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Comparative analysis of riboflavin and thiamine in raw and commercial honey

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Abstract: The paper describes the determination of riboflavin and thiamine in the best-selling types of 13 raw and 8 commercial monofloral and multifloral honeys originating from Serbia. It was found that there is a difference in average riboflavin and thiamine content between raw and commercial honey, as well as between different honey floral varieties. The results showed that forest, meadow and oregano honey had a significantly higher content of both B vitamins compared to acacia, linden and lavender honey. On the other hand, a very low content of riboflavin and thiamine was found in honeydew honey. Among the commercial products, royal jelly was the richest in thiamine and riboflavin, followed by forest honey. In general, the quality of all of the commercial honey samples regarding these vitamins was lower than that of the raw domestic honeys.

Keywords: honeydew; monofloral honey; multifloral honey; royal jelly; vitamins

Honey is a natural product which has been used in human nutrition and cosmetics since ancient times. Due to the variation of botanical and geographical origin, honey differs in appearance, sensory perception and chemical composition. Apart from carbohydrates, fructose and glucose, and about 25 different oligosaccharides honey contains small amounts of proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds, and polyphenols (Bogdanov et al. 2008). For many years, the content of vitamins in honey has been the subject of interest of scientists and experts studying this natural product (Lüttge 1962; Kalimi & Sohonie 1964). More recent researches have shown that vitamins found in honey are mainly of B complex including thiamine (B_1), riboflavin (B_2), nicotinic acid, pantothenic acid, pyridoxine, biotin and folic acid (Da Silva et al. 2016). Also, low amounts of vitamin C were found in honey (Matei et al. 2004; León-Ruiz et al. 2011). Honey is not a rich source of B vitamins. They are mostly from the pollen grains (Da Silva et al. 2016), and their concentrations in a raw honey suspension are low. From the analytical point of view, the determination of B vitamins in this natural product is not so simple. For this reason, studies on the vitamin content in honey are very scarce, and mostly lack information on the amount of both riboflavin and thiamine. Thus, most analyses were done either for riboflavin or thiamine (Vinas et al. 2004; Ciulu et al. 2011; Tuberoso et al. 2012; Yoshida et al. 2012; Kaygusuz et al. 2016). Of the few papers dealing with this topic, only two papers discuss the content of thiamine in honey and royal jelly (Yoshida et al. 2012; León-Ruiz et al. 2013).

Nowadays, in the human diet, there are two main types of honey: raw and commercial honey. Raw honey is usually produced by small farms or households and is left in a natural state without further processing (Blasa et al. 2006). Raw honey is rich in pollen grains and may cause honey allergy in people who are sensitive to bee venom or pollen. Therefore, commercial honey undergoes filtration (ultrafiltration) and pas-

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teurisation. The differences in the processing of raw and commercial honey may lead to differences in nutrient content and their overall quality. Thus, according to Mohapatra et al. (2011), raw honey has higher antimicrobial and antibacterial properties than commercial honey. Another study showed that raw honey contained more antioxidants than the processed type (Blasa et al. 2006). It is already known that the commercial filtration almost completely removes pollen and causes a reduction in vitamin content (Ciulu et al. 2011), but there are very few studies comparing raw and commercial honey.

The aim of this study was to determine both riboflavin (B_2) and thiamine (B_1) in commercial and homemade raw honey and to examine the correlations between their contents.

MATERIAL AND METHODS

Materials and reagents. Thiamine standard (Sigma-Aldrich, USA) and riboflavin standard (Acros Organic, USA) were of analytical grade. HPLC grade methanol was supplied by J.T.Baker (Netherlands).

Apparatus. The Agilent Technologies 1200 Series apparatus (USA) with PDA (photodiode array) detector and Zorbax Eclipse Plus C18 column (3.0 mm × 150 mm, 3.5 μm) was used for the HPLC analysis. Ultrasound-assisted solvent extraction was performed in an ultrasound bath (VWR International, Belgium).

