Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 81 (5) 567–574 (2016)
JSCS–4868
UDC 638.16+543:615.279–188(497.11)
Short communication

SHORT COMMUNICATION

Analytical possibilities for the relative estimation of the antioxidative capacity of honey varieties harvested in different regions of Serbia

UROŠ M. GAŠIĆ[1#], DALIBOR M. STANKOVIĆ[2], DRAGANA Č. DABIĆ[2#], DUŠANKA M. MILOJKOVIĆ-OPSENICA[1#], MAJA M. NATIĆ[1#], ŽIVOSLAV Lj. TEŠIĆ[1#] and JELENA J. MUTIĆ[1]*

[1]*Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158 Belgrade, Serbia and* [2]*Innovative Center, Faculty of Chemistry, Studentski trg 12–16, 11158 Belgrade, Serbia*

(Received 13 March, revised 15 December, accepted 25 December 2015)

Abstract: Two different approaches, spectroscopic and electrochemical, were applied for the rough determination of the antioxidative capacity of honey samples. Honey samples of diverse botanical origin were collected in different geographical regions of Serbia. The total phenolic content (*TPC*) was determined by the Folin–Ciocalteu method. Cyclic voltammograms on a glassy carbon electrode in KCl supporting electrolyte were used to check the electrode sensitivity to the presence of honey. In order to calculate the Trolox equivalent antioxidant capacity (*TEAC*) of the studied honey samples, cyclic voltammograms were recorded for the Trolox standard. The results were expressed as μmol of Trolox equivalents per kg of sample (μmol TE kg^{-1}). Good correlations were observed between the cyclic voltammetry data and the *TPC* determined by the Folin–Ciocalteu method and the radical scavenging activity (*RSA*) determined using the DPPH·(1,1-diphenyl-2-picrylhydrazyl) radical test. Cyclic voltammetry appears to be a highly attractive alternative method for a rapid estimation of the antioxidative capacity of honeys. It was found that polyfloral honey samples had the highest, whereas acacia honey showed the lowest values of *TPC*.

Keywords: antioxidant activity; Folin–Ciocalteu method; radical scavenging activity.

INTRODUCTION

Generally, there is a growing interest on the efficiency of natural antioxidants in food. Polyphenols, *i.e.*, flavonoids and phenolic acids, are considered as

* Corresponding author. E-mail: jmutic@chem.bg.ac.rs
# Serbian Chemical Society member.
doi: 10.2298/JSC150313009G

one of the important groups of components for antioxidant activity identified in honey. The antioxidant activity of honey is closely related to the floral source of the honey. Generally, honeys are classified as monofloral (produced from one plant species) and polyfloral (several plant sources). Different honey types were subjected to antioxidant activity tests and they demonstrated significant potential, comparable to those of the other foodstuff.[1] On the other hand, research on the antioxidant capacity of honey samples originating from Serbia is scarce. Available literature indicates that there have only been a few reports to date on the determination of both total phenolic content and antioxidant activity of Serbian honeys by spectroscopic methods[2–4] and an electrochemical (polarographic) method.[5] Cyclic voltammetry was shown to be sensitive, convenient, rapid and low-cost approach in the quality evaluation of food products beneficial for human health.[6,7] Studies on the evaluation of the antioxidant activity of different food products using electrochemical methods were reported.[8–11] Due to growing interest in the quality of Serbian honey samples and continuing research on these nutritionally important products, the antioxidant potentials of honey samples of diverse botanical origin are communicated in this paper. The samples were obtained and selected directly by the beekeepers from different parts of the territory of Serbia. Two different approaches, spectroscopic and electrochemical, were applied to check rapidly the antioxidative capacity of the selected honey samples. The total phenolic content was determined by the well-established spectrophotometric technique using the Folin–Ciocalteu method. The radical scavenging activity of the honey samples was determined using the 2,2-diphenyl-1--picrylhydrazyl radical (DPPH$^{\bullet}$). Cyclic voltammetry was used to check the electrochemical response of glassy carbon in the presence of the honey samples. To inspect the applicability of cyclic voltammetry for the rather rapid and preliminary determinations, the electrochemically determined results were correlated with the results obtained by spectrophotometric methods already established in the literature for these purposes.

