Using differential scanning calorimetry, laser refractometry, electrical conductivity and spectrophotometry for discrimination of different types of Bulgarian honey

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Abstract. The potential of several physical methods for investigation of the botanical origin of honey has been discussed. Samples from the three most prevalent types of honey in Bulgaria (acacia, linden and honeydew) have been used. They have been examined by laser refractometry, UV, VIS and FTIR spectroscopy, electric conductivity measurement and differential scanning calorimetry. The purpose of this study was to reveal the physical characterizations of honeys from different flora produced in Bulgaria and to identify honeys with a high apitherapy potential for future studies.

1. Introduction

Production of a natural honey is a laborious process, which is time consuming and involves a lot of cost. Therefore, honey is often a subject to falsification by adding sugar and other impurities. Furthermore, from a commercial perspective monofloral honey is preferred over polyfloral one, as well as certain types of monofloral honey are preferred over others. Therefore, it is important to have reliable methods for their discrimination. Several methods have been used for the determination of the floral origin of honey and among them the pollen recognition and sensory analysis are the most popular ones. However, the technique of analysis of honey's pollen content is a long process and has some limitations. The other methods are mainly based on the analysis of honey's aroma compounds, sugar profile, flavonoid pattern, non-flavonoid phenolics, organic acids, isotopic relations, and protein and amino acid compositions and marker presence [1, 2]. However, some of these methods are generally too time-consuming, complex, and labour intensive for routine quality control application or require very specialized personnel to interpret the results [3]. The advantages of the technique of spectroscopy (UV, visible, near and middle infrared, fluorescent) with respect to other methods are the non-invasive approach, the relatively easy and quick data acquisition. Recently, both near infrared and middle infrared spectroscopy have been successfully used for the classification of unifloral and multifloral honeys [4-6]. Some authors have used UV and VIS spectrometry for the same purpose [7-10].

The electrical conductivity is a good criterion for the botanical origin of honey and therefore it is very often used in routine honey control [11, 12]. The moisture content of honey or conversely the soluble solids content, is determined by measuring the refractive index of honey [13, 14]. Differential scanning calorimetry (DSC) is a powerful technique for characterization of the thermal behaviour of honeys and for detecting the effect of adulteration on the physicochemical and the structural properties of samples [15, 16]. Therefore, it is of prime importance to find the best parameters able to distinguish pure honeys from syrups and from adulterated honeys.

The aim of this article is a comparative analysis of several physical methods (UV, VIS and FTIR spectroscopy, laser refractometry, electrical conductivity and DSC analysis) which are widely used for examination of honey in view of its classification on the base of their “botanical origin” and will be applied to three of the most common types of Bulgarian honey – acacia, linden and honeydew honey. For the appropriate visualization of the three classes of honey Principal Components Analysis (PCA) is used. The simulations were carried out in MATLAB environment.

2. Materials and methods

2.1. Samples preparation
Three different types of Bulgarian honey (acacia, linden and honeydew) were purchased from the local market and from private producers. Ten different samples from various manufacturers of each type of honey were purchased. Each of the thirty samples were pre-treated thermally, having been placed in a water bath of 50°C for 30 minutes, after which it was cooled down to room temperature.

2.2. Absorption spectroscopy
The three types of spectrum (UV, VIS, FTIR) were acquired as follows. UV spectra were taken with a spectrophotometer Cary 100 ranging from 190 nm to 380 nm at 1 nm sampling space. VIS spectra were taken with a spectrophotometer Helios Omega ranging from 380 nm to 780 nm at 1 nm sampling space and using the software package VISIONlite ColorCalc [9]. FTIR spectra were taken with Mid-FTIR spectrometer Varian 660 ranging from 600 cm^{-1} to 4000 cm^{-1} at 1.929 cm^{-1} sampling space.

2.3. Electrical conductivity (EC)
The EC was measured using a conductometer DiST by HANNA Instruments in a 20 g/100 ml solution of honey (dry matter basis) in deionised water, at temperatures varying in the range of 5^{o}C to 45^{o}C by step of 5^{o}C. According to the harmonised methods of the European Honey Commission EC is measured at 20°C and expressed in mS/cm [17].

2.4. Differential scanning calorimetry (DSC)
The thermal characteristics of the honey samples were investigated at the temperature range of -70°C to 100°C by differential scanning calorimeter DSC 204 F1 Phoenix (NETZSCH, Germany) equipped with intracooler. The DSC was calibrated with indium. In order to avoid condensation of water, argon gas was used to purge the furnace chamber at 20 ml/min. The samples (5-10 mg) was weighed into 40 µl aluminium standard crucible and hermetically sealed with aluminium standard lead. An empty aluminium crucible was used as a reference. Firstly, the samples were cooled from room temperature to – 70°C at cooing rate of – 40°C/min. The heating was done with standard rate of 5°C/min.

