Production of calcium- and magnesium-enriched caseins and caseinates by an ecofriendly technology

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ABSTRACT

Finding new environmentally friendly ways of producing proteins has never been of such critical public interest, both to meet consumers' needs and to preserve the environment. Milk proteins are among the most attractive protein types due to their high nutritional value and attractive functional properties. In this work, the separation of caseins by conventional chemical acidification was compared with electrodialysis with bipolar membrane coupled to an ultrafiltration module (EDBM-UF), a green process that allows the precipitation of caseins by H^+ generated in situ by the bipolar membrane and, simultaneously, the production of a separated NaOH stream from OH^- electrogenerated by the bipolar membrane. Caseinate production using this NaOH stream by-product and the quantity of NaOH needed to produce caseinates from both methods were also investigated. Hence, the purity and composition of caseins and caseinates were compared in terms of protein, ash, and lactose contents as well as mineral composition. The results showed for the first time that caseinates can be produced by solubilizing caseins with NaOH stream from the EDBM process. Furthermore, the caseins and caseinates produced by EDBM-UF were equivalent in terms of lactose and protein contents to their respective caseins and caseinates that were chemically produced but presented slightly lower sodium content and 3 to 4 times higher magnesium and calcium contents. The fact that calcium and magnesium are likely bound to milk caseins would ensure their favorable absorbability. These caseins or caseinates from the new EDBM-UF process could be suitable as an improved protein-based calcium or magnesium supplement, both for their enhanced nutritional quality and because they are produced by a "green" process.

Key words: caseins, caseinates, bipolar membrane electrodialysis, ultrafiltration, calcium-enriched ingredients

INTRODUCTION

With the growing world population, demand for high-quality protein sources has been increasing steadily. However, the increasing demand in protein production is usually associated with environmental issues (Cao and Li, 2013). Finding new environmentally friendly ("green") ways of producing proteins has never been of such critical public interest, both to meet consumers' needs and to preserve the environment. Milk proteins are among the most attractive protein types because they have high nitrogen content and quality, promoting muscle synthesis (Elliot et al., 2006); contain various bioactive peptides, which aid in glycemic control and cardiovascular health (Korhonen and Pihlanto, 2006); and boast high levels of calcium, known to increase bone health and aid in preventing osteoporosis (Heaney, 2000; Thorpe et al., 2008). Caseins are 1 of the 2 major protein sources derived from milk, and their use as a functional ingredient (e.g., in the form of sodium caseinates) has been increasing steadily in recent years (MarketsandMarkets, 2014). Their most common manufacturing method necessitates the use of chemicals in the form of strong acids to achieve effective separation from milk. Indeed, separation of caseins from whey is most commonly achieved by reducing milk pH to its isoelectric point of 4.6 by acid addition, rendering them insoluble (Carr and Golding, 2016). Other methods of casein isolation include the addition of rennet to skim milk, leading to the precipitation of para- κ -casein and casein macropeptides, and microfiltration and diafiltration, leading to the isolation of micellar casein (or phosphocasein; Carr and Golding, 2016). However, the composition and functional properties of rennet and micellar caseins differ from those of acid casein and caseinate (Mounsey et al., 2005; Augustin et al., 2011).

Nevertheless, a green process, electrodialysis with bipolar membrane (EDBM), can be used to isolate caseins from milk without the use of chemicals and to

