JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 6875−6881 **6875**

Effects of Type of Added Salt and Ionic Strength on Physicochemical and Functional Properties of Casein Isolates Produced by Electroacidification

LAURENT BAZINET,*,† CHRISTINE GENDRON,† DENIS IPPERSIEL,† JOSÉE RENÉ-PARADIS,† CLAUDIA TÉTREAULT,†,§ JOCELYNE BEAUDRY,§ MICHEL BRITTEN,† BEHZAD MAHDAVI,§ JEAN AMIOT,‡ AND FRANÇOIS LAMARCHE†

Centre de Recherche et de Développement sur les Aliments, Agriculture et Agro-Alimentaire Canada, 3600 Boulevard Casavant Ouest, St. Hyacinthe, Québec, Canada J2S 8E3, Centre de Recherche en Sciences et Technologie du Lait (STELA), Pavillon Paul-Comtois, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4, and Laboratoires des Technologies Électrochimiques et des Électrotechnologies d'Hydro-Québec, 600 Avenue de la Montagne, Shawinigan, Québec, Canada G9N 7N5

A procedure developed for soybean protein precipitation which was based on electrodialysis was tested for the production of acid casein from reconstituted skim milk. In a previous paper, the performance of bipolar membrane electroacidification (BMEA) was evaluated under different conditions of ionic strength (μ_{added} = 0, 0.25, 0.5, or 1.0 M) and added salt ($CaCl_2$, NaCl, or KCl) (*1*). The aim of this study, which is the complement of the work on evaluation of BMEA performance, was to evaluate the functionality of the protein isolates produced by BMEA and to compare the BMEA isolates to commercial isolates and an isolate produced by chemical acidification. It was not possible to show differences between the functional properties of isolates produced by BMEA, except at 1 M $CaCl_2$ μ_{added}, due to the variability of the isolates. However, the results showed that it is possible to obtain isolates similar to commercial isolates and that the addition of salt during the process does not induce variations in functional properties. From results on mineral concentrations, it appeared that the addition of monovalent cations did not influence the retention of monovalent or divalent cations in the BMEA isolates, while addition of divalent cations ($CaCl_2$) influenced the retention of magnesium. According to previous results on evaluation of BMEA performances under different conditions of ionic strength and added salt, the difference observed for the BMEA isolate produced at 1.0 M $CaCl_2$ was confirmed.

KEYWORDS: Electrochemical acidification; bipolar membrane; casein; salt; ionic strength; functional properties; physicochemical composition

INTRODUCTION

Bipolar membrane electroacidification (BMEA), a technology using a combination of electrodialytic phenomena and bipolar membranes (BPM), has been used previously to produce soybean protein isolates having chemical compositions and functional properties comparable or superior to those of commercial isolates (*2*). Therefore, BMEA was also studied for the production of casein isolates (*3*).

BMEA uses the property of BPM to split water and the action of cation-exchange membrane (CEM) to demineralize. Briefly, when a current is passed accross a bipolar membrane, electrical conduction is achieved by the transport of H^+ and OH^- generated by electrodissociation of water molecules at the interface of the BPM (*4*). The pH will decrease in a solution circulating in an electrodialysis cell on the cationic side of the BPM, and demineralization will be achieved by the electrodialytic process. Compared to conventional isoelectric precipitation technologies, the generation of H^+ can be precisely controlled in BMEA by the regulation of current density. BMEA also reduces environmental risks related to the handling and transportation of dangerous chemicals such as acids and bases (*5*).

As in any electrochemical process, the products to be treated by BMEA must possess a relatively high mineral content to allow good electrical conductivity and to reduce the global resistance of the electrodialysis cell. Recently, Bazinet et al. (*6*) demonstrated that the electrical efficiency of skim milk electroacidification is decreased because of a lack of sufficiently mobile ions such as potassium. They suggested adding a certain

* Address correspondence to this author at STELA, Pavillon Paul-Comtois, Université Laval, Sainte-Foy, PQ, Canada G1K 7P4 [telephone (418) 656-2131; fax (418) 656-3353; e-mail laurent.bazinet@aln.ulaval.ca]. Fax: 450-773-8461. E-mail: Bazinet_L@yahoo.com.
† Agriculture et Agro-Alimentaire Canada.
§ Hydro-Québec.
‡ Université Laval.

10.1021/jf025650w CCC: $22.00
Published on Web 10/05/2002

amount of salt to the skim milk solution in order to obtain better electrical efficiency. Consequently, in a previous paper the performance of BMEA, in terms of electrodialysis cell parameters, percent protein precipitated, and energy consumption, was evaluated under different conditions of ionic strength (μ_{added} = 0, 0.25, 0.5, or 1.0 M) and added salt ($CaCl_2$, NaCl, or KCl) (*1*). KCl and μ_{added} = 0.5 M was the best combination, with a 45% decrease in energy consumption and no modification in the protein composition of the isolate.

However, according to Cayot and Lorient (*7*), changes in the composition of the surrounding media lead to modifications and to variation in protein functional properties. The functional properties are the microscopic expression of the capacity of a protein to modify its conformation in response to an environmental change. Hence, an increase in ionic strength decreases the ionic interactions and consequently the solubility profile of the protein. In the same way, the presence of divalent ions, in high concentration, may lead to important aggregation during technological treatments with negative technological consequences (*7*).

