Evaluation of Serbian black locust honey quality parameters as a contribution to confirmation of its botanical origin

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Abstract. Acacia honey, like many other specialized kinds of honey, is derived purely from one plant species, in this case, the nectar of black locust (Robinia pseudoacacia) tree flowers. The present study investigated the quality of 270 acacia honeys from the Serbian market, collected during 2017 and 2018. Chemical and physical properties of honey were evaluated according to Serbian regulation. All applied methods were performed according to the Harmonized Methods of the International Honey Commission. Summarizing the results presented, none of the tested acacia honeys exceeded limits of national or EU regulations for moisture, free acid and insoluble matter contents as well as electrical conductivity. However, the most common parameters for non-compliant honeys were hydroxymethylfurfural and sugar contents and diastase activity. Among these parameters, this study shows the fructose to glucose ratio is also an important quality factor, significant for confirming the origin of acacia honey, while the correlation between glycemic index and the fructose to glucose ratio is especially important for individual honey consumers with impaired glucose tolerance or insulin resistance.

1. Introduction

Honey is defined as “the natural sweet substance produced by Apis mellifera bees from the nectar of plants or from secretions of living parts of plants or excretions of plantsucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honey combs to ripen and mature” [1]. The major components of honey are sugars, mostly fructose and glucose, although other minor components such as enzymes, proteins, organic acids, minerals, pollen grains, waxes and phytochemicals are also present [2]. The composition of honey is characterized by the type of plants from which bees collect their nectar, climatic conditions, environmental factors, and bee farming practices [3, 4]. Acacia honey, like many other specialized kinds of honey, is derived purely from one plant, in this case, nectar from flowers of the black locust (Robinia pseudoacacia) tree, which is native to North America, but is also present in Europe, where it is known as a “false acacia”. For clarity reasons, honey originating from Robinia will be, hereinafter, referred to as acacia honey. This variety of honey is almost clear, like liquid glass, and has a mildly sweet flavor.

The Community Directive 2001/110 [1] and national regulation [5] strictly defined and established the general and specific characteristics of honey and the quality indicators which characterize individual honey varieties. Regulative recommendations are that acacia honey should have a moisture content not more than 20%, sugar content not less than 60%, sucrose not more than 10%, free acidity
not more than 50 meq/kg, hydroxymethylfurfural (HMF) content not more than 40 mg/kg, water-insoluble content not more than 0.1%, diastase activity not less than 8 Göthe units and electrical conductivity not more than 0.8 mS/cm.

Limited acacia honey production due to its rarity and insufficient availability has produced heightened interest in its adulteration [6]. Honey adulterants are mainly starch syrup, invert syrup, starch or invert syrup fed to bees, and low quality honey added to high priced honey [7]. The present study shows the results of an investigation into the quality of acacia honey from the Serbian market in 2017 and 2018. Chemical and physical properties of honey were evaluated according to Serbian Regulation [5] and in order to select parameters important for confirming and distinguishing acacia honey from other blossom honeys characteristic of Serbia and the Balkan region.

2. Materials and Methods

2.1. Honey samples
A total of 270 acacia honey samples were obtained from different regions from the Serbian retail market during 2017-2018. All honeys were stored at ambient temperature prior to analysis. In most of the honeys, all parameters of quality defined by the legislation were examined, and in a smaller number, analyses were carried out as per client’s request.

2.2. Methods of physical and chemical analysis
All applied methods were performed according to the Harmonized methods of the International Honey Commission [8]. The moisture content (%) was determined from the refractive index of the honey by reference to a standard table. Free acidity was determined by titration to pH 8.30 and expressed as milli equivalents/kg (meq/kg). Electrical conductivity (mS/cm) was performed using a conductivity meter at 20°C in a 20% (dry matter basis) solution of honey samples prepared with ultrapure water. Content of insoluble matter (%) is defined as that material found by the procedure to be insoluble in water. Determinations of sugars (glucose, fructose and sucrose) (%) were performed with a Waters 2690 high-performance liquid chromatograph equipped with a refractive index (RI) detector (Waters model 2414). Duplicate injections were performed and average peak areas were used for the peak quantification. Glucose, fructose and sucrose, purity ≥99.5 % (Sigma–Aldrich), were used as standards to determine the sugar content of honey. Quantification was performed according to the external standard method on peak areas. Determination of diastase activity after Schade was performed and results are expressed in Göthe units per gram of honey (DN). The concentration of 5-(hydroxymethyl)-furan-2-carbaldehyde (HMF) was determined using reverse phase HPLC equipped with UV detection and the result is expressed in mg/kg. Carbohydrate ratios (F/G) were calculated and evaluated with respect to literature data. All the tests were performed in duplicate and expressed as minimum, maximum, mean and median values.

