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ARTICLES

Micellar Extraction of Active Components from Spices and Evaluation of the Ce(IV)-Based Reducing Capacity of the Extracts

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Abstract—We found the conditions for the micellar extraction of active components from spices using 0.1 M Brij® 35 as an extractant and intensifying the process with ultrasonic treatment. A single extraction for 10 min ensures the maximum recovery of active components. The raw material-to-extractant ratio varies widely depending on the type of the spice. To characterize the extracts obtained, the Ce(IV)-based reducing capacity was used, based on the interaction of the extract components with electrogenerated Ce(IV). Using ascorbic acid as an example, we proved that the Brij® 35 micellar medium does not affect the current efficiency of the coulometric titrant. Stoichiometric coefficients of several biologically active compounds with electrogenerated Ce(IV) were determined. The Ce(IV)-based reducing capacity of 20 spices was determined. The results were compared with total antioxidant parameters.

Keywords: micellar extraction, spices, Ce(IV)-based reducing capacity, coulometric titration, food analysis

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Food is a primary source of biologically active substances for humans; spices exhibiting a wide range of biological activity, including antioxidant properties, should be noted first [1, 2]. These beneficial properties of spices have predetermined their use as food preservatives and flavoring additives. Many biologically active substances used in the production of biologically active supplements or medicines were obtained by isolation from plant materials, including spices. Spice extracts are actively used in the food industry. In this regard, the search for and the development of methods for extracting active components from spices are of theoretical and applied interest. To solve this problem, liquid, solid-phase, and supercritical fluid extraction were used [3, 4]. Ultrasonic treatment is often used to intensify the extraction process. This significantly shortens extraction time and reduces the consumption of the extractant and the number of extraction steps. Together with the relatively mild conditions for the extraction of analytes [3–5], the method corresponds to the concept of "green chemistry." Another way to implement this concept is to switch from extraction with organic solvents to nontoxic aqueous media, which are micellar media based on surfactants. Their primary advantage over other extractants is in the ability to solubilize both polar and nonpolar compounds, which is essential for generalizing the characteristics of the extracts and their further use [3]. Only a few examples of the micellar extraction of active components from spices were described in publications. For example, Cortés-Rojas et al. [6] proposed the extraction of eugenol and polyphenols from cloves by dynamic maceration at 50°C for 40 min with 5% Tween 80 at pH 12, ensuring a higher yield of the analytes compared to conventional extraction with ethanol and water–ethanol mixtures [6].

A combination of extraction with surfactant-containing media and ultrasonic treatment is of practical interest. Micellar media based on ionic liquids under ultrasonic treatment were successfully used to extract tanshinones (bioactive components of salvia) from *Salvia miltiorrhiza bunge* [7]. Ultrasonic treatment helps extracting phenolic antioxidants from spices using 0.25 mM Triton X-100 [8]; however, the approach is not applicable to extracting curcuminoids. This restriction was successfully overcome by the ultrasonic extraction of antioxidants from spices by the Brij® 35 micellar medium [9, 10]. This approach offers a higher yield of phenolic antioxidants compared to extraction with organic solvents [9].

In this work, we used 0.1 M Brij® 35 for the micellar extraction of active components from spices and evaluated the efficiency of this procedure by coulometric titration with electrogenerated Ce(IV). The use of Ce(IV) as a coulometric titrant makes it possible to cover a wide range of biologically active substances capable of oxidation, including antioxidants of various

nature, because Ce(IV) exhibits strong oxidant properties. Ce(IV) is a one-electron oxidant, which to some extent allows the researcher to approach to reaction conditions in living systems with participation of reactive oxygen and nitrogen species. We consider the electrogeneration of Ce(IV) in the presence of Brij® 35 and its effect on the reactions of the titrant with biologically active compounds. The Ce(IV)-based reducing capacity of the obtained micellar extracts of spices was determined and compared with other antioxidant parameters.