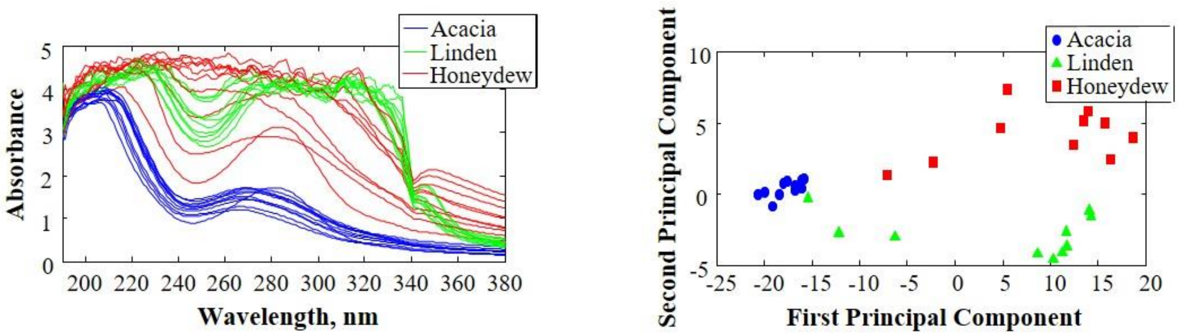
2.5. Laser refractometry
The refractive indices, *n* for all the samples were measured by the method of the disappearing diffraction pattern using a laser refractometer [18] at wavelengths of 405 nm, 532 nm and 635 nm. The total experimental uncertainty of the measured refractive indeces was less than 3×10^{-4}.

2.6. Principal Components Analysis (PCA)

The aim of the method is to reduce the dimensionality of multivariate data (e.g., wavelengths, temperatures) whilst preserving as much of the relevant information as possible. PCA is a linear transformation that transforms the data (observations of possibly correlated variables) to a new coordinate system such as the new set of variables, the principal components (PCs) are linear functions of the original variables. PCs are uncorrelated, and the greatest variance by any projection of the data comes to lie on the first coordinate, the second greatest variance - on the second coordinate, and so on. This is achieved by computing the covariance matrix for the full data set. Then the eigenvectors and eigenvalues of the covariance matrix are computed and sorted according to the decreasing eigenvalue [19, 20]. All principal components are orthogonal to each other. The full set of principal components is as large as the original set of variables. Usually the sum of the variances of the first few principal components exceeds 80% of the total variance of the original data [21].

3. Results and discussion

UV absorbance spectra of the three types of honey in the wavelengths range from 190 nm to 380 nm are shown in figure 1(a). There are two peaks at about (215-240) nm and (284-340) nm with can be attributed with content of hydroxymethylfurfural [22].



(a) (b)

Figure 1. UV absorbance spectra (a), PCA of the spectral characteristics (b).

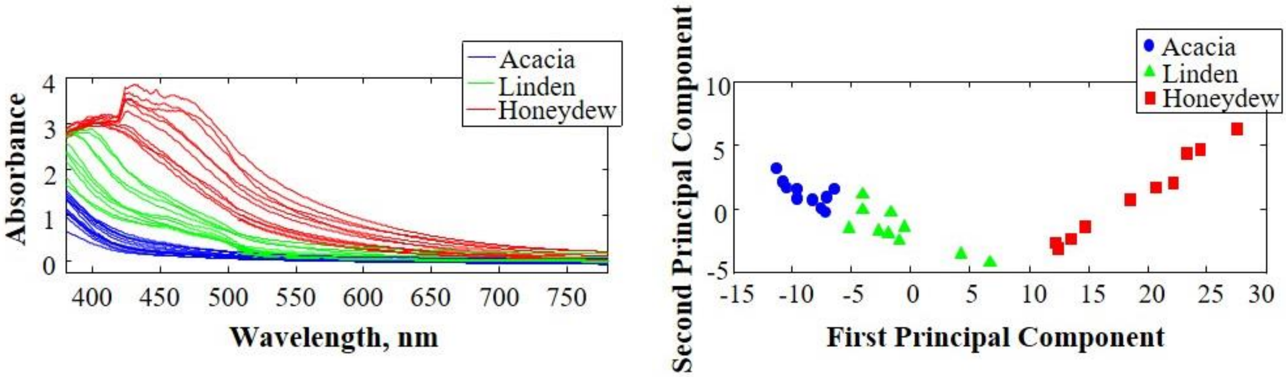
The experimental data are subject to pre-treatment. In order to remove some apparent interference received data are limited from above by a predetermined value (AbsorbMax). The resulting absorbance curves are smoothed by the method of creeping averaging, using the equation:

$$a_{i+l/2} = \frac{1}{l+1}\sum_{k=0}^{l} a_{i+k} , \qquad (1)$$

where l is the width of a linear filter accepting even-numbered values.

