# Effect of Hydrolysis Time on the Determination of Amino Acids in Samples of Soybean Products with Ion-Exchange Chromatography or Precolumn Derivatization with Phenyl Isothiocyanate

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Accurate determination of amino acid levels in soy products facilitates optimum diet formulation and amino acid supplementation. A study was carried out to investigate the effect of hydrolysis time and method of amino acid measurement on amino acid levels. Correction factors to standardize amino acid levels to 24 h of hydrolysis were also determined. Six different soybean products were evaluated. Hydrolysis was carried out for 10 different periods of time. Amino acids were analyzed by both ion-exchange chromatography and precolumn derivatization with phenyl isothiocyanate. Both hydrolysis time and measurement method affected ($P < 0.05$) amino acid levels. Standard hydrolysis conditions (hydrolysis in 6 M HCl at 110 °C for 24 h) rarely provide the maximal amino acid values. Therefore, sequential hydrolyses curves were very useful and should be used.

**Keywords:** *Amino acids; soybean products; measurement methods; hydrolysis time*

## INTRODUCTION

The amino acid levels in feedstuffs and foodstuffs used in animal and human diets must be determined to understand the nutritional value of these ingredients and to optimize diet formulations. Commonly, an acid hydrolysis of the samples is performed. Then, amino acids are measured with high-pressure liquid chromatography using precolumn derivatization with phenyl isothiocyanate (PITC) or ion-exchange chromatography (IEC) with postcolumn ninhydrin reaction (Bidlingmeyer et al., 1984; Elkin and Wasynczuk, 1987).

The release of amino acids from proteins with acid hydrolysis is the most important step in the determination of amino acid concentrations. Standard hydrolysis procedures involve 24-h acid hydrolysis in 6 M HCl at 110 °C (Henrickson and Meredith, 1984; Gehrke at al., 1985; Rowan et al., 1992). Hydrolysis time (16−72 h) has been shown to affect the release and degradation of amino acids in a diet for growing pigs, and standard hydrolysis conditions rarely provide the maximum amino acid concentration (Rowan et al., 1992). Some amino acids require either longer or shorter hydrolysis times to provide the maximum yields (Rowan et al., 1992).

Soybean products are used widely in animal and human nutrition (Emmert and Baker, 1995). However, the effect of hydrolysis time on amino acid concentrations in commonly used soy protein products is not well-known. The purposes of the present study were 3-fold. The first purpose was to determine the effect of hydrolysis time on amino acid concentrations in soybean products. Second, the effect of method (PITC versus IEC) on amino acid concentration was examined. Finally, for amino acids levels that were not maximized with 24 h hydrolysis, correction factors were calculated.

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## MATERIALS AND METHODS

**Procedure.** Representative samples of soybean meal from two crop years, 1996 and 1997 (SBM96, SBM97), soy protein concentrate (SPC; Arcon F 65-301, Archer-Daniels-Midland, Decatur, IL), soy protein isolate (SPI; Ardex 66-960, Archer-Daniels-Midland, Decatur, IL), whole soybeans (WholeSB; Williams 82 variety), and soybean hulls (Soyhulls) were obtained. Homogeneous samples (approximately 10 g) of each were finely ground in a coffee bean grinder (Mr. Coffee, Model # IDS-50, Bedford Heights, OH) with 10 mL of liquid nitrogen for about 20 s, mixed, and stored frozen at −10 °C.

**Chemical Analysis.** Triplicate samples of each soybean product were used to determine dry matter and crude protein ($N \times 6.25$) according to procedures outlined by AOAC (1995). The following chemicals used for amino acid analyses were all purchased from Fisher Scientific, St. Louis, MO: water (HPLC grade, cat. # W5-4), methanol (HPLC grade, cat. # A452-4), acetonitrile (HPLC grade, cat. # A998-4), sodium phosphate dibasic (anhydrous, certified A.C.S., cat. # S374-500), *o*-phosphoric acid (HPLC grade, cat. # A260-500), sodium acetate trihydrate (HPLC grade, cat. # S220-1), concentrated hydrochloric acid (37%, cat. # A144-212), and glacial acetic acid (reagent A.C.S., A38$^{c}$-212). All amino acids, phenyl isothiocyanate (protein sequencing grade, cat. # P-1034), and triethylamine (cat. # 13,206-3) were obtained from Sigma-Aldrich, St. Louis, MO.

Duplicate samples of SBM96, SBM97, WholeSB, and Soyhulls (at least 200 mg) and duplicate samples of SPC and SPI (at least 100 mg) were accurately weighed into screw-capped test tubes (15 mL, Pyrex, cat. # 9826-16x, Corning, NY) with Teflon-lined caps. Twelve milliliters of 6 M HCl was added to the tubes, and the tubes were purged with $N_2$ for 10 s, mixed, and hydrolyzed in an oven (Fisher Scientific, Model # 500 Series, Pittsburgh, PA) at 110 °C. The following hydrolysis times were used: 0, 2, 6, 10, 16, 24, 32, 44, 56, and 72 h, respectively. This was done to construct sequential hydrolysis amino acid curves. After removal from the oven, the samples were allowed to cool. Once the samples had cooled, 0.5 mL of α-aminobutyric acid (AABA, 50 μmol/mL) and 0.5 mL of norleucine (Nor, 50 μmol/mL) were accurately weighed on a balance and added to each tube. The tubes were inverted 200 times and centrifuged at 1100*g* for 10 min to pellet debris.

