Application of Infrared Spectroscopy in Honey Analysis

Lidija SVEČNJAK [1] (✉)
Nikola BILIŠKOV [2]
Dragan BUBALO [1]
Domagoj BARIŠIĆ [1]

Summary

The chemical composition and sensory characteristics vary significantly within different honey types. In order to determine botanical origin of honey, it is necessary to conduct rather complicated and time consuming analytical methods. IR spectroscopy has not yet been experimentally explored for honey analysis in Croatia, so the aim of this study was to determine claimed botanical origin of honey using both, standard and alternative (IR spectroscopy) methods, for the purpose of their comparison, Altogether 144 samples of nine different unifloral honey types (black locust, sweet chestnut, lime, sage, heath, rosemary, lavender, mandarin and strawberry tree) were collected from different Croatian regions directly from the beekeepers. In order to confirm claimed botanical origin of collected honey samples, melissopalinological analysis, moisture and electrical conductivity measurements were conducted. Infrared spectra of honey samples were recorded using the ABB Bomem MB102 Fourier-transform infrared spectrometer (FT-IR spectrometer). Selected IR spectral regions were analyzed by multivariate data analysis, principal components analysis (PCA). Preliminary PCA of IR spectra showed significant clustering of the analyzed samples by botanical origin. The results of this study showed that IR spectroscopy provides reliable results, but also represents rapid and cheap analytical tool in comparison to commonly used standard analytical methods. This research has also provided the first insight in infrared spectra of Croatian honeys.

Key words

honey, botanical origin, standardized analytical methods, infrared spectroscopy

[1] University of Zagreb, Faculty of Agriculture, Department of Fisheries, Beekeeping and Special Zoology
Svetošimunska 25, 10000 Zagreb, Croatia
✉ e-mail: lsvecnjak@agr.hr
[2] Rudjer Boskovic Institute, Division of Organic Chemistry and Biochemistry
Bijenička 54, 10000 Zagreb, Croatia

Received: May 12, 2011 | Accepted: July 11, 2011

Aim

The proportion of nectar or honeydew of certain plant species in honey varies depending on the type of vegetation, the period of plant blooming or the production of honeydew by the insects, as well as on the time when the beekeepers have produced honey. Absolutely unifloral honeys do not exist because the honey bees never visit only one plant species, even though it dominates in a given area. Consequently, the chemical composition and sensory characteristics within different honey types vary significantly. In order to determine botanical origin of honey, it is necessary to conduct melissopalynological analysis, as well as sensory and certain physico-chemical analysis, because only on the basis of aggregate results of mentioned analyses, the final confirmation of the claimed botanical origin can be given. The analytical methods used for honey classification are mostly the same as the ones used for routine quality control (Bogdanov et al., 2004). These methods have been harmonized and described by the International Honey Commission (Bogdanov et al., 1997). However, these methods, especially melissopalynological analysis, require specialised knowledge and expertise, and involve a long counting procedure. Thus, the identification of the botanical origin of honey represents rather difficult and time-consuming process.

