Nutritional Evaluation of Seashore Mallow Seed, *Kosteletzkya virginica*

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Lack of fresh water for irrigation, desertification, and salt buildup in soils have led to increased interest in the potential food or feed value of halophytes. Seeds of seashore mallow (*Kosteletzkya virginica*), a highly salt-tolerant plant, were analyzed for proximate composition, amino acid profile, mineral content, protein quality (PER), and potential toxicants. Hulled seed contained 32% protein and 22% fat. Valine was the limiting amino acid. Protein scores of whole and hulled seed were 64 and 72, respectively. Hulled seed as the protein source supported a PER of 2.0 compared to 3.1 for casein. Both whole and hulled seeds were low in sodium and high in calcium and potassium. Cyclopropenoid fatty acids were detected, but gossypol was not. Seashore mallow seed shows good potential for use as food or feed.

Salinization and desertification are serious threats to global food production (Pirie, 1976). Soil salinity problems have arisen in nearly every part of the world, even on nonirrigated crop lands (Poljakoff-Mayber and Gale, 1975). The area where rainfall is not sufficient to remove salts from the root zone of plants has been estimated to comprise 25% of the earth's surface (Thorne and Peterson, 1954). Arid and semiarid areas and salt marshes have been largely ignored as potential crop lands. Salt marshes are potentially valuable because much of their area is virgin soil and because they receive nutrients deposited with land water runoff and nutrients washed up from deep-sea water.

Somers et al. (1979) have screened more than 350 selections from 60 species of halophytes for vigor of growth in saline habitats, edible yield, characteristics of edible portion, quality, and potential. From their selections, the seed of *Kosteletzkya virginica* (L.) Presl. was chosen for nutritional evaluation. *K. virginica* or seashore mallow (SM) is a highly salt-tolerant plant. It tolerates salt concentrations up to 20 parts per thousand and produces a relatively large (4 mm, 20 mg) seed of 23–25% protein. It is a member of the *Malvaceae* as are okra and cotton. Tidal marshes from Texas to Delaware are its native habitat (Duncan, 1974). Seed yield has been estimated to be 1120 kg/ha (Somers, 1981).

This investigation was designed to assess the nutritional value of SM seeds for use as food or feed. SM seed has a tough hull; utilization of the whole seed would minimize processing steps, but the hull may be an undesirable component. Therefore, analysis of proximate composition, amino acids, and selected minerals and biological evaluation (PER) of both whole seed and hulled seed were conducted. Linoleic acid content of whole seed was determined. Detection of the potential toxicants gossypol and cyclopropene fatty acids was attempted. The possibility that SM seed contains other antinutritional factors was examined by feeding raw- and heated-seed diets in the PER study.

EXPERIMENTAL SECTION

Sample. SM seeds were collected from the brackish marshes of the Broadkill River area near Lewes, DE, in Oct 1979. All seeds were kept in refrigerated storage.

Whole seeds were initially dried under vacuum at 55 °C and then processed into raw, whole seed meal, raw, hulled seed meal, heated, whole seed meal, and heated, hulled seed meal. Meals were ground to pass a 1-mm screen in a Wiley mill. Dry, whole seeds were heated at 177 °C for 15 min. Hulled seeds were obtained by cracking whole seeds in a blender whose blades were covered with vinyl tubing to lessen the impact on the seeds. Fractions were separated by air flow and screens with a Clipper laboratory tester and cleaner (Nasco, Fort Atkinson, WI). Heated seeds were hulled in this way with little difficulty, but removal of hulls from raw seeds was difficult.

Proximate Composition. Moisture, nitrogen, fat, and ash were determined by AOAC (1975) methods. Protein was calculated as $N \times 6.25$. Fiber was determined as acid detergent fiber by using sulfuric acid and cetyltrimethylammonium bromide (AOAC, 1980).

Minerals. Sodium, potassium, and calcium were determined with a Perkin-Elmer 560 atomic absorption spectrophotometer. Digestion with nitric and perchloric acids (Perkin-Elmer Corporation, 1971) preceded spectrophotometry. Lanthanum solution (5%) was added to final solutions during calcium determinations.

