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Carbohydrate and Mineral Removal during the Production of Low-Phytate Soy Protein Isolate by Combined Electroacidification and High Shear Tangential Flow Ultrafiltration

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In this work, soy protein isolates were produced by a combination of electroacidification and high shear tangential flow hollow fiber ultrafiltration with a 100 kDa membrane under constant pressure. The filtration performance was evaluated by comparing the filtration time and the final product composition for an electroacidified (pH 6) and a non-electroacidified (pH 9) soy protein extract. The removal of carbohydrates during the filtration was always consistent with the theoretical predictions (based on free permeability assumption) for both the electroacidified and the non-electroacidified feeds. A higher removal of calcium, magnesium, and phytic acid was achieved during the filtration of the electroacidified feed compared to the non-electroacidified feed. However, the electroacidification pretreatment had a negative impact on the permeate flux and resulted in more significant membrane fouling with correspondingly longer filtration times. A discontinuous diafiltration enhanced the removal of carbohydrates and minerals, thus yielding a product with higher protein content but was unable to improve the permeate flux for the electroacidified feed.

KEYWORDS: Soy proteins; electroacidification; tangential ultrafiltration; high shear; pH; carbohydrates; calcium; magnesium; phytic acid

INTRODUCTION

Plant materials, such as soy, represent an attractive source of dietary protein that is abundant and available at relatively low cost. In terms of nutritional quality, purified soy proteins can be considered equivalent to animal proteins (*1*, *2*). Additional benefits associated with the consumption of soy proteins in sufficient quantities include a lower risk of coronary heart disease, which was recognized by the U.S. FDA in 1999 through the approval of a health claim for soy proteins. This recognition has resulted in an increased demand for soy protein containing products (*3*). Besides the nutritional benefits, soy proteins also provide excellent functional properties, which stabilize and improve the texture of food products. Functional properties are affected by the composition of soy proteins, the presence of antinutritional factors, and processing conditions (*4*, *5*). Soy proteins are mainly composed of storage proteins (∼85%) and tend to precipitate at pH 4.5−4.8 (isoelectric point, p*I*). Their major components are β-conglycinin and glycinin with molecular masses of 180−210 and 300−350 kDa, respectively (*6*).

Soy protein concentrates (minimum 70% protein on dry basis) and isolates (minimum 90% protein on dry basis) are generally obtained by the removal of oligosaccharides and minerals from defatted soy flour. Since the 1970s, membrane ultrafiltration has been investigated for the production of soy protein ingredients with superior properties and low antinutrient content (*7*, *8*). The soy protein extract comes into contact with a membrane that separates the protein from other components based on differences in molecular size. In comparison to isoelectric precipitation, membrane ultrafiltration is considered to be gentler to the proteins and tends to preserve their native structure (*9*). In addition, ultrafiltration recovers proteins that do not precipitate at pH 4.5 (whey proteins), which may also improve the functional properties (*10*, *11*). Rao et al. (*9*) reported improved solubility for soy protein concentrate produced by ultrafiltration when compared to acid-precipitated soy protein isolate. The ultrafiltered soy protein concentrate also produced more stable emulsions, although the ability to form emulsions was less effective.

Membrane filtration systems have also contributed to the understanding of the interactions between phytic acid, calcium, magnesium, and proteins and to the development of low phytic acid soy protein products. Phytic acid is generally considered an important antinutritional factor in soy products as it may limit the bioavailability of minerals and proteins (*12*, *13*). However, recent work attributed also positive effects to phytic acid through anticarcinogenic and antioxidant effects (*14*). Phytic acid contains six phosphate groups that are negatively charged

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over a wide range of pH and can interact with other electrostatically charged species such as multivalent cations, proteins, and starch (*15*). In the context of protein−phytic acid interactions, it appears that pH and the content of calcium and magnesium play an important role in modulating these interactions. A ternary complex between negatively charged protein and phytic acid is facilitated by multivalent cations at pH >6.5, whereas at lower pH the formation of this complex is limited (*16*, *17*). Also at pH >6, phytic acid is known to form insoluble salts with calcium or magnesium depending on their concentration, which may compete with the formation of the ternary complex. The effect of pH on phytate solubility was exploited in the production of soy protein concentrates with enhanced mineral removal and improved solubility profiles (*18*, *19*). Omosaiye et al. (*18*) reported a 65% phytic acid removal at pH 6.7 compared to 43 and 27% at pH 8 and 10, respectively, during direct ultrafiltration to VCR 5. Improved phytic acid removal (92% at pH 6.7 and >80% at pH 8 and 10) was achieved at all three pH values by two-stage discontinuous diafiltration. What differentiates calcium and magnesium interactions with soy proteins is still unknown as limited information on magnesium was found. A study comparing the aggregation−dissociation process of soy protein isolate induced by calcium and/or magnesium chlorides reported a higher turbidity when calcium was used, suggesting different aggregation mechanisms for each cation (*20*).

