

Adulteration Testing of Southwestern Desert Honeys

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(Revised Manuscript Received for Publication Dec. 30, 1990)

ABSTRACT

Honeys from *Prosopis* spp. (U. S. and Mexican mesquite), and *Acacia* spp. (catsclaw, huajillo) are shown to have stable carbon isotope ratio values ranging from *cà.* -21 to -23‰, significantly less negative than the average of nearly all other honey types. Quantitative pollen analysis has been used to identify floral sources; several published identifications based on producer estimates are found to be erroneous.

INTRODUCTION

THE DEVELOPMENT of cheap high-fructose corn syrup in the early 1970's led to its widespread use to adulterate honey. Since existing tests were not effective, a procedure was developed using the ratio of the amount of carbon of atomic weight 13 to that of ordinary carbon of weight 12 (White & Doner, 1978). This value, expressed as $\delta^{13}\text{C}$, was found to differ between corn and cane sugars ($\delta^{13}\text{C} = -9.7\text{‰}$) and honey (average = -25.4‰) enough to allow its use to detect this type of adulteration.

Many authentic U. S. and imported honeys were tested to establish guidelines for using the test. The natural variability of honey again was demonstrated when it was found that for the 119 samples tested the average for the stable carbon isotope ratio was -25.4‰ , but the standard deviation was relatively large, 0.98‰ . This wide range (-22.5 to -27.4‰) reduced the sensitivity more than had been hoped. So as not to discriminate against pure honeys with high ratios (*i.e.* less negative) not included in the research, it was necessary to set the limiting value beyond which a honey was considered to be adulterated at -21.5‰ , four standard deviations (4s) beyond the mean value. It was calculated that there is only one chance in 25,000 that a pure honey would be condemned. Since it is obvious that honeys beyond 2s (-23.4‰) and -21.5‰ could contain as much as 20% added syrup, another test, the TLC test, was developed to be applied to these samples (Kushnir, 1979).

Thanks to the so-called self-policing plan of the American Beekeeping Federation and the cooperation of Federal and State enforcement agencies, honey adulteration appeared to be reduced to a relatively low level within about five years.

During this period the citrus honey of some Florida honey producers and packers was being condemned because of values ranging between -23.4 and -22.0‰ without ap-

plying the TLC test, however. In a study of this problem, White and Robinson (1983) found no difference in isotope ratio values between citrus nectar and honey, showing that these higher values were natural; they did not result from addition of sugars either intentionally or as residues from early stimulative feeding of the colony.

A somewhat similar situation was noted later involving honey imported as "Mexican mesquite." It consistently had isotope ratio values in the range -20.9 to -22.5‰ . Thirteen samples from two years were tested; some were given TLC tests which were negative. Six of these samples were less negative than -21.5‰ , the cut-off beyond which existing rules consider a sample adulterated and no longer require the TLC test. The importer was convinced by personal knowledge of their purity. Attempts to arrange nectar tests or inspect apiaries and collect samples from combs were not fruitful.

A few years ago the ASCS honey price support program began requiring random isotope ratio testing of honey offered for loan and/or acquired by the Government. A limiting value of -23.5‰ was set above which honey was not accepted and was stated to be adulterated. Strict application of this rule caused many problems for producers. Several in Arizona, Hawaii and elsewhere had their honey declared adulterated without recourse. They drew attention to their problems and requested assistance. The floral types of honey being rejected were reported to be mesquite, catsclaw, and algaroba. These had been included in the original research where Hawaiian algaroba had a value of -22.5‰ and Arizona catsclaw, -22.8‰ ; but the value for a huajillo was -24.9‰ and for a Texas mesquite, -26.9‰ . Table 1 provides the botanical names of the plants discussed herein. The floral identification of all of these honey samples was the estimate by the beekeeper. The very limited earlier data for catsclaw (the first sample in Table 3) and mesquite (the first sample in Table 2) are not sufficient to generalize upon. After consultation with ASCS they agreed to allow accused producers to request the additional TLC test to verify purity, at their own expense. It is obvious, however, that additional information is necessary to establish a firmer data base.

