Comparison of Chemical and Bipolar-Membrane Electrochemical Acidification for Precipitation of Soybean Proteins

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We compared chemical and electrochemical acidification in precipitation of soybean proteins, to identify the elements differentiating the two acidification procedures. Chemical acidification and electro-acidification procedures result in differences in 11S precipitation profiles, which would be the consequence of a different solubilization profile of this fraction. At pH 6.0, less of the 11S fraction is precipitated by electro-acidification than by chemical acidification. The conductivity, and consequently the ash content, of the electro-acidified protein solution is decreased while that of the chemically acidified protein solution is increased, depending on the normality of the added HCl.

Keywords: *Chemical acidification; electrochemical acidification; bipolar membrane; protein; soya*

INTRODUCTION

Separation of proteins by isoelectric precipitation from alkaline solution is very common. All commercial soy protein isolates are produced by acid precipitation. The disadvantages of this method include denaturation of protein on exposure to alkali and acid treatment, high ash content, and alteration of protein solubility after rehydration (Nash and Wolf, 1967). Local extremes in pH can cause irreversible denaturation of the proteins, which will alter the precipitation behavior (Fisher et al., 1986; Kilara and Sharkasi, 1986).

Recently, Bazinet et al. (1996, 1997a−c) have developed a procedure called bipolar-membrane electro-acidification (BMEA), which precipitates soya proteins with less denaturation than the conventional isoelectric process: chemical agents are not needed to decrease pH. The bipolar membrane forming the core of this process is composed of three parts: an anion exchange layer, a cation exchange layer, and a hydrophilic interphase at their junction. When a current is passed across this kind of membrane, electrical conduction is achieved by the transport of H^+ and OH^- ions generated by electro-dissociation of water (Mani, 1991). The protons thus generated can come into contact with the proteins, bringing them to their isoelectric point, resulting in selective separation. Centrifugation can then be used, as in the conventional process, for a simple separation of the proteins.

The purpose of this study was to compare chemical and electrochemical acidification, to identify the elements differentiating the two acidification procedures. Both processes were compared in term of conductivity, percentage of proteins precipitated, and protein molecular profiles.

MATERIAL AND METHODS

Material. The crude soybean protein extract (SPE, ∼60% protein) used in this study was obtained as follows: 6 kg of defatted soya flakes (Central Soya, Woodstock, Ontario, Canada) was added to 54 L of distilled water. The mixture was heated to 50 °C, and the pH was adjusted to 8. The mixture was stirred for 30 min, and the insoluble material was removed by basket centrifugation (type SBW11, Western States, Hamilton, OH) and a press filter (1 μm) (Model 6SS4-812-TIHO, StarSystems, Timmonsville, SC). The liquid was then rapidly frozen before being lyophilized. The final product was stored at 4 °C. The SPE contained 57.5% protein, 11.2% carbohydrate, 1.8% fat, and 8.7% ash, expressed as percentage dry matter.

Methods. *(a) Electro-acidification Cell.* The module used was an MP type cell (100 cm^2 of effective electrode surface) purchased from Electrosynthesis Co. Inc. (Lancaster, PA). The cell consists of eight compartments separated by four CR-64-LMP-401 cationic membranes (Ionics Inc., Watertown, MA) and three Neosepta BP-1 bipolar membranes from Tokuyama Soda Ltd. purchased from Electrosynthesis Co. Inc. This arrangement defines three closed loops containing the protein solution, a 2 $g \cdot L^{-1}$ aqueous KCl solution, and a 20 $g \cdot L^{-1}$ Na_2SO_4 solution. Each closed loop was connected to a separate external 10 L reservoir, allowing for continuous recirculation (Bazinet et al., 1997c).

The anode/cathode voltage difference was supplied by a variable 0−100 V power source, Powerstat Model 236BU-2 (The Superior Electric Co., Bristol, CO). The electrolytes were circulated using three centrifugal pumps, Model XVB56C34F2012b-W (Marathon Electric, Wausau, WI), and the flow rate was controlled at 4.5 L min^{-1} using Model FC-FI-C-3/8 flow meters (Filter-Chem, Alhambra, CA). The temperature of the electrolytes was maintained at 25 °C by circulating water inside a stainless-steel coil immersed in each of the reservoirs. The anode, a dimensionally stable electrode (DSA), and the cathode, a 316 stainless-steel electrode, were supplied with the MP cell.

(b) Protocol. For chemical acidification, protein solution volumes of 250 mL were used. HCl solutions of different normality were added to acidify the protein solution to pH 4.4. Electro-acidification was performed in batch process using a constant current of 2.5 A, with solution volumes of 6 L. The electro-acidification was stopped after the pH reached 4.4. The initial pH in both chemical and electrochemical acidification varied between 7.5 and 7.8; to ensure good electrochemical processing, 0.06 M KCl was added.

