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Honey authentication based on physicochemical parameters and phenolic compounds

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ABSTRACT

The aim of this study is to assess the usefulness of physicochemical parameters (pH, water activity, free acidity, refraction index, Brix, moisture content and ash content), color parameters (L*, a*, b*, chroma, hue angle and yellow index) and phenolics (quercetin, apigenin, myricetin, isorhamnetin, kaempherol, caffeic acid, chrysin, galangin, luteolin, *p*-coumaric acid, gallic acid and pinocembrin) in view of classifying honeys according to their botanical origin (acacia, tilia, sunflower, honeydew and polyfloral). Thus, the classification of honeys has been made using the principal component analysis (PCA), linear discriminant analysis (LDA) and artificial neural networks (ANN). The multilayer perceptron network with 2 hidden layers classified correctly 94.8% of the cross validated samples.

1. Introduction

Honey is an ancient food, which is largely consumed due to its nutritional, medicinal and cosmetic properties. The high value of honey is given by its nutritional value, macro and microelements and many other compounds it contains (Jasicka-Misiak et al., 2012). The honey composition (sugars, organic acids, enzymes, vitamins, proteins and phytochemicals) is influenced by the botanical and geographical origin and environmental climatic conditions (Baroni et al., 2015; Solayman et al., 2016). Glucose and fructose are the major sugars present in honey, but there have been reported smaller quantities of twenty-two other compounds (e.g. maltose, sucrose, maltulose, turanose, isomaltose, etc.) (Siddiqui et al., 2017). Honey contains different types of enzymes such as: oxidase, catalyse, acid phospatase, invertase and diastase, which make it unique in the sweeteners domain. Moisture content, reducing sugars, free acids, electrical conductivity, sucrose content and 5-HMF influence nutritional quality, granulation, flavor and texture parameters. In addition to the previously mentioned compounds, phyto-chemical compounds present in honey play a major role in determining the antioxidant activity, which can be correlated with the anti-inflammatory, anti-carcinogenic, anti-thrombotic, anti-atherogenic activity of honey (Piljac-Žegarac et al., 2009). Among the phyto-chemical compounds present in honey, the phenolic compounds play a major role in the antioxidant activity. The phenolic compounds found in honey are free phenols, phenolic acids, polyphenols (usually in the form of flavonoids), anthocyanins, procyanidins and pigments. Their total content depends on the species of plant from which bees collected the nectar and their amount varies from 5 to 1300 mg/kg (Mattonai et al., 2016; Mellen et al., 2015).

The necessity for determining some parameters in terms of the botanical or geographical authentication of honeys derives from the increasing demand for mono-floral honeys on markets. Mono-floral honeys are more expensive than multi-floral ones. A honey can be mono-floral or polyfloral in origin depending on whether it is derived from one or several plant species. According to the international food standards, for a honey to be labelled with floral origin, it must originate wholly or predominantly from a particular floral source and display the corresponding organoleptic, physicochemical and microscopic properties (Codex Alimentarius, 2001). Adulteration of honey can be determined using the quality parameters, and these parameters can confirm the hygiene conditions for the manipulation and storage of honey (da Silva et al., 2016).

The authentication of honey has started with the melissopalynological method, which can be used for botanical authentication (Karabagias et al., 2014). An alternative for honey authentication is the combination of melissopalynological method with physicochemical parameters (Oroian et al., 2015a; Karabagias et al., 2014; Escriche et al., 2014; Juan-Borrás et al., 2014). Over the last decades there have been implemented different methods for the authentication of honey such as: e-tongue and optical

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spectroscopy (Ulloa et al., 2013), potentiometric and voltammetric electronic tongue (Wei and Wang, 2014), headspace volatile profile (Oroian et al., 2015a), phenolic compounds, physicochemical parameters and chemometrics (Karabagias et al., 2014), mineral profile (Oroian et al., 2015b), NIR spectroscopy (Guelpa et al., 2017).

The huge data resulted from the physicochemical properties, volatile fraction, e-tongue, mineral profile, NIR spectroscopy, etc., cannot be used for honey authentication without applying proper statistical methods. The statistical methods have been used to study the usefulness of different parameters in the authentication of honeys. Over the last decades there have been used different statistical methods for the honey authentication such as: principal component analysis (Wei and Wang, 2014; Oroian et al., 2015a, b, Ulloa et al., 2013), discriminant analysis (Wei and Wang, 2014), least square discriminant analysis (Guelpa et al., 2017), cluster analysis (Ulloa et al., 2013), artificial neural networks (Ramzi et al., 2015).

There are only a few systematic studies on the classification of Romanian honeys according to the botanical origin using the mineral content (Oroian et al., 2015b), volatile compounds (Oroian et al., 2015a), stable isotope (Dinca et al., 2015), rheological parameters (Dobre et al., 2012) and physicochemical parameters (Marghitas et al., 2010).