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reduce production of effluents. This process uses anion exchange membranes and cation exchange membranes (**CEM**) to segregate the migration of ions through the cell and bipolar membranes (**BPM**) to produce H^+ and OH^- ions when an electrical current is applied. To reduce the pH of the milk, the H^+ ions electrogenerated by BPM are used (Bazinet et al., 1999; Tanaka, 2010). Another interesting advantage of this process is that a pH of 5.0 is sufficient to precipitate caseins efficiently (Mikhaylin et al., 2016a). Indeed, milk demineralization during the process reduces its ionic strength, facilitating the destabilization of the casein micelles, inducing their precipitation (Bazinet et al., 2000b); this is in contrast with the chemical process in which ions are added, increasing the ionic strength and decreasing the isoelectric point (Bazinet et al., 2000b). However, a major problem of this process is the fouling of the spacers by precipitated caseins during the electroacidification of milk (Bazinet et al., 1999) and mineral scaling (Bazinet et al., 2001). To avoid protein fouling, a UF module was recently coupled to the EDBM cell. This approach allows only the small components of milk (essentially water minerals and lactose) to be recirculated in the EDBM cell (Mikhaylin et al., 2016). In fact, the UF permeate (referred to as the **MUF** in this work) is acidified by the BPM of the EDBM stack and is recirculated in the UF module to reduce the pH inducing casein precipitation. Concerning scaling of membranes, Mikhaylin et al. (2018) proposed a new configuration of the EDBM cell to avoid scaling by separating the mineral concentrate compartment from the basification compartment of the EDBM stack.

However, in all previous studies on electrochemical acidification of caseins, the use of the base produced in parallel during the process has never been tested and evaluated in terms of composition of the final caseinates. Indeed, the final caseins obtained after acidification of milk can be used as caseins (insoluble) or can be further transformed into caseinates (soluble) with the addition of a base. These caseinates have interesting characteristics—namely, highly enhanced solubility in water compared with casein as well as increased foaming and emulsifying capacity (Singh, 2011)—making them a polyvalent functional ingredient with uses ranging from meat emulsifier to dairy foaming agent (Huffman and Harper, 1999). This ingredient has intriguing functional properties and possesses the nutritional qualities shared by all milk proteins products, making it a doubly interesting choice as a food ingredient.

In this work, the separation of caseins by the standard chemical acidification is compared with the green EDBM-UF process. Because the EDBM-UF process also produces (in parallel and in a separate compart-

ment) an NaOH stream from OH^- generated by the BPM, caseinate production using this recycled by-product and the quantity of NaOH needed to produce said caseinates from both methods are investigated. The purity and composition of caseins and caseinates are compared by analyzing their protein, ash, and lactose contents as well as mineral composition to investigate the advantages of the EDBM method over the chemical process.

MATERIALS AND METHODS

Materials

The milk used in this study was a pasteurized skim milk (Pur Filtre; Parmalat, Toronto, ON, Canada). The NaCl (American Chemical Society grade) used for the electrolyte solutions was bought from BDH Chemicals (Radnor, PA). The NaOH, HCl, and HNO_3 were purchased from Anachemia Canada Inc. (Montreal, QC, Canada).

Electroacidification Cell

The EDBM module (Figure 1) used was a laboratory scale cell (model MP; 100 cm² of effective surface) from ElectroCell Systems AB Co. (Täby, Sweden). The cell comprised 6 compartments separated by 3 Neosepta CMX-SB CEM, 1 Neosepta AMX-SB anion exchange membrane, and 1 Neosepta BP-1 BPM (all membranes were food grade and produced by Astom, Tokyo, Japan; Figure 1). The anode, a dimensionally stable electrode, and the cathode, a 316 stainless steel electrode, were supplied with the MP cell. A UF module (filtration model L; GEA Process Engineering, Bönen, Germany) was coupled to the EDBM cell to allow only the mineral fraction of milk (MUF) to flow through the EDBM system and to avoid protein fouling of the spacers and ion-exchange membranes (Figure 1). The UF membrane used was a spiral-wound membrane of 2.14 m² filtration area with a 10-kDa molecular weight cut-off. The pressure used in the UF module during experiments was 30 psi (207 kPa). The volume of milk treated in the UF module was 3 L. The volumes treated in the EDBM cell were 500 mL of MUF, 700 mL of a 30 g/L NaCl solution, and 500 mL of a 2 g/L NaCl solution. The flow rates in the EDBM system were 600 mL/min for the 30 g/L NaCl solution and 200 mL/min for the 2 g/L NaCl solution. The flow rate of the MUF was changed during the experiments to keep a minimal volume of permeate circulating in the EDBM module between 400 and 500 mL (Mikhaylin et al., 2018).

