Specific Rotation and Carbohydrate Profile of Croatian Unifloral Honeys

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Abstract

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Specific rotation and carbohydrate profile of Croatian black locust (*Robinia pseudoacacia* L.), sage (*Salvia officinalis* L.) and chestnut (*Castanea sativa* Mill.) honeys were determined. Fructose, glucose, sucrose, maltose (with cellobiose and trehalose), melezitose (with erlose), raffinose, and xylose were evaluated and quantified by HPLC, while specific rotation was determined by using a polarimeter. The differences in the carbohydrate profile, especially in disaccharide and trisaccharide contents, reflected different specific rotation values of the honey types selected. Weak positive correlations between specific rotation and sucrose, melezitose with erlose, and raffinose contents were found.

Keywords: specific rotation; carbohydrate profile; unifloral honey

Honey is a sweet substance produced by bees from nectar or honeydew. More than 95% of the honey solids are carbohydrates, with monosaccharides (fructose and glucose) predominating. The presence of monosaccharides (fructose, glucose), disaccharides (e.g. maltose, sucrose, isomaltose, turanose, kojibiose), and oligosaccharides (e.g. erlose, melezitose, raffinose) in the most abundantly produced and, on the other hand, also in very specific honeys is documented (Mateo & Bosch-Reig 1997; Da Costa Leite *et al*. 2000; Cote *et al*. 2003; De La Fuente *et al*. 2006; Ouchemoukh *et al*. 2010).

Honey carbohydrates have the ability to rotate linearly polarised light. The direction and degree of rotation are specific for each carbohydrate, the overall optical rotation of honey depending on the contents of different carbohydrates present in it. Fructose, which is the major sugar in nectar honey, has a high negative optical rotation; hence, all nectar honeys have the negative specific rotation. Glucose, disaccharides, and oligosaccharides, present in larger amounts in honeydew honey than in nectar honeys, have positive specific rotations, which results in positive values of honeydew honey specific rotation. The differences in honey specific rotation resulting from different carbohydrate profile are primarily utilised for the differentiation between nectar and honeydew honeys but can also contribute to nectar honey characterisation (Persano Oddo *et al*. 1995; Bogdanov *et al*. 2000).

The main objectives of this work were to determine the specific rotation of the selected Croatian unifloral honeys, black locust (*Robinia pseudoacacia* L.), sage (*Salvia officinalis* L.), and chestnut (*Castanea sativa* Mill.) honeys as well as their carbohydrate profiles. The carbohydrate profiles of the Croatian black locust and chestnut honeys, to the best authors' knowledge, have not been

reported yet and those of sage honey are pioneer results. In addition, the contribution of the carbohydrates determined to overall specific rotation of honey will be evaluated.

MATERIAL AND METHODS

The honey samples were obtained from beekeepers from different regions of Croatia. The samples were collected during several production seasons and the analysis was performed within 6 months after the extraction. Although the beekeepers themselves declared honey as unifloral, all samples were subjected to pollen analysis (Louveaux *et al.* 1978; Ministarstvo Poljoprivrede i Šumarstva 2000), and the honey types were confirmed according to the Croatian regulations (Ministarstvo Poljoprivrede, Ribarstva i Ruralnog Razvoja 2009a). From 75 samples analysed, 41 were sage honey (*S. officinalis* L.) and 17 samples of each black locust (*R. pseudoacacia* L.) and chestnut (*C. sativa* Mill.) honeys.

The carbohydrate content and specific rotation were determined according to the methods prescribed by the International Honey Commission (Bogdanov 2009).

Carbohydrate content was determined by chromatographic (HPLC) method with RI detection. The separation of carbohydrates was achieved on an analytical column containing amine-modified silica gel using the mixture of acetonitrile and water (70:30, v/v) at 1 ml/min as the mobile phase. The separated carbohydrates were identified on the basis of their retention times, and quantification was performed by external calibration. Carbohydrate standards of anhydrous glucose, fructose, sucrose, and melezitose hydrate were purchased from Sigma (St. Louis, USA), xylose and maltose monohydrate from Kemika (Zagreb, Croatia), and raffinose pentahydrate from Fluka (Darmstadt, Germany).

Specific rotation ($[\alpha]_D^{20}$) or optical activity was determined in clear aqueous solution of 1 g/ml of honey, at 20°C by means of the polarimeter.

