

## Production of Soy Protein Concentrates Using a Combination of Electroacidification and Ultrafiltration

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Soy protein concentrates produced by combining electroacidification and dead-end ultrafiltration with a membrane of 100 kDa (pH 7 and 6) were compared with concentrates produced by ultrafiltration (pH 9) and a traditional acid precipitation procedure at pH 4.5. Mineral removal during ultrafiltration (mainly potassium, phosphorus, and calcium) was enhanced for the pH 6 electroacidified extract, compared to the extract at pH 9. This yielded a concentrate with improved solubility characteristics. The solubility for the concentrate prepared at pH 6 was enhanced by as high as 45% when compared to the concentrate at pH 9. The concentrate produced according to the traditional acid precipitation process showed mineral contents and solubility profile similar to those of the pH 6 concentrate, but required twice as much water during the process. The effect of electroacidification treatments on ultrafiltration permeate flux was quantified through the measurement of the different hydraulic resistances. Cake resistance was the main resistance to the permeate flux, and it was minimum at pH 9, maximum at pH 7, and intermediate at pH 6.

**KEYWORDS:** Soy protein; electroacidification; ultrafiltration; minerals content; protein solubility

### INTRODUCTION

Plant protein concentrates (at least 70% protein on a dry weight basis) and isolates (at least 90% protein on a dry weight basis) are valuable ingredients in many foods. They are added to formulated foods, such as meat analogues and dairy and bakery products, to improve their nutritional properties. Soy proteins, in particular, are of great interest to the food industry not only for their nutritional value but also because of their excellent functional properties. They have also found applications in the paper industry, where they are used as co-binders in paper coatings, in fire-fighting foams, in many types of paints and inks, and for the production of plastics, adhesives, and fibers (1).

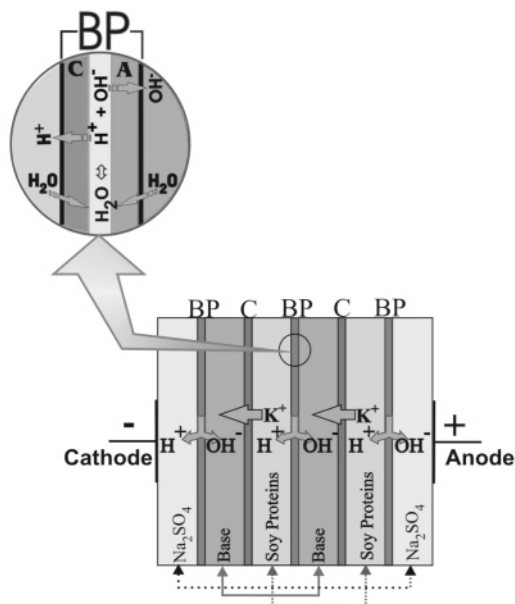
A number of ways have been described for producing soy protein concentrates (SPC) and isolates (SPI), but the only commercial procedure currently being utilized consists of extracting defatted soy flakes with dilute alkali (pH 8.0–9.0), at a temperature of 30–55 °C, followed by centrifugation of the slurry to separate the insoluble materials to obtain a dispersion containing soluble protein and some non-protein solutes. The soy protein extract (SPE) is then adjusted to pH 4.5 to precipitate the proteins, and the recuperated curd is washed to remove non-protein solubles, neutralized (pH 7), and spray-dried to make a protein isolate (2). In the conventional process, the pH is adjusted with a food grade acid such as sulfuric acid, phosphoric acid, or hydrochloric acid. A disad-

vantage of the precipitation with acids is the irreversible denaturation of the proteins on exposure to local extremes in pH and the high ash content of the final product, which alter the precipitation behavior and the protein's solubility after rehydration (3, 4). Other disadvantages include the generation of a high volume of effluents.

Electroacidification of the SPE to pH 4.5 has been considered in the past. A technology known as bipolar membrane electroacidification (BMEA) was developed for soy proteins precipitation (5–8). Electroacidification is based on the production of protons by dissociation of water molecules at the interface of a bipolar membrane (Figure 1), using a sufficiently large cathode/anode voltage difference (9). The protons generated are able to migrate toward the cathode and acidify the protein solution. The gradual acidification of the solution enables protein precipitation, which is then recuperated by centrifugation. BMEA appears to be a promising technology for the production of SPI. The acidification rate can be controlled, by varying the current input, and the volume of effluent generated is significantly lower than that of the traditional process. It was also shown that SPC produced by BMEA has a lower salt content than that separated by the conventional process (5, 6). The application of electroacidification at the industrial scale is limited because of gradual protein precipitation in the cell, which results in an increase of the cell resistance (decrease of the system efficiency) and loss of proteins (decrease in yield).

