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Phospholipid recovery from sweet whey and whey protein concentrate: Use of electrodialysis with bipolar membrane combined with a dilution factor as an ecoefficient method

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ABSTRACT

Electrodialysis with bipolar membrane (EDBM) is promising to recover phospholipids (PLs) from sweet whey and whey protein concentrate (WPC), as it promotes lipoprotein complex formation following a decrease in pH and ionic strength. The aim of this work was to study the impact of dilution factor (without dilution, with a 2X, 4X and 6X dilution) after EDBM on the process performances. For both products, a 4X dilution, which corresponds to a decrease in ionic strength of 81.4 ± 1.5 % for sweet whey and 79.4 ± 0.4 % for WPC, seemed sufficient to maximize lipoprotein complex formation as a plateau was reached in the defatting rates. Furthermore, it was demonstrated that the process could be used to recover PLs. Higher PL contents were found in the precipitates recovered from sweet whey combined with a dilution: highest contents in total PLs for WPC (7.45 ± 0.49 and 8.00 ± 0.49 g/100g with a 4X-6X dilution) were not different from the lowest content for sweet whey (7.50 ± 1.18 g/100 g without dilution). For both products, the main PLs recovered were phosphatidylethanolamine and phosphatidylserine, which respectively represented between 42.0 ± 1.7 and 51.6 ± 2.3 % and 30.1 ± 2.2 to 38.7 ± 0.8 % of total PLs. Furthermore, considering their high ecoefficiency scores, sweet whey combined with a 2X or 4X dilution are the most promising conditions.

1. Introduction

In the last few years, there has been an increasing interest over the dietary phospholipids (PLs) due to the positive impacts they can have on human health. Depending on the source which they are derived (egg yolk, fish, soybean, etc.) and their types, PLs will present different biological activities that are widely linked to their structural particularities (properties of the polar group, length and unsaturation of the fatty alkyl chain) (Sun et al., 2018). Among the food-derived PLs, dairy PLs are particularly interesting as they are a valuable source of sphingomyelin (SM) and phosphatidylserine (PS) (Barry et al., 2020). Even if some confirmations are needed, evidence suggested that these two classes of PLs can have a positive impact on brain, aging and neurodegenerative diseases, as well as on cell growth and development and prevention of colon cancer formation (Contarini and Povolo, 2013; Küllenberg et al., 2012).

Sweet whey, as a by-product of cheese making, as well as derived products, such as whey protein concentrate (WPC), could be attractive sources of dairy PLs. Indeed, the production of a PL enriched fraction would highlight their promising health effects and also limit their negative impacts in these dairy products during their transformation, storing, etc., such as organoleptic defects and technological issues (Morr and Ha, 1991). In skimmed whey and WPC, PLs are referred to as residual lipids and are mainly coming from milk fat globule membrane (MFGM) fragments (Hwang and Damodaran, 1995; Zhu and Damodaran, 2013). Methods have been developed to concentrate PLs from several dairy products and by-products, including solvent extraction, supercritical fluid extraction, ultrafiltration (alone or in combi-

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Table 1
Physicochemical characteristics of the sweet whey and whey protein concentrate.

	Sweet whey	Whey protein concentrate
pH	5.73±0.01[a]*	6.24±0.02[b]
Conductivity (mS/cm)	3.29±0.12[a]	3.67±0.07[b]
Total solid content (g/100g on a dry basis)	95.7 ± 0.6[a]	95.8 ± 2.7[a]
Crude protein content (g/100g on a dry basis)	19.5 ± 0.1[a]	38.5 ± 1.3[b]
True protein content	14.0 ± 0.1	30.6 ± 2.0
Non protein nitrogen content	5.4 ± 0.1	7.9 ± 0.9
Total lipid content (g/100g on a dry basis)	1.30 ± 0.02[a]	2.60 ± 0.15[b]
Phospholipid content (g/100g on a dry basis)	ND	ND
Ash content (g/100g on a dry basis)	8.36 ± 0.01[a]	6.60 ± 0.20[b]
Calcium	0.69 ± 0.02[a]	0.58 ± 0.02[b]
Potassium	1.90 ± 0.04[a]	1.33 ± 0.03[b]
Magnesium	0.10 ± 0.01[a]	0.07 ± 0.02[b]
Phosphorus	0.59 ± 0.01[a]	0.49 ± 0.01[b]
Sodium	0.68 ± 0.01[a]	0.55 ± 0.02[b]
Lactose content (g/100g on a dry basis)	48.8 ± 1.0[a]	38.0 ± 2.7[b]
Moisture content (g/100g on a dry basis)	4.32 ± 0.64[a]	4.16 ± 2.73[a]

ND: not detected.
* Values on the same line with a different letter are significantly different $P < 0.05$ (t-test).

nation) (Price et al., 2020). Nevertheless, in sweet whey and in WPC, methods have also been developed to remove either residual lipids or MFGM fragments, including chitosan precipitation, thermocalcic precipitation, etc. (Damodaran, 2010; Gésan et al., 1995; Hwang and Damodaran, 1995; Maubois et al., 1987; Pereira et al., 2002). These methods are based on the ability of PLs to present a negative charge, depending on the pH, and to interact with positively charged species which strongly suggests that they could also be used to recover PLs (Hwang and Damodaran, 1995). However, they present disadvantages, as the use of components with unclear legislation, their effects on the other components of sweet whey and WPC, etc. Nevertheless, electrodialysis with bipolar membranes (EDBM), a technology derived from electrodialysis, seems promising to isolate PLs, as demonstrated by Faucher et al. (2020) and Lin Teng Shee et al. (2007, 2005). The separation is based on the formation of lipoprotein complexes following simultaneous decreases in pH and ionic strength. Moreover, EDBM is innovative, ecofriendly and does not require chemical reagents during the process itself. EDBM can also be used on larger-scale which would allow it to fit in an industrial context (Bazinet and Geoffroy, 2020). Since electromembrane processes have been used in dairy industry for many years, the use of EDBM to recover PLs seems a suitable method. In previous studies, it has been demonstrated that using a WPC (instead of sweet whey) combined with a dilution after EDBM drastically increased the final defatting rate (Lin Teng Shee et al., 2007). Furthermore, when different concentration factors were tested, combined or not with a dilution, it was demonstrated that dilution was the main factor involved in reaching higher defatting rates (Faucher et al., 2020). Indeed, when a dilution was applied after EDBM, a higher concentration factor allowed to reach only a slightly higher defatting rate, in addition to increase the protein content in the precipitate, which would not fully justify a higher treatment of whey prior to EDBM. Nevertheless, the specific recovery of PLs by EDBM has never been studied, as previous studies only focused on total residual lipids, as well as the impact of the dilution factor.

In this context, this study mainly focused on the impact of the dilution factor after EDBM for sweet whey and WPC, as the decrease of ionic strength would be the key factor to reach higher defatting rates. Furthermore, the specific recovery of PLs after treatment was assessed here and an ecoefficiency analysis was carried out to include and link together environmental impacts and technological considerations. Indeed, the objectives of this work were: (1) to defat sweet whey or WPC by EDBM to study the impact of concentrating whey in WPC prior to EDBM, (2) to test different dilution factor after EDBM treatment, (3) to evaluate the impact of the dilution factor on the process, particularly on the defatting rate and the specific recovery of the PLs, (4) to characterize the composition of the supernatants and the precipitates after treatment and (5) to study the impact on ecoefficiency scores.

2. Materials and methods

2.1. Sweet whey and whey protein concentrate

Fresh sweet whey and WPC were provided by Parmalat (Victoriaville, Qc, Canada). Here, a WPC35 was selected as the WPC, since in a previous study by Faucher et al. (2020), a WPC with a similar crude protein content allowed to reach interesting defatting rate, while minimizing protein complex in the precipitate.

Sweet whey (6 % of dry content) came from a mozzarella cheese production, whereas WPC was obtained from blending and concentration. To produce WPC, sweet whey was mixed with products of reverse osmosis to increase the dry content up to 15%. Then the mix was treated by ultrafiltration (treatment carried out at 45 °C using a polyethersulfone (PES) membrane with a molecular weight cut-off of 10 kDa). Therefore, sweet whey represented 40 % of the dry content of the WPC. Sweet whey and WPC were frozen at -28 °C and thawed at 4 °C before each EDBM treatment. Their physiochemical characteristics are presented in Table 1.

2.2. Electrodialysis cell

To perform the EDBM treatments, a MP type electrodialysis cell (ElectroCell AB, Täby, Sweden) with an effective surface of 100 cm^2 was used, the same cell as Faucher et al. (2020). Briefly, the EDBM cell was composed of bipolar membranes (BMs) (Astom Corporation, Tokyo, Japan) and homogeneous cation exchange membranes (CEMs) (Astom Corporation, Tokyo, Japan) (Fig. 1**a**). placed on 5.49 ± 0.05 mm spacers. Sweet whey/WPC circulated between a BMs and a CEMs. The treatment allowed a simultaneous acidification and demineralization of the sweet whey/WPC, as the BMs allowed the production H^+ ions in this compartment and cations migrated through the CEMs in a recovery compartment. The BMs also allowed the production of OH^- in the recovery compartment. Furthermore, an electrode rising compartment was delimited at the end of the stack due to the presence of CEMs placed next to the electrodes. The cathode was a food grade stainless steel and the anode was a dimensionally stable electrode (DSA-O_2) (ElectroCell AB, Tâby, Sweden). Solutions were allowed to circulate during treatment due to the presence of closed loops, reservoirs and centrifugal pumps (CL3505, Baldor Electric Compagny, AR, USA). Flow-meters were used to control their flow rates (Aalborg Instruments and Control Inc., Orangeburg, NY, USA).

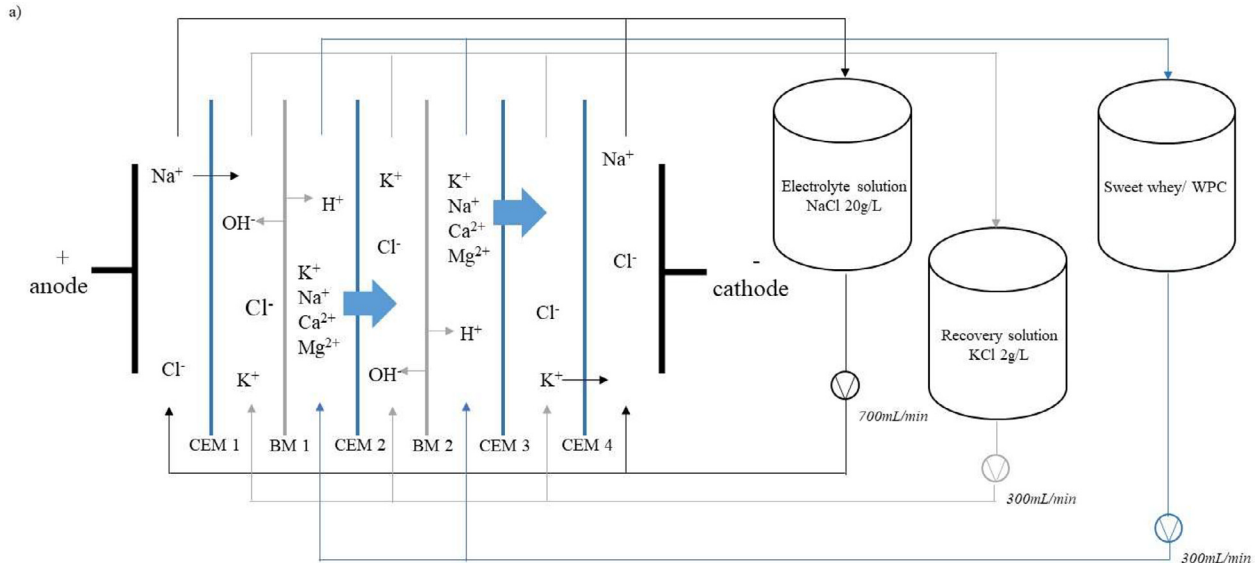

Fig. 1. (a) electrodialysis configuration used to simultaneously acidify and demineralized sweet whey or whey protein concentrate (WPC) and (b) stage of the experimental protocol.

