Organic acids content, sugars content and physicochemical parameters of Romanian acacia honey

Daniela Pauliuc, Mircea Oroian, Paula Ciursă

Stefan cel Mare University, Suceava, Romania

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Corresponding author:

Mircea Oroian
E-mail: m.oroian@fia.usv.ro

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Abstract

Introduction. Some elements of a honey are added by bees; others occur after the maturation of honey. The physicochemical parameters, pollen content, sugars content and organic acids content were determined for Romanian acacia honey.

Materials and methods. 27 samples of acacia honey from 2020 were examined to determine the physicochemical parameters (moisture, electrical conductivity, pH, free acidity, color, HMF content) and sugars content using the methods proposed by the International Honey Commission. Organic acids composition was determined using the method proposed by Ozcelik.

Results and discussion. The botanical origin of all samples was confirmed by melissopalinological analysis, each sample containing more than 45% *Robinia pseudocacia* pollen granules. According to Codex Alimentarius, moisture content should be lower than 20%, and for the analyzed samples the moisture content ranged from 16,66–20,74%. The pH of the acacia honey samples ranged from 3.61 to 5.33. The free acidity of acacia honey analyzed in this study ranged from 0.32 to 4.14 meq/kg. None of the analyzed samples exceeded the limit imposed by legislation. All honey samples showed similar lightness values (29.62–46.57). The maximum content of HMF in the samples was 23.20 mg/kg thus falling within quality requirements. A value of less than 500 µS/cm indicates a pure floral honey, and in this study the samples of acacia honey had electrical conductivity values between 94.8–405 µS/cm. In the acacia honey samples was identified a percentage of 68.35% monosaccharides, and a small percentage of sucrose (maximum 2.093%). The F/G ratio varied between 1.02 and 1.65 for the studied acacia samples and some samples can crystallize quickly because have high glucose content and the F/G ratio is about 1. In the samples with F/G values above 1.3 the tendency of crystallization is slower. Gluconic acid was the main organic acid in all samples (1.916–2.666 g/kg) followed by propionic and acetic acids. Succinic acid has the lowest concentration in the studied acacia honey samples.

Conclusions. All the investigated honey samples (27 samples) met the quality criteria examined (moisture, pH, free acidity, HMF content, color and electrical conductivity) and the high percentage of pollen grains of *Robinia pseudoacacia* confirmed that the samples analyzed were monofloral acacia honey.

Introduction

Honey is the natural sweet substance produced by bees (Codex Alimentarius, 2001) and most of its elements are derived from plants; while some are added by bees and others occur after the maturation of honey (Teklit and Frehiwot, 2016). The main parameters of honey quality, which also influences its price, derive from its botanical origin (Seisonen et al., 2015). Monofloral honey is a high quality product, which has a unique aroma and taste, but is often adulterated by incorrect labeling and mixing with inferior honey (Trifković et al., 2017; White, J. W., 2000). Monofloral honey is known to have specific therapeutic properties and organoleptic attributes, and for this reason is one of the foods preferred by consumers.

Physicochemical parameters and especially the color influence the price of honey but also the preferences of consumers (Visquert et al., 2014). Specific rules and standards are needed to guarantee the identity and quality of honey so that people can consume honey safely and pay the right price (Danezis et al., 2016). Obtaining data on the physicochemical parameters of honey is important both for its characterization and for ensuring the quality of products on the market.

The identification of the botanical and geographical origins of honey are indicators of its authenticity (Jandrić et al., 2017; Karabagias et al., 2018). Ensuring the authenticity and quality of honey is of real interest in the international honey market for both regulators, consumers, traders and beekeepers (Sobrino-Gregorio et al., 2017). It should be noted that each honey has unique organoleptic characteristics, which are closely related to its source: botanical and geographical (Bogdanov et al., 2008).

The quality criteria for honey are set out in the EU Regulation and they include organoleptic characteristics (appearance, color, taste, consistency, aroma and taste) and physicochemical characteristics (moisture, carbohydrate content, pH, acidity, minerals, electrical conductivity, vitamins, organic acids, hydroxymethylfurfural (HMF) content, proteins) (Council, E. U., 2002). These parameters are influenced by the type of nectar, climate, soil but also by handling practices after harvesting honey (Belay et al., 2013). When we refer to the floral origin of honey, we implicitly refer to the geographical area, but also to the flora of that area during the collection period (Kaškonienė and Veskutonis, 2010). The chemical composition of honey even if it has the same floral source can be quite different, due to different climatic conditions, soil characteristics and the presence of different minerals from the soil (Persano Oddo and Piro, 2004). Honey is a complex product, whose composition varies depending on the species of bees, the type of nectar, geographical area, season and storage conditions and requires a characterization that targets various analytes: volatile compounds, phenolic acids, flavonoids, carbohydrates, amino acids and organic acids. The labeling of honey with a certain botanical or geographical origin cannot be carried out taking into account only one type of chemical markers, but rather through a combination of several.

The most known and appreciated assortment of monofloral honey produced in Central Europe is acacia honey (*Robinia pseudocacia*) (Oroian et al., 2015). Acacia honey is available on the European market, being one of the most appreciated assortments for its appearance, light yellow color, delicate fragrance and floral aroma (Schievano et al., 2019).

