Chapter 9

Volatile Constituents of Mesquite (*Prosopis*) Pods

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Mesquite is the common name in North America for leguminous desert plants of the genus *Prosopis* that has about 44 species native to North America, South America, Africa and south Asia. Mesquite pods were a major food source of indigenous people in the semi deserts of North and South America before the arrival of Europeans. The pods, which contain about 10% protein and 10-40% sucrose, have a cinnamon, café mocha and coconut aroma. Volatiles were isolated from mesquite pods by closed loop dynamic headspace sampling. A total of 121 volatiles were identified (14 of which were tentatively identified) using GC and GC/MS.

Mesquite (*Prosopis spp.*) are woody leguminous plants that belong to the family Leguminosae and grow in arid and semiarid regions of America, Africa and Asia. The 44 *Prosopis* species range in size from multi-stemmed, 2-inch trunk diameter, 8 ft. tall shrubs in the deserts of southern New Mexico to 60 ft. tall trees with 6 ft. diameter trunks in the river bottoms of northwestern

Argentina. Most people in the U.S. know mesquite for the flavor the smoke imparts to grilled steaks and seafood. However, the plant has attracted attention due to its ability to tolerate high temperatures and low rainfall, its capacity to grow in saline soil and its ability to fix nitrogen (*1-3*). *Prosopis spp.* produce indehiscent fruit (pods) that contain 13 to 50% sugar, 27 to 32% dietary fiber and 11 to 17% protein (*2, 4*). The sugars in the pod exo mesocarp flour consist of about 92% sucrose, 3% glucose and 5% fructose (*5*).

The majority of the scientific papers reporting on nutritional value and composition of *Prosopis* pods are modifications of (*5*) in which the pods are fractionated by a dry milling process into (a) a high fiber/low protein fraction corresponding to the pod endocarp, (b) a high protein fraction corresponding to the endosperm of the seed, (c) a high galactomannan gum fraction resulting from the seed and (d) a high sucrose fraction resulting from the pod mesocarp. However, several commercial firms (http://www.cocinadevega.com) grind the entire pod to produce a flour. For mesquites of the Sonoran desert, one would expect the proximate compostion of the entire pod to be similar to that of *P. velutina* reported by Meyer et al. (*5*) of 22% sugar, 12% protein, 2.5% fat, 22% crude fiber and 3.5 % ash. The reported proximate composition of the *P. alba* flour was 7.2% protein, 2.2% fat, 3.1% ash, 26.5% total dietary fiber and 59% total sugars and that of the *P. pallida* flour was 8.1% protein, 0.8% fat, 3.6% ash, 32.2% total dietary fibre and 48.5% total sugars (*6*). Thus the mesocarp flours are lower in protein, lower in fiber, lower in fat but higher in sugar.

Meyer et al. (*5*) found that the high protein fraction, mainly resulting from the seeds had 61% protein and 8% fat. Thus the fat content of the seeds was several times greater than that of the flour alone. As mentioned in detail later, hexanal, which is an aldehyde resulting from lipid oxidation, is often used as an assay for rancidity of flour products. Thus the high lipid content of the seeds could result in rancidity due to the oxidation of the several fold higher lipid content than that of the pod mesocarp.

The pods can be milled to produce flour which is sold commercially and is used in pastries and baked goods. The pods and flour have a cinnamon, café mocha and coconut aroma. Our goal was to elucidate what volatiles are responsible for these pleasant sensory attributes.

Experimental

Materials

Classic Sonoran and Sweet Peruvian mesquite meal was purchased from Cocina deVega, Inc. (Golden Valley, MN). *Prosopis alba* pod mesocarp flour (harvested in 2003) was obtained from Roque Sáenz Peña, Chaco, Argentina.

