The Amino Composition of Some Seeds

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The amino acid composition of nine seeds and soybean meal has been determined by ion exchange chromatography. Analyses were made on the material as purchased and after heating. Heating resulted in small losses for most of the amino acids, mainly in tyrosine and the basic acids. The protein score has been calculated according to the Provisional Amino Acid pattern. The quantities of the essential amino acids present at the 20% protein level are compared to those required for optimum chick growth.

KNOWLEDGE of the amino acid composition of common foods and feeds provides a basis for formulating human diets and animal rations with adequate protein content. On the whole, proteins from plant materials are of lower quality or nutritive value than animal proteins, as has been shown by growth studies (*1*, *6*, *15*, *16*, *25*, *26*). A world deficiency of animal proteins exists, which will become more serious with continual increases in world populations (*12*), and, as a result, more and more of the required food protein will have to be supplied by plant proteins, as is already the case in many parts of the world.

As more information becomes available on the amino acid composition of plant proteins, better use can be made of the ones now available for it will then be possible to combine them so as to provide the recommended levels of amino acids and protein intake more efficiently and economically.

The present study was conducted to determine the amino acid composition of some selected seeds and to investigate the effect of heating on their amino acid composition.

Methods and Materials

Single samples of nine different seeds and soybean meal obtained from commercial sources were analyzed for amino acids as received, designated hereafter as untreated, and after heating. The seeds were placed in aluminum trays in $^1/_4$-inch layers and heated as follows: corn, wheat, barley, and rice were autoclaved at 15 pounds pressure for 15 minutes; navy beans (Michelite) and mung beans (Oklahoma Jumbo) for 30 minutes. Millet, Sudangrass seed, and sunflower seeds were heated in an oven at 250° C. for 45 minutes. These treatments were chosen as representative of cooking procedures used in the preparation of these seeds for food purposes, and were also used in formulating diets in rat feeding experiments (*27*).

Commercial soybean meal was autoclaved at 15 pounds pressure for 4 hours. Dried whole egg was included in this study as an example of well balanced protein, and had been fat-extracted before use.

All materials were finely ground in the Wiley mill before analysis, and weighed on the air-dried basis. The sunflower seed meal was fat-extracted after grinding because the oil, which amounted to about 50% by weight, interfered with hydrolysis and analysis.

Duplicate 2.5-gram samples of the finely ground materials were weighed into 125-ml. Erlenmeyer flasks, 50 ml. of 20% hydrochloric acid added to each, and the flasks covered by placing a 50-ml. beaker over the neck. They were then heated in the autoclave for 6 hours at 15 pounds pressure. After this time, the hydrolyzate was transferred to a beaker, the hydrochloric acid was removed by evaporation nearly to dryness on the steam bath, water was added twice, and the evaporation repeated each time. Drying on the steam bath has no effect on the hydrolyzate (*14*). The hydrolyzate was then dissolved in distilled water and made to volume. This method for hydrolyzing proteins was used since it has been determined that autoclaving for 6 hours at 15 pounds pressure gave the same amino acid values as refluxing for 24 hours or autoclaving for 8 or 10 hours (*4*, *30*).

The amino acids were determined by ion exchange chromatography. The acidic and neutral amino acids were determined on a suitable aliquot by the procedure of Moore and Stein (*23*) with the following modifications. Although the Technicon fraction collector was a Time-Flow unit, the volume collected in each tube was kept fairly constant by a constant level arrangement in the funnel holding the eluting solution. All the test tubes were graduated to 10 ml., and about 0.8 ml. of the eluate was collected in each tube. The approximate amount of alkali to give pH 5.0 and 2 ml. of ninhydrin solution, freshly prepared each day according to Moore and Stein (*21*), were added to each tube which was then heated in a water bath (92° C.) for 20 minutes. After cooling, the volume in the test tube was made up to the 10-ml. graduation with a 1:1 solution of isopropyl alcohol and water, thoroughly mixed, and the absorbance read in a Beckman Model B spectrophotometer. Proline was read at 440 mμ, other amino acids were read at 570 mμ. Standard curves had been made for each amino acid, and the micrograms of each per unit of absorbance calculated. The sum of the absorbances of all the tubes for one peak multiplied by the micrograms per unit for that amino acid gives the total amount obtained, and the percentage can then be calculated.