It is clear that the differences between distinctive types of honey are not confined only to their sensory qualities, physicochemical characteristics and the botanical origin (pollen spectrum). One of the methods that provide information on the total chemical composition of any sample is infrared spectroscopy (IR), a type of vibrational spectrosocpy, where molecular vibrations are being analyzed. Infrared spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of all other compounds; only optical isomers absorb in exactly the same way (Skoog and Leary, 1992). Although this method is rather simple and undemanding from the experimental point of view, it has been recognized as very effective in honey analytics only a few years ago. Until recently, IR was considered to be completely inapplicable to samples containing higher water content. However, the development of new methods for spectra recording, of which the most importatnt is attenuated total reflectance (ATR), allowed the obtaining of quantitative information on the chemical composition of water-rich samples. The advantages of this method over commonly used standard ones are that it is fast, reliable and easy to use. Furthermore, vibrational measurements require minimal or no sample preparation, and only a very small amount of the sample, thus allowing *in situ* monitoring of any sample. Based on comparison of several spectroscopic methods used for honey analysis, Ruoff et al. (2006, 2007) have presented comparative advantages of IR spectroscopy over other used methods. In these studies, a large sample of different honey types originating from different European countries, was analyzed using contemporary chemometrics and was compared with standard methods. The results have shown that IR spectroscopy can be considered as a reliable for distinguishing the botanical origin of honey. There are also few other papers indicating that IR spectroscopy can be used for authetication of the botanical origin of honey (Lichtenberg-Kraag et al., 2002; Bertelli et al, 2007; Woodcock et al., 2009) but this method has still not been standardized nor implemented in regulations related to honey quality control. Furthermore, there are still certain aspects of this method that need to be additionally explored, repeated and verified in order to make it outright reliable and applicable as an analytical tool in general practice. IR spectroscopy has not been experimentally explored yet for honey analysis in Croatia, so the aim of this study was to confirm claimed botanical origin of honey samples collected from the beekeepers from different Croatian regions, using both, standard and alternative (IR spectroscopy) methods, for the purpose of their comparison. Moreover, the results of this research will provide the first insight in infrared spectra of Croatian honeys.

Material and methods

Altogether 144 samples of nine different unifloral honey types (black locust, sweet chestnut, lime, sage, heath, rosemary, lavender, mandarin and strawberry tree) were collected from different Croatian regions directly from the beekeepers (Table 1).

In order to confirm claimed botanical origin of collected honey samples, melissopalinological analysis was conducted according to the method of Louveaux et al. (1978) where the identification and counting of pollen grains in prepared insoluble honey sediment was performed at 400x magnification under light microscope. The identification was carried out using reference

Table 1. The number of analyzed samples (N) by honey type

Honey type / botanical species	N
Black locust (*Robinia pseudoacacia* L.)	35
Sweet chestnut (*Castanea sativa* M.)	38
Lime (*Tilia* spp.)	19
Sage (*Salvia officinalis* L.)	20
Lavander (*Lavandula angustifolia* M.)	3
Mandarin (*Citrus unshiu* Marcovitch)	14
Heath (*Erica* spp.)	9
Rosemary (*Rosmarinus officinalis* L.)	3
Strawberry tree (*Arbutus unedo* L.)	3
Total	144

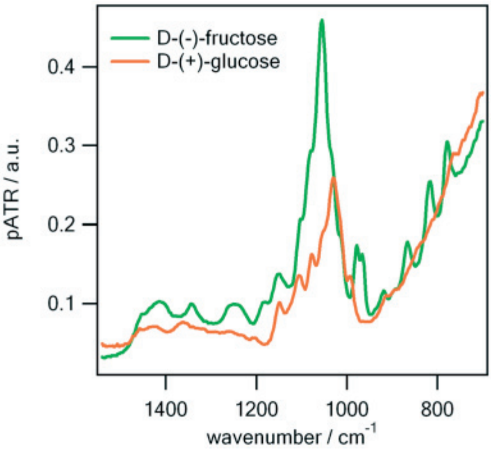

Figure 1. IR spectra of saturated aqueous solutions of D-(-)-fructose and D-(+)-glucose