The average value and standard deviation of each evaluated parameter were calculated and the range values (minimum and maximum) were obtained. The relations between the parameters were evaluated using the Pearson correlation coefficient. For the data analysis, Microsoft Excel 2007 and STATISTICA 8 (Statsoft Inc.) were used.

RESULTS AND DISCUSSION

The results obtained for specific rotation and carbohydrate content are summarised in Table 1. In all honey samples, 7 carbohydrates were evaluated and quantified, i.e. monosaccharides (fructose, glucose, and xylose), disaccharides (sucrose and maltose), and trisaccharides (melezitose and raffinose). The maltose content accounts for the contents of maltose, cellobiose, and trehalose (be-

Table 1. Specific rotation $[\alpha]_D^{20}$ and carbohydrate content (%) in analysed honey types

Parameter	Sage honey (n = 41)	Chestnut honey (n = 17)	Black locust honey (n = 17)
Specific rotation $[\alpha]_D^{20}$	–21–(–11)/–15 ± 3	–25–(–16)/–21 ± 3	–16–(–9)/–13 ± 2
Xylose (%)	0–0.4/0.1 ± 0.1	0.1–0.4/0.2 ± 0.1	0–0.7/0.2 ± 0.2
Fructose, F (%)	38.2–49.9/42.0 ± 3.3	38.7–50.2/42.0 ± 2.8	39.7–49.1/43.0 ± 2.0
Glucose, G (%)	25.7–41.4/32.9 ± 4.1	22.1–31.5/27.4 ± 2.4	24.7–32.7/27.6 ± 2.1
Sucrose (%)	0–3.0/1.8 ± 0.6	1.3–3.4/2.2 ± 0.5	1.6–4.3/2.8 ± 0.7
Maltose (%)	0–5.2/1.9 ± 1.3	0–5.8/2.4 ± 1.1	0–5.4/3.0 ± 1.8
Erlose + Melezitose (%)	0–2.7/0.8 ± 1.0	0–0.8/0.2 ± 0.3	0–4.8/1.7 ± 1.8
Raffinose (%)	0–0.4/0.1 ± 0.1	0	0–0.3/0.1 ± 0.1
F/G	1.0–1.9/1.3 ± 0.3	1.3–1.8/1.5 ± 0.1	1.3–1.9/1.6 ± 0.2
F+G (%)	67.2–81.7/74.8 ± 3.3	62.5–80.4/69.5 ± 4.2	67.7–75.4/70.5 ± 2.3
Total carbohydrates (%)	73.9–84.8/79.4 ± 2.4	69.4–84.1/74.4 ± 3.7	72.0–83.6/78.2 ± 3.9

Data are presented as (min – max/$\bar{x}$ ± SD)

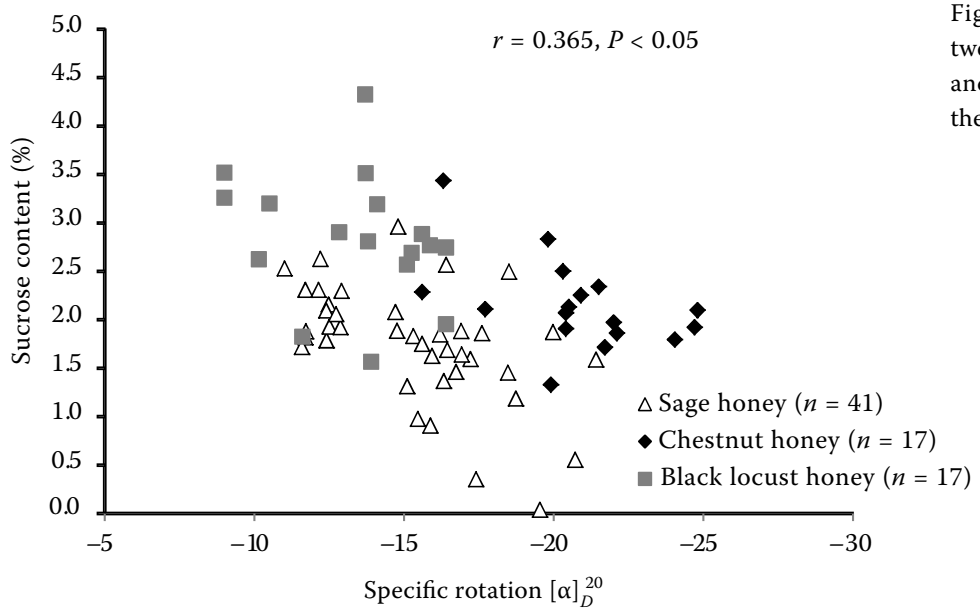

Figure 1. Correlation between specific rotation $[\alpha]_D^{20}$ and sucrose content (%) in the analysed honey types

cause of the peaks overlapping) while melezitose peak involves also erlose.

