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

Table I. Compositions of Kernels, Hulls, and Isolated Protein

Fraction	Weight of Seed, %	Oil[a], %	Nitrogen, %	Protein (N × 6.25), %
Whole seed (kernel and hull)	...	36.3	2.26[a]	14.1
Kernels	55.5	57.8	9.59[b]	59.9
Hulls	44.5	...	0.53[c]	3.1
Isolated protein	...	...	13.44[a]	84.0

[a] Dry Basis. [b] Dry basis and oil free. [c] As is, air-dried.

Table II. Nitrogen Distribution Following Acid Hydrolyses of Kernels, Hulls, and Isolated Protein

(Per cent of total nitrogen in sample)

Distribution	Kernels	Hulls	Isolated Protein
Nitrogen in solution after hydrolysis	100.3	70.0	102.7
Nitrogen in insoluble humin after hydrolysis	1.0	33.3	0.0
Nitrogen as amino acids	77.0	52.0	83.7
Nitrogen as ammonia	13.4	8.2	13.8
Unknown nitrogen by difference	9.6	39.8	2.5

and Stein (*12*). Nitrogen contents of the unhydrolyzed meals, air-dried humin (insoluble after hydrolysis), and an aliquot of the soluble hydrolyzate were determined by the Kjeldahl method. Deviations of soluble plus humin nitrogen from 100% (Table II) provided an indication of the magnitude of experimental error in this step of the analysis.

Extraction of Nitrogen-Containing Material and Preparation of Crude Protein Isolate. Single extractions were made of the hexane-extracted kernel meal at a water to meal ratio of 40 to 1 with pH values between 1 and 9. The pH was adjusted with either hydrochloric acid or sodium hydroxide. Extractions were made in a mechanical shaker for 1 hour at room temperature. The supernatant liquid was recovered from the extracts after centrifugation at 1900 r.p.m. for 10 minutes. The pH shifted toward neutrality during the course of the extraction. For example, the initial pH of a water extract of 6.4 increased to pH 7.03 after 1 hour of extraction. For this reason, periodic pH adjustments were required. Water extracts of soybean meal had a pH of 6.4 to 6.6.

The maximum amount of soluble nitrogen from hexane-extracted kernels was obtained by water extraction at pH 9 as described above. The deep yellow-green extract was acidified to pH 4.0 with 1*N* HCl, and then centrifuged; the supernatant was discarded. The wet curd was washed twice with water and dried in a forced-draft oven at 60° C.

Results and Discussion

Nitrogen Distribution. After acid hydrolysis, essentially all of the nitrogen of the kernel and the isolated crude protein was soluble, but only 70% of the hull nitrogen was in solution (Table II).

Nitrogen present in amino acids and ammonia (Table II) was determined from the ninhydrin color yield of each compound as eluted from the column. More than 90% of the nitrogen in the kernel and isolated crude protein was in this form. In contrast, only 60% of the hull nitrogen was thus accounted for. Almost all the unidentified hull nitrogen was insoluble nitrogen in the humin.

The high nitrogen content of the kernel, of which over 90% is accounted for as amino acids and ammonia, indicates it to be a good source of high protein concentrate for feeds.

Amino Acid Composition. Amino acid content of the kernels and isolated crude protein (Table III) are the average of two or three determinations. Serine and threonine showed some decomposition of prolonged hydrolysis for which a correction was made by extrapolation to zero hydrolysis time.

Comparison of the kernel analyses with recent amino acid compositions reported by Lyman and coworkers (*8*, *9*) for meal from hexane-extracted seed with hull containing 22.1% crude protein (Table III) show that their phenylalanine and tryptophan values are somewhat higher, and the basic amino acids are somewhat lower.

Comparison of the safflower kernel compositions expressed as grams of amino acid per 16 grams nitrogen with the arithmetic mean (cystine and tryptophan excluded) obtained by analysis of defatted seed meals from 200 species of 66 plant families (*21*) showed the following: lysine content of 3.2 was low in comparison with the mean of 4.47; methionine was 1.5 compared with the mean of 1.49. Arginine content of 9.4 and valine of 5.3 were higher than the mean of 8.28 and 4.43, respectively. The remaining amino acids were present in about the same amount as in meal from each of the 200 species.

