Assessment of the Protein Quality of Nine Northern Adapted Yellow and Brown Seed Coated Soybean Cultivars by Amino Acid Analysis

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Accurate and detailed amino acid determinations were carried out on nine northern adapted soybean cultivars to ascertain whether their amino acid profiles could be used as potentially useful indices for assessing their protein quality. The cultivars were Maple Amber, Maple Donovan, Maple Glen, Maple Isle, Maple Presto, Maple Ridge, and three brown seed coat near-isogenic lines, Maple Presto Brown, Maple Ridge Brown, and Maple Arrow Brown. Their total protein and amino acid composition were compared with those of an established cultivar, Maple Arrow. Mean protein values for the new cultivars ranged from 30.1 to 33.1% compared to Maple Arrow, which was 33.2%. The total nitrogen content was also variable among these cultivars, ranging from 5.0 to 5.4%. All nine Maple series soybean cultivars were higher in their essential amino acid (EAA) content, that is, EAA_9 = 45.2−46.5%, than the FAO/WHO reference protein pattern value of EAA_9 = 33.9%, for a 2−5-year-old child. Each of the nine new soybean cultivars was limited only in methionine and to a lesser extent in valine and isoleucine and had a protein digestibility corrected amino acid score of 91% for all cultivars, compared to the value of egg protein (97%). These results suggest that the most accurate evaluation of protein quality in soybeans, and possibly other legumes and cereals, is by the protein digestibility-corrected amino acid score.

Keywords: *Soybeans [Glycine max (L.) Merr.]; assessment; protein quality; amino acids; composition; amino acid score*

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.], a major source of proteins, energy, and other nutrients for both humans and livestock, is an annual leguminous plant that originated in the warm climate of northeastern China (Hymowitz and Singh, 1987; Smith and Huyser, 1987). In recent years considerable efforts have been made by soybean breeders to develop productive soybean cultivars that will germinate, grow, and mature in more northern Canadian latitudes (latitude >45° N), which have long day lengths (>16 h) and short growing seasons (Beversdorf et al., 1995). Genetic improvements have led to the development of early flowering and maturing, cold-tolerant soybean genotypes (Voldeng and Saindon, 1991a,b), with improved yields, pest resistance, and higher seed quality and protein content (Buzzell and Voldeng, 1980; Saindon et al., 1989a,b, 1990; Voldeng and Saindon, 1991a,b; Frederick and Hesketh, 1994). As a result of these breeding efforts, several new cultivars are being widely grown in the more temperate regions of Canada, from Manitoba, through Ontario, and into the Atlantic Provinces.

Most soybean varieties at maturity contain approximately 30−42% protein, 18−20% oil, and 12% nonstructural carbohydrate on a dry weight basis (Burton, 1987; Pazdernik et al., 1996; Brummer et al., 1997). The major reserve proteins of soybean are glycinin and β-conglycinin, which together make up 70−80% of the total protein of the mature seed (Neilsen et al., 1995, 1997). Glycinin and β-conglycinin consist of six and three major subunits, respectively, with the β-conglycinin (Nam et al., 1997; Jung et al., 1997; 1998), which accounts for ∼25% of the total protein in soybeans (Coats et al., 1985), being practically devoid of methionine (Than and Shibasaki, 1978; Holowach et al., 1986). The enzymes involved in metabolism make up another 1% of the total seed protein. The remaining 8−10% is composed of lipoxygenases, the Kunitz trypsin inhibitor, Bowman−Birk and related protease inhibitors, lectin, and urease (Kakade et al., 1973; Nielsen, 1984, 1996; Wolf, 1982, 1992; Wilson, 1987; Liener, 1979, 1995).

Brim and Burton (1979) and Escalante and Wilcox (1993a,b) have indicated that increased protein content in soybean seeds is inversely correlated with yield and that an inverse correlation also exists between oil and protein and between carbohydrate and protein accumulation (Burton, 1987). Hartwig and Hinson (1972) and Werman et al. (1987) have had limited success improving seed protein content using backcrossing, a breeding method used to introduce simply inherited traits into a selected parent. Recurrent selection of soybeans has successfully been used by Brim and Burton (1979) as a procedure for increasing the percent protein in soybean seeds, and they have reported an increase in protein content from 46.3% in the initial parental population to 48.4% after six cycles of selection, without significantly reducing yield. This procedure, however, has not increased methionine levels (Burton et al., 1982). A comparison of the protein content and amino acid composition of several northern adapted

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cultivars has been reported by Zarkadas et al. (1993, 1994, 1997a,b). Analyses showed that total protein content of the Maple, miso and natto type soybeans varied from 30.1 to 42.1%, and all contained an excellent balance of essential amino acids required for both humans and animals. They were limited only in methionine and, to a lesser extent, in valine and isoleucine and had a protein digestibility corrected amino acid score of 91% for all cultivars, compared to the 97% value of egg protein (FAO/WHO, 1991; U.S. Food and Drug Administration, 1993). Recent direct metabolic and nitrogen balance studies in humans using various types of soybean protein products for adults, children, and infants reported values for protein quality for such products ranging from 83 to 96% (average = 93%) compared with milk (Torun, 1992; Fomon and Ziegler, 1992) and >80% of the nutritional value of egg protein (Erdman and Fordyce, 1989; Young, 1992; Young and Pellett, 1990, 1994).

It has been determined that certain of these new early maturing soybean varieties carry the recessive early maturing alleles (e1 to e7) (Buzzell and Voldeng, 1980; Saindon et al., 1989a,b, 1990; Voldeng and Saindon, 1991a,b), and most are homozygous for a dominant form of the pigment inhibitory gene and have yellow seed coats and hila. However, it was noted that some of these soybean genotypes have mutants with brown seed coat color (Voldeng and Saindon, 1991a,b). Other studies have shown that spontaneous mutations from yellow seed to dark colored seed often arise within highly inbred soybean varieties (Wilcox, 1988).

