APPLICATION OF ELECTRONIC NOSE IN HONEY GEOGRAPHICAL ORIGIN CHARACTERISATION

PRIMJENA ELEKTRONSKOG NOSA U ODREĐIVANJU GEOGRAFSKOG PODRIJETLA MEDA

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ABSTRACT

Honey volatile compounds were analysed by means of electronic nose, with the aim to capture honey aroma profile. 49 samples of black locust (Robinia pseudoacacia L.) and 16 of chestnut (Castanea sativa Mill.) honeys were analysed. Their botanical origin and physicochemical properties were analysed in accredited laboratory Geographical origin of samples has been known from interviews with beekeepers. Data obtained by electronic nose analysis were further analysed by principal component analysis (PCA) in order to determine differences among volatile profiles of samples with the same botanical origin but with different geographical origin. PCA result showed that honey samples from geographically close regions tend to group together, while those of distant geographical regions show differences though they are of the same botanical origin. Those results imply possibility to use electronic nose as a tool for honey geographical origin determination.

KEYWORDS: honey, electronic nose, geographical origin

SAŽETAK

Hlapive komponente meda analizirane su pomoću elektronskog nosa s ciljem utvrđivanja aromatskog profila. Ukupno je analizirano 49 uzoraka bagremovog meda (Robinia Pseudoacacia L.) i 16 uzoraka kestenovog (Castanea sativa Mill.) meda. Botaničko podrijetlo kao i fizikalno-kemijski parametri analizirani su u aktreditiranom laboratoriju, a geografsko podrijetlo uzoraka utvrđeno je od samih pčelara kroz razgovor. S ciljem utvrđivanja razlika među uzorcima istog botaničkog, ali različitog geografskog podrijetla podaci dobiveni analizom pomoću elektronskog nosa obrađeni su statistički i metodom analize osnovnih komponenata (PCA). PCA analiza je pokazala da uzorci iz geografsko bliskih regija pokazuju težnju grupiranja, dok uzorci sa udaljenijih lokacija pokazuju različitosti unatoč istom botaničkom podrijetlu. Ovakvi rezultati ukazuju na mogućnost primjene elektronskog nosa kao alata za određivanje geografskog podrijetla meda.

KLJUČNE RIJEČI: med, elektronski nos, geografsko podrijetlo

DETALJNI SAŽETAK

Hlapive komponente meda analizirane su pomoću elektronskog nosa NST 3320 s ciljem utvrđivanja aromatskog profila. Ukupno je analizirano 49 uzoraka bagremovog meda (Robinia Pseudoacacia L.) i 16 uzoraka kestenovog (Castanea sativa Mill.) meda prikupljenog od medara tijekom 2004. godine. Botaničko podrijetlo kao i fizikalno-kemijski parametri analizirani su u aktreditiranom laboratoriju za kontrolu kakvoće meda i drugih pčelinjih proizvoda na Prehrambeno-tehnološkom fakultetu u Osijeku. Geografsko podrijetlo uzoraka utvrđeno je kroz razgovor sa medarima (Tablice 1. i 2.). Zbog jednostavnosti uzorci su grupirani prema županijama iz kojih vode podrijetlo (Slika 1. i 2.). S ciljem utvrđivanja razlika među uzorcima istog botaničkog, ali različitog geografskog podrijetla podaci dobiveni analizom pomoću elektronskog nosa obrađeni su statistički i metodom analize osnovnih komponenata (PCA). Sva mjerenja provedena na elektronskom nosu NST3320 provedena su u triplikatu, te je određena srednja vrijednost mjerenja, koja je podvrgnuta PCA analizi pomoću modula programskog paketata STATISTICA. PCA analiza je pokazala da uzorci iz geografsko bliskih regija pokazuju težnju grupiranja, dok uzorci sa udaljenijih lokacija pokazuju različitosti unatoč istom botaničkom podrijetlu (Slika 3. i 4.). Ovakvi rezultati ukazuju na mogućnost primjene elektronskog nosa kao alata za određivanje geografskog podrijetla meda.