A new testing method has just been developed under a grant from the American Beekeeping Federation to increase the sensitivity of the isotope ratio test and eliminate the need for the TLC test (White & Winters, 1989). In this procedure, the isotope ratio values for a honey and for the protein fraction isolated from the same sample of honey are measured and compared. For a pure honey these two values must not differ by more than 1‰ . Each sample thus pro-

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vides its own standard for comparison, eliminating the need to use an average value for all honeys with the resulting lack of sensitivity. This test makes it possible to demonstrate unequivocally the presence of 7% or more of corn or cane sugars in the honey. During the initial research to establish the method this procedure was applied to the samples of Texas mesquite and huajillo mentioned above and to several Arizona catsclaw honeys.

Pollen contents of honey have long been recognized as one of the important ways to identify the floral sources of honey (Maurizio 1975; Sawyer 1988). According to the International Commission for Bee Botany (ICBB) (Louveau *et al.* 1970) a number of techniques are used to extract pollen from honey samples. We have a variation of one of these extraction methods with modifications similar to the technique mentioned by Low *et al.* (1989). Maurizio (1951), has noted that added information about honey floral types can be derived by conducting pollen concentration tests.

EXPERIMENTAL

Honey samples: The 1975 Texas mesquite, huajillo, and Arizona catsclaw honeys were obtained from the producers

Table 1. Botanical Names of Plants Discussed

Common Name	Botanical Name
Mesquite (Texas)	<i>Prosopis glandulosa</i>
Mesquite (Mexico)	<i>Prosopis</i> spp.
Algaroba	<i>Prosopis juliflora</i>
Catsclaw	<i>Acacia greggii</i>
Huajillo	<i>Acacia berlandieri</i>
Citrus	<i>Citrus</i> spp.
Mexican persimmon	<i>Diospyros texana</i>
Common persimmon	<i>Diospyros virginiana</i>

and certified pure for the original isotope ratio work (White & Doner, 1978). The 1988 honeys (assumed to be catsclaw) from Pima Co., Arizona were provided by a Tucson beekeeper who certified them pure. The Mexican mesquite samples were from a commercial source submitted over the indicated years for adulteration testing. The 1956 California and 1957 Arizona mesquite honeys were collected in those years and used for an analytical study of U. S. honeys (White *et al.*, 1962), and preserved since. The Hawaii algaroba sample is no longer available.

ANALYTICAL METHODS

Stable carbon isotope ratio was determined as directed by the AOAC (1984).

Internal standard isotope ratio analysis was carried out as described by White and Winters (1989).

Pollen Analysis: Our extraction procedure was as follows: 1) each honey sample was heated in a microwave oven until it reached a temperature of 38°C (100°F); 2) the sample was thoroughly stirred before removing 10 g. to a glass beaker; 3) a known quantity (11,000 spores) of an exotic cryptogamic spore (*Lycopodium* sp.) was added and thoroughly mixed with each 10 g. honey sample; 4) 100 ml. of warmed distilled water was mixed with each sample; 5) each diluted honey sample was then centrifuged and the liquid fraction was discarded; 6) the remaining residue of each sample was acetolyzed to remove organic detritus pollen cytoplasm and lipids (Lieux 1980); and 7) the processed residue from each sample was then mixed with glycerin, mounted on glass microscope slides and analyzed.

Pollen counts of from 200-300 grains per sample (excluding the exotic spores that were added) were conducted, as recommended by Vergeron (1964). The added *Lycopodium* spores were counted to determine the ratio of exotic spores to pollen grains in each sample. All counting was con-

Table 2. Stable Carbon Isotope Characteristics of Mesquite Honeys

Sample Number	Production Area	Crop Year	$\delta^{13}\text{C}$		Pollen Content	
			Honey ‰	Protein ‰	<i>Prosopis</i> %	Other
1	Texas	1975	-25.8	-25.5	3.8	62% <i>Dalea</i>
2	California	1956	-23.1		80	12% <i>Brassicaceae</i>
3	Arizona	1957	-22.7		70	21% <i>Salix</i>
4	Arizona	1957	-23.0		84	8% <i>Brassicaceae</i>
5	Mexico	1987	-22.0	-21.9	74	14% Other legumes
6	Mexico	1987	-21.9	-21.8	69	17% Other legumes
7	Mexico	1987	-22.2		78	16% Other legumes
8	Mexico	1982	-22.5			
9-16	Mexico	1981	-21.5, s = 0.47 (Mean of 8 samples)			
17-21	Mexico	1987	-22.2, s = 0.17 (Mean of 5 samples)			

