

Original article

## Physical, chemical and palynological characterization of avocado (*Persea americana* Mill.) honey in Israel

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**Summary** Various methods were used to characterize avocado honey in Israel. Perseitol, a unique sugar present only in avocado honey, served as an indicator for the degree of purity of avocado honey. Low avocado pollen counts made the common melissopalynology method ineffective at predicting the contribution of avocado nectar to honey. It was also found that the levels of fructose, sucrose and glucose do not uniquely characterize avocado honey. Potassium content and eight other minerals were linearly highly correlated to perseitol content. Moreover, pH level, absorbance and degree of darkness were significantly correlated to perseitol content, and can therefore be used to characterize avocado honey.

**Keywords** Absorption, avocado, colour, floral origin, honey, *Persea americana* Mill, perseitol, pollen analysis, potassium, sugars.

### Introduction

The floral origin of honey determines its quality, flavour, colour, etc. Various methods have been used to characterize the specific floral origin of honey. The standard procedure for assessing honey floral origin is melissopalynology, which consists of microscopical analysis of the pollen present in the honey after filtration or centrifugation (Maurizio, 1975). Because of the many limitations of this method – such as being labour intensive, and the low pollen counts in some types of honey – other techniques have been developed. Many of these are based on specific chemical compounds that characterize specific plant origin: flavonoids content was used for identification of eucalyptus honey (Martos *et al.*, 2000), heather honey (Ferrerres *et al.*, 1994a) and sunflower honey

(Sabatier *et al.*, 1992). Degraded carotenoids were used for heather honey (Tan *et al.*, 1989), hesperetin and methyl anthranilate for citrus honey (Ferrerres *et al.*, 1994b), hydrocarbon fraction for chestnut honey (Bonaga *et al.*, 1986), and natural volatiles for orange, eucalyptus, rosemary, lavender and thyme honey (D'Arcy *et al.*, 1997; Pérez *et al.*, 2002). Carbohydrates were used for alfalfa, alsike, canola, trefoil and citrus honeys (Swallow & Low, 1990; Terrab *et al.*, 2003). Floral origin was also recently determined for eucalyptus and sunflower honeys by immunoblot assays (Baroni *et al.*, 2002).

Avocado (*Persea americana* Mill.) is native to the neo-tropics. Cultivars of Guatemalan, Mexican, and West Indian origin have spread, becoming important crops in many tropical and subtropical regions around the world, such as Florida, California, Australia, South Africa, Spain and Israel (Knight, 2002). In the countries where it is grown, it is potentially a rich source for honey

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because of its high nectar production (Ish Am & Eisikowitch, 1998). The unique taste and high quality may make avocado honey desirable for consumers (La-Serna Ramos *et al.*, 1998).

The seven-carbon sugar, D-mannoheptulose, and its polyol form, perseitol, are specific to the avocado tree at all phenological stages (Liu *et al.*, 1999). Perseitol is found in avocado nectar (Ish-Am, 1994; Liu *et al.*, 1995, 2002) and has not been found in the nectar of honey plants that commonly bloom in spring time in the vicinity of avocado orchards and compete with avocado for honeybee visits, such as citrus and wild mustard flowers. Therefore, the presence of perseitol could be used as a marker for avocado honey (Dvash *et al.*, 2002; Dag *et al.*, 2003).

In a previous study, we demonstrated the possible use of NIR (near-infrared) reflectance spectroscopy to identify avocado honey (Dvash *et al.*, 2002). In the current study, we examined other characteristics of avocado honey, physical (darkness, pH and absorption) as well as chemical (minerals and sugars contents) and botanical (melissopalynology) in order to evaluate the potential future use of these properties as indicators of avocado honey.

