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Delipidation of a Whey Protein Concentrate by Electroacidification with Bipolar Membranes

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The separation of residual fats from whey protein concentrates (WPC) results in a better nutritional and functional utilization of this product. Bipolar membrane electroacidification (BMEA) technology allows acidification and demineralization of solutions without any salt addition. The principle of BMEA is based on proton formation from water molecule dissociation at the bipolar membrane interface. The objective of this work was to determine the effect of an electroacidification treatment at pH 4.5 on the precipitation of lipids. WPC electroacidification was carried out with or without preliminary demineralization by conventional electrodialysis. The effect of ionic strength on lipid precipitation rates was also evaluated by dilution of the WPC samples. Lipid precipitation levels of 35−39% were obtained using the electroacidification process without a dilution step, while the combination of BMEA and dilution of the WPC resulted in a decrease in lipid content by six-fold from 0.76 to 0.21%.

KEYWORDS: WPC; delipidation; electroacidification; bipolar membranes; lipids; precipitation

INTRODUCTION

Over the past several years, whey protein concentrates (WPCs) have been largely used in the food industry as nutritional and functional ingredients. WPCs are typically composed of more than 35% protein and have a fat content higher than 4% (*1*). However, the presence of lipids affects the WPC functional properties (*2*) and promotes development of oxidation reactions, which impart off-flavors (*3*). For these reasons, methods to decrease the fat content in WPC products have been developed. For instance, a thermocalcic lipid precipitation method consists of adding divalent calcium ions to the WPC solution, adjusting the pH to a value of 7.3, and heating the final solution (*4*−*6*); this results in aggregation and precipitation of phospholipoprotein complexes, which are removed by microfiltration. WPC produced by this method contains less than 0.5% lipids (*7*). However, the microfiltration process has to be optimized in order to obtain a satisfactory membrane flow and protein permeation. Another method for WPC delipidation consists of precipitating the lipids by chemical acidification in a low ionic strength WPC (*1*, *8*). This method consists of a preliminary whey concentration by ultrafiltration until a total solids value of 23% is obtained; then, the WPC is diluted to decrease the ionic strength and chemically acidified to reach a pH value of 4.3, resulting in the separation of fat compounds by flocculation. After concentration by ultrafiltration and drying by atomization, the final product is composed of less than 1% lipids (*1*). However, the chemical products used for the WPC acidification present the disadvantage of adding organic or mineral salts, which increase the ionic strength of the solution.

Recent works on cheddar cheese whey treatment by bipolar membrane electroacidification (BMEA) have shown partial precipitation of whey lipids (*9*). In this electrochemical method, a 30% precipitation level of the initial lipids was obtained by lowering the pH of the solution down to 3.7, followed by a 5 min centrifugation at 1000*g*. BMEA is a technology that performs both solution acidification and demineralization without any salts addition. Acidification is carried out by H^+ ion production at the cationic interface of bipolar membranes by water dissociation under an electric current (*10*), while partial demineralization is achieved by ion transfer through monopolar membranes in order to maintain electrical neutrality in the solution. In addition to food applications, bipolar membrane electrodialysis (ED) has also been used in other fields such as chemical industries and biotechnologies; for instance, a recent application consisted of using bipolar membrane technology for the regeneration of flue-gas desulfurizing agents (*11*).

The purpose of our study was to evaluate the effects of protein concentration and ionic strength of WPC solutions on lipids precipitation. A WPC with a protein content of 55% (on a dry basis) was used. The ionic strength of the solution was decreased by dilution or by conventional ED. After acidification by BMEA and centrifugation, the protein and lipid precipitation levels were determined by analysis of the supernatants.

MATERIALS AND METHODS

WPC. Fresh WPC was kindly provided by Agropur (Longueuil, QC, Canada). WPC was stored at 4 °C until it was used, and the experiments

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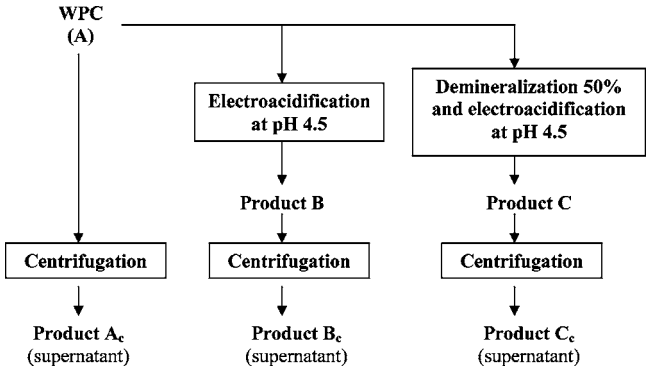

Figure 1. WPC treatment by conventional ED and bipolar membrane electroacidification.