EXPERIMENTAL

Chemicals and materials

Methanol (HPLC grade), sodium carbonate, potassium chloride, hydrochloric acid, Folin–Ciocalteu reagent, and filter paper (Whatman No. 1) were purchased from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma–Aldrich (Steinheim, Germany). 2,2-Diphenyl-1-picrylhydrazyl·(DPPH$^{\bullet}$) was purchased from Fluka (Buch, Switzerland). Ultrapure water (ThermoFisher TKA MicroPure water purification system, 0.055 μS cm^{-1}) was used to prepare the standard solutions, blanks, and artificial honey analogue (30 % glucose, 40 % fructose, 10 % sucrose and 20 % water). An analogue of honey was made to check whether the main sugars in honey could interfere with the proposed electrochemical assay. Sugar standards (glucose, fructose and sucrose) were purchased from Tokyo Chemical Industry (TCI, Europe, Belgium).

Syringe filters (13 mm, PTFE membrane, 0.45 μm) were purchased from Supelco (Bellefonte, PA, USA). Ethanol (96 vol. %) was from J. T. Baker (Deventer, The Netherlands).

Honey samples

A total of 27 honey samples collected from different regions of Serbia (Fig. S-1 of the Supplementary material to this Communication) during the 2009 harvesting season were selected and provided by the Association of the Beekeeping Organizations of Serbia (SPOS, www.spos.info). The botanical origins of the samples were specified by the SPOS based on the information provided by beekeepers and sensory characteristics, and confirmed by physicochemical analyses and chemometrics.[12,13] The honey samples were from: acacia (*Robinia pseudoacacia*), sunflower (*Helianthus annuus*), lime (*Tilia cordata*), giant goldenrod (*Solidago virgaurea*), basil (*Ocimum basilicum*), oilseed rape (*Brassica napus*), buckwheat (*Fagopyrum esculentum*) and polyfloral meadow honey. The honeys were stored at room temperature in the dark before analysis.

Determination of total phenolic content and radical scavenging activity

Samples were prepared according to the slightly modified method proposed by Meda *et al.*[14] Each honey sample (5 g) was mixed with 15 mL ultrapure water, homogenized in ultrasonic bath for 15 min at room temperature, transferred to a 50 mL volumetric flask, and filled to the mark with ultrapure water. The solution was then filtered through 0.45 μm PTFE membrane and subjected to the determination of the total phenolic content (TPC) and radical scavenging activity (RSA). The TPC was spectrophotometrically determined by the Folin–Ciocalteu method reported by Singleton and Rossi,[15] with some modifications. Briefly, 0.3 mL of the sample solution and 6 mL deionized water were mixed with 0.5 mL of Folin–Ciocalteu reagent and solution was incubated for 6 min at room temperature. Then, 3 mL of 20 % sodium carbonate solution was added and after keeping the sample at 40 °C for 30 min, the absorbance was measured at 765 nm. Gallic acid was used as the standard, and the calibration curve of gallic acid was prepared in the concentration range between 50 and 250 mg L^{-1}. A mixture of water and Folin–Ciocalteu reagent was used as the blank. The results are expressed as mg gallic acid equivalent (GAE) per kg of honey.

The RSA of the honey samples was evaluated by a modified method of Li *et al.*[16] An aliquot of 1.0 mL of sample solution was mixed with 3 mL of a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH, 71 mM). The mixture was left for 60 min in the dark (until stable absorption values were obtained). After that, the reduction of the $DPPH^{\bullet}$ absorbance was measured by monitoring continuously the decrease in absorption at 515 nm. The RSA was calculated as a percentage of $DPPH^{\bullet}$ discoloration using the equation:

$$RSA\ (\%) = 100\frac{A_{\mathrm{DPPH}} - A_{\mathrm{sample}}}{A_{\mathrm{DPPH}}}$$

where A_{DPPH} is the absorbance of a methanolic solution of the $DPPH^{\bullet}$, A_{sample} is the absorbance in the presence of a honey extract. The assays were performed in triplicate and the results are expressed as mean values.

For cyclic voltammetry, honey samples, 1 g of each, were mixed with 20 mL 0.1 M KCl, homogenized in an ultrasonic bath for 10 min at room temperature, then filtered through 0.45 μm PTFE membranes and used for cyclic voltammetry measurements. Trolox was used as the standard. In order to achieve better similarity with the honey matrix, the Trolox standard was prepared and recorded in a solution of artificial honey. The solution of artificial honey was prepared in the same manner as were the honey samples (1 g of artificial honey in 20 mL of

supporting electrolyte). The scan was taken in the potential range between –200 mV and 800 mV at a scan rate 100 mV s^{-1}. Cyclic voltammograms were recorded for Trolox standard in the concentration range 10 to 100 μmol L^{-1} In this concentration range, a linear relationship between the response and concentration was obtained. Parameter *Q* was determined as the area under the oxidation voltammetric peak for the Trolox solutions. The obtained calibration curve, $Q = f$(concentration of Trolox) was linear with the correlation coefficient $R^2 = 0.998$ and was used to calculate Trolox equivalent antioxidant capacity (*TEAC*) from the *Q* parameter of the studied honeys. The results are expressed as μmol of Trolox equivalents per kg of sample (μmol TE kg^{-1}).