Therefore, the aim of this work, which is the complement of the previous work on evaluation of BMEA performance (*1*), was (1) to analyze the chemical composition of BMEA isolates produced under different conditions of salts and ionic strength added, (2) after dialysis, to evaluate the effects of increasing ionic strength and added salts during BMEA on the functionality of protein isolates, and (3) to compare the dialyzed BMEA isolates to commercial isolates and to an isolate produced by chemical acidification in our laboratory and prepared under the same conditions as the BMEA isolates.

MATERIALS AND METHODS

Materials. Casein isolates (CAS) were produced by electroacidification. The electroacidifications described in a previous study were carried out under different conditions of added salt ($CaCl_2$, NaCl, or KCl) and ionic strength of the skim milk solution (μ_{added} = 0, 0.25, 0.5, or 1.0 M) (*1*). The raw material used for BMEA was reconstituted milk (10%, w/v) from low-temperature spray-dried skim milk powder (Agropur, Granby, Canada). Two replicates of each combination of added salt and ionic strength were performed in this experiment. At the end of each run, about 2.5 L samples of the electroacidified milk solution were collected. 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.

Three commercial sodium caseinates were purchased: New Zealand isolate (Alanate 180) from New Zealand Milk Products Inc. (Santa Rosa, CA), Sigma isolate (C 3400) from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada), and UFL isolate from Les Aliments UFL Inc. (Montréal, QC, Canada).

Sodium caseinate was produced by chemical acidification with HCl (1.000 N, VWR Canlab, Ville Mont-Royal, QC, Canada) at the laboratory and prepared under the same conditions as the electrochemical CAS.

Methods. *(a) Sample Preparation before Analyses.* Chemical analyses (protein, ash, and lactose contents; moisture; sodium, calcium, magnesium, and potassium concentrations) were performed on each BMEA CAS. For each CAS, 250 mL aliquots of 5% (w/v) protein solutions were dialyzed (1/100) for 18 h against 0.01 M sodium phosphate buffer (pH 6.8) to reduce the influence of salt composition on functional properties. Chemical analyses (ash and lactose contents; sodium, calcium, magnesium, and potassium concentrations) and determinations of the physicochemical (particle size and specific viscosity) and functional properties (foaming capacity and stability, emulsion stability, protein load, and solubility as a function of pH) of each dialyzed CAS were performed. In addition, the properties of BMEA CAS were also compared with those of commercial and chemically produced CAS.

(b) Methods of Analysis. Protein Content. Protein content was determined, using an FP-428 LECO apparatus (LECO Co., Saint-Joseph, MI), according to the conditions and parameters used by Bazinet et al. (*3*), on each of the BMEA, commercial, and chemically produced CAS isolate powders.

Lactose Concentration. Protein solutions (5%, w/v) were acidified with 1.0 N HCl until precipitation of proteins was observed. 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 onto an Ion-300 column (Mandel Scientific Co., Rockwood, ON, Canada) connected to a HPLC (Waters Associates, Milford, MA) equipped with a UV detector (210 nm) (model 490, Waters) and a refractive index detector (model R410, Waters) according to the method of Doyon et al. (*8*). A 0.0054 N H_2SO_4 solution was used as the mobile phase at a flow rate of 0.4 mL min^{-1}. The concentration of lactose was determined using a commercial D-lactose solution (Sigma Chemical Co., St. Louis, MO) of known concentration.

Moisture. Water content was measured according to AOAC method no. 927-05 (*9*).

Ash Content. Ash content was determined according to AOAC methods no. 930-30 (*10*) and 945-46 (*11*).

Potassium, Sodium, Magnesium, and Calcium Concentration. 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 (*6*). The analyses were carried out in radial view. Solutions were prepared from known weight skim milk ash dissolved in 10 mL of HCl (2N) and diluted with HCl (2 N) to be within the calibration ranges for each cation.

Specific Viscosity. Ten milliliters of 4% (w/v) protein solution was introduced into a calibrated viscometer size 100 (Cannon-Fenske routine viscosimeter, Cannon Instrument, VWR, Ville-Mont-Royal, QC, Canada) placed at 25 °C in a thermostated 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 under 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:

$$\eta_{sp} = \frac{\eta_r - 1}{[\text{Prot}]}$$

where η_{sp} is the specific viscosity (milliliters per gram), η_r the relative viscosity of the protein solution, and [Prot] the protein concentration of the solution (grams per milliliter).

Protein Load of the Emulsions. Oil-in water emulsions (33%, v/v) were produced by mixing commercial corn oil (Mazola) and protein solution (4%, w/v) with a Polytron (model PT 10-35, probe PTA 10S, Kinematica AG, Littau, Switzerland) for 30 s at 9000 rpm and homogenizing at a pressure of 70 mPa with an Emulsiflex-C5 valve homogenizer (Avestin, Ottawa, ON, Canada). Protein load was calculated from protein depletion in the serum phase after emulsion formation, according to Britten and Giroux (*12*). The 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 the method of Bradford (*13*), calibrated with a bovine serum albumin (BSA) standard (Biorad Laboratories Canada Ltd., Mississauga, ON, Canada). Protein load results were expressed as milligram per square meter. Protein concentration depletion in the aqueous phase was divided by the interfacial area (IA) of the emulsion. IA of the emulsion was calculated from the turbidity of diluted emulsions (*14*): emulsions were diluted to a final oil volume fraction 6×10^{-5} in sodium phosphate buffer (0.01 M, pH 7.0) containing 0.5%

sodium dodecyl sulfate (SDS, Biorad Laboratories Canada Ltd., Mississauga, ON, Canada) according to Britten and Giroux (*12*).

