Antinutritional Factors in *Cucurbita* Seed Meals

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Trypsin inhibitor, lectin, phytate, and oligosaccharide levels were measured in the defatted decorticated *Cucurbita* seed meals. Species surveyed included potential domesticates *Cucurbita foetidissima* and *Cucurbita digitata* (xerophytic gourds) as well as *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo* (common squashes). Trypsin inhibitor activities in *C. foetidissima* and *C. digitata* samples were 5 times greater than in other cucurbits but were only 17% and 24% of that in soybean, the reference material. Heat treatment reduced the activity in all samples to negligible levels. Lectin activity was greatest in soybean, intermediate in *C. digitata*, and lowest in *C. foetidissima* and the domesticated cucurbits. Heat treatment substantially reduced the activity of lectins in all samples except those in *C. digitata* and soybeans. Levels of phytate and sugars were found to be similar among samples.

Cultivated species of *Cucurbita* have been associated with civilization for centuries (Whitaker and Davies, 1962). Squash, melon, cucumber and other cucurbits are presently supplying variety to human diets. Certain xerophytic *Cucurbita* have been identified as possible food crops for arid and semiarid regions of the world. Buffalo gourd, *Cucurbita foetidissima*, appears to be the most promising and has received considerable attention with respect to domestication and utilization (Bemis et al., 1978; Hogan and Bemis, 1983; Scheerens and Berry, 1986). The species produces starch in roots (52–64% d.w.b.) and oil (24–36% d.w.b.) and protein (30–35% d.w.b.) in seeds (Scheerens and Berry, 1986; Dreher et al., 1983; Vasconcellos et al., 1980; Thompson et al., 1978).

When a plant such as buffalo gourd is investigated as a potential food source, the possible presence of toxic components must be considered. Many naturally occurring toxic substances have been studied extensively (Liener, 1980). Some are associated with individual species (e.g., gossypol in cottonseed), but others are found in a variety of plants. Common antinutritional factors found in legumes, grains, and other seed crops include trypsin inhibitors (Liener and Kakade, 1980), lectins (Jaffe, 1980), phytate (Reddy et al., 1982), and flatulent oligosacchardies (Rackis, 1975). The purpose of this work was to examine seed meals of xerophytic and domesticated *Cucurbita* for the presence of common antinutritional factors and to evaluate seed meals of xerophytic species as potential food sources with respect to inherent levels of these nutritional antagonists.

MATERIALS AND METHODS

Plant Materials and Sample Preparation. Seeds (approximately 1-kg lots) of buffalo gourd (*C. foetidissima*) and coyote gourd (*Cucurbita digitata*) were obtained from plants grown at various University of Arizona Agricultural Centers. Seeds (approximately 2-kg lots) of cultivated squashes (*Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo*) were purchased from local vendors. Soybeans (*Glycine max*), which served as reference material, were harvested from a university-grown stock. Dry, clean seeds were ground with a Wiley mill and defatted by hexane extraction in a Soxhlet apparatus. Separation of the defatted embryo from seed coats using a 100-mesh screen produced a raw seed meal suitable for analysis.

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Trypsin inhibitor and lectin analyses were performed on raw and heat-treated seed meals; phytate, oligosaccharide, and protein contents were determined in raw seed meal only. Heat treatment of raw seed meal was carried out in an autoclave for 30 min at 121 °C (15 psi). All samples were analyzed in duplicate from oil-free meal in sample sizes appropriate to the analytical techniques performed.

Crude Protein. Protein content was determined by the micro-Kjeldahl method (AOAC, 1970) using a conversion factor of 6.25.

Trypsin Inhibitor. Trypsin inhibitor was detected by the enzymatic assay developed by Kakade et al. (1974). One trypsin unit is expressed as an increase of 0.01 absorbance unit/10 mL of the reaction mixture at 410 nm. Trypsin inhibitor activity is defined in terms of trypsin units inhibited.