Linoleic Acid. The linoleic acid content of the ether extract of whole, ground SM seed was determined with a Hewlett-Packard Model 5830A gas chromatograph. SM lipids were methylated by the method described by Tomlins et al. (1972). Aliquots (0.5 μL) of the methyl esters were separated on a column (10% SP-2330 on 100/120 Chromosorb W. AW, $^1/_8$ in. i.d. by 6 ft, stainless steel) under the following conditions: detector temperature, 225 °C; injection port temperature, 250 °C; oven temperature, 200 °C. The carrier gas (nitrogen) flowed at 20 mL/min.

Toxicants. Whole SM meal was subjected to gossypol analysis, and SM oil was subjected to the Halphen test, a qualitative test for cyclopropene fatty acids, according to the methods of the AOCS (1973).

Amino Acids. Amino acids were determined by an ion-exchange, column chromatographic method (Moore and Stein, 1951, 1954), using a Durrum 500 amino acid analyzer. Cystine was determined after performic acid oxidation (Moore, 1963). Tryptophan was determined colorimetrically (Hernandez and Bates, 1969). Protein scores were calculated by expressing the amount of the first limiting amino acid as a percentage of the amount present in the reference protein (FAO/WHO, 1973).

Feeding Trial. Protein efficiency ratio (PER) values of whole and hulled, raw and heated SM seed meals were determined by the AOAC (1975) method. The method's suggested dietary level of fiber could not be obtained because of the high fiber content of the seed meals. Fiber was included in the diets at the expense of cornstarch, and for minimization of the large difference in fiber content, two casein reference diets, one for whole seed meal diets (casein-1) and one for hulled seed meal diets (casein-2), were used. Compositions of the raw, whole SM seed meal; heated, whole SM seed meal; raw, hulled SM seed meal; heated, hulled SM seed meal; and casein reference diets are shown in Table I.

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Table I. Composition and Energy Content of the Feeding Trial Diets

dietary components	raw, whole	heated, whole	casein-1	raw, hulled	heated, hulled	casein-2
protein source, g/100 g	40.2	38.3	10.7	44.4	38.1	10.7
protein, g/100 g	10.0	10.0	10.0	10.0	10.0	10.0
fat, g/100 g	8.0	8.0	9.0	8.0	8.0	8.0
salts, g/100 g	5.0	5.0	5.0	5.0	5.0	5.0
vitamins, g/100 g	1.0	1.0	1.0	1.0	1.0	1.0
fiber, g/100 g	11.3	11.4	13.5	3.8	3.7	3.9
moisture, g/100 g	5.0	5.0	5.0	5.0	5.0	5.0
NFE + starch, g/100 g	8.4 + 51.3	8.4 + 51.2	0.0 + 57.4	7.6 + 59.6	7.6 + 59.7	0.0 + 68.0
energy content, kcal/100 g	351	350	351	381	381	384

Table II. Proximate Compositions of Whole and Hulled Seashore Mallow Seed (Percent)[a]

sample	moisture	protein, N × 6.25	fat	fiber (acid detergent)	ash	NFE[b]
whole SM seed	6.4	24.9	15.4	28.1	4.2	21.0
hulled SM seed	5.2	31.6	21.9	12.0	5.1	24.2

[a] Samples analyzed in triplicate. [b] Nitrogen-free extract, obtained by difference.

Table III. Sodium, Potassium, and Calcium in Seashore Mallow and Selected Foods[a] (Milligrams per 100 Grams)

food	Na	K	Ca
whole SM seed	15	1248	205
hulled SM seed	12	1249	234
rice, brown, raw	9	214	32
barley, pearled, light	3	160	16
oatmeal, dry	2	352	52
Great Northern beans, mature, dry	19	1196	144
raw soybeans, mature, dry	5	1677	226
whole wheat flour, hard wheats	3	370	41
whole, mature okra seed[b]			282

[a] Calculated from Adams (1975) except where otherwise noted. [b] Karakoltsidis and Constantinides (1975).