An attractive alternative to conventional protein precipitation is protein purification using ultrafiltration (UF). Studies on applying membrane ultrafiltration systems to soy protein separa-

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**Figure 1.** Electrodiagnosis cell with bipolar membranes for acidification of the soy protein extracts: BP, bipolar membrane; C, cationic membrane or layer; A, anionic membrane or layer.

tion were first conducted in the early to mid 1970s (10–13). The chief virtues of ultrafiltration are its mild operating conditions and relatively high selectivity. It was demonstrated that by proper selection of membrane characteristics and operating parameters, combining UF and diafiltration steps can be an effective method for removing soybean oligosaccharides, which are relatively small in molecular size compared to the proteins (14–16). In addition, when membranes with a molecular weight cutoff (MWCO) of 50 kDa are used, UF recovers essentially all of the solubilized protein. However, as previously discussed by Lawhon and Lusas (17), membranes with a MWCO of 100 kDa could be more suitable for food applications. Large-pore UF membranes would allow the oil-body molecules associated with small proteins to permeate the membrane. This would result in a slightly lower soy protein rejection (lower protein concentration in retentate) than with the 50 kDa membrane, but a higher permeate flux and an isolate with better organoleptic and off-flavor properties (18).

It has also been observed that ultrafiltration of SPE, without pH adjustment (as is, pH 8.5–9.0), removes an average of <35% of minerals originally present in the soy extract for a volume concentration ratio (VCR) of 2.5 and ~50% for a VCR of 4.5–5 (VCR is calculated as initial volume of feed/volume of retentate) (15, 19). It was postulated that positively charged cations including  $K^+$ , which alone represents 50% of SPE mineral content (20), could interact with the proteins, which are strongly negatively charged at this pH, and, thus, not allow them to permeate the membrane. Furthermore, at this pH, the phosphorus mainly present in soybean in the form of phytic acid (21, 22) interacts with proteins and calcium to form a ternary complex (23), making both phytic acid and calcium together unavailable to permeate the UF membrane. These protein–mineral interactions limit the percent of protein in the final product and affect its properties, including its solubility after rehydration (3, 23). A second limitation is the declining permeate flux with time. This decrease is caused by an accumulation of the feed component in membrane pores as well as on membrane surface. In some instances, a decrease in the flux may be so great as to make membrane processes unattractive for protein isolation.

In this work, we report on the results of a preliminary study that was conducted to estimate the efficiency of combining electroacidification and ultrafiltration for the production of SPC with low ash content. The approach consisted of electroacidification of a SPE to pH 7 or 6 followed by ultrafiltration. Measurements of permeate flux and of the different hydraulic resistances were used to characterize the influence of electroacidification treatments of the SPE on the ultrafiltration step. Final protein, ash (minerals), and carbohydrate contents and protein solubility profiles of the SPC produced with this novel approach were compared with the results obtained for concentrates produced by traditional acid precipitation.

## MATERIALS AND METHODS

**Preparation of Defatted Soy Protein Extract.** One part by weight of mildly toasted soy flakes (Nutrisoy 70, Archer Daniels Midland, Decatur, IL) was suspended with vigorous stirring in 9 parts of water. The mixture was heated to 50–55 °C and the pH adjusted to 8.5 with 1 N NaOH. After 30 min of extraction at 50–55 °C, the insoluble material was removed using vibratory screens (165  $\mu$ m) (model K-18, Kason Division of Separator Engineering, Scarborough, ON) and a basket centrifuge (1  $\mu$ m) (type STM-1000, Western States, Hamilton, OH). The resulting extract was rapidly frozen before being lyophilized. Dry SPE was stored at 4 °C until processed.

**Electroacidification of Soy Protein Extracts.** The SPE pH was adjusted, by electroacidification, as required (pH 7, 6) prior to ultrafiltration. Electroacidification was carried out as a batch process using an Electrocell AB unit (100 cm<sup>2</sup> of effective electrode surface) from Electrocell AB (Taby, Sweden). The anode, a dimensionally stable electrode (DSA), and the cathode, a 316 stainless steel electrode, were supplied with the cell. The cell was assembled in a fashion similar to that illustrated in **Figure 1**. It consisted of a structure with eight compartments separated by four Neosepta CMX cationic membranes (C) and three Neosepta BP-1 bipolar membranes (BP) from Tokuyama Soda Ltd. The electroacidification process was monitored with a YSI conductivity meter (model 35, Yellow Springs, OH) and a pH-meter (model AP61, Fisher Scientific, Montreal, PQ).

The initial concentration of the SPE solutions was 70 g/L, which corresponds to 42 g of protein/L, and the initial pH was between 9.0 and 9.2. Electroacidification was carried out using a current of 1–2 A, with electrolyte volumes of 3.5 L for the protein solution, and 8 L for the KCl (0.1 N) and Na<sub>2</sub>SO<sub>4</sub> (20 g/L) solutions. During a run, each type of fluid was recirculated within its respective compartment in the cell, using recirculating pumps. The recirculation flow rate was controlled at 8 L/min, using panel-mount flow meters, and electrolyte temperature was maintained at 30–35 °C. Electroacidification was stopped when the target pH (6.0 or 7.0) was reached, and the resulting extracts were rapidly frozen before being lyophilized. Dry SPEs were stored at 4 °C until processed.

