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Precipitation of Cheddar Cheese Whey Lipids by Electrochemical Acidification

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Lipid separation from cheddar cheese whey allows a better valorization of protein fractions. In this study, bipolar membrane electroacidification (BMEA) was used to obtain precipitates with a high level of lipids. Whey samples with normal and low (by way of electrodialysis) mineral salt levels have been treated by a BMEA process and centrifuged. The composition of flocs and precipitation yields were determined. The BMEA process increased lipid precipitation rates by almost 50% in comparison with a centrifugation step only whereas a demineralization step prior to electroacidification had a limited effect on the precipitation level. Precipitates obtained were mainly composed of lipids (probably phospholipids) but also contained proteins. BMEA of cheddar cheese whey would allow the production of a lipid-enriched fraction and of a protein-enriched whey.

KEYWORDS: Electroacidification; bipolar membranes; whey; precipitation; lipids; proteins

INTRODUCTION

Dairy whey is a byproduct of cheese and casein production. Whey lipids are composed of about 66% of nonpolar lipids and 33% of polar lipids. Polar lipids are mainly phospholipids distributed as phosphatidylethanolamine (34%), phosphatidylcholine (31%), sphingomyelins (15%), phosphatidylinositol (12%), and phosphatidylserine (8%) (*1*). Moreover, cheese whey contain almost 7 g/L of proteins with a high content in β-lactoglobulin (3 g/L) (*2*).

Lipid separation from cheddar cheese whey allows a better valorization of the protein fractions. In order to improve whey protein fractionation techniques, processes to remove residual fats were proposed. De Wit and Klarenbeek (*3*) have found that a decrease of the ionic strength of solutions combined with a chemical acidification at pH 4.6 allowed the precipitation of lipoproteins which could be separated by decantation. Ionic strength reduction was carried out by demineralization with ion-exchange resins, electrodialysis, or diafiltration. Similar results were obtained by Admundson et al. (*4*). They treated whey solutions by ultrafiltration and acidified the concentrate at pH 4.65 with HCl solutions, which was demineralized by electrodialysis. A precipitate with high levels of lipids and β-lactoglobulin (lipid/protein ratio of 0.60) was then obtained.

Acidification and a decrease in the ionic strenght of the solution might be carried out in one step by bipolar membrane electroacidification (BMEA) technology. BMEA is a technology using the property of bipolar membranes to split water and to produce protons at the cationic interface (*5*). This process was used in the food area for the production of soybean and bovine milk casein isolates (*6*, *7*), for the inhibition of polyphenol-oxidase enzyme in apple juice (*8*), for deacidification of passion fruit juice (*9*), and recently for whey protein isolates fractionation (*10*).

The objective of this work was to evaluate the use of BMEA for simultaneous acidification of cheddar cheese whey and precipitation of lipids. Whey electroacidification was carried out with or without preliminary demineralization by conventional electrodialysis. Lipid and protein precipitation levels were measured after centrifugation of electroacidified whey samples.

MATERIALS AND METHODS

Materials. *Whey.* Fresh cheddar cheese whey was kindly provided by Fromagerie SMA (Quebec, Canada). The whey composition was the following (g/100 g of fresh matter): total solids, 7.2 $\pm$ 0.2; lactose, 6.0 $\pm$ 0.6; total protein, 0.7$\pm$ 0.2; fat, 0.30$\pm$ 0.02; ashes, 0.55 $\pm$ 0.01. Whey initial pH was 6.3.

Electrodialysis Cell. An MP type cell (100 cm^2 of effective surface area) manufactured by ElectroCell Systems AB Company (Täby, Sweden) was used with four Neosepta CMX-SB cationic membranes (Tokuyama Soda, Japan) and two Neosepta AMX-S anionic membranes (Tokuyama Soda, Japan). This system was set up to provide three circuits: the cheddar cheese whey solution (2.5 L), the concentrate, a 2 g/L KCl solution (6 L), and the electrolyte, a 20 g/L NaCl solution (6 L). The flow rate of whey and KCl solutions was set at 2 L/min; the flow rate of electrolyte was 3 L/min. Each closed loop was connected to a separate external reservoir allowing continuous recirculation. The anode, a dimensionally stable electrode (DSA), and the cathode, a 316 stainless steel electrode, were supplied with the MP cell.

