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Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey

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Abstract

Honey samples from the seven most common honey types in Slovenia were screened for total phenolic content by the modified Folin–Ciocalteu method, for potential antioxidant activity using the ferric reducing antioxidant power (FRAP) assay and by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method for antiradical activity. In addition the colour characteristics of honey samples were analysed. The results of the study showed that total phenolic content, antioxidant activity and colour parameters differ widely among different honey types. Phenolic content expressed as gallic acid equivalent ranged from 44.8 mg/kg in acacia honey to 241.4 mg/kg in fir honey. Antioxidant activity was the lowest in the brightest acacia and lime honeys and the highest in darker honeys, namely fir, spruce and forest. The colour of the Slovenian honeys, analysed in this study was very variable and ranged from pale yellow to dark brown. Correlations between the parameters analysed were found to be statistically significant ($p < 0.05$).

Keywords: Honey; Phenolic content; Antioxidant activity; FRAP; DPPH; Colour characteristics

1. Introduction

Honey is a natural product, a highly concentrated solution of a complex mixture of sugars. It also contains small amounts of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals.

Many authors demonstrated that honey serves as a source of natural antioxidants, which are effective in reducing the risk of heart disease, cancer, immune-system decline, cataracts, different inflammatory processes etc. (The National Honey Board, 2003). Honey can also prevent deteriorative oxidation reactions in foods such as enzymatic browning of fruit and vegetables (Chen, Mehta, Berenbaum, Zangerl, & Engeseth, 2000), lipid oxidation in meat (Gheldof & Engeseth, 2002; McKibben & Engeseth, 2002; Nagai, Inoue, Kanamori, Suzuki, & Nagashima, 2006), and inhibit the growth of foodborne pathogens and food spoilage organisms (Mundo, Padilla-Zakour, & Worobo, 2004; Taormina, Niemira, & Beuchat, 2001). The components in honey responsible for its antioxidative effect are flavonoids (chrysin, pinocembrin, pinobanksin, quercetin, kaempferol, luteolin, galangin, apigenin, hesperetin, myricetin), phenolic acids (caffeic, coumaric, ferrulic, ellagic, chlorogenic), ascorbic acid, catalase, peroxidase, carotenoids and products of the Maillard reaction. The quantity of these components varies widely according to the floral and geographical origin of honey. In addition, processing, handling and storage of honey may influence its composition (Gheldof & Engeseth, 2002; Turkmen, Sari, Poyrazoglu, & Velioglu, 2005; Wang, Gheldof, & Engeseth, 2004). In the recent years there has been an increasing interest in determination of the antioxidant activity of honey. Many studies indicated that the antioxidant activity of honey varies widely, depending on the floral source. The botanical origin of honey has the greatest influence on its antioxidant activity, while processing, handling and storage affect honey antioxidant activity only to a minor degree (Al-Mamary, Al-Meeri, & Al-Habori, 2002; Beretta, Granata,

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Ferrero, Orioli, & Maffei Facino, 2005; Frankel, Robinson, & Berenbaum, 1998; Gheldof & Engeseth, 2002; Gheldof, Wang, & Engeseth, 2002). Several studies have shown that antioxidant activity is strongly correlated with the content of total phenolics (Aljadi & Kamaruddin, 2004; Al-Mamary et al., 2002; Beretta et al., 2005; Blasa et al., 2006; Gheldof & Engeseth, 2002; Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Beside this, a strong correlation was found between antioxidant activity and the colour of honey. Many researchers found that honeys with dark colour have a higher total phenolic content and consequently a higher antioxidant capacity (Beretta et al., 2005; Frankel et al., 1998).