Seed coat pigmentation is known to be controlled by at least three genes, *I*, *R*, and *T*. Normally the dominant *R* allele synthesizes anthocyanins, which produce black seed coats (Buzzell et al., 1987), whereas the recessive *r* allele synthesizes proanthocyanidins, which give a brown seed coat (Todd and Vodkin, 1993). Whether or not these colors are expressed is controlled by the *I* locus, which also controls the spatial distribution of anthocyanin and proanthocyanidin pigments in the epidermal layer of the palisade cells of the soybean seed coat during development. Four *I* alleles are known, with the dominant *I* allele inhibiting pigment accumulation in the seed coat, which results in a yellow seed coat color at maturity. Both I^{l} and I^{k} alleles appear to restrict pigments to the hilum and saddle regions, respectively, compared to the homozygous recessive *I* allele, which specifies full pigmentation across the entire seed coat. The *T* gene is pleiotropic and affects both the seed coat color and structure (Bernard and Weiss, 1973; Palmer and Kilen, 1987; Wang et al., 1994; Vodkin, 1996). It has recently been shown that soybean genotypes that carry the recessive allele *t* have defective seed coats due to an epigenetic interaction between the flavonoid pathway and the proline-rich cell wall proteins, which affect the structural integrity of the seed coat (Nicholas et al., 1993; Todd and Vodkin, 1993, 1996; Wang et al., 1994; Vodkin, 1996).

The objectives of the present study were to compare the levels and variation of total protein and the amino acid profiles of the nine northern adapted soybean cultivars, namely, Maple Amber, Maple Donovan, Maple Glen, Maple Isle, Maple Presto, and Maple Ridge, and the three brown seed coat near-isogenic lines, namely, Maple Presto Brown, Maple Ridge Brown, and Maple Arrow Brown, with an earlier release, Maple Arrow (Voldeng et al., 1982, 1985a,b, 1995, 1996; Voldeng and Saindon, 1991a,b), and to assess their protein quality from digestibility and amino acid compositional data (FAO/WHO/UNU, 1985; FAO/WHO, 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. 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. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Selection of Plant Materials and Sample Preparation.* The nine northern adapted Maple soybean genotypes selected for this investigation were Maple Amber, Maple Donovan, Maple Glen, Maple Isle, Maple Presto, Maple Ridge, Maple Presto Brown, Maple Ridge Brown, and Maple Arrow Brown, which were developed at the Plant Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON (Voldeng et al., 1982, 1985a,b, 1995, 1996; Voldeng and Saindon, 1991a,b). All Maple soybean genotypes are well adapted to the more northerly temperate regions of Canada (latitude > 45° N), where the low temperature in May and June ranges from 10 to 15 °C. Their pedigrees are as follows:

Maple Amber = Amsoy/Portage//840-7-3

Maple Donovan = Maple Arrow/Harcor

Maple Isle = PI194.641/2*Harosoy-e_3

Harosoy-e_3 is a Harosoy near-isogenic line carrying the e_3 allele.

Maple Glen is a soybean cultivar intended for production in 2500−2700 crop heat unit areas of eastern Canada. Maple Glen originated from the cross BD22115-13/Premier (Voldeng et al., 1996). The BD22115-13 genotype is a selection from the cross 840-7-3//Portage/Amsoy. Line 840−7-3 is an early-maturing genotype from Sweden.

Maple Ridge was originated as an F_4 plant selection from the cross between Fiskeby III and Evans (Voldeng et al., 1985a). Fiskeby III is a very early maturing cultivar from Sweden. Evans is a cultivar of USDA Maturity Group 0 and originated as an F_4 plant selection from a single-cross Merit/Harosoy.

For purposes of comparison, two established high-yielding soybean cultivars, Maple Arrow and Maple Presto, which were developed by the Agriculture Canada Research Station, Ottawa, ON, were used to evaluate these nine soybean lines. These cultivars have been widely grown in central and eastern Ontario (USDA Maturity Group 00). Maple Arrow (Voldeng and Saindon, 1991a; Zarkadas et al., 1993, 1994) originated as an F_4 plant selection from a single cross Harosoy 63/840-7-3. Line 840-7-3 is an early-maturing line from Sweden. Maple Presto, which originated as an F_4 plant selection (Voldeng et al., 1982; Voldeng and Saindon, 1991a), was identified by the experimental designation BD21117 prior to its release. The name Presto was chosen to denote the rapidity with which the plant develops and matures, with as few as 2200 crop heat units from planting to maturity.

The three cultivars Maple Arrow Brown, Maple Presto Brown, and Maple Ridge Brown have the same pedigrees as Maple Arrow, Maple Presto, and Maple Ridge, respectively, except that these near-isogenic genotypes differ in that they carry the self-color seed coat allele.

Assessment of agronomic performance of all cultivars was carried out at the Plant Research Centre, Central Experimen-

tal Farm, Ottawa, ON, and further tested in five other geographical regions in central and eastern Ontario (USDA Maturity Group 0 or 00) for 5 years, under the Ontario Soybean Variety Trials at Inkerman, Elora, Brussels, Alfred, and Bornholm, ON.

Dried seeds of the four replicate samples were all taken the same year from the same location (Plant Research Centre, Central Experimental Farm, Ottawa, ON). Each of the cultivars selected for this investigation was pulverized in a standard electrically driven end runner mill (Cyclone Sample Mill, U. D. Corp., Fort Collins, CO), passed through a 1.0 mm mesh sieve, lyophilized, and then stored at −20 °C in polypropylene bottles until used.

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on a Beckman System 6300 fully automated high-performance amino acid analyzer using single-column expanded protein hydrolysates methodology (Beckman Bulletin A 6300-AN-007, 1987). 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, as described previously (Zarkadas et al., 1987, 1990).

Preparation of Tissue Hydrolysates. Four replicates ($N = 4$) per cultivar were analyzed for the 15 acid stable basic, acidic, and neutral amino acids. Duplicate samples (0.05 g) from each these replicates were then hydrolyzed in Pyrex (No. 9860) test tubes (18 × 150 mm) under vacuum (<10 mmHg) 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. (1988b). Complete amino acid analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate (64 determinations) according to methods described previously (Zarkadas et al., 1986, 1988b). Norleucine was added in the hydrolysates as an internal standard.

