TRACEABILITY MARKERS IN ITALIAN UNIFLORAL HONEYS

Giulia Colucci[1], Ettore Varricchio[2], Marina Paolucci[2], Elena Coccia[2], Carmina Viola[2], Finizia Russo[2], Maria Carmela Polese[2], Maria Grazia Volpe[1]

[1]Institute of Food Sciences, National Research Council, Avellino, mgvolpe@isa.cnr.it
[2]Department of Sciences and Technologies (University of Sannio), Benevento

Abstract – In this study we carried out the analysis of three varieties of unifloral honey of acacia (*Robinia pseudoacacia*), chestnut (*Castanea sativa*) and sulla (*Hedysarum coronarium*), in order to evaluate the parameters that allow the differentiation of the three varieties of unifloral honey investigated.
The three different varieties of unifloral honey showed clear differences in some parameters, such as the concentration of polyphenols, flavonoids, mineral elements, as well as different profiles of volatile molecules.
Therefore these parameters may allow the discrimination of different varieties of honey, and could be used as markers of product traceability.

Keywords: unifloral honey, sulla, chestnut, acacia, traceability.

1. INTRODUCTION

In recent years, the interest of researchers and consumers has been focusing on product traceability. In fact, consumers claim to know the provenance of foods.
Honey consists essentially of different sugars, mostly fructose and glucose, as well as other substances such as organic acids, enzymes and solid particles derived from honey collection.
The classical approach to the evaluation of botanical origin is based on the integration of pollen, metal content, specific volatile compounds analysis and determination of some physicochemical parameters such as color, pH, sugar contents, and diastase activity [1].
The color of honey varies from nearly colorless to dark brown.
The consistency can be fluid, viscous or partly to entirely crystallized.
The flavor and aroma vary, as they are derived from the plant origin [2].

Mineral composition has also been employed to discriminate honey arising from different geographical areas. Minerals seem to be good candidates for a classification system, mainly because they are stable and can be associated to the soil where melliferous flora grows. Mineral contents range from about 0.04% in pale honeys to 0.2% in some dark honeys.
Specific volatile compounds derived from original nectar sources are in all likelihood responsible for the specific aroma of monofloral honeys. These volatile compounds have been proved to be adequate to authenticate floral origin of honeys and some of them are real markers [3]. The characterization of the volatile profile has been shown to be effective for the evaluation of the botanical origin of honey. In fact, the volatile profile represents the chemical fingerprint of honey of different botanical origin, since the nature and quantity of the volatile compounds depend on the floral source. Specific volatile compounds derived from original nectar sources are in all likelihood responsible for the specific aroma of unifloral honeys. These volatile compounds have been proven to be adequate to authenticate floral origin of honeys [4].
In this work the attention has been focused on the analysis of three varieties of monofloral honey of acacia, chestnut and sulla, in order to verify their quality in terms of assessment of physicochemical and biochemical parameters, nutraceutical properties and antioxidant capacity. In particular, the investigations were carried out to verify the

qualitative differences between the three types of honey and to bring out the peculiarities.

2. MATERIALS AND METHODS

Three different honey samples were used for this study: acacia, chestnut and sulla.
The samples were collected between May and July 2014 in the South of Italy (Benevento area) and they were stored in the dark at room temperature (20°C) until analyses.
Solvents and chemicals were of the highest commercially available purity.
The analyses were carried out to evaluate: (1) chemical and physical parameters (moisture, pH, °Brix and refractive index, ash content, color measurements, determination of the content of mineral by ICP-OES), (2) nutritional peculiarities of each honey, (3) modification of the properties of each cultivar related the freshness and quality, (4) antioxidant activity, (5) micro and macro nutrients, (6) specific polyphenols by highperformance liquid chromatography (HPLC) reverse phase (RP) and (7) profile of volatile molecules by means of GC-MS.

3. RESULTS

In this study we carried out analyses that allow the differentiation of different types of honey.

