Assessing Citrus Honey Quality: Pollen and Methyl Anthranilate Content

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Methyl anthranilate (MA) contents of 159 samples of Florida honey from 10 crop years are summarized (mean, 2.79; range, 0−5.04 ppm). A highly significant relationship between MA and citrus pollen content is shown for 85 samples from 2 crop years. Sixty-three samples of "monofloral" Florida citrus honeys from two crops averaged 64% citrus pollen and 3.1 ppm of MA.

Keywords: *Citrus honey; methyl anthranilate; pollen analysis*

INTRODUCTION

Citrus honey, predominantly from orange and grapefruit, is handled commercially as orange honey. Because of its distinctive and pleasant flavor and aroma, it usually sells at a premium. There are no generally accepted objective criteria for quality of U.S. citrus honey other than color, which is of little real value.

Pollen analysis is frequently used for identification of honey source and quality. This type of analysis has been considered of little value for citrus honey because citrus is one of several types of honey whose pollen is considered "under-represented" by the International Commission for Bee Botany (ICBB) (Loveaux *et al*., 1970). Their guidelines for floral origin state that honeys with only 10−20% citrus pollen may be considered as largely of that origin ("monofloral") compared with the 45% required for most other types of origin. Efforts have been made to develop means other than flavor, aroma, and color for evaluating citrus honey. Nelson (1930) found methyl anthranilate (MA), a known constituent of the oil of orange blossoms, in citrus honey. Lothrop (1932) confirmed the presence of MA in three orange honeys and the absence of MA from 14 other floral types. White (1966) analyzed 33 citrus and non-citrus honeys from 5 crop years and several states. An average MA content of 2.87 ppm [standard deviation (SD) = 0.94] for the 21 citrus honeys and 0.07 ppm for 12 non-citrus honeys was reported. A mean loss of ∼10% per year was found when samples were stored at room temperature; the cause of this loss was not studied. Such a change is not significant because commercial storage of citrus honey to allow sucrose reduction requires only ∼6 weeks and storage in a warehouse is not >6 months. Knapp (1967, 1994) reported analyses of MA in 58 Florida citrus and 14 non-citrus honeys from 4 crop years. The citrus honeys averaged 3.29 ppm of MA (range, 1.6−4.9, SD = 0.57), and of the other honeys, only two (from Florida) contained 0.3 and 0.9 ppm of MA and these two may have had a small amount of citrus honey present.

Serra Bonvehí *et al*. (1987) examined the pollen spectra, physical properties, and chemical composition of 83 Spanish honeys, including 22 from citrus, but did not measure MA. Serra Bonvehí (1988) also examined pollens in 40 honeys from eastern Spain, where parthenocarpic varieties predominate. All varieties were classified as monofloral based on specified "physical and chemical characteristics". Twelve honeys had a citrus pollen content of only 4−8%. The MA values in these varieties (mean, 2.0; SD = 1.1; range, 0.57−4.2 ppm) led the author to consider these low pollen honeys as monofloral and to conclude that a minimum of 0.50 ppm of MA qualified a honey was monofloral citrus, in spite of the data showing only one of the 12 values as <1 ppm. No data for other than these 12 samples were provided. These data allow a comparison of the two analytical methods used in this work: the chemical method of White (1966) and gas−liquid chromatography (GLC). They are in agreement: the regression equation is GLC = −0.0052 + 1.14 chemical (r = 1.00).

Serra Bonvehí and Ventura Coll (1995) examined the effect of the 3-month maturation period required to reduce sucrose to the 5% limit on the quality of Spanish citrus honey. During this period, MA declined from ∼2.2 to 1.9 ppm (four samples). The MA content (fresh) averaged 2.41 ppm (SD = 0.47; range, 1.78−3.60 ppm). The authors concluded that a sucrose content of <10% and an MA content of >1.5 ppm are characteristic of marketable Spanish citrus honey.

