ISSN 1061-9348, Journal of Analytical Chemistry, 2015, Vol. 70, No. 8, pp. 974–982. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © G.K. Ziyatdinova, F. Nguen Cong, H.C. Budnikov, 2015, published in Zhurnal Analiticheskoi Khimii, 2015, Vol. 70, No. 8, pp. 854–862.

ARTICLES

Assessment of the Antioxidant Properties of Micellar Spice Extracts by Galvanostatic Coulometry with Electrogenerated Hexacyanoferrate(III) Ions

G. K. Ziyatdinova, F. Nguen Cong, and H. C. Budnikov

Butlerov Institute of Chemistry, Kazan Federal University, ul. Kremlevskaya 18, Kazan, 420008 Russia

e-mail: Ziyatdinovag@mail.ru

Received July 28, 2014; in final form, December 16, 2014

Abstract—The stoichiometric coefficients of reactions of individual antioxidants of spices—rutin; quercetin; eugenol; curcumin; capsaicin; thymol; tannin; catechol; and gallic, *p*-coumaric, caffeic, chlorogenic, and rosmarinic acids—with electrogenerated hexacyanoferrate(III) ions in a Triton X100 micellar medium are determined; the corresponding reaction schemes are proposed. It is found that the maximum recovery of active components from spices is achieved at a single extraction with a 0.25 mM Triton X100 solution for 10 min upon sonication. The raw material : extractant ratio is 1 : 30 for all spices except for caraway, red sweet pepper, and nutmeg (1 : 40) and cumin and red pepper (1 : 20). The ferric reducing power (FRP) of micellar extracts of 16 spices is evaluated. It is found that the highest FRP is characteristic for cinnamon and clove (122 ± 4 and 106 ± 6 C/g, respectively), while the lowest value of this parameter is typical for cumin and turmeric (3.3 ± 0.3 and 2.5 ± 0.3 C/g, respectively), which is because of the nature of the active components of spices and the extractant. The FRP of spices correlates with their antiradical activity and the total phenolic content with $r = 0.9714$ and 0.9936, respectively.

Keywords: galvanostatic coulometry, micellar media, surfactants, ferric reducing power, antioxidant properties, spices, food analysis

DOI: 10.1134/S1061934815080195

Spices of plant origin are complex in composition and contain a large number of components of different nature, including those possessing antioxidant properties. The antioxidant properties are caused by the presence of compounds of different classes such as vitamins, flavonoids, terpenes, carotenoids, and phytoestrogens, which enable the use of spices as food preservatives [1]. It should be noted that the largest part of antioxidants are phenolic compounds.

In accordance with the database of vegetable raw materials [2], some plants contain up to 40 various antioxidants; for example, fennel, oregano, onion, and thyme bear 35, 34, 32, and 32 different antioxidants. Therefore, approaches enabling an integral assessment, that is, yielding total parameters characterizing the object of study as a whole are the most promising for the evaluation of the antioxidant properties of spices. The screening of integral parameters is often sufficient for routine analysis, which greatly simplifies the procedure and reduces its cost [3]. The ferric reducing power, based on the oxidation of antioxidants under action of Fe(III) compounds, is among these parameters.

The FRP is determined spectrophotometrically by the reduction of Fe(III)–tripyridyltriazine complex to a Fe(II) complex under the effect of antioxidants. The resulting complex has an intense blue color and an absorption band at 593 nm. The amount of antioxidants is proportional to the amount of colored reaction product [4]. Another method to evaluate the FRP is based on the reaction of antioxidants with electrogenerated hexacyanoferrate(III) ions in galvanostatic coulometry [5]. The FRP is determined as the quantity of electricity taken for titration of a sample. In this case, the FRP can be easily expressed in equivalents of any individual antioxidant, knowing the stoichiometric coefficients of its reaction with the titrant.

Spices are added to food in a variety of ways: in the form of plant material or individual components isolated from the corresponding extracts. Conditions and type of extraction are determined by the type of antioxidants, which need to be isolated. The proper selection of the extraction method offers the preconcentration of antioxidants from plant material. To extract active components from spices, organic solvents are commonly used, particularly, methanol, ethanol, acetone, and ethyl acetate [6], which are volatile and toxic. Aqueous micellar media of surfactants are attractive as a substitute of organic solvents: they ensure adequate solubility of both hydrophilic and hydrophobic antioxidants [7] and set up conditions

close to the actual extraction conditions when cooking.

The goal of the present work is to assess the antioxidant properties of micellar spice extracts by galvanostatic coulometry with electrogenerated hexacyanoferrate(III) ions.

