A Compositional Study of Pods of Two Varieties of Mesquite (*Prosopis glandulosa*, *P. velutina*)

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The saccharide composition of the seeds and pericarp of honey and velvet mesquite were determined using gas chromatography and high-performance liquid chromatography. Sucrose was the major saccharide present, occurring mainly in the pericarp. Sucrose, raffinose, stachyose, inositol, and a galactomannan gum were present in the seeds. Autolysis at pH 5.0 and 6.5 for 18 h, resulted in decreased sucrose, raffinose, stachyose, and gum viscosity, while inositol increased. Proximate analysis for protein, fiber, fat, and minerals gave generally expected results. It was concluded that α-galactosidase, β-mannosidase, invertase, and phytase are present in the seeds. The pericarp contained invertase activity. No evidence of cyanogenic glycosides was detected.

Mesquite, a common, thorny and woody leguminous plant, grows in arid and semiarid areas of the world as a shrub or tree. It has attracted interest where its hardiness has made it a pest, as in the southwestern United States where it has extensively invaded grazing lands. The potential economic uses (food, feed, and fuel) of mesquite have been reviewed by Cruse (1973) and by Felker (1979), its biology was reviewed by Simpson (1977), and an annotated bibliography has been prepared by Schuster (1969).

The carbohydrates in the edible pods have not been extensively examined. Walton (1923) found that honey mesquite (*Prosopis glandulosa*) pods contained about 13% protein and 30% sucrose. Figueiredo (1975) examined pods from Algarobeira, a Brazilian mesquite tree (*P. juliflora*), and found similar protein and sucrose levels, a galactomannan polymer of molecular weight about 250000, and some α-galactosidase and β-mannosidase activity in the seeds.

Cyanide, which occurs in some other legumes as a glycoside, was thought to be detected but not quantitated in *P. juliflora* seed by Hegnauer (1958). This apparent cyanide content has been suggested as the cause of some of the problems associated with feeding mesquite seeds to animals (Cruse, 1973), although Pak et al. (1977) found no cyanide in the pods or leaves of *P. tamarugo*.

This paper describes the carbohydrates and their hydrolyzing enzymes in the seed and pods of two North American species, honey and velvet mesquite (*P. glandulosa* and *P. velutina*).

EXPERIMENTAL SECTION

Sample Identification and Preparation. Honey mesquite seed pots (20 lb) were collected in the College Station, TX, area and velvet mesquite (100 lb) in the Tuscon, AZ, area. Both samples had some insect infestation so they were sealed in plastic bags and stored frozen.

Whole mesquite pods (2 lb of honey, 5 lb of velvet) free from insect damage or contamination were selected and separated from two fractions, the seed and the pod pericarp (exocarp, spongy mesocarp, and horny lignified endocarp) by milling in a coffee mill (Hobart Mfg. Co). The mill was set to break the pods while leaving the seeds intact. Most seeds were scarified by the coffee mill; up to 95% hydrated in 18 h and 80-90% germinated in 3-4 days as opposed to 0-1% in hand-separated controls. The honey mesquite pods were dried (forced air oven, 45 °C for 8-16 h) before milling; the velvet mesquite had been adequately sun-dried in Arizona. Seeds used for further studies were ground to 20 mesh in a Wiley mill. The pod pericarp was not further milled.

Morphological composition was obtained by sieving the milled pods through several screen sizes and weighing. Seed morphology was determined by hand dissecting several grams of seeds. The endocarp was further separated from the seed coat by hydration, dissection, lyophilization, and weighing.

Proximate Analysis. Moisture, total nitrogen, ammonia and nitrate nitrogen, fat, fiber, and ash were determined using AOAC (1975) approved methods. Total and reducing sugars were determined as in Potter et al. (1968) and hexose sugars as in McCready et al. (1950). Total and inorganic phosphorus were determined by the phosphomolybdate method of Allen (1940) and other minerals by Perkin-Elmer Corp. methods (1973).

