# Biochemical Characterization and Enzymatic Hydrolysis of Different Commercial Soybean Protein Isolates

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Thirteen commercial soybean protein isolates (SPIs) were characterized and submitted to the same conditions of hydrolysis with pancreatin to the same degree of hydrolysis (DH). The 13 SPIs differed with respect to their phytate contents (7.41−15.62 mg/g of protein), presence of trypsin inhibitor (5.17−94.72 UTI/mg of protein), protein dispersibility index (PDI) (11.7−88.7%), and relative compositions of the 7S subunits and 11S polypeptides present in the soluble fraction: $\alpha'$ (0−100%); $\alpha$ (0−26%); $\beta$ (0−44%); acid polypeptide (50−100%); basic polypeptide (0−50%). The reaction time necessary for the hydrolysis to attain a DH of 21.5% varied from 48 to 252 min and was longer for isolates with complete 7S and 11S globulin fractions and higher PDI values. The 10% TCA soluble nitrogen index of the hydrolysates varied from 61.5 to 100%. The total free amino acids varied between 7.5 and 31.0%, with basic and hydrophobic amino acids being present in greater amounts. Electrophoresis indicated differences in the molecular weight profiles of the hydrolysates. The 13 SPIs analyzed were shown to be different, resulting in different products when submitted to the same conditions of hydrolysis.

**Keywords:** *Protein hydrolysis; soy protein hydrolysates; pancreatin; peptides*

## INTRODUCTION

The production of enzymatic hydrolysates for clinical nutrition formulas gained emphasis in the 1970s as a result of research demonstrating the kinetic advantages of the absorption of di- and tripeptides (Silk et al., 1979). Currently, enzymatically hydrolyzed protein represents a part of innumerable enteral (Mobarhan and Trumbore, 1991) and infant (Milla, 1991) formulas, with various clinical indications. New perspectives contemplate the use of hydrolysates in formulas for individuals requiring protein supplements, such as the aged, athletes, and the obese (Frøkjaer, 1994). In addition to their use as a source of nitrogen, protein hydrolysates can be composed of peptides having amino acid sequences that possess physiological properties (Ariyoshi, 1993; Chen et al., 1995).

In addition to the kinetic advantages of absorbing small peptides, various papers have shown a superior nutritional performance of the enzymatic hydrolysate in both animal and human models, when compared with the intact protein or an equivalent mixture of amino acids.

From the nutritional point of view, soybean protein can be considered a good substrate for the production of hydrolysates (Young, 1991). Clinical nutrition formulas, containing enzymatically hydrolyzed soybean protein as protein sources, are already produced and used, giving excellent clinical results (Ovesen and Allingstrup, 1992).

One of the most important characteristics of the hydrolysates used in nutritional formulas is that of being composed of low molecular weight peptides. However, as shown by Adler-Nissen (1986), an equal degree of hydrolysis of different substrates does not guarantee that the products present the same characteristics with respect to molecular weight. This behavior can be explained on the basis of their primary, secondary, and/or tertiary structures and their protein solubility index (Desphande and Damodaran, 1989; Kim Lee et al., 1990). During the commercial production, the isolates undergo treatments that cause physicochemical alterations in the proteins (Kinsella, 1976; González et al., 1995), denaturation being the most significant effect (Arrese et al., 1991; Petrucellei and Añon, 1994, 1995a,b). Such changes, associated with the presence of other factors such as phytate and trypsin inhibitor, turn the isolates into distinctive substrates and could result in different hydrolysis products.

This work aimed to characterize different commercial soybean protein isolates (SPIs), analyze their behavior when submitted to enzymatic hydrolysis with pancreatin to obtain low molecular weight hydrolysates, and compare the hydrolysis products.

## MATERIALS AND METHODS

**Materials.** Thirteen commercial SPIs were used. The isolates SUPRO 500E, SUPRO 515, SUPRO 590, SUPRO 595, and SUPRO 1751 were supplied by Protein Technologies International (St. Louis, MO). Two batches of the isolates Samprosoy 90 NB, Proteimax 90 HG, Samprosoy 90 SD, and Samprosoy 90 MP were supplied by SAMBRA SA (São Paulo, SP, Brazil). The enzyme used was swine pancreatic pancreatin from Sigma (St. Louis, MO). The remaining reagents were all of analytical grade from various reputable suppliers.

**Proximate composition of the SPIs.** Moisture, ash, and protein (N × 6.25%) were determined using standard methods (AOAC, 1975). Lipids were determined according to the method described by Bligh and Dyer (1959).

**Characterization of the SPIs.** *Phytate* was determined according to the method of Latta and Eskin (1980). A standard

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curve was prepared using sodium phytate (Sigma, catalog no. P3168) at concentrations from 5 to 40 $\mu$g/mL, using the same analytical conditions. Two extractions of phytate were carried out, and the colorimetric analysis was repeated three times for each extract.

