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Nutritive Values of Some Oilseed Proteins[1]

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ABSTRACT

Samples of hexane-extracted peanuts, safflower seed, sesame seed, soybean seed, and sunflower seed were analyzed for their essential amino acid contents, and the nutritive values of seed proteins were determined by growth of rats fed the hexane-extracted seeds as a sole source of protein in their diets and by assay with the microorganism *Tetrahymena pyriformis* W. Peanuts were deficient in methionine, isoleucine, and lysine, but supplementation with these amino acids did not improve the growth-promoting properties of peanuts. Safflower seed was deficient in methionine, lysine, and isoleucine; supplementation with these amino acids improved the nutritive value of safflower seed proteins but did not bring it to that of casein. Sesame seed was deficient in lysine and isoleucine; sesame seed supplemented with these amino acids supported very good growth in rats, as did a mixture of equal parts of sesame and soybean seeds. Sunflower seed proteins were deficient in methionine and lysine and promoted good growth when supplemented with these two amino acids. Heated soybeans were deficient only in the sulfur amino acids; heated soybeans supplemented with methionine promoted very good growth of rats.

Certain seeds are cultivated primarily for the large proportions of oil they contain, but for many years the residues from oil extractions were not considered of much importance. With the impetus of greater need for sources of protein of high nutritive value, however, the oilseeds have been investigated for their composition and nutritive value. Because most oilseeds are relatively rich sources of protein, they have been used extensively as protein supplements in livestock feeds. They also appear to be a possible source of protein in human nutrition.

The purpose of the present experiment was fourfold: to determine the relative nutritive values of the proteins in a number of the common oilseeds; to determine the content of essential amino acids in these seeds; to calculate the amounts of these amino acids that need to be added to the various seeds to furnish dietary requirements; and then to determine whether supplementation with the deficient amino acids will correct the deficiency.

MATERIALS AND METHODS

The oilseeds were obtained from a number of sources during 1963. Acola 4–42 and AXTE cottonseeds came from Shafter, Calif., Delfos 9169

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and Stoneville 7A cottonseeds, from Stoneville, Miss., were grown during the hot, dry 1962 season. Spanish peanuts from Holland, Va., were a mixture of equal quantities of the standard commercial varieties—Starn, Argentine, and Dixie Spanish. NC-4X peanuts from Holland, Va., are a recently introduced variety. One batch of Early Runner peanuts was a 1961 crop from Holland, Va., and the other batch was a 1962 crop from Auburn, Ala. P-1, U.S. 10, and Gila safflower seeds came from Woodland, Calif. American White and American K10 sesame seeds came from Paris, Tex. Renner 15 sesame seeds came from Renner, Tex. Brazilian and Mexican sesame seeds were foreign products. The Chippewa and Harosoy soybeans were grown near Britton, Mich. Arrowhead and Mennonite sunflower seeds were obtained from Crookston, Minn. Greystripe sunflower seed was from Modesto, Calif.

The seeds were ground in a Wiley mill with the 1–mm.–mesh sieve. The ground seed was extracted in large Soxhlet extractors with hexane (Skelly-Solve B, Skelly Oil Co.) to remove most of the oil. Nitrogen determinations were made on the ground extracted seed by the Kjeldahl-Wilfarth-Gunning procedure (1), and crude protein content was calculated by the factor N $\times$ 6.25.

For amino acid analysis, duplicate 2.5-g. portions of finely ground sample were weighed into 125–ml. Erlenmeyer flasks, 50 ml. of 20% hydrochloric acid was added to each flask, and a 50–ml. beaker was placed over the neck of each flask. The flasks and contents were then heated in the autoclave for 6 hr. at 121°C. The hydrolysates were transferred to beakers, the hydrochloric acid was removed by evaporation nearly to dryness on the steam bath, water was added twice, and the evaporation was repeated each time. The hydrolysates were then dissolved in distilled water and made to volume. Amino acid contents of the hydrolysates were determined with the Technicon Amino Acid Analyzer by a procedure based on the Piez and Morris (2) modification of the procedure of Spackman *et al.* (3). One chromatographic run was made every 24 hr.; the long column with Type A chromobeads was used. The ninhydrin reagent was prepared daily for more consistent results.