Foaming Properties. Foaming properties were measured according to Waniska and Kinsella (*15*). 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^{-1} until the foam volume reached 70 mL. Protein solution was added as required to maintain the volume constant at 15 mL. The 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.

Solubility Profile. Hydrochloric acid (0.2 N) was added gradually to 250 mL of 2% (w/v) protein solution. Aliquots (1.5 mL) 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 the method of Bradford (*13*). Nonlinear regression equations were calculated according to Bazinet et al. (*6*):

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

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, 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.

Particle Size of Caseinate Particles. The particle size was determined, in duplicate, on 0.5−1.0 mL of 0.5% (w/v) protein solution in 2 mL of double-distilled water, with a submicron particle analyzer at 22 °C (model N4MD, Beckman Coulter, Miami, FL). Results are expressed in terms of mean diameter (nanometers).

(c) Statistical Analyses. Using SAS software (*16*), the data from the compositional, physicochemical, and functional analyses of isolates produced by BMEA were submitted to an analysis of variance with regression contrasts to examine the effects of interactions between the variables. The data from the compositional, physicochemical, and functional analyses, except for solubility as a function of pH, for BMEA, commercial, and chemical isolates were subjected to an analysis of variance, with a comparison of averages by Duncan tests, to distinguish different groups between the samples. Nonlinear regression equations were calculated for the data for the solubility as a function of pH using Sigmaplot (Version 3.0 for Windows, Jandel Scientific, Corte Madera, CA), and the model curve parameters were compared.

RESULTS AND DISCUSSION

Chemical Composition of BMEA Isolates. The analyses of variance showed significant effects during BMEA of ionic strength ($P < 0.0002$, $P < 0.0015$, $P < 0.0005$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$ for ash, lactose, protein, calcium, potassium, and magnesium, respectively) and type of added salt ($P < 0.0285$, $P < 0.0001$, and $P < 0.0001$ for lactose, calcium, and potassium, respectively) on the lactose, ash, protein, and mineral content of the isolates produced by BMEA.

Protein. The protein content of the isolates decreased with an increase in ionic strength during BMEA (**Table 1**). All added salts data averaged, the protein contents were 86.8, 80.8, 79.9, and 73.0 g per 100 g of dry isolate at 0, 0.25, 0.5, and 1 M μ_{added}, respectively.

Lactose. The lactose content was influenced by the type of added salts (**Table 1**). The lactose contents (all ionic strength data averaged) of CAS produced with addition of KCl and NaCl were similar, with 9.1 and 9.4 g per 100 g of protein, respectively, but different from that for CAS produced with $CaCl_2$, with 12.6 g per 100 g of protein.

Ash. The ash content increased with an increase in ionic strength (**Table 1**). All added salts data averaged, the ash contents were 5.3, 9.2, 10.6, and 20.1 g per 100 g of protein for 0, 0.25, 0.5, and 1 M, respectively.

Table 1. Protein, Ash, and Lactose Contents of Isolates Produced with Addition, during BMEA Process, of Salt ($CaCl_2$, NaCl, or KCl) at Different Ionic Strengths (μ_{added} = 0, 0.25, 0.5, or 1.0 M)[a]

salt	ionic strength (M)	protein (% dry basis)	ash (g per 100 g of protein)	lactose (g per 100 g of protein)
	0.00	86.8 ab	5.3 cd	6.8 bc
$CaCl_2$	0.25	80.8 bc	10.2 bcd	9.4 abc
	0.50	78.5 bc	11.3 bcd	14.4 ab
	1.00	68.0 d	23.7 a	19.7 a
NaCl	0.25	82.2 bc	8.3 cd	10.0 abc
	0.50	81.1 bc	10.8 bcd	10.7 abc
	1.00	76.0 bcd	16.1 abc	10.0 abc
KCl	0.25	79.4 bc	9.1 cd	10.6 abc
	0.50	80.0 bc	9.7 bcd	7.9 abc
	1.00	74.8 cd	20.4 ab	10.9 abc

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

Table 2. Sodium, Potassium, Magnesium, and Calcium Concentrations of Isolates Produced with Addition, during BMEA Process, of Salt ($CaCl_2$, NaCl, or KCl) at Different Ionic Strengths (μ_{added} = 0, 0.25, 0.5, or 1.0 M)[a]

salt	ionic strength (M)	sodium (mg per 100 g of protein)	potassium (mg per 100 g of protein)	magnesium (mg per 100 g of protein)	calcium (mg per 100 g of protein)
	0.00	1729 a	53 b	8.9 bc	162 cd
$CaCl_2$	0.25	3139 a	125 b	9.2 bc	562 c
	0.50	2544 a	235 b	15.4 abc	1037 b
	1.00	3773 a	625 b	30.3 a	4972 a
NaCl	0.25	2649 a	99 b	13.8 abc	273 cd
	0.50	3061 a	166 b	14.3 abc	293 cd
	1.00	5077 a	279 b	16.5 abc	254 cd
KCl	0.25	2299 a	511 b	18.8 ab	293 cd
	0.50	1930 a	712 b	17.3 abc	274 cd
	1.00	2409 a	4980 a	17.7 abc	252 cd

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

Sodium, Potassium, Calcium, and Magnesium Concentrations. For calcium and potassium, the final concentrations in the isolate depended on the ionic strength and type of added salts (**Table 2**). As the ionic strength increased from 0 to 1 M (all added salts data averaged), the final concentrations of calcium and potassium increased highly, but the concentration (all ionic strength data averaged) of calcium in the isolates was higher with addition of $CaCl_2$ (1683 mg per 100 g of protein) than with KCl (245 mg per 100 g of protein) and NaCl (246 mg per 100 g of protein), while potassium concentration was higher with addition of KCl (1564 mg per 100 g of protein) than with $CaCl_2$ (260 mg per 100 g of protein) and NaCl (149 mg per 100 g of protein). For magnesium, the final concentration in the isolate depended on the ionic strength; all added salt data averaged, the magnesium content increased linearly from 8.9 to 21.5 mg per 100 g of protein with an increase in ionic strength from 0 to 1 M. However, this effect is due mainly to the large increase in magnesium retention with the addition of 1 M $CaCl_2$.