**Trypsin Inhibitor Activity (TI).** Activity was determined according to the method of Kakade et al. (1974), using benzoyl-DL-arginine *p*-nitroanilide (BAPA) as substrate. The enzyme was bovine pancreatic trypsin (Sigma, catalog no. T8128). The unit of trypsin was defined arbitrarily as an increase of 0.01 unit of absorbance at 410 nm by 10 mL of reaction mixture. The TI was expressed in terms of the units of trypsin inhibited (TIU). The TI (TIU per milliliter of extract) was considered a function of the volume of extract, and a negative linear correlation was obtained. The curve was extrapolated to a zero degree of inhibitor in the extract, thus providing a value that approached the true value of the inhibitory activity of the SPI (Kakade et al., 1969). The analysis was carried out in duplicate.

**Protein Dispersibility Index (PDI).** Values were determined according to AACC (1983) Method 46-24 with some modifications. A high speed blender was used with a four-blade helix, mixing at 6500 rpm for 10 min. The homogenized sample was transferred to a covered container and left in the refrigerator to allow the suspended material to settle. Aliquots of the decanted liquid were centrifuged at 1800*g* for 10 min. Protein in the supernatant was determined according to the semimicro-Kjeldahl method (AOAC, 1975). PDI is the ratio between the water dispersible protein and the total amount of protein. Two extractions were carried out for each SPI, and the water dispersible protein was determined in triplicate.

*Total amino acids* were determined in duplicate with an automatic analyzer (Alonzo and Hirs, 1968), using the ion exchange resin method of Spackmann et al. (1958) after hydrolysis with 6 N HCl in vacuum-sealed glass ampules incubated at 110 °C for 22 h. Methionine and cysteine were determined as methionine sulfone and cysteic acid, respectively, after oxidation with performic acid (Moore, 1963). Tryptophan was determined after hydrolysis with 4 N LiOH (Lucas and Sotelo, 1980).

*Electrophoresis* was carried out using a gradient in polyacrylamide gel with a concentration from 8 to 25% in the presence of sodium dodecyl sulfate (SDS−PAGE) using method 110 no. 200 of PhastSystem (Pharmacia-LKB Biotechnology, Bromma, Sweden). The samples were prepared according to the method of Arrese et al. (1991) with some modifications: 5 mg of each SPI was dispersed in 5 mL of distilled water and stirred for 1 h at 21 °C; 1.5 mL aliquots of each sample solution were centrifuged at 12000*g* for 20 min at 21 °C. The supernatant was diluted in the following sample buffer: 10 mM Tris-HCl, pH 8.0; 1 mM EDTA; 2.5% SDS; 1% dithiothreitol; and 0.01% bromophenol blue. The mixture was heated in a water bath at 60 °C for 20 min. Pharmacia molecular mass standards varying from 14.4 to 93.0 kDa were used. The densitometric gel analysis was carried out using the densitometric video of Bio-Rad model 620. 1-D Analyst Data Analysis software was used to determine the molecular mass and relative area of each band. The area of the 7S globulin was considered to be the sum of the areas of its component subunits, whereas the sum of the acid and basic polypeptides corresponded to the area of the 11S globulin. The relative composition of the subunits/polypeptides was calculated as the percentage of the area of the subunits with respect to the total area of the 7S fraction and the percentage of the area of the polypeptides with respect to the total area of the 11S fraction.

**Enzymatic Hydrolysis.** The SPIs were hydrolyzed with pancreatin in a batch system using a pH-stat to the preestablished degree of hydrolysis (DH). The reaction was then interrupted by heating at 72 °C for 5 min. The apparatus used consisted of a 500 mL enzyme reactor with a temperature-controlled bath and external circulation. The following conditions were used for the hydrolysis reaction: 5.5% substrate (w/w); ratio enzyme/substrate = 1:15 (w/w); temperature = 40 °C; and pH 8.0, which was maintained by the addition of 4.93 N NaOH. The experiments were carried out in duplicate. The hydrolysates obtained were sampled for the determination of solubility in TCA and the rest frozen and lyophilized for the remaining determinations.

