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Legumes Grown under Nonirrigated Conditions

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The nutritive quality of different ripening soybeans was compared with that of dry beans and various colored seeds and dry peas with regard to their protein and fat contents, amino acid compositions, and trypsin inhibitor activity. The bean protein of three legumes contained the highest levels of methionine, leucine, phenylalanine, and histidine, of the essential amino acids, respectively. The nutritive quality of soy protein, containing high glutamine contents, and dry pea protein, with its large valine contents, exceeded that of the others. Under nonirrigated growing conditions, there were high levels of leucine, valine, glycine, and proline in beans, independent of seed color. The beans with colored seed contained larger concentrations of methionine, cysteine, phenylalanine, and leucine essential amino acids than white bean. The lysine content of white bean protein was as high as that of the pea. The nutritive quality of white bean seeds could be increased with selection for low level of trypsin inhibitors under nonirrigated growing conditions. In water-deficient conditions, the level of valine and proline amino acids increased in soy seed protein, independent of the maturity groups. Irrigation has no effect on the increase, either in protein content or in essential amino acids, in soybeans and, indeed, there were no differences in the above-mentioned among the maturity groups.

Keywords: *Amino acid; nutritive quality; legume; nonirrigation*

INTRODUCTION

Protein quality, which comprises the nutritional value of food, depends on amino acid content and on the physiological utilization of specific amino acids after digestion. The greater the ratio of essential amino acids, the better the biological value of the protein. Thus, proteins deficient in one or more amino acids are of poor quality (Friedman, 1996; Mercer et al., 1989). Amino acids are divided into three categories, based on their rates of protein synthesis in vivo. These are (a) essential, such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; (b) conditionally essential, such as arginine, cysteine, and tyrosine; and (c) nonessential amino acids, alanine, aspartic acid, glutamic acid, glycine, proline, and serine (Mercer et al., 1989). More recent thought on essential amino acids emphasizes their role in promoting growth in the young, preventing diseases, and maintaining a positive nitrogen balance (Bálint, 1986; Laidlaw and Koople, 1987). Human amino acid requirements should be best satisfied by methionine, phenylalanine, valine, and lysine (Young and Pellett, 1991).

Because of their positive dietary effects, the consumption of legumes is highly recommendable for diabetics and those individuals with high blood cholesterol levels (Antal and Bíró, 1991). In different age groups, for example, in children, the proportion of food legumes in the diet is limited by the chemical components in grain, as well as by the amount of antinutritive factor intake (Brandon et al., 1991). The digestibility of crude protein varies with the concentration of protein in the diet. Low digestibility may be due to the occurrence of amino acids in less digestible parts and to protease inhibitors, amylase inhibitors, lectins, phytates, and tannins (McDonough et al., 1990; Sarwar and McDonough, 1990; Sarwar and Paquet, 1989). Protease inhibitors, such as trypsin inhibitors, decrease the digestibility of protein (Van der Poel, 1990), particularly in legume seeds. However, trypsin inhibitor activity can be decreased by heat treatment (Friedman et al., 1991; Genovese and Lajolo, 1996). Various treatments during processing affect the availability of essential amino acids. Heat and alkali treatments caused the formation of toxic compounds, such as lysinoalanine, which formed from lysine in the food protein (Mao et al., 1993; Yin et al., 1994). At high temperature and/or in the presence of carbonyl compounds, tryptophan forms carboline, which is a carcinogen (Friedman, 1996). It is already well-known that the level of L-methionine determines the nutritive quality of legume proteins (Yin et al., 1994; Friedman, 1996); nevertheless, their lysine, isoleucine, and phenylalanine plus tyrosine contents are high (Kochhar et al., 1988).