In this configuration, the 30 g/L NaCl solution (compartments 1, 5, and 6) was demineralized throughout the process, the sodium ions being attracted by the cathode and thus migrating from the first compartment through the CEM 1 to the second compartment. This solution is also demineralized by passing through the fifth compartment and the chloride ions going through the anion exchange membranes, being attracted by the anode. Therefore, this solution is doubly demineralized. This explains the choice of a 30 g/L concentration for this solution of NaCl; a lower concentration could have been too demineralized when approaching the end of the process and thus would have increased the system global resistance. At the same time, the 2 g/L NaCl solution (compartment 2) was supplied in Na^+ ions from the first compartment and in OH^- ions electrogenerated by the BPM, consequently resulting in NaOH. This NaOH was used for the production of caseinates later in the process. The MUF, being the UF permeate of milk, was recirculated in the third compartment of the EDBM, where it was acidified by H^+ produced by the BPM. The MUF is also demineralized by the migration of cations through the CEM 2 to the fourth compartment. The 2 g/L NaCl solution was concentrated in minerals by the migration of cations from the MUF and by the migration of Cl^- from the 30 g/L solution.

The first and sixth compartments were also used as electrode washing compartments.

Protocol

A 2.00-A current was kept constant for EDBM during the experiment. Conductivity in compartments 1 (to check ion concentration; a too-low concentration risked producing a water hydrolysis problem) and 4 (to check demineralization rate of MUF and consequently milk) were recorded throughout the process. The pH was measured in the UF retentate reservoir containing milk (to check milk acidification rate) and in compartments 2 (to check NaOH production) and 4 (to verify that pH did not become too basic, which would have put the membranes at risk of scaling problems; Mikhaylin and Sion, 2016) throughout the process. Conductivity and pH measurements were recorded at the beginning of the experiment, after 5 and 10 min, and then every 10 min until the milk pH decreased to 4.8. Milk samples were taken in the UF retentate reservoir at the beginning of the experiment, at pH 6.2, and then after each successive 0.4 decrease of pH up to the end of the treatment. Conductivity measurement of milk was carried out at the same intervals. Two liters of acidified milk was collected at the end of the experiment for centrifugation to

Figure 1. Configuration of the electrodesialysis with bipolar membrane coupled to a UF module (EDBM-UF) system. C^+ are migrating cations. CEM = cation exchange membrane; BPM = bipolar membrane; AEM = anion exchange membrane; MUF = UF permeate. Color version available online.

Figure 2. Production scheme of the electrodialysis with bipolar membrane (EDBM) process and the chemical process of caseins and caseinates from skim milk.

separate casein curd from whey. In parallel, a chemical acidification was done by decreasing the pH of 2 L of milk with 0.5 *N* HCl (Carr and Golding, 2016) until pH reached 4.6 (casein isoelectric point) in a beaker under agitation. Total added HCl volume was noted. Two liters of acidified milk was then collected for centrifugation (Figure 2).

Casein separation was done by successive centrifugations (Avanti J-26 XP; Beckman Coulter, Brea, CA) for both processes. A first centrifugation at 11,000 × *g* for 15 min and 4°C was carried out to separate the insoluble acid caseins from the liquid whey. The supernatant was then removed, and the remaining casein curd was washed with 1.8 L of distilled water. A second centrifugation with the same parameters was then carried out, followed by a second washing and a third centrifugation. Samples from the supernatants were collected after each centrifugation. The final casein curd was split into 2 equal parts; one was lyophilized for 72 h, and the other underwent a basification and solubilization step to produce caseinates (Figure 2).

