APPLICATION OF NEAR INFRARED SPECTROSCOPY AND CLASSICAL ANALYTICAL METHODS FOR THE EVALUATION OF HUNGARIAN HONEY

Short running title: **EVALUATION OF HUNGARIAN HONEY WITH NIRS**

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Abstract

People have started to pay more attention on the healthier lifestyle recently, which also includes the consumption of more natural and less processed food products. Honey as one of the most often used natural sweetener has been also reconsidered and more commonly used. However, honey has been also target of food adulteration due to its emerging use and relatively high price. Therefore, there is an increasing need to develop rapid evaluation methods for identification of honey from different sources. Experiments have been performed with 79 authentic honey samples of different floral and geographical origins, mainly from Hungary. The standard analytical parameters used to characterize the nutritional values of honey such as antioxidant capacity, polyphenol content, ash content, pH, conductivity have been determined. The samples were also analyzed with a bench top near infrared (NIR) spectrometer to record their NIR spectra. The data acquired with NIR spectroscopy measurements were evaluated with various univariate and multivariate statistical methods. Results show that NIR spectroscopy can be used for the identification of floral and geographical origin of honey samples. Further experiments are proposed to build a robust database, which could support the use of NIR spectroscopy as a quick alternative for honey authentication.

Keywords: Hungarian honey, chemometrics, authentication

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1. Introduction

Whether from flower nectar or the juice secreted from leafs under the action of insects, honey is a particularly valuable natural product, rich in nutritional components such as vitamins, minerals and antioxidants, organic acids (Czipa, 2010). On the other hand, its composition and quality depends on the floral and geographical origin, having an important impact on price. As a consequence, honey is prone to adulteration, methods ranging from feeding bees with sugar syrups to blending honeys from different floral or geographical sources. Detection of adulteration includes various analyses from chromatographic techniques to nuclear magnetic resonance spectroscopy. The limitation of these methods is that they time-consuming and expensive. Generally, a wide range of quality indicators are needed in order to detect honey adulteration, but new methods are also necessary (Zábrodská & Vorlová, 2015). As cheap and fast techniques, specific physicochemical and antioxidant properties can represent a good choice, especially when combined with other target methods (Cimpoiu et al., 2013) (Jose et al., 2009). Near infrared spectroscopy (NIRS) can be a fast and simple tool for identifying adulterated honey (Bazar et al., 2016). Data processing with different statistical analyses - for instance discriminant analysis (DA) or principal component analysis (PCA) – are suitable for detecting differences between floral and geographical origins. These can also support identification of adulterated honeys measured by NIR spectroscopy (Guelpa et al.). Although Hungary is one of the main honey importer countries, data referring to Hungarian honeys is very limited.

2. Materials and methods

2.1 Honey samples

In this study 79 honey samples of different floral and geographical origin were analyzed. To ensure authenticity, honeys were collected mainly from the producers but we have also procured some commercial products for comparison. All of the 60 Hungarian honeys are from honey producers, including samples of Acacia, False Indigobush, Honeydew, Pine, Chestnut, Buckwheat, Linden, Linden-chestnut, Raspberry, Milk Thistle, Mustard, Sunflower, Canola, Canola-fruit,

Figure 1. Map of the geographical origin of Hungarian honeys

Canola-Linden, Bear's garlic, Meadow Sage, Silkgrass and multiflora honeys. We also tested some samples from other European countries and samples of non-European origin. Hungarian honeys were collected from different parts of Hungary (Figure 1). The samples were stored at room temperature.

2.2 Methods

2.2.1 Physicochemical properties

Classical honey analytical parameters (ash, pH, conductivity, total water soluble matter by refractometry) were determined according to the International Honey Committee methods (Bogdanov et al., 2002).

2.2.2 Total polyphenol content and antioxidant capacity

Sample preparation

~1 g of honey sample was diluted 10 times with distilled water and this solution was used for antioxidant measurements. Spectrophotometric measurements were performed on a Thermo Helios Alpha UV-VIS spectrophotometer (±0.001 au), using cells of 1 cm path.