EXPERIMENTAL

Reagents. We used 95% rutin trihydrate (Fluka, Germany); 98% quercetin dihydrate (Sigma, Germany); 98% catechol hydrate (Sigma, Germany); tannin (pharmacopoeial grade; Fluka, Germany); the following acids: 99% gallic acid (Sigma, Germany), 98% caffeic acid (Sigma, Germany), 95% chlorogenic acids (Aldrich, Germany), 98% rosmarinic acid (Sigma, China), and 98% *p*-coumaric acid (Sigma, Germany); 70% curcumin from *Curcuma longa* (Sigma, Germany); 50% capsaicin (Sigma, India); 99% eugenol (Aldrich, Germany); and 99.5% thymol (Sigma, Germany). Other reagents were of cp grade; we also used methanol (cp grade) and rectified ethanol.

Stock 0.01 M solutions of antioxidants were prepared by dissolving their accurately weighed portions in 10 mL of a 0.25 mM solution of Triton X100 (Sigma, Germany). Dilute solutions were prepared immediately before the measurements in 10.0-mL flasks, by diluting the stock solutions with a 0.25 mM Triton X100 solution up to the mark.

Measurement procedure. *Coulometric determinations* were performed using an Ekspert-006 analyzer (Ekoniks-Expert). Ions $Fe(CN)_6^{3-}$ were electrogenerated from 0.1 M $K_4Fe(CN)_6$ in a 0.5 M NaOH solution with a platinum electrode ($S = 50$ mm^2) at a constant current intensity of 5.0 mA. The cathode was a coiled platinum wire (l = 2.0 cm). A cathode chamber, wherein the auxiliary electrode was set, was separated from the anode chamber by a porous glass septum. The titration end-point was determined by amperometry with two polarized platinum electrodes (ΔE = 200 mV).

Twenty milliliters of a supporting electrolyte was inserted in a 50-mL electrochemical cell; then, the working (generator), auxiliary, and indicator electrodes were placed into the cell. The aliquot portions were selected so that the titration time took no more than 5 min.

The end-point was determined by an inflection in the titration curves. The theoretically calculated mass (g) of the substance released at the electrode during electrolysis was found by Faraday's law.

Photometric measurements were performed using a PE-5300 VI spectrophotometer (Ekros, Russia).

The ferric reducing power was found as the quantity of electricity required for titration of a sample recalculated per 1 g of dry spice:

$$\mathrm{FRP} = \frac{QV_{\mathrm{extr}}1}{V_{\mathrm{al}}m_{\mathrm{s}}},$$

where Q was the amount of electricity required for titration, C; V_{extr} was the volume of extract, mL; V_{al} was the aliquot volume of the extract, mL; m_{s} was the weight of spice taken for extraction, g; and 1 g was the weight of spices, for which the FRP was recalculated, g.

Extraction with a Triton X100 solution. An accurately weighed portion of spices (0.1000 ± 0.0005 g) was placed in a 5.0-mL flask, 2.0 to 4.0 mL of a 0.25 mM Triton X100 solution was added, and the mixture was placed in a Sonorex Super RK 100 100H ultrasonic bath for 10 min. The resulting extract was filtered and used for the evaluation of the antioxidant properties.

Antiradical activity was evaluated by the reaction with 2,2'-diphenyl-1-picrylhydrazyl (DPPH; Sigma, Germany) [8]. A standard 61-μM solution of DPPH was prepared by dissolving its accurately weighed portion in methanol. To evaluate the antiradical activity, 3.0 mL of a DPPH solution and 5 μL of a spice extract were placed in a test tube, mixed thoroughly, and incubated in the dark at room temperature for 30 min. The absorbance of the solution was then measured at 515 nm against methanol (3.0 mL). The antiradical activity was calculated as the ratio of the absorption intensities of DPPH before and after the reaction with antioxidants of the spice extract and expressed as the weight of gallic acid per 1 g of dry spices (mg/g).

Total phenolic concentration by Folin–Ciocalteu was determined spectrophotometrically and recalculated for gallic acid [9]. The method is based on the reaction of phenolic compounds with the Folin–Ciocalteu reagent (Sigma-Aldrich, Germany), in which phenolic groups are oxidized and the reagent is reduced to a mixture of molybdenum and tungsten oxides, colored blue. The color intensity is proportional to the concentration of phenolic compounds. The total content of phenolic antioxidants is expressed in units of gallic acid recalculated per 1 g of spice.

The statistical treatment of results was carried out for three or five measurements at a confidence level of 0.95. Results are presented as $X \pm \Delta X$, where X is the mean value and ΔX is the confidence interval. The corresponding values of the relative standard deviation (RSD) were also calculated.