In order to be able to classify a sample of honey as authentic acacia honey, it must contain at least 45% granules of acacia pollen, as specified in the regulations on the quality of honey (Soares et al., 2017). Analysis of pollen grains in honey sediments is used as a benchmark to identify the botanical origin of honey (Pires et al., 2009). The main components of honey are sugars and their use as floral or geographical markers of honey is not common due to the difficulties of specifying one or more carbohydrates present in honey that can serve this purpose (Kaškonienė et al., 2010). However, some authors have suggested the use of the amount and ratio of specific carbohydrates (fructose and glucose), as well as oligosaccharides, as useful indicators for the recognition of monofloral honey (Cotte et al., 2004). Organic acids are present in small quantities in honey but are important in establishing the freshness and authenticity of honey (Pauliuc and Oroian, 2020).

The purpose of this work was to determine for acacia honey the physicochemical parameters (melissopalynological analysis, moisture, color, pH, free acidity, electrical conductivity), hydroxymethylfurfural content, sugars content and organic acids content.

This is the first study aimed at analyzing the organic acids found in acacia honey in the N-E area of Romania.

Materials and methods

Samples

The acacia honey samples (27 samples) were purchased from the N-E part of Romania and come from the production of 2020. The samples were purchased directly from beekeepers and were liquefied at 50 ° C to be prepared for analysis.

Melissopalynological analysis

To identify the type of pollen that predominates in the honey sample, the pollen granules on a sediment spread were counted at ×40 magnification using an light microscope (Motic). Sediment spread was obtained by dissolving 10 g of honey in 40 mL of distilled water, followed by centrifugation at 4500 rpm for 15 minutes. The centrifugation process was repeated for another 15 minutes after the supernatant was removed and water was added (Von Der Ohe et al., 2004).

Physicochemical analysis

In order to determine the physicochemical parameters of acacia honey, the methods proposed by the International Honey Commission (Harmonised Methods Of The International Honey Commission, 2008) were used. With the help of these methods the following parameters were determined: moisture content (using Abbe refractometer, Leica Mark II Plus), electrical conductivity (using portable conductometer HQ14d, HACH, USA), pH (using pH meter Mettler Toledo FiveGo, Mettler Toledo, USA), free acidity (using TITROLINE easy, Schott Instruments, Germany) and color (using portable chromameter CR-400, Konica Minolta, Japan). The hydroxymethylfurfural (HMF) content was determined using a UV-VIS-NIR spectrophotometer SCHIMADZU UV-3600 (Schimadzu Corporation, Japan), according to the method proposed by White (White, 1979).

Determination of sugars composition

The sugars content was determined with a HPLC instrument (Schimadzu, Kyoto, Japan). Before being injected into the HPLC instrument the samples were prepared as follows: 5 g of each acacia honey sample were dissolved in 40 mL distilled water, mixed with 25 mL of methanol (in a 100 mL volumetric flask) and then brought to volume with distilled water (Harmonised Methods Of The International Honey Commission, 2008). The

HPLC instrument was equipped with a LC-20 AD liquid chromatograph, SIL-20A auto sampler, CTO-20AC column oven, and RID-10A refractive index detector. The separation was performed on a Phenomenex Luna® Omega 3 µm SUGAR 100 Å HPLC Column 150 x 4.6 mm. The samples were filtered through 0.45 µm PTFE membrane filters and then injected in the HPLC instrument. The sample volume injection was 10 µL. The flow rate was 1.3 mL/min and the mobile phase was acetonitrile: water (80:20, v/v); the temperature of the column and detector was 30 °C.

Determination of organic acids

Organic acids were determined using the same instrument as in the case of the sugar content. The method of analysis was described previously by Ozcelik et al., (2016). 0.5 g of acacia honey were mixed with 2.5 mL of 4% metaphosphoric acid (w/v), then the samples were vortexed. Then, the samples were centrifuged for 5 min at 3500 rpm using a Z216-MK refrigerated centrifuge (Hermle Labortechnik, Wemingen, Germany) The sample was injected in the HPLC instrument (Schimadzu, Kyoto, Japan) with a diode array detector.

Results and discussion

Melissopalynological analysis

Since the last century, the analysis of honey pollen has been used as a method of authentication, but the methodology has improved and harmonized several times over the years (Ruoff et al., 2006).

The botanical origin of all samples was confirmed using melissopalinological analysis, each sample containing more than 45% *Robinia pseudocacia* pollen granules. The percentage of 45% pollen grains is the indicator by which honey can be classified as monofloral (Consonni and Cagliani, 2015). Ma et al., (2019) detected a content of 80% pollen granules of *Robinia pseudoacacia* in honey samples from China. Dobre et al., (2013) reported a percentage of 58% *Robinia pseudoacacia* pollen in acacia honey samples from Romania.

Pollen analysis is not suitable for cases where the honey has been incorrectly filtered or has been adulterated by the addition of pollen. Therefore, melisopalinology should usually be supplemented by physicochemical and organoleptic analyzes. Thus, in order to classify honey according to its botanical origin, a global interpretation of all results is needed (Bogdanov et al., 2008).

