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Original scientific paper

Classification of Honeys From Three Geographical Regions Based on Their Quality Control Data

Slavica Grujić[1], Jasmin Komić[2]
[1]Faculty of Technology, University of Banja Luka, Bosnia and Herzegovina,
[2]Faculty of Economics, University of Banja Luka, Bosnia and Herzegovina

Abstract: Characterisation of commercial honeys is a hard task initiated in response to consumers' demands. They demand basic quality level and determination of geographical and botanical origin. During processing and bottling of honey, there is a possibility of mixing honeys from various origins and quality. The processing could cause honey alterations that can affect the properties and quality, due to the adulteration of high quality honey types with a lower price natural honey. Control of honey requires determination of parameters that could unequivocally establish origin and improve honey characterisation. Nineteen physicochemical parameters (water content, ash, content of Zn, Cd, P, Fe, Cu, Mn, Mg, K, Na, refraction index, density, electrical conductivity, pH, free-acidity, percentage of total sugars, fructose and glucose, and sucrose) were analyzed in 194 samples of 7 nectar honey types (Amorpha, Black Locust, Black Locust and Multifloral, Chestnut, Chestnut and Multifloral, Bast-small-leaved lime and White Linden, Multifloral) from three geographical regions of the North-West Bosnia and Herzegovina. The aim of the study was to find which physicochemical parameters correlated to the geographical origin of the honey. After applying the one-way analysis of variance - ANOVA statistical comparison of analytically measured physicochemical parameters for a given honey types and Turkey's Multiple Comparison HSD Test, the results showed that the content of ash, Cd, Mn, Mg, K, pH and electrical conductivity was useful for discriminating honeys from three geographical regions of the North-West Bosnia and Herzegovina.

Key words: honey, geographical origin, physicochemical parameters

Introduction

The increase of a number of products that are available on the market makes it more and more difficult for consumers to choose which product satisfies quality characteristics. The system of protecting geographical indication, designation of origin and authenticity is an efficient way for food products quality managing. The region of origin of food products can affect consumers' evaluation according to the expected specific sensory characteristics and value of the food, due to its symbolic or affective role. Affective aspect refers to the consumers who appreciate tradition and authenticity of products, as food with strong regional identity (Verlegh & Itterisum, 2001) (Stefani et al., 2006). Characterisation of commercial honeys is a hard task initiated in response to consumers' demands. Those have demanded basic quality level and determination of geographical and botanical origin (Golob & Plestenjak, 1999) (Terrab et al., 2004) (Piazza & Persano, 2004) (Šarić et al., 2008).

Food law lays down definition and rules for composition, production and analytical methods for quality control of honey (*Sluzbeni glasnik BiH*, 37/2009; Council Directive 2001/110/EC). During processing and bottling of honey, there is a possibility of mixing honeys from various origins and quality. The processing could cause honey alterations that can affect the properties and quality, due to the adulteration of high quality honey types with a lower price natural honey. Control of honey requires determination of parameters that could unequivocally establish origin and improve honey characterisation. It is important to establish analytical methods, which allow the determination of the botanical and geographical origin of honey (Terrab et al., 2004) (Anklam, 1998) (Ashurts & Dennis, 1998) (Karoui et al., 2007) (Grujić & Popov-Raljić, 2007) (Lušić et al., 2007).

The botanical origin of honey is one of its main quality parameters for consumers. Determining honey floral origin, usually complemented with sensory analysis, was evaluated by identification and counting of pollen grains contained in honey sediment by microscopic analysis (Mateo & Bosch-Reig, 1998) (Ouchemoukh et al., 2007) (Castro-Vázquez et al., 2009). However, sometimes shape and pollen grain type identification can be difficult. Different plant species produce different proportions of pollen, the amount of pollen can vary from season to season, bees can collect pollen without nectar or honey could be filtered (Anklam, 1998) (Ashurts & Dennis, 1998). Honey can never be derived from a single botanical source. The term 'unifloral' honey is used to describe honey produced mostly from one plant specie. Results of pollen grain identification depend mainly on the operator experience. Some unifloral honeys have specific chemical or physical properties, which may be used to confirm the results of microscopic analysis. So, physicochemical characteristics of honey may be used to confirm the results of microscopic analysis and authenticity of honey (Popek, 2002) (Terrab et al., 2002) (Grujić et al., 2003) (Nanda et al., 2003) (Devillers et al., 2004) (Cotte et al., 2007) (Bertelli et al., 2007) (Cuevas-Glory et al., 2007).

