Determination of the Protein Quality of Three New Northern Adapted Cultivars of Common and Miso Type Soybeans by Amino Acid Analysis†

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The total protein and amino acid contents of three new northern adapted soybean cultivars, namely Apache, Baron, and a miso type Maple Belle and its older male recurrent parent Evans, were compared as potentially useful indices for assessing their protein quality from their FAO/WHO amino acid scoring pattern. The total protein contents, although similar, were statistically significantly different ($P > 0.05$), varying from 30.1% in Baron to 31.2% in Evans, 32.3% in Apache, and 31.5% in Maple Belle. All four soybean cultivars contained an excellent balance of essential amino acids (EAA), i.e., $EAA_9 = 46-46.6\%$ compared to the FAO/WHO reference protein pattern value of 33.9% for a 2−5-year-old child. All were limited only in methionine, and to a lesser extent in isoleucine and valine, and had a protein digestibility corrected amino acid score of 91% compared to the values obtained for hen's whole egg (97%). These results indicate that an accurate calculation of protein quality of soybean seeds and other legumes can be made from their amino acid composition.

Keywords: *Soybeans; miso type soybean; assessment; protein quality; amino acids; composition; amino acid score*

INTRODUCTION

High-quality protein soybean [*Glycine max* (L.) Merr.] cultivars are an important source of edible vegetable protein and are used in many countries of the Pacific Rim today for the production of several basic foods. Such soybean cultivars are used to manufacture fermented soybean products such as miso, soy sauce, shoyu, natto, suffu, and tempeh. Soybean miso is the Japanese name for a commercial fermented soybean paste (Fukushima, 1981; Lin, 1991).

Miso is also produced throughout the Orient, including Indonesia, China, Korea, the Philippines, and Indochina, under various names. Although innumerable miso cultivars are possible, depending upon substrates and amount of salt used and the length of fermentation, the basic processes include the production of starter culture mass, called koji, followed by fermentation in brine solution and finishing. Cereals are added to the soybeans as fermentation substrates. Typically, the starter mass for miso is made from either rice or barley, cooked and inoculated with a mixture of *Aspergillus oryzae* or *Aspergillus soyae*, *Pediococcus*, and *Saccharomyces rouxii* (Hesseltine, 1983b, 1989), which is then mixed with whole soybean mash, adjusted to 48% moisture, and further inoculated with a salt-tolerant yeast and lactic acid bacteria to accelerate the fermentation (Snyder and Kwon, 1987; Hesseltine,1983a,b, 1989). Although some destruction of protein and other nitrogenous compounds may occur during fermentation, both protein and carbohydrate soybean components are hydrolyzed into smaller fragments, which are more digestible.

Breeding miso type soybean cultivars in Canada has received increased attention from soybean breeders, producers (Frederick and Hesketh, 1994; Beversdorf et al., 1995), and exporters for the premium Pacific Rim market. Until recently, the soybean breeding objectives have been directed primarily toward the selection of the most productive early maturing varieties with increased disease resistance. Soybean breeders are also directing their emphasis toward the development of soybean cultivars with improved protein quantity and quality.

Soybean seeds contain an average of 40% protein and 21% oil on a dry weight basis (Hartwig, 1969) and have a protein efficiency ratio (PER) value of 1.7−1.9 (Steinke, 1992). Such PER bioassay values, however, tend to seriously underestimate the nutritional quality of the soybean proteins for both children and adults (Torun et al., 1981; Steinke, 1992; Young and Pellett, 1994). Recent direct metabolic and nitrogen balance studies in humans using various types of soybean protein products for adults, children, and infants reported much higher values for protein quality for such products, ranging from 83 to 96% (average = 93%) in relation to 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, 199, 1994).