This basification was done with a 0.5 *N* NaOH solution for the chemical process, and the NaOH produced in the second EDBM compartment was used for the electrodialytic method. The NaOH produced in the EDBM stack was titrated with a 0.5 *N* HCl solution using a phenolphthalein indicator to determine its concentration. The NaOH solution was progressively added to the acid casein curd under constant agitation on a laboratory stirrer and hot plate (model PC-420; Corning, New York, NY) with a magnetic stirrer (speed 5) at a temperature of 70 to 75°C until a pH of 6.7 to 6.8 was reached and the complete dissolution of the caseins was observed. A temperature of 70 to 75°C was used to facilitate solubilization of caseins during NaOH addition. As reported by Carr and Golding (2016), a localized viscous layer tends to appear on the surface of the casein particles, preventing sodium hydroxide action; hence, an increase in temperature reduced this problem. The volume of NaOH added was noted. The alkalized casein fraction (caseinate) was then lyophilized for 72 h. Both lyophilized acid casein and casein-

ate fractions obtained from the chemical and electrodialytic methods were collected to determine their ash, moisture, protein, and lactose contents and to analyze their mineral composition.

Analyses

Theoretical Casein Mass in Milk. Theoretical mass of caseins in milk was calculated using values from Miller et al. (2000). The following formula was used:

$$\begin{aligned} & (\text{Theoretical g of protein in skim milk /} \\ & \text{L of skim milk}) \times \text{Theoretical ratio of casein} \\ & \text{to total skim milk protein} \times \text{L of skim milk used} = \\ & \text{Theoretical casein mass in skim milk.} \end{aligned}$$

Titration of Base Produced by EDBM. The titration of produced NaOH in the second compartment of EDBM was carried out using a 0.5 *N* HCl solution with a phenolphthalein indicator. The HCl was gradually added using a burette to 10 mL of the base solution until color change. The result was expressed as milliliters of HCl per liter.

Quantity of H^+ and OH^- Used for Casein and Caseinate Production. The theoretical concentration of H^+ and OH^- produced by EDBM was calculated by Faraday's law for electrolysis, $n = \frac{I \times t}{F}$, where n is the number of moles produced, I is the current applied, t is the time in seconds, and F is the Faraday constant. In addition, a conservative current efficiency of 75% was used. Typical values of current efficiency for laboratory scale electrodialysis cells are about 75 to 90% (Leiva, 1988; Mafart and Béliard, 1992; Kaveh et al., 2009).

The H^+ needed for casein production were calculated by comparing the number of moles of H^+ added to the milk (chemically or electrochemically) to the theoretical amount of casein contained in milk calculated as previously from Miller et al. (2000). The OH^- needed for

soluble caseinate production (by basification of caseins) was found by calculating total moles of OH^- from NaOH volume added to the lyophilized caseins powder separated previously from milk. For electrochemical acidification, the theoretical value for OH^- was calculated by Faraday's law but also compared with the value obtained for the base titration using phenolphthalein.

Ash Content. Ash content of the lyophilized samples of caseins and caseinates obtained after centrifugation of acidified skim milk was determined using the modified AOAC method 945.46 (AOAC International, 2006). Crucibles were soaked in a solution of 5% HNO_3 for 1 h to solubilize residual ashes that could be present in the crucibles. Crucibles were then washed with ultrapure water and conditioned at 550°C in an oven for 18 h to remove volatile compounds and obtain an accurate mass measurement. The crucible's mass was then noted. About 1 g of sample was added to each crucible before placing it in a stove at 105°C for 24 h. The sample-containing crucible was then incinerated at 550°C for 18 h. The crucible's mass with sample before and after incineration was noted to acquire the ash content according to the following equation, where CrM = crucible mass:

% ash =

$$\frac{\text{CrM with sample after incineration} - \text{CrM}}{\text{CrM with sample before incineration} - \text{CrM}} \times 100.$$

Moisture Content. Moisture content of the lyophilized caseins and caseinates was determined by placing 1 g of sample in an aluminum cup in a 105°C stove for 36 h to evaporate the water in the sample. Mass of cups and mass before and after evaporation of the samples were noted to acquire the moisture content according to the following equation, where CM = cup mass:

% moisture =

$$\left(1 - \frac{\text{CM with sample after drying} - \text{CM}}{\text{CM with sample before drying} - \text{CM}} \right) \times 100.$$

Protein Content. Protein content of the lyophilized casein and caseinate samples was determined using a Leco Truspec device (Leco Corp., Saint Joseph, MI). This device uses the Dumas combustion method, which consists of transforming organic nitrogen to molecular nitrogen and routing it to a detector (method 14891, ISO, 2002). Parameters used were a furnace temperature of 950°C, a postcombustion temperature of 850°C, incineration steps of 90 s with a high furnace flow, and gas purity of 99.993% for helium and 99.995% for oxygen. Nitrogen conversion factor was 6.38 (IDF, 2016).

