Amino Acids in Soybean Hulls and Oil Meal Fractions

J. J. RACKIS, R. L. ANDERSON, H. A. SASAME,[1] A. K. SMITH, and C. H. VanETTEN

Northern Regional Research Laboratory, Peoria, Ill.

Amino acids were determined by ion exchange chromatography on soybean hulls, dehulled soybean meal, isolated soybean protein, and the residue; on whey proteins obtained by isolation of the acid-precipitated protein; and on the hypocotyl and the acid-precipitated protein of the hypocotyl. Pretreatment of meal and other fractions prior to colorimetric determination of tryptophan is described. Essential amino acid composition of soybean meal fractions varied considerably indicating that biological values of the protein in these fractions also differ. Soybean hulls contained unusually large amounts of glycine and hydroxyproline. Dehulled meal and residue contained no hydroxyproline.

NEARLY 9 million tons of high-protein meal, out of a total supply of 16.7 million tons available for animal feeding in the 1958–1959 crop year, came from soybeans. Production of soybean meal for feeds will continue to increase if the demand for meat, milk, and eggs by a growing population is satisfied.

While the main outlet for soybean meal in this country is for high-protein feeds, an infant industry converts the meal into protein concentrates for utilization in food preparations as reviewed recently by Smith and Wolf (*17*). The United States has a substantial export market for soybeans and the portion going to the Orient is used almost entirely for food (*4*, *14*). Foreign markets for our soybeans for food are much larger than the domestic ones. Approximately 460 million bushels of U. S. soybeans were used for feed and food in the approximate ratio of 91:9 in 1959.

Commercial processing of soybeans into protein concentrates for feeds and food gives several fractions for which there is no available amino acids information. In processing 50% protein meal for poultry and other animal feeds and for food uses, an estimated 350,000 tons of seed coat, or hulls, are removed which are used mainly in cattle feed. When meal is processed into acid-precipitated or isolated soybean protein for food and industrial uses, two by-product fractions result. One is an insoluble residue, which accounts for 25 to 30% of the protein of the original meal according to the procedure described below; the other is a whey solution (*16*), which contains about 6% of the original protein and all the nonprotein nitrogen and other water-soluble constituents in the bean. The residue is used in animal feeds, but at present the whey solution is a waste product that must be treated as sewage.

Previous workers have reported amino acid analysis of whole soybean meal, acid-precipitated protein, heat-coagulable protein of the whey, and soybean hemagglutinin (*1*, *5*, *7*, *20*, *21*). Krober (*6*) reported on the variables that influence the methionine content of whole soybeans. No reports are known on the amino acid composition of hulls, the residue, whole whey protein, or of the hypocotyl and its fraction. The amino

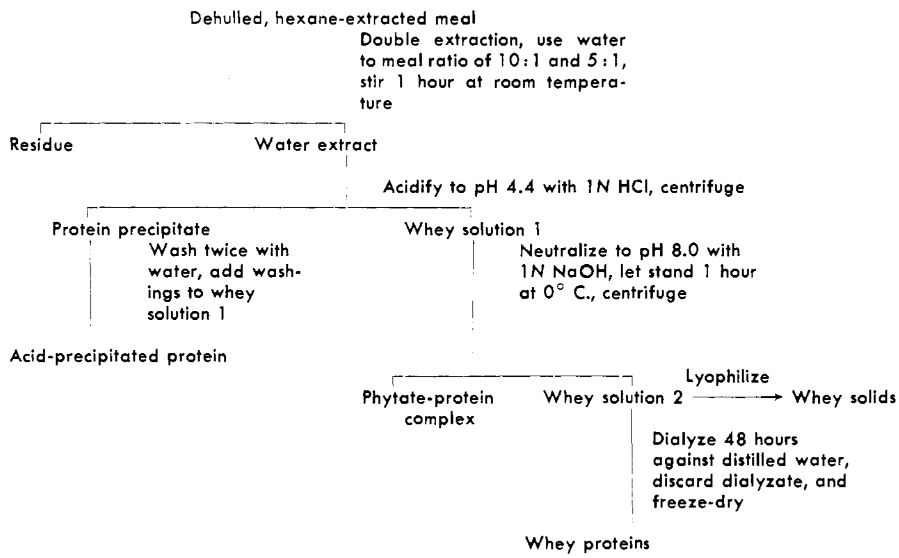

Figure 1. Preparation of soybean meal fractions

[1] Present address, Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

acid composition of these meal fractions is needed for a better evaluation of their use in feeds and foods and as a basis for further improving their usefulness in such products. This report is concerned with the amino acid analysis of the soybean seed coat and of the dehulled and defatted soybean oil meal and its fractions.