INTRODUCTION

Volatile compounds analysis by analytical methods with purpose of foods characterization mostly involves extraction followed by chromatographic separation [3,8,13]. Volatile compounds define the nature of a food and therefore contribute to consumer's preference of some product from a group of similar ones, and can be described as a flavour, which is generally accepted as the most important sensory characteristic of foods [5].

Still, sensorial analysis has certain limitations derived from the subjectivity of humans gathered into panels, and their possibility to percept slight differences [9].

Electronic nose, sensor array that mimics human olfactory system showed to be useful in food characterization and classification on the basis of non-specific volatile compounds profile. Different types of foods (vegetable oils, fruit juices, fruits, cheese, wines, chocolates) have been characterized by this method [4,5,7,10,12,16]. Application of electronic nose is also successfully been used for honey botanical origin determination and classification [1,2,6].

Honeys of same botanical origin have the same dominant volatile compounds originating from main nectar in honey, which is the basis for application of electronic nose for this purpose. But, besides the volatile compounds originating from the honey type declared floral source, unifloral honeys contain also nectar, and therefore volatile compounds, of other floral sources represented in smaller amounts.

These compounds differ on the basis of floral sources represented in geographical area of nectar collecting due to climatic and other conditions. Though human olfactory organs cannot differ among the same honey type samples from the various geographical regions, it is justified to expect that all samples from some region will have similar minor volatile compound profile traceable by electronic nose, which will differ from other regions.

Therefore, the purpose of this research was to evaluate the applicability of electronic nose on honey geographical origin determination.

MATERIALS AND METHODS

Honey samples

Study was conducted on 65 honey samples produced in season 2004 which were purchased directly from the beekeepers. Their botanical origin, as well as their physicochemical properties were analysed in accredited laboratory for the analyses of honey and other bees products at the Faculty of Food Technology in Osijek. 49 samples were declared as black locust (Robinia pseudoacacia L.) honey, and 16 samples as chestnut (Castanea sativa Mill.) honey, and all samples complied with the values prescribed by Croatian and International regulations [11,15].

Data on the geographical origin of samples have been collected from beekeepers through interview (Tables 1 and 2) and grouped according to the county of production (Figures 1 and 2).

Samples, once received, were stored at 4 °C until analysed and none of them was exposed to any treatment that might alter their composition.

Electronic nose analysis

Aromatic profile analyses were performed using electronic nose model 3320 (Applied Sensor Lab Emission Analyser; Applied Sensor Co., Linkoping, Sweden) consisted of an automatic sampling apparatus, a detector unit containing the array of sensors, and software for pattern recognition. The automatic sampling system supported a carousel of 12 sites for loading the samples under controlled temperature, and random sampling for analysis. The sensor array was composed of 22 different sensors ten of which were Metal Oxide Semiconductor

Table 1. Black locust honey samples
Tablica 1. Uzorci bagremovog meda

Sample Code Šifra uzorka	Sample production origin[a] Geografsko podrijetlo uzorka[a]	Area code Šifra geog. lokacije	Sample Code Šifra uzorka	Sample production origin[a] Geografsko podrijetlo uzorka[a]	Area code Šifra geog. lokacije
m32	Zagreb	1	m98	Ludbreg	42
m33	Zagreb	1	m107	Varaždin	42
m54	Vrbovec	1	m116	Varaždin	42
m123	Zagreb	1	m96	Garešnica	43
m02	Beli Manastir	31	m126	Veliki Zdenci	43
m22	Donji Miholjac	31	m13	Moslavačka gora	44
m37	Gajić	31	m84	Moslavačka gora	44
m57	Čeminac	31	m93	Kutina	44
m114	Beli Manastir	31	m101	Sisak	44
m115	Beli Manastir	31	m34	Križevci	48
m118	Bilje	31	m74	Bilogora	48
m129	Osijek	31	m26	Konjšćina	49
m45	Stari Jankovci	32	m78	Konjšćina	49
m81	Lovas	32	m83	Konjšćina	49
m88	Lovas	32	m94	Konjšćina	49
m137	Novigrad	32	m95	Bedekovčina	49
m49	Orahovica	33	b59	Bribir	51
m04	Pivnica	33	b62	Završje	51
m05	Nova Bukovica	33	m79	Motovun	52
m06	Nova Bukovica	33	m09	Karojba	52
m30	Našice	34	m10	Pazin	52
m132	Požega	34	m77	Livade	52
m76	Čakovec	40	m103	Grožnjan	52
m18	Varaždin	42	m141	Buje	52
m92	Ključ	42			