Glutamic acid, aspartic acid, and alanine are said to be the precursors of many amino acids in protein

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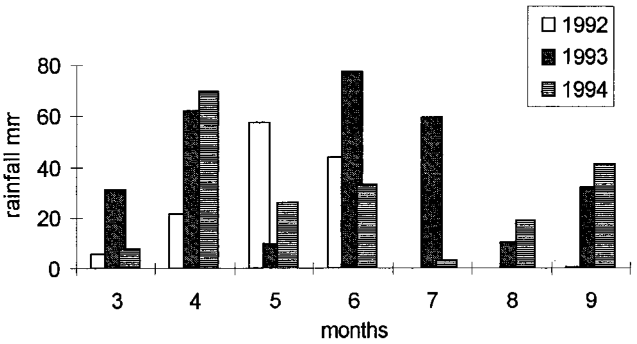

Figure 1. Distribution of rainfall during the vegetation from 1992 to 1994.

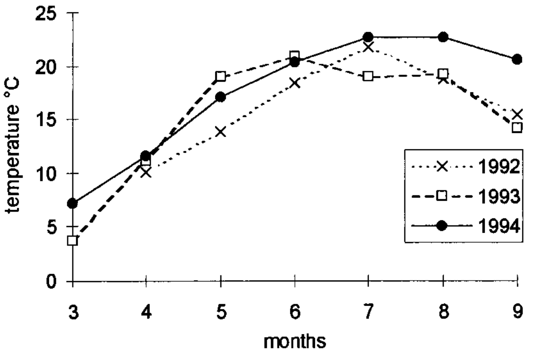

Figure 2. Average temperature of months during the vegetation from 1992 to 1994.

synthesis. The accumulation of asparagine is very intensive in young lupin crops, due to the high asparaginase enzyme activity (Ratajczak, 1986). The concentration of glutamine was the largest in pea seeds by the 31st and 32nd days after flowering (Murray, 1987), and, in the early stages of development, seedcoat secretion is enriched with histidine (Peoples et al., 1985). Mossé and Baudet (1977) demonstrated a direct relationship between the contents of lysine plus histidine plus arginine and crude protein contents for broad beans (*Vicia faba* L.). An increase in protein content does not necessarily ensure good nutritive quality. Low negative correlations have been emphasized between the percentage of protein and methionine (Bliss and Hall, 1977; Ma and Bliss, 1978) and between protein and yield of soybean, respectively (Smith et al., 1989; Hartwig and Kilen, 1991; Shannon et al., 1972; Thorne and Fehr, 1970). Protein-bound methionine is poorly utilized, presumably because of its poor digestibility (Begbie and Pusztai, 1989; Wu et al., 1996). Protein content showed a very high negative correlation with carbohydrate content in cowpea (Nielsen et al., 1993), similar to that in French beans, the protein content of which decreased while its sugar content increased during seed development (Nemeskéri et al., 1994).

In this study, we made a comparison of nutritive quality, on the basis of such criteria as protein and fat contents, amino acid composition, and trypsin inhibitor activity, in various maturing soybean varieties and dry beans with variously colored seeds as well as dry peas. We analyzed the effect of drought on compositions of amino acids in soy and bean protein produced under nonirrigated growing conditions.

MATERIALS AND METHODS

From 1992 to 1994, we investigated 11 white and 11 colored dry bean seed lines, early, mid-early, and late maturing soybean varieties and dry peas, respectively. The white-seeded breeding lines can be sorted into navy types, while the color-seeded varieties represented both dark red kidney types and those which were beige with visible red markings. McCall and BS-38 varieties presented the early maturing group of soy, Evans and Eszter varieties the mid-early ones, and Borza, Panther, and K-5846 the late maturing groups of soy. Smaragd and LU-70 dry peas with green seed and Romeo, Bohatyr, LU-254, and Y-228 of yellow-seeded dry peas were also investigated.

The varieties and breeding lines were planted in four random repetitious field experiments on chernozem soil, under both irrigated and nonirrigated conditions. Hungary extends from 46° to 48° latitude, and the hot and dry periods often occur during the vegetation (as seen in Figure 1). The sowing of soybeans is carried out between the 27th and 29th of April, and the beans were planted in the first half of May every year, so that the flowering of both species would be at the end of June or the first of July. In 1992 and 1993, the low rainfall (0−59 mm) in July was not favorable for fertilization of soybeans and beans (Figure 1). The hot and dry climate in August hampered the seed development of the plants (Figure 2).

