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Food Chemistry 102 (2007) 683–690
Food Chemistry
www.elsevier.com/locate/foodchem

Flavonoid profile of *Robinia* honeys produced in Croatia

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Received 23 February 2006; received in revised form 12 May 2006; accepted 29 May 2006

Abstract

In this work specific pollen content, selected physicochemical parameters and flavonoid profile of 40 Croatian *Robinia* honeys from two production seasons were analysed. Results showed good compliance with national and international regulatory requirements, as well as with values typical for *Robinia* monofloral honey. All analysed samples showed same, typical flavonoid profile. Flavonoid content was different for two seasons, but rates of individual compounds remained unchanged. Higher concentrations of flavonoids were found in samples produced during dry season with high temperatures.

Keywords: Honey; Croatia; Quality control; Flavonoids; Characterisation

1. Introduction

Considering the number of the possible floral sources it is understandable that no honey is completely the same as another one. Though this is a problematic issue from the aspect of market requirements, at the same time this variability allows to the consumer the possibility of choosing the type of honey which has, according to its own preferences, the best characteristics. Scientifically, this variability could be a result of different factors, but in most cases it is in connection with the floral origin of honey. Some unifloral honeys are regarded as a more valuable class of honey, and botanical designations are widely employed on the European as well as Croatian market, often achieving higher prices than honey blends. Of course, these denominations must be verifiable, in order to protect consumer and to preserve the reputation of the denomination (Persano Oddo & Bogdanov, 2004). Namely, limited availability and the increased price of some honey types have provided major stimulant for falsification not only in respect of honey production, but also in respect of the description of botanical and geographical origin. Since the melissopalinological analysis is rather subjective method the study of phytochemical constituents of honeys, as markers for their floral origin, has been widely researched during the past decade (Martos et al., 2000).

Different compounds have been researched and with more or less efficacy related to the floral origin (Ampuero, Bogdanov, & Bosset, 2004; Benedetti, Mannino, Sabatini, & Marcazzan, 2004; Devillers, Morlot, Pham-Delègue, & Doré, 2004; Hermosín, Chicón, & Cabezudo, 2003; Iglesias, De Lorenzo, Del Carmen Polo, Martín-Álvarez, & Pueyo, 2004; Mateo & Bosch-Reig, 1997; Popek, 2002; Prodolliet & Hischenhuber, 1998).

Flavonoid patterns and phenolic acids were studied for more types of honey (Ferreres, García-Viguera, Tomás-Lorente, & Tomás-Barberán, 1993; Gil, Ferreres, Ortiz, Subra, & Tomás-Barberán, 1995; Martos, Ferreres, & Tomás-Barberán, 2000; Tomás-Barberán, Martos, Ferreres, Radovic, & Anklam, 2001), always with the aim of finding the specific compounds which could be used as a floral marker. The aim of the present work was to analyse the flavonoid content of Croatian *Robinia* honeys and to

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doi:10.1016/j.foodchem.2006.05.055

compare them with those found in *Robinia* honeys from other countries. Furthermore, since the compounds which are about to be used as floral markers should be unaffected by the ecological factors the impact of climatic conditions on flavonoid content and composition was studied.

2. Materials and methods

2.1. Honey samples

Forty samples of *Robinia* honey from two production seasons (20 samples produced 2002 and 20 samples produced 2003) were provided by the beekeepers from different parts of Republic Croatia.

The characterisation of the samples as *Robinia* was achieved by the combination of physicochemical attributes and pollen analysis in compliance with Croatian Regulations (Ministry of Agriculture & Forestry, 2000) and Harmonised methods of the European Commission (Bogdanov, Martin, & Lüllmann, 1997). Afterwards, samples were stored till the flavonoid analysis. Since flavonoids are relatively stable compounds, resistant to heat, oxygen and moderate degrees of acidity honey samples were prior to analysis stored in dark place but at room temperature (Peterson & Dwyer, 1998).

2.2. Pollen analysis

Though beekeepers themselves declared honey as monofloral, all the samples were subjected to pollen analysis using method of Louveaux, Maurizio, and Vorwohl (1978) with the aim of confirming honey type according to the Croatian regulations which prescribes minimum of 20% of *Robinia pseudoacacia* pollen in indissoluble matter if honey is about to be declared as *Robinia* honey (Ministry of Agriculture & Forestry, 2000).

2.3. Physicochemical analysis

Physicochemical parameters were determined according the methods prescribed by the Croatian Regulations (Ministry of Agriculture & Forestry, 2000) and European Honey Regulative (Bogdanov et al., 1997). Moisture content was determined using refractometric method, free acidity by titration of honey sample solution with 0.1 M sodium hydroxide to pH 8.30, and electrical conductivity was measured at 20.0 °C in a 20% (w/v) solution of honey (dry matter basis) in a water with conductivity <1 μS/cm.