Recently, Mondor et al. (*19*, *21*) and Alibhai et al. (*22*) developed a novel approach integrating electroacidification and membrane ultrafiltration for the production of soy protein concentrates. In electroacidification, hydrogen ions are generated by electrodissociation of water and can be used to acidify the soy protein extract solution. The pH can be lowered at a controlled rate without the use of concentrated acids, which was shown as a more protein friendly approach (*23*). In the work of Mondor et al. (*19*, *21*), the proteins, initially extracted from defatted soy flakes at pH 9, were subjected to electroacidification for which the pH was adjusted from 9 to 7 or 6. Subsequent ultrafiltration of the soy protein extracts in a dead-end system with a 100 kDa membrane was associated with membrane fouling being maximum at pH 7, intermediate at pH 6, and minimum at pH 9. Analysis of the hydraulic resistance revealed that most of the fouling (92−98%) was due to the cake layer formation, suggesting that operation in a tangential flow ultrafiltration system, where the cake buildup is minimal, would be more suitable. Despite higher membrane fouling, electroacidification to pH 6 enhanced the mineral and phytic acid removal, thus producing a soy protein concentrate with superior composition and improved solubility (*19*). The mineral removal was 72.9% at pH 6 compared to 55.1% at pH 9. The phytic acid content decreased at pH 6, whereas it increased for both pH 7 and 9 final products.

In this study, the potential of a high shear tangential flow hollow fiber system operated in a concentration or a discontinuous diafiltration mode was investigated for the production of soy protein isolates (minimum 90% protein dry basis). The effect of electroacidification on the filtration performance was assessed by comparing the most promising electroacidified soy protein extract at pH 6 (SPE 6) to the non-electroacidified soy protein extract at pH 9 (SPE 9). The efficiency of carbohydrate removal, mineral removal, and protein retention is presented. Particular emphasis is given to the phytic acid, calcium, and magnesium contents of the final product. The permeate flux profile as the filtration proceeded is also reported and discussed in the context of fouling behavior, feed pretreatment, and protein retention.

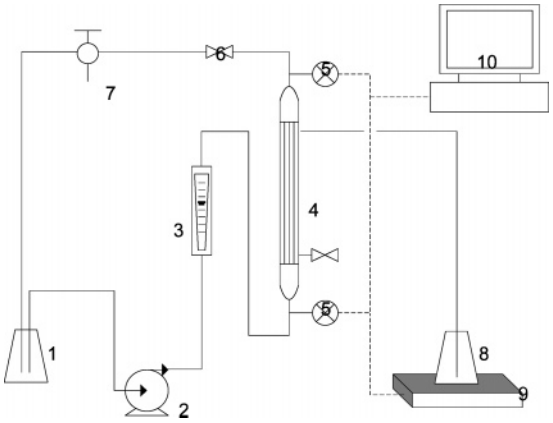

Figure 1. Schematic diagram of the filtration unit: 1, feed tank; 2, pump; 3, flow meter; 4, membrane; 5, pressure transducers; 6, pinch valve; 7, sampling valve; 8, permeate container; 9, balance; 10, PC/software.

MATERIALS AND METHODS

Feed Preparation. Lyophilized soy protein extracts containing approximately 60% protein, 30% carbohydrates, and 10% ash were provided by Agriculture and Agri-Food Canada (Saint-Hyacinthe, QC, Canada). Details on the preparation of the soy protein extracts and the electroacidification process can be found in Mondor et al. (*19*). The filtration feed consisting of SPE 9 or SPE 6 was obtained as follows: 2% (w/w) SPE solution was prepared by mixing a preweighed amount of SPE powder with Nanopure water (resistivity > 17.5 MΩ-cm) from a Barnstead water purification system (Dubuque, IA). The suspended SPE was stirred at room temperature for 1−2 h to allow rehydration. The suspension was then centrifuged at 15300*g* for 15 min using a Beckman Coulter L7-35 ultracentrifuge (Mississauga, ON, Canada) to remove any insoluble solids, and the supernatant was used as the feed solution for subsequent filtration. All experiments were performed with a feed volume of approximately 1.5 L.

Experimental Setup. Ultrafiltration experiments were performed with a hollow fiber membrane unit purchased from GE Healthcare (Baie d'Urfe, QC, Canada). The membrane was made of polysulfone with a nominal molecular weight cutoff (MWCO) of 100 kDa. The module was 30 cm long and consisted of 50 fibers, each having an inner diameter of 1 mm. The membrane area was 420 cm^2. The feed was pumped into the system (**Figure 1**) by a progressing cavity pump (Moyno Inc., Springfield, OH), and the flow rate was measured by a flowmeter. The pressure was monitored at the feed and the retentate side with two pressure transducers. The transmembrane pressure (TMP) was controlled on the retentate side by a manual pinch valve. The flowmeter, pressure transducers, and manual pinch valve were purchased from Cole Parmer Canada Inc. (Anjou, QC, Canada). A sampling valve was installed on the retentate side to allow for sample collection during the filtration experiments. The permeate was collected in a reservoir, and the flux was measured by weighing the permeate at specified time intervals. The balance (Ohaus Corp., Pine Brooks, NJ), and the pressure transducers were connected to a PC running Labview 7.1. To compensate for the temperature increase due to pumping, the feed tank was placed in an ice bath and the temperature of the feed was maintained constant (25 ± 1°C).

