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*Original scientific paper*

# PHYSICOCHEMICAL PROPERTIES OF CHESTNUT HONEY (*CASTANEA SATIVA MILL.*) PRODUCED IN THE POLOG REGION OF THE REPUBLIC OF NORTH MACEDONIA

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**Abstract**

In this study, we investigated physicochemical properties of chestnut honey produced in the Polog region in Republic of North Macedonia, and compared them to the properties of chestnut honey produced in other European countries, outlined in the descriptive sheet for chestnut honey by Persano Oddo et Piro (2004). The monofloral status of the honey samples was confirmed through melissopalynological analysis by Loveaux et al. (1978). The research included the determination of electrical conductivity, water content, free acidity, fructose, glucose, sucrose, and HMF content, using the methods outlined by the AOAC Official Methods (2005) and the International Honey Commission (IHC, 2009). Based on the obtained values for water, fructose and glucose content, the ratios of F/G and G/W, as well as the sum of F+G, were calculated. The electrical conductivity of the samples ranged from 1.04 to 1.45 mS/cm, the water content ranged from 16.7–17.7 %, the free acidity ranged from 15.5 to 19.5 meq/kg, the HMF content ranged from 2.0 to 5.2 mg/kg, the fructose content ranged from 38.9 to 42.7 %, the glucose content ranged from 25.4 to 30.5 %, the sucrose content ranged from 0.0 to 0.1 %, the sum of fructose + glucose ranged from 66.3 to 70.5 %, the F/G ratio ranged from 1.34 to 1.64 and G/W ratio ranged from 1.43 to 1.74. These findings demonstrate that chestnut honey produced in the Polog region meets the standards established by the European Directive concerning honey (2001/110/CE).

*Keywords*: chestnut honey, melissopalynological analysis, physicochemical properties, Polog reion, Republic of North Macedonia.

## Introduction

Bee honey is defined as "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, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature." (Council Directive 2001/110/EC, 2001).
Depending on its origin, honey is classified as monofloral (obtained primarily from the nectar of one plant species) or polyfloral (obtained from the nectar of multiple plant species). Bee honey intended for human consumption should meet a sufficient level of quality. In order for a product to be labelled as honey, it must adhere to certain requirements as described in Directive 2001/110/EC, such as meeting the values for specific physicochemical parameters. In addition, the content and type of pollen grains present in honey must be analyzed to determine if it is monofloral. More than 100 botanical species that produce monofloral honey are known in Europe Persano Oddo et Piro (2004). While most of them are produced occasionally or are only of local interest, others are part of the import-export market between different European countries. In 1998, the International Apimondia Honey Commission (IHC) established a working group comprising of 28 researchers who specialized in honey analysis from 20 different laboratories in 11 countries, with the aim of collecting analytical data on the

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main European monofloral honeys in order to establish quality criteria for each significant type of honey. Fifteen types of honey were selected as the most significant based on their abundance of production or commercial importance in European countries. One of those fifteen types of monofloral honey is chestnut honey, obtained from the Castanea sativa plant, which is one of the best sources of nectar and pollen for honey bees. Chestnut trees can be found in a wide range of geographical locations, spanning from southern Europe (Iberian Peninsula, Italy, the Balkans, Mediterranean Islands) and North Africa (Morocco), to northwestern Europe (England, Belgium) and east to western Asia (Northeastern Turkey, Armenia, Georgia, Azerbaijan, Syria), at altitudes ranging from 200 to 1,800 m (D. Avanzato, 2009). In Europe, the chestnut trees cover an area of over 2.5 million hectares, roughly the size of the island of Sardinia. Most of the area (89 %) is concentrated in a few countries, namely France, Italy, Spain, Portugal and Switzerland (M. Conedera et al., 2004). A part of the natural distribution area of chestnut trees also extends to the territory of the Republic of North Macedonia, where it covers an area of 11,080 ha
in four out of eight regions (State Statistical Office, RSM). This gives the possibility of producing monofloral chestnut honey. The aim of this paper is to evaluate some physicochemical properties of the chestnut honey produced in the Polog region in the Republic of North Macedonia and compare them to the properties of chestnut honey produced in other European countries, outlined in the descriptive sheet for chestnut honey by Persano Oddo et al. 2004.