Numerous investigations have been published by many scientists related to physical properties and chemical composition of honey as complex function of botanic and geographical origin. The composition of honey depends highly on the type of flowers utilized by the bee as well as climatic conditions. (Pena & Herrero, 1993) showed that multivariate statistical methods applied to physicochemical parameters could be successfully used in order to achieve a correct geographical classification of honey samples from different origins. They analyzed 11 parameters of legal quality control honeys from two production areas of Spain and classified these honeys according to their geographical origin. Water content and free acidity were found to be the most important parameters for the classification. They concluded that the use of pollen data to achieve a correct geographical classification of the honey samples is not necessary. The same, author (Latorre et al., 1999) used metal composition of honey samples for chemometric classification of honeys according to their type. It was shown that minerals are good parameters for classification system, as they are stable.

Several studies have been published on the use of physicochemical parameters for characterisation of honeys from different production regions (Piazza & Persano, 2004) (Felsner et al., 2004) (Lušić et al., 2007) (Mărghitaş et al., 2009) (Grujić et al., 2011). Modern statistical data evaluation techniques could be a useful tool for detection of the honey geographical origin. As the result of comparing quality characteristics of honey from three geographical areas in Spain, (Sancho et al. 1991) selected five parameters related to the geographical origin of honey: total acidity, formol index, sucrose, and fructose/glucose and glucose-water/fructose ratios. Using discrimination of honey from mountain and valley areas of La Roja region in Spain, authors (Sanz et al. 1995) found free acidity, pH, mineral content - ash content and electrical conductivity to be the most important parameters for classification of analysed honeys according to their geographical origin. Authenticity and geographical origin of honey could be determined by qualitative and quantitative flavonoids and phenols content (Martos et al., 1997) (Tomas-Barberan et al., 2001), pH, water content, electrical conductivity, colour and sugar in honey (Mateo & Bosch-Reig, 1998) or pH and electrical conductivity (Acquarone et al., 2007).

Some authors published results of honey mineral content analysis in order to confirm the geographical origin of honey (Lopez-Garcia et al., 1999) (Nanada et al., 2003) (Felsner et al., 2004) (Conti, 2000) (Golob et al., 2005) (Lachman et al., 2007) (Pisani et al., 2008), or geographical discrimination of honey by using mineral composition and common chemical quality parameters (Paramas et al., 2000) (Persano et al.,

2004) (Kropf et al., 2008). The mineral content of honey reflects the presence of specific minerals within the forage area of the hive. An excess or insufficiency of certain chemical elements in soil, rocks or water, as well as other phytological, environmental or seasonal factors, reflects on the mineral composition of the plants and the nectar, pollen or honeydew. Taking this as a basis, it is possible to define natural geographical honey production regions or zones (Sanz et al., 1995) (Anklam, 1998) (Paramas et al., 2000) (Lušić et al., 2007).

The present work represents the study of the quality of honeys from three geographical regions of the North-West Bosnia and Herzegovina. These three regions have production of honey from wild plants and of great biodiversity. The objective of this study was to determine whether it would be possible to differentiate honeys produced in the three ecological-vegetation geographical regions in Bosnia and Herzegovina. If there is a difference, it is important to find which physicochemical parameters are in correlation with the geographical origin of the honey, applying the statistical comparison of analytically measured physicochemical parameters for a given honeys types.

Materials and Methods

Honey samples

Totally 194 samples of seven nectar honeys types were collected: Amorpha (*Amorpha Fruticosa l., Fam. Fabaceae*); Black Locust (*Robinia Pseudoacacia l., Fam. Fabaceae)*; Black Locust and Multifloral; Chestnut *(Castanea Sativa Mill. Fam. Fagaceae)*; Chestnut and Multifloral; Bast-small-leaved lime and White Linden; Multifloral. (Table 1). Collected samples were from three geographical regions, eleven locations of municipality area of the North-West Bosnia and Herzegovina: Banja Luka, Gradiška, Kostajnica, Prijedor, Kotor Varoš, Mrkonjić Grad, Novi Grad, Prnjavor, Srbac, Brod and Teslić. All the data of interest (beekeeper's name, location, geographical area, date of sampling, data of harvest of honey, botanical data, flowers from which that honey could have been elaborated, location of the hives, extraction procedure, storage location and conditions) were recorded.