Like other leguminous proteins, soybean proteins have a low content of sulfur-containing amino acids, with methionine, and then cysteine and threonine, being the most significant limiting amino acids (Eggum and Beames, 1983). Genetic improvements to increase the methionine levels in miso type soybeans could be made, first, by reduction of β-conglycinin, which accounts for approximately 25% of the total soybean protein and is practically devoid of methionine (Thanh and Shibasaki, 1978; Holowach et al., 1984); second, by increasing the proportion of other soybean storage proteins, i.e., glycinin, protein inhibitors, lectins, and urease, which have higher levels of methionine and tryptophan (Bowman,

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1946; Kunitz, 1947; Frattali, 1969; Kakade et al., 1973; Birk, 1985; Kollipara and Hymowitz, 1992; Liener, 1979, 1995); or, third, by a combination of the two (Burton et al., 1982; Grabau et al., 1986; de Lumen and Kho, 1987; de Lumen, 1990; George and de Lumen, 1991). An attempt to improve the nutritional quality of soybeans by introduction of the gene encoding a methionine-rich 2S albumin (molecular weight 9000) (Sun et al., 1987) from Brazil nut (*Bertholletia excelsa*) was made (Townsend and Thomas, 1994) without adversely affecting their agronomic performance. However, the soybeans proved to be highly allergenic and the research was terminated (Nordlee et al., 1996; Nestle, 1996).

The present study was designed to quantitatively measure the total protein and the amino acid contents of the three new northern adapted cultivars of soybean, namely Apache, Baron, and a miso type soybean cultivar Maple Belle and its male recurrent parent Evans, and to assess their protein quality from their FAO/WHO/UNU (1985) and FAO/WHO (1991) protein digestibility corrected amino acid scoring patterns.

MATERIALS AND METHODS

Materials. The amino acid standards were obtained as follows: the standard amino acid calibration mixture from Beckman Instruments, Inc., Palo Alto, CA., norleucine from Pierce Chemical Co., Rockford, IL; and 3-nitrotyrosine from Aldrich Chemical Co., Milwaukee, WI. 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 three new northern adapted soybean cultivars selected for this investigation were Apache, Baron, and Maple Belle (2500−2650 crop heat unit zones; U.S. Maturity group 00), all developed at the Plant Research Centre, Agriculture Canada, Ottawa, ON. Their pedigrees are as follows:

Apache = T8106::PI.232997/(Altona/Calland)

Baron = T8009::Harosoy 63/Fiskeby V

Maple Belle = OT85-5,X1322-B-7YH::840-7-3/3 × Evans-e_3(X833A-)/4/Evans-e_3

Evans-e_3 is an Evans near-isogenic line carrying the e_3 allele.

For purposes of comparison, an established high-yielding soybean cultivar, Evans, was used. Evans originated as an F_4 plant selection from a single-cross Merit/Harosoy and was used as the male recurrent parent of Maple Belle. Before its release (Lambert and Kennedy, 1975), Evans was identified by the experimental designation M61-94. It is classed as U.S. Maturity group 0, and it is intended for the 2500−2700 crop heat unit zones.

Assessment of agronomic performance of all cultivars was carried out at the Plant Research Centre, Central Experimental Farm, Ottawa, ON, and, for purposes of registration, the cultivars were further tested in three other geographical regions in central and eastern Ontario (USDA Maturity Group 00) for 3 years, between 1988 and 1991.

Representative samples of seed of the four 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 1991.

The dried seed samples were pulverized in a standard electrically driven end runner mill (Cyclone sample mill, Udy Corp., Fort Collins, CO), passed through a 1.0 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 mmHg) with 3.0 mL of triple-glass-distilled constant-boiling HCl (6.0 M) containing 0.2% (v/v) phenol at 110 ± 0.5 °C for periods of 24, 72, and 96 h with the usual precautions described by Zarkadas et al. (1988b, 1990). Analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate according to methods described previously (Zarkadas et al., 1986, 1988a,b).

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 hydrolysate methodology (Beckman Bulletin A 6300-AN-007,1987). For high-sensitivity standard amino acid analyses, three sodium citrate Beckman HPLC microcolumn buffers recommended for ninhydrin analysis were used (sodium citrate buffers E, F, and D). 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 (486-DX series) compatible personal computer. The incorporation of these components into 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, 1990).