Instrumentation

A UV/Vis spectrophotometer (GBC UV/Visible Cintra 6) was used for the absorbance measurements and spectra recording, using optical cuvettes of 1 cm optical path.

Cyclic voltammograms were recorded on a CHI760B instrument (CH Instruments, Austin, TX, USA). The cell was equipped with a GC electrode, 3 mm in diameter (model CHI104), an auxiliary platinum electrode of a larger area (model CHI221, a cell top including a Pt wire counter electrode) and an Ag/AgCl reference electrode (model CHI111; all potentials in the paper are referred to Ag/AgCl). All measurements were performed at ambient temperature. Prior to each run, the surface of the glassy carbon electrode was freshly polished with 1.0, 0.3 and 0.05 μm alumina powder, rinsed with redistilled water and degreased in ethanol in an ultrasonic bath.

Statistical analysis

Data of all measurements were obtained in triplicate and are expressed as the mean values. Statistical analyses were performed by the NCSS software package.[17]

RESULTS AND DISCUSSION

The results on the total phenolic content (TPC) and of the radical scavenging activiry (RSA) of the honey samples are presented in Table I, together with the *Q* parameter derived from cyclic voltammograms (CV) as Trolox equivalents.

Different values for the CV charge correspond to oxidation of low-formal potential antioxidants and could be an indicator of the antioxidant potential of a sample.[18] The highest values were registered for the polyfloral honey samples from South–West Serbia, whereas the lowest CV charge values were generally obtained for the acacia honey samples, as well as for honey from basil harvested in North Serbia.

All honey samples were characterized with *TPC* values ranging between 127.76 mg (acacia – H1) to 887.18 mg (polyfloral – H22) of gallic acid per kg of honey, Table I. The average content of total phenolics was in a good agreement with the values given in the literature for honeys of the surrounding regions.[19–21] Generally, polyfloral honey samples had the highest values of TPC, while acacia samples showed the lowest values. Such findings are consistent with literature data. Here, just as an example, we cite a paper published by Bertoncelj *et al.*[22] who reported higher *TPC* values of polyfloral honeys in comparison to monofloral honeys (lime and sunflower) is cited. As is visible from Table I, the results of *RSA* ranged from 1.86 % (acacia honey – H4) to 23.20 % (polyfloral honey –

H22). Among all monofloral honey samples, buckwheat was found to have the highest *TPC* and *RSA* values. This was also found in a study of different monofloral honeys when buckwheat honey was reported to have the highest antioxidant activity.[23] In general, the results indicated that samples from the Zlatibor region (H5, H20, H22, H23, H25 and H26) were characterized with high *TCP* and *RSA* values. It was observed that polyfloral honey samples originating from this region showed different physico-chemical properties than those from the rest of Serbia.[24]

TABLE I. Total phenolic content (*TPC*), radical scavenging activity (RSA), CV charge derived from cyclic voltammograms and *Q* parameter derived from CV of monofloral and polyfloral honey samples

Sample	Botanical origin	*TPC* mg GAE kg^{-1}	*RSA* %	*Q* μC	*Q* μmol TE kg^{-1}
H1	Acacia	127.76	2.18	0.041	9.01
H2		279.20	2.93	0.074	16.26
H3		328.22	3.34	0.080	17.58
H4		281.35	1.86	0.084	18.46
H5		368.69	2.51	0.110	24.18
H6	Sunflower	362.42	5.04	0.112	24.62
H7		465.16	9.65	0.168	36.92
H8		246.99	5.95	0.080	17.58
H9		451.19	10.64	0.124	27.25
H10	Lime	320.84	4.04	0.100	21.98
H11		373.49	4.95	0.123	27.03
H12		474.19	6.43	0.090	19.78
H13		483.03	10.84	0.170	37.36
H14	Giant goldenrod	467.11	6.69	0.110	24.18
H15		414.99	5.72	0.120	26.37
H16	Basil	379.63	8.76	0.070	15.39
H17		395.46	4.33	0.130	28.57
H18	Oilseed rape	513.64	13.51	0.180	39.56
H19		372.47	9.45	0.110	24.18
H20	Buckwheat	668.58	14.44	0.210	46.15
H21	Polyfloral	496.40	5.82	0.125	27.47
H22		887.18	23.20	0.300	65.93
H23		782.16	13.81	0.280	61.54
H24		540.63	7.74	0.170	37.36
H25		432.29	5.89	0.140	30.77
H26		631.85	11.86	0.230	50.55
H27		688.81	18.67	0.220	48.35