Table 1. Honey types and botanical origin of samples distributed according to the production regions in the North-West Bosnian region (GROUP 1); in the Northern Bosnian region (GROUP 2); in the West-Bosnian limestone-dolomite region (GROUP 3)

	Number of samples			
Honey types (floral sources)	GROUP 1	GROUP 2	GROUP 3	TOTAL
Amorpha (*Amorpha Fruticosa l., Fam. Fabaceae*)	2	16	0	18
Black Locust (*Robinia Pseudoacacia l., Fam. Fabaceae)*	9	18	15	42
Black Locust (*Robinia Pseudoacacia l., Fam. Fabaceae)* and Multifloral	8	2	6	16
Chestnut *(Castanea Sativa Mill. Fam. Fagaceae)*	11	2	0	13
Chestnut (*Castanea Sativa Mill. Fam. Fagaceae)* and Multifloral	9	2	2	13
Bast-small-leaved lime *(Tilia Cordata Mill.*) and White Linden *(Tilia Tomentosa Moench*, *Fam. Tiliaceae)*	2	2	6	10
Multifloral	31	15	36	82
TOTAL	72	57	65	194

Description of geographical regions of honey production

All the honey samples, collected for the study, were produced on the territory of the North-West Bosnia and Herzegovina. Stefanović, Beus, Burlica, Dizdarević and Vukorep (1983) argue that, based on the analysis and consideration of the entire territory of the North-West Bosnia and Herzegovina and according to the differences in regional, geographical, climate factors and composition of soil, it is scientifically justified to create three climate ecological-vegetation geographical regions (territorial units) in the North-West part of Bosnia and Herzegovina. Observed ecological and vegetation regions are located in areas where, under the influence of complex geophysical factors, on a relatively small area, there are conflict impacts from the north and south air flow influencing on the specific variation of the basic climatic elements. In those regions, optimal conditions for honey collecting are from beginning of April, to the end of September.

North-West Bosnian region (1) is located from the river Una to the southeast part of the region, sneaking in up to the river Vrbanja above the town of Kotor Varoš. This region mostly belongs to the hilly-zone, and a smaller part belongs to the valley-zone, with height above sea-level from 130 to 500 m. This slightly hilly land and ore rich in medium-high mountain was built in the alluvial plain in the valley of the river Una, Sana and Vrbas, and of the Tertiary sediments, and less of Paleozoic sandstone and shale, and limestone eruptives. The low basin, river valleys and hills, filtered soil characteristics, thus affecting the climate and hydrological potential of the region and created favourable conditions for the growth of various wild honey plants. This region has characteristics of medium-continental climate with signs of the Atlantic climate impact. Vegetation period lasts from 195 to 200 days.

Northern Bosnian region (2) is located in the middle part of the North Bosnia and Herzegovina, from the river Sava to the slopes of hilly-mountain territory of the inland Dinarids. In east, it includes territory following the bordering line connecting town Brčko - mountain ridge of Majevica - east brow of Spreča field, and in west and southwest bordering with the northwest region of this territory. This region is located on height above sea-level from 80 to 980 m. In this region, there are alluvial plain and diluvial terrace with raised hilly relief covered with sediments and carbonateless rocks, with unfavourable characteristics and forest bare, due to heavy texture and excessive moisture. More favourable is the land on higher slopes and smaller areas, dominated by forest vegetation, meadows and pastures. Climate of this area has expressed moderate continental character. Vegetation period lasts from 180 to 200 days.

As the third part of this phyto-geographical and ecological territory, it is possible to separate **West-Bosnian limestone-dolomitic region (3).** This region is a part of the territory of the inland Dinarids. It takes possession of wide geographical space of limestone-dolomite areas and mountains massifs; from the river Una in northwest to (including) Glamoč-Kupres plateau in the southeast of the territory. It is bordering with Mediterranean-Dinarids region in the southwest, reaching valley of the river Vrbas, including limestone massifs of Vlašić and Čemernica. Most part of this region belongs to the mountain and subalpe zones height above sea-level from 800 to 1900 m, and a smaller part of this region belongs to the hilly-zones with height above sea-level from 300 to 700 m. This region is characterised with divided mountain systems of different geological structure, relief and direction of mountains spreading. This region consists of a few limestone-dolomite massifs and plateaus with traces of glacial activity and with uneven penetration of Mediterranean and continental climate. Land is shallow, dry, hard texture and high surface stoniness. Characteristics of mountain climate in this region depend on altitude areas and have impact on the growing period. Only a small amount of total annual precipitation falls in the growing season.

