Table V. Amino Acid Score of Wild Rice Protein

Amino acid	FAO pattern, mg/g of N	Wild rice, mg/g of N	Amino acid score, wild rice[a]
Isoleucine	250	275	110
Leucine	440	456	104
Lysine	340	281	82
Methionine + cystine	220	275	125
Phenylalanine + tyrosine	380	637	167
Threonine	250	225	90
Valine	310	375	120
Tryptophan	60	ND[b]	

[a] Calculated as amino acid in wild rice protein divided by amino acid in reference pattern and multiplied by 100 (FAO-WHO, 1973). [b] ND, not determined.

glutamic acid and proline and more arginine and aspartic acid than many of the cereal grains. The lysine content of wild rice was comparable to that of high-lysine corn (Wu and Sexson, 1976). Table V shows the amino acid scores of wild rice protein calculated based on the FAO pattern (FAO-WHO, 1973). Of the essential amino acids analyzed, lysine yielded the lowest score, 82. With the exception of lysine and threonine, the amount of each of the other essential acids matched or exceeded the FAO pattern.

Cereal grains are regarded primarily as energy sources rather than as sources of proteins. However, both the magnitude of protein malnutrition and the food consumption patterns have stimulated research effort to utilize cereals to help satisfy human protein needs. Insofar as PER and amino acid composition are concerned, wild rice has much to offer as a potentially excellent source of high-quality cereal protein.

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Comparative Nutritive Value, Amino Acid Content, Chemical Composition, and Digestibility in Vitro of Vegetable- and Grain-Type Soybeans

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Four vegetable-type and six grain-type varieties have been analyzed for their nutritive value, i.e., PER (protein efficiency ratio), NPR (net protein retention), chemical composition, and in vitro digestion. The protein quality index based on PER at 10% protein level was found to be highest in vegetable-type varieties Coker Stuart, 28-1-2, and Coker-240. Grain-type varieties were generally rich in crude fat, whereas vegetable-type varieties showed superiority over grain type in respect of iron content. A chemical score based on the essential amino acid content of egg protein and FAO pattern (1973) has indicated the level of first limiting amino acid methionine and cysteine (sulfur amino acids) in vegetable- and grain-type varieties of soybean. The EAAI (essential amino acid index) and BV (biological value) were calculated and found to be well correlated to PER values in the case of vegetable- and grain-type varieties. The study on in vitro digestion using trypsin enzyme revealed wide variation in different varieties. The high nutritive value, i.e., PER and NPR, obtained in the case of vegetable and some of the grain varieties is due to low trypsin inhibitor activity in the raw seeds and also to the inactivation of trypsin inhibitor on autoclaving.

Soybean and soybean products have been consumed over the years both separately and in food blends to increase protein intake and amino acid balance in the diet of man and animals in many oriental countries, but numerous attempts to introduce it in India have not succeeded very well because of an unpleasant beany flavor and difficulty in cooking (Kanthamani, 1970; Rathod and Williams, 1973). However, the vegetable varieties have been found to be superior to grain-type varieties in flavor, texture, and cooking (Morse, 1950).

The amino acid composition of a protein seldom gives its true nutritional value (Eggum, 1970). The nutritive value depends upon the presence of inhibitors of digestive enzymes, digestibility and absorption, toxic factors, and

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chemical composition, particularly in the case of legumes. Biological evaluation, i.e., PER, NPR, BV combined with chemical composition and amino acid composition, has been recognized as a test criteria in evaluation of nutritive value. The chemical composition of soybean is further governed by hereditary and environmental characters. Although many workers have reported on the protein quality (Kapoor and Gupta, 1975), chemical composition (Mustakas et al., 1970), and amino acid composition (Singh, 1975) of soybean, such information regarding vegetable- and grain-type varieties is very limited (Gupta et al., 1976a). The present study was therefore taken in hand to find out and compare the chemical composition, amino acid composition, nutritive value, and in vitro digestibility in vegetable- and grain-type varieties.