**Dead-end Ultrafiltration of Soy Protein Extracts.** Dead-end ultrafiltration experiments were carried out with a laboratory scale Amicon stirred cell model 8050 (Millipore Canada, Toronto, ON) with a capacity of ~50 mL and a membrane area of 13.4 cm<sup>2</sup>. The filtration experiments employed YM 100 regenerated cellulose hydrophilic membranes with a MWCO of 100 kDa (Millipore Canada). Each experiment was conducted in duplicate.

The clean membrane was placed in the stirred cell and its resistance evaluated from data for the water flux, measured via timed collection, as a function of the applied pressure. Clean membrane resistance was estimated using the equation

$$R_{m_0} = \frac{\Delta P}{\mu_w J_{w_0}} \quad (1)$$

where  $R_{m_0}$  is the clean membrane resistance,  $\Delta P$  is the transmembrane pressure, which corresponds to the applied nitrogen pressure,  $\mu_w$  is the water viscosity, and  $J_{w_0}$  is the water flux.

The stirred cell was then carefully emptied and refilled with 45 mL of the SPE solution (pH 9, 7, or 6) at a concentration of 70 g of extract/

L. The cell was nitrogen pressurized (30 PSI) and the stirring speed adjusted to 535 rpm with a tachometer (model Solomat MPM 500) from ITM Instruments Inc. (Ste Anne de Bellevue, PQ). SPE solution was ultrafiltered until 60% of the initial water content was removed (VCR = 2.5). Permeate flux was estimated, as a function of time and VCR, from the mass of permeate and the permeate density. The mass of permeate was recorded with a Mettler balance (model AE166) from Fisher Scientific (Montreal, PQ). Permeate density was determined, at room temperature, using 10 mL picnometers. For some experiments, a discontinuous diafiltration step was also carried out following the ultrafiltration step. For these experiments, double-distilled water was added to bring the SPE solution volume to its initial value. The solution was re-ultrafiltered to VCR = 2.8; that is, until 64% of the water content had been removed. Theoretically, assuming that all non-protein solutes in the extract are freely permeable, combining UF (VCR = 2.5) and diafiltration (VCR = 2.8) should result in a final product with at least 90% db protein.

Global resistance of the system at the end of the ultrafiltration,  $R_G$ , which is equal to the sum of the clean membrane resistance,  $R_{m_0}$ , the resistance due to irreversible fouling (pore plugging and adsorbed material together),  $R_f$ , and the resistance due to the cake layer,  $R_c$ , was estimated from

$$R_G = R_{m_0} + R_f + R_c = \frac{\Delta P}{\mu_p J_p^f} \quad (2)$$

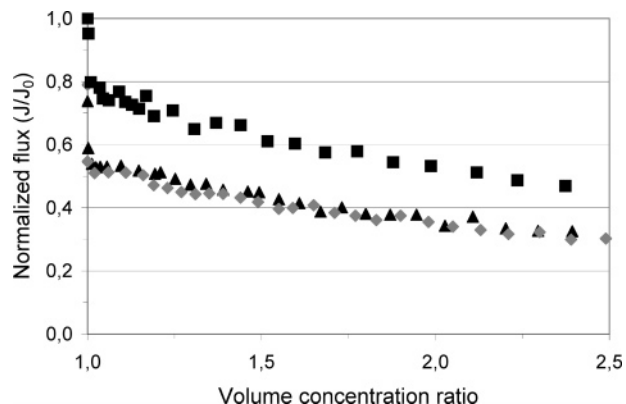
where  $\mu_p$  is the permeate viscosity and  $J_p^f$  is the final permeate flux. Permeate viscosity was estimated at room temperature using a size 100 Cannon-Fenske routine viscometer.

At the completion of SPE ultrafiltration, the stirred cell was carefully emptied and rinsed with double-distilled water to remove any deposited protein. SPC was rapidly frozen before being lyophilized and stored at 4 °C until analyzed. The stirred cell was filled with double-distilled water, and the flux was re-evaluated. Membrane resistance was estimated from eq 1, where the initial water flux,  $J_{w_0}$ , was replaced by the final water flux,  $J_w$ . Membrane resistance was reported as the sum of the clean membrane resistance plus resistance due to irreversible fouling. The above-mentioned approach leads to the estimation of the different individual hydraulic resistances.