Bipolar Membrane Electroacidification Cell. The MP type cell was also used for the bipolar membrane electroacidification of the whey solution. The cell consisted of three closed loops separated by four Neosepta CMX-SB cationic membranes (Tokuyama Soda, Japan) and two Neosepta BP-1 bipolar membranes (Tokuyama Soda, Japan) containing the same solutions as previously described for an electrodialysis cell (**Figure 1**).

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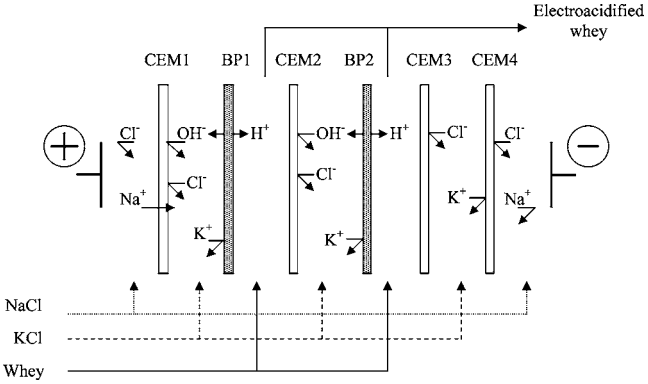

Figure 1. Configuration of bipolar membrane electrodialysis cell used for whey electroacidification. CEM: cation exchange membrane. BP: bipolar membrane.

Protocol. Three processes were tested in this study. In process 1, whey solutions were only centrifuged, whereas in process 2, they were electroacidified using bipolar membranes prior to centrifugation. Process 3 consisted of a conventional electrodialysis step of whey solution to decrease demineralization levels to 60% of the original values prior to bipolar membrane electroacidification and centrifugation. Precipitates obtained during the different processes were freeze-dried for 48 h (Freezone Model 4.5; Labconco Corporation, Kansas City, MO), weighed, and kept at −20 °C until analysis. Total protein, fat, lactose, ash, and dry matter analyses were performed on dry samples. Each process or combination of processes was repeated three times, and results are the average of these data.

Centrifugation. Whey (1 L) was centrifuged for 5 min at 1000*g* (Biofuge centrifuge model 22R, Heraeus Instruments, Germany).

Conventional Electrodialysis. Conventional electrodialysis was carried out in batch processes under constant voltage of 10 V. Electrodialysis process was stopped after a 40% whey demineralization was reached, as calculated from the conductivity decrease.

Bipolar Membrane Electroacidification. BMEA was carried out in batch processes under constant current density of 20 mA/cm^2. The electroacidification processes were stopped after a pH value of 3.7 had been reached.

Analysis Methods. *Electrodialytic Parameters.* The duration, pH and electrical conductivity of cheddar cheese whey solutions, voltage and current intensity applied at the electrodes were recorded throughout electrodialysis and electroacidification treatments. The global system resistance (Ω) was calculated, using Ohm's law, from the ratio of voltage (V) over current intensity (A) read directly from the indicators on the power supply. The pH of whey solutions was measured with a pH-meter VWR (model SP 20, VWR International, Beverly, MA). Conductivity was measured with a VWR conductivity meter model SP 40 with a cell constant = 1/cm (VWR International, Beverly, MA, electrode no. 14002-802).

Total Protein. The total protein content of freeze-dried precipitates was determined by an FP-528 Leco apparatus (Leco Corp., St. Joseph, MI) according to the method of Bazinet et al. (*7*). The instrument was calibrated with ethylenediaminetetraacetic acid (EDTA) as a nitrogen standard. The protein level was calculated from nitrogen data using a coefficient of 6.38.