Methionine and cyst(e)ine were determined separately in each of the four replicates per cultivar as their oxidation products according to the performic acid procedure of Moore (1963). Triplicate samples (0.05 g) from each of the above replicates were first oxidized by performic acid, dried, and hydrolyzed under vacuum (<10 mmHg) with triple-glass-distilled constant-boiling HCl (6.0 M) for 24 h. Each of the hydrolysates was then analyzed in duplicate as described previously (Zarkadas et al., 1988a,b). 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. Norleucine was added in the hydrolysate as an internal standard. The data were then normalized relative to alanine, valine, leucine, and isoleucine present in the sample and represent the average of 24 determinations. The yields obtained following performic acid oxidation of these amino acid calibration standards were 105.9% for cysteic acid and 89.0% for methionine *S,S*-dioxide.

Tryptophan in soybean samples (50.0 mg) was also determined separately after alkaline hydrolysis (Hugli and Moore, 1972) on a Beckman Spinco Model 121 MB fully automated amino acid analyzer using a single-column methodology as described previously (Zarkadas et al., 1986). Triplicate samples (0.05 g) from each of the above replicates were hydrolyzed under vacuum (<10 mmHg) with 4.2 M NaOH for 24 h. Each of the dried alkaline hydrolysates was analyzed in duplicate as described previously (Zarkadas et al., 1988a,b). 3-Nitrotyrosine was used as the internal standard. The data presented in Tables 1 and 2 represent the average of 24 determinations.

Protein Determination. Precise quantitation of the protein mass in each soybean acid hydrolysates was carried out according to the method described by Horstmann (1979), Nguyen et al. (1986), and Zarkadas et al. (1988a,b). The mean residue weight, WE (in micrograms per nanomole), was calculated as

$$\mathrm{WE} = \sum_{i=1}^{18} (a_i b_i) \qquad (1)$$

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).

A conversion factor, CF (in micrograms per nanomole), for determining the protein mass in each sample analyzed in the absence of tryptophan, methionine, and cyst(e)ine was also calculated as described previously (Horstmann, 1979; Zarkadas et al., 1988a,b) as

$$\mathrm{CF} = \mathrm{WE}/[1 - (a_{\mathrm{Trp}} + a_{\mathrm{Cys}} + a_{\mathrm{Met}})] \qquad (2)$$

The protein content, P (in micrograms), of each sample was calculated by multiplying CF by the nanomoles of total amino acids in each acid hydrolysates as

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

where χ_i is the nanomoles of each amino acid i found in the analyzed aliquot. The values reported in Table 1 for the content of total protein in each of the 10 soybean cultivars investigated are the averages of 48 determinations.

Predicting Properties of Proteins from Amino Acid Compositions. Barantes (1973, 1975) has grouped the amino acids into four classes, (I) total charged, (ii) hydrophilic, (iii) hydrophobic, and (iv) apolar, and compared the ratio (R) of the frequencies of occurrence (χ) of whatever particular side chains of proteins one wishes to stress. Using the following formulas he grouped the amino acids as

$$R = \sum_k \chi_k / \sum_j \chi_j \qquad (4)$$

where k can be hydrophilic (polar) and j hydrophobic (nonpolar) side chains.

(i) Total charged = basic + acidic. The basic amino acids are histidine, lysine, and arginine. The acidic amino acids are aspartic acid, glutamic acid, asparagine, and glutamine.

(ii) Hydrophilic = total charged + threonine + serine.

(iii) Hydrophobic = valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and tryptophan.

(iv) Apolar = hydrophobic minus tyrosine.

Barrantes (1973, 1975) suggested that using the following four ratios would give an indication of shifts in the protein fractions present in the samples being compared: ratio 1 (R_1), hydrophobic/hydrophilic; ratio 2 (R_2), hydrophilic/apolar; ratio 3 (R_3), total charged/hydrophobic; ratio 4 (R_4), total charged/apolar.

For example, the side chains of charged or very hydrophilic (polar) amino acids tend to be located on the outside of the molecule. They are highly soluble in water. At the opposite end of the polarity scale are the apolar or hydrophobic side chains, which tend to have low solubility in water and are located on the inside of the protein molecule (Bigelow, 1967; Nozaki and Tanford, 1971). These ratios have also been used to measure actual differences and predict characteristic properties of proteins in plant tissues from their amino acid composition (Khanizadeh et al., 1989, 1992; Zarkadas et al., 1994).

Statistical Analysis. Data processing of the results was carried out by an EXCEL version 5 for Windows spreadsheet 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 using SAS under the windows operating system, release 6.2 (SAS, 1991), and represents the average values from eight subsamples per genotype.

RESULTS AND DISCUSSION

Accurate and detailed amino acid determinations were carried out on nine new northern adapted soybean cultivars to ascertain whether the amino acid profiles

Table 1. Comparison of the Amino Acid (AA) Composition and Protein Contents (Grams of AA per Kilogram of Total Protein; Mean ± SEM) of Nine New Northern Adapted Soybean Cultivars