Many different methods are appropriate for assessing the antioxidant activity of a substance and in most cases it is necessary to use several tests to obtain good reliability (Fukumoto & Mazza, 2000; Miquel Becker, Nissen, & Skibsted, 2004; Roginsky & Lissi, 2005). There is no official method for honey antioxidant activity determination. Various tests are in use, each based on different principles and experimental conditions; the FRAP assay (ferric reducing antioxidant power), the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, ORAC (oxygen radical absorbance capacity), superoxide radical-scavenging activity, TEAC (Trolox equivalent antioxidant activity). And even when investigators use the same method, different modifications are often included. Thus the results of different studies are hard to compare. A step forward regarding this problem was made by the work of Beretta et al. (2005), where a practical analytical approach for standardization of the antioxidant properties of honey was set. Their finding was that it is necessary to use a combination of antioxidant tests, comparative analyses and statistical evaluation to determine the antioxidant behaviour of honey.

The colour of honey, beside flavour and aroma, is one of the characteristics that serve to indicate the plant source. It ranges from very pale yellow through amber and dark reddish amber to nearly black (Mateo Castro, Jimenez Escamilla, & Bosch-Reig, 1992; Terrab, Escudero, González-Miret, & Heredia, 2004). It is related to the content of minerals, pollen and phenolics, and is characteristic of floral origin (Baltrusaitytė, Venskutonis, & Čeksterytė, 2007; González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005; Lazaridou, Biliaderis, Bacandritsos, & Sabatini, 2004). Darkening of honey during storage may occur because of Maillard reactions, fructose caramelization and reactions of polyphenols. The degree of darkening depends on the temperature and/or time of storage (Pereyra Gonzales, Burin, & Pilar Buera, 1999). In Slovenia the wide assortment of honeys available is a result of production in different regions with specific climatic conditions and a wide range of floral sources (Golob, Bertoncelj, & Škrabanja, 2002). Different authors use different approaches for evaluation of honey colour and the procedures can be classified into subjective (sensory) and objective (instrumental) methods (Terrab, Diez, & Heredia, 2002). The instrumental methods most commonly used for honey colour evaluation are the CIE $L^*a^*b^*$ tristimulus method, absorbance measurements and simple colour grading after Pfund (Bogdanov, Ruoff, & Persano Oddo, 2004; Terrab et al., 2002).

Until now, there has been no research to determine the phenolic content, antioxidant activity and colour of Slovenian honeys. In the present study we investigated the abovementioned parameters of seven different types of honey; acacia, lime, chestnut, fir, spruce, multifloral and forest honey, seventy samples in all. In addition correlations between all the analysed parameters were evaluated.

2. Materials and methods

2.1. Chemicals and instruments

All of the chemicals and reagents used were of analytical grade. DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), sucrose, fructose, glucose, maltose, and gallic acid were purchased from Sigma (St. Louis, MO, USA). Folin–Ciocalteu's phenol reagent, HCl, $FeSO_4 \cdot 7H_2O$ and $FeCl_3$ were obtained from Merck (Darmstadt, Germany).

A CECIL CE 2021 UV–Vis spectrophotometer (2000 series from CECIL instruments, Cambridge, England) was used for absorbance measurements and a Minolta Chromameter CR-200B was used for determination of colour parameters in the CIE $L^*a^*b^*$ system.

2.2. Samples

Seventy honey samples were obtained directly from beekeepers during the 2004 harvest from different locations across Slovenia. The floral origin of the samples was specified by the beekeepers regarding hive location, season and available floral sources, and further confirmed by means of sensory analysis, where the aroma, taste and colour characteristics of each honey were analysed. The honey samples were classified into seven groups, namely acacia (*Robinia pseudoacacia*), lime (*Tilia* spp.), chestnut (*Castanea sativa*), fir (*Abies alba*), spruce (*Picea abies*), multifloral and forest honey (honeydew honey from latifoliae and coniferous trees).

Honey samples were stored at 4 °C in the dark until analysed. A sugar analogue (an artificial honey whose composition reflects the approximate sugar composition of honey), consisting of 40% fructose, 30% glucose, 8% maltose and 2% sucrose, was prepared to check whether the main sugar components of honey could interfere in the assays. All tests were performed in triplicate.