Moisture content

The moisture of honey depends on several factors, namely: environmental factors, the moisture of the plant visited by the bees, the degree of maturity of the honey reached in the hive and the handling of beekeepers during honey harvest (Flores et al., 2015). According to Codex Alimentarius (Codex Alimentarius, 2001) moisture content should be lower than 20%. Exceeding the maximum allowed value leads to a deterioration by fermentation, due to the presence of yeasts and bacteria in honey (Sakač et al., 2019). The fermentation process results in alcohol, which in the presence of oxygen will decompose into acetic acid and water, honey thus having a sour taste (Prica et al., 2014). This parameter is important in determining quality and stability by limiting degradation during the fermentation process (El Sohaimy et al., 2015). It is considered that a moisture content lower than 18% prevents the fermentation process. However, this possibility cannot be ruled out even when honey has a moisture content of less than 17.1%, because there are certain predisposing factors such as: yeast content, honey temperature but also the availability and distribution of water after crystallization (Prica et al., 2014). For the analyzed samples the moisture content ranged from 16.66-20.74% (table 1). The moisture content of samples of acacia honey studied by Ahamed et al., (2017) ranged between 8.8-13.85, values which were also in accordance with the Codex Alimentarius standard. In another study, Liberato et al., (2013) examined 22 samples of honey of different botanical origins from Nothern Brazil and found that the moisture percentage range was 13.63-20.4%. Juan-Borrás et al., (2014) studied acacia honey from 3 different countries and reported a moisture content of 16.9% for Romanian acacia honey, 15.9% for Spanish honey and 17% for Czech honey.

pH

The pH of honey is an important parameter because an acidic pH inhibits both the presence and growth of microorganisms and can also influence the texture of honey, its stability and shelf life (Karabagias et al., 2014). Organic acids are the compounds responsible for protecting against microbial attacks, the pH of honey beeing normally between 3.5 and 5.5. Besides the fact that pH is an indicator of a possible microbial growth (Da Silva et al., 2016) it also has a role in identifying the botanical origin of honey (Sanz et al., 2005). The geographical and floral origin influences the pH values. The acidic pH of honey depends largely on the amount of gluconic acid resulting from the oxidation of glucose under the action of glucose oxidase. In addition, different aromatic and non-aromatic acids can affect the pH of honey (Khalafi et al., 2016). The pH of acacia honey samples ranged from 3.61 to 5.33 (table 1). The values were similar to those reported by Karabagias et al., (2018) for eucalyptus, chestnut and heather honey (pH 3.62 to 4.42). Cimpoiu et al., (2013) reported that the average pH value is 4.23 for acacia honey and 4.36 for polyfloral honey.

Table 1

Physical-chemical parameters, sugars content and organic acids content of acacia honey

Variable	Minimum	Maximum	Mean	Std. deviation
pH	3.610	5.330	3.980	0.365
Acidity (meq / kg)	0.320	4.140	2.377	0.828
Conductivity µS/cm	94.800	405.000	212.322	84.159
Moisture (%)	16.660	20.740	18.093	1.126
HMF (mg / kg)	0.150	23.204	4.136	5.012
Fructose	31.986	39.818	37.062	2.148
Glucose	20.662	33.684	25.796	2.621
Sucrose	0.000	2.093	0.450	0.555
F+G	53.084	68.352	62.858	3.510
F/G	1.021	1.655	1.449	0.150
Gluconic acid (g/kg)	1.916	2.666	2.206	0.202
Formic acid (g/kg)	0.030	0.175	0.119	0.032
Acetic acid (g/kg)	0.038	0.180	0.096	0.035
Propionic acid (g/kg)	0.004	0.196	0.032	0.036
Lactic acid (g/kg)	0.000	0.041	0.002	0.008
Butiric acid (g/kg)	0.000	0.045	0.012	0.014
Succinic acid (k/kg)	0.000	0.027	0.002	0.006

Free acidity

Free acidity is given by the presence of organic acids and is the parameter that indicates the beginning of the honey fermentation process, therefore the maximum allowed value for free acidity is 50 meq acid/kg (Oroian et al., 2017; Da Silva et al., 2016). The acidity of honey is caused, in addition to the presence of organic acids (tartaric, citric, oxalic, acetic, etc.), by nectar or by secretions from bees (Yadata, 2014). The natural acidity of honey increases during the storage and maturation of honey, as well as during the fermentation of honey. The value of acidity, which is related to organic acids naturally present in honey, varies depending on the floral source and the bee species (de Sousa et al., 2016). Free acidity is also used to differentiate nectar honey from honeydew (Sanz et al., 2005). High values of free acidity indicate the sugar fermentation with the formation of acetic acid by hydrolysis of alcohol (Geană et al., 2020).

The free acidity of acacia honey analyzed in this study ranged from 0.32 to 4.14 meq/kg. (table 1) None of the analyzed samples exceeded the limit imposed by legislation. Fuentes Molina et al., (2020) reported values for free acidity of 9.5–46 meq/kg when studying polyfloral honey from Chile. Balos et al., (2018) reported values for free acidity of 5.44 meq/kg (acacia honey) – 19.33 meq/kg (forest honey).

Color

Honey color analysis is a valuable method of classifying honey as monofloral. The color varies from light yellow to dark brown with reddish hue or greenish hue, this color variation being influenced by the source of honey (Mărghitaş, 2005) The values of color parameters are presented in Figure 1.

