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Cucurbit Seeds. II. Nutritive Value of Storage Protein Isolated from *Cucurbita foetidissima* (Buffalo Gourd)

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The storage globulin of *Cucurbita foetidissima* H.B.K. seed was isolated and evaluated nutritionally. Arginine, aspartic acid, and glutamic acid were the most abundant amino acids. Ratios of the content of each essential amino acid to the content of total essential amino acids indicated that lysine, cysteine, and tryptophan were low and methionine and isoleucine were borderline. With respect to total protein nitrogen furnished by essential amino acids, the globulin was between soybean protein and proteins of cottonseed, sunflower, and peanut. The corrected protein efficiency ratio was 1.66 compared with casein at 2.50 g of weight gain of weanling rats per g of protein intake. In these respects, *C. foetidissima* globulin resembled globulins of other oilseeds.

In view of the increasingly large demand for protein to support a burgeoning world population, oilseeds are being considered as primary nutritional and economic sources of edible protein for foodstuffs (Altschul, 1962). In this regard, *Cucurbita foetidissima* H.B.K. should be investigated as a valuable source of oilseed protein. *C. foetidissima* (Buffalo Gourd) is a wild xerophilous species that potentially could yield 3000 lb of seeds/acre in desert regions (Curtis, 1946). Decorticated seeds, which comprise about 70% of the weight of whole seeds, contain about 50% oil and 35% protein. (For a review of cucurbit seed composition, see Jacks *et al.* (1972).) Since the oil is edible (Bolley *et al.*, 1952; Shahani *et al.*, 1951) and since large scale isolation of purified oilseed protein is readily accomplished by current technology (Meyer, 1971), examination of the nutritional aspects of *C. foetidissima* seed protein was of interest. Amino acid composition, amount of each essential amino acid relative to the total amount of essential amino acids (A:E ratios), amount of protein nitrogen supplied by essential amino acids (E:T ratio), amount of weight gain of weanling rats per unit of protein intake (PER), and digestibility of crystallized *C. foetidissima* globulin are reported here.

MATERIALS AND METHODS

C. foetidissima seeds, obtained from W. P. Bemis, University of Arizona, Tucson, Ariz., were dehulled in a Bauer mill. Hulls were removed with a Bates laboratory aspirator and then by hand-picking. Decorticated seeds were pulverized with a pin mill, and 960 g of powder was extracted with 2.5 l. of hexane–acetone (3:2, v/v) three times by methods described previously (Jacks *et al.*, 1970). Globular protein was isolated from dried meal by the procedure of Vickery *et al.* (1952). The yield from 535 g of oil-free meal, prepared from 960 g of powdered whole seed, was 205 g of crystallized globulin. This globulin con-

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Table I. Composition of Diets

Ingredients	Control, %	Experimental, %
Casein	11.10	
C. foetidissima protein		11.03
Corn oil	8.00	8.00
H_2O	1.35	1.35
Mineral mixture[a]	4.74	4.75
Cellulose	3.00	3.00
Vitamin mixture[b]	2.00	2.00
Corn starch	20.00	20.00
Dextrose	49.81	49.87

[a] Salt mixture U.S.P. XIV fortified with $ZnSO_4 \cdot 7H_2O$ (548 mg/kg) and $CoCl_2 \cdot 6H_2O$ (23 mg/kg). [b] Each kilogram of mixture contained the following vitamins, triturated in dextrose (in grams): vitamin A concn (200,000 units/g), 4.5; vitamin D concn (400,000 units/g), 0.25; α-tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; riboflavin, 1.0; menadione, 2.25; *p*-aminobenzoic acid, 5.0; niacin, 4.5; pyridoxine·HCl, 1.0; thiamine·HCl, 1.0; Ca pantothenate, 3.0; and (in micrograms) biotin, 20; folic acid, 90; and vitamin B_{12}, 1.35 (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp.).

tained 14.5% nitrogen, whereas the oil-free meal contained 10% nitrogen (w/w).

Nitrogen was determined by the procedure of Minari and Zilversmit (1963). Amino acid analyses were conducted by Worthington Biochemical Corp., Freehold, N. J., according to their protocol. Protein efficiency ratio was determined as described by Derse (1965). The assay utilized a 28-day feeding period with five weanling male Sprague–Dawley rats per test group. Table I shows the compositions of the diets.

RESULTS AND DISCUSSION

The isolated globulin was crystalline, as judged by birefringence of the material observed microscopically with crossed polars.