## Materials and methods

**Honey samples** - This research has been conducted with the collection of 10 samples of chestnut honey that were collected directly from beekeepers in the Polog region of North Macedonia, which is divided into nine municipalities - 1- Bogovinje, 2 - Brvenica, 3 - Gostivar, 4 - Jegunovce, 5 - Mavrovo and Rostushe, 6 - Tearce, 7 - Tetovo, 8 - Vrapcishte and 9 - Zelino (as shown in Map 1).


**Map 1** Study area (Polog region)

The apiaries were stationed at different locations within four municipalities - two each in Tetovo and Gostivar, five in Tearce and one in Mavrovo and Rostushe (Table 1). The honey samples were collected immediately after extraction during the 2024 production year, then properly packaged, labeled, and stored until analysis, which was conducted one month later. The monoflorality of the honey samples was verified through melissopalynological analysis.
**Melissopalinological analysis** - Honey samples were analysed by qualitative melissopalynological analysis according to the method recommended by the International Commission of Bee Botany (ICBB), Louveaux et al 1978. Microscopic examination and counting of pollen grains was performed on a Motic BA 210 light microscope (400×). 500

pollen grains were counted on 2 slides for each honey sample, and chestnut pollen as dominant was presented as a percentage in relation to the total number of pollen grains. The identification of chestnut pollen grains was carried out by comparison using photographs taken from Major European Monofloral Honeys: Description Sheets (Persano Oddo L. et al. 2004).

**Table 1.** Locations of apiaries

| *Municipality* | *Location* | *Coordinates* | *Altitude (m)* |
|---|---|---|---|
| *Tetovo* | Dzepciste | 42°1′58″N 21°0′0″E | 443 |
| | Setole | 42°3′16″N 21°3′20″E | 960 |
| | Neproshteno | 42°3′11″N 21°1′9″E | 457 |
| *Tearce* | Tearce | 42°4′27″N 21°3′9″E | 540 |
| | Odri | 42°07′31″N21°05′19″E | 612 |
| | Prsovce | 42°5′0″N 21°3′41″E | 493 |
| | Dobroste | 42°6′11″N 21°4′40″E | 494 |
| *Gostivar* | Zdunje | 41°47′39″N20°52′32″E | 548 |
| | Peckovo | 41°46′53″N20°49′54″E | 930 |
| *Mavrovo and Rostushe* | Mavrovo | 41°40′32″N 20°44′3″E | 1230 |

**Physicochemical analysis:**

**Electrical conductivity (EC)** – The electrical conductivity of the honey samples was determined using a standard conductometric method according to Harmonized methods of the International Honey Commission (IHC, 2009). The results are expressed as mS/cm.

**Water content** – The water content of the honey samples was determined using a refractometric method according to Harmonized methods of the International Honey Commission (IHC, 2009). The results are expressed as g/100 g of honey (%).

**Sugars in honey -** The content of sucrose, fructose, and glucose was determined according to AOAC (2005) Official Method 977.20 (HPLC_IR). The results are expressed as g/100 g of honey (%).

**Free acidity** – The content of free acids in the honey samples was determined according to AOAC (2005) Official Method 962.19. The results are expressed as meq/kg of honey.

**Hydroximethylfurfural (HMF)** - The content of HMF was determined according to AOAC (2005) Official Method 980.23. The results are expressed as mg/kg of honey.

**The Fructose/Glucose (F/G) ratio, Glucose/Water (G/W) ratio, and the sum of Fructose + Glucose (F+G)** were calculated from the water, fructose, and glucose content of the samples.