Lectin. Lectin activities were quantified by using a microtiter hemagglutination method described by Jaffe et al. (1972). Serial dilutions of seed meal extracts were made in microtitration multiwell plates (Flow Laboratories, McLean, VA) containing saline solutions of bovine erythrocytes treated with Pronase (Sigma Chemical Co., St. Louis, MO) and trypsin or saline solutions of rabbit and hamster erythrocytes treated with Pronase. Each dilution in series reduced the lectin titer level by half. Lectin content was expressed in terms of the dilution value, 2^n, where n represents the number of dilutions performed effecting agglutination (i.e., dilution value equals the reciprocal of the titer level).

Phytate. Phytic acid was estimated by the chromatographic procedure of Harland and Oberleas (1977). By an anion-exchange technique, inorganic phosphorus was separated from the organic form; after isolation, phytate phosphorus was determined colorimetrically.

Oligosaccharides. Chromatographic determination of oligosaccharides was accomplished by HPLC. Preparation of sugar samples followed the procedure of Black and Glover (1980). Sugars were eluted from a carbohydrate analysis column (Waters Associates, Milford, MA) with a solvent mixture of 70/30 acetonitrile/water delivered at 2.0 mL/min. Individual sugars were quantified by using a repetitive injection technique similar to that described by Black and Bagley (1978). Sample peak areas were ascertained by triangulation and compared to standard response curves.

RESULTS AND DISCUSSION

Crude Protein. The protein contents of xerophytic and domesticated *Cucurbita* seed meals were comparable and varied from 55.9 to 71.4% (Table I). All cucurbit protein

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Table I. Trypsin Inhibitor Activity in *Cucurbita* Seed Meals[a]

species	protein, %	trypsin inhib units (TIU)/mg of protein	
		raw[b]	heat treated[c]
Cucurbita foetidissima	70.3	20.2	1.2
Cucurbita digitata	55.9	28.6	1.5
Cucurbita maxima	71.4	5.1	1.2
Cucurbita moschata	65.8	3.4	1.0
Cucurbita pepo	69.2	6.3	1.6
Glycine max	49.9	120.1	1.6

[a] Defatted, decorticated seed embryo; dry basis, measured in duplicate. [b] Mean of duplicate analyses. [c] Heat treated by autoclave, 30 min at 121 °C and 15 psi.

Table II. Lectin Activity in *Cucurbita* Seed Meals[a,b]

species	erythrocyte source					
	bovine[c]		rabbit		hamster	
	raw	HT[d]	raw	HT	raw	HT
Cucurbita foetidissima	2	1	2	1	2	1
Cucurbita digitata	128	16	128	32	256	1
Cucurbita maxima	1	1	1	1	4	1
Cucurbita moschata	2	1	2	1	4	1
Cucurbita pepo	4	1	8	2	64	1
Glycine max	1	1	8192	256	512	1

[a] Defatted, decorticated seed embryo; dry basis, measured in duplicate. [b] Dilution values 2^n, where n represents the number of dilutions performed effecting agglutination. [c] Bovine erythrocytes treated with trypsin and Pronase; rabbit and hamster erythrocytes treated with Pronase only. [d] Heat treated by autoclave, 30 min at 121 °C and 15 psi.

values were greater than that associated with soybean (49.9%).

Trypsin Inhibitor. The apparent levels of trypsin inhibitor in seed meals of domesticated cucurbits (5.1–6.3 TIU/mg of protein) were about one-fifth of those associated with xerophytic gourds (20.2 and 28.6 TIU/mg of protein). However, samples of the xerophytes respectively possess only 17 and 24% of the activity found in soybean.

Virtually all of the cucurbits exhibit lower inhibitor activity when compared with various edible legumes. Al-Bakir et al. (1982) have determined levels in chickpea (*Cicer arientinum*), lentil (*Lens esculenta*), and black-eyed pea (*Vigna unguiculata*) of 46.5, 40.0, and 33.9 TIU/mg of protein, respectively. Trypsin inhibitor activities of legumes indigenous to the Southwest have been reported to be 75.5 and 61.7 TIU/mg of protein in pinto bean (*Phaseolus vulgaris*) and tepary bean (*Phaseolus acutifolius*), respectively (Thorn et al., 1983).