Lipids. The lipid content of freeze-dried precipitates was determined using a modified Mojonnier method (*11*). Two extractions were carried out on 4 g samples using 0.3 mL of NH_4OH, 2 mL of ethanol, 5 mL of diethyl ether, and 5 mL of petroleum ether. Samples were stirred for 1 min after each solvent addition.

Lactose. Lactose content was determined with a spectrophotometric method using sulfuric phenol (*12*). The sample solution (1 mL) was mixed with a 5% phenol solution (1 mL) and with concentrated H_2SO_4 (5 mL). Lactose concentration was determined using lactose solutions at known concentrations to obtain a standard curve. The absorbance of solutions was read at 490 nm.

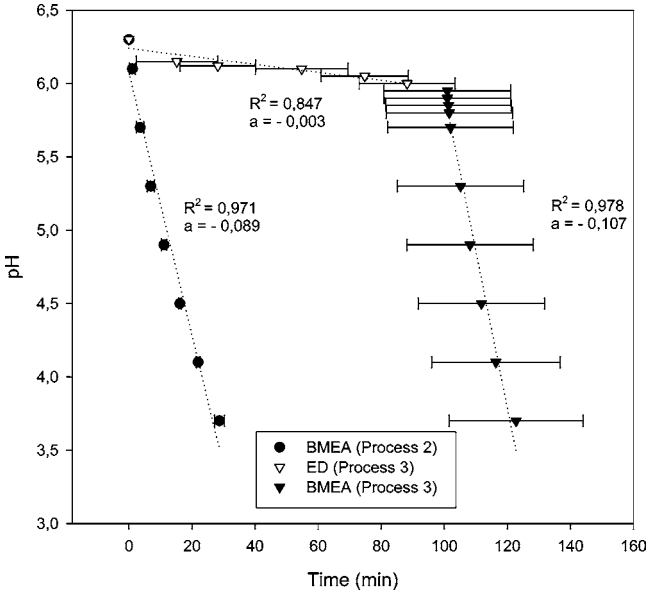

Figure 2. Evolution of pH as a function of time during whey electroacidification with or without preliminary demineralization by conventional electrodialysis.

Dry Matter and Ash Content. Dry matter content was determined by drying samples overnight in an oven at 95 °C. The samples were then ashed at 550 °C for 20 h (*13*) for ash measurement.

Statistical Analyses. Data for lipid, protein, lactose, and total solid precipitation yields and composition of precipitates in lipids, proteins, ashes, and lactose were subjected to a one-way analysis of variance using SigmatStat software (version 2.03, Jandel Scientific, Corte Madera, CA). Regression equations and curve fitting were calculated for the system resistance and the solution conductivity as a function of pH and for the duration of electroacidification and electrodialysis processes as a function of time using SigmaPlot (version 3.0 for Windows, Jandel Scientific, Corte Madera, CA). Analysis of precipitation yield and precipitate composition were completed by an all pairwise multiple comparison procedure (Tukey test) to determine the significance of differences between processes.

RESULTS AND DISCUSSION

Electrodialysis Parameters: Duration, Resistance, and Conductivity. *Duration.* A whey electroacidification process (process 2) was carried out to decrease the solution pH from 6.3 to 3.7 (**Figure 2**). The mean time required to electroacidify whey solutions was 29 ± 2 min. The duration of the global process (BMEA plus electrodialysis) in process 3 was 123 ± 22 min. A small decrease in whey pH, from 6.3 to 6.0, was noticed during the demineralization step, which had a duration of 88 ± 15 min whereas the electroacidification step lasted for 35 ± 22 min. Electroacidification reactions for both processes 2 and 3 appear similar since their acidification curves in the electroacidification phase are almost identical (**Figure 2**), in agreement with results in the literature (*15*).