Table 1. WPC composition (% w/w on Wet Basis), pH, and Conductivity

dry matter (%)	15.0 ± 0.1
protein (%) ($N \times 6.38$)	9.3 ± 0.1
fat (%)	0.76 ± 0.02
ash (%)	0.69 ± 0.01
pH	6.2 ± 0.1
conductivity (mS/cm)	4.4 ± 0.1

were carried out within 3 days following the WPC production. The composition of the initial WPC and its characteristics are presented in **Table 1**.

ED Cell. WPC demineralization was carried out using an ED MP type cell (100 cm^2 of effective surface) equipped with a dimensionally stable anode and a 316 SS cathode from ElectroCell AB (Täby, Sweden). The cell included four CMX-SB cationic membranes and two AMX-SB anionic membranes manufactured by Tokuyama Soda Ltd. (Japan) and was purchased from Ameridia (Somerset, NJ). This arrangement set up three circuits: the demineralized WPC solution (1.5 L); the concentrate, a 2 g/L KCl solution (6 L); and the electrode rinse solution, a 20 g/L NaCl solution (6 L). The flow rates of WPC and KCl solutions were set at 2 L/min; the flow rate of NaCl electrolyte was 3 L/min. Each closed loop was connected to a separate external reservoir allowing continuous recirculation. A voltage of 10 V was maintained throughout the treatment.

BMEA Cell. The MP type cell was also used for BMEA of WPC solutions. The cell consisted of three closed loops separated by four CMX-SB cationic membranes and two Neosepta BP-1 bipolar membranes (Tokuyama Soda Ltd.). The cell configuration was similar to the one used in our previous work on delipidation of cheddar cheese whey (*9*). The cell included the same solutions as previously described for WPC demineralization. BMEA was conducted using a constant current density of 20 mA/cm^2.

Protocol. Fresh WPC (product A) was treated by BMEA at pH 4.5 to provide acidified WPC (product B). Product A was demineralized at 50% (based on conductivity decrease) by conventional ED and acidified electrochemically to yield product C (**Figure 1**). During electroacidification and ED treatments, the pH and conductivity of WPC solutions were recorded as well as the voltage and current intensity. Products A, B, and C were centrifuged at 10000*g* at 20 °C for 20 min, and the supernatants were collected to obtain, respectively, products A_c, B_c, and C_c (**Figure 1**). Products A, B, C, A_c, B_c, and C_c were also diluted six-fold (1 mL of WPC in 5 mL of distilled water), decanted overnight at 4 °C, and centrifuged at 1000*g* for 5 min (Biofuge 22R, Heraeus Instruments, Germany) to yield supernatants A_d, B_d, C_d, $A_{c/d}$, $B_{c/d}$, and $C_{c/d}$, respectively (**Figure 2**). All products were analyzed for their fat, nitrogen, ash, and total solids contents.

Analytical Methods. *Physicochemical Parameters.* The pH of the WPC solutions was measured with a pH meter model SP 20 from VWR International (Plainfield, NJ). The conductivity was measured with a conductivity meter model SP 40 with a cell constant of 1 cm^{-1} (VWR International).

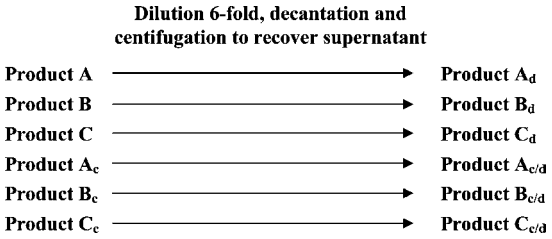

Figure 2. WPC delipidation process by dilution of samples.

Electrical Resistance. The global system resistance (Ω) was calculated using Ohm's law, from the ratio of voltage (V) over current intensity (A). This parameter was useful for scaling-up and further pilot or industrial development.

Total Solids and Ash Content. The total solids content was determined by drying WPC samples at 95 °C overnight. The ash content was measured by heating the dried samples at 550 °C for 20 h according to the AOAC method (*12*).

Protein Content. The total nitrogen content was determined by a FP-528 Leco apparatus (Leco Corp., St Joseph, MI) with similar operating conditions used in a previous study by Lin Teng Shee et al. (*9*). The soluble protein content was calculated from nitrogen data using a conversion factor of 6.38.

