Nutritive Value of Legume Seed Proteins

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Samples of beans, peas, cowpeas, soybeans, lupines, and vetches were analyzed for content of essential amino acids, and nutritive values of the proteins were determined by feeding to rats as a sole source of protein (10%) and by assay with *Tetrahymena pyriformis* W. All legume seeds studied were deficient in the sulfur-containing amino acids as shown by a comparison of methionine and cystine contents with the FAO pattern of amino acid requirements. Most of the legumes contained growth-inhibitory substances that were destroyed by heat. Rats fed heated legume seed supplemented with methionine grew normally in most cases, although Blanco beans and *L. hartweigii* appeared to contain toxic materials not destroyed by heat.

Production of animal proteins is relatively inefficient when contrasted with production of plant proteins, and the animal proteins are an expensive source of dietary protein. More seed protein than animal protein can be produced on a unit of land. Legumes are some of the higher protein-containing seeds, but the seeds are generally poor sources of dietary protein unless they have been heated and supplemented with one or more amino acids.

The purpose of the present investigation was to determine the relative nutritive values of the proteins of selected legume seeds and to study the influence of heating and of amino acid supplementation on the nutritive values.

Experimental

Legume seeds were obtained during 1963 from a number of sources. Sanilac and Charlevoix red kidney beans were grown in Michigan. Black beans, red beans, and mung beans were obtained from Thailand. Alaska peas and First and Best peas were grown in Washington. Cowpeas or blackeye beans came from California. Chippewa and Harosoy soybeans were grown in Michigan. Small samples of soybean and of soybean curd were obtained from Thailand. Auburn Wooly Pod and Borro Sweet Blue were 1959 seed grown on the Michigan State Agricultural Experiment Station farm. Blanco was breeders seed from the 1959 harvest and was from the Coastal Plain Experiment Station in Tifton, Ga. Oregon Commercial Vetch was 1960 seed and *V. sativa* was 1961 seed, both grown in East Lansing, Mich. *L. hartwegii* was 1960 seed grown at Lake City, Mich. Both *L. albus* and *L. mulabalis* were 1961 seed, from the Michigan Agricultural Experiment Station.

The seeds were ground in a Wiley mill with the 1-mm. mesh sieve. Ground soybeans were extracted in a large Soxhlet extractor using hexane (Skellysolve B) to remove the oil. Nitrogen determinations were made by the Kjeldahl-Wilfarth-Gunning procedure (Association of Official Agricultural Chemists, 1950), and crude protein content was calculated by multiplying nitrogen by 6.25. For amino acid analyses, duplicate 2.5-gram samples were weighed into 125-ml. Erlenmeyer flasks, 50 ml. of 20% hydrochloric acid were added to each flask, and the flasks were covered by placing a 50-ml. beaker over the neck. They were then heated in the autoclave for 6 hours at 15 pounds' pressure, which has been shown by Schweigert *et al.* (1944), Riesen *et al.* (1946), and work done in this laboratory (unpublished) to give hydrolysis equivalent to that obtained by 24 hours' refluxing. The hydrolyzate was transfered to a beaker, evaporated nearly to dryness on the steam bath to remove the hydrochloric acid, water was added twice, and the evaporation repeated each time. The hydrolyzate was then dissolved in distilled water and made to 50 ml. Amino acid content of the hydrolyzates was determined with the Technicon amino acid analyzer using a procedure based on the Piez and Morris (1960) modification of the Spackman, Stein, and Moore (1958) method. One chromatographic run was made every 24 hours using the long column with Type A chromobeads. The ninhydrin reagent was prepared daily for more consistent results. Cystine was determined by the procedure of Schram, Moore, and Bigwood (1954) as modified by Bandemer and Evans (1960).

Tryptophan was determined by the procedure of Roth and Schuster (1939).

