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Analysis of the botanical origins of monofloral honey types

1. Summary

At the Institute of Food Science of the University of Debrecen, we have been analyzing honey for ten years. In our study, the proline and phenolic compound contents of 70 types of monofloral honey (acacia, linden, rape, sunflower, milkweed, chestnut and forest) were examined. During the study, the answer was sought to the question whether it was possible, based on these two parameters, to differentiate monofloral honey types from each other or, in other words, was there an effect of the botanical origin on the amounts of these two compounds.

With the help of linear discriminant analysis, it was determined that groups of monofloral honey could be clearly differentiated from each other. Differentiation of the two groups was not unambiguous in the case of forest and chestnut honey, so the analysis of a third characteristic could be necessary in the case of these two monofloral honeys.

2. Introduction

Honey is a natural, sweet substance, produced by the *Apis mellifera* bees. In terms of origin, it can come from two sources, from the nectar secreted by the plants (honey of nectar origin), or from the substance secreted by insects, e.g., aphids (honeydew honey). In Hungary, mainly honey of nectar origin is produced, in an amount of roughly 17,000 tons, most of which is exported. With this quantity, in terms of European Union member states, we are ranked third on the list of honey exporters behind Spain and Romania [1]. In Hungary, acacia, linden, sunflower, rape and milkweed honeys are typically consumed, but chestnut, wild tobacco and lavender honeys play significant roles as well.

Honey is a complex food, containing a number of beneficial compounds, helping to preserve human health, and so a significant role is attributed to it not only in the human diet, but also in medicine. Its antibacterial properties are due to its high sugar content, pH and hydrogen peroxide content, among other things [2]. In addition, moderate consumption of honey also provides a protection against gastrointestinal infections [3].

Honey composition is largely dependent on the plant it is derived from, and it can also be influenced by soil properties and post-collection treatment [4]. To determine the botanical origin of honeys, the most widely used method is pollen analysis, however, using this method, added pollen grains cannot be differentiated from those of natural origin. Even though type identification of honey is based on the pollen ratio, it has been shown by previous studies that the values of certain physico-chemical parameters are characteristic of different monofloral honeys [5], [6]. In our research, the answer was sought to the question whether the different monofloral honeys could be differentiated, based on the parameters chosen by us.

3. Materials and methods

3.1. Honey samples

In our study, monofloral honeys from the year 2015 were used, which were as follows: acacia honey (*Robinia pseudoacacia*), linden honey (*Tilia sp.*), rape honey (*Brassica napus*), sunflower honey (*Helianthus annuus*), milkweed honey (*Asclepias syriaca*), chestnut honey (*Castanea sativa*) and forest honey. 10 samples each were selected from each monofloral honey. Analyses were performed within 3 months after reception of the samples. Until the beginning

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of the analysis, samples were stored in sterile glass containers, in the dark, at room temperature. Analyses were performed in 2015 at the Institute of Food Science of the University of Debrecen.

3.2. Determination of the proline content and the total phenolic compound content

Measurement of the proline content of honeys was performed according to the method issued in 2009 by the International Honey Commission [7], which is based on the method of Ough [8]. This procedure is suitable for the determination of the amount of proline found in the honey. The principle of the method is that a colored compound is formed by the proline in the honey with ninhydrin, and the absorbance of this compound is measured at 510 nm. Results are given in mg/kg.

Determination of TPC (total phenolic content) was performed according to the method of Singleton [9], using Folin-Ciocalteu reagent, and measuring the absorbance of the resulting colored compound at 760 nm. Results are given in mg GAE/100 g.

3.3. Statistics

Each analytical measurement was run in triplicate. For the evaluation of the measurement results, SPSS statistical software was used (version 13; SPSS Inc. Chicago, Illinois, USA), with which basic statistical parameters (mean, standard deviation, minimum and maximum values) were calculated and linear discriminant analysis (LDA) was performed.

4. Results

4.1. Proline content

Proline is a non-essential amino acid, making up approximately 50-85% of the amino acid content of honey [10]. Its quantity decreases over time, therefore, the measurement could be suitable to determine the maturity of honey [11]. Currently, there is no clear regulation on the proline content of honeys, therefore, the minimum value of 180 mg/kg, adopted in Germany, was taken as a basic value.

