SUGARS AND SUGAR PRODUCTS

$^{13}C/^{12}C$ Ratios of Citrus Honeys and Nectars and Their Regulatory Implications

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Application of isotope ratio testing for adulteration to predominantly citrus honeys without using the recommended confirmatory testing has caused regulatory difficulties for several Florida honey packers. It is demonstrated that $\delta^{13}C$ of citrus honey is significantly less negative than that of other honey types. Analysis of citrus nectar shows that the less negative values are not due to the inadvertent mixing of cane or corn syrups used for spring feeding of the bee colonies previous to the citrus nectar flow. Means for identifying citrus honey and specific limiting values of $\delta^{13}C$ for adulteration testing of this honey type are proposed.

Use of the stable carbon isotope ratio to demonstrate the addition to honey of cane or corn syrups is now well established (1). The average $\delta^{13}C$ value for 119 honey samples (84 United States, 35 imported) was −25.4‰ (s = 0.98‰) and for corn syrup, −9.7‰ ($\delta^{13}C = [(^{13}C/^{12}C\ \text{sample}/^{13}C/^{12}C\ \text{std}) - 1] \times 10^3$). Values for mixtures of honey and corn syrup are the weighted sum of the value for each component. Individual values have been published (2). Although the composition of a mixture can be established within a few percent when the $\delta^{13}C$ value of the honey component is known (ref. 1, Table 5), the variability encountered among the authentic samples precludes accurate calculation of the composition of unknown mixtures. In fact, samples with values between −23.5‰ (2s) and −21.5‰ (4s) should be confirmed as adulterated by an independent test (3). Nonetheless, at least one laboratory has regularly declared samples to be adulterated when such values were obtained, without additional testing. During the 1980 season, this practice caused some marketing difficulty with the Florida citrus honey crop. In one instance a citrus sample with $\delta^{13}C$ = −23.8‰ was said to be 10% adulterated, resulting in considerable confusion and economic loss.

Many $\delta^{13}C$ values for citrus honey were encountered that were within the range requiring additional testing; in all cases, the result was negative. It thus seemed advisable to establish the isotope ratio ranges to be expected with authentic citrus honey, with the hypothesis that this floral type differs from other U.S. honeys in this regard. Accordingly, samples of "orange" (citrus) honey from bulk shipments to a Florida packer from long-established producers were analyzed for stable carbon isotope ratio. Results confirmed the less negative nature of citrus honey $\delta^{13}C$ compared with averages for all honey determined in an earlier study. However, the origin of this difference was not resolved because stimulative feeding was a common practice for most producers.

Because the citrus nectar flow comes so early in the year, it is frequently necessary to feed honeybee colonies with honey or sugar syrup to ensure that they are strong enough to take full advantage of the usually heavy nectar flow. This stimulative feeding is a necessary and normal management practice, and beekeepers are careful to avoid mixing stores from such feeding with surplus honey removed for sale. The recent high cost of sugar has encouraged substitution by high-fructose corn syrup for bee feeding. Hence, it is conceivable that the less negative $\delta^{13}C$ values usually found for citrus honey actually represent a consistent, inadvertent admixture of corn or cane syrup used for the bee feeding before the flow.

To resolve this question, isotope ratio measurements were made on a series of citrus nectars collected directly from the blossoms, thus eliminating any possible contamination by other sugars. We found that $\delta^{13}C$ values of citrus nectars are identical with those found earlier for citrus honey, showing that no appreciable admixture with feeding sugars took place under the normal beekeeping practice, and that the dif-

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Table 1. $\delta^{13}C$ values (‰) of honeys and nectars

Sample	No. samples	Mean	Range High	Range Low	SD	CV, %
Citrus honey [a]	15	−23.8	−22.1	−25.5	0.96	4.03
Citrus nectar	17	−23.3	−22.0	−24.6	0.74	3.17
U.S. honey [b]	84	−25.2	−22.5	−27.4	0.94	3.73

[a] Negative TLC tests were obtained on each of the 5 samples tested (see text).
[b] From ref. 1.

fering $\delta^{13}C$ values of citrus honey are indeed characteristic of that type of honey.

Experimental

Samples

Citrus honey.—Samples were taken from stored bulk Florida citrus honey (1980 crop) as supplied to a Florida packer by 15 producers who regularly sell honey to this packer. Stable carbon isotope ratio was determined for each sample and in addition, the 5 samples with $\delta^{13}C$ values less negative than −23.1‰ were tested for high fructose corn syrup by the charcoal column–TLC method (4).