It appears that the chemical composition of the isolates produced by BMEA varied mainly according to the ionic strength of the skim milk solution treated. At high concentrations of $CaCl_2$, there was a retention of salts and lactose in the isolates,

thus reducing the total protein content of these isolates. As the isolates were washed under the same conditions whatever the ionic strength, these washing conditions in the case of high calcium and potassium concentration isolates were not sufficient to allow diffusion of most of the salts and lactose from the coagulum. Moreover, the concentration of lactose in the isolates increased with the ionic strength; ionic strength might reduce porosity and increase particle size of the coagulum, thereby decreasing surface contact area with washing solution and thus the effectiveness of lactose removal. Many authors have pointed out that processing variables such as number of washes to which the curd is subjected influence the quality of the rennet casein (*17*−*19*). Moreover, Munro et al. (*17*) noted that poorly washed casein contains a very high lactose content. In addition, for mineral concentrations, the values presented in **Table 2** showed high standard deviations. These high standard deviations were not due to the ICP method, which was very accurate and reproducible, as will be shown later for the mineral content of the dialyzed isolates, but were due to the variability of the isolates produced by BMEA. Since the washing step was not efficient enough, the mineral composition of BMEA isolates was very variable. However, from these results some conclusions could be drawn about the influence of added minerals on monovalent and divalent cation retention in the BMEA isolates. It appeared that the addition of monovalent cations did not influence the retention of monovalent or divalent cations in the BMEA isolates. The addition of divalent cations influenced the retention of divalent cations. More precisely, the magnesium concentration in the BMEA isolate increased with addition of $CaCl_2$. These results mean that during BMEA treatment there was exchange of divalent cations with monovalent cations when KCl or NaCl was added, but exchange of divalent cations, especially magnesium, was lower or absent when $CaCl_2$ was added.

Under such conditions, dialysis of BMEA isolates was necessary to decrease the difference in salt and lactose concentrations between isolates, to study the effects of BMEA conditions on their functional and physicochemical properties.

Effects of Added Salts and Ionic Strength on BMEA Isolate Functionality. After dialysis, the chemical composition and the physicochemical and functional properties of the BMEA isolates were reanalyzed.

(a) Chemical Composition. The analyses of variance showed a significant effect of ionic strength ($P < 0.0001$, $P < 0.0015$, and $P < 0.0005$ for ash, lactose, and calcium, respectively) and type of added salt ($P < 0.0001$, $P < 0.0285$, and $P < 0.0001$ for ash, lactose, and calcium) on the lactose, ash, and mineral content of the isolates produced by BMEA. The regression contrast results demonstrated the significant double effects of added salts and ionic strength on calcium ($P < 0.0001$) and ash ($P < 0.0001$) contents.

Lactose. The lactose content was influenced by the type of added salts (**Table 3**). The lactose contents (all ionic strength data averaged) of CAS produced with addition of KCl and NaCl were similar, with 1.2 and 1.1 g per 100 g of protein, respectively, but different from that of CAS produced with $CaCl_2$, with 1.4 g per 100 g of protein.

Ash. The ash content increased with an increase in ionic strength (**Table 3**). All added salts data averaged, the ash contents were 0.9, 1.2, 1.1, and 1.7 g per 100 g of protein for 0, 0.25, 0.5, and 1 M, respectively.

Sodium, Potassium, Calcium, and Magnesium Concentrations. For calcium, the final concentration in the isolate always depended on the ionic strength and type of added salts (**Table 4**). As the ionic strength increased from 0 to 1 M (all added salts data averaged), the final concentration of calcium increased from 1.3 to 7.7 mg per 100 g protein, but its concentration in the isolates (all data of added ionic strength) was higher with addition of $CaCl_2$ (7.9 mg per 100 g of protein) than with NaCl (1.7 mg per 100 g of protein) and KCl (1.5 mg per 100 g of protein). For sodium, calcium, and magnesium, no more differences were observed for the isolates.

Dialysis of BMEA isolates was very effective since ash and lactose contents, except at high concentrations of $CaCl_2$ added, were decreased to 0.054 and 1.2 g per 100 g of protein, respectively. Dialysis of isolates produced with high concentra-

Table 3. Comparison after Dialysis of Ash and Lactose Contents of BMEA Isolates Produced under Different Conditions of Ionic Strength ($\mu_{added} = 0$, 0.25, 0.5, or 1.0 M) and Added Salt ($CaCl_2$, NaCl, or KCl), of Commercial Isolates (New Zealand, Sigma, and UFL), and of an Isolate Produced by Chemical Acidification and Washed under the Same Conditions as the BMEA Isolates[a]

salt	ionic strength (M)	ash (g per 100 g of protein)	lactose (g per 100 g of protein)
	0.00	0.056 c	0.9 ab
$CaCl_2$	0.25	0.057 bc	1.2 ab
	0.50	0.065 b	1.3 ab
	1.00	0.090 a	2.1 a
NaCl	0.25	0.053 cd	1.2 ab
	0.50	0.054 cd	1.2 ab
	1.00	0.053 cd	1.2 ab
KCl	0.25	0.050 cde	1.1 ab
	0.50	0.052 cde	0.9 ab
	1.00	0.057 cb	1.8 a
chemical		0.052 cde	1.1 ab
New Zealand		0.053 cd	0.1 b
Sigma		0.043 e	0.0 b
UFL		0.046 de	0.2 b