MATERIALS AND METHODS

Four vegetable type varieties, namely BB-4-7-1, Coker-240, 28-1-2, Coker Stuart, and six grain type varieties, namely Hill, Bragg, JS-17, Pb-1, Kalitur, and JS-2, were selected for the study. The samples were obtained from 1973–1974 field trials conducted at J.L.N. Agricultural University, Jabalpur, India. The samples were analyzed for moisture content, crude fat, ether extract, ash content, phosphorus, calcium, iron, amino acid content, PER, NPR, and in vitro digestion. The standard methods of analysis used in the study are described below.

Methods. The soybean seeds were dry heated in an electric oven at 50–60 °C for 2 h to inactivate lipoxidase (Mustakas et al., 1967), cooled and cracked into two parts in an electric grinding machine, dehulled, and dehusked by blowing air into the husks. The dehulled samples were powdered in an electric grinding mill mesh No. 60. The powdered samples were used for proximate determination and making up diets.

The methods of the Association of Official Agricultural Chemists (AOAC, 1955) were used to determine ash, nitrogen, iron, and calcium content. Oil was determined as in the AOAC method for oil in cotton seed except that the samples were extracted for 8 h with petroleum ether, bp 40–60 °C. Phosphorus was determined by King's (1932) method, a modification of the method of Fiske and Subbarow (1925). The protein content of the samples was calculated by multiplying the Kjeldahl N by 6.25. The amino acid composition was studied using a Technicon automatic amino acid analyzer [details given in the study of Sikka et al. (1975)], according to the method of Moore and Stein (1954). Tryptophan was determined by the method of Spies and Chambers (1949).

Diets. The powdered samples of soybean as obtained above were autoclaved with water (w/v) (250 g of powdered sample in 250 mL of water) in a 1-L conical flask for 20 min at 15 lb of atmospheric pressure, cooled, and then air-dried. The dried samples were then used for making diets.

The diets for all the biological experiments were prepared at a 10% protein level and 10% fat level after determining crude fat in the sample. The composition of 100 g of diet was as follows: test sample flour calculated weight to give 10% protein; groundnut oil calculated weight to give 10% fat (containing 1 mg or 100 IU of vitamin E); 4% mineral mixture (USP XVII 4) composition as per Sikka et al. (1975); 5 g of glucose and 5 g of complete vitamin mixture (Manna and Hauge, 1953); and 2 drops of adoxline containing vitamin A (12 000 IU) and vitamin D_2 (I.P. 2000 IU/g) was fed orally twice a week.

Protein Efficiency Ratio (PER). PER was determined by the method of Osborne et al. (1919). Weanling albino rats about 22 days old and weighing 20–40 g were divided into 14 groups. All groups within each experiment had the same average initial weight. Each group consisted of three males and two females.

The rats were placed in individual all-wire cages with a raised platform. Water was available to them at all times. Food intake was measured every day, and spilled food was collected daily and used to correct the amount of food intake. The animals were weighed twice a week for 4 weeks or 28 days.

Net Protein Retention (NPR). NPR was determined by the method of Bender and Doell (1957). One-month-old albino rats, three males and two females in each group (14 groups in all), having the same initial weight were used. All soybean flours in the diet were at a 10% protein level. A nonprotein diet was prepared by replacing soybean flour with protein-free starch in the diet. Ten groups were fed with soybean flours, two groups with nonproteinous diet, and two groups with a standard casein diet. Two sets of experiments were run, one having 10 groups (eight of test diet, one of nonprotein diet, and one of standard diet) and the other set having four groups (two of test diet, one of nonprotein diet, and one of standard diet). The experiment was continued for 10 days. The weight of each rat was recorded every third day. NPR was calculated as follows:

$$\text{NPR} = \frac{\text{weight gain of TPG} + \text{weight loss of NPG}}{\text{weight of protein consumed}}$$

where TPG represents the test protein group (soybean flour) and NPG represents the nonprotein group.

Biological Value (BV). BV was determined by calculation, according to the regression equation given by Duggal and Eggum (1977), for proteins with methionine + cystine as limiting amino acids.

In Vitro Digestion. Soybean flour in raw and autoclaved forms was digested with proteolytic enzyme trypsin according to the method of Villegas et al. (1968) and Borcheres and Ackerson (1947), with slight modification. The digestibility was expressed in terms of milligram of tyrosine released during the enzyme action.