The casien control diets were formulated with ANRC casein (Humko Sheffield, Union, NJ). Fat, fiber, and starch were added in the forms of cottonseed oil, Celufil, and cornstarch, respectively (U.S. Biochemical Corp., Cleveland, OH). Salts were added as Jones Foster Salt Mix (U.S. Biochemical Corp., Cleveland, OH), and the vitamin mix was prepared according to the AOAC (1975) method for PER.

Weanling (21 days) male rats (Sprague-Dawley) were fed a casein acclimation diet for 3 days after which they were divided into groups and fed the test diets for 28 days. The number of animals fed hulled seed diets and the number fed the casein control diet differed because of the limited supply of seed and the difficulty of preparing hulled seed. Eight rats per treatment were fed the raw, whole SM, heated, whole SM, and the whole seed casein control diets. The number of rats fed the raw, hulled SM, heated, hulled SM, and hulled seed casein control diets were 4, 7, and 12, respectively. Body weight and food intake were recorded every other day. Fecal pellets were collected and pooled by group. Fecal nitrogen was determined in triplicate by the Kjeldahl method (AOAC, 1975).

Data were subjected to analyses of variance and Duncan's multiple range tests (Duncan, 1955; Kramer, 1956).

RESULTS AND DISCUSSION

Proximate Composition. The proximate composition of whole and hulled SM seed is shown in Table II. The protein content of whole SM (24.9%) is greater than the 20–21% protein of whole okra seed (Karakoltsidis and Constantinides, 1975:; Savello et al., 1980). Removal of hulls resulted in a SM meal with 27% more protein, 42% more lipid, 21% more ash, 15% more NFE, and 57% less acid detergent fiber.

Minerals. SM seeds contained abundant amounts of potassium and calcium (Table III). The concentration of sodium in SM, 12–15 mg/100 g, is similar to that for several of the foods listed for comparison. In contrast, the potassium level of SM seed (1250 mg/100 g) was higher than that of most of the foods with the exception of soybeans. SM seeds contained rich amounts of calcium, more than 200 mg/100 g.

In higher plants, salt resistance has been found to involve an ability to either tolerate or modify internal salinity (Rains, 1979). Some halophytes, such as *Atriplex* species, have salt glands in the leaves that collect salt from surrounding tissue (Rains, 1979). The mechanism by which seashore mallow tolerates salinity has not been elucidated. Analysis of the sodium concentrations of the seashore mallow plant structures has not been done, but the present report indicates that its seed does not have a sodium content that differs from that of conventional seeds and grains.

From the standpoint of human nutrition, the abundance of calcium and potassium and the dearth of sodium in SM seed may be useful. SM seed could make a significant contribution to the recommended dietary allowance of 800 mg/day calcium and to the estimated safe and adequate intake of 1875–5625 mg/day potassium (NRC, 1980). The value of low-sodium, high-potassium legumes in the diets of individuals taking diuretics for the control of hypertension has been noted (Haytowitz et al., 1981). SM seeds may also be of value in such diets.

Linoleic Acid Content. SM lipids contained 55.2% by weight linoleic acid. This is a greater proportion of linoleic acid than is found in soybean oil (50.7%), corn oil (34.3%), cottonseed oil (47.8%) (Weast and Astle, 1980), or okra seed oil (31.5%) (Karakoltsidis and Constantinides, (1975).

Toxicants. Gossypol, the toxic phenolic compound found in cottonseed (Singleton and Kratzer, 1973) and okra seed (Karakoltsidis and Constantinides, 1975), was not detected in SM seed. SM oil was Halphen-positive for cyclopropene fatty acids. Most Malvaceae have been found to contain these fatty acids. Cyclopropene fatty acids in SM oil must be quantified before a judgement about their significance can be made. Cottenseed oil contains concentrations of these acids of 0.6–1.2% in the crude oil and 0.1–0.5% in the processed oil (Mattson, 1973).