2.3. Methods

2.3.1. Total phenolic content

The Folin–Ciocalteu method as modifed by Beretta et al. (2005) was used to determine total phenolic content. Each honey sample (5 g) was diluted to 50 ml with distilled water and 100 μl of the solution obtained, corresponding to

10 mg of fresh honey, were added to 1 ml of 10% Folin–Ciocalteu reagent. The mixture was vortexed for 2 min and the absorbance was determined after 20 min at 750 nm against the sugar analogue. Gallic acid (0–200 mg/ml) was used as a standard to derive the calibration curve. Total phenolic content was expressed as mg of gallic acid (GA) per kg of honey.

2.3.2. FRAP – the ferric reducing/antioxidant power assay

The procedure described by Benzie and Strain (1996) was used with minor modification. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous, coloured form (Fe^{2+}-TPTZ) in the presence of antioxidants. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6. It was prepared daily and warmed to 37 °C. Aliquots of 200 μl of sample were mixed with 1.8 ml of FRAP reagent and the absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubation at 37 °C for 10 min against the sugar analogue. Aqueous standard solutions of $FeSO_4 \cdot 7H_2O$ (100–1000 μM) were used for the calibration curve and the results were expressed as the FRAP value (μM Fe(II)) of the 10% honey solution.

2.3.3. DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

The antiradical activity of honey samples was estimated according to the procedure of Brand-Williams, Culivier, and Berset (1995) with some modification as made by Beretta et al. (2005). Honey samples were dissolved in water at concentrations from 30 to 600 mg/ml and 0.1 ml of each solution was mixed with 1.9 ml of 130 μM DPPH (final concentration 83.3 μM) dissolved in absolute ethanol and 1 ml of acetate buffer solution (100 mM, pH 5.5). The mixtures were shaken vigorously and left for 90 min at room temperature in the dark, after which the absorbance of the remaining DPPH was measured at 517 nm against a blank to eliminate the influence of honey colour. The blank was honey, at the same concentration as described above, and acetate buffer without DPPH. For each honey concentration tested, the percentage of DPPH remaining was calculated. The radical scavenging activity was expressed as IC_{50} (the concentration of the honey sample (mg/ml), required to scavenge 50% of DPPH), calculated by a linear regression analysis.

2.3.4. Colour analysis

Colour characteristics were assessed by the CIE $L^*a^*b^*$ method where lightness L^* and two colour coordinates, a^* and b^*, were defined by means of a Minolta CR-200B Chromameter. Honey samples were heated to 50 °C to dissolve sugar crystals and then placed in a plastic container 7 cm in diameter and covered with a plastic plate. The initial pretreatment of the honey samples did not alter their colour. The measured layer was 1 cm thick. L^*, a^* and b^* parameters were measured against a white background and were directly obtained from the apparatus.

In addition colour was determined by spectrophotometric measurement as described by Beretta et al. (2005), where the net absorbance of a 50% honey solution (w/v) was defined as the difference between the absorbances at 450 and 720 nm. The results are expressed as mAU. Before making measurements, honey solutions were filtered for removal of any coarse particles.

2.4. Statistical analysis

All analyses were carried out in triplicate and the data were expressed as means ± standard deviations (SD). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare the phenol contents, FRAP values, IC_{50} values and colour parameters of different honey types. Differences between means at the 95% ($p < 0.05$) confidence level were considered statistically significant. Correlations were obtained by Pearson's correlation coefficient (r) in bivariate linear correlations.

3. Results and discussion

The results obtained showed that the total phenolic content ($mg_{gallic\ acid}$/kg of honey) determined by the modified Folin–Ciocalteu method varied greatly among the honey types, as is apparent from Table 1. The lowest value was determined in acacia honey, where the average result of ten samples was 44.8 mg/kg, rising further in lime, multifloral, chestnut and spruce honey. The highest values were obtained for fir and forest honey, 241.4 and 233.9 mg/kg, respectively, and were approximately 5-fold higher than that for acacia honey. The average total phenolic content was in close agreement with the results reported by Beretta et al. (2005) for acacia, chestnut and multifloral honey.