The amino acid composition of *C. foetidissima* protein is given in Table II. These data indicate that the globulin of *C. foetidissima*, like oilseed globulins in general, was rich in arginine, aspartic acid, and glutamic acid. Weber *et al.* (1969), using microbiological assays, reported that oil-free meal of *C. foetidissima* was not particularly rich in arginine. However, arginine was the most abundant amino acid of the eight amino acids assayed in *C. foetidissima* meal by Lyman *et al.* (1956). In addition, a recent survey of amino acid compositions of meals and seed globulins of several cucurbits showed that arginine, aspartic acid, and glutamic acid were the most plentiful amino acids (Jacks *et al.*, 1972).

To assess *C. foetidissima* protein for potential food uses, the amino acid composition was evaluated according to FAO/WHO (1965). The A:E ratios are shown in Table II. These data indicate that, with respect to the FAO provisional pattern (FAO/WHO, 1965), lysine, cysteine, and tryptophan are low and methionine and isoleucine are borderline.

To determine whether *C. foetidissima* protein would provide sufficient nitrogen for metabolic processes without obligatory catabolism of essential amino acids, the proportion of total nitrogen intake furnished by essential amino acids was calculated. This proportion, the E:T ratio (FAO/WHO, 1965), was 2.34 g of essential amino acids/g of nitrogen. The value is lower than the mean value for globulins of other cucurbits (2.67 g/g of N) reported by Jacks *et al.* (1972) and falls between soybean protein (2.58 g/g of N) and proteins of cottonseed, sunflower, and peanut (2.15, 2.11, and 2.08 g/g of N, respectively) given by FAO/WHO. The FAO pattern is 3.22 g/g of N for whole hen's egg protein.

Table II. Amino Acid Content[a] and A:E Ratio[b] of *C. foetidissima* Globulin

Amino acid	Globulin		FAO pattern[c]	
	Content	A:E ratio	Content	A:E Ratio
Lysine	3.3	89	4.2	134
Methionine	2.5	67	2.2	71[d]
Cysteine	0.7	18	2.0	62[d]
Isoleucine	4.1	111	4.2	134
Leucine	7.8	210	4.8	152
Phenylalanine	5.9	158	2.8	89
Tyrosine	3.9	104	2.8	89
Threonine	3.5	94	2.8	89
Valine	5.0	134	4.2	134
Tryptophan	0.6	17	1.4	45[d]
Arginine	16.8			
Glycine	4.8			
Histidine	2.5			
Alanine	5.0			
Aspartic acid	10.9			
Glutamic acid	21.5			
Proline	4.5			
Serine	5.9			

[a] Values are g of amino acid/16 g of nitrogen. [b] Values are mg of amino acid/g of total essential amino acids. [c] From FAO/WHO (1965). [d] Probably too high (FAO/WHO, 1965).

Table III. Effect of Dietary Source of Protein on Growth of Weanling Rats

Dietary source of protein[a]	Final body weights[b]	PER[c]		Digestibility %[d]	
		Actual	Corrected	Diet	Nitrogen
Casein	172 ± 29	3.56 ± 0.22	2.50	95	95
C. foetidissima	116 ± 6[e]	2.36 ± 0.18[e]	1.66	95	93

[a] Diets contained 10% protein. [b] Values are means in grams ± standard deviations of five rats per group. Mean initial weight was 57 g/rat. [c] Protein efficiency ratio (PER) = g of weight gain/g of protein intake. Values represent means in grams ± standard deviations. [d] Digestibility (%) = 100 (g of feed intake − g of fecal weight)/g of feed intake. [e] Value is significantly less than value for casein ($p < 0.01$).

To estimate experimentally the nutritive value of *C. foetidissima* protein, the ability of the globulin to support growth of weanling rats was determined. Results presented in Table III show that the corrected PER for *C. foetidissima* globulin was 1.66 compared with casein at 2.50 g of weight gain/g of protein intake. Weber *et al.* (1969) found that the protein quality of crude meal was also lower than that of whole egg for feeding weanling mice.

During the second week of the assay, feces were collected quantitatively and analyzed for nitrogen content. This allowed calculations of the digestibility of the diet and of the nitrogen in the diet. The results (Table III) indicate that nitrogen digestibility of *C. foetidissima* protein was almost as good as the value for casein.