The basic amino acids were determined by chromatography on Amberlite IR-120 (*20*). Cystine was determined on a separate sample by the chromatographic method of Schram, Moore, and Bigwood (*29*), as modified by Bandemer and Evans (*3*). Trytophan was not determined.

Nitrogen content was determined on the ground, air-dried samples by the standard Kjeldahl procedure. Protein content was calculated by multiplying the nitrogen value by 6.25. Duplicate determinations were made on each sample, and the values presented are the average of these determinations.

Results and Discussion

The amino acid contents of the seeds and the whole egg sample, which was included as representative of a "protein of high nutritive value" (*13*), are presented in Table I, and are based on the grams of amino acid in 16 grams of nitrogen in the original sample. Correc-

Table I. Amino Acid Content of Some Seeds

(Grams of amino acid per 16 grams of nitrogen)

	Wheat		Corn		Rice		Barley		Sudangrass Seed	
	Untreated	Heated	Untreated	Heated	Untreated	Heated	Untreated	Heated	Untreated	Heated
Alanine	3.5	3.5	7.5	7.1	5.2	5.0	3.9	3.7	8.2	7.8
Arginine	3.8	3.7	4.5	4.4	9.6	8.7	4.1	3.9	4.0	3.5
Aspartic acid	6.2	5.8	6.8	6.5	9.5	10.1	6.5	5.6	7.1	6.8
Cystine	2.1	1.9	2.0	1.9	1.5	1.2	2.3	1.9	1.4	1.2
Glutamic acid	26.6	25.1	16.6	16.3	14.2	14.6	22.9	21.6	17.2	17.0
Glycine	3.9	3.9	3.2	3.2	3.8	4.2	4.0	3.9	2.8	2.6
Histidine	1.4	1.4	2.4	2.5	2.1	1.9	1.4	1.4	1.7	1.5
Isoleucine	3.7	3.4	3.7	3.4	3.7	3.7	3.0	2.7	3.4	3.5
Leucine	5.6	5.9	11.4	11.0	6.9	6.7	6.0	5.6	10.6	10.8
Lysine	3.2	3.0	2.7	2.6	3.5	3.4	3.5	3.5	2.5	2.0
Methionine	1.6	1.6	2.0	1.7	2.1	2.3	1.2	1.0	1.4	1.3
Phenylalanine	4.0	4.2	4.6	4.4	4.3	4.6	4.2	4.2	4.4	3.8
Proline	7.2	7.2	8.5	7.2	3.2	3.5	7.8	7.5	6.1	5.2
Serine	4.9	4.8	5.1	4.5	3.9	3.7	4.4	3.9	3.9	3.6
Threonine	2.7	2.8	3.2	3.0	2.5	2.8	3.5	2.8	3.2	2.8
Tyrosine	1.8	1.6	2.9	2.1	2.4	2.1	2.1	1.4	1.9	2.3
Valine	3.8	3.7	4.2	4.2	4.3	4.4	3.9	3.5	4.0	4.0
Ammonia	2.6	2.7	3.2	3.7	2.7	2.2	2.2	2.5	3.0	2.6