Amino Acid Composition. Amino acid compositions of the seed are presented in Table IV. The limiting amino acid for both seed forms is valine, which sets amino acid scores at 64 (whole seed) and 72 (hulled). Both seed forms have adequate concentrations of sulfur-containing amino acids, but isoleucine, leucine, and lysine are present in lesser amounts, 70%, 81%, and 82%, respectively, than those suggested by FAO/WHO (1973).

Table IV. Amino Acid Composition (Milligrams per Gram of N) of Seashore Mallow Seeds

amino acid[a]	whole SM	hulled SM	FAO/WHO (1973)	amino acid score whole	amino acid score hulled
Asp	598	528			
Thr	224	263	250	90	100
Ser	321	287			
Glu	1045	1022			
Pro	230	255			
Gly	336	261			
Ala	261	281			
Cys	166	170			
Met	93	98			
total S-containing AA's	259	268	220	100	100
Val	199[b]	223[b]	310	64	72
Ile	175	193	250	70	77
Leu	357	350	440	81	80
Tyr	187	185			
Phe	283	310			
total aromatic AA's	470	495	380	100	100
His	172	172			
Lys	278	273	340	82	80
Arg	657	737			
Trp	258	87	60	100	100

[a] Samples analyzed in duplicate. [b] Denotes limiting amino acid.

Table V. Responses of Rats to Seashore Mallow and Casein Diets

diet	no. of rats	wt gain, g/28 days[a]	food intake, g/28 days[a]	PER[a,d]	adjusted[b] PER	% fecal N	apparent digestibility,[c] %
raw, whole	8	27.0 ± 6.8	219.0 ± 42.8	0.98 ± 0.12[a]	0.77	2.4[e]	66.0
heated, whole	8	42.9 ± 14.6	287.0 ± 62.9	1.47 ± 0.12[b]	1.15	2.5	65.2
casein-1	8	152.8 ± 21.3	477.7 ± 53.1	3.19 ± 0.12[d]	2.50	0.9	89.0
raw, hulled	4	57.1 ± 10.1	250.8 ± 52.3	2.07 ± 0.18[c]	1.67	3.7	76.4
heated, hulled	7	51.3 ± 16.5	274.2 ± 24.2	2.01 ± 0.13[c]	1.63	3.6	72.0
casein-2	12	123.2 ± 21.7	397.2 ± 54.6	3.09 ± 0.10[d]	2.50	1.9	91.5

[a] Mean ± standard deviation. [b] PER values adjusted on the basis of casein = 2.50. [c] Apparent digestibility = (N intake − fecal N)/N intake × 100. [d] PER values not followed by a common letter are significantly different ($P < 0.05$) as determined with Duncan's multiple range test. [e] Values are means of triplicate determinations on fecal samples pooled by group.

The amino acid pattern is similar to that of okra seed. Savello et al. (1980) reported the limiting amino acids in hulled okra seed meal. These were valine (amino acid score 55), isoleucine (69), and lysine (87).

Feeding Trial. The responses of rats to SM and casein reference diets are shown in Table V. The PER's of the casein reference diets were signifiicantly greater ($P < 0.05$) than the PER's of the SM diets. Given the superior amino acid composition of casein (FAO, 1970), this result was expected.

Comparisons among the SM diets showed that hulled seed diets were superior to whole seed diets in PER values ($P < 0.05$). Heating produced a difference in PER's in the whole seed but not the hulled seed diets. The PER of the heated, whole SM was significantly greater ($P < 0.5$) than that of the raw seed.

The apparent nitrogen digestibility data provide another reason, besides amino acid composition, for the difference in PER's between casein reference and SM diets and suggest why hulled seed SM diets were superior to whole seed diets. The casein reference diets were highest in nitrogen digestibility, hulled seed diets were second, and whole seed diets were lowest. Heating did not produce substantial differences in digestibility compared with raw seeds.