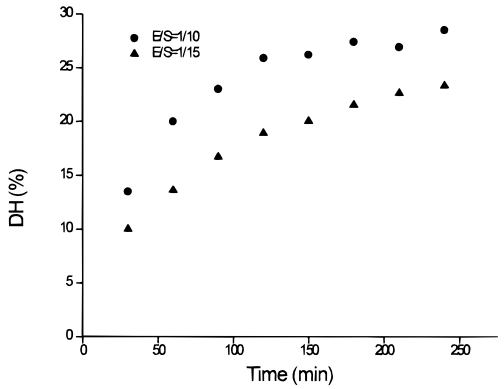


**Figure 1.** Hydrolysis curve of SPI with pancreatin for different enzyme substrate ratios (E/S).

The DH is a measure of the extent of cleavage of the peptide bonds. The number of bonds hydrolyzed can be determined with the trinitrobenzenesulfonic acid (TNBS) method, the reaction being detected spectrophotometrically (Adler-Nissen, 1979). The pH-stat can also be used to determine DH. In this technique the DH is determined on the basis of the number of titratable free amino groups produced by the hydrolysis of the peptide bonds (Adler-Nissen, 1986). To determine the desired DH, a hydrolysis curve was established using a protein isolate and pancreatin, the TNBS method being used to follow the reaction. Figure 1 shows the results obtained for two different enzyme/substrate ratios (1:10 and 1:15). Because the hydrolysis of the 13 isolates should be interrupted when the reaction reaches the desired DH, it is necessary to establish a marker of this moment. The TNBS method is inadequate for monitoring this reaction, due to the long time required to obtain the results. Thus, the pH-stat technique was used, because it is easily adapted for use on-line. Although the different methods for determining DH are not directly comparable, Adler-Nissen (1979) showed good correlation between the pH-stat technique and the increase in concentration of free α-amino groups as determined by the TNBS method. To establish the relationship between the results obtained with the TNBS reaction and the pH-stat technique used in this research, two series of reactions were carried out for the hydrolysis reaction under the same conditions cited above. In the first series, the volume (milliliters) of NaOH used to titrate the free amino groups formed was registered at intervals of 30 min (pH-stat method). In the second series, also at intervals of 30 min, aliquots of hydrolysate were taken for the determination of the free α-amino groups using TNBS and the DH calculated. Correlations between the two methods ($r^2$) were 0.9982 and 0.9945 for enzyme/substrate ratios of 1:15 and 1:10, respectively. Using the hydrolysis curves as a base, and knowing that it was necessary to reach a high DH to obtain low molecular weight hydrolysates, it was decided to interrupt the reaction when it reached 21.5% DH, using an enzyme/substrate of 1:15. This DH corresponded to a consumption of 4 mL of 4.93 N NaOH according to the results of the correlation established between the methodologies.

**Characterization of the Hydrolysates.** *Nitrogen Soluble in 10% Trichloroacetic Acid (TCA Index).* After heating to interrupt the hydrolysis reaction, a solution of TCA was added to aliquots of the hydrolysate such that a final concentration of 10% was obtained, to precipitate proteins and high molecular weight peptides. This solution was kept at room temperature for 1 h and the precipitate removed by filtration through Whatman No. 42 filter paper. Soluble nitrogen was determined in the supernatant according to the semimicro-Kjeldahl method (AOAC, 1975). The solubility index in TCA is given by the following formula:

$$\text{TCA index} = \frac{\text{10\% TCA soluble N}}{\text{total N}} \times 100$$

For each hydrolysis reaction, the TCA index was determined in triplicate.

*Free Amino Acid (FAA) Composition of the Hydrolysates.* As described in the methodology for the determination of total amino acids, one aliquot of the material not hydrolyzed by 6 N HCl was used to quantify the FAAs. The determination was carried out in duplicate.

*Electrophoresis of the Hydrolysates.* The hydrolysates obtained enzymatically from the 13 SPIs were examined by high-density SDS-PAGE, according to Method 112 of the Phast-System of Pharmacia. The gels were stained and unstained according to Method 200 of the PhastSystem. The samples were prepared as follows: The hydrolysates were dissolved in the sample buffer, which consisted of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2.5% SDS, 5% 2-mercaptoethanol, and 0.01% bromophenol blue. The mixture was heated at 100 °C for 5 min and applied to the gels. The molecular mass standards used were from Bio-Rad, with molecular masses from 6.5 to 200.0 kDa.

*Statistical Analysis.* The means of the results obtained were submitted to analysis of variance, and when differences were shown among the results, the study was completed using the Tukey test for contrasts between the means (Cockran and Cox, 1957).

## RESULTS AND DISCUSSION

The factors that affect the enzymatic hydrolysis of proteins include enzyme specificity, extent of protein denaturation, enzyme/substrate concentration, pH, ionic strength, temperature, and presence of inhibitory substances (Kilara, 1985; Panyam and Kilara, 1996). Thus, if the operational process parameters are controlled, the substrate characteristics will define not only the characteristics of the product but also the development of the hydrolysis reaction.

**Characterization of the SPIs.** The protein content of the SPIs studied varied from 87.9% (NB′) to 94.1% (590) (db), the lipid content from 3.0% (HG′) to 4.7% (515) (db), and the ash content from 3.5% (590) to 5.0% (1751) (db).

Table 1 shows the characterization of the 13 SPIs with respect to phytate content, trypsin inhibitor, PDI, and relative composition of the water soluble proteins. The phytate content varied from 7.41 to 15.62 mg/g. According to Brooks and Morr (1984), under alkaline conditions and in the presence of divalent cations such as calcium, magnesium, and zinc, the phytate binds strongly to the protein. If the protein is precipitated under acid conditions, as used during the production of protein isolates, the phytate coprecipitates, resulting in final concentrations of 2−3% (Brooks and Morr, 1982).

The different isolates presented antitryptic activity varying from 5.17 to 94.72 UTI/mg of protein, isolates HG and HG′ showing the highest values. This variation in the values is possibly due to differences in the thermal treatment received by each product. The thermal inactivation is never complete, since this would require drastic treatments that would affect the nutritional and/or functional qualities of the protein (Savage et al., 1995). Thus, the commerically available soybean products present residual activities on the order of 5−20% of the antitryptic activity of the raw soybean (Liener, 1986). The trypsin inhibitor content can also be affected by the presence of salt in the extracting solution and also by the pH used to precipitate the protein (Honig et al., 1977).

