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MOISTURE AND ACIDITY AS INDICATORS OF THE QUALITY OF HONEY ORIGINATING FROM VOJVODINA REGION

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Abstract

The color, aroma and flavor are major sensory characteristics of honey, which are mainly determined by the botanical origin of honey as well as by processing and storage conditions. Increased moisture content above the maximum permitted level could result in honey spoilage, which affects its sensory properties. The higher water-in-honey content, the greater possibility of yeast fermentation and thus the change of the flavor and color of honey. Fermentation process results in alcohol formation and, in the presence of oxygen, the alcohol will break down to acetic acid and water, which causes honey to have sour taste. Thus, moisture content of honey is a critical parameter for its quality as it affects the stability of honey and its resistance to microbial spoilage during storage. Physicochemical analysis of moisture content and acidity of honey play an important role in determining the overall characteristic of honey and final assessment of its quality. In this study, the investigation of aforementioned parameters resulted in positive quality assessment for 48 of 50 examined honey samples produced in 2013 in the territory of Vojvodina.

Keywords: honey, quality, moisture, acidity

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SADRŽAJ VODE I KISELOST KAO POKAZATELJI KVALITETA MEDA SA PODRUČJA VOJVODINE

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Kratak sadržaj

Boja, ukus i miris su važna senzorna svojstva meda i najviše zavise od biljnog porekla meda, a zatim i od uslova prerade i čuvanja. Ukoliko je procenat vode u medu veći od dozvoljenog, postoji mogućnost da dođe do vrenja i da se med pokvari, što utiče na njegova senzorna svojstva. Što je veći sadržaj vode u medu veća je verovatnoća da će kvasci fermentisati med i tako prouzrokovati gubitak ukusa. Fermentacijom nastaje alkohol koji se u prisustvu kiseonika razgradi na sirćetnu kiselinu i vodu što takvom medu daje kiseo ukus. Stoga se može reći da je sadržaj vode važan parametar kvaliteta meda obzirom da određuje stabilnost meda i otpornost na mikrobiološko kvarenje tokom čuvanja. Fizičko-hemijske analize parametara kao što su sadržaj vode i kiselost meda imaju značajnu ulogu u definisanju ukupnih svojstava meda i proceni kvaliteta meda. U ovom radu ispitivanjem navedenih parametara procenjen je odgovarajući kvalitet 48 od 50 ispitanih uzoraka meda iz 2013. godine sa područja Vojvodine.

Cljučne reči: med, kvalitet, sadržaj vode, kiselost

INTRODUCTION

What is it that makes honey so special foodstuff? An answer to this question is highly complex, same as its extraordinary taste is. Honey is durable food, it never spoils and has virtually unlimited shelf life. The production of honey involves a wide range of factors acting together in perfect harmony.

Probably the most important property describing the chemical composition of honey is its diversity, that is, one could not find even two fully identical honey samples (Rogulja et al., 2009). However, huge body of information available to modern consumers results in their increased expectations and demands in view of the safety and quality of food (Prica et al., 2009). Therefore, there is a need to find the answer to the question: What is the quality of honey, how is it assessed and graded? One of the possible approaches to evaluate the quality of honey includes passing of relevant regulations establishing minimum

and maximum levels of particular substances and ingredients in foodstuffs. In Serbia, the quality requirements for honey are stipulated in the Regulation on quality and other requirements for honey, other bee products, products based on honey and other bee products (Sl. list, 2003).

According to the Regulation (Sl. list SCG, 2003), honey is defined as “sweet, dense, crystallized, viscous product produced by honeybees from the nectar of honeyplant 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, some originate from honeyplants, whereas some are produced during the maturation process in the honeycomb (Krell, 1996).

Honey types, as well as the individual samples within particular type, differ by their composition according to their floral and geographic origin, climatic conditions, honeybee species as well as processing and storage conditions (Škenderov and Ivanov, 1986).

The average composition of honeys includes some 17% water, 38.19% fructose, 31.28% glucose, 1.31% saccharose, 7.31% maltose, 7.11% lactose, 0.04% nitrogen and some 0.169% ash. After the carbohydrates, water is the second most important component of honey. Its content ranges between 15 and 23% (Krell, 1996). The moisture content substantially affects some physical properties of honey (crystallization, viscosity, specific weight) and is influenced by climatic factors, bee species, bee-colony's strength, humidity and air temperature in the hive, processing and storage conditions as well as by the honeyplant species. However, there are no substantial differences in water content between individual honey types (Škenderov and Ivanov, 1986).