The data from Table I show that *TPC*, *RSA* and *Q* are in acceptable agreement and bring similar information about the relative antioxidant capacity of the investigated honey samples. The correlation matrix for these variables, presented in Table II, shows large positive correlations between all the values. The *Q* para-

meter derived from the CV charge was strongly correlated with the *TPC*, with a correlation coefficient of 0.946. The level of significance for each correlation was $p < 0.000001$. Such statistically significant correlations clearly indicate that CV is a potentially applicable fast and informative experimental tool for the relative estimation of the antioxidant capacities of honeys.

TABLE II. Correlation coefficients between *TPC*, *RSA* and *Q*

	TPC	*RSA*	*Q*
TPC	1		
RSA	0.879	1	
Q	0.946	0.859	1

CONCLUSION

Serbian honey samples from different botanical origins and geographical regions were studied for their relative antioxidant capacity. Two methods, spectroscopic and electrochemical, were used for this purpose. Polyfloral honey samples had the highest values of *TPC*, while acacia samples showed the lowest values. Cyclic voltammetry was shown to be a highly attractive alternative method for the rapid relative checking of the antioxidant capacity of honeys. The linear dependence between this method and the commonly used Folin–Ciocalteu method was high with $r = 0.946$. The *TPC* values were compared with the antioxidant activities of the honey samples and a good correlation was obtained. Such a simple electrochemical technique could be considered as a valuable method for quality control, not only of honey but also for plant-derived food products in general.

SUPPLEMENTARY MATERIAL

A map of the geographical regions of Serbia from where the 27 honey samples under study were collected is available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. This work was performed within the framework of the research projects Nos. 172017 and 172030, supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

ИЗВОД

АНАЛИТИЧКЕ МОГУЋНОСТИ ЗА РЕЛАТИВНУ ПРОЦЕНУ АНТИОКСИДАТИВНОГ КАПАЦИТЕТА РАЗЛИЧИТИХ СОРТИ МЕДА ПРИКУПЉЕНИХ ИЗ РАЗЛИЧИТИХ РЕГИОНА СРБИЈЕ

УРОШ М. ГАШИЋ[1], ДАЛИБОР М. СТАНКОВИЋ[2], ДРАГАНА Ч. ДАБИЋ[2], ДУШАНКА М. МИЛОЈКОВИЋ-ОПСЕНИЦА[1], МАЈА М. НАТИЋ[1], ЖИВОСЛАВ Љ. ТЕШИЋ[1] и ЈЕЛЕНА Ј. МУТИЋ[1]

[1]Хемијски факултет Универзитета у Београду, Студентски трг 12–16, п. пр. 158, 11000 Београд и [2]Иновациони центар Хемијског факултета Универзитета у Београду, Студентски трг 12–16, п. пр. 158, 11000 Београд

Помоћу два различита приступа, спектроскопског и електрохемијског, тестиране су могућности релативне процене антиоксидативног капацитета узорака меда. Узорци меда различитог ботаничког порекла прикупљени су из различитих географских региона Србије. Садржај укупних фенола (*TPC*) одређен је применом Folin–Ciocalteu методе. Циклична волтаметрија (CV) са електродом од стакластог угљеника коришћена је као индикатор антиоксидативног потенцијала кроз промену волтаметријског наелектрисања у присуству узорака меда. Ради израчунавања Тролокс еквивалента антиоксидативног капацитета (*TEAC*) испитиваног меда, снимани су и циклични волтамограми са Тролокс стандардом. Резултати су изражени као μmol Тролокс еквивалента по kg узорка (μmol TE kg^{-1}). Показано је да су резултати цикличне волтаметрије у доброј корелацији са резултатима који се добијају применом Folin–Ciocalteu реагенса, као и са антиоксидативним потенцијалом (*RSA*) који је одређен употребом 1,1-дифенил-2-пикрилхидразил ($DPPH^{\bullet}$) радикала. Резултати указују на то да је циклична волтаметрија ефикасна метода и да може бити алтернативна метода за брзу релативну процену антиоксидативног капацитета меда. Нађено је да највеће вредности *TPC* имају полифлорни узорци меда, док најмање вредности показује багремов мед.

