

Phenolic Profile of Honeydew Honeys from the North-East Part of Romania

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Abstract

The aim of this study was to determine the physicochemical (moisture content, pH, free acidity, electrical conductivity, colour (L*, a*, b*, chroma, hue angle), ash content, fructose and glucose content) and to determine the phenolic profile (quercetin, apigenin, myricetin, isorhamnetin, kaempferol, caffeic acid, chrysin, galangin, luteolin, *p*-coumaric acid, gallic acid and pinocembrin) of five samples of honeydew honeys from the North-East part of Romania. All analysed honey samples had a moisture content below the maximum level of 20% established by the European Directive 110/2001 regarding honey. The acidic nature of the honeydew was confirmed by the level of the pH and free acidity of the samples, and is influenced mainly by the organic acids; all the samples had a free acidity lower than 50 meq acid/kg. The honey colour was dark which is confirmed by the level of the CIE L*a*b* parameters (lower values of L*, a* and b*). The inverted sugar level (was higher than 60 g/100g). The myricetin ranged between 0 – 0.37 mg/100 g honey, *p*-coumaric acid ranged between 0-4.35 mg/100 g honey, chrysin ranged between 0-0.16 mg/100 g honey, caffeic acid ranged between 0-1.92 mg/100 g honey, pinocembrin ranged between 0.27-4.36 mg/100 g honey, quercetin ranged between 0.10 – 2.79 mg/100 g honey, apigenin ranged between 0-1.10 mg/100 g honey, kaempferol 0-0.60 mg/100 g honey, isorhamnetin 0-0.12 mg/100 g honey, luteolin ranged between 0-0.11 mg/100 g honey, gallic acid ranged between 0.02-0.26 mg/100 g honey and galangin 0.02-0.49 mg/100 g honey, respectively.

Keywords: *honeydew honey, phenolic profile, physicochemical parameters*

INTRODUCTION

Honey is a natural product proved to provide beneficial effects to the human health, due to a high concentration of sugars, water, proteins, organic acids, minerals, phenolic acids, flavonoids and enzymes (Halouzka *et al.*, 2016). According to the present European Directive 110/2001, there are three types of honeys with regard to their origin: (1) nectar honey – made from plant nectar that can be monofloral or multifloral, (2) honeydew – made mostly from the secretion of insects feeding on plant juices or plant secretion, and (3) mixed honey of honeydew and nectar honey.

Honeydew honey refers to honey produced by bees that collect the excretion of plant-sucking insects on the living parts of plants. Honeydew itself has been historically considered as delightful

syrup used as food and medicine by people, to the extent that the famous term “manna” often refers to the crystallized honeydew produced by scale insects feeding on the tree (de Miguel *et al.*, 2014).

Phenolic compounds that can be found in honey are free phenols (volatile compounds), phenolic acids, polyphenols (usually in the form of flavonoids), anthocyanins, procyanidins and pigments. Lately, the phenolic compounds have been intensively used by researchers to determine the botanical and geographical origin of honeys (Escriche *et al.* 2014, Halouzka *et al.*, 2016, Gomes *et al.* 2010; Bertoncelj *et al.* 2011; Manzanares *et al.* 2011; Juan-Borras *et al.* 2014; Karabagias *et al.* 2014). The kaempferol has been established as marker for rosemary honeys, quercetin for

sunflower honey, while hesperitin for citrus honey (Thomas-Barberan *et al.*, 2001)

The aim of this study was to analyse from melissopalynological point of view and to establish the phenolic profile (quercetin, apigenin, myricetin, isorhamnetin, kaempferol, caffeic acid, chrysin, galangin, luteolin, *p*-coumaric acid, gallic acid and pinocembrin) of the honeydew honeys from the North-East part of Romania using an HPLC-UV methods; to our knowledge, no other studies have been reported on the phenolic profile determination of the honeydew honeys from the North-East part of Romania.

MATERIALS AND METHODS

Materials. The honeydew honeys (5 samples, each sample of 500 g) were purchased in September 2015 from the local beekeepers from the Suceava county, Romania. The samples were preserved at 20 °C until they were analysed.