A 5 × 2 factorial array was set up: 5 acidification procedures (addition of 0.25, 0.5, 1, and 2 N HCl and electro-acidification) and 2 concentrations of soya protein extract (15 and 60 $g \cdot L^{-1}$)

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were tested. Three replicates of each combination of factors were performed in this experiment.

During each treatment, samples of the protein solution were taken at the following pH readings: initial pH (about 7.6), 7.2, 6.8, 6.4, 6.0, 5.6, 5.2, 4.8, and 4.4. During acidification treatment, the conductivity and the temperature were recorded. On freshly acidified samples, the concentration of soluble protein and the characterization of 11S and 7S protein fractions were performed. In chemical acidification, the volume of added HCl was noted at each pH reading. In electro-acidification, the time required to reach pH 4.4 was recorded as well as the anode/cathode voltage difference as the treatment progressed.

(c) Analysis Methods. (i) pH Measurement. The pH of the protein solution was measured with a pH meter Model Φ11 (Beckman Instruments Inc., Fullerton, CA).

(ii) Anode/Cathode Voltage Difference. The voltage was read directly from the indicators on the power supply.

(iii) Conductivity. A YSI conductivity meter, Model 35, was used with a YSI immersion probe, Model 3417, cell constant $K = 1\ cm^{-1}$ (Yellow Springs Instrument Co.,Yellowsprings, OH), to measure the conductivity of the protein solutions. The measured conductivity was normalized to a constant volume in order to decrease the effect of dilution by HCl addition: conductivity normalized = conductivity measured × (250 mL + V_{HCl})/250 mL where V_{HCl} represents the volume of HCl added.

(iv) Soluble Protein. The concentration of soluble protein was measured using the method of Bradford (1976). Samples were centrifuged at 10000*g* for 10 min at 20 °C; 100 μL of the supernatant, containing 10−100 μg of protein, was added to 5 mL of one-fifth dilution of the dye reagent concentrate (protein assay dye reagent concentrate, 500−0006; Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). After vortexing, the absorbance at 595 nm (Model DU 640 spectrophotometer; Beckman Instruments, Fullerton, CA) was measured after 5 min and before 1 h in 4.5 mL cuvettes against a reagent blank prepared from 0.1 mL of distilled water and 5 mL of protein reagent. The method was calibrated each time with a bovine serum albumin standard (Protein Standard II, 500−0007; Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) from 0 to 1.4 $g \cdot L^{-1}$. The determined concentration of soluble protein was transformed into percentage to compare the protein solution at the different SPE concentrations and to diminish the effect of dilution in the case of chemical acidification.

(v) Molecular Weight Profiles. The molecular weight profiles were determined by size-exclusion high-performance liquid chromatography (SE-HPLC) according to a procedure slightly modified from that described by Musakhanian and Alli (1987). Chromatographic analysis was performed using a WISP work station (Model 712, Waters, Milford, MA) equipped with a dual pump system (Model 510, Waters) and a variable wavelength detector (Model 490, Waters) set at 280 nm. A TSK-G3000PW exclusion column (7.5 mm i.d. × 30 cm) (Model 8-05672, Supelco, Mississauga, ON, Canada) was used along with a Progel-TSK PWXL (6 mm ID × 4 cm) guard column (Model 8-08033, Supelco). Analyses were performed isocratically by eluting a 0.1 M phosphate buffer (pH 6.8) mobile phase at a flow rate of 0.8 mL/min, at room temperature and under a pressure of 100 psi. The same centrifuged sample was used for soluble protein determination and HPLC analysis. A 15 μL aliquot of each protein solution supernatant sample diluted in the mobile phase was injected.

The gel exclusion chromatographic procedure was calibrated, for estimating the average molecular weight of the protein fraction and to determine the 11S and 7S fractions, using a set of six protein standards (chymotrypsinogen A, MW = 25 000; bovine serum albumin, MW = 66 000; aldolase, MW = 158 000; catalase, MW = 232 000; apoferritin, MW = 443 000; thyroglobulin, MW = 669 000). The average molecular weights of the proteins in the sample which were chromatographed were estimated using the equation of Andrews (1964).

The chromatographic data were collected by means of a personal computer (NEC Powermate, Boxborough, MA). Integration of the peaks was carried out with the aid of Millipore chromatography software (Maxima 820 Dynamics solutions, Millipore, Bedford, MA). For both fractions, the quantification was expressed relative to the respective initial peak area, and calculated as a percentage of the initial area of the unacidified protein solutions.