**Production of Soy Protein Concentrates by Traditional Acid Precipitation.** SPCs were also produced using the traditional acid precipitation method. For the first concentrate (pH 7<sub>chemical</sub>), a volume of 150 mL of a 10% w/w (pH 9) protein solution in double-distilled water was used and HCl solution (1 N) was added to acidify the protein solution to pH 4.5. This resulted in protein precipitation. The proteins were recuperated by centrifugation at 13000g for 5 min and resuspended in double-distilled water, and the pH was adjusted to 7, using 1 N NaOH. The resulting SPC was rapidly frozen before being lyophilized. The second concentrate (pH 7<sub>chemical+wash</sub>) was produced using the same method except that a washing step (protein resuspension in 150 mL of double-distilled water for 30 min and centrifugation) was performed following the precipitation step at pH 4.5 and prior to adjustment of the pH to 7. Dry SPCs were stored at 4 °C until processed.

**Analytical Methods.** *Total Protein, Moisture, and Ash Contents.* The total protein content of the SPE and SPC was expressed as total nitrogen  $\times$  6.25. Nitrogen was determined using an FP-428 LECO apparatus (LECO Corp., St. Joseph, MI). The instrument was calibrated each time with EDTA as nitrogen standard. Moisture and ash contents were respectively determined using methods derived from AOAC methods 925.09 and 923.03 (24). Carbohydrate content was calculated by difference.

*Mineral Contents.* The residues resulting from the ash measurement procedure were dissolved in 10 mL of 1 M HCl solution, and portions were used for the mineral content determination by flame atomic absorption spectrometry using a Spectra A-100 from Varian (Palo Alto, CA). Sodium, potassium, and calcium contents were determined at 589, 766.5, and 422.7 nm, respectively (25). Phosphorus content was estimated by spectrophotometry at 400 nm according to a method derived from *Analytical Methods Manual* (26). The method is based on the formation of a molybdivanadophosphoric complex, which



**Figure 2.** Experimental normalized permeate fluxes as a function of VCR: ▲, pH 6; ◆, pH 7; ■, pH 9. A YM 100 regenerated cellulose membrane was used.

absorbs in the visible region of the electromagnetic spectrum. A monobasic potassium phosphate solution was used as standard.

**Protein Solubility Profiles.** Protein solubility profiles of the SPC produced by combining electroacidification and ultrafiltration (pH 9, 7, and 6) and of the concentrates obtained by traditional acidification (pH 7<sub>chemical</sub> and pH 7<sub>chemical+wash</sub>) were determined as follows: 40 mL of double-distilled water was added to each sample (0.5 g) and samples were stirred to allow rehydration. The pH was then adjusted to 9.0, and the weight of the solution was adjusted to 50 g to give a 1% w/w solution. Samples were acidified using 0.1 N HCl to pH values ranging from 9 to 2 and then centrifuged, at 13000g for 5 min at room temperature, to separate the insoluble proteins from the soluble proteins. Proteins remaining in solution under the above conditions were considered to be soluble proteins. Total and soluble proteins were determined using Bradford's method (7).

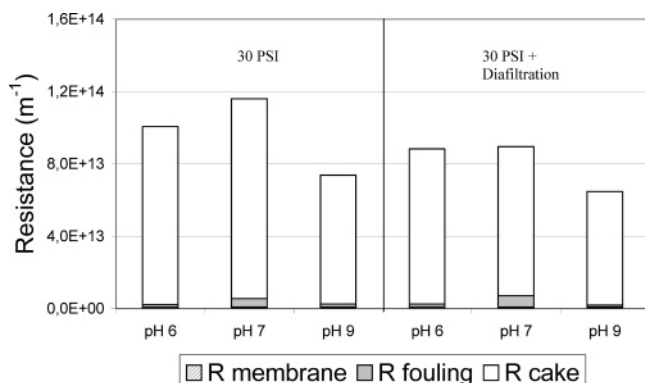
**Statistical Analyses.** Experimental data including the different hydraulic resistances, protein content, and percent of soluble protein were submitted to a *t* test analysis to determine whether the different treatments give equivalent results. The test procedure was applied, as described by Montgomery (27). Each experiment was conducted in duplicate.

## RESULTS AND DISCUSSION

**Ultrafiltration Parameters.** A typical SPE direct UF permeate flux curve (30 PSI) plotted as a function of VCR is shown in **Figure 2**. Permeate fluxes are shown as normalized permeate fluxes, where  $J_0$  is the initial flux obtained with the original pH 9 extract. As expected, permeate flux declined as ultrafiltration progressed for all pH values studied. The flux decline may be associated with internal fouling and cake formation. Effects of membrane fouling and cake formation on the permeate flux can be quantified via the different hydraulic resistances.

Results for the YM 100 regenerated cellulose membranes are presented in **Figure 3**. After processing of the SPE, the resistance due to cake formation was the main resistance to liquid permeation. At the end of the ultrafiltration process, the resistance due to cake formation accounted for 92–98% of the global final resistance, whereas the resistance due to irreversible fouling showed contributions of 1–7%. This suggests that minimization of permeate flux decline could be achieved using tangential flow ultrafiltration, which is less prone to cake formation.