Ferreres *et al*. (1993) proposed the use of the flavonoid hesperetin as a "marker" for citrus honey, having found it present by HPLC analysis in each of 20 citrus honey samples and absent from all of the 14 other honey types examined (no quantitation was done). Hesperetin, one of 17 flavanones identified in the citrus honey, made up 1−5% of the total flavonoid content. No individual data were provided on MA or citrus pollen content. Hesperidin was the major flavanoid detected when orange anthers were analyzed; however, because there were no significant differences in hesperetin content of honeys with (total) pollen between 95 and 75 000 grains/10 g, it was concluded that nectar is the source of the hesperetin. The presence of hesperetin appears to be a specific attribute of citrus honey, but does not seem to be useful as a quality index because no significant correlation was found between hesperetin and MA or citrus pollen content. Later, Ferreres *et al*. (1994) measured MA and hesperetin in 17 Spanish citrus honeys, but no pollen counts were made. The mean MA content was 2.35 ppm (SD = 0.54; range, 1.44−3.60 ppm), and the mean hesperitin content was 0.60 ppm (SD = 0.16; range, 0.28−0.84 ppm). There was no consistent relationship between the two parameters, as

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Table 1. Methyl Anthranilate Analyses of 18 Samples of 1983 Crop Honey

	MA content (ppm)		
year of analysis	mean	SD	range
1986	1.51	1.09	0.20−3.30
1992	0.84	0.63	0−1.95

Table 2. Methyl Anthranilate and Citrus Pollen Content of Honeys

	no. of	MA, ppm			citrus pollen, %		
year	samples	mean	SD	range	mean	SD	range
1983[a]	61	2.10	1.51	0−5.04	46.5	33.40	0−95.5
1993	24	3.15	0.86	0.58−4.80	53.9	15.1	8−77
both	85	2.40	1.44	0−5.04	48.6	29.5	0−95.5

		correl coeff	analysis of variance		
year	regression eq	(r)	F	DF	p
1983	MA = 0.26 + 0.40 × (pollen)	0.88	190	60	<0.00009
1993	MA = 0.80 + 0.043 × pollen)	0.76	29.8	23	<0.0009
both	MA = 0.39 + 0.412 × pollen)	0.85	208.7	84	<0.00009

[a] Adjusted values (see text).

confirmed by the correlation coefficient (r) of 0.19. The stability of the flavonoid was proposed as a complementary test.

MATERIALS AND METHODS

Honey. *1983 Crop.* Of 103 citrus and non-citrus samples from Michigan, Georgia, and Florida, for which pollen composition was known, 63 were selected with the following citrus pollen content: 10 containing 0−2%; 10 containing 2−10%; and 43 with >10%.

1993 Crop. Twenty samples of high-grade Florida citrus honey and four of lower quality, as subjectively judged by flavor, color, and aroma, were used.

Pollen Analysis. *Extraction Procedure.* Each honey sample was heated in a microwave oven to 38 °C and thoroughly stirred before removing 10 g to a glass beaker. After dilution with 100 mL of warmed, distilled water, the sample was centrifuged and the liquid fraction was discarded. The residue was acetolyzed to remove organic detritus, cytoplasm, and lipids (Lieux, 1980). The processed residue was mixed with glycerin, mounted on glass microscope slides, and analyzed.

Counting. Pollen counts of 200−300 grains/sample were conducted, as recommended by Vergeron (1964). All counting was conducted with a mechanical stage microscope at magnifications of 400×. Occasionally, a higher magnification was used to resolve the identification of specific pollen taxa. 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.

Determination of MA. A photometric method developed specifically for determination of MA in honey (White, 1966), in which MA is obtained from a honey solution by steam distillation with a standard microkjeldahl distilling unit, was used. After diazotization, the color is measured at 500 nm. Recovery of added MA averaged 94.5% in the 0.6−3.8-μg/g of honey range. In a preliminary study, conducted in 1986, 19 of these samples were analyzed. In 1992, 61 of the 63 (including the 19 stored frozen since 1986) were analyzed for MA by the same method. In 1993, 24 samples of new-crop Florida citrus honeys were analyzed for pollen and MA contents by the same procedures.

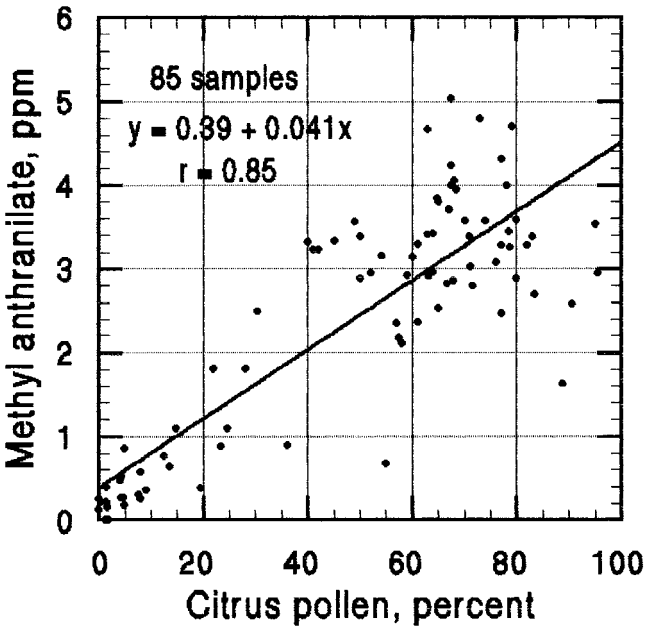

Figure 1. Relation between MA content and amount of citrus pollen in U.S. honey.