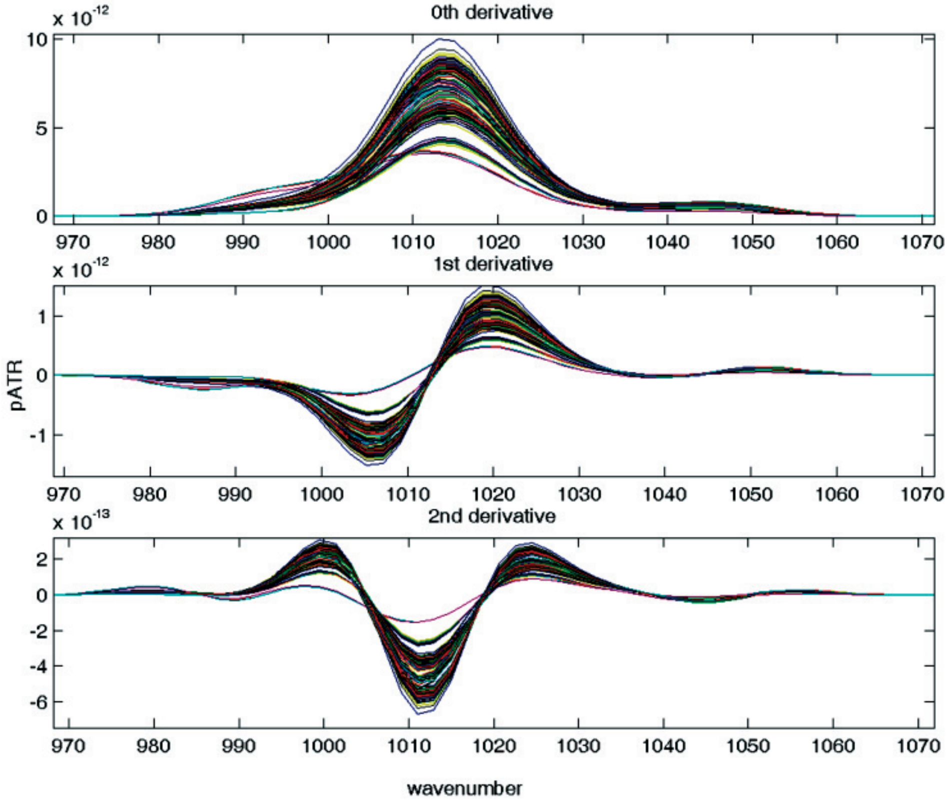

Figure 2. Input IR spectra as prepared for PCA

samples of pollen in the form of native preparations (reference collection of the University of Zagreb, Faculty of Agriculture) and literature (Von der Ohe and Von der Ohe, 2000). A standard sample of 500 pollen grains was used for pollen and honey type determination (Von der Ohe et al., 2004). Unifloral types of honey were determined in accordance with Croatian regulations given in Ordinance on the quality of unifloral honey (Ministry of Agriculture, Fisheries and Rural Development, 2009). Physico-chemical parameters (moisture, electrical conductivity) were determined according the methods officially prescribed by the Croatian Regulations (Ministry of Agriculture and Forestry, 2000) and in accordance with Harmonised methods of the European Honey Commission (Bogdanov et al.1997). Moisture (water content) was determined from the refractive index of the honey obtained by Abbé refractometer (AOAC 969.38). Electrical conductivity was measured by a multirange conductivity meter HI 8733 (Hanna Instruments) (Bogdanov et al., 1997).

Infrared spectra of honey samples were recorded using the ABB Bomem MB102 Fourier-transform infrared spectrometer (FT-IR spectrometer) with a DTGS detector and CsI optics. Single reflection attenuated total reflection (ATR) technique was used to obtain spectra. For this purpose, a MKII Golden Gate^{TM} ATR accessory (Specac Inc., Woodcock GA, USA) was used. Each spectrum was recorded as the ratio of the spectrum of ATR element containing sample to the spectrum of the empty ATR plate. Since model MB102 is a single-beam spectrometer, a background was taken with the empty ATR plate before measuring each sample. Each spectrum represents an average of 100 Fourier-transformed interferograms. The nominal resolution was 4 cm^{-1}, which gives a distance between two points in the resulting spectrum $\Delta\tilde{\nu} = 1.2\,\mathrm{cm}^{-1}$. The honey samples were recorded as obtained.

The spectral range from 1200 to 700 cm^{-1} (maximum absorbance established at 1026 cm^{-1}) was subjected to further statistical analysis since the greatest alterations in honey have been identified in this particular spectral region (Figure 1).