Cystine was determined by the procedure of Schram *et al.* (4) as modified by Bandemer and Evans (5). Tryptophan was determined by the procedure of Roth and Schuster (6).

Protein nutritive values of the proteins of the ground and fat-extracted seeds were determined by means of rat-growth experiments. Weanling albino rats, 21–24 days of age, were divided into groups of six animals per group 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 lib.* Rats were weighed weekly. The diets fed consisted of casein or ground fat-extracted seeds to furnish 10% crude protein, 6% corn oil (Mazola), 4% salt mixture (Nutritional Biochemicals Corp., Hegsted Salt Mixture (7)), 2% vitamin mixture,[2] 30% sucrose, and corn starch

[2]Nutritional Biochemicals Corp., vitamin diet fortification mixture: contains 4.5 g. vitamin A concentrate, 200,000 units/g.; 0.25 g. vitamin D concentrate, 400,000 units/g.; 5.0 g. alpha

to make to 100%. Usually six to twelve groups of rats were put on experiment at one time, and one group was fed casein as a control. Rats were kept on the experimental diets for 6 weeks. Protein nutritive value for this experiment was calculated by dividing the average gain in weight of rats fed the seed by the average gain in weight of rats fed casein and multiplying by 100. It is therefore a percentage.

Diets containing the oilseeds were in some cases supplemented with amino acids before they were fed to the rats; the amino acids replaced the seed and starch, so that the crude protein (N $\times$ 6.25) content was not changed from that of the unsupplemented diet.

Arrowhead and Mennonite sunflower seed samples were heated for 15 or 30 min. The Harosoy and Chippewa soybean samples were heated for 15 min. in the autoclave at 121°C. to study the effect of heat on the protein nutritive values of these seeds. The ground seed was placed on an enamel pan to a depth of about 1 in. and then heated for the required time after the autoclave temperature reached 121°C.

Some of the fat-extracted oilseeds were also assayed for protein quality by the microbiological assay procedure on the unhydrolyzed ground seeds with *Tetrahymena pyriformis* W. Assays were performed essentially by the procedure of Pilcher and Williams (8). Finely ground seed was suspended (1.0 mg. nitrogen/ml.) in a 0.25% solution of sodium hydroxide and kept at room temperature overnight. After adjustment of the pH to 7.0, basal medium was mixed with aliquots of the seed suspension, autoclaved for 15 min., and incubated with a culture of the organism. The tubes were then incubated at 25° for 72 hr., triphenyl tetrazolium chloride solution was added, and the tubes were incubated for 15 min. at 37°C. The reaction was stopped by the addition of acidic $HgCl_2$. The precipitate was recovered by centrifugation and 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 contents of the fat-extracted oilseeds are presented in Table I on the basis of precentage in the crude protein. The reference pattern of essential amino acid requirements of the Food and Agriculture Organization (FAO) (9) is also given for comparison. However, FAO did not give a requirement for arginine or histidine; the requirement listed for arginine is taken from the pattern proposed by Rose (10), and that for histidine is one given by the Food and Nutrition Board of the National Academy of Sciences (11) for both human infants and albino rats. The value for the most limiting amino acid in each seed protein is underlined. Methionine (or total sulfur-containing amino acids) and lysine are the most limiting amino acids in the seeds studied.

None of the oilseed proteins except soybeans had really high nutritive

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tocopherol; 45.0 g. ascorbic acid; 5.0 g. inositol; 75.0 g. choline chloride; 2.25 g. menadione; 5.0 g. paraaminobenzoic acid; 4.5 g. niacin; 1.0 g. riboflavin; 1.0 g. pyridoxine hydrochloride; 1.0 g. thiamine hydrochloride; 3.0 g. calcium pantothenate; 20 mg. biotin; 90 mg. folic acid; and 1.35 mg. vitamin B_{12} in 1,000 g.