Complete amino acid analyses were carried out on each of the four replicate soybean samples (0.05 g) according to the standard procedures described previously (Zarkadas et al., 1988a,b, 1993a,b, 1994). Each of the four replicates was then hydrolyzed in duplicate for 24, 72, and 96 h as described previously (Zarkadas et al., 1988a,b). Analyses of individual acid hydrolysates were performed in duplicate. The data reported for serine and threonine in Tables 1 and 2 represent the average values of 32 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 24 values obtained from the 72 and 96 h of hydrolysis. All others are reported as the average values of 32 determinations from 24, 72, and 96 h of hydrolysis.

Methionine and cyst(e)ine were determined separately in each fraction (0.05 g 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 amino acid calibration standards and solutions of the sulfur amino acids, and 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 single-column methodology as described previously (Zarkadas et al., 1986). 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 hydrolysate was carried out according to the method described by Horstmann (1979), Nguyen et al. (1986), and Zarkadas et al. (1988a,b) as follows:

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

a_i is the mole fraction of an amino acid i found in the analyzed aliquot, b_i is the molecular weight of amino acid residue i (in micrograms), and WE (in micrograms per nanomole) is the

Table 1. Comparison of the Amino Acid (AA) Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein) of Three New Northern Adapted Cultivars of Soybean (Namely Apache, Baron, and a Miso Type Maple Belle and Its Male Recurrent Parent Evans)