Materials and Procedure

Preparation of Soybean Fractions. Hawkeye soybeans, 1958 crop, were used throughout this study. Defatted and dehulled meal was prepared by cracking whole beans between corrugated rolls into six to eight parts and removing about 95% of the hulls and 50% of the hypocotyl by aspiration in a Eureka seed cleaner; the resulting grits were pressed into flakes between smooth rolls and the oil was removed by extraction with hexane (b.p. 30° to 60° C.). Acid-precipitated protein, residue, and whey proteins were prepared from this meal by the procedure shown in Figure 1.

Hulls and hypocotyl fractions were separated by hand; they comprise 8 and 2% of the whole bean, respectively. The acid-precipitated protein of the hypocotyl was prepared from hexane-extracted hypocotyl following a procedure similar to that outlined in Figure 1, except that a single extraction of the defatted meal was made at a water-to-meal ratio of 40:1 and the protein was precipitated at pH 4.0. Isolation and solubility characteristics of the hypocotyl protein have been reported previously (*12*).

Except for the hulls, which were analyzed on an "as is" basis, all other analyzed samples were washed with cold (0° C.) absolute alcohol, followed by anhydrous ether at room temperature, and dried in a vacuum oven at 40° C. Yield, nitrogen, and protein content of meal and seed fractions are given in Table I. The washing procedure had no measurable effect on the nitrogen content of the meal fractions.

Method of Hydrolysis. Meal fractions, 50 mg. each, were hydrolyzed for 24 and 72 hours by heating in a bath held at 125° to 135° C. with 25 ml. of constant-boiling hydrochloric acid, which was redistilled in an all-glass apparatus. Hydrochloric acid was removed from the hydrolyzate in a rotary evaporator under vacuum; the residue was then made up to 10 ml. with water. Hull fractions, 1500 mg. each, were

Table I. Yield, Nitrogen, and Protein Content of Soybean Oil Meal Fractions

Fraction	Yield,[a] G./100 G. Meal	Nitrogen,[a] %	Protein,[b] %	Percentage Total N[c]
Soybean meal	100	9.83	61.4	...
Acid-precipitated protein	36.9	16.29	101.9	61.1
Residue	30.3	8.31	52.0	25.6
Total whey solids	31.9	2.86	17.9	9.3
Isolated whey protein	3.9	16.23	101.4	6.4
Phytate-protein complex	0.93	2.98	18.6	0.3
Seed coat	8.0[d]	1.53	9.56	...
Hypocotyl	2.0[d]	7.90	49.40	...
Acid-precipitated protein of hypocotyl	...	15.19	95.15	...

[a] Dry basis.
[b] N × 6.25.
[c] Total nitrogen recovered 96.3%.
[d] Approximate for whole seed.

Table II. Tryptophan Content and Recovery of Added Tryptophan to Meal Fractions

Fraction	μg./10-Mg. Sample	Tryptophan Added, μg.[a]	Total Found	% Recovery[b]	G./16 G. N
Soybean meal	60.0	13.5	66.5	48	0.80
Treated soybean meal[c]	92.1	13.3	105.8	102	1.28
Residue	56.0	13.3	66.7	81	1.23
Treated residue[c]	62.4	13.3	73.3	82	1.31
Acid-precipitated protein	118.0	13.5	131.5	100	1.01
Whey proteins	90.0	13.3	100.0	75	1.33
Treated whey proteins[d]	125.0	13.3	138.5	101	1.29

[a] Added as crystalline bovine serum albumin.
[b] Based on added tryptophan.
[c] Methanol-chloroform (v./v.) and 80% ethyl alcohol extraction.
[d] Precipitation with 80% saturated $(NH_4)_2SO_4$.

