at an early period of demineralization, the chlorine and potassium ions are mainly removed, and later the remaining phosphoric acid radicals or calcium and magnesium ions are mainly removed. However, because their hydration radius is large, it is difficult for these ionic species to pass through the membrane. For these reasons, water is dissociated into OH^- and H^+, the OH^- easily passing through the membrane but the H^+ remaining in the deashing solution, resulting in a lowering in pH (*22*). Nevertheless, it was possible to perform BMEA at a very low conductivity level, but of course the energy efficiency was decreased with an increase in the demineralization level.

Precipitation Kinetics during Acidification. The repeated measure analyses of variance of the data showed that the pH ($P < 0.0001$), and the double interaction pH/demineralization rate ($P < 0.0001$) had a significant effect on the precipitation of milk proteins. The nonlinear regression sigmoidal curves produced coefficients of determination ranging from 0.984 to 0.999 (Table 1).

Table 1. Parameters of the Model Sigmoidal Curves Describing the Soluble Protein Evolution during Chemical and Electrochemical Acidifications of 0, 25, 50, and 75% Demineralized Skim Milk

	0%	25%	50%	75%
BMEA				
amplitude (% unit)	79.8	80.9	79.1	76.7
soluble protein at pH_i (%)	20.6	20.2	18.5	20.7
inflection point	4.96	5.06	5.09	5.29
transition width (pH unit)	0.0115	0.0309	0.0324	0.0315
R^2	0.998	0.998	0.984	0.993
chemical acidification				
amplitude (% unit)	78.6	81.5	80.9	77.5
soluble protein at pH_i (%)	19.7	17.7	17.2	20.9
inflection point	4.89	4.91	5.05	5.13
transition width (pH unit)	0.0442	0.0419	0.0346	0.0262
R^2	0.995	0.998	0.998	0.999

Soluble protein evolution during the pH decrease revealed differences between the different demineralization rates. From pH 6.6 to 5.4, the percent soluble protein was the same, ranging from 95 to 100% whatever the demineralization rate. At pH 5.2 the 75% demineralized milk showed the lower soluble protein with 25% followed by the 50, 25, and 0% demineralized milk with respective soluble protein levels of 95, 100, and 100%. As the electoacidification progressed from 5.2 to 5.0, 75 and 50% demineralized milk showed similar low percentages of soluble protein with 20.7 and 23.1%, respectively, whereas the 25 and 0% demineralized milks had soluble protein values of 29.5 and 98.1%, respectively. At pH 4.8 and after, the soluble protein levels were similar for the demineralized and nondemineralized milks, with an averaged value of 20.1%. The demineralization rate did not influence the final precipitation extent of protein. These different precipitation profiles were confirmed by model sigmoidal curves generated to mathematically describe the evolution of the soluble protein content; as shown in Table 1 the inflection points of the model curves were influenced by the demineralization rates: pH 4.96, 5.06, 5.09 and 5.29, respectively, for 0, 25, 50, and 75% demineralization rates.

The differences observed between milks electroacidified at different demineralization rates can be explained mainly by an increase in electrostatic repulsion (salting-in) due to a decrease in ionic strength. Delay in protein precipitation was observed previously between the chemical and electrochemical acidification and should be due to a salting-in effect by the addition of HCl (*24*). Therefore, to confirm that observation milk solutions demineralized at the different rates were chemically acidified. For each demineralization rate, a delay in precipitation was observed: the inflection points calculated by model sigmoidal curves for the chemical acidifications were 4.89, 4.92, 5.05, and 5.13 for 0, 25, 50, and 75% demineralization rates, respectively. As hypothesised by Bazinet et al. (*24*) for HCl chemical acidification, the salts added would conduct to a salting-in effect and consequently to a delay in precipitation, whereas in the case of electroacidification, the migration of salts from the protein solution by electrochemical demineralization would favor the precipitation of proteins.