Nutritive value of the proteins was estimated in rat growth experiments. Weanling albino rats, 20 to 24 days of age, were divided into groups of six animals each and were housed in individual wire-bottomed cages. The groups were equalized as nearly as possible with respect to sex and weight. Food and water were supplied *ad libitum*. Rats were weighed weekly. Their diets consisted of casein or ground seed to furnish 10% crude protein, 6% corn oil (Mazola), 4% salt mixture (Hegsted, Nutritional Biochemical Corp.) (Hegsted *et al.*, 1941), 2% vitamin mixture (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp., containing 4.5 grams of vitamin A concentrate, 200,000 units per gram, 0.25 gram of vitamin D concentrate, 400,000 units per gram, 5.0 grams of α-tocopherol, 45.0 grams of ascorbic acid, 5.0 grams of inositol, 75.0 grams of choline chloride, 2.25 grams of menadione, 5.0 grams of *p*-aminobenzoic acid, 4.5 grams of niacin, 1.0 gram of riboflavin, 1.0 gram of pyridoxine hydrochloride, 1.0 gram of thiamine hydrochloride, 3.0 grams of calcium pantothenate, 20 mg. of biotin, 90 mg. of folic acid, and 1.35 mg. of vitamin B_{12} in 1000 grams), 30% sucrose, and cornstarch to make to 100%. Usually six to 12 groups were put on experiment at one time,

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Table I. Essential Amino Acid Content
(Grams of amino acid per

Amino Acid	FAO[a]	Sanilac Beans	Kidney Beans	Black Beans	Red Beans	Mung Beans	Alaska Peas	First and Best Peas	Cowpeas
Arginine	2.0[b]	5.1	5.1	6.7	7.2	6.6	9.5	8.8	6.4
Histidine	2.4[c]	2.0	2.6	2.9	3.2	2.8	2.2	2.4	2.9
Isoleucine	4.2	4.3	4.2	4.1	3.8	4.1	3.7	3.9	4.0
Leucine	4.8	7.5	8.1	7.7	7.7	8.4	6.5	7.0	7.6
Lysine	4.2	5.7	6.7	6.7	7.2	8.0	6.9	7.0	6.8
Methionine	2.2[d]	1.3	0.9[e]	2.0	1.3	1.6	0.7	0.9	1.0
Methionine + cystine	4.2	2.0	1.9	3.0	2.2	2.4	1.8	2.0	2.0
Phenylalanine	2.8	5.0	5.3	5.1	5.4	5.6	4.1	4.2	5.3
Threonine	2.8	5.1	4.2	3.1	3.5	3.4	3.6	3.4	3.7
Tryptophan	1.4	1.8	1.5	1.5	1.7	1.9	1.9	1.8	1.4
Valine	4.2	4.7	5.1	4.9	5.0	5.3	4.1	4.4	4.8

[a] FAO pattern of amino acid requirements (Food and Agr. Org., 1957).
[b] Amino acid requirements suggested by Rose (1937).
[c] Requirement of infant or albino rat (Food and Nutrition Board, 1963).
[d] FAO pattern of amino acid requirements (FAO/WHO Expert group, 1965).
[e] The underlined value is for the most limiting amino acid in each particular seed using the FAO pattern as optimal.

and one group was fed casein as a control. Rats were kept on the experimental diet for 6 weeks. Protein nutritive value for this experiment was calculated by dividing the average gain in weight of rats fed the seed by average gain in weight of rats fed casein and multiplying by 100 and is, therefore, a percentage of the value for the casein control group.

Legume seeds were fed to rats both in a raw state and after the ground seed had been heated as follows. Ground seed was spread on an enamel pan to a depth of about 1 inch and then heated in the autoclave at 121° C. for 15 to 30 minutes, except for special heating studies where time of heating was variable.

Some of the legume seeds were also assayed for protein quality by a microbiological assay procedure on the intact ground seeds using *Tetrahymena pyriformis* W. Assays were performed essentially by the procedure of Pilcher and Williams (1954). Finely ground seed (1.0 mg. of N per ml.) was suspended in a 0.25% solution of sodium hydroxide overnight. After adjusting the pH to 7.0, basal medium was mixed with aliquots of the seed suspension, autoclaved for 15 minutes, and inoculated with a culture of the organism. The tubes were then incubated at 25° C. for 72 hours, triphenyl tetrazolium chloride solution was added, and the tubes were incubated for 15 minutes at 37° C. The reaction was stopped by the addition of acidic $HgCl_2$. The precipitate was recovered by centrifugation, extracted with acetone, and the absorbance of the acetone solution of precipitated triphenylformazan was determined with the Beckman Model B spectrophotometer at 485 mμ.