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Then, the hydrolysates were diluted in a 1:2.5 ratio (v/v) by adding 300 μL of distilled, deionized water and a 200 μL aliquot of hydrolysate to a 1.5 mL microfuge tube (Fisher Scientific, cat. # 05-408-10, Pittsburgh, PA). Amino acid standards (2.5 μmol/mL) were prepared by weighing out individual amino acids into a 250 mL volumetric flask and dissolving them in 0.1 M HCl. Three standards were used. Standard 1 consisted of Asp, Ser, Gln, citrulline, Arg, AABA, Val, Ile, Nor, and Trp. Standard 2 consisted of Glu, Asn, taurine, Thr, Pro, AABA, Met, Leu, Nor, and ornithine. Standard 3 consisted of hydroxyproline, Gly, His, Ala, AABA, Tyr, Cys, Nor, Phe, Lys, and homoarginine. Standards were prepared to accommodate analyses of hydrolysates and physiological samples. Samples and standards were prepared for PITC and IEC analysis.

The procedures for amino acid determination with PITC were the following: 20 μL of diluted hydrolysates or 10 μL of either standard 1, 2, or 3 was pipetted into polypropylene tubes (Fisher Scientific, cat. # 1495910AA, St. Louis, MO). The aliquots were allowed to dry under vacuum overnight in a freeze-drier (Labconco, Model # 77500, Kansas City, MO). The samples were redried by adding 20 μL of 1:1:1 (v/v/v) methanol:water:triethylamine to each sample. The tubes were held at a 45° angle and turned several times to resolubilize amino acids. Next, they were allowed to vacuum-dry for 4−6 h. Finally, the samples were derivatized by adding 20 μL of 7:1:1:1 (v/v/v/v) methanol:water:triethylamine:phenyl isothiocyanate and mixed by holding the tubes at a 45° angle and turning them several times. Derivatization was allowed to occur for 35 min at room temperature (22 °C). The samples were vacuum-dried for 4−6 h following derivatization. The samples were then reconstituted by the addition of 200 μL of sample diluent, which contained a mixture of 95:5 (v/v) phosphate buffer (5 mM sodium phosphate dibasic, pH 7.4):acetonitrile. The samples were vortexed (Barnstead/Thermolyne, Type 16700 Mixer, Dubuque, IA), allowed to stand for approximately 15 min, and then vortexed again. A pipet was used to transfer most of the liquid to a polypropylene HPLC vial (Bio-Rad Laboratories, cat. # 223-9471, Hercules, CA), while any debris was left at the bottom of the microfuge tube. The injection volume used ranged from 30 to 90 μL depending on the protein content of the sample.

The Waters HPLC system consisted of either a 712 WISP or a 700 Satellite WISP autosampler, two 510 pumps, a column heater (46 °C), and a 484 tunable absorbance detector set at 254 nm. Peaks were identified and integrated with Waters Maxima 820 software. The HPLC column was a Waters Pico-Tag 3.9 mm × 30 cm reverse-phase column (Waters, cat. # WAT010950, Milford, MA). The packing consisted of 4 μm Silica/C18 beads. A 4.6 mm × 5 cm Supelcosil reverse-phase C18 guard column with 40 μm Pellicular packing (Sigma-Aldrich, Supelco, cat. # 5-8232, Bellefonte, PA) was used. Two eluents were used:eluent A consisted of 70 mM sodium acetate, pH 6.55, 2.5% (v/v) acetonitrile and eluent B consisted of 50:35:15 (v/v/v) acetonitrile:water:methanol. Both eluents were vacuum-filtered through a 0.45 μm nylon filter before use. The flow rate began at 1.0 mL/min. At 75 min, the flow rate was increased to 1.3 mL/min. The flow rate was returned to 1.0 mL/min at 76 min. The gradient which was run for the separation consisted of 100% eluent A until 13.5 min, at which point the level of eluent A was decreased to 97% and eluent B increased to 3% (vertical change, Waters No. 11). The level of eluent A continually decreased while eluent B increased, and this pattern is indicated in the following: 24 min, concave curve, Waters No. 9/(A, 95%; B, 5%); 30 min, convex curve, Waters No. 5/(A, 91%; B, 9%); 50 min, linear change, Waters No. 6/(A, 66%; B, 34%); 65 min, linear change, Waters No. 6/(A, 0%; B, 100%). The column was reequilibrated with 100% eluent A at 76 min until 89 min.

The procedures for amino acid determination with IEC were the following: Duplicate samples of SBM97 and SPC were utilized. Samples of hydrolysate were taken from the same tubes that were used for the PITC procedure. Following hydrolysis, addition of internal standards, and centrifugation at 1100*g* for 10 min, as previously described above, the samples were diluted 1:5 (v/v) with distilled, deionized water. The diluted samples were then neutralized with a buffer solution (pH 2.0) of 0.1 g of NaOH in 10 mL of sodium citrate (2%, Beckman Instruments, Inc., Palo Alto, CA) in a ratio of 1:5 (v/v) diluted sample:buffer solution. The pH of the samples was measured with litmus paper and found to be approximately 2. The samples were then analyzed using postcolumn detection with ninhydrin with procedures that have been described previously (Spitz, 1973). It should be noted that the internal standards were not used for calculations of amino acid concentrations with IEC but were used for PITC calculations only. External standards were used for the IEC procedure.

