Assessment of the Protein Quality of a New High-Protein Soybean Cultivar by Amino Acid Analysis†

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The contents of total protein, amino acids, and 4-hydroxyproline-rich glycoproteins of a widely grown soybean cultivar, Maple Arrow, and a newly released high-protein genotype, OT89-16, developed by back crossing to Maple Arrow, were compared as potentially useful and practical indices for evaluating their protein quality. The significant increase ($P < 0.01$) in protein from 33.7% (cv. Maple Arrow) to 42.1% (cv. OT89-16) and the respective decrease in methionine of 9.9% suggest that a large increase in protein was accompanied by a small but not significant decrease in the most limiting essential amino acid. Both cultivars contained an excellent balance of essential amino acids (EAA) limited only in methionine, followed by tryptophan, and their amino acid scores, adjusted for digestibility, were almost as high as those of milk and egg proteins. Mean values for total EAA_7 and EAA_{10} ranged from 33.7 to 35.4% and from 46.1 to 47.8%, respectively, and both had mean calculated PER values of 2.7. The 4-hydroxyproline glycoproteins found in the extracellular matrices of soybean seeds ranged from 2.98 to 2.32 g/kg of total protein in Maple Arrow and OT89-16, respectively, which corresponded to 0.10% on a dry weight basis.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is a major source of vegetable protein for human and animal nutrition in many countries today. Soybean is an annual leguminous plant that originated in northeastern China (Smith and Huyser, 1987) and is now grown in many parts of the world, including the more northerly temperate regions. Genetic improvements of soybean cultivars have played a key role in developing adapted varieties for these regions and in establishing soybean as the eighth largest agricultural commodity in the world (Swaminathan, 1983; Toenniessen, 1985; Smith and Huyser, 1987).

Until recently, soybean breeding has been directed primarily toward the selection of soybean varieties with high yield performance and increased disease resistance (Fehr, 1987). Now, however, plant breeders are also directing their emphasis toward the development of soybean varieties with improved protein quantity and quality (Doll, 1984; de Lumen, 1990), lower levels of digestive enzyme inhibitors (Hymowitz, 1986; Friedman et al., 1991), and reduced linoleic acid content and lipoxygenase activity (Hilderbrand and Hymowitz, 1981; Hildebrand, 1989; Siedow, 1991).

Soybean seeds contain approximately 40% protein (Wolf, 1982; Kinsella, 1979; Berkowitz and Webert, 1987) and have an average protein efficiency ratio (PER) of 2.3 (Torun et al., 1981; Bodwell et al., 1980; Wayler et al., 1983). Such PER values, however, are determined by rat bioassays, which tend to underestimate the protein quality of soybean for humans because the rat has higher relative requirements for sulfur-containing amino acids (Bodwell, 1979; Torun et al., 1981). Human nutritional studies, which focused upon the utilization of various types of soybean products for adults, children, and full-term and premature infants, reported values for protein quality of adequately processed soybean protein ranging from 62 to 92% of casein (Torun et al., 1981; Rackis et al., 1975; Fomon and Ziegler, 1979; Bodwell, 1979; Scrimshaw and Young, 1979; Erdman and Fordyce, 1989).

Soybeans, like most leguminous plants, have seed proteins with a low content of sulfur-containing amino acids, with methionine being considered as the most significant limiting amino acid, followed by cyst(e)ine and threonine (Eggum and Beames, 1983). Two proteins, glycinin and β-conglycinin, account for about 70% of the storage proteins in soybean on a dry weight basis (Koshiyama, 1983; Wilson, 1987). Both proteins consist of three major subunits which are encoded by a large gene family clustered in several DNA regions (Harada et al., 1989). β-Conglycinin, which accounts for approximately 25% of the total protein in soybeans (Hill and Briedenbach, 1974a,b; Beachy et al., 1981; Coats et al., 1985), is practically devoid of methionine (Than and Shibasaki, 1978; Holowach et al., 1986). As a result, the overall methionine content of soybean protein is only 1.4% of the total amino acid content (de Lumen, 1990). Improvements in the quality of soybean proteins (Kelley, 1973; Cook et al., 1988), therefore, will necessitate either a reduction in this storage protein or an increase in methionine-containing proteins or a combination of the two (Jaynes et al., 1986; de Lumen and Kho, 1987; de Lumen, 1990; George and de Lumen, 1991). Recurrent selection of soybeans has been successfully used by Brim and Burton (1979) as a procedure for increasing the percent protein in soybean seed without significantly decreasing yields. This procedure, however, has not increased methionine levels (Burton et al., 1981).

In the present study an attempt was made to establish the levels and variation of total proteins as well as the individual amino acids, including 4-hydroxyproline [Pro-(4-OH)] found in extensin (Varner and Lin, 1989), in a widely grown soybean cultivar, Maple Arrow, and a newly released high-protein genotype, OT89-16, developed by three cycles of crossing and back crossing to Maple Arrow

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(Voldeng and Saindon, 1991). Further aims of this study were (1) to determine whether the amino acid composition and protein contents of these two cultivated varieties could be correlated with their protein quality, (2) to establish whether perceived differences in protein contents between these two cultivars could be explained by differences in amino acid composition determined according to the procedures developed by Zarkadas et al. (1986, 1987), and (3) to ascertain whether or not similar genetic changes occur in the total amount of 4-hydroxyproline-rich glycoproteins found in the extracellular matrices of the primary cell walls of soybean seeds (Hong et al., 1987; Cassab and Varner, 1987; 1988; Averyart-Fullard et al., 1988; Ye and Varner, 1991).

MATERIALS AND METHODS

Materials. Type DC-5A (lot 746) cation-exchange spherical resin, sized to 6.0 × 0.5 mm, was purchased from Dionex Chemical Co., Sunnyvale, CA. The amino acid standards were obtained as follows: 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; norleucine from Pierce Chemical Co., Rockford, IL; 3-nitrotyrosine from Aldrich Chemical Co., Milwaukee, WI; and the standard amino acid calibration mixture from Beckman Instruments, Inc., Palo Alto, CA. Highly purified ninhydrin and hydrindantin (Nin-Sol AF) dissolved in sequenal grade dimethyl sulfoxide was purchased from Pierce Chemical Co. Octanoic acid was obtained from Eastman Kodak Co., Rochester, NY, and phenol was a product of J. T. Baker Chemical Co., Phillipsburg, NJ. Hydrochloric acid (Analar), hydrobromic acid (Aristar), formic acid (88.0%), and hydrogen peroxide (30.0%) were purchased from BDH Inc., Poole, England. High-purity sodium hydroxide (50.0% w/w), which was used to prepare all buffers and reagents, was a product of Allied Fisher Scientific, Fair Lawn, NJ. The three highly purified microcolumn citrate buffers (pH 3.295, 0.20 M; pH 4.10, 0.20 M; pH 6.40, 1.0 M) and sample dilution buffer (pH 2.2, 0.20 M) recommended for high-sensitivity single-microcolumn analysis were used as described previously (Zarkadas et al., 1987). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Plant Materials and Sample Preparation.* Two genotypes, cv. Maple Arrow and line OT89-16, were selected for this investigation. Maple Arrow has been widely grown in central and eastern Ontario (USDA Maturity Group 00). Line OT89-16 was developed by three cycles of crossing and back crossing to Maple Arrow with selection of the highest protein F_3 bulks in each cycle as the nonrecurrent parent. After the second back cross, bulk selection in the F_3 for protein was followed by pedigree selection and yield evaluation of F_5-derived bulks. The high-protein line used for the first cross to Maple Arrow (DU-41) was selected from the cross of PI 189950 to a high-protein selection from cv. Merit × PI 153293. Full details are given by Voldeng and Saindon (1991).