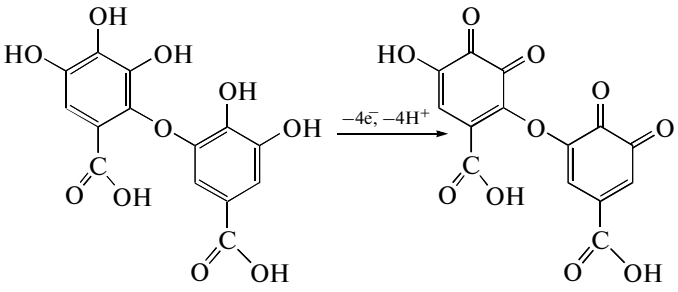
RESULTS AND DISCUSSION

Reactions of phenolic antioxidants of spices with electrogenerated $Fe(CN)_6^{3-}$ ions in the Triton X100 medium. Spices contain a large number of components that exhibit antioxidant properties. Electrogenerated $Fe(CN)_6^{3-}$ ions are capable of oxidizing mainly phenolic compounds [10]; therefore, the reactions of individual phenolic antioxidants of spices with the

above titrant are studied under galvanostatic coulometry in a Triton X100 micellar solution.

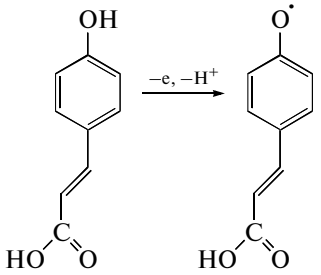
To determine the stoichiometry of the reactions, standard solutions of rutin, quercetin, catechol, tannin, thymol, eugenol, curcumin, capsaicin, *p*-coumaric acid, caffeic acid, chlorogenic acid, and rosmarinic acid were coulometrically titrated in a 0.25 mM Triton X100 solution. At this concentration of the surfactant, a 100% current yield of the titrant is observed in the cell [11]. It is found that all the antioxidants react with $Fe(CN)_6^{3-}$ ions rapidly and quantitatively, with the exception of curcumin, which is poorly soluble in a Triton X100 micellar medium due to its high hydrophobicity, which inhibits the evaluation of its reactivity with respect to the titrant. The stoichiometric coefficients of reactions are presented in Table 1.

The oxidation of antioxidants under the action of $Fe(CN)_6^{3-}$ ions proceeds with the involvement of hydroxyl groups. In an alkaline medium at pH > 11 and under the effect of atmospheric oxygen, gallic acid is dimerized with the formation of dehydrodigallic acid [12], which is then oxidized by $Fe(CN)_6^{3-}$ ions to the corresponding di-*o*-quinone (Eq. (1)).



(1)

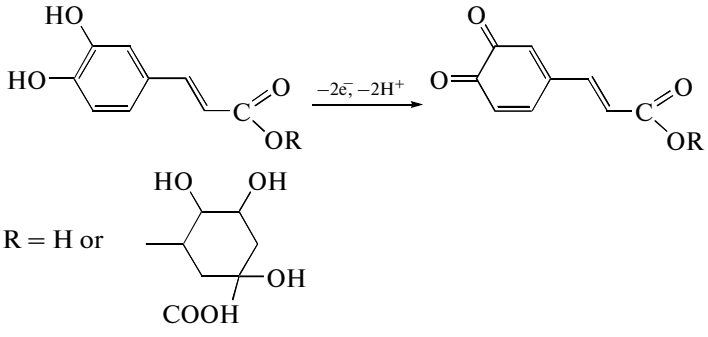
In the case of monophenols (thymol, eugenol, capsaicin, and *p*-coumaric acid), one electron participates in the reaction with $Fe(CN)_6^{3-}$ ions, and a relatively stable phenoxyl radical is formed (Eq. (2)).



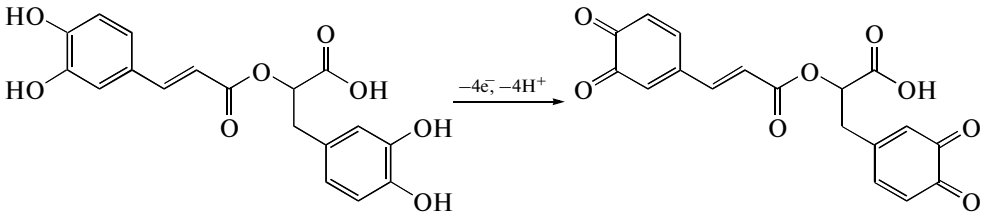
(2)

The number of electrons involved in the reaction of tannin coincides with the number of hydroxyl groups in its molecule. Flavonoids are oxidized by hydroxyl groups in aromatic rings. In a rutin molecule, hydroxyl groups bonded to the benzene ring are only oxidized. The glycosidic moiety does not react with the titrant, which was confirmed by titration of standard solutions of glucose and rhamnose.