(d) Statistical Analyses. The duration of the electro-acidification and the voltage as a function of pH were subjected to an analysis of variance. Regression equations were calculated for the voltage and duration as a function of pH using SigmaPlot (version 3.0 for Windows, Jandel Scientific, Corte Madera, CA). The conductivity and the percent initial fraction area for 11 S and 7 S as the pH decreased were analyzed with a multivariate analysis of variance since the Huynh−Feldt condition was not met. The Huynh−Feldt condition is a mathematical condition to be met in order to test the sphericity of a set of orthogonal components (Huynh and Feldt, 1970). It tests the hypothesis that the orthogonal components are uncorrelated and have equal variance.On the other hand, the percent soluble protein as a function of pH was analyzed by a split-plot analysis of variance, since the Huynh−Feldt condition was met. Regression contrasts were calculated by the univariate analysis of variance, using SAS software (SAS, 1989), to examine the effect of interaction between the variables. The nonlinear and linear regression coefficients and the parameters of the model sigmoidal equations were calculated with Sigmaplot software. The percentage of initial 11S fraction area data obtained as the pH decreased were examined by Duncan tests at the different pH values in order to determine the significance of differences between the different acidification procedures.

RESULTS AND DISCUSSION

Electro-acidification Parameters: Duration and Anode/Cathode Voltage Difference. Analysis of the variance of the duration of electro-acidification and of the anode/cathode voltage difference indicated that the SPE concentration ($P < 0.0001$) had a significant effect on the rate of decrease of pH. The second-order regression curves calculated for the duration and voltage as a function of pH produced coefficients of determination on the order of 0.91−0.99.

Duration of Electro-acidification. The time required to lower the pH from its initial value of 7.6 to 4.4 is largely influenced by the SPE concentration. The time required to reach pH 4.4 increases from about 19.3 min at 15 $g \cdot L^{-1}$ to 69.9 min at 60 $g \cdot L^{-1}$. A larger quantity of H^+ is required from the dissociation of water by the bipolar membranes to lower the pH of the solution, when the protein concentration is increased, which results mostly from the buffering capacity of the proteins in solution (Cheftel et al., 1985; Prakash and Narasinga Rao, 1990). These results confirm those obtained by Bazinet et al. (1997a) with 14.7 and 54.9 min, respectively, for 15 and 60 $g \cdot L^{-1}$. The difference can be explained by the fact that in our past experiment values were calculated for averaged KCl concentrations and the decrease in pH was from 7.4 to 4.5.

Anode/Cathode Voltage Difference. During pH decrease, the voltage decreased to a minimum and then increased back to the initial value or higher. The evolution of anode/cathode voltage difference depended greatly on the initial SPE concentration (Figure 1); at 15 $g \cdot L^{-1}$, the voltage decreased, to a minimum of 47 V at pH 5.6, and then rose to 51 V at pH 4.4. However, at 60 $g \cdot L^{-1}$, it decreased from 52 to 40 V, in the same pH range, and then increased from 40 to 57 V, again in the pH range from 5.6 to 4.4. At high concentrations of SPE, the decrease and increase in anode/cathode voltage differences are more marked. The decrease in

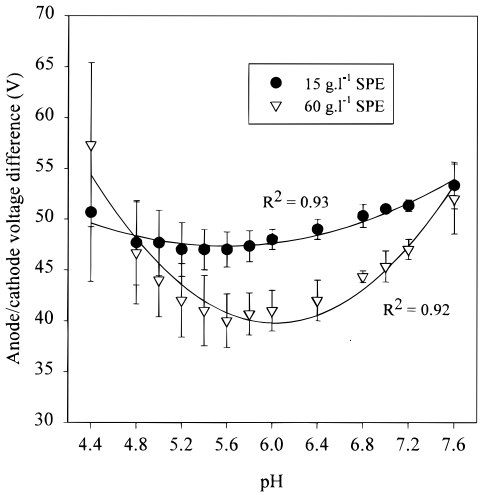

Figure 1. Effect of the SPE concentration on the anode/cathode voltage difference observed during bipolar-membrane electro-acidification of a soybean protein solution with 0.06 M KCl, run at 25 °C with a 2.5 A constant current.

