Comparison of the Protein Quality of Five New Northern Adapted Natto Soybean Cultivars by Amino Acid Analysis

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The total protein and amino acid compositions of five newly released northern adapted natto soybean cultivars, namely Canatto, TNS, Nattosan, AC T2653, AC Pinson, and their recurrent parent, Nattawa, have been determined. Mean protein values ranged from 30.4% in AC T2653 to 31.0% (AC Pinson), 31.0% (Nattawa), 32.1% (TNS), and 32.3% (Canatto), compared to the protein content of Nattosan, which was 34.2%. The total nitrogen content was also variable among these cultivars, ranging from 5.15% to 5.92%. All five new natto soybean cultivars were similar in their essential amino acid (EAA) content, i.e., $EAA_9 = 45.0-47.0\%$, compared to the FAO/WHO reference protein pattern value of $EAA_9 = 33.9\%$ for a 2−5-year-old child. Each of the six 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 natto soybeans, and possibly other legumes and cereals, is by the protein digestibility corrected amino acid score.

Keywords: *Natto soybeans; assessment; protein quality; amino acids; composition; amino acid score*

INTRODUCTION

The development of early-maturing high-protein soybean cultivars [*Glycine max* (L.) Merr.], for the manufacture of fermented soybean products in Japan, is receiving increased attention from Canadian soybean breeders. One such product is natto, which is the Japanese name for fermented whole soybeans. It is produced from the fermentation of small-size natto soybeans, using primarily a *Bacillus natto* starter culture (Fukushima, 1981; Hesseltine, 1983a, 1989). Natto is normally eaten with rice, but it can also be used as an ingredient for sauce production or as a flavoring agent (Winarno, 1979; Snyder and Kwon, 1987; Hesseltine, 1983b, 1989; Lin, 1991).

To develop small-size yellow natto type soybean cultivars in Canada for use in the Pacific Rim export market, breeding varieties that are more productive and that will germinate, grow, and mature in more northern latitudes (latitude >45° N), with long daylengths (>16 h) and a short growing season (2300−2700 corn heat unit zone), are required (Beversdorf et al., 1995; Frederick and Hesketh, 1994). Extensive field trials have already been carried out on five new northern adapted small-size natto soybean cultivars to identify their agronomic characteristics, which include improved yields, pest and lodging resistance, and seed quality. Attempts have also been made to improve the protein quantity and quality of these natto type soybean cultivars. As a result, five northern adapted natto type soybean cultivars have been released, namely Canatto, Nattosan, TNS, AC T2653 and AC Pinson, along with an earlier cultivar, Nattawa, which has been used as the recurrent parent of both AC T2653 and AC Pinson (Voldeng et al., 1996a,b, 1997).

Most soybean varieties contain approximately 30−40% protein on a dry weight basis (Wolf, 1982, 1992; Nielsen, 1984) and have an average protein efficiency ratio (PER) of 1.8 relative to casein, which has a PER of 2.5 (Kakade et al., 1973; Bodwell et al., 1980; Torun et al., 1981; Steinke, 1992). Human nutritional studies have shown that diets containing adequately heat processed soybean protein have high protein quality, ranging from 83% to 96% (average 93%) in relation to casein, and >80% of the nutritional value of egg protein, due to the increased availability of the sulfur-containing amino acids (Torun et al., 1981; Torun, 1992; Fomon and Ziegler, 1979, 1992). According to Erdman and Fordyce (1989) the methionine content of soybean foods is sufficient to maintain nitrogen balance and support growth in adults, children, and full-term infants and must be supplemented only in feeding premature infants. The two objectives of the present study were, first, to compare the levels and variation of total protein and the amino acid profiles of the five new northern adapted natto soybean cultivars Canatto, TNS, Nattosan, AC T2653, and AC Pinson along with an earlier release, Nattawa; and, second, 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

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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 new northern adapted soybean genotypes selected for this investigation, cv. Nattawa, Canatto, TNS, AC T2653, AC Pinson, and Nattosan, were developed by Dr. H. D. Voldeng, at the Plant Research Centre and their pedigrees are as follows:

Canatto was developed by three cycles of crossing and back-crossing X655-2 × Evans e3 with selection of the highest protein F_3 bulks in each cycle. Line X655-2 was a small-seeded selection from the cross DW-1-15-1 × BD22115. Line DW-1-15-1 was also a small-seeded selection from the cross of *Glycine soja* (USDA Genetic Type Collection T106) and the Minnesota line M62-173. Line BD22115 (USDA Maturity Group 000) is a sister line of the cultivar Maple Presto (Voldeng et al., 1982) from the cross (Amsoy × Portage) × (P1438477). After the second back-cross, bulk selection F_3 for protein was followed by pedigree selection and yield evaluation of F_5 and F_6 derived bulks. Canatto was tested as OT82-2 and X702-7-3 Ontario soybean trials from 1982 to 1984 and in the Ontario natto variety trials from 1991 to 1995.

Nattosan was selected from the cross of X655-5 × DH-3-10-1. The line DH-3-10-1 is a semideterminate selection from the cross (0-52-903 × OX27-8) × (P71-39). Line 0-52-903 is a selection made at CEF in Ottawa from a Swedish introduction (USDA PI 194654). Line OX27-8 is a semideterminate isoline of Harosoy 63, and line P71-39 is a high-protein experimental line from CEF in Ottawa. Nattosan was tested as OT82-2 and 702-7-3 in the Ontario soybean variety trials from 1985 to 1988 and again between 1991 and 1995.

TNS soybean originated from the cross OX611/X393-23 made at the CEF in Ottawa in 1979. Line OX611 was selected from the cross SRF200 (Harosoy-In)/OX708 (Harosoy-Dt_2). X393-23 was selected from the cross Altona/DW-1, while DW-1 was selected from a cross between *Glycine soja* (USDA Genetic Type Collection T106) and M62-173. TNS was tested as OT85-5-BR-92 in the 1991−1995 Ontario natto variety trials (Voldeng et al., 1997).

AC T2653 soybean was developed from the cross TNS/X1428-2-B-B-32 made at Ottawa in 1985. Line X1428-2-B-B-32 was selected from the cross H-24/Nattawa. Line H-24 is a small-seeded soybean (USDA Maturity Group V). AC T2653 was tested as X2653-12-1-S2-6 in Ottawa in 1990 and in the Ontario natto variety trials from 1991 to 1995. Full details are given by Voldeng et al. (1996b).

AC Pinson was developed from the cross X947-24-B-B-B-55/X1428-2-B-B-32 (Voldeng et al., 1996a). Line X947-24-B-B-B-55 was selected from the cross OX611/X393-23. OX611 was selected from the cross SRF200 (Harosoy-In)/OX708 (Harosoy-Dt_2). X1428-2B-B-32 was selected from the cross H-24/Nattawa. AC Pinson was tested as X2668-15-1-4 in Ottawa in 1990 and in the Ontario natto variety trials from 1991 to 1995 (Voldeng et al., 1996a).

For comparison, an established natto soybean cultivar, Nattawa, was used. Nattawa was developed at the Agriculture Research Station, Ottawa, from an F_4 plant selection from the DW-5 × Acme. Line DW-5 was an early-maturiing F_2 plant with small, round, yellow seeds from the cross of the wild soybean *Glycine soja* × M62-173. The wild soybean strain used was the USDA Genetic Type Collection T106. M62-173 is a USDA Maturity Group 0 line developed at the University of Minnesota from the cross M387 (Renville × Capital) × 406 (Harosoy × Norchief). Nattawa was identified by the experimental designation X390-73.

Assessment of agronomic performance of all cultivars was carried out at the Plant Research Centre, Central Experimental Farm, Ottawa, ON, and further tested in five other geographical regions in central and eastern Ontario (USDA Maturity Group 00) for 5 years, under the Ontario natto soybean variety trial grown between 1991 and 1995 at Inkerman, Elora, Brussels, Alfred, and Bornholm, ON.

Dried seed of the four replicate samples taken from each of the six cultivars selected for this investigation (Canatto, Nattosan, TNS, AC T2653, AC Pinson, and Nattawa) were 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.