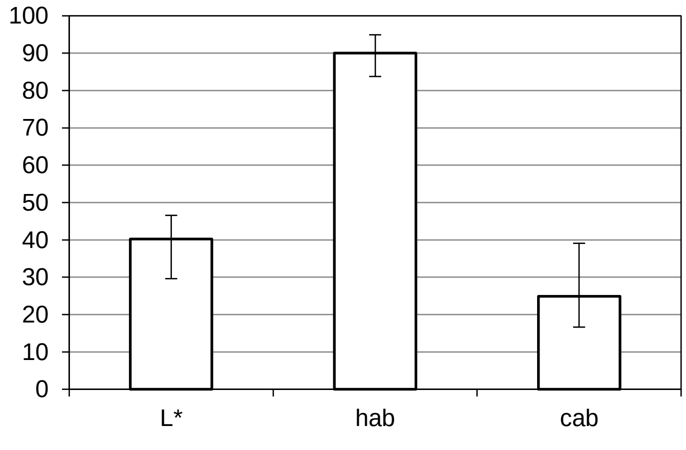

Figure 1. Color parameters (L*, h_{ab} and c_{ab}) for acacia honeys

Depending on the floral source, honey has a specific color; for example, acacia and citrus flowers produce straw-colored honey, while tilia flower produces a darker honey with a reddish hue (Siddiqui et al., 2017). The carotenoid, flavonoid and xanthophyll pigments come from nectar and pollen. The color of the pollen can vary from shades of pale yellow to dark brown, depending on the botanical species, this significantly influencing the color of honey (dos Santos Scholz et al., 2020). Besides its botanical origin, the color of honey can also be influenced by the mineral content, the climate but also by the storage conditions (Sakač et al., 2019). The price of honey depends largely on its color, acacia honey and citrus honey obtaining the highest prices (Bogdanov et al., 2004). All honey samples showed similar lightness values (29.62–46.57). Mădaș et al., (2019) reported values for L* of 50.19-64 for *Robinia pseudoacacia* honey from Romania. Dos Santos Scholz et al., (2020) reported that the mean values of L* in Ortigueira honey was 52.65.

HMF content

The hydroxymethylfurfural (HMF) content is a parameter that indicates the degree of freshness of the honey and consequently its degree of deterioration (Onur et al., 2018). Cyclic aldehyde HMF is absent in fresh and untreated foods. HMF content is formed by caramelization and Maillard reactions or by the dehydration reaction of hexose in acidic medium (Bouhlali et al., 2019). The main causes that can lead to increased HMF in food are aging, the amount of water, free acidity, botanical origin, and stress during storage (Apriceno et al., 2018). Therefore, HMF is a compound that occurs in large quantities in damaged honey, in improperly stored honey and also in honey that has undergone a strong or prolonged heat treatment (Önür et al., 2018). The maximum limit allowed by law is 40 mg/kg (Council, E. U., 2002). The maximum limit of HMF in the samples analyzed in this study was 23.20 mg/kg thus falling within quality requirements (table 1). Juan Borras et al., (2014) identified a maximum value of 7.1 mg/kg of HMF content in tilia honey from Romania.

Electrical conductivity

Electrical conductivity is a parameter used to control the quality of honey and to differentiate honeydew from floral honey. It can be used in determining the botanical origin by correlating with the pollen content of honey (the mineral content being brought into honey along with the pollen) (Kaskoniene et al., 2010). The analysis of this parameter is very often used, being considered a good criterion to be able to identify the botanical origin and implicitly the purity of honey (Balos et al., 2018). Components of honey such as organic and mineral acids have the ability to dissociate into ions when they are in an aqueous solution or conduct electricity. Light honey usually indicates a lower conductivity value than darker honey (Kropf et al., 2008). The maximum allowed value for floral honey is 800 µS/cm and values higher than 800 µS/cm are specific to honeydew (Karabagias et al., 2018). The electrical conductivity increases with the amount of ash and acid in the honey (El Sohaimy et al., 2015). A value of less than 500 µS/cm indicates a pure floral honey, with exceptions (Saxena et al., 2010). In this study the samples of acacia honey had values between 94.8 and 405 µS/cm and this value confirm that the analyzed samples are pure floral honey (table 1). Vranić et al., (2017) reported values of electrical conductivity between 160 µS/cm and 450 µS/cm for acacia and blossom honey samples. Mărghitaș et al., (2010) reported an average value of 150 µS/cm for ten samples of *Robinia pseudoacacia* honey.

Sugars

The sugar content of honey depends on the type of flowers visited by bees and thus varies with the botanical and geographical origin of honey and climatic conditions, processing and storage (Kaškonienė et al., 2010). The sugar content of honey and even the ratios between sugars are important indicators for classifying honey according to its botanical origin (Nozal et al., 2005). The predominant profile of sugars such as glucose, fructose, sucrose and maltose have been associated with a wide variety of properties of honey, such as viscosity, hygroscopy, granulation and energy value (Ouchemouk et al., 2009). As shown in Table 1, in the studied acacia honey a percentage of 68.35% monosaccharides was identified, with fructose reaching a maximum of 39.81% and glucose a maximum of 33.68%. All the analyzed samples showed a higher fructose content. The high proportion of d-fructose than d-glucose is related to the nectar source and suggests the possible existence of a low glycemic index (Bouhlali et al., 2019). Kamboj et al., (2020) analyzed cotton honey and reported a fructose content of 36.98% and a glucose content of 33.91%. The analyzed acacia honey samples contain a small percentage of sucrose (maximum 2.093%) which is below the 5% limit specified by Codex Alimentarius (Codex Alimentarius, 2001). A high sucrose content indicates a premature harvest of honey, which means that sucrose has not been completely transformed by the action of the enzyme invertase into glucose and fructose (do Nascimento et al., 2015). The concentration of sucrose varies with the degree of maturity and the source of nectar (Kamboj et al., 2013). Marghitaș et al., (2010) reported for Romanian acacia honey a concentration of fructose between 41.12–44.52% and for glucose 26–31.41%.