Results

The essential amino acid content of the legume seeds is presented in Table I on a percentage basis in the crude protein. The Food and Agriculture Organization (FAO) (1957) reference pattern of essential amino acid requirements is given for comparison. However, FAO did not give a requirement for arginine or histidine, and the requirement listed for arginine is taken from the pattern proposed by Rose (1937) and that for histidine is one given by the Food and Nutrition Board of the National Academy of Sciences (1963) for infants or rats. All of the legumes studied were most deficient in methionine or the sulfur amino acids, using the FAO pattern as the guide for requirements. Most of the legumes are very good sources of lysine, and if all of the lysine is available, they should be good as supplements for lysine-deficient proteins. Some of the legume seeds appear to be slightly deficient in isoleucine, tryptophan, or valine, but the deficiencies appear to be small. All of the legume seeds studied were very deficient in histidine according to the standard given by Rose.

The protein nutritive values of most of the legume seeds studied by the rat growth method were improved by heat (Table II). Sanilac and kidney beans promoted no growth unless they were heated before feeding, and heating in the autoclave markedly improved the growth promoting properties of cowpeas, fat-extracted soybeans, and Auburn Wooly Pod. Heat did not improve the poor nutritive values of Blanco beans, Borro Sweet Blue, or *L. hartweigii*, and in the first 2 seeds, heat actually decreased the protein nutritive value.

Supplementation of Alaska peas and First and Best peas, autoclaved Chippewa soybeans, and autoclaved Borro Sweet Blue with methionine improved their growth-promoting values (Table III). Blanco beans were not improved by supplementation with methionine. Supplementation of Alaska peas with either threonine and methionine or valine and methionine gave no more improvement in nutritive value than methionine supplementation alone.

The protein nutritive value of a mixture of navy beans and Gary oats was greater than that of either one alone when each supplied 5% crude protein in the mixture (Table IV). The mixture of Alaska peas and Clintland

of Some Legume Seed Proteins
16 grams of nitrogen)

Chippewa Soybeans	Harosoy Soybeans	Thailand Soybeans	Soybean Curd	Auburn Wooly Pod	Blanco	Borro Sweet Blue	Oregon Vetch	Vetch Sativa	*Lupine hartweigii*	*L. albus*	*L. mulabalis*
6.5	7.5	6.3	6.6	7.0	9.6	11.2	6.4	7.8	7.1	9.4	8.9
2.3	2.6	2.9	1.7	2.6	2.5	2.8	2.4	2.4	2.8	2.3	2.8
4.2	4.2	3.8	4.3	3.8	3.8	3.7	3.1	3.8	4.0	4.0	4.4
7.4	8.0	7.3	7.6	6.8	6.8	7.1	6.6	7.1	6.5	7.0	6.5
6.4	6.5	7.0	5.0	6.2	4.9	5.1	6.0	6.7	5.6	5.0	5.3
1.1	1.0	1.6	1.2	1.7	1.0	1.0	0.2	1.7	0.6	0.5	1.4
2.3	2.2	2.4	2.4	2.5	1.9	2.5	1.2	2.4	2.2	1.5	2.8
4.5	4.9	5.1	4.8	4.1	3.6	3.7	3.7	3.9	3.2	3.5	3.6
3.6	3.7	3.2	3.2	3.4	3.4	3.3	3.4	3.2	3.7	3.7	3.2
1.7	1.8	1.9	1.0	1.2	1.4	1.5	1.4	1.6	1.5	1.6	1.4
4.3	4.6	4.4	4.0	4.1	3.8	3.6	4.2	4.2	3.5	3.9	3.9

Table II. Relative Nutritive Values of Seed Proteins

(Gain in weight of rats fed seed × 100/gain in weight of rats fed casein at same protein level)

Seeds	Unheated	Heated[a]
Sanilac beans	0	38
Kidney beans	0	51
Alaska peas	44	51
First and Best peas	41	40
Cowpeas	38	72
Chippewa soybeans	34	87
Harosoy soybeans	35	99
Auburn Wooly Pod	17	44
Blanco beans	23	0
Borro Sweet Blue	0	0
Oregon vetch	21	32
L. hartweigii	0	0

[a] Heated in autoclave at 121° C. for 15 to 30 minutes.

60 oats gave better growth than either one alone did, but growth was further increased by the addition of methionine. A mixture of Chippewa soybeans and Mexican sesame seed where each supplied 5% protein also promoted more growth than either one alone or than a mixture of soybeans and sesame seed where soybeans furnished 2.5% protein and sesame seed 7.5% protein.

Peas were not greatly influenced by mild heat treatment but drastic heating in the autoclave decreased their protein nutritive value in the same way that it decreased that of Sanilac beans (Table V). The optimal time of heating in the autoclave at 121° C. was between 5 and 15 minutes; more drastic heating decreased protein nutritive values.