CONCLUSION

Storage protein purified from *C. foetidissima* seed resembles oilseed globulins with respect to amino acid composition and nutritive value.

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Gel Chromatography of Sunflower Proteins

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The salt-extractable proteins in sunflower flour were characterized by gel chromatography, disk electrophoresis, and amino acid composition. The proteins from Commander, Majak, and Valley sunflower and a soybean control were 69–70% dispersible in 2.5% neutral salt solution. These proteins were separated into five fractions by gel chromatography on a standardized Sephadex G-200 column. The fractions I–V contained about 5, 49, 9, 24, and 12% of the extracted meal proteins, respectively, in the three sunflower varieties. After dialysis, fraction I contained a large proportion of nucleic acids, while chlorogenic acid appeared to be bound to only fraction V proteins. Molecular weight estimations indicated that, on the average, the five sunflower protein fractions were similar in molecular weight to the five soybean protein fractions. Sedimentation analyses of Valley fraction II showed that the major protein component in this fraction had a sedimentation coefficient of 12.1 S. The Valley proteins demonstrated fewer bands on disk electrophoresis at pH 8.9 than the soybean proteins. Amino acid analysis indicated that soybean was higher in lysine but lower in methionine than sunflower. Majak proteins were higher in lysine and methionine than Commander and Valley proteins. The major protein fraction II contained high proportions of isoleucine, phenylalanine, threonine, and nonessential amino acids. The fraction IV proteins were very rich in lysine and methionine, while the fraction V in each sunflower variety was very deficient in these essential amino acids.

In recent years, sunflower has become an important oilseed crop in many countries and ranks second in importance as a world source of vegetable oil. In addition, the defatted meals from dehulled seeds contain a higher content of protein than some other oilseed meals (Sosulski and Bakal, 1969). On the basis of their solubility in dilute salt solutions, the major portion of the sunflower proteins was shown to be globulins (Osborne and Campbell, 1897). Their low water solubility but high dispersibility in neutral salt solutions suggested that sunflower proteins may differ from soybean proteins in biochemical characteristics and functional properties (Sosulski and Bakal, 1969). While sunflower meals are presently fed to livestock, the functional properties of the protein could form the basis for utilizing sunflower flours and protein concentrates in new food products.

Few investigations have been conducted on the nature and biochemical properties of the globular proteins in sunflower meal (Gheyasuddin *et al.*, 1970; Hohlenko, 1960; Joubert, 1955). The objective of the present study was to characterize the salt-extractable proteins from sunflower flour by gel chromatography, disk electrophoresis, and amino acid analysis. The proportions of protein, nucleic acid, and chlorogenic acid were determined on the protein fractions as well as their molecular weights (*MW*). Soybean flour proteins were used as reference proteins in this study.

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EXPERIMENTAL SECTION

Seeds of Commander, Majak, and Valley sunflowers and Altona soybeans were obtained from varietal tests grown at the Research Station, Canada Department of Agriculture, Morden, Manitoba. After dehulling the seeds, the lipids were extracted from the ground sunflower kernels with *n*-hexane for 16 hr at room temperature. The solubility of the sunflower flour proteins was determined by two extractions with 0.02 *M* sodium phosphate buffer, pH 7.0, containing 2.5, 5.0, and 10% sodium chloride. Each extraction was conducted at room temperature for 45 min at a meal to solvent ratio of 1:15 (w/v). After centrifugation at 10,000 × *g* for 10 min, the yields of salt-soluble nitrogen (N) were determined by the micro-Kjeldahl procedure (AOAC, 1970).

For chromatography, the proteins were extracted from the soybean and sunflower flours by the above procedure with the 2.5% sodium chloride solution buffered to pH 7.0 and containing 0.01 *M* 2-mercaptoethanol in order to maintain the proteins and phenolic acids in a reduced state. After centrifugation, the combined supernatants were applied directly to the Sephadex gel column. In an alternate procedure, the sunflower extracts were dialyzed before column chromatography. These dialyses were conducted against distilled water containing 0.01 *M* 2-mercaptoethanol for 72 hr at 4°. The contents of the dialyses bags were freeze-dried for storage and, before chromatography, were resolubilized in the sodium chloride solution.

The salt-soluble proteins were fractionated at 4° on a 2.5 × 83 cm column packed with Sephadex G-200. The proteins were eluted from the column by upward flow of the above sodium chloride solution containing 0.02% sodium azide (a bacteriostatic agent). The flow rate was