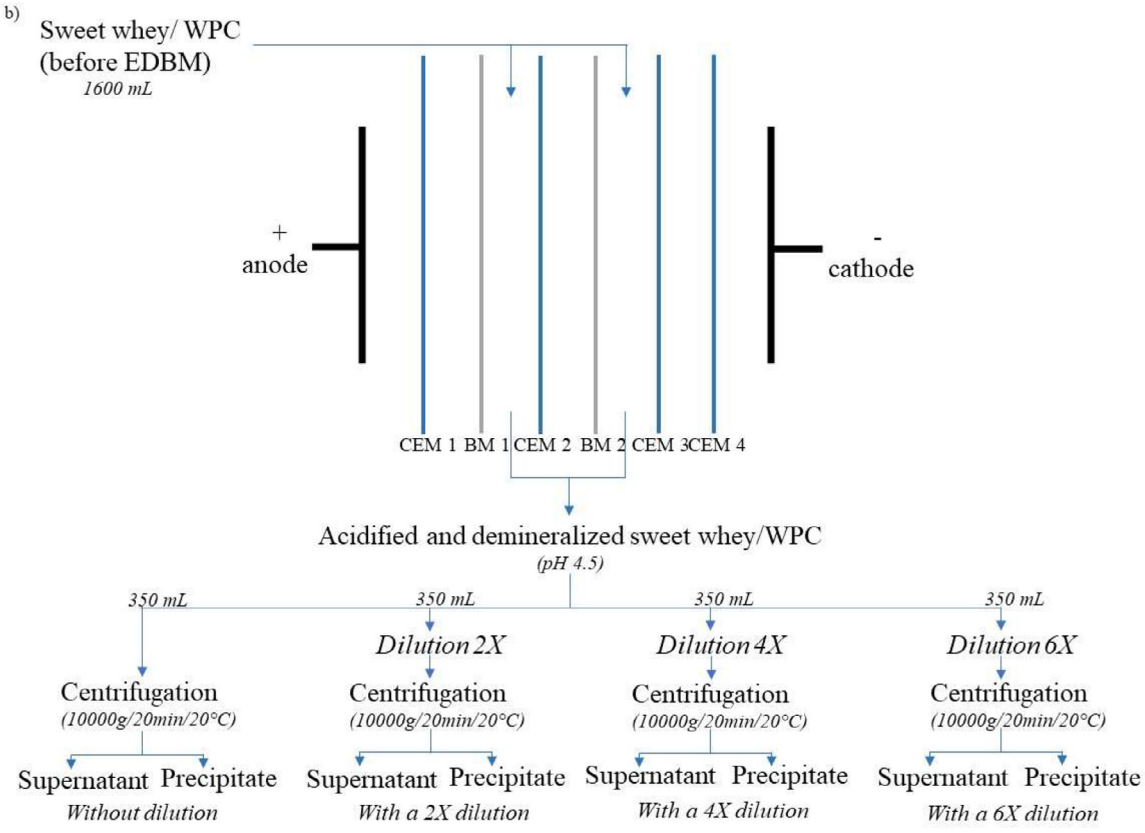

Fig. 1. Continued

2.3. Protocol

The parameters used for EDBM treatments were similar to those used previously by Faucher et al. (2020), except for the volume of the solutions that were 1600 mL for sweet whey/WPC and 2000 mL for recovery and electrode rinsing solutions. Briefly, treatments were performed at room temperature, under a constant current of 1.5 A and until the pH of sweet whey or WPC reached 4.5. The flows were 300 mL/min for sweet whey/WPC, 300 mL/min for the 2 g/L KCl recovery solution and 700 mL/min for the 20 g/L NaCl electrode rinsing solution. Furthermore, the pH and conductivity of sweet whey/WPC and KCl recovery solution were measured every 5 min during EDBM treatments, as well as, the voltage applied to the EDBM cell.

After treatment, 1400 mL of acidified and demineralized sweet whey or WPC were collected and divided in four equal volumes of 350 mL. A first 350 mL was centrifugated at 10000 g, for 20 min at 20 °C, as

is (without dilution). The others were whereas diluted 2X, 4X or 6X in deionized water (2X dilution, 4X dilution and 6X dilution respectively) before being centrifuged as described previously (Fig. 1**b**). All supernatants were recovered and measured, before being freeze-dried. The precipitates for all dilution were washed two times in deionized water (centrifugation between each washing step: 10000 g, 20 min, 20 °C) before being recovered, freeze-dried and weighted. The freeze-dried supernatants and precipitates were used in further analyses.

2.4. Analyses

2.4.1. Electrodialytic parameters and membrane characteristics

2.4.1.1. Global resistance. During EDBM treatments, the voltage (U, in V) and current intensity (I, in A) were directly obtained on the power supply (60V Multi Range DC Power Supply model 9110, BK Precision, Yorba Linda, CA, USA). They were used to calculate the system global resistance R, in Ω) with Ohm's law (U=RI).

2.4.1.2. Membranes conductivity and thickness. A conductivity clip (Laboratoire des Matériaux Échangeurs d'Ions, Créteil, France) was used to measure the membrane conductance, before and after each EDBM treatment, which was the average of six measurements at different locations on the membranes. The conductivity clip was connected to a conductivity meter (Model 3100, Yellow Springs Instrument, Yellow Spring, OH, USA) and had a distance of 1 cm between the electrode.

Therefore, membrane transversal resistance (R_m, in Ω) was calculated as proposed by Lebrun et al. (2003) and Lteif et al. (1999) as:

$$R_m = \frac{1}{G_m} = \frac{1}{G_{m+s}} - \frac{1}{G_s} = R_{m+s} - R_s \quad (1)$$

Where G_m is the membrane conductance (S), G_{m+s} is the conductance of the membrane and the NaCl 0.5M solution used for measurement (S), G_s is the conductance of the NaCl solution (S), R_{m+s} is the resistance of the membrane and the NaCl solution (Ω) and R_s is the resistance of the NaCl solution (Ω).

The membrane thickness (L, in cm) was measured with an electronic micrometer (Marathon Watch Company Ltd., Richmond Hill, ON, Canada) and was averaged from six measurements, as for the membrane conductance.

With the membrane thickness and electrical resistance, the conductivity of the membrane (K, in mS/cm) was calculated as Lteif et al. (1999):

$$K = \frac{L}{R_m \times A} \quad (2)$$

Where A is the electrode surface ($1cm^2$)

2.4.2. Physicochemical parameters

2.4.2.1. pH. To follow the evolution of the pH of the KCl recovery solution and sweet whey or WPC during EDBM treatments, a VWR Symphony pH-meter model SP20 (Thermo Orion, West Chester, PA, USA) was used.

2.4.2.2. Conductivity. A YSI conductivity meter (Model 3100, Yellow Springs Instrument, Yellow Spring, OH, USA) with a YSI immersion probe (Model 3252, cell constant K=1 cm^{-1}) was used to measure the conductivity of sweet whey or WPC and KCl recovery solution.

2.4.2.3. Mass of precipitates. An AB204-S/FACT Mettler Toledo analytical scale (Columbus, OH, USA) was used to weight the freeze-dried precipitates (without dilution and with a 2X, 4X and 6X dilution for sweet whey and WPC).

2.4.2.4. Lactose. Sweet whey before EDBM, WPC before EDBM and all supernatants (without dilution and with a 2X, 4X and 6X dilution for sweet whey and WPC) lactose content was analyzed through high-performance liquid chromatography (HPLC) (Organization of International Standardization, 2007). Such analysis was not performed on the precipitates, since some quantities recovered after treatment were too small.

Briefly, prior to HPLC analysis, 0.2 g freeze-dried sample were solubilized in HPLC grade water and treated with Biggs-Szijarto solution (Organization of International Standardization, 2007). The solution was centrifuged (5000 g, 5 min, 10 °C) and the supernatant was recovered, before being diluted 10 times in HPLC grade water and filtered using a 0.45 μm nylon filter (CHROMSPEC Syring Filter, Chromatographic Specialties, Brockville, ON, Canada). An Agilent 1100 Series chromatograph (Santa Clara, CA, USA) equipped with an Agilent 1260 Infinity refractive index detector (Santa Clara, CA, USA), a column oven and a cooled 717 Plus autosampler was used as the HPLC system for the measurement. 15 µL of the prepared liquid sample were injected on an ICSep-ICE-ION-300 column (Transgenomic, Omaha, NE, USA), kept under a constant temperature of 40 °C, with a mobile phase of a 6.5 mM of H_2SO_4 (180 μL H_2SO_4/L) solution at a flow rate of 0.4 mL/min. The run time was set at 45 min and a standard of lactose anhydrous (Sigma Company, Saint-Louis, MO, USA) was used as the external standard to perform quantification.

2.4.2.5. Moisture, ashes and mineral composition. The AOAC methods 927.05 and 930.30 (International Association of Official Analytical Chemists, 1995) were used to determine the moisture and ash contents of sweet whey before EDBM, WPC before EDBM and all supernatants (without dilution and with a 2X, 4X and 6X dilution for sweet whey and WPC). To perform the measurements, 0.50 g of freeze-dried sample was weighed and dried (100 °C, 5 h) into a vacuum oven (Isotemp Vacuum Oven Model 280A, Thermo Fisher Scientific, Waltham, MA, USA). After drying, the samples were weighed again, before calcination (550 °C) overnight in a furnace (Lindberg/Blue M Moldatherm Box Furnaces, Thermo Fisher Scientific, Waltham, MA, USA). The ashes were recovered and let cooled before being weighed.

The moisture content (g/100g on a dry basis) was calculated as:

$$\left(\frac{Mass_{dry}}{Mass_{ini}}\right) \times 100 \quad (3)$$

Where $Mass_{dry}$ is the mass of the sample (g) after being dried in the vaccum oven and $Mass_{ini}$ is the initial mass of the sample (g).

The ash content (g/100g on a dry basis) was calculated as:

$$\left(\frac{Mass_{ash}}{Mass_{ini}}\right) \times 100 \quad (4)$$

Where $Mass_{ash}$ is the mass of ashes (g) after cooling and $Mass_{ini}$ is the initial mass of the sample (g).

The ashes were then solubilized in 10 mL of a 25 % V/V nitric acid solution and the solution obtained was filtered with a 0.45μm PTFE filter (CHROMSPEC Syring Filter, Chromatographic Specialties, Brockville, ON, Canada) and used to analyze the mineral composition of the sample. Calcium, potassium, sodium, magnesium and phosphorus were determined using an Agilent 5110 SVDV ICP-OES (Agilent Technologies, Victoria, Australia) at the following wavelengths: 588.995; 589.592 (Na), 393.366; 396.847; 422.673 (Ca), 279.553; 280.270; 285.213 (Mg), 766.491 (K), 177.434; 178.222; 213.618; 214.914 (P). All ion analyses were performed in axial and/or radial view.

This analysis was not performed on any precipitate, as for some conditions, the quantities recovered were too small.

2.4.2.6. Total lipids. The total lipid content was determined on sweet whey before EDBM, WPC before EDBM and all supernatants (without dilution and with a 2X, 4X and 6X dilution for sweet whey and WPC) using the same modified Mojonnier method used by Faucher et al. (2020). As

for the lactose and mineral contents, the total lipid content of the precipitates was not analyzed due to too small quantities recovered to perform this analysis.

2.4.2.7. Phospholipids. PL analysis was carried out on sweet whey before EDBM, WPC before EDBM and for this specific analysis on precipitates (without dilution and with a 2X, 4X and 6X dilution for sweet whey and WPC), according to Ferreiro et al. (2017) with some modifications. First, for PL extraction, the samples were dissolved in chloroform:methanol (2:1, v/v) at a concentration of 10 mg/mL, and the mixture was heated in a shaking water bath at 25 °C for 30 min at 75 rpm. Then, the mixture was filtered using a 0.45 µm PTFE syringe filter (Fisher Scientific, Pittsburgh, PA, USA), and then transferred into an amber vial.

PL composition in the extracted samples was determined using an ultra-performance liquid chromatography (UPLC) (Agilent 1290 Infinity II UPLC, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a Sedex 85 evaporative light scattering detector (ELSD) (Sedere, Alfortville Cedex, France). The drift tube temperature was at 80 °C, the nebulizer gas pressure was 3.2 bar, and the flow rate of nitrogen was set at 0.9 standard liters per minute (SLM). The samples were separated on a µPorasil silica column (3.9 × 30 mm, 10 µm particle size; Waters Corporation, Milford, MA, USA) using a mobile phase of A: chloroform, B: methanol and C: 1 % acetic acid with ammonium hydroxide at pH 6.0. The gradient elution was 80:19.5:0.5 (A:B:C, v/v/v) at the beginning, and changed to 60:33:7 (A:B:C, v/v/v) within 17 min, and then changed back to 80:19.5:0.5 (A:B:C, v/v/v) within 5 min, and kept constant for 12 min. The flow rate of the mobile phase was 0.7 mL/min and the temperature of the column oven was set at 40 °C. The injection volume was 20 µL. PLs were identified by comparison of the retention data with those of authentic PL standards and the PLs were quantified using the external calibration curves that were prepared using the authentic PL standards.