Lactose Content. Lactose content was determined by HPLC method. Liquid samples were diluted and then placed in the HPLC device (Waters Corp., Milford, MA) with an ICSEP-ION-300 column (Tansgenomic, Omaha, NE). A refractive index detector (Hitachi, Foster City, CA) was used to quantify lactose. For caseins, a dilution step was performed as well as a resolubilization step using 0.5 N NaOH until pH 6.7; a filtration step using 0.45- μm -pore filters was then made before passing the solubilized caseins into the HPLC. Lyophilized caseinate samples were solubilized in water and then diluted before passing them in HPLC.

Mineral Composition. Mineral composition of caseins and caseinates was determined using the ash samples obtained during the ash determination step. These ashes samples were solubilized in 25% HNO_3 for 1 h and then diluted and filtered with 0.2- μm -pore filters before being analyzed by optical emission spectrometry with inductively coupled plasma ionization (Optima 4300, Dual View, Perkin-Elmer, Waltham, MA). The wavelengths used were 285.219, 317.933, 589.592, and 766.490 nm for magnesium, calcium, sodium, and potassium, respectively. These cation analyses were carried out in radial view.

RESULTS AND DISCUSSION

Milk pH Evolution During EDBM Process

Milk pH decreased from an initial pH of about 6.7 to a final pH of 4.8 throughout the EDBM process (Figure 3) by H^+ electrogenerated on the cationic side of the BPM adjacent to the MUF compartment. The slow acidification was due to the buffer capacity of milk (e.g., proteins, weak acids) and the release of phosphate anions from the caseins, neutralizing the acidification effect of the H^+ addition (Buchanan and Peterson, 1927). As pH decreased, protein-bound compounds of calcium (or magnesium) phosphates (or citrates) converted to the soluble ionic form and remained in the whey fraction (Lucey and Horne, 2009). The phosphor-containing anions along with citrate anions consumed a part of the H^+ produced by the BPM, decreasing the rate of acidification. Milk pH decreased in a similar way to the one observed previously by Bazinet et al. (2000b).

Milk Conductivity Evolution During EDBM Process

Conductivity of milk decreased throughout the EDBM process (Figure 3). As the EDBM process progressed, cations present in the MUF were transported to the adjacent concentrate compartment (compart-

ment 4), being attracted by the cathode. This migration of cations from milk to the adjacent compartment through the cation exchange membrane was responsible for the decrease in conductivity over time (Bazinet et al., 2000a). The nonlinear nature of the curve was attributed to the release of cations and anions bound to caseins micelles (mostly colloidal tricalcium phosphate) into the soluble whey phase (Bazinet et al., 2000a). This increase of soluble ionic content in the MUF would have then increased its conductivity and then partially counteracted the inherent demineralization effect of the EDBM configuration through the CEM. Milk conductivity decreased in a way similar to Bazinet et al. (2000a).

Relative H^+ Addition to Produce Insoluble Acidified Caseins

It was found that the EDBM process necessitated less H^+ than the chemical process, with values of 0.00124 ± 0.00010 compared with 0.00194 ± 0.00005 mol/g of caseins ($P < 0.0002$). The higher value for the chemical process was due to the fact that casein precipitation was carried out at a lower pH in comparison with the EDBM process (4.6 compared with 4.8), which consequently increased the number of H^+ required for acidification.

These values are lower than the ones obtained by Whittier (1929), who obtained an adjusted value of 0.00280 mol/g of caseins. This difference was probably caused by the fact that the acidification of milk was made from pH 7.0 to pH 4.0, a higher pH range than the one used in this study, thereby necessitating higher quantities of acid to decrease pH. In addition, the milk composition may vary according to the cow breed, diet, lactation period, and season (White and Davies, 1958).