Although drought conditions prevailed between 1992 and 1993, during the plants' generative periods, we did not irrigate so that we could test the effect of drought on the nutritive quality of the crop. In 1994, the climatic conditions were similar to those in previous years, but this time we irrigated the plants in 30 mm water doses during flowering. The dry pea varieties investigated represented the only nonirrigated field experiments. After harvesting, we measured the weight of 1000 grains from the four repetitions, and then 100 g seed samples were subjected to chemical analysis.

Chemical Analysis. The measurement of seed protein was carried out using the Kjel-Foss Automatic 16200 (A/S N. Foss-Electric, Hillerod, Denmark) apparatus according to the automated Kjeldahl method (AOAC, 1995), based on the weighing of 1 g of air-dried material, and supplied on dry matter.

The crude fat contents of seeds were dissolved in petroleum ether at a 40−70 °C boiling point, and samples of 0.5 g were analyzed by Soxtec System HT 1043 (Tecator AB, Höganäs, Sweden) equipment (AOAC, 1995).

Amino Acid Analysis. After hydrochloric acid hydrolysis of the samples, the content of amino acids in the protein was determined by using a Biotronik LC 3000 (Biotronik, Maintal, Germany) amino acid analyzer (AOAC, 1995). It is well-known that the cystine and cysteine decompose during acid hydrolysis. Thus, the cystine of protein was oxidized by performic acid to cysteic acid, and then it was followed by hydrolysis by hydrochloric acid. The results refer to cysteine.

Trypsin Inhibitor Activity. We weighed 10 g of full grits of soybean, bean, and pea seeds, and then the homogeneous samples were degreased by hexane. We used 0.1 mm granulated material for further examinations to determine the amount of trypsin inhibitors. The determination of the degree of enzyme inhibition, based on the MSZ-21175-1988 standard method (Hungarian Standard), coincides with the methods of Smith et al. (1980). After the addition of bovine trypsin at pH 9.5, the hydrolysis rate of the natural or artificial substrate decreased. Using as the substrate *N*-α-benzoyl-DL-arginine *p*-nitroanilide hydrochloride (DL-BAPA), a yellow *p*-nitroaniline compound was released, which can be measured by spectrophotometer at 410 nm. Enzyme activity directly correlates with the amount of colored compounds present; thus, the degree of inhibition based on the remaining trypsin can be expressed. The degree of enzyme inhibition was expressed in trypsin inhibition units (TIU) per milligram of fatfree dry matter. The results have been appraised by ANOVA statistical methods.

RESULTS AND DISCUSSION

Nutritive Quality. From 1992 to 1994, the results concerning the nutritive value of seeds in different

Table 1. Nutritive Quality of Seed of Soybean, Dry Pea, and Dry Bean under Nonirrigated Growth Conditions[a]

variety	group	1000 grain wt (g)	TIU/ mg[b]	fat[c]	protein[c]
soybean	early maturing	152	37.40	19.87	33.98
	mid-early maturing	148	41.23	18.53	33.48
	late maturing	153	29.82	17.66	36.57
dry bean	white-seeded	190	16.66	1.82	30.39
	color-seeded	430	18.95	1.47	28.20
dry pea	green-seeded	278	*d*	1.47	25.59
	yellow-seeded	251	*d*	1.01	23.52

[a] The values are the average of 3 years of experimental data. [b] The amount of trypsin inhibitor is expressed in trypsin inhibitor units (TIU) per milligram of fatfree dry matter. [c] Fat and protein contents are expressed as percentage of dry matter. [d] The amount of TIU was not detected.