Selected IR spectral regions were analyzed by multivariate data analysis, principal components analysis (PCA). Statistical analysis was performed using the statistical software package Matlab 6.0. Preparation of spectra for PCA was performed as follows: all spectra were smoothed using the Savitzky-Golay procedure by fitting 4th order polynomial to every 13 spectral points. First and second derivative spectra were calculated. After that, each absorbance was multiplied by corresponding overall standard deviation. In this way spectral ranges that change significantly were accented. Spectra were cut to 1200 – 700 cm^{-1} range and they were normalised to unit area. Principal component analysis was applied to in this way prepared spectra (Figure 2).

Results and discussion

The results of conducted standardized methods have confirmed analyzed samples as unifloral, for each honey type. Average values of electrical conductivity of analyzed honey types were ranged within the chemical quality criteria for unifloral honeys (Table 2), established for each honey type (Accorti et al., 1986;

Table 2. Electrical conductivity range (min, max) for unifloral honey types and average values of analyzed samples

Honey type / botanical species	Min/max (mS/cm)	$\overline{x}$ (mS/cm)
Black locust (*Robinia pseudoacacia* L.)	0.09-0.23	0.15
Sweet chestnut (*Castanea sativa* M.)	0.86-1.91	1.22
Lime (*Tilia* spp.	0.37-0.87	0.65
Sage (*Salvia officinalis* L.)	0.15-0.52	0.36
Lavander (*Lavandula angustifolia* M.)	0.12-0.31	0.29
Mandarin (*Citrus unshiu* Marcovitch)	0.11-0.31	0.27
Heath (*Erica* spp.)	0.50-0.85	0.64
Rosemary (*Rosmarinus officinalis* L.)	0.08-0.23	0.23
Strawberry tree (*Arbutus unedo* L.)	0.60-0.90	0.84

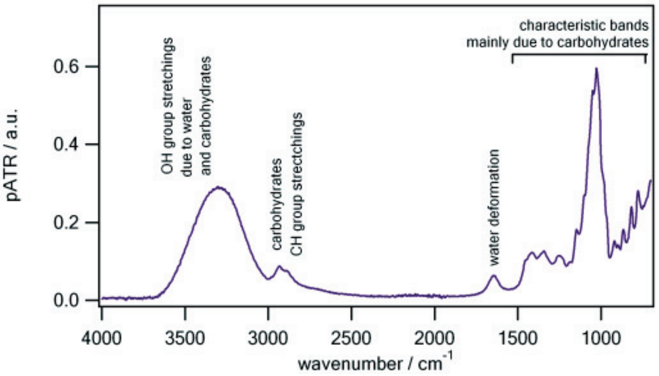

Figure 3. Typical FT-IR spectrum of honey with arbitrary assignation of the IR absorption bands to corresponding vibrations

Persano Oddo and Piro, 2004; Kenjerić et al., 2006). The authentication of the botanical origin was also conformed by melissopalynological analysis. Pollen spectrum was determined on the basis of the share of specific pollen grains in the insoluble honey sediment.

Honey is mostly consisted of hydrated glucose and fructose, together with higher carbohydrates in smaller amount. Thus, typical IR spectrum of honey is dominated by the features corresponding to hydrogen-bonded hydroxyl groups of carbohydrates (Figure 3), together with the bands due to the skeletal vibrations of carbohydrates. The absorption due to the other, less abundant, honey components is mainly overlapped.

Preliminary principal component analysis (PCA) of IR spectra showed reliable and significant clustering of the analyzed samples by botanical origin. The samples were grouped close together creating uniform clusters for each of nine analyzed honey types in the PCA scatter plot (Figure 4). Applied PCA of IR spectra showed that IR spectroscopy provides results equally reliable to those achieved by standard analytical methods. Clusters of nine different unifloral honey types obtained by PCA correspond to the results of their clasiffication confirmed by standardized physico-chemical and melissopalynological analysis.