Heat treatment caused a substantial decrease in trypsin inhibitor activity in all test samples; extracts of heat-treated meals exhibited levels of activity below 2.0 TIU/mg of protein. Levels of this magnitude may challenge the sensitivity of the assay procedure. In addition to the destruction of inhibitor activity, heat treatment of seed proteins may also render these materials more digestible by increasing their susceptibility to enzymatic attack.

Lectin. In general, cucurbit seed meal extracts exhibited lower levels of lectin activity than did soybean extracts (Table II). Buffalo gourd and domesticated cucurbit samples effected agglutination only at low-dilution (high titer) values whereas coyote gourd extracts were found to possess intermediate levels of lectin activity. Cucurbit extracts were most reactive when combined with hamster erythrocytes; soybean extracts appeared to be most sensitive to the assay employing rabbit erythrocytes. Heat treatment reduced lectin activity in almost all cucurbit samples to negligible levels. However, heat-treated coyote gourd seed meal displayed considerable resistance to heat denaturation.

Table III. Phytate, Glucose, and Oligosaccharide Levels in *Cucurbita* Seed Meals[a]

species	phytate,[b] %	glucose, %	oligosaccharides, %		
			sucrose	raffinose	stachyose
Cucurbita foetidissima	1.9	0.6	2.3	1.1	0.9
Cucurbita digitata	3.1	1.2	1.9	1.2	0.7
Cucurbita maxima	1.1	1.7	2.0	0.7	1.7
Cucurbita moschata	2.0	1.0	2.6	0.6	1.7
Cucurbita pepo	2.0	0.8	2.5	0.8	1.1
Glycine max	1.4	3.9	4.6	2.9	2.9

[a] Defatted, decorticated seed embryo; dry basis, measured in duplicate. [b] Mean of duplicate analyses.

Phytate. Phytate levels in cucurbit seed meals ranged from 1.1 to 3.1%, with *C. maxima* and *C. digitata* exhibiting the highest and lowest levels, respectively (Table III). The value for *C. foetidissima* was intermediate in this range. Most cucurbits seed meals contained higher levels of phytate than did the soybean control. However, cucurbit seeds and common beans appear to contain similar quantities of phytate. Lolas et al. (1975) reported phytate contents in four dry bean cultivars to range from 0.65 to 1.30% of whole seeds. As phytate in dicotyledonous seeds is concentrated in the embryo (Lott and Vollmer, 1979), preparation of defatted and decorticated cucurbit seed meals may have enhanced apparent phytate levels. In ground, whole seed samples, phytate contents would constitute a smaller percentage of the dry weight.

Oligosaccharides. Cucurbit seed meals were found to contain lower levels of raffinose, stachyose, and other free sugars than were uncovered in the defatted soybean sample. It is noteworthy that soybean contained 2.9% stachyose, the oligosaccharide indicated to be a predominent cause of flatus (Rackis, 1974). The domesticated cucurbits generally exhibited higher stachyose levels (1.1–1.7%) than did xerophytic species (0.7–0.9%). Conversely, raffinose levels were greater in the xerophytes (1.1–1.2%) than in the domesticates (0.6–0.8%).

Using a GLC technique, Fleming (1981) measured the raffinose content of several beans: red kidney bean (*P. vulgaris*), 0.37%; chickpea (garbanzo bean), 0.67%; lentil, 0.60%; and navy bean (*P. vulgaris*), 0.67%. The stachyose contents of these beans were reported to be 4.05, 2.16%, 1.70%, and 3.53%, respectively. Stachyose levels were generally higher in these grain legumes than in cucurbit seed meals (particularly the xerophytes). However, raffinose levels in xerophytic cucurbit seed meals surpassed those found in the dry bean cultivars. As with phytate contents, oligosaccharide levels in cucurbits would be reduced if they were expressed on a whole seed basis.