[a] Geographical origin of the honey as denominated by the beekeepers according to the location of hives / Geografsko podrijetlo meda na temelju iskaza pčelara o lokaciji košnica

Field Effect Transistors (MOSFET) and twelve Taguchi type sensors (Metal Oxide Semiconductors – MOS). The MOSFET sensors were divided into two arrays of five sensors each, one array operating at 140 °C and the other at 170 °C, while the MOS sensors were mounted in a separate chamber, and were kept at 400–500 ° C through all process phases.

Measurements were performed according the method previously created by Benedetti et al. [2]. Three grams of honey were placed in 40mL Pyrex® vials with silicone caps and then introduced inside the automatic sampling carousel of the electronic nose. After an equilibration time of 20 minutes at room temperature, the measurement sequence started with the following temperature phases: standby at 20 °C for 10 min followed by incubation at 40 °C for 5 min.

The measurement procedure consisted of pumping reference air (room air filtered through active carbon) over the sensors at a constant flow rate (1cm^3/s) for 10 s, in order to have a stable baseline. Then honey gas headspace sampled by an automatic syringe was pumped over the sensor surfaces for 30 s. The sensors were then exposed to reference air to recover the baseline. The total cycle time for each measurement was 5 min. No sensor drift during the measurement period was experienced.

Each sample was analysed 3 times and the average of the results was used for subsequent analysis.

Statistical analysis

The data obtained from the sensor array were analysed

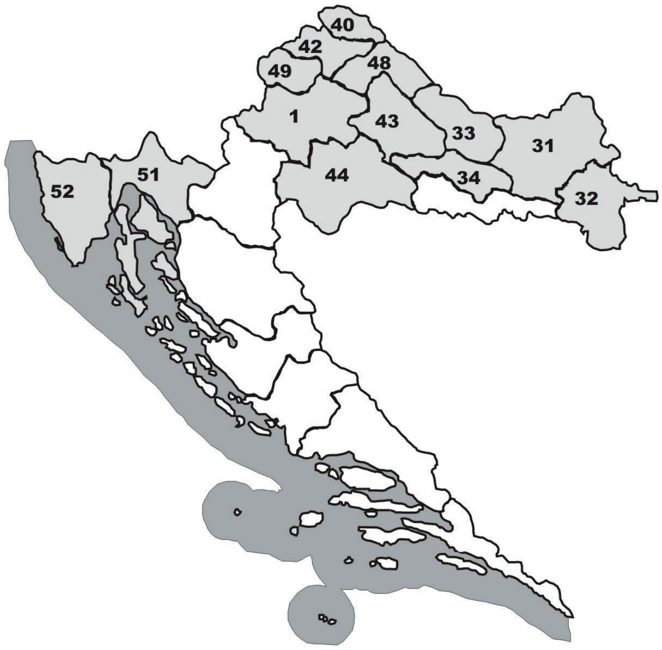

Figure 1. Geographical origin of black locust honey samples as declared by the beekeepers on the basis of the location of hives. Counties from which samples were originating are light grey coloured and have area code.
Slika 1. Geografsko podrijetlo uzoraka bagremova meda prema iskazu pčelara s obzirom na lokacije košnica. Županije podrijetla uzoraka označene su svijetlo sivom bojom i numeričkim kodom.

