Abstract

Honey, one of the most valued foods, has been on the market since ancient times. Due to its popularity and lack of definition, it is often the target of falsification or modification of botanical and geographical origin of the final product.

In this doctoral thesis modern analytical techniques were used for developing simple and fast methods without any special preparation of samples for determining markers for distinguishing between Slovenian chestnut, linden, acacia, silver fir, spruce, flower and forest honey. Markers were investigated and determined among volatile organic compounds, metals and other elements and kynurenic acid in honey. Analyses were performed on GC-MS, ICP-MS and HPLC-MS/MS systems. Due to the selectivity of methods, special pre-preparation of the samples was not necessary, which reduces the cost and time of the analysis.

Volatile organic compounds, specific and characteristic, were determined for each honey type. Specific coupounds were suggested as specific markers. Volatile organic components were extracted from the headspace by SPME followed by analysis with a GC-MS method. Specific markers were determined for each honey type namely twelve for linden, six for chestnut, four for acacia, five for spruce and nine for silver fir honey. Five characteristic compounds were determined for each honey type (compounds with the highest content) which can serve us as supporting markers.

After microwave digestion, 33 elements were simultaneously qualitatively and quantitatively analyzed in honey using the ICP-MS method. It turned out that the median total content of analyzed elements is the highest in chestnut honey with $4{,}4\times10^{3}$ μg/g in range $3{,}3\times10^{3}$–$6{,}0\times10^{3}$ μg/g, and the lowest in acacia honey with $4{,}02\times10^{2}$ μg/g in range $2{,}0\times10^{2}$–$7{,}6\times10^{2}$ μg/g; both results could be, in comparison with results of other honey types, proposed as markers. After analyzing the data and processing the results with the PCA method, the highest number of markers were proposed for chestnut honey, which is characterized by the presence of Ga, which is absent in other honey types, and a significantly high content of B, Na, K, Ca, Mn and Ba. Significantly low contents of K, Mn and Rb were found in acacia. For purposes of geographical origin 11 metals (and their contents) have been proposed as characteristic for Slovenian honey.

Kynurenic acid (KYNA) is credited with many positive health effects and changes in its concentration in the human body, are attributed to certain medical conditions. The need for an optimized method for direct analysis of KYNA has led us to develop a rapid and simple HPLC-MS/MS method with a detector in SRM mode that allows selective analysis of KYNA, requiring

no extraction or other special sample preparation. At the same time, it proved to be a marker for chestnut honey, namely the content of KYNA in chestnut honey (328–1,02×10^3 μg/g) is significantly higher than in the other analyzed types of honey. A higher content was also detected in linden honey (24,6–189 μg/g) and was also suggested as marker for linden honey.

The level of 5-hydroxymethylfurfural (HMF) in honey is an indicator of quality. Elevated levels indicate excessive heat treatment or the addition of sugar syrups. Slovenian acacia honey was found to be more susceptible to HMF formation than other types of honey, as they exceeded the legal limit of 40 μg/g. We found that it was prone to HMF formation even at room temperature. We also confirmed the expected low HMF content in fresh honey.

With these fast, simple and relatively inexpensive methods, we identified markers for a specific type of honey. With this, we set new milestones that could potentially be used in practice in determining botanical and geographical origin, as well as determining quality and counterfeits.

Keywords: honey, marker, GC-MS, volatile organic compounds, ICP-MS, metals, HPLC-MS/MS, kynurenic acid, 5-hydroxymethylfurfural