Table III. Amino Acid Composition of Soybean Meal Fractions

Amino Acid	Whole Meal	Residue	Acid-Precipitated Protein	Whey Protein	Hulls	Hypocotyl Meal	Acid-Precipitated Protein of Hypocotyl
			Grams/16 Grams N				
Arginine	8.42 ± 0.00	7.44 ± 0.50	9.00 ± 0.00	6.64 ± 0.00	4.38 ± 0.12	8.32 ± 0.01	6.38 ± 0.42
Histidine	2.55 ± 0.05	2.70 ± 0.20	2.83 ± 0.10	3.25 ± 0.15	2.54 ± 0.11	2.60 ± 0.02	2.65 ± 0.14
Lvsine	6.86 ± 0.10	6.14 ± 0.54	5.72 ± 0.08	8.66 ± 0.03	7.13 ± 0.17	7.45 ± 0.13	7.80 ± 0.34
Tyrosine	3.90 ± 0.44	3.30 ± 0.10	4.64 ± 0.03	4.67 ± 0.05	4.66 ± 0.40	3.48 ± 0.07	3.78 ± 0.04
Tryptophan	1.28 ± 0.05	...	1.01 ± 0.01	1.28 ± 0.09	...	...	...
Phenylalanine	5.01 ± 0.35	5.24 ± 0.44	5.94 ± 0.08	4.46 ± 0.03	3.21 ± 0.11	3.88 ± 0.01	4.22 ± 0.06
Cystine	1.58 ± 0.03	0.71 ± 0.06	1.00 ± 0.01	1.82 ± 0.02	1.66 ± 0.10	1.24	...
Methionine	1.56 ± 0.08	1.63 ± 0.16	1.33 ± 0.08	1.92 ± 0.10	0.82 ± 0.04	1.72 ± 0.07	1.79 ± 0.03
Serine	5.57[a]	5.97[a]	5.77[a]	7.62[a]	7.02[a]	4.90[a]	4.50[a]
Threonine	4.31 ± 0.43	4.67 ± 0.33	3.76 ± 0.20	6.18 ± 0.18	3.66[a]	4.00 ± 0.22	3.82 ± 0.06
Leucine	7.72 ± 0.32	8.91 ± 0.13	7.91 ± 0.40	7.74 ± 0.21	5.93 ± 0.25	6.62 ± 0.02	7.22 ± 0.02
Isoleucine	5.10 ± 0.22[b]	6.02 ± 0.03[b]	5.03 ± 0.25	5.06 ± 0.09	3.80 ± 0.08	4.11 ± 0.09	4.53 ± 0.11
Valine	5.38 ± 0.07	6.37 ± 0.20	5.18 ± 0.17	6.19 ± 0.19	4.55 ± 0.00	4 82 ± 0.12	5.28 ± 0.27
Glutamic acid	21.00[a]	17.76[a]	23.40[a]	15.64[a]	8.66 ± 0.22	13.78 ± 0.08	14.12 ± 0.17
Aspartic acid	12.01 ± 0.25	12.39 ± 0.45	12.87[a]	14.08[a]	10.05[a]	9.74 ± 0.09	9.84 ± 0.22
Glycine	4.52 ± 0.38	5.21 ± 0.33	4.56 ± 0.17	5.74 ± 0.02	11.05 ± 0.73	4.25 ± 0.17	4.93 ± 0.02
Alanine	4.51 ± 0.31	5.73 ± 0.19	4.48 ± 0.49	6.16 ± 0.12	3.98 ± 0.13	4.69 ± 0.07	4.47 ± 0.23
Proline	6.28 ± 0.54	5.35 ± 0.30	6.55 ± 0.51	6.66 ± 0.09	5.76[a]	4.23 ± 0.23	4.38 ± 0.11
Hydroxyproline	0	0	...	...	7.57[a]	Trace	0
Ammonia	2.05[a]	2.61[a]	2.20[a]	1.53[a]	1.55[a]	1.40[a]	1.20[a]

[a] Value obtained by extrapolation to zero-hydrolysis time.
[b] Data from 72-hour hydrolysis time.

hydrolyzed with 250 ml. of hydrochloric acid using the same conditions. To determine cystine, the fractions were treated with performic acid (*13*) before hydrolysis.

Amino Acid Analysis Procedures. Amino acids in the meal, residue, acid-precipitated protein, and whey protein fractions were determined by the procedures of Moore, Spackman, and Stein (*9*). Hulls and hypocotyl fractions were analyzed by the automatic recording apparatus procedure (*18*); the long column was operated at 30° and 50° C. for resolving hydroxyproline. For a comparison of methods, the amino acid content of soybean meal and residue was determined by both procedures (*9, 18*). Recoveries of known amino acids from the columns were within 5% of the known amount of amino acid used to test each procedure.