Ultrafiltration Experiments. Both SPE 6 and SPE 9 were subjected to two types of experiments, direct ultrafiltration (UF) and two-stage discontinuous diafiltration (DDF). The volume concentration ratio (VCR) given by eq 1 was used as a governing parameter to control the concentration extent during the filtration:

$$\mathrm{VCR} = \frac{V_F}{V_R} \tag{1}$$

V_F and V_R are the feed and the retentate volumes, respectively. VCR was determined by weighing the feed and the retentate and using the density of water to convert to volume.

In direct UF, the feed (~1.5 L) was concentrated in one step to VCR 4.5. In DDF, the feed (~1.5 L) was concentrated to VCR 2 (stage 1), then diluted to original volume with Nanopure water, and reconcentrated to VCR 4 (stage 2). The filtrations were performed under identical operating conditions: temperature, 25 °C; TMP, 6 psi (41.4 kPa); flow rate, 2.4 L/min, corresponding to the shear rate of 8000 s^{-1}. Therefore, stage 1 of DDF is a replicate of direct UF up to VCR 2. The permeate flux profile and the composition of the retentate samples collected up to VCR 2 should be the same for both filtrations. Assuming all nonprotein solutes were freely permeable, direct UF (VCR 4.5) and DDF (VCR 2, dilution, VCR 4) should result in a removal of 78 and 88% of these solutes, respectively. During filtrations, samples of the retentate were taken at specific time points to investigate the changes in the composition. At the end of the filtration, samples of the final retentate and permeate were collected, lyophilized (Super-Modulyo, Thermo Electron Corp., Waltham, MA), and kept for further analysis.

Analytical Methods. *Total Solids and Carbohydrates.* The concentration of total solids (TS) was determined by gravimetric analysis. The carbohydrate concentration was determined by phenol−sulfuric acid assay modified from Fox and Robyt (*24*) using glucose standards (20−160 μg/mL). The procedure consisted of adding 25 μL of standard and sample into separate microtiter plate wells and then adding 25 μL of 5% (w/w) of phenol solution and 125 μL of concentrated sulfuric acid to each well. The microtiter plate was wrapped in DuraSeal (Diversified Biotech, Boston, MA) and placed in an 80 °C oven for 30 min. The absorbance was read on Multiskan Ascent microtiter plate reader (Labsystems, Helsinki, Finland) at 492 nm. The carbohydrate content on dry basis was determined as a percentage of the carbohydrates to the TS concentration.

Protein. The protein concentration in the retentate samples collected during the filtration and the final permeate was determined according to the Bradford protein assay (Standard Procedure for Microtiter Plates, Bio-Rad Laboratories, Mississauga, ON, Canada) using purified β-conglycinin and glycinin as protein standards (provided by M. Corredig, University of Guelph, ON, Canada). The standard solutions were prepared by combining β-conglycinin and glycinin in a ratio of 1:1.4, which corresponds with the actual distribution of these two protein fractions in the soy proteins (*6*). The Bradford assay enabled fast analysis of the retentate samples (liquid) collected during the filtration (>10). It was also used to analyze the permeate samples, which contained only small amounts of protein. The protein content (dry basis) was determined as a percentage of the protein to the TS concentration. The results obtained from the Bradford assay were verified for freeze-dried feed and final retentate samples with a LECO FP-428 nitrogen analyzer (LECO Corp., St. Joseph, MI). The combustion unit was calibrated with EDTA as nitrogen standard. The nitrogen content was determined using a sample size of 50−125 mg, and the protein content was expressed as total nitrogen (N) × 6.25 (*19*).

Moisture, Ash, and Mineral Contents. Freeze-dried samples of the feed (samples collected before filtration) and the final retentate and permeate were analyzed for moisture and ash contents. The moisture and ash contents were determined according to the methods derived from AOAC (25). The ash residues were dissolved in 1.86 M HCl solution, and portions were used for mineral content analysis. Phosphorus was determined by using the phosphovanadomolybdate spectrophotometric method described in ref *26* with monobasic potassium phosphate standard. Magnesium and calcium contents were determined by inductively coupled plasma-optical emission spectrometry using Spectroflame Modula (FSM-08, Spectro Analytical Instruments, Kleve, Germany).

Unless stated otherwise, all analytical assays were performed at least in triplicate for each sample.

Numerical Analysis. The increase of the protein concentration in the retentate during the filtration was quantified by fitting a first-order kinetics equation to the experimental data

$$c_R(t) = c_F \exp(kt) \quad (2)$$

where $c_R(t)$ is the protein concentration in the retentate at time t, c_F is the feed concentration (at time 0), and k is the rate constant, representing the increase in protein concentration per unit time. Model fitting was performed with Microsoft Excel Solver function by minimizing the residual sum of squares.