Saccharide Analysis. Weighed amounts of milled powders were mixed with 70% ethanol (v/v) to give 100 mg/mL, refluxed 1 h, cooled, and centrifuged. Aliquots of the supernatant were evaporated to dryness at 60 °C under nitrogen and silylated, overnight at room temperature with shaking, using Tri-Sil reagent. This simplified procedure gave essentially the same results as refluxing and centrifuging three times. The saccharides were separated on a Hewlett Packard 5830A gas chromatograph with dual flame ionization detectors. The unit was equipped with dual $^{1}/_{8}$ in. × 6 ft stainless steel columns packed with 3% OV-1 on Chromosorb W (HP 80-100). For the di- and polysaccharides the GC was temperature programmed from 180 to 330 °C at 15 °C/min (Becker et al., 1977). Monosaccharides were separated by temperature programming from 100 to 300 °C at 20 °C/min. The injector and detectors were at 330 °C. Sucrose, raffinose, stachyose, and the monosaccharides were identified by their retention times and were quantified by comparison of their peak areas with standard curves obtained using known amounts of sugar. The inositol peak was similarly identified and quantified using known amounts of myoinositol. The reported results are averages of two or more separate determinations that agreed within 10%.

Identities of the sugars were confirmed by comparison of their retention times using high-performance liquid chromatography (LC). Filtered aliquots were isocratically chromatographed in either 85:15 or 60:40 acetonitrile/water (v/v) using a Waters Associates carbohydrate column, injector, pump, and refractive index detector (Conrad and Palmer, 1976).

The quantity of water-soluble gum was determined by centrifuging and decanting water extracts (three times) of

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Table I. Morphological Composition of Mesquite Fruit

honey mesquite	%	velvet mesquite	%
seeds	15.0	seeds	24.6
seed coat (testae)	7.0	seed coat (testae)	10.6
sclereid	3.6	sclereid	5.5
endosperm	2.4	endosperm	5.1
cotyledon	7.9	cotyledon	14.0
pericarp	85.0	pericarp	75.4
endocarp	36.8	endocarp	11.7
mesocarp + exocarp	48.2	mesocarp + exocarp	63.7

slurries prepared by mixing seed powders with 10 parts water and autoclaving 20 min. The combined water extracts were made to 60% ethanol and centrifuged, and the precipitate was lyophilized and weighed. Gum aliquots were hydrolyzed with 0.02 or 2.0 N H_2SO_4 for 30 min at 80 °C and neutralized with $BaCO_3$, and the free sugars were analyzed. These hydrolysis conditions would liberate any easily degraded furanosides as well as the more resistant pentosans.

Autolysis. Weighed amounts of ball-milled mesquite powders were mixed with 0.2 M Tris buffer (pH 6.5) or 0.2 N acetate buffer (pH 5.0) in glass stoppered flasks to make 100 mg/mL, 2 drops of toluene were added, and the flasks were incubated at 37 °C for 16–18 h. The samples were cooled to room temperature, made to 80% ethanol, and centrifuged, and aliquots were evaporated to dryness under nitrogen at 60 °C and assayed for their sugar content by GLC and LC as described above.

Ball-milled seeds were dialyzed for 18 h against running distilled water. The retentate was transferred to Erlenmeyer flasks and known amounts of raffinose, sucrose, or phytic acid were added. The samples were then autolyzed and assayed for sugar content.

Cyanide Analysis. Weighed amounts of freshly milled powders were mixed with water, β-glucosidase (10 mg of enzyme/100 mg of sample) at pH 5, or dilute H_2SO_4, and held at 37 °C while being swept with air. The evolved gasses were trapped in 0.1 N NaOH and assayed colorimetrically with picric acid (Egli, 1977) or by the König reaction (Lambert et al., 1975).

RESULTS AND DISCUSSION

Morphology and Proximate Composition. The morphological composition of mesquite pods (fruit) is shown in Table I. Because the pod pericarp is hygroscopic and sticky, separations and weights are somewhat arbitrary. The apparently greater proportion of seeds in velvet mesquite probably reflects differences in moisture content of the pericarp as well as agronomical conditions. Honey mesquite seeds contained amounts of testae, sclereid, endosperm, and cotyledon fractions similar to velvet mesquite.