Sample Preparation

Dynamic Headspace Sampling. The powdered mesquite pods (30 g) were placed in a 1 L round-bottomed flask along with 54 g NaCl (previously heated to 150 °C to remove volatiles) and 150 mL purified water (Milli-Q Plus, Millipore Corporation, Bedford, MA). The flask was fitted with a Pyrex head to allow the sweep gas to enter the top of the flask (via a Teflon tube) and exit out of a side arm through a Tenax trap (ca. 10 g of Tenax [Alltech Associates, Deerfield, IL], fitted with ball and socket joints). The system was purged with purified nitrogen (200-400 mL/min) for 2 min and immediately connected to an all Teflon diaphragm pump that recirculated nitrogen around the loop (closed loop sampling) at 6 L/min for 3 h. The sample was continuously stirred during the sampling period with a magnetic stirrer. After sampling, the Tenax trap was removed and the volatiles eluted with 70 mL of freshly distilled diethyl ether containing ca 0.001% Ethyl antioxidant 330 (1,3,5-trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene). The ether was carefully concentrated to ca. 50 µL using a warm water bath (50-60 °C) and a Vigreux column.

Capillary Gas Chromatography

A Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) was used. A 60 m × 0.32 mm i.d. DB-WAX fused silica capillary column (d_f = 0.25 µm; J&W Scientific, Inc. Folsom, CA) was employed. The injector and detector temperatures were 180 °C and 290 °C, respectively. The oven temperature was programmed from 30 °C (4 min isothermal) to 200 °C (held for 25 min at final temperature) at 2 °C/min. Helium carrier gas linear velocity was 36 cm/s (30 °C).

Capillary Gas Chromatography/Mass Spectrometry (GC/MS)

The system consisted of an HP 6890 gas chromatograph coupled to an HP 5973 quadrupole mass spectrometer (capillary direct interface). A 60 m × 0.25 mm i.d. DB-WAX fused silica capillary column (d_f = 0.25 µm) was used. Helium carrier gas was used at a headpressure of 22 psi. The oven temperature was programmed from 30 °C to 200 °C (held for 35 min at the final temperature) at 2 °C/min.

Odor Threshold Determinations

Odor thresholds were determined in water with reference standards (purified by preparative gas chromatography) with a panel of 16-22 members using procedures described previously (*7*).

Results and Discussion

Volatile constituents of ground mesquite pods were isolated by dynamic headspace sampling. Sample constituents were identified by comparison of the compound's Kovats index, I (*8*), and mass spectrum with that of a reference compound.

We investigated three mesquite pod samples. The first sample was *Prosopis alba*, pod mesocarp flour produced in Chaco, Argentina in 2003. The second and third flours were obtained commercially from Cocina deVega, a Classic Sonoran and Sweet Peruvian. The flours were raw though they were dried at between 38 and 54 °C. We identified a total of 121 compounds, 14 of which were tentatively identified. To our knowledge mesquite volatiles have not been previously studied with the exception of the identification of maltoxazine in *Prosopis tamarugo* pods by Schmeda-Hirschmann and Jakupovic (*9*). These researchers found that maltoxazine is a DNA binding constituent with almost the same activity as the standard inhibitor vinblastine. Maltoxazine was reported to occur at a concentration of 1 ppm and was said to contribute to the characteristic odor of *P. tamarugo* pods. This compound has been previously identified in beer and malt (*10*). We could not detect this constituent in any of the three samples we investigated.