2.3. Statistical analysis
For statistical evaluation of data, Microsoft Excel with Data Analysis Tool Pack from MS Office was used.

3. Results and Discussion
The results of physicochemical analysis expressed as minimum, maximum, mean and median as well as number of tested and compliant honeys are presented in Table 1. All studied acacia honeys were within standard limits of moisture, free acidity, electrical conductivity and water insoluble content [5]. None of the honeys exceeded the maximum allowed moisture value of 20% [1, 5], levels above which can elevate the honey’s ability to resist fermentation and granulation and impede longer shelf life during storage [8]. These values agree with the results obtained in 201 acacia honeys from Serbia, tested in 2009 (average moisture content 16.12%) [9] and 132 acacia honeys in 2014-2016 (moisture content in all examined honeys were lower than the permitted value) [10]. Slightly higher mean values
for moisture content (16.00±1.33%) were reported in examined acacia honeys from Bosnia and Herzegovina [11], Spain (17.5%) [12], and Romania (17.9%) [13].

Table 1. Descriptive statistics for acacia (*Robinia pseudoacacia*) honey quality parameters and number and percent of compliant honeys

| Parameter                             | Number of samples examined | Min     | Max     | Mean     | Median   | Complianent (number) | Complianent (%) |
|---------------------------------------|----------------------------|---------|---------|----------|----------|----------------------|-----------------|
| Moisture (%)                          | 196                        | 13.30   | 20.00   | 16.31    | 16.22    | 196                  | 100             |
| Free acidity (meq/kg)                 | 180                        | 3.00    | 45.90   | 10.78    | 9.89     | 180                  | 100             |
| Electrical conductivity (mS/cm)       | 222                        | 0       | 0.66    | 0.17     | 0.16     | 222                  | 100             |
| Water-insoluble content (%)           | 191                        | 0       | 0.06    | 0.01     | 0.01     | 191                  | 100             |
| Glucose and fructose content (%)      | 196                        | 32.62   | 80.55   | 69.59    | 70.23    | 190                  | 96.94           |
| Sucrose (%)                           | 200                        | 0.97    | 42.12   | 6.37     | 5.95     | 196                  | 98.00           |
| Fructose/glucose ratio                | 196                        | 0.44    | 1.91    | 1.52     | 1.60     | 149*                 | 76.02           |
| Diastase activity (DN)                | 197                        | 0       | 50.16   | 15.70    | 13.98    | 191                  | 96.95           |
| HMF (mg/kg)                           | 210                        | 0.16    | 295.2   | 13.18    | 4.49     | 197                  | 93.81           |

*Honeys with fructose to glucose ratio over 1.4 were considered as compliant [14]*

Free acidity is a quality parameter related to honey fermentation [15]. However, the presence of different organic acids, geographical origin and harvest season can affect the honey’s acidity [16]. National regulation permits a maximum value of 50 meq/kg [5]. The results obtained (mean value was 10.78 meq/kg) showed a similar trend to data from a study of quality in honey from the Serbian retail market, 2014-2016 [10], when reported mean values for free acidity for acacia honeys were 10.82 meq/kg (2014); 10.87 meq/kg (2015) and 8.23 meq/kg (2016). A slightly higher mean free acidity (12.43±7.13meq/kg) was shown for acacia honey from Bosnia and Herzegovina [11], while free acidity was lower (6.45meq/kg) in acacia honeys from Romania [13].

Electrical conductivity is often used in the quality control of honey to distinguish blossom honey from honeydew [17] and is considered a good criterion for the botanical origin of honey [18]. The Official Regulation on quality of honey [5] recommends a maximum value of 0.8 mS/cm for acacia and blossom honey and minimum value of 0.8 mS/cm for honeydew. The mean electrical conductivity value of 0.17 mS/cm was similar to our previously reported electrical conductivity levels of honey from Serbia [10], (acacia honey during 2016 (0.16 mS/cm) as well as reported results for honey from Bosnia and Herzegovina, 2016-2017 (0.13±0.15 mS/cm) [11]. Our results agreed with those reported for acacia honeys from Romania (mean value 0.150 mS/cm) [13].