	Millet		Mung Bean		Navy Bean		Sunflower Seed		Soybean Meal		Egg
	Untreated	Heated	Untreated	Heated	Untreated	Heated	Untreated	Heated	Untreated	Heated	Whole
Alanine	9.9	9.6	4.1	4.1	4.0	4.0	3.9	3.5	3.8	3.6	6.2
Arginine	3.2	3.1	6.7	6.4	5.6	4.2	10.0	8.7	6.2	4.4	6.5
Aspartic acid	6.2	5.8	13.9	11.6	13.5	12.5	9.8	8.9	12.0	11.0	10.9
Cystine	1.7	1.3	0.6	0.5	1.1	1.1	1.7	1.4	1.5	0.8	2.3
Glutamic acid	21.9	21.6	16.6	16.0	15.3	14.9	19.9	19.4	18.2	16.5	12.5
Glycine	2.3	2.1	3.7	3.7	3.8	4.0	5.6	5.1	4.2	3.7	3.4
Histidine	1.8	1.8	2.1	2.2	2.1	2.2	2.3	1.8	2.0	1.7	2.4
Isoleucine	3.4	3.4	4.6	3.8	4.5	4.5	4.1	3.9	5.1	4.6	6.0
Leucine	11.1	10.7	7.2	6.4	7.7	7.7	5.5	5.3	7.4	6.8	8.1
Lysine	1.6	1.5	7.3	6.6	6.5	6.3	3.6	2.1	5.6	4.1	7.2
Methionine	2.3	2.5	1.1	1.0	1.2	0.9	1.5	1.3	0.9	0.9	3.5
Phenylalanine	5.3	4.5	5.9	5.4	5.1	4.6	4.6	4.0	4.6	4.3	6.9
Proline	5.4	5.0	5.0	4.5	4.0	3.6	3.6	3.6	5.5	5.4	3.7
Serine	7.1	6.7	6.1	5.4	6.7	6.4	4.5	4.1	6.0	5.5	8.2
Threonine	2.8	2.7	3.4	3.1	4.7	4.6	3.4	3.2	4.0	3.8	5.1
Tyrosine	1.9	1.9	1.7	1.3	3.0	1.7	1.6	1.4	2.7	2.7	3.4
Valine	4.3	4.1	5.1	5.0	4.2	4.2	4.0	4.2	4.1	3.9	5.9
Ammonia	3.3	3.0	2.0	1.7	2.1	1.7	2.4	2.6	2.1	2.1	1.6

Table II. Distribution of Nitrogen in Hydrolyzates

	Untreated			Heated		
		N as % Total N			N as % Total N	
Material	Protein, %[a] (N × 6.25)	Amino Acid N	Ammonia N	Protein, %[a] (N × 6.25)	Amino Acid N	Ammonia N
Wheat	9.9	67.7	13.7	10.3	66.0	14.0
Corn	10.8	74.6	16.5	10.8	70.5	19.2
Rice	6.6	75.0	14.0	8.0	73.6	11.3
Barley	9.6	67.8	11.5	10.0	63.3	12.8
Sudangrass seed	10.3	67.8	15.4	10.9	63.6	13.1
Millet	14.0	72.4	17.2	14.2	69.0	15.7
Mung bean	24.3	81.2	10.2	24.9	75.6	8.7
Navy bean	24.0	78.1	10.9	24.0	72.8	8.8
Sunflower seed	66.2	80.6	12.3	49.9	72.2	13.3
Soybean meal	49.8	78.7	10.6	51.5	68.0	10.9
Whole egg	72.8	86.4	8.5	...	...	...

[a] Nitrogen determined on ground, air-dried sample.

tions for the hydrolytic losses have been applied to serine and threonine (*31*) and aspartic and glutamic acids (*22*). Methionine values are the sums of the values for methionine and methionine sulfoxide, which is formed from the methionine on acid hydrolysis (*28*).

The amino acids most frequently deficient in animal nutrition are lysine, methionine, threonine, tryptophan (*12*), and, particularly with chicks, arginine and glycine (*1*, *2*). On comparison with the values of whole egg, some of the seed proteins contain inadequate amounts of one or more of the amino acids essential in animal nutrition.

When the amino acid contents of the untreated seeds (Table I) are compared to the amino acid proportions required to give optimum growth of chicks fed these seeds at the 20% level in a ration, according to the recommendations of the National Research Council (*24*), all seeds contained adequate amounts of histidine, phenylalanine, leucine, isoleucine, threonine, and valine. The amounts of the other essential amino acids present in the seeds in proportion

