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Inhibition of Oxidation of Unsaturated Fatty Acid Methyl Esters by Essential Oils

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Abstract—The essential oils from 16 various spice plants were studied as natural antioxidants for the inhibition of autooxidation of polyunsaturated fatty acids methyl esters isolated from linseed oil. The content of methyl oleate, methyl linoleate, and methyl linolenoate after 1, 2, and 4 months of autooxidation were used as criteria to estimate the antioxidant efficiencies of essential oils. In 4 months, 92% of the methyl linolenoate and 79% of the methyl linoleate were oxidized in a control sample of a model system. It was found that the most effective antioxidants were essential oils from clove bud, cinnamon leaves, and oregano. They inhibited autooxidation of methyl linolenoate by 76–85%. The antioxidant properties of these essential oils were due to phenols—eugenol, carvacrol, and thymol. Essential oil from coriander did not contain phenols, but it inhibited methyl linolenoate oxidation by 38%. Essential oils from thyme, savory, mace, lemon, and tea tree inhibited methyl linolenoate oxidation by 17–24%. The other essential oils had no antioxidant properties.

Keywords: essential oils, autooxidation, polyunsaturated fatty acid methyl esters, linseed oil, gas-liquid chromatography

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INTRODUCTION

Linseed oil contains 90% unsaturated fatty acids, 50–60% of which are an essential linolenic acid that belongs to the ω-3 polyunsaturated fatty acids (PUSFA). The main limitation on the nutritive utilization of linseed oil is its fast oxidation. Hence, it is necessary to use antioxidants in order to inhibit oxidative processes in this oil. To pursue this goal, we chose natural preparations of plant derived essential oils and spice-aromatic plant extracts. Essential oils (EOs) and some spice-aromatic plant extracts are known to possess antioxidant (AO) and antiradical activity [1–3]. The high AO activity of EOs is due to the presence of substances capable of interacting with oxygen and nitrogen radicals, the major oxidative agents inducing lipid autooxidation. These include plant derived mono- and polyphenols: eugenol, thymol, carvacrol, phenolic acids, flavonoids, carotinoids, antacyanids, coumarins, tannins, alkaloids, and a variety of other substances contained in plants and plant derived products, such as EOs and extracts [4–6].

We previously showed that clove bud EO demonstrated a higher AO efficiency than extracts from black pepper, allspice tree, and ginger [7]. A 1% addition of this EO to linseed oil preserved 96% of the linoleic acid and 88% of the linolenic acid. Next in order of AO efficiency was ascorbylpalmitate, which preserved 83% of linoleic acid and 74% of linolenic acid. Extracts of allspice tree and ginger failed to protect di- and triunsaturated acids from oxidation and thus were not recommended as AOs. However, it is known that AO efficiency increases alongside an increase in the AO concentration in a model system. Therefore, it appears that the concentration used in our study was not sufficient to reveal their AO capacities.

The present work was aimed at the study of autooxidation inhibition of linseed-derived polyunsaturated fatty acid by EOs isolated from 16 spice-aromatic plants at 4% of their content with respect to the acid mass.

EXPERIMENTAL

In the present research we studied EOs isolated from clove tree buds (*Eugenia caryophyllata* Thumb.), leaves and bark of cinnamon (*Cinnamomum zeylanicum* Bl.), dried leaves and flowers of oregano (*Oreganum majorana* L.), thyme (*Thymus vulgare* L.), savory (*Satureja hortensis* L.), coriander seeds (*Coriandrum sativum* L.), mace fruits (*Myristica fragrans* Houtt.), lemon rind (*Citrus limon* L.), tea tree (*Melaleuca alternifolia* Maid.), celery seeds (*Apium graveolens* L.), cardamom seeds (*Elettaria cardamomum* L.), ginger root (*Zingiber officinale* L.), juniper berries (*Juniperus comminis* L.), and caraway seeds (*Carvum carvi* L.). The EOs were produced by Plant Lipids Ltd. (India). Each EO was characterized and its content was estimated. The EO characteristics are shown in Table 1.

Table 1. Concentration of the basic components (%) of EOs: 1—clove bud, 2—cinnamon leaves, 3—oregano, 4—thyme, 5—savory, 6—mace, 7—nutmeg, 8—coriander, 9—lemon, 10—tea tree, 11—cinnamon bark, 12—cardamom, 13—celery, 14—ginger, 15—juniper, 16—caraway