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

Table 4. Comparison after Dialysis of Sodium, Potassium, Magnesium, and Calcium Concentrations of BMEA Isolates Produced under Different Conditions of Ionic Strength ($\mu_{added} = 0$, 0.25, 0.5, or 1.0 M) and Added Salt ($CaCl_2$, NaCl, or KCl), of Commercial Isolates (New Zealand, Sigma, and UFL), and of an Isolate Produced by Chemical Acidification and Washed under the Same Conditions as the BMEA Isolates[a]

salt	ionic strength (M)	sodium (mg per 100 g of protein)	potassium (mg per 100 g of protein)	magnesium (mg per 100 g of protein)	calcium (mg per 100 g of protein)
	0.00	15.7 a	0.54 a	< dl[b] a	1.3 c
$CaCl_2$	0.25	11.8 ab	0.61 a	< dl a	3.6 bc
	0.50	12.3 ab	0.57 a	0.06 a	7.3 b
	1.00	10.9 ab	0.73 a	< dl a	19.5 a
NaCl	0.25	12.5 ab	0.54 a	< dl a	1.7 c
	0.50	14.9 ab	0.51 a	0.02 a	1.7 c
	1.00	13.9 ab	0.89 a	0.03 a	1.9 c
KCl	0.25	13.8 ab	0.96 a	< dl a	1.6 c
	0.50	14.3 ab	0.70 a	< dl a	1.4 c
	1.00	13.9 ab	0.96 a	< dl a	1.7 c
chemical		13.5 ab	0.84 a	0.06 a	1.7 c
New Zealand		15.2 ab	0.64 a	< dl a	1.1 c
Sigma		11.2 ab	0.51 a	< dl a	0.7 c
UFL		10.6 b	1.17 a	< dl a	0.5 c

[a] Means within a column followed by different letters are significantly different ($P < 0.05$). [b] dl = detection limit.

Table 5. Comparison after Dialysis of Viscosity and Particle Size of BMEA Isolates Produced under Different Conditions of Ionic Strength (μ_{added} = 0, 0.25, 0.5, or 1.0 M) and Added Salt ($CaCl_2$, NaCl, or KCl), of Commercial Isolates (New Zealand, Sigma, and UFL), and of an Isolate Produced by Chemical Acidification and Washed under the Same Conditions as the BMEA Isolates[a]

salt	ionic strength (M)	particle size (nm)	specific viscosity (mL g^{-1} of protein)
	0.00	291.0 ab	60.62 b
$CaCl_2$	0.25	259.0 abcd	53.60 b
	0.50	185.0 d	48.88 b
	1.00	341.5 a	79.43 a
NaCl	0.25	278.0 abc	56.84 b
	0.50	269.2 abc	57.69 b
	1.00	262.0 abcd	51.20 b
KCl	0.25	277.5 abc	57.14 b
	0.50	280.0 abc	58.48 b
	1.00	254.2 bcd	52.69 b
chemical		285.8 ab	57.46 b
New Zealand		270.5 abc	55.11 b
Sigma		198.2 cd	55.73 b
UFL		286.2 ab	63.26 ab

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

Table 6. Comparison after Dialysis of Foaming Properties and Protein Load of BMEA Isolates Produced under Different Conditions of Ionic Strength (μ_{added} = 0, 0.25, 0.5, or 1.0 M) and Added Salt ($CaCl_2$, NaCl, or KCl), of Commercial Isolates (New Zealand, Sigma, and UFL), and of an Isolate Produced by Chemical Acidification and Washed under the Same Conditions as the BMEA Isolates[a]

salt	ionic strength (M)	foaming capacity (min)	foaming capacity (mL)	foam stability (mL)	protein load (mg m^{-2})
	0.00	3.24 a	13.65 a	4.03 a	17.4 ab
$CaCl_2$	0.25	3.80 a	13.55 a	3.66 ab	14.5 bc
	0.50	3.60 a	14.15 a	3.89 a	18.7 ab
	1.00	3.22 a	11.85 a	3.60 ab	24.8 a
NaCl	0.25	3.67 a	13.10 a	3.86 a	14.3 bc
	0.50	3.65 a	13.80 a	3.97 a	13.7 bc
	1.00	3.64 a	12.75 a	3.84 a	16.9 ab
KCl	0.25	3.54 a	12.05 a	3.64 ab	6.9 c
	0.50	3.28 a	13.30 a	4.09 a	15.6 b
	1.00	3.58 a	12.70 a	3.89 a	14.2 bc
chemical		3.27 a	12.30 a	3.72 ab	12.5 bc
New Zealand		3.47 a	9.97 ab	3.25 ab	15.5 b
Sigma		3.71 a	10.14 ab	3.17 ab	18.5 ab
UFL		3.62 a	6.45 b	2.53 b	10.7 bc

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

tion of $CaCl_2$ was incomplete. This could be due to the large size of casein aggregates, which prevented rapid diffusion of lactose and ions during the process.