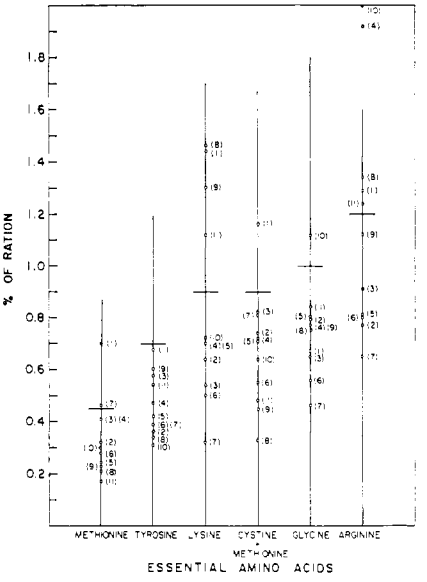

Figure 1. Comparison of amino acid concentration of seeds with chick requirements at 20% protein level

(1) Whole egg
(2) Wheat
(3) Corn
(4) Rice
(5) Barley
(6) Sudangrass seed
(7) Millet
(8) Mung bean
(9) Navy bean
(10) Sunflower seed
(11) Soybean meal

(Cross bars indicate % of ration necessary at 20% level of protein to provide the amino acid requirement)

Table III. Protein Scores of Seeds Compared to Provisional Amino Acid Pattern Values[a]

(Underlined are lowest with regard to Provisional pattern and indicate protein score)

Material	Lysine	Tyrosine	Phenyl-alanine	Total S Amino Acids	Methionine	Threonine	Leucine	Isoleucine	Valine	Protein Score
Provisional Amino Acid Pattern	270	180	180	270	144	180	306	200	270	100
				UNTREATED						
Wheat	200	112	250	231	100	169	350	231	238	74
Corn	169	181	288	250	125	200	712	231	262	63
Rice	219	150	269	225	131	156	439	231	269	81
Barley	219	131	262	219	75	219	375	188	244	81
Sudangrass seed	156	119	275	169	88	200	662	212	250	58
Millet	100	119	331	250	144	175	694	212	269	37
Mung bean	456	106	369	106	69	212	450	288	319	39
Navy bean	406	138	319	144	75	294	481	281	262	53
Sunflower seed	225	100	288	200	94	212	344	256	250	74
Soybean meal	350	169	288	150	56	250	462	319	256	56
				HEATED						
Wheat	188	100	262	219	100	175	369	212	231	70
Corn	162	131	275	225	106	188	688	212	262	60
Rice	212	131	288	219	144	175	419	231	275	78
Barley	219	88	262	181	62	175	350	169	219	67
Sudangrass seed	125	144	238	156	81	175	675	219	250	46
Millet	94	119	281	238	156	169	669	212	256	35
Mung bean	412	81	338	94	62	194	400	238	312	35
Navy bean	394	106	288	125	56	288	481	281	262	46
Sunflower seed	131	88	250	169	81	200	331	244	262	48
Soybean meal	256	169	269	106	56	238	425	288	244	39

[a] Milligrams of amino acid per gram of nitrogen.

to the amount required at the 20% level in a ration for chicks are shown in Figure 1. The heated seeds at the 20% protein level follow the same pattern shown in Figure 1 for the untreated seeds, but the deficiencies would be aggravated, since heating caused a loss of the essential amino acids in all but a few instances.

Heating the seeds resulted in small losses of most of the amino acids (Table I), with the over-all loss ranging from about 0.5% for rice, to more than 10% for soybean meal, which had undergone a more severe heating. The losses occurred primarily in the basic amino acids, although there was considerable loss of tyrosine with some of the seeds.

The distribution of the amino acid nitrogen and ammonia nitrogen as per cent of the total nitrogen in the ground sample is presented in Table II. Heating seems to have little effect on the ammonia concentration. Except for corn and navy bean where it was unchanged, and for the fat-extracted sunflower seed where it was about 25% less, the protein content of the seeds was slightly higher after heating. The amino nitrogen as per cent of the total nitrogen was higher in every case for the untreated indicating a loss of amino acids on heating which is unaccounted for in the ammonia. The three legumes, navy bean, mung bean, and soybean, were similar in the untreated state, but the heated soybean meal experienced much greater loss because of the more severe treatment (*8–11*).

In Table III, the protein scores of the seeds are compared to the Provisional Amino Acid pattern (*13*). The amino acid with the lowest value with respect to the Provisional pattern is considered the limiting acid, and proved to be either lysine or the sulfur-containing amino acids. Of the seeds, rice has the best score for both the untreated and the heated.