The probable physical and chemical differences between the fiber constituents of SM meal and purified cellulose, and the inherent nature of the fiber in the SM diets, most likely account for the disparity in digestibility between the SM and the casein diets. The digestibility of casein in vitro has been shown to be reduced to a greater extent by highly branched and/or highly ionized types of fiber than by cellulose, which is linear and lacking in ionizable groups (Acton et al., 1982).

Hulled seed diets seem to have been superior to whole seed diets because of their lower fiber content, but the possibility that this effect was due to a reduction in the concentration of some other hull component cannot be eliminated.

The mean food intake of animals fed the raw, whole SM diet was much lower than that of animals fed the heated, whole SM diet (Table V). This lower food intake supported a lower weight gain. Although the destruction of an antinutritional factor in the hull cannot be discounted as an explanation for the results obtained with feeding heated whole SM, it is also possible that improved palatability of the heated seed promoted greater food consumption and weight gain.

Comparison of the PER of hulled SM with average PER values (unadjusted) of several grains and oilseeds (FAO, 1970) shows that hulled SM is superior in unadjusted PER to corn and whole wehat, 1.18 and 1.53, respectively, but somewhat inferior to rice, soybeans, and cottonseed, 2.18, 2.32, and 2.25, respectively, for the rat. Karakoltsidis and Constantinides (1975) reported that the unadjusted PER of whole okra seed meal was 3.45, comparable to the PER of their casein reference.

The SM seeds used in this study were gathered from the wild, whereas the grains and oilseeds whose PER's were cited for comparison are cultivated crops and the protein quality of such crops is known to vary with such factors as cultivar, fertilization, and growing conditons. The results of this initial nutritional evaluation of SM seed are therefore encouraging. SM has been shown to be a good

candidate for the production of a salt-tolerant food plant through selection, breeding and development of cultivation techniques. Also, improved processing of SM seed, such as more complete hull removal, represents a potential means of improving the nutritional quality of SM seed.

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Studies on the Phenolase Enzymatic System in Durum Wheat

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Ammonium sulfate fractionation of crude preparations of durum wheat (cv. Valnova) *o*-diphenolase, followed by treatment with calcium phosphate gel, ion-exchange chromatography, and column isoelectric focusing, gave an 885-fold increase in specific activity. In the first purification steps, process activation of the enzyme was observed, thus revealing a latent enzymatic activity. The purified enzyme showed activity toward di- and polyphenolic substrates but it did not catalyze the oxidation of monophenols. With 4-methylcatechol as the substrate, the enzyme displayed two pH optima of 5.3 and 7.3. The main maximum was located on a broad plateau of 0.4 pH unit. Isoelectric focusing and ion-exchange chromatography revealed three isoenzymes with different specific activities. Some significant differences between the *o*-diphenolase studied here and that previously isolated by us from a common wheat sample were emphasized.

Browning reactions which occur in some wheat products are believed to be related to the enzyme *o*-diphenolase (*o*-diphenol:O_2 oxidoreductase, EC 1.14.18.1). Besides this well-known function, the enzyme is also believed by some authors to have properties that might be useful in identifying wheat varieties (Lamkin et al., 1981), particularly in the identification of common wheats used in the production of pasta products (Feillet and Kobrehel, 1974; Kobrehel and Feillet, 1976) that in some countries, like France and Italy, is an adulteration. Our previous reports described some properties of *o*-diphenolase isolated from a common wheat sample (Interesse et al., 1980, 1981) and displayed the existence of multiple forms of that enzyme. Wheat isoenzymes have been revealed by many workers using acrylamide gel electrophoresis (Kruger, 1976; Singh and Sheoran, 1972; Taneja et al., 1974; Taneja and Sachar, 1974, 1977a,b; Tikoo et al., 1973). The number of isoenzymes and their properties may be different in each wheat variety. In this study we have isolated and characterized the *o*-diphenolase from a durum wheat sample as well as separated enzymatically active proteins by ion-exchange chromatography and isoelectric focusing. Fur-

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