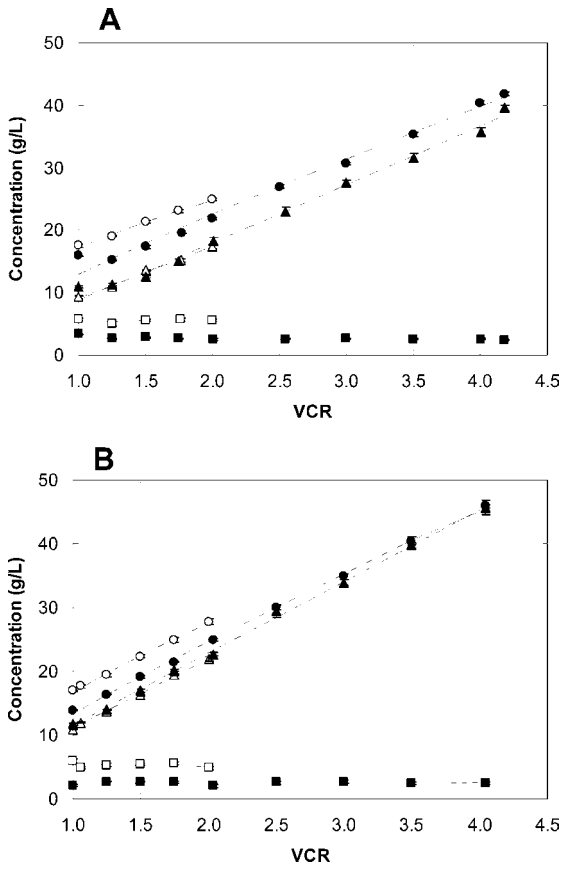

Figure 2. Effect of VCR on the retentate concentration of total solids (○, ●), protein (△, ▲), and carbohydrates (□, ■) in DDF during stage 1 (open symbols) and stage 2 (solid symbols): (**A**) SPE 6; (**B**) SPE 9. Protein concentrations determined by Bradford are presented as means ± SE, $n = 3$. MWCO = 100 kDa, TMP = 6 psi, shear rate = 8000 s^{-1}, $T = 25$ °C.

Statistical Analysis. The removal of total minerals (ash), calcium, phosphorus, and magnesium was compared for the direct UF/DDF of SPE 6 and SPE 9 using a two-sample t-test analysis described in ref *27* to determine whether there were any significant differences between the two feeds.

RESULTS AND DISCUSSION

For this study, the operating TMP was selected from the permeate flux−TMP profile for total recycle mode (both retentate and permeate recycled to the feed tank). During this operation, a typical concentration polarization effect was observed with an initial linear increase of the flux with TMP followed by a leveling off of the flux at TMP above 6 psi (41.4 kPa, data not shown). On the basis of this analysis, the operating TMP of 6 psi was selected.

Composition Profile during Filtration. The concentration of TS, protein, and carbohydrates during stages 1 and 2 (DDF) for SPE 6 and SPE 9 is illustrated in **Figure 2** as a function of VCR. For clarity, the results of the direct UF of SPE 6 and SPE 9 are not shown as the composition profiles were similar to those observed in stage 1 of DDF (up to VCR 2). A 100 kDa membrane was used so that all proteins would be retained, whereas carbohydrates and minerals, the remaining components of the feed mixture, should be free to permeate through the

Table 1. Composition of the Feed, Final Retentate, and Final Permeate for Direct UF and DDF of SPE 6 and SPE 9[a]

				% dry basis			
expt	sample	vol[b] (L)	TS concn (g/L)	protein[c,d]	carbohydrates[c]	ash[c]	av total (%)
SPE 6 direct UF	feed	1.421	18.0	55.2	33.3 ± 2.4	7.9 ± 0.1	96.4
	retentate	0.315	52.0	91.6 ± 3.9	12.0 ± 2.3	4.2 ± 0.3	107.8
	permeate	1.106	7.9	2.0 ± 3.0	73.8 ± 5.2	14.9 ± 5.0	90.7
SPE 6 DDF	feed	1.406	18.0	55.2	32.4 ± 3.9	7.9 ± 0.1	95.5
	retentate	0.336	42.0	94.6 ± 3.8	5.8 ± 3.3	3.5 ± 1.5	103.9
	permeate 1	0.703	7.3	1.5 ± 2.9	55.5 ± 2.8	14.4 ± 0.9	71.4[e]
	permeate 2	1.070	4.0	3.1 ± 5.6	51.1 ± 0.4	14.2 ± 3.7	68.4[e]
SPE 9 direct UF	feed	1.400	17.0	59.9	31.0 ± 1.7	9.4 ± 0.8	100.3
	retentate	0.309	50.0	90.7 ± 3.1	10.4 ± 3.5	6.2 ± 4.9	107.3
	permeate	1.091	6.7	0.7 ± 5.0	81.2 ± 4.1	18.5 ± 2.1	100.4
SPE 9 DDF	feed	1.467	17.0	59.9	35.2 ± 4.8	9.4 ± 0.8	104.5
	retentate	0.361	46.0	91.5 ± 1.7	5.3 ± 7.3	5.1 ± 0.1	101.9
	permeate 1	0.738	6.7	1.1 ± 3.5	80.8 ± 2.3	16.6 ± 1.2	98.5
	permeate 2	1.106	3.7	1.3 ± 6.2	55.9 ± 7.5	16.0 ± 1.5	73.2[e]