Ključ-Petrovac region is a part of this area, where honey samples from municipality area of Novi Grad were collected. Ključ-Petrovac region extends over parts of the left side of the river Vrbas and masses of Manjača and Zmijanje in the southeast of the Grmeč and the river Una canyon in the northwest, including the massive of Plješevica, and mountains Lunjevača and Jadovnik in the south. Tracts of this district are mostly covered with forests and mostly belong to the highlands, to a lesser extent subalpine zone, with height above sea-level from 300 to about 2000 m. This part is mainly built of limestone and less than dolomite, and the soil is shallow and dry, mostly covered with forests. Mountain depression biogeographical region are plant communities of forest and grass, adapted to relatively low temperatures and significant rainfall, with moderate-continental climate.

Classification of honey samples according to the geographical origin

According to the information from the beekeepers regarding the geographical origin, the location of production and belonging to the ecological-vegetation regions (60), analyzed honey samples were classified in three groups. Honey samples collected on locations of municipality area of Kostajnica, Prijedor, Banja Luka and Kotor Varoš participate in the **North-West Bosnian region,** designated as GROUP (1). As GROUP (2) are designated honey samples produced inside the **Northern Bosnian region**, at the locations of honeys collecting and producing in municipality area of Gradiška, Prnjavor, Srbac, Brod and Teslić. Inside the west part of **West Bosnian limestone-dolomite region** there is Ključ-Petrovac region, designated as GROUP (3), with locations of honey samples collecting in municipality area of Novi Grad and Mrkonjić Grad.

Physicochemical parameters

Samples of honey were collected from individual producers and members of the Beekeepers Association of the Republic of Srpska, according to the Association of Official Analytical Chemists (AOAC) Official Method (2000) for sampling, and stored short time on 15°C in the dark, prior to the analysis. Samples were screened by microscopic and sensory analysis assessment after receipt to the laboratory.

Physicochemical analyses were done according to the AOAC Official methods (2000) or official methods for honey quality control (*Sluzbeni glasnik BiH*, **37/2009)**. All analyses were performed in triplicate at the same time and the results are given as mean values. Nineteen physicochemical parameters were analysed.

Water content (moisture) was determined according to the AOAC Offical Method (969.38); content of total sugars (AOAC Offical Method 920.183); fructose and glucose (AOAC Offical Method 920.183); sucrose (AOAC Offical Method 920.184); total acidity (AOAC Offical Method 962.19); active acidity (pH) on pH Meter (Model 3310, Jenway, Engl.) (AOAC Offical Method 962.19); specific electrical conductivity (on Palinest Conductivity Meter, PT 115, Palinest Instruments); refractive index (AOAC Offical Method 920.182) on Leica Abbe Mark II Refractometer Model 10480, Leica, USA); density of honey (expressed in g/cm^3) measured in accordance with the standard methods (*Sluzbeni glasnik BiH*, 37/2009). Ash content was determined by overheating honey sample in furnace at 550°C to constant mass (AOAC Offical Method 920.181). Trace elements in honey (Zn, Cd, Fe, Cu, Mn, Mg, K, Na) were analysed after the samples were digested by the wet oxidation method, by treating the samples with HNO_3 and H_2O_2, and analyzed on atomic absorption spectrometer with flame (oxygen – acetylene) detector (AAS Unicam 969) (Golob et al., 2005) and working parameters of the atomic absorption spectrometer are defined by the manufacturer. Spectophotometric measurements of phosphorus (P) content were performed at the wavelength of 420 nm on UV-VIS Spectrophotometer (Milton Roy Spectronic 1201) in accordance with AOAC (2000) methods.

Statistical analysis

Basic statistics (median, standard error, standard deviation, minimum, maximum and range of values) and multivariate statistical analysis were carried out using statistical package StatistiXL Toolpak SPSS 1.8 for Microsoft Excel Analysis (StatistiXL Toolpak SPSS 1.8 for Microsoft Excel Analysis) (Komić, 2000). To verify if there is variability between honey samples as a result of the impact of geographical origin, one-way analysis of variance (ANOVA) was realized and appropriate F-tests applied. To determine if the difference between the GROUPS in the mean values of analysed parameters is significant, Turkey's Multiple Comparison HSD Test was also performed (Komić, 2000).