**Statistical analysis -** The results obtained for the tested characteristics are expressed as mean values ± standard deviation (SD) and min-max values. One-way analysis of variance (ANOVA) was used to compare the values of Macedonian honeys to European honeys (Persano Oddo and Piro 2004). Differences between mean values were considered statistically significant at the 95 % confidence level ($p < 0.05$).

## Results

**Melissopalinological analysis -** The monofloral status of chestnut honey was confirmed in all 10 samples of honey. According to Von der Ohe W. et al (2004), a honey sample can be classified as monofloral chestnut honey if at least 85 % of the counted pollen grains are Castanea sativa Mill. pollen grains (Figure 1). In the honey samples analyzed, the percentage of Castanea sativa Mill. pollen grains ranged from 86.9 % to 98.5 % of the total pollen spectrum.

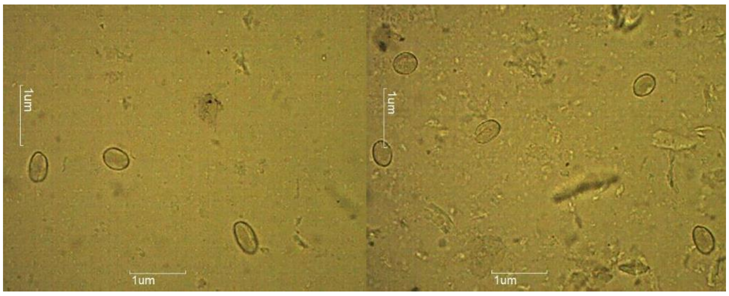


**Figure 1** Photomicrographs of pollen grains in sample of chestnut honey

**Table 2.** Physicochemical properties of chestnut honey from Polog region and European chestnut honey

| *Parametar* | *Chestnut honey from Polog region* | | | *European chestnut honey* | | | *ANOVA test* |
|---|---|---|---|---|---|---|---|
| | **X̄** | **Sd** | **Min-max** | **X̄** | **Sd** | **Min-Max** | |
| *Electrical conductivity (/kg)* | 1.26 | 0.14 | 1.04-1.45 | 1.38 | 0.27 | 0.86-1.91 | p=0.2418 |
| *Water content (%)* | 17.15 | 0.38 | 16.7-17.7 | 17.5 | 1.2 | 15.2-19.8 | p=0.2283 |
| *HMF (mg/kg)* | 2.96 | 1.32 | 2.0-5.2 | 2.0 | 1.6 | <10 | p=0.1154 |
| *Free acidity (meq/kg)* | 17.21 | 1.41 | 15.5-19.5 | 13.0 | 3.5 | 6.2-20.0 | p=0.0017 |
| *Sucrose content (%)* | 0.1 | 0.4 | 0.0-0.6 | 0.2 | 0.3 | 0.0-0.8 | p=0.3103 |
| *Fructose content (%)* | 40.93 | 1.18 | 38.9-42.7 | 40.8 | 2.6 | 35.8-45.9 | p=0.8751 |
| *Glucose content (%)* | 27.39 | 1.72 | 25.4-30.5 | 27.9 | 2.5 | 22.9-32.8 | p=0.5233 |
| *Fructose + Glucose (%)* | 68.32 | 1.63 | 66.3-70.5 | 68.7 | 2.5 | 63.8-73.6 | p=0.6341 |
| *G/W ratio* | 1.60 | 0.09 | 1.43-1.74 | 1.62 | 0.13 | 1.37-1.88 | p=0.6332 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***F/G ratio*** | 1.50 | 0.11 | 1.34-1.64 | 1.48 | 0.19 | 1.11-1.85 | p=0.7413 |

**Physicochemical properties** - The results of the physicochemical properties of the analyzed chestnut honey samples, along with data on European chestnut honeys, are presented in Table 2.