2.4. Flavonoids isolation

Flavonoids were determined according to the previously developed method (Ferreres et al., 1994). Honey sample (*ca.* 50 g) was diluted with five parts of acidified water (pH adjusted on 2–3 with HCl). Solution was than passed through a glass column (25×2 cm) filled with Amberlite XAD-2 resins (pore size 9 nm, particle size 0.3–1.2 mm, Supelco, Bellefonte). During this passing the various phenolic compounds remained in the column, while sugars as well as other polar compounds were eluted with the aqueous solvent. Further, the column was washed with 100 mL of acidified water, and 300 ml of distilled water. The whole phenolic fraction was eluted with *ca.* 300 ml of methanol and taken to dryness under the reduced pressure. The dry residue was redissolved in 5 mL of distilled water and partitioned with ethyl ether (3×5 mL). The ether extracts were combined and ether removed under the reduced pressure. At the end of the extraction procedure, dry residue containing flavonoid fraction was redissolved in 0.5 mL of methanol and analysed by HPLC.

2.5. HPLC analysis of honey flavonoids

For this purpose liquid chromatographic system consisting of Varian ProStar 230 Solvent Delivery Module, Varian ProStar 500 Column Valve Module, Varian Pro Star 310 UV/Vis Detector and Varian ProStar 330 Photodiode Array Detector coupled to a computer with the ProStar 5.5 Star Chromatography Workstation and PolyView 2000 Ver. 6.0 Software was used. LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany, 12.5×0.4 cm ID, 5 μm particle size) was used for separation of sample flavonoid components. The mobile phase consisted of a mixture of water and formic acid (95:5) (solvent A) and methanol (solvent B) at a flow rate of 1 mL/min. To achieve better separation gradient elution was used starting with 30% of methanol which remained isocratic for the first 15 min, and than followed by gradient to obtain 40% of methanol at 20 min, 45% of methanol at 30 min, 60% of methanol at 50 min, 80% of methanol at 52 min, and which than again become isocratic until the end of analysis in the 60 min. Chromatograms were recorded at two wavelengths (340 and 290 nm). The injection volume was 10 μL.

The flavonoid identification was achieved through comparison of chromatographic data (retention times and UV spectra) with authentic markers, while quantification was performed through external calibration data with the same compounds.

Authentic markers available at the market were used for chromatographic comparison of data. Quercetin (3,3′,4′,5,7-pentahydroxyflavone), luteolin (3′,4′,5,7-tetrahydroxyflavone) and myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone) were supplied by Sigma, while chrysin (5,7-dihydroxyflavone), apigenin (4′,5,7-trihydroxyflavone), kaempferol (3,4′,5,7-tetrahydroxyflavone) and galangin (3,5,7-trihydroxyflavone) were by Fluka (Buchs/Schweiz, Switzerland). *p*-Coumaric acid (*trans*-4-hydroxycinnamic acid), ellagic acid (4,4′,5,5′,6,6′-hexahydroxydiphenic acid dilactone) and caffeic acid (3,4-dihydroxycinnamic acid) were also supplied by Fluka. Formic acid (Fluka) and methanol (Merck) were HPLC grade, while other chemicals were of analytical grade.

2.6. Data analysis

Statistical calculations were performed using computer programs Statistica 7.0 (Statsoft Inc.) and Microsoft Excel 2000 (Microsoft Corp.). Association between variables was evaluated using the Pearson correlation coefficient. Comparison of the flavonoid content between two seasons of production (2002 and 2003) was performed using ANOVA.

3. Results and discussion

The results of physicochemical and pollen analysis, presented in Table 1, show that all samples comply with the prescribed minimum of 20% *R. pseudoacacia* pollen grains (Ministry of Agriculture & Forestry, 2000). Average share of *R. pseudoacacia* pollen grains was statistically lower ($p < 0.05$) in samples produced in 2002 (40%, range 20–57%), than in samples produced in 2003 (average 48%, range 23–69%). Considering the fact that *Robinia* pollen is under-represented both groups have relatively high percentages of *R. pseudoacacia* pollen grains. Mean value of *Robinia* pollen grains found in 288 samples encompassed in unifloral honey descriptive sheets (Persano Oddo & Piro, 2004) was lower (28.1%), but for the purpose of these sheets samples with less than 20% of *Robinia* pollen grains were also take into consideration.

Lower mean value of moisture content was found in samples from 2002 (15.6% in contrast to 16.0% from 2003) though range of values was practically the same in both years (14.0–19.2 in 2002, and 14.1–19.0% in 2003). The moisture content has a minor importance for the characterisation of unifloral honeys, but it is very important quality parameter for shelf life (Bogdanov, Ruoff, & Persano Oddo, 2004). Relatively low moisture content in both groups is a positive characteristic while honeys containing less water are not likely to get spoiled by the fermentation.