Fructose contents in all three honey types were similar while sage honey had a higher glucose content than those of chestnut and black locust and, consequently, had a lower fructose/glucose (F/G) ratio and a higher fructose + glucose (F+G) content (Table 1). The results obtained are similar to the literature data (Persano Oddo *et al.* 1995; Cote *et al.* 2003; Persano Oddo & Piro 2004; Kenjerić *et al.* 2006). Due to the high F/G ratio, black locust and chestnut honeys remain liquid for a long time, while sage honey can be characterised by a moderate crystallisation rate. F+G content is in compliance with the national (Ministarstvo Poljoprivrede, Ribarstva i Ruralnog Razvoja 2009b) and international (Council of the European Union 2002) demands for F+G content of nectar honeys. Xylose content was negligible in all honey types. Significant differences can be noticed between the analysed honey types in disaccharide and trisaccharide contents. Sage honey had the lowest content of sucrose (mean 1.8%), maltose with cellobiose, and trehalose (mean 1.9%) while chestnut honey had the lowest content of melezitose with erlose (mean 0.2%) and no raffinose. Cote *et al.* (2003) characterised chestnut honey by a low trisaccharide content, opposite to black locust honey that is characterised by high di- and trisaccharide contents which is in compliance with the results obtained for our chestnut and black locust honeys. Ouchemoukh *et al.* (2010) reported

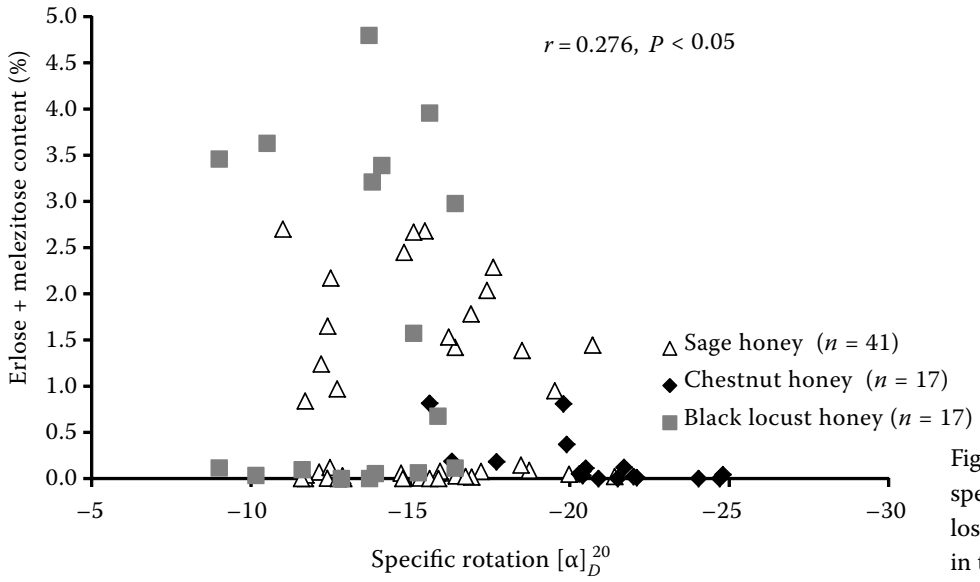

Figure 2. Correlation between specific rotation $[\alpha]_D^{20}$ and erlose + melezitose content (%) in the analysed honey types