2.4.2.8. Crude protein, non-protein nitrogen and true protein. The AOAC 991.20 Kjeldahl method (International Association of Official Analytical Chemists, 1995) was used to quantified the total nitrogen content of sweet whey before EDBM, WPC before EDBM, supernatants (without dilution and with 2X, 4X and 6X dilution for sweet whey and WPC) and precipitates (without dilution and with a 2X, 4X and 6X dilution for sweet whey and WPC). BÜCHI equipment (Flawil, Swizterland) were used to perform digestion and distillation. A nitrogen-to-protein conversion factor of 6.38 was used to calculate the crude protein content which was averaged from a duplicat. For sweet whey and WPC before EDBM, as for all supernatants (without dilution and with a 2X, 4X and 6X dilution), it was also possible to analyze the non-protein nitrogen (NPN) content (991.21 AOAC method (International Association of Official Analytical Chemists, 1995)) and the true protein content of the samples. For the precipitates, such analysis was not performed, due to too small quantities recovered after treatment.

With the crude protein and the NPN contents, it was possible to calculate the true protein content as:

$$Proteins_{true} = Proteins_{crude} - NPN \quad (5)$$

Where $Proteins_{true}$ is the true protein content, $Proteins_{crude}$ is the crude protein content and NPN is the non-protein nitrogen.

2.4.2.9. Ionic strength provided by cations. Ionic strength provided by cations (Na^+, K^+, Ca^{2+}, Mg^{2+} and P^{5+}) was calculated for sweet whey before EDBM, WPC before EDBM and all supernatants (without dilution and with a 2X, 4X and 6X dilution for sweet whey and WPC) as:

$$I = \frac{1}{2}\sum_i c_i z_i^2 \quad (6)$$

Where I is the ionic strength ($mol \cdot L^{-1}$), c_i is the molar concentration ($mol \cdot L^{-1}$), and z_i is the charge of an ionic species.

2.5. Ecoefficiency analysis

Ecoefficiency (EE) can be used as an instrument to increase value of product, while minimizing its environmental impact, and that throughout the product life cycle (Verfaillie and Bidwell, 2001). Hence, EE can be defined as:

$$EE = \frac{value\ of\ the\ product}{environmental\ impacts\ of\ the\ product} \quad (7)$$

As part of this work, an indicator of the environmental impacts was used to calculate the EE scores of all scenarios, as it has previously been carried out by Agoua et al. (2020). Here, the volume of water and effluents involved in the process, that include the water needed to perform the dilution after EDBM, the washing of the precipitate and the permeate recovered from the ultrafiltration treatment that need to be treated (when applicable), was used as the indicator. This indicator was chosen since the use of water and the treatment of effluents is one of the main environmental consideration of the process assessed here. Furthermore, it was important to mention that the energy consumption of both products during EDBM was not included in the indicator of the environmental impacts. Indeed, they were not different when reported to the same amount (g) of protein treated, since the quantity of H^+ electrogenerated is dependent of the protein content (Faucher et al., 2020).

To determine the value of the product, three different approaches were evaluated: (1) g of PLs recovered/g of crude protein treated ratio (to compare sweet whey and WPC on the same basis), (2) PL/crude protein ratio in the precipitate (as a high PL content is desired in the precipitate) and (3) a combination of approaches 1 and 2, expressed as the product between both ratios.

2.6. Statistical analyses

Data obtained were reported as mean value ± standard deviation. The physicochemical characteristics of the sweet whey and WPC, as the electrodialytic parameters, were subjected to a t-test. When the impact of the product (either sweet whey or WPC) and the dilution was assessed, the values obtained were then subjected to a two-way ANOVA. SigmaPlot software (version 12, Systat Software, San Jose, CA, USA) was used and the statistical difference were analyzed by Tukey test ($P < 0.05$).

3. Results and discussion

3.1. Electrodialysis treatment

3.1.1. pH and conductivity of the solutions

During EDBM treatments, the evolution of pH and conductivity of sweet whey and WPC followed the same tendencies for both products. As expected, their pH decreased linearly (Fig. 2**a**) due to the production of H^+ ions by the BMs and the duration needed to reach pH 4.5 was longer for WPC than for sweet whey ($P < 0.001$), as they have different protein content (Table 1). Indeed, the number of electrogenerated H^+ ions is linearly dependent of protein content which explains the different treatment duration (Faucher et al., 2020). Concerning the conductivity (Fig. 2**b**), despite the fact that demineralization occurred during treatment due to the migration of ionic species, such as K^+, Na^+, etc. through the CEMs, for both products, during the first 1000 s of treatment, conductivity increased due to the production of H^+ ions by the BMs. Since their electrical conductivity is higher than the conductivity of other species found in the system (Lin Teng Shee et al., 2008), the impact of the cation (K^+, Na^+, etc.) migration was less visible. However, after 1000–1200 s, for WPC, seemed to reach a plateau due to the simultaneous generation of H^+ ions and their migration through the CEMs (Faucher et al., 2020). Such tendency was not observed for sweet whey, as the treatment duration was probably too short.

During treatment, pH and conductivity of the KCl solution evolved in a similar way for sweet whey and WPC and their evolution followed ten-

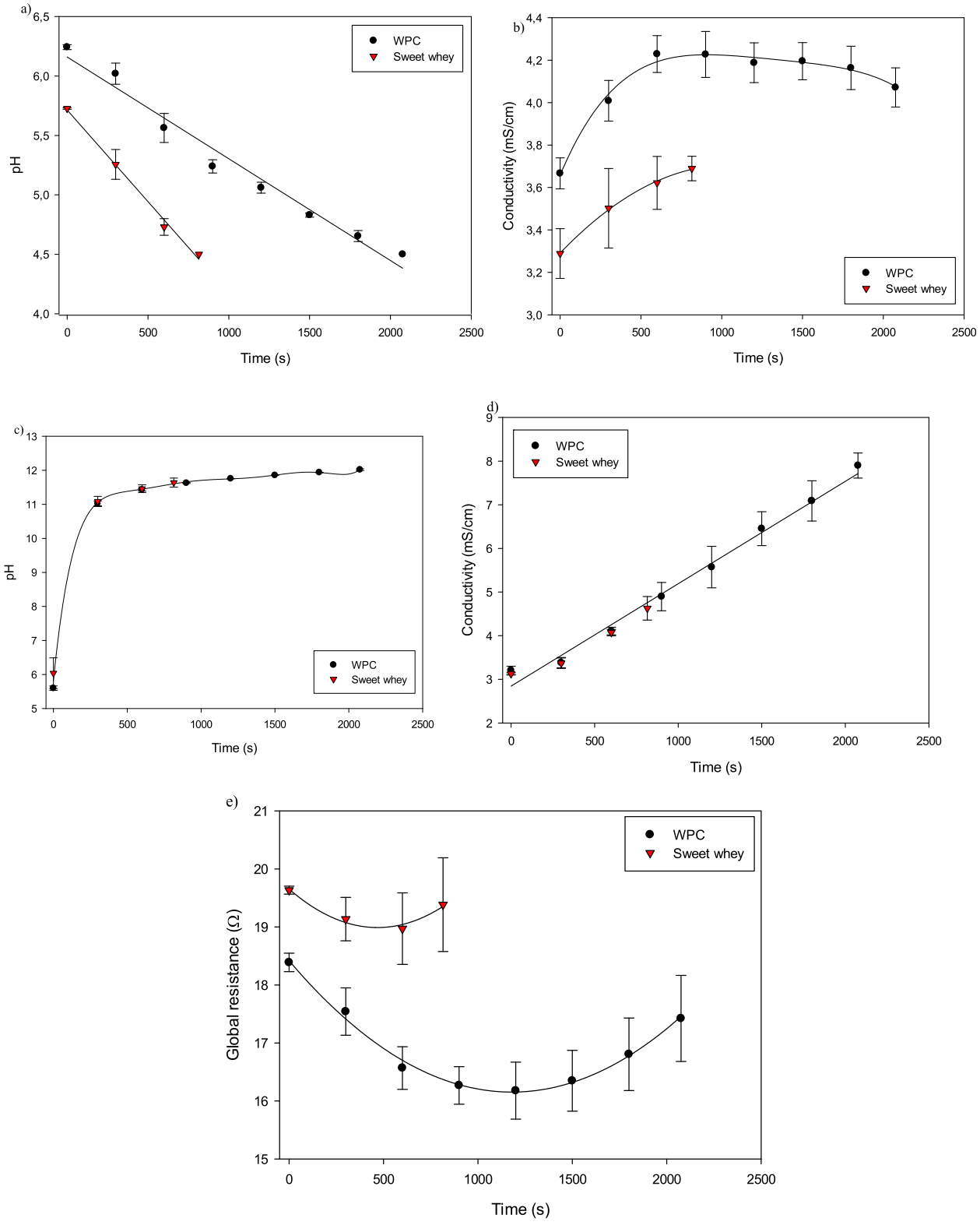

Fig. 2. Evolution as a function of time of a) pH in sweet whey and whey protein concentrate (WPC), (b) conductivity in sweet whey and WPC, (c) pH in the KCl solution, (d) conductivity in the KCl solution and (e) global resistance of the system during treatments.

dencies that were highlighted in a previous work (Faucher et al., 2020). Briefly, the pH increased drastically (Fig. 2**c**) due to the production of OH^- ions by the BMs and conductivity increased (Fig. 2**d**) because of cation migration and OH^- ion electrogeneration.

3.1.2. *System global resistance*

System global resistance evolution for sweet whey and WPC followed the same tendencies (Fig. 2**e**): a decrease of resistance at the beginning of treatment, followed by a slowly increase as the treatment continued.

Table 2
Mineral content (g/100g on a dry basis) of the sweet whey and whey protein concentrates before EDBM and the supernatants at different dilution factors.

	Sweet whey					Whey protein concentrate				
	Before EDBM	Supernatant without dilution	Supernatant with a 2X dilution	Supernatant with a 4X dilution	Supernatant with a 6X dilution	Before EDBM	Supernatant without dilution	Supernatant with a 2X dilution	Supernatant with a 4X dilution	Supernatant with a 6X dilution
Calcium	0.69±0.02[a]	0.62±0.01[b]	0.62±0.01 [b]	0.64±0.01 [b]	0.64±0.01 [b]	0.58±0.02[a]	0.50±0.01[b]	0.50±0.01 [b]	0.52±0.01 [b]	0.52±0.01 [b]
P value	0.001					<0.001				
Potassium	1.90±0.04[a]	1.58±0.05[b]	1.57±0.04 [b]	1.53±0.07 [b]	1.61±0.10 [b]	1.33±0.03[a]	0.76±0.15[b]	0.83±0.08 [b]	0.85±0.05 [b]	0.84±0.10 [b]
P value	<0.001					<0.001				
Magnesium	0.10±0.01[a]	0.11±0.00 [a]	0.10±0.01 [a]	0.11±0.01 [a]	0.11±0.00 [a]	0.07±0.02 [a]	0.09±0.00 [a]	0.09±0.0 0 [a]	0.09±0.00 [a]	0.09±0.00 [a]
P value	0.229					0.212				
Phosphorus	0.59±0.01 [a]	0.60±0.01 [a]	0.58±0.01 [a]	0.59±0.02 [a]	0.61±0.00 [a]	0.49±0.01 [a]	0.50±0.01 [a]	0.49±0.01 [a]	0.50±0.00 [a]	0.50±0.01 [a]
P value	0.182					0.488				
Sodium	0.68±0.01 [a]	0.59±0.02[b]	0.59±0.02b	0.60±0.03 [a]	0.58±0.04[b]	0.55±0.02 [a]	0.45±0.02[b]	0.45±0.01 [b]	0.46±0.02 [b]	0.48±0.02 [b]
P value	<0.001					<0.001				

Letter: difference between before EDBM and the supernatants at different dilution factor either for sweet whey or whey protein concentrate $P < 0.05$ (Tukey test).

More details on the system global resistance can be found in the **Appendix**.