**Data Analysis.** The data were analyzed statistically to determine if hydrolysis time affected amino acid yield determined by PITC using the PROC GLM procedures of SAS (1990). The means of each amino acid level at the various points in time were compared according to the Student−Newman−Keuls multiple range test (Ott, 1993). For isoleucine in SPC, as well as histidine and threonine in Soyhulls, the data were compared using Fisher's least significant difference procedure (pdiff procedure, SAS; Milliken and Johnson, 1984). This was done because, for three of the concentrations at different hydrolysis times, one value was considered an outlier and removed from the data set. This procedure (Fisher's least significant difference) does not allow for multiple comparisons of means unless a significant *F*-value is detected ($P < 0.05$). Means for SBM97 and SPC obtained with 24 h hydrolysis and determined using PITC and IEC were compared with PROC GLM procedures of SAS (1990) with method as the source of variation.

## RESULTS

The dry matter and crude protein (%, dry matter basis) contents, respectively, of the soybean samples are the following: SBM96 (88.8%, 53.6%); SBM97 (88.5%, 51.2%); SPC (92.7%, 68.2%); SPI (90.7%, 82.7%); WholeSB (93.1%, 41.0%); and Soyhulls (88.1%, 11.6%).

Mean amino acid contents and standard errors of the different soybean samples determined with PITC at the different times are shown in Figures 1−4. Regardless of soybean product, amino acid concentrations were affected by hydrolysis time ($P < 0.01$). For all samples, a tremendous increase in amino acid concentration was observed from 0 to 6 h of hydrolysis. From 6 to 72 h of hydrolysis, the amino acid concentrations either increased, decreased, or remained relatively constant. Maximum ($P > 0.05$) valine and isoleucine concentrations were obtained with hydrolysis times greater than 24 h for all soybean samples, with the exception of isoleucine in SBM97. Glutamic acid, glycine, histidine, alanine, arginine, proline, leucine, phenylalanine, and lysine tended ($P > 0.05$) to increase over time in most samples. Serine concentrations were maximized ($P > 0.05$) at hydrolysis times of less than 24 h, with the exception of SPI. Aspartic acid, threonine, and tyrosine tended ($P > 0.05$) to increase or remain constant from 6 to 16 or 24 h, and then degradation exceeded the rate of release. There was also degradation ($P > 0.05$) at hydrolysis times greater than 24 h for glutamic acid, histidine, valine, isoleucine, leucine, phenylalanine, and lysine in SBM97. The same patterns were not observed, or were seen to a lesser degree, in SBM96. Also, there was considerable degradation ($P > 0.05$) after 24 h hydrolysis for many amino acids in SPI and for some amino acids in SPC. Threonine and alanine degradation ($P > 0.05$) in SPC occurred before 24 h hydrolysis. For most amino acids, the maximum concentrations obtained were similar ($P > 0.05$) or equal to the amino acid concentration with 24 h hydrolysis.

The mean amino acid concentrations at 24 h hydrolysis for SBM97 and SPC are shown in Table 1. With the