***Cucurbita* Seed Meals as Potential Food Sources.** Data indicating the food and feed potential of xerophytic cucurbits (especially buffalo gourd) have been recently reviewed (Scheerens and Berry, 1986); seed and seed fractions have been considered as possible sources of protein in the diets of both monogastric and ruminant animals. Amino acid analysis of the defatted embryo revealed it to be a typical plant protein, limiting in several essential amino acid constituents (especially tryptophan and the sulfur amino acids). Defatted buffalo gourd meal was found to be similar in nutritional value to that of soybean meal when protein efficiency ratios were calcu-

lated by using mice as the test animal. The performance of mice improved when buffalo gourd protein diets were supplemented with methionine (Thompson et al., 1978). Favorable performance of mice on diets containing defatted buffalo gourd seed meal suggested a lack of endogenous antinutritional compounds in this material. However, a quantitative determination of nutritional antagonists was necessary prior to their use as a protein source. In this study, the levels of antinutritional factors found in the seed of arid-adapted cucurbit species, particularly buffalo gourd, were similar to those in domesticated cucurbits and were comparable to or less than those in soybean. Evidence proffered in this study strengthens previous data indicating the suitability of buffalo gourd seed protein for incorporation in the diets of monogastric animals. If xerophytic cucurbits are to be grown extensively as crops, there appears to be little concern in the use of whole seed, seed meal, and/or seed protein fractions as food sources.

Registry No. Trypsin inhibitor, 9035-81-8; phytate, 83-86-3; sucrose, 57-50-1; raffinose, 512-69-6; stachyose, 470-55-3.

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Chromium in Kale, Wheat, and Eggs: Intrinsic Labeling and Bioavailability to Rats

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Retention of ^{51}Cr was measured in rats fed 3-g test meals containing 63% sucrose, 10% corn oil, and 27% of a test food radiolabeled intrinsically or extrinsically. The test foods were egg yolk, kale, and wheat radiolabeled intrinsically and egg yolk, kale, wheat, and casein radiolabeled extrinsically. Five-week-old male rats were fed a Cr-deficient semisynthetic diet for 2 weeks prior to and 9 days following the 3-g test meals containing ^{51}Cr-labeled foods. By day 9, only 1.1–2.3% of the ^{51}Cr in the test meal remained in the animal. Retention of ^{51}Cr from casein (2.3%) was not significantly different from retention from egg yolk (1.7%) but was significantly higher than that from kale (1.1%) and from wheat (1.5%). These differences may have reflected dietary Cr content rather than a difference in form. There were no significant differences in the retention of ^{51}Cr due to method of labeling ($p \leq 0.05$). Preparation of foods intrinsically labeled with ^{51}Cr was difficult because of a root–shoot barrier in the case of plants and poor absorption or tissue retention by animals. Several approaches to endogenous labeling were attempted. An aqueous extract of kale subjected to gel permeation chromatography showed a low molecular weight chromium complex similar to that found in alfalfa (M_r 2900).

INTRODUCTION

Although chromium has been recognized as an essential trace element and appears to function in glucose utilization (Gurson and Saner, 1971), its physiological function remains unclear. Deficiency symptoms of glucose intolerance, neuropathy, weight loss, and metabolic encephalopathy occurring in patients receiving long-term parental nutrition were reversed upon supplementation with chromium (Jeejeebhoy et al., 1977; Freund et al., 1979). The recommended safe and adequate range of dietary chromium is 50–200 μg/day, and a daily infusion of 15–20 μg of chromium for patients on total parental nutrition has been proposed (Howard and Michalek, 1984).

Little is known about the bioavailability of chromium from foods. Oral Cr^{3+} is poorly absorbed (<1%) and appears mostly in the feces, regardless of the dose and dietary

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