## Materials and methods

### Samples

Honey samples were obtained from beehives during the years 2001–2003. In early April each year, at the beginning of the avocado bloom, we transferred beehives to six avocado orchards in different locations in Israel. The area of each orchard ranged from 15 to 45 ha. All the orchards were dominated by the ‘Ettinger’ cultivar, but other cultivars – such as ‘Pinkerton’, ‘Fuerte’, and ‘Reed’ – were also present. The orchards were adjacent to large citrus (mainly grapefruit) groves, and to fields of wildflowers (mainly Brassicaceae) that bloom simultaneously with the avocado.

After the beehives were placed in the orchards, a second super was added to each hive, above a queen excluder, according to standard apicultural methods. The honey supers were collected at the end of April, after the citrus and early-blooming cultivars (i.e. ‘Ettinger’, ‘Pinkerton’, and ‘Fuerte’) finished blooming. The honey was extracted from

each beehive separately, using a ten-frame manual honey extractor, and 0.5 kg of honey was sampled from each colony for analysis. All samples were kept in sealed glass jars at room temperature until analysed.

### Sugar composition

Approximately 200 mg of each honey sample was solubilized in 1.5 mL double-distilled water at 70 °C for 2 h. The sample was vortexed vigorously and filtered through a 0.2- $\mu$ m Nylon filter prior to HPLC analysis. Soluble sugars were separated using an Alltech 700CH carbohydrate column (300  $\times$  6.5 mm) at 90 °C (Schaffer *et al.*, 1991). The mobile phase consisted of double-distilled water at a flow rate of 0.5 mL min<sup>-1</sup>, and detection was performed by differential refraction (Shimadzu RID-10A). A standard solution containing 0.25% (w/v) each of sucrose, glucose, fructose, and perseitol was used to identify and quantify the individual sugar components in the honey samples. We examined 350 samples collected in the years 2001–2003.

### Pollen

We sampled eight samples from each of three locations in 2001 and in 2003, for a total of forty-eight samples from five different locations (one location was common to both years). At each location, we chose four samples with the highest perseitol concentration and four samples with the lowest perseitol concentration. Subsamples of 10 g each were examined. The samples were diluted with 20 mL of distilled water and centrifuged for 10 min at 2500 rpm. The sediment was transferred to three different slides and was examined under a light microscope at magnification of  $\times$  400. We counted 100 pollen grains on each slide for a total of 300 pollen grains per sample and calculated the percentage of avocado pollen grains (Louveaux *et al.*, 1978).

### Minerals

Mineral content analysis was conducted on the same samples used for pollen analysis. Subsamples of 0.5 g were analysed. The samples were dissolved in 5 mL nitric acid 65%. After dissolving, the

samples were placed in a microwave (Milestone, Sorisole, BG, Italy) in 500 W radiation intensity. Afterward, each sample was mixed with 20 mL distilled water. The contents of twenty-four minerals was examined with a flame-photometer (Spectro, Kleve, Germany).

### Acidity

Effective acidity level (pH) was examined in the same honey samples used for the pollen analysis (forty-eight samples). Subsamples of 2 mg honey were dissolved in 15 mL distilled water (Diez *et al.*, 2004) and pH level was measured with a pH-meter (Cyberscan 510, Eutech Instruments, Singapore).

### Absorption

We measured the absorption of each honey sample in a spectrophotometer (Uvikon 810, Kontron, Zurich, Switzerland) as an index of honey darkness. We tested the absorption of different concentrations of honey diluted with distilled water to find which yielded the greatest difference between dark and light honeys. The greatest difference in absorption was at a honey:water ratio of 1:8, with peak absorption at 260 nm. Accordingly, we collected two samples from each honey jar, diluted them with distilled water at 1:8, and read their absorption at 260 nm. We then averaged the readings of the two samples from each hive prior to statistical analysis. Fifty-three samples were collected and examined in the year 2001.