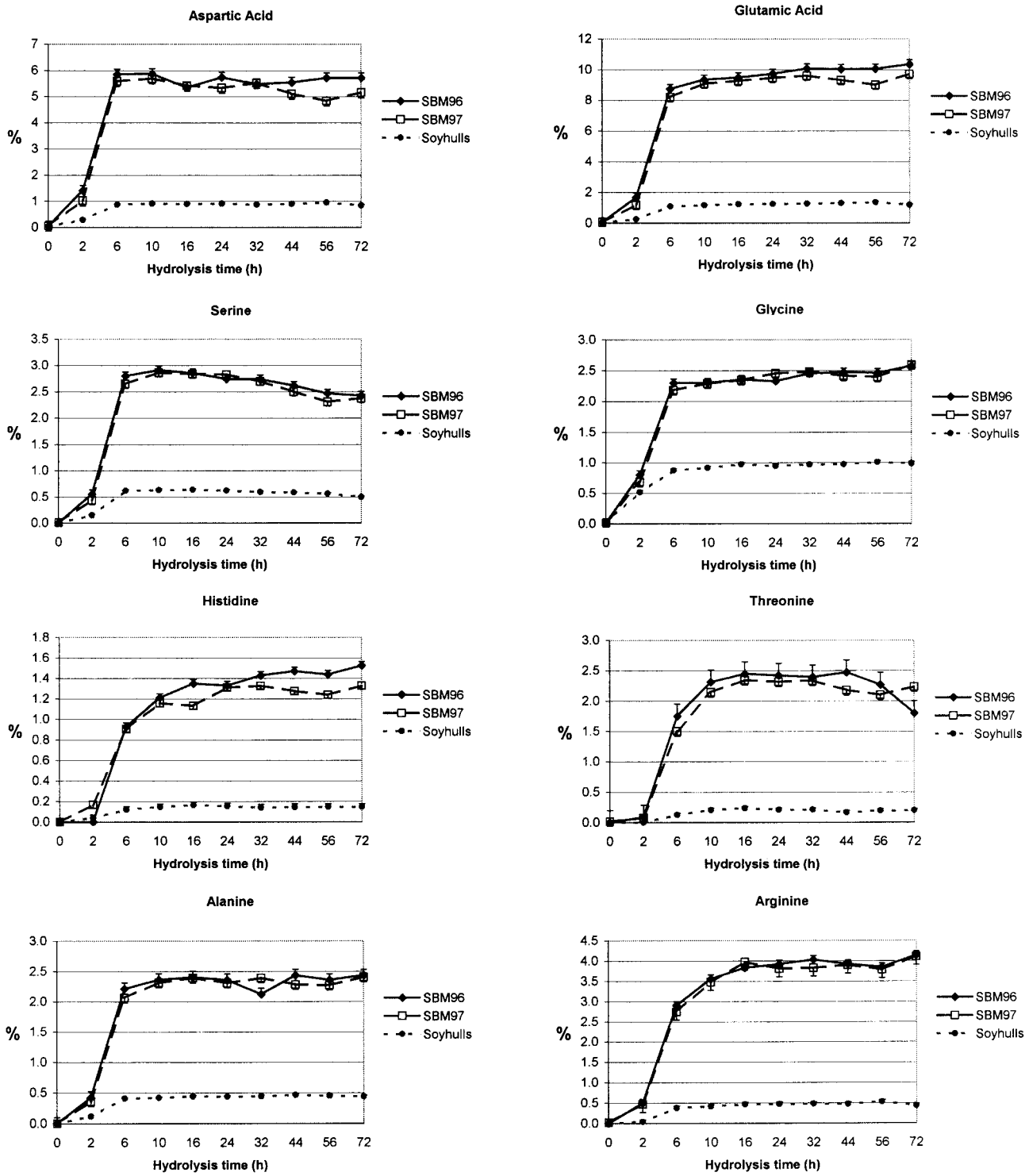


**Figure 1.** Effect of hydrolysis time on the mean yield of amino acids (*Y* axis, % dry matter basis) from soybean meal from 1996 and 1997 and soybean hulls. Error bars indicate sample standard error ($n = 2$; $n = 1$ for histidine at 32 h and threonine at 72 h in soyhulls).

exception of aspartic acid in SPC, the amino acid concentrations were higher ($P < 0.05$) when determined with PITC than when determined with IEC.

Correction factors for all soybean samples are presented in Table 2. The correction factors were determined by dividing the maximum amino acid concentration by the value obtained with 24 h hydrolysis. Most of the correction factors were relatively small (1.00−1.31). However, the correction factors for isoleucine, phenylalanine, and lysine in SPI were greater than 1.31.

## DISCUSSION

It is well know that valine and isoleucine are released slowly during acid hydrolysis, while serine and threonine are continually degraded (Rees, 1946; Gehrke et al., 1985; Rowan et al., 1992). Valine and isoleucine were released slowly during hydrolysis, while valine was liberated faster than isoleucine in the present study (Figures 1−4). Rowan et al. (1992) reported similar observations. Serine was progressively destroyed from 10 h hydrolysis in SBM96 and SBM97, from 16 h in