Lipid Content. The lipid content was measured by nuclear magnetic resonance according to the American Oil Chemists' Society Official Method Cd 16b-93 (*13*) using a NMR minispec MQ20 Bruker analyzer (Billerica, MA). Precipitation rates of fat were determined by calculation taking into account the original and final fat contents as well as the volumes of the recovered products after treatment.

Absorbance at 500 nm. WPC samples were diluted six-fold with distilled water, and their absorbance was read at 500 nm with a UV−visible Hewlett-Packard 8453 spectrophotometer (Mississauga, ON, Canada). Because the turbidity of whey was mainly due to light scattering by milk fat globule membrane fragments, the absorbance reading at 500 nm was used as an alternate method to detect the presence of residual fat in treated samples (*14*).

Statistical Analysis. Three repetitions were carried out, and all measurements on WPC samples were subjected to analysis of variance using SigmatStat software (Windows version 2.03, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

ED Parameters. *Duration.* Electroacidification processes were carried out to acidify WPC solutions from pH 6.2 to 4.5. The mean duration required to electroacidify 1.5 L of WPC was almost 45 min at 20 mA/cm^2 using two bipolar membranes (**Figure 3**). In comparison, Lin Teng Shee et al. (*9*) obtained a process duration of 30 min for the acidification of 2.5 L of whey from pH 6.3 to pH 3.7. The acidification rate was lower for WPC in comparison to whey because of a higher protein content in WPC (9.3%) than in whey (0.7%) (*15*). A small decrease in WPC pH (0.62 pH unit) was observed during the conventional ED step. Such variations had already been reported by Hiraoaka et al. (*16*) and Delbeke (*17*) for cheese whey with decreases ranging from 0.39 and 1.39 pH units from 70 to 90% demineralization. Perez et al. (*18*), with demineralized whey permeates and retentates obtained by ultrafiltration using ED, also observed similar pH decreases ranging from 0.13 to 0.55 pH units for 40−65% demineralization rates. The duration of the demineralization/BMEA process was 270 min (**Figure 3**), while it was 30 min for the BMEA of the demineralized whey. Whether the WPC had been demineralized or not, the kinetics of WPC electroacidification were very similar (**Figure 3**). The demineralization step had no significant effect on the electroacidification duration since BMEA was carried out at constant current, as previously observed by Bazinet et al. (*19*).

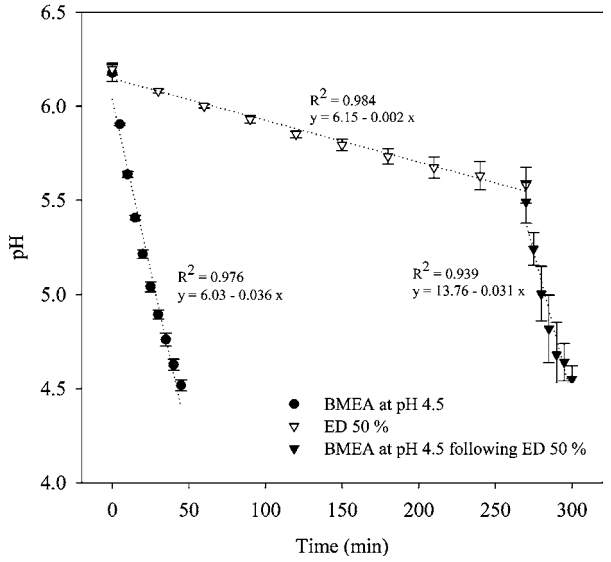

Figure 3. Evolution of pH as a function of time during WPC electroacidification with or without preliminary demineralization.

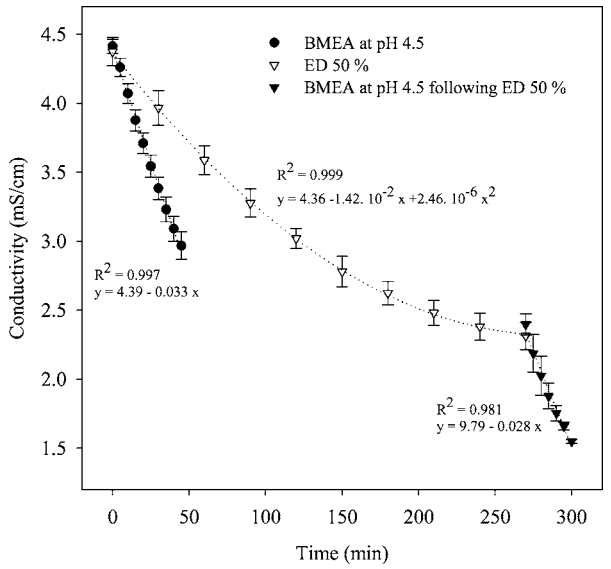

Figure 4. Evolution of WPC conductivity during BMEA with or without preliminary demineralization.