Results and Discussion

Honey and regional characteristics of production territory

Soil composition, regional-geographical position and local climatic conditions have important influence on natural selection of wild-growth plants in that region. Cold winters with medium precipitation amount and warm summers, create optimal conditions for a different aromatic wild plants growth and for great biodiversity in the North-West part of Bosnia and Herzegovina. In those territories, optimal conditions for honey production are from beginning of April, to the end of September and thereby meeting basic prerequisites for beekeeping development and honey collecting. (Stefanović et al. 1983) concluded that it is scientifically justified and appropriately to create three territorial areas or regions in the North-West part of Bosnia and Herzegovina. Within this relative small geographical territory there are clearly expressed differences of the soil composition, geographical position and climatic factors of the three studied honey production geographical regions which have a direct influence on natural selection and diversity of wild-growth honey plants adapted to the conditions of that territory.

Physicochemical characterization of honey

Honey samples were classified by beekeepers, regarding botanical origin and production regions and after the microscopic analysis as Amorpha, Black Locust, Black Locust and Multifloral, Chestnut, Chestnut and Multifloral, Bast-small-leaved lime and White Linden, Multifloral. Distribution of studied honey samples according to the type and three geographical production regions in the North-West part of Bosnia and Herzegovina are summarised in Table 1. Means, median, minimum, maximum and range of 19 physicochemical parameters determined for studied honey types produced on three different geographical regions listed in Tables 2-7. show and confirm their mutual difference.

Table 2. Physicochemical parameters of honey samples from GROUP (1)

Parameters	Fructose + Glucose (g/100 g)	Sucrose (g/100 g)	Total sugars (g/100 g)	Refraction index	Density (g/cm^3)	Electrical conductivity (mS/cm)	pH	Total acidity (mmol/kg)	Water (g/100g)
Mean	71.81	2.97	74.78	1.4923	1.419	0.40	3.96	12.44	17.55
Median	71.95	2.52	74.57	1.4925	1.420	0.35	3.92	11.19	17.60
Std Error	0.173	0.140	0.209	0.0003	0.001	0.025	0.040	0.607	0.108
Std Dev.	1.464	1.190	1.774	0.0024	0.008	0.210	0.341	5.149	0.880
Minimum	68.25	1.18	71.40	1.4852	1.389	0.09	3.49	4.09	15.80
Maximum	74.70	8.68	80.84	1.4972	1.432	0.91	5.05	34.00	19.40
Range	6.45	7.50	9.44	0.0120	0.043	0.82	1.56	29.91	3.60

Table 3. Physicochemical parameters of honey samples from GROUP (2)

Parameters	Fructose + Glucose (g/100 g)	Sucrose (g/100 g)	Total sugars (g/100 g)	Refraction index	Density (g/cm^3)	Electrical conductivity (mS/cm)	pH	Total acidity (mmol/kg)	Water (g/100g)
Mean	71.26	3.47	74.73	1.4924	1.420	0.26	3.90	11.20	17.23
Median	71.00	2.90	74.58	1.4934	1.420	0.24	3.87	10.90	17.20
Std Error	0.212	0.182	0.202	0.0011	0.001	0.015	0.030	0.426	0.133
Std Dev.	1.604	1.376	1.524	0.0087	0.010	0.118	0.239	3.409	1.039
Minimum	68.25	1.66	72.00	1.4270	1.395	0.10	3.45	4.08	15.00
Maximum	74.65	8.41	80.61	1.4991	1.442	0.64	4.56	22.39	20.20
Range	6.40	6.75	8.61	0.0721	0.047	0.54	1.11	18.31	5.20

Table 4. Physicochemical parameters of honey samples from GROUP (3)