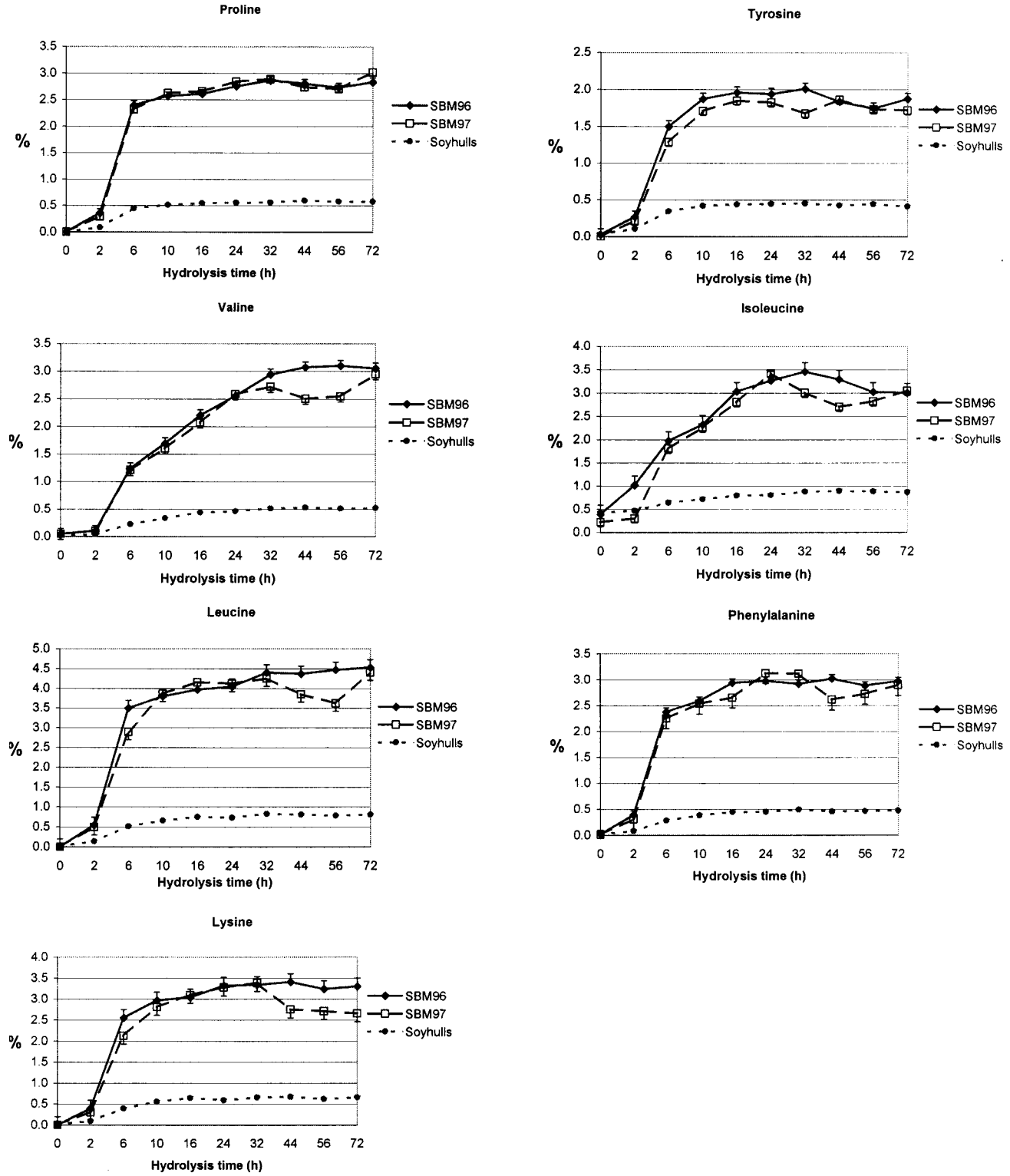


**Figure 2.** Effect of hydrolysis time on the mean yield of amino acids (*Y* axis, % dry matter basis) from soybean meal from 1996 and 1997 and soybean hulls. Error bars indicate sample standard error ($n = 2$).

SPC, WholeSB, and Soyhulls, and from 24 h in SPI, respectively. Threonine was degraded from 16 h hydrolysis in SBM97, SPC, and Soyhulls and from 24 h in SPI and Soyhulls. Threonine was degraded from 16 h in SBM96, but was maximized at 44 h before declining again. The differences in degradation of serine and threonine are, at least in part, dependent on protein source. Glazer et al. (1976) and Rowan et al. (1992) attributed differences in degradation and release of amino acids to protein source. Tyrosine, though susceptible to oxidation (Finley, 1985; Gehrke et al., 1985), remained relatively stable from 6 or 10 h to 24 h hydrolysis, after which degradation occurred in the present study. Rowan et al. (1992) reported similar observations concerning tyrosine and attributed the stability to the addition of phenol. In the present study, however, phenol was not added prior to hydrolysis, and accurate tyrosine values were obtained with 24 h hydrolysis.