**Table 1. Phytate Content, TI, PDI, and Relative Composition of the Water Soluble Proteins of 13 Commercial SPIs**

| | | | | 7S[e] | | | 11S[e] | |
|---|---|---|---|---|---|---|---|---|
| SPI | phytate[a,b] | TI[a,c] | PDI[a,d] | α′ | α | β | acidic | basic |
| NB | 15.16[de] (±0.33)[f] | 17.86[d] (±0.19) | 88.2[f] (±0.2) | 70.0 | 20.0 | 10.0 | 71.4 | 28.6 |
| HG | 14.90[de] (±0.14) | 94.72[h] (±2.30) | 84.4[ef] (±1.5) | 66.7 | 22.2 | 11.1 | 50.0 | 50.0 |
| SD | 14.61[de] (±0.21) | 13.93[bc] (±0.32) | 14.4[a] (±0.4) | 100.0 | | | 100.0 | |
| MP | 15.62[e] (±0.44) | 21.50[ef] (±0.56) | 69.0[d] (±1.6) | 66.7 | 19.0 | 14.3 | 75.0 | 25.0 |
| NB′ | 12.52[c] (±0.29) | 17.86[d] (±0.33) | 85.4[ef] (±0.2) | 62.2 | 21.6 | 16.2 | 60.0 | 40.0 |
| HG′ | 15.36[e] (±0.26) | 86.40[g] (±0.85) | 52.7[c] (±1.0) | 66.7 | 20.0 | 13.3 | 66.7 | 33.3 |
| SD′ | 12.28[c] (±0.32) | 11.62[b] (±0.32) | 11.7[a] (±0.1) | | | | 100.0 | |
| MP′ | 11.94[c] (±0.25) | 23.78[f] (±0.17) | 81.3[e] (±2.2) | 56.4 | 25.6 | 18.0 | 61.3 | 38.7 |
| 500E | 13.98[d] (±0.34) | 14.58[c] (±0.40) | 23.3[b] (±0.4) | 56.4 | | 43.6 | 90.5 | 9.5 |
| 515 | 10.61[b] (±0.30) | 17.92[d] (±0.13) | 67.1[d] (±3.5) | 70.0 | | 30.0 | 76.9 | 23.1 |
| 590 | 7.41[a] (±0.28) | 19.37[de] (±0.44) | 22.9[b] (±0.2) | 85.7 | | 14.3 | 100.0 | |
| 595 | 15.36[e] (±0.35) | 11.86[bc] (0.16) | 22.5[b] (±0.3) | 60.0 | | 40.0 | 100.0 | |
| 17.51 | 14.56[de] (±0.20) | 5.17[a] (±0.20) | 88.7[f] (±0.6) | | | | | |

[a] Duplicate analysis. Dry weight basis. Values with the same letter in the same column show no difference in Tukey's test ($p \leq 0.05$). [b] Milligrams per gram of protein. [c] Inhibitor per milligram of protein. [d] Water soluble protein as percentage of total protein. [e] The values represent a percentage of the area of the subunits as related to the total area of the 7S globulin and the percentage of the polypeptides as related to the total area of the 11S globulin, calculated by densitometry of the electrophoresis gels. [f] Standard deviation.

The PDI values varied from 11.7 to 88.7%. Solubility is one criterion most frequently used by the industry to evaluate the effects of the process heat treatment on the soybean proteins, since the thermal treatment causes a variable degree of denaturation and may result in a decrease in protein solubility in water and salt solutions (Wolf, 1970; Snyder and Kwon, 1987; Kilara and Sharkasi, 1990). However, solubility is not considered to be a sensitive method for evaluating protein denaturation. Isolates with a high degree of denaturation, as shown by differential scanning calorimetry (DSC), showed the same solubility as native or only slightly denatured proteins (Wagner and Añon, 1990; Arrese et al., 1991).

The main reserve proteins present in the SPIs are the globulins: β-conglycinin (7S), composed of the subunits α′, α, and β; and glycinin (11S), consisting of acid and basic polypeptides. Arrese et al. (1991) studied 19 different SPIs and observed that the percentages of each protein were similar in all samples, 36.2 ± 2.3% for 7S and 47 ± 2.3% for 11S. However, the authors determined a decrease in the proportion of the β subunit of 7S and the basic peptide of 11S in the soluble fraction of isolates that also exhibited a greater degree of denaturation. The relative composition of the subunits/polypeptides in the soluble fractions of the isolates studied varied from 0 to 100% for the α′ subunit, from 0 to 25% for the α subunit, and from 0 to 43.6% for the β subunit, whereas the acid and basic polypeptides varied from 50 to 100% and from 0 to 50%, respectively (Table 1). Considering that only the water soluble