	soybean cultivars[a]										signif levels among[b] cultivars			
											overall			brown vs not
AA	Maple Amber	Maple Donovan	Maple Glen	Maple Isle	Maple Presto	Maple Presto Brown	Maple Ridge	Maple Ridge Brown	Maple Arrow	Maple Arrow Brown	SEM	CV	*F*	*F*
aspartic acid	115.16 ± 1.8	110.81 ± 0.8	111.17 ± 3.3	113.83 ± 0.8	108.57 ± 2.3	108.87 ± 0.9	109.96 ± 1.6	111.22 ± 1.6	115.35± 1.8	108.81 ± 3.6	3.37	3.02	*	ns
threonine	42.86 ± 4.1	46.81 ± 2.1	43.16 ± 1.9	44.92 ± 2.1	45.06 ± 1.2	45.06 ± 4.9	43.91 ± 1.8	46.75 ± 2.9	42.44 ± 0.9	48.40 ± 2.2	5.20	11.4	ns	*
serine	46.50 ± 6.7	51.06 ± 3.7	50.75 ± 2.4	49.30 ± 3.3	43.99 ± 3.5	56.61 ± 7.4	52.98 ± 1.7	48.81 ± 4.6	48.12 ± 3.1	58.98 ± 5.6	9.42	18.5	ns	ns
glutamic acid	193.16 ± 4.0	177.61 ± 1.9	180.08 ± 3.6	178.36 ± 2.2	175.95 ± 6.0	175.17 ± 3.1	174.87 ± 2.7	180.06 ± 2.7	189.05± 5.6	175.17 ± 0.4	5.8	3.22	***	ns
proline	50.47 ± 0.6	48.31 ± 1.4	50.37 ± 1.9	47.59 ± 1.3	51.20 ± 2.8	47.21 ± 0.2	46.88 ± 0.1	47.14 ± 0.6	50.14 ± 3.6	46.76 ± 1.1	2.57	5.29	ns	*
glycine	37.63 ± 0.6	36.51 ± 0.3	37.17 ± 0.5	37.25 ± 09	36.37 ± 0.6	36.33 ± 0.5	36.89 ± 0.1	37.41 ± 0.6	37.30 ± 0.2	36.51 ± 0.9	1.03	2.79	ns	ns
alanine	40.91 ± 0.6	39.59 ± 0.3	39.68 ± 0.5	39.99 ± 0.7	39.26 ± 0.5	39.62 ± 0.3	39.59 ± 0.1	40.23 ± 0.61	40.61 ± 0.4	39.58 ± 1.0	0.98	2.47	*	*
cysteine	20.85 ± 0.8	18.88 ± 0.3	18.88 ± 0.4	22.07 ± 0.8	19.92 ± 1.3	23.17 ± 1.2	23.96 ± 1.8	22.27 ± 0.3	21.77 ± 0.5	20.87 ± 0.5	1.80	8.39	*	ns
valine	51.14 ± 0.8	53.32 ± 0.4	53.32 ± 1.1	52.79 ± 1.2	51.17 ± 0.6	51.32 ± 0.8	53.19 ± 0.7	52.41± 1.1	51.99 ± 0.8	51.53 ± 1.5	1.86	3.58	ns	ns
methionine	21.30 ± 0.9	19.43 ± 0.3	19.43 ± 0.9	22.22 ± 1.1	20.07 ± 0.7	21.32 ± 0.4	21.57 ± 0.4	22.30 ± 0.4	20.96 ± 1.4	20.92 ± 0.7	1.45	6.89	ns	ns
isoleucine	50.36 ± 0.8	48.41 ± 0.3	48.41 ± 0.5	48.77 ± 1.0	47.74 ± 0.6	47.43 ± 0.7	48.03 ± 0.1	48.24 ± 0.9	50.46 ± 0.8	47.70 ± 1.1	1.23	2.54	***	ns
leucine	79.82 ± 1.3	78.59 ± 0.4	78.59 ± 0.7	77.45 ± 1.2	75.21 ± 1.1	75.23 ± 0.9	75.79 ± 0.2	76.51 ± 1.3	79.88 ± 0.4	75.94 ± 1.7	1.85	2.41	***	ns
tyrosine	39.97 ± 0.8	39.79 ± 0.5	39.79 ± 0.6	39.12 ± 09	38.95 ± 0.4	38.47 ± 0.7	39.41 ± 0.3	39.04 ± 0.8	38.99 ± 0.6	38.35 ± 1.2	1.16	2.95	**	ns
phenylalanine	51.59 ± 1.8	53.92 ± 0.8	53.67 ± 0.5	52.69 ± 1.5	52.39 ± 0.6	52.45 ± 0.8	53.13 ± 0.4	53.27 ± 0.9	52.48 ± 1.5	52.78 ± 1.4	1.61	3.05	*	ns
histidine	28.21 ± 0.4	28.93 ± 0.3	28.92 ± 1.1	28.10 ± 0.4	27.09 ± 03	26.56 ± 0.2	26.28 ± 0.8	27.01 ± 0.5	28.22 ± 0.3	26.75 ± 0.4	1.11	4.07	*	ns
lysine	66.56 ± 1.0	66.52 ± 0.4	66.53 ± 1.6	64.83 ± 0.7	63.71 ± 0.8	63.97 ± 0.7	64.89 ± 0.2	64.97 ± 0.8	63.83 ± 1.3	63.83 ± 1.2	1.97	3.05	ns	ns
arginine	66.33 ± 6.3	75.71 ± 0.9	75.71 ± 1.0	74.73 ± 1.2	71.64 ± 0.6	73.57 ± 2.9	74.51 ± 0.8	77.66 ± 1.7	62.14 ± 5.4	78.05 ± 1.8	5.81	7.97	**	**
tryptophan	12.14 ± 0.3	11.92 ± 0.5	11.92 ± 1.1	13.45 ± 0.8	11.62 ± 0.4	13.45 ± 0.6	14.12 ± 0.9	14.18 ± 0.8	13.91 ± 0.6	16.51 ± 2.3	1.96	14.5	*	ns
ammonia	13.12 ± 4.6	13.20 ± 1.1	13.20 ± 1.3	11.54 ± 1.6	8.23 ± 3.1	12.43 ± 0.3	10.09 ± 1.3	12.44 ± 1.1	16.22 ± 2.1	10.47 ± 1.3	4.3	36.5	ns	ns
basic	161.11	171.17	171.17	167.67	162.46	164.11	165.67	169.64	154.18	168.64	7.28	4.41	ns	ns
acidic	308.33	291.25	291.25	292.19	284.52	284.04	284.84	291.28	304.40	283.97	8.70	2.98	**	ns
charged	469.43	462.42	462.42	459.86	446.98	448.16	450.52	460.93	458.58	452.62	12.04	2.63	ns	ns
hydrophilic	558.79	556.56	556.56	554.08	536.04	553.97	547.41	556.49	549.15	560.02	12.53	2.27	ns	*
hydrophobic	306.33	305.14	305.14	306.50	297.18	299.69	305.25	305.96	308.69	303.74	8.37	2.75	ns	ns
apolar	266.36	265.34	265.34	267.38	258.22	261.22	265.84	266.91	269.69	265.39	7.52	2.84	ns	ns
R1[c]	0.548	0.55	0.55	0.55	0.55	0.54	0.55	0.55	0.56	0.54	0.02	3.98	ns	ns
R2[c]	2.099	2.09	2.09	2.07	2.07	2.12	2.05	2.08	2.03	2.02	0.08	3.94	ns	ns
R3[c]	1.532	1.51	1.52	1.50	1.50	1.4	1.47	1.51	1.48	1.49	0.04	2.97	ns	ns
R4[c]	1.762	1.74	1.74	1.72	1.73	1.7	1.69	1.72	1.70	1.71	0.05	3.10	ns	ns
WE[d]	0.11360	0.11351	0.11351	0.11361	0.11277	0.11309	0.11337	0.11376	0.11286	0.11371	0.006	0.61	ns	ns
CF[d]	0.11632	0.11603	0.11603	0.11649	0.11528	0.11588	0.11622	0.11672	0.11564	0.11672	0.0007	0.76	ns	ns
total protein[d] (g/kg of dry matter)	321.91 ± 4.8	321.71 ± 4.1	321.71 ± 3.8	300.11 ± 7.9	331.00 ± 9.5	304.94 ± 0.2	313.12 ± 4.7	303.06 ± 4.3	332.31± 5.7	326.42 ± 6.25	11.75	3.71	**	**