[a] Permeates 1 and 2 were collected in DDF during stages 1 and 2, respectively. [b] Determined from mass using the density of water. [c] Mean ± relative standard error ($n = 3$). [d] Feed and retentate determined by LECO ($n = 2$); permeate determined by Bradford assay ($n = 3$). [e] Indicates samples in which the total composition differs from 100% by >10%.

membrane. A linear increase of the TS concentration in the retentate was observed for both the electroacidified and the non-electroacidified feeds. This increase was expected because most of the TS were proteins (60% initially in the feed). The TS concentration was lower for stage 2 of the DDF operation because about half of the carbohydrates was removed in the permeate during stage 1. The concentration of the carbohydrates (smaller than the membrane MWCO) remained constant through the course of the filtration, indicating that the permeation of carbohydrates across the membrane was not affected by the retention of the proteins. In contrast, the protein concentration increased with increasing VCR. This increase profile was similar for both stages of DDF as the proteins were rejected by the membrane. The total rejection of the proteins by the membrane was confirmed by the analysis of the permeate in which the protein concentration ranged between 0.049 and 0.155 mg/mL, accounting for about 0.7−3.1% of TS in the permeate (**Table 1**).

The retention of proteins and the removal of carbohydrates during the filtration were also assessed through their respective mass fraction of the total solids. As expected, the protein content increased while the carbohydrate content decreased in direct UF and stage 1 of DDF (**Figure 3**). For both SPE 6 and SPE 9, the protein content appeared to level off above VCR 2.5 (direct UF). Such an observation agrees with previous results (*11*) when the amount of the permeable components remaining in the retentate decreases with the filtration progress, resulting in a smaller change in the protein content. An additional removal of the nonprotein solutes was achieved by DDF (stage 2) and resulted in a higher final protein content and a lower final carbohydrate content when compared to direct UF.

When considering the evolution with time of the protein concentration in the retentate for the direct UF operation, an exponential relationship was observed for both SPE 6 and SPE 9 (**Figure 4**). Similar behavior was also observed during the DDF operation (data not shown). The rate of the protein concentration increase was modeled using the first-order kinetics (eq 2), and the estimated rate constants (k) are presented in **Table 2**. A good fit was found between the model and the experimental data (R^2 values being in the range of 0.95−0.99). The rate constant, k, provides an estimate of how quickly the protein concentration increased during the filtration of SPE 6 and SPE 9 in direct UF or DDF modes. The values of rate constant k for the electroacidified feed were always approximately half of those obtained for the non-electroacidified feed for both direct UF and DDF. In other words, the filtration

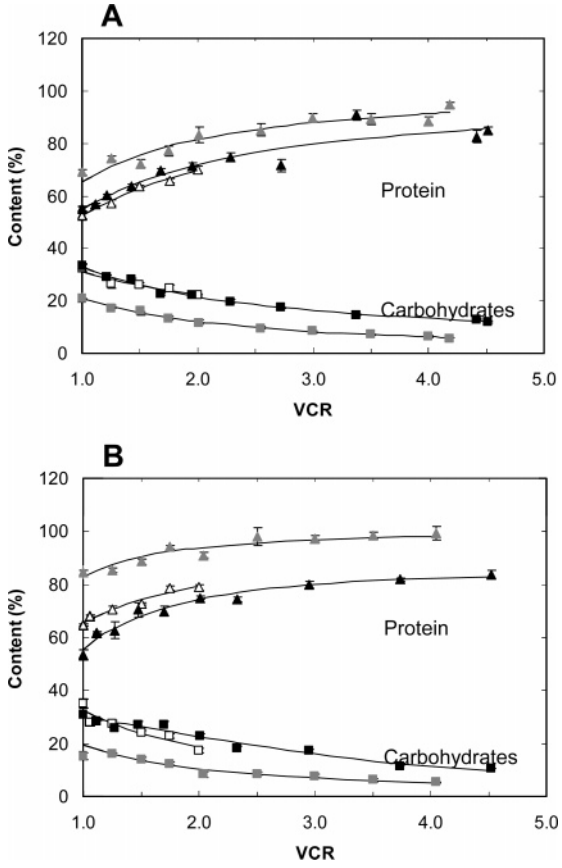

Figure 3. Protein and carbohydrate content in retentate during direct UF (solid triangles and squares), stage 1 of DDF (open triangles and squares), and stage 2 of DDF (gray triangles and squares): (**A**) SPE 6; (**B**) SPE 9. Data are presented as means ± SE, $n = 3$. MWCO = 100 kDa, TMP = 6 psi, shear rate = 8000 s^{-1}, $T = 25$ °C.