Honey in its natural form is characterized by extremely low moisture content, thus very few bacteria and microorganisms can survive in such environment, which is essential for its resilience (Geiling, 2013). Yet, honey is highly hygroscopic substance and its moisture content may vary depending on air humidity during storage. The higher moisture-in-honey content, the greater is the possibility that the yeasts will ferment and change the flavor. Namely, fermentation process results in alcohol formation and, in the presence

of oxygen, the alcohol will break down to acetic acid and water, which causes honey to have sour taste and to spoil (Rogulja et al., 2009).

It is well established that molasses, a byproduct of cane sugar, is similar to honey by its properties, yet—although it has a long shelf life molasses can eventually spoil. The durability of honey is partly to be attributed to the bees themselves. Nectar, the first component collected by bees to make honey, is by its nature highly humid with a moisture content ranging from 60-80%. Throughout the process of making honey, the bees dry out much of this moisture by flapping their wings. The chemical composition of bees' stomach significantly contributes to honey's resilience to spoilage. Bees' stomach produces an enzyme called glucose oxidase, which mixes with the nectar, breaking it down into two by-products: gluconic acid and hydrogen peroxide, the latter one being of crucial importance for the maintenance of quality of honey (Geiling, 2013).

Honey is naturally highly acidic. Its pH is extremely low, ranging between 3 and 4.5, which inhibits the growth of bacteria and other spoil-ready organisms (Geiling, 2013). During a long time, formic acid has been considered major (if not the only one) acid in the honey. Nowadays, it is well established that honey contains a wide range of organic acids. Besides the formic acid, honey contains oxalic acid, butyric acid, citric acid, 2,3-dihydroxybutanedioic acid, malic acid, pyroglutamic acid, lactic acid, benzoic acid, maleic acid, gluconic acid, isobutyric acid, succinic acid, pyruvic acid, α -ketoglutaric acid and glycolic acid. Out of these, gluconic acid, a byproduct of enzymatic activity of glucose oxidase, predominates. According to the data from the literature, the content of organic acids in honey ranges between 0.17 and 1.17% (average range 0.57%). Most of organic acids are present in honey in the form of esters, which contributes to its characteristic flavor and aroma. Some of the acids are introduced into honey via the nectar, i.e., their contents depends on the type of the honey, whereas some are produced during storage process and are influenced by storage temperature and processing conditions. The acidity of *honey* can range from 8.7 to 59.5 meq/kg, with an average of 29.1 meq/kg. Increased acidity of honey is an indicator for a fermentation process and transformation of alcohol into organic acid (Rogulja et al., 2009). It is believed that moisture content less than 18% will prevent the fermentation. However, this possibility cannot be absolutely excluded even in honeys with moisture content below 17.1% since the potential effects of yeast content and temperature of honey as well as distribution and availability of water after crystallization have to be taken into consideration (Krell, 1996).

Moisture content can be considered the most important parameter of

honey quality as it determines its stability and resistance towards microbial spoilage (fermentation) during storage (Bogdanov et al., 1999). The influence of acid content on fermentation processes, flavor and aroma as well as bactericidal properties of honey make the total acidity an important indicator of quality of honey. To that end, the objective of this study was to investigate these quality parameters in honey samples collected during 2013 in the territory of Vojvodina.

MATERIAL AND METHODS

To the purpose of determining the moisture content and total acidity, 50 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 (Sl. list SFRJ, 1985), using an Abbe refractometer (Model RMT, Optech, Italy). All measurements were performed at 20°C after equilibrium. The corresponding % 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 (Sl. list 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/dm³ NaOH. Acidity (milimol of formic acid per kg of honey) was determined as 10 times the volume of NaOH used in titration.

RESULTS AND DISCUSSION

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

Moisture content in the examined samples ranged between 14.2 and 20.2%, with an average of 16.5±1.01%. Pursuant to relevant Regulation in Serbia (Sl. list SCG, 2003), which is harmonized with the EU Directive (EU Council 2002), maximum moisture content in honey put in the market is fixed to 20%. According to the obtained results, moisture content exceeded maximum permitted value in only one sample of sunflower honey.