The insoluble matter is important to detect honey impurities, which includes wax, pollen, honeycomb debris, bees and filth particles. The water-insoluble contents in all honeys ranged from 0.00% to 0.06% with a mean value of 0.01%, and all these values were lower than the permitted maximum limit (0.1%). Similar results of water-insoluble contents were reported in the study of acacia honey from Serbian [10] and from Bosnia and Herzegovina markets [11].

Essentially, honey contains a concentrated water solution of two main sugars, fructose and glucose, with small amounts of various complex sugars. Sugars contribute nearly 95% of honey’s dry weight [18], of which 75% is composed of monosaccharides (fructose and glucose), as well as minute amounts of disaccharides (sucrose) and 10-15% of other types of sugars (oligosaccharides and tetrasaccharides). Fructose is always the most important sugar in honey, quantitatively, followed by glucose. Sugars in honey are used for energy supply, as well as contributing to the observed physical
characteristics of honey such as viscosity, hydroscopicity and granulation [19]. Sugar content depends mostly on botanical and to a lesser extent on climate conditions, geographical origin, and on seasonal, processing, and storage conditions [20, 21].

According to [2], the mineral content and the sugar profile have been suggested as criteria for the characterization of monofloral honeys. The sum of glucose plus fructose is a discriminatory variable used to distinguish between blossom and honeydew honeys [1, 5]. The average total value of glucose plus fructose was 69.59% (minimum amount of reducing sugars is 60%). Altogether, 96.94% of tested honeys were in accordance with national regulation [5]. The results were slightly lower than those obtained in studies [11, 22], where the average total value of glucose plus fructose was 75.47±0.29 and 72.26±6.53%, respectively. Our previously reported results for acacia honey during 2014, 2015 and 2016 showed the total content of glucose plus fructose ranged from 24.54-71.56%, 23.97-79.13% and 33.42-76.23%, respectively [10].

Besides the reducing sugars analysis, the amount of sucrose is a very important parameter in evaluating the honey maturity. This parameter is analyzed with the purpose of identifying improper manipulation of honey. Inadequate maturation or artificially feeding bees with sucrose syrups can cause high levels of sucrose [22], as does early harvest, which indicates the sucrose was not completely transformed into glucose and fructose [23]. Sucrose was present in all the honeys analyzed, ranging between 0.97 and 42.12%. Only four of the acacia honeys contained higher sucrose content than is recommended (10%) [5]. The mean sucrose content (6.37%) was higher than was reported for other acacia honeys, 2.3% and 2.55%, respectively [11, 12]. According to [24], the reason for the variable levels of sucrose could be due to the transglucosylation reaction initiated by transference of the α-D-glucopyranosil unit from sucrose to an acceptor molecule.

The time required for honey to crystallize depends mostly on the ratio of fructose to glucose (F/G), [25]. The F/G ratio is a typical feature of honey types [26]. Honeys that do not crystallize for a long time have F/G ratios greater than 1.33, and if the ratio is less than 1.11, the honey crystallizes quickly [28]. The rate at which glucose crystallization occurs in honey also depends on the glucose/water ratio (G/W), and according to reported data [28, 20], slow crystallization of honey occurred when G/W was less than 1.7, but when it is greater than 2.0, this phenomenon is fast and complete. Glucose is less water soluble than fructose, and therefore, this makes it an important parameter to predict the crystallization tendency of honey [29]. Furthermore, honey crystallization depends on other factors such as the presence of other sugars (e.g. sucrose, maltose), insoluble substances (e.g. dextrin, colloids, pollen), and storage temperature that can influence the crystallization process [30, 31]. The F/G ratio of the examined honeys ranged from 0.44 to 1.91 (Table 1). The mean F/G ratio (1.52) obtained in this study was similar to those reported in other studies [22, 12, 32, 13] (1.29±0.00; 1.40; 1.69-1.82 and 1.50, respectively).