EXPERIMENTAL

Reagents and solutions. We used 97% rutin trihydrate (Alfa Aesar, United Kingdom), 98% quercetin dihydrate, 98% catechin hydrate (Sigma, Germany), pharmacopoeial tannin (Fluka, Germany), 99% gallic acid, 98% caffeic acid, 98% rosmarinic acid, and 98% *p*-coumaric acid (Sigma, Germany), 70% curcumin from *Curcuma longa*, 96% α-tocopherol and 95% retinol (Sigma, Germany), 50% capsaicin (Sigma, India), 95% chlorogenic acid, 99% ferulic acid, 99% eugenol, and 98% syringaldehyde (Aldrich, Germany), and 99.5% thymol (Sigma, Germany). Their standard 0.40–10 mM solutions were prepared by dissolving accurately weighed portions in 0.1 M Brij® 35 (Sigma, Germany) under ultrasonic treatment in a WiseClean WUC-A03H apparatus (DAIHAN, South Korea) for 10 min. Other reagents were of cp grade.

Coulometric determinations were performed using an Ekspert-006 analyzer (Econix-Expert, Russia). Ce(IV) was electrochemically generated from a 0.1 M solution of cerium(III) nitrate in 3 M H_2SO_4 at a platinum electrode at a constant current of 5.0 mA, ensuring a 100% current efficiency of the titrant. Coiled platinum wire was used as the cathode. The cathode chamber with the auxiliary electrode was separated from the anode chamber by a porous glass partition. The titration endpoint was determined biamperometrically with platinum electrodes (ΔE = 200 mV). The surface of platinum electrodes was cleaned with nitric acid, followed by rinsing with distilled water.

Twenty milliliters of a supporting electrolyte was added to a 50-mL electrochemical cell; electrodes were placed in the cell, and the generator circuit was turned on. When the indicator current reached a specified value, the generator circuit was automatically turned off and turned on again simultaneously with the timer after adding an aliquot portion of a test compound or a spice extract. The aliquot portions were selected so that the titration time took no more than 2 min. The titration endpoint was set when the indicator current reached the initial value. The titration time was used to calculate the amount of electricity consumed for the titration of the extract or the number of electrons according to Faraday's law for individual compounds.

The total antioxidant capacity and the Ferric reducing power were determined by the coulometric titration of the extracts using electrogenerated bromine and hexacyanoferrate(III) ions, respectively [8, 10].

Extraction with Brij® 35 micellar medium. An accurately weighed portion (0.1000 ± 0.0005 g) of commercial spice samples was placed in a 15.0-mL test tubs, and from 2.0 to 9.0 mL of a 0.1 M Brij® 35 solution was added. The test tubes were placed in an ultrasonic bath for 10 min [9]. The extracts were filtered and used to assess the completeness of extraction and the cerium-based reducing capacity.

The statistical processing of the results was carried out for five measurements (three measurements to assess the efficiency of titrant generation) with a confidence level of 0.95. The results were presented as $X \pm \Delta X$, where X was the mean value and ΔX was the confidence interval. The random error of the determination was estimated by the magnitude of the relative standard deviation (RSD). Correlation analysis was performed using OriginPro 8.0 software (OriginLab, United States).

RESULTS AND DISCUSSION

Micellar extraction with 0.1 M Brij® 35 was proposed to isolate active components from spices, because the nonionic surfactant ensures the maximum recovery compared to cationic or anionic surfactants [9].

Electrogeneration of Ce(IV) in the presence of Brij® 35. A prerequisite for the implementation of the coulometric titration method is the quantitative current efficiency of the titrant. Surfactant-containing media have different solution viscosity, which decreases the diffusion rate of ions from which the titrant is generated in the course of the electrochemical reaction, and, consequently, the rate of electron transfer [11, 12]. Therefore, surfactant concentration in the titrant generation medium is important.

