Abstract. Honey is a viscous, aromatic, sweet food that is consumed and enjoyed by people around the world due to its unique nutritional and medicinal properties. The physicochemical parameters of natural honeys, such as moisture, reducing sugars, sucrose, hydroxymethylfurfural (HMF), free acidity, diastase activity, water-insoluble content and electrical conductivity are strictly defined and constitute the quality indicators which characterize individual honey varieties. The present study shows results of honey quality investigation from various regions of Serbia, which were evaluated according to the Serbian Regulation. A total of 372 honey samples (132 acacia honey, 221 blossom honey and 19 honeydew honey) were obtained from the Serbian market. All applied methods were performed according to the Harmonized Methods of the International Honey Commission. Summarizing the results presented, the most important parameters for detecting honey that was non-compliant with the regulation were HMF content along with diastase activity and sugar content. Results show that in 2014 and 2015, a great number of honey samples were of insufficient quality to satisfy regulatory requirements. In 2016, the situation on the Serbian honey market improved and became more under control.
of honey quality investigation from various regions of Republic of Serbia. Chemical and physical properties of honey are evaluated according to the Serbian Regulation [5].

Table 1. Regulatory values of chemical and physicochemical parameters for honey [4, 5]

| Chemical and physicochemical parameters | Composition criteria |
|-----------------------------------------|-----------------------|
| Moisture                                | In general: Not more than 20% |
|                                         | Heather and baker’s honey: Not more than 23% |
|                                         | Baker’s honey from heather: Not more than 25% |
| Free acidity                            | In general: Not more than 50meq/kg |
|                                         | Baker’s honey: Not more than 80meq/kg |
| Diastase activity                       | In general, except baker’s honey: Not less than 8 |
|                                         | Not less than 3* |
| HMF                                     | In general, except baker’s honey: Not more than 40mg/kg |
|                                         | Not more than 80 mg/kg** |
| Glucose and fructose content            | Blossom honey 60≤ |
|                                         | Honeydew honey, blends of honeydew honey with blossom honey 45%≤ |
| Sucrose                                 | In general: Not more than 5% |
|                                         | False acacia, alfalfa, Menzies Banskia, French honeysuckle, red gum, leatherwood, Citrus spp.: Not more than 10% |
|                                         | Lavender, borage: Not more than 10% |
| Electrical conductivity                 | Honey not listed below, and blends of these honeys: Not more than 0.8 mS/cm |
|                                         | Honeydew and chestnut honey and blends of those except with those listed below: Not less than 0.8 mS/cm; exceptions: strawberry tree, bell heather, lime, ling heather, manuka or jelly bush, tea tree |
| Water-insoluble content                 | In general: Not more than 0.1% |
|                                         | Pressed honey: Not more than 0.5% |

*honey with low natural enzyme content (e.g. citrus honeys) and an HMF content of not more than 15 mg/kg. **honey of declared origin from regions with tropical climate and blends of these honeys.

2. Materials and methods

2.1. Honey samples

The total of 372 honey samples (132 acacia honey, 221 blossom honey and 19 honeydew honey) were obtained from different regions from the Serbian market. The sample distribution by the year was: 2014: 21 samples of acacia honey, 39 samples of blossom honey and 4 samples of honeydew honey; 2015: 35 samples of acacia honey, 74 samples of blossom honey and 9 samples of honeydew honey; 2016: 76 samples of acacia honey, 108 samples of blossom honey and 6 samples of honeydew honey. All samples were stored at ambient temperature prior to analysis.

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 milliequivalents/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. Determination 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 DN after Schade was performed and results are
expressed in Göthe units per gram of honey. The concentration of 5-(hydroxymethyl)-furan-2-carbaldehyde (HMF) was determined using reverse phase HPLC equipped with UV detection and result is expressed in mg/kg.

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

3. Results and discussion
Analysis of the water content showed that all samples were in accordance with the regulation, and the maximum value of 20% was not exceeded [4,5]. The results obtained were similar compared to data reported in studies of 201 honey samples originating from the entire territory of Serbia which was performed during 2009 (average moisture content ranged from 16.12% in acacia honey samples to 17.98% in sunflower honey samples), [9]. In 187 evaluated honeys harvested in Northwest Spain, the average water content was 17.6% [10], and in 39 pine honey samples in Greece, water content was between 10.50% and 20.50% [11].

The water-insoluble content in all honey samples were similar and ranged from 0.01% to 0.04% and all these values were lower than the permitted limit (at most 0.1%). The water-insoluble content is an indicator of purity of honey, because it is defined as that matter which is not soluble in water, such as pollen, honeycomb and mineral particles, etc. [12]. Similar results for the water-insoluble content in honey were reported in the study of acacia and linden honey samples in Croatia [13].