Melissopalynological analysis. The pollen analysis was performed according to the method of Louveaux *et al.* (1970), using a non-acetolytic method. Ten grams of honey were mixed with about 40 ml of distilled water; then, centrifuged at 4500 rpm (3383 × g) for 15 minutes and the supernatant was carefully removed. The residue was re-dissolved again and centrifuged for other 15 minutes. The full sediment was used to prepare the slide. The pollen spectrum of each honey sample was determined by a light microscopy (Motic × 40) by counting at least 800 pollen grains. Quantitative evaluation of each pollen type was determined and expressed as percentage according to Dobreet *al.* 2012.

Physicochemical properties determination. The physicochemical parameters (moisture content, pH, free acidity, electrical conductivity, colour (L*, a*, b*, chroma, hue angle), fructose and glucose content) have been made based on the method described by Bogdanov *et al.* (2002).

Phenolics extraction. The phenolics extraction was realized using the method described by Baltrušaitytė *et al.* (2007) and Escriche *et al.* (2014). Sixty grams of Amberlite XAD-2 resin, pore size 9 nm, and particle size 0.3–1.2 mm were soaked in methanol for 10 minutes, then, the most of methanol was decanted and replaced by distilled water. The mixture was stirred, allowed to stand for 5–10 min and packed into a glass column, 25×2 cm. The honey samples (25 g) were

thoroughly mixed with 250 mL of distilled water and adjusted to pH 2 with concentrated HCl. The solution was slowly filtered through the column packed as previously described. The column was washed with 250 mL of acidified water (pH 2 with HCl) and subsequently rinsed with 300 mL of neutral distilled water to remove all sugars and other polar compounds of honey. The flavonoids and phenolic compounds were eluted from the sorbent with 250 mL of methanol. The methanol extracts were concentrated under vacuum at 40°C in rotary evaporator HS-2005S-N (HAHN VAPOR, China). The residue was dissolved in 5 mL of distilled water and extracted three times with 5 mL of diethyl ether. The dried residue was then re-dissolved in 1 mL of methanol (HPLC grade) and filtered through a membrane filter with a 0.45 µm pore size. Three replicate extractions were performed for each sample.

HPLC analysis of phenolics. The phenolic compounds were separated and quantified using the method described by Coneac *et al.* (2008). A High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan) system equipped with a LC-20 AD liquid chromatograph, SIL-20A auto sampler, CTO-20AC auto sampler and a SPD-M-20A diode array detector was used. The separation was carried out on a Zorbax SP-C18 column, with 150 mm length, 4.6 mm i.d., and 5 µm-diameter particle was used; the phenolics detection was set at 200 nm and 210 nm. The mobile phase was acetonitrile: water ratio 48:52, temperature was of 25°C, with a flow of 0.3 ml/min, the injected sample volume was of 20 µl. The diluted standard solutions of quercetin, apigenin, myricetin, isorhamnetin, kaempferol, caffeic acid, chrysin, galangin, luteolin, *p*-coumaric acid, gallic acid and pinocembrin were analyzed under the same HPLC conditions and furthermore the calibration of the detector response was made. Data collection and subsequent processing were performed using the LC solution software 1.21 version (Shimadzu, Kyoto, Japan). The quantitative results were expressed as mg of compound per 100 g honey.

RESULTS AND DISCUSSION

The honey samples were submitted to the melissopalynological analysis for confirming the authenticity of it. The range of pollen grains number was between 1650 to 2450. The honeydew honey was poor in pollen, recording an average of 2083

Tab. 1. The main pollen types in honeydew honeys samples

Honey type	Samples	Principal pollen type	Secondary pollen types	Other significant pollen types
Honeydew	5	<i>Brassica napus</i> (18.1%)	<i>Quercus</i> (14.3%) <i>Trifolium repens</i> (11.8%) <i>Castanea sativa</i> (8.6%)	<i>Helianthus annuus</i>