We considered the electrogeneration of Ce(IV) in the presence of Brij® 35. A comparison of the titrant generation times showed that the one-electron oxidation of Ce(III) to Ce(IV) is not complicated in the presence of Brij® 35 in the range of its concentrations from 0.050 to 4 mM. The efficiency of Ce(IV) electrogeneration was estimated by the titration of ascorbic acid as a standard. The titrant was generated for a time of 90–95% of the theoretically required in the absence of the titrated substance; then, a specific amount of ascorbic acid was introduced into the cell and titrated to the equivalence point.

Ascorbic acid is oxidized by electrogenerated Ce(IV) to dehydroascorbic acid with the participation of two electrons. We calculated the efficiency of titrant generation for different amounts of ascorbic acid as the ratio of the theoretical and experimental values of

Table 1. Efficiency of the Ce(IV) generation current in the presence of Brij® 35 according to the coulometric titration of ascorbic acid ($n = 3$, $P = 0.95$)

$c_{Brij® 35}$, mM	Q_{theor}, C	Q_{exp}, C	RSD, %	Titrant current yield, %
0.25	0.102	0.102 ± 0.004	1.5	100 ± 1
0.50	0.205	0.205 ± 0.001	0.28	99.8 ± 0.3
0.75	0.307	0.306 ± 0.005	0.68	99.6 ± 0.7
1.0	0.409	0.408 ± 0.002	0.25	99.9 ± 0.3

the quantity of electricity, expressed in percentages (Table 1). The electrogeneration of the titrant in the presence of Brij® 35 proceeds with a 100% current efficiency, which ensures the use of Ce(IV) as a titrant for solving analytical problems.

Reactions of electrogenerated Ce(IV) with components of spices. The coulometric titration of standard solutions of individual biologically active spices components showed that all compounds under consideration were oxidized by Ce(IV). The number of electrons participating in the reaction varied with the nature of compounds under consideration and the strength of the titrant-oxidizing agent (in an acidic medium, Ce(IV) exhibits strong oxidant properties). For example, ascorbic, caffeic, chlorogenic, ferulic, and *p*-coumaric acids and α-tocopherol were oxidized by a two-electron mechanism. For gallic acid and rutin, the reaction proceeded with the participation of three electrons, and for quercetin, eugenol, rosmarinic acid, and retinol, the mechanism involved four electrons. The oxidation of catechin and thymol proceeded with the transfer of five electrons, and curcumin and capsaicin were oxidized in six-electron reactions. Tannin was oxidized with the participation of 150 electrons, which indicated the deep oxidation of