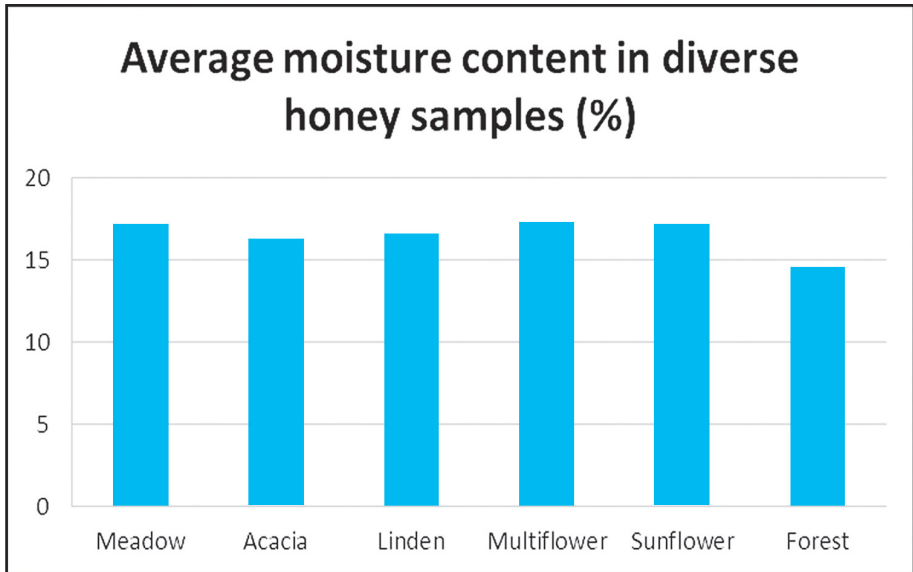
The acidity value in the same samples ranged from 7.75 mmol/kg to 44

mmol/kg, with an average of 17.38 ± 6.79 mmol/kg. Pursuant to EU Council (2002), the maximum permitted acidity of honey is 50 meq/kg (the unit meq/kg is identical with mmol/kg since the acidity is expressed as the content of formic acid). Maximum value permitted by Serbian Regulation (Sl. list SCG, 2003) is somewhat lower, being 40 mmol of formic acid per 1000 g of the sample. Our results revealed that acidity was higher than the maximally permitted level (according to Serbian Regulation) in only one sample of linden honey.

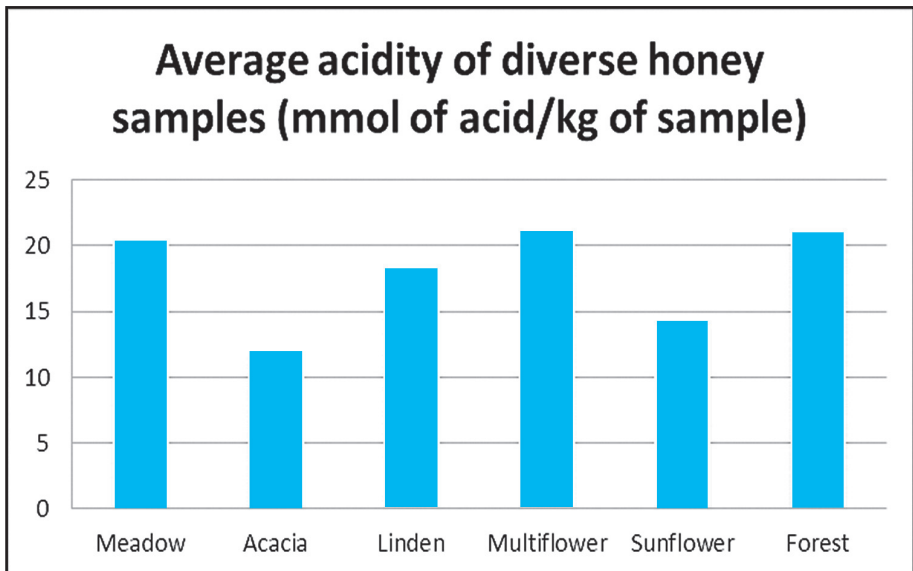
Table 1 Results of determining moisture content and acidity in diverse honey samples

| TYPE OF HONEY | No. of samples | Moisture content (%) | | Acidity (mmol of acid/1000 g) | |
|---------------|----------------|----------------------|------------------------|-------------------------------|------------------------|
| | | Range | Average value \pm SD | Range | Average value \pm SD |
| Meadow | 12 | 14.6–18.2 | 17.1 \pm 1.3 | 13.75–26.00 | 20.39 \pm 3.81 |
| Acacia | 14 | 14.2–18.4 | 16.3 \pm 1.3 | 7.75–18.75 | 12.08 \pm 3,29 |
| Linden | 14 | 14.8–18.6 | 16.5 \pm 7.0 | 10.50–44.00 | 18.26 \pm 9.90 |
| Multi-flower | 4 | 16.0–19.2 | 17.2 \pm 1.6 | 12.50–27.70 | 21.12 \pm 6.60 |
| Sunflower | 5 | 16.0–20.2 | 17.2 \pm 1.7 | 10.00–19.90 | 14.25 \pm 3.54 |
| Forest | 1 | 14.6 | 14.6 | 21,00 | 21.00 |