The proline contents of the monofloral honeys studied can differ significantly [7], which was proven by our measurements (**Table 1**). The lowest values were obtained for acacia honeys (245±25 mg/kg), while the highest ones were shown by forest honeys (1042±44 mg/kg). By comparing our results to those of other studies it can be seen that very similar results were obtained by Can et al. [12] in the case of honeys of Turkish origin, however, lower values were measured for Italian and Slovenian monofloral honeys by Truzzi et al. [13] and Kropf et al. [14], respectively. Compared to the values obtained for acacia honeys, the proline content of linden honeys was twice as high, that of milkweed honeys was three times, of sunflower and chestnut honeys three and a half times, and of forest honeys four times higher. Based on the proline content, the order of the monofloral honeys analyzed is as follows: acacia honey < rape honey < linden honey < milkweed honey < sunflower honey < chestnut honey < forest honey.

4.2. Total phenolic content

The total phenolic content of different monofloral honeys ranges from 5.6 to 50.0 mg/100 g [15], however, **Table 2** clearly shows that higher values were measured in the case of forest and chestnut honeys analyzed by us (65.5±3.7 and 71.0±4.8 mg GAE/100 g). Higher values were also measured by Can et al. [12] in the case of Italian chestnut honeys, and similar values were obtained by Kowalski [16] when analyzing Polish linden and forest honeys.

Based on our results it was determined that the lowest values were shown by acacia honeys (17.6±1.0 mg GAE/100 g), while chestnut honeys showed the highest values (71.0±4.8 mg GAE/100 g). Similarly to proline content, compared to the values obtained for acacia honeys, one and a half times higher values were measured in rape and milkweed honeys, two times higher in linden honeys, almost two and a half times higher in sunflower honeys, and close to four times higher in forest and chestnut honeys. Based on the total phenolic content, the order of the monofloral honeys analyzed is as follows: acacia honey < milkweed honey ≤ rape honey < linden honey < sunflower honey < forest honey < chestnut honey.

4.3. Results of discriminant analysis

In the discriminant analysis, all seven groups had the same weight. For both parameters, the value of Wilks' lambda was 0.023, and both variables are significant, and based on this it can be stated that the variables had a significant effect on belonging to the group. The value of the canonical correlation was high for both functions (0.994 and 0.933), meaning that a significant part of the total variance is explained by both functions. Based on the values calculated from this it can be stated that 98.8% of the first function and 87.0% of the second function is explained from the variance of the dependent variable.

In the case of acacia, linden, sunflower, rape and milkweed honeys, all 10 samples got into the same group. In the case of forest honeys, only 9 samples got into the same group, but one sample was included in the group of chestnut honeys. In the case of chestnut honeys, 7 samples were in the same group, and 3 samples were included in the group of forest honeys. So, in the case of forest and chestnut honeys, there was mixing between the groups, while the other monofloral honey groups could be differentiated from each other unambiguously. In terms of percentage values, in the case of the acacia, linden, sunflower, rape and milkweed honey groups, the proportion of correctly categorized cases was 100%, for forest honeys this value was 90%, while for chestnut honeys it was only 70%.

Based on **Figure 1** it can be stated that there is a significant difference between the mean values of the types in the first dimension. The highest mean values were shown by forest and chestnut honeys, while the lowest ones were shown by acacia honeys. The mean values of forest and chestnut honeys did not show a significant difference in this dimension (12.0

and 11.0), similarly to linden and milkweed honeys (-3.32 and -2.53). There is a difference in the second dimension as well, but its extent is smaller than in the first dimension. The highest mean values, very similar to each other, were shown by sunflower (3.52) and milkweed honeys (3.48), while the lowest ones were presented by chestnut honeys (-3.46). In this dimension, the mean values of linden and forest honeys were similar (-0.14 and 0.03).

Analyzing the two dimensions together it can be stated that the lowest mean values were presented by acacia, rape and linden honeys, followed by milkweed and chestnut honeys. The highest mean values were shown by sunflower and forest honeys in both dimensions.

Overall, it was found that acacia, linden, sunflower, rape and milkweed honeys differed from each other, and from forest and chestnut honeys, significantly, while in the case of the latter two monofloral honeys, the difference is much smaller, based on the two parameters examined.

5. Conclusions

In our study, the proline and total phenolic contents of 70 monofloral honeys were investigated, and the principle formulated by us, that monofloral honeys can be differentiated based on these parameters, could be proven. Linear discriminant analysis clearly showed that the two parameters chosen by us were suitable for proving botanical origin. The only exceptions were forest and chestnut honeys, where mixing within the groups was found, so it could not be determined, based on these parameters, whether the sample analyzed was a forest honey or a chestnut honey. Nevertheless, these two monofloral honeys could be clearly differentiated from the other ones, so the determination of proline and total phenolic contents are definitely a good starting point for the confirmation of botanical origin. A further goal is to determine whether the inclusion of a third parameter (e.g., electric conductivity) in the analysis could facilitate the differentiation of these two monofloral honeys.

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