(b) Physicochemical and Functional Properties. Results of analyses of variance performed on foaming capacity ($P > 0.5489$ for foaming capacity expressed in min. and $P > 0.4578$ in mL) and foaming stability ($P > 0.3754$) showed no significant effect of added salts or ionic strength. The analyses of variance showed significant effects of ionic strength ($P < 0.0001$, $P < 0.0011$, and $P < 0.0029$ for viscosity, particle size, and protein load) and of added salts ($P < 0.025$ and $P < 0.035$ for viscosity and protein load) on some functional properties of the isolates produced by BMEA. The regression contrast results demonstrated the significant double interaction between added salts and ionic strength ($P < 0.0006$ and $P < 0.002$ for viscosity and particle size, respectively).

Particle Size. For KCl and NaCl, the particle size decreased in a similar manner with an increase in ionic strength from 0 to 1 M, by 12.7% and 10.0%, respectively (**Table 5**). With addition of $CaCl_2$, the particle size decreased by 36.4% between 0 and 0.5 M μ_{added} and increased back at 1 M μ_{added}.

Specific Viscosity. The viscosity was influenced by the type of added salt and the ionic strength (**Table 5**). The viscosities of isolates electroacidified with addition of NaCl and KCl were similar, with averaged values of 56.6 and 57.2 mL g^{-1}, respectively, while the viscosity for isolates produced with addition of $CaCl_2$ decreased by 19.4% with an increase in ionic strength from 0 to 0.5 M, and increased by 62.5% between 0.5 and 1 M μ_{added}.

Protein Load. The protein load was influenced by the type of added salt and the ionic strength (**Table 6**). With addition of NaCl, KCl, and $CaCl_2$, the protein load (all ionic strength data averaged) were different, with averaged values of 15.6, 13.5, and 18.8 mg m^{-2}, respectively. Furthermore, the isolates produced with addition of $CaCl_2$ at 1 M μ_{added} and KCl at 0.25 M μ_{added} presented higher and lower portein loads, respectively, while the others isolates showed similar protein load values of 15.7 mg m^{-2}.

Table 7. Model Curve Parameters of Solubility as a Function of pH after Dialysis of BMEA Isolates Produced under Different Conditions of Ionic Strength and Added Salt ($CaCl_2$, NaCl, or KCl), of Commercial Isolates (New Zealand, Sigma, and UFL), and of an Isolate Produced by Chemical Acidification and Washed under the Same Conditions as the BMEA Isolates

salt	ionic strength (M)	soluble protein at pH_i	amplitude	center	width	R^2
	0.00	0.181	17.7	5.18	0.102	0.997
$CaCl_2$	0.25	0.213	16.8	5.12	0.095	0.999
	0.50	0.289	17.3	5.10	0.094	0.998
	1.00	0.905	11.5	5.21	0.013	0.982
NaCl	all ionic strengths averaged	0.314	16.8	5.15	0.101	0.994
KCl	all ionic strengths averaged	0.250	16.5	5.14	0.108	0.986
chemical		0.104	15.4	5.05	0.148	0.960
New Zealand		0.690	17.0	5.19	0.013	0.991
Sigma		0.159	18.0	5.16	0.083	0.998
UFL		0.149	17.1	5.18	0.099	0.999

Solubility. The addition of KCl or NaCl prior to BMEA treatment influenced the solubility of the protein solution in a similar way whatever the ionic strength (**Table 7**). Soluble protein at the isoelectric point, amplitude, inflection point, and width of the curves were similar for both salts. With addition of $CaCl_2$, the solubility profiles were different according to the ionic strength. For ionic strength ranging from 0 to 0.5 M, the curves were similar, with curve parameters comparable to those of monovalent salts (**Table 7**). However, the solubility profile at 1 M μ_{added} was different. Soluble protein at isoelectric point, amplitude, inflection point, and width of the curves were 0.227 vs 0.905, 17.3 vs 11.5, 5.13 vs 5.21, and 0.097 vs 0.013, respectively, for averaged μ_{added} parameters from 0 to 0.5 and 1 M.

In this study, dialysis of isolates produced by BMEA was efficient to standardize the mineral composition of caseinate and allowed the production of sodium caseinate, except in the case of addition of $CaCl_2$ at 1 M μ_{added}. For this particular isolate, the higher concentration of calcium explained the formation of aggregate responsible for the lower solubility of the isolate and the higher viscosity and particle size. The relatively high content of calcium after dialysis decreased the solubility of phosphoproteins and consequently of the final isolate (*7*). This result confirmed the fact, previously noted, that the coagulum of the BMEA isolate produced with addition of $CaCl_2$ at 1 M μ_{added} was more difficult to wash since the viscosity and the particle size of the caseinate were higher, consequently decreasing the salt diffusion.

These results agree with data in the literature. As the isolate produced by BMEA with addition of 1 M $CaCl_2$ had a high calcium content, this caseinate showed functional and physicochemical properties similar to those of a calcium/sodium mixed caseinate (*20*). It is recognized that calcium or mixed caseinates form colloidal dispersions in water rather than solutions, with a lower solubility than sodium and potassium caseinates (*21*). Mulvihill and Fox (*22*) reported that calcium caseinate exists in water as large aggregates (artificial casein micelles) that are stable at pH > 5.5, if the calcium concentration is not excessive. In the same way, Bastier et al. (*20*) showed that the calcium content was negatively correlated to the solubility index, rate of water absorption, and apparent viscosity.

Comparison of BMEA Isolates with Commercial and Chemically Produced Isolates. The chemical compositions and the physicochemical and functional properties of the dialyzed BMEA isolates were compared to those of commercial and chemically produced isolates.