Representative samples of seed of the two cultivars were taken from each of the four replicates of the Ontario soybean variety trial grown at four different sites at Agriculture Canada's Central Experimental Farm, Ottawa, in 1989. The dried seed samples were then pulverized in a standard electrically driven end runner mill (Cyclone sample mill, U. D. Corp., Fort Collins, CO), passed through a 0.5-mm mesh sieve, lyophilized, and then stored at −20 °C in polypropylene bottles until used.

Preparation of Tissue Hydrolysates. Duplicate samples (0.05 g) were hydrolyzed in Pyrex (No. 9860) test tubes (18 × 150 mm) under vacuum (below 10 μmHg) with triple-glass-distilled constant-boiling HCl (6.0 M) containing 0.2% (v/v) phenol at 110 ± 0.5 °C for periods of 24, 48, 72, and 96 h with the usual precautions described by Zarkadas et al. (1988c) and Ozols (1990). Analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate by methods described previously (Zarkadas et al., 1986, 1988b,c).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a Model 120C conventional or a Beckman Spinco Model 121 MB fully automated amino acid analyzer using single-column methodology (Zarkadas et al., 1986, 1987, 1990). The conventional instrument was equipped with a module control (Autolab Spectra-Physics GmbH, Darmstadt, Germany) and a companion Autolab System AA (Beckman Methodology Bulletins AA-TB-001–AA-TB-014) for computing peak concentrations (Zarkadas, 1975). The automated instrument was equipped with a Beckman Model 406 analog interface module, the system Gold (Beckman Instrument, Inc., Altex Division, San Ramon, CA) chromatographic data reduction system, and an IBM (AT series) compatible personal computer, which was obtained from Microcom AL Computer, Ottawa, ON. The incorporation of these components to the system increased the sensitivity of the analysis and enabled quantitation of amino acids at the picomole level as described previously (Zarkadas et al., 1987).

Complete amino acid analyses were carried out on each of the four soybean replicate samples (50.0 mg) according to the standard procedures described previously (Zarkadas et al., 1986, 1987). Each of the four replicates was divided into two subsamples, i.e., A and B, which were then hydrolyzed in duplicate for 24, 48, 72, and 96 h as described previously (Zarkadas et al., 1988a–c). Analyses of individual acid hydrolysates were performed in duplicate. The data reported for serine and threonine in Table I represent the average values of 72 determinations extrapolated to zero time of hydrolysis by linear regression analysis of the results. The values for valine, isoleucine, leucine, and phenylalanine are the average of 48 values obtained from the 48, 72, and 96 h of hydrolysis. All others are reported as the average values of 72 determinations from 24, 48, 72, and 96 h of hydrolysis.

Methionine and cyst(e)ine were determined separately (50.0-mg samples) according to the performic acid procedure of Moore (1963). Norleucine was added in the hydrolysate as an internal standard. Recoveries of cyst(e)ine as cysteic acid and methionine as methionine *S,S*-dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids, relative to alanine, valine, leucine, and isoleucine present in the sample, and represent the average of 24 determinations. Tryptophan in soybean samples (50.0 mg) was also determined separately after alkaline hydrolysis (Hugli and Moore, 1972) on a single column as described previously (Zarkadas et al., 1986), using 3-nitrotyrosine as the internal standard, and the data presented in Table I represent the average of 24 determinations.

4-Hydroxyproline (Berg, 1982) was determined separately from a concentrated 24-h hydrolysate (equivalent to 50.0 μg of protein/analysis) using a single column (21 × 0.6 cm) packed with Dionex DC-6A resin (Zarkadas et al., 1986). Recoveries of Pro(4-OH) were calculated relative to alanine, isoleucine, and leucine. The Pro(4-OH) data represent the average values of 24 determinations.

Protein Determination. The content of total protein in each of these soybean products was determined according to the procedure described by Horstmann (1979) and Zarkadas et al. (1988a,c) as follows:

$$\mathrm{WE} = \sum_{i=1}^{19} (a_i b_i) \tag{1}$$

The mean residue weight (WE in micrograms per nanomole) and conversion factor CF (in micrograms per nanomole) for determining the protein mass in each sample analyzed in the absence of tryptophan and cyst(e)ine was calculated as described previously (Horstmann, 1979; Zarkadas et al., 1988a). A conversion factor, CF′ (in micrograms per nanomole), was also calculated according to the method of Horstmann (1979) for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and/or Pro(4-OH)

$$\mathrm{CF}' = \frac{\sum_{i=1}^{15} (a_i b_i)}{1 - [a_{\mathrm{Trp}} + a_{\mathrm{Cys}} + a_{\mathrm{Pro}} + a_{\mathrm{Pro(4\text{-}OH)}}]} \tag{2}$$

where a_i is the mole fraction of an amino acid i found in the analyzed aliquot and b_i is the molecular weight of amino acid residue i (in micrograms). The conversion factors, CF and CF′, were used in all subsequent quantitations of a given sample. The protein content of each sample calculated by multiplying CF

Table I. Comparison of the Amino Acid Composition of a New High-Protein Soybean Genotype with a Standard (Grams of Anhydrous Amino Acid Residues per Kilogram of Total Protein)