For determination of the antioxidant capacity we used the FRAP assay (ferric reducing/antioxidant power), a simple direct test that is widely used for antioxidant activity determination in many different samples, including honey (Aljadi & Kamaruddin, 2004; Benzie & Strain, 1996; Beretta et al., 2005; Blasa et al., 2006; Küçük et al., 2007; Taormina et al., 2001). As shown in Table 1, there were significant differences ($p < 0.05$) among the types of honey. The antioxidant activity for different types increased in the order: acacia < lime < multifloral < chestnut < spruce < forest < fir honey. Acacia honey had an average FRAP value of only 71.0 μM Fe(II), while the highest FRAP values were reached by Slovenian fir and forest honey, 478.5 and 426.4 μM Fe(II) for the 10% honey solution, respectively. These results are similar to those obtained by Beretta et al. (2005) for chestnut and acacia honey; their finding was that the least active honeys are those of monofloral origin (acacia, sulla, dandelion and clover). A positive linear correlation between the total antioxidant activity, determined by the FRAP method, and phenolic content (Fig. 1) was observed. The high correlation

Table 1
Phenol content, FRAP values and antiradical power (DPPH) of analysed honeys

Parameter	Statistics	Type of honey						
		Acacia ($n = 10$)	Lime ($n = 10$)	Chestnut ($n = 10$)	Fir ($n = 10$)	Spruce ($n = 10$)	Multifloral ($n = 10$)	Forest ($n = 10$)
Phenol content ($mg_{gallic\ acid}$/kg)	Mean ± SD	44.8 ± 14.8[a]	83.7 ± 14.3[b]	199.9 ± 34.1[d]	241.4 ± 39.5[f]	217.5 ± 20.6[d,e]	157.3 ± 20.9[c]	233.9 ± 21.7[e,f]
	Range	25.7–67.9	90.0–159.0	146.8–272.3	163.4–285.7	185.7–239.0	126.8–194.6	192.3–270.1
FRAP value (μM Fe(II))	Mean ± SD	71.0 ± 10.2[a]	118.8 ± 20.3[a]	360.1 ± 66.5[c]	478.5 ± 95.5[e]	395.3 ± 69.6[c,d]	224.8 ± 24.7[b]	426.4 ± 41.5[d]
	Range	56.8–86.0	94.6–155.1	238.3–469.5	320.8–582.2	277.5–495.4	181.1–262.9	371.6–494.1
DPPH–IC_{50} (mg/ml)	Mean ± SD	53.8 ± 8.5[a]	28.8 ± 5.4[b]	10.0 ± 1.8[c]	8.2 ± 1.7[c]	7.4 ± 1.5[c]	10.7 ± 2.2[c]	7.2 ± 1.2[c]
	Range	33.9–63.9	20.6–36.1	7.8–14.0	6.4–11.7	5.4–9.7	8.1–13.9	5.3–8.7

[a,b,c,d,e,f] Means in the same row with different letters are significantly different according to Duncan's test ($p < 0.05$).

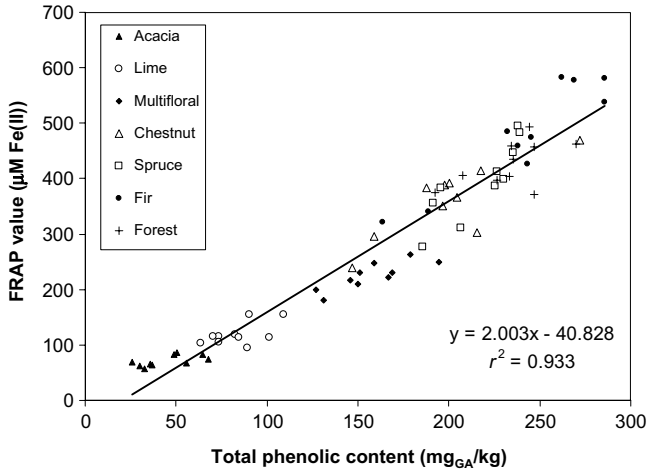

Fig. 1. Correlation between total phenolic content and total antioxidant capacity (FRAP) of honey.

coefficient ($r = 0.966$) indicates that phenolics are one of the main components responsible for the antioxidant behaviour of honey. This statistically significant correlation was in agreement with the findings of other authors (Beretta et al., 2005; Blasa et al., 2006), who also found a strong relationship between antioxidant capacity determined by the FRAP assay and the phenolic content of honey. Gheldof et al. (2002) stated that phenolic compounds significantly contribute to the antioxidant activity of honey, but in spite of this, it seems that antioxidant activity appears to be a result of the combined activity of honey phenolics, peptides, organic acids, enzymes and Maillard reaction products.