Total polyphenol content

Total polyphenol content (TPC) was determined by the Folin–Ciocalteu method, following a procedure adapted from (Singleton & Rossi, 1965). 1 ml of the honey sample solution was put in a test tube, and 7.5 ml distilled water was added. Then 0.5 ml of the Folin–Ciocalteu reagent was given to each tube and after 3 minutes 1 ml Na_2CO_3 solution was added. Absorbances were read at 750 nm after a 30-minutes incubation period in the dark. Results were expressed as mg equivalents of gallic acid (GAE) per 100 g honey.

FRAP- Ferric Reducing Antioxidant Power assay

In FRAP experiments, the reduction power of honeys was estimated according to the procedure described by Benzie & Strain. (1996). The freshly prepared FRAP reagent contained 25.0 ml of 300 mM acetate buffer, 2.5 ml of 10 mM TPTZ solution in 40mM HCl, 2.5 ml of 20 mM iron (III) chloride solution. For the measurements 500 µl honey stock solution was given to 15 ml of FRAP reagent, rigorously shaken, incubated at 37 °C for an hour and measured at 653 nm against a blank. Results were reported as µmol ascorbic acid equivalent (ASE) /g honey.

2.2.3 NIR spectrometer

NIR absorption spectra of honey samples were recorded by Spectralyzer (PMC Spectralyzer 10-25 infrared spectrophotometer) in the range between 1000 and 2500 nm with 2 nm spectral resolution. Illumination and observation geometry is 0/45°. The holographic grating of the instrument includes color filter two ranges (1000-1600 nm and 1600-2500 nm).

Two series were analyzed by the NIR instrument. Reference spectrum was taken before every sample. Each honey sample was measured with three consecutive measurement. The layer thickness of the tested honey sample was 0.4 mm.

Statistical analysis

The R-project software was applied for data processing and analysis of the absorption spectra of the samples using the spectral range between 1100 and 1800 nm. Smoothing with Savitzky-Golay smoothing filter (Press & Numerical Recipes, 1992) (2nd order polynomial and 21 points) and multiplicative scatter correction (MSC) (Næs et al., 2002) were used as pretreatment before the multivariate data evaluation. The second derivative spectra were also calculated with Savitzky-Golay smoothing filter (21 points) for visualization of the absorption peaks.

Principal component analysis (PCA) (Cowe & McNicol 1985) was used to detect patterns and to visualize the information present in the multivariate spectral dataset. Linear discriminant analyses (LDA) was used to develop classification models for identification of floral and geographical origin of the honey samples.

3. Results and discussion

3.1 Results of the classical analytical methods

These indicators, obtained in the preliminary measurements of the honey samples, are used for characterization of honeys and interpretation of the data obtained by NIR.

FRAP-Ferric Reducing Antioxidant Power results

The antioxidant capacity and polyphenol content are belonging to important properties of honey. According to the literature for honeys from the same floral origin similar values were shown, but significant variances were found between honeys from different botanical sources, especially honeydew honeys. Low ferric reducing antioxidant power were measured in acacia and canola honeys (average: 6.576 mg ± 1.450 ASS/100g, respectively 18.036± 2.643 mg ASS/100g). Higher capacity