	soybean cultivars[a]								signif levels among cultivars		isolated soy[f] protein, g
	Apache		Baron		Evans		Maple Belle (miso)				
AA	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	CV	*F*	
aspartic acid	108.45 ± 1.47[e]	2.71	110.43 ± 0.88[d,e]	1.60	113.44 ± 1.75[d]	3.09	112.69 ± 1.49	2.65	1.98	4.02*	116
threonine	45.82 ± 2.95	12.86	41.87 ± 0.42	2.04	44.11 ± 2.81	12.73	43.15 ± 3.02	14.02	12.16	0.52ns	38
serine	42.67 ± 1.66	7.81	42.02 ± 1.72	8.19	52.23 ± 2.05	7.86	40.14 ± 7.2	35.88	14.97	2.65ns	52
glutamic acid	183.37 ± 4.34	4.73	175.90 ± 3.34	3.81	177.34 ± 1.97	2.22	180.35 ± 2.12	2.35	3.58	0.90ns	191
proline	49.44 ± 2.87	11.59	48.28 ± 0.64	2.65	49.43 ± 0.44	1.79	51.94 ± 2.08	8.03	6.87	1.16ns	51
glycine	36.89 ± 0.53	2.29	35.17 ± 0.31	1.76	37.83 ± 0.38	2.01	36.35 ± 1.14	6.27	3.69	1.86ns	42
alanine	39.74 ± 0.51	2.56	39.57 ± 0.25	1.28	40.16 ± 0.37	1.86	38.73 ± 0.93	4.82	2.33	2.49ns	43
cysteine	20.42 ± 0.53	5.19	22.61 ± 0.66	5.88	21.17 ± 0.37[e]	3.46	20.29 ± 0.68	6.74	5.37	231ns	13
valine	53.74 ± 0.84[e]	3.12	53.92 ± 0.37[e]	1.38	54.44 ± 0.87[e]	3.19	56.88 ± 0.80[d]	2.82	2.30	4.01*	50
methionine	21.07 ± 0.67	6.39	21.47 ± 0.41	3.81	20.93 ± 1.00	9.59	19.43 ± 1.10	11.35	6.65	2.34ns	13
isoleucine	48.47 ± 1.03	4.26	48.50 ± 0.72	2.99	48.34 ± 1.05	4.36	48.65 ± 0.37	1.54	2.47	2.49ns	49
leucine	76.99 ± 1.41	3.68	75.81 ± 1.07	2.82	76.96 ± 1.40	3.64	77.36 ± 0.68	1.77	1.77	5.02ns	82
tyrosine	39.07 ± 0.73	3.77	38.63 ± 0.11	0.58	39.02 ± 0.41	2.09	39.27 ± 0.40	2.05	1.66	3.05ns	38
phenylalanine	53.01 ± 0.70	2.64	53.35 ± 0.61	2.29	53.07 ± 0.68	2.57	53.79 ± 0.61	2.29	1.84	2.28ns	52
histidine	28.09 ± 0.29	2.13	28.68 ± 0.15	1.06	28.12 ± 0.23	1.66	28.05 ± 0.32	2.30	1.83	1.13ns	26
lysine	64.15 ± 0.86	2.67	63.75 ± 0.70	2.20	63.95 ± 0.55	1.74	64.83 ± 0.64	2.09	1.98	1.20ns	63
arginine	74.37 ± 1.97[e]	5.31	79.32 ± 1.37[d]	3.47	72.22 ± 2.19[e]	6.08	73.76 ± 0.75[e]	2.05	3.67	4.97*	76
tryptophan	14.14 ± 0.80	11.32	13.21 ± 0.69	10.54	14.71 ± 0.74	10.09	14.29 ± 0.77	10.83	8.47	2.26ns	
ammonia	14.21 ± 3.76	53.02	14.51 ± 4.48	61.87	9.43 ± 3.99	84.64	16.35 ± 2.12	25.97	50.80	0.73ns	
basic[b]	166.61 ± 2.94	3.53	171.72 ± 1.58	1.84	164.29 ± 2.84	3.45	166.64 ± 1.54	1.84	2.37	2.46ns	
acidic[b]	291.81 ± 5.04	3.45	286.21 ± 2.62	1.83	290.78 ± 3.71	2.55	293.05 ± 3.58	2.44	2.37	0.72ns	
charged[b]	458.42 ± 6.90	3.01	458.07 ± 2.79	1.22	455.08 ± 6.55	2.87	459.69 ± 5.01	2.18	1.96	0.19ns	
hydrophobic[b]	306.49± 3.29	2.15	304.91 ± 3.13	2.05	307.48 ± 4.60	2.99	309.69 ± 3.92	2.53	1.29	1.02ns	
hydrophilic[b]	546.92 ± 8.21	3.00	541.96 ± 4.51	1.66	551.42 ± 2.26	0.83	542.98 ± 5.28	1.95	2.11	0.55ns	
apolar	267.42 ± 2.59	1.94	266.27 ± 3.04	2.28	268.46 ± 4.31	3.21	270.41 ± 3.63	2.69	1.33	0.97ns	
R1[b]	0.56 ± 0.004	1.46	0.56 ± 0.009	3.27	0.56 ± 0.012	2.29	0.57 ± 0.01	4.41	2.48	0.65ns	
R2[b]	2.04 ± 0.01	1.51	2.03 ± 0.03	3.52	2.05 ± 0.05	2.51	2.01 ± 0.04	4.62	2.53	0.57ns	
R3[b]	1.49 ± 0.004	0.58	1.50 ± 0.021	2.82	1.48 ± 0.004	0.544	1.48 ± 0.009	1.22	1.61	0.74ns	
R4[b]	1.71 ± 0.009	1.13	1.72 ± 0.026	3.05	1.69 ± 0.006	0.74	1.70 ± 0.012	1.47	1.68	0699ns	
WE,[c] g/nmol	0.11363 ± 0.0006	1.15	0.11374 ± 0.0002	0.34	0.11332 ± 0.0005	0.88	0.11370 ± 0.0003	0.55	0.58	0.32ns	
Cf,[c] g/nmol	0.11636 ± 0.0006	1.19	0.11655 ± 0.0002	0.45	0.11618 ± 0.0006	1.12	0.11641 ± 0.0004	0.68	0.57	0.21ns	
total protein, g/kg of dry matter	322.66 ± 9.05[d]	5.61	301.48 ± 6.12[e]	4.06	311.79 ± 6.72[d,e]	4.31	315.30 ± 8.59[d,e]	5.45	3.44	3.96*	

[a] Mean values and standard error of measurements (SEM) for 4 replicates ($N = 4$) and 32 determinations. Significance: *F*, values from analysis of variance among cultivars, $*P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Calculated according to the method of Barrantes (1973, 1975) using eq 4. [c] Computed according to the method of Horstmann (1979) and Zarkadas et al. (1988a,b). [d,e] Means along a row with different superscripts are significantly different (Duncan, 1955). [f] Adapted from Steinke (1992).

mean residue weight. 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 calculated as described previously (Horstmann, 1979; Zarkadas et al., 1988a,b, 1993a):

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

The amount of protein content, *P* (in micrograms), in each sample can then be calculated by multiplying WE or CF by the total nanomoles of the amino acids present in each acid hydrolysate as follows:

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

X_i is the nanomoles of each amino acid *i* found in the analyzed aliquot. The values for the content of total protein in each of the four soybean cultivars investigated are the average of 32 determinations.