The values for the amino acids of seeds presented in Table I agree satisfactorily with those reported in the literature (*5, 7, 19, 32*). The values in the literature cited include determinations made by microbiological methods, chemical precedures, and both ion exchange and paper chromatography. Different varieties of the same plant material or the same kind of seeds planted in different localities will give different amino acid values (*17, 18*). Complete agreement cannot, therefore, be expected.

Literature Cited

(1) Almquist, H. J., *J. Nutr.* **34,** 543 (1947).
(2) Almquist, H. J., Grau, C. R., *Ibid.*, **28,** 325 (1944).
(3) Bandemer, S. L., Evans, R. J., *J. Chromatog.* **3,** 431 (1960).
(4) Bandemer, S. L., Evans, R. J., unpublished data.
(5) Block, R. J., Bolling, D., "Amino Acid Composition of Proteins and Foods," 2nd ed., pp. 487, 490–2, Charles C Thomas, Springfield, Ill., 1951.
(6) Block, R. J., Mitchell, H. H., *Nutr. Abstr. Rev.* **16,** 249 (1946–47).
(7) Commonwealth Bur. Animal Nutr. Rowett Inst., Blackburn, Aberdeenshire, Scot., "Tables of the Amino Acids in Foods and Feeding Stuffs," Tech. Commun. No. **19,** 1956.
(8) Evans, R. J., Butts, H. A., *Food Res.* **16,** 415 (1951).
(9) Evans, R. J., Butts, H. A., *J. Biol. Chem.* **175,** 15 (1948).
(10) Evans, R. J., Groschke, A. C., Butts, H. A., *Arch. Biochem.* **30,** 414 (1951).
(11) Evans, R. J., McGinnis, J., *J. Nutr.* **35,** 477 (1948).
(12) Flodin, N. W., J. AGR. FOOD CHEM. **1,** 222 (1953).
(13) *Food Agr. Organ. U. N. FAO Nutr. Studies*, No. **16,** p. 28, Rome, 1957.
(14) Fox, S. W., Warner, C., Hurst, T. L., J. AGR. FOOD CHEM. **3,** 704 (1955).
(15) Goettsch, M., *Federation Proc.* (Abstracts) **11,** 44 (1952).
(16) Hegsted, D. M., Worcester, J., *J. Nutr.* **33,** 685 (1947).
(17) Krober, O. A., J. AGR. FOOD CHEM. **4,** 254 (1956).
(18) Kuiken, K. A., Lyman, C. M., *J. Biol. Chem.* **177,** 29 (1949).
(19) Lyman, C. M., Kuiken, K. A., Hale, F., J. AGR. FOOD CHEM. **4,** 254 (1956).
(20) Moore, S., Spackman, D. H.,

Stein, W. H., *Anal. Chem.* **30,** 1185 (1958).
(21) Moore, S., Stein, W. H., *J. Biol. Chem.* **176,** 367 (1948).
(22) *Ibid.*, **178,** 53 (1949).
(23) *Ibid.*, **192,** 663 (1951).
(24) *Natl. Acad. Sci. Natl. Res. Council Publ. No.* **301,** p. 6, Washington, D. C., January 1954.
(25) Osborne, T. B., Mendel, L. B., *J. Biol. Chem.* **20,** 35 (1915).
(26) *Ibid.*, **25,** 1 (1916).
(27) Piedad, F. F., M.Sc. Thesis, Michigan State University, East Lansing, Mich., 1956.
(28) Schram, E., Dustin, J. P., Moore, S., Bigwood, E. J., *Anal. Chim. Acta* **9,** 149 (1953).
(29) Schram, E., Moore, S., Bigwood, E. J., *Biochem. J.* **57,** 33 (1954).
(30) Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., Strong, F. M., *J. Biol. Chem.* **155,** 183 (1944).
(31) Stein, W. H., Moore, S., *Ibid.*, **178,** 79 (1949).
(32) Williams, H. H., *Cornell Univ. Agr. Expt. Sta. Bull. Memoir No.* **337,** 1955.