Honey products. Twenty honey samples and one royal jelly product were collected and analysed. The examined honey was declared by the producers as acacia (*Robinia pseudoacacia* L.), linden (*Tilia* sp.), lavender (*Lavandula* sp.), oregano (*Origanum vulgare* L.), forest, meadow and honeydew honey. Thirteen homemade raw honey samples of different floral origin were obtained by individual beekeepers from different locations in the mountainous area of southeastern Serbia (Suva planina, Stara planina, Svrljiške planine and Sićevo). The samples were harvested during 2016. The botanical origin of the examined raw honey samples was confirmed by the melissopalynological analysis. Eight commercially processed honeys and royal jelly included in this study were purchased from the local market. The type of commercial honey was also confirmed by the melissopalynological analysis. Sensory characteristics of undiluted honey samples were obtained by subjective assessment.

Melissopalynological analysis of honey samples. The melissopalynological analysis described by Louveaux et al. (1978) was used for the classification of honey samples. Different pollen morphology guides (Petersen & Bryant 2011; Shubharani et al. 2013) and websites were used to identify the botanical affinity of the pollen types [Palynological Database (PalDat); the Council for Agricultural Research and Economics (CREA) Pollen Atlas (Available at http://www.pollenatlas.net/index.php)]. To determine the frequency classes, 300 pollen grains were counted from each sample and expressed as percentages. The pollen types from different honey samples were identified, counted, and classified, according to their percentages, as dominant pollen (> 45% of the total grains), accessory pollen (16–45%), important isolated pollen (3–15%), and occasional isolated pollen (< 3%).

Sample preparation and HPLC procedure. One gram of honey was dissolved in 1–2 mL of deionised water (depending on the sample viscosity) and vortexed for 1 min. Ultra-sonication was conducted for 10 min at a frequency of 45 kHz at 20 ± 1 °C. Samples were centrifuged at 200 rpm at 5 °C for 10 min. For the riboflavin analysis, an aliquot of the obtained supernatant was filtered through a cellulose membrane filter before injection into the HPLC column. For the thiamine analysis, 650 μL of the obtained supernatant was first derivatized with 130 μL of 1% potassium ferricyanide in a 15% sodium hydroxide mixture. After vortex mixing for 1 min, the insoluble precipitate was removed by centrifugation. An aliquot of the obtained supernatant was filtered prior to the HPLC analysis.

HPLC analysis. The chromatographic conditions were similar to those in the study of Sunarić et al. (2012) and Lalić et al. (2014). The mobile phase consisted of 30.0% (v/v) methanol and 70.0% (v/v) 0.005 M NH_4Ac (pH 5.0). The fluorescence detector was programmed to the excitation wavelength of 370 nm and the emission wavelength of 435 nm until 5.5 min of the analysis (optimum wavelengths for thiochrome) and 440 nm excitation wavelength and 520 nm emission wavelength after 5.5 min (optimum wavelengths for riboflavin) (Sunarić et al. 2020).

Statistical analysis. All the analyses for each sample were carried out in triplicate and the results were expressed as mean value ± standard deviation (SD). The one-way analysis of variance (ANOVA) was used to test significant differences between mean values. The Games-Howell post-hoc test and Tukey's honestly significant difference (HSD) were used for pairwise comparisons. Experimental data were also analysed using the Spearman rank correlation test. The differences were accepted as significant for $P < 0.05$. The IBM Corp. SPSS 21.0 statistical software was used.

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Table 1. Melissopalynological analysis and sensory characteristics of honey samples