The DPPH method with the stable organic radical 1,1-diphenyl-2-picrylhydrazyl is used for determination of free radical scavenging activity, usually expressed as IC_{50}, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. This means that the lower is the IC_{50} value of the sample, the higher is its antioxidant activity (Molyneux, 2004). Our results (Table 1) showed that the monofloral honeys, acacia and lime were the least active. Their IC_{50} values were 53.8 and 28.8 mg/ml, respectively, and were significantly higher ($p < 0.05$) than the IC_{50} values of the remaining types of honey. No statistically significant differences were found among forest, spruce, fir, chestnut and multifloral honey with IC_{50} values from 7.2 to 10.7 mg/ml. The results obtained for the scavenging ability of honey were in close agreement with the IC_{50} values reported by Beretta et al. (2005). For correlation analysis the IC_{50} values were transformed into their reciprocal values ($1/IC_{50}$). The correlation between the free radical scavenging activity and total phenolic content was highly statistically significant; the correlation coefficient was equal to 0.932. The relation between the two methods for determination of antioxidant activity, FRAP and DPPH, was also significant, with a correlation coefficient equal to 0.894 (Table 3).

The colours of Slovenian honeys defined by visual observation were noticeably different and varied from almost colourless to dark brown. The brightest were acacia and lime honeys, almost colourless to pale yellow and white to cream or ivory, with a yellow or green tinge, respectively. Multifloral honeys were very colourful, from yellow to brown; depending on the plant species and the presence of honeydew. The colour of chestnut honeys was amber, more or less dark, with a reddish tinge. Spruce honeys were medium to dark amber, with a reddish tinge; the speciality of this honey type is the glitter of the surface. Fir honey had a specific dark grey-brown colour, with a dark green tinge. The colour of forest honeys was light to dark amber, with reddish or green tinge. The colour characteristics are presented in Table 2 which summarizes the means, standard deviations and ranges of the parameters L^*, a^* and b^*, obtained with the Minolta chromameter for seven types of Slovenian honey. Acacia and lime honeys had the highest average values of parameter L^* that indicates lightness, 64.60 and 63.24, respectively. No statistically significant difference existed between these two types of honey and they were also found to be the lightest by visual comparison. The L^* value decreased further in multifloral (53.87) and chestnut honey (48.11). Fir, spruce and forest honeys were the darkest, with very similar L^* values of 43.17, 43.48 and 42.12, respectively. González-Miret et al. (2005) classified honey samples into two groups regarding their lightness value: light honeys (citrus, rosemary, lavender, eucalyptus and thyme) with $L^* > 50$ and dark honeys (honeydew, heather, chestnut and avocado)

Table 2
Colour characteristics of Slovenian honeys

Colour parameter	Statistics	Type of honey						
		Acacia ($n = 10$)	Lime ($n = 10$)	Chestnut ($n = 10$)	Fir ($n = 10$)	Spruce ($n = 10$)	Multifloral ($n = 10$)	Forest ($n = 10$)
L^*	Mean ± SD	64.60 ± 0.63^{d}	63.24 ± 1.26^{d}	48.11 ± 4.27^{b}	43.17 ± 2.61^{a}	43.48 ± 2.28^{a}	53.87 ± 2.41^{c}	42.12 ± 2.27^{a}
	Range	63.53–65.67	61.38–65.57	42.30–53.35	38.85–46.42	39.60–47.10	50.30–57.30	37.97–45.56
a^*	Mean ± SD	-2.82 ± 0.36^{a}	-3.41 ± 0.26^{a}	7.66 ± 2.71^{c}	$8.18 \pm 1.80^{c,d}$	$9.66 \pm 1.59^{d,e}$	2.25 ± 2.21^{b}	10.14 ± 1.39^{e}
	Range	−3.51 to (−2.40)	−3.79 to (−3.01)	3.79–10.82	5.67–11.12	7.10–12.20	−0.90 to 5.77	7.59–12.31
b^*	Mean ± SD	17.95 ± 3.12^{a}	25.74 ± 4.47^{b}	41.28 ± 5.55^{d}	34.95 ± 3.80^{c}	34.98 ± 3.42^{c}	46.45 ± 2.12^{e}	32.88 ± 3.33^{c}
	Range	13.49–22.67	20.93–33.36	33.32–49.36	29.10–39.77	29.80–40.90	43.10–49.22	27.81–37.86
Net absorbance (mAU)	Mean ± SD	70 ± 15^{a}	123 ± 25^{b}	495 ± 73^{e}	405 ± 60^{d}	417 ± 35^{d}	344 ± 57^{c}	467 ± 74^{e}
	Range	44–95	90–159	376–613	310–486	358–478	248–431	351–546

