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PHYSICOCHEMICAL ANALYSIS AS AN INDICATOR OF THE QUALITY OF HONEY ORIGINATING FROM VOJVODINA REGION

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Abstract

Physicochemical analysis of honey plays an important role in determining the overall characteristic of honey and final assessment of its quality. This study was aimed at investigating the physicochemical properties of local honeys collected from different flora from Vojvodina. The physicochemical parameters such as moisture content, titratable acidity, HMF determination and ash content were analyzed. The physicochemical characteristics of 35 out of 40 honey samples (87.5%) analyzed in this study completely correspond with the national Regulation No. 45/2003 and Codex Alimentarius, indicating adequate processing, good maturity and freshness. Five samples (12.5%) did not meet standards established in the Regulation No. 45/2003 and Codex standards. In 40 samples analyzed, the HMF content was elevated in one sample (2.5%) exceeding the limit of 40 mg/kg, and 29 samples (72.5%) revealed values lower than 10 mg/kg, which is typical for fresh unheated honeys, according to the current quality criteria. The moisture content exceeded the maximum level permitted by the Regulation in only one of 40 analyzed honey samples (2.5%). Moreover, in only three samples (7.5%), ash content exceeded the maximum level permitted by the Regulation. The titratable acidity of all samples was lower than the limit of 40 mmol of acid per 1000 g of sample. Research findings pointed out that the physicochemical properties of local honey from Vojvodina were in accordance with the Codex standard and the products meet significant quality criteria for a high-quality honey.

Key words: honey, quality, moisture content, titratable acidity, HMF content, ash content

Introduction

Honey is a sweet liquid produced by honeybees using nectar from flowers through a process of regurgitation and evaporation**.** The possible health benefits of consuming honey have been documented in early Greek, Roman, Vedic, and Islamic texts and the healing qualities of honey were referred to by philosophers and scientists all the way back to ancient times, such as Aristotle (384 - 322 BC) and Aristoxenus (320 BC). Modern science is finding that many of the historical claims that honey can be used in medicine may indeed be true. In the Bible (Old Testament), King Solomon said, "My son, eat thou honey, for it is good", and there are a number of reasons why it may be good (Nordqvist, 2014).

Honey also possesses antiseptic and antibacterial properties. In modern science, we have managed to find useful applications of honey in chronic wound management. However, it should be noted that many of honey's health claims still require further rigorous scientific studies to confirm them (Nordqvist, 2014).

According to the Regulation ("Official Gazette of SCG", No. 45/2003), honey is defined as "sweet, dense, crystallized, viscous product produced by honeybees from the nectar of honey plant flowers or from secretions of living parts (conifer or hardwood species), which the bees collect, transform by combining with specific substances of their own, and deposit in honeycombs to mature". In

Codex standard (2001), honey is defined as "natural sweet substance produced by honey 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, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in the honey comb to ripen and mature". Chemical composition of honey implicates highly complex mixture of more than 200 different substances (Ferreira et al., 2009). Some of these substances are produced by honeybees, and some originate from honey plants, whereas some are produced during the maturation process in the honeycomb (Krell, 1996).

Serbia has a very long tradition of beekeeping. Its favourable climate, good geographical condition and a variety of botanical species provide great potential for the development of apiculture (Mačukanović-Jocić, 2008).

Serbian honey could potentially be very interesting for the EU market, so it is very important to verify its compliance with the quality specifications of the European Union (European Economic Community, 2002).

Material and methods

To the purpose of determining the moisture content, ash content, hydroxyl methyl furfural (HMF) content and total acidity, 40 samples of different honeys originating from Vojvodina region were collected. All samples were in their original packages and were transferred to the laboratory and stored in a cold and dark place. The investigated samples included 12 samples of meadow honey, 14 samples of acacia honey, 14 samples of linden honey, 4 samples of multiflower honey, 5 samples of sunflower honey and 1 sample of forest honey.

Moisture content was determined by the refractometric method (Off.Gazette of SFRJ, 1985), using an Abbe refractometer (Model RMT, Optech, Italy). All measurements were performed at 20^0C after equilibrium, and obtaining the corresponding % of moisture from the refractive index of the honey sample was calculated by consulting a standard table for this purpose.

The acidity of honey was determined by volumetric method (Off. Gazette of SFRJ, 1985). Ten grams of honey were dissolved in 75 ml of distilled water and alcoholic solution of phenolphthalein was added. The solution was titrated with 0.1 mol/dm^3 NaOH. Acidity (milimol of formic acid per kg of honey) was determined as 10 times the volume of NaOH used in titration.