Parameters	Fructose + Glucose (g/100 g)	Sucrose (g/100 g)	Total sugars (g/100 g)	Refraction index	Density (g/cm^3)	Electrical conductivity (mS/cm)	pH	Total acidity (mmol/kg)	Water (g/100g)
Mean	71.77	3.32	75.09	1.4922	1.418	0.24	3.76	10.91	17.62
Median	72.30	2.82	75.14	1.4919	1.418	0.24	3.75	10.49	17.60
Std Error	0.250	0.159	0.164	0.0004	0.001	0.012	0.021	0.383	0.225
Std Dev.	2.014	1.279	1.325	0.0029	0.009	0.096	0.168	3.014	1.333
Minimum	63.45	1.42	68.00	1.4861	1.388	0.08	3.47	5.10	15.30
Maximum	74.90	6.00	77.25	1.4978	1.438	0.54	4.17	22.16	20.00
Range	11.45	4.58	9.25	0.0117	0.050	0.46	0.70	17.06	4.70

Table 5. Content of Zn, Cd, P, Fe, Cu, Mn, Mg, K, Na and ash in honey samples from GROUP (1)

Parameters	Zn mg/100g	Cd mg/100g	P mg/100g	Fe mg/100g	Cu mg/100g	Mn mg/100g	Mg mg/100g	K mg/100g	Na mg/100g	Ash g/100g
Mean	1.280	0.193	5.940	0.559	0.179	0.364	2.227	11.089	6.099	0.33
Median	1.008	0.169	5.896	0.490	0.100	0.212	2.702	11.022	6.122	0.27
Std Error	0.102	0.017	0.304	0.037	0.041	0.045	0.140	0.227	0.052	0.024
Std Dev.	0.828	0.142	2.469	0.299	0.330	0.366	1.139	1.841	0.419	0.193
Minimum	0.466	0.036	2.178	0.194	0.025	0.017	0.050	6.292	5.041	0.08
Maximum	4.038	0.980	14.022	2.279	2.520	2.123	3.782	17.157	6.979	0.80
Range	3.572	0.944	11.844	2.085	2.495	2.106	3.732	10.865	1.938	0.72

Table 6. Content of Zn, Cd, P, Fe, Cu, Mn, Mg, K, Na and ash in honey samples from GROUP (2)

Parameters	Zn mg/100g	Cd mg/100g	P mg/100g	Fe mg/100g	Cu mg/100g	Mn mg/100g	Mg mg/100g	K mg/100g	Na mg/100g	Ash g/100g
Mean	1.280	0.100	5.636	0.426	0.586	0.152	2.121	10.202	6.012	0.19
Median	0.846	0.086	5.285	0.427	0.077	0.115	2.273	10.187	6.046	0.16
Std Error	0.124	0.008	0.339	0.013	0.210	0.018	0.129	0.213	0.043	0.014
Std Dev.	0.968	0.060	2.651	0.100	1.642	0.139	1.004	1.667	0.336	0.106
Minimum	0.464	0.004	1.392	0.247	0.024	0.056	0.142	7.035	5.166	0.04
Maximum	4.415	0.294	17.139	0.775	8.256	0.845	3.752	17.497	6.542	0.69
Range	3.951	0.290	15.747	0.528	8.232	0.789	3.610	10.462	1.376	0.65

Table 7. Content of Zn, Cd, P, Fe, Cu, Mn, Mg, K, Na and ash in honey samples from GROUP (3)

Parameters	Zn mg/100g	Cd mg/100g	P mg/100g	Fe mg/100g	Cu mg/100g	Mn mg/100g	Mg mg/100g	K mg/100g	Na mg/100g	Ash g/100g
Mean	1.266	0.180	4.992	0.594	0.149	0.182	1.639	9.944	6.161	0.16
Median	1.145	0.190	5.009	0.434	0.097	0.134	1.808	10.206	6.115	0.14
Std Error	0.088	0.008	0.357	0.172	0.022	0.031	0.200	0.217	0.084	0.012
Std Dev.	0.537	0.051	2.171	1.046	0.131	0.187	1.215	1.319	0.513	0.073
Minimum	0.489	0.077	1.007	0.208	0.036	0.016	0.048	7.219	5.334	0.06
Maximum	3.160	0.294	10.068	6.763	0.610	0.736	3.445	12.803	8.713	0.34
Range	2.671	0.217	9.061	6.555	0.574	0.720	3.397	5.584	3.379	0.28

Quality control data statistical analysis

Characterisation of honey samples produced in three regions in the North-West part of Bosnia and Herzegovina grouped regarding their geographical origin and designated as GROUP (1), GROUP (2) and GROUP (3), performed the data set consisting of 19 measured and compared physicochemical parameters for honeys, as follow: water content, ash, content of Zn, Cd, P, Fe, Cu, Mn, Mg, K, Na, refraction index, density, electrical conductivity, pH, acidity, percentage of total sugars, fructose and glucose, and sucrose. As deduced from application one-way analysis of variance (ANOVA) for a given honeys produced in three geographical production regions, differences were considered statistically significant ($p<0.05$) for ash content, content of minerals Cd, Mn, Mg, K, electrical conductivity and pH, of the parameters under consideration (Table 8). The differences could be associated with the influence of regional, geographical and climate factors in honey production regions in the North-West part of Bosnia and Herzegovina.