	soybean genotype				
	Maple Arrow		OT89-16		signif levels
amino acid	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	between genotypes,[a] F
aspartic acid	68.86 ± 3.39	9.85	81.77 ± 4.52	11.06	2.76[ns]
threonine	41.94 ± 1.79	8.53	42.05 ± 1.22	5.80	0.00[ns]
serine	54.05 ± 1.66	6.13	58.13 ± 2.12	7.29	2.43[ns]
glutamic acid	190.16 ± 3.42	3.60	200.07 ± 3.34	3.34	4.14[ns]
proline	52.91 ± 2.38	8.98	51.76 ± 1.18	4.59	0.16[ns]
glycine	36.72 ± 0.15	0.82	35.97 ± 1.23	6.85	0.33[ns]
alanine	40.23 ± 1.11	5.53	38.04 ± 2.07	10.89	0.63[ns]
cyst(e)ine	25.00 ± 0.67	5.35	23.25 ± 1.03	8.89	1.75[ns]
valine	54.27 ± 0.32	1.20	51.08 ± 0.23	0.91	98.67**
methionine	10.70 ± 0.31	5.82	9.64 ± 0.50	10.51	3.10[ns]
isoleucine	51.58 ± 0.50	1.96	50.05 ± 1.37	5.46	0.96[ns]
leucine	81.69 ± 0.73	1.78	78.83 ± 2.67	6.77	1.42[ns]
tyrosine	41.55 ± 0.64	3.10	38.83 ± 1.42	7.32	5.64[ns]
phenylalanine	56.29 ± 0.63	2.25	52.62 ± 1.90	7.21	7.08[ns]
histidine	34.38 ± 5.65	32.85	32.28 ± 4.93	30.56	0.28[ns]
lysine	68.37 ± 1.06	3.10	62.06 ± 2.09	6.74	5.39[ns]
arginine	77.16 ± 2.35	6.10	80.21 ± 4.63	11.55	0.34[ns]
tryptophan	12.73 ± 0.41	6.54	12.20 ± 0.47	7.67	0.55[ns]
4-hydroxyproline	1.40 ± 0.02	3.09	1.12 ± 0.08	14.86	13.85*
ammonia	11.98 ± 0.82	13.75	10.62 ± 0.92	17.36	0.65[ns]
total AA N					
g of N/kg of protein	169.61 ± 1.46	1.73	168.15 ± 0.50	0.59	0.74[ns]
g of N/kg of sample	56.68 ± 1.35	4.76	70.29 ± 2.62	7.45	25.31**
	Essential Amino Acids (EAA)				
total EAA,[b] g/g of N	3074.3 ± 38.826	3.17	2979.2 ± 93.07	6.24	0.99[ns]
EAA_7,[c] % of total protein	35.42 ± 0.06	0.34	33.67 ± 0.69	4.07	6.27[ns]
EAA_{10},[c] % of total protein	47.84 ± 0.59	2.46	46.14 ± 0.68	2.97	2.06[ns]
	Calculated Protein Efficiency Ratio (PER)				
predicted by[d]					
eq 5 (PER_7)	2.75 ± 0.004	0.35	2.61 ± 0.05	4.24	6.27[ns]
eq 6 (PER_{10})	2.87 ± 0.037	2.59	2.76 ± 0.04	3.14	2.06[ns]
	Protein Mass and Mean Residue Weight Constants				
total protein,[e] g/kg of dry wt	336.69 ± 5.33	3.17	421.05 ± 14.78	7.02	30.21**
WE,[e] μg/nmol	0.113500 ± 0.0003	0.55	0.113500 ± 0.0004	0.86	0.00[ns]
CF,[e] μg/nmol	0.114375 ± 0.0003	0.55	0.114300 ± 0.0005	0.93	0.00[ns]
CF′,[f] μg/nmol	0.112150 ± 0.0003	0.64	0.121900 ± 0.0005	0.44	0.13[ns]

[a] Mean values and standard error of measurements (SEM) for four replicates and 64 determinations. The values for valine, isoleucine, leucine, and phenylalanine are the average of 32 determinations. The values for tryptophan and 4-hydroxyproline represent the average of 24 determinations. Significance: F, values from analysis of variance between cultivars; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Computed from reference protein standards (FAO/WHO, 1965, 1973). [c] Calculated according to the method of Lee et al. (1978). EAA_7: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}: EAA_7 plus histidine, arginine, and tryptophan. [d] PERs were calculated according to the method of Lee et al. (1978) from eq 5 [$PER_7 = 0.08084(EAA_7) - 0.1094$] and eq 6 [$PER_{10} = 0.06320(EAA_{10}) - 0.1539$]. [e] The WE and CF constants were calculated according to the method of Horstmann (1979). [f] The conversion factor CF′ (g) was also calculated according to eq 2 but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

and CF′ by the nanomoles of total amino acids in each acid hydrolysate was calculated as follows:

$$P = (\mathrm{CF}')\sum_{i=1}^{15}\chi_i \qquad (3)$$

Determination of 4-Hydroxyproline-Rich Glycoproteins. In this study an attempt was also made to relate the amounts of protein-bound 4-hydroxyproline, which occurs exclusively in the 4-hydroxyproline-rich glycoproteins of the primary cell walls of the angiosperms, i.e., extensin, arabinogalactan protein, and salt-extractable glycoproteins (Lamport, 1977; Fincher et al., 1983; McNeil et al., 1984; Cooper et al., 1987; Cassab and Varner, 1988), to the contents of these extracellular matrix proteins in soybean seeds. Previous studies (Zarkadas et al., 1988c, 1990) have shown that a general method to calculate the amount of a specific protein j present in animal or plant tissue from the quantitative determination of a given unique amino acid i known to occur exclusively in the specific protein (j) was

$$P_j = C_i\frac{1000}{n'_i}\frac{\mathrm{WE}(P_j)}{M_r(i)} \qquad (4a)$$

where P_j is the concentration of a specific primary cell wall glycoprotein j, i.e., extensin (expressed in grams per kilogram of total protein), C_i is the mean concentration of a unique protein-bound amino acid, i, [i.e., Pro(4-OH), in grams per kilogram of total protein], $\mathrm{WE}(P_j)$, is the weight equivalent of a specific protein j determined from its known amino acid composition according to the method of Horstmann (1979), and n'_i is the number of residues of a unique amino acid residue i per 1000 amino acid residues.

Since the 86-kDa carrot extensin monomer has been the best characterized (Cooper et al., 1987; Cassab and Varner, 1988), its amino acid composition as reported by Stuart and Varner (1980) and Van Holst and Varner (1984) was used as a standard for quantitating the 4-hydroxyproline-rich glycoprotein content in soybean seeds. This quantitation is based on three major findings: first, the 86-kDa carrot monomer (ext-1) consists of 35% protein and 65% carbohydrate; second, the 30-kDa protein moiety contains 302 amino acids in its primary sequence (Chen and Varner, 1985a,b; Smith et al., 1986) and has a calculated mean residue weight of WE = 0.1095 μg/nmol (Horstmann, 1979); third, Pro(4-OH) makes up 45.5% of the polypeptide backbone, corresponding to 455 4-hydroxyproline residues/1000 amino acid residues. The anhydrous $M_r(i)$ of Pro(4-OH) is 113.12. Substituting the computed parameters for extensin in eq 4a, the total 4-hydroxyproline-rich glycoproteins in grams per kilogram of total protein in soybean seeds was calculated according to the

method of Khanizadeh et al. (1989) by the following convention:

$$\text{amt of extensin } (P_{ext\text{-}1}) = \text{amt of Pro(4-OH)} \times 2.128 \quad (4b)$$

Statistical Analysis. Data processing of the results was carried out by a FORTRAN computer program developed for this purpose. Analysis of variance, conducted on the amino acid data, for a completely randomized block design (factorial) was done by the general linear model procedure (SAS, 1982), and represents the average values from eight subsamples per genotype.

