



Tracing the geographical origin of honey using contemporary analytical methods and chemometric approach

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INTRODUCTION

Honey is recognized from the past as high-quality food which is why it is more and more often target of different types of adulteration. Assessing the authenticity of honey is the goal of many scientists worldwide for which simple analytical methods have not been sufficient for a long time. One of the promising analytical approaches in determination of honey authenticity is usage the combination of stable isotopes and mineral composition together with chemometrics.

In Croatia, production of multifloral and different unifloral honey types is possible because of its diverse vegetation and climate. One of the well-known honey types, not only in Croatia but also more widely, are black locust (*Robinia pseudoacacia* L.) and lime (*Tilia* spp.) honey. Both of these plants could be found in continental and coastal region of Croatia.

The aim of this work was to achieve distinction of honey samples of the same botanical origin produced in different geographical regions of Croatia by using stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratio and mineral composition.

MATERIALS AND METHODS

□ Honey samples

- 29 black locust (*Robinia pseudoacacia* L.) honey samples
 - 9 Istria County (coastal region)
 - 20 in the area extending from Krapina-Zagorje County to Vukovar-Srijem County (continental region)
- 12 lime (*Tilia* spp.) honey samples
 - 4 Primorje-Gorski Kotar County (coastal region)
 - 8 in the area extending from Bjelovar-Bilogora County to Vukovar-Srijem County (continental region)

□ Botanical origin confirmation

- melissopolynological and physico-chemical analyses (water content, electrical conductivity, HMF content, diastase activity, carbohydrate content by HPLC)
- EA-IRMS analysis of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratio
 - isolation of proteins from honey samples according to AOAC method 998.12 (AOAC, 2000)
 - weighted the samples of honey and honey proteins into tin containers placed in solids autosampler (MAS 200R, Thermo Scientific, UK) connected with EA-IRMS (FlashEA 2000 HT with NoBlank device-Thermo-Electron Delta V Advantage Isotope Mass Spectrometer, Thermo Scientific, UK) device via ConFlow IV Interface
 - reference standards Pee Dee Belemnite (PDB) for carbon and IAEA-N1 and IAEA-N2 for nitrogen

□ ICP-OES analysis

- digestion of honey samples in microwave digestion system (ETHOS 1 Advanced Microwave Digestion System; Milestone, Sorisole, Italy)
- quantification of 20 elements (Fe, Mg, Al, Cr, Mn, Zn, Cu, B, Cd, Ba, Ni, Co, As, Pb, Ca, K, Na, P, S, and Hg) was carried out in triplicate by ICP-OES (iCAP 6500 Duo; Thermo Scientific, Loughborough, United Kingdom)

□ Data analysis

- Principal Component Analysis (PCA) used for potential classification of honey according to geographical origin
- statistical analysis was conducted in Statistica 13 (Stat. Soft. Inc., USA), Microsoft Office Excel and R-3.5.2 software.

RESULTS AND DISCUSSION

Figure 1 shows the classification of black locust honey samples according to two regions. For this PCA analysis, the main components PC1 and PC2 were functions of all three IRMS variables ($\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{15}\text{N}_{\text{protein}}$), and obtained PCA model explain 86.47% of the total variability among the data, and the classification success of the model was 88.89% (Table 1). The two samples of black locust honey overlapped due to similar isotope ratio values (Figure 1). Sample from Virovitica - Podravina County overlapped with the area of the coastal region, and its values of $\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{15}\text{N}_{\text{protein}}$ were -24.99‰, -24.75‰ and 3.99‰. Similar values were found in the black locust honey sample from the coastal region (-25.01‰, -24.76‰, 3.82‰). In order to determine the botanical and geographical origin, in addition to the stable isotope ratio, many authors have used a larger number of parameters, especially mineral composition of honey. The success of the correct classification of black locust honey according to the region of origin increased when the principal components PC2 and PC3 were a function of the IRMS ($\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{15}\text{N}_{\text{protein}}$) and ICP (Mn, Mg, Zn, Pb, P, K) (Figure 2) variables and amounted 100% (Table 1). The model successfully managed to group the samples according to the region, and PC2 and PC3 explain 35.97% of the total variability of the data.

The same was done for linden honey (Figure 3) where a successful 100% classification (Table 1) with respect to the region of origin of the honey was obtained using only the IRMS variables ($\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{15}\text{N}_{\text{protein}}$). The first two principal components explain 91.07% of the total variability among the data.



CONCLUSION

PCA analysis showed that only IRMS parameters were not sufficient for discrimination of black locust honey according to geographical origin due to data overlapping. Despite the separation of lime honey from the coastal and continental regions was achieved only by IRMS parameters, the separation of black locust honey samples was successful after inclusion of ICP parameters.