[a,b,c,d,e] Means in the same row with different letters are significantly different according to Duncan's test ($p < 0.05$).

with $L^* \leqslant 50$. Considering this classification Slovenian acacia, lime and multifloral honeys can be placed in the group of light honeys, while other types belong to dark honeys.

The plot of parameters a^* and b^* of honeys, grouped by botanical origin, is shown in Fig. 2. It can be seen that the honey samples analysed had red, yellow and green components. Green components (negative a^* values) were present in all samples of acacia and lime honey and in one multifloral honey sample. From the diagram it is apparent that the lightest acacia and lime honeys were clearly distinguished from all the other honey types. Honeydew types of honey (fir, spruce and forest) with a^* and b^* values in the range from 8.18 to 10.14 and 32.88 to 34.98, respectively, overlapped extensively in the (a^*,b^*) diagram. No statistically significant differences were found for these three types of honey in the values of their a^* and b^* parameters.

In general the parameters L^*, a^* and b^* of the Slovenian honey samples analysed were in a similar range as previously reported data (González-Miret et al., 2005; Lazaridou et al., 2004; Mateo Castro et al., 1992).

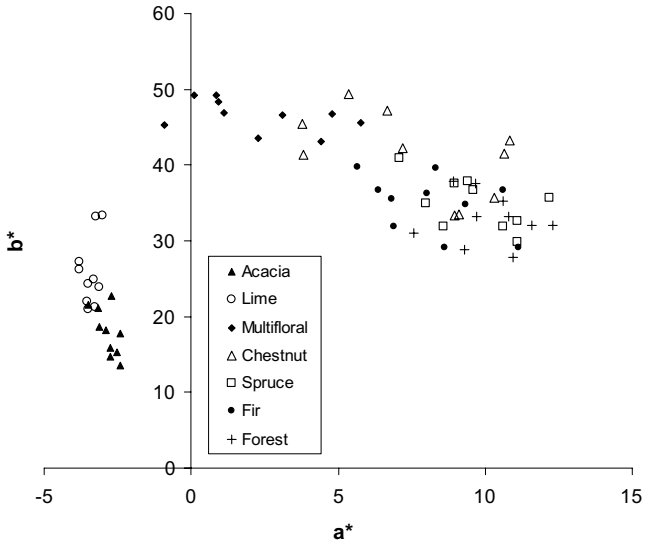

Fig. 2. Localization area of analysed honey samples on an (a^*,b^*) diagram.

In addition, the colour intensity of a 50% honey solution (w/v) was measured spectrophotometrically. Net absorbance varied from 70 mAU in the brightest acacia honey to 495 mAU in chestnut honey (Table 2). Net absorbances of different honey types increased in the order: acacia < lime < multifloral < fir < spruce < forest < chestnut honey. The results were in close relationship with the L^* value of honey, with a correlation coefficient equal to −0.887. This means that net absorbance increased with decreasing lightness value.