Table 2. Amino Acid Composition of Seed Protein in Different Legumes

amino acid	soybean[a]	dry pea[a]	dry bean[a]
Asp	11.026 bc	10.430 c	11.586 a
Thr	3.682 ab	3.392 c	3.67 a
Ser	4.816 b	4.278 c	5.645 a
Glu	18.041 a	16.250 b	15.585 bc
Gly	3.818 ab	3.482 b	3.996 a
Ala	4.254	3.972	4.176
Cys	0.296 a	0.266 ab	0.173 b
Val	3.214 bc	3.702 a	3.095 c
Met	1.013 bc	0.798 c	1.288 a
Ile	3.052 ab	3.438 a	2.245 c
Leu	5.820 bc	5.508 c	6.310 a
Tyr	2.852	3.020	2.888
Phe	3.914 bc	3.912 c	4.582 a
His	3.65 bc	2.834 c	5.797 a
Lys	5.825 ab	6.098 a	5.458 c
Arg	6.373	6.838	6.491
Pro	4.802 ab	4.612 b	3.682 c
protein %	34.822 a	24.440 c	28.02 b

[a] The average were data based on 3 years of nonirrigated experiments. The concentration is expressed as grams per 100 g of protein; the values in a row having different letters are significantly ($P < 0.05$) different.

maturing soybean, dry bean with different colored seeds, and dry pea varieties are shown in Table 1. The values are the average of 3 years of experimental data. The experiments were carried out under nonirrigated conditions. The trypsin inhibitor activity is expressed in TIU. Fat and protein contents were the highest in soybean. The late maturing soybean varieties seemed to have the best nutritive quality because of their high protein content and relatively low TIU. The breeding bean lines selected for low trypsin inhibitor activity represented the white-seeded groups. The decrease of trypsin inhibitors and slightly increased levels of protein were due to selection in the white-seeded group, which differs from the results in colored-seeded groups, which were not selected. Our results concerning the trypsin inhibitors of various seedcoat bean are inconsistent with those of Wyckoff et al. (1983), who measured high TIU in white-seeded (small white) beans. In the colored-seeded beans, not using selection, the trypsin inhibitor activity was high, particularly that of dark red kidney types (Nemeskéri, 1997). Investigating protein availability, others (Koehler et al., 1987) determined that the best protein quality was found in pinto beans, whereas the poorest proved to be that of dark colored beans, such as red kidney and black turtle soup beans. Obviously, there exists a correlation with seedcoat color.

In dry peas trypsin inhibitor activity could not be detected by spectrophotometrical methods at 410 nm, contrary to findings from other studies (Vetter et al., 1984; Ferrasson et al., 1997). Six protease inhibitors were isolated from winter pea seeds that were found to be similar to those of *Vicia faba* inhibitors and belong to the Bowman−Birk class of trypsin inhibitors (Ferrason et al., 1997). The dry pea seemed to be more favorable for the human diet because of its minimum trypsin inhibitors and lower fat contents than the other two, although it has a low protein content (Table 1).

Because of the large fat content of soybean, its seeds contained much more energy than the other beans or pea seeds. Soybean seeds provided both significant energy and protein sources, but there is a strong inverse relationship between the seed protein and oil contents (Wilcox, 1995). When compared with the maturity group of soybeans, the fat content was significantly different only in the early and late maturity groups, independent of growing conditions. Oil accumulation was greater in early soybean groups grown under irrigation than in late maturity groups (Nemeskéri, 1997).

Amino Acid Composition. The requirements for essential amino acids vary in different age groups, the highest being particularly those of leucine, tyrosine plus phenylalanine, lysine, and valine in up to 5-year-old children, whereas it decreases by 3-fold in adults (FAO/WHO, 1991). The need for leucine and tyrosine plus phenylalanine amino acids in adults should be similar to that of preschool children, according to Young and Pellett (1991). In the diet of these groups, the bean protein may be important because of its essential amino acids.

We compared the amino acid composition of soybean with that of dry bean and dry pea seeds; the averages were data based on 3 years of nonirrigated experiments (as seen in Table 2).

Dry bean protein was rich in sulfurous amino acids (methionine) and had significantly higher levels of serine, leucine, phenylalanine, and histidine amino acids than dry pea and soy protein. However, isoleucine and lysine contents of bean protein were less than in the others.

