

Quality assessment of different types of Croatian honey between 2012 and 2016



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Introduction

Honey is 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 plant-sucking insects on the living parts of plants. These fluids are collected by bees, transformed by combination with specific substances of their own, deposited, dehydrated, stored and left in the honey comb to ripen and "mature" (Croatian Regulation, 2015). Honey is recognized as a food with nutritional properties, and as a natural product with valuable therapeutic applications. It contains many different substances, mainly sugars such as fructose, glucose and sucrose (65–75% of total soluble solids) in addition to various organic and inorganic acids, enzymes, vitamins, flavonoids, proteins, amino acids and elements. Redundant, honey from each species is a different type vary in their composition, depending on the crop and geographical origin, climatic conditions, the breed of bees and the ability of the beekeeper (honey processing and storage methods).

Honeys are usually classified by the pollen spectra, which can allow for the

determination of both the botanical and geographical origin of products (Golob and Plestenjak, 1999). Although Croatia is a small country, differences in climate, soil and plants provide a solid base for the production of different types of honey. The most common types are acacia (*Robina pseudoacacia*), meadow, floral, sage (*Salvia officinalis*), chestnut (*Aesculus hippocastantum*) and lime (*Tilia cordata*), though there are also rarer and more specific varieties, such as rosemary (*Rosmarinus officinalis*), lavender (*Lavandula officinalis*), and heather (*Calluna vulgaris*) (Šarić et al., 2008). There are several reports on the physical, chemical, sensory and melissopalynological analysis of Croatian honey (Mandić et al., 2006; Kenjerić et al., 2007; Šarić et al., 2008; Bilandžić et al., 2011; Sabo et al., 2011; Čalopek et al., 2016).

The aim of this study was to present the analytical data related to various honey types produced in Croatia, especially rare honey types such as sunflower (*Helianthus annuus*), bearberry (*Arbutus unedo*), rapeseed (*Brassica napus*), hawthorn (*Crataegus*) and goldenrod (*Solidago virga aurea*) for which, to our

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knowledge there are very little or no reported data.

Materials and methods

In total, 227 honey samples of varying botanical origin (83 acacia, 46 chestnut, 36 floral, 15 lime, 12 meadow, 11 honeydew, 6 amorphia, 6 sunflower, 2 sage, 2 mandarin, 1 heather, 1 rapeseed, 1 lavender, 1 willow, 1 hawthorn, 1 Christ's thorn, 1 goldenrod, 1 bearberry) produced in different parts of Croatia were collected during five harvesting seasons (2012, 2013, 2014, 2015 and 2016).

All samples were subjected to pollen analysis with the aim of confirming honey type according to the Croatian regulations (Official Gazette 2009, 2015). Moisture content was determined by a refractometer using the AOAC Official Method in a laboratory accredited according ISO

17025 (AOAC, 2005). Physicochemical parameters were determined according to the methods prescribed by International Honey Commission (IHC, 2009). Electrical conductivity was measured in a solution of 20 g honey dry matter in demineralized low conductivity water at 20 °C (IHC, 2009). Diastase activity was determined using the method after Schade proposed by the IHC (IHC, 2009).

Hydroxymethylfurfural (HMF) was determined by HPLC with UV detection at 285 nm using water-methanol (90+10 by volume) as the mobile phase on the column Hypersil ODS 5 µm, 250×4 mm. Sample preparation prior to HPLC analysis is described by IHC.

The obtained results were evaluated using Croatian and international honey-profiling criteria (Official Gazette, 2015). Statistical analysis of the results of physicochemical parameters was