Preparation of Raw Sample. The whole seed sample was heated in an electric oven to 50–60 °C, cooled, dehulled, and then ground to fine powder, 100 mesh. The sample was defatted with petroleum ether, bp 40–60 °C. After removal of ether, the sample was air-dried and used in the study.

Preparation of Autoclaved Sample. The above defatted sample was mixed with an equal amount of water (w/v) in a 1-L conical flask, autoclaved at 15 lb of pressure for 20 min, cooled, air-dried, ground to fine powder (100 mesh), and used in the study.

Defatted raw or autoclaved sample containing 400–500 mg of protein was suspended in 45 mL of 0.1 M sodium phosphate buffer (pH 7.6). To the above suspension was added 5 mL of trypsin (2000 units/g, Merck) enzyme solution (2%). The incubation was done for 1 h at 37 °C with intermittent shaking in a BOD incubator. The reaction was stopped by the addition of an equal volume of 5% TCA and centrifuged at 5000 rpm for 30 min. The tyrosine liberated during the reaction was determined as below. A 0.5-mL aliquot was mixed with 5 mL of 2.8 N sodium carbonate and 1.5 mL of 1 N phenol reagent in a 10-mL volumetric flask and made to volume. The intensity of color that developed was read at 640 mμ in a photoelectric colorimeter, and in vitro digestibility was expressed as milligram of tyrosine released/100 g of protein. Due care was taken for the determination of tyrosine present in the sample before enzyme reaction. A suitable tyrosine

Table I. Mean Values of Food Intake, Protein Intake, Gain in Weight, and PER of Different Varieities of Soybean and Casein at 10% Protein Level

Protein source	Food intake, g/28 days per animal	Protein intake, g/28 days per animal at 10%	Mean wt gain, g/28 days per animal	PER	F value obsd
Hill	141[b]	14.1 ± 1.4[a]	27.5 ± 5.2[a]	1.94 ± 0.025[a]	20.2[d]
Kalitur	144	14.4 ± 1.4	26.0 ± 4.8	1.80 ± 0.022	
B.B.4-7-1	110	11.0 ± 0.8	16.5 ± 4.9	1.50 ± 0.015	21.3[d]
Bragg	121	12.1 ± 1.0	20.2 ± 2.9	1.66 ± 0.014	
Coker-240	152	15.2 ± 1.6	32.0 ± 7.6	2.10 ± 0.030	
28-1-2	187	18.7 ± 2.4	40.6 ± 12.2	2.17 ± 0.033	7.4[d]
JS-17	149	14.9 ± 1.4	20.9 ± 3.5	1.40 ± 0.013	
Pb-1	116	11.6 ± 0.9	14.8 ± 1.6	1.27 ± 0.011	
JS-2	187	18.7 ± 1.9	26.9 ± 4.0	1.60 ± 0.014	
Coker Stuart	205	20.5 ± 2.3	42.2 ± 10.0	2.31 ± 0.028	20.2[d]
Casein	143	14.3 ± 1.5	38.3 ± 11.0	2.68 ± 0.049	

[a] Standard error. [b] Average value of one animal. [c] F value, significant at P = 0.05. [d] F value, significant at P = 0.01.

Table II. Mean Values of Food Intake, Protein Intake, Gain in Weight, and NPR of Different Varieties of Soybean and Casein at 10% Protein Level

Protein source	Food intake, g/10 days per animal	Protein intake, g/10 days per animal at 10%	Mean wt gain, g/10 days per animal	Loss in wt, g	NPR
Hill	44.5[b]	4.45 ± 0.7[a]	9.5 ± 3.0[a]	7.0	3.7 ± 0.45[a]
Kalitur	43.0	4.30 ± 0.6	8.6 ± 2.4	7.0	3.6 ± 0.43
B.B.4-7-1	29.0	2.90 ± 0.3	3.3 ± 0.4	7.0	3.5 ± 0.40
Bragg	39.0	3.90 ± 0.5	7.9 ± 2.1	7.0	3.8 ± 0.48
Coker-240	34.0	3.40 ± 0.4	2.8 ± 0.2	7.0	2.9 ± 0.27
28-1-2	44.0	4.40 ± 0.6	7.8 ± 2.0	7.0	3.4 ± 0.37
JS-17	39.0	3.90 ± 0.5	6.3 ± 1.3	7.0	3.4 ± 0.38
Pb-1	33.0	3.30 ± 0.4	1.3 ± 0.6	7.0	2.5 ± 0.20
JS-2	62.0	6.20 ± 1.2	16.2 ± 3.4	7.4	3.2 ± 0.55
Coker Stuart	68.0	6.80 ± 1.5	16.0 ± 3.5	7.4	3.9 ± 0.55
Casein	62.4	6.24 ± 1.2	16.1 ± 3.4	7.4	3.8 ± 0.54