Mateo Castro et al. (1992) stated that colour assessment using visual comparisons, spectrophotometric measurements and CIE $L^*a^*b^*$ parameters can be a useful complementary tool for determining the botanical origin of honey. In the case of Slovenian honeys we found that we could distinguish between acacia, lime, chestnut, multifloral and the group of honeydew honeys. Honeydew types of honey, namely spruce, fir and forest, showed very similar colour characteristics that prevented their differentiation.

The relationships between honey colour and the other parameter analysed in this study (total phenolic content and antioxidant activity, determined by FRAP and DPPH assays) are presented in Table 3. The correlation matrix showed that all the relationships were statistically significant at $p < 0.05$. The significant correlation between total phenolic content and parameter L^* of honey colour is presented in Fig. 3. The correlation was negative which means that lighter honeys have higher L^* values because of their smaller content of total phenolics. Since the colour of honey partly reflects the content of pigments with antioxidant properties (Frankel et al., 1998), we expected a significant correlation between antioxidant activity and the colour of honey. The highest correlation coefficient found was for the relationship between antioxidant activity assessed by the FRAP method and the L^* value of honey colour ($r = -0.924$), while the correlation coefficient for the relation between antioxidant activity and net absorbance was a little bit lower, equal to 0.853. The connection

Table 3
Correlation matrix (Pearson correlation coefficients)

	Phenol content	FRAP value	DPPH ($1/IC_{50}$)	Net absorbance	L^*	a^*
FRAP value	0.966					
DPPH ($1/IC_{50}$)	0.932	0.894				
Net absorbance	0.908	0.853	0.432			
L^*	−0.943	−0.924	−0.884	−0.887		
a^*	0.907	0.900	0.868	0.905	−0.964	
b^*	0.553	0.453	0.581	0.667	−0.446	0.443

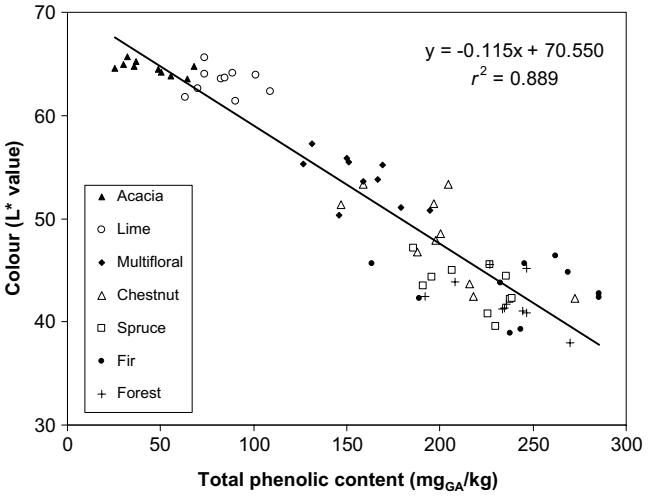

Fig. 3. Correlation between total phenolic content and parameter L^* of honey colour.

between antiradical activity (DPPH assay) and lightness value of honey colour was also statistically significant. Correlations between honey colour and its antioxidant activity were also studied by other researchers. Beretta et al. (2005) demonstrated a close correlation between the net absorbance and the total antioxidant activity ($r_{\text{net absorbance/FRAP}} = 0.918$). Frankel et al. (1998) found a significant correlation between the antioxidant capacity determined by the spectrophotometric DPPH method and honey colour measured with a Pfund honey grader, while Taormina et al. (2001) demonstrated a significant correlation between the antioxidant capacity determined by the FRAP assay and honey colour (absorbance of a 10% honey solution at 593 nm).

4. Conclusions

In the present study it was established that all types of honey contained phenolic compounds and possessed antioxidant activity. The total phenolic content and antioxidant activity varied greatly among different types of honey and were found to be the highest in darker types, namely fir, spruce and forest, while the lightest honey types, acacia and lime, showed low total phenolic content and consequently lower antioxidant capacity. Phenolic compounds appear to be responsible for the antioxidant activity of honey; a significant correlation was found between the antioxidant activity as determined by the FRAP assay and the phenolic content. However, further studies of the antioxidative components of Slovenian honeys are required, especially identification and quantification of individual flavonoids and phenolic acids.

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