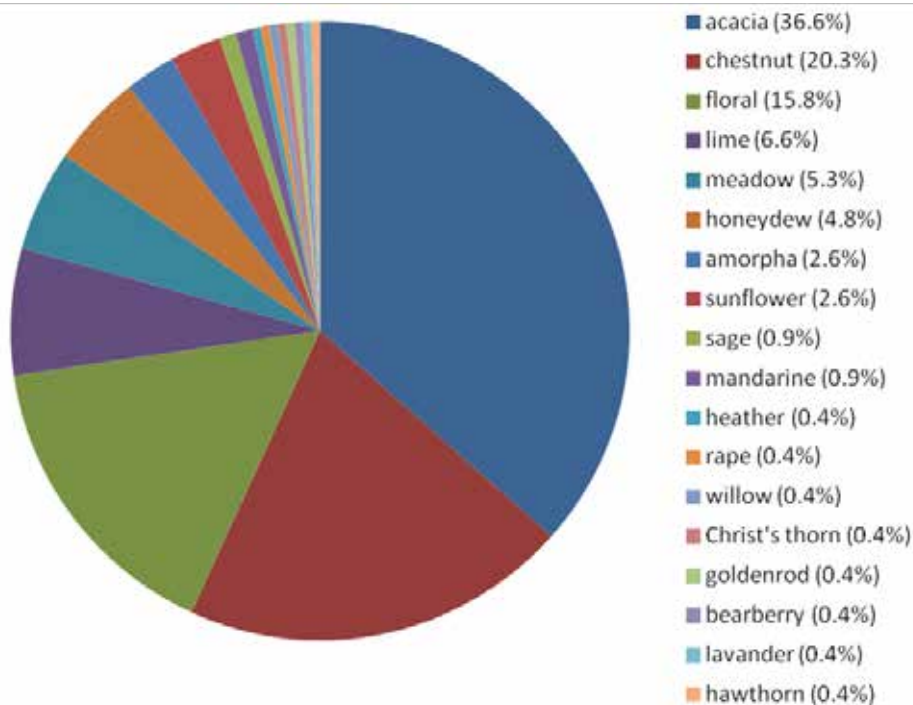


Figure 1. The percentage of individual honey types within 227 analyzed samples.

performed using the Microsoft Excel program. Calculated statistical values included the mean and standard deviation (SD), and range (minimum - maximum).

Results and discussion

The percentage of individual honey types within the 227 analysed samples is shown in Figure 1. The most common types of analysed honeys were acacia, chestnut and floral honey, constituting 72.6% of the total samples.

Honey is a natural food and its use and demand is growing in different aspect of human life. Therefore, quality control is required, primarily to protect consumers. Physicochemical and sensory characteristics are the main features in understanding honey quality. This study analysed the physicochemical parameters of Croatian honey. Table 1 shows the results of the analyses of mass fraction of water, diastase activity, electrical conductivity and mass fraction of HMF.

Due to its high sugar content, honey is highly hygroscopic and can absorb a certain amount of water in contact with moist air (Čalopek et al., 2016). Higher percentages of water in honey can cause problems in processing and storage (Vahčić and Matković, 2009). The moisture content of honey depends on climatic conditions, the season and the degree of maturity of honey. A high moisture content makes it susceptible to fermentation, deterioration and loss of taste, resulting in a significant drop in quality (Čalopek et al., 2016). While most bacteria grow in neutral and slightly alkaline medium, yeasts and moulds can grow in an acid medium (pH 4.0-4.5).

In all the honeys analysed in the present study, the average mass fraction of water was below the limit set by the Regulation (20%). The mean values of moisture obtained from different honey types were very similar and ranged from

16.0% for sage to 17.4% for sunflower. Croatian honeys have a slightly higher water content than honeys from Slovenia (15.36%–16.60%) (Golob and Plestenjak, 1999). In comparison with other studies on Croatian honeys, the water content in different types of honey harvested during 2003, 2004 and 2005 and 2011 was similar to those values presented here, i.e. from 15.4% to 17.5% (Šarić et al., 2008; Čalopek et al., 2016). Reported water content for Dalmatian sage honeys produced during the period 1999–2005 was 16.0% (Kenjarić et al., 2006), which corroborates the present results. Sabo et al. (2008) reported a water content of 18.8% in amorphous honey, which is much higher than the results reported here for the same honey type (16.2%). The same variation was noticed with goldenrod honey (16.0% vs. 18.8%). On the other hand, a decreased water content was observed for willow honey (18.4% vs. 16.5%). The obtained values for Christ's thorn honey, 15.2% to 18.0%, are in agreement with previous reports (Kenjarić et al., 2008). Water contents in honeydew honeys from Macedonia were in the same range as the obtained values in honeydew honeys in this study (Primorac et al., 2009). In an international evaluation of sunflower honey, a water content of 19.4% was reported (Vahčić and Matković, 2009), which is 2% higher than those reported in the present study. To our knowledge there are no reported data for the water content of bearberry and hawthorn honeys.

Electrical conductivity is a property that depends largely on the concentration of mineral salts, proteins and organic acids in the samples. Therefore, the total content of mineral substances, the higher the conductivity. On the other hand, electrical conductivity is negatively correlated to the moisture content in the samples. The measurement of electrical conductivity in honeys gives an indication regarding its origin (nectar