Conclusions

Being very sensitive to the overall chemical composition of the sample, infrared spectroscopy, especially when coupled with multivariate statistical analysis, provides reliable results in this regarding determination of botanical origin of honey. Vibrational spectroscopy enables a non-destructive chemical characterization of honey based on identifiable spectral features. The authentication of the botanical origin of nine unifloral honey types was confirmed using both, standard and alternative (FT-IR spectroscopy) methods. The results of this study showed that IR spectroscopy provides equally reliable results, but also represent rapid and cheap analytical tool in comparison to commonly used standard analytical methods. Therefore, it is expected that IR spectroscopy will contribute to authentication and quality control of Croatian honey.

References

Accorti M., Persano Oddo L., Giola Plazza M., Sabatini A.G. (1986). Schede di caratterizzazione delle principali qualitadi miele Italiano. Apicoltora, 2:20-21.

AOAC (1996). Official methods of analysis (16th ed.). Washington, DC: Association of Official Analytical Chemists. Methods: Official method 969.38.

Bertelli D., Plessi M., Sabatini A.G. Lolli M. Grillenzoni F. (2007). Classification of Italian honeys by mid-infrared diffuse reflectance spectroscopy (DRIFTS). Food Chemistry 101:1565-1570.

Bogdanov S., Martin P., Lullman C. (1997) Harmonised methods of the European honey commision. Apidologie, extra issue, 1-59.

Bogdanov S., Ruoff K., Persano Oddo L. (2004). Physico-chemical methods for the characterisation of unifloral honeys: a review. Apidologie 35:S4-S17.

Kenjerić D.,Primorac Lj., Mandić M.L., Bubalo D., Perl Pirički A., Flanjak I. (2006). Dalmatian sage (*Salvia officinalis* L.) honey characterization. Deutsche Lebensmitte-Rundschau 10:479-484.

Lichtenberg-Kraag B., Hedtke C., Bienefeld K. (2002). Infrared spectroscopy in routine quality analysis of honey. Apidologie 33:327–337.

Louveaux J., Maurizio A., Vorwohl G. (1978). Methods of melissopalynology. Bee World. 59: 139-157.

Ministry of Agriculture and Forestry (2000). Quality standard of honey and other bee products.

Ministry of Agriculture, Fisheries and Rural Developement (2009). Ordinance on the quality of unifloral honey.

Persano Oddo L., Piro R. (2004). Main European unifloral honeys: descriptive sheets. Apidologie 35:S38–S81.

Ruoff K., Luginbühl W., Künzli R., Iglesias M. T., Bogdanov S., Bosset J. O., von der Ohe K., von der Ohe W., Amadò R. (2006). Authentication of the Botanical and Geographical Origin of Honey by Mid-Infrared Spectroscopy. J. Agric. Food Chem. 54:6873-6880.

Ruoff K., Luginbühl W., Bogdanov S., Bosset J.O., Estermann B., Ziolko T., Kheradmandan S., Amadò R. (2007). Quantitative determination of physical and chemical measurands in honey by near-infrared spectrometry. Eur Food Res Technol. 225:415-423.

Skoog D.A., Leary J.J. (1992). Introduction to Instrumental Analysis, 4thed., Saunders College Publishing, New York.

Von der Ohe K., Von der Ohe W. (2000). Celler Melissopalynologische Sammlung, Niedersächsisches Lamdesinstitut für Bienenkunde, Celle.

Von der Ohe W., Persano Odo L., Piana M.L., Morlot M., Martin P.. (2004). Harmonized methods of melissopalinology. Apidologie 35: 18-25.

Woodcock T., Downey G., O'Donnell C.P. (2009). Near infrared spectral fingerprinting for confirmation of claimed PDO provenance of honey. Food Chemistry 114:742-746.