(a) Chemical Composition. The analyses of variance of the data for lactose ($P < 0.0001$), ash ($P < 0.0001$), potassium ($P < 0.0001$), and calcium ($P < 0.0001$) contents indicated at least one significant difference between the isolates.

Lactose. Two main groups could be distinguished (**Table 3**), indicating a difference in final lactose concentration. The three commercial isolates had similar lactose content, at 0.1 g per 100 g of protein, while chemical and BMEA isolates had higher lactose concentrations, at about 1.3 g per 100 g of protein. In the second group of BMEA and chemical isolates, the 1 M KCl and $CaCl_2$ conditions gave the highest concentrations of lactose.

Ash. Four main groups could be distinguished (**Table 3**), indicating a difference in ash content. The Sigma and UFL isolates had the lowest ash content (0.045 g per 100 g of protein), the 1 M $CaCl_2$ isolate had the highest ash content (0.090 g per 100 g of protein), the 0.5 M $CaCl_2$ isolate had an intermediate ash content (0.065 g per 100 g of protein), and the other isolates appeared to have the same ash content, at about 0.054 g per 100 g of protein.

Sodium, Potassium, Calcium, and Magnesium Concentrations. Three main groups could be distinguished (**Table 4**), indicating a difference in calcium final concentration. The 1 M $CaCl_2$ isolate had the highest calcium content (19.5 mg per 100 g of protein), the 0.5 and 0.25 M $CaCl_2$ had intermediate calcium concentrations (7.3 and 3.6 mg per 100 g of protein, respectively), and the other isolates appeared to have the same calcium concentration, at about 1.4 mg per 100 g of protein.

(b) Physicochemical and Functional Properties. The analyses of variance of the particle size ($P < 0.0004$), viscosity ($P < 0.0001$), foaming capacity (mL) ($P < 0.0001$), protein load ($P < 0.0055$), and solubility ($P < 0.0001$) data indicated at least one significant difference between the isolates. No significant differences were observed between the isolates in terms of the foaming capacity (minutes) ($P > 0.3170$) and foam stability ($P > 0.1660$).

Particle Size. Three main groups could be distinguished (**Table 5**), indicating a difference in particle size. The 1 M $CaCl_2$ isolate had the highest particle size (341.5 nm), the Sigma and 0.5 M $CaCl_2$ had the lowest particle size (191.6 nm), and all the other isolates had similar intermediate particle size (about 274.0 nm).

Specific Viscosity. Two main groups could be distinguished (**Table 5**). The 1 M $CaCl_2$ isolate had the highest viscosity (79.4 mL g^{-1}), while all other isolates had similar viscosity of about 56.0 mL/g. Moreover, it appeared that the 1 M KCl and NaCl and 0.5 M $CaCl_2$ had the lowest viscosities of the second group.

Protein Load. Three main groups could be distinguished (**Table 6**), indicating a difference in protein load. The 1 M $CaCl_2$ isolate had the highest protein load (24.8 mg m^{-2}), the 0.25 M KCl had the lowest protein load (6.9 mg m^{-2}), and all the other isolates had similar intermediate protein load (approximately 15.2 mg m^{-2}).

Solubility. Three main groups could be distinguished (**Table 7**). The 1 M μ_{added} $CaCl_2$ isolate had the lowest solubility (amplitude of 11.5 mg mL^{-1} and soluble protein at the pH_i of 0.905 mg mL^{-1}), the chemical isolate had intermediate solubility (amplitude of 15.4 mg mL^{-1} and soluble protein at the pH_i of 0 mg mL^{-1}), and all the other isolates had higher solubility (averaged amplitude of 17.2 mg mL^{-1} and soluble protein at pH_i of 0.280 mg mL^{-1}).

Foaming Properties. The foaming capacity, expressed in minutes, was the same for all the isolates, with a duration of 3.63 min (**Table 6**). In the same way, the foam stability was similar for all the isolates with a quantity of protein solution withdrawn of 3.80 mL. However, the UFL isolate had a lower foam stability in comparison with the others. For the foaming capacity (milliliters), three main groups could be distinguished. The UFL isolate had the lowest foaming capacity (6.45 mL), the Sigma and New Zealand isolates had an intermediate foaming capacity (10.05 mL), and all the other isolates had an averaged foaming capacity of 13.0 mL.

Although the chemical compositions of the isolates were different, three main groups could be defined on the basis of all data concerning the functional properties. The first group is composed of the 1 M $CaCl_2$ μ_{added} isolate with high viscosity, low solubility, high protein load, and intermediate foaming properties. The second group is composed of the UFL isolate with intermediate viscosity, high solubility, high foaming properties, and intermediate protein load. The third group is composed of all other isolates with intermediate viscosity, high solubility, and intermediate foaming properties and protein load.

CONCLUSIONS

The isolates produced by BMEA with different added salts and ionic strengths, except at 1 M $CaCl_2$ μ_{added}, showed physicochemical and functional properties similar to those of the chemical, New Zealand, and Sigma isolates. However, the small differences in foaming properties in comparison with the UFL isolate seem to result from a different mineral content, coming from a different isolate preparation procedure. It was not possible to show differences between functional properties of isolates produced by BMEA due to the variability of the isolates. However, the results showed that it is possible to obtain isolates similar to commercial isolates and that the addition of salt was not directly linked to variations in functional properties. An interesting complementary finding from this study is that

the addition of monovalent and divalent cations influenced their retention in the BMEA isolates. During BMEA treatment, there was exchange of divalent cations with monovalent cations when KCl or NaCl was added, but exchange of divalent cations, especially magnesium, was low or absent when $CaCl_2$ was added.