The ratio of fructose to glucose (F/G) is used to discriminate honeydew and floral honey (Dobre et al., 2012) and to predict the crystallization potential of honey (Laos et al., 2011). All types of honey that crystallize quickly have high glucose content and the F/G ratio is about 1 (Rajs et al., 2017) but the tendency of crystallization is slower with F/G values above 1.3 (Dobre et al., 2012). As shown in Table 1, the F/G ratio varied between 1.02 and 1.65 for the studied acacia samples. Juan Boras et al., (2014) reported an F/G ratio of 1.6 for acacia honey and lower values of this ratio for lime honey (1.3) and sunflower (1.06), which shows that acacia honey crystallizes more slowly.

Organic acids content

Organic acids represent a small percentage of the total components of honey (<0.5%) but define the aroma, color, pH and acidity and also play an important role in the antimicrobial and antioxidant activities of honey (Da Silva et al., 2016). The origin of aliphatic organic acids in honey is partially known, although many of them can be natural intermediates through the metabolic pathways of microorganisms, the Krebs cycle (acids: citric, succinic, glutaric, fumaric and oxaloacetic) or enzymatic reactions. The acids can also be synthesized from glucose, fructose and sucrose in the nectar, by the enzymatic action of bees or can come directly from the secretion of plants and also from the excretion of insects that reach the plants (Mato et al., 2003; Brugnerotto et al., 2019).

Determining the composition of organic acids in honey can be an important parameter used to discriminate the botanical origin of honey (Daniele et al., 2012). The acidity of honey is given by the more than 30 organic acids that are obtained directly from nectar or are formed when nectar is transformed into honey (Da Silva et al., 2016; Mato et al., 2003). Fermentation and aging processes that can occur during storage lead to an increase of total acid content (Mato et al., 2006). The citric acid content is an essential parameter in differentiating floral honey from honeydew (Suarez-Luque et al., 2012).

The non-aromatic organic acid that is predominant in the composition of honey is gluconic acid and is formed by the activity of glucose oxidase during maturation or by the metabolic activity of certain *Gluconobacter spp.* (Mato et al., 2003). Gluconic acid is also the main organic acid in the case of the samples analyzed in this study (1.916–2.666 g/kg), followed by propionic and acetic acids. Suto et al., (2020) studied acacia honey and reported a content of 1.575 g/kg gluconic acid. In a previous study, a higher amount of gluconic acid

(5.62 g/kg) was identified on sunflower honey (Pauliuc and Oroian, 2020), and the same was observed for chestnut honey (8.90 g/kg) (Sahin and Erim, 2011). In conclusion, acacia honey has a lower content of gluconic acid. As shown in Table 1, succinic acid has the lowest concentration in the studied acacia honey samples. Suto et al., (2020) reported that succinic acid was detected in 16 of 25 samples (average succinic acid concentration of 0.028 g/kg).

Conclusion

The content of pollen, the physicochemical parameters, the organic acids content and the sugar composition of Romanian acacia honey were analyzed in this study in order to classify this type of honey as monofloral honey.

All the investigated honey samples (27 samples) met the examined quality criteria (moisture, pH, free acidity, HMF content, color and electrical conductivity) and the high percentage of pollen grains of *Robinia pseudoacacia* confirmed that the analyzed honey samples were samples of monofloral acacia honey.

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References

Ahamed M.M.E., Abdallah A., Abdalaziz A., Serag E., Atallah A.B.E.H. (2017), Some physiochemical properties of Acacia honey from different altitudes of the Asir Region in Southern Saudi Arabia, *Czech Journal of Food Sciences*, 35(4), pp. 321–327

Apriceno A., Bucci R., Girelli A. M., Marini F., Quattrocchi L. (2018), 5–Hydroxymethyl furfural determination in Italian honeys by a fast near infrared spectroscopy, *Microchemical Journal*, 143, pp. 140–144.

Baloš M. Ž., Popov N., Vidaković S., Pelić D. L., Pelić M., Mihaljev Ž., Jakšić S. (2018), Electrical conductivity and acidity of honey, *Archives of Veterinary Medicine*, 11(1), pp. 91–101.

Belay A., Solomon W.K., Bultossa G., Adgaba N., Melaku S. (2013), Physicochemical properties of the Harenna forest honey, Bale, Ethiopia, *Food chemistry*, *141*(4), pp. 3386–3392.

Bogdanov S., Jurendic T., Sieber R., Gallmann P. (2008), Honey for nutrition and health: a review, *Journal of the American college of Nutrition*, *27*(6), pp. 677–689.

Bogdanov S., Ruoff K., Oddo L.P. (2004), Physico-chemical methods for the characterisation of unifloral honeys: a review, *Apidologie*, *35*(Suppl. 1), pp. S4–S17

Bouhlali E.D. T., Bammou M., Sellam K., El Midaoui A., Bourkhis B., Ennassir J., ... Filali-Zegzouti Y. (2019), Physicochemical properties of eleven monofloral honey samples produced in Morocco, *Arab Journal of Basic and Applied Sciences*, 26(1), pp. 476–487.