When a diafiltration step was carried out after the direct UF step, a decrease of the aforementioned resistance was observed. This decrease was significant at a level of  $\alpha = 0.05$  [100(1 -  $\alpha$ )% of the time this decrease is significant] for the pH 7 extract and at a level of  $\alpha = 0.10$  for the pH 6 extract. The decrease of the cake layer resistance, using diafiltration, could be attributed to a decrease of the particle convection to the membrane surface, resulting from SPE dilution.



**Figure 3.** Distribution of different hydraulic resistances for dead-end ultrafiltration experiments. A YM 100 regenerated cellulose membrane was used.

**Table 1.** Protein, Ash, and Carbohydrate Contents of Soy Protein Concentrates Produced by Dead-end Ultrafiltration (100 kDa Regenerated Cellulose Membrane)

exptl parameter <sup>a</sup>	% dry basis		
	protein content	ash content	carbohydrate content <sup>b</sup>
extract, pH 9	59.9	10.4	29.7
ultrafiltration, 30 PSI	76.8	8.4	14.8
ultrafiltration + diafiltration, 30 PSI	87.6	7.5	4.9
electroacidified, pH 7	57.6	11.9	30.5
ultrafiltration, 30 PSI	74.2	8.9	16.9
ultrafiltration + diafiltration, 30 PSI	86.3	6.9	6.8
electroacidified, pH 6	55.2	8.0	36.8
ultrafiltration, 30 PSI	71.7	5.7	22.6
ultrafiltration + diafiltration, 30 PSI	84.7	3.8	11.5

<sup>a</sup> Starting material reconstituted from lyophilized extract. <sup>b</sup> By difference.

Global resistance was also observed to be a function of pH. The global resistance was minimum at pH 9, maximum at pH 7, and intermediate at pH 6. Differences in global resistance between pH 6 and 9 ( $\alpha = 0.10$ ) and between pH 7 and 9 ( $\alpha = 0.05$ ) were significant for all conditions. For pH 6 and 7, the differences in global resistance were significant at a level of  $\alpha = 0.10$  for 30 PSI and not significant for the diafiltration step. These observations are in agreement with the permeate flux curves reported in **Figure 2**. Permeate flux, which is proportional to the inverse of the resistance, was maximum at pH 9, minimum at pH 7, and intermediate at pH 6. Although the difference in permeate flux between pH 6 and 7 was small, it was significant ( $\alpha = 0.10$ ).

**Soy Protein Concentrates Produced from pH 9 Soy Extracts.** Theoretically, for the pH 9 SPE, the direct UF step (VCR = 2.5) and the combined UF–diafiltration steps (VCR = 2.5 and 2.8) should result in a product with protein contents of 78.9 and 91.2% db, respectively. As reported in **Table 1**, protein, ash, and carbohydrate contents of the SPC differ slightly from theoretical predictions. Protein content was 2–3% lower than expected ( $\alpha = 0.05$ ). This value is very close to the theoretical values calculated. SPC protein content increased by ~11% when a diafiltration step was carried out following the direct UF step (**Table 1**).

As reported in **Table 2** for the YM 100 membrane, it was observed that the percent of carbohydrate removal was 20–23% higher than the percent of mineral removal for direct UF.

**Table 2.** Percentage of Ash and Carbohydrate Removal after Dead-end Ultrafiltration (100 kDa Regenerated Cellulose Membrane)

exptl parameters <sup>a</sup>	ash removal <sup>b</sup> (%)	carbohydrate removal <sup>b</sup> (%)
extract, pH 9		
ultrafiltration, 30 PSI	41.1	63.6
ultrafiltration + diafiltration, 30 PSI	55.1	93.2
electroacidified, pH 7		
ultrafiltration, 30 PSI	46.5	60.2
ultrafiltration + diafiltration, 30 PSI	64.5	86.5
electroacidified pH 6		
ultrafiltration, 30 PSI	51.4	58.4
ultrafiltration + diafiltration, 30 PSI	72.9	82.3

<sup>a</sup> Starting material reconstituted from lyophilized extract. <sup>b</sup> Ash or carbohydrate removal (%) = [ash or carbohydrate content before UF (% dry basis) – ash or carbohydrate content after UF (% dry basis)]/ash or carbohydrate content before UF (% dry basis).