RESULTS AND DISCUSSION

The average protein content and amino acid composition of Maple Arrow, a cultivar with protein levels typical of most adapted soybeans, and OT89-16, an experimental high-protein line developed by three cycles of crossing and back crossing a high-protein nonrecurrent parent to Maple Arrow, and the levels of statistical significance obtained from the analyses of variance are summarized in Table I and represent the average values of four replicates ($N = 4$). The least variability in amino acid content was found when the results were expressed as grams of anhydrous amino acid residues per kilogram of protein, since the influences of both fat and moisture were eliminated (Tristram and Smith, 1963; Eastoe, 1967; Zarkadas et al., 1988a,b). This method of expressing results allows comparisons to be made between the results from this study and those given in food-compositional tables and is in accord with the recommendations of the Joint FAO/WHO Expert Consultation Group (FAO/WHO, 1990), who have suggested that amino acid data be reported as milligrams of amino acids per gram of protein or as grams of amino acids per gram of nitrogen. The best estimate of the protein content in each of these two soybean cultivars was therefore made by the summation of the weights of their amino acid residues. The average weight equivalent (WE, in micrograms per nanomole) and conversion factors CF and CF′ obtained (in micrograms per nanomole) are also listed in Table I. These values can be used in subsequent quantitations of such plant tissues since the values for these constants of both genotypes do not vary significantly.

The data presented in Table I indicate that an increase in seed protein from 33.67 to 42.11% was accomplished in three cycles of crossing and back crossing, which represents an increase of 25.1% in protein over the recurrent parent, Maple Arrow. This corresponds to an increase of 8.4 g of protein/100 g of soybean sample, which is significantly higher than the 3.3% protein increase reported by Brim and Burton (1979) in five cycles of recurrent selection for high protein.

Brim and Burton (1979) and Burton et al. (1982) reported an increase in soybean protein content from 42.8 to 46.1%. These values are considerably higher than the 33.63% seed protein found for Maple Arrow in this study. However, their values were determined using the conventional Kjeldahl nitrogen procedure or by infrared grain quality analysis calibrated with Kjeldahl nitrogen determinations. Large differences in reported seed protein content were noted previously when other leguminous seeds and soybean protein products were analyzed according to the Kjeldahl nitrogen method and quantitative amino acid analyses (Zarkadas et al., 1988c). For example, the protein content of protein concentrates and isoelectric isolates, two commercial soybean protein products as determined by the Kjeldahl nitrogen procedure, were 70 and 90% total protein, respectively. Precise quantitation of the protein contents of these soybean products by amino acid analysis, however, indicated that soybean protein concentrate and isolate contained only 59.6 and 76.2% protein, respectively (Zarkadas et al., 1988a). The most likely explanation for the large differences in these values is that the Kjeldahl nitrogen procedure does not differentiate between the nitrogen derived from proteins and that originating from the nonprotein nitrogenous compounds present in these soybean products (Benedict, 1987). For this reason it has been suggested that because the conventional Kjeldahl nitrogen procedure tends to overestimate the protein content of both plant and animal tissues, it should be used only for estimates of crude protein content (Morries, 1983). It appears from this study that the best estimate of the protein content in each of these two soybean cultivars can be made by the summation of the weights of the amino acid residues in these cultivars, as described by Horstmann (1979).

The amino acid profiles of the two soybean cultivars evaluated in this study (Table I) appeared to be very similar in composition, although significant differences were noted for a few amino acids. The following features were found to be common to the total protein content of both cultivars: (1) Glutamic acid is the most abundant amino acid, followed by aspartic acid, and when added together these two amino acids represent 25.9–28.2%. (2) The acidic amino acids constitute approximately one in four of the total amino acids present, compared to the basic amino acids which constitute one in five. (3) The amino acids with hydrophobic side chains account for a further 19.0–20.0% of the total protein compared to the mean values for total aromatic amino acids, which ranged from 9.1 to 9.8%. (4) Mean values for proline accounted for a further 5.2–5.3%. The mean values for the above amino acids in the present study are in agreement with those reported by Cavins et al. (1972) and Kellor (1974) for defatted soybean flour and grits, with respect to both the amino acid composition as a whole and many of the individual values reported. Moreover, these results indicate that the effect of genotypes in soybean seed amino acid content was not statistically significant for the above amino acids. However, when comparisons were made of the weighted mean values of the amino acid composition between the two genotypes evaluated, it was found that between genotypes variation was highly significant ($P < 0.01$) only for valine and that the variation in the 4-hydroxyproline concentration of the total protein was statistically significant at the $P < 0.05$ level. The mean value obtained for 4-hydroxyproline in Maple Arrow exceeded that of the high-protein genotype by 20.0%, whereas the valine levels of Maple Arrow were some 5.9% higher than that of the OT89-16 line.

The data presented in Table I indicate that as a result of three cycles of crossing and selection, the methinoine content of the seed protein decreased from 10.7 to 9.64 g/kg of soybean protein in OT89-16, a decrease of 9.9% compared to the recurrent parent, Maple Arrow. At present it is not known whether the observed reduction in methionine content was a result of a decrease in methionine-containing proteins or a reduction in methionine in the polypeptide chain of other storage proteins in the soybean seeds or both. Further studies will be needed to explain these results at the molecular level.

The levels of methionine found in this study are in agreement with those reported by Kellor (1974) for defatted soybean flour and grits and by Burton et al. (1982) from their recurrent selection studies for increasing percent protein in soybeans. However, Burton et al. (1982) reported that from recurrent cycles from four soybean populations grown in two consecutive years methionine concentration per 100 g of protein in one year ranged from

Table II. Comparison of the Essential Amino Acid (EAA) Composition of Two Soybean Genotypes, Two Soybean Products, and Two High-Quality Animal Proteins with the Suggested EAA Requirements for Humans and Broiler Chickens

	EAA requirements		essential amino acids					
			soybean line		soybean product		animal product	
amino acid	preschool child[a] (2–5 years)	broiler chickens[b]	Maple Arrow	OT89-16	concentrate	isolate	egg[a]	cow's milk[a]
			Milligrams of Amino Acid per Gram of Total Protein[d]					
histidine	19	<19	34	32	29	32	22	27
isoleucine	28	<27	51	50	54	49	54	47
leucine	66	<58	82	79	82	81	86	95
lysine	58	48	68	62	64	65	70	78
methionine + cyst(e)ine	25	32	36	33	27	23	57	33
phenylalanine + tyrosine	63	<61–62	98	91	88	86	93	102
threonine	34	28–29	42	42	38	34	47	44
tryptophan	11	<7.8	13	12	13	11	17	14
arginine		<42						
valine	35	38–39	54	51	63	56	66	64
total	339		479	453	458	437	512	504
			Percent Protein Digestibility in Man[d]					
			86	86	95	98	97	95
			Amino Acid Score Adjusted for Digestibility					
			99	93			119	119

[a] Data from FAO/WHO (1985). [b] Data from Woodham and Deans (1975). According to these authors the avian species has an additional requirement for glycine (27–28 mg/g of dietary protein) as an essential amino acid. [c] Data from Zarkadas et al. (1988c). [d] Calculation of protein rating was carried out by comparison of the amino acid composition of the soybean cultivars with that of the reference pattern established by FAO/WHO (1985) from eq 7 [100 × concentration of AA in product (mg/g of protein)/concentration of AA in FAO/WHO pattern (mg/g of protein)]; the EAA are isoleucine, leucine, lysine, methionine and cyst(e)ine, phenylalanine and tyrosine, threonine, tryptophan, valine, and histidine.

