ASSESSING CITRUS HONEY QUALITY:

POLLEN AND METHYL ANTHRANILATE CONTENT

JONATHAN W. WHITE

Honeydata Corporation

217 Hillside Drive

Navasota TX 77868

Indicated Corrections Made. (1996)
J Ag Fd Ch, 44 (11) 3423-5

VAUGHN M. BRYANT JR.

Palynology Laboratory

Texas A&M University

College Station TX 77843-4352

ABSTRACT

Methyl anthranilate contents of 159 samples of Florida honey from 10 crop years are summarized: mean 2.79, range 0 - 5.04ppm. A highly significant relationship between methyl anthranilate and citrus pollen content is shown for 85 samples from 2 crop years in which both were studied. Sixty-three samples of "monofloral" Florida citrus honeys from 2 crops averaged 64% citrus pollen and 3.1 ppm methyl anthranilate.

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 acccepted 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. It has been considered of little value for citrus honey, since citrus is one of several types of honey whose pollen is considered "under-represented" by the International Commission for Bee Botany (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"), rather than the 45% required for most other types. Efforts have been made to develop other means 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 its presence in three orange honeys and absence 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 (s = 0.94) for the 21 citrus and 0.07 ppm for 12 non-citrus honeys was reported. A mean loss of ca. 10% per year was found when samples were stored at room temperature; its cause was not studied. Such change is not significant since commercial storage of citrus honey to allow sucrose reduction requires only about 6 weeks and warehousing not over 6 months. Knapp (1967, 1994) reported MA analyses of 58 Florida citrus and 14 non-citrus honeys from 4 crop years. The citrus honeys averaged 3.29 ppm, (range 1.6 - 4.9, s = 0.57); of the other honeys, only 2 (from Florida) contained 0.3 and 0.9 ppm and may have had a small content of citrus honey.

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) examined pollens in 40 honeys from eastern Spain. where parthogenic varieties predominate. All were classified as monofloral based upon specified "physical and chemical characteristics". However 12 had citrus pollen content only between 4 and

8%.Their MA values (mean 2.0, s = 1.1, range 0.57 - 4.2 ppm) led him to consider these low-pollen honeys as monofloral, and to conclude that a minimum of 0.50 ppm MA qualified a honey as monofloral citrus, in spite of the data showing only 1 of the 12 values as less than 1 ppm. No data for other than these 12 samples were provided. These data allow a comparison of the two analytical methods, which 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 *ca.* 2.2 ppm to 1.9 pm (4 samples). MA content (fresh) averaged 2.41 ppm, s = 0.47, range 1.78 - 3.60 ppm. They concluded that a sucrose content <10% and MA of >1.5 ppm is characteristic of marketable Spanish citrus honey.

Ferreres *et al.* (1993) have 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 from 1 to 5% of total flavonoid content. No individual data were provided on methyl anthranilate or citrus pollen content. Hesperidin was the major flavanoid detected when orange anthers were analyzed; however since no significant differences in hesperetin content of honeys with (total) pollen between 95 and 75000 grains /10 g. it was concluded that nectar is the source. The presence of hesperetin appears to be a specific attribute of citrus honey, but does not seem to be useful as a quality index since no significant correlation was found with MA or citrus pollen content. Later Ferreres *et al.* (1994) measured MA and hesperetin in 17 Spanish citrus honeys. No pollen counts were done. The mean MA content was 2.35 ppm, s = 0.54, range 1.44 -3.60 ppm; hesperitin mean 0.60 ppm, s = 0.16, range 0.28 - 0.84 ppm. There was no consistent relationship between the two parameters; this is confirmed

by the correlation coefficient (r = 0.19). The stability of the flavonoid was cited in proposing it 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 over 10%.

1993 crop. 20 samples of high-grade Florida citrus honey and 4 of lower quality, as subjectively judged by flavor, color and aroma.

Pollen Analysis

Extraction procedure. Each honey sample was heated in a microwave oven to 38°C , thoroughly stirred before removing 10 g. to a glass beaker. After dilution with 100 ml. of warmed distilled water it was centrifuged and the liquid fraction 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 from 200-300 grains per sample were conducted, as recommended by Vergeron (1964). All counting was conducted using a mechanical stage microscope at magnifications of 400x. 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), was used, in which MA is obtained from honey solution by steam distillation using a standard microkjeldahl distilling unit. After diazotiztaton the color is measured at 500 nm. Recovery of added MA averaged 94.5% in the 0.6 - 3.8 μg range per g honey. A preliminary study in 1986 analyzed 19 of these samples. In 1992 61 of the 63, including these 19 stored frozen since, were analyzed for MA using the same method. In 1993, the 24 samples of new-crop Florida citrus honeys were analyzed for pollen and MA contents by the same procedures.

RESULTS AND DISCUSSION

Results of the analyses in 1986 and 1992 of 18 of the 19 1983-crop honeys are summarized in Table 1. As shown there, the same 18 honeys analyzed in a different laboratory in 1992, after extended (6-year) freezer storage (*ca.* -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 + 0.996x(1986 values). $r = 0.95$, F (17DF) = 145 , $p = <0.0001$.

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 the table.