[a] Mean values and standard error of measurements (SEM) for 4 replicates ($N = 4$) and 64 determinations. The values for cyst(e)ine, methionine, and tryptophan represent the average of 24 determinations. [b] Significance: *F*, values from analysis of variance among cultivars; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [c] Calculated according to the method of Barrantes (1973, 1975). [d] Computed according to the method of Horstmann (1979) and Zarkadas et al. (1988a,b).

Table 2. Comparison of the Amino Acid (AA) Composition and Nitrogen (N) Contents (Grams of AA per 16 Grams of N; Mean ± SEM) of Nine New Northern Adapted Soybean Cultivars

	soybean cultivars[a]										signif levels among[b] cultivars			
											overall			brown vs not
AA	Maple Amber	Maple Donovan	Maple Glen	Maple Isle	Maple Presto	Maple Presto Brown	Maple Ridge	Maple Ridge Brown	Maple Arrow	Maple Arrow Brown	SEM	CV	*F*	*F*
aspartic acid	11.11 ± 0.21	10.67 ± 0.0	10.83 ± 0.72	10.92 ± 0.08	10.88 ± 0.21	10.46 ± 0.03	10.46 ± 0.14	11.51 ± 0.13	10.97 ± 0.05	10.51 ± 0.09	0.53	4.97	ns	ns
threonine	4.66 ± 0.11	4.51 ± 0.21	4.51 ± 0.09	4.08 ± 0.39	4.58 ± 0.09	4.47 ± 0.43	4.18 ± 0.22	4.44 ± 2.9	4.29 ± 0.20	4.64 ± 0.23	0.48	10.7	ns	ns
serine	5.01 ± 0.30	4.92 ± 0.37	4.59 ± 0.22	4.27 ± 0.75	4.47 ± 0.53	4.69 ± 17.07	5.05 ± 0.24	4.81 ± 4.6	4.84 ± 0.42	4.72 ± 0.41	0.85	18.2	ns	ns
glutamic acid	18.63 ± 0.37	17.11 ± 0.10	16.75 ± 0.21	17.12 ± 0.26	17.63 ± 0.28	16.74 ± 0.09	16.63 ± 0.22	18.06 ± 2.7	17.97 ± 0.38	17.01 ± 0.11	0.52	3.04	***	ns
proline	4.86 ± 0.07	4.65 ± 0.15	5.35 ± 0.31	4.57 ± 0.17	5.12 ± 0.21	4.51 ± 0.02	4.46 ± 0.09	4.71 ± 0.61	4.77 ± 0.02	4.42 ± 0.01	0.25	5.47	***	**
glycine	3.63 ± 0.07	3.52 ± 0.03	3.62 ± 0.22	3.57 ± 0.06	3.64 ± 0.05	3.47 ± 0.01	3.51 ± 0.06	3.74 ± 0.62	3.54 ± 0.02	3.44 ± 0.02	0.17	4.73	ns	ns
alanine	3.95 ± 0.07	3.81 ± 0.03	3.86 ± 0.24	3.83 ± 0.05	3.93 ± 0.07	3.79 ± 0.01	3.77 ± 0.07	4.02 ± 0.61	3.86 ± 0.03	3.74 ± 0.03	0.18	4.81	ns	ns
cysteine	2.01 ± 0.07	2.09 ± 0.04	1.85 ± 0.15	2.11 ± 0.06	1.99 ± 0.06	2.22 ± 0.14	2.29 ± 0.22	2.23 ± 0.3	2.07 ± 0.07	1.97 ± 0.02	0.21	10.1	ns	ns
valine	4.94 ± 0.17	4.95 ± 0.03	5.19 ± 1.1	5.06 ± 0.08	5.13 ± 0.11	4.91 ± 0.02	5.06 ± 0.12	5.24 ± 1.1	4.94 ± 0.11	4.87 ± 0.10	0.27	5.43	ns	ns
methionine	2.05 ± 0.08	2.08 ± 0.04	1.92 ± 0.2	2.13 ± 0.06	2.01 ± 0.06	2.03 ± 0.04	2.05 ± 0.07	2.23 ± 0.4	1.99 ± 0.07	1.97 ± 0.03	0.17	8.61	ns	ns
isoleucine	4.86 ± 0.08	4.56 ± 0.02	4.71 ± 0.27	4.68 ± 0.06	4.78 ± 0.09	4.53 ± 0.01	4.57 ± 0.01	4.82 ± 0.9	4.79 ± 0.02	4.51 ± 0.04	0.21	4.52	ns	*
leucine	7.69 ± 0.13	7.29 ± 0.03	7.61 ± 0.43	7.43 ± 0.07	7.54 ± 0.11	7.19 ± 0.01	7.72 ± 0.11	7.65 ± 1.3	7.59 ± 0.02	7.17 ± 0.04	0.32	4.28	ns	ns
tyrosine	4.98 ± 0.24	3.75 ± 0.05	3.95 ± 0.21	3.75 ± 0.07	3.91 ± 0.11	3.67 ± 0.02	3.75 ± 0.07	3.90 ± 0.8	3.68 ± 0.05	3.62 ± 0.04	0.17	4.59	ns	ns
phenylalanine	4.98 ± 0.25	5.02 ± 0.07	5.24 ± 0.32	5.05 ± 0.12	5.25 ± 0.13	5.01 ± 0.02	5.05 ± 0.07	5.33 ± 0.9	4.98 ± 0.13	4.98 ± 0.05	0.27	5.33	ns	ns
histidine	2.72 ± 0.05	2.61 ± 0.03	2.81 ± 0.17	2.69 ± 0.02	2.72 ± 0.05	2.54 ± 0.01	2.52 ± 0.12	2.70 ± 0.5	2.67 ± 0.02	2.53 ± 0.03	0.14	5.59	ns	ns
lysine	6.42 ± 0.14	6.13 ± 0.03	6.47 ± 0.37	6.22 ± 0.04	6.38 ± 0.8	6.11 ± 0.01	6.18 ± 0.10	6.50 ± 0.8	6.07 ± 1.3	6.03 ± 0.04	0.30	4.89	ns	ns
arginine	6.42 ± 0.69	7.24 ± 0.11	7.37 ± 0.42	7.17 ± 0.09	7.18 ± 0.17	7.03 ± 0.20	7.09 ± 0.18	7.77 ± 1.7	5.19 ± 0.14	7.38 ± 0.18	0.58	8.38	***	**
tryptophan	1.17 ± 0.02	1.39 ± 0.05	1.17 ± 0.16	1.29 ± 0.07	1.16 ± 0.4	1.28 ± 0.02	1.34 ± 0.07	1.42 ± 0.8	1.32 ± 0.05	1.55 ± 0.20	0.17	13.8	ns	ns
ammonia	1.25 ± 0.41	1.01 ± 0.11	1.14 ± 0.06	1.10 ± 0.17	0.98 ± 0.18	1.18 ± 0.15	1.25 ± 0.37	1.24 ± 1.1	1.81 ± 0.11	1.29 ± 0.02	0.51	40.9	ns	ns
total AAN[c]														
g/kg of protein	164.94 ± 4.8	166.06 ± 0.8	173.52 ± 4.7	166.78 ± 1.6	164.07 ± 0.9	167.35 ± 1.9	168.24 ± 3.3	170.14 ± 1.88	168.31 ± 1.17	169.41 ± 3.49	47.15	26.8	ns	ns
g/kg of dry matter	53.44 ± 1.4	51.41 ± 0.8	53.37 ± 3.05	50.01 ± 0.9	52.85 ± 1.8	52.32 ± 1.7	51.55 ± 0.6	53.55 ± 1.7	54.06 ± 0.9	54.06 ± 0.97	3.395	6.46	ns	ns
g/16 g of N	88.98 ± 8.8	96.36 ± 0.5	97.50 ± 6.3	95.96 ± 0.9	100.37 ± 2.9	95.64 ± 0.2	95.21 ± 1.87	94.07 ± 1.0	95.07 ± 0.6	94.56 ± 0.6	7.39	7.74	ns	ns