### Darkness

We assessed the colour of the honey in each jar using the orange-to-brown colour plate from the colour standards of Kornerup & Wanscher (1967). Three observers categorized the honey jars in random order by matching the most similar colour in the colour plate. After identifying the colour in the plate, the 'Munsell notation' value (position on a lightness-to-darkness scale) was recorded. Observations were made under sunlight with a white background behind each honey jar. Fifty-three samples were collected and examined in the year 2001 (the same samples used for the absorption analysis).

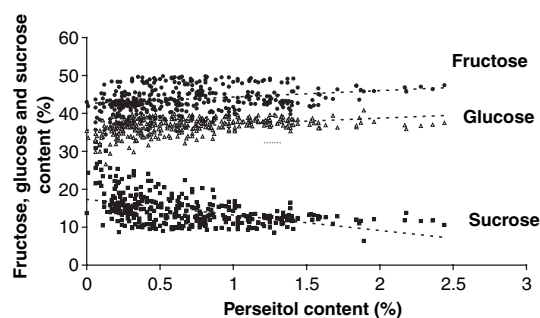
### Data analysis

Linear and logarithmic correlations between perseitol concentration and other parameters were tested by ANOVA, using JMP 5.0.1 software (SAS Institute Inc., Cary, NC, USA).

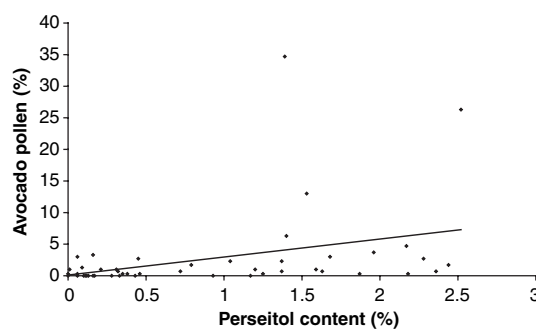
### Results

Figure 1 presents the correlation between perseitol (as an indicator for avocado honey) and the sugars – sucrose, glucose and fructose. Fructose was the most prevalent sugar and sucrose the least prevalent. There was a significant correlation between perseitol content and each of the other examined sugars ( $P < 0.0001$ ) but the  $R^2$  values were low; 0.06 for fructose, 0.14 for glucose and 0.22 for sucrose.

The percentage of avocado pollen in the different samples ranged from 0 to 35%. Only in four honey samples was the avocado pollen percentage above 5%; in these samples perseitol level was relatively high – above 1.4% (Fig. 2). The



**Figure 1** The correlation between perseitol contents and fructose, glucose and sucrose in honey samples.



**Figure 2** The correlation between perseitol contents and avocado pollen percentage in honey samples.

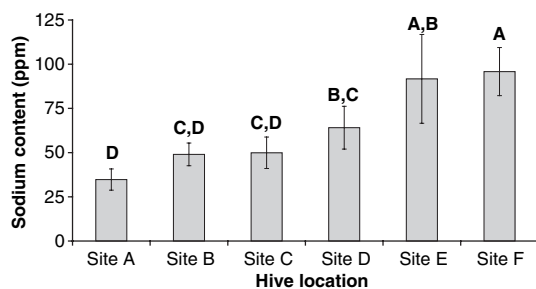
Mineral	Range and (average) content in ppm	Correlation coefficient ( $R^2$ )	Probability
Potassium	189.0–3768.3 (1153.7)	0.93	<0.0001
Phosphorous	47.0–651 (184.7)	0.91	<0.0001
Calcium	58.4–137.1 (84.5)	0.003	N.S.
Sulphur	22.4–188.3 (72.2)	0.92	<0.0001
Magnesium	18.5–204.6 (64.0)	0.88	<0.0001
Sodium	26.6–132.5 (61.6)	0.08 (negative)	0.05
Silicon	4.2–18.0 (8.7)	0.40	<0.0001
Boron	1.7–12.6 (6.3)	0.46	<0.0001
Zinc	0.8–11.5 (4.1)	0.42	<0.0001
Iron	0.9–9.3 (2.9)	0.76	<0.0001
Lead	0.15–8.22 (2.56)	0.02	N.S.
Copper	0.009–3.18 (0.66)	0.83	<0.0001

**Table 1** The concentrations of different minerals and the correlation between their content and the perseitol content in the honey samples

correlation between perseitol level and percentage of avocado pollen was significant ( $F_{1,46} = 6.62$ ,  $P = 0.013$ ) but low ( $R^2 = 0.13$ ).