Free acidity and electrical conductivity values were slightly higher in 2002 (10.6 mmol/kg and 0.130 mS/cm respectively) than in 2003 samples (10.1 mmol/kg and 0.118 mS/cm respectively). The differences between samples from two production seasons were not statistically significant ($p > 0.05$) for any of the three mentioned parameters. The results for all three mentioned parameters were in compliance with the European Community Directive (The Council of the European Union, 2002), and in comparison with *Robinia* honeys reported in descriptive sheets, where mean values are 17.1%, 11.2 meq/kg and 0.16 mS/cm, for moisture content, free acidity and electrical conductivity respectively, results are slightly lower (Persano Oddo & Piro, 2004).

The RP-HPLC analysis of isolated *Robinia* honey fraction showed that all the samples have similar flavonoid profile as presented in Figs. 1 and 2. Quercetin, luteolin, kaempferol, apigenin chrysin and galangin were identified at 340 nm, while presence of phenolic acids was confirmed at 290 nm. Flavonoid myricetin was not found in any of analysed samples, but this was not surprising since it was not found in any of previously reported acacia honey samples (Tomás-Barberán et al., 2001), and it was even reported as a floral marker of heather honey (Soler, Gil, Garcia-Viguera, & Tomás-Barberán, 1995). Results of quantitative flavonoid analysis are presented as follows

Table 1
Selected physicochemical parameters and specific pollen content of *Robinia* honey samples produced in 2002 and 2003

Sample code		Moisture content (%)		Free acidity (mmol/kg)		Electrical conductivity (mS/cm)		Specific pollen share (%)	
2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
M-02/02	M-03/03	15.4	17.2	13.6	10.2	0.132	0.129	56	39
M-06/02	M-06/03	15.5	17.6	12.5	7.2	0.123	0.101	41	50
M-10/02	M-09/03	14.0	15.7	15.1	13.7	0.215	0.129	29	40
M-15/02	M-10/03	15.0	16.3	8.4	8.0	0.113	0.106	57	51
M-17/02	M-15/03	14.9	14.1	10.9	9.9	0.271	0.118	39	48
M-19/02	M-20/03	15.3	15.4	11.9	9.7	0.218	0.114	36	64
M-25/02	M-25/03	15.4	14.5	9.1	8.6	0.092	0.107	56	45
M-26/02	M-29/03	19.2	15.9	7.7	9.6	0.104	0.106	40	39
M-29/02	M-30/03	14.2	15.7	8.9	14.5	0.115	0.146	50	23
M-32/02	M-32/03	15.6	17.3	9.1	15.6	0.117	0.100	37	54
M-34/02	M-59/03	15.5	19.0	13.3	9.2	0.115	0.111	34	47
M-36/02	M-69/03	15.8	16.6	10.8	12.5	0.109	0.135	34	35
M-39/02	M-79/03	17.1	15.4	7.9	8.6	0.101	0.113	32	68
M-40/02	M-82/03	16.9	17.6	7.2	9.7	0.097	0.124	54	53
M-52/02	M-85/03	14.9	15.4	7.9	8.3	0.095	0.103	43	49
M-62/02	M-88/03	15.4	15.9	12.0	7.5	0.103	0.105	26	69
M-74/02	M-92/03	15.6	14.4	11.7	9.3	0.111	0.121	34	50
M-86/02	M-100/03	16.7	15.2	10.7	9.9	0.139	0.137	45	45
M-89/02	M-103/03	14.8	15.2	15.4	9.2	0.131	0.123	30	42
M-93/02	M-104/03	15.5	15.7	6.9	11.1	0.098	0.124	20	58
Mean		15.6	16.0	10.6	10.1	0.130	0.118	40	48
Range/Min.–Max.		14.0–19.2	14.1–19.0	6.9–15.4	7.2–15.6	0.092–0.271	0.100–0.146	20–57	23–69

