PROCEEDINGS

27th International Conference Ecological Truth and Environmental Research

EDITOR

Prof. Dr Snežana Šerbula

18-21 June 2019, Hotel Jezero, Bor Lake, Serbia

PROCEEDINGS

27th INTERNATIONAL CONFERENCE

ECOLOGICAL TRUTH AND ENVIRONMENTAL RESEARCH – EcoTER'19

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Publisher: University of Belgrade, Technical Faculty in Bor

For the Publisher: Dean Prof. Dr Nada Štrbac

Printed: TERCIJA DOO, Bor, 150 copies

Year of publication: 2019

ISBN 978-86-6305-097-6

CIP - Каталогизација у публикацији - Народна библиотека Србије, Београд

502/504(082)(0.034.2)
613(082)(0.034.2)

МЕЂУНАРОДНА конференција Еколошка истина и истраживање животне средине (27 ; 2019 ; Бор)
Proceedings [Elektronski izvor] / 27th International Conference Ecological Truth and Environmental Research - EcoTER'19, 18-21 June 2019, Bor Lake, Serbia ; editor Snežana Šerbula. - Bor : University of Belgrade, Technical faculty, 2019 (Bor : Tercija). - 1 USB fleš memorija ; 9 x 6 cm (u obliku kartice)

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 150. - Bibliografija uz svaki rad.

ISBN 978-86-6305-097-6

a) Животна средина - Заштита - Зборници b) Здравље - Заштита - Зборници
COBISS.SR-ID 277159692

27th International Conference
Ecological Truth and Environmental Research 2019

is organized by:

UNIVERSITY OF BELGRADE, TECHNICAL FACULTY IN BOR (SERBIA)

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FLUORESCENCE SPECTROSCOPY AND PRINCIPAL COMPONENT ANALYSIS IN THE HONEY SAMPLES CLASSIFICATION

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Abstract

Steady state fluorescence spectroscopy in combination with Principal Component Analysis (PCA) for spectral analysis was used to differentiate multifloral honeys from different parts of Serbia. The emission spectra were recorded in the wavelength range 280 – 550 nm, after excitation in the 270 – 370 nm range. After normalization of the spectra, chemometric evaluation of the spectral data was carried out using principal component analysis (PCA). This study indicates that front-face fluorescence spectroscopy is a promising technique for the authentication of geographical origin of honey and may also be useful for determination of the botanical origin within the same unifloral honey type.

Keywords: honey, spectrofluorometry, Principal Component Analysis

INTRODUCTION

Honey is a pure natural food produced by bees from the nectar of flowers. Honey can be considered as sugar syrup, mainly composed of fructose and glucose, along with some proteins, free amino acids, enzymes, vitamins, polyphenols and minerals.

Honey has intrinsic emission properties, which are reportedly attributed to a mixture of fluorophores, like amino acids, vitamins and polyphenols. The positions of emission maxima of the phenolic components vary for various honey samples, but they are in the same emission range 415–450 nm. Component related to the proteins and syringic acid emit at 340 nm and 370 nm, respectively [1–3].

Spectroscopic techniques are fast, relatively low–cost, and provide considerable information about the sample with only one test. They are considered as sensitive, non–destructive, rapid, environmentally friendly, and non–invasive.

The fluorescence spectra, in combination with appropriate statistical methods, may provide useful fingerprints in food analysis [4]. Steady state fluorescence spectroscopy in combination with Principal Component Analysis (PCA) for spectral analysis has been applied to differentiate samples of honey. We have chosen the samples of multifloral honey from different parts of Serbia.

MATERIALS AND METHODS

In this pilot study, eight honey samples were analyzed. All samples are multifloral honeys, collected in 2013, and were obtained from 8 beekeepers from different parts of Serbia. Samples were stored at room temperature in the dark before analysis.

Fluorescence spectroscopy

The fluorescence spectra of the honey samples were recorded using a Fl3–221 P spectrofluorimeter (JobinYvon, Horiba, France), equipped with a 450W Xe lamp and a photomultiplier tube. The sample was placed in solid sample holder, in front-face configuration. The illumination's incident angle was set to 35°C, to minimize light reflections, scattered radiation and depolarization phenomena. The Reyleigh masking was applied in order to reduce Rayleigh scattering from the solid sample which limits the sensitivity and accuracy of the measurement. The fluorescence emission spectra in range from 280 to 550 nm, were recorded with excitation wavelengths of 270 to 370 nm. The integration time was 0.1 s, and the wavelength increment in excitation measurements was 5 nm, and emission increment was 1 nm. A spectral band width of 2 nm was employed for both the excitation and emission slits.

Statistical analysis

The PCA was used to classify honey samples according to the differences in characteristic emission spectra. Principal Component Analysis (PCA) is an application of chemometrics used as a tool in exploratory analysis, which applies algorithms and being designed to reduce large complex data sets, or rearranges the data to exploit linear structure. PCA is a technique using mathematical procedures, such as orthogonal linear transformation from original data. The transformation of new data must have correlation between the new variable, called Principal Components (PCs). PCA was performed by using Unscrambler software (X10). For each sample the average of the 18 emission spectra recorded for various excitation wavelengths was used as the input value in PCA, in order to take into account contribution of all fluorophores present in the sample.

RESULTS AND DISCUSSION

The excitation-emission spectral series were recorded for various excitation wavelengths for each sample. Figure 1a shows excitation–emission spectral series for one of the honey samples. The averaged normalized emission spectra for different samples (Figure 1b), enabled the study of the main emitting compounds in honey, which are the base for estimation of differences between the samples. The spectral shapes, number, and positions of the emission maxima differed among the samples.