Table 8. Results of one-way analysis of variance (ANOVA) for determined quality control parameters, for honeys grouped in accordance to the production regions[a]

Variable	F-value	p[b]
fructose + glucose	1.781	0.171
sucrose	2.627	0.075
total sugars	0.922	0.399
water	2.027	0.135
ash	19.755	0.000*
Zn	0.002	0.998
Cd	15.381	0.000*
P	1.717	0.183
Fe	1.469	0.233
Cu	3.101	0.048
Mn	11.470	0.000*
Mg	4.643	0.011*
K	7.257	0.001*
Na	1.068	0.346
refraction index	0.037	0.963
density	0.947	0.390
conductivity	22.462	0.000*
pH	10.087	0.000*
acidity	2.782	0.064

[a] *fructose + glucose , sucrose, total sugars, water, ash, Zn, Cd, P, Fe, Cu, Mn, Mg, K, Na, refraction index, density, conductivity, pH, acidity*

[b] *the mean difference is significant at the p<0.05 level.*

** statistically significant difference for mean values considered at the p<0.05 confidence level*

After applying one-way ANOVA for content of total sugars, fructose and glucose, and sucrose, water, content of minerals Zn, P, Fe, Cu and Na, for refraction index, density, free-acidity in analysed honey samples produced in three regions in the North-West part of Bosnia and Herzegovina, it was considered that there are no statistically significant differences ($p<0.05$) between analysed honey samples grouped in accordance to the production regions, relatively to the geographical origin (Table 8).

Our results can be confirmed by similar results of Mateo and Bosch-Reig (1998) published for honeys from Spain for which as the most discriminant variables electrical conductivity, colour (*x,y,L*), water content, fructose and sucrose were selected. After a stepwise discriminant analysis was performed on the corresponding matrix consisted of common physicochemical characteristics, authors (Devillers et al., 2004) selected electrical conductivity, pH, free-acidity, percentage of fructose, glucose and raffinose as variables required to obtain the best classification honeys according to their botanical origin. These results are similar to those obtained for honeys from different production zones (Sanz et al, 1995).

Our results are in agreement with the results reported by (Paramas et al., 2000). They analysed common legal physicochemical parameters and individual mineral elements in order to test their geographical classification. As the most discriminant variable for 6 zones of the province of Salamanca they find: conductivity, ash content and content of major mineral elements Na and K, and minor mineral elements Al, Cd, Co, Cr, Fe, Mn and Ni. For extremely high values of Cd content in 3 honey samples, authors considered it to be the result of contamination by equipment. The greatest variability among all samples, as could be expected, would be attributable to the different compositions of the soils of the various zones and their vegetation, without ignoring the contribution of contamination by equipment (Paramas et al., 2000). (Latorre et al.,1999) classified honeys according to their type and origin on the basis of the three chemical data, Cu, Mn and Li content. (Sanz et al., 1995) found that free acidity, pH, mineral content - ash content, electrical conductivity, (Acquarone et al., 2007) found that pH and electrical conductivity are the most important parameters for classification of analysed honeys according to their geographical origin, or mineral elements (Lopez-Garcia et al., 1999) (Conti, 2000) (Nanada et al., 2003) (Lachman et al., 2007).

Comparison between geographical production regions

The results of ANOVA lead us to the consideration of statistical differences between three observed groups for analysed physicochemical parameters, ash content, content of minerals Cd, Mn, Mg, K, electrical conductivity and pH for honey grouped in accordance to the geographical production regions in the North-West part of Bosnia and Herzegovina. We would like to know between which regions there are significant difference. One of the ways to get precise answer is to apply Turkey's Multiple Comparison HSD Test.