Table 1. Physicochemical parameters of the honey samples

Type of honey	Descriptive statistics	Water content (%)	Electrical conductivity (mS/cm)	HMF (mg/kg)	Diastase activity (DN)
Acacia (n=83)	Mean	16.4	0.22	2.5	16.4
	SD	1.11	0.54	3.12	2.29
	Min-max	14.1-18.2	0.10-0.51	0.1-19.4	11-21
Chestnut (n=46)	Mean	16.6	1.33	1.8	29.3
	SD	1.23	0.30	5117	3.32
	Min-max	15.0-19.9	0.75-2.16	0.1-34.5	25-36
Floral (n=36)	Mean	17.0	0.47	13.0	21.9
	SD	1.27	0.17	15.5	3.29
	Min-max	14.4-19.2	0.18-0.80	0.1-62.1	18-34
Lime (n=15)	Mean	16.7	0.69	2.3	18.3
	SD	4.32	0.14	2.33	5.41
	Min-max	15.3-19.3	0.47-0.96	14-29	0.1-7.1
Meadow (n=12)	Mean	16.8	0.51	4.0	22
	SD	1.97	0.24	3.28	2.53
	Min-max	14.3-19.4	0.32-1.17	0.1-11.8	20-27
Honeydew (n=11)	Mean	16.8	1.14	2.2	40.7
	SD	1.28	0.14	4.90	8.10
	Min-max	15.3-18.7	0.96-1.33	0.1-16.0	29-52
Amorpha (n=6)	Mean	16.2	0.23	4.2	28.3
	SD	0.45	0.04	4.32	8.17
	Min-max	15.8-16.3	0.18-0.28	0.1-11.1	21-44
Sunflower (n=6)	Mean	17.4	0.57	3.9	NA
	SD	0.83	0.07	4.08	NA
	Min-max	16.4-18.9	0.49-0.68	1.8-12.0	NA
Sage (n=2)	Mean	16.0	0.55	3.2	27
	SD	1.13	0.12	4.03	2.83
	Min-max	15.2-16.8	0.46-0.63	0.3-6	25-29
Mandarine (n=2)	Mean	17.2	0.35	10.5	12.5
	SD	1.20	0.11	11.60	0.71
	Min-max	16.3-18.0	0.27-0.43	2.3-18.7	12-13
Hawthorn (n=1)		18.2	0.63	0.99	NA
Rape (n=1)		16.1	0.20	32.4	NA
Goldenrod (n=1)		16.0	0.69	4.0	NA

Christ's thorn (n=1)		15.7	0.75	0.1	23
Willow (n=1)		18.4	0.3	1.0	NA
Lavander (n=1)		20.0	0.44	0.55	NA
Bearberry (n=1)		17.0	0.89	6.8	25
Heather (n=1)		18.3	0.31	8.4	25

or honeydew), the source of nectar and whether bees have been fed with sugar (Golob and Plestenjak, 1999).

Honeydew and chestnut honeys show higher values of electrical conductivity than other nectar honey types (Šarić et al., 2008). Therefore, the Croatian and European regulations prescribe electrical conductivity greater than 0.80 mS/cm for these two honey types, while for other nectar honey types this is lower than 0.8 mS/cm. The situation is different with lime, bearberry and heather which are exceptions, as they may be blended with honeydew honey. Only two analysed samples failed to meet the regulation requirements. Measured values for lime honey samples ranged from 0.47 mS/cm to 0.96 mS/cm with an average value of 0.69 mS/cm, which is lower than the reported values for Slovenian lime honey (0.815 mS/cm) (Golob and Plestenjak, 1999). Electrical conductivity measured for amorpha, willow and goldenrod honey was similar to those reported by Sabo et al. (2008). Primorac et al. (2009) reported a mean value of 1.00 mS/cm in Croatian honeydew honey, while in this study, the values were nearer those measured in Macedonian honeydew honey (1.14 mS/cm; Primorac et al., 2009).

The mass fraction of HMF (cyclic aldehyde formed by the dehydration of fructose and glucose) was originally used as an indicator of honey counterfeiting, as its concentration increases in proportion to a rise in temperature. However, HMF is present in small amounts in natural honey and its mass fraction in honey is dependent on honey type, specifically pH

value, acidity, moisture content and light exposure. In certain types of honey, such as chestnut that has a high pH, no HMF occurs, even during prolonged heating at 50 °C. Today, the proportion of the substance used as an indicator of energy and overheating of honey, a value higher than 100 mg/kg can still indicate honey counterfeiting. The Croatian regulations sets the maximum HMF content at 40 mg/kg. In this study, the mass fraction of HMF ranged from less than 0.1 mg/kg (LOQ) to the highest value of 62.1 mg/kg. Among the analysed samples, only two samples of floral honey did not meet this requirement. Excluding these two samples, the maximum value of the mass fraction of HMF would be 38.5 mg/kg for floral honey. Previous studies have shown that differences in the concentration of HMF in honey samples collected during the same period may be due to variations in climate among areas (Čalopek et al., 2016).