All honey samples have maximum in the range 400–450 nm. As phenolic and polyphenolic compounds have been described as reliable indicators of botanical and geographical origin of honeys [5–8] the fluorescence properties of these intrinsic and unique fluorophores may inform identification of floral source reliably.

The PCA was used to classify honey samples according to the differences in characteristic emission spectrum. The dependent variables were honey samples of different geographic origin. The independent variables were the recorded fluorescence emission spectra.

Principal component analysis of honey samples suggested that a two–component model explains 98% of total variance (PC1 accounted for 86% and PC2 for 12%). The scores plot is shown in Figure 2. The PCA scores plot discriminated four groups of samples, which correspond to their geographic origin. This grouping is based on the similarity of the spectra of the samples in corresponding groups. Zlatar, Zlatibor and Tara (Western Serbia) samples have emission maxima at 430nm; Avala and Šumadija (Central Serbia) have emission maxima at 438nm; Deliblatska peščara and Fruška gora (Vojvodina) have emission maxima at 425nm and sample from Kučevo (Eastern Serbia) has emission maxima at 420nm. Since all honey samples are multifloral, similarities of the spectra within the groups on PCA plot indicate differences in plant species at particular geographic regions.

a) *b)*

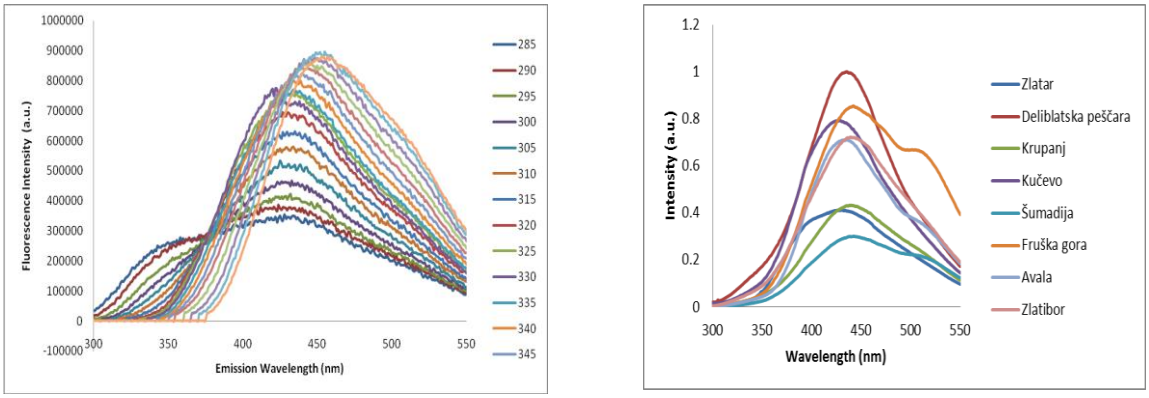

Figure 1 *a) Excitation-emission matrix for the raw spectra b) The normalized emission spectrum of each honey sample is an average of the 18 spectra recorded for various excitation wavelengths*

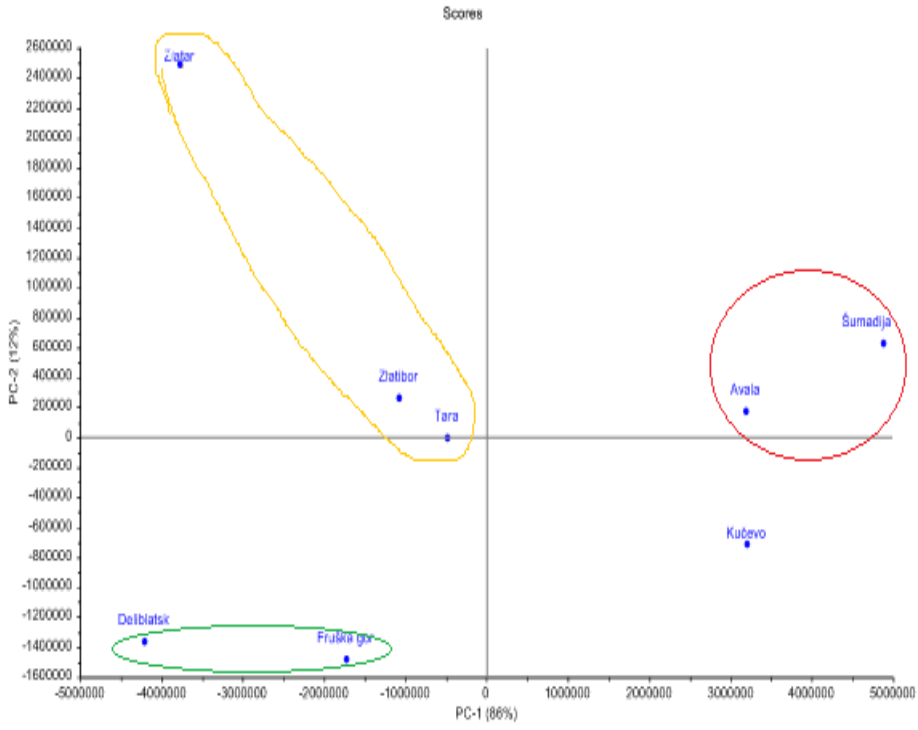

Figure 2 *Principal component analysis of the fluorescence emission spectra*

CONCLUSION

This preliminary study shows that front–face fluorescence spectroscopy combined with chemometrics offers a promising approach for the authentication of the geographical origin of honey. The technique is non-destructive, rapid, easy to use, and not expensive. It does not need any particular sample preparation. The measurements that we performed focused on the fluorescence of a small set of pure honey samples. However these preliminary findings should be confirmed with a larger set of samples and additional honey types.

ACKNOWLEDGEMENT

This work was financed by the grants OI173017 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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