Conductivity. A drop in conductivity from 4.4 to 3.0 mS/cm occurred during WPC acidification, which represented a 0.03 unit decrease per minute (**Figure 4**). A similar decrease in conductivity was observed with BMEA of the 50% demineralized WPC, and the slopes of their respective regression curves were −0.033 and −0.028 (**Figure 4**). The kinetic curves for conductivity were different during conventional ED in comparison with BMEA process: The decrease in conductivity was lower and nonlinear during ED (**Figure 4**). This could be explained by the higher current density used during BMEA (20 mA/cm^2) than in conventional ED (2−4 mA/cm^2). Because the migration of ions is determined by the current intensity, this may explain the faster conductivity decrease during BMEA than in conventional ED.

Electrical Resistance of the System. The resistance of the system increased during both BMEA and conventional ED processes. During BMEA, at 0% demineralization, electrical resistance increased from 18 to 26 Ω, and at 50% demineralization, it increased from 21 to 37 Ω (**Figure 5**). The higher resistance observed during BMEA of WPC solutions with

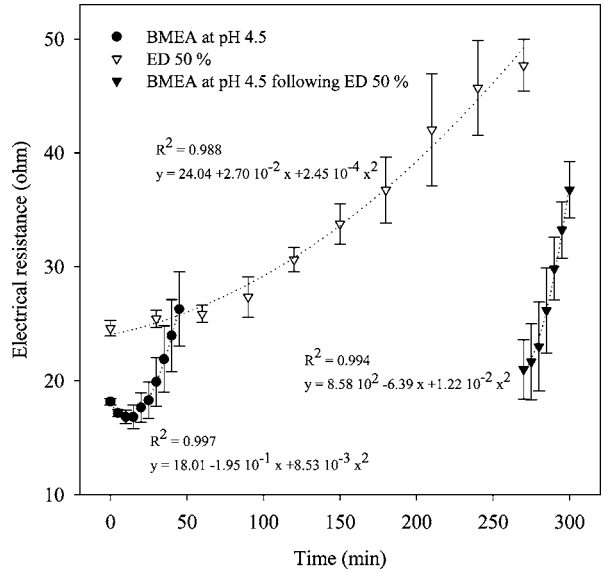

Figure 5. Evolution of electrical resistance during BMEA of WPC with or without preliminary demineralization.

preliminary demineralization may be explained by a decrease, by ED, in mineral salt levels, which results in an increase of the global resistance. During conventional ED, the resistance increased from 25 to 48 Ω. This phenomenon was in agreement with previous observations on the duration and conductivity evolution during ED. Indeed, the high resistance at the end of the ED process resulted in the lower ion migration rate and explained the long duration to reach a 50% level of demineralization as well as the low decrease of the conductivity at the end of the treatment. In addition, a resistance decrease of 27 Ω was observed between the end of ED at 50% and the following BMEA process. This difference might be explained by the different current density used during ED and BMEA: Indeed, during electroacidification, the current density was 20 mA/cm^2, whereas it was in the range of 2−4 mA/cm^2 in the conventional ED. Under the limiting current density, the resistance drops in an almost linear way with a decrease of the reciprocal current 1/*I* (*20*). The current intensity of the system was higher in electroacidification than in ED, resulting in a lower system resistance.

Effect of WPC Electroacidification and Demineralization. Products A_c, B_c, and C_c are the supernatants coming from centrifugation of products A, B, and C (**Figure 1**). Their total solids ($P = 0.232$) and proteins contents ($P = 0.317$) were not significantly different. However, the fat contents were significantly lower in products B_c and C_c ($P < 0.001$). Indeed, lipid contents in supernatants A_c, B_c, and C_c were 0.63, 0.55, and 0.47%, respectively (**Table 2**). Products B_c and C_c have conductivity values of 2.9 and 1.3 mS/cm, respectively. Demineralization was carried out during electroacidification and conventional ED. The final pH of products B_c and C_c was 4.5. The decrease in fat content of these products showed that there was precipitation of the WPC lipids. This phenomenon may occur by the combined effects of acidification and a decrease of the ionic strength of the solution (*8*). The rates of lipid precipitation were significantly different between products A_c and B_c ($P < 0.01$) and not significantly different between B_c and C_c products ($P = 0.181$) (**Table 2**). Then, the BMEA process resulted in an increase in the level of lipid precipitation (35% yield) in comparison with a single centrifugation step. The demineralization process carried out under the given conditions did not induce a destabilization of the lipids matter,