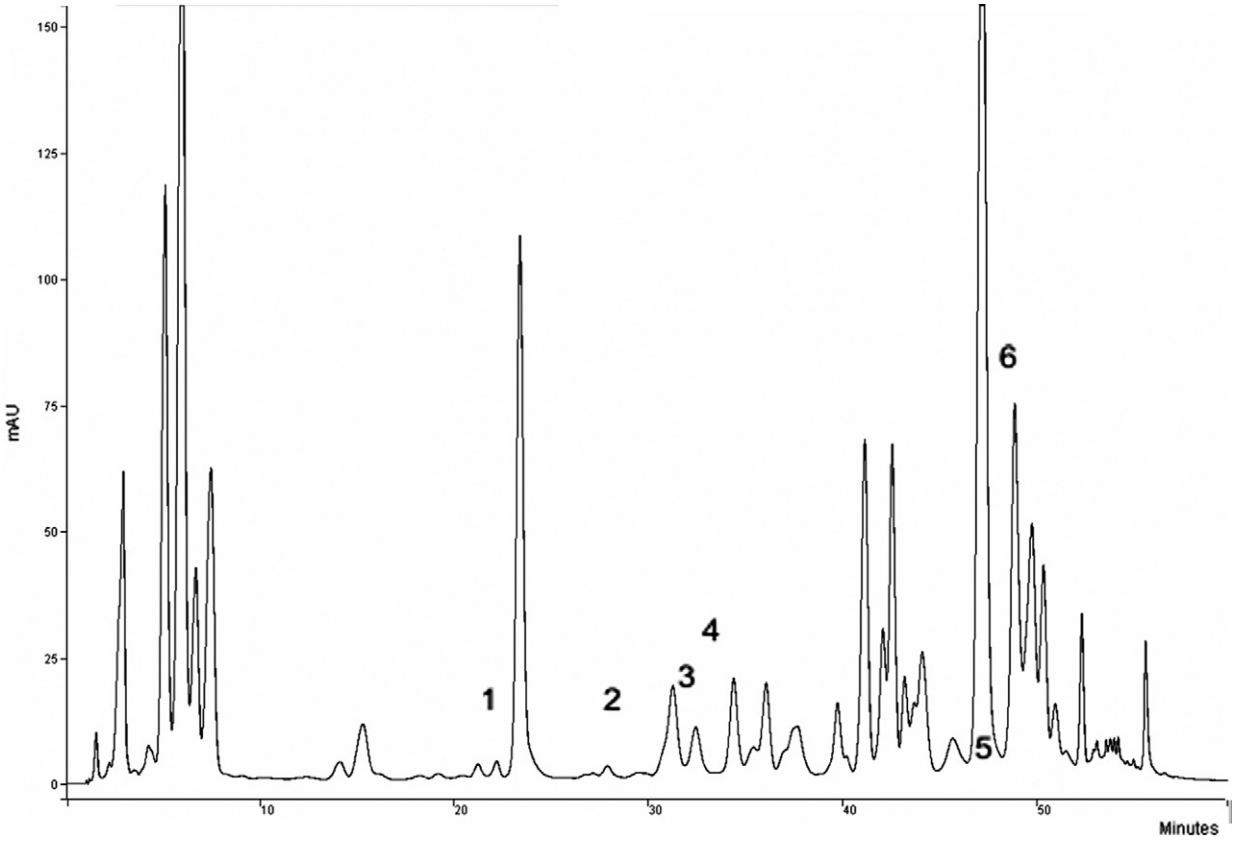

Fig. 1. Typical HPLC chromatogram of flavonoids in Croatian *Robinia* honeys (340 nm). Peaks: quercetin (1), luteolin (2), kaempferol (3), apigenin (4), chrysin (5) and galangin (6).