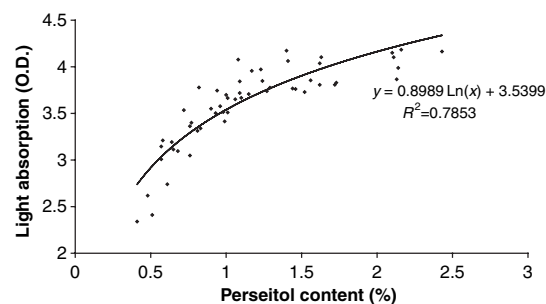
Among the twenty-four minerals examined, eleven had an average concentration above 1 ppm in the examined honey samples. The predominant mineral was potassium, with a concentration that ranged from 189 to 3768 ppm. A positive high correlation was found between perseitol and potassium (Table 1). Except for potassium, a positive correlation was found between perseitol and phosphorous, magnesium, sulphur, silicon, boron, zinc, iron and copper (Table 1). A low and negative correlation was found between perseitol and sodium and it seems that this mineral represents the geographical source of honey rather than its botanical origin (Fig. 3).

A significant positive correlation was also found between perseitol content and absorption readings (logarithmic fit,  $R^2 = 0.79$ ,  $P < 0.0001$ , Fig. 4),

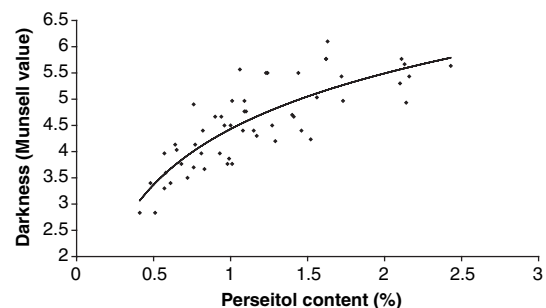


**Figure 3** The effect of geographical origin of honey samples on their sodium content (mean  $\pm$  SE).

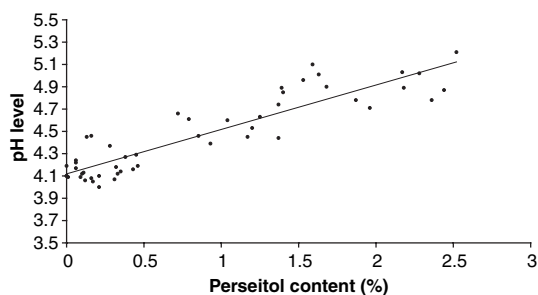
and darkness in colour, as reflected by Munsell notation values (logarithmic fit,  $R^2 = 0.68$ ,  $P < 0.0001$ , Fig. 5). A significant positive correlation was also found between the pH level and the perseitol level of the honey samples (linear fit,  $R^2 = 0.82$ ,  $P < 0.0001$ , Fig. 6).



**Figure 4** The correlation between perseitol contents and light absorption of honey samples.



**Figure 5** The correlation between perseitol contents and darkness of honey samples.



**Figure 6** The correlation between perseitol contents and pH level of honey samples.

## Discussion

Beehives located in avocado orchards during their blooming often prefer to collect nectar from alternative flora surrounding the orchard (Dag *et al.*, 2003). Hence, honey collected from such hives will contain different degrees of avocado honey. Rather than collecting honey from beekeepers and classifying it in accordance to their statement regarding its origin, in the current study, we placed hives in the avocado orchards and harvested the honey. This approach provided us with a high degree of confidence regarding the flora foraging area of the hives. Despite our effort, a wide variation was found in levels of avocado honey in our samples. This variation may be partly because of environmental factors (e.g. amount of competing flora) and to genetic differences between colonies (Dag *et al.*, 2003).