The mean free acidity values of honey ranged from the lowest 10.82 meq/kg (2014), 10.87 meq/kg (2015) and 8.23 meq/kg (2016) for acacia honey; 17.44 meq/kg (2014), 14.65 meq/kg (2015) and 16.46 meq/kg (2016) for blossom honey to the highest 26.03 meq/kg (2014), 18.53 meq/kg (2015) and 23.59 meq/kg (2016) meq/kg for honeydew honey. Free acidity values of all examined honeys were below the legal limit (lower than 50 meq/kg). Free acidity is an important parameter which is characterised by the presence of organic acids in equilibrium with lactones, internal esters and some inorganic ions such as phosphates, sulphates and chlorides [14]. Although higher values for free acidity can be indicative of fermentation of sugars into organic acids [6] and related to the deterioration of honey, variation in free acidity among different honeys can be explained by blossom origin, the presence of different organic acids or some inorganic ions, geographical origin or harvest season [15,16]. The results obtained in the current study agree with data in the literature [9,17,18,19].

Diastases (α- and β-amylases) are enzymes naturally present in honey. Diastase content depends on the blossom and geographical origins of the honey. They are sensitive to heat (thermolabile) and consequently are able to indicate overheating of the product and the degree of preservation [20]. Similar to HMF content, the activity of diastase (DN) can be used as an indicator of aging and increased temperature, because the activity of this enzyme can be reduced during storage or when the product is subjected to heating above 60°C [18]. The current law stipulates a minimum DN value of 8.00 Göthe units. However, honeys with naturally lower DN tolerate a minimum of 3 Göthe units if honeys have up to 15 mg/ kg of HMF [5]. Hence, where DN is low, it is essential that this honey sample contain a maximum of 15 mg/kg of HMF, because as DN is low, it is necessary to prove that honey has not undergone heat treatment or prolonged storage [6]. The lowest mean DN values were in acacia honey samples (in 2014, 2015 and 2016 were 13.05; 8.86 and 11.60, respectively), while the highest mean DN values were in samples of honeydew honey (18.72; 15.63 and 23.50, respectively). The mean DN values for during 2014, 2015 and 2016 in blossom honeys were 14.30, 11.86 and 16.96, respectively. In 2014, analysis showed non-compliant DN levels in 13 acacia honey samples (61.90%), 16 blossom honey (42.11%) and 1 honeydew honey (25%); in 2015: 20 samples of acacia (57.14%), 41 blossom (56.16%) and 8 honeydew honey samples (88.89%); in 2016: 13 acacia honey samples (17.11%) and 10 blossom honey (9.26%). All these honey samples contained low diastase activity (DN), which were below a minimum value of 8.00. According to the results of a study in which the bees were fed with commercial glucose [21], bees should not be fed glucose in excessive
amounts, as this could promote an enzyme deficiency (especially diastase) among enzymes which convert glucose and fructose. Honeyes with lower enzyme content are produced from young nectars in early spring and a low enzyme concentration is caused by a low concentration of nectar and higher sugar content with reduced activity of the bees during their growth [22].

The range of HMF content in acacia honeys was 3.07-140.06 mg/kg (2014); 2.11-387.43 mg/kg (2015) and 0.57-211.35 mg/kg (2016), while HMF in blossom honeys was 1.15-234.15 mg/kg (2014); 0.58-392.81 mg/kg (2015) and 0.18-265.59 mg/kg (2016). In analysed honeydew honeys, except the control in 2015 (range 21.31-363.13 mg/kg), all HMF levels were lower than the permitted limit (max. 40 mg/kg), and so the honeys can be considered as fresh honeys (2014: range 3.84-33.06 mg/kg and 2016: 1.57-32.81 mg/kg). The HMF results obtained, in general, were higher than reported in literature for honeys from Spain (6.80 mg/kg), [23], from Turkey (2.52 mg/kg), [24] and from Argentina (8.98 mg/kg), [19]. The Regulations [4, 5] set a maximum HMF value of 40.00 mg/kg for honey mixtures or processed honey and a maximum value of 80.00 mg kg/kg if the honey and honeyblends contain a declared origin from regions with a tropical climate. Among the acacia honey samples analysed, 21 of them had HMF values above the maximum stipulated [5], 2014: 9 samples (42.86%), 2015: 7 (21.21%) and 2016: 5 (6.58%). The number of blossom honeys with HMF values above the maximum permitted value (51) was higher (2014: 10 samples (25.64%), 2015: 34 (45.95%) and 2016: 7 (6.48%) than the number of acacia honeys that exceeded the limit. For honeydew honeys, only 7 samples from 2015 had HMF values above the maximum permitted value (although this was 77.78% of these honeys in that year). HMF is formed by the decomposition of monosaccharides when honey is heated or stored for a long time [6]. High HMF content in honeys can also indicate falsification by adding invert syrup, because HMF can be produced by heating sugars in the presence of an acid to the inversion of sucrose [18, 25].