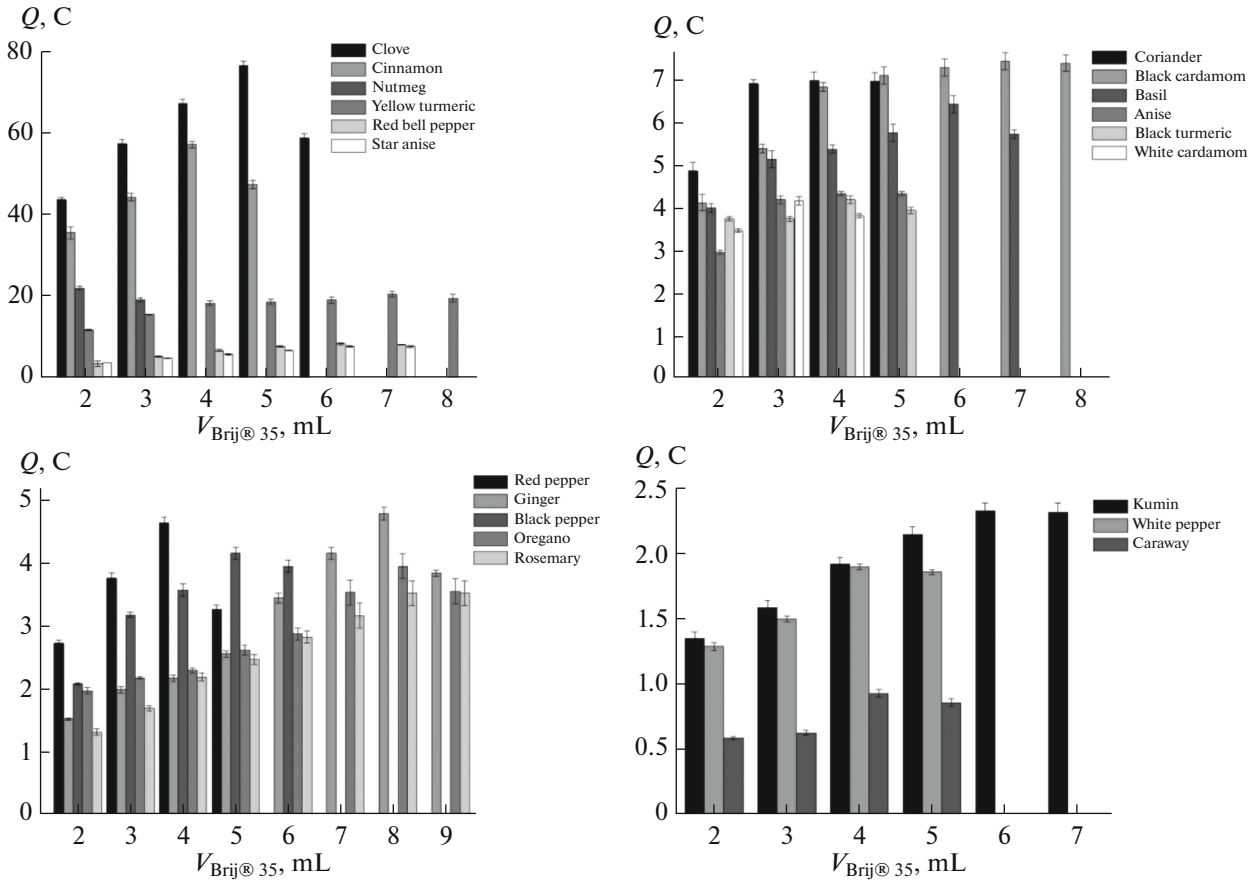

Fig. 1. Effect of extractant volume (0.1 M Brij® 35) on the recovery of active components of spices.

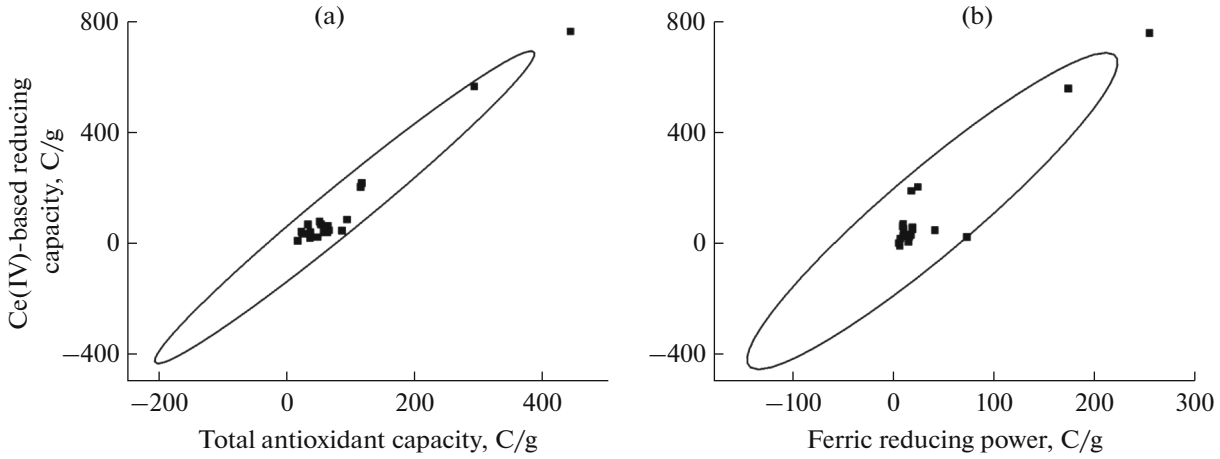

Fig. 2. Correlation of the Ce(IV)-based reducing capacity of micellar extracts of spices with (a) total antioxidant capacity and (b) Ferric reducing power.