Investigators have attempted to associate botanical origin with sugar composition, since more than 95% of honey solids are carbohydrates. For example, gas chromatography analysis of the composition of sugars in honey (trimethylsilyl oxime derivatives: fructose, glucose, sucrose and maltose) has allowed reliable classification of some, but not all, Spanish unifloral honeys (Mateo & Bosch-Reig, 1997). Similarly, a study in the UK concluded that honey oligosaccharide profiles, determined by HPLC, have a potentially valuable role to play in the assessment of floral origin in honey, although it is unlikely that this procedure alone will allow unambiguous determination of all floral types (Goodall *et al.*, 1995). One of the unique characteristics of avocado nectar is that it contains the rare sugar perseitol (Ish-Am, 1994; Liu *et al.*, 1995). Therefore, honey that is pre-

dominantly from avocado nectar source is expected to have relatively high perseitol content, and could be identified by its sugar composition. Avocado nectar also contains a very high proportion (generally higher than 90%) of the translocated disaccharide sucrose (Liu *et al.*, 1995; Ish-Am, 1994). In general, in the final honey product, the sucrose is cleaved by hydrolysis, leaving approximate equimolar concentrations of the hexoses glucose and fructose. However, in the current study, there is a consistent trend of fructose levels somewhat higher than glucose levels (Fig. 1). We observed a negative correlation between sucrose and perseitol and a constant proportion between fructose and glucose with c. 10% higher fructose. This general trend of higher fructose ratios in honey from different sources has been well documented (White, 1980).

Unlike many other honey types, it seems that the palynological characteristic of honey does not allow reliable criteria for classification of avocado honey. Usually, a honey is considered unifloral if the pollen grain proportion of that plant is above 45% (Maurizio, 1975). Many of our samples that had high levels of perseitol, minerals, pH, and absorbance – as well as dark colour – contained very little (below 5%) avocado pollen. Very low levels of avocado pollen grains in Spanish honey considered to be avocado honey were recently reported by Terrab *et al.* (2004), who found that only 2–14% of the pollen grains belonged to avocado. These findings also support the findings of La-Serna Ramos *et al.* (1998) for avocado honey that was harvested in the Canary Islands; even in the ‘avocado honey’ samples, avocado was not the dominant pollen.

We may explain the low pollen counts by the fact that approximately half of the nectar produced by avocado is secreted from flowers in the female stage (Ish Am & Eisikowitch, 1998) and by the fact that flowers produce relatively low amounts of pollen grains (Gazit & Degani, 2002).

The dark colour of avocado honey found in the current study has been previously reported by La-Serna Ramos *et al.* (1998). They describe avocado honey as ‘very dark brown’. They also describe its taste, under the ‘intensity of flavour’ criterion, as ‘high and strong’. Terrab & Heredia (2004) describe avocado honey colour as between very dark to dark/amber colour. Colour, in general, is

related to flavour in that many light honeys are of mild flavour and dark honeys have a more pronounced taste (White, 1992). Another desirable characteristic of dark honeys, such as avocado is their high antioxidant capacity (Frankel *et al.*, 1998; Gheldof & Engeseth, 2002). The correlation found in the current study between honey colour (Fig. 5) and its absorption (Fig. 4) level, has been documented before (Fasler, 1975). However, the common recommended wavelength is 560 nm, while our study found that 260 nm provided the most reliable measure. Absorption in this wavelength is usually correlated to the presence of phenols.