For determination of ash content, the method Off. Gazette of SFRJ, 1985, was followed. According to the method, 5 g of each sample was weighed in a ceramic plate. The plate was heated in a muffle furnace for about 3 to 5 h at 600°C. It was cooled in desiccators and weighed. The weight of ash gave the ash content and was calculated by the following formula:

$$Ash\ (\%) = Weight\ of\ sample\ after\ ashing \times 100 / Weight\ of\ fresh\ sample\ taken$$

For the HMF determination use was made of an HPLC Dionex UltiMate 3000 Series system with a diode array detector (DAD-3000, Thermo Scientific, Germany), consisting of an autosampler WPS-3000, degasser, quaternary pump, and Hypersil GOLD column (150×3 mm, particle size 3 μm). The system was controlled by Chromeleon® 7 software (Thermo Scientific). The mobile phase was MeOH−water (10+90, *v/v*) filtered through 0.22-μm membrane filter, at a flow rate of 1.0 ml/min.

Results and discussion

The obtained results on moisture content, total acidity, HMF content and ash content in the examined honey samples are displayed in Table 1.

Moisture content is one of the most important compositions to be considered as a quality parameter of honey. The maximum moisture content was found to be 34.00 mmol of acid/1000 g in linden samples, whereas 2.2 mmol of acid/1000 g moisture content was observed in multiflower variety.

Table 1. Results of determining moisture content, HMF, ash content and total acidity in diverse honey samples

TYPE OF HONEY	No. of samples	Moisture content (%)		Acidity (mmol of acid/1000 g)		Ash content (%)		HMF content (mg/kg)	
		Range	Average ±SD	Range	Average ±SD	Range	Average ±SD	Range	Average ±SD
Meadow	12	14.6 – 18.6	17.33 ± 1.13	13.75 – 26	20.34 ± 3.79	0.07 – 2.96	0.48 ± 0.81	3.84 – 34.75	10.81 ± 8.20
Acacia	10	14.2 – 18.4	16.2 ± 1.42	9.5 – 18.75	13.17 ± 3.07	0.0 – 0.41	0.09 ± 0.13	6.78 – 43.78	15.87 ± 12.64
Linden	8	15 – 18.6	16.67 ± 1.36	10.5 – 34.0	15.9 ± 7.44	0.04 – 1.27	0.38 ± 0.40	4.69 – 16.13	8.40 ± 3.54
Multi flower	7	15.8–21.4	17.66±1.93	2.2 –27.7	18.98 ±9.10	0.07 –-0.36	0.22 ± 0.10	5.69 –10.7	7.48 ±1.75
Sunflower	3	16 – 16.8	16.33 ± 0.42	10 – 15.00	12.42 ± 2.50	0.02 –0.275	0.11 ± 0.15	5.23 – 7.45	6.47 ± 1.13

Average values obtained in our research (Table 1.) are in agreement with the findings of Cantarelli et al. (2008), who reported that the moisture content in honey was in the range of 14 to 18%; however, it depends upon the season and geographic condition. Furthermore, these results are also in agreement with those of Nuru (2002) and Downey et al. (2005), who reported that the range of moisture content of pure honey is 16.10 to 23.36%. Fredes and Montenegro (2006) reported that honey with lower moisture content would have a longer shelf life. The maximum ash content (2.96%) was found in one sample of meadow honey, another sample of meadow honey revealed ash contents of 0.86%, which exceeds the maximum permitted level of 0.50%. Total ash contents measured in other honey samples were in accordance with the limits prescribed by relevant Regulation. , These findings are in agreement with those of Ihtisham-ul-haq (1997), who analyzed different varieties of honey and determined the ash content range of 0.008 to 0.49% in honey samples. These results are also in agreement with those of White (1975a), who worked on different varieties of honey and obtained ash content in the range of 0.020 to 1.028%. The variation may be due to many factors such as soil conditions, atmospheric conditions and physiology of each plant. The composition of organic acids in honey has not yet been adequately investigated; however, some evidence (Rogulja et al., 2009) suggest that acacia, chestnut and meadow honeys are characterized by particularly low contents of organic acids, whilst darker honeys in general appear to be higher in acidity.