the molecule and was consistent with a large number of phenolic hydroxyl groups in its structure. Ce(IV) is a one-electron oxidant, which minimizes the probability of the occurrence of competing reactions. Thus, electrogenerated Ce(IV) is highly reactive and can be used to assess the concentration of active species components.

Micellar extraction of active components from spices and the Ce(IV)-based reducing capacity of the obtained extracts. We evaluated the efficiency of micellar extraction with the ultrasonic treatment of active components of spices using 0.1 M Brij® 35 as an extractant. For this, the coulometric titration of the extracts with electrogenerated Ce(IV) was carried out.

Table 2. Ce(IV)-based reducing capacity of micellar extracts of spices; extractant, 0.1 M Brij® 35 ($n = 5$, $P = 0.95$)

Spice	$V_{\text{Brij® 35}}$ per 1 g of spice, mL	Ce(IV)-based reducing capacity, C/1 g	RSD, %
Clove	50	768 ± 8	1.0
Cinnamon	40	569 ± 7	1.1
Nutmeg	20	219 ± 4	1.4
Yellow turmeric	70	205 ± 7	2.0
Red bell pepper	60	87 ± 3	1.2
Star anise	60	79 ± 1	1.1
Black cardamom	70	74 ± 2	1.2
White cardamom	80	69 ± 4	2.5
Coriander	30	69 ± 1	0.83
Basil	60	64 ± 1	1.6
Ginger	90	47.6 ± 0.7	0.92
Red pepper	40	46.5 ± 0.5	0.9
Anise	40	43.0 ± 0.4	0.38
Black turmeric	40	42.0 ± 0.7	1.0
Black pepper	60	41 ± 3	2.9
Oregano	80	40 ± 1	1.2
Rosemary	80	35.2 ± 0.9	1.8
Kumin	60	23.3 ± 0.5	1.7
White pepper	40	18.8 ± 0.30	0.62
Caraway	40	9.3 ± 0.6	2.5

Table 3. Biologically active components of spices [2, 3, 14, 16–18]