Chlorogenic, caffeic, and rosmarinic acids are oxidized with hydroxyl groups to form the corresponding *o*- and di-*o*-quinones (Eqs. (3) and (4)).



(3)



(4)

Table 1. Stoichiometric coefficients of the reactions of phenolic antioxidants of spices with $Fe(CN)_6^{3-}$ ions

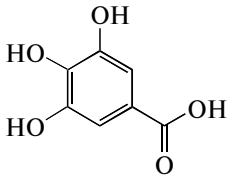
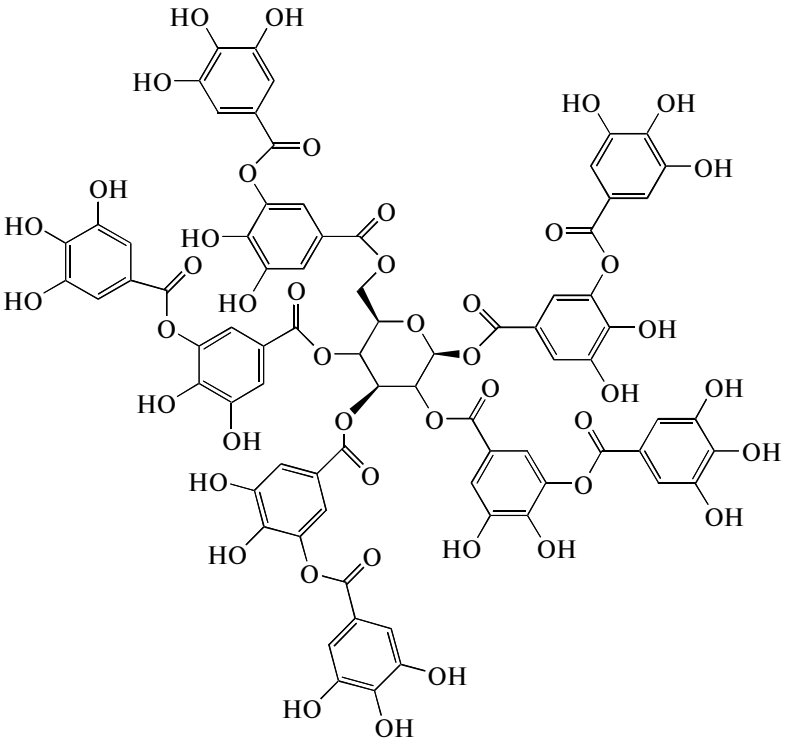
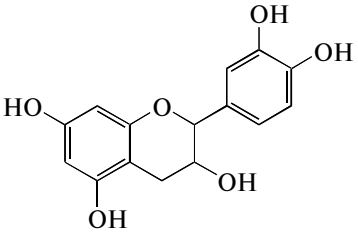
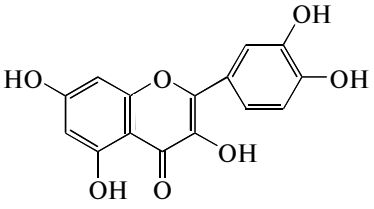
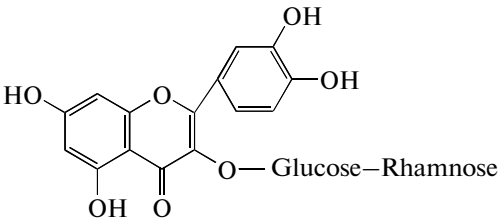
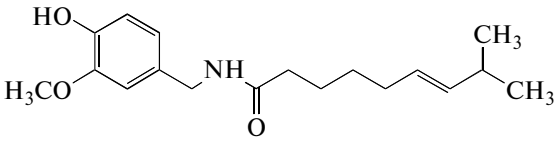
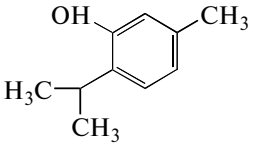
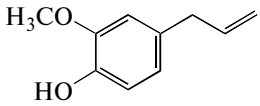
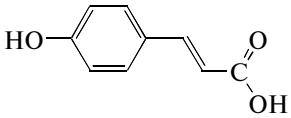
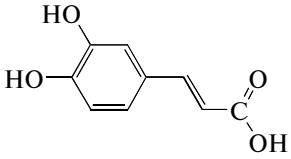
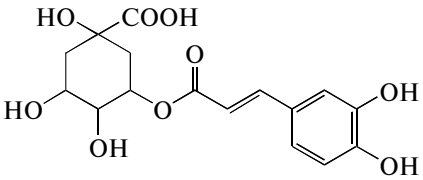
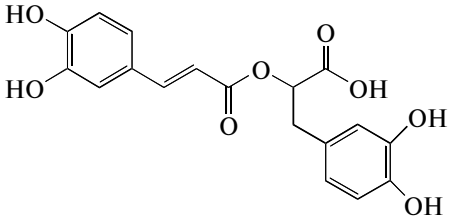
Compound	Chemical structure	ν(compound) : ν(titrant)
Gallic acid		1 : 4
Tannin		1 : 25
Catechin		1 : 4
Quercetin		1 : 4

Table 1. (Contd.)