The proximate and mineral composition of the mesquite seeds, pericarp, and pods are shown in Table II and III. The seeds are very rich in protein while the pericarp contains most of the fiber and free sugars. The iron values are lower than those reported by Figueiredo (1975) but near other legume seeds [i.e., soybean, 84 ppm Fe; Watt and Merrill (1963)]. All other results in Table I, II, and III are near previously reported values for other *Prosopis* species (Walton, 1923; Earle and Jones, 1962; Jones and Earle, 1966; Figueiredo, 1975).

Saccharide Composition. Sucrose is by far the most abundant free sugar in both species of mesquite. It occurs in over ten times greater amounts than raffinose, the next most abundant sugar (Table IV). This is about twice the amount of sucrose found in sugar cane or sugar beets. Most sucrose is concentrated in the pericarp, which appears to be typical of *Prosopis* species (Walton, 1923; Figueiredo, 1975; Felker, 1979). The other free sugars in the pericarp were raffinose, inositol, fructose, and trace amounts of galactose. The disappearance of sucrose and raffinose during pericarp autolysis, with an accompanying increase in glucose, fructose, and melibiose (data not shown), indicates invertase activity. The absence of the release of significant amounts of inositol, coupled with the phosphorus data, suggests an absence, or low level, of phytic acid and phytase in the pericarp. Proximate analysis indicates 20–25% additional pericarp carbohydrate is probably occurring as acid- or base-soluble fiber.

The seeds differ from the pericarp both in the kinds and amounts of saccharides and in their hydrolyzing enzymes; the major differences being the greatly decreased sucrose level and the occurrence of stachyose in the seed. Autolysis of the seed for as little as 6 h results in the disappearance of all raffinose and much of the stachyose; this indicates high α-galactosidase activity in the seeds. The absence of melibiose and the rather slow disappearance of sucrose

Table II. Proximate Composition of Mesquite[a]

sample	total solids	% N	% NH_3	% NO_3N	% crude protein[b]	% fat	% fiber	% ash	% total sugars	% hexose sugars	% reducing sugars
honey mesquite seeds	92.88	5.08	0.09	0.00	31.19	4.32	6.99	3.42		4.21	0.00
pericarp	91.47	1.28	0.08	0.11	6.81	2.79	26.57	3.44	31.60		3.04
whole pods	92.45	1.79	0.07	0.22	9.38	2.66	21.68	3.27	26.40		1.92
velvet mesquite seeds	97.58	5.13	0.04	0.38	29.44	5.68	7.12	3.83		3.90	0.00
pericarp	93.96	1.13	0.01	0.02	6.88	2.24	23.48	5.52	31.60		1.42
whole pods	92.73	1.95	0.04	0.02	11.81	2.36	22.61	4.83	22.20		1.65

[a] As received. [b] N × 6.25.

Table III. Mineral Composition of Mesquite

mineral	honey mesquite			velvet mesquite		
	seed	pericarp	whole pod	seed	pericarp	whole pod
calcium, %	0.28	0.42	0.30	0.26	0.63	0.53
magnesium, %	0.37	0.06	0.08	0.18	0.09	0.09
sodium, %	0.04	0.08	0.09	0.06	0.04	0.03
potassium, %	0.70	1.03	1.02	0.68	1.61	1.27
copper, ppm	16.1	3.1	4.6	13.6	6.4	8.3
zinc, ppm	74.1	9.9	18.8	49.6	9.6	26.4
manganese, ppm	23.0	6.1	8.4	24.2	11.6	14.5
iron, ppm	94.2	18.2	32.4	46.6	48.8	40.4

Table IV. Percent Saccharides in Mesquite Pods before and after Autolysis

sample	autolysis pH	sucrose	raffinose	stachyose	inositol
honey mesquite					
pericarp		27.02	1.17	0.00	0.03
	5.0	20.16	0.24	0.00	0.06
	6.5	21.87	0.16	0.00	0.06
seed		1.81	1.70	0.96	0.03
	5.0	1.97	0.01	0.05	0.23
	6.5	1.77	0.01	0.57	0.08
velvet mesquite					
pericarp		32.08	0.54	0.00	0.00
	5.0	10.91	0.14	0.00	0.00
	6.5	6.21	0.08	0.00	0.00
seed		1.83	1.85	0.60	0.06
	5.0	1.57	0.00	0.00	0.30
	6.5	1.70	0.00	0.04	0.09