Predicting Properties of Proteins from Amino Acid Compositions. Barrantes (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 follows:

$$R = \sum_k X_k / \sum_i X_j \qquad (4)$$

In eq 4 *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 prop-

Table 2. Amino Acid (AA) Composition and Nitrogen Contents (Grams of Amino Acids per 16 g of Nitrogen) of Three New Northern Adapted Cultivars of Soybean (Namely Apache, Baron, and a Miso Type Maple Belle and Its Male Recurrent Parent Evans)

	soybean cultivars[a]								signif levels among cultivars	
	Apache		Baron		Evans		Maple Belle (miso)			
AA	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	CV	*F*
aspartic acid	10.25 ± 0.18[e]	3.57	10.39 ± 0.23[e]	4.44	11.27 ± 0.10[d]	1.82	10.58 ± 0.18[e]	3.41	2.63	10.48**
threonine	4.37 ± 0.32	14.63	3.94 ± 0.04	2.32	4.40 ± 0.37	16.96	4.05 ± 0.28	14.03	14.19	0.61[ns]
serine	4.68 ± 0.43	18.37	4.67 ± 0.53	22.82	5.34 ± 0.22	8.46	4.78 ± 0.12	5.42	10.29	0.06[ns]
glutamic acid	17.31 ± 0.38	4.39	16.54 ± 0.37	4.42	17.63 ± 0.23	2.66	16.93 ± 0.23	2.69	13.45	0.91[ns]
proline	4.66 ± 0.19[d,e]	8.21	4.22 ± 0.27[e]	13.14	4.92 ± 0.08[d]	3.27	4.87 ± 0.14[d]	6.15	7.15	3.61*
glycine	3.75 ± 0.04	2.36	3.41 ± 0.15	9.01	3.76 ± 0.08	4.58	3.42 ± 0.13	7.87	6.57	2.04[ns]
alanine	3.76 ± 0.04	2.36	3.72 ± 0.08	4.39	3.99 ± 0.08	3.97	3.64 ± 0.11	6.32	4.14	3.76[ns]
cysteine	2.01 ± 0.11	10.74	2.12 ± 0.14	6.86	2.11 ± 0.06	6.09	1.90 ± 0.05	5.59	7.99	1.56[ns]
valine	5.08 ± 0.07	2.78	5.07 ± 0.09	3.83	5.41 ± 0.17	6.37	5.34 ± 0.09	3.56	4.14	2.70[ns]
methionine	1.91 ± 0.05	5.07	2.02 ± 0.05	5.55	2.08 ± 0.08	7.97	1.82 ± 0.11	12.97	7.14	2.58[ns]
isoleucine	4.58 ± 0.08	3.49	4.56 ± 0.12	5.26	4.80 ± 0.03	1.62	4.57 ± 0.03	1.45	2.26	4.91[ns]
leucine	7.28 ± 0.13	3.70	7.13 ± 0.19	5.55	7.64 ± 0.06	1.52	7.26 ± 0.09	2.63	2.46	5.95[ns]
tyrosine	3.70 ± 0.05	2.97	3.63 ± 0.07	4.14	3 .88 ± 0.07	3.61	3.68 ± 0.03	2.16	2.99	2.15[ns]
phenylalanine	5.01 ± 0.08	3.19	5.02 ± 0.12	5.11	5.27 ± 0.05	2.23	5.05 ± 0.07	2.95	3.03	3.61[ns]
histidine	2.67 ± 0.03[e]	2.45	2.69 ± 0.04[d,e]	3.42	2.78 ± 0.05[d]	3.31	2.63 ± 0.02[e]	1.84	2.16	4.89*
lysine	6.15 ± 0.15	4.83	5.99 ± 0.07	2.26	6.35 ± 0.11	3.51	6.09 ± 0.06	2.08	3.33	2.26[ns]
arginine	7.16 ± 0.28[d,e]	7.90	7.46 ± 0.18[d]	4.88	7.17 ± 0.05[e]	1.63	6.92 ± 0.09[e]	2.51	3.12	3.80*
tryptophan	1.29 ± 0.02[e]	2.79	1.24 ± 0.07[e]	12.04	1.45 ± 0.05[d]	6.35	1.34 ± 0.08[e]	12.38	4.72	8.65**
ammonia	1.33 ± 0.33	50.13	1.34 ± 0.38	57.80	0.89 ± 0.36	82.73	1.52 ± 0.18	24.19	48.61	0.76[ns]
total AAN										
g of AAN/kg of protein	169.32 ± 3.07	3.62	170.28 ± 3.71	4.36	161.09 ± 3.92	4.87	170.42 ± 1.69	1.98	3.18	2.81[ns]
g of AAN/kg of dry mass	54.57 ± 1.06	3.88	51.34 ± 1.59[e,f]	6.23	50.15 ± 0.61[f]	2.45	53.44 ± 1.69[d,e]	6.32	3.01	6.78[ns]
g of AAN/16 g of N	94.59 ± 1.71	3.62	94.09 ± 2.02	4.28	99.49 ± 2.36	4.73	93.91 ± 0.93	1.99	3.19	3.04[ns]