Compound	Chemical structure	ν(compound) : ν(titrant)
Rutin		1 : 4
Capsaicin		1 : 1
Thymol		1 : 1
Eugenol		1 : 1
p-Coumaric acid		1 : 1
Caffeic acid		1 : 2
Chlorogenic acid		1 : 2
Rosmarinic acid		1 : 4

Based on the results, electrogenerated $Fe(CN)_6^{3-}$ ion is proposed as a reagent for evaluating the antioxidant properties of micellar spice extracts.

Extraction of active components of spices by a Triton X100 micellar solution. A micellar medium of Triton X100 nonionic surfactant is selected as the extractant. Triton X100 is an easily available and inexpensive surfactant; it solubilizes well a wide range of compounds of different nature. We found the extraction conditions of the active components of spices using a 0.25 mM Triton X100 solution with sonication. It is determined that the maximum extraction is achieved with a single extraction for 10 min.

The ratio of raw material–extractant was set for each individual spice. The extraction efficiency was evaluated coulometrically by the reaction with electrogenerated $Fe(CN)_6^{3-}$ ions and expressed as a quantity of electricity required for the titration of the extract (Fig. 1). For the majority of spices, the maximum recovery is observed at raw material–extractant ratio of 1 : 30, except for caraway, red sweet pepper, and nutmeg (1 : 40) and cumin and red pepper (1 : 20). For further measurements, the extraction was carried out again at the ratio of raw material–extractant, ensuring the maximum recovery.

The FRP of the spice extracts was evaluated (Table 2). Cinnamon and clove demonstrated the highest FRP, which is because of high concentrations of hydroxycinnamic acid and eugenol in cinnamon [13] and gallic acid and eugenol in clove [14, 15]. These results agree with the data [14] on their antioxidant activity. Juniper berries containing a wide range of phenolic antioxidants, in particular, flavonoids, stilbenes [16], and phenolic acids [17], have a rather high FRP. This is confirmed by the data on total phenolic content [17] and the antiradical activity and reducing power of the methanol extracts of juniper berries [18].

The next group of spices with comparable values of FRP combines basil, oregano, and rosemary. Basil and oregano contain rosmarinic and hydroxycinnamic acids. Oregano also contains some flavonoids such as luteolin, apigenin, dihydrokaempferol, and dihydroquercetin [19, 20]. Rosemary contains carnosol and carnosic acid [21–23], rosmanol and rosmarinic acid [24, 25], and a number of related compounds, for example, epi- and isorosmanol [26], rosemarydiphenol [27], and rosmadial [28]. All of them react with electrogenerated $Fe(CN)_6^{3-}$ ions and contribute to the FRP.

For other spices, rather moderate values of FRP are observed because of the low concentrations of phenolic antioxidants and relatively high concentrations of unsaturated lipophilic compounds in them, poorly extracted with Triton X100 and nonreactive with the titrant. The lowest FRP was obtained for turmeric because of a high concentration of curcumin [29],