(Примљено 13. марта, ревидирано 15. децембра, прихваћено 25. децембра 2015)

REFERENCES

1. D. D. Schramm, M. Karim, H. R. Schrader, R. R. Holt, M. Cardetti, C. L. Keen, *J. Agr. Food Chem*. **51** (2003) 1732
2. U. Gašić, S. Kečkeš, D. Dabić, J. Trifković, D. Milojković-Opsenica, M. Natić, Ž. Tešić, *Food Chem*. **145** (2014) 599
3. V. T. Tumbas, J. J. Vulić, J. M. Čanadanović-Brunet, S. M. Đilas, G. S. Ćetković, S. Stajčić, D. I. Štajner, B. M. Popović, *Acta periodica technol.* **43** (2012) 293
4. S. M. Savatović, D. J. Dimitrijević, S. M. Đilas, J. M. Čanadanović-Brunet, G. S. Ćetković, V. T. Tumbas, D. I. Štajner, *Acta periodica technol.* **42** (2011) 145
5. S. Ž. Gorjanović, J. M. Alvarez-Suarez, M. M. Novaković, F. T. Pastor, L. Pezo, M. Battino, D. Ž. Sužnjević, *J. Food Compos. Anal.* **30** (2013) 13
6. V. Roginsky, E. A. Lissi, *Food Chem.* **92** (2005) 235
7. S. Chevion, M. A. Roberts, M. Chevion, *Free Radical Bio. Med.* **28** (2000) 860
8. J. N. Veljković, A. N. Pavlović, S. S. Mitić, S. B. Tošić, G. S. Stojanović, B. M. Kaličanin, D. M. Stanković, M. B. Stojković, M. N. Mitić, J. M. Brcanović, *J. Food Nutr. Res.* **52** (2013) 12
9. J. Piljac-Žegarac, L. Valek, T. Stipčević, S. Martinez, *Food Chem.* **121** (2010) 820
10. R. Bhattacharyya, B. Tudu, S. C. Das, N. Bhattacharyya, R. Bandyopadhyay, P. Pramanik, *J. Food Eng.* **109** (2012) 120

11. R. Keyrouz, M. L. Abasq, C. Le Bourvellec, N. Blanc, L. Audibert, E. ArGall, D. Hauchard, *Food Chem*. **121** (2010) 820
12. S. Kečkeš, U. Gašić, T. Ćirković Veličković, D. Milojković-Opsenica, M. Natić, Ž. Tešić, *Food Chem*. **138** (2013) 32
13. K. Lazarević, F. Andrić, J. Trifković, Ž. Tešić, D. Milojković-Opsenica, *Food Chem.* **132** (2012) 2060
14. A. Meda, C. E. Lamien, M. Romito, J. Millogo, O. G. Nacoulma, *Food Chem.* **91** (2005) 571
15. V. L. Singleton, J. A. Rossi, *Am. J. Enol. Viticul.* **16** (1965) 144
16. H. Li, X. Wang, P. Li, Y. Li, H. Wang, *J. Food Drug Anal.* **16** (2008) 67
17. J. Hintze, *NCSS and PASS Number Cruncher Statistical Systems*, Kaysville, UT, 2001
18. J. Sochor, J. Dobes, O. Krystofova, B. Ruttkay-Nedecky, P. Babula, M. Pohanka, T. Jurikova, O. Zitka, V. Adam, B. Klejdus, R. Kizek, *Int. J. Electrochem. Sci*. **8** (2013) 8464
19. P. A. Kilmartin, *Antioxid. Redox. Sign.* **6** (2001) 941
20. B. Dobre, G. Gâdei, L. Pătraşcu, A. M. Elisei, R. Segal, *Ann. Univer. Dunarea Jos Galati, Fascicle VI*, *Food Technol*. **34** (2010) 67
21. J. Piljac-Žegarac, T. Stipčević, A. Belščak, *J. ApiProduct ApiMedical Sci.* **1** (2009) 43
22. J. Bertoncelj, U. Doberšek, M. Jamnik, T. Golob, *Food Chem.* **105** (2007) 822
23. N. Gheldof, X. Wang, N. J. Engeseth, *J. Agr. Food Chem.* **50** (2002) 5870
24. K. Lazarević, J. Trifković, F. Andrić, Ž. Tešić, I. Anđelković, D. Radović, N. Nedić, D. Milojković-Opsenica, *J. Serb. Chem. Soc.* **78** (2013) 1875.