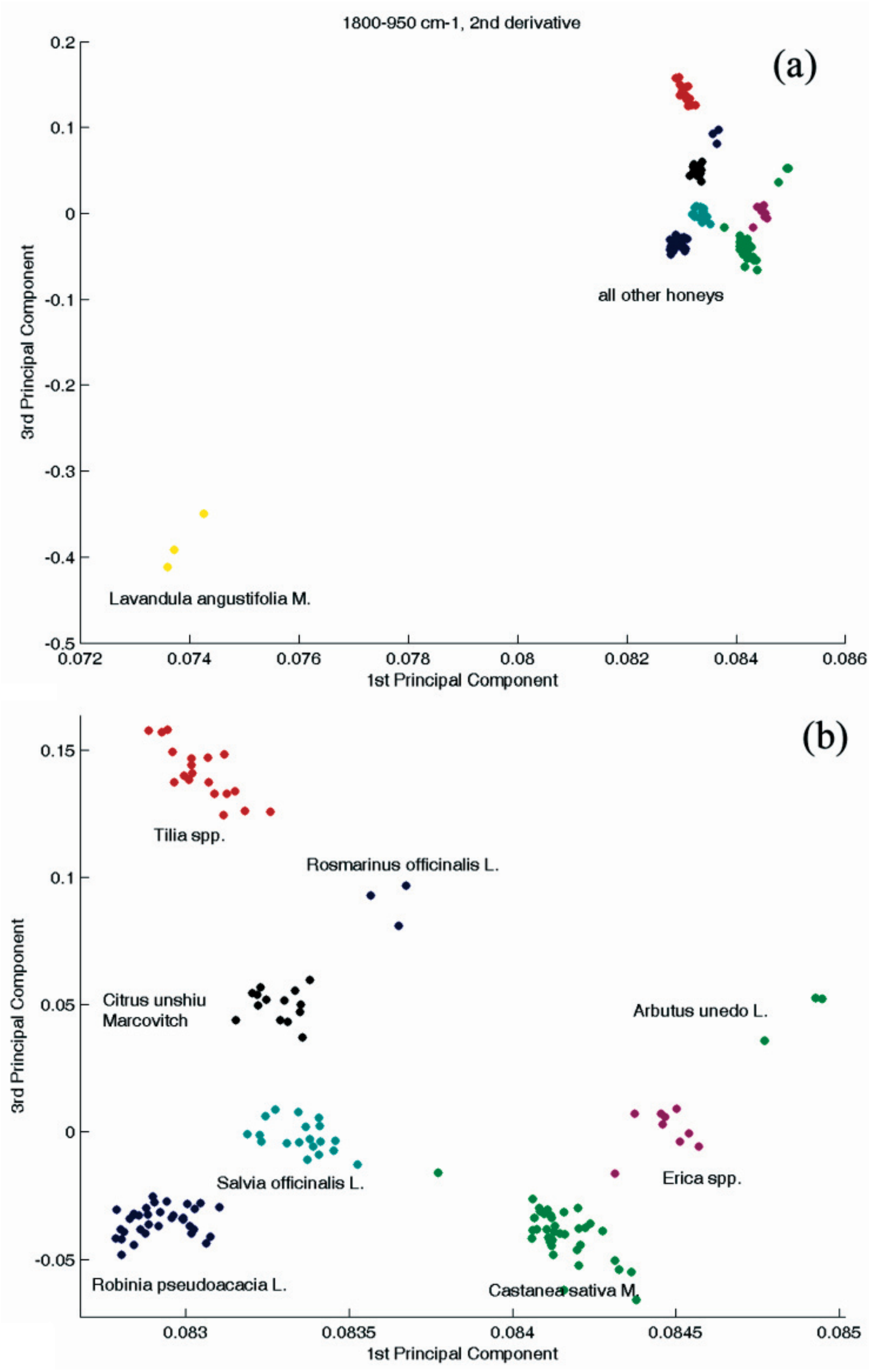

Figure 4. (a) Clustering of 9 unifloral honey types by botanical origin obtained using principal component analysis (PCA) of IR spectra; (b) increased region of clusters of all honey types except lavender