RESULTS AND DISCUSSION

Results of the analyses conducted in 1986 and 1992 of 18 of the 19 1983-crop honeys are summarized in Table 1. The same 18 honeys analyzed in a different laboratory in 1992, after extended (6-year) freezer storage (~ −12 °C), averaged 56% of their earlier MA content. By multiplying the individual 1992 results by the ratio of the means of the two sets of analyses (1.51 ÷ 0.84 = 1.80), these results may be adjusted to the time of the 1986 analyses. The regression equation for the two sets of analyses (1986 and adjusted 1993) is (1993 values) = 0.012 + 0.996 × (1986 values); r = 0.95, F (17 df) = 145, and p = <0.0001.

The MA values for all 61 of the 1983 honeys (43 not analyzed in 1986 but analyzed in 1993 after the extended storage, plus the 18 in Table 1 as analyzed in 1992), were multiplied by 1.80 to give the data in Table 2. Results of the pollen analyses of these two groups of samples are also shown. A highly significant relationship between MA content and citrus pollen content is indicated by the regressions in Table 2.

A summary of the MA contents of all of the U.S. honeys discussed here from 10 seasons from 1956 to 1993 is given in Table 3. The individual values and the regression line that indicates that a honey with ≥20% citrus pollen (considered "monofloral citrus" by ICBB standards) should have a minimum MA content of 1.2 ppm are shown in Figure 1. To indicate the distributions of MA and citrus pollen in such honey from Florida, all samples with <20% citrus pollen were removed from those honey samples described in Table

Table 3. Methyl Anthranilate Content of Florida Honeys

		MA (ppm)			
crop years	no. samples	mean	SD	range	ref
1956, 1957, 1963, 1964	14	3.15	0.94	1.42−4.37	White, 1966
1964, 1965, 1966, 1967	58	3.29	0.57	1.6−4.9	Knapp, 1968
1983, 1993	87	2.40	1.44	0−5.04	this paper

Table 4. Pollen and Methyl Anthranilate in 63 Samples of 1983 and 1993 Crop "Monofloral"[a] Florida Citrus Honey

MA, ppm			citrus pollen, %		
mean	SD	range	mean	SD	range
3.10	0.91	0.68−5.04	64	17	21.9−95.5

[a] Containing >20% citrus pollen.

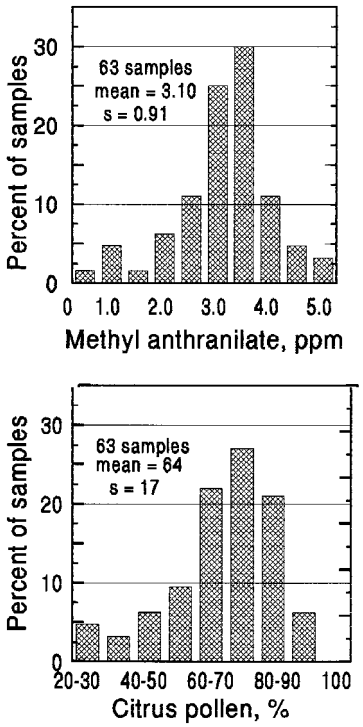

Figure 2. Distributions of MA content and citrus pollen content of monofloral Florida citrus honeys.

3, leaving 63. These 63 honey samples are summarized in Table 4 and are described in Figure 2. The minimum of 0.68 ppm of MA is comparable to a minimum of 0.50 ppm adopted for Spanish "monofloral" citrus honey (Serra Bonvehí, 1988).

CONCLUSION

Examination of the MA content of 159 samples of Florida honey from 10 crop years, together with citrus pollen contents for 85 samples, shows that for "monofloral" Florida citrus honey (citrus pollen ≥20%), the mean MA content was 3.10 ppm (SD = 0.91) and the mean citrus pollen content was 64% (SD = 17).

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