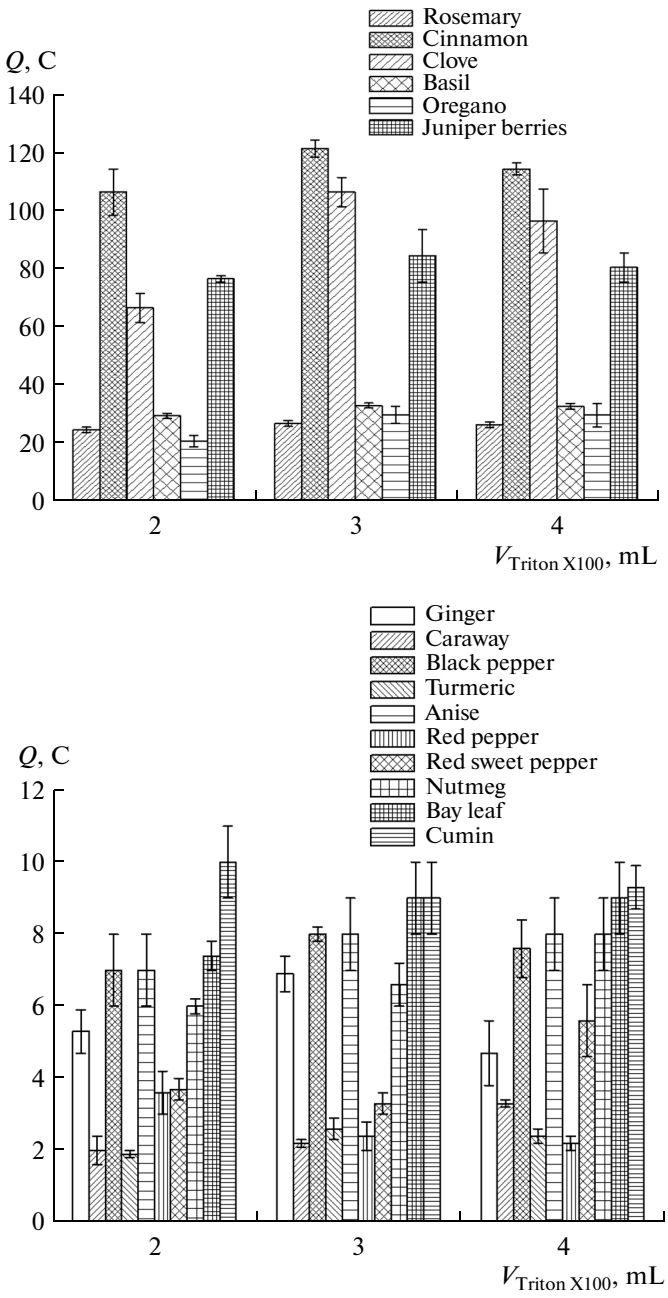


Fig. 1. Effect of the extractant volume on the extraction efficiency of active components from spices.

which is virtually not extracted by the Triton X100 micellar solution.

Currently, the conventional way of evaluating the antioxidant properties is the spectrophotometric determination of the antiradical activity and total phenolic content. Since gallic acid (GA) is one of the most common standards, the parameters are expressed in weight units recalculated for 1 g of this spice. For this purpose, the calibration curves described by Eqs. (5) and (6) were preplotted for the antiradical activity

Table 2. Ferric reducing power of the spice extracts; extractant, 0.25 mM Triton X100 ($n = 5$, $P = 0.95$)

Spice	Brand	FRP, C/g	RSD, %
Cinnamon	Appetita	122 ± 4	2.8
Clove	″	106 ± 6	4.5
Juniper berries	″	82 ± 3	3.1
Basil	″	34 ± 2	3.6
Oregano	Galeo	30 ± 1	3.7
Rosemary	Appetita	26.8 ± 0.9	2.7
Cumin	Magiya vostoka	10.3 ± 0.8	6.0
Bay leaf	″	9.2 ± 0.6	5.2
Anise	Appetita	8.4 ± 0.2	1.5
Nutmeg	Interjarek	8.2 ± 0.6	5.9
Black pepper	Volshebnoe derevo	7.7 ± 0.9	9.1
Ginger	″	6.9 ± 0.2	2.7
Red sweet pepper	″	5.6 ± 0.6	8.8
Red pepper	Galeo	3.7 ± 0.1	2.8
Coriander	Appetita	3.5 ± 0.2	3.7
Caraway	Volshebnoe derevo	3.3 ± 0.3	6.6
Turmeric	M&S	2.5 ± 0.3	8.4

Table 3. Antiradical activity of spices and total phenolic content in them ($n = 3$, $P = 0.95$)

Spice	AA, mg GA/g	RSD, %	TPh, mg GA/g	RSD, %
Cinnamon	17 ± 4	10.6	54 ± 3	2.1
Clove	50 ± 5	7.9	111 ± 6	2.0
Juniper berries	30 ± 4	10.5	73 ± 2	0.9
Basil	12 ± 3	10.2	33 ± 2	2.1
Oregano	12 ± 3	10.7	31 ± 1	1.9
Rosemary	10 ± 3	10.7	24 ± 1	1.7
Cumin	1.4 ± 0.1	7.9	12 ± 1	3.4
Bay leaf	10 ± 1	7.8	14.6 ± 0.8	2.1
Anise	12 ± 2	10.9	6.7 ± 0.4	2.3
Nutmeg	6 ± 2		9.4 ± 0.5	2.0
Black pepper	2.2 ± 0.3	10.9	6.7 ± 0.3	2.1
Ginger	8 ± 2	9.6	11.0 ± 0.6	2.1
Red sweet pepper	0.8 ± 0.1	12.6	7.5 ± 0.4	2.1
Red pepper	0.36 ± 0.04	7.9	4.7 ± 0.3	2.2
Coriander	15 ± 2	7.8	5.6 ± 0.3	2.2
Caraway	0.54 ± 0.05	7.9	2.5 ± 0.1	2.0
Turmeric	0.58 ± 0.06	8.1	4.5 ± 0.2	1.9