3.1.3. Thickness and conductivity of the membranes

Following the EDBM treatments, the thickness of the membranes did not evolve for both sweet whey and WPC. However, there were some variations in the membrane conductivity, particularly for CEM 2 and CEM 3 for both sweet whey and WPC, due to change in the membrane transversal resistance. More details on the evolution of thickness and conductivity of the membranes, as well as some figures, and can be found in the **Appendix**.

3.2. Mass of the precipitates

The mass of the washed and freeze-dried precipitates recovered from treated sweet whey and WPC are presented in Table A1 (**Appendix**). There were differences in the mass of the precipitates recovered depending on the product (sweet whey or WPC) and the dilution factor (without dilution and with a 2X, 4X and 6X dilution) applied after EDBM treatment. First, for the same dilution factor, a higher mass was recovered for WPC than for sweet whey ($P < 0.001$ for all) (ex.: 0.08 ± 0.01 g for sweet whey and 0.56 ± 0.03 g for WPC without dilution). When looking at the effect of the dilution factor, it was possible to observe different behaviors for sweet whey and WPC. Indeed, for sweet whey, dilution led to a higher mass recovered, but there was no difference in the mass of the precipitates recovered for dilution factors 2X, 4X and 6X (0.08 ± 0.01 g without dilution and around 0.20 g with a 2X, 4X and 6X dilution). However, for WPC, a higher dilution factor led to a higher mass of precipitate recovered ($P < 0.001$) (ex.: 0.56 ± 0.03 g without dilution and 1.75 ± 0.06 g with a 6X dilution).

Whatever the product, sweet whey or WPC, dilution led to a higher mass of precipitate, possibly due to a greater formation of complexes between lipids and proteins, since the ionic strength was further decreased. Indeed, the formation of lipoprotein complexes seemed to be mainly affected by a dilution (Faucher et al., 2020). However, for sweet whey and WPC, the dilution factor (2X, 4X or 6X) did not have the same effect on the mass recovered. That could be related to their protein content: a product with a higher protein content and diluted after EDBM could further promote protein precipitation along with lipoprotein complex formation. Such phenomenon was already reported in a previous work on WPCs with high protein content (Faucher et al., 2020) and could explain the different masses observed.

3.3. Physicochemical composition of the supernatants and precipitates

3.3.1. Lactose

As mentioned previously, the lactose content of sweet whey before EDBM, WPC before EDBM and the supernatants were analyzed and are presented in Fig. 3**a**. For both sweet whey and WPC, there was no significant difference in the lactose content between the product before EDBM and the supernatants whatever the dilution factor ($P = 0.793$ for sweet whey and $P = 0.182$ for WPC). Lactose was not impacted during EDBM since it has no charge and has a high molecular weight (342.3 g/mol) (Lide, 2005) which do not allow its migration through the membrane. Nevertheless, since the results are presented in g/100g on a dry basis and other component contents changed, it was surprising that the lactose content did not change after treatment.

3.3.2. Ashes and mineral composition

For sweet whey and WPC, there was a significant decrease in ash content of the supernatants (without dilution and with a 2X, 4X and 6X dilution), compared to their content before EDBM (Fig. 3**b**) ($P = 0.008$ and $P < 0.001$ for sweet whey and WPC respectively). This was due to the partial demineralization that occurred during EDBM treatments, since the configuration was designed to promote cation migration from the sweet whey/WPC compartment to the KCl recovery compartment through CEMs. Nevertheless, the ash content of the supernatants for all dilution factors, either for sweet whey or WPC, was not different (Fig. 3**b**). Indeed, they were generated from the same treated product and the results are expressed on a dry basis. Furthermore, the demineralization rate of sweet whey (10.0 ± 1.9 %) and WPC (23.1 ± 3.0 %) were significantly different ($P < 0.001$), due to their different EDBM treatment durations (Section 3.1.1), as a longer treatment duration further promoted migration.

Regarding the mineral composition and content of the samples, few observations can be made. First, as for the ash content and for the same reasons explained previously, there was no difference between the supernatants whatever the dilution factor, either for sweet whey or WPC (Table 2). Secondly, for K^+, Na^+ and Ca^{2+} cations, there were significant differences in their contents between the value before EDBM and the supernatants for both sweet whey and WPC (Table 2). Furthermore, when calculating the demineralization rate specific to each of these cations, the demineralization rate followed the order $K^+ > Na^+ > Ca^{2+}$ (K^+: 17.4 ± 1.8 % and 36.9 ± 0.5 %, Na^+: 13.2 ± 0.0 % and 16.5 ± 2.2 %, Ca^{2+}: 9.0 ± 1.0 % and 11.7 ± 2.0 % for sweet whey and WPC respectively). Thus, it is possible to see that cation migration did not take place in the same way for all cations during EDBM treatment (for both sweet whey and WPC), since different contents after treatment and specific demineralization rates were observed. Indeed, some cations are more favorable to migrate due to different phenomena: (1) they have different electrical mobility and conductivity which modulate their migration (for example, K^+ has a high electrical mobility and conductivity in comparison with other cations, which could explain why its content decreased the most during treatment (Lemay et al., 2020) and (2) some species, such as calcium, can have a tendency to interact with other components of sweet whey or WPC, such as proteins, which could limit

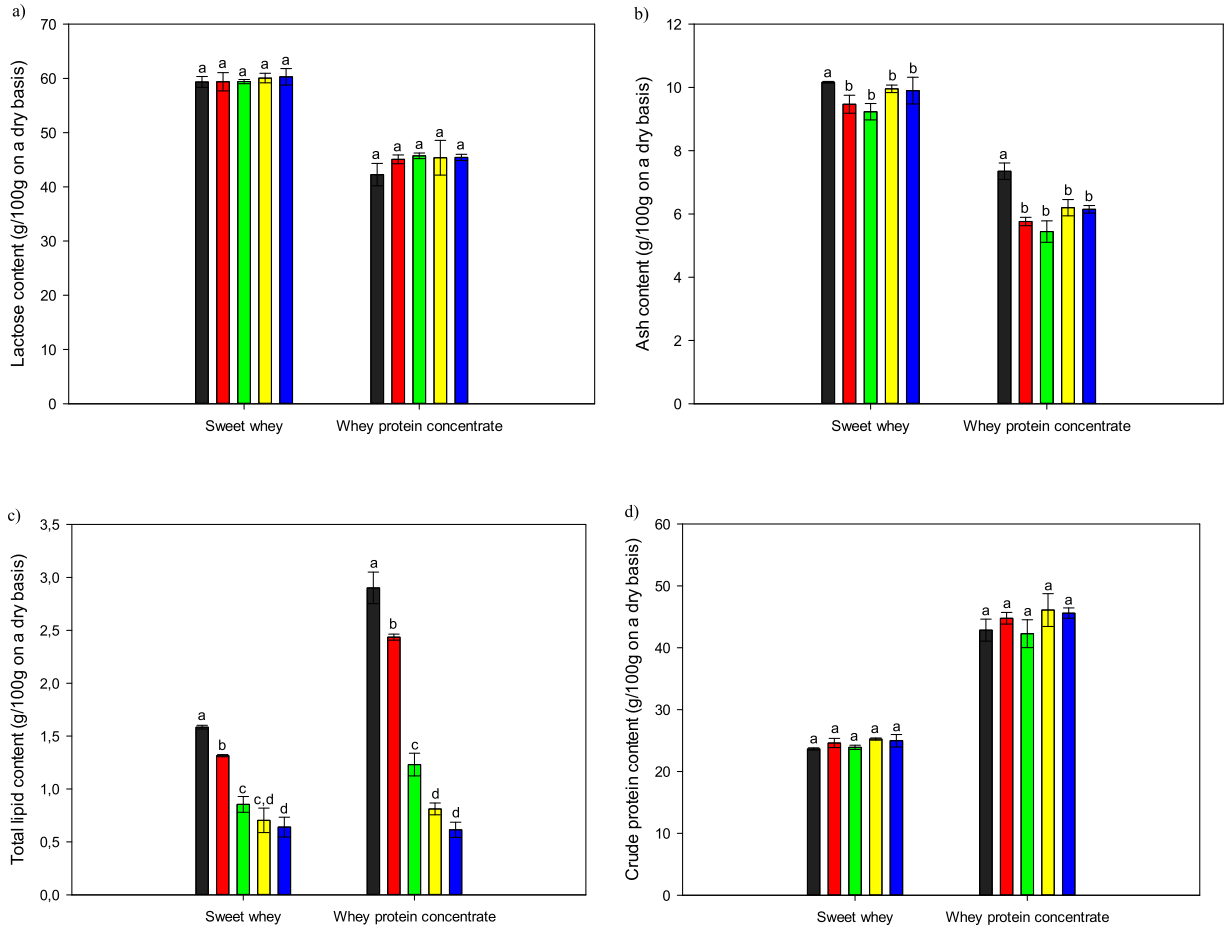

Fig. 3. (a) lactose, (b) ash content, (c) total lipid, d) crude protein, (e) non-protein nitrogen, (f) true protein and (g) moisture contents of sweet whey and whey protein concentrate before EDBM and the supernatants at different dilution factors.
The results were normalized according to the mass balance to highlight the tendencies observed
Different letters for a same product (either sweet whey or WPC) and a same component are significantly different $P < 0.05$ (Tukey test)

their migration. Such phenomena were already reported and described in a previous work Faucher et al. (2020). Furthermore, it is important to mention that the difference in the cation migration may also be the result of Donnan theory. Indeed, CEMs have a different permselectivity to counterions (cations), as multivalent counterions are more strongly absorbed to the CEM than monovalent ones (Strathmann, 2010). Nevertheless, it was important to mention that in a previous work with a WPC of similar crude protein content (Faucher et al., 2020), there was a tendency to have less calcium in the supernatants, whereas here, the decrease is significant. This may be only due to the different batches of WPC of the two works and their different treatment duration needed to reach pH 4.5.

3.3.3. Total lipids

The total lipid content of sweet whey before EDBM, WPC before EDBM and all supernatants are presented in Fig. 3**c**. For both products, the content is significantly different between the initial value (before EDBM) and the supernatants ($P < 0.001$ for sweet whey and WPC). Indeed, the process allowed to decrease the lipid content of the supernatants compared to its corresponding product before EDBM and this decrease was even more marked with an increase in the dilution factor. The defatting rates associated with these lipid contents were calculated and are presented in Table 3.

When a dilution was applied after EDBM, the defatting rates reached were higher for WPC than for sweet whey ($P = 0.700$ for without dilution, $P = 0.031$ for a 2X dilution, $P = 0.029$ for a 4X dilution and $P = 0.007$ for a 6X dilution). That phenomenon can be explained by the different protein content of sweet whey and WPC (Table 1). Indeed, in this process, the defatting mechanism exploited is the formation of lipoprotein complexes following a decrease of pH and ionic strength; phenomena that occurred during EDBM treatment. The literature suggests that the PLs would be involved in these lipoprotein complexes, as they can be negatively charged and can interact with proteins positively charged. However, when ionic strength is further lowered, such as following a dilution after EDBM, the protein content also impacts the complex formation: a higher protein content further favorizes lipoprotein complex formation and allows to reach higher defatting rates (Faucher et al., 2020). However, as previously reported Faucher et al. (2020), the increase in defatting rate here was not proportional to the protein content (when a dilution was applied) since WPC has 1.97 times more crude protein and 2.18 times more true protein than sweet whey but the defatting rates reached for WPC were only 1.23 ± 0.14 to 1.30 ± 0.13 times higher than for sweet whey.