The gradual increase in concentration of glutamic

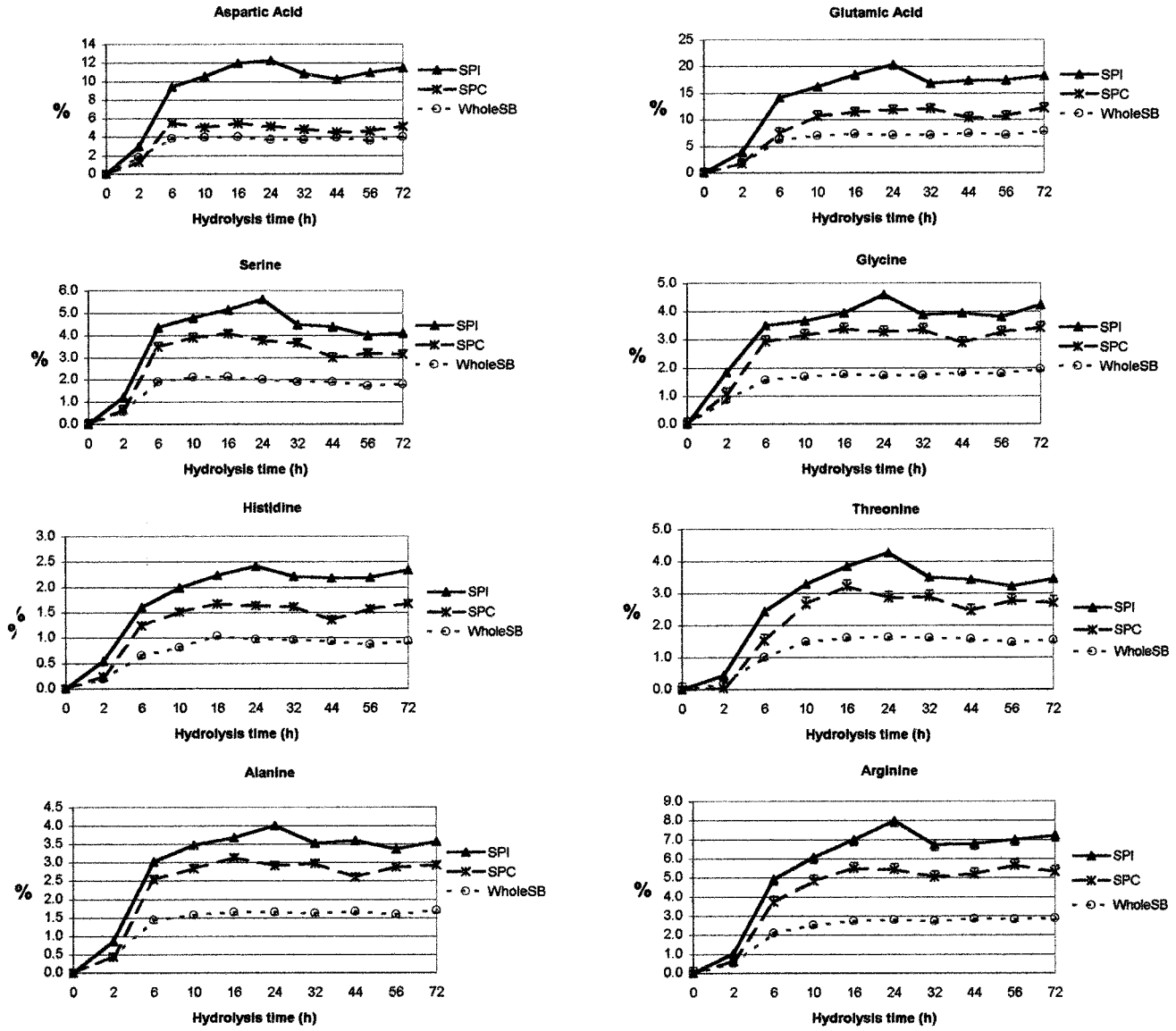


**Figure 3.** Effect of hydrolysis time on the mean yield of amino acids (*Y* axis, % dry matter basis) from soy protein isolate, soy protein concentrate, and whole soybeans. Error bars indicate sample standard error ($n = 2$).

acid, glycine, histidine, alanine, arginine, proline, leucine, phenylalanine, and lysine over time observed during this study has also been reported by Rowan et al. (1992). Aspartic acid remained constant over time in this study, and similar results were found by Rowan et al. (1992). This is the first study designed to investigate the effects of hydrolysis time on amino acid concentrations in soybean product samples using standard hydrolysis procedures.

Different investigators have evaluated the two amino acid determination methods used in this study, and they have reported that the two procedures provide very similar results (Bidlingmeyer et al., 1984; Heinrikson and Meredith, 1984; Elkin and Wasynczuk, 1987). However, in this study, measurement method affected amino acid concentration. For almost every amino acid, in both SBM97 and SPC using 24 h hydrolysis, the PITC procedure provided significantly higher concentrations. Also, it should be noted that the hydrolysis curves for SBM97 and SPC determined with IEC follow the same patterns of release as those shown in Figures 1−4 (data not shown). Comparisons with published amino acid values indicate that the concentrations determined with PITC are more precise than IEC concentrations. For example, using PITC and IEC in this study, the lysine content of SBM97 was 3.27% and 2.77%, respectively (dry matter basis). Other publications (Cavins et al., 1972; Rudolph et al., 1983; Emmert and Baker, 1995; NRC, 1998) have reported lysine concentrations in soybean meal of 3.36%, 3.15%, 3.46%, and 3.36%, respectively. The lysine content of SPC using PITC and IEC was found to be 4.47% and 3.83%, respectively. Emmert and Baker (1995) and NRC (1998) reported lysine concentrations in SPC of 4.35% and 4.67%, respectively. According to a recent study (Cromwell et al., 1999) designed to investigate the variability of nutrient analyses among sources and laboratories, most of the lower amino acid values for soybean meal obtained with IEC in the present study could not be explained by simple laboratory or sample variation. The study supports the values obtained with PITC in the present study. There are several possible explanations for the differences in amino acid concentrations determined with the two methods. Many steps of the IEC procedure demand accurate volumetric measurements. Before amino acids are determined with the IEC pro-