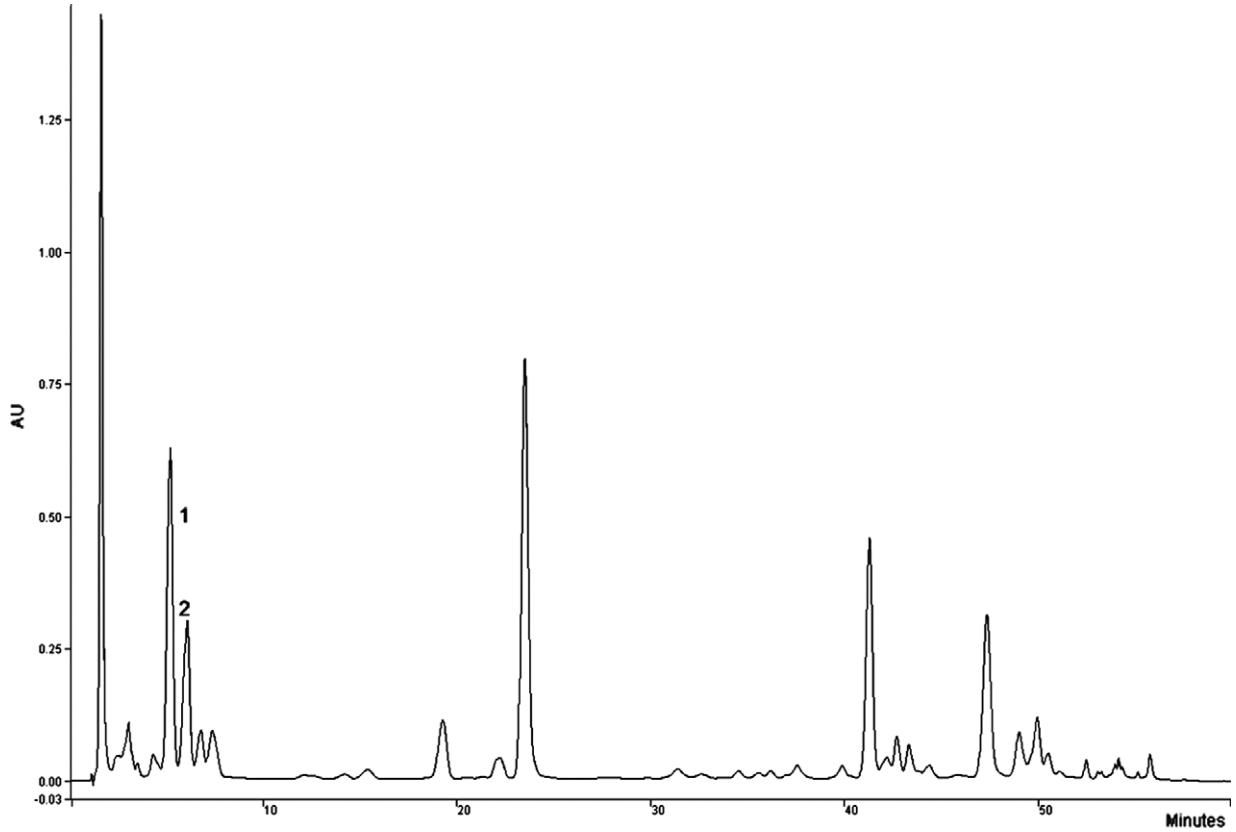

Fig. 2. Typical HPLC chromatogram of phenolic acids in Croatian *Robinia* honeys (290 nm). Peaks: caffeic acid (1) and *p*-coumaric acid (2).

in Tables 2 and 3. Concentrations of specific, as well as total identified flavonoids, are statistically significant ($p < 0.001$) higher in samples produced in 2003, with the exception of flavone luteolin which was present in slightly, but not statistically significant ($p > 0.05$) higher concentrations in 2002 samples. Tomás-Barberán et al. (2001) in their research on flavonoid profiles of different European unifloral honeys typified acacia honey as monofloral honey with no specific flavonoid markers in HPLC chromatogram. Their research encompassed seven acacia honey samples, two of which were from Germany, four from Italy and one sample from France. As mentioned

Table 2
Flavonoid content (μg/100 g of honey) of the analysed honey samples produced in 2002

Sample code	Myricetin	Quercetin	Luteolin	Kaempferol	Apigenin	Chrysin	Galangin	Total
M-02/02	–	6.6	3.2	11.2	4.5	57.9	14.2	97.6
M-06/02	–	3.2	1.5	14.1	4.2	24.7	14.7	62.3
M-10/02	–	6.0	2.9	8.1	7.0	82.7	32.6	139.3
M-15/02	–	8.1	2.6	9.3	7.5	56.8	18.2	102.5
M-17/02	–	8.5	3.2	11.8	6.8	101.0	31.7	163.0
M-19/02	–	5.5	2.5	23.1	7.1	37.0	17.7	92.9
M-25/02	–	20.9	6.5	20.8	15.2	231.1	50.4	344.8
M-26/02	–	3.0	1.4	6.1	2.6	24.2	11.1	48.4
M-29/02	–	4.7	1.5	9.4	4.6	111.2	54.9	186.3
M-32/02	–	6.8	4.0	20.0	7.7	55.1	25.5	119.1
M-34/02	–	6.0	2.8	9.6	6.2	84.8	34.7	144.1
M-36/02	–	4.3	2.0	10.5	3.2	60.7	20.4	101.1
M-39/02	–	6.7	4.0	17.6	6.9	85.8	31.8	152.8
M-40/02	–	2.9	1.6	9.9	3.5	33.2	10.8	61.9
M-52/02	–	5.0	3.1	10.7	5.4	89.6	28.4	142.1
M-62/02	–	6.4	2.8	23.8	6.5	74.9	29.9	144.5
M-74/02	–	6.2	4.1	23.8	8.6	82.9	33.3	158.8
M-86/02	–	3.8	2.1	13.5	6.2	21.9	11.8	59.3
M-89/02	–	5.8	4.1	10.6	7.6	49.0	21.6	98.6
M-93/02	–	6.1	2.4	10.1	7.8	125.3	52.3	204.0
Mean	–	6.3	2.9	13.7	6.5	74.5	27.3	131.2
SD	–	3.8	1.2	5.7	2.7	47.3	13.5	66.4

– Compound not detected.