Table 2. Chemical Composition of Casein Isolates Produced at Different Demineralization Rates

	0%	25%	50%	75%
protein (% dry wt)	92.1 ± 0.9	92.1 ± 2.1	93.4 ± 1.3	90.5 ± 3.3
ash (g/100 g of protein)	4.52 ± 0.25	4.24 ± 0.13	4.22 ± 0.24	4.85 ± 0.75
lactose (g/100 g of protein)	4.07 ± 0.75	2.19 ± 0.94	1.72 ± 0.25	1.67 ± 0.94
sodium (mg/100 g of protein)	1333 ± 137	1335 ± 18	1153 ± 127	1555 ± 179
potassium (mg/100 g of protein)	39 ± 3	26 ± 6	21 ± 3	28 ± 3
magnesium (mg/100 g of protein)	9 ± 3	7 ± 3	11 ± 1	9 ± 4
calcium (mg/100 g of protein)	197 ± 40	177 ± 40	306 ± 24	279 ± 105
κ-casein (% of total peak area)	14.7 ± 0.3	14.4 ± 1.0	16.6 ± 2.4	13.9 ± 0.5
α_s-casein (% of total peak area)	37.3 ± 0.1	37.0 ± 0.2	36.8 ± 1.0	37.9 ± 0.5
β-casein (% of total peak area)	48.0 ± 0.4	48.1 ± 0.5	46.6 ± 1.5	47.8 ± 0.6
whey protein (% of total peak area)	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.4 ± 0.4

Table 3. Physicochemical and Functional Properties of Casein Isolates Produced at Different Demineralization Rates and Relative Energy Consumption

	0%	25%	50%	75%
viscosity (mL/g)	61.2 ± 5.1	60.4 ± 5.7	73.5 ± 21.0	60.8 ± 10.5
foaming capacity (min)	3.51 ± 0.31	3.20 ± 0.26	3.19 ± 0.28	3.62 ± 0.55
foaming capacity (mL added)	13.3 ± 0.6	13.2 ± 0.5	13.8 ± 0.2	13.7 ± 0.7
foam stability (mL recovered)	3.5 ± 0.3	3.7 ± 0.4	3.7 ± 0.7	3.5 ± 0.3
protein load (mg/m^2)	10.6 ± 2.2	10.0 ± 0.9	11.5 ± 1.3	25.9 ± 0.1
interfacial area (m^2/mL of emulsion)	0.63 ± 0.02	0.59 ± 0.02	0.63 ± 0.03	0.62 ± 0.02
emulsion stability (m^2/mL emulsion)	1.01 ± 0.09	1.01 ± 0.01	1.42 ± N/A	0.92 ± 0.22
solubility as a function of pH				
amplitude (% unit)	95.5	99.7	96.5	94.3
soluble protein at pH_i (%)	0.82	0.22	−0.04	0.72
inflection point	4.97	4.98	5.01	5.00
transition width (pH unit)	0.085	0.074	0.081	0.082
R^2	0.991	0.994	0.995	0.987
energy (kWh/kg of isolate)	1.02	1.61	1.92	3.08

Protein Profile. The repeated measure analyses of variance showed a highly significant effect of pH on κ-casein ($P < 0.0001$), α_s-casein ($P < 0.0001$), β-casein ($P < 0.0001$), and whey protein concentrations ($P < 0.0001$) and of double-interaction pH/demineralization rate on κ-casein ($P < 0.0001$), α_s-casein ($P < 0.0001$), and β-casein ($P < 0.0001$).

For casein fractions, their percentages in the supernatant decreased as the acidification progressed and were influenced by the demineralization rate. The precipitation of these fractions took place at a higher pH value when the demineralization rate was increased. For the whey protein fraction, its percentage was stable until the casein precipitated, and a small part of the whey protein fraction coprecipitated with the casein. The precipitation of this small part with casein would also be influenced by the demineralization rate because the probability level of the double-interaction pH/demineralization rate ($P > 0.0540$) was close to the 5% acceptance level. This coprecipitation was the result of the precipitation of a β-lactoglobulin−α-lactalbumin−κ-casein complex formed during pasteurization heat treatment (*25*, *26*).

These results were in accordance with previous results obtained for the precipitation kinetics and gave more information on each casein fraction precipitation.

Casein Isolate Composition. The analyses of variance showed no significant effect of demineralization rate on protein ($P > 0.4731$), ash ($P > 0.2831$), magnesium ($P > 0.3688$), and calcium concentrations ($P > 0.0908$), whereas a significant effect was shown on lactose ($P < 0.0179$), potassium ($P < 0.0019$), and sodium concentrations ($P < 0.0331$). The analyses of variance showed no significant effect of demineralization rate on percent κ-casein ($P > 0.1406$), α_s-casein ($P < 0.1265$), β-casein ($P > 0.2046$), and whey protein fractions ($P < 0.3764$).