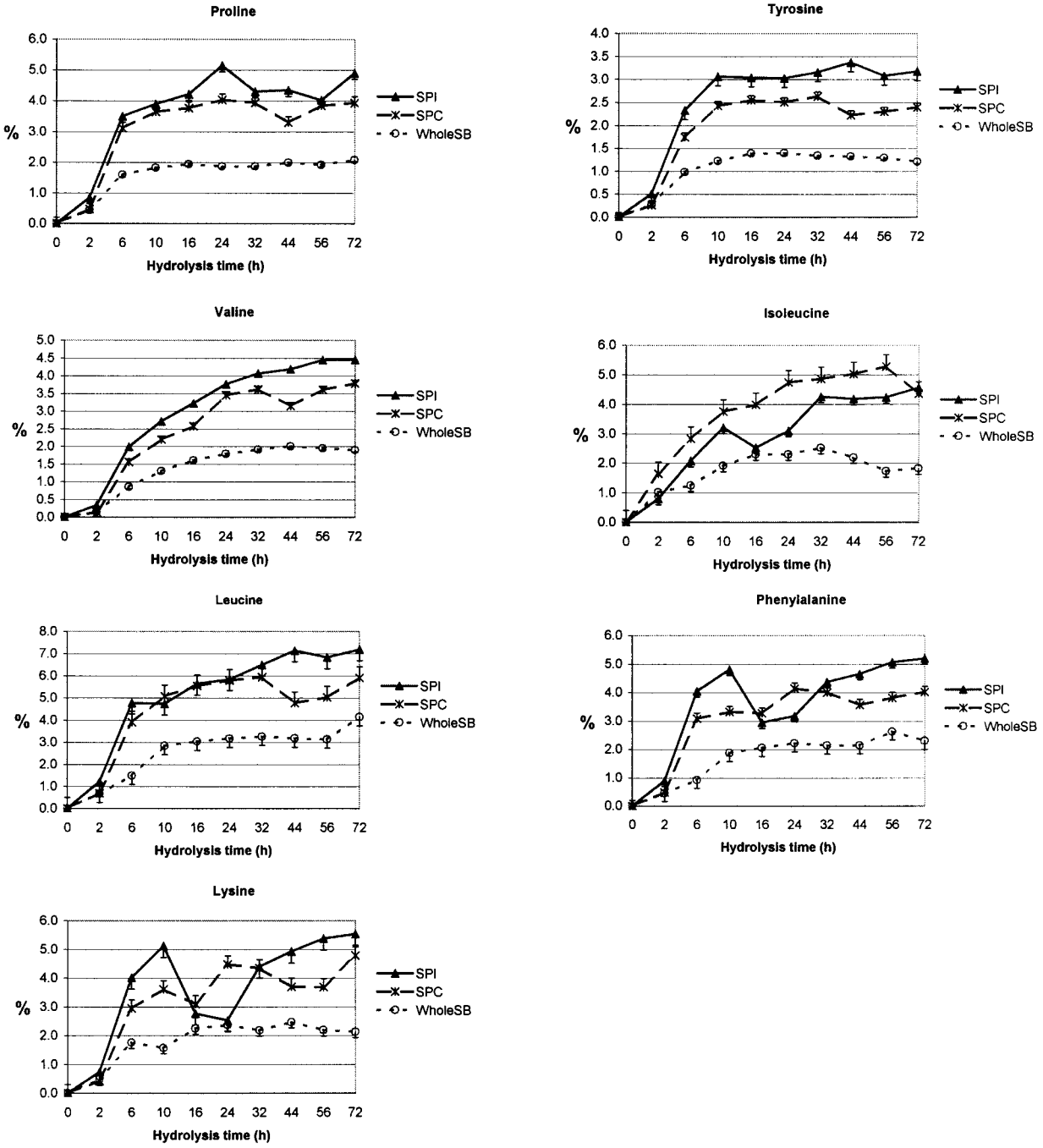


**Figure 4.** Effect of hydrolysis time on the mean yield of amino acids (*Y* axis, % dry matter basis) from soy protein isolate, soy protein concentrate, and whole soybeans. Error bars indicate sample standard error ($n = 2$; $n = 1$ for isoleucine at 16 h in SPC).

cedure, the hydrolysate needs to be diluted and buffered. This is done in two different steps. As part of this procedure, external standards were used and therefore, dilution of standards and injection volumes need to be accurate. The PITC procedure does not require accurate volumetric measurements because internal standards and tube weighing are used. Therefore, more opportunities for error exist with the IEC procedure than when the PITC procedure is used.

Garnett (1985) suggested the use of generalized correction factors as a simple approach to correcting amino acid concentrations. Correction factors have been calculated by others (Kohler and Palter, 1967; Tkachuk and Irvine, 1969; Slump, 1980; Rowan et al., 1992) to correct amino acid concentrations determined with 24 h hydrolysis to the maximum values. Recently, research projects have begun to use correction factors to obtain amino acid concentrations that are more accurate (Lenis et al., 1990; Mroz et al., 1994). Correction factors for serine, isoleucine, and threonine in foods have been reported (Kohler and Palter, 1967; Tkachuk and Irvine, 1969; Slump, 1980; Rowan et al., 1992) and ranged from 1.04 to 1.14, 1.02 to 1.21, and 1.02 to 1.08, respectively. In the present study, correction factors for serine, isoleucine, and threonine ranged from 1.01 to 1.08, 1.06 to 1.11, and 1.01 to 1.13, respectively. Slump (1980) and Rowan et al. (1992) have reported correction factors for valine of 1.08 and 1.20, respectively. In the present study, valine correction factors ranged from 1.10 to 1.21. Most of the correction factors in this study were relatively small. For isoleucine, phenylalanine, and lysine in SPI, the correction factors were 1.47, 1.63, and

**Table 1. Amino Acid Levels (%, dry matter basis) in Soybean Meal (SBM97) and Soy Protein Concentrate (SPC) after 24 h of Acid Hydrolysis Determined Using Precolumn Derivitization with Phenyl Isothiocyanate (PITC) or Postcolumn Detection with Ninhydrin (IEC)**