Sample A represents the *Prosopis alba* sample while sample B and C represent the commercial Classic Sonoran and Sweet Peruvian samples. The *Prosopis alba* sample contained a much higher proportion of pyrazines than the commercial samples. Since our quantification studies have not yet been completed we can only speculate on the contribution of volatiles at this point. Methylpyrazine has a very high odor threshold of 60 ppm and probably doesn't contribute to the odor. 2,5-Dimethylpyrazine constitutes almost 12% of the sample A. Though it has a relatively high odor threshold of 1.7 ppm it may contribute to the odor with its chocolate, roasted nuts and earthy aroma. There is also a large proportion of 2,6-dimethylpyrazine at 3.22%. This compound has an odor of chocolate and roasted nuts. Ethylpyrazine has an odor threshold of 6.0 ppm and is present at relatively low levels in the samples. 2,3,5-Trimethylpyrazine has a nutty, roasted peanut, cocoa aroma. It constitutes about 1% of sample A and may contribute to the aroma due to its odor threshold of 91 ppb. 2,5-Dimethyl-3-ethylpyrazine has cocoa, chocolate, burnt almond and filbert-hazelnut aroma notes. This constituent is likely an important contributor to the aroma since it is a major constituent at 4.83% and has a low odor threshold of 0.4 ppb. Odor threshold values were not available for 2-methyl-6-vinylpyrazine and 2-propyl-3,6-dimethylpyrazine. γ-Hexalactone has a rather high odor threshold of 1600 ppb and probably doesn't contribute to the aroma. Though there are moderate levels of γ-pentalactone and γ-butyrolactone we suspect that these lactones have high odor thresholds like γ-hexalactone and don't contribute much to the aroma. γ-Octalactone and γ-nonalactone have lower odor thresholds (7 and 30 ppb, respectively) and might be expected to contribute

to the coconut aroma of mesquite flour. γ-Octalactone has a sweet, creamy, dairy, coconut aroma while γ-nonalactone has strong, sweet, soft coconut aroma. γ-Nonalactone is used in the formulation of synthetic coconut flavors (*11*). δ-Octalactone has an odor threshold of 0.5 ppm and has sweet, fatty, coconut, tropical, dairy notes. Massoia lactone has a coconut, creamy, fatty, sweet aroma (*11*). It has a similar structure to δ-decalactone except that it has a double bond in the 2 position of the ring. δ-Decalactone has an odor threshold of 100 ppb so we might expect that massoia lactone would have an odor threshold of a similar magnitude.

The Classic Sonoran sample had an abundance of aldehydes. Hexanal was the major volatile constituting almost 22% of the volatiles. Given its low odor threshold of 5 ppb it probably contributes to the aroma of sample B. While the lipid composition of the mesocarp flour and the whole pod are rather similar in both being about 2-2.5 %, evidently the type of fat in the seeds is more predisposed to oxidation to produce hexanal than is the fat in the mesocarp. The sample had 1.24% (*E*)-2-hexenal which probably contributed to the aroma with its low odor threshold of 17 ppb. Given the high sugar level in the samples it seems reasonable to have moderate levels of furfural ranging from 1.73% to 0.17%. The *Prosopis alba* sample also had moderate levels of 5-methylfurfural of almost 1%. Samples A and B had moderate levels of benzaldehyde, 1.7% and 4.13%, respectively. (*E,E*)-2,4-Decadienal probably contributes to the odor of sample B due to its very low odor threshold of 0.07 ppb.

There were relatively low levels of these four ketones, 2-pentanone, 2,3-butanedione, 2-methyl-3-pentanone, and 4-methyl-2-pentanone and their contribution is probably low. 3-Penten-2-one is a likely contributor to mesquite flavor with its low odor threshold of 1.5 ppb, particularly in sample B where it constituted 0.9%. There was a moderate level of 3-hydroxy-2-butanone in sample B but its odor threshold is relatively high at 800 ppb. Other possible contributors are 6-methyl-5-hepten-2-one and 3,5-octadien-2-one which have odor thresholds of 50 and 150 ppb, respectively. The highest levels were found in sample B with 0.96% and 0.89%. There were moderate levels of 6-methyl-3,5-heptadien-2-one but its odor threshold was 380 ppb.

The *Prosopis alba* sample had 0.39% and 0.97% of 2-acetylfuran and 5-methylfurfural, respectively. However, 2-acetylfuran and 5-methylfurfural have high odor thresholds, 10 ppm and 1.3 ppm and probably don't contribute to the odor. Acetophenone has a sweet, hawthorn, floral, almond aroma and could contribute to the odor with its odor threshold of 65 ppb. Furfuryl alcohol has an odor threshold of 2400 ppb. We don't know the odor threshold of 5-methyl-2-furfuryl alcohol but it is probably quite high too so these compounds probably have little if any contribution. Methyl salicylate has a minty, sweet, wintergreen aroma and an odor threshold of 40 ppb. A likely contributor is 2-methoxyphenol or guaiacol. This compound has a relatively low odor threshold of 3 ppb and has a sweet, smoky odor. Sample B contained 0.88%. Compounds such as benzyl alcohol, 2-phenylethanol, 2-acetylpyrrole, and methyl cinnamate have high odor