Table V. Millimoles of Phytic Acid in Mesquite

	calcd from phosphorus determinations (total − inorganic)	calcd from inositol values after pH 5.0 autolysis (liberated inositol − free inositol)
honey mesquite		
seeds	2.90	1.28
pericarp	0.22	0.00
velvet mesquite		
seeds	2.47	1.33
pericarp	0.38	0.00

suggest only low levels of invertase activity. Autolysis of dialyzed seed powders containing added raffinose or sucrose confirmed these observations (data not shown).

The liberation of inositol during autolysis of seeds at pH 5.0 indicates the presence of indigenous phytase, the phytic acid hydrolyzing enzyme. Incubation of cooked seed powders with commerical phytase also liberated inositol. Phytase was found in mesquite seeds but not in the pericarp. The detection of less than stoichiometric amounts of inositol in the seeds (Table V) could be due to incomplete phytic acid hydrolysis. Inositol monophosphate, for example, would not be detected by this assay.

Cyanogenic glycosides are probably not present in the *P. velutina* or *P. glandulosa* seed or pericarp analyzed (Table VI). It is thought that interferences are virtually nonexistent in reactions based on the König reaction (Lambert et al., 1975), which gave negative results for all mesquite samples tested and positive results for samples known to contain cyanide, lima beans, and apricot kernels. The picric acid test, which indicated low cyanide levels in *P. velutina* seed, is known to give positive results with many reducing compounds other than cyanide. Varietal or agronomic differences, or unknown reducing compounds, could explain the positive cyanide results reported by others.

About 25% of the seed is a water-soluble, high-molecular-weight endosperm gum which, on acid hydrolysis, gave nearly equal amounts of galactose and mannose. An additional carbohydrate must be present which is not water soluble and not detected by crude fiber determinations (residue after hot 0.3 N H_2SO_4, 0.3 N NaOH washes). Autolysis of dialyzed seed powders results in a large decrease in viscosity after 24 h, but only trace amounts of free monosaccharides (data not shown). This indicates the presence of a gum endohydrolase in the seeds. The galactomannan gum may serve as the reserve carbohydrate since the seeds contain only about 5–6% fat, and no starch was observed, or maltose detected, after autolysis.

Table VI. Estimation of Cyanogenic Glycosides in Mesquite

sample	mg of CN^-/100-g sample: picric acid	Konig reaction
P. velutina, seed		
autolyzed 16 h	3.0	0.0
autolyzed 6 h	3.1	0.0
acid hydrolyzed 16 h	2.7	0.0
β-glucosidase, 16 h	3.3	0.0
sprouted 5 days, autolyzed 6 h		0.0
P. velutina, pericarp		
autolyzed, 16 h	0.0	0.0
acid hydrolyzed, 16 h	0.0	0.0
β-glucosidase, 16 h	0.0	0.0
P. glandulosa, seed		
autolyzed, 16 h	0.0	0.0
acid hydrolyzed, 16 h	0.0	0.0
β-glucosidase, 16 h	0.0	0.0
P. glandulosa, pericarp		
autolyzed, 16 h	0.0	0.0
acid hydrolyzed, 16 h	0.0	0.0
β-glucosidase, 16 h	0.0	0.0
lima beans		
autolyzed 6 h	14.0	17.3
apricot kernels		
autolyzed 6 h	300	225

CONCLUSIONS

Mesquite pods contain large amounts of sucrose, located mainly in the pericarp. The seeds contain much smaller amounts of sucrose and also contain raffinose and stachyose. Seeds are rich in α-galactoside sugars and a galactose–mannose gum.

Autolysis of ground mesquite seeds demonstrated the presence of high α-galactosidase activity, lesser invertase and phytase activity, and a gum endohydrolase. Only low levels of invertase activity were found in the pericarp. The mesquite seed pod composition and autolytic changes resemble germinating guar and carob (McCleary and Matheson, 1974).