Cystine after oxidation to cysteic acid was determined by the method of Schram, Moore, and Bigwood (*13*). A forerun of 100 ml. of $0.01N$ acid was used. Cysteic acid was also determined on the columns employed by Moore and coworkers (*9*). The cystine contents of the meal fractions of Figure 1 were much higher when determined by the latter procedure. Cystine values for a meal hydrolyzate were 1.58 and 2.59 grams per 16 grams of nitrogen, respectively, for the two procedures. Since the lower cystine values agree very closely with those reported by many previous investigators, the procedure of Schram, Moore, and Bigwood (*13*) was used for all fractions.

Tryptophan Analysis. The method of Spies and Chambers (*19*) to determine tryptophan was evaluated for soybeans by determining the recovery of tryptophan in bovine serum albumin in the presence of various untreated meal fractions as shown in Table II. Recovery of added tryptophan was 100% only for the acid-precipitated protein fraction. Recoveries for the other meal fractions were only 48 to 81%. Furthermore, reproducibility was poor because of very high blanks.

Substances responsible for the high blank values and the apparent destruction of tryptophan in soybean meal were removed by extraction with a methanol-chloroform mixture (v./v.) followed by 80% ethyl alcohol. Nearly 1.5% of the total nitrogen of the meal, representing only nonprotein nitrogen components, was removed by these solvents. No protein was extracted from either the meal or residue fractions. This extraction failed to remove interfering substances from the residue.

One hundred per cent recovery of added tryptophan in the presence of the whey protein fraction was obtained by precipitation of whey proteins with 80% saturated ammonium sulfate, followed by dialysis and lyophilization. Nitrogen recovery was 95%. For maximum color yields, 0.08% of sodium nitrite was used with all meal fractions analyzed for tryptophan.

Results and Discussion

Amino Acid Composition of Fractions. Data are reported in Table III as grams of amino acid per 16 grams of nitrogen. Average deviation values, except for hypocotyl, represent averages of three to four determinations on both the 24- and 72-hour hydrolyzates. Single runs on 24- and 72-hour hydrolyzates were made on both hypocotyl protein and hulls, with an added run at 90 hours for hulls. When values from the 72-hour hydrolysis were higher than the 24-hour hydrolysis, figures from the longer hydrolysis are reported. When values for the 72-hour hydrolysis were consistently lower, the values given in the table were obtained by extrapolation to zero hydrolysis time (*1*).

There was evidence that extensive destruction of hydroxyproline and moderate destruction of proline occurred in the 72- and 90-hour hydrolyzates of hulls. An unknown peak having a maximum absorption at 440 mμ was eluted in the area just before methionine sulfoxide (*18*) from the 72- and 90-hour hydrolysis. The rate of increase in the area of this unknown peak did not parallel the rate of destruction of either hydroxyproline or proline. A similar peak labeled X has been reported for collagens of sponges (*11*). Miyada and Tappel (*8*) reported on the degradation

Table IV. Relative Distribution and Recovery of Essential and Related Amino Acids in Soybean Meal Fractions

	% of Total Amino Acid in Soybean Meal			
Amino Acid	*Residue*	*Acid-precipitated protein*	*Whey protein*	*Recovery of Amino Acid, %*
Arginine	22.3	65.4	5.6	92
Histidine	26.7	67.8	6.4	101
Lysine	23.0	51.0	9.0	83
Tyrosine	21.0	72.8	8.5	102
Tryptophan	26.1	48.3	7.1	82
Phenylalanine	25.2	72.6	6.3	104
Cystine	15.9	53.5	12.3	82
Methionine	26.9	52.2	7.7	87
Threonine	27.3	53.4	10.2	91
Leucine	29.7	62.7	7.1	100
Isoleucine	30.3	60.4	7.0	98
Valine	30.4	59.0	8.2	98

Table V. Protein Scores of Soybean Meal Fractions Compared to Provisional Amino Acid Pattern