[a] Mean values and standard error of measurements (SEM) for 4 replicates ($N = 4$) and 64 determinations. The values for cyst(e)ine, methionine, and tryptophan represent the average of 24 determinations. [b] Significance: *F*, values from analysis of variance among cultivars; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [c] Total amino acid nitrogen (N) was determined according to the methods of Heidelbaugh et al. (1975), Horstmann (1979), and Zarkadas et al. (1988a,b, 1993, 1997a,b).

and/or protein contents in such soybean genotypes could be used as potentially useful indices for assessing their protein quality (FAO/WHO/UNU, 1985; FAO/WHO, 1991; U.S. Food and Drug Administration, 1993). The cultivars were Maple Amber, Maple Donovan, Maple Glen, Maple Isle, Maple Presto, Maple Ridge, and three brown seed coat cultivars, namely, Maple Presto Brown, Maple Ridge Brown, and Maple Arrow Brown. Their total protein and amino acid composition were compared with those of an established cultivar, Maple Arrow (Zarkadas et al., 1993).

Results of the amino acid compositions of the nine selected soybean cultivars and the levels of statistical significance obtained from analysis of variance are presented in Table 1. The data are expressed as grams of anhydrous amino acid per kilogram of anhydrous, fat- and ash-free seed tissue protein and represent the average values of four replicates ($N = 4$). Duplicate 24-, 48-, 72-, and 96-h hydrolysates were prepared, and each was analyzed in duplicate (64 determinations). The method of reporting amino acid composition, as presented in Table 1, allows comparisons to be made between the results from this study and the recommended FAO/WHO (1991) reference amino acid patterns for humans and enables the calculation of total protein and percentage recovery of the amino acids by simple summation (Tristram and Smith, 1963).

Another method for expressing amino acid content is based on grams of amino acid per 16 g of total nitrogen, as recommended by FAO/WHO (1991). This method was first introduced by Block and Weiss (1956) for rapid calculation of the amino acid content of nutritional studies, and for purposes of comparison, the data from this study have been calculated in this way and are presented in Table 2. The weighted mean nitrogen contents of the selected new soybean cultivars, calculated according to the method of Heidelbaugh et al. (1975) by the summation of the amino acid nitrogen contents of each soybean cultivar, are also presented in Table 2.

Protein determinations in each acid hydrolysate were carried out according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a,b, 1997a,b), and the results are summarized in Table 1. This method of calculating the protein mass in seeds or tissues is based upon the knowledge of the amino acid composition of the protein in the soybean cultivars and yields accurate estimates of the amount of protein present as determined by eqs 1−3. The mean residue weight (WE, micrograms per nanomole) and conversion factor (CE, micrograms per nanomole), given in Table 1, can be used in all subsequent protein quantitations as described previously by Horstmann (1979) and Zarkadas et al. (1988a, 1993). Significant variations ($P < 0.01$) in the protein content were found among the nine new northern adapted soybean cultivars. Their protein contents varied from 30.1% (Maple Isle and Maple Ridge Brown) to 30.5% (Maple Presto Brown), 31.3% (Maple Ridge), 32.1% (Maple Amber, Maple Donovan, and Maple Glen), 32.6% (Maple Arrow Brown), and 33.1% (Maple Presto), compared to the protein content of an earlier release, Maple Arrow, which contained 33.2%. These data correspond closely to those reported previously by Zarkadas et al. (1993, 1994, 1997a,b) for Maple Arrow and a variety of new miso (30.1−32.3%) and natto type (30.4−34.2%) soybean cultivars. Mean total nitrogen content among these cultivars ranged from 5.01 to 5.41% (Table 2). These data suggest that the best estimate of the protein content in each of these genotypes was made by the summation of the weights of the amino acids of which each of these cultivars are composed, as described by Hortsmann (1979). The results summarized in Table 1 show that this method yields accurate estimates of the absolute amount of protein present among the cultivars evaluated.