The water content of honey is related to its botanical origin, atmospheric and environmental conditions during the production and storage conditions. When the water content is greater than 20%, it may cause fermentation phenomena. The optimal value of the water content can be defined at around 17%, but we can find honeys with a value even higher than 21%.[16] In this work it was found a difference between the chestnut honey and the other two types. In fact, the first has a value of moisture of 21.2 ± 1.79, while the other two types have a moisture value near to the optimal one, that is of 16.4 ± 1.81 for the honey of acacia and 17.6 ± 1.78 for the honey of sulla.

The acidity of honey is mainly due to the presence of various organic acids, such as gluconic , formic and malic acid. These acids contribute to the formation of the aroma of the honey and favor the stability of the product against the bacterial load.

Usually, the pH, influenced by the botanical origin of the honey, is between 3.5 and 4.5, a range that can vary due to added substances, such as organic acids. The results obtained in this work show a significant difference between the chestnut honey and the other two types. The honey of acacia and sulla showed a pH of 3.5 and 3.4 respectively, while the honey of chestnut showed a pH of 6.7. As reported in the literature by Chiş *et al.* [5] chestnut honey presents pH values generally around 6.5, then outside the range between 3.5 and 4.5. This aspect represents a peculiarity of this type of honey.

The total content of soluble solids (°Brix) is represented by all the dissolved substances in the water such as salts, acids, sugars, proteins, phenols and other organic molecules. Conventionally, the Brix (%) is calibrated on the number of grams of brown sugar contained in 100 g of a solution. When measuring a pure sugar solution, the Brix level corresponds exactly to the real content and can be considered an index of the quality and sweetness of the product. Directive 2001/110/EC established a range for °Brix from 76.5 to 81. In agreement with , the analyzed samples showed values of 78.2 ± 0.8 for acacia honey, 75.1 ± 0.7 for chestnut honey and 78.7 ± 0.8 for sulla honey.

The total ash contained in the product depends on the raw material of which is composed of a honey. The ash content in honey is generally small and depends on the composition of the nectar of the predominant plants. The variability in the ash content of honey has been associated in a qualitative way with the different botanical origin and the geographical characteristics. Both are interesting parameters, considering the production of a wide range of types of honey and the need for traceability markers. The ash content found in honey samples analyzed in this study was in agreement with the *'International Honey*

Commission' which provides a maximum value of 0.6 g 100 g^{-1}.

The different types of honey can be discriminated by means of the profile of volatile compounds and

the different concentration of some mineral elements, so some of these parameters can be used as markers of product traceability.

The sulla honey, for example, shows higher concentrations of polyphenols, while chestnut honey has a higher content of potassium and exhibits some volatile molecules not present in the other honeys analyzed.

Specifically, potassium is present in honey in significant amount. In fact, it is the mineral with the highest concentration present in honey, in particular the high concentration of potassium in chestnut honey, is a feature that can be used to differentiate it from the other type of honey.

Additional elements detected in high concentrations, are sodium, calcium and magnesium.

Concerning the volatile profiles, about 300 aroma compounds have been separated by gas chromatography, but only 100 were well identified in chestnut, acacia and Sulla honey.

Chestnut honey is characterized by high amounts of acetophenone. Indeed Guyot et al. [6] proposed the acetophenone as a compound 'guide' typical of this honey. This suggests that the chestnut honey differs from the other two types that no specific markers of the honey botanical origin were found.

The polyphenol test employs the Folin-Ciocalteu, a mixture of phosphotungstic acid and phosphomolybdic acid which is reduced by the oxidation of phenols. The reagent is presented as a yellow solution after the reaction in the presence of phenolic compounds is reduced to a mixture of the blue oxides of tungsten and molybdenum. The analysis provides a figure which corresponds to the total content of polyphenols in relation to this colorimetric change, which is measured by reading absorbance at a wavelength of 760 nm.