Sample	Type	Honey type	Dominant pollen (> 45% of the total grains)	Accessory pollen (16–45%)	Important isolated pollen (3–15%)	Occasional isolated pollen (< 3%)	Sensory characteristics (colour, consistency)
H1	raw	acacia			*Robinia pseudoacacia* L. (12%)		pale yellow, liquid
H2	raw	acacia		*Robinia pseudoacacia* L. (42%)			pale white, smooth, jelly
H3	commercial	acacia	*Robinia pseudoacacia* L. (55.33%)				yellow, liquid
H4	raw	linden				*Tilia* sp. (1.66%)	pale white, liquid
H5	raw	linden		*Tilia* sp. (43%)			yellow, solid, fine granulated
H6	commercial	linden	*Tilia* sp. (83.33%)				pale yellow, crystalised
H7	raw	meadow		*Trifolium* sp. (25%), *Rubus* sp. (18%)	*Asteraceae* (5%)		dark yellow, crystallied
H8	raw	meadow		*Rubus* sp. (20%)	*Trifolium* sp. (7.66%)	*Asteraceae* (2.33%)	yellow, solid
H9	raw	meadow			*Rubus* sp. (2.6%), *Trifolium* sp. (4.66%)	*Asteraceae* (1%)	amber, solid
H10	raw	meadow	*Rubus* sp. (76%)		*Trifolium* sp. (14.67%)		dark yellow, solid, fine granulated
H11	commercial	meadow	*Trifolium* sp. (60%)	*Asteraceae* (24.70%), *Rubus* sp. (18.67%)			dark yellow, cream
H12	commercial	meadow	–	–	–	–	pale yellow, liquid
H13	commercial	meadow	–	–	–	–	dark yellow, solid
H14	raw	forest		*Fagus* sp. (26.42%)			dark amber, solid
H15	raw	forest		*Quercus* sp. (18.66%)	*Fagus* sp. (5.33%)		amber, solid, fine granulated
H16	commercial	forest	*Asteraceae* (75%)		*Rubus* sp. (3.3%)		pale amber, crystalised
H17	commercial	forest	*Fagus* sp. (59%)	*Rubus* sp. (23.33%)			amber, solid
H18	raw	honeydew	–	–	–	–	dark amber, solid
H19	raw	lavender		*Lavandula* sp. (39%)			dark amber, solid
H20	raw	oregano	*Origanum vulgare* L. (70.66%)				amber, solid, fine granulated

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RESULTS AND DISCUSSION

The results from the melissopalynological analysis and sensory characteristics for the twenty honey samples are summarized in Table 1. As can be seen, pollen grains were not found in two of the tested commercial meadow honey samples or in raw honeydew honey. The chromatograms of B_1 and B_2 vitamins in raw forest honey are shown in Figures 1 and 2. The results of the average vitamin content in raw and commercial honey classified according to floral varieties, as well as statistical data, are presented in Table 2. Data obtained for B_2 content did not meet the homogeneity of variances assumption, and we used the Games-Howell post-hoc test for their analysis. On the other hand, data for B_1 content met the assumption of homogeneity of variances, and we used the HSD post-hoc test. As can be seen, large inter-individual variations were found in the examined raw honey samples. Most of the forest, meadow and oregano honey had a significantly higher content of both B vitamins compared to acacia, linden and lavender honey. Linden and acacia honeys are the most popular and most selling types of honey in the world, primarily because of their pleasant taste and reduced presence of pollen which may cause an allergic reaction in sensitive people. a low quantity of pollen consequently causes low vitamin B levels. On the other hand, very low content of riboflavin and thiamine in honeydew was surprising as it was in contrast with reports by Tuberoso et al. (2012). There is a difference in the origin of blossom honey and honeydew honey. Honeydew is a sweet secretion originated from a passage of juice through the insect's intestine (Delabie 2001). Therefore, a considerable difference in the chemical composition of these two natural products can be expected.

Among the commercial products, royal jelly was the richest in B_1 and B_2 vitamins, followed by forest honey. This confirms the centuries-old knowledge of the nutritional value of royal jelly (Pasupuleti et al. 2017). Acacia and meadow commercial honey had the lowest B_1 and B_2 levels. In one of the commercial samples (H12) neither thiamine nor riboflavin was detected. Since no pollen content was found in this sample (Table 1), it can be concluded that it was most likely adulterated honey or honey product of very low quality.

Other authors reported contents of B_1 and B_2 vitamins ranging between 0.1 mg kg^{-1} and 6.1 mg kg^{-1} in different honey types (Vinas et al. 2004; Ciulu et al.

