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Classification of raw Ethiopian honeys using front face fluorescence spectra with multivariate analysis

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ABSTRACT

Front face fluorescence measurements were carried out to classify raw honeys as such based on their floral origins. The excitation-emission matrix patterns of mixed flower, pseudoacacia, arabica, fialsindica, and amygdalina raw honeys along with fake honey sample from the market were examined by recording emission wavelength from 250 to 600 nm with excitation wavelength in the range of 200 −550 nm. The spectra of fake honey samples demonstrated low intensity and did not fit within any one of the classified raw honeys. The multivariate analyses of the spectra were performed using principal component analysis and soft independent modeling of class analogy (SIMCA). The SIMCA model showed that the adulterate honey samples were detected with 100% sensitivity and specificity.

1. Introduction

Honey is a natural sweet substance produced by bees from the nectar of flowers. It consists mostly of sugars, water and a large number of minor constituents like pollen grains, various amino acids, lipids, and flavoring components (Cereser Camara & Laux, 2010). Natural honey can be defined as either polyfloral, if it contains nectar from different plant species or monofloral, if it consist of more than 45% of pollen concentration from one plant species (Bryant & Jones, 2001).

The quality of honey samples is currently judged based on the physicochemical parameters such as acidity, electrical conductivity, water content, hydroxymethylfurfural concentration, and sugar composition (Codex Alimentarious & EU Directive 110/2001). These parameters are determined by using various analytical methods with different instruments such as pH meter, conductometer, refractometer and high performance liquid chromatography (HPLC) which are laborious and time consuming, and consequently limiting the number of honey-sample analysis.

Authenticity problem in the honey market has prompted establishment of an easy and fast instrumental techniques such as FT-Raman (Ozbalci, Boyalci, Topcu, Kadilar, & Tamer, 2013; Pierna, Abbas, Dardenne, & Baeten, 2011), Near IR (Chen, Huang, & Chen, 2014; Chen et al., 2012; Zhelyazkova, Atanasova, & Elencheva-Karaneycheva, 2013), and FT-IR (Gok, Severcan, Goormaghtigh, Kandemir, & Severcan, 2015; Ruoff, Iglesias, Luginbuhl, Bosset, Bogdanov, & Amado, 2006; Svecnjak, Bubalo, Baranovic, & Novosel, 2015). However, these techniques are prone to low sensitivity, low practicality and spectral interferences. The presence of fluorophores in honey samples gives rise to fluorescence spectroscopy as an alternative technique to check honey adulteration and classify honey samples based on their botanical origins.

Fluorescence spectra obtained from biological samples are often distorted by strong light absorption and scattering-inner filter effect. In order to suppress this inner filter effect, several fluorescence spectroscopic techniques based on front face modes have been developed for honey analysis (Ruoff et al., 2005; Ruoff, Luginbuhl, Kunzli, Bogdanov, Bosset, Von der Ohe, & Amado, 2006; Karoui, Dufour, Bosset, & Baerdemaeker, 2007). All of them have shown the potential of front face fluorescence as rapid screening tool for honey samples in correlating their botanical origins. Synchronous fluorescence coupled with multivariate analysis was used to classify honey samples based on their floral origin (Lenhardt, Zekovic, Dramicanin, Dramicanin, & Bro, 2014). Recently a three dimensional excitation emission matrix (EEM) combined with parallel

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factor analysis (PARAFAC) has been developed for characterization and classification of honeys (Lenhardt, Bro, Zekovic, Dramicanin & Dramicanin, 2015). However, the analysis of the honey samples was performed after liquefying them at elevated temperature (40 °C) in the front face fluorescence measurements which could limit on-line monitoring of honey samples in the market. Hence, fluorescence measurement of honey without any sample processing is needed for its practical characterization and classification.

The aim of this study is to explore the potential front face fluorescence measurements to classify raw honey samples collected from tropical region of various floral origins and check the authenticity of honeys in Addis Ababa market, Ethiopia. The front face fluorescence measurements were carried out without treatment of the semi-solid honey samples to elevated temperature with incidence angle of the excitation radiation set at 30°. In order to cope up with honey matrix complexity effectively, multivariate analysis was used in this investigation.