[a] Standard error. [b] Average value of one animal.

standard estimation was also run along with the sample.

RESULTS AND DISCUSSION

Table I shows food intake, protein intake, weight gain, and PER. The PER values reveal that vegetable-type varieties "Coker Stuart", "28-1-2", and "Coker 240" had better protein quality than seed type soybean varieties "Hill", "Kalitur", "Bragg", "JS-17", "JS-2", and "PB-1". Among the vegetable-type varieties, Coker Stuart PER value of 2.31 approaches very closely the values of casein PER (2.68). Among seed-type varieties, Hill seemed to be better in protein quality than the other ones. The gain in weight and food intake of rats fed on vegetable-type soybean varieties were also higher than the grain-type varieties. The analysis of variance shows that PER values were significant in case of Coker Stuart, 28-1-2, BB 4-7-1 (all vegetable type), and Hill (grain type) (Table I).

Similar PER values were reported in full fat soy flour by Bookwalter et al. (1975), Bressani et al. (1974), Mustakas et al. (1970), Sidwell et al. (1970), McLaughlon et al. (1967), Maneepum et al. (1974), and Kakade et al. (1972). However, Kapoor and Gupta (1975) and Henry (1965) obtained higher PER values for soybean flour. The higher weight gain and hence higher PER values obtained by Kapoor and Gupta (1975) may be due to the fact that fat content of the soybean variety was not taken into account, whereas in this study the fat content was also fixed at 10% protein level after duely taking into account the fat content of the sample. Further, it may be due to variation in the chemical composition in the varieties grown at New Delhi and Jabalpur because of agricultural practices applied, i.e., fertilizer doses, environmental factors, and soil.

The NPR values of vegetable- and grain-type varieties are presented in Table II. The values listed in Table II are highest for vegetable-type variety Coker Stuart and lowest for grain-type variety Pb-1. Bender and Doell (1957) reported that NPR is far more accurate measure of protein value, since it measures protein efficiency based on both growth and maintenance. The results of NPR values of this study agree very well with those of Kapoor and Gupta (1975) for the Bragg variety but they obtained higher NPR value for Pb-1. The higher PER values of vegetable-type soybean varieties are probably due to high threonine and valine content, the second limiting amino acids of these varieties (Table III).

Table III. Amino Acid Composition of Vegetable- and Grain-Type Varieties (g/16 g of N)

Amino acid	Vegetable type		Grain type	
	Coker-240	Coker Stuart	Bragg	Pb-1
Aspartic acid	11.89	11.92	11.86	11.28
Threonine	4.12	4.23	3.98	3.68
Serine	4.89	4.90	4.84	4.38
Glutamic acid	20.11	20.23	19.71	19.25
Proline	5.01	4.98	5.07	5.20
Glycine	4.27	4.32	4.25	4.02
Alanine	4.44	4.60	4.39	4.06
Valine	5.18	5.32	5.12	4.77
Isoleucine	5.00	5.20	4.80	4.57
Leucine	7.25	7.21	7.65	7.10
Tyrosine	4.15	4.25	4.05	3.73
Phenylalanine	5.23	5.47	5.22	4.87
Lysine	6.45	6.80	6.30	6.21
Histidine	2.59	2.65	2.58	2.55
Ammonia	1.34	1.30	1.46	1.55
Arginine	7.90	7.94	7.84	7.55
Methionine	1.32	1.50	1.32	1.45
Cystine	1.50	1.55	1.33	1.65
Tryptophan	1.60	1.64	1.52	1.64