A summary of the MA contents of all of the US honeys discussed here from 10 seasons from 1956 to 1993 is given in Table 3. Figure 1 provides the individual values and the regression line which indicates that a honey with ≥20% citrus pollen (considered "monofloral citrus" by IUBB standards) should have a minimum MA content of 1.2 ppm. To indicate the distributions of MA and citrus pollen in such honey from Florida, all samples with less than 20% citrus pollen were removed from those described in Table 3, leaving 63. These are summarized in Table 4 and described in Figures 2 and 3. The minimum of 0.68 ppm MA is comparable to a minimum of 0.50 ppm adopted for Spanish "monofloral" citrus honey (Serra Bonvehí 1988).

CONCLUSION

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

ACKNOWLEDGEMENT

Permission to use pollen data obtained from the 1983 samples under a contract with the Office of Inspector General, USDA was granted in 1984, for which we are grateful. We thank Dutch Gold Honey Co., Lancaster PA, for the 1993 samples, whose pollen analysis was supported by part of a grant from the National Honey Board. We are indebted to J. L. Probst, W. Huser, and

M. Mallett, Sioux Honey Association, for the MA analyses of some of the honey samples and appreciate the cooperation of George Latimer, Texas State Chemist, and J. E. Balthrop Jr. for the 1992 and 1993 MA analyses.

LITERATURE CITED

DESHUSSES, J. and GABBAI, A.

1962 Recherche de l'anthranilate de méthyle dans les mieles espagnols de fleur d'oranger par chromatographie sur couche mince. *Mitteilungen aus dem Gebeite der Lebensmittleuntersuchung und Hygene*, 53: 408-411.

FERRERES, F., GARCIA-VIGUERA, C., TOMÁS-LORENTE, F. and TOMÁS-BARBARÁN, F. A.

1993 Hesperetin: A marker of the floral origin of citrus honey. *Journal of the Science of Food and Agriculture*. 64: 121-123.

FERRERES, F., GINER, J. M., AND TOMÁS-BARBERÁN, F. A.

1994 A comparative study of hesperetin and methyl anthranilate as markers of the floral origin of citrus honey. *Journal of the Science of Food and Agriculture*. 1994, 65: 371-372

KNAPP, F. W.

1967 Methyl anthranilate content of citrus and non-citrus honeys. Abstracts of Papers, *XXI International Beekeeping Congress. Preliminary Scientific Meeting Summary Paper 34* Beltsville MD.

KNAPP, F. W. Dept. of Food Science, Univ. of Florida (Retired). Unpublished text of 1967 presentation. Personal communication.

LIEUX, M. H

1980 Acetolysis applied to microscopical honey analysis. *Grana*, 19: 57-61.

LOVEAUX, J.; MAURIZIO, A.; VORWOHL, G.

1970 Methods of melissopalynology, by International Commission for Bee Botany of IUBS. *Bee World* 51: 125-138.

MAURIZIO, A.

1975 Microscopy of honey. Chapter 7 In: *Honey: a Comprehensive Survey.* Crane, E., ed; Wm. Heinemann Ltd., London, 1975.

SERRA BONVEHÍ, J.

1988 Determinación de antranilato de metilo en la miel de cítricos *(Citrus sp.)* del Levante Español y su influencia en la actividad diastásica de la miel. *Alimentaria* 25: 37,39-40.

SERRA BONVEHÍ, J., GÓMEZ PAJUELO, A.; GONELL GALINDO, J.

1987 Composición, propiedades físico-químicas y espectro polinico de algunas mieles monoflorales de España. *Alimentaria*: 24: 61-84.

SERRA BONVEHÍ, J., VENTURA COLL, F.

1995 Characterization of citrus honey (*Citrus* spp.) produced in Spain. *Journal of Agricultural and Food Chemistry* 43: 2053-2057.

VERGERON, P.

1964 Interprétation statistique des résultats en matière d'analyse pollinique des miels. *Annales Abeille* 1964, 7, (4), 349-364.

WHITE, J. W. JR.

1966 Methyl anthranilate content of citrus honeys. *Journal of Food Science* 1966, 31: 102-104.

Legends for figures

Figure 1. Relation between methyl anthranilate content and amount of citrus pollen in United States honey.

Figure 2. Distributions of methyl anthranilate content and citrus pollen content of "monofloral" Florida citrus honeys.

Table 1. Methyl anthranilate analyses of 18 samples of 1983-crop honey.

Year of analysis	MA content (ppm)		
	Mean	s	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.

Year	No. of samples	MA, ppm			Citrus pollen, %		
		Mean	s	Range	Mean	s	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

	Regression equations	Correlation coefficient (r)	Analysis of variance F	DF	p
1983	MA=0.26+0.40x(pollen)	0.88	190	60	<0.00009
1993	MA=0.80+0.043x(pollen)	0.76	29.8	23	<0.0009
Both	MA=0.39+0.412x(pollen)	0.85	208.7	84	<0.00009

1983 data are adjusted values (see text).

Table 3. Methyl anthranilate content of Florida honeys.

Crop years	No. samples	MA (ppm)			Reference
		Mean	s	Range	
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" (containing >20% citrus pollen) Florida citrus honey

Methyl anthranilate, ppm			Citrus pollen, %		
Mean	s	Range	Mean	s	Range
3.10	0.91	0.68-5.04	64	17	21.9-95.5