voltage would be the result of the higher conductivity of H^+ generated at the bipolar membrane in replacement of K^+, the main ionic species in solution (Waggle and Kolar, 1979), to maintain the electrical neutrality of the solution: the molar conductivity of H^+ is 349.6 $S{\cdot}cm^2{\cdot}mol^{-1}$ compared to 73.5 $S{\cdot}cm^2{\cdot}mol^{-1}$ for K^+ (Brett and Oliveira-Brett, 1994). This replacement of K^+ by H^+ in the protein solution, and the migration of K^+ from the protein compartment to KCl and Na_2SO_4 compartments across the cationic membrane, coupled in the KCl compartment with generation of OH^- (molar conductivity of 199.1 $S{\cdot}cm^2{\cdot}mol^{-1}$) at the anionic exchange layer of the bipolar membrane, induces a decrease in the overall resistance of the system, and consequently a decrease in the anode/cathode voltage difference. This phenomenon is in progress during all of the process, but when the protein begins to precipitate, the overall resistance of the system is increased by a slight fouling in the spacers of the cell. The amount of fouling is greater at high SPE concentrations, explaining the higher increase in voltage after pH 5.6 was reached at 60 $g{\cdot}L^{-1}$. This result confirms that of Bazinet et al. (1997a), who observed the same effect with increasing SPE concentration.

Conductivity. The multivariate analysis of variance of the data shows that the pH ($P < 0.0001$), the SPE concentration ($P < 0.0001$), and the acidification ($P < 0.0001$) have a highly significant effect on the conductivity. These three factors also act in concert doubly and triply. The MANOVA results demonstrate the significant effect of SPE concentration and pH ($P < 0.0001$); acidification and pH ($P < 0.005$); SPE concentration and acidification ($P < 0.0008$); and SPE concentration and acidification and pH ($P < 0.009$).

Interaction between pH and Acidification. During chemical acidification of protein solution, the conductivity increased from 9.6 to 10.9 $mS{\cdot}cm^{-1}$ (all acidification levels and SPE concentrations averaged), and decreased from 8.9 to 7.4 $mS{\cdot}cm^{-1}$ in electro-acidification (all SPE concentrations averaged). In fact, the addition of acid to decrease the pH corresponds to an addition of H^+ and Cl^- ions: their respective conductivities are 349.6 and 76.4 $S{\cdot}cm^2{\cdot}mol^{-1}$ (Brett and Oliveira-Brett, 1994). Consequently, this addition of ionic species contributes to an increase in the overall conductivity of protein solution (Figure 2). On the other hand, in electro-acidification, the conductivity of the solution decreases due to the desalination phenomenon by electrodialysis (Lopez Leiva, 1988a,b; Pérez et al., 1994) (Figure 2). In the bipolar membranes electro-acidification (BMEA) configuration cell, the electric field generated by the anode/cathode voltage difference generates a flow of cations migrating from the protein solution.

A difference in the changes of conductivity during chemical acidification can be noted between the different normalities of acid used. For both SPE concentrations averaged, the conductivity increased with the normality of the added acid (Figure 2). When the normality of the added acid was increased from 0.25 to 2 N, the variation of conductivity increased from 8.1 to 18.2%: the higher the normality, the higher the variation of conductivity. This difference is due to a dilution factor induced by the volume of added HCl. For example, to 250 mL of protein solution at a SPE concentration of 60 $g{\cdot}L^{-1}$ is added 50.93 mL of 0.25 N HCl versus 6.42 mL of 2 N HCl. Calculation with the corresponding dilution factor shows no difference in conductivity variation between the different levels of acidification.

Interaction between pH and SPE Concentration. In chemical acidification, the variation of conductivity increased with the SPE concentration. At a SPE concentration of 15 $g{\cdot}L^{-1}$, all acidification levels averaged, the conductivity increased from 8.5 to 9.1 $mS{\cdot}cm^{-1}$ (+7.0% variation) while at 60 $g{\cdot}L^{-1}$ it increased from 10.7 to 12.6 $mS{\cdot}cm^{-1}$ (+18.0% variation) (Figure 2). This increase in conductivity depending on the SPE concentration must be related to the buffering capacity of the protein solution. The buffering capacity increases with the concentration of protein (Cheftel et al., 1985; Kinsella et al., 1985). On the other hand, in electrochemical acidification, the conductivity decreased from 7.9 to 7.2 $mS{\cdot}cm^{-1}$ (−8.5% variation) and from 10.0 to 7.6 $mS{\cdot}cm^{-1}$ (−23.1% variation), respectively, for SPE concentrations of 15 and 60 $g{\cdot}L^{-1}$. As the protein concentration increases, more H^+ production is required to lower the pH. To maintain the protein solution electrically neutral, one cation must cross the cationic membrane for each H^+ produced at the bipolar membrane (Bazinet et al., 1997a,c). This results in a lower mineral content, and therefore lower conductivity by removing salt from the protein compartment (Houldsworth, 1980; Brun, 1989).

Interaction between pH and SPE Concentration and Acidification. As the pH decreases, the variation of conductivity depends both on the SPE concentration and on the acidification type. As the pH dropped from an initial pH of ∼7.6 to 4.5, the decrease in conductivity increased with the SPE concentration in electro-acidification while the increase in conductivity in chemical acidification increases (Figure 2). These effects are the results of a coupled action of cationic species migration and an increase in the buffering capacity of the protein solution, in the case of electro-acidification, and of ionic species addition and an increase in the buffering capacity of the protein solution.