1.06 to 1.15 g compared to values ranging from 1.21 to 1.33 g determined the following year. These large differences in methionine content were not statistically significant, and the authors suggested that such differences from year to year could be a result of environmental factors and not genetic differences. They also suggested that the lack of correlation between percent protein in the seeds and the methionine concentration in the protein is evidence that protein quality is not likely to decrease significantly as a result of selection for higher protein.

The presence of small amounts (1.40 and 1.09 g/kg of protein for Maple Arrow and OT89-16, respectively) of the unique amino acid Pro(4-OH) in the acid hydrolysates of both soybean cultivars (Table I) is highly significant, since it may reflect compositional differences in the primary cell walls of these cultivars. 4-Hydroxyproline was once thought to be unique to collagen and elastin (Eastoe, 1967). However, there is evidence to suggest that Pro(4-OH) is a constituent of the 4-hydroxyproline-rich glycoproteins found in the primary cell walls of plants (angiosperms) (Lamport, 1977; McNeil et al., 1984; Cooper et al., 1987; Cassab and Varner, 1988; Varner and Lin, 1989) and seeds including the cell walls of soybean seed coats (Cassab et al., 1985; Cassab and Varner, 1987, 1988), developing soybean tissues (Ye and Varner, 1991), and the cell walls of wounded and infected plants (Corbin et al., 1987). The Pro(4-OH) in the primary sequence of the 30-kDa glycoprotein moiety of carrot extensin (Stuart and Varner, 1980; Van Holst and Varner, 1984; Chen and Varner, 1985a,b) has been used as a standard for comparison in the present study (Cassab and Varner, 1988; Varner and Lin, 1989). Using this, it has been possible to calculate the content of 4-hydroxyproline-rich glycoproteins of soybean seeds by multiplying the amounts of Pro(4-OH) found in their acid hydrolysates by 2.128 (eq 4b), as described previously (Khanizadeh et al., 1989). The concentration of the 4-hydroxyproline glycoproteins found in soybean seeds was very small, ranging from 2.32 to 2.98 g of the glycoprotein/kg of total protein in OT89-16 and Maple Arrow, respectively. The actual 4-hydroxyproline-rich glycoprotein content in both cultivars corresponds to 0.10% on a dry weight basis.

The present data (Table I) also indicate that both soybean cultivars evaluated in this study contain all of the essential amino acids (EAA) required for human or animal nutrition with methionine, followed by tryptophan, as the major limiting amino acid. Mean values for total EAA ranged from 2980 mg of EAA/g of N (N calculated from amino acid nitrogen) in the high-protein genotype, OT89-16, to 3074 mg of EAA/g of N found in Maple Arrow, which are lower than the values for cow's milk (3200 mg/g of N) and hen's whole egg (3215 mg/g of N) (FAO/WHO, 1965, 1973). Similar results were obtained from the EAA indices and chemical scores, determined according to the methods of Block and Mitchell (1946) and Oser (1951).

Lee et al. (1978) and Pellett and Young (1984, 1988) attempted to classify the EAA for predicting the nutritional quality of meats and meat products. They grouped the EAA either as 7 or 10 amino acids. The 7 (EAA_7) were isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine. The 10 (EAA_{10}) were these 7 plus tryptophan, histidine, and arginine. Mean values for total EAA_7 and EAA_{10} ranged from 33.2 to 35.4% and from 45.8 to 47.8%, respectively, in the selected soybean genotypes evaluated (Table I). These results are consistent with those listed by Pellett and Young (1984) for soybean products. The protein efficiency ratios (PER) of soybean seeds were also calculated from amino acid data (Table I). Using the prediction, eqs 5 (EAA_7) and 6 (EAA_{10}) both showed mean PER values close to a calculated value of 2.7 for soybean proteins, which is considerably higher than the average PER of 2.3 reported by others for soybeans (Torun et al., 1981; Bodwell et al., 1980; Wayler et al., 1983).

However, as these predictive tests fail to take into account differences in the digestibility and availability of individual amino acids, more reliable methods (Alsmeyer et al., 1974; McLaughlan et al., 1980; Sarwar, 1984; Expert Work Group (FSIS), 1984; FAO/WHO/UNU, 1985; Young, 1987; Young et al., 1989; Millward et al., 1989) have been developed to assess the nutritive value of proteins. The FAO/WHO/UNU Expert Consultant Group (FAO/WHO/UNU, 1985; FAO/WHO, 1990) adapted some of these approaches and recommended that an amino acid scoring

procedure, based on the amino acid composition and corrected for true digestibility of protein or bioavailability of amino acids, should be the preferred method for assessing protein quality of plant and animal proteins. In this study the protein ratings of the soybean cultivars were calculated and compared with that of the reference pattern established by FAO/WHO/UNU (1985) for four different age groups (infants, 2–5-year-old children, 10–12-year-old children, and adults). They recommended that, in conjunction with in vivo protein digestibility data, the most appropriate approach would be to use amino acid values for the 2–5-year-old child as the reference pattern (Table II) in the evaluation of mixed diets for all persons except infants. The essentiality of amino acids is dependent upon the requirements of the specific animal species in question. This amino acid scoring method is based on the nine essential amino acids (EAA_9) required by humans: histidine, isoleucine, leucine, lysine, methionine and cyst(e)ine, phenylalanine and tyrosine, threonine, tryptophan, and valine. Since cyst(e)ine and tyrosine can partially replace methionine and phenylalanine, respectively, the two sulfur-containing [methionine plus cyst(e)ine] and two aromatic amino acids (phenylalanine and tyrosine) are usually considered together. The data of Table II compare these requirements with those of avian species, which include these nine amino acids plus glycine and arginine. The results indicate that soybean proteins are a good source of all amino acids with the exception of methionine and that the amino acid score adjusted for digestibility is almost as high as that of milk and egg proteins (Table II).

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NOTE ADDED IN PROOF

The new high-protein soybean cultivar, OT89-16, has now been registered as AC Proteus.

LITERATURE CITED

Alsmeyer, R. H.; Cunningham, A. E.; Happich, M. L. Equations Predict PER from Amino Acid Analysis. *Food Technol.* **1974**, *28*, 34–40.