**Table 3.** Mineral Contents of Soy Protein Concentrates Produced by Dead-end Ultrafiltration (100 kDa Regenerated Cellulose Membrane)

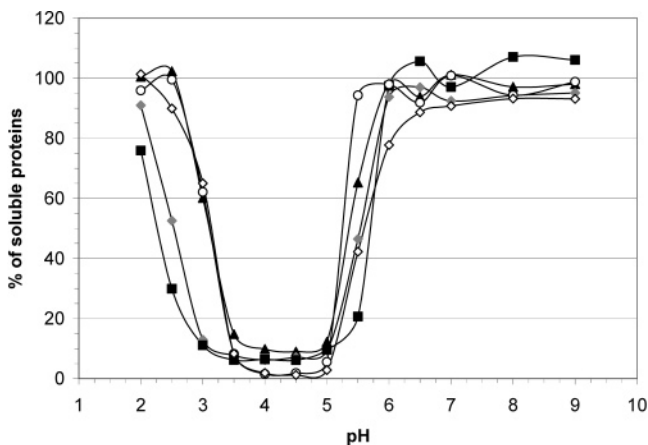
exptl parameters	mg/g of dry powder			
	Na content <sup>a</sup>	K content <sup>a</sup>	Ca content <sup>a</sup>	P content <sup>a</sup>
extract, pH 9	7.1	29.0	3.95	8.99
ultrafiltration, 30 PSI	5.6	23.5	4.61	10.47
ultrafiltration + diafiltration, 30 PSI	3.3	17.5	5.39	11.57
electroacidified, pH 7	7.0	64.5	3.81	8.73
ultrafiltration, 30 PSI	4.6	27.7	4.56	9.83
ultrafiltration + diafiltration, 30 PSI	3.0	17.3	4.98	10.50
electroacidified, pH 6	6.3	24.6	4.25	8.57
ultrafiltration, 30 PSI	4.5	12.3	3.58	7.48
ultrafiltration + diafiltration, 30 PSI	1.9	8.3	2.80	5.98

<sup>a</sup> Mineral contents were determined from the residues resulting from the ash measurement procedure.

When the combined UF–diafiltration steps were carried out, the difference was increased to 38%. Mineral removal was not as efficient as carbohydrate removal for the pH 9 SPE. Concentrations of sodium and potassium, after direct UF and UF–diafiltration, still represent a significant fraction of the initial mineral concentrations. For phosphorus and calcium, their concentrations are even increased during the UF and UF–diafiltration treatments (**Table 3**). These results are in agreement with observations previously reported in the literature (15, 16, 19).

**Soy Protein Concentrates Produced from the Electroacidified (pH 7 and 6) Soy Extracts.** Protein content of extracts resulting from the electroacidification of the SPE averaged 57.6% db (pH 7) and 55.2% db (pH 6). The ash content of the extracts decreased from 10.4% db, for the pH 9 SPE, to 8.0% db, for the pH 6 SPE ( $\alpha = 0.05$ ) and was 11.9% db at pH 7. As presented in **Table 3**, this increase of the ash content, when the pH is decreased from 9 to 7, is due to an increase of potassium. The reason for this increase is unclear but could be due to the migration of potassium ions from the KCl compartment into the protein compartment. Bazinet et al. (28) reported a similar observation for electroacidification of SPE from pH 7.4 to pH 4.5.

Electroacidified extracts were ultrafiltered in the same way as for the pH 9 SPE. The theoretical protein contents for pH 7



**Figure 4.** Solubility profiles of soy protein concentrates as a function of pH:  $\blacktriangle$ , pH 6;  $\blacklozenge$ , pH 7;  $\blacksquare$ , pH 9;  $\diamond$ , pH 7<sub>chemical</sub>;  $\circ$ , pH 7<sub>chemical+wash</sub>.

and 6 SPC were 77.3 and 75.5% db, for direct UF, and 90.6 and 89.5% db, for the combined UF–diafiltration steps. For both pH values (Tables 1 and 2), the SPC protein contents obtained were 2–5% lower than expected (pH 7,  $\alpha = 0.05$ ; pH 6,  $\alpha = 0.10$ ). These values are still very close to theoretical values. It was also observed that decreasing the SPE pH with electroacidification (pH 7 or 6), prior to the ultrafiltration and diafiltration steps, increased mineral removal efficiency (Table 2). For sodium and potassium, the mineral concentration after direct UF and UF–diafiltration represents a less significant fraction of the initial mineral concentration than for the pH 9 SPE (Table 3). A possible explanation is that the decrease of the protein charge with decreasing pH results in fewer electrostatic interactions between proteins and minerals, such as  $\text{Na}^+$  and  $\text{K}^+$ , making a larger part of the minerals available for permeation through the ultrafiltration membrane.

This improvement in mineral removal was also observed for phosphorus and calcium, but only at pH 6. This may be due to the fact that at pH values  $> 6.5$ , phytic acid (the main source of phosphorus in soy) and calcium are present in the form of a ternary complex (phytic acid–protein–calcium) that is unable to permeate the UF membrane, whereas between pH 4.5 and 6.5 both minerals are in free form and are available to permeate the membrane (23, 29). A decrease of pH also resulted in a slight decrease of the percent of carbohydrate removed; the most likely explanation for this could be an increase in their rejection due to increased cake formation.