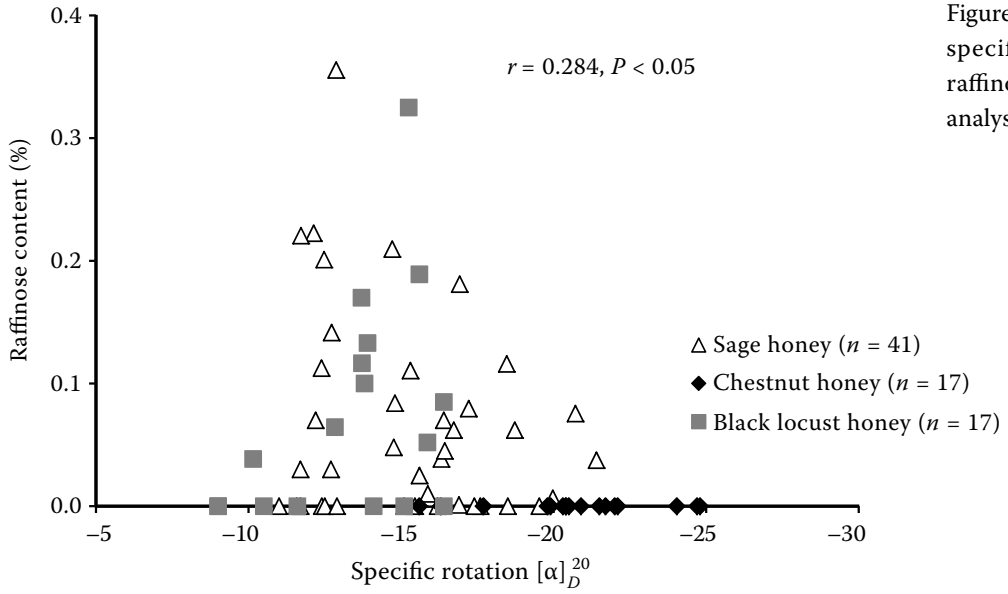

Figure 3. Correlation between specific rotation $[\alpha]_D^{20}$ and raffinose content (%) in the analysed honey types

that honey types cannot be distinguished by their sugar contents, however, Mateo and Bosch-Reig (1996) reported 77.9% successful classification of unifloral honeys on the basis of selected sugars by discriminate analysis. Da Costa Leite *et al.* (2000) reported that the oligosaccharide profile could be useful for the identification of the production region and authenticity.

Specific rotation of honey is the result of carbohydrates ability to rotate linear polarised light. Negative specific rotation of nectar honeys results from the predominance of fructose which has a high negative specific rotation ($[\alpha]_D^{20} = -92.3°$), while honeydew honeys have positive values due to the lower content of fructose and higher contents of di- and oligosaccharides that have positive specific rotation (e.g. maltose $[\alpha]_D^{20} = +137.0°$, melezitose $[\alpha]_D^{20} = +88.2°$, erlose $[\alpha]_D^{20} = +121.8°$, raffinose $[\alpha]_D^{20} = +104.1°$). In addition, specific rotation can be a useful parameter for unifloral honeys differentiation even though a notable overlapping occurs with different honey types (Bogdanov *et al.* 2004; Bogdanov 2009). All honey samples analysed had negative values of specific rotation (Table 1). The highest negative value was revealed by chestnut honey, with mean value (–21) which is in compliance with the data obtained by Persano Oddo *et al.* (1995) and Persano Oddo and Piro (2004) for Italian and European chestnut honeys, respectively. Šarić *et al.* (2008) found considerably lower negative values for specific rotation of chestnut honey as well as for sage and black locust honeys. The results for sage honey specific rotation (–21° to –11°) were in agreement with those reported in our previous paper (Kenjerić *et al.* 2006). The lowest negative results were obtained for black locust honey, similarly to the results reported by Krpan *et al.* (2009) for Croatian black locust honey, and were slightly lower than the results reported by Přidal and Vorlová (2002) for Czech and Persano Oddo and Piro (2004) for European black locust honeys.

Although chestnut and black locust honeys had similar fructose and glucose contents and, consequently, F/G ratios, the differences in specific rotation of those honey types were considerable. Black locust honey contained more sucrose, maltose, erlose + melezitose and raffinose than chestnut honey which contributed to the higher values of black locust specific rotation. The correlations between specific rotation and each determined carbohydrate content were calculated and very weak positive correlations were found only between the specific rotation and content of sucrose ($r = 0.365$, $P < 0.05$), melezitose with erlose ($r = 0.276$, $P < 0.05$), and raffinose ($r = 0.284$, $P < 0.05$) with all samples (Figures 1–3).

CONCLUSION

Black locust and chestnut honeys had similar fructose and glucose contents, but the higher disaccharide and trisaccharide contents in black locust honey were expressed in higher specific rotation values. The higher glucose content as well as lower sucrose and maltose with celobiose and trehalose contents with respect to chestnut

and black locust honeys were reflected in the sage honey specific rotation value. The results obtained demonstrated the relationship between the carbohydrate profile and specific rotation of the determined honey types.

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