The kernel meal has a protein score by the method of the Food and Agriculture Organization of the United Nations (FAO) (*4*) of 74 in terms of either lysine or methionine plus cystine. The protein score in terms of tryptophan is 63.

Comparison of the nitrogen, accounted for as amino acids and ammonia from the isolated crude protein, with that of the kernel (Table II) indicates the nitrogen solution after precipitation of the crude protein is derived in part from compounds which on acid hydrolysis do not form amino acids or ammonia. The percentage of lysine in the protein of the kernel is greater and of the remaining amino acids the same or less than that in the isolated protein.

Hydroxyproline of the hulls was similar in amount to that previously reported for seed coat and pericarp from other angiospermous seeds (*20*). Glycine content, however, was about one third that of soybean hulls (*14*).

The only unidentified elution peaks observed in the ion-exchange chromatogram from the kernel and the isolated crude protein were assigned to levulinic acid and its associated compounds (*23*), and a small peak emerging before lysine was attributed to a decomposition product of tryptophan (*11*).

Extractable Nitrogen and Crude Protein Isolate. Solubility of the nitrogen of the safflower kernel at pH's below 4.0 is lower than that of defatted soybean meal (Figure 1) and that of defatted radish seed meal (*18*), but similar to that of navy bean seed (*13*). A possible explanation of the low solubility under acid conditions is the formation of insoluble protein phytate complexes (*2*, *3*, *17*).

At pH 7.0 and above, the supernatant solutions were deep green and cloudy. At lower pH, the solutions were nearly water-clear. On standing for 24 hours, appreciable amounts of nitrogen-containing material precipitated from extracts that were above pH 5. The cause of this precipitation was not determined; however, a phytate-protein interaction similar to that found with soybean meal extracts (*17*) may be the explanation.

Yield of crude protein isolate from 100 grams of defatted kernel meal (moisture-free basis) was 45 grams of dark gray material compared to a yield of 37 grams of isolated soybean protein prepared in the same manner (*16*). However, the isolated safflower protein contained less nitrogen.

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Table III. Amino Acid Composition[a]

(Grams of amino acid per 16 grams of nitrogen)

Amino acid	Seed with Hull[b]	Kernel[c]	Hull	Isolated Protein[c]
Lysine	2.7	3.2 ± 0.04	4.1	2.9 ± 0.02
Methionine	1.5	1.5 ± 0.02	0.8	1.6 ± 0.00
Cystine	ND[d]	1.7 ± 0.02	ND	ND
Arginine	7.8	9.4 ± 0.13	3.1	9.7 ± 0.13
Glycine	ND	5.0 ± 0.06	4.2	5.1 ± 0.05
Histidine	2.0	2.6 ± 0.01	2.4	2.6 ± 0.00
Isoleucine	3.8	3.7 ± 0.08	3.0	4.4 ± 0.10
Leucine	5.5	6.0 ± 0.02	4.9	6.8 ± 0.01
Phenylalanine	5.2	4.3 ± 0.15	3.2	5.1 ± 0.03
Tyrosine	2.8	2.9 ± 0.11	1.4	3.6 ± 0.06
Threonine	2.9	3.2 ND	3.4	3.1
Tryptophan	1.2	0.9 ± 0.04	ND	ND
Valine	4.9	5.3 ± 0.11	3.9	6.2
Alanine	ND	3.9 ± 0.14	3.5	4.6 ± 0.10
Aspartic acid	ND	9.2 ± 0.34	7.0	10.9 ± 0.09
Glutamic acid	ND	18.4 ± 0.40	8.0	20.2 ± 0.10
Hydroxyproline	ND	0.0	5.6	0.0
Proline	ND	3.9 ± 0.23	3.9	4.4 ± 0.02
Serine	ND	4.3	4.3	4.5

[a] For conversion to mg. of amino acid per gram of nitrogen, an expression used by nutritionists, divide each value by 0.016. [b] Data taken from Lyman *et al.* (*7*, *8*). [c] ± Represents average deviation. [d] Not determined.