Table 3
Flavonoid content (μg/100 g of honey) of the analysed honey samples produced in 2003

Sample code	Myricetin	Quercetin	Luteolin	Kaempferol	Apigenin	Chrysin	Galangin	Total
M-03/03	–	21.2	1.0	27.0	8.5	80.5	39.4	177.6
M-06/03	–	14.6	0.7	22.1	4.6	110.8	40.1	192.8
M-09/03	–	14.5	1.7	32.7	16.0	233.0	57.2	355.0
M-10/03	–	15.1	3.7	31.6	8.4	174.4	54.5	287.6
M-15/03	–	34.3	2.2	36.3	21.7	214.9	76.9	386.1
M-20/03	–	32.3	6.9	29.3	11.4	241.9	65.0	386.7
M-25/03	–	87.5	1.9	18.2	16.5	212.3	56.1	392.4
M-29/03	–	86.2	0.9	21.2	9.3	168.1	42.3	327.9
M-30/03	–	46.6	2.7	24.4	13.1	77.9	18.6	183.2
M-32/03	–	52.4	1.1	27.0	8.0	63.1	21.4	172.9
M-59/03	–	17.5	1.7	19.3	12.9	53.2	15.7	120.1
M-69/03	–	19.0	1.4	24.6	16.2	299.5	96.6	457.3
M-79/03	–	17.5	1.7	18.8	16.1	147.1	40.4	241.5
M-82/03	–	29.6	3.8	60.6	21.1	190.3	78.9	384.2
M-85/03	–	23.1	1.6	33.9	28.6	184.5	64.6	336.1
M-88/03	–	17.6	1.6	19.5	12.7	168.7	51.5	271.4
M-92/03	–	17.0	1.6	19.2	12.8	212.9	57.7	321.0
M-100/03	–	18.4	1.2	23.9	9.1	143.2	32.6	228.3
M-103/03	–	24.5	4.5	38.8	12.4	162.9	46.0	288.9
M-104/03	–	16.5	1.9	21.3	1.4	122.6	36.8	200.5
Mean	–	30.2	2.2	27.5	13.0	163.1	49.6	285.5
SD	–	21.4	1.5	10.0	6.2	64.8	20.7	93.2

– Compound not detected.

myricetin was not detected in their samples, apigenin and galangin were not analysed, while quercetin, luteolin, kaempferol and chrysin were detected. Amounts of identified compounds in their samples varied in a wide range (for example concentration of quercetin varied from minimum 5.8 μg/100 g of honey in one of German samples up to 510 μg/100 g of honey in the sample from France), and therefore comparison with our results is rather aggravated. However, these results point at the fact that variability of flavonoid concentrations is to be expected in analysis of different samples. Furthermore, some authors confirmed that flavonoid content depends on climate conditions (Edreva, 2005). Flavonoids are secondary plant metabolites and according to some authors they have protective function for plant survival under the adverse environmental conditions (Winkel-Shirley, 2002). Since the compounds which are about to be used as floral markers should be unaffected by the ecological factors, if analysed from this aspect, flavonoids are inconvenient for botanical origin determination in every case except if there are specific markers connected with the plant source. On the other hand, polyphenols have been used as chemotaxonomic markers in plant systematics for more than 30 years (Iwashina, 2000; Kataliníć, 1997), and are proved to be very useful for those purposes. In this case, the samples were collected during two seasons of opposite climatically conditions and noticed differences in flavonoid content are probably due to those differences. Namely, according to the data available in the literature the production of flavonoids widely varies, and seasonal variations in the phenolic compounds production have been previously reported (Chaves, Escudero, & Gutierrez-Merino, 1993). Bell (1980) proposed that flavonoids synthesis should be considered as a plant defence mechanism against stress. Among the recognized stressors and flavonoid synthesis inducers in plants are bacterial and fungal infections, mechanical wounding, and physicochemical conditions (Gottstein & Gross, 1992; Winkel-Shirley, 2002). According to the report of Croatian Meteorological and Hydrological Service season 2002 was in Croatia extremely warm and 80% of area was very rainy, while season 2003 was the opposite according to the rainfalls and 80% of Republic area was extremely dry in combination with extremely high temperatures (Meteorological & Hydrological Service, Republic of Croatia, 2003, 2004). Absence of rainfalls and extremely high temperatures in the year 2003 were combined by sunny weather, and increased UV-B radiation is also proved to enhance flavonoids production of plants (Tevini, Iwanzik, & Thoma, 1981). Furthermore, Balakumar, Vincent, and Paliwal (1993) reported that multiple stress conditions (for example, high levels of UV light irradiation are usually accompanied by drought and high temperatures) could have synergistic and/or antagonistic responses. Raised concentration of particular as well as total flavonoids in honey samples produced in the year 2003 is therefore something that can be expected, considering how honey is produced.