Soluble Protein. The analysis of variance of the data shows that the pH ($P < 0.0001$) has a highly significant effect on the percentage of soluble protein. The SPE concentration ($P > 0.14$) and the acidification ($P > 0.05$) have no effect on the percentage of soluble protein. However, the regression contrast results demonstrate significant multiple effects of the initial SPE

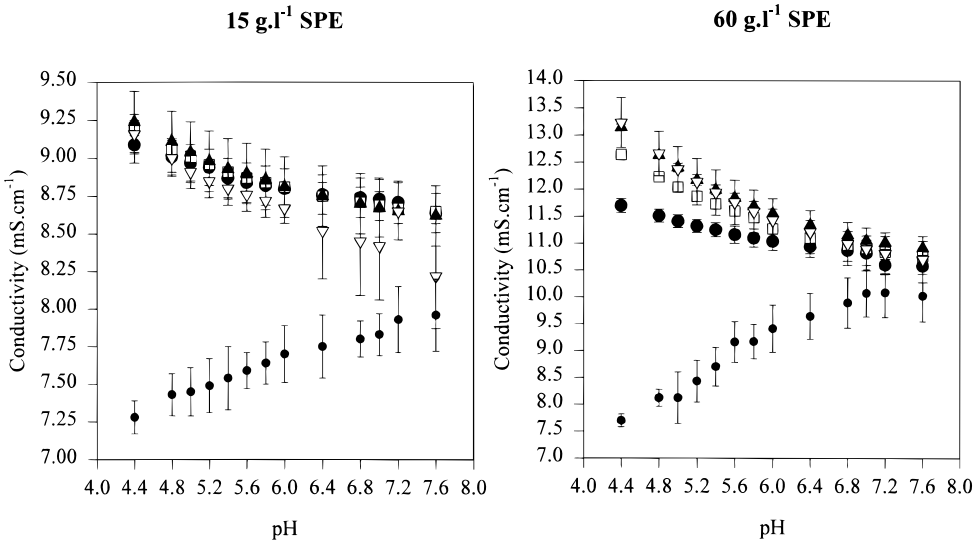

Figure 2. Effect of SPE concentration, 15 and 60 g·L^{-1}, and acidification procedure, 0.25 N HCl (●), 0.5 N HCl (□), 1.0 N HCl (▲), 2.0 N HCl (▽), and electro-acidification (•), on the conductivity measured during the pH decrease of electrochemical and chemical acidifications of soybean protein solutions, with 0.06 M KCl added and maintained at 25 °C.

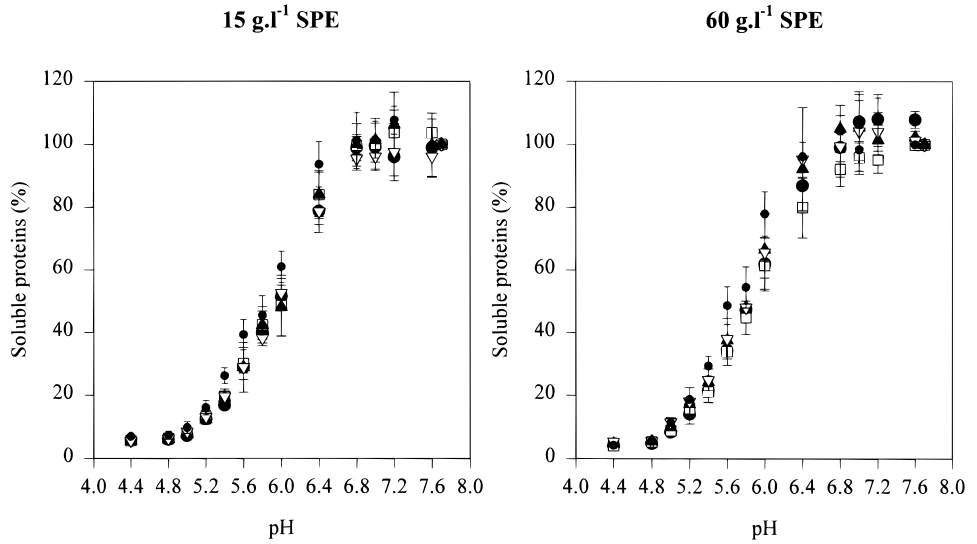

Figure 3. Effect of SPE concentration, 15 and 60 g·L^{-1}, and acidification procedure, 0.25 N HCl (●), 0.5 N HCl (□), 1.0 N HCl (▲), 2.0 N HCl (▽), and electro-acidification (•), on the percentage of soluble proteins measured during the pH decrease of electrochemical and chemical acidifications of soybean protein solutions, with 0.06 M KCl added and maintained at 25 °C.

concentration and pH ($P < 0.001$), and acidification and pH ($P < 0.001$). Nonlinear regression models of both double interactions produced coefficients of determination on the order of 0.993 to 0.998.