Brugnerotto P., Della Betta F., Gonzaga L.V., Fett R., Costa A.C.O. (2019), A capillary electrophoresis method to determine aliphatic organic acids in bracatinga honeydew honey and floral honey, *Journal of Food Composition and Analysis*, *82*, pp. 103243.

Cimpoiu C., Hosu A., Miclaus V., Puscas A. (2013), Determination of the floral origin of some Romanian honeys on the basis of physical and biochemical properties, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 100, pp. 149–154.

Codex Alimentarius Commission (2001), *Codex alimentarius: fats, oils and related products, Vol. 8*, FAO.

Consonni R., Cagliani L.R. (2015), Recent developments in honey characterization, *RSC Advances*, 5(73), pp. 59696–59714,

Cotte J.F., Casabianca H., Chardon S., Lheritier J., Grenier-Loustalot M.F. (2004), Chromatographic analysis of sugars applied to the characterisation of monofloral honey, *Analytical and bioanalytical chemistry*, 380(4), pp. 698–705.

Council E. U. (2002), Council Directive 2001/110/EC of 20 December 2001 relating to honey, *Official Journal of the European Communities L*, *10*, pp. 47–52.

da Silva P.M., Gauche C., Gonzaga L.V., Costa A.C.O., Fett R. (2016), Honey: Chemical composition, stability and authenticity, *Food Chemistry*, *196*, pp. 309–323.

Danezis G.P., Tsagkaris A.S., Camin F., Brusic V., Georgiou C.A. (2016), Food authentication: Techniques, trends emerging approaches, *TrAC Trends in Analytical Chemistry*, *85*, p. 123

Daniele G., Maitre D., Casabianca H. (2012), Identification, quantification and carbon stable isotopes determinations of organic acids in monofloral honeys. A powerful tool for botanical and authenticity control, *Rapid Communications in Mass Spectrometry*, *26*(17), pp. 1993–1998

de Sousa J.M.B., de Souza E.L., Marques G., de Toledo Benassi M., Gullón B., Pintado M.M., Magnani M. (2016), Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi–arid region, *LWT–Food Science and Technology*, *65*, pp. 645–651.

do Nascimento A.S., Chambó E.D., de Jesus Oliveira D., Andrade B. ., Bonsucesso J.S., de Carvalho C.A.L. (2018), Honey from stingless bee as indicator of contamination with metals, *Sociobiology*, *65*(4), pp. 727–736.

do Nascimento A. ., Marchini L. C., de Carvalho C.A.L., Araújo D.F.D., de Olinda R. A., da Silveira T.A. (2015), Physical-chemical parameters of honey of stingless bee (Hymenoptera: Apidae), American Chemical Science Journal, 7(3), pp. 139–149.

Dobre I., Alexe P., Escuredo O., Seijo C.M. (2013), Palynological evaluation of selected honeys from Romania, *Grana*, 52(2), 113–121.

Dobre I., Georgescu L. A., Alexe P., Escuredo O., Seijo M.C. (2012), Rheological behavior of different honey types from Romania, *Food Research International*, *49*(1), pp. 126–132.

dos Santos Scholz M.B., Júnior A. Q., Delamuta B. H., Nakamura J. M., Baudraz M. C., Reis M. O., ... Bianchini F.P. (2020), Indication of the geographical origin of honey using its physicochemical characteristics and multivariate analysis, *Journal of food science and technology*, *57*(5), pp. 1896–1903

El Sohaimy S.A., Masry S.H.D., Shehata M.G. (2015), Physicochemical characteristics of honey from different origins, *Annals of Agricultural Sciences*, *60*(2), pp. 279–287.

Flores M.S.R., Escuredo O., Seijo M.C. (2015), Assessment of physicochemical and antioxidant characteristics of Quercus pyrenaica honeydew honeys, *Food Chemistry*, *166*, pp. 101–106.

Fuentes Molina O., Alizadeh K., Bucarey S.A., Castaneza Zúñiga, E., Vásquez-Quitral P. (2020), Analysis of organic molecules, physicochemical parameters, and pollen as indicators for authenticity, botanical origin, type and quality of honey samples examined, *International Journal of Food Properties*, *23*(1), pp. 2242–2256.

Geană E. I., Ciucure C. T., Costinel D., Ionete R. E. (2020), Evaluation of honey in terms of quality and authenticity based on the general physicochemical pattern, major sugar composition and δ13C signature, *Food Control*, 109, pp. 106919.

Harmonised Methods of the International Honey Commission, (2008).

Jandrić Z., Frew R. D., Fernandez-Cedi L. N., Cannavan A. (2017), An investigative study on discrimination of honey of various floral and geographical origins using UPLC-QToF MS and multivariate data analysis, *Food Control*, *72*, pp. 189–197

Juan-Borrás M., Domenech E., Hellebrandova M., Escriche I. (2014), Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys, *Food Research International*, *60*, pp. 86–94.

Kamboj R., Bera M., Nanda V. (2013), Chemometric classification of Northern India unifloral honey, *Acta Alimentaria*, *42*(4), pp. 540–551.

Kamboj R., Nayik G.A., Bera M. B., Nanda V. (2020), Sugar profile and rheological behaviour of four different Indian honey varieties, *Journal of Food Science and Technology*, pp. 1–9.