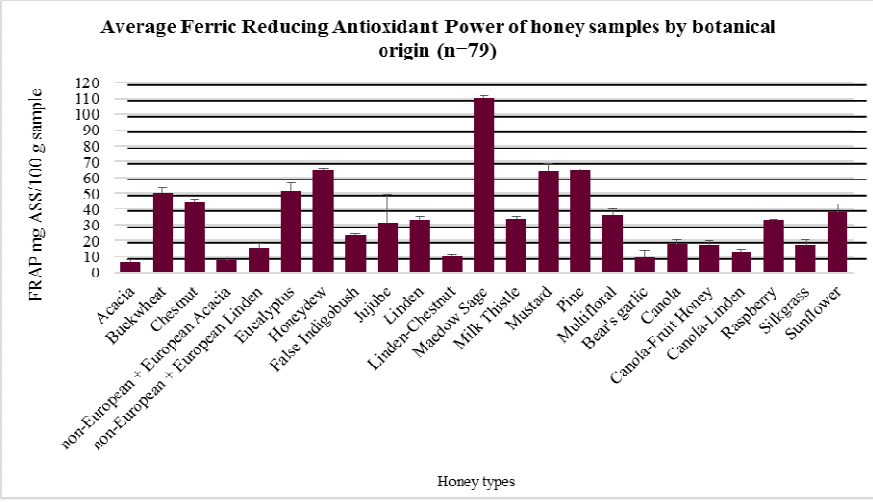

Figure 2. Average and standard deviation of FRAP antioxidant capacity of the tested honey samples grouped by botanical origin (n = 79)

were found in honeydew and chestnut honeys (average: 44.86 ±1.393 mg ASS/100g, respectively 64.808 ±1.5 mg G ASS/100g) ( ). Comparable trends were observed in total polyphenol content values.

Physicochemical properties

Target physicochemical parameters used for honey characterization. Average values for some selected honeys are given in Table 1.

Table 1. Physicochemical property averages for honey samples

	Ash content %	Refraction/total dry material %	pH	Electrical conductivity (µS/cm)
Acacia	0.041 (±0.003)	81.78 (±0.016)	3.932 (±0.007)	155.9 (±0.298)
Honeydew	0.449 (±0.149)	81.42 (±0.311)	4.024 (±0.003)	573.0 (±0.842)
Linden	0.168 (±0.003)	81.53 (±0.035)	4.110 (±0.030)	416.5 (±2.775)
Sunflower	0.155 (±0.006)	80.84 (±0.017)	3.762 (±0.008)	479.9 (±0.833)

Results of NIR spectroscopy

Smoothed and msc transformed and 2nd derivative absorption spectra of the tested honey samples are shown in Figure 3. The peaks of the 2nd derivative graphs reveal the underlying peaks of the overlapped bands presenting the peaks of water (1358, 1580 nm (Segtnan et al., 2001) and C–H stretching in sugars (1690, 1732, 1780 nm (Golic et al., 2003)

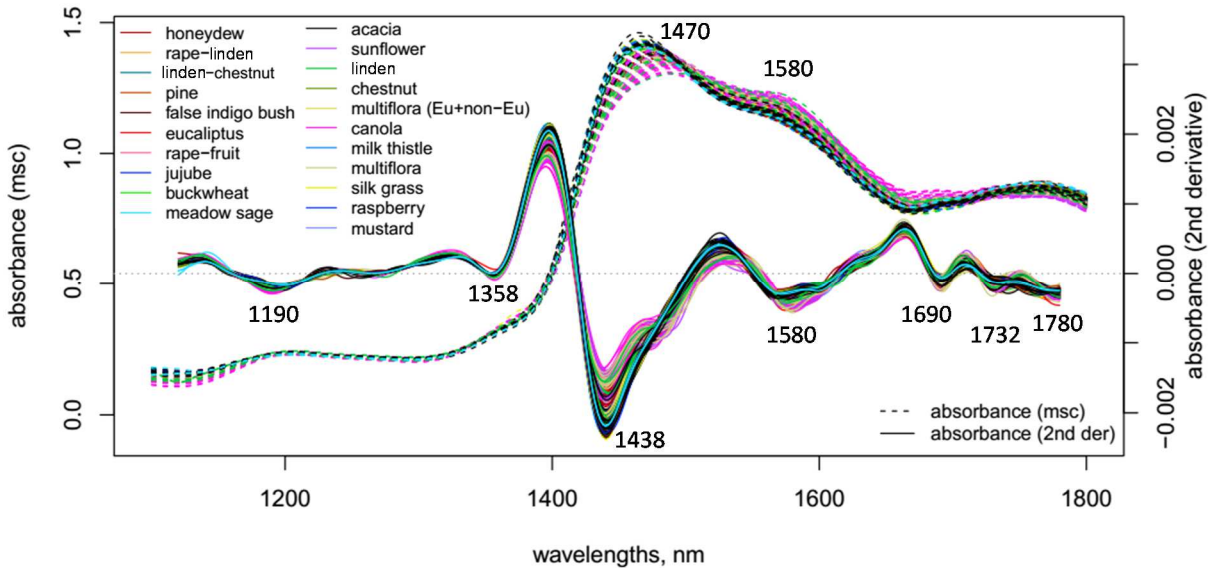

Figure 3. Smoothed (Savitzky-Golay 21 points) and MSC transformed spectra and 2nd derivative (Savitzky-Golay 21 points) spectra of the analyzed honey samples (n = 309)