TABLE I

ESSENTIAL AMINO ACID CONTENT OF SOME OILSEED PROTEINS
(g. amino acid per 16 g. nitrogen)

Amino Acid	FAO[a]	Cottonseed				Peanuts				P-1
		Acola 4-42	AXTE	Delfos	Stoneville 7A	Spanish	NC-4X	Virginia Early Runner	Alabama Early Runner	Safflower
Arginine	2.0[b]	10.0	10.6	10.2	11.2	11.6	11.3	11.6	10.5	8.5
Histidine	2.4[c]	2.6	2.7	2.7	2.7	2.3	2.2	2.4	2.1	2.2
Isoleucine	4.2	2.8	3.0	2.9	2.7	3.4	3.2	3.2	3.1	3.5
Leucine	4.8	5.3	5.4	5.9	5.9	6.6	6.4	6.7	6.2	6.1
Lysine	4.2	4.2	4.2	4.5	4.7	3.6	3.7	3.6	3.4	3.0
Methionine	2.2[d]	0.9[e]	1.0	1.2	1.1	0.9	1.4	0.8	1.3	0.6
Methionine + cystine	4.2	2.2	2.4	2.5	2.7	2.0	2.5	2.0	2.4	2.2
Phenylalanine	2.8	4.6	4.6	4.9	4.8	4.9	4.7	5.1	4.6	3.9
Threonine	2.6	3.2	3.3	3.3	3.2	2.5	2.8	2.5	2.8	2.8
Tryptophan	1.4	1.3	1.5	1.5	1.8	1.7	1.5	1.7	1.2	3.5
Valine	4.2	4.1	4.2	4.1	5.0	4.1	3.8	3.8	3.6	4.8

Amino Acid	Safflower		Sesame					Sunflower			Soybeans	
	U.S. 10	Gila	American K10	American White	Brazilian	Mexican	Renner 15	Arrow-head	Mennonite	Greystripe	Chippewa	Harosoy
Arginine	7.7	8.3	12.4	12.9	12.0	13.0	12.3	7.1	8.1	7.2	6.5	7.5
Histidine	2.2	2.5	2.5	2.8	2.4	2.5	2.3	2.2	2.3	1.9	2.3	2.6
Isoleucine	3.5	3.4	3.6	3.5	3.5	3.6	3.3	3.9	4.1	3.8	4.2	4.2
Leucine	5.9	6.1	6.6	7.0	6.5	6.9	6.6	6.1	6.5	5.9	7.4	8.0
Lysine	3.0	3.3	2.6	3.0	2.8	2.8	2.5	3.6	3.8	3.1	6.4	6.5
Methionine	1.1	0.4	4.0	3.0	2.5	2.7	2.9	1.7	1.9	1.3	1.1	1.0
Methionine + cystine	2.3	1.8	5.5	4.1	3.8	4.5	5.1	3.4	3.4	2.8	2.3	2.2
Phenylalanine	3.5	3.9	4.3	4.4	4.2	4.5	4.2	4.2	4.3	3.9	4.5	4.9
Threonine	2.9	3.2	3.6	3.8	3.8	3.4	3.5	3.6	3.8	3.2	3.6	3.7
Tryptophan	4.0	2.9	2.1	2.2	2.1	2.4	2.0	1.9	1.2	1.0	1.7	1.8
Valine	4.7	4.9	4.4	4.4	4.2	4.4	4.2	4.8	4.9	4.4	4.3	4.6

[a] FAO pattern of amino acid requirements (ref. 9).
[b] Amino acid requirements suggested by Rose (ref. 10).
[c] Requirement of albino rat or human infant (ref. 11).
[d] FAO pattern of amino acid requirements (ref. 12).
[e] The underlined value is the most limiting amino acid in each particular seed, with the FAO pattern as optimal.