Small but significant differences in protein content were also found between the brown and yellow seed coated soybeans. Although from these data it is not possible to determine which specific proteins have been affected, further detailed studies to ascertain their identity and biological significance, and location in the plant seeds, could prove to be a very fruitful area for future research. Lindstrom and Vodkin (1991) and Nicholas et al. (1993) have shown that increased levels of a saline-soluble proline-rich cell wall protein, PRP1, are found in the developing seed coats of the yellow-seeded cultivars, compared to the pigmented seed coat varieties. Their procedure yielded between 25 and 50 μg of purified PRP1 protein from 150 mg of freeze-dried seed coats, corresponding to 30 seeds. The 35 kDa PRP1 protein was composed primarily of proline, 4-hydroxyproline, valine, tyrosine, and lysine. In contrast, a closely related proline-rich cell wall protein, PRP2, was synthesized later in seed coat development and was affected by the genotype of the *i* logus. Another difference was noted by Wang et al. (1994) and Todd and Vodkin (1996), who reported a 7−10-fold decrease in activity in one of the key enzymes of the flavonoid pathway, chalcone synthase, in yellow seed coated soybeans compared to the pigmented seed coated soybeans. Chalcone synthase activity in yellow seed coats (*I)* was 0.037 pmol of deoxychalcone per total protein compared to 0.432 pmol of deoxychalcone per total protein in the pigmented seed coats that have the homozygous recessive *i* allele.

The overall amino acid composition of the nine northern adapted soybean cultivars and levels of statistical significance obtained from analysis of variance, expressed as grams of amino acid per kilogram of total proteins, are presented in Table 1. The main advantage of this unit of expressing the composition of soybeans is that it reflects the relative amounts of the amino acids present (Tristam and Smith, 1963; Zarkadas et al., 1988a,b) because the influence of fat, ash, and moisture is eliminated. In addition, the data from this study have also been calculated as grams of amino acid per 16 g of total nitrogen, as recommended by FAO/WHO (1991), and the results are summarized in Table 2. The total amino acid nitrogen contents of these soybean cultivars were calculated from their amino acid nitrogen levels as described by Heidelbaugh et al. (1975). The total amino acid nitrogen per 100 g of soybean protein among the nine genotypes ranged from 16.4 to 17.4%, with the Maple Glen containing the highest nitrogen (17.4%) content.

The amino acid profiles of the nine Maple type soybean cultivars investigated appeared to be very similar. Glutamic acid was the most abundant amino acid in all cultivars, followed by aspartic acid, with a frequency of carboxyl groups of ~28.3−30.8%. The variation noted for glutamic acid among the nine soybean cultivars evaluated was statistically highly significant ($P < 0.001$), with Maple Arrow and Maple

Table 3. EAA Scores of Nine New Soybean Cultivars and Hen's Whole Egg and the EAA Requirements of a Preschool 2−5-Year-Old Child

EAA	EAA[a] requirements for a preschool child	soybean cultivars										
		Maple Amber	Maple Donovan	Maple Glen	Maple Isle	Maple Presto	Maple Presto Brown	Maple Ridge	Maple Ridge Brown	Maple Arrow	Maple Arrow Brown	egg
		Milligrams of AA per Gram of Total Protein[b]										
histidine	19	28	29	29	28	27	26	26	27	28	27	22
isoleucine	28	50	48	49	48	47	48	48	48	50	48	54
leucine	66	79	78	79	77	75	75	76	75	80	76	86
lysine	58	66	66	66	65	64	64	65	65	64	64	70
methionine + cyst(e)ine	25	42	38	38	44	40	45	46	45	43	42	57
phenylalanine + tyrosine	63	92	94	93	92	91	91	93	92	91	91	93
threonine	34	43	47	43	45	45	45	44	47	42	48	47
tryptophan	11	12	12	12	13	12	13	14	14	14	16	17
valine	35	51	53	53	53	51	51	53	52	52	51	66
% total protein, EAA_9[a]	33.9	46.3	46.5	46.2	46.5	45.2	45.8	46.5	46.5	46.5	45.8	51.2
EAA index[c] (%)		86	90	84	90	89	89	89	88	86	89	
total EAA[d] (mg/g of N)		2988	3065	2923	3061	3169	3045	3050	3013	2988	2918	3215
		Percent True Protein Digestibility[e] in Man										
		91	91	91	91	91	91					97
		Protein Digestibility Corrected AA Score[e]										
		91	91	91	91	91	91					97

[a] Data from FAO/WHO/UNU (1985) and FAO/WHO (1991). [b] Calculation of protein ratings of natto soybean cultivars was carried out by comparison of the AA composition of hen's whole egg with that of the reference pattern established by FAO/WHO/UNU (1985) for a preschool child (2−5 years old). [c] Calculated according to the methods of Block and Mitchell (1946) and Oser (1951). [d] Computed from reference protein standards (FAO/WHO, 1965). [e] True protein digestibility values were taken from the U.S. Food and Drug Administration (U.S. FDA, 1993) *Federal Register*, Appendix B.

Amber being much higher in total glutamic acid (18.9−19.3%) compared to mean values (17.5−18.0%) found in the other cultivars. Serretti et al. (1994) reported that the glutamic acid content of high-protein soybean lines averaged 19.0−19.7%. The variation noted for leucine, which is the next most abundant amino acid (7.5−7.9%), and isoleucine (4.7−5.1%) was highly significant ($P < 0.001$) among the nine cultivars analyzed. These values are higher than those of Serretti et al. (1994), which ranged from 7.1 to 7.3% for leucine and from 3 to 3.1% for isoleucine. The variation found for the content of arginine among these cultivars, which is the third most abundant amino acid, was highly significant at the $P < 0.01$ level, and the variation in the aspartic acid, alanine, cysteine, histidine, and tryptophan contents was statistically significant at the $P < 0.05$ level. Maple soybean genotypes are also a good source of aromatic amino acids, tyrosine and phenylalanine, which vary significantly among these nine soybean cultivars ($P < 0.01$ to $P < 0.05$). These data correspond closely to those reported by Steinke (1992) and Zarkadas et al. (1993, 1994). The mean amino acid values in the present study, summarized in Table 1, indicate they are in close agreement with those reported previously by Zarkadas et al. (1993, 1994, 1997a,b) for Maple Arrow and a variety of new miso and natto type soybean cultivars. These results, however, are considerably different from those reported for high-protein soybean cultivars by Pazdernick et al. (1997), using near-infrared reflectance spectroscopy.