Averyart-Fullard, V.; Datta, K.; Marcus, A. A Hydroxyproline-Rich Protein in the Soybean Cell Wall. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1082–1085.

Beachy, R. N.; Jarvis, N. P.; Barton, K. A. Biosynthesis of Subunits of the Soybean 7S Storage Protein. *J. Mol. Appl. Genet.* **1981**, *1*, 19–27.

Benedict, R. C. Determination of Nitrogen and Protein Content of Meat and Meat Products. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 69–74.

Berg, P. A. Determination of 3- and 4-Hydroxyproline. *Methods Enzymol.* **1982**, *82*, 372–398.

Berkowitz, D. B.; Webert, D. W. Determination of Soy in Meat. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 85–90.

Block, R. J.; Mitchell, H. H. The Correlation of the Amino Acid Composition of Proteins with their Nutritive Value. *Nutr. Abstr. Rev.* **1946**, *16*, 249–278.

Bodwell, C. E. Human Animal Assays. In *Soy Protein and Human Nutrition*; Wilke, H. L., Hopkins, D. T., Waggle, D. H., Eds.; Academic Press: New York, 1979; pp 331–339.

Bodwell, C. E.; Satterlee, L. D.; Hackler, L. R. Protein Digestibility of the Same Protein Preparations by Humans and Rat Assays and by *in Vitro*, Enzymic Digestion Methods. *Am. J. Clin. Nutr.* **1980**, *33*, 677–686.

Brim, C. A.; Burton, J. W. Recurrent Selection in Soybeans. II. Selection for Increased Percent Protein in Seeds. *Crop Sci.* **1979**, *19*, 494–498.

Burton, J. W.; Purcell, A. E.; Walter, W. M., Jr. Methionine Concentration in Soybean Protein from Populations Selected for Increased Percent Protein. *Crop Sci.* **1982**, *22*, 430–432.

Cassab, G. I.; Varner, J. E. Immunocytolocalization of Extensin in Developing Soybean Seed Coats by Immunogold-Silver Staining and by Tissue Printing on Nitrocellulose Paper. *J. Cell Biol.* **1987**, *105*, 2581–2588.

Cassab, G. I.; Varner, J. E. Cell Wall Proteins. *Annu. Rev. Plant Physiol. Mol. Biol.* **1988**, *39*, 321–353.

Cassab, G. I.; Nieto-Sotelo, J.; Cooper, J. B.; Van Holst, G. J.; Varner, J. E. A Developmentally Regulated Hydroxyproline-Rich Glycoprotein from the Cell Walls of Soybean Seed Coats. *Plant Physiol.* **1985**, *77*, 532–535.

Cavins, J. F.; Kwolek, W. E.; Inglett, G. E.; Cowan, J. C. Amino Acid Analysis of Soybean Meal: Interlaboratory Study. *J. Assoc. Off. Anal. Chem.* **1972**, *55*, 686–691.

Chen, J.; Varner, J. E. Isolation and Characterization of cDNA Clones for Carrot Extensin and a Protein-Rich 33 kDa Protein. *Proc. Natl. Acad. Sci. U.S.A.* **1985a**, *82*, 4399–4403.

Chen, J.; Varner, J. E. An Extracellular Matrix Protein in Plants: Characterization of a Genomic Clone for Carrot Extensin. *EMBO J.* **1985b**, *3*, 2145–2152.

Coats, J. B.; Medeiros, J. S.; Than, V. H.; Neilsen, N. C. Characterization of the Subunits of β-Conglycinin. *Arch. Biochem. Biophys.* **1985**, *243*, 184–194.

Cook, D. A.; Jensen, A. H.; Fraley, J. R.; Hymowitz, Y. Utilization by Growing and Finishing Pigs of Raw Soybeans of Low Kunitz Inhibitore Content. *J. Anim. Sci.* **1988**, *66*, 1686–1691.

Cooper, J. B.; Chen, J. A.; Van Holst, G. J.; Varner, J. E. 4-Hydroxyproline-Rich Glycoproteins of Plant Cell Wall. *Trends Biochem. Sci.* **1987**, *12*, 24–27.

Corbin, D. R.; Sauer, N.; Lamb, C. J. Differential Regulation of a Hydroxyproline-Rich Glycoprotein Gene Family in Wounded and Infected Plants. *Mol. Cell. Biol.* **1987**, *7*, 4337–4344.

de Lumen, B. O. Molecular Approaches to Improving the Nutritional and Functional Properties of Plant Seeds as Food Sources: Developments and Comments. *J. Agric. Food Chem.* **1990**, *38*, 1779–1788.

de Lumen, B. O.; Kho, C.-J. Identification of Methionine-Containing Proteins and Quantitation of their Methionine Contents. *J. Agric. Food Chem.* **1987**, *35*, 688–691.

Doll, H. Nutritional Aspects of Cereal Properties and Approaches to Overcome Their Deficiencies. *Philos. Trans. R. Soc. London* **1984**, *B304*, 373–380.

Eastoe, J. E. *Composition of Collagen and Allied Proteins*; Ramachandran, G. H., Ed.; Treatise on Collagen I; Academic Press: New York, 1967; pp 1–72.

Eggum, B. O.; Beames, R. M. The Nutritive Value of Seed Proteins. In *Seed Proteins, Biochemistry, Genetics, Nutritive Value*; Gottschalk, W., Muller, H. P., Eds.; Martinus Nijhoff/Junk: The Hague, 1983; pp 499–531.

Erdman, J. W.; Fordyce, E. J. Soy Products and the Human Diet. *Am. J. Clin. Nutr.* **1989**, *49*, 725–737.

Expert Work Group of the Food Safety and Inspection Service (FSIS), USDA. Final Report and Recommendations. *Am. J. Clin. Nutr.* **1984**, *40*, 675–684.

FAO/WHO. *Protein Requirements*; FAO/WHO Nutrition Meeting, Report Series 37; Food and Agriculture Organization/World Health Organization: Rome, 1965.

FAO/WHO. *Energy and Protein Requirements*; FAO/WHO Nutrition Meetings, Report Series 522; Food and Agriculture Organization/World Health Organization: Rome, 1973.

FAO/WHO/UNU Expert Consultation. *Energy and Protein Requirements*; FAO/WHO Nutrition Meetings, Report Series 724; Food and Agriculture Organization/World Health Organization: Geneva, 1985.

FAO/WHO Expert Consultation. *Protein Quality Evaluation*; FAO/WHO Nutrition Meetings, Report Series 51; Food and Agriculture Organization/World Health Organization: Rome, 1990.

Fehr, W. R. Breeding Methods for Cultivar Development. In *Soybeans: Improvement, Production and Uses*, 2nd ed.; Wilcox, J. R., Ed.; Agronomy Monograph 16; American Society of Agronomy: Madison, WI, 1987; pp 249–293.