TABLE II

RELATIVE NUTRITIVE VALUES OF SEED PROTEINS FOR RATS

Seeds	Protein Nutritive Value[a]
Peanuts	
Spanish	44 ± 5[b]
NC-4X	49 ± 8
Virginia Early Runner	45 ± 9
Alabama Early Runner	32 ± 13
Safflower	
P-1	49 ± 9
U.S.-10	63 ± 20
Gila	38 ± 8
Sesame	
American K10	15 ± 3
American White	25 ± 13
Sesame	
Brazilian	42 ± 6
Mexican	34 ± 11
Renner 15	30 ± 6
Sunflower	
Arrowhead	45 ± 15
Mennonite	44 ± 10
Greystripe	48 ± 13
Soybeans[c]	
Chippewa	93 ± 14
Harosoy	99 ± 16

[a] Protein nutritive value = weight gain × 100/weight gain of rats fed casein.
[b] Standard error of mean.
[c] Heated in autoclave at 121°C. for 15 min.

values (Table II). This was to be expected from the fact that all were deficient in one or more amino acids.

Supplementation of peanut proteins with the amino acids they were deficient in did not improve their protein nutritive values (Table III). Supplementation of safflower seed with methionine and lysine increased its protein nutritive value, but further supplementation with isoleucine had no additional beneficial effect, and the nutritive values were not brought to the level of casein. Sesame seed proteins were improved markedly by supplementation with lysine, and the nutritive value was equal to that of casein when supplemented with lysine, methionine, and isoleucine. Growth was good with sunflower seed supplemented with methionine and lysine.

Soybean proteins are deficient primarily in the sulfur amino acids (13), and sesame seed proteins have an abundance of the sulfur amino acids but are deficient in lysine (Table I), which soybean proteins contain in excess (Table I). A diet containing 5% protein supplied by Mexican sesame seed and 5% supplied by Chippewa soybeans promoted better growth than one containing 10% protein furnished by either Mexican sesame or Chippewa soybeans (Table IV). When only one-fourth of the protein was furnished by sesame seed and three-fourths by soybeans, growth was not as good as when soybeans were the sole source of protein.

Protein nutritive value of fat-extracted sunflower seeds was decreased by heating in the autoclave at 121°C. for 15 or 30 min. (Table V).

Rats grew better on peanuts than did the microorganism *T. pyriformis* W.; supplementation of the peanuts with methionine, isoleucine, and lysine improved microbial growth very much but had no effect on rat growth. Both rats and *T. pyriformis* W. grew better on Gila safflower seed supplemented with methionine, lysine, and isoleucine than they did on the unsupplemented seed. On the other hand, *Tetrahymena* grew poorly on Mexican sesame

TABLE III

RELATIVE NUTRITIVE VALUES FOR RATS OF SEED PROTEINS SUPPLEMENTED WITH AMINO ACIDS

SEEDS AND AMINO ACIDS	PNV[a]
Spanish peanuts	43 ± 3[b]
+ 0.3% methionine	44 ± 7
+ 0.3% methionine + 0.1% isoleucine	42 ± 8
Spanish peanuts	32 ± 11
+ 0.3% methionine	31 ± 11
+ 0.3% methionine + 0.1% isoleucine + 0.1% lysine	33 ± 14
Gila safflower	38 ± 8
+ 0.4% methionine	31 ± 3
+ 0.4% methionine + 0.2% lysine	59 ± 7
+ 0.4% methionine + 0.2%lysine + 0.1% isoleucine	55 ± 12
Gila safflower	43 ± 8
+ 0.4% methionine	31 ± 11
+ 0.4% methionine + 0.1% lysine + 0.1% isoleucine	61 ± 12
Mexican sesame	31 ± 5
+ 0.2% lysine + 0.3% methionine	63 ± 8
Mexican sesame	47 ± 5
+ 0.2% lysine	94 ± 13
+ 0.1% methionine	46 ± 6
+ 0.2% lysine + 0.1% methionine	93 ± 16
+ 0.2% lysine + 0.1% methionine + 0.1% isoleucine	102 ± 12
American White sesame	36 ± 7
+ 0.2% lysine + 0.2% methionine	91 ± 14
Mennonite sunflower	34 ± 7
+ 0.3% methionine	40 ± 5
Greystripe sunflower	59 ± 12
+ 0.17% methionine	63 ± 14
+ 0.17% methionine + 0.17% lysine	85 ± 9
Chippewa soybeans[c]	93 ± 14
+ 0.2% methionine	109 ± 17

[a] Protein nutritive value = weight gain × 100/weight gain of rats fed casein.
[b] Standard error of mean.
[c] Heated in autoclave for 15 min. at 121°C.