Interaction between pH and SPE Concentration. When the initial SPE concentration is increased from 15 to 60 g·L^{-1}, a change in the percentage of soluble protein as a function of pH is observed (Figure 3). From the initial pH to 6.8, no real difference is noted between the two concentrations; the percentage of soluble protein was about 100%. At pH 6.4, 6.0, and 5.6, differences are noted with respectively 81.6, 56.9, and 29.6% of soluble protein at 15 g·L^{-1}, and 88.7, 66.7, and 37.1% at 60 g·L^{-1}. At pH 5.2, 4.8, and 4.4, no real difference is noted between both concentrations with respective soluble protein percentages of 12, 4.3, and 1.5% at 15 g·L^{-1} compared to 15.1, 5.2, and 1.7% at 60 g·L^{-1}. These results are confirmed by sigmoidal models (Table 1). For both concentrations, with all types and levels of acidification averaged, the precipitation curves take on a sigmoidal form, but the inflection points or centers are not the same: at 15 and 60 g·L^{-1} SPE, the inflection points are respectively at pH 5.93 and 5.80. A high protein concentration slows the acidification as a result of the intrinsic buffering capacity of the protein, which increases with the concentration (Cheftel et al., 1985; Kinsella et al., 1985). Bazinet et al. (1997a,c) observed the same phenomenon during the electro-acidification process.

Interaction between pH and Acidification. A comparison of the soluble protein evolution (all SPE concentrations averaged) during the pH decrease, for the different levels of chemical acidification and electro-acidification, revealed that there is no difference between the chemical acidification level, but there is a difference between the chemical acidification and the electro-acidification (Figure 3 and Table 1). The protein precipitation curve obtained during electro-acidification shows a shift in

Table 1. Parameter Calculated Values of the Modelized Sigmoidal Curves for pH and SPE Concentration Interaction and pH and Acidification Interaction on Soluble Protein

interactions	levels	parameters	calculated values
pH/SPE	15 g·L^{-1} SPE	amplitude	103.78
		center	5.93
		width	0.36
		R^2	0.995
	60 g·L^{-1} SPE	amplitude	103.89
		center	5.80
		width	0.34
		R^2	0.997
pH/acidification	0.25 N HCl	amplitude	104.76
		center	5.92
		width	0.36
		R^2	0.997
	0.5 N HCl	amplitude	102.96
		center	5.91
		width	0.38
		R^2	0.998
	1.0 N HCl	amplitude	104.94
		center	5.88
		width	0.35
		R^2	0.995
	2.0 N HCl	amplitude	102.61
		center	5.87
		width	0.36
		R^2	0.996
	electro-acidification	amplitude	104.28
		center	5.75
		width	0.34
		R^2	0.993

comparison with those of chemical acidification levels; from the initial pH to pH 6.8, the soluble protein percentage was about 100% for chemical and electrochemical acidification. At pH 6.4, a difference appears between the two procedures of acidification, with 85.1 and 94.9% of soluble protein, respectively, for chemical acidification (all levels averaged) and electro-acidification. This difference increases between pH 6.0 and 5.6 with respectively 57.1 and 32.6% of soluble protein for chemical acidification (all levels averaged) compared to 69.4 and 43.9% for electro-acidification. Then, the difference greatly decreases until it disappears; at pH 5.2, 4.8, and 4.4, the soluble protein percentages were 14.5, 5.7, and 5.1 compared to 17.4, 6.4, and 5.5, respectively, for chemical and electrochemical acidification. The acidification procedures do not seem to influence the final precipitation extent of protein, but the precipitation is slower during electro-acidification. The model sigmoidal curves confirm this result: for chemical acidification, the inflection points for 0.25, 0.5, 1, and 2 N added HCl curves are respectively pH 5.92, 5.91, 5.88, and 5.87, while for electro-acidification the inflection point of the curve is at pH 5.75 (Table 1). There is a slight difference between the inflection points of two ways of acidification, but it is real. The difference in precipitation of protein between chemical and electrochemical acidification could be related to a lower local excess of acid in electro-acidification. In fact, the conventional chemical acidification process to produce protein isolates is known to denature protein by local excesses of acid (Kilara and Sharkasi, 1986; Fisher et al., 1986) while electro-acidification was demonstrated to precipitate protein with a low extent of protein denaturation (Bazinet et al., 1996, 1997b).