## Discussion

**Sugars in honey** – Honey is primarily composed of sugars, with monosaccharides (fructose and glucose) and disaccharides (sucrose) being the essential components. The predominant profile of sugars such as glucose, fructose, sucrose, and maltose have been associated with several properties of honey, including viscosity, hygroscopy, granulation and energy value (Ouchemouk et al., 2009). As shown in Table 2, the chestnut honey samples analyzed in this study exhibited an average of 68.32 % reducing sugars (glucose + fructose), with fructose reaching a maximum of 42.7 % and glucose a maximum of 30.5 %. All samples showed higher fructose content. Aboud et. al. (2011) and Beşir et.al. (2017) reported reducing sugars values in chestnut honey ranging from 58.3 % to 63.6 % and from 67.80 % to 75 %, respectively. The reducing sugars values of all the samples analyzed were found to be within the acceptable range as per the international regulations for honey quality. The sucrose content in honey is evaluated as it can be an indication of potential manipulation. High levels of sucrose may be the result of several adulterations, such as mixing with low-cost sweeteners like sugar cane, or feeding the honeybees with sucrose syrups (Escuredo et al. 2012). The chestnut honey samples analyzed in this study contained a small percentage of sucrose (maximum 0.6 %), which is below the 5 % limit specified by EU Council 2002. The low sucrose content in the honey samples indicated no adulteration (addition of low-cost sweeteners e.g. cane or refined sugars) and early harvest, indicating that the sucrose in these samples has been fully converted into glucose and fructose. For comparison, previous studies by Ruoff et al. (2007) and Beşir et.al. (2017) reported a maximum value of sucrose content in chestnut honey of 3.6 % and 3.0 %, respectively, which are also within the permitted range.

**Fructose/glucose (F/G) ratio and glucose/water (G/W) ratio** - The concentrations of fructose and glucose, as well as their ratio (F/G), and glucose/water ratio (G/W) are crucial indicators of honey quality (Nour, 1988; Persano Oddo and Piro, 2004; Soria et al., 2004, Buba et al., 2013). The F/G ratio in particular is a good indicator of honey's ability to crystallize, as glucose is less soluble in water than fructose. Honey crystallization is faster when the F/G ratio is less than 1.11, and it is slower when this ratio is greater than 1.33 (Smanalieva and Senge, 2009). Additionally, the rate of glucose crystallization in honey also depends on the ratio of glucose to water (G/W) (Escuredo et al., 2014). Slow crystallization occurs when the G/W ratio is less than 1.7, whereas when the ratio is greater than 2.0, rapid and complete crystallization will occur. As shown in Table 2, the F/G ration in the investigated honey samples varied between 1.34 and 1.64, and the G/W ratio varied between 1.43 and 1.74. These results indicated that the chestnut honey samples had a low to moderate ability to crystallize. The comparison of the sugar content values (sucrose, fructose and glucose), as well as the calculated F/G ratio and G/W ratio, in chestnut honey produced in Polog with the values of chestnut honeys produced in Europe outlined in the descriptive sheet for chestnut honey by Persano Oddo et al. 2004, did not show statistically significant differences.

The **water content** in honey is strongly influenced by factors such as the floral source, climatic conditions, handling techniques, and maturity period (Nayik et al. 2018). Information about the moisture content is critical in preventing mold growth in honey, for improving conservation and storage, which are associated with the maturity of honey (Singh and Singh 2018). High moisture content can alter various physicochemical parameters (Escuredo et al. 2013) and is often the cause of undesirable fermentation of honey during storage. The moisture content of the analyzed honey was found to be well below the imposed limit (< 20%) set by the EU Council 2002, with values ranging from 16.7 to 17 %, or an average of 17.15 %. These values were similar to the values reported by other authors, such as Aboud et al. (2011), who determined a water content of 13.9 % and 17 % in two samples of chestnut honey from Sicily; Czipa (2010), who determined an average water content of 17.2 ± 0.2 % in chestnut honey from Hungary; Truzzi et al. (2014), who determined an average water content of 17.5 ± 0.9% in Italian chestnut honey; and Beşir et.al. (2017), who determined an average water content of 18.13 ± 1.53 % in chestnut honey produced in Black Sea Region of Turkey. The comparison of the water content in Macedonian chestnut honey with chestnut honeys produced in Europe outlined in the descriptive sheet for chestnut honey by Persano Oddo et al. 2004, did not show a statistically significant difference.