Global Resistance of Electrodialysis System. The global resistance of the conventional electrodialysis system was constant during the process with a value of 18 ± 2 Ω. During the BMEA process (**Figure 3**), for pH values between 6.0 and 3.7, resistances dropped from 17.5 ± 0.5 to 14.8 ± 0.3 Ω for process 2 and from 16.0 ± 0.0 to 15.0 ± 0.0 Ω for process 3. The decrease in resistance is caused by H^+ ions produced by the bipolar membrane. When H^+ ions are generated at the bipolar membrane, whey cationic charges (calcium, sodium, potassium, magnesium, and so on) cross the cation exchange membrane to keep the solution electrically neutral. Intrinsic

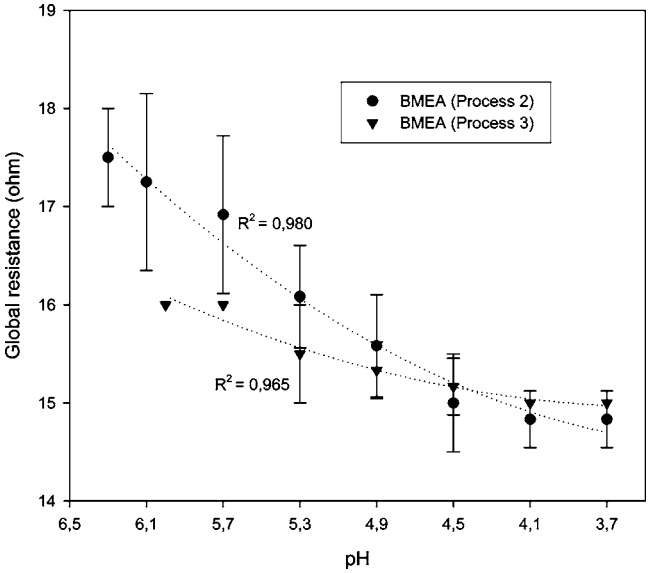

Figure 3. Evolution of the system global resistance as a function of pH during whey electroacidification processes.

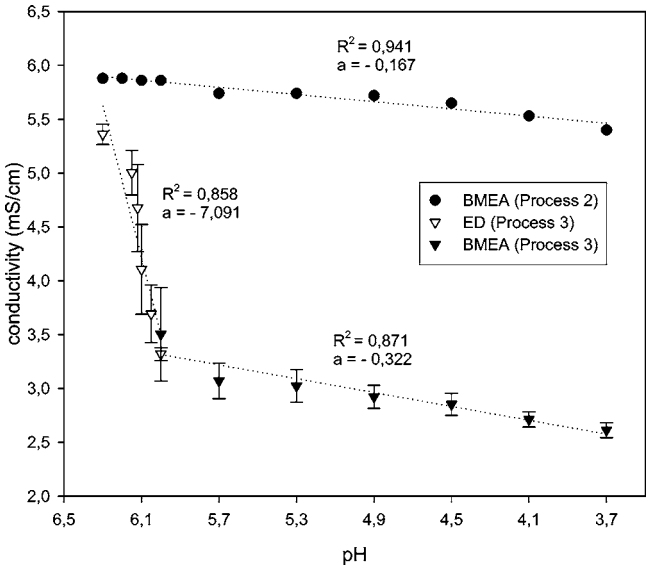

Figure 4. Evolution of the conductivity as a function of pH during whey electroacidification with or without preliminary demineralization by conventional electrodialysis.

cationic charges of whey are then replaced by H^+ ions, which have a higher electrical mobility. Due to their higher mobility, H^+ ions improved the flow of the electrical current and thus decreased the global resistance of the system (*14*). This drop was also due to the fact that ionic concentration increased in the adjacent compartment, contributing to a global decrease in resistance.

Conductivity. Conductivity data for whey solutions during electrodialysis and electroacidification processes are presented in **Figure 4**. During the conventional electrodialysis process, conductivity dropped from 5.36 ± 0.1 to 3.32 ± 0.06 mS/cm, which corresponds to a 40% whey solution demineralization. Partial demineralization of whey solutions occurred during electroacidification processes, which implies a 16% decrease in conductivity (0.5 to 0.9 mS/cm decrease). In comparison, Bazinet et al. (*7*) observed a smaller conductivity drop of 0.1 to 0.2 mS/cm during skim milk (1.1 L) electroacidification with three bipolar membranes and an electrical current of 2 A, with a final pH value of 4.5. The drop in conductivity during whey electroacidification was higher than the decrease observed in milk because of the lower buffering capacity of the whey solutions in comparison with milk. With a higher protein and salt content, more H^+ ions had to be generated by the bipolar membranes to decrease the pH by one unit. Thus the electroacidification process takes more time for solutions having a high protein content (*16*). Furthermore, as previously mentioned, H^+ ions which replaced the main cationic species in the treated solution contribute more to conductivity in comparison to others cations (*17*).