Citrus nectar.—All nectar samples were collected from mature but unopened citrus buds. By selecting buds whose petals were just beginning to separate, a slight pressure on the tip of the bud would cause the petals to open fully providing easy access to the accumulated nectar, which was collected in 10 or 20 μL capillary pipets. After being filled, the ends of the pipets were flame-sealed, and the sealed tubes were placed in boiling water to sterilize the nectar sample to prevent fermentation.

Determination of $\delta^{13}C$

Stable carbon isotope ratio was determined generally according to method **31.150** (4) by Coastal Science Laboratories, Inc., Port Aransas, TX. Combustion was carried out according to Sofer (5) in a tube in which the sample had been dried. Copper oxide was added, the air was evacuated, the tube was sealed and heated in a furnace, and the CO_2 was purified as specified in **31.150** (4).

Results and Discussion

The results of the analyses of the 1980 citrus honeys and the 1981 citrus nectars are summarized in Table 1, which also shows data for 84 U.S. honeys studied earlier (1). Table 2 shows the individual results for the nectar samples.

Application of the t-test showed that the mean values for the citrus honey and for the citrus nectar do not differ significantly (t = 1.66, 30 DF; $t_{0.10}$, 30 DF = 1.697). The generally less negative $\delta^{13}C$ values for citrus honey in comparison with other floral types of honey therefore do not arise from inadvertent contamination with sugar used before the nectar flow for spring build-up of the bee colony, but are characteristic of the plant.

The mean values for citrus honey and all U.S. honey are significantly different (t = 5.11, 97 DF; $t_{0.01}$, 100 DF = 2.64). This implies that use of isotope ratio values for judgment of adulteration of honey known to be of citrus origin should properly be based on mean and variability data

Table 2. $\delta^{13}C$ values of Florida citrus nectars

Variety	Location	Collection date	$\delta^{13}C$, ‰
Orange			
Parson Brown	Clermont	3/19/81	−23.2[a], −22.3, −23.2
Valencia	Clermont	3/19/81	−23.6, −23.2, −22.7
	Lake Placid	3/08/81	−22.4, −23.0
	St. Petersburg	3/23/81	−23.4
Temple	Clermont	3/17/81	−23.3
	Clermont	3/19/81	−24.4
Tangelo	Clermont	3/19/81	−23.3, −22.0, −24.2
	Clermont	3/17/81	−23.6
	Clermont	3/25/81	−24.6
Grapefruit	Clermont	3/30/81	−24.3

[a] Each value represents a single determination on a different sample.

for citrus honey, not all honey in general. The 4s limit proposed earlier (1) for declaring honey to be unquestionably adulterated (−21.5‰) should logically be replaced for citrus honey by −20.0‰. This value was obtained by subtracting 4s (4 times the standard deviation of 0.96‰) from the mean value for citrus honey of −23.8‰ (Table 1). Confirmation by the TLC test should be required for a citrus sample if the value is between −21.9‰ (2s) and −20.0‰ (4s). The use of 4s (assuming a normal distribution for $\delta^{13}C$) means that less than 1 time out of 10 000 would a pure sample falsely be declared adulterated. Such conservatism could lead to frequent acceptance of adulterated samples as unadulterated unless accompanied by the recommended TLC and/or methyl anthranilate testing described below.

Application of these criteria requires that a honey be identifiable as principally citrus in origin. This is not difficult because the flavor and aroma of such honey is quite distinctive and is easily recognized. Furthermore, citrus honeys are unique among U.S. honeys in containing methyl anthranilate (6–8). (Lavender honey, not produced in the United States, it also reported to contain this substance (9)), Knapp (8) has stated that only 1 in 1000 citrus honeys would be expected to contain less than 1.5 ppm. The 80 citrus honeys studied (7, 8) averaged 3.8 ppm (range 0.84–4.9). White (7) reported an apparent methyl anthranilate content averaging 0.07 ppm for 12 non-citrus honeys.

It may reasonably be concluded that Florida citrus honey has significantly less negative $\delta^{13}C$ values than does other U.S. honey, and requires different standards for the determination of adulteration by this measurement. These values are not less negative than those of other honey because of possible contamination with feeding sugar, but are characteristic of the plant species.

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