Results of the PCA calculation based on the spectral data of the entire sample set (Figure 4) provides some separation of the different sample groups based on floral and geographical origin as well.

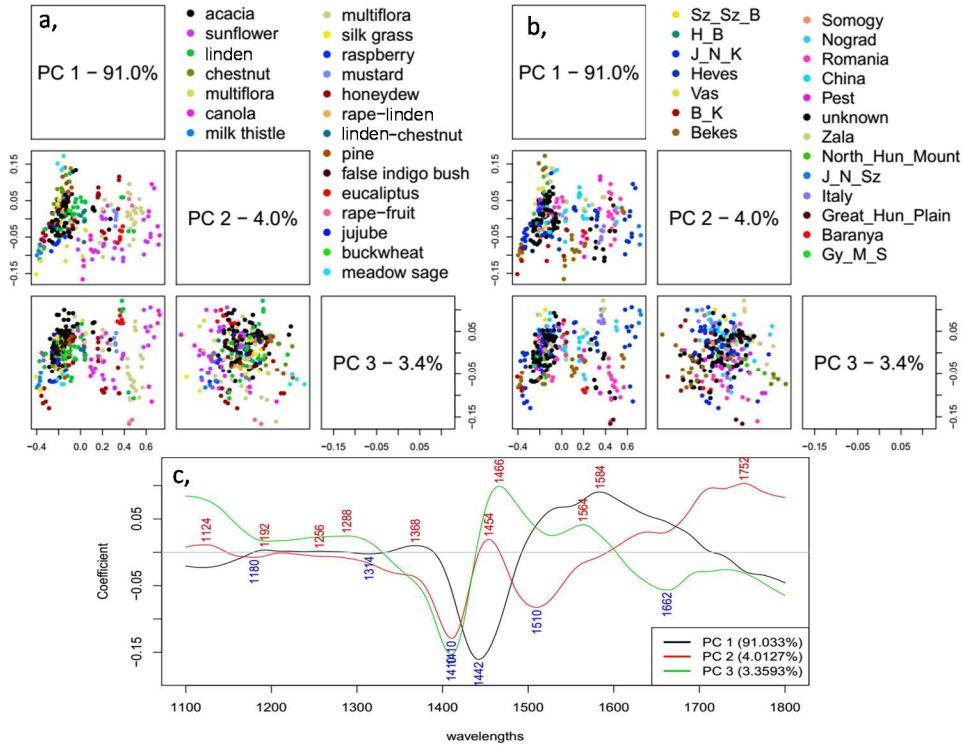

Figure 4. PCA score plots presenting the separation of the different (a) floral and (b) geographical groups and loadings (c) of PC1-PC3 calculated on the entire sample set after eliminating the outliers (n = 309)

PC1 presenting 91% of the spectral variation shows the biggest difference between the groups of canola and sunflower samples from the rest of the samples.

Floral types with at least 5 samples were selected to build classification models with LDA to test the ability of NIRS for floral origin identification.