Table 1. Recovered Solids (% w/w) of Whey Components in Centrifugation Pellets

	process 1	process 2	process 3
lipids	20.8 ± 3.5	32.1 ± 4.7	29.7 ± 2.3
proteins	1.1 ± 0.7	1.9 ± 0.5	3.3 ± 0.4
lactose	0.6 ± 0.4	0.9 ± 0.2	1.1 ± 0.1
total solids	1.5 ± 0.6	2.3 ± 0.4	2.6 ± 0.2

Table 2. Cheddar Cheese Whey and Pellet Composition, Protein/Lipid Ratios, and Ash/Lipid Ratios (% of Dry Matter)

	whey	process 1 pellet	process 2 pellet	process 3 pellet
lipids	4.0 ± 0.1	59.2 ± 9.1	57.3 ± 1.7	49.8 ± 4.0
proteins	9.6 ± 0.7	6.8 ± 1.8	8.0 ± 0.7	12.7 ± 1.1
lactose	79.5 ± 1.2	30.5 ± 7.2	32.5 ± 1.2	35.7 ±3.1
ashes	6.9 ± 0.5	3.6 ± 0.2	2.2 ± 0.1	1.7 ± 0.1
protein/lipid	2.40	0.11	0.14	0.26
ash/lipid	1.72	0.06	0.04	0.03

Yield and Composition of Precipitates. Whey centrifugation at 1000*g* during 5 min (process 1) allowed a 20.8% recovery of cheddar cheese whey initial lipids (**Table 1**). The other constituents, proteins and lactose, were recovered at low levels of 1.1% and 0.6%, respectively. Whey lipid precipitation amounted to 32.1% for process 2 (electroacidification to pH level 3.7 and centrifugation). This represents a 54% increase in precipitation level in comparison with process 1. Proteins ($P > 0.282$) and lactose ($P > 0.073$) recovery rates (1.9% and 0.9%, respectively) were quite similar to those obtained in process 1. Similar recovery rates for lipids ($P > 0.713$) and lactose ($P > 0.088$) were also obtained in process 3 in comparison with process 2 values whereas conventional electrodialysis resulted in an increase in protein recovery from 1.9% to 3.3% ($P < 0.034$) (**Table 1**).

Results on recovery yield and composition of centrifugation pellets were compared to values for raw cheddar cheese whey (**Table 2**). As expected the composition of the pellets in comparison with raw whey was different in lipid ($P < 0.001$), protein ($P < 0.001$), ash ($P < 0.001$), and lactose ($P < 0.001$) content. However, the pellet composition was different between the three processes. It appeared that the composition in lipids and lactose was not statistically different for the three processes ($P > 0.198$ and $P > 0.415$, respectively). Although the lipid composition was not significantly different due to the large standard deviation of the process 1 pellet, a trend appeared with a decrease in the lipid content of 15.9% from process 1 to process 3. For protein and ash contents, the composition was significantly different between the processes. The protein content was quite doubled from process 1 to process 3 and with an intermediary value for process 2. In the opposite way, the ash content decreased 52.8% from process 1 to process 3 and still with an intermediary value for process 2. The fact that lipid percent in pellets would decrease while previous precipitation

yields indicated an increase was due to the difference in recovery yield: for example, between processes 1 and 3, the protein and lactose recovery rates increased by 3 and 2 respectively, while for lipids the yield increased only by 1.5. The decrease in ash content was due to the demineralization effect of the conventional electrodialysis, accentuated by the demineralization during the electroacidification process. The lower ash content decreased the ionic strength of the solution, which may have caused an increase in protein recovery. The nature of the protein fraction was not determined, but this fraction could be thermally denatured protein or lipoprotein complex.