The obtained results on moisture content and total acidity of honey samples are presented in Graphs 1 and 2.



Graph 1. Moisture content in diverse honey samples



Graph 2. Total acidity of diverse honey samples

Analysis of the results obtained for the investigated parameters in honey samples revealed the lowest average values for water content and acidity in samples of forest honey and acacia honey, respectively. The highest average values for both parameters were established in multiflower honey samples.

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*. *Our results 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.*

Determination of physicochemical parameters in different honeys has been the topic of numerous researches both in Serbia and worldwide. The investigation of different quality parameters in 226 honey samples originating from Braničevo and Podunavlje regions during 2010-2012 revealed that honey in this region of Serbia is of good quality. Namely, all samples were characterized by adequate moisture content, and only one sample of acacia honey demonstrated increased acidity (Milošević et al., 2013). Examination of 201 honey samples originating from the entire territory of Serbia (acacia, sunflower and linden) was performed during 2009. The average moisture content ranged from 16.12% in acacia honey samples to 17.98 in sunflower honey samples. Free acidity differed widely among the three studied botanical samples, ranging from 11.20 in acacia honey samples to 25.65 meq/kg in sunflower honey samples (Lazarević et al., 2012). The investigation of the quality of diverse honeys produced in Montenegro (Đuričković et al., 2012) revealed moisture contents ranging from 17.0% in acacia honey to 19.2 in sage honey. The lowest and highest total acidity was determined in acacia honey (10 mmol/kg) and sage honey (40.0 mmol/kg), respectively.

Moisture content reported for five honey samples from Portugal ranged from 15.9 to 17.2%, whereas free acidity was within the range 16.0–32.0 meq/kg (Gomes et al., 2010). Determination of moisture content in 70 honey samples in Turkey revealed as much as 10% of inadequate samples, whereas the acidity values ranged between 6.94 and 29.6 meq/kg (Kahraman et al., 2010). In honey samples originating from India, the highest average values for water content were obtained for mustard honey (21.75 %), whereas eucalyptus and clover honeys had somewhat lower moisture contents (19.4 and 18.7 %, respectively) (Singh and Bath, 1997). The acidity level of the examined samples ranged between 29.5 and 41.5 meq/kg. By analyzing the samples of multifloral

honey collected in Venezuela during rainy and dry seasons, De Rodriguez et al. (2004) concluded that climatic conditions are of no importance for moisture content in honey. Namely, one of two honey samples with moisture content above 20% originated from dry season. The authors are of the opinion that increased moisture content is more likely associated with insufficient maturity of honey rather than with climatic conditions (De Rodriguez et al., 2004). Similar rates of moisture content in honey were reported in Argentina. The moisture content in 143 analyzed samples was within a range 16.4–18.1 % (Malcalza et al., 2005). Moisture content in Brazilian honey was somewhat higher, ranging between 18.59 and 19.58 % (Azeredo et al., 1999). The investigation including 73 samples of different honey types from Poland revealed moisture contents of 15.93–17.96 % (Popek, 2002).

As obvious from a brief review of quality control of honey in Serbia and worldwide, the analysis of physicochemical parameters is of vital importance in quality assessment. Although the aforementioned researches encompassed different types of honey, our research demonstrated that the quality of honey from Vojvodina corresponds to that of honeys available in international market.

CONCLUSION

The moisture content exceeded the maximum level permitted by the Serbian Regulation in only one of 50 analyzed honey samples. Moreover, in only one sample, the acidity was above the upper limit of 40 mmol of acid per 1000 g of sample (Sl. list SCG, 2003). We can conclude that 96% of investigated samples corresponded with the prescribed quality parameters, which may be taken as indicative of freshness of all honey samples. Nevertheless, potential effects of storage conditions on the quality of honey strongly suggest the necessity of continuous monitoring of the aforementioned parameters throughout the year.

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