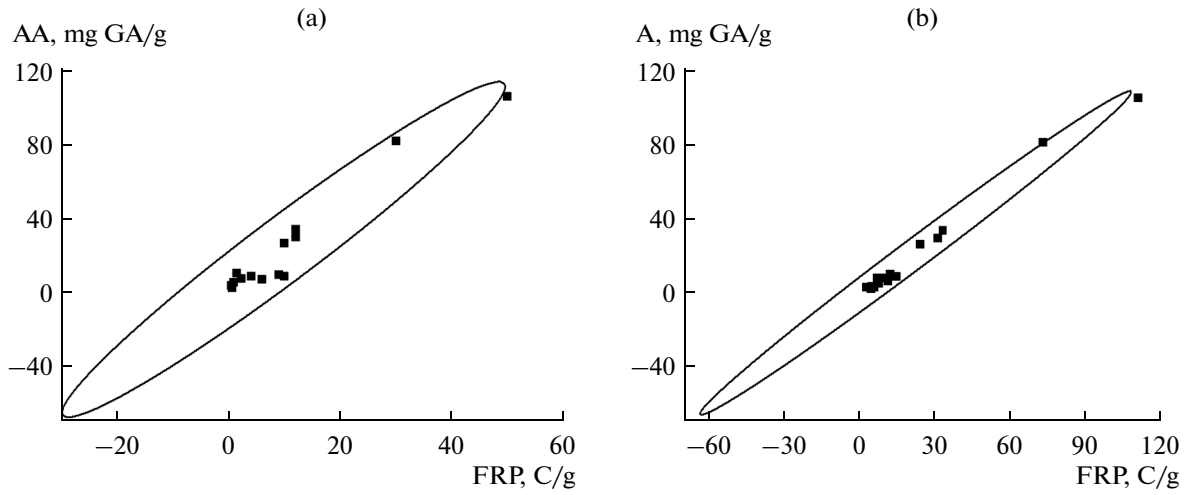


Fig. 2. Correlation of the FRP of spices with (a) the antiradical activity and (b) total phenolic content.

(AA) and the total phenolic content (TPh), respectively.

$$\mathrm{AA}[\%] = (5 \pm 5) + (101 \pm 11)c_{\mathrm{GA}}[\mathrm{mg/mL}], \quad R^2 = 0.9576, \tag{5}$$

$$\dot{\mathrm{A}}[\mathrm{rel.\ units}] = (-0.015 \pm 0.006) + (0.48 \pm 0.01)c_{\mathrm{GA}}[\mathrm{mg/mL}], \quad R^2 = 0.9987. \tag{6}$$

The results of the spectrophotometric evaluation of parameters characterizing the antioxidant properties of spices are shown in Table 3. The general trend of the antioxidant properties of spices is maintained with a few exceptions. The difference for some spices is due to the nature of chemical reactions forming the basis of the determination. Thus, the presence of components capable of undergoing radical reactions is of key significance for the antiradical activity. Note that DPPH is a bulk radical, and, therefore, the nature and rate of its interaction differ from the reactions involving oxygen radical species characterized by a high penetration and reaction ability [30].

The main contribution to the total concentration of phenols is introduced by compounds with aromatic hydroxyl groups, while the hydroxyl groups of reducing sugars and ascorbic acid can also be oxidized, which can lead to overstated results [31].

The comparative analysis of FRP and conventional parameters showed a correlation (Fig. 2) with the coefficients 0.9714 and 0.9936 for the antiradical activity and the total phenolic content, respectively.

Thus, the coulometric titration with electrogenerated $Fe(CN)_6^{3-}$ ions allows an adequate assessment of the FRP of spices; the method can be recommended for the rapid screening of their antioxidant properties.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 14-03-31173-mol_a.