Sanilac beans, red kidney beans, First and Best peas, and Chippewa soybeans were assayed for protein nutritive value with *Tetrahymena pyriformis* W., and the results are presented in Table VI. Except for soybeans the results agree well with the data obtained by rat

Table III. Relative Nutritive Values of Seed Proteins Supplemented with Amino Acids

(Gain in weight of rats fed seed × 100/gain in weight of rats fed casein at the same protein level)

Seeds	Amino Acids Supplemented	
Alaska peas		51
	0.2% methionine	102
	0.5% methionine	124
	0.5% methionine + 0.2% threonine	100
	0.5% methionine + 0.2% valine	93
First and Best peas		41
	0.3% methionine	91
Chippewa soybeans (heated)		87
	0.2% methionine	109
Blanco beans (heated)		0
	0.25% methionine	0
Borro Sweet Blue (heated)		0
	0.3% methionine	85

growth with the unsupplemented and unautoclaved seeds. Apparently, the treatment used in preparing the sample of beans for assay did not destroy the growth-inhibitory materials completely, so that supplementation did not give normal growth.

Discussion

The adequacy of a protein for man or farm animals depends upon the content of essential amino acids in the protein, the availability of the amino acids, and the presence of growth inhibitors in the protein or the concentrate that carries the protein. The present studies were undertaken to determine the amino acid content and the relative growth-promoting value of the proteins

Table IV. Relative Nutritive Values of Seed Proteins and Mixture with Other Seeds

(Gain in weight of rats fed seeds × 100/gain in weight of rats fed casein at same protein level)

Seeds	
Navy beans[a]	53
Gary oats	79
Navy beans[a] + Gary oats	97
Harosoy soybeans[a,b]	99
Harosoy soybeans[a,b] + Gary oats	93
Alaska peas	44
Clintland 60 oats	67
Alaska peas + Clintland 60 oats	77
Alaska peas + Clintland 60 oats + 0.4% methionine	99
Chippewa soybeans[a,b]	87
Mexican sesame[b]	47
Chippewa soybeans[a,b] + Mexican sesame (1 to 1)	99
Chippewa soybeans[a,b] + Mexican sesame (1 to 3)	78

[a] Beans and soybeans were heated in autoclave at 121° C. for 15 minutes.
[b] Soybeans and sesame extracted with hexane to remove the oil before feeding.

Table V. Effect of Heating in the Autoclave at 121° C. on the Nutritive Value of Seed Proteins

(Gain in weight of rats fed seeds × 100/gain in weight of rats fed casein at same protein level)

Time Heated, Minutes	Alaska Peas	First and Best Peas	Sanilac Beans[a]
0	44	41	0
5	49		36
15	51	41	26
30	31		21
60	16		12

[a] Calculated from Table I of Kakade and Evans (1965a).

in selected legume seeds and to determine the extent of growth inhibitors in these seeds.

The legume seeds studied contained between 21.8 and 41.2% crude protein, except that the oil-free soybeans grown in Michigan contained 47.0 to 49.2% crude protein. However, the soybean sample from Thailand contained only 26.9% protein. The legumes are, therefore, of intermediate protein content, with the cereal seeds containing less and the oil seeds for the most part containing more protein.

The Food and Agriculture Organization of the United Nations (1957) has set a pattern of the minimum quantities of most of the essential amino acids that a protein should contain to meet dietary requirements. According to this pattern the legumes that the authors studied were all most deficient in the sulfur amino acids (methionine and cystine) and were borderline with regard to isoleucine; they either contained the minimal level or were slightly deficient in isoleucine (Table I). Auburn Wooly Pod was also deficient in tryptophan.

Because the sulfur amino acids are limiting in the proteins of these seeds, one would expect that the rate of growth of rats fed the seeds should be proportional to the sulfur amino acids or methionine in the seeds. Such was not the case, because heated soybeans produced about twice as much growth as heated Sanilac beans, First and Best peas, or Auburn Wooly Pod, but contained very little more methionine or total sulfur amino acids. Methionine or cystine must have been more available in soybeans than in the peas, because when peas were supplemented with sufficient methionine, the protein nutritive value was as good as when soybeans were supplemented with methionine (Table III). Even Borro Sweet Blue, which supported no growth in the raw or autoclaved condition, when supplemented with 0.3% of methionine supported 85% as good growth of rats as did casein. Proteins of heated soybeans, beans (Kakade and Evans, 1965a), and peas when supplemented with methionine supplied all amino acids needed by the growing rat.