| | SBM97 | | | SPC | | |
|---|---|---|---|---|---|---|
| amino acid | PITC | IEC | sig[a] | PITC | IEC | sig |
| aspartic acid | 5.33 ± 0.27[b] | 4.88 ± 0.14 | NS | 5.13 ± 0.43 | 6.75 ± 0.05 | * |
| glutamic acid | 9.47 ± 0.24 | 8.00 ± 0.21 | * | 11.82 ± 0.91 | 10.99 ± 0.12 | NS |
| serine | 2.83 ± 0.09 | 2.30 ± 0.06 | * | 3.75 ± 0.08 | 3.19 ± 0.02 | ** |
| glycine | 2.45 ± 0.09 | 1.85 ± 0.05 | * | 3.28 ± 0.07 | 2.57 ± 0.01 | ** |
| histidine | 1.31 ± 0.07 | 1.16 ± 0.04 | NS | 1.63 ± 0.02 | 1.63 ± 0.03 | NS |
| threonine | 2.31 ± 0.13 | 1.77 ± 0.05 | * | 2.86 ± 0.11 | 2.47 ± 0.04 | * |
| alanine | 2.31 ± 0.01 | 1.92 ± 0.05 | ** | 2.92 ± 0.06 | 2.67 ± 0.03 | * |
| arginine | 3.81 ± 0.01 | 3.11 ± 0.11 | * | 5.44 ± 0.01 | 4.36 ± 0.05 | ** |
| proline | 2.84 ± 0.03 | 2.27 ± 0.05 | ** | 4.03 ± 0.08 | 3.19 ± 0.17 | * |
| tyrosine | 1.82 ± 0.02 | 1.54 ± 0.03 | ** | 2.51 ± 0.25 | 2.24 ± 0.02 | NS |
| valine | 2.59 ± 0.19 | 1.97 ± 0.05 | * | 3.45 ± 0.05 | 2.75 ± 0.05 | ** |
| isoleucine | 3.42 ± 0.06 | 1.91 ± 0.05 | ** | 4.74 ± 0.53 | 2.66 ± 0.04 | * |
| leucine | 4.12 ± 0.30 | 3.36 ± 0.08 | NS | 5.79 ± 0.44 | 4.64 ± 0.04 | NS |
| phenylalanine | 3.13 ± 0.13 | 2.24 ± 0.04 | * | 4.15 ± 0.14 | 3.07 ± 0.03 | ** |
| lysine | 3.27 ± 0.03 | 2.77 ± 0.07 | * | 4.47 ± 0.01 | 3.83 ± 0.10 | * |

[a] NS, nonsignificant $P > 0.05$; *, significant, $P < 0.05$; **, significant, $P < 0.01$. [b] Standard deviation ($n = 2$).

**Table 2. Correction Factors[a] for Amino Acids in Soybean Meal (SBM96, SBM97), Soy Protein Concentrate (SPC), Soy Protein Isolate (SPI), Whole Soybeans (WholeSB), and Soybean Hulls (Soyhulls)**

| amino acid | SBM96 | SBM97 | SPC | SPI | WholeSB | Soyhulls |
|---|---|---|---|---|---|---|
| aspartic acid | 1.02 | 1.07 | 1.07 | 1.00 | 1.09 | 1.04 |
| glutamic acid | 1.06 | 1.03 | 1.03 | 1.00 | 1.11 | 1.07 |
| serine | 1.06 | 1.01 | 1.08 | 1.00 | 1.07 | 1.03 |
| glycine | 1.11 | 1.06 | 1.04 | 1.00 | 1.13 | 1.06 |
| histidine | 1.15 | 1.01 | 1.02 | 1.00 | 1.07 | 1.06 |
| threonine | 1.02 | 1.01 | 1.12 | 1.00 | 1.00 | 1.13 |
| alanine | 1.03 | 1.04 | 1.07 | 1.00 | 1.03 | 1.06 |
| arginine | 1.06 | 1.08 | 1.04 | 1.00 | 1.03 | 1.11 |
| proline | 1.04 | 1.06 | 1.00 | 1.00 | 1.11 | 1.07 |
| tyrosine | 1.04 | 1.02 | 1.05 | 1.11 | 1.00 | 1.02 |
| valine | 1.21 | 1.14 | 1.10 | 1.18 | 1.12 | 1.14 |
| isoleucine | 1.06 | 1.00 | 1.11 | 1.47 | 1.09 | 1.11 |
| leucine | 1.12 | 1.07 | 1.03 | 1.23 | 1.31 | 1.12 |
| phenylalanine | 1.01 | 1.00 | 1.00 | 1.63 | 1.19 | 1.10 |
| lysine | 1.03 | 1.03 | 1.07 | 2.18 | 1.04 | 1.14 |

[a] Correction factors determined by expressing the maximum amino acid yield as a proportion of the yield at 24 h.

2.18, respectively. These factors should be used with caution, as comparison correction factors and hydrolysis curves are not available for SPI. However, there is nothing to indicate that analytical error produced abnormal concentrations of these amino acids in SPI. Soy protein isolate is a purified protein source that has undergone extensive processing, and little is known concerning the ideal measurement conditions for amino acids in purified, high-protein sources.

In conclusion, determination of amino acid concentrations in protein sources is affected by acid hydrolysis of the samples and the use of different methods. Ion-exchange chromatography has been shown to provide similar results when compared to precolumn derivatization with phenyl isothiocyanate, but the findings of this study indicate that the two methods do not always provide similar concentrations. Internal standards, and not external standards, should be used in future research to calculate amino acid concentrations determined by ion-exchange chromatography. Using standard hydrolysis procedures (24 h hydrolysis) usually does not provide the maximum amino acid concentrations in samples. Correction factors are necessary to more accurately determine amino acid concentrations (i.e., for serine, threonine, isoleucine, valine) in protein sources. The amino acid pattern of release is dependent on protein source, and therefore, further research is necessary with more feedstuffs and foodstuffs, as well as a range of diets, to determine correction factors. Also, further research needs to be conducted with SPI and other purified, high-protein sources to determine the proper conditions for their amino acid analysis.

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