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 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). Analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate according to methods described previously (Zarkadas et al., 1986, 1988b).

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). The automated instrument was equipped with a Beckman Model 406 analog interface module and the system Gold (Beckman Instrument, Inc., Altex Division, San Ramon, CA) chromatographic data reduction system as described previously (Zarkadas et al., 1987, 1990).

Complete amino acid analyses were carried out on each of the four replicate soybean samples (50.0 mg) according to the standard procedures described previously (Zarkadas et al., 1988a,b, 1993a). Each of the four replicates was divided into two subsamples, i.e., A and B, which were then hydrolyzed under vacuum (below 10 μmHg) with 10 mL of triple glass-distilled constant-boiling HCl (6.0 M; 20.5% v/v) at 110.0 ± 0.5 °C in duplicate for 24, 72, and 96 h as described previously (Zarkadas et al., 1988a,b).

Methionine and cyst(e)ine were determined separately in each cultivar (50.0 mg samples) according to the performic acid procedure of Moore (1963) as described previously (Zarkadas et al., 1988a,b). Norleucine was added in the hydrolysate as an internal standard. The data were 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 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). 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) as

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

Table 1. Comparison of the Amino Acid (AA) Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein; Mean ± SEM) of Five New Northern Adapted Natto Cultivars of Soybean, Namely Canatto, TNS, Nattosan, AC T2653, and AC Pinson, and Their Recurrent Parent Nattawa