**Table 2. Total Amino Acid Content of 13 Commercial SPIs[a]**

| | NB | HG | SD | MP | NB′ | HG′ | SD′ | MP′ | 500E | 515 | 590 | 595 | 1751 | av | SD[b] | CV[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trp | 0.78 | 0.74 | 0.85 | 0.99 | 0.81 | 0.62 | 0.88 | 0.77 | 0.83 | 0.81 | 0.85 | 0.89 | 0.90 | 0.83 | 0.09 | 11.09 |
| Lys | 4.94 | 5.02 | 5.21 | 5.76 | 5.48 | 4.05 | 5.46 | 5.15 | 5.51 | 5.54 | 4.95 | 5.28 | 8.40 | 5.44 | 0.44 | 8.14 |
| His | 2.25 | 2.07 | 2.15 | 2.63 | 2.37 | 1.61 | 2.26 | 2.12 | 2.30 | 2.50 | 1.97 | 2.20 | 2.28 | 2.21 | 0.26 | 11.77 |
| Arg | 5.97 | 5.76 | 5.89 | 6.39 | 5.94 | 4.73 | 6.19 | 5.66 | 5.61 | 5.79 | 5.38 | 5.41 | 5.71 | 5.73 | 0.43 | 7.51 |
| Asx | 10.75 | 10.86 | 10.55 | 10.74 | 11.02 | 11.47 | 10.41 | 11.43 | 11.48 | 11.70 | 11.08 | 10.51 | 10.49 | 10.96 | 0.44 | 3.97 |
| Thr | 4.08 | 4.01 | 3.97 | 4.07 | 4.20 | 4.09 | 4.14 | 4.11 | 4.54 | 4.30 | 4.34 | 4.33 | 3.99 | 4.17 | 0.17 | 4.01 |
| Ser | 6.69 | 6.39 | 6.03 | 6.50 | 6.75 | 6.17 | 6.71 | 6.60 | 6.85 | 6.62 | 6.23 | 6.41 | 6.16 | 6.47 | 0.26 | 3.94 |
| Glx | 17.91 | 17.34 | 16.83 | 15.75 | 17.21 | 17.55 | 17.34 | 18.02 | 15.19 | 17.25 | 17.13 | 16.63 | 15.25 | 16.88 | 0.83 | 4.91 |
| Pro | 6.49 | 6.98 | 6.60 | 6.63 | 5.58 | 5.65 | 7.01 | 6.21 | 6.82 | 5.81 | 6.00 | 6.66 | 7.03 | 6.42 | 0.51 | 7.89 |
| Gly | 7.27 | 6.76 | 6.82 | 6.75 | 7.09 | 7.08 | 6.86 | 7.08 | 6.90 | 7.08 | 7.41 | 7.13 | 6.79 | 7.00 | 0.21 | 2.94 |
| Ala | 6.02 | 6.00 | 5.92 | 5.47 | 5.72 | 6.32 | 5.80 | 6.22 | 6.03 | 6.07 | 5.83 | 6.44 | 5.67 | 5.96 | 0.27 | 4.47 |
| half-Cys | 0.80 | 1.19 | 1.05 | 0.78 | 1.03 | 1.10 | 1.14 | 1.07 | 0.89 | 1.15 | 1.10 | 0.85 | 0.82 | 1.00 | 0.14 | 14.34 |
| Val | 5.21 | 5.46 | 5.55 | 4.81 | 5.21 | 6.28 | 5.04 | 5.39 | 5.19 | 5.15 | 5.92 | 5.36 | 5.23 | 5.37 | 0.40 | 7.38 |
| Met | 1.65 | 1.43 | 1.34 | 1.69 | 1.44 | 1.09 | 1.42 | 1.17 | 0.91 | 1.37 | 1.52 | 1.39 | 1.48 | 1.38 | 0.22 | 16.17 |
| Ile | 4.53 | 4.91 | 4.89 | 4.42 | 4.65 | 5.57 | 4.55 | 4.39 | 4.90 | 4.43 | 5.00 | 4.77 | 4.57 | 4.74 | 0.34 | 7.11 |
| Leu | 7.92 | 8.08 | 8.22 | 8.64 | 8.20 | 8.76 | 7.89 | 7.65 | 8.17 | 7.71 | 8.01 | 8.36 | 7.89 | 8.12 | 0.34 | 4.14 |
| Tyr | 2.57 | 2.79 | 3.49 | 3.27 | 2.83 | 2.93 | 2.69 | 2.79 | 3.14 | 2.52 | 3.03 | 2.98 | 3.36 | 2.95 | 0.29 | 9.66 |
| Phe | 4.17 | 4.22 | 4.65 | 4.70 | 4.48 | 4.92 | 4.24 | 4.19 | 4.74 | 4.21 | 4.23 | 4.42 | 3.99 | 4.40 | 0.26 | 5.95 |
| %AAE[d] | 35.53 | 35.94 | 36.83 | 37.71 | 36.84 | 36.99 | 35.88 | 34.94 | 37.09 | 36.02 | 36.79 | 37.00 | 38.73 | 36.64 | 0.98 | 2.67 |

[a] Average of duplicate analysis. Expressed in $\mu$mol %. [b] Standard deviation. [c] Coefficient of variation. [d] Percent of essential amino acids (Trp, Lys, His, Thr, Val, Met, Ile, Leu, Phe).