Fincher, G. B.; Stone, B. A.; Clarke, A. E. Arabinogalactan Proteins: Structure, Biosynthesis, and Function. *Annu. Rev. Plant Physiol.* **1983**, *34*, 47–70.

Fomon, J. J.; Ziegler, E. E. Soy Protein Isolates in Infant Feeding. In *Soy Protein and Human Nutrition*; Wilke, H. L., Hopkins, D. T., Waggle, D. H., Eds.; Academic Press: New York, 1979; pp 79–96.

Friedman, M.; Bandon, D. L.; Bates, A. H.; Hymowitz, T. Comparison of a Commercial Soybean Cultivar and an Isoline Lacking the Kunitz Trypsin Inhibitor: Composition, Nutritional Value, and Effects of Heating. *J. Agric. Food Chem.* **1991**, *39*, 327–335.

George, A. A.; de Lumen, B. O. A Novel Methionine-Rich Protein in Soybean Seed: Identification, Amino Acid Composition, and N-Terminal Sequence. *J. Agric. Food Chem.* **1991**, *39*, 224–227.

Harada, J. J.; Barker, S. J.; Goldberg, R. B. Soybean β-Conglycinin Genes Are Clustered in Several DNA Regions and Are Regulated by Transcriptional and Posttranscriptional Processes. *Plant Cell* **1989**, *1*, 415–425.

Hildebrand, D. F. Lipoxygenases. *Physiol. Plant* **1989**, *76*, 249–253.

Hildebrand, D. F.; Hymowitz, T. Two Soybean Genotypes Lacking Lipoxygenase-1. *J. Am. Oil Chem. Soc.* **1981**, *58*, 583–586.

Hill, J. E.; Breidenbach, R. W. Proteins of Soybean Seeds. I. Isolation and Characterization of the Major Components. *Plant Physiol.* **1974a**, *53*, 742–746.

Hill, J. E.; Breidenbach, R. W. Proteins of Soybean Seeds. II. Accumulation of the Major Components During Seed Development and Maturation. *Plant Physiol.* **1974b**, *53*, 747–751.

Holowach, L. P.; Madison, J. T.; Thompson, J. F. Studies on the Mechanism of Regulation of the mRNA level for a Soybean Storage Subunit by Exogenous L-Methionine. *Plant Physiol.* **1986**, *80*, 561–567.

Hong, J. C.; Nagao, R. T.; Key, J. L. Characterization and Sequence Analysis of a Developmentally Regulated Putative Cell Wall Protein Gene Isolated from Soybean. *J. Biol. Chem.* **1987**, *262*, 8367–8376.

Horstmann, J. H. A Precise Method for the Quantitation of Proteins Taking Into Account Their Amino Composition. *Anal. Biochem.* **1979**, *96*, 130–138.

Hugli, T. E.; Moore, S. Determination of the Tryptophan Content of Proteins by Ion Exchange Chromatography of Alkaline Hydrolysates. *J. Biol. Chem.* **1972**, *247*, 2828–2834.

Hymowitz, T. Genetics and Breeding of Soybeans Lacking the Kunitz Trypsin Inhibitor. In *Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods*; Friedman, M., Ed.; Plenum Press: New York, 1986; pp 291–298.

Jaynes, J.; Yang, M.; Espinoza, M.; Dodds, J. H. Plant Protein Improvement by Genetic Engineering: Use of Synthetic Genes. *Trends Biotechnol.* **1986**, *4*, 314–320.

Kelley, J. F. Increasing Protein Quantity. In *Nutritional Improvement of Food Legumes by Breeding*; Milner, M., Ed.; Protein Advisory Group of the United Nations System, UN: New York, 1973; pp 179–184.

Kellor, R. L. Defatted Soy Flour and Grits. *J. Am. Oil Chem. Soc.* **1974**, *51*, 77A-80A.

Khanizadeh, S.; Buszard, D.; Zarkadas, C. G. Seasonal Variation of Proteins and Amino Acids in Apple Flower Buds (*Malus pumila* Mill., cv. McIntoch/M7). *J. Agric. Food Chem.* **1989**, *37*, 1246–1252.

Kinsella, J. Functional Properties of Soy Proteins. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242–258.

Koshiyama, I. Storage Proteins of Soybean. In *Seed Proteins: Biochemistry, Genetics, Nutritive Value*; Gottschalk, W., Muller, H. P., Eds.; Martinus Nijhoff/Junk: The Hague, 1983; pp 427–450.

Lamport, D. T. A. Structure, Biosynthesis and Significance of Cell Wall Glycoproteins. In *Recent Advances in Phytochemistry*; Loewus, F. A., Runeckless, V. C., Eds.; Plenum Press: New York, 1977; Vol. II, pp 79–115.

Lee, Y. B.; Elliott, J. G.; Rickansrud, D. A.; Hagberg, E. C. Predicting Protein Efficiency Ratio by the Chemical Determination of Connective Tissue Content in Meat. *J. Food Sci.* **1978**, *43*, 1359–1362.

McLaughlan, J. M.; Anderson, G. H.; Hackler, L. R.; Hill, D. C.; Jansen, G. R.; Keith, M. O.; Sarwar, G.; Sosulski, F. W. Assessment of Rat Growth Methods for Estimating Protein Quality: Interlaboratory Study. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 462–467.

McNeil, M.; Darville, A. G.; Fry, S. C.; Albersheim, P. Structure and Function of The Primary Cell Walls of Plants. *Annu. Rev. Biochem.* **1984**, *53*, 625–663.

Millward, D. J.; Jackson, A. A.; Price, G.; Rivers, J. P. W. Human Amino Acid and Protein Requirements: Current Dilemmas and Uncertainties. *Nutr. Res. Rev.* **1989**, *2*, 109–132.

Moore, S. On the Determination of Cystine as Cysteic Acid. *J. Biol. Chem.* **1963**, *238*, 235–237.

Morries, P. A Century of Kjeldahl (1883–1983). *J. Assoc. Public Anal.* **1983**, *21*, 53–58.

Oser, B. L. Method for Integrating Essential Amino Acid Content in the Nutritional Evaluation of Protein. *J. Am. Diet. Assoc.* **1951**, *27*, 396–402.

Ozols, J. Amino Acid Analysis. *Methods Enzymol.* **1990**, *182*, 587–601.

Pellett, P. L.; Young, V. R. Background Paper 4: Evaluation of the Use of Amino Acid Composition Data in Assessing the Protein Quality of Meat and Poultry Products. *Am. J. Clin. Nutr.* **1984**, *40*, 718–736.

Pellett, P. L.; Young, V. R. Protein and Amino Acid Needs for Adults. *Ecol. Food Nutr.* **1988**, *21*, 321–330.

Rackis, J. J.; McGhee, J. E.; Booth, A. N. Biological Threshold Levels of Soybean Trypsin Inhibitor by Rat Bioassay. *Cereal Chem.* **1975**, *52*, 85–92.

Sarwar, G. Available Amino Acid Score for Evaluating Protein Quality of Foods. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 623–626.