Lipid recuperation by centrifugation in process 1 was due to residual fat particules. The increase of lipid precipitation levels in processes 2 and 3 may be explained by the formation of lipid/protein complexes during electroacidification (*3*). This complex formation may be characterized by the protein/lipid ratio: The more important is the ratio, the more important is the level of proteins which could bind with lipids. The protein/lipid association was probably enhanced (**Table 2**) by process 3 conditions (protein/lipid ratio of 0.26) in comparison with processes 1 and 2 (ratios of 0.11 and 0.13, respectively).

The protein−lipid interactions that exist in food systems involve hydrophobic interactions between apolar aliphatic chains of the lipid and the apolar regions of the protein. In model systems, the energy of the protein lipid interaction reaches a maximum in the neighborhood of the isoelectric point of the protein. The associations between lipids and proteins are probably electrostatic interactions involving the phospholipids. The isoelectric point of the β-lactoglobulin, the main whey protein, is at pH 5.5. So at pH 3.7, the protein carries a positive electric charge (*18*). However, some phospholipids have a negative charge at acidic pH like the phosphatidylserine, which has, at pH 3.7, two negatively charged groups: the phosphoric acid group and the carboxylic acid group (*19*). An equation, including calcium ion level, that represents the mechanism of protein/lipid complex formation was proposed by Cornell et al. to explain such a mecanism (*20*). At high calcium level, eq 1 would be moved to the left, while at low calcium concentration, it would move to the right, resulting in formation of a phospholipid complex. In eq 1, P is protein with a charge *Z*, Ca is calcium, L is phospholipid carrying a negative charge, and *n* is the number of phospholipids bound per protein group.

$$P^{Z+} + n\mathrm{CaL}^{+} \leftrightarrows \mathrm{PL}_n^{(Z-n)+} + n\mathrm{Ca}^{2+} \qquad (1)$$

Lau et al. (*21*) have shown that calcium ions are linked to phospholipids. The binding of calcium with lipids inhibits the formation of lipid/protein complexes. The calculation of ash/lipid ratio allowed the evaluation of the level of interactions between the calcium ion and the lipid components. The ash/lipid ratios were 0.06, 0.04, and 0.03 respectively for processes 1, 2, and 3 (**Table 2**). The decrease in mineral salts, particularly in magnesium and calcium ions, during processes 2 and 3 promotes the lipid/protein complex formation. This phenomenon was confirmed by higher precipitation levels for lipids and proteins in process 3 in comparison with process 1 (**Table 1**). Equation 1 also means that an increase of P^{Z+} components should improve the lipid/protein complex formation. This increase of P^{Z+} may be obtained by a concentration step of whey solutions by ultrafiltration.

Conclusion. The BMEA process prior to a centrifugation step at 1000*g* during 5 min allowed a 32.1% lipid precipitation level of initial fresh cheddar cheese whey solutions. This corresponds to a 54% increase of the lipid precipitation level in comparison with the centrifugation step alone. The whey demineralization process before the BMEA would allow an increase of protein recovery but would have no effect on lipid precipitation. The lipid and protein precipitation phenomenon was probably based on the formation of lipid/protein complexes due to a combination of acidification and to a decrease in the ionic strength of the medium. Acid pH enhances the electrostatic interactions between negatively charged compounds for lipids and positively charged compounds for proteins. The decrease in mineralization level allows the liberation of ionized zones in phospholipids and thus enhances the lipoprotein complexe formation.

This new process would have two advantages, the production of a phospholipid-enriched fraction which could be used in cosmeceutics and a purified (demineralized and delipidized) and more valuable protein fraction after concentration of the whey. Further work is currently in progress to evaluate the effect of protein concentration on lipid precipitation, and to confirm the phospholipid nature of the precipitated lipids.

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