Table IV. E:N, E:P, and E:T Ratios, Chemical Score, EAAI, and BV of Vegetable- and Seed-Type Varieties of Soybean

Feed source	E:N,[b] mg/g	E:P,[c] mg/g	E:T,[d] mg/g	EAAI,[a] %	Chemical score, %	BV	Chemical score, %
Coker-240	881	482	468	85.59	52.6	50.70	80.1
Coker Stuart	898	496	473	88.16	56.8	53.50	86.6
Bragg	884	478	469	83.77	49.4	48.49	75.2
Pb-1	869	456	465	82.22	57.8	54.29	88.0
EGG				100.00	100.00	100.00	
FAO/WHO (1973)							100.00

[a] EAAI (essential amino acid index) is based upon the ratios of the amounts of essential amino acids in a protein relative to their amount in whole egg protein (Oser, 1951). Chemical score is the percentage of the most deficient essential amino acid in the protein as compared to the requirement pattern (Mitchell and Block, 1946). [b] E:N = ratio of essential amino acids to nonessential amino acids. [c] E:P = ratio of essential amino acids to protein (100 g). [d] E:T = ratio of essential amino acids to total amino acids.

Table V. Distribution of Chemical Constituents in Soybean Varieties (Results Expressed at 10% Moisture Free Basis)

Variety	Ash	Protein N × 6.25	Ether extract crude fat	Phosphorus, g/100 g	Calcium, g/100 g	Iron, mg/100 g
Hill	5.14	44.45	25.41	0.65	0.37	8.9
Kalitur	5.07	49.71	20.96	0.64	0.34	8.9
BB.4-7-1	5.52	45.14	23.64	0.74	0.42	10.4
Bragg	5.55	47.57	26.66	0.65	0.45	10.2
Coker-240	4.99	50.66	23.60	0.47	0.36	12.0
28-1-2	5.21	46.81	23.53	0.95	0.45	9.9
JS-17	5.29	45.15	26.04	0.69	0.41	7.2
Pb-1	5.23	49.01	22.67	0.56	0.44	15.5
JS-2	5.19	49.97	24.98	0.67	0.47	7.2
Coker Stuart	5.53	46.98	23.20	0.51	0.51	10.2

Amino Acid Composition, Chemical Score, BV, and EAAI. The amino acid composition of two varieties each of vegetable- and grain-type soybean was determined (Table III). It is observed that soybean approaches the level of amino acids to a great extent as compared to the FAO pattern or amino acids in egg. The first limiting amino acids in all the four varieties studied are methionine + cystine (sulfur-containing amino acids). The second limiting amino acids were found to be threonine and valine. This further confirms the findings of earlier workers: Kapoor and Gupta (1975), Coppock (1974), Panemangalore et al. (1964), and Bookwalter et al. (1975). The amino acid composition of soybean varieties reported agrees very closely with that of Singh (1975).

The E:N ratio (ratio of essential amino acid to nonessential amino acids), E:P ratio (ratio of essential amino acids to protein), E:T ratio (ratio of essential amino acids to total amino acids), chemical score, EAAI, and BV in these four varieties are presented in Table IV. The results show that the E:N, E:P, and E:T ratios are highest in vegetable type soybean, i.e., "Coker Stuart", followed closely by Bragg and Coker-240, which are very close to one other. The lowest results were in the case of variety Pb-1.

Coker Stuart has given the highest value for EAAI, whereas Pb-1 has given the highest value for chemical score and BV, followed closely by Coker Stuart. Although the value for chemical score and BV are highest for Pb-1, Pb-1 has given the lowest PER and NPR values which may probably be due to high trypsin inhibitor activity.

Chemical Composition. Results of the distribution of chemical constituents in soybean varieties (Table V) show that Coker-240 contains the maximum amount of protein, followed closely by JS-2 and Kalitur.

The maximum amount of iron was present in the grain-type variety Pb-1 which was an exception from other grain-type varieties, which were comparably low in iron. The variation is between 7.2 mg/100 g to 15.5 mg/100 g. Similarly, phosphorus and calcium varies from 0.47/100 g to 0.95 g/100 g and 0.36 g/100 g to 0.51 g/100 g, respectively. The vegetable-type varieties contain lower amounts of fat than grain-type varieties. The results agree very well with those reported by Gupta et al. (1976a) and Bookwalter et al. (1975). The general trend is the same as obtained by Gupta et al. (1976a), although the percentage obtained by us is slightly higher. This may be due to seasonal variation.