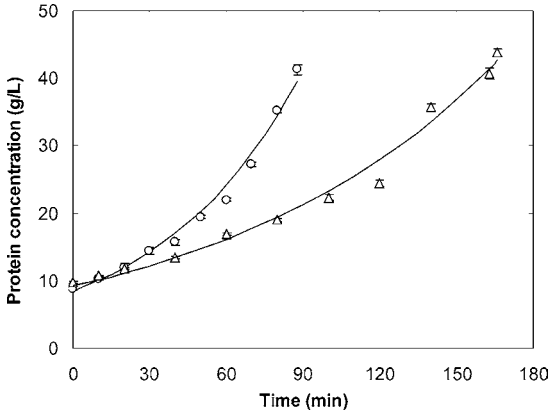

Figure 4. Protein concentration in retentate during direct UF and DDF as a function of time for SPE 6 (△) and SPE 9 (○). Solid line shows the fit based on the first-order kinetics. Protein concentrations determined by Bradford are presented as means ± SE, $n = 3$. MWCO = 100 kDa, TMP = 6 psi, shear rate = 8000 s^{-1}, $T = 25$ °C.

Table 2. Rate Constants (k) Describing Increase in Protein Concentration during Direct UF and DDF of SPE 6 and SPE 9

	rate constant, k (10^{-3} min^{-1})	
expt	SPE 6	SPE 9
direct UF	9.2	17.6
DDF stage 1	7.8	13.7
DDF stage 2	7.8	15.0

time to reach the same final protein concentration (~40 g/L) for the electroacidified feed was twice as long compared to the non-electroacidified feed, suggesting significant interactions between the proteins and the membrane at lower pH of the feed as will be discussed in subsequent sections.

Composition Analysis of Electroacidified and Non-electroacidified Soy Protein Isolates. In this study, proteins, carbohydrates, and ash were estimated by independent analytical techniques. The accuracy of the respective analytical techniques was assessed by computing the total material balance for the feed, the retentate, and the permeate streams. As seen in **Table 1** (last column), the total material balance accounted for 100 ± 10% of the composition for most operations. Significant discrepancies for the permeate streams of the DDF operation are associated with the determination of the carbohydrate content. Assuming that the carbohydrates are freely permeable, an assumption supported by the concentration profile of the carbohydrates presented in **Figure 2**, the theoretical content of carbohydrates in the permeate should be at least 75%. The carbohydrate content was calculated as the ratio of the carbohydrate concentration to the TS concentration. However, the TS concentrations in the feed or retentate were much higher compared to the permeates (**Table 1**). For this reason, the carbohydrate content is associated with higher experimental error compared to the feed or the retentate data. The removal of carbohydrates based on the retentate composition, indicates ~78% for direct UF and ~89% for DDF of both SPE 6 and SPE 9, which agrees with the theoretical expectations and results previously observed (*7*, *11*).

The effect of the feed electroacidification pretreatment and the mode of operation can be compared on the basis of the carbohydrate analysis for the retentate stream. The electroacidification pretreatment had a negligible effect on the carbohydrate content of the retentate for both direct UF and DDF. For both feeds, the carbohydrate content of the retentate for the DDF operation was reduced by 50% when compared to the direct UF operation.

Of utmost importance in this study is that soy protein isolate (90% protein on dry basis) was obtained for both operations, direct UF and DDF, for a high shear tangential flow system and for both the electroacidified and the non-electroacidified feeds. Previous work with a dead-end system was unable to produce soy protein isolates after an electroacidification to pH 6 and 7 (*19*). The protein content in the retentate determined by LECO analysis was on average >90%, but the experimental error was significant (**Table 1**). Such estimates are higher than theoretical predictions (~86% for direct UF and ~92% for DDF on dry basis) calculated using the protein content of the feed and assuming that all nonprotein solutes are free to permeate. Although the protein content should be improved by ~7% in DDF due to additional removal of carbohydrates and minerals, the analysis was unable to detect such differences in the two modes of operation.

The ash content (minerals intrinsically present in the SPE composition) was analyzed only at the end of the filtration when sufficient material was collected for the analysis. For both SPE 6 and SPE 9, the ash content in the retentate produced by direct UF decreased (**Table 1**), corresponding to a minerals removal of 64.1% for SPE 6 and 59.0% for SPE 9. By conducting two-stage DDF, the ash removal was enhanced to 71.8% for SPE 6 and 63.8% for SPE 9. For both direct UF and DDF, the mineral removal was lower than theoretical (based on the free permeability assumption), indicating that the minerals were partially retained by the membrane. Although the mineral removals were similar for SPE 6 and SPE 9 during direct UF, a significantly higher mineral removal was achieved for SPE 6 during DDF compared to SPE 9 ($\alpha = 0.05$, one-sided t test). This could be attributed to the differences in the surface charge on the proteins at pH 6 and 9. Proteins at pH 9 possess a higher negative net charge, being further away from their isoelectric point, which would lead to a higher degree of electrostatic attraction to positively charged ions (minerals), compared to conditions at pH 6. A similar mineral removal effect was observed during the dead-end ultrafiltration of soy protein extracts with different pH values by Mondor et al. (*19*). Kumar et al. (*11*) also indicated that during ultrafiltration of soy flour suspension at pH 8, the minerals were partially retained, suggesting that they were either bound to the protein or in an insoluble form. In their approach, a continuous diafiltration with 5 diavolumes (corresponding to >99% removal of all permeable components) was unable to achieve complete removal of minerals, suggesting there could be a limit to mineral removal if the minerals were firmly bound to the protein or insoluble.