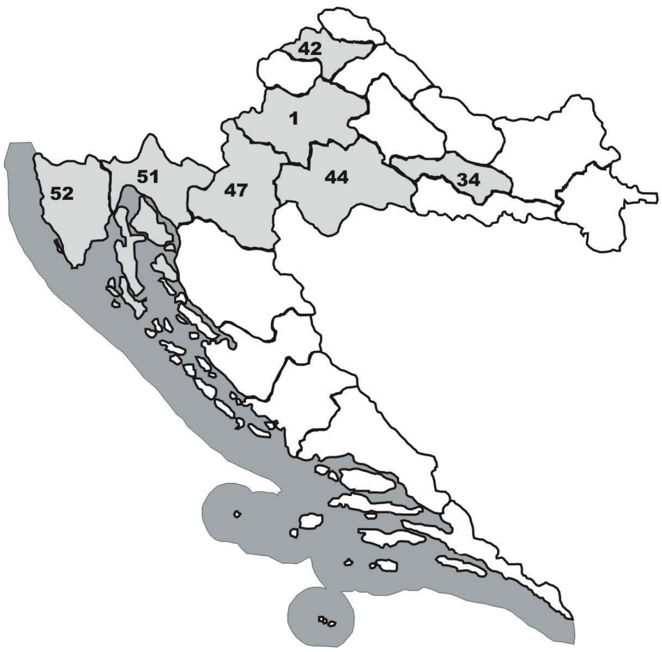

Figure 2. Geographical origin of chestnut honey samples as declared by the beekeepers on the basis of the location of hives. Counties from which samples were originating are light grey coloured and have area code.
Slika 2. Geografsko podrijetlo uzoraka kestenova meda prema iskazu pčelara s obzirom na lokacije košnica. Županije podrijetla uzoraka označene su svijetlo sivom bojom i numeričkim kodom.

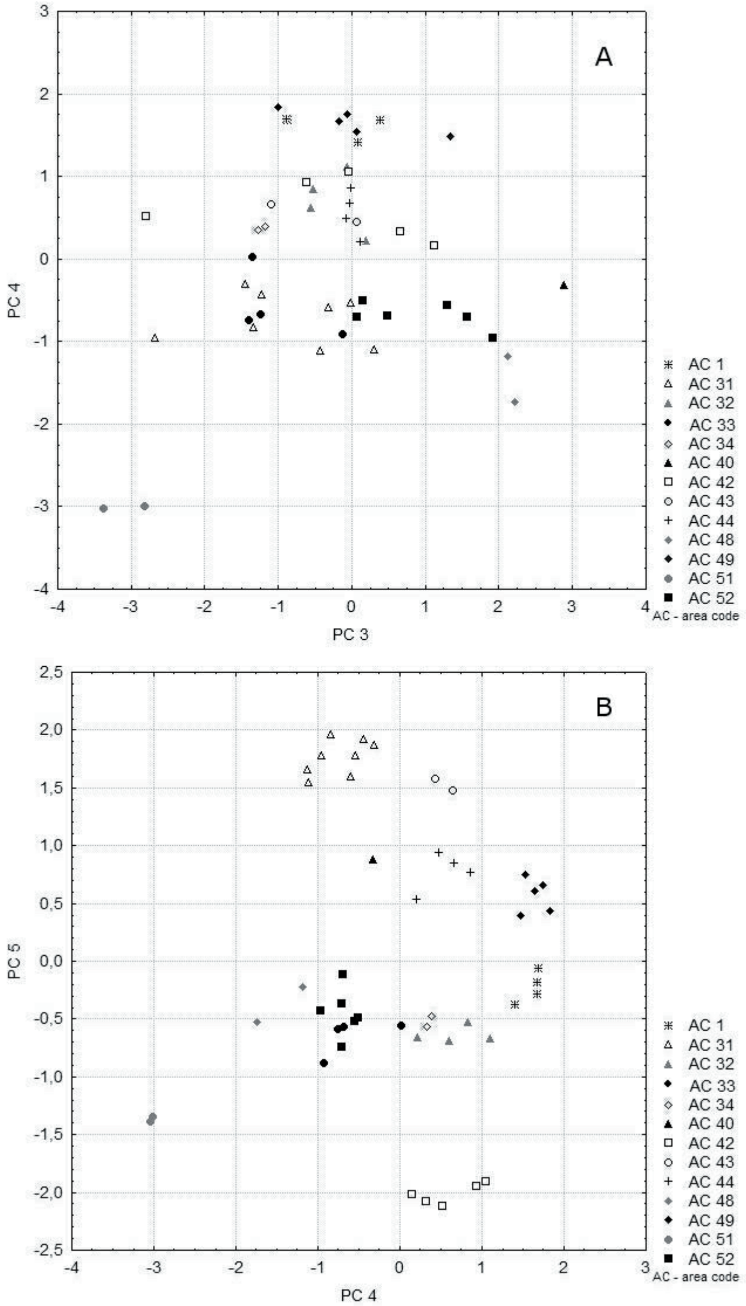

Figure 3. PCA results of black locust samples in PC3-PC4 (A) and PC4-PC5 (B) space.
Slika 3. Rezultati PCA analize uzoraka bagremovog meda u PC3-PC4 (A) i PC4-PC5 (B) ravninama.