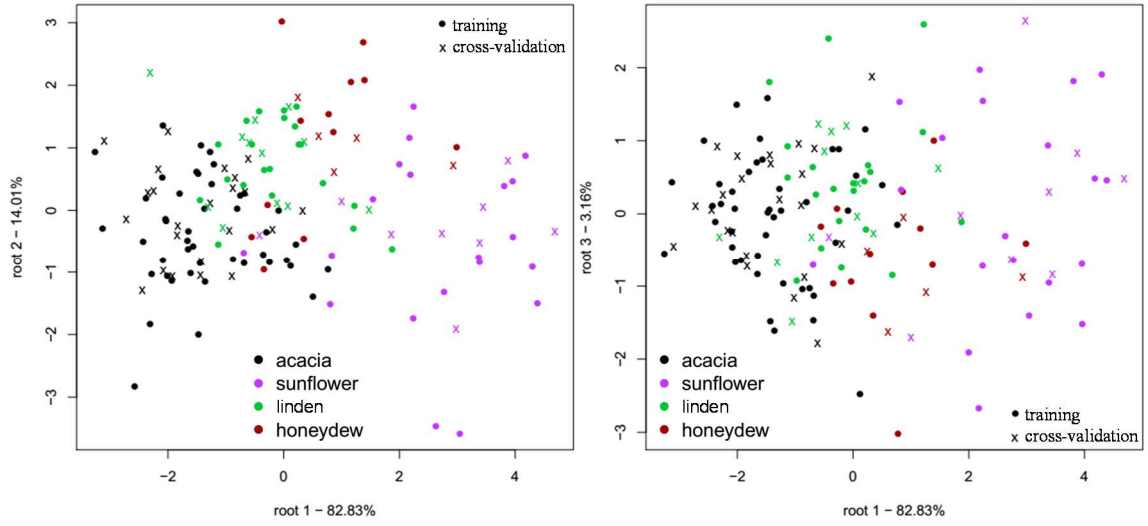

Figure 5. LDA score plots presenting the separation of the different (a) floral and (b) geographical groups and loadings (c) of PC1-PC3 calculated on the entire sample set after eliminating the outliers (n = 309)

Groups of sunflower honey samples showed the best separation from the rest of the sample groups based on root 1, presenting nearly 83% of between group variance (Figure 5). The LDA model presented average recognition and prediction abilities of 81% and 75%, respectively (Table 2) for the classification of Acacia, Honeydew, Linden and Sunflower samples.

Table 2. LDA results for discriminating honey floral origin using NIRS

MODEL BUILDING (%)				
	Acacia	Honeydew	Linden	Sunflower
Acacia	**97.02**	17.65	16.68	6.88
Honeydew	0.74	**64.70**	1.50	5.17
Linden	2.24	8.83	**77.27**	3.47
Sunflower	0.00	8.83	4.55	**84.48**
MODEL VALIDATION (%)				
	Acacia	Honeydew	Linden	Sunflower
Acacia	**89.53**	11.82	18.17	13.77
Honeydew	3.00	**64.73**	6.09	6.94
Linden	4.48	17.64	**69.66**	3.42
Sunflower	3.00	5.82	6.09	**75.88**

Classification models were also developed for the discrimination of the honey samples based on their geographical origin for the individual floral types. Results summarized in Table present the classification performance for sunflower samples providing average recognition and prediction abilities of 93% and 86%, respectively.

Table 3. LDA results for discriminating sunflower honey geographical origin using NIRS

MODEL BUILDING (%)			
	Bekes	Heves	Nograd
Bekes	**100.00**	0.00	0.00
Heves	0.00	**96.19**	16.75
Nograd	0.00	3.81	**83.25**
MODEL VALIDATION (%)			
	Bekes	Heves	Nograd
Bekes	**100.00**	0.00	0.00
Heves	0.00	**92.38**	33.50
Nograd	0.00	7.62	**66.50**

4. Conclusion

The high nutraceutical value of Hungarian honeys was proven by presenting their high antioxidant activities. It has been shown that there are significant differences between samples of different botanical origin. These origin-related differences are in good correlation with those reported for honeys from other geographical regions and showed the same trends as those observed in NIR data.

NIR spectra of the honey samples provide information about the chemical composition of honey which can be used for identification of the samples originating from different floral or geographical sources. Classification model built for the identification of the floral origin of

the main honey types provided average recognition and prediction abilities of 81% and 75%, respectively. Identification of geographical origin of the honey samples was also possible with satisfactory accuracy for the individual floral types.

Our results show the application of NIR spectroscopy might have a great importance in the authentication of honeys, however, further experiments are proposed to build up a robust spectrum library which can serve as a base of more sophisticated chemometrics models.

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