Spice	Biologically active components
Clove	Eugenol and its derivatives, gallates, flavonoids, vanillin, sesquiterpenoids
Cinnamon	Eugenol and its derivatives, cinnamaldehyde, monoterpenes, linalool, β-caryophyllene, flavonoids (catechins, procyanidins, quercetin, kaempferol), tannins
Nutmeg	Eugenol and its derivatives; flavonoids (catechins, myricetin, orgentin), γ-terpenes; oleic, caffeic, palmitic, and myristic acids; terpene alcohols; camphene; myrcene
Yellow turmeric	Curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin, and tetrahydrocurcumin); ascorbic, syringic, vanillic, and hydroxycinnamic acids; eugenol; β-carotene
Black turmeric	Estragol, eugenol derivatives, terpenoids (camphor, 1,8-cineole, turmerone, ocimene, borneol), flavonoids
Red bell pepper	Ascorbic, palmitic, myristic, and hydroxycinnamic acids; γ-terpenes; terpene alcohols; eugenol; flavonoids; α-tocopherol; β-carotene; β-sitosterol; camphene
Red pepper	Capsaicinoids; terpene alcohols; ascorbic, palmitic, myristic, and hydroxycinnamic acids; flavonoids; β-carotene
Black pepper	Piperine and its derivatives; phenolic amides; terpene alcohols; ascorbic, lauric, palmitic, and myristic acids, flavonoids; β-carotene; camphene
Cardamom	Terpenoids (limonene, 1,8-cineole, myrcene, terpinolene), caffeic acid, flavonoids (quercetin, kaempferol, luteolin), anthocyanins (pelargonin)
Coriander	Ascorbic, vanillic, myristic, protocatechic, and hydroxycinnamic acids; flavonoids; terpene alcohols; γ-terpenes; palmitic acid; β-carotene; β-sitosterol; camphene; myrcene
Basil	Flavonoids (apigenin, catechins, quercetin, rutin, kaempferol), anthocyanins, eugenol, terpenoids (linalool, pinene, ocimene), tannins, phenolic acids (caffeic, vanillic, *p*-coumaric, rosmarinic), ursolic acid, methylchavicol
Ginger	Zingeron, shogaols, paradols, diarylheptanoids, γ-terpenes, terpene alcohols, flavonoids, myristic and hydroxycinnamic acids, β-carotene, β-sitosterol, camphene, capsaicin, curcumin, myrcene
Anis	Chlorogenic acids, *trans*-anethole, anise aldehyde, flavonoids, estragole, terpenoids
Star anis	*cis*- and *trans*-Anethole, shikimic and hydroxybenzoic acids, flavonoids, lignans, sesquiterpenoids, phenylpropanoids
Oregano	Phenolic acids (rosmarinic, protocatechuic, *p*-coumaric, caffeic) and their derivatives, flavonoids (apigenin, quercetin, luteolin, myricetin, diosmetin, and eriodictiol), isopropyl methyl phenols (carvacrol and thymol), tocopherols, γ-terpenes
Rosemary	Carnosine; rosmarinic, ursolic, ascorbic, and hydroxycinnamic acids; carnosol; rosmanol; γ-terpenes; terpene alcohols; flavonoids (apigenin; diosmin; luteolin); tannins; eugenols; oleic acid; β-carotene; β-sitenosterol; camphene, pinene 1,8-cineole
Cumin	Phenolic monoterpenoids (thymol, α-terpineol, cumin alcohol), terpenoids (limonene, 1,8-cineole, myrcene, terpinolene)
Caraway	β-Carotene; camphene; myrcene; γ-terpenes; myristic, palmitic, and lauric acids; quercetin; tannin

The amount of electricity consumed for the titration of an extract was used as a parameter to evaluate extraction efficiency. For clove, varying extraction time in the range 5–15 min showed that 10 min was sufficient for the extraction of active components. The raw materials-to-extractant ratio, ensuring the maximum recovery, was determined for each spice individually (Fig. 1). This parameter varied within wide limits (from 1 : 20 to 1 : 90), depending on the nature of the spice, which was consistent with the literature data [8–10].

We determined the Ce(IV)-based reducing capacity of extracts from 20 spices (Table 2), which was expressed as the amount of electricity spent for the titration of the extract recalculated per 1 g of a dry spice. The main components contributing to the Ce(IV)-based reducing capacity were mainly terpenoids and various classes of phenolic antioxidants (Table 3). The maximum values of the Ce(IV)-based reducing capacity were obtained for clove and cinnamon and were in good agreement with the antioxidant

parameters of their alcohol extracts [10, 13–15] and micellar extracts with Triton X-100 [8].

The results for the Ce(IV)-based reducing capacity of micellar extracts from spices were compared with their total antioxidant capacity by the reaction with electrogenerated bromine and ferric reducing power by the reaction with electrogenerated hexacyanoferrate(III) ions. Positive correlations with the coefficients of correlation 0.9826 and 0.9382 were found (Fig. 2) for the total antioxidant capacity and ferric reducing power, respectively, confirming the accuracy of the results obtained. The proposed approach can be successfully used for screening spices and other plant materials.