Table 3. Stable Carbon Isotope Characteristics of Honeys from *Acacia* species*

Sample Number	Production Area	Crop Year	$\delta^{13}\text{C}$		Pollen Content	
			Honey ‰	Protein ‰	<i>Acacia</i> %	Other
22	Arizona	1975	-22.0	-21.9		
23	Arizona	1988	-22.9	-22.9	0	89% <i>Prosopis</i>
24	Arizona	1988	-22.4	-22.0	45	44% <i>Prosopis</i>
25	Arizona	1988	-22.3	-22.8	0.8	91% <i>Prosopis</i>
26	Texas	1975	-23.6	-23.8	15	47% <i>Diospyros</i>

*Samples 22-25 identified as catsclaw and 26 as huajillo by their producers.

ducted using a mechanical stage microscope at magnifications of 400x. Occasionally a higher magnification was used to resolve the identification of specific pollen taxa. We have followed the reporting technique recommended by Louveaux *et al.* (1970) where pollen results are listed according to percentage classes rather than actual percentages because, as they have noted, actual percentages for honey samples should be used only when counts of 1200 + grains per sample are conducted.

Pollen concentrations per 10 g. of honey were calculated by computing the ratio of exotic spores to pollen encountered during the analysis of each honey sample (Maurizio 1975).

Identification of pollen types from these samples was based on comparisons with known pollen types in the Texas A&M Palynology Modern Pollen Reference Collection. Pollen produced by each plant genus is generally unique, and different from those found in other plant genera. Thus, in most cases identification of pollen to the genus level is possible. However, in some plant families (*i.e.*, Poaceae, Chenopodiaceae, etc.) all genera appear very similar and generally it is not possible to distinguish one genus from another without extensive comparative studies using the refinement of the scanning electron microscope. Therefore, we have identified some pollen only to the family level because they represent pollen grains that we could not distinguish beyond the family. In addition, in some instances, such as in the group listed as Brassicaceae, certain pollen types were included of genera we knew belonged in this specific family, but we were not able to determine the precise genus.

Because the majority of the pollen taxa in the Asteraceae family can not be identified precisely to genus using only a light microscope, except for *Artemisia* and a few other genera, the pollen in this family were combined. Also combined were various pollen taxa in the Fabaceae, other than the specific genera we could identify and therefore listed separately (*Acacia*, *Dalea*, *Prosopis*, etc.). Because the pollen of genera in the Chenopodiaceae and those in the genus *Amaranthus* appear nearly identical, they were combined into a single group. Finally, the category listed as unknown represents grains that we were unable to identify to either the family or genus level using our available reference collection. The number of unknown pollen types was less than 3% in each sample.

RESULTS AND DISCUSSION

Results of the analyses are shown in Tables 2, 3 and 4. All of the 26 samples of honey in the tables (except sample 1) would be considered contaminated with corn or cane sugars under the guidelines of the AOAC (1984) unless a TLC test were found negative. Those less negative than -21.5‰ (all were Mexican mesquite) would not normally even be given the TLC test, this being the presumptive limit under the information then available. The availability of the new internal standard procedure provided an opportunity to characterize these desert honeys.

The considerably different $\delta^{13}\text{C}$ value found for the Texas mesquite as compared with those from Mexican mesquite required an explanation. Pollen analyses were done on the Texas sample, three other U. S. mesquite samples, and three 1987 Mexican mesquite honeys to determine if significant amounts of nectar from other desert plants might be present. Such plants (cacti and succulents) are known (Bender, 1971) to use the Crassulacean acid photosynthetic pathway (CAM) rather than the Calvin cycle common to honey sources. The $\delta^{13}\text{C}$ values for CAM plants range between about -12 and -20‰ . Ziegler *et al.* (1979) have suggested that inclusion of nectar from such plants

could complicate isotope ratio analysis of honey for adulteration.