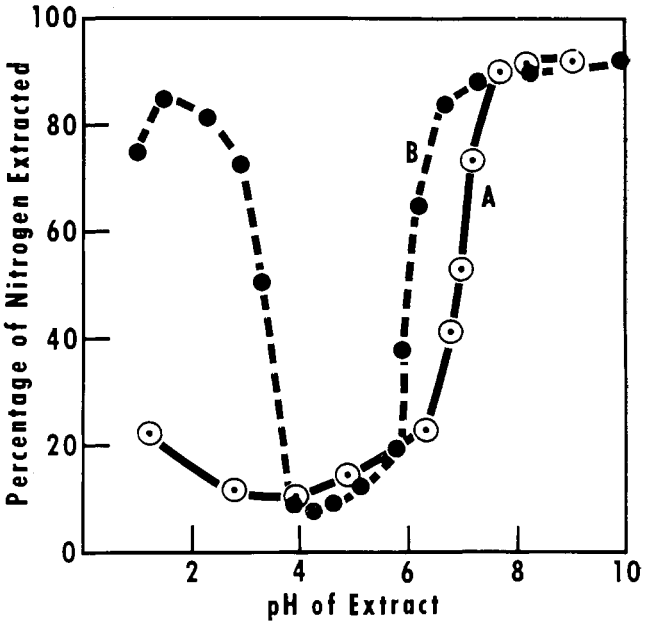

Figure 1. Solubility of the nitrogen in safflower kernel meal (*A*) and soybean meal (*B*) as a function of pH

[Data of Smith and Circle (*15*)]

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Literature Cited

(1) Baker, M. L., Baker, G. N., Matsushima, J. K., *Nebraska Univ. Agr. Expt. Sta. Bull.* **SB 447,** 1959.
(2) Craine, E. M., Fahrenholtz, K. E., *Cereal Chem.* **35,** 245 (1958).
(3) Fontaine, T. D., N. A., Jr., Irving, G. W., Jr., *J. Biol. Chem.*, **164,** 487 (1946).
(4) *Food Agr. Organ. U. N. FAO Nutr. Studies* **16,** Rome, 1957.
(5) Holloran, H. R., Kneeland, J. A., *Western Feed and Seed*, **16,** 23–4,70 (1961).
(6) Kneeland, J. A., "Processed Plant Protein Foodstuffs," A. M. Altschul, Ed., Chap. 22, Academic Press, New York, 1958.
(7) Knowles, P. F., *Econ. Botany* **9,** No. 3, 273 (1955).
(8) Lyman, C. M., Camp, B. J., Tiller, H. M., J. Agr. Food Chem. **6,** 767 (1958).
(9) Lyman, C. M., Kuiken, K. A., Hale, F., *Ibid.*, **4,** 1008 (1956).
(10) Milner, R. T., Hubbard, J. E., Wiele, M. B., *Oil & Soap* **22,** 304 (1945).
(11) Moore, S., Rockefeller Institute for Medical Research, New York, N. Y., private communication, September 1959.
(12) Moore, S., Stein, W. H., *J. Biol. Chem.* **178,** 53 (1949).
(13) Powrie, W. D., J. Agr. Food Chem. **9,** 67 (1961).
(14) Rackis, J. J., Anderson, R. L., Sasame, H. A., Smith, A. K., VanEtten, C. H., *Ibid.*, **9,** 409 (1961).
(15) Schram, E., Moore, S., Bigwood, E. J., *Biochem. J.* **57,** 33 (1954).
(16) Smith, A. K., Circle, S. J., *Ind. Eng. Chem.* **30,** 1414 (1938).
(17) Smith, A. K., Rackis, J. J., *J. Am. Chem. Soc.* **79,** 633 (1957).
(18) Smith, C. R., Earle, F. R., Wolff, I. A., Jones, Q., J. Agr. Food Chem. **7,** 133 (1959).
(19) Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30,** 1190 (1958).
(20) VanEtten, C. H., Miller, R. W., Earle, F. R., Wolff, I. A., Jones, Q., J. Agr. Food Chem. **9,** 433 (1961).
(21) VanEtten, C. H., Miller, R. W., Wolff, I. A. Abstracts of Papers, page 12A, Division of Agricultural and Food Chemistry, ACS, 141st Meeting, Washington, D. C., 1962.
(22) VanEtten, C. H., Miller, R. W., Wolff, I. A., Jones, Q., J. Agr. Food Chem. **9,** 79 (1961).
(23) Zacharius, R. M., Talley, E. A., *J. Chromatog.* **7,** 51 (1962).

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