[a] Mean values and standard error of measurements (SEM) for 4 replicates ($N = 4$) and 32 determinations. Significance: *F*, values from analysis of variance among cultivars, $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Calculated according to the method of Barrantes (1973, 1975) using eq 4. [c] Computed according to the method of Horstmann (1979) and Zarkadas et al. (1988a,b). [d,e] Means along a row with different superscripts are significant different (Duncan, 1955).

erties of proteins in plant tissues from their amino acid compositions (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 the SAS system under the Windows operating system, release 6.2 (SAS, 1992), and represents the average values from four subsamples per genotype.

RESULTS AND DISCUSSION

An accurate determination of all of the amino acids in three new northern adapted soybean cultivars, namely Apache, Baron, and a miso type Maple Belle along with the older male recurrent parent of Maple Belle called Evans (Lambert and Kennedy, 1975), was carried out to evaluate their overall protein quality.

The results of the amino acid analysis, total protein contents, and levels of statistical significance obtained from analysis of variance among the selected soybean cultivars are summarized in Tables 1 and 2. The data represent the average values of four replicates ($N = 4$) and are expressed as grams of amino acids per kilogram of protein (Table 1). These results show deviations of <2.5% from the average values among the replicates of each cultivar.

The data presented in Table 1 indicate that on a dry weight basis the actual protein contents of the four soybean cultivars, although similar, were statistically significantly different ($P < 0.05$), varying from 30.1% in Baron to 31.2% in Evans, 31.5% in Maple Belle, and 32.3% in Apache. These data suggest that the best estimate of the protein content in each of these soybean cultivars was made by the summation of the weights of the amino acid residues of which each of these soybean cultivars is composed, as described by Horstmann (1979). The mean residue weight equivalent (WE, micrograms per nanomole) and conversion factor (CE, micrograms per nanomole) given in Table 1 were determined using eqs 1 and 2, respectively, and can be used in all subsequent protein quantitations. The results summarized in Table 1 show that this method of protein determination yields accurate estimates of the absolute amount of protein present among the cultivars investigated.

These data allow a comparison to be made between the present results and those recommended by FAO/WHO (1991) and enable the calculation of total protein and percentage recovery of the amino acids by simple summation. In addition, the data from this study have also been calculated as grams of amino acid per 16 g of total nitrogen, and the results are presented in Table 2. The total amino acid nitrogen contents for these soybean cultivars were calculated from their amino acid nitrogen levels as described by Heidelbaugh et al. (1975). The total nitrogen of these samples ranged from 16.10 to 17.04%, with the miso type soybean Maple Belle containing the highest nitrogen (17.04%) content. On the basis of amino acid compositional data, Tkachuk (1969, 1977) reported a nitrogen to protein conversion factor of 5.69 for soybean meal, while Sosulski and Holt (1980) obtained a conversion factor of 5.63 for soybean meal. This value was 5.22 if corrected for nonprotein nitrogen (4.45%) present in soybean seeds. Krober and