Karabagias I. K., Badeka A., Kontakos S., Karabournioti S., Kontominas M.G. (2014), Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics, *Food chemistry*, *146*, pp. 548–557

Karabagias I. K., Louppis A. P., Kontakos S., Drouza C., Papastephanou C. (2018), Characterization and botanical differentiation of monofloral and multifloral honeys produced in Cyprus, Greece, and Egypt using physicochemical parameter analysis and mineral content in conjunction with supervised statistical techniques, *Journal of analytical methods in chemistry*, *2018*.

Karabagias I.K., Maia M., Karabagias V.K., Gatzias I., Badeka A.V. (2018), Characterization of eucalyptus, chestnut and heather honeys from Portugal using multi–parameter analysis and chemo-calculus, *Foods*, 7(12), pp. 194.

Kaškonienė V., Venskutonis P.R., eksterytė V. (2010), Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania, *LWT–Food Science and Technology*, *43*(5), pp. 801–807.

Khalafi R., Goli S.A.H., Behjatian M. (2016), Characterization and classification of several monofloral Iranian honeys based on physicochemical properties and antioxidant activity, *International journal of food properties*, 19(5), pp. 1065–1079.

Kropf U., Jamnik M., Bertoncelj J., Golob T. (2008), Linear regression model of the ash mass fraction and electrical conductivity for Slovenian honey, *Food Technology and Biotechnology*, *46*(3), pp. 335–340.

Laos K., Kirs E., Pall R., Martverk K. (2011), The crystallization behaviour of Estonian honeys, *Agron. Res.*, *9*, pp. 27–432.

Liberato M.C.T.C., Morais S.M. (2013): Physicochemical properties and mineral and protein content of honey samples from Ceará State, Northeastern Brazil, *Food Science and Technology (Campinas)*, 33, pp. 38–46

Ma T., Zhao H., Liu C., Zhu M., Gao H., Cheng N., Cao W. (2019), Discrimination of natural mature acacia honey based on multi-physicochemical parameters combined with chemometric analysis, *Molecules*, *24*(14), pp. 2674.

Mădaş N.M., Mărghitaş L.A., Dezmirean D.S., Bonta V., Bobiş O., Fauconnier M.L., ... Nguyen K.B. (2019), Volatile profile and physico–chemical analysis of acacia honey for geographical origin and nutritional value determination, *Foods*, *8*(10), p. 445.

Marghitas L.A., Dezmirean D.S., Pocol C.B., Marioara I.L.E.A., Bobis O., Gergen I. (2010), The development of a biochemical profile of acacia honey by identifying biochemical determinants of its quality, *Notulae Botanicae Horti Agrobotanici Cluj–Napoca*, *38*(2), pp. 84–90

Marghitas A.L. (2005), *Bees and their products, publishing house Ceres*, Bucharest, Romania

Mato I., Huidobro J. F., Simal-Lozano J., Sancho M.T. (2003), Significance of nonaromatic organic acids in honey, *Journal of food protection*, *66*(12), pp. 2371–2376.

Mato I., Huidobro J. F., Simal-Lozano J., Sancho M. T. (2006), Rapid determination of nonaromatic organic acids in honey by capillary zone electrophoresis with direct ultraviolet detection, *Journal of agricultural and food chemistry*, 54(5), pp. 1541–1550.

Nozal M. J., Bernal J. L., Toribio L., Alamo M., Diego J. C., Tapia J. (2005), The use of carbohydrate profiles and chemometrics in the characterization of natural honeys of identical geographical origin, *Journal of agricultural and food chemistry*, *53*(8), pp. 3095–3100.

Oddo L.P., Piro R., Bruneau É., Guyot-Declerck C., Ivanov T., Piskulová J., ... Ruoff K. (2004), Main European unifloral honeys: descriptive sheets, *Apidologie*, 35(Suppl. 1), pp. S38–S81

Ouchemouk S., Schweitzer P., Bey M.S., Djoudad-Kadji H., Louaileche H. (2009), HPLC sugar profiles of Algerian honeysn, *Food Chemistry*, 121, pp. 561–568

Önür İ., Misra N. N., Barba F. J., Putnik P., Lorenzo J. M., Gökmen V., Alpas H. (2018), Effects of ultrasound and high pressure on physicochemical properties and HMF formation in Turkish honey types. *Journal of Food Engineering*, *219*, pp. 129–136.

Oroian M., Amariei S., Rosu A., Gutt G. (2015) Classification of unifloral honeys using multivariate analysis, *Journal of Essential Oil Research*, 27(6), pp. 533–544.

Oroian M., Ropciuc S., Buculei A. (2017), Romanian honey authentication based on physico–chemical parameters and chemometrics, *Journal of Food Measurement and Characterization*, *11*(2), pp. 719–725.

Özcelik S., Kuley E., Özogul F. (2016), Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria, *LWT*, *73*, pp. 536–542.

Pauliuc D., Oroian M. (2020), Organic acids and physico–chemical parameters of romanian sunflower honey, *Food and Environment Safety Journal*, *19*(2),

Pires J., Estevinho M.L., Feás X., Cantalapiedra J., Iglesias A. (2009), Pollen spectrum and physico-chemical attributes of heather (Erica sp.) honeys of north Portugal, *Journal of the Science of Food and Agriculture*, 89(11), pp. 1862–1870.