by Principal Component Analysis (PCA) performed with STATISTICA (v. 7.1 Statsoft Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Data available in literature [1,2] as well as our own research (unpublished data) have shown that honeys can be characterised according their botanical origin on the basis of volatile compounds profile obtained by electronic nose. Therefore, to validate the applicability of the same method on the geographical origin differentiation, each honey type in this research was considered separately. In that way, differences caused by the honey type were neutralised, and all obtained differences are attributed by the differences originating from the geographic origin of the samples. PCA analysis of data set obtained for acacia volatile compound profiles by electronic nose resulted with large number of principal components (17) which explain 97.96 % of total variance of original data set, what points out very complex model for determination of underlying patterns. To simplify data presentation, based on eigenvalue screen plot first 5 principal components were used. They explain 66.26 % variability of original data set. First two components PC1 (gives 41.62 % of the variability to the original data set) and PC2 (gives 12.75 % of the variability to the original data set) as two-dimensional plot do not result with good separation of samples in respect to their geographic origin. All samples form compact cloud in the centre of a plot, with some samples displaced, but there is no grouping that can be connected with their geographical origin. Similar results are obtained with PC2 and PC3. When PC3, PC4 and PC5 were used (Figure 3), samples form clusters that resemble their geographical origin. Also, though not regularly, samples originating from the geographically close counties form clusters close to one another in the plot, while clusters of the samples from more distant counties are more distant in the plot as well. Namely, black locust is rather common botanical species in Croatia and very abundant in all regions, and collected samples originated from practically all parts of Croatia.

Similar results were obtained for chestnut samples. Smaller number of samples and smaller number of originating counties resulted in simpler model with less PC's. PCA analysis showed that 7 principal components give 96.42% variance of original data. PC1 (gives 50.38% of the variability to the original data set) and PC2 (gives 21.89% of the variability to the original data set) together explain 72.27% of total variance. Despite the fact that first two principal components contribute in large to the variance in original dataset, as in previous case with black locust samples, their graphic presentation does not result in geographical origin related clusters. Good graphical separation, with respect to geographical origin, is achieved in a plot defined by PC3-PC4, and PC4-PC5 which are shown on Figure 4. Chestnut honey samples originated mostly from same small geographic region of Croatia, known as Bania. Majority originated from relatively small area located near town of Sisak and Petrinja in central Croatia in county labelled by area code 44, where are chestnut forest are mainly situated (Figure 2). These samples are well grouped and separated from samples originating from other counties (Figure 4).

On both group of samples results obtained by PCA analysis on the results of electronic nose analysis showed good separation of samples according the area code. Since, within each group of samples, only the samples of the same botanical origin were considered, differences, which have resulted in cluster formation, can be interpreted as geographical origin caused variability. Similar results were obtained earlier by S. Benedetti et al. [2]. Namely, they have reported differences in volatile compound profile captured with e-nose of black locust honey samples from Italy and Hungary, which enabled discrimination between the samples. Our results have proven that this method can be successfully used to differentiate samples even from close geographical regions (within one country), and not just in cases of distant regions (as Italy and Hungary). Any other cases of electronic nose application on geographical origin of honey were not found in literature available to authors. Data on the application of other methods and their results on geographical origin application are also scarce. Sanz et al. reported in 83 % cases accurate determination of the geographical origin of a La Rioja honey samples based on multivariate analysis of 14 legally required chemical and physical parameters of quality [14]. Still, it should be considered that their research was performed on samples of different botanical origin what could have contributed to the obtained differences and in final to correct classification of samples more than geographical origin.