The purpose of this paper is to investigate the usefulness of phenolics and physicochemical parameters for the authentication of acacia, tilia, sunflower, polyfloral and honeydew from Romania.

2. Materials and methods

2.1. Materials

50 honey samples of five different botanical origins (acacia, tilia, sunflower, honeydew and polyfloral) have been purchased from local beekeepers from Suceava County, Romania. Quercetin, apigenin, myricetin, isorhamnetin, kaempherol, caffeic acid, chrysin, galangin, luteolin, *p*-coumaric acid, gallic acid and pinocembrin have been purchased from Plant MetaChem (Germany). Amberlite XAD-2 resin, methanol, HCl, diethyl ether, membrane filter 0.45 μm have been purchased form Sigma Aldrich (Germany).

2.2. Methods

2.2.1. Melissopalynological analysis

The pollen analysis was made according to the method of Louveaux et al. (1970), using a non-acetolytic method. Ten grams of honey were mixed with about 40 ml of distilled water; then, centrifuged at 4500 rpm (3383*g*) for 15 min and the supernatant was carefully removed. The residue was re-dissolved again and centrifuged for other 15 min. The full sediment was used to prepare the slide. The pollen spectrum of each honey sample was determined by a light microscopy (Motic × 40) by counting at least 800 pollen grains. For all pollen types the individual occurrence was expressed as percentage (Dobre et al., 2012).

2.2.2. Physicochemical properties

Moisture content, pH, refraction index, Brix concentration, electrical conductivity and ash content have been determined using the Harmonised methods of the international honey commission (Bogdanov et al., 2002). Water activity was measured using a water activity meter AquaLab Lite (Decagon, USA).

Colour has been determined using a Konica CR400 cromameter (Konica Minolta, Japan). The samples were placed in a 20 mm vat and they were measured to a white spectrum. The color intensity, hue angle and yellow index (YI) were computed as follows:

$$c^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h* = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

$$YI = \frac{142.86 \cdot b*}{L*} \quad (3)$$

2.2.3. Phenolics extraction

The phenolics extraction was made using the method described by Baltrušaitytė et al. (2007) and Escriche et al. (2014). Sixty grams of Amberlite XAD-2 resin, pore size 9 nm, and particle size 0.3–1.2 mm were soaked in methanol for 10 min, then, the most of methanol was decanted and replaced by distilled water. The mixture was stirred, allowed to stand for 5–10 min and packed into a glass column, 25 × 2 cm. The honey samples (25 g) were thoroughly mixed with 250 mL of distilled water and adjusted to pH 2 by concentrated HCl. The solution was slowly filtered through the column packed as previously described. The column was washed with 250 mL of acidified water (pH 2 with HCl) and subsequently rinsed with 300 mL of neutral distilled water to remove all sugars and other polar compounds of honey. The flavonoids and phenolic compounds were eluted from the sorbent with 250 mL of methanol. The methanol extracts were concentrated under vacuum at 40 °C in a rotary evaporator. The residue was dissolved in 5 mL of distilled water and extracted three times with 5 mL of diethyl ether. The dried residue was then re-dissolved in 1 mL of methanol (HPLC grade) and filtered through a membrane filter with a 0.45 μm pore size. Three replicate extractions were performed for each sample.

2.2.4. HPLC analysis of phenolics

The phenolic compounds were separated and quantified using the method described by Coneac et al. (2008). A High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan) system equipped with a LC-20 AD liquid chromatograph, SIL-20A auto samples, CTO-20AC auto sampler and a SPD-M-20A diode array detector was used. The separation was carried out on a Zorbax SP-C18 column, with 150 mm length, 4.6 mm i.d., and 5 μm-diameter particle was used; the phenolics detection was set at 200 nm and 210 nm. The mobile phase was acetonitrile: water ratio 48:52, temperature was of 25 °C, with a flow of 0.3 ml/min, the injected sample volume was of 20 μl. The diluted standard solutions of quercetin, apigenin, myricetin, isorhamnetin, kaempherol, caffeic acid, chrysin, galangin, luteolin, *p*-coumaric acid, gallic acid and pinocembrin were analyzed under the same HPLC conditions and furthermore the calibration of the detector response was made. Data collection and subsequent processing were performed using the LC solution software 1.21 version (Shimadzu, Kyoto, Japan).

The phenolics were identified by comparing the chromatographic retention times and UV spectra of each compound. The calibration curves were constructed via least-squares linear regression analyses of the ratio of the peak area of each representative compound versus the respective concentration. The regression analysis (n = 5) showed higher correlation coefficients (R^2) higher than 0.99 for all the compounds. The quantitative results were expressed as mg of compound per 100 g honey.

2.3. Statistical analysis

The statistical analysis was performed using Unscrambler X 10.1 software system (Camo, Norway). The data corresponding to each variable were analyzed by one-factor analysis of variance