PCA method of multivariate analysis was applied to distinguish honey. The calculations and visualization were carried out in MATLAB environment. Figure 1(b) shows the scattered plot of the first and the second PCs of UV spectral characteristics. As evident, the three types of honey are well distinguished from PCA of the UV spectra.

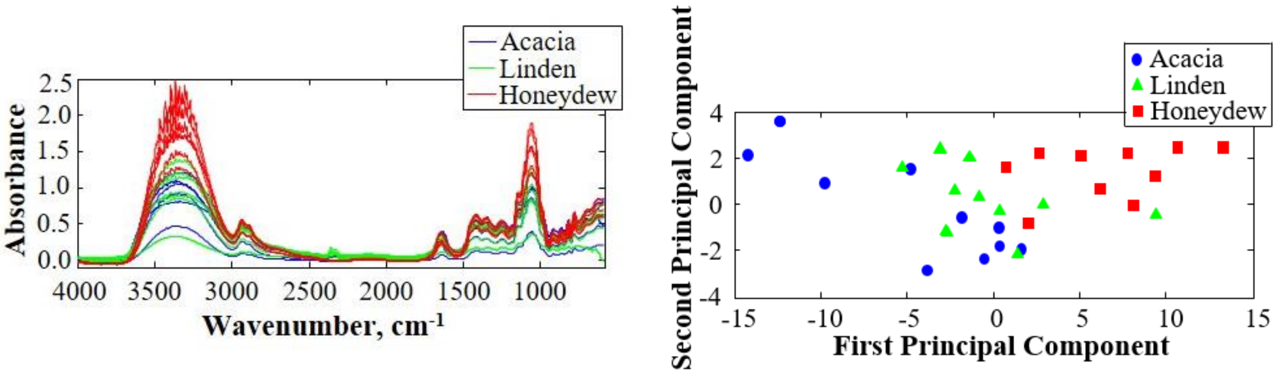
VIS absorbance spectra of the three types of honey in the wavelengths range from 380 nm to 780 nm are shown in figure 2(a) and PCA of the VIS characteristics – in figure 2(b). The spectra present wide peak at the band of (400-500) nm that can be connected with the colour of the samples [22]. The honeydew has the absorbance peak at about (450-470) nm [23]. As it can be seen the three types of honey are well distinguished from PCA of the VIS spectra.



(a) (b)

Figure 2. VIS absorbance spectra (a), PCA of the spectral characteristics (b).

FTIR absorbance spectra of the three brands of honey in the wave numbers range from 4000 cm^{-1} to 600 cm^{-1} are shown in figure 3a and PCA of the FTIR characteristics – in figure 3(b).The mid-infrared bands could be interpreted using previous reported studies [24]. The first peak (3600-3000) cm^{-1} corresponds to O-H stretching of water and carbohydrates, and the second peak at (2950-2800) cm^{-1} − to the C-H stretching bound of carbohydrates (methyl and methylene groups). The scattered plot in figure 3(b) shows the mixing of the three classes of honey. The infrared spectroscopy allows distinguishing honeydew honey from monofloral honey, but cannot differentiate different monofloral honey groups.



(a) (b)

Figure 3. FTIR absorbance spectra of honey (a), PCA of the spectral characteristics (b).

The dependence of PCA of the EC characteristics is shown in figure 4. EC values (recorded at 20ºC) of honey samples tested in our study are 0.248 mS/cm for acacia honey samples, (0.499-0.640) mS/cm - for linden honey and (1.100-1.291) mS/cm - for honeydew honey samples. The EC value depends on the ash and acid content in honey: the higher their content, the higher the resulting conductivity [25]. The EC is a good criterion for the botanical origin of honey. A lower limit has been proposed for blossom than for honeydew honeys [25]. Our investigation on the EC of Bulgarian honey shows a good distinguishing of the three types of honey, based on this metric.

The information on honey, which is derived from DSC, is not sufficient for the reliable determination of the botanical origin of honey, but it can be used for quality estimation. A linear relation between the glass transition temperature and the moisture content was established, which is in a good agreement with the numerous reports about the water activity (a_w) effect on T_g and the plasticizing properties of water [26].