AA	soybean cultivars						signif levels among cultivars		isolated soybean protein[h]
	Canatto	Nattawa	TNS	Nattosan	AC T2653	AC Pinson	CV	*F*	
aspartic acid	109.13 ± 3.3	114.34 ± 3.4	111.97 ± 2.1	115.56 ± 1.9	107.73 ± 1.3	108.78 ± 1.3	4.43	1.70^{ns}	116
threonine	31.49 ± 2.0^{f}	38.61 ± 2.8^{f}	38.25 ± 3.4^{f}	36.48 ± 2.7^{e,f}	53.86 ± 1.98^{e}	49.83 ± 1.5^{e}	12.83	10.38***	38
serine	36.17 ± 5.3	40.23 ± 7.3	37.57 ± 5.7	44.41 ± 6.7	61.19 ± 3.5	53.17 ± 4.4	26.24	32.72^{ns}	52
glutamic acid	181.32 ± 1.5^{e}	174.52 ± 4.1^{e,f}	172.91 ± 2.0^{f}	181.22 ± 2.4^{e}	165.34 ± 0.6^{g}	164.62 ± 0.8^{g}	2.70	9.57***	191
proline	60.81 ± 1.5^{e}	50.53 ± 2.3^{g}	55.71 ± 0.1^{f}	50.89 ± 1.1^{g}	46.8 ± 0.3^{g}	50.92 ± 0.3^{g}	4.84	15.53***	51
glycine	35.07 ± 0.8^{f}	38.85 ± 0.9^{e}	37.98 ± 0.4^{e}	37.23 ± 0.4^{e,f}	38.63 ± 0.7^{e}	38.84 ± 1.0^{e}	4.31	3.22*	42
alanine	38.48 ± 3.1	38.15 ± 0.8	37.07 ± 0.3	37.33 ± 0.3	40.12 ± 0.5	41.02 ± 0.8	7.54	1.15^{ns}	43
cysteine	22.69 ± 0.6^{e,f}	24.66 ± 0.5^{e}	22.90 ± 0.5^{e,f}	23.51 ± 0.8^{e,f}	18.80 ± 1.0^{g}	21.34 ± 0.2^{f}	6.13	8.83***	13
valine	53.63 ± 2.8	51.36 ± 0.7	48.97 ± 1.4	51.77 ± 1.2	51.15 ± 0.4	52.57 ± 0.5	5.71	1.13^{ns}	50
methionine	20.26 ± 0.7	22.08 ± 0.6	20.78 ± 0.2	21.33 ± 0.7	21.25 ± 1.4	20.54 ± 0.2	7.25	0.73^{ns}	13
isoleucine	52.94 ± 2.7	48.04 ± 1.5	47.57 ± 0.9	50.18 ± 1.1	46.64 ± 0.4	47.73 ± 0.3	6.12	1.77^{ns}	49
leucine	77.07 ± 0.55	78.23 ± 2.0	76.88 ± 1.1	77.84 ± 0.4	75.68 ± 1.1	75.64 ± 0.8	3.21	0.75^{ns}	82
tyrosine	44.18 ± 1.3^{e,f}	45.34 ± 1.2^{e}	42.62 ± 1.5^{e,f,g}	42.16 ± 0.8^{e,f,g}	39.32 ± 0.5^{g}	40.43 ± 0.3^{f,g}	5.60	3.67*	38
phenylalanine	56.22 ± 1.1^{e}	55.31 ± 1.2^{e}	54.45 ± 198^{e,f}	54.30 ± 0.5^{e,f}	50.77 ± 0.4^{g}	51.56 ± 0.2^{f,g}	4.03	3.90**	52
histidine	25.49 ± 1.2^{g}	30.65 ± 0.8^{e}	30.87 ± 0.6^{e,f}	27.71 ± 0.3^{f}	29.34 ± 0.1^{e,f}	29.20 ± 0.2^{e,f}	5.18	7.24***	26
lysine	65.61 ± 3.9	67.79 ± 1.8	67.62 ± 06	66.16 ± 0.8	62.73 ± 0.5	67.32 ± 1.8	6.04	0.91^{ns}	63
arginine	72.85 ± 2.5^{f}	81.60 ± 2.4^{e}	72.44 ± 0.8^{f}	72.93 ± 1.1^{f}	75.71 ± 1.0^{f}	71.89 ± 1.2^{f}	4.49	4.86**	76
tryptophan	15.08 ± 1.06	13.71 ± 0.5	13.73 ± 0.3	13.64 ± 0.7	13.78 ± 0.1	14.52 ± 0.1	9.86	0.72^{ns}	12
ammonia	8.14 ± 1.4	20.13 ± 8.1	9.23 ± 2.7	27.25 ± 7.2	11.38 ± 5.6	12.65 ± 3.1	72.14	1.93^{ns}	
basic	163.96 ± 6.9	180.05 ± 4.3	170.94 ± 1.9	166.81 ± 2.0	167.77 ± 0.7	168.42 ± 0.5	4.34	2.29^{ns}	
acidic	290.45 ± 4.6^{e}	288.86 ± 7.3^{e}	284.87 ± 4.1^{e,f}	296.79 ± 3.0^{e}	273.38 ± 2.3^{f}	273.41 ± 2.1^{f}	3.25	4.24**	
charged	454.41 ± 11.5	468.92 ± 11.1	455.82 ± 4.9	463.59 ± 4.1	441.16 ± 2.4	441.83 ± 1.6	3.21	2.37^{ns}	
hydrophilic	522.08 ± 10$^{'}$	547.76 ± 8.5^{e,f}	531.65 ± 6.2^{f,g}	544.49 ± 8^{e,f,g}	556.22 ± 5.4^{e}	544.85 ± 4^{e,f,g}	2.57	3.09*	
hydrophobic	318.40 ± 9.6	314.17 ± 5.1	305.22 ± 2.7	311.23 ± 5.1	298.61 ± 3.94	303.03 ± 2.4	3.68	1.72^{ns}	
apolar	274.21 ± 8.7	268.73 ± 4.5	262.39 ± 2.2	269.07 ± 4.4	259.29 ± 3.4	262.59 ± 2.1	3.81	1.20^{ns}	
R_1[b]	0.61 ± 0.02	0.57 ± 0.01	0.57 ± 0.01	0.57 ± 0.014	0.54 ± 0.01	0.55 ± 0.01	5.81	2.20^{ns}	
R_2[b]	1.91 ± 0.09	2.04 ± 0.05	2.02 ± 0.09	2.02 ± 0.05	2.14 ± 0.05	2.07 ± 0.03	5.45	1.89^{ns}	
R_3[b]	1.43 ± 0.07	1.49 ± 0.03	1.49 ± 0.01	1.49 ± 0.02	1.48 ± 0.02	1.45 ± 0.01	4.84	0.46^{ns}	
R_4[b]	1.66 ± 0.09	1.75 ± 0.03	1.73 ± 0.01	1.72 ± 0.02	1.70 ± 0.02	1.68 ± 0.01	5.10	0.75^{ns}	
WE[c]	0.114383	0.114872	0.113835	0.113855	0.112467	0.112657	0.73	5.13**	
CF[c]	0.117104	0.117836	0.116628	0.116193	0.115237	0.115427	0.83	4.20**	
total protein,[c] g/kg of dry matter	323.08 ± 12^{e,f}	309.66 ± 7.8^{f}	321.16 ± 4.2^{e,f}	341.63 ± 15^{e}	304.48 ± 4.9^{f}	310.45 ± 1.0^{f}	4.97	2.89*	