2. Experimental

2.1. Samples

The honey samples used in this project were collected from different parts of Ethiopia during two harvest sessions from November 2015 to early March 2016. The botanical origin was determined based on the information of the small-scale honey producers. Preliminary tests on adulterate samples were performed by doing NMR experiment whether sucrose was artificially added to the samples. It was found that there was not much difference in the sucrose contents between the market honeys and raw honeys, indicating that the samples may be adulterated by sugar feeding. The samples were mixed flower (n = 36), pseudoacacia (*Robinia pseudoacacia*) (n = 10), arabica (*Coffee Arabica*) (n = 24), fials-indica (*Opuntia fials-indica*) (n = 10), amygdalina (*Vernonia amygdalina*) (n = 20) and adulterate honey sample (n = 20). Prior to fluorescence measurement, the honey samples were stored at 21± 2 °C for several days to reach equilibrium temperature. The experiments were performed with five common varieties of Ethiopian raw honey shown in Fig. 1.

The images of different types of honey with color camera (Canon, Japan) were taken using image acquisition setup (Fig. 2). Here polarizing (PL) filters in front of the color camera (F no. 4.5, shutter speed 1/15, focal length 55) as well as halogen lamp equipped with polarizing filter were used in order to allow only diffused light on the honeys enters into the camera so that image acquisition of honey samples without halation is efficient and the various colors of honey samples are clearly depicted.

2.2. Spectral acquisition

The EEM spectra were measured using front face fluorescence spectroscopy on JASCO spectrofluorometer (FP-8300, Japan) at room temperature. A 0.312 ± 0.04 g of semi-solid honey sample as such was placed in a sample holder and the incidence angle of the excitation light was set at 30° to avoid specular reflection from the detector. The spectra were obtained by recording the emission wavelength (from 250 to 600 nm at 1 nm intervals) and excitation wavelength (from 200 to 550 nm at 10 nm) with low sensitivity. Spectral correction was made in order to suppress the instrumental distortion in excitation using a rhodamine cell in the reference channel and triplicate spectra were recorded for different samples. The fluorescence signals in all measured wavelengths were calibrated to Raman unit (R.U.) (Lawaetz & Stedmon, 2009). In brief, Raman scatter band of pure water was measured by the spectrofluorometer and then the integral of the measured Raman peak was used to normalize the excitation-emission intensity of the fluorescence spectra. This helps to minimize the matrices of different floral sources of the various honey samples.

2.3. Multivariate analysis methods

Principal component analysis (PCA) and Soft independent modeling of class analogy (SIMCA) was carried out using Unscrambler software package (Version 9.7, CAMO, Norway). Prior to the classification, all samples were divided randomly into training set 60% (72 samples) and prediction set 40% (48 samples). PCA is a

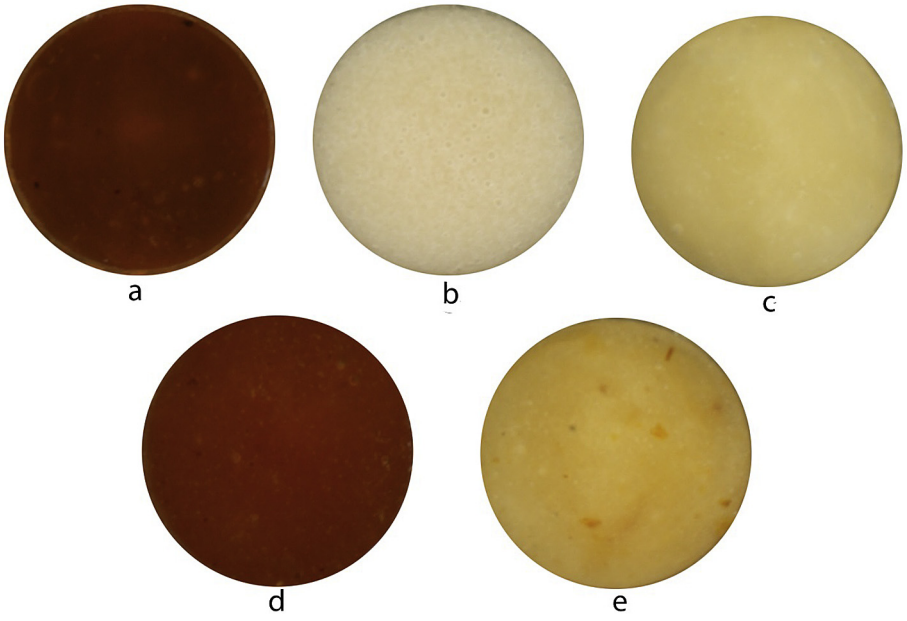

Fig. 1. Typical raw honey samples: a) amygdalinar, b) arabica, c)fials-indica, d) mixed flower, and e) pseudoacacia.