Concerning the effect of the dilution factor on the defatting rates, for both sweet whey and WPC, different defatting rates were reached for the different dilution factors (without dilution and with a 2X, 4X and 6X dilution) ($P < 0.001$ for both sweet whey and WPC). Some interesting observations can also be highlighted regarding the impact of the dilution factor on both sweet whey and WPC. First, a dilution was needed to reach a higher defatting rates, as the demineralization that occurred

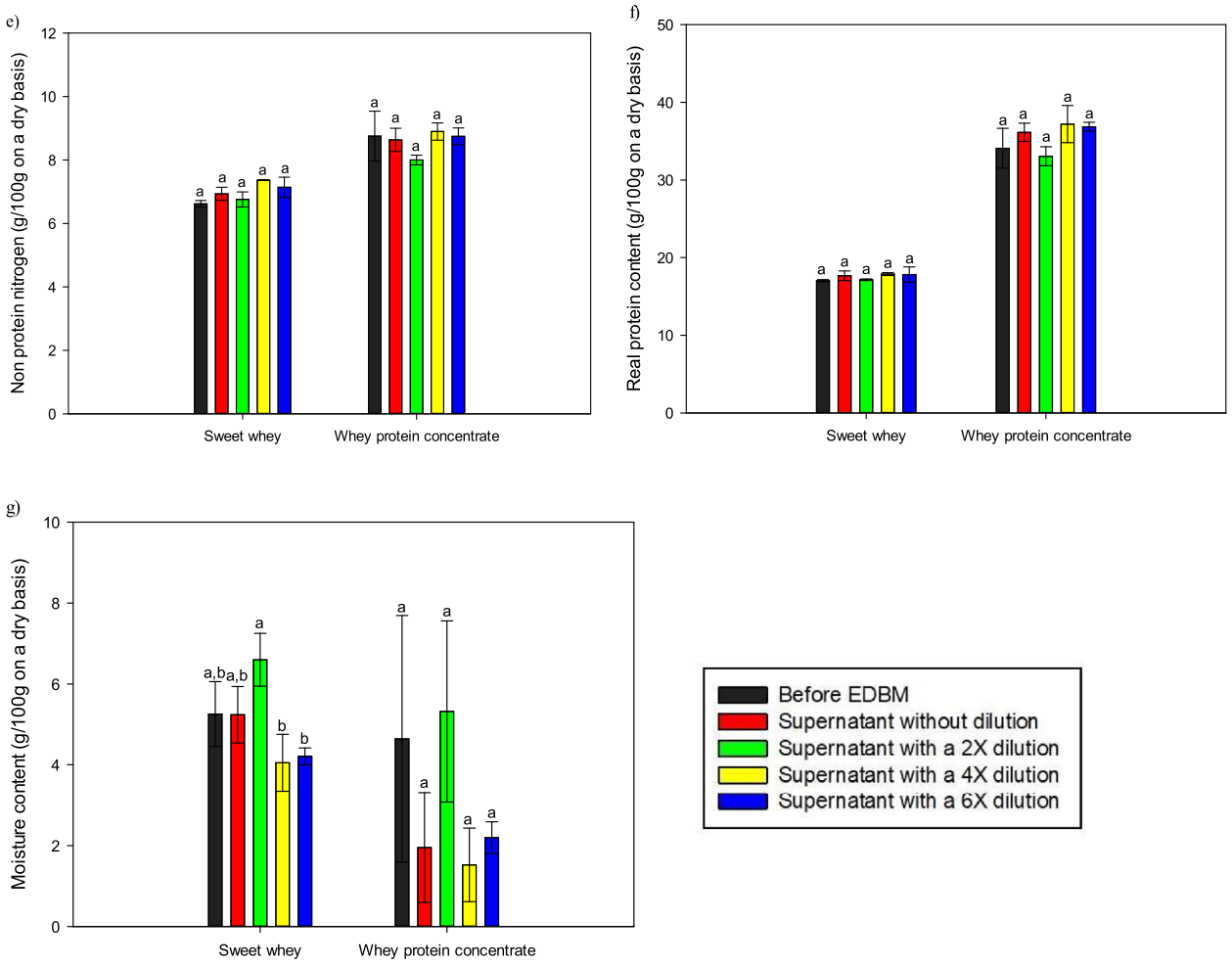

Fig. 3. Continued

Table 3
Defatting rate (%), ionic strength provided by cations (mol•L^{-1}) and ionic strength decrease (%) in the supernatant according to the value before EDBM for sweet whey and WPC.

	Defatting rate		Ionic strength		Decrease	
	Sweet whey	Whey protein concentrate	Sweet whey	Whey protein concentrate	Sweet whey	Whey protein concentrate
Before EDBM	N/A	N/A	0.17 ± 0.01$^{a, A*}$	0.22 ± 0.00$^{b, A}$	N/A	N/A
Supernatant without dilution	20.9 ± 1.4$^{a, A*}$	19.2 ± 4.7$^{a, A}$	0.14 ± 0.00$^{a, B}$	0.20 ± 0.01$^{b, B}$	19.9 ± 3.4$^{a, A}$	11.7 ± 0.9$^{b, A}$
Supernatant with a 2X dilution	47.1 ± 5.2$^{a, B}$	57.6 ± 2.1$^{b, B}$	0.07 ± 0.00$^{a, C}$	0.09 ± 0.00$^{b, C}$	61.5 ± 2.2$^{a, B}$	58.9 ± 1.6$^{a, B}$
Supernatant with a 4X dilution	58.8 ± 7.0$^{a, B, C}$	73.7 ± 3.3$^{b, C}$	0.03 ± 0.00$^{a, D}$	0.05 ± 0.00$^{b, D}$	81.5 ± 1.5$^{a, 5C}$	79.4 ± 0.4$^{a, C}$
Supernatant with a 6X dilution	62.1 ± 5.2$^{a, C}$	80.1 ± 3.3$^{b, C}$	0.02 ± 0.00$^{a, E}$	0.03 ± 0.00$^{b, E}$	87.3 ± 1.3$^{a, C}$	86.5 ± 0.5$^{a, D}$

N/A: not applicable

* Capital letter: difference between the initial value and dilution factors for either sweet whey or whey protein concentrate P <0.05 (Tukey test)Small letter: difference between sweet whey and whey protein concentrate for the initial value or for the same dilution factor P <0.05 (Tukey test)

during EDBM was not sufficient on its own to lower enough the ionic strength to promote the lipoprotein complex formation (Faucher et al., 2020). However, the defatting rates reached did not necessarily increased with the dilution factor. Indeed, the defatting rates seemed to reach a plateau after a 4X dilution. Moreover, for both sweet whey and WPC, there is a quadratic relation between the dilution factor and the defatting rate (y=-1.43x^2+15.34x+21.22, R^2=0.9983 for sweet whey and y=-2.01x^2+21.91x+19.80, R^2=0.9964 for WPC). As mentioned, a dilution is needed to further decrease the ionic strength and to promote the lipoprotein complex formation, but it seems that only a minimal value needs to be reached. Eq. (8) presents the mechanism suggested to explain the lipoprotein complex formation (Cornell and Patternson, 1989) and highlights the important role of ionic strength in it. At high ionic strength, the equation is moving towards the left side and at lower ionic strength concentration, the equation moves to the right, promoting the complex formation.

$$P^{z+} + nCaL^{+} \rightleftarrows PL_n^{(z-n)^{+}} + nCa^{2+} \quad (8)$$

Where P are the proteins with a charge of z$^+$, Ca is the calcium, L are the lipids with a negative charge and n are the lipids that can interact per protein groups.

Ionic strength provided by cations (Na^+, K^+, Ca^{2+}, Mg^{2+} and P^{5+}) of sweet whey before EDBM, WPC before EDBM and all supernatants (without dilution and with a 2X, 4X and 6X dilution) were calculated (Table 3). As expected, the ionic strength decreases after EDBM treatment (before EDBM compared with supernatant) without a dilution, due to the demineralization that occurred during EDBM treatment. Furthermore, ionic strength of the supernatants further decreased with the dilution factor for both sweet whey and WPC. Ionic strength of the supernatants without dilution are 0.14 ± 0.00 mol•L^{-1} and 0.20 ± 0.00 mol•L^{-1} for sweet whey and WPC and represent a decrease of 19.9 ± 3.5 % and 11.7 ± 0.9 % compared to the initial ionic strength of sweet whey and WPC respectively. For both products, since the defatting rate associated with this condition is the lowest, ionic strength value must further favor the left side of Eq. (8) and consequently more or less promotes lipoprotein complex formation. However, when a dilution is applied after EDBM treatment, the ionic strength of the supernatants (with a 2X, 4X and 6X dilution) further decreased which would favor the right side of Eq. (8) and the formation of the complexes. However, for both sweet whey and WPC, the higher defatting rates observed with 4X and 6X dilutions led to a decreasing ionic strength of at least 79 %. Thus, a dilution factor that would allow a decrease of ionic strength around 80 % would be enough to led to higher defatting rates after process.

3.3.4. Crude proteins, non-protein nitrogen, true proteins

Crude protein, non-protein nitrogen and true protein contents for sweet whey before EDBM, WPC before EDBM and supernatants without dilution and with a 2X, 4X or 6X dilution are presented in Fig. 4**d–f**, respectively. For all analyses and for both sweet whey and WPC, there are no significant difference between the content before EDBM and the supernatants (sweet whey: P =0.057 for crude proteins, P =0.09 for non-protein nitrogen, P =0.244 for true proteins, WPC: P =0.108 for crude proteins, P =0.325 for non-protein nitrogen and P =0.212 for true proteins). This result is different of what was observed previously (Faucher et al., 2020), particularly for WPC, where there was a tendency to have less proteins (crude proteins) in the supernatants was observed compared to the value before EDBM. This loss should be normal, since proteins are involved in lipoprotein complexes precipitate after treatment.

Nevertheless, proteins (crude proteins) were found in the precipitates (**Appendix:** Table A2) and their contents varied depending on the product considered (sweet whey or WPC) and the dilution factors (without dilution and with a 2X, 4X or 6X dilution). Previously it has been demonstrated that (1) proteins others than proteins involved in the lipoprotein complexes could precipitate and (2) this phenomenon was greatly dependent on the fact that a dilution was applied after EDBM treatment and the protein content of the considered product. Here, since, WPC has a higher protein content than sweet whey (Table 1), this phenomenon could explain why for all dilution factors (with and without dilution) WPC precipitates have a higher protein content than sweet whey precipitates (ex.: 46.7 ± 0.6 g/100g for sweet whey and 58.3 ± 0.7 g/100g for WPC with a 2X dilution) (P =0.003 without dilution, P <0.001 for a 2–4X dilution and P =0.004 for a 6X dilution): more proteins precipitated in protein complexes. For both, sweet whey and WPC, there are differences in the protein content according to the dilution factor (without dilution and with a 2X, 4X and 6X dilution) (P <0.001 for sweet whey and WPC). The protein content is lower in the precipitates with a dilution compared to the precipitates without a dilution since they technically content more lipids, as the defatting rates of the supernatants are higher (ex.: 50.0 ± 0.2 g/100g with a 6X dilution and 58.3 ± 6.3 g/100g without dilution for sweet whey). For WPC, the protein content of the precipitate with a dilution (2-4-6X) are not different, but for sweet whey, it slightly increases with the dilution factor. Probably, to favor a maximum of protein-protein interactions, the basis for the formation of protein complexes, a minimal threshold must be reached and that threshold also depends on the protein concentration of the solution.

3.3.5. Phospholipids

The PL composition and content of sweet whey before EDBM, WPC before EDBM, precipitates without dilution and precipitates with dilution (2X, 4X and 6X dilution) are presented in Table 4. Here, the main classes of PLs found in dairy products were analyzed, namely, PS, SM, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI). One can notice that in sweet whey before EDBM and WPC before EDBM, PLs were not detected by the analytical method; they would therefore be present in these products in trace amounts. Indeed, as PLs are residual lipids in sweet whey and WPC, they would possibly be in very small quantities compared to the other components. However, PLs were found in the precipitates, which implies that the process actually made it possible to recover and concentrate PLs in the precipitates. It was proven for the first time that PLs are actually found in the precipitate after EDBM treatment combined or not with a dilution; all previous works on this process focused on residual lipids and only hypotheses were made towards PL recovery in the precipitates. Moreover, these results regarding the presence of PLs in the precipitates reinforce the idea that they are involved in the formation of lipoprotein complexes. Furthermore, it is possible to observe that the precipitates contained SM and PS, the two PL classes associated with the health benefices of dairy PLs.

For all dilution conditions (without dilution and with a 2-4-6X dilution), the precipitates recovered from treated sweet whey had a higher total PL content that the precipitates recovered from treated WPC (Table 4). Depending on the dilution factor, the content was 1.47 to 2.51 times higher in sweet whey compared to WPC. This phenomenon was also observed for most classes of PLs. That could be explained by the fact that proteins can be recovered with the PLs and their content in the precipitate is more important for WPC compared to sweet whey (Table A2 **(Appendix) and** Section 3.3.4). Furthermore, a dilution after EDBM treatment led in an improvement of the content in total PLs and in most classes of PLs in the precipitate, which is in accordance with the results obtained for the defatting rates (Table 3). Depending on the product and the dilution factor, this increase reached up to more than 2 times. Those results reinforce the fact that a minimal ionic strength value must be reached to favor the lipoprotein complex formation without higher protein precipitation.