[a] Mean values and standard error of measurements (SEM) for 4 replicates ($N = 4$) and 48 determinations. The values for cyst(e)ine, methionine, and tryptophan represent the average of 24 determinations. 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. [b] Calculated according to the method of Barrantes (1973, 1975). [c] Computed according to the method of Horstmann (1979) and Zarkadas et al. (1988a,b). [e−g] Means along a row with different superscripts are significantly different (Dungan, 1955). [h] Adapted from Steinke (1992).

The protein content *P* (in micrograms) of each sample was calculated by multiplying CF by the nanomoles of total amino acids in each acid hydrolysate as follows:

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

In eq 3 X_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 six 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 (*X*) of whatever particular side chains of proteins one wishes to stress. Using the following formulas he grouped the amino acids as

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

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 − 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 spread-sheet 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

Natto type soybean is a relatively new agricultural crop in Canada, cultivated primarily in the more

Table 2. Comparison of the Amino Acid (AA) Composition and Nitrogen (N) Contents (Grams of Amino Acids per 16 g of Nitrogen; Mean ± SEM) of Five New Northern Adapted Natto Cultivars of Soybean, Namely Canatto, Nattosan, TNS, AC T2653, and AC Pinson, and Their Recurrent Parent Nattawa

AA	soybean cultivars[a]						signif levels among cultivars		AC Proteus[b]
	Canatto	Nattawa	TNS	Nattosan	AC T2653	AC Pinson	CV	*F*	
aspartic acid	11.58 ± 0.68	10.48 ± 0.44	11.16 ± 0.12	10.27 ± 0.36	10.28 ± 0.48	10.33 ± 0.05	8.08	1.65^{ns}	7.78
threonine	3.32 ± 0.17^{e}	3.87 ± 0.37^{e,f}	3.83 ± 0.37^{d,e}	3.37 ± 0.32^{f}	5.13 ± 0.30^{d,e}	4.74 ± 0.19^{d,e}	15.61	5.47***	4.00
serine	3.78 ± 0.43	3.92 ± 0.75	3.76 ± 0.60	4.19 ± 0.63	5.82 ± 0.36	5.07 ± 0.48	27.47	1.91^{ns}	5.53
glutamic acid	19.22 ± 0.81^{d}	15.99 ± 0.51^{e}	17.25 ± 0.14^{e}	16.27 ± 0.61^{e}	15.79 ± 0.65^{e}	15.64 ± 0.16^{e}	6.93	5.55**	19.04
proline	6.44 ± 0.21^{d}	4.62 ± 0.18^{e}	6.54 ± 0.56^{d}	4.66 ± 0.12^{e}	4.46 ± 0.17^{e}	4.84 ± 0.09^{e}	11.19	10.61***	4.93
glycine	3.72 ± 0.2^{f}	3.55 ± 0.11	3.79 ± 0.09	3.31 ± 0.09	3.67 ± 0.11	3.69 ± 0.07	7.10	1.77^{ns}	3.42
alanine	3.79 ± 0.31	3.50 ± 0.15	3.70 ± 0.07	3.42 ± 0.11	3.82 ± 0.13	3.90 ± 0.04	7.84	2.71^{ns}	3.62
cysteine	2.32 ± 0.12^{d}	2.25 ± 0.05^{d}	2.28 ± 0.07^{d}	2.03 ± 0.02^{e}	1.86 ± 0.08^{e}	2.02 ± 0.04^{e}	6.42	6.78**	2.21