Relative OH^- Addition to Produce Soluble Basified Caseinates

Following caseins extraction, OH^- additions to produce caseinates from both processes were calculated. The NaOH generated by the electrochemical process was titrated and presented a titration value of 0.212 ± 0.001 mol/L. Both processes needed a similar quantity of NaOH to produce caseinates, with values of 0.00070 ± 0.00002 mol/g of caseins for the EDBM process and 0.00073 ± 0.00002 mol/g of caseins for the chemical process ($P > 0.05$). This result was unexpected because the final EDBM pH was lowered to 4.8 instead of the standard 4.6 for the chemical process: consequently, fewer OH^- ions would have been needed to reach the final pH of 6.7 to 6.8 in the basification step.

Figure 3. Milk pH and conductivity evolutions during the electrodialysis with bipolar membrane (EDBM) process. Error bars represent SD.

Figure 4. Ash content of caseins and caseinates from the electro-dialysis with bipolar membrane (EDBM) and chemical acidification processes. The error bar represents SD, and data with different letters (a,b) are significantly different at $P < 0.05$. Color version available online.

The NaOH addition requirements were found to be higher in the present study compared with the value of 0.00058 mol/g of caseins obtained by Zittle et al. (1959). This difference of values could probably be attributed to the different conditions of basification used (e.g., temperature, pH, particle size; Carr and Golding, 2016).

Mineral Composition

Following electrodialysis, it was found that the caseins obtained had a higher ash content than those from the conventional chemical acidification, with a value of $1.34 \pm 0.05\%$ compared with $1.01 \pm 0.05\%$ ($P < 0.005$; Figure 4). The higher ash content value could be attributed to the different final pH between both processes, as the complete precipitation of caseins was obtained at pH 4.8 from the EDBM process compared with the standard pH 4.6 in the chemical process.

These ash content values are quite similar to the ones obtained by Bazinet et al. (2000b), with values of 1.20% for the EDBM process and 1.28% for the chemical process. The small difference in ash content could be attributed to the different centrifugation methods, as Bazinet et al. (2000b) used a centrifugation step at $500 \times g$ for 10 min compared with the $11,000 \times g$ for 15 min in the present study. This variable could have influenced ash retention. Of interest is that the higher final pH would have allowed a greater quantity

of divalent cations (Ca^{2+} , Mg^{2+}) to remain in the casein matrix by being bound to phosphoserine residues (Le Graet and Brulé, 1993).

Ash content for caseinates was unsurprisingly higher than for caseins due to the increased amount of Na^+ from the addition of NaOH to caseins, with values of $4.21 \pm 0.04\%$ for the EDBM process and $4.30 \pm 0.07\%$ for the chemical process (Figure 4). These caseinate ash content values were nevertheless not significantly different between both processes ($P > 0.05$). These similar values, in contrast to the different ash values of caseins from both methods, were due to the slightly higher quantity of NaOH added in the case of chemically produced caseinates. Indeed, as the final pH for casein production was 4.6 compared with 4.8 for the EDBM process, a higher quantity of NaOH was added to the chemically produced caseins for soluble caseinate production at a pH of 6.7 to 6.8. These ash contents were similar to the ash content observed in standard caseinates of 3.98% of ash on a dry basis (3.8% wet basis; Huffman and Harper, 1999).