Since 2015, as a result of harmonization of national legislation with that of the EU, measurement of the electrical conductivity of honey has been expanded from only honeydew honey [26] to the other types of honey with few exceptions [5]. The electrical conductivity is often used in the quality control of honey to distinguish blossom honey from honeydew honey [11]. The Serbian 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 honey. Results of the electrical conductivity of honeydew samples, analysed during 2014 and 2015 varied in the range 0.40-2.27 mS/cm (mean 1.15 mS/cm) and 0.12-1.10 mS/cm (mean 0.33 mS/cm), respectively. Mean values and ranges of conductivity for examined honeys in 2016 were 0.16 mS/cm (0.06-0.52) mS/cm; 0.45 mS/cm (0.06-2.05) mS/cm and 0.94 mS/cm (0.46-1.83) mS/cm for acacia, blossom and honeydew samples, respectively. Two samples of honeydew honey (50%) in 2014 and eight samples (88.89%) in 2015 had values below the minimum value (1.00 mS/cm), [26], while during 2016, 2 samples of blossom honey (2.35%) and 2 samples of honeydew honey (33.33%) had values higher than 0.80 mS/cm and lower than 0.80 mS/cm for blossom and honeydew honey, respectively, as defined in the current national Regulation [5].

Monosaccharides make up about 75% of the sugars found in honey, along with 10-15% disaccharides and small amounts of other sugars [6]. Sugar composition depends mainly on the honey’s botanical and geographical origin, and is affected by climate, processing and storage [15, 27]. In 2014, the ranges of reducing sugars expressed as sum of glucose and fructose, and sucrose contents in acacia honeys were 24.54-71.56% and not detected (n.d.)-22.67%, respectively; in 2015, 23.97-79.13 and 0.99-26.23% and, in 2016, 33.42-76.23 and 0.96-30.72%, respectively, while the range of these parameters in blossom honeys were 24.50-79.96% and n.d.-28.18%, respectively in 2014; 20.60-84.47% and 0.98-48.18%, respectively in 2015 and 36.42-82.80 and 0.63-29.81%, respectively in 2016. The results for these quality parameters for honeydew honey were 49.97–69.93% and 1.85-6.13%, respectively in 2014; 33.87-66.36% and 1.11-22.34% in 2015 and 58.03-65.62% and 2.47-4.98%, respectively in 2016. According to results in 2014, a total of 11 honey samples were non-compliant for lower content of reducing sugars and for higher content of sucrose than legally allowed, which was 52.38% of all examined acacia honeys; 14 samples (35.90%) of blossom honey (reducing
sugars) and 5 blossom honey samples (12.82%) (sucrose), plus 2 samples (50%) (reducing sugars) of honeydew honey were non-compliant; in 2015 there were 18 non-compliant acacia honeys (51.43%) (lower content of reducing sugars) and 20 (57.14%) (higher content of sucrose); for blossom honey, 33 samples (44.59%), (reducing sugars) and 24 samples (32.43%) (sucrose) were non-compliant, and 8 honeydew samples (88.89%) (reducing sugars) and 6 samples (66.67%) (sucrose) were non-compliant; in 2016, there were 10 non-compliant acacia honey samples (13.16%) (lower contents of reducing sugars) and 5 (6.58%), (higher content of sucrose); among blossom honey, 8 samples (7.41%) (reducing sugars) and 5 samples (4.63%) (sucrose) were non-compliant. The results obtained for reducing sugars and sucrose contents in all examined samples of honeydew honey during 2016 were in accordance with composition criteria defined in the current national regulation [5].

Summarizing the results presented, the most important parameters for detecting honey that was non-compliant with the law were HMF content along with diastase activity and sugar content. Moisture, water-insoluble matter and free acidity alone were not relevant factors for evaluation of honey quality. Previous articles on the same topic were of local significance and did not cover the entire market of Serbia, or they used insufficient parameters for checking compliance with regulations [17,28]. The number of non-compliant honey samples by year and type is shown on Figure 1. Control of honey in 2014 and 2015 showed that the quality of honey on the Serbian market was seriously affected during this period. These results might indicate possible adulteration [29]. The drastic decrease of non-compliant honey among all honey types in 2016 is noticeable. That is the most likely consequence of tightening control by government authorities.

4. Conclusion
Quality control of honey from the entire Serbian market during the period 2014-2016 showed that the most relevant parameters for detecting non-compliant honeys were determination of HMF, sugar content and diastase activity. Electrical conductivity was relevant only for determining whether honeydew honey conformed with regulation, but free acidity, moisture and water-insoluble matter were of no significance for determining compliance of honey with legislation.

Results show that in 2014 and 2015, a great number of honey samples were of insufficient quality to satisfy regulatory requirements. In 2015, for example, the number of non-compliant honeys culminated with almost 83% of acacia honey, 58% of blossom honey and 89% of honeydew honey being non-compliant. After government action early in 2016, the situation on the Serbian honey market improved and became more under control. The number of non-compliant honey samples decreased to under 20% for both acacia and blossom honey. In interpreting the results for the non-
compliant samples of honeydew honey, it should be noted that relatively few of these were sampled and, therefore, the trend must be taken with the reserve.

Serbia exports honey, especially rare and valuable honey like acacia, sunflower and linden honey. Adulteration and degradation of the Serbian honey quality has a significant impact on the public health and economy of the country. Therefore, it is necessary to emphasise the importance of honey quality control over the whole territory of Serbia. Positive results of the government action on enhancing the quality control of honey show that this control must be continued and maintained.

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