The proportion of each class of PLs found in the precipitate varied for sweet whey and WPC (Table 4). However, regardless of the products and the dilution factors, some classes of PLs tended to be found at very low quantities in the precipitates (particularly SM) and others were presented in large quantities. Thereby, PE and PS were the main PLs found in the precipitates whatever the dilution condition (without dilution or with a 2X, 4X and 6X dilution). Depending on the product and the dilution factor, PE represented between 42.0 ± 1.7 and 51.6 ± 2.3 % of the total PLs recovered in the precipitate and PS, between 30.1 ± 2.2 and 38.7 ± 0.8 % (Table 4). As it was not possible to determine the content of the products before EDBM, it can be difficult to know if the treatment allowed specifically to recover these PLs in the precipitates, compared to other classes of PLs that were present in lower proportion. Nevertheless, to compare the PL composition of the precipitates with the composition of whey or WPC in the literature would be more or less accurate as the origin of the whey/WPC and the type of process can impact the composition (Boyd et al., 1999). However, in previous studies on the clarification on WPC, based on the formation of complexes with PLs with other defatting methods, it has been demonstrated that the recovery of some PLs, such as PE, could be specific (Vaghela and Kilara, 1996). Furthermore, PS is likely to further interact with protein to form the lipoprotein complexes, as, at pH 4.5, it has 2 negatively charged groups (Tatullan, 1993). That could explain why this PLs could be preferentially found in the precipitates if a specific precipitation actually occurred.

Nevertheless, it was important to mention that the phospholipid content in the precipitates is interesting, but due to the volumes that have been treated here, the mass of phospholipids recovered (in grams) are

Table 4
Phospholipid content (in g/100g on a dry basis) and proportion of each class of phospholipids compared to the total phospholipid content (%) for sweet whey and whey protein concentrate (WPC) before EDBM and for the precipitates at different dilution factors.

		PE		PI		PC		PS		SM		Total	
		Sweet whey	WPC	Sweet whey	WPC	Sweet whey	WPC	Sweet whey	WPC	Sweet whey	WPC	Sweet whey	WPC
Phospholipid content	Before EDBM	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Precipitate without dilution	3.53±0.49$^{a, A}$*	1.41±0,04$^{b, A}$	0.90±0.29^{A}	ND	0.81±0.18$^{a, A}$	0.44±0.02$^{b, A}$	2.24±0.22$^{a, A}$	1.14±0.05$^{b, A}$	0.02±0.01$^{a, A}$	0.01±0.00$^{a, A}$	7.50±1.18$^{a, A}$	2.99±0.07$^{b, A}$
	Precipitate with a 2X dilution	5.48±0.55$^{a, B}$	2.32±0.03$^{b, B}$	0.67±0.05$^{a, A}$	0.60±0.02$^{a, A}$	0.92±0.06$^{a, A}$	0.57±0.02$^{b, B}$	3.52±0.06$^{a, B}$	2.02±0.19$^{b, B}$	0.02±0.00$^{a, A}$	0.02±0.01$^{a, A, B}$	10.6±0.6$^{a, B}$	5.53±0.18 $^{b, B}$
	Precipitate with a 4X dilution	6.46±1.00$^{a, B}$	3.21±0.33$^{b, C}$	0.98±0.25$^{a, A}$	0.67±0.09$^{a, A}$	1.20±0.12$^{a, A}$	0.67±0.05$^{b, C}$	4.13±0.46$^{a, B}$	2.88±0.19$^{b, B}$	0.02±0.00$^{a, A}$	0.02±0.00$^{a, B}$	12.8±1.2 $^{a, B}$	7.45±0.49 $^{b, C}$
	Precipitate with a 6X dilution	5.79±0.27$^{a, B}$	3.63±0.58$^{b, C}$	0.96±0.31$^{a, A}$	0.63±0.04$^{a, A}$	1.17±0.25$^{a, A}$	0.69±0.02$^{b, C}$	3.77±0.42$^{a, B}$	3.02±0.02$^{b, B}$	0.02±0.00$^{a, A}$	0.03±0.01$^{a, B}$	11.7±1.2 $^{a, B}$	8.00±0.49 $^{b, C}$
Proportion	Before EDBM	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Precipitate without dilution	47.2±1.1$^{a, A}$*	47.0±1.5 $^{a, A}$	11.8±2.2^{A}	N/A	10.7±0.8$^{a, A}$	14.7±0.4$^{b, A}$	30.1±2.2$^{a, A}$	38.0±1.0$^{b, A}$	0.22±0.05$^{a, A}$	0.33±0.01$^{b, A}$	N/A	N/A
	Precipitate with a 2X dilution	51.6±2.3 $^{a, A}$	42.0±1.7$^{b, B, C}$	6.31±0.73$^{a, A}$	10.8±0.1$^{b, A}$	8.69±0.63 $^{a, A}$	10.4±0.7$^{b, B}$	33.2±1.3 $^{a, A}$	36.5±2.3 $^{a, A}$	0.19±0.01 $^{a, A}$	0.30±0.10 $^{a, A}$	N/A	N/A
	Precipitate with a 4X dilution	50.3±3.2$^{a, A}$	43.0±0.9$^{b, B}$	7.84±2.50 $^{a, A}$	9.03±0.97$^{a, B}$	9.41±1.24 $^{a, A}$	9.00±0.56$^{b, B, C}$	32.3±0.6 $^{a, A}$	38.7±0.8 $^{b, A}$	0.16±0.01 $^{a, A}$	0.27±0.02 $^{b, A}$	N/A	N/A
	Precipitate with a 6X dilution	49.6±3.3 $^{a, A}$	45.2±3.5$^{a, A, C}$	8.06±1.87 $^{a, A}$	7.93±0.32 $^{a, B}$	9.93±1.19 $^{a, A}$	8.61±0.45$^{b, C}$	32.2±0.5 $^{a, A}$	37.9±2.9 $^{b, A}$	0.17±0.02 $^{a, A}$	0.33±0.06 $^{b, A}$	N/A	N/A

N/A: not applicable, ND: not detected, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PC: phosphatidylcholine, PS: phosphatidylserine, SM: sphingomyelin.

* Capital letter: difference between the dilution factor for either sweet whey or WPC $P < 0.05$ (Tukey test).Small letter: difference between sweet whey and whey protein concentrate for the same dilution factor $P < 0.05$ (Tukey test).

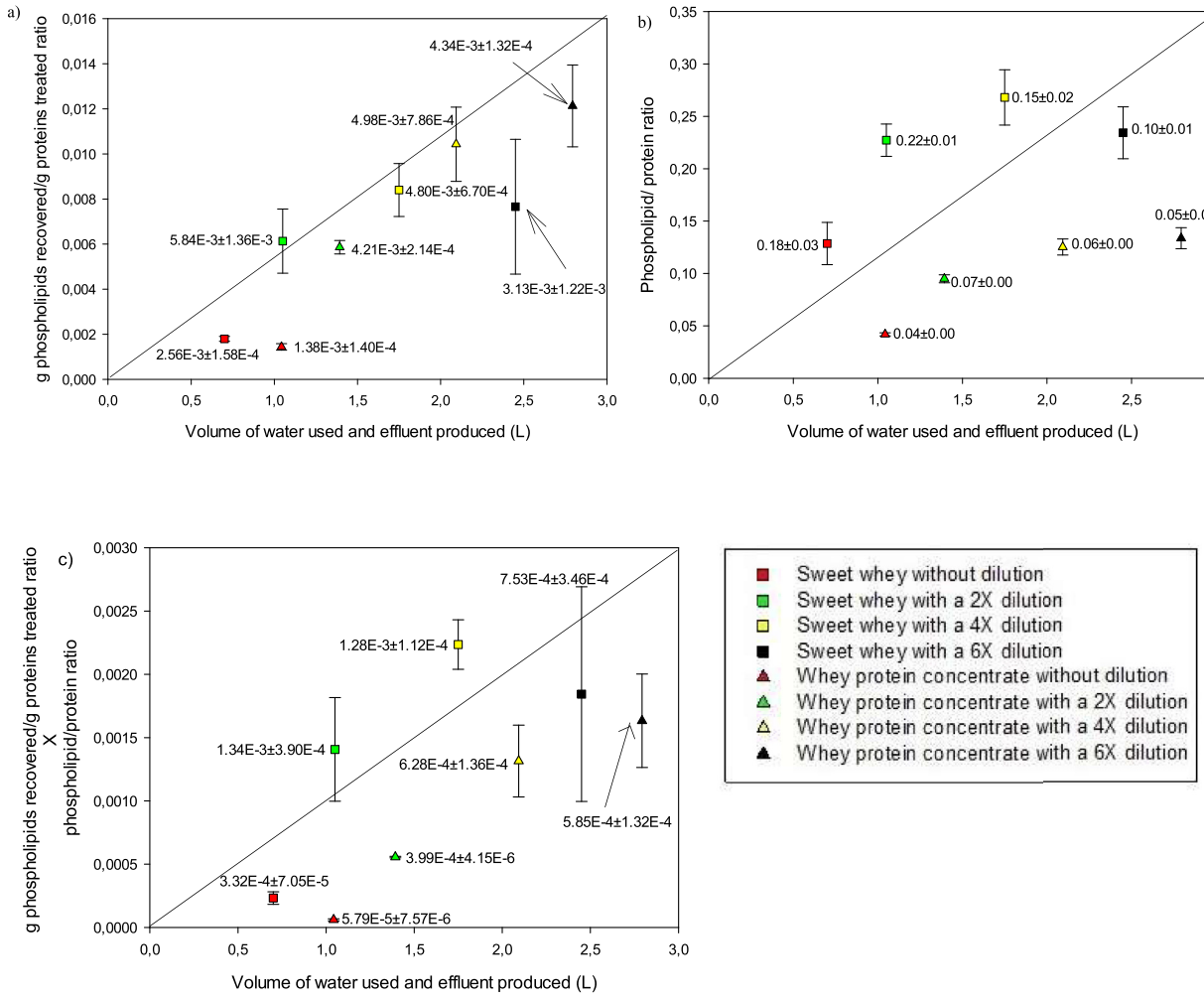

Fig. 4. Ecoefficiency scores with the value being (a) g of phospholipids recovered/g of crude proteins treated ratio (approach 1), (b) phospholipid/crude protein ratio in the precipitate (approach 2) and (c) a combination of g of phospholipids recovered/g of crude proteins treated ratio and phospholipid/crude protein ratio in the precipitate (approach 3) for sweet whey and whey protein concentrate at different dilution factors (without dilution and with a 2X, 4X and 6X dilution).

small. This could explain why they constitute a minor component of sweet whey and WPC before EDBM and they would be found in trace amounts for these products. Indeed, the grams of PLs recovered represent a very small portion of the total solids. On the other hand, when mass balances were carried out on the precipitates without and with dilution (2X, 4X and 6X) for both products, it is possible to see that proteins and PLs do not constitute the total mass of the precipitates. Therefore, other components of sweet whey or WPC must be present. Since the precipitates were washed before being recovered and freeze-dried, it is most likely that these components are not soluble components such as lactose and minerals. Nevertheless, when trying to recover PLs, MFGM fragments are also recovered, as PLs are found in them. MFGM, in addition of being composed of PLs, also contains proteins and other non-polar lipids such as triglycerides, sterols, esters, etc. (Conway et al., 2014). The presence of these other lipids in the MFGM and therefore in the precipitates could be hypothesized to explain the differences in the mass balances. Moreover, as these lipids could also precipitate with the PLs, that could also be a possible explanation for the different defatting rate observed (Table 3). However, to confirm these hypotheses it would be essential to analyze the total lipids of the precipitates, but here, that was not possible, since some precipitates were recovered in too small quantities to perform Mojonnier analysis.