As found in the current study, for avocado honey, darkness of honey was positively correlated with potassium and other mineral content. Schutte & Woessner (1939) also found that potassium levels in light honey vary from 100 to 558 ppm (mean 205 ppm) and in dark honey they vary from 115 to 4733 ppm (mean 1676 ppm). Similarly, Petrov (1970) showed a relationship between mineral content of honey and degree of pigmentation; he found 441 and 1241 ppm potassium in light and dark honey, respectively. In general, potassium is the most dominant mineral in honey (Petrov, 1970). Very high levels of potassium (3800 ppm, Table 1), as found in the current study for the honey with the highest perseitol content, which may be considered the most monofloral avocado honey, were also found for onion, carrot, and salt cedar (Waller *et al.*, 1972). High levels of potassium were recently reported as one of the characteristics of avocado honey, with an average of 1762 ppm (Terrab *et al.*, 2004). Except for potassium, relatively high levels of phosphorous, magnesium, sulphur, silicon, boron, zinc, iron and copper also characterize avocado honey (Table 1). Terrab & Heredia (2004) measured the concentrations of Ca, Na, K, Mg, S and P in avocado honeys from Spain. According to our analysis, calcium and sodium concentrations are not informative in discriminating avocado honey from other honeys; the concentration of sodium, in particular, varies greatly between geographic regions (Table 1, Fig. 3). The higher sodium concentrations (135–694 ppm) reported by Terrab & Heredia (2004), relative to our results, may reflect geographic differences between Spain and Israel. For the other four minerals, which we

found to be indicative of avocado honey, there was some overlap in the range of concentrations between our honeys and those of the Spanish honeys. Similarly for pH, which we found to be relatively high in avocado honey (Fig. 6), the range in our samples was similar to that of the Spanish honeys (Terrab & Heredia, 2004).

In conclusion, avocado honey may be characterized by perseitol content and NIR analysis, as we previously reported (Dvash *et al.*, 2002; Dag *et al.*, 2003). Analysis of the following minerals may also be used: potassium, phosphorous, magnesium, sulphur, silicon, boron, zinc, iron and copper. Physical characteristics such as absorption, darkness and pH are also reliable characteristics of avocado honey. These parameters can be used to categorize avocado honey. Honey is considered to be unifloral if the proportion of pollen grains from that plant is above 45% (Maurizio, 1975). Similarly, we may assume that a honey is monofloral if at least 45% of the components of nectar origin are from that plant. In Israel, most of the avocado honey flow is probably from 'Ettinger', with 5% perseitol (Ish-Am, 1994). Hence, monofloral avocado honey must contain at least 2.25% perseitol. From our analyses, we calculate that this value corresponds to minimum values of 2560 ppm potassium, 410 ppm phosphorous, 141 ppm sulphur, 135 ppm magnesium, 11.9 ppm silicon, 9.3 ppm boron, 6.8 ppm zinc, 5.2 ppm iron, 1.7 ppm copper, absorption of 4.25 O.D., darkness of 5.67 (Munsell value) and pH 5.01.

Only a few of the samples in our study would be classified as monofloral avocado honey based on these criteria, despite the fact that all the samples were from beehives located in blooming avocado orchards. We may explain this fact by the tendency of honeybees to prefer alternative blooms to that of avocado (Ish Am & Eisikowitch, 1998). Similarly, based on these criteria, the avocado honey samples tested by Terrab & Heredia (2004) would not be classified as monofloral. The range of values for the important minerals was similar between the Israeli and Spanish avocado honeys, suggesting that our criteria may apply to other regions and cultivars. However, the generality of these criteria needs to be tested in areas where other avocado cultivars dominate.

For commercial purposes, we may consider reducing these criteria since avocado honey is dominant in its colour and flavour (La-Serna Ramos *et al.*, 1998). Honeys with less than 45% of their nectar origin being from avocado, may still possess the organoleptic and visual characteristics of avocado. Sugar composition (level of fructose, glucose and sucrose) does not provide reliable characterization of honey collected from avocado flowers; pollen counts may be similarly problematic, because of the low presence of pollen in avocado honey.

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