Dry pea protein was the richest in valine and lysine amino acids, and soy protein was outstanding in its high glutamine contents, compared to the others. Comparing the bean protein to the soy protein, it had a larger isoleucine and lysine content; nevertheless, its high serine contents differentiate it from pea protein (Table 2).

Reoccurrences of drought during seed development and crop ripening change the quality of crops and their nutritive values. Under nonirrigated (A) growth conditions, the amino acid composition of dry pea protein was compared to beans with white and colored seeds (Table 3). The pea protein is much richer in isoleucine and valine contents and poorer in histidine than the bean protein under nonirrigated conditions. Pea protein is richer in arginine and lysine contents than that of beans with colored seeds, but sulfurous amino acids, such as methionine or cysteine, were found at lower levels than in the protein of beans with colored seeds. According to these results, the lysine content of white bean protein is similar to that in the pea protein.

Comparing the protein quality of beans in white and colored seeds grown under nonirrigated conditions, the colored beans with large seed contain more methionine, cysteine, phenylalanine, and leucine than small white-seeded beans. In this last group, the arginine and histidine levels are significantly higher than in colored-seeded beans. However, these are not considered to be

Table 3. Amino Acid Composition of Dry Bean and Dry Pea Seed Protein under Nonirrigated (A) and Irrigated (B) Growth Conditions

amino acid	A			B	
	dry pea seed[a]	dry bean seed		dry bean seed	
		colored[a]	white[a]	colored[a]	white[a]
Asp	10.43 c	10.988 b	10.944 bc	12.574 a*	12.168 a*
Thr	3.392 c	3.833 ab	3.403 c	3.730 ab	3.822 b*
Ser	4.278 d	5.279 c	5.65 b	5.905 a*	5.904 a*
Glu	16.250 ab	16.549 a	14.244 d	15.948 c*	15.347 c*
Gly	3.482 d	4.177 ab	4.227 a	3.776 c*	3.689 c*
Ala	3.972 b	4.541 a	4.040 b	4.027 bc*	3.956 bc
Cys	0.266 b	0.333 a	0.205 c	0.076 d*	0.000
Val	3.702 a	3.275 b	3.327 b	2.875 c*	2.79 c*
Met	0.798 b	1.847 a	1.124 b	1.045 b*	0.916 b
Ile	3.438 a	2.779 b	2.112 c	1.868 d*	2.016 cd
Leu	5.508 c	6.915 a	6.546 b	5.668 c*	5.828 c*
Tyr	3.020	2.970	3.008	2.808	2.708
Phe	3.912 b	4.713 a	3.943 b	4.914 a	4.786 a*
His	2.834 d	5.336 c	7.059 a	5.59 b*	5.201 b*
Lys	6.098 a	4.972 d	6.002 ab	5.537 c*	5.447 c*
Arg	6.838 b	6.009 c	6.898 b	5.903 d*	7.374 a*
Pro	4.612 a	4.609 a	4.112 a	2.772 b*	2.774 b*
protein %	24.44 b	29.84 a	28.76 a	24.095 b*	28.73 a

[a] The values in a row having different letters are significantly ($P < 0.05$) different between dry peas and white and colored beans under the same conditions. An asterisk indicates significant difference between the irrigated (B) and nonirrigated (A) groups of beans.

Table 4. Amino Acid Composition of Seed Protein in Different Ripening Soybean Varieties under Nonirrigated (A) and Irrigated (B) Growth Conditions