Table VI. Relative Growth of *Tetrahymena pyriformis* W. Fed Ground Seeds

(Absorbance at 485 mμ for seeds × 100/absorbance at 485 mμ for casein)

Seeds	Amino Acids Supplemented	Relative Growth
Sanilac beans		14
	2.2% methionine	38
	2.2% methionine + 2.0% histidine	40
Red kidney beans		3
	2.4% methionine	12
	2.4% methionine + 1.4% histidine	12
First and Best peas		34
	2.5% methionine	68
	2.5% methionine + 0.3% isoleucine	86
Chippewa soybeans		12
	1.8% methionine	81
	1.8% methionine + 0.2% isoleucine + 0.1% valine	96

Osborne and Mendel (1917) observed that while raw soybeans failed to support growth of animals, previous cooking of the soybeans increased their growth-promoting properties to what would be expected from the previously determined amino acid composition of the soybean glycinin (Osborne and Clapp, 1907). Trypsin inhibitor (Ham and Sandstedt, 1944) and hemagglutinin (Liener and Pallansch, 1952) are believed to be the heat-labile growth inhibitors of raw soybeans. There is an optimal amount of heating of soybeans that gives maximum nutritive value, and when soybeans are heated more than this there is a loss of nutritive value of the proteins (Evans and McGinnis, 1946) and a decreased content (Clandinin *et al.*, 1947) and availability (McGinnis and Evans, 1947) of lysine. Raw beans do not support any growth of rats even when supplemented with methionine (Kakade and Evans, 1965a), but beans that have been autoclaved for 5 minutes at 121° C. and supplemented with methionine support normal growth (Kakade and Evans, 1965a). More drastic autoclaving of beans decreased growth (Kakade and Evans, 1965a). Beans also contain heat labile growth inhibitors, including the trypsin inhibitor (Bowman, 1944), hemagglutinin (Honavar *et al.*, 1962), and an unidentified substance (Kakade and Evans, 1965b). The protein nutritive value of peas was not appreciably increased by autoclaving for 5 minutes at 121° C., but more drastic autoclaving decreased rat growth (Table V).

There are also heat-stable toxic substances found in some of the legumes. Most of the lupines contain alkaloids, which are toxic. Presence of such alkaloids may account for the failure of *L. hartweigii* to promote growth of rats. Harper and Arscott (1962) observed the common vetch to be toxic for chicks. The presence of toxic materials was probably responsible for the poor growth of rats fed Blanco beans.

Bell (1963) has pointed out that many seeds contain nonprotein amino acids, which act as antimetabolites for microorganisms and which also may be toxic for higher animals. Canavanine has been shown to be an antimetabolite for arginine. Auburn Wooly Pod and Oregon Commercial vetch seeds contained canavanine (Table VII), which might account for their poor protein nutritive values (Table IV). Beans contained appreciable quantities of *S*-methylcysteine (Table VII), and because of the relation of *S*-methylcysteine to methionine and cystine, the authors considered the possibility that it might act as an antimetabolite for cystine or methionine. Accordingly *S*-methylcysteine was fed to growing rats but appeared to have no harmful effects on the rats.

Except for dry peas (*Pisum sativum*), all of the raw legume seeds studied appeared to contain toxic or growth-inhibitory substances, but in most cases these substances were destroyed by heat. All of the legumes were deficient in methionine, and when the heated seeds were supplemented with adequate levels of methionine the seeds promoted normal growth of rats.

Table VII. Content of Some Nonprotein Amino Acids in Legume Seeds

(Grams of amino acid per 16 grams of nitrogen)

Seeds	Canavanine[a]	*S*-Methylcysteine[a]
Sanilac beans		1.0
Kidney beans		1.0
Black beans		0.8
Red beans		0.8
Mung beans		0.6
Cowpeas		0.9
Harosoy soybeans		0.7
Auburn Wooly Pod	8.7	
Oregon commercial vetch	6.7	

[a] Canavanine and *S*-methyl cysteine were determined with amino acid analyzer with authentic canavanine and *S*-methylcysteine as standards. No detectable amounts of canavanine or *S*-methylcysteine were observed in legume seeds other than those presented in this table.

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