Prica N., Baloš M. Ž., Jakšić S., Mihaljev Ž., Kartalović B., Babić J., Savić S. (2014), Moisture and acidity as indicators of the quality of honey originating from Vojvodina region, *Archives of veterinary medicine*, 7(2), pp. 99

Rajs B. B., Flanjak I., Mutić J., Vukojević V., Đurđić, S., Primorac, L. (2017), Characterization of Croatian Rape (Brassica sp.) Honey by Pollen Spectrum, Physicochemical Characteristics, and Multielement analysis by ICP–OES, *Journal of AOAC International*, *100*(4), pp. 881–888

Ruoff K., Luginbühl W., Künzli R., Iglesias M. T., Bogdanov, S., Bosset, J. O., ... Amado, R. (2006), Authentication of the botanical and geographical origin of honey by mid–infrared spectroscopy, *Journal of agricultural and food chemistry*, *54*(18), pp. 6873–6880.

Sahin F.T.S.K.H., Erim E.U.F.B. (2011), Evaluation of organic acid, saccharide composition and antioxidant properties of some authentic Turkish honeys, *Journal of Food and Nutrition Research*, 50(1), pp. 33–40.

Sakač M.B., Jovanov P.T., Marić A.Z., Pezo L.L., Kevrešan Ž.S., Novaković A.R., Nedeljković, N.M. (2019), Physicochemical properties and mineral content of honey samples from Vojvodina (Republic of Serbia), *Food chemistry*, *276*, pp. 15–21

Sanz M. L., Gonzalez M., De Lorenzo C., Sanz J., Martınez-Castro I. (2005), A contribution to the differentiation between nectar honey and honeydew honey, *Food chemistry*, 91(2), pp. 313–317.

Saxena S., Gautam S., Sharma A. (2010), Physical, biochemical and antioxidant properties of some Indian honeys, *Food Chemistry*, 118(2), pp. 391–397

Schievano E., Stocchero M., Zuccato V., Conti I., Piana L. (2019), NMR assessment of European acacia honey origin and composition of EU–blend based on geographical floral markers, *Food chemistry*, *288*, pp. 96–101.

Seisonen S., Kivima E., Vene K. (2015), Characterisation of the aroma profiles of different honeys and corresponding flowers using solid-phase microextraction and gas chromatography-mass spectrometry/olfactometry, *Food chemistry*, 169, pp. 34–40.

Siddiqui A.J., Musharraf S.G., Choudhary M.I. (2017), Application of analytical methods in authentication and adulteration of honey, *Food chemistry*, 217, pp. 687–698

Soares S., Amaral J. S., Oliveira M.B.P., Mafra I. (2017), A comprehensive review on the main honey authentication issues: Production and origin, *Comprehensive Reviews in Food Science and Food Safety*, 16(5), pp. 1072–1100

Sobrino-Gregorio L., Vargas M., Chiralt A., Escriche I. (2017), Thermal properties of honey as affected by the addition of sugar syrup, *Journal of food engineering*, 213, pp. 69–75.

Suárez-Luque S., Mato I., Huidobro J.F., Simal-Lozano J., Sancho M.T. (2002), Rapid determination of minority organic acids in honey by high–performance liquid chromatography, *Journal of Chromatography A*, 955, pp. 207–214

Suto M., Kawashima H., Nakamura Y. (2020), Determination of Organic Acids in Honey by Liquid Chromatography with Tandem Mass Spectrometry, *Food Analytical Methods*, 13(12), pp. 2249–2257.

Teklit G., Frehiwot M. (2016), Phytochemical and biochemical composition of wild honey: A Case study in Eastern Zone areas in Tigray Ethiopia, *Journal of Nutritional Health Food Engineering*, 4(4), pp. 141–146

Trifković J., Andrić F., Ristivojević P., Guzelmeric E., Yesilada, E. (2017), Analytical methods in tracing honey authenticity, *Journal of AOAC International*, 100(4), p. 827

Visquert M., Vargas M., Escriche I. (2014), Effect of postharvest storage conditions on the colour and freshness parameters of raw honey, *International journal of food science & technology*, 49(1), pp. 181

Von Der Ohe W., Oddo L.P., Piana M.L., Morlot M., Martin P. (2004), Harmonized methods of melissopalynology, *Apidologie*, 35(Suppl. 1), S18–S25.

Vranić D., Petronijević R., Stojanović J.Đ., Korićanac V., Milijašević J.B., Milijašević M. (2017, September), Physicochemical properties of honey from Serbia in the period 2014–2016. In *IOP Conference Series: Earth and Environmental Science*, 85(1), 012058, IOP Publishing.

White J.J. (1979), Spectrophotometric method for hydroxymethylfurfural in honey, Journal–Association of Official Analytical Chemists, 62(3), 509–514 work of the International Honey Commission, *Mitt. Lebensm. Hyg*., 90, pp. 108–125.

White J. W. (2000), Isotope ratio testing of honey: demystifying the Internal standard test, *American Bee Journal*, 140(4), p. 318

Xagoraris M., Lazarou E., Kaparakou E.H., Alissandrakis E., Tarantilis P.A., Pappas C.S. (2020), Botanical origin discrimination of Greek honeys: physicochemical parameters versus Raman spectroscopy, *Journal of the Science of Food and Agriculture*.

Yadata D. (2014), Detection of the electrical conductivity and acidity of honey from different areas of Tepi, *Food Science and Technology*, 2(5), 5, pp. 9–63.