proteins were submitted to electrophoresis, this variability suggests the formation of insoluble aggregates between the different components of the globulins. The following factors can affect the degree of aggregation of the subunits and polypeptides of the 7S and 11S globulins: acid precipitation of the isolates, heat treatment, presence of calcium ions, addition of salts and reducing agents, and pH used in the neutralization of the isolates (Wolf, 1970; Petrucelli and Añon, 1994, 1995a−c). Although it has been shown that water solubility is not a good parameter to evaluate protein denaturation, it was observed in this work that the isolates with lower PDI values (SD, 14.30%; SD′, 11.47%; 500E, 23.20%; 590, 22.94% and 595, 22.5%) were those in which one or more components of the 7S and/or 11S fractions were absent. These results indicate that low PDI values could correspond to the degree of denaturation of these isolates, because the greater the level of denaturation of a protein, the greater its tendency to form insoluble aggregates.

Table 2 shows the total amino acid composition of the 13 isolates. For 13 of the 18 amino acids analyzed, the variability between the SPIs was within the limits of experimental error suggested for this methodology (±8%). However, the amino acids Trp, His, Cys, Met, and Tyr presented coefficients of variation between 10 and 16%. With respect to Trp, the losses of this amino acid during analysis would be due to the presence of oxygen, being of the order of 10−20% even under controlled conditions (Landry et al., 1988). The variability between the values cited for Met and Cys could be due to incomplete oxidation, because it is necessary to convert them to sulfonic methionine and cysteic acid, respectively, by oxidation with performic acid, before the acid hydrolysis (Moore, 1963).

**Enzymatic Hydrolysis and Characterization of the Hydrolysates.** Table 3 shows the reaction time necessary for the 13 SPIs to reach 21.5% DH and the TCA index of the hydrolysates. The average reaction time varied from 48 min for the isolate SD to 252 min for the isolate MP′. From an analysis of the hydrolysis time with respect to some characteristics presented by the isolates such as the relative composition of the water soluble proteins and the PDI (Tables 1 and 3), it can be seen that the SPIs having hydrolysis times >150 min are those with PDI >50% and whose water soluble fraction contains all the components of the $\beta$-conglycinin and glycinin. Isolates 515 and 590 were the exceptions to this rule. The isolates with hydrolysis times <150 min had PDIs <50%, with the exception of SPI 1751, and lacked one or more elements of the globulins 7S and 11S. With respect to SPI 1751, the electrophoretic behavior (results not shown) suggests that it was partially hydrolyzed. The results indicate that the SPIs with higher PDI values and containing all of the subunits of the polypeptides of the water soluble fraction contain native protein in their composition, which would require longer hydrolysis time (Alder-Nissen, 1986). Thus, this variation in reaction time is probably due mainly to conformational differences between the component proteins of the SPIs. Proteins submitted to treatments that result in rupture of the tertiary structure, especially the more compact ones like in soybean protein, with a consequent unraveling of the molecules, present a more rapid and effective enzymatic hydrolysis (Kella et al., 1986; Deshpande and Damodaran, 1989).

**Table 3. Hydrolysis Reaction Times of 13 Commercial SPIs and TCA Index of the Hydrolysates Obtained[a]**

| SPI | time,[b] min | TCA index[c,d] | SPI | time,[b] min | TCA index[c,d] |
|---|---|---|---|---|---|
| NB | 212.0$^{ef}$ (±4.24)[e] | 89.6$^{c}$ (±0.6) | 500E | 123.0$^{b}$ (±7.07) | 61.5$^{a}$ (±0.7) |
| HG | 240.0$^{gh}$ (±7.07) | 76.3$^{b}$ (±1.3) | 515 | 188.0$^{de}$ (±5.66) | 93.2$^{cde}$ (±1.1) |
| SD | 48.0$^{a}$ (±0.00) | 90.8$^{cd}$ (±1.8) | 590 | 220.0$^{fg}$ (±9.90) | 95.0$^{def}$ (±1.4) |
| MP | 176.5$^{cd}$ (±4.95) | 98.5$^{ef}$ (±3.0) | 595 | 98.0$^{b}$ (±5.66) | 90.1$^{cd}$ (±1.6) |
| NB′ | 184.0$^{d}$ (±8.48) | 101.1$^{f}$ (±2.3) | 1751 | 50.0$^{a}$ (±0.00) | 88.1$^{c}$ (±1.3) |
| HG′ | 153.0$^{c}$ (±7.07) | 80.7$^{b}$ (±1.8) | | | |
| SD′ | 53.0$^{a}$ (±4.24) | 92.2$^{cde}$ (±1.9) | | | |
| MP′ | 252.0$^{h}$ (±8.48) | 98.4$^{ef}$ (±2.0) | | | |

[a] Hydrolysis conditions were [S] = 5.5% (w/w), E/S = 1/15 (w/w), pH 8.0, and temperature = 40 °C; final degree of hydrolysis (DH) = 21.5%. [b] Average of two hydrolysis reactions. Values with different letters in the same column differ significantly by Tukey's test ($p \leq 0.05$). [c] Calculated using the following equation: (N sol TCA 10%/N total) × 100. N was determined by semimicro-Kjeldahl method (AOAC, 1975). [d] Average of two hydrolysis reactions. Three repetitions of 10% NTCA were made for each hydrolysis. [e] Standard deviation.