The obtained results of differentiating the geographical origin of honey samples within one botanical species are encouraging, but considering that a narrow geographical area was encompassed, it would be interesting to expand the further research to more botanical species of honey coming from more distant geographical regions.

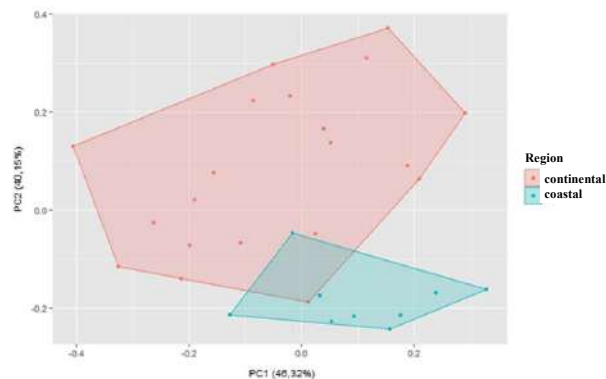


Figure 1 Classification of black locust (*Robinia pseudoacacia* L.) honey by regions using PC1 and PC2 as functions of IRMS variables

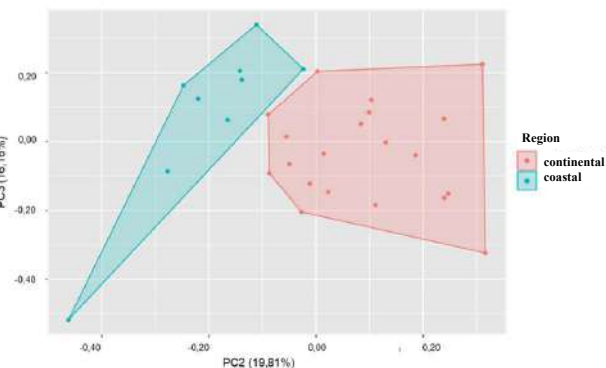


Figure 2 Classification of black locust (*Robinia pseudoacacia* L.) honey by regions using PC2 and PC3 as functions of IRMS and ICP variables

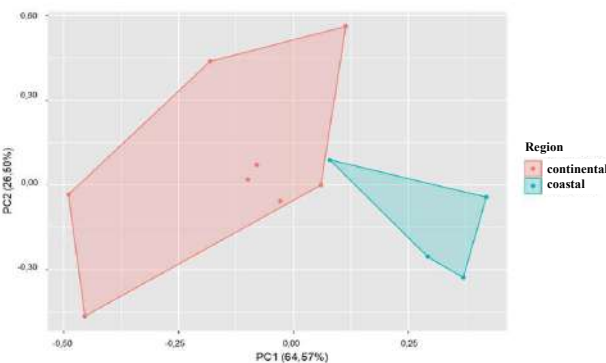


Figure 3 Classification of lime (*Tilia* spp.) honey by regions using PC1 and PC2 as functions of IRMS

Table 1 PCA model classification success (cutoff=0.6)

Figure	Total percentage of correct classification of the model (%)	Accurate classification of honey from the continental region (%)	Accurate classification of honey from the coastal region (%)
Figure 1	93.10	95.00	88.89
Figure 2	100.00	100.00	100.00
Figure 3	100.00	100.00	100.00

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Honey is recognized from the past as high-quality food which is why it is more and more often target of different types of adulteration. Assessing the authenticity of honey is the goal of many scientists worldwide for which simple analytical methods have not been sufficient for a long time. One of the newer analytical approaches in determination of honey authenticity is usage the combination of stable isotopes and mineral composition together with chemometrics. In Croatia production of multifloral and different unifloral honey types is possible because of its diverse vegetation and climate. One of the well-known honey types, not only in Croatia but also more widely, are black locust (*Robinia pseudoacacia* L.) and lime (*Tilia* spp.) honey. Both of these plants could be found in continental and coastal region of Croatia. The aim of this work was to achieve distinction of honey samples of the same botanical origin produced in different geographical regions of Croatia by using stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratio and mineral composition. Lime and black locust honey samples were collected in coastal and continental parts of Croatia. Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were determined in honey and isolated honey proteins by elemental analyser - isotope ratio mass spectrometer (EA-IRMS). Inductively coupled plasma - optical emission spectrometry (ICP-OES) was analytical technique for mineral elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Zn) determination, before which the microwave digestion of the samples was carried out. Taking into account the geographically relatively small distance of locations where honey samples of the same botanical origin were collected, quite successful differentiation was obtained according the geographical origin of these two honey types. PCA results showed that 87% of black locust honey samples from two regions were distinguished by using only IRMS parameters, while in combination with ICP parameters classification was completely successful. IRMS parameters were sufficient for lime honey samples classification according to region of origin. Combination of used analytical techniques together with PCA analyses could be useful tool in assessing honey authenticity.