amino acid	A, soybean			B, soybean		
	early[a]	mid-early[a]	late[a]	early[a]	mid-early[a]	late[a]
Asp	10.30 b	10.67 b	10.62 b	11.56 a*	11.77 a*	11.72 a*
Thr	3.70 a	3.85 a	3.81 a	3.53 ab	3.50 ab	3.42 b*
Ser	4.52 b	4.63 b	4.65 b	5.15 a*	5.08 ab	5.10 ab
Glu	16.86	18.71	18.31	17.46	17.75	17.77
Gly	3.54	3.64	3.57	4.16	4.09	4.16
Ala	4.43 ab	3.897 b	3.93 b	4.65 a	4.67 a*	4.62 a*
Cys	0.33	0.31	0.32	0.28	0.25	0.25
Val	3.64 a	3.66 a	3.69 a	2.57 b*	2.57 b*	2.49 b*
Met	0.97	0.95	1.05	1.17	0.96	1.03
Ile	3.64 a	3.63 a	3.64 a	2.21 b*	2.25 b*	2.09 b*
Leu	5.67	5.99	5.73	5.73	5.78	5.73
Tyr	2.94	2.89	2.92	2.82	2.76	2.74
Phe	3.63	4.02	3.73	3.93	3.98	3.97
His	3.20 b	3.11 b	3.25 b	4.41 a*	4.20 a*	4.48 a*
Lys	5.93 a	6.43 a	5.89 a	5.31 b*	5.34 b*	5.16 b*
Arg	6.54	6.63	6.43	5.97	6.17	6.14
Pro	5.59 a	5.11 a	5.48 a	4.08 b*	3.79 b*	4.28 b*
protein %	33.91	34.74	35.29	33.22	35.69	36.17

[a] The values in a row having different letters are significantly ($P < 0.05$) different under the same conditions. An asterisk indicates the significant difference between irrigated (B) and nonirrigated (A) groups.

essential amino acids (Mercer et al., 1989). The glutamic and aspartic acids and alanine are the starting point in the synthesis of further amino acids. The alkaloids concerning their biosyntheses are amino acids such as lysine, phenylalanine, tyrosine, and tryptophan (Hess, 1979). The alkaloids have various effects on animal and human organisms through the nervous system. Lysine is a precursor of biosynthesis of lupin alkaloids. Papaverine and morphine alkaloids are formed throughout different reactions from dihydroxyphenylalanine (DOPA) and tyrosine (Hess, 1979)

In both bean groups grown without irrigation (A), increased levels of leucine, valine, glycine, and proline were found, compared with those from irrigated experiments (B). In both white- and colored-seeded bean groups, the proline level dropped, which is due to the effect of irrigation (Table 3). The breeding lines with drought tolerance could be selected under dry growing conditions, because the increasing proline levels correlated with tolerance to drought (Singh et al., 1972, 1973; Venekamp et al., 1987, 1989).

Irrigation brought about a decrease in the protein content of colored-seeded beans, but it did not in white beans. We detected an increasing level of asparagine and serine in both groups. Whereas the histidine and lysine contents of colored beans increased under irrigation, these decreased in white beans. In addition, the concentrations of phenylalanine, threonine, glutamine, and arginine in nonessential amino acids increased in white beans. The protein of the colored-seeded bean produced without irrigation seemed to have a better nutritive quality than that of the white-seeded ones, but its availability may have been decreased by large antinutritive factors.

We investigated whether irrigation had an effect on the protein quality of different maturity soybeans. Differences were detected only in asparagine and glutamine between soybean breeding lines selected for high protein content, according to the results of Serretti et al. (1994). According to our results, there were no significant differences in either protein content or amino acid concentration between the maturating groups of soy, independent of growth conditions (Table 4).

In all ripening groups, irrigation increased the concentrations of histidine and asparagine. Simultaneously, the level of proline significantly decreased, particularly in the mid-ripening soy group. Irrigation increased only the content of serine in the early ripening group. In water efficiency (A), independent of maturity, the valine, lysine, and proline levels of soy protein were high, when compared with irrigated growth conditions.

In summary, the essential amino acid composition of soy protein produced under nonirrigated conditions is more favorable, but the amount of trypsin inhibitor is higher than under irrigated growth conditions (Nemeskéri, 1997). The protein quality of bean seeds produced without irrigation, based on essential amino acids—particularly of colored-seeded beans—is more favorable than that of beans produced with good water supply.

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