Mineral Characterization

The calcium and magnesium contents were higher for caseins produced by the EDBM process than for those produced by the chemical process, with values of $0.22 \pm 0.02\%$ compared with $0.057 \pm 0.004\%$ for calcium (about 4 times higher; $P < 0.001$) and $0.011 \pm 0.0002\%$ compared with $0.0032 \pm 0.0008\%$ for magnesium (about 4 times higher; $P < 0.001$; Figure 5). As stated previously, these results are due to the higher final pH of EDBM, inducing a lower solubilization of Ca^{2+} and Mg^{2+} from the caseins. Indeed, those minerals are bound to the phosphoserine groups of different individual caseins and remain as colloidal phosphocalcic (phosphomagnesian) bridges inside the casein micelles and are released as pH decreases (Le Graet and Brulé, 1993). As the proportion of bound calcium and magnesium decreases in favor of the soluble form as the pH decreases, a higher proportion of those minerals was extracted from the casein curd in the centrifugation step of production, consequently reducing their contents in the final casein fraction. Sodium and potassium contents were found to be not significantly different between both processes. Sodium values were $0.59 \pm 0.04\%$ for EDBM and $0.47 \pm 0.06\%$ for the chemical process ($P > 0.05$), whereas values for potassium were $0.13 \pm 0.004\%$ for the EDBM process and $0.10 \pm 0.02\%$ for the chemical process ($P > 0.05$; Figure 5). These results are probably due to the fact that sodium and potassium are mostly present in unbound form in caseins, each being present in the colloidal bound form at only 8% (Le Graet and Brulé,

Figure 5. Mineral composition of caseins from the electrodialysis with bipolar membrane (EDBM) and chemical acidification processes. The error bar represents SD, and data with different letters (a,b) are significantly different at $P < 0.05$. Color version available online.

1993). These minerals are easily rejected from caseins toward the whey in the centrifugation step of production.

Calcium and magnesium contents from chemically acidified caseins were found to be somewhat similar to those of Noël et al. (2008), who obtained values of 0.0382 and 0.0054%, respectively, whereas sodium and potassium contents were found to be much lower, with values of 0.0040% for sodium and 0.0026% for potassium. These different values are attributable to the centrifugation procedures used in both studies having the potential of increasing or decreasing the final soluble mineral retention.

Similar trends were found for caseinates (Figure 6). Calcium and magnesium contents were found to be greatly higher following the EDBM process compared with the standard chemical process, with values of $0.18 \pm 0.005\%$ compared with $0.062 \pm 0.02\%$ for calcium (about 3 times more; $P < 0.005$) and $0.010 \pm 0.001\%$ compared with $0.0036 \pm 0.001\%$ for magnesium (about 3 times more; $P < 0.005$; Figure 6). Once again, the presence of more calcium and magnesium in caseinates was due to the fact that they were more present in bound form. Because no calcium or magnesium were added or removed following the basification step of caseinate production from caseins, their values were similar before and after conversion. Sodium content was lower for the EDBM process than for the chemical process, with values of $2.3 \pm 0.02\%$ compared with $2.6 \pm 0.1\%$ ($P < 0.05$; Figure 6). The lower value for the

EDBM process, as explained previously, was attributed to the fact that slightly less NaOH was added to the caseins for this treatment compared with the chemical process for caseinate production. Sodium content was higher for caseinates compared with caseins because a high Na^+ ion content was added in the form of NaOH to convert the acid-insoluble caseins to soluble caseinates. Potassium contents were not found to be different between the treatments, with values of $0.16 \pm 0.02\%$ for the EDBM process and $0.13 \pm 0.002\%$ for the chemical process ($P > 0.05$; Figure 6). This result can be attributed to the fact that most of the potassium in caseins is found in soluble form, which is easily withdrawn from the caseins curd into the whey during centrifugation. As expected, potassium content in caseinates was similar to potassium content in caseins considering that no potassium ions were added or removed in the basification process to produce caseinates.

Calcium content was found to be somewhat similar to the standard content for chemically produced caseinates of 0.03% (Huffman and Harper, 1999), whereas the sodium content in the present study was lower than that in the work of Huffman and Harper (1999), with values of 1.2%. Differences in ash content could be attributed to an excess of NaOH that could have been added to produce the caseinates in the present study, as NaOH addition was performed until a pH of 6.7 to 6.8. This difference in final pH could bring variation in the final ash content. Concerning magnesium and

Figure 6. Mineral composition of caseinates from the electrodialysis with bipolar membrane (EDBM) and chemical acidification processes. The error bar represents SD, and data with different letters (a,b) are significantly different at $P < 0.05$. Color version available online.

potassium, their standard content values in caseinates were not found in the literature.