Phytic Acid, Magnesium, and Calcium Removal. The antinutritional nature of phytic acid and its known interactions with protein and divalent cations led us to investigate the removal of these components from SPE 6 and SPE 9 during direct UF and DDF. We decided to focus on magnesium (Mg) and calcium (Ca), because both are among the most abundant divalent minerals in soy products (*1*) and are known to form salts with phytic acid (phosphorus, P) (*16*, *28*). Direct UF of the electroacidified feed (SPE 6) reduced Mg, Ca, and P contents in the retentate when compared to the feed by a factor of 2.0, 1.5, and 1.4, corresponding to removals of 63, 55, and 50%, respectively (**Table 3**). Two-stage diafiltration further improved the removal of all three components by 8, 11, and 7% (for Mg, Ca, and P, respectively) when compared to direct UF. In contrast, both direct UF and DDF of the non-electroacidified

Table 3. Magnesium, Calcium, and Phosphorus (Phytic Acid) Contents in Feed and Final Retentates Produced by Direct UF and DDF

			content (mg/g of dry powder)			removal[a] (%)		
extract	sample	VCR	Mg	Ca	P	Mg	Ca	P
SPE 6	feed	1.0	4.40	5.98	7.54			
	retentate (UF)	4.5	2.26	3.99	5.53	63.1	54.9	50.3
	retentate (DDF)	4.0	2.06	3.65	5.38	71.6	65.9	57.2
SPE 9	feed	1.0	2.80	3.34	4.91			
	retentate (UF)	4.5	2.54	4.20	6.85	40.9	21.0	11.0
	retentate (DDF)	4.0	2.57	4.67	6.51	41.3	14.4	13.7

[a] Expressed as mean ± range based on the mass balance calculations.

feed (SPE 9) resulted in an increase of Ca and P contents in the retentate when compared to the feed. The overall removal of both components was marginal (11−21%), indicating either that the components are insoluble at pH 9 or that the degree of interaction between proteins, Ca, and P is higher at this pH. The difference in Ca and P removal between SPE 6 and SPE 9 was significant for both direct UF and DDF at $\alpha = 0.005$ (one-sided *t* test). Higher removal of Ca and P when the feed was electroacidified to pH 6 was previously observed by Mondor et al. (*19*) for a dead-end UF system. The effects of phytate−calcium−soy protein interactions on the pH solubility profiles of soy protein isolates were reported by Grynspan et al. (*16*), who noted an increased solubility of calcium, phytic acid, and protein just above pH 6.5. This solubility increase was attributed to the formation of a ternary complex. With increasing pH the solubility of protein kept on increasing while the solubility of calcium and phytic acid decreased, depending on their molar ratio. This observation suggests that for SPE 9, calcium and phytic acid are either associated with the protein via electrostatic forces or form an insoluble salt, both cases leading to the incapability of permeating through the membrane. In contrast, for the electroacidified feed at pH 6, the conditions for the formation of a ternary complex are limited (the proteins are negatively charged but less than at pH 9) and both calcium and phytic acid should be relatively soluble. The removal of Ca and P at pH 6 was still lower than the theoretical expectations based on free permeability (78 and 88% for direct UF and DDF, respectively), indicating that both components are somewhat retained by the membrane but to a much lower extent than at pH 9.

In the context of the magnesium content, a higher reduction of Mg was achieved in the final retentate for SPE 6 produced by direct UF and DDF when compared to SPE 9. The removal of Mg was by 22 (direct UF) and 31% (DDF) higher for SPE 6 than for SPE 9 (**Table 3**). The difference in Mg removal between SPE 6 and SPE 9 was not as pronounced compared to the removals of Ca and P, which were closely correlated. Although both Mg and Ca have been implicated in the context of protein−phytic acid interactions, our observations suggest that Mg and Ca behave differently when contained in a soy protein mixture. This is especially obvious for the filtrations at pH 9 where the difference in the removal of Mg compared to Ca and P becomes significant ($\alpha = 0.025$, one-sided *t* test). This could imply that calcium has a higher affinity toward protein and/or phytic acid, which leads to more magnesium ions in a free form, able to permeate through the membrane.

Permeate Flux Characteristics of the Electroacidified Soy Protein Extract. The permeate flux profile and the filtration time to reach a desired VCR was used to investigate the process