REFERENCES

1. Calucci, L., Pinzono, C., Zandomeneghi, M., Capocchi, A., Ghiringhelli, S., Saviozzi, F., Tozzi, S., and Galleschi, L., *J. Agric. Food Chem.*, 2003, vol. 51, no. 4, p. 927.
2. USDA, ARS, National Genetic Resources Program. Phytochemical and Ethnobotanical Databases. [Online Database]. National Germplast Resources Laboratory, Beltsville, Maryland, 25 June 2003.
3. Chernysheva, N.N., Ziyatdinova, G.K., and Budnikov, G.K., *Vestnik Tatarst. Otd. Ros. Ecol. Akad.*, 2004, no. 1, p. 54.
4. Benzie, I.F. and Strain, J.J., *Anal. Biochem.*, 1996, vol. 239, no. 1, p. 70.
5. Ziyatdinova, G., Nizamova, A., and Budikov, H., *Food Anal. Methods*, 2011, vol. 4, no. 3, p. 334.
6. Suhaj, M., *J. Food Compos. Anal.*, 2006, vol. 19, nos. 6–7, p. 531.
7. Ziyatdinova, G.K., Ziganshina, E.R., and Budnikov, H.C., *J. Anal. Chem.*, 2012, vol. 67, no. 11, p. 869.
8. Arteaga, J.F., Ruiz-Montoya, M., Palma, A., Alonso-Garrido, G., Pintado, S., and Rodriguez-Mellado, J.M., *Molecules*, 2012, vol. 17, no. 5, p. 5126.
9. Singleton, V.L. and Rossi, J.A., *Am. J. Enol. Vitic.*, 1965, vol. 16, no. 3, p. 144.
10. Ziyatdinova, G.K., Nizamova, A.M., and Budnikov, G.K., *Butlerovsk. Soobshch.*, 2011, vol. 24, no. 4, p. 72.
11. Ziyatdinova, G., Ziganshina, E., and Budnikov, H., *Anal. Chim. Acta*, 2012, vol. 744, p. 23.
12. Oniki, T. and Takahama, U., *J. Wood Sci.*, 2004, vol. 50, no. 6, p. 545.

13. Wu, T.S., Leu, Y.L., Chan, Y.Y., Yu, S.M., Teng, C.M., and Su, J.D., *Phytochemistry*, 1994, vol. 36, no. 3, p. 785.

14. Parthasarathy, V.A., Chempakam, B., and Zachariah, T.J., *Chemistry of Spices,* CABI, 2008.

15. Kramer, R.E., *J. Am. Oil Chem. Soc.*, 1985, vol. 62, no. 1, p. 111.

16. Miceli, N., Trovato, A., Dugo, P., Cacciola, F., Donato, P., Marino, A., Bellinghieri, V., La Barbera, T.M., Guvenc, A., and Taviano, M.F. *J. Agric. Food Chem.*, 2009, vol. 57, no. 15, p. 6570.

17. Miceli, N., Trovato, A., Marino, A., Bellinghieri, V., Melchini, A., Dugo, P., Cacciola, F., Donato, P., Mondello, L., Guvenc, A., De Pasquale, R., and Taviano, M.F., *Food Chem. Toxicol.*, 2011, vol. 49, no. 10, p. 2600.

18. Taviano, M.F., Marino, A., Trovato, A., Bellinghieri, V., Melchini, A., Dugo, P., Cacciola, F., Donato, P., Mondello, L., Guvenc, A., De Pasquale, R., and Miceli, N., *Food Chem. Toxicol.*, 2013, vol. 58, p. 22.

19. Ziyatdinova, G.K. and Budnikov, H.C., *J. Anal. Chem.*, 2014, vol. 69, no. 10, p. 990.

20. Skerget, M., Kotnik, P., Hadolin, M., Hras, A.R., Simovic, M., and Knez, Z., *Food Chem.*, 2005, vol. 89, no. 2, p. 191.

21. Aruoma, O.I., Halliwell, B., Aeschbach, R., and Loliger, J., *Chem. Unzerer Zeit*, 1992, vol. 22, no. 2, p. 257.

22. Bracco, U., Loliger, J., and Viret, J.-L., *J. Am. Oil Chem. Soc.*, 1981, vol. 58, no. 6, p. 686.

23. Chen, Q., Shi, H., and Ho, C.-T., *J. Am. Oil Chem. Soc.*, 1992, vol. 69, no. 10, p. 999.

24. Nakatani, N. and Inatani, R., *Agric. Biol. Chem.*, 1981, vol. 45, no. 10, p. 2385.

25. Gerhardt, U. and Schroter, A., *Fleischwirtschaft*, 1983, vol. 63, p. 1628.

26. Nakatani, N. and Inatani, R., *Agric. Biol. Chem.*, 1984, vol. 48, no. 8, p. 2081.

27. Houlihan, C.M., Ho, C.-T., and Chang, S.S., *J. Am. Oil Chem. Soc.*, 1984, vol. 61, no. 6, p. 1036.

28. Nakatani, N. and Inatani, R., *Agric. Biol. Chem.*, 1983, vol. 47, no. 2, p. 353.

29. Chattopadhyay, I., Biswas, K., Bandyopadhyay, U., and Banerjee, R.K., *Curr. Sci.*, 2004, vol. 87, no. 1, p. 44.

30. Magalhaes, L.M., Segundo, M.A., Reis, S., and Lima, J.L.F.C., *Anal. Chim. Acta*, 2008, vol. 613, no. 1, p. 1.

31. Waterhouse, A.L., in *Current Protocols in Food Analytical Chemistry*, Wrolstad, R.E., Ed., New York: Wiley, 2002, vol. 247, p. 237.

Translated by O. Zhukova