Table 3. Essential Amino Acid (EAA) Scores of Selected Soybean Cultivars, a High-Quality Animal Protein, Hen's Whole Egg, and the EAA Requirements of a Preschool 2−5-Year-Old Child

EAA	EAA[a] requirements for a preschool child (2−5 years old)	EAA scores						
		soybean cultivars				soybean product		
		Apache	Baron	Evans	Maple Belle miso	concentrate	isolate	egg[a]
	Milligrams of Amino Acid per Gram of Total Protein[b]							
histidine	19	28	29	28	28	29	32	22
isoleucine	28	49	49	48	49	54	49	54
leucine	66	77	76	77	77	82	81	86
lysine	58	64	64	64	65	64	65	70
methionine + cyst(e)ine	25	41	44	42	40	27	23	57
phenylalanine + tyrosine	63	92	92	92	93	88	86	93
threonine	34	46	38	44	43	38	34	47
tryptophan	11	14	13	14	14	13	11	17
valine	35	54	54	55	57	63	56	66
% total protein								
EAA_9[c]	33.9	46	46	46.5	46.6	45.8	43.7	
EAA index[d] (%)	88	88	93	88				
total EAA, mg/g of N[e]	3007	3013	3163	3003	2822	2789	3215	
	Percent True Protein Digestibility[c] in Man							
		91	91	91	91			97
	Protein Digestibility Corrected Amino Acid Score[c]							
		91	91	91	91			97

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

Gibbons (1962) found that in very immature soybean seeds over 30% of the nitrogen present was nonprotein nitrogen compared to 4 or 5% in mature seeds (Tkachuk, 1969; Sosulski and Holt, 1980). These results give further support to the recommendations of Benedict (1987), Zarkadas et al. (1988a), and Khanizadeh et al. (1992, 1995) that the protein conversion factor of 6.25 be used only for calculating the crude protein content of different foods.

A comparison between the amino acid profile of the miso type soybean Maple Belle and the other three soybean cultivars investigated, Apache, Baron, and Evans, as presented in Tables 1 and 2, showed that they are very similar. Certain characteristic features of the overall composition may be noted. All cultivars were found to contain high levels of glutamic acid, glutamine, aspartic acid, and asparagine, which account for almost 28.5−29.6% of all residues. The frequency of occurrence of the total basic amino acids is considerably lower and accounts for approximately 16.4−17.2% compared to the acidic amino acids. Leucine, the next most abundant amino acid, accounts for a further 7.6−7.8%. The present mean values for total hydrophobic amino acids ranged from 29.9% in Apache to 30.6% in Evans, 29.8% in Baron, and 30.5% in Maple Belle compared to 54.1−54.9% for hydrophilic amino acids. The amino acids in smallest amount in these four cultivars are tryptophan (1.32−1.47%), cysteine (2.02−2.26%), and methionine (1.94−2.14%), which exceeds slightly the amounts reported previously for either Maple Arrow or AC Proteus (Zarkadas et al., 1993b, 1994).

The present data (Table 3) also indicate that the miso type as well as the other soybean cultivars evaluated in this study contain all of the essential amino acids (EAA) and nitrogen required for human and animal nutrition with methionine as the limiting amino acid. Mean values for total EAA ranged from 2998 mg of EAA/g of nitrogen (N) in Apache to 3001 mg of EAA/g of N in Maple Belle, 2999 mg of EAA/g of N in Baron, and 3091 mg of EAA/g of N in Evans (N calculated from amino acid nitrogen), which closely approaches that of proteins that have been traditionally been used as references sources, such as hen's whole egg (3215 mg of EAA/g of N) (FAO/WHO, 1965). These data support the view that an accurate evaluation of the protein quality of soybeans and other legumes can be made from their amino acid composition.

Block and Mitchell (1946) were first to introduce the use of amino acid compositional data for the evaluation of protein quality of plant and animal proteins. Their amino acid scoring concept was based on the observed linear relationship between the biological value of proteins and their limiting amino acid. In their scoring procedure, termed chemical score, egg protein was used as a standard. According to these authors, the chemical score is the content of each essential amino acid in a dietary protein as a percentage of the same amino acid in the selected standard. Therefore, the chemical score depends upon the standard chosen. Mean values for percent chemical score were low and ranged from 62% in Maple Belle and 67% in its male recurrent parent Evanss to 64.8% and 68.3% in Apache and Baron, respectively.