Table I. Volatile Constituents of Mesquite Pods

Constituent	$I^{DB\text{-}WAX}$ exp.	ref.	% area[a] A	B	C
2-pentanone	979	973	-[b]	-	0.04
2,3-butanedione	981	966	-	-	0.05
pentanal	983	974	-	0.53	-
methyl butanoate	987	980	-	-	0.01
2-methyl-3-pentanone	998	998	-	0.04	-
4-methyl-2-pentanone	1005	1005	-	-	0.01
methyl 2-methylbutanoate	1011	1006	-	-	0.02
2-methylpropyl acetate	1016	1008	-	-	0.03
methyl 3-methylbutanoate	1021	1014	-	-	0.11
chloroform	1024	1012	-	-	0.01
toluene	1041	1034	-	0.01	0.01
2-methyl-3-buten-2-ol	1055	1032	-	0.17	-
2,3-pentanedione	1064	1050	-	-	0.06
dimethyl disulfide	1074	1078	0.06	0.02	0.14
hexanal	1081	1077	0.43	21.75	0.25
methyl pentanoate	1087	1080	-	-	0.01
(2-methyl-2-butenal)[c]	1088		-	-	0.03
2-methylpropanol	1098	1084			1.90
3-penten-2-one	1123	1121 (lit.)	0.28	0.90	0.15
methyl 4-methylpentanoate	1147	1137			0.01
butanol	1153	1138	0.06	0.30	0.08
1-penten-3-ol	1171	1152	0.10	2.75	0.11
2-heptanone	1181	1178	-	-	0.10
pyridine	1181	1181	1.03	0.05	-
heptanal	1184	1180	-	0.09	0.02
(1,3-dimethylbenzene)	1186		-	0.32	-
methyl hexanoate	1188	1181	-	-	0.09
limonene	1196	1197	-	-	0.02
2-methyl-1-butanol	1214	1203	-	comb.	0.34
3-methyl-1-butanol	1215	1205	0.71	4.79	0.55
(*E*)-2-hexenal	1218	1214	-	1.24	-
2-pentylfuran	1236	1224	-	0.04	-
thiazole	1253	1262	-	0.04	-
3-methyl-3-buten-1-ol	1255	1244	-	-	0.84
pentanol	1259	1246	0.35	2.91	0.13
(2-methyltetrahydrofuran-3-one)	1261		0.26	-	0.78
methylpyrazine	1265	1262	0.93	0.16	0.05
3-hydroxy-2-butanone	1281	1278	0.22	0.76	0.24
3-hepten-2-one	1299	1297	-	0.17	-

Continued on next page.