The research of Prica et al. (2014.) also demonstrated low acidity of acacia honey as compared with other examined honey types. The results obtained for meadow honey do not correspond with the aforementioned evidence, yet the acidity was within the proper range. Our results correspond with these reports. HMF represents the freshness of honey and depends on adequate beehives and harvest practice. The majority of honey samples had HMF contents in line with the maximum permitted levels prescribed by the Regulation ("Official Gazette of SCG", No. 45/2003); maximum HMF content in honey put in the market is fixed to 40 mg/kg. According to the obtained results, HMF in honey content exceeded maximum permitted value in only one sample of acacia honey.

Conclusion

The physicochemical characteristics of 35 out of 40 honey samples (87.5%) analyzed in this study completely correspond with the national Regulation No. 45/2003 and Codex Alimentarius, indicating adequate processing, good maturity and freshness. Five samples (12.5%) did not meet characteristics established in the Regulation No. 45/2003 and Codex standards. In 40 samples analyzed, the HMF content was elevated in one sample (2.5%) exceeding the limit of 40 mg/kg, and 29 samples (72.5%) revealed values lower than 10 mg/kg, which is typical of fresh unheated honeys, according to the current quality criteria.

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Research findings pointed out that the physicochemical properties of local honey from Vojvodina were in accordance with the Codex standard and it meets significant quality criteria for a high-quality honey.

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References

1. Cantarelli M.A., Pellerano R.G., Marchevsky E.J., Camiña J.M.: Quality of honey from Argentina: Studi of chemical composition and trace elements. *The Journal of the Argentine Chemical Society*, 96, 1/2, 33-41, 2008
2. Codex Alimentarius Commission: Revised Codex Standard for Honey, Codex STAN 12-1981, 2001.
3. Downey G., Hussey K., Jelly J.D., Walshe T.F., Martín P.G.: Preliminary contribution to the characterization of artisanal honey produced on the island of Ireland by palynological and physicochemical data. *Food Chemistry*, 91: 347-354. 2005
4. European Economic Community: EEC Council Directive of 20 December 2001 relating to honey. *Official Journal of the European Communities*, 110, 47-50, 2002
5. Ferreira I.C.F.R., Aires E., Barreira J.C.M., Estevinho L.M.: Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, 114, 1438-1443, 2009
6. Fredes C., Montenegro G.: Heavy metal and other trace elements contents in honey bee in Chile. *Cien. Inv. Agric.*, 33, 50-58, 2006
7. Ihtisham-ul-haq: Effect of floral type, geographical locations and honey bee species on the water activity and other physico-chemical parameters of honey. M.Sc. Thesis. Deptt. Food Sci. Technol., Faculty of Nutrition Sciences, NWFP Agricultural University, Peshawer, Pakistan, 1997
8. Krell R.: Value-added products from beekeeping, *FAO Agricultural Services Bulletin* No. 124, Food and Agriculture Organization of the United Nations Rome 1996, Dostupno na www.fao.org/docrep/w0076e/w0076e00.htm. Pristupljeno 12.11.2014.
9. Mačukanović-Jocić M.: The biology of melliferous plants with an atlas of Serbian apiflora. Faculty of Agriculture (on Serbian), Beograd, Serbia, 2008
10. Nordqvist J., Reviewed by Megan Ware, RDN, LD, registered dietitian and nutritionist, What are the health benefits of honey?; 26.09.2014. Dostupno na:

    http://www.medicalnewstoday.com/articles/264667.php

11. Nuru A.: Geographical races of the Honeybees (Apis mellifera L.) of the Northern Regions of Ethiopia. Ph.D. dissertation. Rhodes University, South Africa. 2002
12. Prica N., Živkov-Baloš M., Jakšić S., Mihaljev Ž., Kartalović B., Babić J., Savić S.: Water content and acidity as an indicator of the quality of honey originating from Vojvodina region, *Archives of Veterinary Medicine*, 2, vol 7, 99-110, 2014
13. Rogulja D., Vahčić N., Matković D.: Kemijske, fizikalne i senzorske značajke meda, 2009, Dostupno na www.pcelinjak.hr/OLD/index.php/Prehrana-i-biotehnologija/kemijske-fizikalne-i-senzorske-znaajke-med.html. Pristupljeno 12.11.2014.
14. Sl. list SCG, br. 45/2003, Pravilnik o kvalitetu i drugim zahtevima za med, druge pčelinje proizvode, preparate na bazi meda i drugih pčelinjih proizvoda.
15. White JW : Composition of Honey in: Honey A Comprehensive Survey (Ed. Crane E); Heinemann, London, pp. 157-206,1975a