Scrimshaw, N. S.; Young, V. R. Soy Protein in Adult Human Nutrition: A Review With New Data. In *Soy Protein and Human Nutrition*; Wilke, H. L., Hopkins, D. T., Waggle, D. H., Eds.; Academic Press: New York, 1979; pp 121–143.

Siedow, J. N. Plant Lipoxygenase: Structure and Function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 145–188.

Smith, J. J.; Muldoon, E. P.; Willard, J. J.; Lamport, D. T. A. Tomato Extensin Precursors P1 and P2 are Highly Periodic Structures. *Phytochemistry* **1986**, *25*, 1021–1030.

Smith, K. J.; Huyser, W. World Distribution and Significance of Soybean. In *Soybeans, Improvement, Production, and Uses*; Wilcox, J. R., Ed.; Agronomy Monograph 16; American Society of Agronomy: Madison, WI, 1987; pp 1–22.

SAS (Statistical Analysis System). *User's Guide: Basics*; SAS Institute: Cary, NC, 1982.

Stuart, D. A.; Varner, J. E. Purification and Characterization of Salt Extractable Hydroxyproline-Rich Glycoproteins from Aerated Carrot Discs. *Plant Physiol.* **1980**, *66*, 787–792.

Swaminathan, M. S. Relevance of Protein Improvement in Plant Breeding. In *Seed Proteins: Biochemistry, Genetics, Nutritive Value*; Gottschalk, W., Muller, H. P., Eds.; Martinus Nijhoff/Junk: The Hague, 1983; pp 1–23.

Than, V. H.; Shibasaki, K. Major Proteins of Soybean Seeds: Subunit Structure of β-Conglycinin. *J. Agric. Food Chem.* **1978**, *26*, 692–695.

Toenniessen, G. H. Plant Molecular Biology and the International Agricultural Research System. *Plant Mol. Biol. Rep.* **1985**, *3*, 1–11.

Torun, B.; Vitery, F. E.; Young, V. R. Nutritional Role of Soya Protein for Humans. *J. Am. Oil Chem. Soc.* **1981**, *58*, 400–406.

Tristram, G. R.; Smith, R. H. The Amino Acid Composition of Some Purified Proteins. *Adv. Protein Chem.* **1963**, *18*, 227–328.

Van Holst, G. J.; Varner, J. E. Reinforced Polyproline II Conformation in Hydroxyproline-Rich Cell Wall Glycoproteins from Carrot Root. *Plant Physiol.* **1984**, *73*, 247–251.

Varner, J. E.; Lin, L.-S. Plant Cell Wall Architecture. *Cell* **1989**, *56*, 231–239.

Voldeng, H. D.; Saidon, G. Registration of a High Protein Soybean Gerplasm Line OT89-16. *Crop Sci.* **1991**, *31*, 1100.

Wayler, A.; Queiroz, E.; Scrimshaw, N. S.; Steinke, F. H.; Rand, W. M.; Young, V. R. Nitrogen Balance in Young Men to Assess the Protein Quality of an Isolated Soy Protein in Relation to Meat Proteins. *J. Nutr.* **1983**, *113*, 2485–2491.

Wilson, R. F. Seed Metabolism. In *Soybean: Improvements, Production and Uses*, 2nd ed.; Wilcox, J. R., Ed.; Agronomy Monograph 16; American Society of Agronomy: Madison, WI, 1987; pp 643–686.

Wolf, W. J. Edible Soybean Protein Products. In *Handbook of Processing and Utilization in Agriculture*; Wolff, I. A., Ed.; CRC Boca Raton, FL, 1982; Vol. II, pp 23–55.

Woodham, A. A.; Deans, P. S. Amino Acid Requirements of Growing Chickens. *Br. Poult. Sci.* **1975**, *16*, 269–287.

Yang, V. R.; Espinoza, N. O.; Nagpala, P. G.; Dodds, J. H.; White, F. F.; Schnorr, K. L.; Jaynes, J. M. Expression of a Synthetic Gene for Improved Protein Quality in Transformed Potato Plants. *Plant Sci.* **1989**, *64*, 99–111.

Ye, Z.-H.; Varner, J. E. Tissue-Specific Expression of Cell Wall Proteins in Developing Soybean Tissues. *Plant Cell* **1991**, *3*, 23–37.

Young, V. R. Kinetics of Human Amino Acid Metabolism: Nutritional Implications and Some Lessons. *Am. J. Clin. Nutr.* **1987**, *46*, 709–725.

Young, V. R.; Bier, D. L.; Pellett, P. L. A Theoretical Basis for Increasing Current Estimates of the Amino Acid Requirements in Adult Man, with Experimental Support. *Am. J. Clin. Nutr.* **1989**, *50*, 80–92.

Zarkadas, C. G. A Simple Chromatographic Method for the Determination of the Methylated Basic Amino Acids in Proteins. *Can. J. Biochem.* **1975**, *53*, 96–101.

Zarkadas, C. G.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D.; Nguyen, Q. Rapid Methods for Determining Desmosine, Isodesmosine, 5-Hydroxylysine, Tryptophan, Lysinoalanine and the Amino Sugars in Proteins and Tissues. *J. Chromatogr.* **1986**, *378*, 67–76.

Zarkadas, C. G.; Rochemont, J. A.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D. Determination of Methylated Basic, 5-Hydroxylysine, Elastin Crosslinks, Other Amino Acids, and the Amino Sugars in Protein and Tissues. *Anal. Biochem.* **1987**, *160*, 251–266.

Zarkadas, C. G.; Drouliscos, N.; Karatzas, C. J. Comparison of the Total Protein, Nitrogen and Amino Acid Composition of Selected Additives and Ingredients Used in Composite Meat Products. *J. Agric. Food Chem.* **1988a**, *36*, 1131–1146.

Zarkadas, C. G.; Karatzas, C. N.; Khalili, A. D.; Khanizadeh, S.; Morin, G. Quantitative Determination of the Myofibrillar Proteins and Connective Tissue Content in Selected Porcine Skeletal Muscle. *J. Agric. Food Chem.* **1988b**, *36*, 1131–1146.

Zarkadas, C. G.; Meighen, E. A.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D.; Rochemont, J. A.; Berthelet, M. The Determination of the Myofibrillar and Connective Tissue Proteins in the Bovine Diaphragm. *J. Agric. Food Chem.* **1988c**, *36*, 1095–1109.

Zarkadas, C. G.; Meighen, E. A.; Rochemont, J. A.; Zarkadas, G. C.; Khalili, A. D.; Nguyen, Q. Determination of Methylated Basic Amino Acids, 5-Hydroxylysine, and Elastin Crosslinks in Proteins and Tissues. In *Amino Acids: Chemistry, Biology and Medicine*; Lubec, G., Rosenthal, G. A., Eds.; ESCOM Science Publishers: Leiden, The Netherlands, 1990; pp 201–216.

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