As a result of applying Turkey's Multiple Comparison HSD Test, for mean values of ash content statistically significant difference was considered ($p<0.05$) between honey samples designated as GROUP (1) and honey samples designated as GROUP (2). The same, differences for ash content between honey samples designated as GROUP (1) and honey samples designated as GROUP (3) were considered (Table 9). The differences found between compared groups could be associated with the geographical origin of the honeys. Comparing mean values for electrical conductivity and content of minerals Mn and K, took identical results, so we could conclude that the geographical origin of honey has influence on appearing of statistically significant difference ($p<0.05$) of this physicochemical parameters. Statistically significant difference was considered ($p<0.05$) for mean values of Cd content, between honey samples designated as GROUP (1) and honey samples designated as GROUP (2). The same differences were found between honey samples designated as GROUP (2) and honey samples designated as GROUP (3) (Table 9).

Applying Turkey's Multiple Comparison HSD Test for mean values of analysed physicochemical parameters, Mg content and pH, it was considered that there are statistically significant difference ($p<0.05$) between honey samples designated as GROUP (1) and honey samples designated as GROUP (3) (Table 9). The same difference for mean values of Mg content and for pH for honeys grouped in accordance to the geographical origin was considered between honey samples designated as GROUP (2) and honey samples designated as GROUP (3). Established differences could be associated with the influence of the regional, geographical and climate factors of the three honey production regions in the North-West part of Bosnia and Herzegovina.

Table 9. Results of differences for comparison between observed groups for the determined quality control parameters, for honeys grouped in accordance to the production regions, by applying Turkey's Multiple Comparison HSD Test [b]

Parameter Dependent Variable	**I** group [a]	**J** group [a]	p	Parameter Dependent Variable	**I** group [a]	**J** group [a]	p
fructose + glucose	1	2	0.191	ash	1	2	0.000*
	1	3	0.990		1	3	0.000*
	2	3	0.256		2	3	0.588
sucrose	1	2	0.073	Zn	1	2	1.000
	1	3	0.248		1	3	0.998
	2	3	0.790		2	3	0.998
total sugars	1	2	0.994	Cd	1	2	0.000*
	1	3	0.478		1	3	0.933
	2	3	0.462		2	3	0.000*
water	1	2	0.218	P	1	2	0.771
	1	3	0.934		1	3	0.153
	2	3	0.189		2	3	0.423
refraction index	1	2	0.989	Fe	1	2	0.347
	1	3	0.989		1	3	0.934
	2	3	0.960		2	3	0.286
density	1	2	0.542	Cu	1	2	0.068
	1	3	0.955		1	3	0.991
	2	3	0.396		2	3	0.116
conductivity	1	2	0.000*	Mn	1	2	0.000*
	1	3	0.000*		1	3	0.003*
	2	3	0.731		2	3	0.851
pH	1	2	0.331	Mg	1	2	0.849
	1	3	0.000*		1	3	0.009*
	2	3	0.009*		2	3	0.037*
acidity	1	2	0.172	K	1	2	0.008*
	1	3	0.072		1	3	0.002*
	2	3	0.914		2	3	0.675
				Na	1	2	0.367
					1	3	0.999
					2	3	0.520

[a] *honey produced in the North-West Bosnian region designated as GROUP 1.*
[a] *honey produced in the Northern Bosnian region designated as GROUP 2.*
[a] *honey produced in the West-Bosnian limestone-dolomitic region designated as GROUP 3.*
[b] *the mean difference is significant at the 0.05 level.*
* *statistically significant differences for mean values at p<0.05 confidence level*

Conclusion

Different wild plants are growing in the North-West part of Bosnia and Herzegovina. During the time, after natural selection, they have adapted to that specific regional, geographical and climate factors. As a result, today, there is a wide range of wild-growth plants used by honeybees for honey production. Control of honey requires determination of parameters that could unequivocally establish origin and improve honey characterisation. Chemical composition and quality of honey depend on chemical composition of the plants and nectar or honeydew collected from the plants. After applying statistical analysis and comparison of analytically measured physicochemical parameters for studied honey types, it was proved that there are statistically significant differences between certain physicochemical quality characteristics of honeys produced in the three studied geographical regions in Bosnia and Herzegovina, in the North-West Bosnian, in the Northern Bosnian and in the West-Bosnian limestone-dolomite regions. These comprehensions could be useful for implementation of further activities on preparing for the protection of quality and geographical origin of honeys produced in Bosnia and Herzegovina.

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