At the same time, though concentrations of analysed flavonoid compounds have changed, their share in total concentration has stayed practically unchanged as showed in Fig. 3. Such results are in compatibility with obtained chromatograms, which pointed at the fact that botanical origin of *Robinia* honey could be identified from the typical flavonoids profile even if it does not have a specific marker compound. Namely, *Robinia* flavonoid profile shows in global different pattern than flavonoid profiles of other monofloral honey types analysed in our laboratory, and/or found in the literature (Tomás-Barberán et al., 2001). In both groups of samples chrysin was dominant compound, making 57% of total flavonoids in samples produced in the year 2002 and 56% of total flavonoids in honey samples produced in the year 2003. Comparison of chrysin share in the total flavonoids revealed that obtained difference between two years of production is not statistically significant ($p > 0.05$). Share of pollen/nectar sourced kaempferol and apigenin remained unchanged with averages 10% and 5% respectively. At the similar principle, as a ratio of individual sugar compounds, results of honey sugar analysis are usual presented and used as a characterisation criterion (Persano Oddo & Piro, 2004).

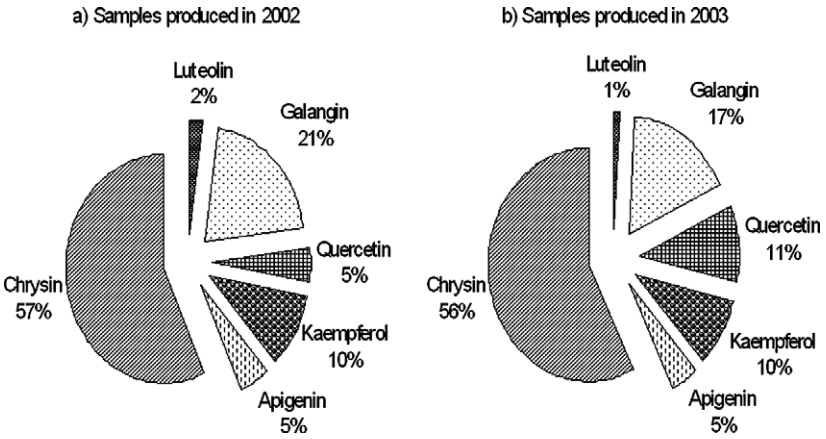

Fig. 3. Mean share (%) of individual flavonoid compounds in total identified flavonoids.

Though both parameters, specific pollen share and particular or total flavonoids content, were higher in the samples produced in the year 2003, data analysis revealed that there is no statistically significant correlation between them. The similar results, pointing that there is no connection between total content and specific pollen grains share, were reported previously by Ferreres et al. (1998). They researched rosemary honey, and concluded that there was no correlation between the kaempferol content in honey and pollen content though total pollen number varied from less than 2000 pollen grains up to 50,000 pollen grains per gram of honey. Similarly, Ferreres et al. (1993) have found no significant differences in the relative amounts of flavonoid hesperetin in citrus honey and total pollen number of pollen grains varied from only 95 pollen grains per 15 g of honey up to 75,000 pollen grains per 15 g of honey. In both mentioned examples it was concluded that nectar, and not pollen, is the main source of flavonoids in honey.

4. Conclusion

The analysis of Croatian *Robinia* honey samples showed that all samples are in agreement with Croatian, as well as international legislative. The values of physicochemical parameters were, furthermore, in a range of values characteristic for this type of monofloral honey. Flavonoid analysis showed that shares of individual compounds have stayed unchanged, though their concentrations varied as a consequence of climatically conditions. In combination with obtained chromatographic profile of samples, even in the absence of specific marker compound, they could be used as a differentiation criterion for *Robinia* honey.

Acknowledgements

This research is conducted as a part of the technological project TP-02/0113-07 financed by the Ministry of science and Technology of the Republic of Croatia.

References

Ampuero, S., Bogdanov, S., & Bosset, J.-O. (2004). Classification of unifloral honeys with an MS-based electronic nose using different sampling modes: SHS, SPME and INDEX. *European Food Research and Technology, 218*, 198–207.

Balakumar, T., Vincent, H. B., & Paliwal, K. (1993). On the interaction of UV-B radiation (280–315 nm) with water stress in crop plants. *Physiologia Plantarum, 87*, 217–222.

Bell, E. A. (1980). The possible significance of secondary compounds in plants. In E. A. Bell & B. V. Charlwood (Eds.), *Secondary plant products* (pp. 11–21). New-York: Springer-Verlag.

Benedetti, S., Mannino, S., Sabatini, A. G., & Marcazzan, G. L. (2004). Electronic nose and neural network use for classification of honey. *Apidologie, 35*, 397–402.

Bogdanov, S., Martin, P., & Lüllmann, C. (1997). Harmonised methods of the European Commission. *Apidologie*(extra issue).

Bogdanov, S., Ruoff, K., & Persano Oddo, L. (2004). Physico-chemical methods for the characterisation of unifloral honeys: a review. *Apidologie, 35*, S4–S17.

Chaves, N., Escudero, J. C., & Gutierrez-Merino, C. (1993). Seasonal variations of exudate of *Cistus ladanifer*. *Journal of Chemical Ecology, 19*(11), 2577–2591.