					Sulfur Amino Acids						
Item	*Lysine*	*Tyrosine*	*Tryptophan*	*Phenylalanine*	*Total*	*Methionine*	*Threonine*	*Leucine*	*Isoleucine*	*Valine*	*Protein Score*
Provisional amino acid pattern[a]	270[b]	180	90	180	270	144	180	306	270	270	100
Soy flour[c]	395	201	86	309	197	86	247	484	333	328	73
Soluble protein[d]	431	238	94	351	171	84	244	512	431	...	63
Soybean meal	429	244	80	313	198	98	269	483	319	337	73
Soybean residue	383	206	81	327	146	102	292	557	376	398	54
Soybean acid-precipitated protein	358	290	63	371	146	83	235	495	314	324	54
Soybean acid-precipitated protein[d]	380	244	91	335	164	88	225	570	570	340	61
Soybean whey proteins	541	292	80	279	234	115	387	484	316	387	87
Soybean hulls	446	291	..	201	155	51	239	370	237	284	57
Soybean hypocotyl	465	218	..	242	186	108	250	414	257	301	69

[a] Food and Agricultural Organization of the United Nations.
[b] Milligrams amino acid per gram of nitrogen.
[c] Data from amino acid content of foods (*10*).
[d] Data from (*20*).

of hydroxyproline during acid hydrolysis. This unknown peak was not present in the chromatograms of soybean meal, although its proline content was similar to that of hulls. Hydroxyproline, based on its elution position and absorption at 440 mμ however, was present only in hull hydrolyzates. This finding would suggest that the unknown peak is probably a degradation product of hydroxyproline.

Serine values for all soybean fractions were corrected for losses occurring during hydrolysis, whereas threonine values were corrected only in the hull fraction. Correction for losses of glutamic acid were made for the meal, acid-precipitated protein, and whey protein fractions and of aspartic acid for the acid-precipitated protein, whey protein, and hull fractions. Proline and hydroxyproline destruction was found in the hull fraction only. Recovery of nitrogen after 24- and 72-hour hydrolysis as soluble nitrogen was 98% for all fractions except the residue and hulls. Recovery of nitrogen from the residue and hull fractions was 90 and 85%, respectively.

Comparison of Soybean Fractions. Cystine and tyrosine are important nutritionally because of their sparing action on methionine and phenylalanine. The first two limiting amino acids in toasted soybean meal are methionine and tryptophan. Borchers (*2*) reported that rats fed raw soybean meal, supplemented with a mixture of tyrosine, methionine, threonine, and valine, showed as good growth as rats fed a toasted soybean meal diet.

Residue. Essential and related amino acids varied considerably when compared with meal values (Table III). Leucine, isoleucine, and valine values of the residue fraction compared to meal are 15, 18, and 19% higher, respectively. Histidine, phenylalanine, methionine, and threonine values were slightly higher; whereas values for arginine, lysine, and tyrosine were about 12 to 15% lower. Cystine and cystine plus methionine content of the residue proteins are 55 and 25% lower, respectively.

Acid-Precipitated Protein. This fraction gave lower essential amino acid values than did the meal for lysine, tryptophan, cystine, methionine, and threonine (Table III). Values for tryptophan, cystine, and cystine plus methionine were lower by 20% or more. The rest of the essential and related amino acids were about the same or somewhat higher, except for tyrosine and phenylalanine. These values were 19% higher for the acid-precipitated protein.

Whey Proteins. Histidine, lysine, tyrosine, threonine, cystine, and methionine values were at least 20% higher than the meal. In contrast, arginine values were at least 20% lower. The other essential and related amino acid values remained about the same.

Hulls. Most of the essential and related amino acid values are much lower than those for the meal, particularly arginine, phenylalanine, methionine, isoleucine, and leucine. These values are at least 20% lower. Lysine values are slightly higher and tyrosine values, 20% higher. Hulls also have an unusually high glycine and hydroxyproline content. The other meal fractions did not contain hydroxyproline.

Hypocotyl. Nearly all essential amino acids in the hypocotyl meal and acid-precipitated protein fraction were lower than in the meal, particularly phenylalanine and cystine. These values were lower by 23 and 22%, respectively. Lysine and methionine values were about 10% higher.

In commercial processing, about 50% of the hypocotyl and small pieces of cotyledon are aspirated off along with the hulls. The much higher protein content and better balance of amino acids in the hypocotyl and cotyledons increase the protein and essential amino content of a commercial hull fraction.

Distribution and Recovery of Amino Acids in Soybean Meal Fractions. Relative distribution and percentage recovery of essential and related amino acids in residue, acid-precipitated, and whey protein fractions obtained from dehulled hexane-extracted soybean meal are given in Table IV. The residue fraction accounts for 16 to 30% of the total amino acids originally present in soybean meal; distribution in the acid-precipitated protein fraction is 48 to 73%, and in the whey protein fraction, 6 to 10%.