**Table 4. FAA Composition of 13 Hydrolysates Obtained from the Enzymatic Hydrolysis of 13 Commercial SPIs[a]**

| amino acid | NB | HG | SD | MP | NB′ | HG′ | SD′ | MP′ | 500E | 515 | 590 | 595 | 1751 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trp | 26 | 31 | 24 | 38 | 35 | 30 | 31 | 41 | 32 | 40 | 28 | 33 | 57 |
| Lys | 10 | 9 | 26 | 29 | 12 | 11 | 26 | 12 | 16 | 9 | 16 | 22 | 45 |
| His | 4 | 5 | 5 | 16 | 4 | 11 | 6 | 6 | 6 | 5 | 8 | 8 | 25 |
| Arg | 17 | 14 | 46 | 34 | 20 | 15 | 37 | 20 | 26 | 14 | 17 | 49 | 48 |
| Asx | 1 | 1 | 1 | 1 | 1 | ND | ND | 1 | ND | ND | 8 | ND | 7 |
| Thr | 5 | 4 | 2 | 11 | 3 | 4 | 2 | 5 | 4 | 4 | 31 | 5 | 38 |
| Ser | 4 | 3 | 4 | 15 | 3 | 5 | 5 | 5 | 6 | 3 | 13 | 9 | 50 |
| Glx | ND[b] | ND | ND | 1 | ND | ND | ND | ND | 1 | ND | 8 | 1 | 9 |
| Pro | ND | ND | ND | ND | ND | 2 | ND | ND | ND | ND | 6 | ND | 20 |
| Gly | ND | ND | 1 | 7 | ND | 1 | 2 | 1 | 2 | 1 | 13 | 2 | 21 |
| Ala | 3 | 2 | 3 | 9 | 1 | 6 | 4 | 4 | 7 | 2 | 22 | 5 | 33 |
| half-Cys | 11 | 6 | 11 | ND | ND | 6 | 7 | 5 | 9 | 5 | 10 | 10 | 19 |
| Val | 18 | 14 | 6 | 24 | 14 | 11 | 9 | 15 | 13 | 11 | 19 | 7 | 48 |
| Met | 17 | 11 | 17 | 31 | 20 | 6 | 23 | 25 | 31 | 16 | 48 | 20 | 40 |
| Ile | 19 | 17 | 7 | 26 | 16 | 11 | 9 | 18 | 14 | 12 | 28 | 8 | 50 |
| Leu | 19 | 25 | 14 | 26 | 22 | 16 | 18 | 23 | 19 | 15 | 14 | 21 | 47 |
| Tyr | 53 | 56 | 31 | 57 | 52 | 39 | 48 | 49 | 46 | 51 | 28 | 38 | 46 |
| Phe | 37 | 39 | 26 | 47 | 40 | 30 | 36 | 37 | 34 | 32 | 12 | 34 | 40 |
| total FAA, % | 9.2 | 9.2 | 9.6 | 16.3 | 9.5 | 8.2 | 10.4 | 9.8 | 10.4 | 7.5 | 15.0 | 12.0 | 30.8 |

[a] Expressed as percent free amino acid as related to the total amount of that amino acid present in the SPI, calculated from Table 2. Hydrolysis conditions: [S], 5.5%; E/S, 1/15; pH 8.0; temp, 40 °C; DH, 21.5%. [b] ND, not detected.

The nature and extent of denaturation can also cause different types of aggregation, resulting in more or less sites for enzymatic attack being blocked by inter- and/or intramolecular interactions (Fukushima, 1969; Adler-Nissen, 1986; Savoie et al., 1991).

The TCA index of the hydrolysates obtained was between 61.5 and 100%, 10 showing values >88%. According to Adler-Nissen (1986), protein hydrolysis can proceed either sequentially, releasing one peptide at a time, or through the formation of intermediates, which are further hydrolyzed to small peptides as proteolysis progresses. The variability between the values suggests that differences between the SPIs determined a distinct pattern of enzymatic cleavage, with a consequent modification of the molecular distribution profile. Adler-Nissen (1986) also observed different TCA solubility values in casein and SPI hydrolysates with the same DH. The TCA solubility of the hydrolysates does not appear to correlate with any of the characteristics of the SPI studied. For example, the hydrolysates obtained from the isolates HG and MP′, the PDIs of which did not vary statistically, possessed TCA indices as different as 76.3 and 98.4% (Tables 1 and 3). SPIs like NB, SD, SD′, 515, 595, and 1751, for example, had different protein solubility patterns (relative compositions of the subunits/polypeptides of the globulins 7S and 11S) but showed the same solubility in 10% TCA. These results possibly indicate that other aspects of the substrates determine the differences in TCA index.