Lactose Content

Lactose content of caseins from both processes was similar, with values of $0.25 \pm 0.06\%$ for EDBM and $0.18 \pm 0.01\%$ for the chemical process ($P > 0.05$; Figure 7). Because no lactose removal steps were applied in either process, these similar results were expected. For the same reasons, similar lactose contents were found in caseinates, with $0.18 \pm 0.03\%$ for EDBM and $0.19 \pm 0.05\%$ for the chemical process ($P > 0.05$; Figure 7).

The standard lactose concentration for caseins and caseinates is 0.1% (Huffman and Harper, 1999), which is lower than the one found in this study. These results could be attributed to standard lactose variation in cow milk or differing centrifugation steps, which would influence lactose retention in the casein curd.

Protein Concentration

The protein concentration in chemically produced caseins was higher than the caseins produced by the EDBM process, with respective values of $96.3 \pm 0.3\%$ and $95.3 \pm 0.5\%$ ($P < 0.005$; Figure 8). This difference was attributable to the higher ash content obtained in the EDBM-produced caseins, consequently reducing the overall protein content of the product. These results for casein protein content are somewhat similar to the

Figure 7. Lactose content of caseins and caseinates from the electrodialysis with bipolar membrane (EDBM) and chemical acidification processes. The error bar represents SD, and data with different letters are significantly different at $P < 0.05$. Color version available online.

Figure 8. Protein content of caseins and caseinates from the electrodialysis with bipolar membrane (EDBM) and chemical acidification processes. The error bar represents SD, and data with different letters (a,b) are significantly different at $P < 0.05$. Color version available online.

Huffman and Harper (1999) results of 96.6% (87.3% wet basis).

The protein contents of caseinates were unsurprisingly lower than for caseins due to the addition of ash content from NaOH. Nevertheless, protein concentration was found to be the same for both processes, with a value of $92.3 \pm 0.8\%$ and $92.7 \pm 0.5\%$ for the EDBM and chemical processes, respectively ($P > 0.05$; Figure 8). The difference in protein content in caseins due to the presence of more calcium and magnesium was, however, masked by the high quantity of sodium added, leading to no difference in ash content between caseinates. These values are similar to the standard caseinate protein content of 94.8% (90.4% wet basis) obtained by Huffman and Harper (1999).

The results obtained also show that the caseins and caseinates produced by EDBM were equivalent in terms of lactose and protein contents to their respective chemically produced caseins and caseinates but presented lower sodium content and higher magnesium and calcium contents. The higher calcium content and, to a lesser extent, magnesium content are very interesting because these high concentrations induced by the new EDBM process add increased value to the final product. Indeed, the high calcium content of the produced caseins and caseinates could justify their use as a calcium-rich protein source, notably in osteoporosis patients, because calcium is essential to bone health (Heaney, 2000). Furthermore, the fact that the calcium

is likely bound to milk protein makes it somewhat pH resistant and ensured its absorbability (Guéguen and Pointillart, 2000). This could make these caseins or caseinates from the new EDBM process suitable as an improved protein-based calcium supplement both for its enhanced nutritional quality and for the fact that it is produced by a green process. The reduced sodium content of caseinates from the EDBM process (around 12%) is also interesting because higher sodium contents in food can lead to high blood pressure, bringing different health issues (Havas et al., 2007). Furthermore, the new food policies tend to decrease the sodium content in food. All these characteristics give the EDBM caseins and caseinates enhanced nutritional qualities. In addition, a recent life cycle analysis considering both processes demonstrated that the EDBM was more environmentally friendly than the standard chemical acidification–basification (Mikhaylin et al., 2018). Further research will aim to evaluate the possibility of adding a diafiltration step to reduce lactose content in the different protein isolates, which would increase their purity.

CONCLUSIONS

The results obtained in this study show for the first time that caseinates can be produced by solubilizing produced caseins with recycled NaOH stream from the EDBM process. This could lead to a process that produces caseins and caseinates with high calcium content and a whey by-product with low lactose content. In addition, research on functional and nutritional properties of the EDBM-produced caseinates will be carried out.

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