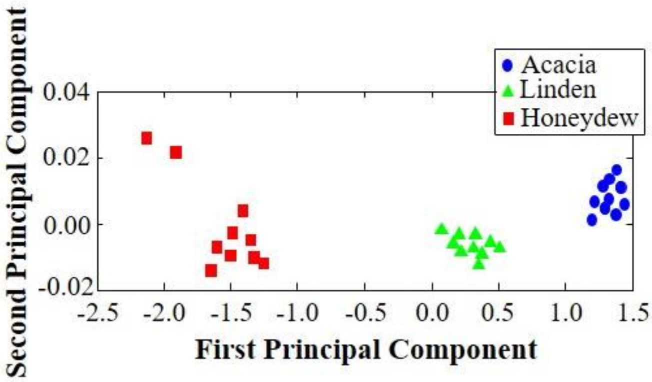


Figure 4. PCA of the EC characteristics of honey.

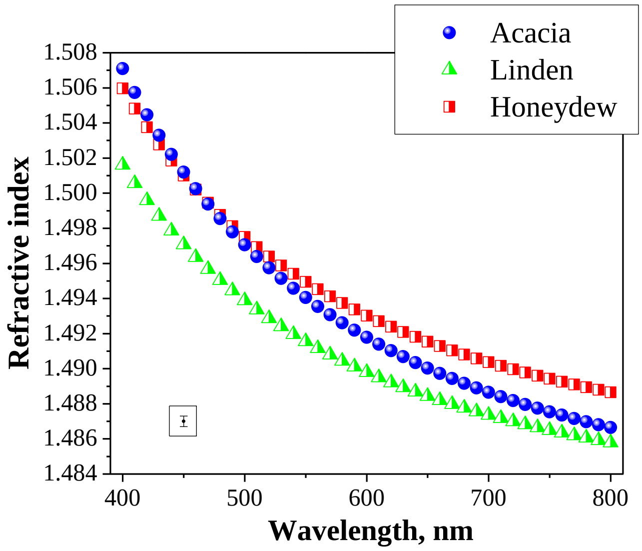


Figure 5. Dispersion dependences for the honey samples.

The refractive indices values for the all samples are accurately measured at 25°C at three different laser wavelengths – 405 nm, 532 nm and 635 nm. Far from the fundamental absorption band, the most appropriate dispersion relation for liquids is the one term Sellmeier relationship:

$$n^2 - 1 = \frac{s\lambda^2}{\lambda^2 - \lambda_s^2}, \tag{2}$$

where n is the refractive index at wavelength λ, s and λ_s are the Sellmeier's coefficients.

By applying equation (2) for three combinations of refractive indices pairs, we determined the Sellmeier's coefficients (s and λ_s) [27]. The measured average values of the n at the used three wavelengths and the obtained values of the Sellmeier coefficients are listed in table 1.

Table 1. Average values of the refractive indices and obtained values of the Sellmeier coefficients for three types Bulgarian honey.

Honey type	n (λ=405 nm)	n (λ=532 nm)	n (λ=635 nm)	s	λ_s
Linden	1.5021	1.4921	1.4892	1.1926	89.07
Acacia	1.5056	1.4953	1.4902	1.1910	100.56
Honeydew	1.5045	1.4961	1.4916	1.1997	92.77

Using the already determined dispersion coefficients, the dispersion dependences are built in the range (400-800) nm and plotted in figure 5. The sign of error bar in the lower left of the figure 5 represents the experimental uncertainty of the refractive indices values. The n is the highest for honeydew and it is the lowest for linden honey.

It can be seen from figure 5 that the most appropriate values for the discrimination between a linden honey and an acacia honey are the refractive indices values in the range (400-550) nm while the n values for acacia and honeydew are almost the same. A good separation between acacia honey and honeydew, but not between acacia honey and linden honey, can be made by measurement of the n in the range (700-800) nm. The honeydew and the linden honey are well separated by their refractive indices in the whole visible range. The results show that the refractive indices gives a good opportunity for distinguishing the botanical origin of honey. But it should be noted that for good separation between different types of honey a measurement of the refractive index at one wavelength is not sufficiently secure. Our studies indicate that the refractive index value can do sufficiently good separation of the investigated three types of honey using precision laser refractometer at one wavelength in the range (550-700) nm.

The obtained experimental results have shown that not all the presented investigations are suitable for discrimination of three different types of Bulgarian honey. Such separation can be done only by PCA of UV and visible spectra and PCA of EC characteristics. It should be noted that the honeys cannot be divided by group according to its botanical origin by using only peaks in the visible and UV spectra. This can be obtained by PCA. Another suitable method for good separation among investigated types of honey is to obtain the dispersion dependencies in the visible range by the laser refractometry.