Table VI. Tyrosine Released during in Vitro Digestion of Raw and Autoclaved Soybean Proteins by Trypsin Enzyme

Variety	Raw form, tyrosine released, mg/100 g of protein	Autoclaved form, tyrosine released, mg/100 g of protein	Autoclaved form, mg of tyrosine/1 PER unit
Hill	0.42	1.44	0.74
Kalitur	0.41	1.42	0.78
BB.4-7-1	0.32	0.96	0.64
Bragg	0.30	1.22	0.73
Coker-240	0.43	1.46	0.69
2B-1-2	0.42	1.39	0.64
JS-17	0.15	0.70	0.56
Pb-1	0.13	0.62	0.48
JS-2	0.40	1.00	0.62
Coker Stuart	0.32	1.44	0.53

In Vitro Digestion of Soybean. An experiment was conducted to determine the trypsin inhibitor activity in the grain- and vegetable-type soybean varieties both in raw and autoclaved seeds using trypsin enzyme. The release of tyrosine under assay conditions was taken as index of total amino acid release and as measure of trypsin inhibitor activity (Table VI). It is quite evident from the results (Table VI) that tyrosine released in raw soybean seeds is very low as compared to autoclaved soybean seeds. There is wide variation between different varieties analyzed; the percentage variation of tyrosine released in raw soybean seeds is between 0.13 to 0.43 mg and in autoclaved seeds is between 0.62 to 1.46 mg. The results agree very well with the findings of earlier workers (Gupta et al., 1976b). It appears from the results that the high nutritive value, i.e., PER obtained in the case of vegetable-type varieties, is due

to low trypsin inhibitor activity in the raw seeds and also to the inactivation of trypsin inhibitor on autoclaving.

A significant positive correlation (0.82) was observed between tyrosine released on autoclaving and average values of PER for different varieties. The grain-type variety Pb-1 has given the lowest PER and NPR values and this is due to high trypsin inhibitor activity in the raw seeds. Further, better nutritive values of vegetable-type varieties appear to be due to better amino acid patterns as a whole rather than any specific amino acid (Table III).

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Oligosaccharides in Pulses: Varietal Differences and Effects of Cooking and Germination

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Oligosaccharide content of four commonly used Indian pulses [red gram or pigeon pea (*Cajanus cajan*), Bengal gram or chick pea (*Cicer arietinum*), black gram (*Phaseolus mungo*), and green gram (*Phaseolus aureus*)] was estimated. Red gram and green gram had significantly higher amounts of verbascose and stachyose than did Bengal gram and black gram. Bengal gram had higher amounts of raffinose. There were significant variations within varieties of the same pulse. Cooking brought about a significant increase in the oligosaccharide content of all the pulses. However, the increase was highest in Bengal gram and least in green gram. Germination decreased the oligosaccharide content in all the pulses. A systematic study needs to be undertaken to understand the precise role of oligosaccharides in flatus production.

Habitual Indian diets contain pulses, and the average per capita consumption is around 40 g daily (Gopalan et al., 1971). While they are good sources of protein, many common varieties of pulses are known to contain antinutritional factors such as trypsin inhibitors (Borchers et al., 1947), hemagglutinins (Huprikar and Sohonie, 1961), cyanogenic agents (Sharpless et al., 1939), and saponins (Birk et al., 1963) which may affect the utilization of pulse protein. Many of these factors are heat labile and may therefore have little relevance in human nutrition. Oligosaccharides of the raffinose family, in which galactose is present in α-linkage, are present in mature legumes (Shallenberger and Moyer, 1961) and have been shown to be responsible for flatulence following the consumption of these beans (Steggerda, 1968). Unlike trypsin inhibitor and other antinutritional factors, oligosaccharides are heat stable. Isolated oligosaccharides have been shown to induce flatulence in experimental animals and in man (Calloway, 1973).

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