Diastase activity (diastase number - DN) is one of the main parameters utilised in the determination of the intensity of heating to which honey is exposed during processing and storage. In this study, honey samples showed DN values ranging from 16.4 to 40.7. The average DN for all types of honey studies was higher than 8, which is in accordance with the Regulation (diastase activity > 8.0 DN). The lowest DN was determined in mandarin honey, and the highest in honeydew, with a mean of 40.7, which is in agreement with earlier studies (Primorac et al., 2008; Šarić et al., 2008). In study of Golob and Plestenjak (1999), Slovenian

honey samples had a DN ranging from 5.36 to 27.3, which were lower than the values obtained in this study. The reported DN value for Dalmatian sage is 21.9, which is comparable to reported values (Kenjerić et al., 2006). Christ's thorn honey is characterised by high enzymatic activity in comparison with other types of Mediterranean honey, with the reported values ranging from 25.39 to 50.51 DN (Kenjerić et al., 2008), while in the present study, values were lower, at 23 DN.

Conclusions

This study gave the physicochemical profile of 227 honey samples originating from all parts of Croatia collected between 2012 and 2016, and including 18 different honey types: acacia, chestnut, floral, lime, meadow, honeydew, amorphous, sunflower, sage, mandarin, hawthorn, rape, goldenrod, Christ's thorn, willow, lavender, bearberry and heather. Of the total number of honey samples studied and harvested during five study seasons, only 1.8% failed to meet the requirements prescribed by the Croatian Regulation.

Abstract

In this study, a total of 227 honey samples of varying types were collected from different areas in Croatia. All samples were subjected to pollen analysis and assorted into several groups including rare honey types such as amorphous, sunflower, Christ's thorn, bearberry, heather, mandarin, willow, goldenrod, lavender, sage, rape and hawthorn. The following groups of honey were also analysed: acacia, floral, chestnut, meadow, lime, honeydew. The following physicochemical properties were tested: electrical conductivity, diastase number, water content and hydroxymethylfurfural content. All samples met the criteria for moisture content (< 20%) and ranged from 14.1% to 20.0%. Hydroxymethylfurfural concentrations ranged from 0.1 to 62.1 mg/kg

and two samples did not meet the prescribed value (< 40 mg/kg). Electrical conductivity ranged from 0.10 to 2.16 mS/cm. Increased conductivity was determined for chestnut and honeydew honey, with average values of 1.33 and 1.14 mS/cm, which meets the requirements (> 0.8 mS/cm). In all other honey types, except lime, heather and bearberry, values must be below the prescribed 0.8 mS/cm, and only two samples did not meet the prescribed criteria. The lowest mean conductivity of 0.10 mS/cm was determined for acacia honey.

Key words: honey, Croatia, electrical conductivity, diastase number, water content, hydroxymethylfurfural

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Procjena kakvoće različitih hrvatskih vrsta meda u periodu od 2012. do 2016.

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U ovome istraživanju ukupno je sakupljeno 227 različitih vrsta meda u razdoblju od 2012. do 2016. godine iz različitih područja Hrvatske. Svi uzorci podvrgnuti su peludnoj analizi na temelju koje su razvrstani u nekoliko skupina: amorfa, suncokret, drača, planika, vriješak, mandarina, vrba, zlatošipka, lavanda, kadulja, repica, glog, bagrem, cvjetni, kesten, livada, lipa, medljika. Određeni su osnovni fizikalno-kemijski pokazatelji: električna vodljivost, aktivnost dijastaze, maseni udio vode i udio hidrosimetilfurfurala. Svi uzorci zadovoljavaju kriterij masenog udjela vode ispod 20%, a određeni su u rasponu od 14,1% do 20,0%. Koncentracije hidrosimetilfurfurala kretale su se u rasponu od 0,1 do 62,1 mg/kg, a dva uzorka bila su iznad propisane vrijednosti od 40 mg/kg. Vrijednosti električne vodljivosti kretale su se u rasponu od 0,10 do 2,16 mS/cm. Povećane vodljivosti određene su za kestenv med i medljikovac sa srednjim vrijednostim od

1,33 i 1,14 mS/cm što udovoljava zahtjevima Pravilnika o medu (> 0,8 mS/cm). Za sve ostale vrste meda, uz izuzetak meda lipe, planike i vrijeska, određena vodljivost mora biti ispod propisane vrijednosti od 0,8 mS/cm, a postavljeni kriterij nisu zadovoljila dva uzorka meda. Najniža vrijednost vodljivosti od 0,1 mS/cm određena je u medu bagrema. Vrijednosti električne vodljivosti za med lipe kretale su se u rasponu od 0,47 do 0,96 mS/cm, sa srednjom vrijednošću od 0,69 mS/cm. Aktivnost dijastaze uzoraka meda obuhvaćenih ovom studijom kretala se od 16,4 do 40,7. Prosječne vrijednosti aktivnosti dijastaze svih vrsta meda u ovoj studiji, bile su iznad 8 što je u skladu sa zahtjevom Pravilnika o medu (> 8). Pokazano je da 98,2% testiranih uzoraka odgovara granicama prihvatljivosti propisanih Hrvatskom legislativom.

Ključne riječi: med, Hrvatska, električna vodljivost, voda, aktivnost dijastaze, hidrosimetilfurfural