TABLE IV

NUTRITIVE VALUES OF MIXTURES OF DIFFERENT OILSEED PROTEINS

SEEDS	PROTEIN NUTRITIVE VALUE[a]
Mexican sesame	47 ± 5[b]
Chippewa soybeans[c]	93 ± 14
Mexican sesame + Chippewa soybeans,[c] 1:1	99 ± 12
Mexican sesame + Chippewa soybeans,[c] 1:3	78 ± 13

[a] Protein nutritive value = weight gain × 100/weight gain of rats fed casein.
[b] Standard error of mean.
[c] Soybeans were heated in autoclave at 121°C. for 15 min.

TABLE V

EFFECT OF HEATING SUNFLOWER SEEDS IN AUTOCLAVE AT 121°C. ON NUTRITIVE VALUE OF PROTEINS

Time of Autoclaving	Protein Nutritive Value[a]	
	Arrowhead Sunflower	Mennonite Sunflower
min.		
0	58 ± 7[b]	55 ± 6[b]
15	36 ± 5	35 ± 4
30	27 ± 7	38 ± 6

[a] Protein nutritive value = weight gain × 100/weight gain of rats fed casein.
[b] Standard error of mean.

even when it was supplemented with lysine or lysine and isoleucine, whereas lysine-supplemented sesame promoted very good growth of rats.

DISCUSSION

Rats fed cottonseed did not grow, because of gossypol or other toxic pigments which were not removed from the seeds by hexane extraction. No studies other than amino acid analyses were made with the cottonseed because of the extensive studies made by the cottonseed industry and the Southern Regional Research Laboratory.

According to Table I, peanuts are deficient in methionine, lysine, and isoleucine, but supplementation with these amino acids did not improve the protein nutritive values of the Spanish peanuts. According to McOsker (14) and Anderson and Warnick (15), peanuts also are deficient in threonine. On the other hand, Waldroup and Harms (16) found peanut meal deficient in lysine, methionine, and tryptophan but not in threonine for chicks. Data in Table I indicate that threonine and tryptophan may be slightly deficient in peanuts, but not nearly so deficient as methionine, lysine, and isoleucine. Evidently either the threonine or tryptophan of peanuts, or both, are poorly utilized.

There is a discrepancy in the results obtained by feeding different safflower seeds and feeding amino acid-supplemented seeds. Safflower seed appears to be most deficient in methionine; U.S. 10 safflower seed contained 1.1% of methionine and had a protein nutritive value of 63, whereas Gila safflower, which had a protein nutritive value of 38, contained but 0.4% methionine (Tables I and II). Therefore, one would expect that supplementation of Gila safflower with methionine would increase the protein nutritive value, but it did not. Supplementation with both methionine and lysine was required to bring the protein nutritive value of Gila safflower seed to that of U.S. 10 safflower seed (Table III), even though Gila safflower seed contained more lysine than did U.S. 10 safflower seed (Table I). Evidently the lysine of Gila safflower seed was poorly utilized by the rats. Safflower seed was also deficient in isoleucine, but supplementation with methionine, lysine, and isoleucine failed to give growth equivalent to that given by casein. Kratzer and Williams (17) and Valadez *et al.* (18) found that safflower seed protein was deficient in lysine.