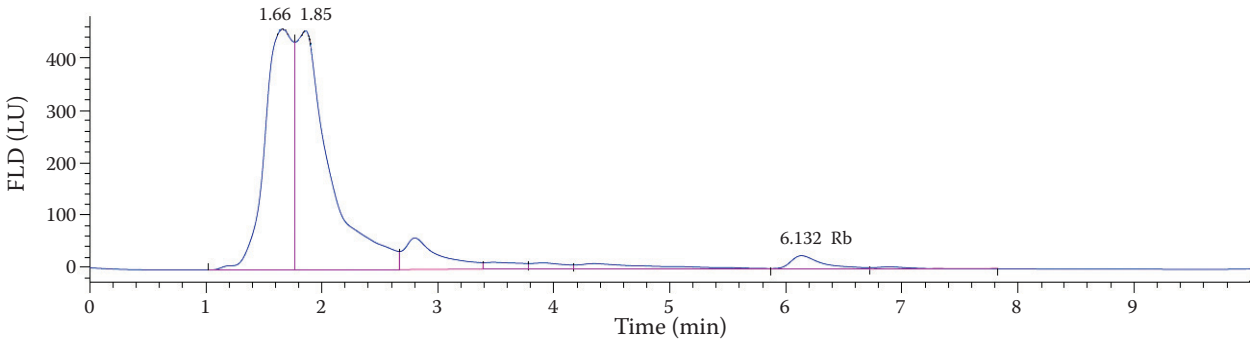

Figure 1. HPLC analysis of riboflavin (Rb) in raw forest honey
FLD – fluorescence detection; LU – luminescence units

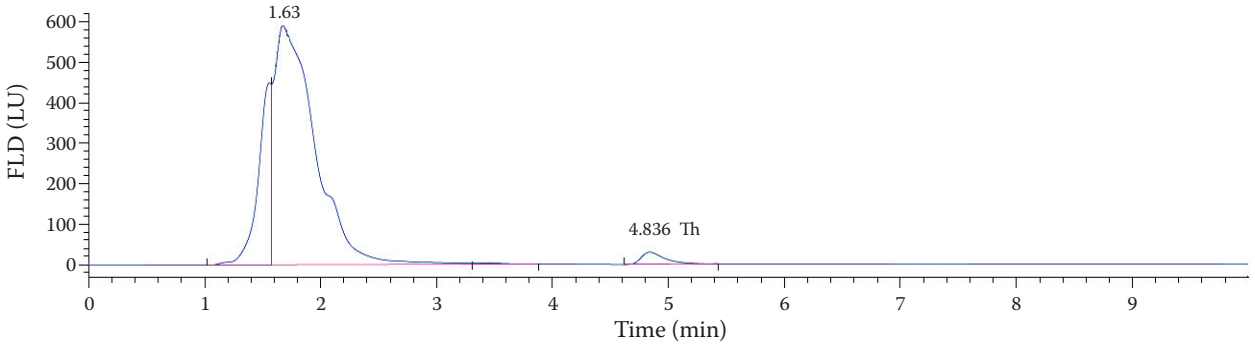

Figure 2. HPLC analysis of thiamine (Th) in raw forest honey
FLD – fluorescence detection; LU – luminescence units

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Table 2. Riboflavin and thiamine in raw and commercial honey (n = 3)