Table I. *Continued.*

Constituent	I^{DB-WAX} exp.	ref.	% area[a] A	B	C
4-penten-1-ol	1306	1299	0.19	-	0.31
((*Z*)-2-penten-1-ol)	1331		-	1.61	-
2,5-dimethylpyrazine	1321	1320	11.92	-	0.11
2,6-dimethylpyrazine	1327	1326	3.22	0.04	-
ethylpyrazine	1332	1331	0.08	0.04	-
6-methyl-5-hepten-2-one	1337	1333	0.39	0.96	0.08
2,3-dimethylpyrazine	1345	1344	0.09	0.06	-
(methylcyclohexylketone)	1360		-	0.11	-
hexanol	1361	1350	0.60	5.06	-
(2-methyl-2-cyclopenten-1-one)	1374		-	0.06	-
dimethyl trisulfide	1375	1374	0.01	0.02	0.18
2-ethyl-6-methylpyrazine	1384	1383	0.20	-	0.03
(2-ethyl-3-methylpyrazine)	1388	-	-	0.02	-
2-ethyl-5-methylpyrazine	1389	1390	0.02	0.04	0.20
(*Z*)-3-hexen-1-ol	1392	1381	-	0.34	-
2,3,5-trimethylpyrazine	1402	1404	1.04	-	0.05
(*E,E*)-2,4-hexadienal	1402	1388	-	0.02	-
(*E*)-2-hexen-1-ol	1413	1404	-	0.09	-
(3-ethyl-2-methyl-1,3-hexadiene)	1416		-	0.04	-
5-hexen-1-ol	1418	1408	0.17	0.32	0.13
linalool oxide A (*trans*-THF)	1443	1442	0.05	0.40	-
2,5-dimethyl-3-ethylpyrazine	1447	1446	4.83	-	-
acetic acid	1452	1475	0.88	-	-
1-octen-3-ol	1458	1448	0.29	0.61	0.10
furfural	1461	1456	1.73	0.79	0.17
6-methyl-5-hepten-2-ol	1467	1462	0.15	0.11	-
linalool oxide B (*cis*-THF)	1477	1470	0.12	0.18	-
methyl 3-hydroxybutanoate	1483	1477	-	-	0.03
2-methyl-6-vinylpyrazine	1486	1485	0.11	-	-
(*E,E*)-2,4-heptadienal	1494	1489	-	0.07	-
2-acetylfuran	1499	1500	0.39	0.44	0.09
2-propyl-3,6-dimethylpyrazine	1509	1514	0.09	-	-
camphor	1510	1513	-	-	0.02
benzaldehyde	1514	1516	1.72	4.13	0.58
undecanal	1537	1601	-	0.20	-
propanoic acid	1540	1535	0.45	-	-
2,3-butanediol (threo)	1547	1539	0.59	0.42	0.43
linalool	1556	1546	-	0.02	0.04
5-methyl-2-furfural	1569	1567	0.97	-	-
2-methylpropanoic acid	1571	1568	-	-	1.00
3,5-octadien-2-one	1575	1566	-	0.89	-

Table I. *Continued.*

Constituent	I^{DB-WAX} exp.	Ref.	% area[a] A	B	C
2,3-butanediol (meso)	1581	1576	0.23	0.29	0.05
6-methyl-3,5-heptadien-2-one	1590	1589	0.59	0.85	0.11
(2,6,6-trimethyl-2-hydroxycyclohexanone)	1600		-	0.38	-
γ-pentalactone	1605	1605	0.81	0.81	0.20
methyl benzoate	1614	1616	0.13	-	0.03
γ-butyrolactone	1618	1623	1.06	0.54	0.39
(2-acetyl-5-methylfuran)	1615		-	0.06	-
butanoic acid	1630	1650	0.65	0.24	0.10
acetophenone	1644	1645	0.62	0.28	0.15
(4-methylthiazole)	1662		-	0.27	-
furfuryl alcohol	1665	1656	1.30	-	0.24
(4-methyl-4-vinylbutyrolactone)	1669		-	0.15	-
3-methylbutanoic acid	1672	1680	6.80	4.50	4.35
(3,5,5-trimethyl-2-cyclohexene-1,4-dione)	1686		0.15	0.39	0.05
γ-hexalactone	1693	1699	0.25	0.46	0.09
(5-methyl-2-furfuryl alcohol)	1714		0.06	-	-
pentanoic acid	1745	1750	0.87	0.85	0.20
(5-ethyl-2(5*H*)-furanone)(2-hexen-4-olide)	1751		0.08	0.22	0.03
methyl phenylacetate	1755	1755	0.06	-	0.04
methyl salicylate	1768	1771	0.21	0.10	0.04
δ-hexalactone	1783	1801	0.10	-	0.05
γ-heptalactone	1795	1801	0.07	0.14	0.05
(*E,E*)-2,4-decadienal	1810	1808	-	0.18	-
(unknown)	1825		4.73	0.67	3.63
hexanoic acid	1849	1825	3.92	2.37	1.36
2-methoxyphenol	1859	1855	0.24	0.88	0.27
benzyl alcohol	1878	1874	0.89	0.87	0.22
2-phenylethanol	1912	1910	0.63	-	-
γ-octalactone	1917	1920	-	0.39	-
heptanoic acid	1957	1925	0.66	-	0.28
benzothiazole	1961	1954	-	0.35	-
δ-octalactone	1962	1970	0.31	-	0.27
2-acetylpyrrole	1976	1970	0.96	0.29	1.77
(unknown)	2003		17.61	2.21	19.19
(methyl ester diethylcarbamodithoic acid)	2010		-	0.19	-
2-pentadecanone	2012	2020	0.10	-	-
(unknown) (base peak 97; 68)	2025		-	-	0.64