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Evaluation of the Resistance of Lysine Sulfite to Maillard Destruction

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Lysine sulfite (a salt consisting of 2 mol of lysine and 1 mol of sulfurous acid) was found to be very resistant to nonenzymic browning when heated in a glucose solution. As measured by loss of chemically available lysine, it was also found to be more resistant to Maillard destruction when compared with lysine or lysine plus sodium bisulfite. However, heat treatment of lysine-supplemented wheat–glucose diets resulted in losses of bioavailable lysine which were equally severe when either lysine sulfite or lysine hydrochloride was used as the lysine source. Thus, lysine sulfite, although quite resistant to color formation, is very susceptible to loss of nutritionally available lysine due to Maillard destruction.

Cereal grains are limiting in lysine (Howe et al., 1965). Because lysine is also susceptible to destruction via the Maillard reaction, heat treatment of proteins in the presence of reducing sugars often reduces the protein quality still further. Although supplementing the processed protein with lysine generally counteracts loss in quality, postprocessing supplementation is impractical. Addition of lysine prior to heat processing, however, also poses problems since dark colors and off-flavors sometimes occur. Moreover, protein quality may even be reduced in some instances upon heating. Adrian and Frangne (1969), for example, demonstrated that supplementing an unbaked cookie with lysine in the form of skim milk powder increased the PER from 1.09 to 2.10, but after baking, the PER dropped from 0.19 for the unsupplemented cookie to 0.02 for the lysine-supplemented cookie. Thus, it is critical to supplement with a form of lysine which is reasonably resistant to the Maillard reaction.

Kawashima et al. (1978) recently proposed that lysine sulfite (a salt consisting of 2 mol of lysine and 1 mol of sulfurous acid) would be a useful form of lysine for preprocessing supplementation. However, evidence presented for lysine sulfite's resistance to Maillard destruction was restricted to its resistance to color formation. This is a critical point since sulfites generally act on coloration without markedly lowering amino acid loss (Friedman and Kline, 1950).

Our objective was to further evaluate the resistance of lysine sulfite to Maillard destruction using a bioassay technique.

EXPERIMENTAL SECTION

Lysine sulfite was prepared by the method of Kawashima et al. (1978). Final lysine content was determined using a Beckman 119CL amino acid analyzer. Sulfurous acid content was determined by the conventional iodometric titration technique. The final product contained 76.1% L-lysine and 22.0% sulfurous acid.

To test the stability of the reaction mixtures, 2.5 mmol of lysine base (United States Biochemical Corporation, Cleveland, OH), lysine hydrochloride, or lysine sulfite was dissolved in 10 mL of 0.5 M glucose with or without the addition of 1.25 mmol of sulfite as either sodium sulfite or sodium bisulfite. The solutions were heated in capped tubes at 100 °C and the resultant colors were read at 490 nm in a spectrophotometer.

To test the resistance to Maillard destruction, lysine base (5 mmol), lysine base plus sodium bisulfite (5 mmol and 2.5 mmol, respectively), or lysine sulfite (5 mmol) was dissolved in 10 mL of 0.5 M glucose. The solutions were heated at 100 °C for 40 min. after which remaining free lysine was determined on an amino acid analyzer.

New Hampshire × Columbian male chicks were fed a crystalline amino acid diet (Table I) to assess the bioavailability of lysine in lysine sulfite (experiment 1). The

Table I. Composition of the Purified Crystalline Amino Acid Diet

basal diet	%	amino acid mixture	%
cornstarch	to 100.00	L-Arg·HCl	1.15
amino acid mixture	19.84	L-Lys·HCl	0.50
corn oil	10.00	L-His·HCl·H_2O	0.45
cellulose	3.00	L-Tyr	0.45
mineral premix[a]	5.37	L-Phe	0.50
sodium bicarbonate	1.50	L-Thr	0.65
choline chloride	0.20	L-Trp	0.15
vitamin premix[a]	0.20	DL-Met	0.35
DL-α-tocopheryl acetate (20 mg/kg)	+	L-Cys_2	0.35
ethoxyquin (125 mg/kg)	+	L-Leu	1.00
		L-Val	0.69
		L-Ile	0.60
		Gly	0.60
		L-Pro	0.40
		L-Glu	12.00
		total	19.84

[a] Baker et al. (1979).

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