valine	5.66 ± 0.26^{d}	4.71 ± 0.13^{e}	4.89 ± 0.29^{e}	4.63 ± 0.10^{e}	4.87 ± 0.17^{e}	4.99 ± 0.05^{e}	7.13	4.38**	4.86
methionine	2.14 ± 0.10	2.02 ± 0.09	2.07 ± 0.04	1.87 ± 0.03	2.02 ± 0.16	1.95 ± 0.01	8.77	1.17^{ns}	0.92
isoleucine	5.48 ± 0.23^{d}	4.39 ± 0.11^{e}	4.75 ± 0.10^{e}	4.47 ± 0.09^{e}	4.44 ± 0.17^{e}	4.53 ± 0.06^{e}	6.48	7.41***	4.76
leucine	8.16 ± 0.28^{d}	7.16 ± 0.12^{e}	7.66 ± 0.09^{e}	7.15 ± 0.27^{e}	7.21 ± 0.27^{e}	7.18 ± 0.06^{e}	6.08	3.37*	7.50
tyrosine	4.68 ± 0.27^{d}	4.16 ± 0.14^{d,e}	4.25 ± 0.15^{d,e}	3.83 ± 0.14^{e}	3.74 ± 0.16^{e}	3.84 ± 0.04^{e}	8.80	3.90**	3.69
phenylalanine	5.94 ± 0.17^{d}	5.27 ± 0.21^{e}	5.43 ± 0.16^{d,e}	4.97 ± 0.19^{e}	4.84 ± 0.19^{e}	4.89 ± 0.06^{e}	7.11	5.13**	5.01
histidine	2.99 ± 0.47	2.81 ± 0.08	3.07 ± 0.11	2.49 ± 0.09	2.79 ± 0.10	2.77 ± 0.04	15.62	0.85^{ns}	3.078
lysine	6.98 ± 0.64	6.21 ± 0.17	6.75 ± 0.16	5.82 ± 0.13	5.98 ± 0.21	6.41 ± 0.25	10.60	1.78^{ns}	5.91
arginine	7.72 ± 0.43	7.47 ± 0.19	7.23 ± 0.16	6.75 ± 0.34	7.21 ± 0.30	6.83 ± 0.07	8.12	1.63^{ns}	7.64
tryptophan	1.59 ± 0.10^{d}	1.25 ± 0.03^{e}	1.37 ± 0.08^{e}	1.17 ± 0.04^{e}	1.31 ± 0.06^{e}	1.38 ± 0.02^{e}	10.46	4.23**	1.16
ammonia	1.21 ± 0.44	1.75 ± 0.67	0.88 ± 0.26	2.30 ± 0.55	1.14 ± 0.62	1.18 ± 0.27	73.73	1.06^{ns}	11.01
total AAN[c]									
g of AAN/ kg of protein	162.98± 0.77	175.64 ± 6.80	161.80 ± 2.45	176.32 ± 6.95	168.62 ± 6.69	168.55 ± 2.67	6.33	1.26^{ns}	168.35
g of AAN/ kg of dry matter	51.61 ± 1.51^{e}	54.12 ± 1.48^{d,e}	51.51 ± 0.57^{e}	59.19 ± 0.81^{d}	51.34 ± 2.24^{e}	52.17 ± 0.58^{e}	6.17	3.37*	70.28
g of AA/ 16 g of N	101.67± 1.69	91.77 ± 3.58	99.80 ± 1.78	90.96 ± 3.37	95.32 ± 3.61	94.99 ± 1.47	5.95	2.24^{ns}	95.15

[a] Mean values and standard error of measusremrents (SEM) for 4 replicates ($N = 4$) and 32 determinations. The values for cyst(e)ine, methionine, and tryptophan represent the average of 24 determinations. Significance: *F*, values from analysis of variance among cultivars; ***, $P < 0.001$; **, $P < 0.01$; ns, notsignificant; CV, coefficient of variation. [b] Values were taken from Zarkadas et al. (1994). [c] Computed according to the methods of Heidelbaugh et al. (1975), Horstmann (1979), and Zarkadas et al. (1988a,b). [d−f] Means along a row with different superscripts are significantly different (Dungan, 1955).

temperate regions of southwestern Ontario. Most of the production is sold to Japan for the manufacture of natto, a fermented food product made from whole soybeans. For this product, uniformly small yellow natto type soybeans, which soak, ferment, and cook evenly, are highly desirable.