REFERENCES

1. Rubio, L., Motilva, M.-J., and Romero, M.-P., *Crit. Rev. Food Sci. Nutr.*, 2013, vol. 53, no. 9, p. 943. https://doi.org/10.1080/10408398.2011.574802
2. Yashin, A., Yashin, Y., Xia, X., and Nemzer, B., *Antioxidants*, 2017, vol. 6, no. 3, 70. https://doi.org/10.3390/antiox6030070
3. Ziyatdinova, G.K. and Budnikov, H.C., *J. Anal. Chem.*, 2018, vol. 73, no. 10, p. 946. https://doi.org/10.1134/S106193481810012X
4. Ajila, C.M., Brar, S.K., Verma, M., Tyagi, R.D., Godbout, S., and Valero, J.R., *Crit. Rev. Biotechnol.*, 2011, vol. 31, no. 3, p. 227. https://doi.org/10.3109/07388551.2010.513677
5. Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., and Abert-Vian, M., *Ultrason. Sonochem.*, 2017, vol. 34, p. 540. https://doi.org/10.1016/j.ultsonch.2016.06.035
6. Cortes-Rojas, D.F., Souza, C.R.F., and Oliveira, W.P., *Sep. Sci. Technol.*, 2015, vol. 50, no. 2, p. 207. https://doi.org/10.1080/01496395.2014.952305
7. Wu, K., Zhang, Q., Liu, Q., Tang, F., Long, Y., and Shouzhuo, Y., *J. Sep. Sci.*, 2009, vol. 32, nos. 23–24, p. 4220. https://doi.org/10.1002/jssc.200900398
8. Ziyatdinova, G.K., Nguen Cong, F., and Budnikov, H.C., *J. Anal. Chem.*, 2015, vol. 70, no. 8, p. 974. https://doi.org/10.1134/S1061934815080195
9. Ziyatdinova, G., Ziganshina, E., Nguyen Cong, Ph., and Budnikov, H., *Anal. Methods*, 2016, vol. 8, no. 39, p. 7150. https://doi.org/10.1039/c6ay02112c
10. Ziganshina, E.R., Ziyatdinova, G.K., Nguen Kong, F., and Budnikov, H.C., *Butlerov. Soobshch.*, 2015, vol. 42, no. 5, p. 56.
11. Rosen, M.J. and Kunjappu, J.T., *Surfactants and Interfacial Phenomena*, Hoboken: Wiley, 2012, 4th ed. https://doi.org/10.1002/9781118228920
12. Ziyatdinova, G., Ziganshina, E., and Budnikov, H., *Anal. Chim. Acta*, 2012, vol. 744, p. 23. https://doi.org/10.1016/j.aca.2012.07.023
13. Ziyatdinova, G.K. and Budnikov, H.C., *J. Anal. Chem.*, 2014, vol. 69, no. 10, p. 990. https://doi.org/10.1134/S1061934814100189
14. *Chemistry of Spices*, Parthasarathy, V.A., Chempakam, B., and Zachariah, T.J., Eds., CABI, 2008.
15. Mansour, A.F., Ramadan, M.M., Fekry, R.M., Salem, M.T., Mohammad, A.A., Ali, M.M., and Mohammed, N.S., *Int. J. Biol. Chem.*, 2017, vol. 11, no. 1, p. 9. https://doi.org/10.3923/ijbc.2017.9.16
16. Embuscado, M.E., *J. Food Bioact.*, 2019, vol. 6, p. 68. https://doi.org/10.31665/JFB.2019.6186
17. Shan, B., Cai, Y.Z., Sun, M., and Corke, H., *J. Agric. Food Chem.*, 2005, vol. 53, no. 20, p. 7749. https://doi.org/10.1021/jf051513y
18. Embuscado, M.E., *J. Funct. Foods*, 2015, vol. 18, p. 811. https://doi.org/10.1016/j.jff.2015.03.005

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