Molecular Profile Analysis. With our HPLC fractionation method, the average molecular mass of the 11S fraction was estimated between 359 000 and 506 300 Da and that of 7S between 32 600 and 63 200 Da. These results are in agreement with those of the literature (Kinsella et al., 1985; Cheftel et al., 1985) with an average molecular mass of 320 000−350 000 Da for the 11S fraction and an average molecular weight of 61 000−175 000 Da for the 7S fraction.

Table 2. Parameter Calculated Values of the Modelized Sigmoidal Curves for pH Effect and Interaction between pH and SPE Concentration on Molecular Profile Analysis

effect or interaction	levels	parameters	calculated values
pH	11S	amplitude	105.88
		center	6.09
		width	0.22
		R^2	0.996
	7S	amplitude	97.99
		center	5.67
		width	0.22
		R^2	0.995
11 S/SPE	15 g·L^{-1}	amplitude	105.37
		center	6.19
		width	0.24
		R^2	0.995
	60 g·L^{-1}	amplitude	107.16
		center	6.01
		width	0.19
		R^2	0.994

The MANOVA showed a significant effect of pH ($P < 0.0001$ for the 11S and 7S fractions), SPE concentration ($P < 0.0004$ for the 11S fraction), and pH/SPE concentration interaction ($P < 0.003$ for the 11S fraction) on the fraction molecular profiles. The acidification had no effect on the solubility profile of the 11S and 7S fractions during acidification ($P > 0.23$ for the 7S fraction and $P > 0.21$ for the 11S fraction). Nonlinear regression curves were calculated for the solubility profile of the 11S and 7S fractions, for all conditions averaged, as a function of pH (R^2 respectively 0.996 and 0.995) and for the solubility profile of the 11S fraction, for both SPE concentrations, as a function of pH ($R^2 = 0.995$ at 15 g·L^{-1} SPE and $R^2 = 0.994$ at 60 g·L^{-1}).

Single Effect of pH. Whatever the acidification conditions, both fractions have the same precipitation profile but with a shift for the 7S fraction (Table 2). From pH 7.6 to 6.8, both fractions remain in solution. At pH 6.4, the area of the 11S and 7S fractions represents respectively 86.5 and 89.3% of their initial fraction area: there is no difference between the two fractions. The difference is maximum at pH 6.0 and 5.6 with respectively 39.3 and 12.3% of the 11S initial area compared to 79.4 and 42.9% of the 7S initial area. This differentiation continues but diminishes after pH 5.6. At the end of the acidification, the percentage of the initial area for both fractions is the same with 1.5% for the 11S fraction and 5.7% for the 7S fraction. These results are confirmed by a sigmoidal model (Table 2). For both fractions, the precipitation curves take on a sigmoidal form, but with different inflection points: for the 11S and 7S fractions, the inflection points are respectively at pH 6.09 and 5.67. These results are in agreement with those of Thanh and Shibasaki (1976), who observed that the 7S and 11S fractions had slightly different precipitation curves, with the maximum for the 7S fraction at about pH 5 and that for 11 S at pH 5.8 in a low ionic strength solution (0.03 M). As the presence of salts slightly raises the isoelectric point of soy protein components, our values obtained at an ionic strength