Devillers, J., Morlot, M., Pham-Delègue, M. H., & Doré, J. C. (2004). Classification of monofloral honeys based on their quality control data. *Food Chemistry, 62*, 305–312.

Edreva, A. (2005). The importance of non-photosynthetic pigments and cinnamic acid derivatives in photoprotection. *Agriculture, Ecosystems and Environment, 106*, 135–146.

Ferreres, F., García-Viguera, C., Tomás-Lorente, F., & Tomás-Barberán, F. A. (1993). Hesperetin: a marker of the floral origin of citrus honey. *Journal of the Science of Food and Agriculture, 61*, 121–123.

Ferreres, F., Juan, T., Pérez-Arquillué, C., Herrera-Marteache, A., Garcia-Viguera, C., & Tomás-Barberán, F. A. (1998). Evaluation of pollen as a source of kaempferol in rosemary honey. *Journal of the Science of Food and Agriculture, 77*, 506–510.

Ferreres, F., Tomás-Barberán, F. A., Soler, C., García-Viguera, C., Ortiz, A., & Tomás-Lorente, F. (1994). A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie, 25*, 21–30.

Gil, M. I., Ferreres, F., Ortiz, A., Subra, E., & Tomás-Barberán, F. A. (1995). Plant phenolic metabolites and floral origin of rosemary honey. *Journal of Agricultural and Food Chemistry, 43*, 2833–2838.

Gottstein, A., & Gross, D. (1992). Phytoalexins of woody plants. *Trees, 6*, 55–68.

Hermosín, I., Chicón, R. M., & Cabezudo, M. D. (2003). Free amino acid composition and botanical origin of honey. *Food Chemistry, 83*, 263–268.

Iglesias, M. T., De Lorenzo, C., Del Carmen Polo, M., Martín-Álvarez, P. J., & Pueyo, E. (2004). Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. *Journal of Agricultural and Food Chemistry, 52*, 84–89.

Iwashina, T. (2000). The structure and distribution of the flavonoids in plants. *Journal of Plant Research, 113*, 287–299.

Katalinić, V. (1997). High-performance liquid chromatographic determination of flavan fingerprints in plant extracts. *Journal of Chromatography A, 775*, 359–367.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee World, 59*, 139–157.

Martos, I., Ferreres, F., & Tomás-Barberán, F. A. (2000). Identification of flavonoid markers for the botanical origin of *eucalyptus* honey. *Journal of Agricultural and Food Chemistry, 48*, 1498–1502.

Martos, I., Ferreres, F., Yao, L., D'Arcy, B., Caffin, N., & Tomás-Barberán, F. A. (2000). Flavonoids in monospecific *eucaliptus* honeys from Australia. *Journal of Agricultural and Food Chemistry, 48*, 4744–4748.

Mateo, R., & Bosch-Reig, F. (1997). Sugar profiles of Spanish unifloral honeys. *Food Chemistry, 60*, 33–41.

Meteorological and Hydrological Service, Republic of Croatia (2003). Reviews No. 13 Climate monitoring and assessment for 2002.

Meteorological and Hydrological Service, Republic of Croatia (2004). Reviews No. 12 Climate monitoring and assessment for 2003.

Ministry of Agriculture and Forestry (2000). Quality standard of honey and other bees products. *Narodne Novine – Official Journal of The Republic of Croatia, 20*, 642–652.

Persano Oddo, L., & Bogdanov, L. (2004). Determination of honey botanical origin: problems and issues. *Apidologie, 35*, S2–S3.

Persano Oddo, L., & Piro, R. (2004). Main European unifloral honeys: descriptive sheets. *Apidologie, 35*, S38–S81.

Peterson, J., & Dwyer, J. (1998). Flavonoids: dietary occurrence and biochemical activity. *Nutrition Research, 18*(2), 1995–2018.

Popek, S. (2002). A procedure to identify a honey type. *Food Chemistry, 79*, 401–406.

Prodolliet, J., & Hischenhuber, C. (1998). Food authentication by carbohydrate chromatography. *Zeitschrift für Lebensmittel Untersuchung und Forschung A, 207*(1), 1–12.

Soler, C., Gil, M., Garcia-Viguera, C., & Tomás-Barberán, F. A. (1995). Flavonoid patterns of French honeys with different floral origin. *Apidologie, 26*(1), 53–60.

Tevini, M., Iwanzik, W., & Thoma, U. (1981). Some effects of enhanced UV-B irradiation on the growth and composition of plants. *Planta, 153*(4), 388–394.

The Council of the European Union (2002). Council Directive 2001/110/ec of 20 December 2001 relating to honey. *Official Journal of the European communities, 10*, 47–52.

Tomás-Barberán, F. A., Martos, I., Ferreres, F., Radovic, B. S., & Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture, 81*(5), 485–496.

Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology, 5*, 218–223.