Considering F/G ratio, beside its importance for predicting crystallization, this ratio is also important in testing honey quality as well as authenticity. F/G ratios higher than 1.4 were found to be essential for differentiation between authentic acacia honey and other declared, but unauthentic acacia honeys. Regarding the correlation between glycemic index (GI) and F/G ratio [14], the significance of being able to unequivocally confirm the origin of acacia honey is of great importance, especially for individuals with impaired glucose tolerance or insulin resistance. Sucrose could be partially or totally replaced with honey, particularly low GI honeys, such as acacia, for improving postprandial metabolic response [33].

HMF and diastase activity are routinely used to evaluate honey freshness, providing information about inadequate processing and/or inappropriate storage conditions [34]. Therefore, high quality honey should present high diastase and low HMF contents. The current law stipulates a minimum diastase activity of 8.00 Göthe units. Diastase is a quality factor for determining honey freshness and is influenced by botanical origin, the climate of the region, storage and heating [35]. The diastase activity in the examined honeys ranged between 0.00 and 50.16 Göthe units (mean value 15.79 Göthe units). Lower mean values for diastase activity in acacia honeys were reported in Bosnia and Herzegovina (10.19±8.33) [11] and for those in Serbia in 2014, 2015 and 2016 (13.05; 8.86 and 11.60,
respectively) [10]. HMF is widely known as the most consistent indicator of honey freshness. It is absent in fresh honeys and tends to increase during processing and/or aging [36]. For instance, honeys stored for more than 12-24 months contained 128-1131 mg/kg of HMF and according to study [37], honey should be consumed within one year of storage. Also, high HMF in honeys can be an indication of adulteration by adding invert syrup [38, 39]. Results (mean value 13.18 mg/kg, Table 1) showed that the content of HMF in 93.81% of analyzed honeys were below 40 mg/kg and so complied with national and European regulations [5, 1], as well as with some European bee federations that consider a “quality honey” has HMF content lower than 15 mg/kg [40]. However, HMF content in 13 honeys exceeded the legal limit. The mean HMF value was higher than reported data (6.79±1.56 mg/kg) for China black locust honey [22] and lower than results for acacia honeys from Bosnia and Herzegovina (31.36 mg/kg) [11] and Serbia in 2014, 2015 and 2016 (3.07-140.06 mg/kg; 2.11-387.43 mg/kg and 0.57-211.35 mg/kg, respectively [10]).

4. Conclusion

Government action during 2014-2015 and enhanced quality control probably influenced the better honey quality measured during recent years (2016, 2017 and 2018). Serbia is a honey exporting country, especially of rare and valuable honeys like acacia, sunflower and linden. Adulteration and degradation of the quality of Serbian honey has a significant impact on public health and the economy of the country.

In general, the present results on quality of acacia honeys during 2017-2018, from the entire Serbian market, showed a significant drop of non-compliant honeys (<7% were non-compliant) compared to 2016 and particularly 2014-2015, when the percentage of non-compliant honeys was approximately 20% and 80%, respectively [10].

None of the tested acacia honeys exceeded limits of national or EU regulations [1, 5] for moisture, free acids, insoluble matter, or electrical conductivity. However, similarly to our previous examinations [10], the most relevant parameters for non-compliant honey detection were determinations of HMF, sugar contents and diastase activity. Among these parameters, this study shows the F/G ratio is also an important quality factor and should be defined and taken into consideration for legal regulations on honey quality. Additionally, it could be a cornerstone for rapid confirmation of the authenticity of a honey’s floral origin, especially for acacia honey.

Therefore, further research on acacia and other kinds of honey, including on their physicochemical properties and composition is required to guarantee the quality, safety and authenticity of this product and verify its functional and health properties. This work should contribute to current knowledge of acacia honey, and consequently, will support future research to correlate the examined parameters with botanical origin, climate, bee forage, the effects of environment, and production technology on quantity and quality of honey.

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References

[1] Council directive 2001/110/EC 2002 O.J. L 10 47
[2] Manzanares A B, Garcia Z H B, Galdon R, Rodriguez E R and Romero C D 2014 LWT-Food Sci. Technol. 55 572
[3] Anklam E 1998 Food Chem. 63 549
[4] Meo S A, AL-Asiri S A, Mahesar A I and Ansari M J 2017 Saudi J. Biol. Sci. 24 975
[5] Off. Gazette RS 2015 101
[6] Wang Y, Juliani R, Simon J E and Ho C 2009 Food Chem. 115 233
[7] Zabrodoska B and Vorlova L 2015 Acta Vet. Brno 83 85
[8] http://www.bee-hexagon.net/en/network.htm
[9] Lazarevic K B, Andrić F, Trifković J, Tešić Ž and Milojković-Opšenica D 2012 *Food Chem.* **132** 2060