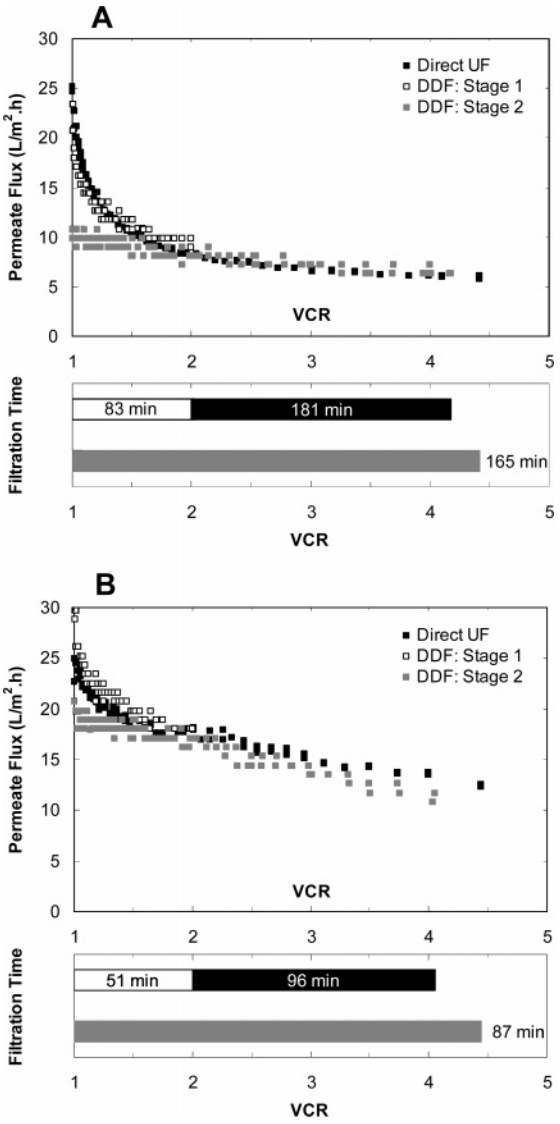

Figure 5. Permeate flux decline in direct UF and DDF as a function of VCR, with the filtration time indicated below: (**A**) SPE 6; (**B**) SPE 9. MWCO = 100 kDa, TMP = 6 psi, shear rate = 8000 s^{-1}, $T = 25$ °C.

performance of the soy protein concentration. In direct UF, significant permeate flux decline was observed up to VCR 1.5 for both SPE 6 and SPE 9, indicating that most of the membrane fouling took place in the initial part of the filtration. After reaching VCR 1.5, the flux continued declining but at lower rate. The permeate flux declined more seriously for SPE 6, with the final flux of ~6 $L/m^2 \cdot h$ (**Figure 5A**), which was half of the flux for SPE 9 reached at VCR 4.5 (**Figure 5B**). A more pronounced permeate flux decline for SPE 6 was also observed when using a dead-end and a low shear UF system (*19*, *22*) and was attributed to the lower net charge of the proteins at pH 6 (closer to p*I*) compared to pH 9. As a result, the electrostatic repulsion forces become weaker, which would promote the formation of protein aggregates. Further analysis using a force balance on a given particle concluded that larger particles are more likely to deposit on the membrane surface and contribute to the cake layer formation (*21*). Thus, the use of a high shear tangential flow hollow fiber system was unable to eliminate the higher fouling observed when the feed was electroacidified. The potential to improve the flux performance by a two-stage DDF was explored. It was hoped that a dilution step would reduce interactions between the proteins and other feed com-

ponents involved in the membrane fouling (*8*). Also, it was expected that lowering the concentration in the bulk solution could lead to solute desorption from the membrane surface, which would improve the permeate flux. However, the permeate flux did not increase after the water addition (stage 2 in DDF) regardless of whether the feed had been subjected to electroacidification or not. Due to more serious membrane fouling, both direct UF and DDF filtrations of SPE 6 required significantly more time compared to SPE 9 to achieve the same VCR (**Figure 5**, lower part). The total filtration time of DDF operation was 264 min for SPE 6 compared to 147 min for SPE 9. These times were 1.6 and 1.7 times longer compared to direct UF of SPE 6 and SPE 9, respectively. Thus, the DDF operation with the dilution of the retentate at VCR 2 could not disrupt the cake deposit on the membrane surface irrespective of the feed investigated in this study.

Conclusion. In this study, we investigated the possibility of using electroacidification for the production of soy protein isolates. We have demonstrated that soy protein isolates were obtained using a high shear tangential flow ultrafiltration system for a feed with or without electroacidification pretreatment and whether direct UF or discontinuous diafiltration was used. Carbohydrate analysis during the course of the filtration confirmed that carbohydrates behaved as freely permeable solutes. In contrast, magnesium, calcium, and phytic acid (antinutrient) were partially retained by the membrane. Electroacidification to pH 6 was beneficial in providing a higher removal of magnesium, calcium, and phytic acid when compared to non-electroacidified feed at pH 9. A two-stage discontinuous diafiltration improved the carbohydrate removal (and thus increased the protein content) and the ash removal (including Mg, Ca, and P) for the electroacidified feed. Despite the use of a high shear ultrafiltration configuration, the electroacidified feed at pH 6 was still characterized by higher fouling when compared to the non-electroacidified feed at pH 9. Future work will investigate the mechanisms responsible for the higher fouling observed for the electroacidified feed at pH 6 and the potential fouling minimization using a continuous diafiltration.

ABBREVIATIONS USED

DDF, discontinuous diafiltration; MWCO, molecular weight cutoff; p*I*, isoelectric point; SPE 6, electroacidified soy protein extract (pH 6); SPE 9, non-electroacidified soy protein extract (pH 9); TMP, transmembrane pressure; TS, total solids; UF, ultrafiltration; VCR, volume concentration ratio.

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