Sesame seed protein is deficient primarily in lysine (Table I), and when it was supplemented with lysine, growth was optimum (Table III). One would suspect that if sesame seed (a good source of methionine but a poor source of lysine) and soybean seed (a good source of lysine but a poor source of methionine) were mixed, they would supplement each other to produce a protein mixture of high nutritive value. Such, indeed, did occur when a mixture of Chippewa soybeans and Mexican sesame seed was fed to growing rats; growth was as good as with 10% casein when each furnished 5% protein. Grau and Almquist (19) obtained good growth of chicks fed sesame seed supplemented with lysine. Patrick (20) showed sesame seed to be deficient in lysine and some other factor present in milk albumin or soybean meal. No evidence was found in the present study for the need of a supplement other than lysine to convert sesame seed into a good protein for growing rats.

Amino acid analyses of sunflower seeds show deficiencies of lysine and the sulfur amino acids (Table I), and supplementation of the seed with these two amino acids increased its protein nutritive value from 59 to 85 (Table III). Previous analysis of sunflower seed had indicated that it is most deficient in the sulfur amino acids (21). Grau and Almquist (22) found sunflower seed to be a complete single source of amino acids for growth of the young chick at a level to provide 20% protein in the diet, but McGinnis *et al.* (23) found it deficient in lysine. Even very mild heating (121°C. for 15 min. in autoclave) reduced the protein nutritive value of sunflower seeds (Table V). Bandemer and Evans (21) observed that whereas unheated sunflower seed is most deficient in the sulfur amino acids, the heated meal is most deficient in lysine, and the lysine content of the protein was reduced from 3.6 to 2.1%. Morrison *et al.* (24,25) also observed a detrimental effect of heat on sun-

TABLE VI

RELATIVE GROWTH OF *Tetrahymena pyriformis* W. GROWN ON GROUND OILSEEDS

SEEDS	AMINO ACIDS[a]	PROTEIN NUTRITIVE VALUE[b]
Spanish peanuts		26
	+ 3.3% methionine	26
	+ 3.3% methionine + 0.7% isoleucine	29
	+ 3.3% methionine + 0.7% isoleucine + 0.5% lysine	81
	+ 3.3% methionine + 0.7% isoleucine +0.5% lysine + 0.2% valine + 0.2% threonine	45
Gila safflower		12
	+ 2.4% methionine	9
	+ 2.4% methionine + 0.8% isoleucine	16
	+ 2.4% methionine + 0.8% isoleucine + 0.9% lysine	56
Mexican sesame		5
	+ 1.4% lysine	16
	+ 1.4% lysine + 0.6% isoleucine	19
Chippewa soybeans		12
	+ 1.8% methionine	81
	+ 1.8% methionine + 0.2% isoleucine + 0.1% valine	96

[a] Amino acids were added as percent of crude protein.

[b] Protein nutritive value = A at 485 mμ for seeds $\times$ 100/A at 485 mμ for casein.

flower seed meal, and Renner *et al.* (26) observed destruction of lysine in heated sunflower seed meals.

The poor agreement between values obtained for the nutritive values of some of the proteins by assay with *T. pyriformis* W. and those obtained by assay with growing albino rats raises a question as to the value of the *Tetrahymena* assay. Rats utilized the proteins of Spanish peanuts, Gila safflower, Mexican sesame, and Chippewa soybeans much better in relation to the utilization of casein protein than did the microorganism. Apparently the rat and the microorganism have different amino acid requirements, or else the rat is better able to utilize some of the amino acids present in the seeds. For example, the rats grew much better than *Tetrahymena* on unsupplemented soybeans, but when the soybeans were supplemented with methionine, growth of *Tetrahymena* was greatly increased whereas that of rats was changed very little. Rats fed peanuts grew 43% as well as rats fed casein, and *Tetrahymena* fed peanuts grew only 26% as fast as that fed casein. Rats fed peanuts supplemented with methionine, isoleucine, and lysine did not grow any better than rats fed the unsupplemented peanuts, but *Tetrahymena* fed peanuts supplemented with methionine, isoleucine, and lysine grew three times as fast as that fed the unsupplemented peanuts (Table VI).

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