Continued on next page.

Table I. *Continued.*

Constituent	$I^{DB\text{-}WAX}$ exp.	Ref.	% area[a] A	B	C
γ-nonalactone	2028	2030	0.51	1.62	-
3-phenylpropanol	2033	2046	0.08	-	-
methyl cinnamate	2065	2076	0.44	-	0.28
4-methylphenol	2073	2078	0.10	-	-
3-methylphenol	2081	2085	0.06	-	-
4-phenyl-3-buten-2-one	2111	2117	0.02	-	-
γ-decalactone	2154	2147	-	-	0.05
octanoic acid	2172	2120	0.49	0.37	0.33
4-vinyl-2-methoxyphenol	2194	2180	0.10	0.03	0.13
massoia lactone	2228	2227	0.31	0.08	1.19
(2-heptadecanone)	2231		0.08	-	-

[A]*Prosopis alba*, pod mesocarp, Argentina, 2003; [B]Classic Sonoran (Cocina deVega, Inc.); [C]Sweet Pervian (Cocina deVega, Inc.). [a]Peak area percentage of total FID area excluding the solvent peaks (assuming all response factors of 1). [b]Not detected. [c]Tentative or partial identifications enclosed in parentheses.

thresholds, 10 ppm, 1.1 ppm, 170 ppm and 70 ppm, respectively, and probably don't contribute to the odor. 4-Vinylguaiacol has a low odor threshold of 3 ppb. This compound ranged from 0.03% to 1.3% in the samples and could contribute to the odor.

A variety of free fatty acids ranging from acetic acid to octanoic acid were identified. Acetic acid probably doesn't contribute to the odor with its high odor threshold of 22 ppm. Propanoic acid and 2-methylpropanoic acid also have high odor thresholds of 2.2 ppm and 3 ppm, respectively, and are unlikely odor contributors. Butanoic acid and 3-methylbutanoic acid have odor thresholds of 240 ppb and 250 ppb, respectively. The latter compound could contribute to the odor since it occurs at high levels ranging from 4.35% to 6.80%. Another possible contributor is hexanoic acid which despite its high odor threshold of 3 ppm occurs at levels ranging from 1.4% to 3.9%. The other acids such as pentanoic acid, heptanoic acid and octanoic acid probably don't contribute to the odor due to their high odor thresholds and low % areas.

Other compounds that may contribute to the odor include dimethyl disulfide, dimethyl trisulfide and 1-octen-3-ol. Dimethyl trisulfide has a very low odor threshold of 0.003 ppb or 3 ppt. 1-Octen-3-ol has an odor threshold about 433 times higher than dimethyl trisulfide but it still has a low odor threshold of 1.3 ppb and was present at 0.29% and 0.61% in samples A and B, respectively.

The unknown compound that was the major volatile in *P. alba* (17.61%) and *P. pallida* (19.19%) but only 2.21% of the *P. velutina* volatiles is apparently new to flavor chemistry. We are in the process of synthesizing this compound in order to definitively characterize its structure and this will be reported in a later communication.

In summary, we have studied the composition of three different samples of mesquite flours. The samples had surprisingly different compositions and we found the *Prospopis alba* sample had a more pleasant aroma than the two commercial samples. The supplier of the two commercial samples would not reveal their species and we suspect that these samples are mixtures of *Prosopis* species. We have received pods and flours of known species from southern California and South America. We will be analyzing these samples and completing our quantification studies.

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