Tab.2. Physicochemical parameters of the honeydew honeys

Variable	Minimum	Maximum	Mean	Std. deviation
Moisture content (%)	14.44	17.04	16.13	1.04
pH	4.19	5.16	4.75	0.48
Free acidity(meq acid/kg)	11.80	20.00	15.50	3.49
Electrical conductivity ($\mu\text{S}/\text{cm}$)	923.3	1276.8	1077.3	149.6
Ash (%)	0.45	0.62	0.52	0.07
L*	19.59	21.52	20.41	0.82
a*	4.16	7.68	5.55	1.38
b*	4.15	6.90	5.45	1.06
Chroma	6.53	9.47	7.86	1.26
Hue-angle	0.15	1.15	0.67	0.45
Fructose (%)	35.96	40.98	38.73	2.03
Glucose (%)	32.98	36.54	35.06	1.50

pollen grains, similar to other honeydew honeys reported into the literature (Dobre *et al.* 2013). The principal pollen types presented into the honeydew honeys are shown in Tab. 1. The palynological analysis provides useful information regarding the vegetation where the honey was collected. The presence of *Brassica napus*, *Trifolium repens*, *Castanea sativa*, *Quercus* and *Helianthus annuus* are giving information regarding the time of year of the honey production and it can be considered a spring honey.

In the case of honeydew samples, they can be authenticated using the electrical conductivity parameter (EC 110/2001) If the electrical conductivity is higher than 800 $\mu\text{S}/\text{cm}$, then the honey is a honeydew one (EC 110/2001). The analyzed honeys had electrical conductivity higher than 800 $\mu\text{S}/\text{cm}$ (Tab. 2).

In the Tab. 2 are presented the physicochemical parameters (moisture content, pH, free acidity, electrical conductivity, colour (L*, a*, b*, chroma, hue angle), fructose and glucose content) of the five samples of honeys analysed.

One of the most important parameter for the honey quality is the moisture content. Regarding this parameters, the UE (2001) has established a threshold of 20% in the case of honeys. A higher content of water in honey will promote the fermentation processes (Escriche *et al.*, 2014). In the case of the honeys analysed all the samples respected the regulation established by the UE (EC 110/2001).

Honey contains less than 0.5% of organic acids. They are a group of constituents that contribute to several properties of this food, such as its color, aroma, taste, pH, acidity, and to

a lesser extent, electrical conductivity (Sancho *et al.*, 2013). Small variations in the range of pH in relation to the large swings in the free acid values were attributed to the buffer properties of honey, due to mineral salts such as phosphates, carbonates and other (Bogdanov 2009, Sancho *et al.*, 2013). In the case of the present study, the pH ranged between 4.19 to 5.16 being in agreement with those reported in the case of Spanish honeys (Oroian *et al.*, 2013, Escriche *et al.*, 2014) and Romanian honeys (Oroian 2012).

The honey acidity is influenced in principal by the organic acids (White 1979). Lactones are internal esters of organic acids and do not contribute to honey's active acidity (Bogdanov 2009). Lactones hydrolyze over time, therefore increasing honey free acid. Total acidity is the sum of free acid and lactones. In terms of free acidity the Codex Alimentarius (2001) established a level of 50 meq acid/kg for honeydew honeys respectively. All the samples analysed respected the regulation established by Codex Alimentarius (2001).

Electrical conductivity is a physical property of honey mainly related to the content of mineral salts, and to a lesser extent to the content of organic

acids, proteins, sugars, and polyols (Crane 1990). It was found that the electrical conductivity was directly proportional to ash content and acidity of honey (Sancho *et al.*, 2013). The electrical conductivity ranged between 923.3 to 1276.8 $\mu\text{S}/\text{cm}$, being higher than 800 $\mu\text{S}/\text{cm}$ recommended by the literature in the case of honeydew honeys (EC 110/2001).

Color is an optical property of honey, described as the result of different degrees of absorption of light at different wavelengths by honey compounds (Sancho *et al.*, 2013). The honeydew honeys are dark honeys (L^* values are lower) due to the high mineral content and pigments (Oroian 2012). The lower the L^* values are, the honey is darker. Due to the opacity of the honey the a^* and b^* values are close to 0. The taste of honeydew honeys is stronger and tend to have relatively more maltose, minerals, acids and antioxidant flavonoids (Stanway 2012).