Table 2. Composition of Products A_c, B_c, and C_c

	A_c	B_c	C_c
	composition		
dry matter (%)	15.5 ± 0.2	16.1 ± 0.1	15.2 ± 0.9
protein (%)	9.0 ± 0.2	9.6 ± 0.2	9.0 ± 0.2
fat (%)	0.63 ± 0.03	0.55 ± 0.03	0.47 ± 0.02
	physicochemical parameters		
pH	6.2	4.5	4.5
conductivity (mS/cm)	4.4 ± 0.1	2.9 ± 0.1	1.3 ± 0.1
conductivity decrease (%)	0	34	70
	precipitation rate		
proteins (%)	4 ± 2	6 ± 2	6 ± 2
fat (%)	18 ± 1	35 ± 3	39 ± 3

Table 3. Protein Content of Initial Samples (A, B, C, A_c, B_c, and C_c) and of Their Diluted Samples Supernatants

products	protein content (%)	diluted products	protein content (%)	protein content (%) × 6	decrease of protein content in supernatant (%)
A	9.3 ± 0.1	A_d	1.5 ± 0.1	9.3 ± 0.4	0 ± 5
B	9.7 ± 0.1	B_d	1.5 ± 0.1	9.6 ± 0.3	2 ± 4
C	8.9 ± 0.2	C_d	1.3 ± 0.1	8.1 ± 0.3	10 ± 6
A_c	9.0 ± 0.2	$A_{c/d}$	1.4 ± 0.1	9.0 ± 0.2	0 ± 3
B_c	9.6 ± 0.2	$B_{c/d}$	1.4 ± 0.1	8.8 ± 0.1	9 ± 2
C_c	9.0 ± 0.2	$C_{c/d}$	1.3 ± 0.1	8.2 ± 0.1	8 ± 2

Table 4. Lipids Content of Initial Samples (A, B, C, A_c, B_c, and C_c) and of the Diluted Samples Supernatants

products	fat content (%)	products	fat content (%)	fat content (%) × 6	decrease of fat content in supernatant (%)
A	0.76 ± 0.02	A_d	0.09 ± 0.01	0.54 ± 0.03	28 ± 8
B	0.78 ± 0.02	B_d	0.03 ± 0.01	0.21 ± 0.05	73 ± 10
C	0.72 ± 0.02	C_d	0.04 ± 0.01	0.24 ± 0.06	66 ± 10
A_c	0.63 ± 0.02	$A_{c/d}$	0.08 ± 0.01	0.50 ± 0.02	21 ± 5
B_c	0.55 ± 0.04	$B_{c/d}$	0.05 ± 0.01	0.28 ± 0.02	49 ± 13
C_c	0.47 ± 0.02	$C_{c/d}$	0.04 ± 0.01	0.26 ± 0.02	45 ± 8

which could have resulted in a higher lipid precipitation. However, a higher decrease of ionic strength could have a significant effect on lipids precipitation. This was the object of the second part of this study on the decrease of the ionic strength of solutions by dilution of the acidified WPC.

Effect of WPC Dilution. Products A, B, C, and their supernatants were diluted six-fold and centrifuged to recover the supernatants (**Figure 2**). The resulting products were subjected to protein and fat content determination (**Tables 3** and **4**). Protein and fat content values were corrected by a factor of 6 corresponding to the dilution factor. Decreases in protein and lipid contents were calculated from the initial composition of samples. Hence, significant drops in protein ($P = 0.020$) and lipid levels ($P < 0.001$) are observed in comparison with the initial product compositions.