The protein and ash content of isolates produced in the different conditions were the same with respective values of 92.0 ± 2.1 and 4.45 ± 0.44% (Table 2). In the same way, the mineral composition of the isolates in magnesium and calcium were the same whatever the conditions in which they were produced with respective averaged concentrations of 9.1 ± 3.0 and 239 ± 77 mg/100 g of protein for magnesium and calcium (Table 2). The lactose content was decreased by 58.9% with an increase in demineralization from 0 to 75%. Sodium and potassium concentrations first decreased by 13.5 and 46.1%, respectively, with an increase in demineralization rate from 0 to 50%, and thereafter increased by 34.8 and 33.4%, respectively, with a 75% demineralization rate (Table 2). In the isolates produced after BMEA of the different demineralized skim milk solutions, the percentages of κ-casein, α_s-casein, β-casein, and whey protein fractions were the same with respective values of 14.9, 37.3, 47.6, and 0.2% total peak area (Table 2).

The protein content of the isolates was not influenced by the demineralization rate, and monovalent cations, which are the more mobile ions, were removed efficiently from the skim milk solution. However, sodium and potassium content increased in the isolate after a demineralization rate >50%. Moreover, the efficiency of lactose removed during curd washing was increased with an increase in demineralization rate. During the precipitation of the casein relatively large amounts of lactose are trapped within the curd, and this prevents their removal during washing of the curd (*27*). Sufficient holding time during each washing stage is therefore required to allow diffusion of the lactose from the curd into the wash water (*5*). Because the size of the casein

micelles was reduced during demineralization (*28*), lactose diffusion during the washing step of the curd was facilitated and explained the lower content of lactose for a 75% demineralized BMEA isolate.

Physicochemical and Functional Properties of Casein Isolates. Results of analyses of variance showed that the viscosity ($P > 0.5334$), interfacial area ($P > 0.1638$), emulsion stability ($P > 0.0651$), foaming capacity expressed in minutes ($P > 0.4292$), foaming capacity expressed in milliliters of protein solution added ($P > 0.4578$), and foam stability ($P > 0.8521$) were unchanged whatever the demineralization rate. The analyses of variance showed a significant effect of demineralization rate on protein load ($P < 0.0015$). The repeated measure analyses of variance indicated no significant effect of demineralization rate ($P > 0.1960$) on the solubility of isolate produced as a function of pH.

Except for protein load, the emulsifying properties of the isolates produced from milk solutions demineralized at different rates were unchanged (Table 3). Averaged interfacial area was 0.617 ± 0.027 m^2/mL, and averaged emulsion stability was 1.04 ± 0.17 m^2/mL. The protein load of the isolates produced at different demineralization rates increased in an quadratic fashion ($P < 0.0032$); the protein load was stable between 0 and 25, with respective values of 10.5 and 10.0 mg/m^2, increased slightly to 11.5 mg/m^2 from 25 and 50% demineralization, and reached a value of 25.9 mg/m^2 at 75% demineralization. It can then be hypothesized that the demineralization rate would allow a more complete unfolding of the protein.

Demineralization by electrodialysis prior to bipolar membrane electroacidification, except on protein load, did not influence the functional and physicochemical properties of isolates produced by BMEA.

CONCLUSION

The ED phase to demineralize the skim milk solution was very efficient. However, the electroacidification parameters were modified by the demineralization level of the skim milk solution: the energy efficiency was decreased with an increase in demineralization, but it was still possible to perform BMEA at a very low conductivity level.

The differences observed between milks electroacidified at different demineralization rates can be explained mainly by an increase in electrostatic repulsion due to a decrease in ionic strength. These results confirm that in the case of HCl chemical acidifications, the salts added would conduct to a salting-in effect, and consequently to a delay in precipitation, whereas in the case of electroacidification, the migration of salts from the protein solution by electrochemical demineralization would favor the precipitation of proteins.

The isolates produced by BMEA after electrodialysis demineralization at different rates showed similar chemical composition, except on potassium and lactose contents for 75% demineralized isolate. These isolates, except on protein load for 75% demineralization rate, showed similar physicochemical and functional properties, whatever the demineralization rate.

Further works are currently under way and focus on whey composition and the economic feasability of demineralizing milk before electroacidification.

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