**Protein Solubility Profile.** Protein solubility is an important functional property of soy proteins. In general, proteins with low solubility indices have limited functional properties and more limited uses. Soy protein solubility profiles of the concentrates produced with the novel approach and with traditional acid precipitation at pH 4.5 are presented in Figure 4. For SPC produced using a combination of electroacidification and ultrafiltration, the solubility profiles vary with pH. The broad minimum between pH 3 and 5, in the pH 9 profile, is changed to a sharper minimum, between pH 4 and 5, in the pH 6 profile. Furthermore, between pH 2 and 3, an increase in solubility (from 25 to 70%) is observed for the pH 6 SPC when compared to the pH 9 SPC. For pH varying between 5 and 9, the increase in solubility for the pH 6 SPC, when compared to the pH 9 SPC, is as high as 45%. These increases were significant at a level of  $\alpha = 0.0025$ .

The pH 6 SPC produced using a combination of electroacidification and ultrafiltration was also more soluble than the pH 7<sub>chemical</sub> SPC produced by traditional acid precipitation. The

difference in solubility was most significant between pH 5.5 and 7 and was as high as 23% in favor of the pH 6 SPC ( $\alpha = 0.0025$ ). An increase of protein solubility was observed for the pH 7<sub>chemical+wash</sub>, which had a higher protein content and a lower mineral content than the pH 7<sub>chemical</sub> (83.3% protein and 6.2% ash for the pH 7<sub>chemical</sub> SPC and 86.9% protein and 4.1% ash for the pH 7<sub>chemical+wash</sub> SPC). As a result, the pH 6 SPC and the pH 7<sub>chemical+wash</sub> SPC show similar solubility profiles. However, the volume of water needed with the new process is only 1.6  $V_0$  (1 initial volume + 0.6 initial volume required during the discontinuous diafiltration step) as compared to at least 3  $V_0$  for the traditional process (1 initial volume + 1 initial volume for the wash step + 1 initial volume for the pH adjustment step). This represents a volume of water close to 2 times less with the new approach than with the traditional one. This is a significant advantage of the new process as compared to the traditional acid precipitation process using inorganic acid.

On the basis of the results presented in Figure 4 and the aforementioned observations, it appears that protein solubility increases with a decrease of the mineral contents. This suggests that SPC produced by combining electroacidification and ultrafiltration (especially at pH 6) would be easier to incorporate into food than SPC produced by ultrafiltration alone (pH 9) or by traditional acid precipitation process (pH 7<sub>chemical</sub>). In addition, the protein content of the SPC produced by combining both technologies was close to 90%, and an increase of the diafiltration volume permeated ratio would result in the production of a protein isolate.

These results illustrate the feasibility and advantages of combining both electroacidification and ultrafiltration in the production of SPC and SPI. Effective application of this technology in industry would require the use of a tangential ultrafiltration system. Tangential flow ultrafiltration provides better control of cake layer formation and would thus help to improve permeate flux. The extension of these experimental studies to tangential flow ultrafiltration is currently in progress.

#### ABBREVIATIONS USED

BMEA, bipolar membrane electroacidification; DSA, dimensionally stable electrode; db, dry basis; MWCO, molecular weight cutoff; SPC, soy protein concentrate; SPE, soy protein extract; SPI, soy protein isolate; UF, ultrafiltration; VCR, volume concentration ratio.

#### ACKNOWLEDGMENT

We thank Lucie Masse for assistance with atomic absorption.

#### LITERATURE CITED

- (1) Johnson, L. A.; Myers, D. J. Industrial uses for soybeans. In *Practical Handbook of Soybean Processing and Utilization*; Erickson, D. R., Ed.; AOCS Press: Champaign, IL, 1995; pp 380–427.
- (2) Mounts, T. L.; Wolf, W. J.; Martinez, W. H. Processing and utilization. In *Soybeans: Improvement, Production, and Uses*; Wilcox, J. R., Ed.; American Society of Agronomy: Madison, WI, 1987; pp 819–860.
- (3) Nash, A. M.; Wolf, W. J. Solubility and ultracentrifugal studies on soybean globulins. *Cereal Chem.* **1967**, *44*, 183–192.
- (4) Fisher, R. R.; Glatz, C. E.; Murphy, P. A. Effects of mixing during acid addition on fractionally precipitated protein. *Biotechnol. Bioeng.* **1986**, *28*, 1056–1063.
- (5) Bazinet, L.; Lamarche, F.; Labrecque, R.; Toupin, R.; Boulet, M.; Ippersiel, D. *Systematic Study on the Preparation of a Food Grade Soybean Protein*; No. 9326 U 987; The Canadian Electricity Association, Research and Development: Montreal, Canada, May 1996.