3.4. Ecoefficiency analysis

In Fig. 4, each point represents the relation between the environmental impact and the value (for all value evaluation approaches) of sweet whey and WPC in all dilution conditions. Furthermore, next to each point is also presented the EE score of this scenario and visually the most ecoefficient scenario (compared to the other scenarios) is the scenario, which point can be found above the line and for which the orthogonal distance from the line is the smallest. When calculating the EE for the value approach 1, sweet whey with a 2X and 4X dilution and WPC with a 4X dilution had the highest EE scores. For value calculation approaches 2 and 3, it was rather sweet whey without dilution and with a 2X and 4X dilution that had the highest scores. One can observe that, whatever the dilution condition, WPC had a higher environmental impact compared to its sweet whey equivalent. Indeed, for a dilution condition, the same volume of water was needed to dilute and to wash the precipitates for both products, but WPC generated permeate (effluent) after UF treatment (prior to EDBM) that had to be treated before being reused or rejected, as it is rich in minerals, lactose, etc. Furthermore, concerning the value, for all approaches, the highest values were obtained after a dilution step was performed following EDBM treatment (approach 1 (Fig. 4**a**): 4X and 6X dilution, approach 2

(Fig. 4**b**): 2X, 4X and 6X dilution, approach 3 (Fig. 4**c**): 4X and 6X dilution) since, as discussed previously, a dilution after EDBM treatment increased the recovery of PLs (Section 3.3.5). Moreover, depending on the approach, the highest value was reached by either sweet whey (approaches 2 and 3) or WPC (approach 1). Hence, considering these EE scores, using sweet whey combined with a 2X or 4X dilution after EDBM seems advantageous, as for each scenario, these conditions had the highest EE scores and were more ecoefficient compared to the other scenarios considered.

4. Conclusion

The aim of this work was to recover PLs from sweet whey and WPC by EDBM, to assess the impact of the dilution factor (without dilution and with a 2X, 4X or 6X dilution) on the performances of the process and to carry out an ecoefficiency analysis between the different condition studied.

As previously reported, it appeared that a dilution was necessary after EDBM to reach higher defatting rates, but also high PL recovery, as demonstrated in the present study, since the demineralization that occurred during EDBM was not sufficient to decrease ionic strength enough to promote lipoprotein complex formation. However, when a dilution was applied after EDBM, the increase in the dilution factor did not result in higher performances, as the defatting rate and PL recovery seemed to reach a plateau after a 2X or 4X dilution depending on the conditions. Indeed, a decrease in ionic strength around 80 % would be necessary to further favor lipoprotein complex formation, and thus PL precipitation and recovery. Concerning the product, when diluted after EDBM, WPC allowed to reach higher defatting rates whereas, higher PL content and lower protein content were analyzed in the precipitates recovered from sweet whey. Regarding PL recovery, the treatment allowed to concentrate them in the precipitates and the major classes of PLs found in the precipitates, whatever the conditions, were PS and PE. To the best of our knowledge, it was the first time that the specific recovery of PLs in sweet whey and WPC by EDBM was assessed. Thus, EDBM combined with a dilution appears to be a promising process to recover and precipitate PLs from sweet whey and WPC. However, when an ecoefficiency analysis was performed to compare the different scenarios (sweet whey and WPC for all dilution conditions), whatever the approach used to evaluate the value, sweet whey combined with a 2X or 4X dilution after EDBM was more ecoefficient compared to the other scenarios, as these conditions had the highest score for all approaches (from 1.40 ± 0.37 to 3.37 ± 1.00 times higher than WPC).

Nevertheless, in the future, it would be interesting to treat higher volumes of sweet whey or WPC in order to produce more precipitate. That could allow a more complete characterization of the precipitates and also confirm the nature of the other components found in the precipitates (in addition to proteins and PLs). Furthermore, it would be interesting to test *in vitro* different bioactivities on the precipitates to verify if they demonstrate the positive effects normally associated with the presence of dairy PLs, particularly PS and SM.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix

A.1. System global resistance

The decrease in resistance observed at the beginnings of treatment was due to the production of H^+ and OH^- ions by the BMs and their impact on the conductivity. Indeed, the electrical conductivity of these ions is higher than the conductivity of the other species found in the system (Lin Teng Shee et al., 2008). Therefore, their electrogeneration decreased the system global resistance which was related to a lower voltage that had to be applied during treatment. Afterwards, the increase in resistance, corresponding to higher voltage applied to the cell, was rather due to cations migrations, particularly H^+ ions migration, that occurred during treatment due to the EDBM cell configuration.

A.2. Thickness and conductivity of the membranes

Hence, variations of membrane conductivity were noticed after treatment for both products: for sweet whey there were only significant difference for CEM 2 and CEM 3 ($P < 0.001$ and $P = 0.007$, respectively) (Fig. A1**c**), while after treatment of WPC, all membrane conductivity values were different ($P < 0.001$ for CEM 1, $P = 0.003$ for CEM 2, $P = 0.004$ for CEM 3, $P = 0.002$ for CEM 4, $P < 0.001$ for BM 1 and $P = 0.004$ for BM 2) (Fig. A1**d**). For both products, the loss of conductivity of CEM 2 and CEM 3, that ranged from 19,9 % to 35.4 %, could be explained by a replacement of counterions with a lower electrophoretic mobility due to the cell configuration, a phenomenon that was previously for electrodialysis process (Aspirault et al., 2020; Dufton et al., 2019; Faucher et al., 2020). For the significant loss of conductivity of CEM 1, CEM 4, BM 1 and BM 2 for WPC, such phenomenon was not observed in the previous study of Faucher et al. (2020) for a WPC with a similar crude protein content, even if there was a tendency for membranes to have a lower conductivity after treatment. However, despite

Table A1
Mass of the precipitates (in g) and crude protein content of the precipitates (in g/100g on a dry basis) at different dilution factors.

	Mass		Protein content	
Dilution	Sweet whey	Whey protein concentrate	Sweet whey	Whey protein concentrate
Without dilution	$0.08 \pm 0.01^{a, A*}$	$0.56 \pm 0.03^{b, A}$	$58.3 \pm 6.3^{a, A}$	$71.5 \pm 1.7^{b, A}$
With a 2X dilution	$0.19 \pm 0.03^{a, B}$	$1.23 \pm 0.04^{b, B}$	$46.7 \pm 0.6^{a, B}$	$58.2 \pm 0.7^{b, B}$
With a 4X dilution	$0.22 \pm 0.04^{a, B}$	$1.62 \pm 0.04^{b, C}$	$47.7 \pm 0.4^{a, B, C}$	$59.5 \pm 1.3^{b, B}$
With a 6X dilution	$0.21 \pm 0.06^{a, B}$	$1.75 \pm 0.06^{b, D}$	$50.0 \pm 0.2^{a, C}$	$59.8 \pm 0.4^{b, B}$

* Capital letter: difference between the dilution factors for either sweet whey or WPC $P < 0.05$ (Tukey test)Small letter: difference between sweet whey and whey protein concentrate for the same dilution factor $P < 0.05$ (Tukey test)

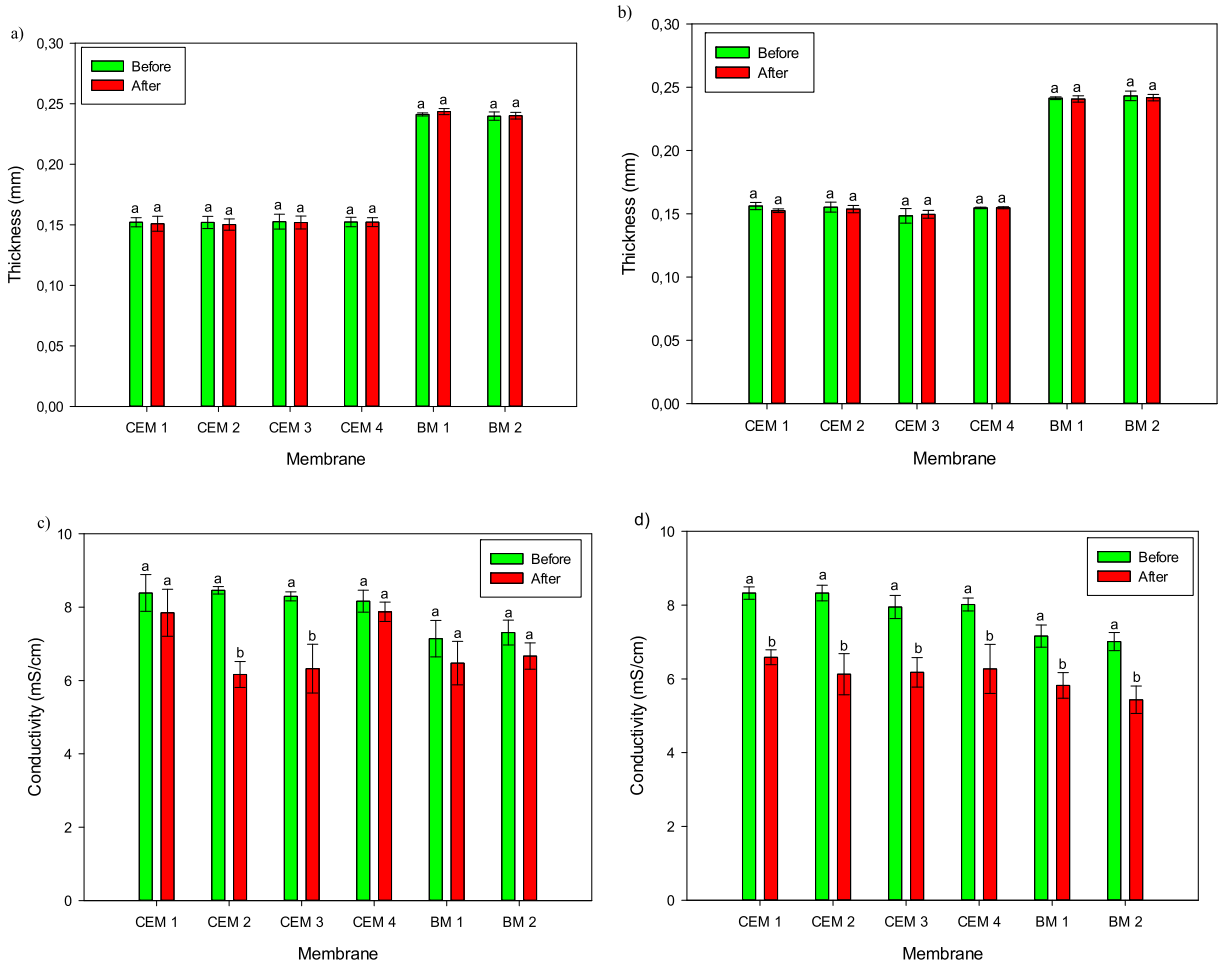

Fig. A1. Evolution of (a) membrane thickness (mm) before and after treatment of sweet whey, (b) membrane thickness (mm) before and after treatment of whey protein concentrate, (c) membrane conductivity (mS/cm) before and after treatment of sweet whey and (d) membrane conductivity (mS/cm) before and after treatment of whey protein concentrate. Different letters for a same membrane are significantly different P <0.05 (Tukey test)

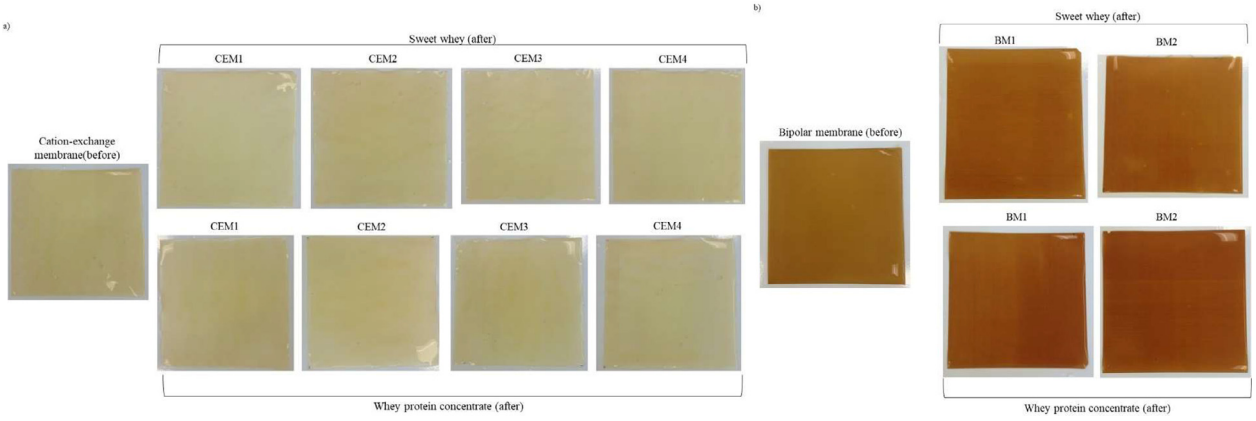

Fig. A2. Photographs of (a) cation-exchange membranes (CEMs) and (b) bipolar membranes (BMs) before and after treatment for both sweet whey and whey protein concentrate.

the fact that the configuration used in this work could allow the interaction between divalent cations and OH^- ions produced by the bipolar membrane, the loss of conductivity of the membranes was not likely due to scaling. The demineralization that occurred during process mainly favors K^+ and Na^+ migration, due to their higher electrophoretic mobility and conductivity. Ca^{2+} and Mg^{2+} migration rates were either very low or zero (due to their electrophoretic mobility or their tendency to interact with other components of sweet whey or WPC) (Section 3.3.2), so interactions between divalent cations and OH^- ions was not likely to happened. Furthermore, no visual scaling was observed on the membranes after treatment (Fig. A2) which explains why no further analyses were made on the membranes. Nevertheless, it was important to men-

Table A2
Physicochemical characteristics of the sweet whey before EDBM, whey protein concentrate before EDBM and the supernatants (without and with a 2X, 4X, 6X dilution) (in g/100g on dry basis).