According to Meda et al. [7], the total phenolic content in honey (mg GAE 100 g^{-1} of honey) is comprised between 32.59 and 114.75 mg with an average of 74.38 ± 20.54 mg, using the standard curve of gallic acid (R2 = 0.9990). The results obtained in this work are in agreement with those reported in literature.

In fact the concentration of the polyphenols in honey is 134.2 ± 0.95 mg GAE 100 g^{-1}. The honey of acacia and chestnut fall perfectly in the reference range with values of 118.6 ± 0.87 for the first and 95.6 ± 0.80 for the second. Interestingly, the honey of sulla shows a value of polyphenols higher than the average value reported in literature.

Flavonoids in the diet can be classified as flavonols, flavanones, flavones, anthocyanidins and isoflavones. They feature a wide range of biological effects, such as antibacterial, anti-inflammatory, anti-allergic and anti-thrombotic effects.

The flavonoids act as antioxidants in a variety of ways, including the direct capture of reactive oxygen species, inhibition of enzymes responsible for the production of superoxide anions, chelation of transition metals involved in the processes of the formation of radicals and prevention of peroxidation process by reducing alkoxy radicals and peroxidic.

The method of aluminum chloride, introduced by Smirnova et al. [8], allows the determination of the content of total flavonoids, separating their contribution from that of the polyphenols. The reagent AlCl3 is presented as a colorless mixture that following the reaction with the flavonoids, is reduced to a mixture of straw yellow color. According to Meda et al. [7], the total content of flavonoids of honey samples (mg QE 100 g^{-1}) varies between 0.17 and 8.35 mg with mean value 2.57 ± 9.2 mg. The results of this research are reported in the graphic 5 that shows the high content of these bioactive molecules in chestnut honey, followed by Sulla and acacia honey.

4. CONCLUSIONS

The different concentration of polyphenols and flavonoids, the profile of volatile compounds and the presence of different concentration of some mineral elements allow discriminating different varieties of honey, and could be used as markers of product traceability.

Moreover, the high concentration of some macro elements such as potassium, remarkably high in the chestnut honey, makes it especially useful in individuals with deficiency of these mineral or practicing sports.

ACKNOWLEDGMENTS

We would like to thank "GAL Alto Tammaro-Terre dei Tratturi" - PSR-2007-2013 – Regione Campania for their financing.

REFERENCES

[1] P. Truchado, F. Ferreres, L. Bortolotti, A.G. Sabatini, F.A. Tomas-Barberan, *Nectar Flavonol Rhamnosides Are Floral Markers of Acacia (Robinia pseudacacia) Honey*. J. Agric. Food Chem., 2008.

[2] S. Bogdanov. *Honey Quality and International Regulatory Standards: review by The International Honey Commission.* Bee World, 1999.

[3] Schievano E, Morelato E, Facchin C, Mammi S, Characterization of Markers of Botanical Origin and Other Compounds Extracted from Unifloral Honeys. J Agric Food Chem 61:1747–1755 (2013).

[4] Bianchi F, Mangia A, Mattarozzi M, Musci M, Characterization of the volatile profile of thistle honey using headspace solid-phase microextraction and gas chromatography–mass spectrometry. Food Chem 129:1030–1036 (2011).

[5] Chiş A and Cornelia Purcărea, Quality Of Chestnut Honey Modified By Thermal Treatment Department Of Food Control, University Of Oradea, Environment Protection Faculty 21:573–579 (2011).

[6] Guyot C, Bouseta A, Scheirman V, Collin S, Floral Origin Markers of Chestnut and Lime Tree Honeys. J Agric Food Chem 46:625–633 (1998).

[7] Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG, Determination Of The Total Phenolic, Flavonoid And Proline Contents In Burkina Fasan Honey, As Well As Their Radical Scavenging Activity. Food Chem 91:571–577 (2005).

[8] Smirnova LP, Pervykh LN, Quantitative determination of the total content of flavonoids in the flowers of immortelle Helichrysum arenarium. Pharm Chem J 32,6:35–38 (1998).