[10] Vranic D, Petronijevic R, Djinovic-Stojanovic J, Koricanac V, Babic Milijasevic J and Milijasevic M 2017 59th Int. Meat Industry Conf. MEATCON2017, October 1-4, Zlatibor, Serbia. IOP Conf. Series: *Earth Environ. Sci.* **85** 012058

[11] Ciric J, Sando D, Spiric D, Janjic J, Boskovic M, Glisic M and Baltic MZ 2018 *Meat Technol.* **59**(1) 46

[12] Escuerdo O, Dobre I, Fernandez-Gonzales M and Carmen Seijio M 2014 *Food Chem.* **149** 84

[13] Alexandru Marghitas L, Severus Dezmirein D, Bianca Pocul C, Ilea M, Bobis O and Gergen I 2010 *Not. Bot. Hort. Agrobot.* **32** 84

[14] Deibert P, Koenig D, Kloock B, Groenefeld M and Berg A 2010 *Eur. J. Clin. Nutr.* **64** 762

[15] Silvano M F, Varela M S, Palacio M A, Ruffinengo S and Yamul D K 2014 *Food Chem.* **152** 500

[16] Tornuk F, Karaman S, Ozturk I, Toker O S, Tastemur B, Sagdic O, Dogan M et al. 2013 *Ind. Crops. Prod.* **46** 124

[17] Karabagias IK, Badeka A, Kontakos S, Karabournioti S and Kontominas MG 2014 *Food Chem.* **146** 548

[18] Pita-Calvo C and Vazquez M 2017 *Trends Food Sci. Tech.* **59** 79

[19] Kemal M A and Klein P 2011 *Saud. J. Biol. Sci.* **18** 17

[20] Dobre I, Georgescu L A, Escuerdo O A P and Seiji M C 2012 *Food Res. Int.* **49** 126

[21] Khalil M I, Alan N, Moniruzzaman M, Sulaiman SA and Gan S H 2011 *J. Food Sci.* **76** C921

[22] Guo P, Deng Q and Lu Q 2019 *Food Chem.* **286** 608

[23] Escuerdo O, Miguez M, Fernandez-Gonzales M and Seijo MC 2013 *Food Chem.* **146** 851

[24] Leite Da Costa J M, Trugo L C, Costa L S M, Quinteiro L M C, Barth O M and Dutra V M L 2000 *Food Chem.* **70** 93

[25] Gleiter R, Horn H and Isengard HD 2006 *Food Chem.* **96** 441

[26] Czipta N 2010 Ph Thesis

[27] Smanalieva J and Senge B 2009 *Eur. Food Res. Technol.* **229** 107

[28] Manikis I and Thrasivoluou A 2001 *Apiacta* **36** 106

[29] Laos K, Kris E and Pall R 2011 *Agron. Res.* **9** 427

[30] Buba F, Gidado A and Shugabu A 2013 *Biochem Anal. Biochem.* **2** 139

[31] El-Metwally AAE 2015 PhD Thesis, *Fac. Agric. Cairo. Univ.*

[32] Adriana C, Szabó E, Borbély M, Czipta N and Purcărea C 2012 *Zootehnie si Tehnologii de Industrie Alimentara* p 325

[33] Flint A, Moller B K, Raben A, Sloth B, Pedersen D, Tetens I et al 2006 *Am. J. Clin. Nutr.* **84** p 1365

[34] Soares S, Pinto D, Rodrigues F, AlvesRC and Oliveira B 2017 *Molecules* **22** 1338

[35] Singh N and Barth P K 1997 *Food Chem.* **58** 129

[36] da Silva P M, Gauche C, Gonzaga L V, Oliveira C A C and Fett R 2016 *Food Chem.* **196** 309

[37] Khalil M I, Sulaiman S A and Gan S H 2010 *Food Chem. Toxicol.* **48** 2388

[38] Yücel Y and Sultanoglu P 2013 *Food Biosci.* **1** 16

[39] Capuano E and Fogliano V 2011 *Food Sci. Technol.* **44** 793

[40] Silvano M F, Varela M S, Palacio M A, Ruffinengo S and Yamul D K 2014 *Food Chem.* **152** p 500