Natto soybeans are a very good source of protein, and fermentation further enhances their protein quality by inactivating the trypsin inhibitors and lipoxygenases (Standal, 1963; Hesseltine, 1983b; Lin, 1991). In addition, fermentation reduces the undesirable beany flavors of soybeans. The average protein content of the six natto soybean cultivars investigated, calculated according to the method of Horstmann (1979), are summarized in Table 1 and represent the mean of 48 determinations. The weighted mean nitrogen contents of natto soybeans, calculated according to the method of Heidelbaugh et al. (1975) by the summation of the amino acid nitrogen contents of individual natto cultivars, are presented in Table 2. Significant variations ($P < 0.05$) in the protein content among these cultivars were noted. Values for protein content ranged from 30.4% in AC T2653 to 31.0% (AC Pinson), 31.0% (Nattawa), 32.1% (TNS), and 32.3% (Canatto), compared to the higher protein content of Nattosan, which was 34.2%. There was also a small but significant variation ($P < 0.05$) in the total nitrogen content, on a dry weight basis, among these cultivars ranging from 5.15% to 5.92%. These data compared favorably with those of Zarkadas et al. (1993a,b, 1994) reported for Maple Arrow.

The overall amino acid composition of the new northern adapted natto soybean cultivars, expressed as grams of amino acid per kilogram of anhydrous fat- and ash-free plant tissue protein, and as grams of amino acid per 16 g of total nitrogen as recommended by FAO/WHO (1991), are presented in Tables 1 and 2, respectively. These results show that all new natto cultivars were high in several amino acids, including glutamic acid (16.5−18.1% of the total amino acids), aspartic acid (10.7−11.5%), leucine (7.6−7.8%), arginine (7.1−8.2%), lysine (6.5−6.8%), phenylalanine (5.3−5.6%), and valine (4.7−5.3%). Thus, seven amino acids account for 59.3−63.7% of the total amino acids. The total basic amino acids, which include lysine, histidine, and arginine, constituted 16.5−18.2% of the total amino acids. These values are considerably lower than the acidic amino acids, which represent 28.1−29.9% of the total amino acid residues. Significant variations ($P < 0.01$) in acidic and hydrophilic amino acid content were found among the natto cultivars evaluated. Natto soybeans contained high levels of glutamic acid, reflecting its major role in seed metabolism. The variation noted for proline among the six natto soybean cultivars evaluated was statistically significant ($P < 0.001$). These results are in accord with those of Steinke (1992), who has reported a proline value of 5.1% for isolated soybean protein. Natto soybeans are also a good source of aromatic amino acids, which vary significantly among the six natto soybeans ($P < 0.01$ to $P < 0.001$). These data correspond closely to those reported by Steinke (1992) and Zarkadas et al. (1993a, 1994), but are much higher than those of Hesseltine (1983a).

The amino acid residues present in lowest amounts are methionine (2.03−2.21%), tryptophan (1.36−1.51%), and cysteine, which accounted for a further 1.88−2.47% of the total. The levels of methionine found in this study are higher than those reported by Cavins et al. (1972) and Kellor (1974) for defatted soybean flour and grits, by Hesseltine (1983b) for natto soybeans, and by Burton