CONCLUSION

Results presented in this work indicate that electronic nose could be useful in honey geographic origin determination if combined with simple statistical analysis. Volatile profile data obtained by electronic nose analysis and analysed using PCA showed that samples of the same botanical, but different geographical origin have tendencies of grouping in PC spaces. Therefore aromatic profile of honeys obtained by electronic nose can be used

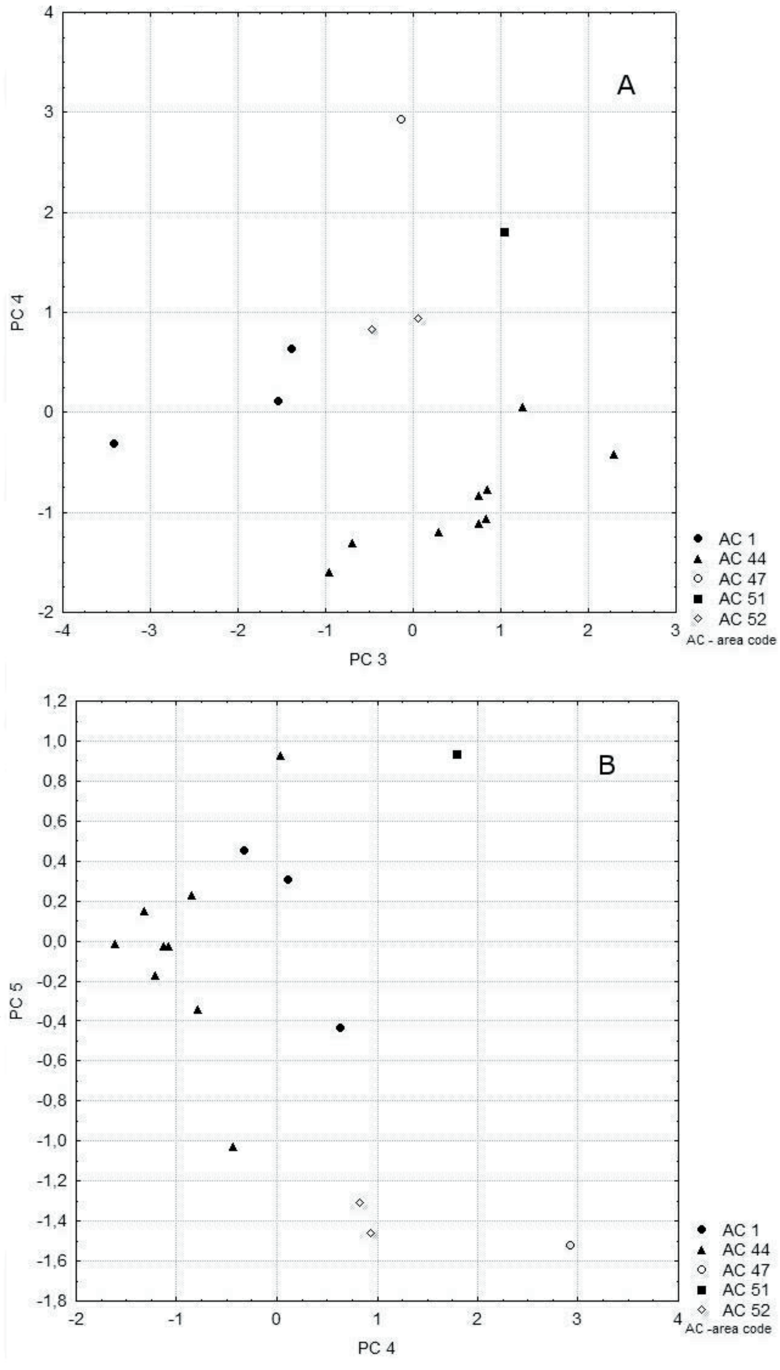

Figure 4. PCA results of chestnut samples in PC3-PC4 (A) and PC4-PC5 (B) space.
Slika 4. Rezultati PCA analize uzoraka kestenovog meda u PC3-PC4 (A) i PC4-PC5 (B) ravninama.

for geographical origin characterisation. Further studies should include other honey types, as well as honey from different years to verify obtained results.

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