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SAFFLOWER AMINO ACIDS

Amino Acid Composition of Safflower Kernels, Kernel Protein, and Hulls, and Solubility of Kernel Nitrogen

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Safflower seed kernel (seed without seed coat) and hull (seed coat and pericarp) containing 60% and 3% crude protein, respectively, and isolated crude kernel protein were analyzed for amino acid composition by column chromatographic methods. Methionine, cystine, lysine, and tryptophan were present in the kernel in amounts of 1.5, 1.7, 3.2, and 0.9 gram per 16 grams of nitrogen, respectively. Comparison of the seed kernel with seed from other plant species showed arginine and valine content to be high and lysine content low. Hydroxyproline was found in the hull. An isolated crude protein was prepared by precipitation of alkali-soluble protein at pH 4. One hundred grams of dry, defatted, kernel meal gave a laboratory yield of 45 grams of crude protein. Amino acid composition of this crude protein was similar to that of the kernel. The solubility of the kernel nitrogen as related to the pH of the extract was determined.

In recent years, safflower (*Carthamus tinctorius*) has become a profitable oilseed crop for western areas of the United States. Top ranking states in production are California, Montana, and Nebraska. Future plantings will likely be greater than the estimated 360,000 acres grown in 1961.

As an industrial raw material, the oil is used for the manufacture of protective coatings, ink vehicles, putty, linoleum, and similar products. As a food, it has attracted special attention for use in diets as an alleged preventive of atherosclerosis because of its high linoleic acid content. Meal from the seed including hull after oil extraction is used for cattle feed (*1*), and meal from a partly dehulled, oil-extracted seed containing 44% protein (*5*) is being introduced into poultry feeds. Kneeland (*6*) and Knowles (*7*) have reviewed production, processing, and utilization of safflower.

Milner, Hubbard, and Wiele (*10*) have reported the following average composition for the seed including hull for eight safflower varieties: oil, 32.8%; protein, 13.5%; sugar, 1.6%; and ash, 3.2%. The average composition of the oil was linoleic acid, 77.9%; oleic acid, 16.4%; and saturated acids, 5.7%. Average hull content of the eight varieties was 49%, range 47.6 to 50.4%. Average oil content of the kernel was 62.6%, range 58.6 to 64.1%. Crude protein content of the defatted kernel may be as high as 60%.

By microbiological assay, Lyman and coworkers (*8, 9*) have determined the amount of each of the 10 essential amino acids and tyrosine in the seed. No information was found in the literature concerning the amount of the remaining amino acids present, including nutritionally important cystine and glycine.

To assist in further evaluation of safflower protein for animal feeds, a more complete amino acid composition of safflower kernels and hulls is reported in this communication. In view of the interest in low-cost proteins for food and industrial uses, preliminary tests are reported on extraction, isolation, and amino acid composition of the protein.

Materials and Methods

Preparation of Safflower Kernels and Hulls. A new variety known as P-1, having a very light-colored hull, was selected for the study. The sample was a composite of the 1960 commercial crop grown in a number of locations in Northern California. Hexane-extracted meal from the kernel was prepared by cracking the hull from the kernel by passage between corrugated rolls after which most of the hulls were removed by aspiration in a Eureka seed cleaner. The remaining hulls were removed by hand. Kernels were ground in a mortar, extracted with hexane, and air dried, after which each of these three steps was repeated before analysis of the resulting meal. The hulls used for the amino acid assay were removed from the kernel by hand. Proximate compositions of these samples are given in Table I.

Amino Acid Analysis Procedures. Kernel samples were hydrolyzed with constant boiling hydrochloric acid by the method previously described (*22*) for 24, 48, and 72 hours; isolated proteins, for 24 and 52 hours; and hulls, for 24 hours. Acid-stable amino acids were determined by the method of Spackman, Stein, and Moore (*19*) with a Model MS Beckman Spinco automatic amino acid analyzer. Cystine content of the meals was determined after oxidation to cysteic acid as described by Schram, Moore, and Bigwood (*15*). Tryptophan estimations were made by alkaline hydrolysis of the meals in 4*N* sodium hydroxide for 24 hours in an autoclave at 15 p.s.i., followed by separation of the compound on a starch column as described by Moore