For protein content, only samples $B_{c/d}$ and $C_{c/d}$ have a protein content significantly different from the other samples (**Table 3**). The decrease in protein content was of 9 and 8%, respectively, for these products, showing that part of the protein in the supernatants has precipitated. This can be explained by the lower conductivity of the diluted samples (conductivity of 0.4−0.8 mS/cm). Products A_d, B_d, C_d, and $A_{c/d}$ had similar protein contents. The acidification by BMEA followed by a six-fold dilution has left in solution more than 90% of the protein initially present. For the fat content, the highest decreases were

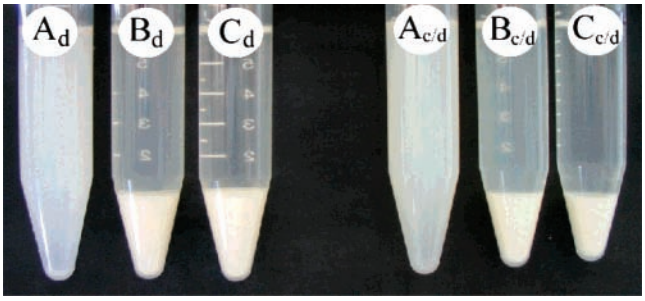

Figure 6. WPC diluted samples following a 24 h decantation.

Table 5. Absorbance at 500 nm of Diluted Samples Supernatants

products	absorbance at 500 nm
A_d	0.93 ± 0.07
B_d	0.14 ± 0.02
C_d	0.11 ± 0.02
$A_{c/d}$	0.62 ± 0.02
$B_{c/d}$	0.18 ± 0.01
$C_{c/d}$	0.14 ± 0.02

Table 6. Conductivity of Initial Samples (A, B, C, A_c, B_c, and C_c) and of Their Diluted Samples Supernatants

products	conductivity (mS/cm)	products	conductivity (mS/cm)	decrease in conductivity (%)
A	4.4 ± 0.1	A_d	1.1 ± 0.1	74 ± 2
B	2.9 ± 0.1	B_d	0.9 ± 0.1	71 ± 3
C	1.5 ± 0.1	C_d	0.5 ± 0.1	69 ± 2
A_c	4.4 ± 0.1	$A_{c/d}$	1.1 ± 0.1	73 ± 3
B_c	2.9 ± 0.1	$B_{c/d}$	0.9 ± 0.1	71 ± 2
C_c	1.3 ± 0.1	$C_{c/d}$	0.5 ± 0.1	71 ± 4

observed for products B_d and C_d with respective reductions of their lipids contents of 73 and 66%. For products $B_{c/d}$ and $C_{c/d}$, 49 and 45% reductions of lipids levels were obtained, respectively (**Table 4**). These results show that electroacidified products have the lowest fat contents (0.21−0.28%), corresponding to a decrease of nearly 73% of initial content in WPC lipids. It also resulted in the obtention of clarified supernatants with a low level in lipids and with the majority of the proteins initially present.

Electroacidified products (B_d, C_d, $B_{c/d}$, and $C_{c/d}$) were less turbid than products A_d and $A_{c/d}$ (**Figure 6**). Another indicator of the fat content is the turbidity of the solution, which was evaluated by the absorbance measurement at 500 nm (*14*). Products A_d and $A_{c/d}$ had the highest absorbances, 0.93 and 0.62, respectively, in comparison with the supernatants of the electroacidified products, which had absorbance readings from 0.11 to 0.18 (**Table 5**). These results are in agreement with fat contents measured by nuclear magnetic resonance. The turbidity reading might be a fast and simple method applicable in the industry to characterize the fat content of WPC solutions.

As for conductivity of diluted samples, no significant difference was observed between the diluted WPC samples (**Table 6**). The conductivity decreased by nearly 70% for all products because of the six-fold dilution. However, the final conductivities of acidified and diluted samples vary from 0.5 to 0.9 mS/cm in comparison with the control sample (1.1 mS/cm). These results show that it is necessary to decrease the conductivity of the acidified WPC below 1 mS/cm to obtain a precipitation level of WPC lipids between 45 and 73%.

The electroacidification process without dilution of the WPC resulted in a precipitation of 35−39% of initial lipids content

(vs 18% for the control) and confirmed previous results obtained on cheese whey (*9*). The combination of the bipolar membrane acidification process with the dilution of the WPC allowed the decrease of the WPC fat content from 0.78 to 0.21%, a 73% decrease from the initial fat content. A dilution step is necessary to increase the lipid precipitation rate. The six-fold dilution contributed to the significant decrease of the ionic strength of the medium, while the ED process at a demineralization level of 50% was not sufficient to increase lipid precipitation.

The BMEA process may be the first step of WPC treatment for whey valorization in various lipidic fractions. In comparison with the chemical acidification, the BMEA process has the advantage of offering continuous acidification without salt addition. This could be useful, in an industrial process, to maintain a low ionic strength in the solution prior to or following WPC dilution and to produce a purified WPC with a low ash level.

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