	Sweet whey					Whey protein concentrate				
	Before EDBM	Supernatant without dilution	Supernatant with a 2X dilution	Supernatant with a 4X dilution	Supernatant with a 6X dilution	Before EDBM	Supernatant without dilution	Supernatant with a 2X dilution	Supernatant with a 4X dilution	Supernatant with a 6X dilution
Crude protein content	38.5 ± 1.4[a]	38.6 ± 0.0[a]	37.9 ± 1.1[a]	38.7 ± 1.0[a]	38.3 ± 0.4[a]	19.5 ± 0.1[a]	19.3 ± 0.2[a]	19.3 ± 0.2[a]	19.3 ± 0.2[a]	19.3±0.2[a]
P value	0.635					0.854				
True protein content	14.0±0.1[a]	13.9±0.2 [a]	13.8±0.0 [a]	13.6±0.2 [a]	13.8±0.3 [a]	30.6±2.0[a]	31.2±0.5 [a]	30.6±1.2 [a]	31.2±1.0 [a]	30.9±0.3 [a]
P value	0.187					0.955				
Non protein nitrogen content	5.44±0.07 [a]	5.44±0.11 [a]	5.45±0.17 [a]	5.62±0.06 [a]	5.52±0.32 [a]	7.87±0.87[a]	7.46±0.46 [a]	7.28±0.17 [a]	7.48±0.02 [a]	7.34±0.14 [a]
P value	0.308					0.637				
Total lipid content	2.60±0.15[a]	2.10±0.02[b]	1.10±0.10[c]	0.68±0.05[d]	0.52±0.06[d]	1.30±0.02[a]	1.03±0.03[b]	0.69±0.06[c]	0.54±0.09[c, d]	0.49±0.07[d]
P value	<0.001					<0.001				
Ash content	8.36±0.01[a]	7.42±0.10 [b]	7.44±0.17 [b]	7.59±0.14 [b]	7.64±0.15 [b]	6.60±0.20[a]	4.97±0.01[b]	4.88±0.19 [b]	5.21±0.07 [b]	5.16±0.13 [b]
P value	<0.001					<0.001				
Lactose content	46.2±1.6[a]	47.9±1.9 [a]	46.9±2.7 [a]	47.2±2.5 [a]	47.4±1.5 [a]	35.7±2.2 [a]	39.4±0.4 [a]	39.4±1.9 [a]	39.7±2.3 [a]	40.1±1.3 [a]
P value	0.889					0.074				
Moisture content	4.32±0.64[a, b]	4.10±0.48 [a, b]	5.32±0.55[a]	3.09±0.51[b]	3.23±0.14 [b]	4.16 ± 2.73[a]	1.70 ± 1.20 [a]	4.80 ± 2.10 [a]	1.28 ± 0.78 [a]	1.85±0.35 [a]
P value	0.002					0.088				

The values presented here are the raw values; they were not normalized according to the mass balance. Letter: difference before EDBM and the supernatants at different dilution factor either for sweet whey or whey protein concentrate P<0.05 (Tukey test)

tion that if EDBM treatment was carried out in such a way to increase the demineralization, scaling could probably occur with this cell configuration. In that case, Ca^{2+} and Mg^{2+} migration would be more important, since sweet whey or WPC would be further depleted in K^+ and Na^+ cations, and that phenomenon could lead to complex formation, of these divalent ions with OH^- ions electrogenerated on the membrane interfaces or inside. In this case, it would be important to implement different approaches to decrease scaling and ensure membrane stability, such as the use of pulsed electric fields.

References

Agoua, R.S., Bazinet, L., Vorobiev, E., Grimi, N., Mikhaylin, S., 2020. Substantial improvement of tryptic and chymotryptic hydrolysis of β - lactoglobulin pretreated with high voltage electrical treatments`. ACS Sustan. Chem. Eng. 8, 14775–14785. doi:10.1021/acssuschemeng.0c03904.

Aspirault, C., Doyen, A., Bazinet, L., 2020. Impact of preheating temperature on the separation of whey proteins when combined with chemical or bipolar membrane electrochemical acidification. Int. J. Mol. Sci. 21, 2792.

Barry, K.M., Dinan, T.G., Kelly, P.M., 2020. Preparation and applications of polar milk lipids/MFGM. In: Truong, T., Lopez, C., Bhandari, B., Prakash, S. (Eds.), Dairy Fat Products and Functionality. Springer, pp. 67–90.

Bazinet, L., Geoffroy, T.R., 2020. Electrodialytic processes : market overview, membrane phenomena, recent developments and sustainable strategies. Membranes 10. doi:10.3390/membranes10090221.

Boyd, L.C., Drye, N.C., Hansen, A.P., 1999. Isolation and characterization of whey phospholipids. J. Dairy Sci. 82, 2550–2557. doi:10.3168/jds.S0022-0302(99)75509-8.

Contarini, G., Povolo, M., 2013. Phospholipids in milk fat : composition, biological and technological significance, and analytical strategies. Int. J. Mol. Sci. 14, 2808–2831. doi:10.3390/ijms14022808.

Conway, V., Gauthier, S.F., Pouliot, Y., 2014. Buttermilk : much more than a source of milk phospholipids. Anim. Front. 4, 44–51. doi:10.2527/af.2014-0014.

Cornell, D.G., Patternson, D.L., 1989. Interaction of phospholipids in monolayer with b-lactoglobulin adsorbed from solution. J. Agric. Food Chem. 37, 1455–1459. doi:10.1021/jf00090a001.

Damodaran, S., 2010. Zinc-induced precipitation of milk fat globule membranes: A simple method for the preparation of fat-free whey protein isolate. J. Agric. Food Chem. 58, 11052–11057. doi:10.1021/jf101664j.

Dufton, G., Mikhaylin, S., Gaaloul, S., Bazinet, L., 2019. Positive impact of pulsed electric field on lactic acid removal, demineralization and membrane scaling during acid whey electrodialysis. Int. J. Mol. Sci. 20. doi:10.3390/ijms20040797.

Faucher, M., Perreault, V., Gaaloul, S., Bazinet, L., 2020. Defatting of sweet whey by electrodialysis with bipolar membranes : effect of protein concentration factor. Sep. Purif. Technol. 251, 117248. doi:10.1016/j.seppur.2020.117248.

Ferreiro, T., Gayoso, L., Rodriguez-Otero, J.L., 2017. Determination of phospholipids in milk by HPLC with evaporative light scattering detector: Optimization and validation. J. Dairy Vet. Sci. 1, 555562.

Gésan, G., Daufin, G., Merin, U., Labbe, J.P., Quemerais, A., 1995. Microfiltration performance : physicochemical aspects of whey pretreatment. J. Dairy Res. 62, 269–279.

Hwang, D.C., Damodaran, S., 1995. Selective precipitation and removal of lipids from cheese whey using chitosan. J. Agric. Food Chem. 43, 33–37. doi:10.1021/jf00049a008.

International Association of Official Analytical Chemists, 1995. AOAC. Official Methods of Analysis of AOAC International, Washington, DC, USA.

Küllenberg, D., Taylor, L.A., Schneider, M., Massing, U., 2012. Health effects of dietary phospholipids. Lipids Health Dis. 11, 1–16. doi:10.1186/1476-511X-11-3.

Lebrun, L., Silva, E.Da, Pourcelly, G., Métayer, M., 2003. Elaboration and characterization of ion-exchange films used in the fabrication of bipolar membranes. J. Memb. Sci. 227, 95–111. doi:10.1016/j.memsci.2003.07.022.

Lemay, N., Mikhaylin, S., Mareev, S., Pismenskaya, N., Nikonenko, V., Bazinet, L., 2020. How demineralization duration by electrodialysis under high frequency pulsed electric field can be the same as in continuous current condition and that for better performances? J. Memb. Sci. 603, 117878. doi:10.1016/j.memsci.2020.117878.

Lide, R.D., 2005. CRC Handbook of Chemistry and Physics : A Ready-Reference Book of Chemical and Physical Data, 86th ed. CRC Press, Boca Raton.

Lin Teng Shee, F., Angers, P., Bazinet, L., 2007. Delipidation of a whey protein concentrate by electroacidification with bipolar membranes. J. Agric. Food Chem. 55, 3985–3989. doi:10.1021/jf070191q.

Lin Teng Shee, F., Angers, P., Bazinet, L., 2005. Precipitation of cheddar cheese whey lipids by electrochemical acidification. J. Agric. Food Chem. 53, 5635–5639. doi:10.1021/jf047959g.

Lin Teng Shee, F., Joseph, A., Serge, B., Bazinet, L., 2008. Performing a three-step process for conversion of chitosan to its oligomers using a unique bipolar membrane electrodialysis system. J. Agric. Food Chem. 56, 10019–10026.

Lteif, R., Dammak, L., Larchet, C., Auclair, B., 1999. Conductivité électrique membranaire : étude de l'effet et de la concentration de la nature de l'électrolyte et de la structure membranaire. Eur. Polym. J. 35, 1187–1195.

Maubois, J.L., Pierre, A., Fauquant, J., Piot, M., 1987. Industrial fractionation of main whey proteins. Bull. Int. Dairy Fed. 212, 154–159.

Morr, C.V., Ha, E.Y.W., 1991. Off-flavors of whey protein concentrates : a literature review. Int. Dairy J. 1, 1–11.

Organization of International Standardization, 2007. ISO 22662: Milk and milk products-Determining of lactose content by high-performance liquid chromatography (Reference method).

Pereira, C.D., Diaz, O., Cobos, A., 2002. Valorization of by-products from ovine cheese manufacture: clarification by thermocalcic precipitation/microfiltration before ultrafiltration. Int. Dairy J. 12, 773–783. doi:10.1016/S0958-6946(02)00070-5.

Price, N., Wan, Z., Fei, T., Clark, S., Wang, T., 2020. Development of industrially scalable method for phospholipids and branch-chain fatty acids of dairy by-product. J. Am. Oil Chem. Soc. 1043–1053. doi:10.1002/aocs.12377.

Strathmann, H., 2010. 2.14 Electromembrane processes: basic aspects and applications. In: Drioli, E., Giorno, L. (Eds.), Comprehensive Membrane Science and Engineering Volume 2. Elsevier.

Sun, N., Chen, J., Wang, D., Lin, S., 2018. Advance in food-derived phospholipids : Sources, molecular species and structure as well as their biological activities. Trends Food Sci. Technol. 80, 199–211. doi:10.1016/j.tifs.2018.08.010.

Tatullan, S., 1993. Ionization and ion binding. In: Cevc, G. (Ed.), Phospholipids Hanbook. CRC Press, pp. 511–552.

Vaghela, M., Kilara, A., 1996. Lipid composition of whey protein concentrates manufactured commercially and in the laboratory. J. Dairy Sci. 79, 1172–1183. doi:10.3168/jds.S0022-0302(96)76471-8.

Verfaillie, H., Bidwell, R., 2001. Measuring eco-efficiency: a guide to reporting compagny performances. Geneva, Switzerland. World Bussiness Council on Sustainable Development. p2-37 ISBN 2-940240-14-0.

Zhu, D., Damodaran, S., 2013. Dairy lecithin from cheese whey fat globule membrane : its extraction, composition, oxidative stability, and emulsifying properties. J. Am. Oil Chem. Soc. 90, 217–224. doi:10.1007/s11746-012-2152-5.