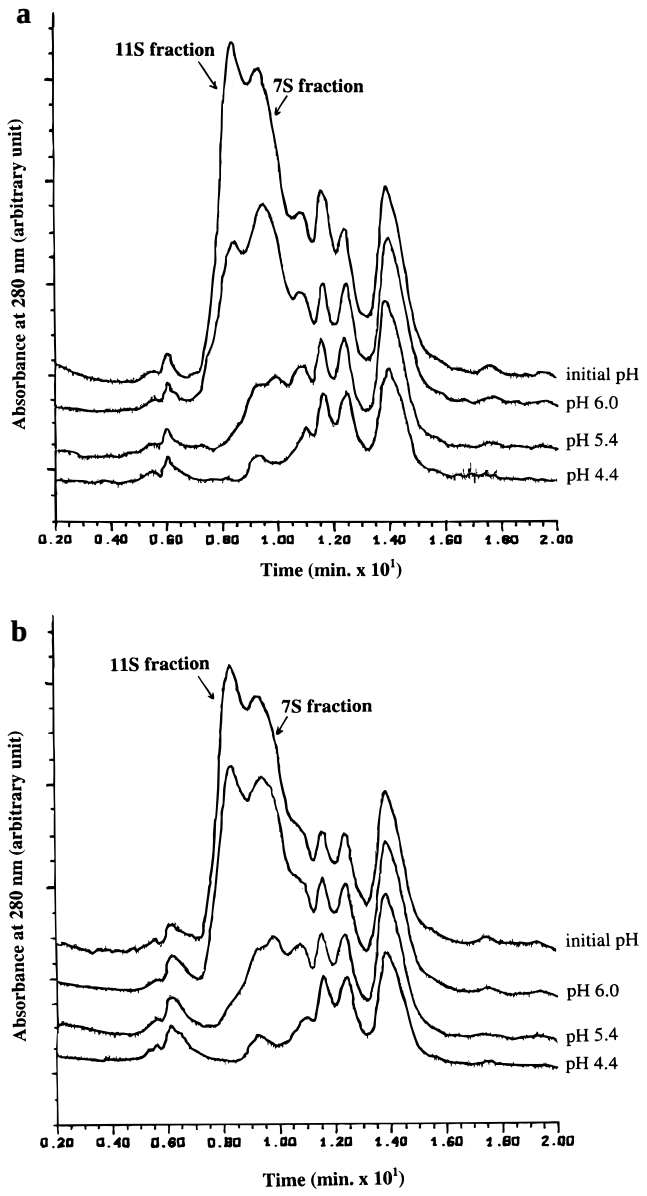

Figure 4. Size-exclusion HPLC chromatograms of (a) chemically and (b) electrochemically acidified soybean protein solutions, with 0.06 M KCl added and maintained at 25 °C, at different pH values.

higher than 0.03 M (addition of 0.06 M KCl) seem to be reasonable for both fractions.

Interaction between pH and SPE Concentration. For all acidification procedures averaged, it appears that the solubility profile of the 11S fraction during pH decrease is different in relation to the SPE concentration (Table 2). The 11S fraction curve presents a slight shift in precipitation as the pH is lowered when the SPE concentration is increased from 15 to 60 g·L^{-1}. In fact, from the initial pH to pH 6.8, the percentage of initial 11S fraction area was about 100% for the both SPE concentrations. At pH 6.4, a difference appears between the two concentrations, with 75.6 and 97.5% of the initial 11S fraction area. This difference is stable at pH 6.0 with 29.5 and 49.2% of the initial 11S fraction area respectively for 15 and 60 g·L^{-1} of SPE. Below this pH, the difference greatly decreases and disappears; at pH 5.6, 5.2, 4.8, and 4.4, the percentages of the initial area were 11, 4.8, 2.1, and 1.6% compared to 13.6, 5.0, 1.8, and 1.3%, respectively, for 15 and 60 g·L^{-1}. The sigmoidal model confirms this difference in precipitation with different inflection points; at pH 6.19 and pH 6.01, respectively, at 15 and 60 g·L^{-1} (Table 2).

Duncan's tests performed at pH 6.0 on the 11S fraction data show a significant difference between the acidification procedures (all SPE concentrations averaged) ($P < 0.001$). The four levels of normality were demonstrated to have a similar percentage of initial 11S fraction precipitated, while the electro-acidification was different from the others; at pH 6.0, the percent of initial 11S fraction area was 33.2, 34.4, 34.6, 40.3, and 52.8%, respectively, for 0.5 N, 0.25 N, 2 N, 1 N, and electro-acidification (Figures 3 and 4a,b).

The electro-acidification process has an effect on the precipitation curve of the 11S fraction; the electro-acidified 11S fraction precipitation curve presents a slight shift in comparison with the chemical acidification curves (Figure 4a,b). This would confirm the result obtained for the soluble protein demonstrating a shift of the precipitation curves between the electrochemical and the chemical acidification. The low precipitation for the electro-acidified proteins would be the result of the lower extent of precipitation obtained for the 11S fraction.

CONCLUSION

Results obtained in this study show that chemical acidification and electro-acidification procedures present some differences in their acidification profile of soya proteins. The difference in precipitation profile would be the consequence of a different solubilization profile of the 11S fraction during chemical and electrochemical acidification. At pH 6.0, less of the 11S fraction is precipitated by electro-acidification than by chemical acidification.

The conductivity, and consequently the ash content, of the electro-acidified protein solution is decreased while that of the chemical acidified protein solution is increased, depending on the normality of the added HCl. The electrochemical procedure for precipitation of protein is a good compromise between environmental and industrial considerations. Electro-acidification requires more time than chemical precipitation, which is instantaneaous, but allows for less denaturation of the proteins. The alkali and the acid needed in the conventional protein isolate process can be generated in situ by electrochemical acidification, and can be very precisely controlled, as electro-acidification and electro-alkalinization rates are regulated following the effective current density in the cell. Moreover, the water consumption is decreased by reusing the effluents generated, and the chemical composition of the electrodialyzed samples was demonstrated to have a lower salt content than proteins separated by the conventional isoelectric process (Bazinet et al., 1996, 1997b).

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