The chemical score method was improved by Oser (1951), who introduced the EAA index procedure. It is based on the ratios of the amounts of EAA in a protein, or protein mixture, relative to their amounts in standard hen's whole egg. Oser's (1951) method computes values that integrate the amino acid contents of proteins expressed as percentages of the corresponding values of a standard protein chosen for its high nutritive value. When the present data were calculated according to the method of Oser (1951), the mean values for the EAA indices were 88 in Apache and 89 in Maple Belle, compared to 88 and 91 found for Baron and Evans, respectively. However, because of the relatively high amounts of EAA present in egg proteins, both the chemical scoring method and the EAA index procedure could undervalue the protein quality of plant proteins for human or animal nutrition.

A more accurate procedure for evaluating protein quality, which was adopted recently by the Expert

Consultation Group of FAO/WHO (1991), is based on the concept of specific amino acid requirements as the basis for subsequent amino acid scoring system. This new amino acid score, corrected for digestibility, uses the essential amino acid requirements for the 2−5-year-old child as the reference pattern (FAO/WHO/UNU, 1985), since their protein per kilogram requirements are the greatest, except for infants. The nine EAA (EAA_9) include histidine, isoleucine, leucine, lysine, methionine and cystine, phenylalanine and tyrosine, threonine, tryptophan, and valine (FAO/WHO/UNU, 1985). Since cystine and tyrosine can replace methionine and phenylalanine, respectively, the two sulfur-containing (methionine plus cystine) and the two aromatic amino acids (phenylalanine plus tyrosine) are usually considered together.

The FAO/WHO/UNU (1985) and FAO/WHO (1991) 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:

$$\text{PDCAAS} = [\text{AA content (mg/g of protein) of food protein} \times \text{digestibility}]/[\text{AA content of FAO/WHO/UNU (1985) pattern for 2−5-year-old child}] \quad (5)$$

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 limiting 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, except infants (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. In general, animal proteins are more easily digested than plant proteins. 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).

The FAO/WHO Expert Consultation Group (1991) on protein quality evaluation recommended that this method be used as the procedure of choice for international use, for routine regulatory and labeling purposes, and for the determination and control of the protein quality of common and processed foods (Expert Work Group, FSIS, 1984; Pellett and Young, 1984). This amino acid scoring procedure is now required by the U.S. Food and Drug Administration (1993) as the official procedure 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 U.S. Food and Drug Administration (1993) also acknowledges that the PDCAAS procedure, based on human requirements, is an inherently more appropriate method for assessing protein quality of foods intended for human consumption than the PER bioassay procedure, which is based on the amino acid requirements of the rat.

The calculated amino acid scores for the miso type soybean Maple Belle and the other three soybean cultivars investigated, Apache, Baron, and Evans, are very similar in their essential amino acid contents (Table 3). These soybean proteins contain all of the EAA_9, ranging from 46.0 to 46.6% compared to the 33.9% reference protein pattern value given by FAO/WHO/UNU (1985) for a 2−5-year-old child. As a result, the soybean amino acid profile gives a good balance of total essential amino acids, limited only in methionine, and has an amino acid score, adjusted for digestibility, of 91% for all soybean cultivars, compared to the value of egg protein (97%). The data presented in this paper show that the soybean seeds of both the miso type Maple Belle cultivar and its recurrent parent Evans, as well as the new northern adapted cultivars Apache and Baron, contained an excellent balance of most essential amino acids and can be considered as a good source of high-quality plant proteins that would provide adequate amounts of nitrogen and all of the EAA needs for human and animal nutrition, when supplied at significant intake levels of total protein. Their amino acid scores, corrected for digestibility, exceed the suggested pattern of requirement for the preschool child and adult human as recommended by FAO/WHO/UNU (1985) and FAO/WHO (1991). From these results, it became apparent that an accurate evaluation of protein qualty of new soybean cultivars can be made from their amino acid composition.

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