The results show that although the amino acid profiles of soybean seeds at maturity appeared to be similar and highly characteristic of this plant tissue, there was a preferential accumulation of individual amino acids in soybean seeds during development. It would therefore be useful if the constituent amino acids of soybean seeds could be grouped into classes with distinct properties so that such classes correlate to some extent with the general properties of the proteins in this plant tissue. Barrantes (1973, 1975) has grouped the amino acids into four classes, totally charged, hydrophilic, hydrophobic, and apolar, and simply compared the ratio (R) of the frequencies of occurrence (χ) of whatever particular side chains of proteins one wishes to stress. This method of amino acid classification was used, using eq 4, and the results are summarized in Table 1. The variation of amino acids in soybean seeds among these cultivars was found to be highly significant ($P < 0.01$) for acidic amino acids in accord with the assumption that this variation reflects genetic changes among these cultivars.

Comparisons of the variation in amino acid composition between the yellow and brown seed coated genotypes showed statistically highly significant difference ($P < 0.01$) only for arginine. The values reported in Table 1 for proline between the yellow and brown seed coated cultivars, although they differed significantly at the $P < 0.05$ level, are in accord with the proline value (5.1%) of Steinke (1992) and slightly lower than those obtained from *p*-toluenesulfonic acid hydrolyzed high-protein soybeans by Serretti et al. (1994), which ranged from 5.8 to 6.3%. The only other variables that showed significant effects were the threonine and alanine contents and total hydrophilic amino acids between the yellow and brown seed coat soybeans at the $P < 0.05$ level.

The data presented in Table 1 indicate that the levels of methionine in both the yellow and brown seed coated soybeans are similar, ranging from 1.94 to 2.23% of the total protein. This is of interest because this sulfur amino acid is considered to be the most significant limiting amino acid in soybeans, followed by cyst(e)ine (Eggum and Beames, 1983). The levels of methionine reported in this study are in close agreement with those reported by Zarkadas et al. (1997a,b) for miso and natto type soybean cultivars but are higher than those reported previously for Maple Arrow and AC Proteus (Zarkadas et al., 1993, 1994). The present results are also higher than those reported by Cavins et al. (1972) and Kellor (1974) for defatted flour and grits, by Seretti et al. (1994) for high-protein soybean varieties, which ranged from 1.08 to 1.26%, and by de Lumen (1990) for high-protein soybeans (1.4%). However, Burton et al. (1982) suggested that such differences in methionine

content could be the result of environmental factors and not genetic differences. These authors found that the overall methionine content of soybean protein is only 1.4−1.6% of the total amino acid content and that it should be increased to 3.0% of total protein to provide the methionine equivalent of egg protein, the standard or reference methionine pattern recommended by FAO/WHO (1965).

The essential amino acid (EAA) profiles of the nine soybean cultivars ranged from 2918 to 3169 mg of EAA/g of dietary nitrogen (Table 3). The data indicated that these cultivars contained high amounts of all essential amino acids required for both human and animal nutrition compared to whole egg (3215 mg of EAA/g of nitrogen) (FAO/WHO, 1965). Similar results were obtained from the essential indices of these soybean cultivars, calculated from their amino acid composition according to the method of Block and Mitchell (1946) and Oser (1951). The essential amino acid profiles and protein ratings of the nine soybean cultivars investigated are compared with those of the reference pattern (FAO/WHO/UNU, 1985; FAO/WHO, 1991) for a 2−5-year-old child and with hen's whole egg, and the results are summarized in Table 3. The proposed method for calculating the protein digestibility corrected amino acid score (PDCAAS) of foods can be defined according to Young and Pellett (1994) as follows:

$$\text{PDCAAS} = \frac{\text{AA content (mg/g of protein) of food protein} \times \text{digestibility}}{\text{AA content of FAO/WHO (1991) pattern for 2−5-year-old child}} \quad (5)$$

These authors have defined the amino acid score as the concentration of the limiting amino acid in the food protein, which is expressed as a proportion or percentage of the concentration of the same amino acid in a standard or reference amino acid pattern. In this case the amino acid requirement pattern for the 2−5-year-old child has been adopted as that to be used for assessing protein nutritional quality by the amino acid scoring procedure for all ages shown in Table 3 (Young, 1992; Young and Steinke, 1992). Digestibility is included in this amino acid scoring procedure (eq 5) to allow for differences in the digestibility between plant and animal sources. The true protein digestibility values for soybeans and other foods quoted in this study were taken from the U.S. *Federal Register's* Appendix B, pp 2193−2195 (U.S. Food and Drug Administration, 1993). This amino acid scoring procedure is now required by the U.S. Food and Drug Administration (1993) as the official method for routine food quality evaluation and regulatory control of protein foods and for the nutrition label declaration of protein content of foods in the United States.

The calculated amino acid scores for the Maple type soybeans are very similar in their EAA contents (Table 3). 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. These soybean proteins contain all of the EAA_9, ranging from 45.2 to 46.5% compared to the 33.9% reference protein pattern value given by FAO/WHO/UNU (1985) and FAO/WHO (1991) for a 2−5-year-old child. As a result, the nine soybean amino acid profile gives a good balance of total EAA, limited only in methionine, and has an amino acid score adjusted for digestibility of 91% for all soybean cultivars, compared to the value of whole egg protein (97%). The data presented in this study show that both yellow and brown seed coated soybeans are very good sources of high-quality plant proteins for human and animal nutrition. According to Young and Steinke (1992) and Young and Pellett (1990, 1994) soybean proteins would satisfy the EAA needs of both children and adults. These authors also indicated that for practical human nutrition, under conditions of normal usage of soybean proteins in the diet, methionine supplementation is not only unnecessary but may even be undesirable, except for the feeding of the newborn (Nestle, 1996), for whom modest supplementation of soybean-based formulas with methionine may be beneficial (Erdman and Fordyce, 1989).

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