- (6) Bazinet, L.; Lamarche, F.; Labrecque, R.; Toupin, R.; Boulet, M.; Ippersiel, D. Electro-acidification of soybean proteins for the production of isolate. *Food Technol.* **1997**, *51*, 52–56, 58, 60.
- (7) Bazinet, L.; Lamarche, F.; Ippersiel, D. Comparison of chemical and bipolar membrane electrochemical acidification for precipitation of soybean proteins. *J. Agric. Food Chem.* **1998**, *46*, 2013–2019.
- (8) Bazinet, L.; Lamarche, F.; Ippersiel, D. Bipolar membrane electrodialysis: Applications in the food industry. *Trends Food Sci. Technol.* **1998**, *9*, 107–113.
- (9) Mani, K. N. Electrodialysis water splitting technology. *J. Membrane Sci.* **1991**, *58*, 117–138.
- (10) Porter, M. C.; Michaels, A. S. Applications of membrane ultrafiltration to food processing. Presented at the 3rd International Congress of Food Science and Technology, Washington, D.C., Aug 9–14, 1970.
- (11) Okubo, K.; Waldrop, A. B.; Iacobucci, G. A.; Myers, D. V. Preparation of low-phytate soybean protein isolate and concentrate by ultrafiltration. *Cereal Chem.* **1975**, *52*, 263–271.
- (12) Goodnight, K. C.; Hartman, G. H.; Marquardt, R. F. Aqueous purified soy protein and beverage. U.S. Patent 3 995 071, 1976.
- (13) Lawhon, J. T.; Hensley, D. W.; Mulsow, D.; Mattil, K. F. Optimization of protein isolate production from soy flour using industrial membrane systems. *J. Food Sci.* **1978**, *43*, 361–364.
- (14) Omosaiye, O.; Cheryan, M.; Mathews, E. Removal of oligosaccharides from soybean water extracts by ultrafiltration. *J. Food Sci.* **1978**, *43*, 354–360.
- (15) Nichols, D. J.; Cheryan, M. Production of soy isolates by ultrafiltration: factors affecting yield and composition. *J. Food Sci.* **1981**, *46*, 367–372.
- (16) Kumar, N. S. K.; Yea, M. K.; Cheryan, M. Soy protein concentrates by ultrafiltration. *J. Food Sci.* **2003**, *68*, 2278–2283.
- (17) Lawhon, J. T.; Lusas, E. W. New techniques in membrane processing of oilseeds. *Food Technol.* **1984**, *38*, 97–106.
- (18) Samoto, M.; Miyazaki, C.; Kanamori, J.; Akasaka, T.; Kawamura, Y. Improvement of the off-flavor of soy protein isolate by removing oil-body associated proteins and polar lipids. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 935–940.
- (19) Lawhon, J. T.; Mulsow, D.; Cater, C. M.; Mattil, K. F. Production of protein isolates and concentrates from oilseed flour extracts using industrial ultrafiltration and reverse osmosis systems. *J. Food Sci.* **1977**, *42*, 389–394.
- (20) Pearson, A. M. Soy proteins. In *Development in Food Proteins—2*; Hudson, B. J. F., Ed.; Applied Science Publishers: London, U.K., 1983; p 88.
- (21) Snyder, H. E.; Kwon, T. W. Morphology and composition. In *Soybean Utilization*; Van Nostrand Reinhold: New York, 1987; p 60.
- (22) Garcia, M. C.; Torre, M.; Marina, M. L.; Laborda, F. Composition and characterization of soyabean and related products. *Crit. Rev. Food Sci. Nutr.* **1997**, *37*, 361–391.
- (23) Grynspan, F.; Cheryan, M. Phytate-calcium interactions with soy protein. *J. Am. Oil Chem. Soc.* **1989**, *66*, 93–97.
- (24) AOAC International. Methods 923.03 and 925.09. In *Official Methods of Analysis*, 16th ed.; Cunniff, P., Ed.; AOAC: Washington, DC, 1995.
- (25) Varian Australia Pty Ltd. *Flame Atomic Absorption Spectrometry: Analytical Methods*; Publication 85-100009-00; Varian Australia Pty Ltd.: Mulgrave, VIC, Australia, 1989.
- (26) Leatherhead Food Research Association. Method 2: Determination of total phosphorus (phospho-vanado-molybdate colorimetric method). In *Analytical Methods Manual*, 2nd ed.; Leatherhead, U.K., 1987.
- (27) Montgomery, D. C. Simple comparative experiments. In *Design and Analysis of Experiments*, 3rd ed.; Wiley: New York, 1991; p 38.
- (28) Bazinet, L.; Lamarche, F.; Ippersiel, D. Ionic balance: a closer look at the K<sup>+</sup> migrated and H<sup>+</sup> generated during bipolar membrane electro-acidification of soybean proteins. *J. Membrane Sci.* **1999**, *154*, 61–71.
- (29) Shallo, H. E.; Rao, A.; Ericson, A. P.; Thomas, R. L. Preparation of soy protein concentrate by ultrafiltration. *J. Food Sci.* **2001**, *66*, 242–246.

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Received for review February 25, 2004. Revised manuscript received June 23, 2004. Accepted July 14, 2004. The financial support of Agriculture and Agri-Food Canada and of the Natural Sciences and Engineering Research Council of Canada is acknowledged.

JF0400922