4. Conclusion

In this work, the several physical methods (UV, VIS and FTIR spectroscopy, laser refractometry, electrical conductivity and DSC analysis) for discrimination of three of the most common types of Bulgarian honey – acacia, linden and honeydew honey are applied. It was shown that the most appropriate methods, which enable differentiation in botanical origin among investigated types of honey, are PCA of UV and visible spectra, PCA of EC characteristics and laser refractometry in visible range. It was established that PCA of FTIR spectra and DSC method could not be used to differentiate botanical origin of honey.

Acknowledgements

The paper presents research and development, supported by the Scientific Fund of Internal Competition of the University of Food Technologies – Plovdiv under the Research Project No.7/16-H.

References

[1] Anklam E 1998 *Food Chem.* **63** 549
[2] Hermosin I, Chicon R and Cabezudo M 2003 *Food Chem.* **83** 263
[3] Bogdanov S and Martin P 2002 *Mitt. Geb. Leb. Hyg.* **93** 232
[4] Davies A, Radovic B, Fearn T and Anklam E 2002 *J. Near Infrared Spectrosc.* **10** 121
[5] Ruoff K, Luginbühl W, Bogdanov S, Estermann B, Ziolko T and Amado R 2003 *Eur. Congres for Authenticity of Food*, Nyon, Switzerland
[6] Lichtenberg-Kraag B 2003 *Apidologie* **34** 479
[7] Li Y and Yang H 2012 *Int. Sch. Res. Network, ISRN Spectrosc.* **2012** 4
[8] Tsankova D and Lekova S 2015 *J. Chem. Technol. Met.* **50** 638
[9] Tsankova D, Lekova S, Nikolova K and Terziiski G 2015 *Annals of Faculty Eng. Hunedoara – Intern. J. Eng.* **XIII** 275
[10] Tsankova D and Lekova S 2015 *Intern. Conf. "Automatics and Informatics'2015"* 107
[11] Kaskoniene V, Venskutonis P and Ceksteryte V 2010 *Food Sci. Technol.* **43** 801
[12] Lašáková D, Nagy J and Kasperová J 2009 *Folia veter.* **53** 31
[13] Ouchemoukh S, Louaileche H and Schweitzer P 2007 *Food Control* **18** 52
[14] Nyau V, Mwanza E P and Moonga H B 2013 *African J. Food, Agricult. Nutrit. Devel.* **13** 7416
[15] Cordella C, Faucon J, Cabrol-Bass D and Sbirrazzuoli N 2003 *J. Thermal Anal. Calor.* **71** 279
[16] Tomaszewska-Gras J, Bakier S, Goderska K and Mansfeld K 2015 *J. Apic. Scie.* **59** 109
[17] Bogdanov S, Martin P and Lullmann C 1997 *Apidologie (extra issue)* 1
[18] Bodurov I, Yovcheva T and Sainov S 2015 *Opt. Appl.* **XLV** 199
[19] Hotelling H 1933 *J. Educational Psychology* **24** 498
[20] Jolliffe I 2002 *Principal Component Analysis* 2nd ed (Springer Series in Statistics, New York: Springer-Verlag New York)
[21] Statistics Toolbox™ User's Guide, R2014a, Copyright 1993–2014 (The MathWorks, Inc.)
[22] Escuredo O, Fernández-González M and Seijo María C 2012 *Agriculture* **2** 25

IOP Conf. Series: Journal of Physics: Conf. Series **794** (2017) 012034 doi:10.1088/1742-6596/794/1/012034

[23] Sun D-W 2008 *Modern Techniques for Food Authentication* 1st ed (San Diego, California, USA: Academic Press Elsevier) p 201

[24] Domínguez-Martínez I, Meza-Márquez O, Osorio-Revilla G, Proal-Nájera J and Gallardo-Velázquez T 2014 *J. Korean Soc. Appl. Biol. Chem.* **57** 133

[25] Bogdanov S 2002 Harmonized methods of the International Honey Commission, *International Honey Commission* p 1

[26] Costa PA, Moraes ICF, Bittante AMQB, Sobral PJA, Gomide CA and Carrer CC 2013 *Int. J. Food Stud.* **2** 118

[27] Tan WC, Koughia K, Singh J and Kasap SO 2006 Fundamental optical properties of materials, *Optical Properties of Condensed Matter and Applications*, ed Jai Singh (New York: Wiley-Interscience) p 7