The pollen analysis for the 1974 Texas mesquite (Table 2) implies that it is not a mesquite honey, but is largely from *Dalea* species which provide honey of splendid quality (Pellet 1976). This resolves the apparent discrepancy among Texas, Arizona, and Mexican mesquite honeys. The agreement between $\delta^{13}\text{C}$ values for honey and protein in the three samples so tested in Table 2 show that they are pure honey. All of the other samples in the Table tested for pollen content show mesquite pollen predominating. Therefore, one can conclude that mesquite honeys normally have isotope ratio values in the range -21.5 to -23.0‰ .

In Table 3 are shown data on Arizona "catsclaw" honey. These values are in the same range as mesquite. The 1988 samples were provided by an Arizona beekeeper to represent honey that had been declared "adulterated" by ASCS. The small differences between $\delta^{13}\text{C}$ of honey and protein show that these honeys are pure.¹ It was our impression that these samples were catsclaw, and they were so labelled in an earlier paper (White & Winters, 1989). However, the pollen analysis shows that only one of them contains any appreciable amount of *Acacia* pollen; two of the three must be considered mesquite. This does not of course affect their status as pure honey. Sample 26, said by the producer to be a Texas huajillo, can be considered a unifloral persimmon; this may account for the rather more negative isotope ratio value.

Table 4 provides complete data on the pollen analyses of all samples. Little published data are available on the expected ratios of mesquite or catsclaw pollen in honey samples (Crane *et al.* 1984). What data do exist in Crane's report state only that one should expect a "high" ratio of certain kinds of mesquite pollen in honey produced from those floral sources. However, there is no clarification as to what is meant by "high." In the ICCB monograph on melissopalynology, (Louveaux *et al.* 1970) there is no mention of the levels of mesquite or catsclaw pollen that should be present in honey classified as these unifloral types. However, they state that for a honey sample to be classified as having a "predominant" type, the pollen of one plant taxa must represent more than 45% of the total pollen present. Earlier, Maurizio (1951) noted that for a honey to be classified as a unifloral type, its pollen should consist of at least 50% from one plant taxa. The ICBB (Louveaux *et al.* 1970) refined this classification by saying that for some unifloral honey types pollen percentages of a single taxa as low as 10-30% may reflect sufficient evidence that a single taxa was the dominant nectar sources. Thus an under-represented nectar source (*Salvia*, *Robinia*, *Tilia*, *Citrus*, and *Medicago*, etc.) can often confirm a honey sample as unifloral with lower pollen concentration levels than most other sources.

By using Maurizio's (1951) suggestions as a guide, we can classify all but three of the 11 samples listed in Table 4 as having mesquite pollen as the predominant taxa and also as being mesquite unifloral types. The actual percentages of mesquite pollen in each of the eight samples classified as mesquite unifloral honey (Samples 2, 3, 4, 5, 6, 7, 23, and 25) ranged from a low of 69% to a high of 91%.

Sample 1 was listed as "Texas mesquite honey" by the producer who certified it to be a pure honey. However, our pollen analysis shows that it was not a unifloral mesquite honey, but rather a unifloral *Dalea* honey with the percentage of *Dalea* pollen reaching over 60%. According to Correll and Johnson (1979) there are 23 known species of

¹A difference of 1‰ , the 4s limit, is equivalent to 7% added corn/cane sugars.

Dalea in Texas, many of which grow in the same geographical regions with mesquite. As mentioned earlier, Pellett (1976) notes that it is a good source of nectar. Sample 26, thought to be a Texas huajillo honey, had a persimmon pollen as the predominant type. The pollen in this sample is similar to that produced by both the common persimmon and the Mexican persimmon. Of the two, only the Mexican persimmon is known to grow in the natural vegetation of Atascosa County, Texas, the region where the honey was produced (Correll and Johnson 1979).

However, the common persimmon has been reported from area less than 50 miles northwest of the region and it is possible that it could have been cultivated near the hives that produced sample 26. In two of these three samples (Sample 24 and 26) pollen from *Acacia* species was one of the dominant secondary types, but neither could be classified as an *Acacia* unifloral type.

As noted above, pollen concentration levels sometimes provide additional information helpful in determining the certainty of unifloral types. However, no published information is available on the expected pollen concentration levels in either mesquite or catsclaw honey. As noted in Table 4, pollen concentration levels of the eight mesquite unifloral samples range from a low of 8,780 to a high of 80,190 per 10 g. of honey. The pollen concentration values

of the other three non-mesquite honey samples had pollen concentration levels ranging between 9,850-90,720 per 10 g. of honey.