The sugar composition of honeys is influenced by the honey origin. The main components of honey are D-glucose and D-fructose which are originated from the honeydey and from the enzymatic hydrolysis of sucrose and other sugars from the

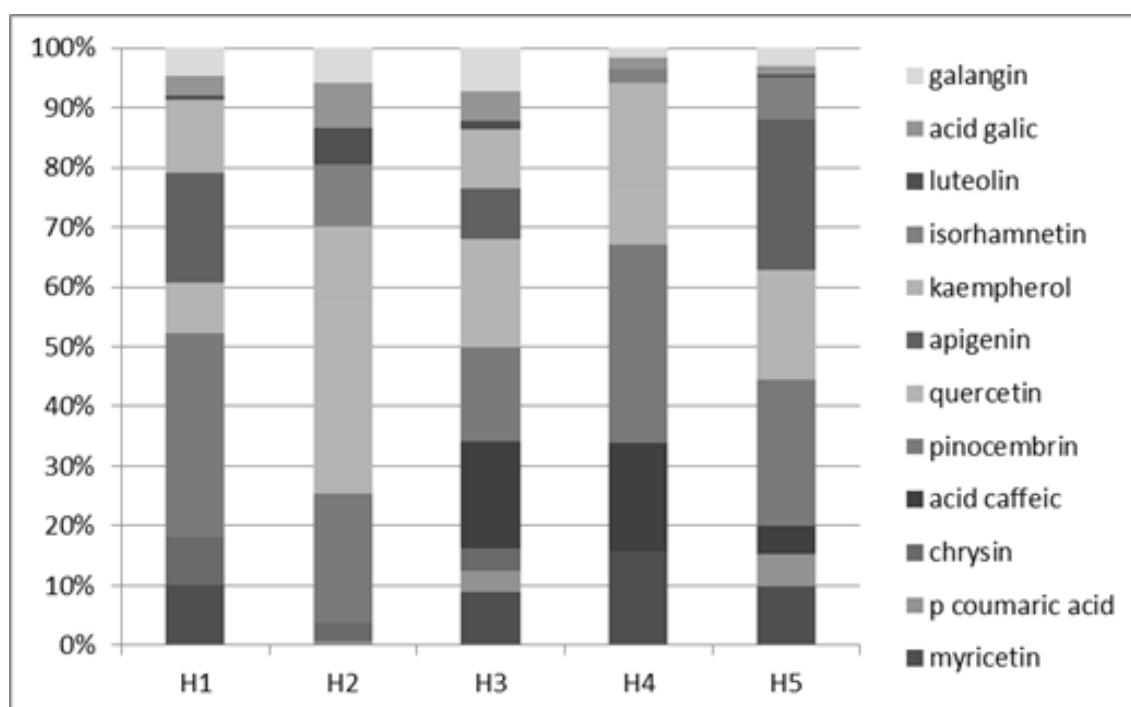


Fig.1.Relative quantities of analysed phenolics in honeydew honey samples

nectar (Tomasik 2003). The concentration of glucose and fructose in honey must be higher than 60 g/100 g honey (EC 110/2001). All the analysed honeys met this requirements.

Polyphenols are another important group of compounds with respect to the appearance and the functional properties of honey. Polyphenols in honey are mainly flavonoids (e.g. quercetin, luteolin, kaempferol, apigenin, chrysin and galangin), phenolic acids, and phenolic acid derivates (Saad & Said 2011). In the case of the analysed honeydew honeys the following polyphenols were identified and quantified: myricetin (0 – 0.37 mg/100 g honey), *p*-coumaric acid (0-4.35 mg/100 g honey), chrysin (0-0.16 mg/100 g honey), caffeic acid (0-1.92 mg/100 g honey), pinocembrin (0.27-4.36 mg/100 g honey), quercetin (0.10 – 2.79 mg/100 g honey), apigenin (0-1.10 mg/100 g honey), kaempferol (0 – 0.60 mg/100 g honey), isorhamnetin (0-0.12 mg/100 g honey), luteolin (0-0.11mg/100 g honey), gallic acid (0.02-0.26 mg/100 g honey) and galangin (0.02-0.49 mg/100 g honey).

The comparison of relative quantities of phenolic acids in different honeydew honey samples demonstrated significant ($P < 0.05$) variations of their quantitative composition (Fig. 1). From the figure 1 it can be observed that the major polyphenols presented into the honeydew honeys are quercetin and pinocembrin.

CONCLUSION

The honey samples analysed respected the regulation of the European Union and Codex Alimentarius in terms of moisture content and glucose and fructose content. The honeydew colour parameters were closed to the origin of the three axes (L^* , a^* and b^*) due to their chemical composition. The phenolic profile of the honeydew samples do not presented one compound that can be considered a chemical marker. From the 12 individual phenolics studie, we observed that quercetin and pinocembrin were in the highest concentration. The honeydew honeys analysed had a high content of phenolics and it can be considered a source of antioxidants.

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