If these data on physicochemical and functional properties are related to the BMEA performances under the different conditions of ionic strength and added salt, the difference observed for the BMEA isolate produced at 1.0 M $CaCl_2$ is confirmed. Furthermore, since the protein compositions of the BMEA isolates produced with KCl and NaCl, and the ash and lactose contents of these isolates, were similar after dialysis, this explains why no real differences were observed in the functionalities of these isolates.

These results confirm that BMEA is a "gentle" and an environmentally friendly technology with a wide-ranging application potential, and they give a further explanation and more information about this technology. Furthermore, to obtain functional property values comparable with those of commercial standards, it will be necessary to add one or more steps of washing of BMEA isolates in order to obtain their real and representative chemical composition and functional properties.

ACKNOWLEDGMENT

The authors thank Mr. Christopher Barr for reviewing the manuscript.

LITERATURE CITED

(1) Bazinet, L.; Ippersiel, D.; Gendron, C.; Mahdavi, B.; Amiot, J.; Lamarche, F. Effect of added salt and increase in ionic strength on skim milk electroacidification performances. *J. Dairy Res*. **2001**, *68* (2), 237−250.

(2) Bazinet, L.; Lamarche, F.; Labrecque, R.; Toupin, R.; Boulet, M.; Ippersiel, D. Electro-acidification of soybean proteins for the production of isolate. *Food Technol*. **1997**, *51* (9), 52−56, 58, 60.

(3) Bazinet, L.; Lamarche, F.; Ippersiel, D.; Amiot, J. Bipolar membrane electroacidification to produce bovine milk casein isolate. *J. Agric. Food Chem*. **1999**, *47*, 5291−5296.

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

(5) Bazinet, L.; Lamarche, F.; Ippersiel, D. Bipolar-membrane electrodialysis: Application of electrodialysis in the food industry. *Trends Food Sci. Technol*. **1998**, *9*, 107−113.

(6) Bazinet, L.; Lamarche, F.; Ippersiel, D.; Gendron, C.; Beaudry, J.; Mahdavi, B.; Amiot, J. Cationic balance in skim milk during bipolar membrane electroacidification. *J. Membr. Sci*. **2000**, *173*, 201−209.

(7) Cayot, P.; Lorient, D. *Structures et technofonctions des protéines du lait, Arilait Recherches*; Technique et Documentation Lavoisier; Paris, 1998; p 363.

(8) Doyon, G.; Gaudreau, G.; St-Gelais, D.; Beaulieu, Y.; Randall, C. J. Simultaneous HPLC determination of organic acids, sugars and alcohols. *Can. Inst. Sci. Technol. J*. **1991**, *24*, 87−94.

(9) AOAC International. Method 927-05: Moisture of milk. In *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC: Gaithersburg, MD, 1995; Vol. 2.

(10) AOAC International. Method 930-30: Ash of dried Milk. In *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC: Gaithersburg, MD, 1995; Vol. 2.

(11) AOAC International. Method 945-46: Ash of milk. In *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC: Gaithersburg, MD, 1995; Vol. 2.

(12) Britten, M.; Giroux, H. Interfacial properties of milk protein-stabilized emulsions as influenced by protein concentration. *J. Agric. Food Chem*. **1993**, *41*, 1187−1191.

(13) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem*. **1976**, *72*, 248−254.

(14) Pearce, K. N.; Kinsella, J. E. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem*. **1978**, *26*, 716−723.

(15) Waniska, R. D.; Kinsella, J. E. Foaming properties of proteins; evaluation of a column aeration apparatus using ovalbumin. *J. Food Sci*. **1979**, *44*, 1398−1402, 1411.

(16) SAS Institute Inc. *SAS/Stat user's guide*, 4th ed.; SAS Institute Inc.: Cary, NC, 1989; Vol. 2, version 6, 846 pp.

(17) Munro, P. A.; Southward, C. R.; Elston, P. D. The effect of casein manufacturing variables on the properties of rennet casein plastics. *N. Z. J. Dairy Sci. Technol*. **1980**, *15* (2), 177−190.

(18) McDowell, A. K. B.; Southward, C. R.; Elston, P. D. The Effect of ion on the colour of rennet casein plastic. *N. Z. J. Dairy Sci. Technol*. **1976**, *11* (1), 40−45.

(19) Weal, B. C.; Southward, C. R. Recent developments in casein manufacture. I. The production of rennet casein by a "continuous cook". *N. Z. J. Dairy Sci. Technol*. **1974**, *9* (1), 2−5.

(20) Bastier, P.; Dumay, E.; Cheftel, J. C. Physico-chemical and functional properties of commercial caseinates. *Lebensm. Wiss. Technol*. **1993**, *26*, 529−537.

(21) Modler, H. W. Functional properties of non-fat dairy ingredients—A Review. Modification of products containing casein. *J. Dairy Sci*. **1985**, *68*, 2195−2205.

(22) Mulvihill, D. M.; Fox, P. F. Physico-chemical and functional properties of milk proteins. In *Developments in dairy Chemistry—4*; Fox, P. F., Ed.; Elsevier Applied Science Publishers: London and New York, 1989; Vol. 4, pp 131−172.

Received for review May 4, 2002. Revised manuscript received August 13, 2002. Accepted August 14, 2002. Financial support of this research furnished by Novalait Inc., Québec, Canada, is gratefully acknowledged.

JF025650W