Table 3. Essential Amino Acid (EAA) Scores of Six Natto 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 (2−5 years old)	soybean cultivars						natto[b]	egg[a]
		Canatto	Nattawa	TNS	Nattosan	AC T2653	AC Pinson		
		Milligrams of Amino Acid per Gram of Total Protein[c]							
histidine	19	25	31	31	28	29	29		22
isoleucine	28	53	48	47	50	47	48	51	54
leucine	66	77	78	77	78	76	76	75	86
lysine	58	66	68	68	66	63	67	56	70
methionine + cyst(e)ine	25	43	47	44	45	40	42	23	57
phenylalanine + tyrosine	63	100	101	97	96	90	92	75	93
threonine	34	31	39	38	36	53	50	41	47
tryptophan	11	15	14	14	14	14	14	12	17
valine	35	54	51	49	52	51	53	51	66
% total protein									
EAA_9[a]	33.9	46.6	47	46	45	46	47		512
EAA index,[d] %		98	86	93	83	90	90		
total EAA, mg/g of N[e]		3383	3002	3157	2826	3041	3048		3215
		Percent True Protein Digestibility[f] in Man							
		91	91	91	91	91	91		97
		Protein Digestibility Corrected Amino Acid Score[f]							
		91	91	91	91	91	91		97

[a] Data from FAO/WHO/UNU (1985) and FAO/WHO (1991). [b] Taken from Hesseltine (1983b). [c] Calculation of protein ratings of natto soybean cultivars was carried out by comparison of the amino acid 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). [d] Calculated according to the methods of Block and Mitchell (1946) and Oser (1951). [e] Computed from reference protein standards (FAO/WHO, 1965). [f] True protein digestibility values were taken from the U.S. Food and Drug Administration (1993; *Federal Register*, Appendix B).

et al. (1982) from their recurrent selection studies for increasing percent protein in soybeans. These results were also higher than those reported by Zarkadas et al. (1993a, 1994) for normal soybeans (cv. Maple Arrow and AC Proteus). However, Burton et al. (1982) suggested that such differences could be a result of enviromental rather than genetic factors. In all of the natto soybean cultivars studied, cyst(e)ine varied significantly ($P < 0.001$) among these genotypes. The lowest mean values were found in the AC T2653 and AC Pinson genotypes, and the results reported in Table 1 were very similar to those of Kellor (1974), Burton et al. (1982), and Zarkadas et al. (1993a, 1994), but were considerably higher than those of Steinke (1992). Moreover, these results indicate that the effect of genotype in soybean seed amino acid content was statistically highly significant (Tables 1 and 2) for the above amino acids.

A comparison of the essential amino acid (EAA) patterns (milligrams per gram of dietary nitrogen) of the five new soybean cultivars (Table 3) indicates that natto type soybeans contain significant amounts of all EAA required for both human and animal nutrition (Block and Mitchell, 1946; Oser, 1951) and are limited only in methionine and, to a lesser extent, in valine and isoleucine. Mean values for total EAA calculated according to the procedure of FAO/WHO (1965) show that natto soybeans have slightly lower EAA content (from 2838 to 3123 mg of EAA/g of N) than milk or egg proteins (from 3200 to 3215 mg of EAA/g of N). Similar results were obtained from the EAA indices (Oser, 1951), which show that natto soybeans provide from 83% to 91% of the essential amino acids compared to hen's egg protein.

A comparison of the EAA pattern of the natto soybean proteins with that of the reference amino acid pattern (Table 3) indicates that natto soybean proteins meet this pattern and contain all of the EAA_9, ranging from 45% to 47% compared to the 33.9% reference protein pattern value given by the FAO/WHO/UNU (1985) for a 2−5-year-old child. The amino acid profile of natto soybeans indicates that they provide a good balance of total EAA and have a protein digestibility corrected amino acid score of 91% for all cultivars, compared to the value of egg protein (97%). Young and Steinke (1992) and Young and Pellett (1990, 1994) showed that at protein intakes needed to meet the total nitrogen requirements, soybean proteins would satisfy the EAA needs of both childen 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 which modest supplementation of soybean-based formulas with methionine may be beneficial (Erdman and Fordyce, 1989).

The data presented in this paper show that the five new northern adapted soybean cultivars, as well as the earlier cultivar Nattawa, contained an excellent balance of EAA and can be considered as a good source of high-quality plant proteins for both human and animal nutrition. From these results, it is evident that a potentially useful means for evaluating the protein quality of different soybean cultivars would be based on an accurate quantitation of their amino acid composition, corrected for protein digestibility, as recommended by the FAO/WHO (1991) and by the U.S. Food and Drug Administration (1993).

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