Sample	Honey type	Riboflavin found (mg kg^{-1} ± SD)	Thiamine found (mg kg^{-1} ± SD)
H1	acacia	ND	0.0045 ± 0.0005^{h}
H2	acacia	ND	0.01 ± 0.0008^{f,g,h}
H3 (commercial)	acacia	ND	0.0084 ± 0.0008^{g,h}
H4	linden	0.002 ± 0.0003^{i}	0.013 ± 0.001^{e,h}
H5	linden	0.0045 ± 0.0005^{g}	0.023 ± 0.002^{e}
H6 (commercial)	linden	0.02 ± 0.002^{c,f,h}	0.0077 ± 0.0006^{g,h}
H7	meadow	0.40 ± 0.01^{a}	0.08 ± 0.005^{b,c}
H8	meadow	0.30 ± 0.008^{b}	0.04 ± 0.003^{d}
H9	meadow	0.003 ± 0.0005^{g,i}	0.019 ± 0.0015^{e,f}
H10	meadow	0.20 ± 0.03^{b,c,d,e,f,h}	0.08 ± 0.005^{b,c}
H11 (commercial)	meadow	0.002 ± 0.0003^{i}	0.022 ± 0.0015^{e}
H12 (commercial)	meadow	ND	ND
H13 (commercial)	meadow	0.0016 ± 0.0002^{i}	0.0053 ± 0.0005^{h}
H14	forest	0.34 ± 0.01^{b}	0.075 ± 0.005^{c}
H15	forest	0.017 ± 0.001^{c,f,h}	0.12 ± 0.008^{a}
H16 (commercial)	forest	0.03 ± 0.004^{c,e,f,h}	0.017 ± 0.001^{e,g}
H17 (commercial)	forest	0.09 ± 0.004^{c,d}	0.09 ± 0.006^{b}
H18	honeydew	ND	0.008 ± 0.001^{g,h}
H19	lavender	ND	0.017 ± 0.002^{e,g}
H20	oregano	0.045 ± 0.004^{c,e,f}	0.034 ± 0.002^{d}
H21 (commercial)	royal jelly	0.22 ± 0.01	0.17 ± 0.015

Data are means ± standard deviation (SD); means within a column sharing the same letter are not significantly different (P < 0.05); ND – not detected

2011; Tuberoso et al. 2012; Yoshida et al. 2012; León-Ruiz et al. 2013; Kaygusuz et al. 2016). However, such high concentrations of riboflavin were found only in some of the samples. As stated above, due to the high limit of detection of the analytical methods used in most of these papers, riboflavin could not be determined in many samples in which it is present at very low concentrations. Thus, the authors reported that riboflavin content in many honey samples was below the limit of detection (< 0.2–0.25 mg kg^{-1}). In most of these papers, thiamine was not determined. Only two papers had the data about thiamine content in honey (León-Ruiz et al. 2013) or royal jelly (Yoshida et al. 2012), and they found higher thiamine concentrations than in our equivalent samples.

Table 3. Mean values of riboflavin and thiamine

	Raw honeys (n = 14)	Commercial honeys (n = 6)
Riboflavin found (mg kg^{-1} ± SD)	0.094 ± 0.150	0.024 ± 0.035*
Thiamine found (mg kg^{-1} ± SD)	0.038 ± 0.037	0.025 ± 0.032^{ns}

SD – standard deviation; *statistically significant at P < 0.05; ^{ns}not statistically significant (P > 0.05)

The correlations between vitamin contents were calculated. A strong correlation (P < 0.01) between B_2 and B_1 content (r_s = 0.772) was found, where r_s is the Spearman coefficient.

The mean values of riboflavin and thiamine contents were further compared by using the two-sample test for variances. The results are shown in Table 3. The average B_2 level in raw honey was significantly higher than that in commercial honey. Average B_1 content in raw honey was also higher than in commercial samples, but the difference was not significant.

Considering the RDI (Reference Daily Intake) values for B_1 vitamin (1.2 mg/day for adults) and for B_2 vitamin (1.3 mg/day for adults) (Bogdanov 2017), it can be concluded that a daily dose of 30 g of the examined

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domestic honey (Table 3) covers 0.1% of RDI for B_1 vitamin and 0.22% of RDI for B_2 vitamin.

CONCLUSION

Botanical origin of honey has a great influence on the vitamin content. In general, multifloral honeys had a higher content of B_1 and B_2 vitamins compared to the monofloral ones. Further, all of the raw honey samples contained thiamine, while riboflavin was not present in all samples. Another important finding of this study is that the average riboflavin and thiamine levels in domestic raw honey were higher than those in highly processed commercial honey. This clearly indicates that honey processing influences nutrient content.

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