For most amino acids, good recoveries of 91 to 104% of the total present in soybean meal were achieved. Recovery of tryptophan, lysine, cystine, and methionine varied between 82 and 87%. These amino acids are susceptible to destruction during acid hydrolysis and lower recoveries were expected.

Protein scores given in Table V are based on the system followed by the Food and Agricultural Organization of the United Nations (FAO) (*3*). If the provisional mixture of pure amino acids proposed under the FAO system is used to compare nutritive value of the proteins in these meal fractions, scores indicate that the whey proteins have the highest nutritive value, hypocotyl and meal proteins have an intermediate value, while the residue, acid-precipitated protein, and hull fractions have a much lower nutritive value.

The lower biological value for the acid-precipitated protein value compared to soybean meal is substantiated by unpublished rat feeding tests (*15*). Biological value of raw whey proteins is very low, but is considerably improved after toasting (*15*). In these tests, acid-precipitated protein and soybean meal were the sole source of nitrogen in the ration, whereas whey proteins replaced 20% of casein nitrogen. The diets contained 15% protein. Biological value refers to the differences in growth rate, feed and protein efficiencies as compared to toasted soybean meal which served as control diet.

Literature Cited

(1) Block, R. J., Weiss, K. W., "Amino Acid Handbook," Charles C Thomas, Springfield, Ill., 1956.
(2) Borchers, R., *Federation Proc.* **18,** 517 (1959).
(3) Food and Agr. Organization U. N., Protein Requirements, FAO Nutritional Studies, No. 16, Rome, 1957.
(4) Hayashi, S., *Soybean Dig.* **20,** No. 7, 26 (1960).
(5) Hopper, T. H., "Processed Plant Protein Foodstuffs," A. M. Altschul, ed., chap. 33, Academic Press, New York, 1958.
(6) Krober, O. A., J. Agr. Food Chem. **4,** 254 (1956).
(7) Lyman, C. M., Kuiken, K. A., Hale, F., *Ibid.*, **4,** 1008 (1956).
(8) Miyada, D. S., Tappel, A. L., *Anal. Chem.* **28,** 909 (1956).
(9) Moore, S., Spackman, D. H., Stein, W. H., *Ibid.*, **30,** 1185 (1958).
(10) Orr, M. L., Watt, B. K., Amino Acid Content of Foods, U. S. Dept. Agr. Home Econ. Research Rept. **4,** 1957.
(11) Piez, K. A., Gross, J., *Biochim. et Biophys. Acta* **34,** 24 (1959).
(12) Rackis, J. J., Smith, A. K., Sasame, H. A., *Arch. Biochem. Biophys.* **78,** 180 (1958).
(13) Schram, E., Moore, S., Bigwood, E. J., *Biochem. J.* **57,** 33 (1954).
(14) Smith, A. K., "Uses of U. S. Soybeans in Japan," U. S. Dept. Agr. ARS-**71-12** (April 1958).
(15) Smith, A. K., Rackis, J. J., Northern Laboratory; Booth, A. N., Western Regional Laboratory, Albany, Calif., unpublished data.
(16) Smith, A. K., Schubert, E. N., Belter, P. A., *J. Am. Oil Chemists' Soc.* **32,** 274 (1955).
(17) Smith, A. K., Wolf, W. J., *Food Technol.* (in press).
(18) Spackman, D. W., Stein, W. H., Moore, S., *Anal. Chem.* **30,** 1190 (1958).
(19) Spies, J. R., Chambers, D. C., *Ibid.*, **21,** 1249 (1949).
(20) VanEtten, C. H., Hubbard, J. E., Mallan, J. M., Smith, A. K., Blessin, C. W., J. Agr. Food Chem. **7,** 129 (1959).
(21) Wada, S., Pallansch, M. J., Liener, I. E., *J. Biol. Chem.* **233,** 395 (1958).

Received for review October 10, 1960. Accepted January 12, 1961. Division of Agricultural and Food Chemistry, 138th Meeting ACS, New York, September 11–16, 1960. The Northern Laboratory is a part of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Mention in this article of commercial equipment or materials does not constitute endorsement by the U. S. Department of Agriculture over those of other manufacturers.