It is tempting to state that our study has provided a data base for established pollen concentration ranges for mesquite unifloral honey. However, until pollen concentration tests of a larger number of samples are available, we will not know whether or not our concentration values are characteristic for unifloral mesquite honeys.

CONCLUSION

We conclude that the high $\delta^{13}\text{C}$ value of the honey from mesquite is typical of the species. Very limited data imply that this may also be typical of honey from the two *Acacia* species examined. A previous record for Texas mesquite has been shown by pollen analysis to be in error, as have previously published floral source designations for two so-called catsclaw honeys and a Texas huajillo honey.

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Table 4. Pollen Content of Honeys from *Acacia* and *Prosopis* species

Pollen type	Sample number											
	1	2	3	4	5	6	7	23	24	25	26	
Concentration ¹	10630	8780	38420	62430	34050	80190	56500	23230	9850	36460	90720	
<i>Acacia</i>						M			P	M	S	
<i>Agave</i>									M			
<i>Artemisia</i> ²	M	M	M							M		
ASTERACEAE ³	I	M	M	M	I	M	M ⁴	M	I		I	
BRASSICACEAE	I	I	M	I	I	I	M		M			
CACTACEAE				M ⁶								
<i>Caesalpinia</i>							M					
<i>Celtis</i> ⁵										M		
CENOPODIACEAE ² + <i>Amaranthus</i> ²			M	M				M	M	M	M	
<i>Dalea</i>	P											
<i>Diospyros</i>											P	
<i>Eriogonum</i>								M				
EUPHORBIACEAE				M	M						M	
FABACEAE ⁶	I	I	M	M	I	S	S	M	M		M	
<i>Juglans</i> ²				M				M				
LAMIACEAE	M					M	M	M		M	M	
<i>Leucaena</i>										M		
LILIACEAE											M	
<i>Melilotus</i>	I									M		
<i>Mimosa</i>											I	
NYCTAGINACEAE								M				
<i>Pinus</i> ²										M		
<i>Plantago</i> ⁷				M								
POACEAE ²			M					M	M	M		
<i>Prosopis</i>	I	P	P	P	P	P	P	P	S	P	I	
<i>Quercus</i> ²	M			M	M			I	M	M	M	
<i>Rhus</i>	M											
ROSACEAE			M					M			M	
<i>Salix</i> ⁷	M		S	M	I	M					M	
<i>Ulmus</i> ²	M											
VERBENACEAE			M									
<i>Yucca</i>	I		M									
Unknown	M	M	M	M	M	M	M	M	M	M	M	

¹Grains per 10 grams.

²Wind-pollinated types.

³All except *Artemisia*.

⁴*Ambrosia* type.

⁵Resembles *Mammillaria*.

⁶Other than Fabaceae genera listed.

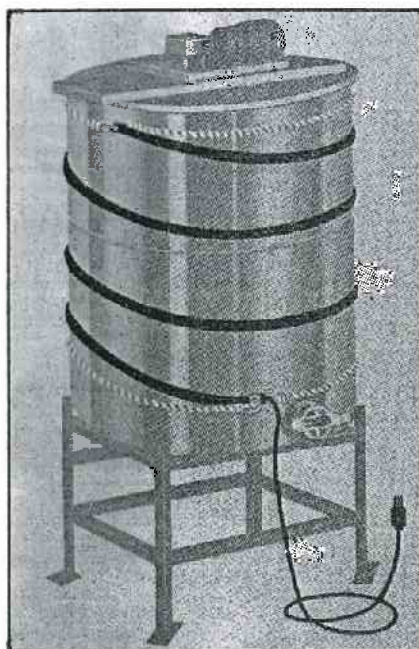
⁷Wind- and insect-pollinated.

Pollen frequency classes (Loveaux *et al.* (1970)

P	> 45%	Predominant type
S	16-45%	Secondary type
I	3-15%	Important minor type
M	< 3%	Minor type

ably excellent for AHB; another 40% is good enough to maintain either permanent or temporary populations of feral colonies. At most only 10% of Mexico is unsuitable for these honey bees.

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