No correlation was observed between the phytate or TI contents (Table 1) and the hydrolysis time or the TCA index (Table 3). For instance, the isolates MP, and MP′, and 590, showing practically the same TI contents, required different times to be hydrolyzed, from 177 to 252 min. Isolates HG and HG′, having the same activity, exhibited hydrolysis times as different as 240 and 153 min. The same was observed for the presence of phytate, where, for example, the isolates HG and SD, with the same contents of this component, presented significantly different hydrolysis times (240 and 48 min, respectively). Boonvisut and Whitaker (1976) have shown that the presence of TI correlated negatively with soy protein digestibility. On the other hand, whereas others suggest interference by phytate in the hydrolysis of the protein, probably due to the formation of a phytate−protein complex less susceptible to enzymatic attack (Singh and Krikorian, 1982; Knuckles et al., 1985; Ritter et al., 1987), the results obtained by Reddy et al. (1988) showed no correlation between phytate and enzymatic hydrolysis.

Table 4 shows the FAA composition of the 13 hydrolysates, expressed as a percentage of each FAA with respect to the total content of that amino acid (TAA) in that isolate. The total FAA content ($\mu$mol %) present in the hydrolysates varied from 7.5% (515) to 31% (1751), the majority being between 8.0 and 12.0%. Only the hydrolysates MP and 590 showed higher values, 16.0 and 15.0%, respectively. The elevated amount of FAAs in hydrolysate 1751 could be due to the fact that the original isolate was already partially hydrolyzed. With the exception of hydrolysates 590 and 1751, the remaining hydrolysates liberated the amino acids Trp, Lys, Arg, Val, Met, Ile, Leu, Tyr, and Phe in greater amounts than the remaining amino acids. This profile is in agreement with the enzymatic activity of the endo- and exopeptidase pairs of pancreatin:chymotrypsin/carboxypeptidase A that liberate aromatic and hydrophobic amino acids and trypsin/carboxypeptidase B which liberate basic amino acids (Palmer, 1985). However, variations were observed between the hydrolysates in the percentages of these amino acids liberated: Lys, 9−29%; Arg, 14−49%; Trp, 26−41%; Tyr, 28−57%; Phe, 12−47%; Val, 6−24%; Met, 6−48%; Ile, 7−28%; Leu, 14−26% (sample 1751 was not considered due to being orginally partially hydrolyzed). These differences suggest that some aspects, probably conformational, prevent equal access by the pancreatin to the specific amino acids to allow for enzymatic action during the hydrolysis process. A further analysis of Table 4 shows that in the majority of the hydrolysates, the percentage of the amino acids Arg and Lys is lower than that of Trp, Tyr, and Phe. The smaller contribution of the basic amino acids in the FAA composition could indicate a lower activity of the pair trypsin/carboxypeptidase B. This result could possibly be explained by the presence of protease inhibitors in the SPIs, since the Kunitz inhibitor establishes a 1:1 stoichiometric reaction with trypsin, binding strongly to the enzyme at a specific reaction site. The complex formed with chymotrypsin is, however, weaker (Liener, 1986).

Electrophoresis of the enzymatically hydrolyzed SPIs was carried out with the objective of following the changes occurring in the proteins as well as the molecular mass profile of the hydrolysates. The results showed that the SPIs were extensively hydrolyzed, as expected. However, although the samples were submitted to the same DH (21.5%), differences between some of the hydrolysates were observed. The electrophoretic profile of the hydrolysate HG presented well-defined bands in the molecular mass region from 31 000 to 21 500. This material could represent either nonhydrolyzed subunits or partially hydrolyzed material of a molecular mass higher than that of the remaining hydrolysis products. The TCA index of this hydrolysate (76.3%) was one of the lowest as compared to the others (Table 3). The original SPI showed a high PDI value (84.4%) and contained all of the 7S subunits and 11S polypeptides, suggesting a native protein, which could have determined a different pattern of enzymatic cleavage. However, SPIs such as NB and NB′, which showed similar characteristics, did not show the same behavior. On the other hand, the hydrolysate 515, which also presented bands in the higher molecular mass region (21 500 to 14 400), had a high TCA index (93.2%) and was obtained from an SPI with a medium PDI value (67.1%). The SPIs SD, SD′, MP′, 500E, and 590 seemed to be composed of a greater quantity of small peptides, being less stained in the molecular mass region below 6500 than the other hydrolysates, suggesting that these peptides were not fixed by the gel. The most weakly stained hydrolysate was 1751, suggesting, once again, that this one was partially hydrolyzed before being submitted to the enzymatic treatment used in this work.

**Conclusions.** In general, the present work showed that the 13 SPIs analyzed, although submitted to the same conditions of enzymatic hydrolysis, behaved as distinct raw materials in the production of hydrolysates, since differences were observed in the hydrolysis reaction as well as in the products obtained. This fact is relevant in the production of protein hydrolysates, since the choice of raw material determines both the process and product characteristics, which is of great importance with respect to their nutritional application. However, not all of the differences found in the hydrolysates could be explained by the determinations carried out on the SPIs, indicating the need for further studies.

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