**Free acidity -** The free acidity in honey is caused by the presence of polyphenol, ascorbic acid, and amino groups. The natural acidity of honey increases during storage, maturation, and fermentation. High values of free acidity indicate sugar fermentation and formation of acetic acid through the hydrolysis of alcohol (Geană et al., 2020). Free acidity serves as an indicator of the beginning of the honey fermentation process, and the maximum allowed value for free acidity is 50 meq acid/kg of honey. The free acidity of chestnut honey analyzed in this study ranged from 15.5 to 19.5 meq/kg or 17.21 meq/kg in average (Table 2). None of the analyzed samples exceeded the International limits, but the values for free acidity in the honeys analyzed are higher than those of European honeys. For this parameter, a statistically significant difference was determined by analysis of variance. Other authors (Aboud et al. 2011, Ruoff et al. 2007 and Czipa 2010), have reported the following values for the content of free acids in chestnut honey: 13.4 and 18.4 meq/kg for two honey samples, from 4 to 30 meq/kg for 60 honey samples and 23,5 ± 5,1 meq/kg average value, respectively.

**Hydroxymethylfurfural (HMF)** - HMF is a compound that is formed through the breakdown of fructose, one of the main sugars in honey, in the presence of acid. The formation of HMF occurs slowly during storage and rapidly when honey is heated, making it a great indicator of honey quality. The use of the HMF value alone is sufficient to provide information required to estimate the total heat exposure of honey, making it a commonly used test for assessing honey quality. The HMF content is a parameter that indicates the degree of freshness and deterioration of honey (Onur et al., 2018). According to EU Council 2002, honey must meet a limit of 40 mg/kg HMF after processing and blending. The analysis of variance revealed that there is no statistically significant difference between the average HMF values in the Macedonian honey samples and in the European honey sample, which are 2.96 mg/kg and 2.0 mg/kg, respectively.

**Electrical conductivity** - Electrical conductivity is a parameter used to control the quality of honey and differentiate honeydew from floral honey. It can be used for determining botanical origin by correlating it with the pollen content of honey (the mineral content being brought into honey along with the pollen) (Kaskoniene et al., 2010). Organic and mineral acids present in honey have the ability to dissociate into ions, when they are in an aqueous solution, or conduct electricity. Light honey typically has a lower conductivity value compared to darker honey (Kropf et al., 2008). The electrical conductivity of honey increases with the amount of ash and acid present (El Sohaimy et al., 2015). The maximum allowed value for floral honey is 0.8 mS/cm, with values higher than this being specific to honeydew and chestnut honey (EU Council 2002). In this study, the samples of Macedonian chestnut honey had conductivity values between 1.04 and 1.45 mS/cm and the samples of European chestnut honey had values between 0.86 and 1.91 mS/cm. This confirms that the samples analyzed were pure chestnut honey (Table 2). Other studies by Aboud et al. (2017), Ruoff et al. (2007) and Czipa (2010) have reported values of electrical conductivity for chestnut honey samples between 1.30 mS/cm and 1.60 mS/cm, between 0.62 mS/cm and 1.70 mS/cm, and an average value of 0,584±0,112 mS/cm, respectively.

## Conclusion

To better understand the potential differences and specific characteristics of chestnut honey from North Macedonia, further research should include samples from the other three chestnut honey-producing regions in North Macedonia - Southwestern, Pelagonia, and Southeastern. Future studies should also be expanded to analyze a greater number of samples collected over multiple production seasons and stored for varying durations. This will allow for the assessment of climatic influences in individual years, as well as the evaluation of honey quality preservation over time. Additionally, further investigation is needed to determine whether the higher acidity observed in chestnut honey from the Polog region represents a distinctive trait that differentiates it from chestnut honey produced in other regions of North Macedonia and in other European countries.

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