Composition	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
α-Tujene		0.13	0.39	1.02	1.00	3.85	3.38		0.06		0.64	0.20		0.10	0.82	
α-Pinene	0.16	1.23	2.06	1.46	0.70	21.22	20.57	4.00	0.29	3.52	1.92	1.90	0.25	2.08	42.81	0.32
Camphene		0.35	0.58	1.15	0.53	0.25		0.90			1.85	0.15		6.41	0.15	0.20
Sabinene			0.50	0.42	0.62	20.46	24.70	0.21	0.59		0.82	2.26		0.02	1.18	0.12
β-Pinene		0.38	1.55	1.94	0.23	16.00	16.78	0.47	3.13	0.23	1.01	0.36	1.42	0.20	1.35	
β-Myrcene	0.26	0.15			1.70	2.71	3.00	0.65	0.87			1.53	1.86	0.75	32.48	0.61
α-Phellandrene		1.46		0.24	0.20	1.16	0.59			0.12	0.10			0.20		0.02
3-Carene						2.17	0.95			0.10	0.02				1.05	
α-Terpinene			0.92	0.81	1.91	2.97	1.37		0.10	8.65						
p-Cymene		1.96	13.00	20.75	10.73	1.62	1.72	0.66	0.11	4.16	0.42	1.15			0.71	
1,8-Eucalyptol	3.06	1.10	0.22	1.50	0.86							41.17		9.63		
Limonene					1.02	8.14	7.82	2.27	62.91	4.42	5.30		42.62		10.39	40.38
γ-Terpinene	0.10		8.73	10.49	11.48	5.76	1.59	10.16	9.15	20.96	0.35	0.20			0.43	
Sabinene hydrate							1.73			0.57	0.34	0.19			0.30	
α-Terpinolene						2.17		0.47	0.54		0.12		1.96	0.58	0.63	
Linalool	0.45	2.45	2.38	5.21	0.54		2.06	72.03	0.40	2.50	0.85	0.76	3.89	0.56	0.53	0.97
Camphor								4.05								
Isoborneol			0.32	1.94	1.86						0.51			1.21		
4-Terpineol	0.14	0.07		1.20	0.88	4.54	3.72	0.02	0.19	37.78	1.05	1.08		0.10		0.36
α-Terpineol	0.15	0.23		0.50	0.18	0.37	0.32	0.05	0.65	5.13	0.75	1.20		0.53		0.53
Neral									3.25			0.40		0.79		
Carvone																54.73
Cinnamal											65.94					
Geranial		0.82							5.06			0.51		0.55		
Linalyl acetate										0.45		1.23				
Bornyl acetate	0.45			0.62	5.93											
Safrole		0.80				1.56	1.15					0.64				
Thymol			4.23	45.11	17.48											
Carvacrol			63.28	2.36	32.23											
Neryl acetate									1.72							
Terpenyl acetate												40.61			0.23	
Eugenol	73.08	65.38														
Geranyl acetate						0.92	0.21	2.64	0.43			0.66		0.42		
Methyl eugenol	0.54	0.56				0.31					5.76			0.70		
β-Caryophyllene	10.39	5.48	1.64	1.72	4,.9	0.20	1.31		0.81		2.49	0.82			0.55	
α-Bergamotene	3.07				0.52				1.52							
Eugenyl acetate	5.30	3.40			0.31											
Curcumene														10.19		
Gingeron														1.23		
Myristicin					0.74	2.80	4.22				0.60					
Zingiberene														31.06		
α-Selinene														3.36		
β-Selinene													35.61	5.29		
Elemicin						0.35	1.52									
Bicyclogermacrene	0.10	0.25			1.82											
Sedanolid													8.12			
Butyl phthalide													2.65			

Raw linseed oil obtained by cold pressing from Sotsservis Agro Ltd. (Moscow region, Russia) and purchased in retail sale was used for the experiments. Methyl esters of fatty acids (FAMEs) were obtained by methanolysis of 1.0 g of linseed oil as described in [6]. The FAME content in the obtained hexane extract was brought to a final concentration of 10 mg/mL with hexane on the basis of gas-chromatographic analysis. The obtained FAME solution was aliquoted by 5 mL and supplemented with 2 μL (4%) of 16 EOs. The tubes with the samples were closed tightly and incubated in light under room temperature. Once in 7 days, the tubes were open for 30 min. After 1, 2 and 4 months, the amount of FAME was measured in both the control and experimental samples.

Gas-chromatographic analysis of all samples was performed on a Crystal 2000M chromatographer (Russia) equipped with a flame-ionizing detector and a SPB-1 quartz capillary column 50 m × 0.32 mm; the phase layer was 0.25 μm (Supelco, United States). The FAMEs were analyzed by column programming from 120 to 270°C at a rate of 4°C/min. The rate of helium carrier gas through the column was 1.5 mL/min. The components were identified on the basis of value retention indices by comparison with data obtained from the literature and our own experimental data. The content of each FAME was assessed as the ratio of the acid peak area to the area of palmitic acid C16:0 peak, and the relative amount was then calculated by comparison with an original FAME sample obtained from fresh linseed oil.

Mathematical data processing was carried out in Microsoft Excel 2007 and Sigma Plot 10. The standard deviation of the mean values of three measurements did not exceed 5% (relative).

RESULTS AND DISCUSSION

Linseed oil is a mixture of triglycerides that includes the following acids: saturated palmitic (3.8%) and stearic (2.7%) acids, monounsaturated oleic acid (16.7%), diunsaturated linoleic acid (18.3%), and triunsaturated linolenic acid (58.2%). Unsaturated fatty acids autooxidation is a complex multistage process that develops in several directions [8]. One of these is the formation of peroxides, which are further cleaved to form low molecular weight products, such as volatile alcohols, ketones, and aldehydes, that provide the specific flavor of the oxidized oil. The addition of oxygen to this radical is followed by transformation of *cis*-bonds into *trans*-bonds in the forming of primary radicals. Simultaneously, oxidized polyunsaturated fatty acids and products of their cleavage get involved in polymerization [8]. It is noteworthy that polymerization is the main process during the oxidation of linseed oil. It was shown that long-term (90 days) autooxidation of linseed oil resulted in the formation of a polymer film on its surface,as a result of polymerization, and the viscosity of the oil samples was significantly increased. This film prevented the absorption of oxygen and inhibited its diffusion through the oil volume. Therefore, the obtained data on the AO efficiency of the preparations in such systems may mirror real properties of AOs. In our study we used a system that consisted of a hexane solution of methyl esters of linseed oil–derived fatty acids in order to assess the ability of EO to inhibit the autooxidation of polyunsaturated fatty acids.

The level of each FAME was estimated as the ratio of an acid ester peak area to the methyl palmitate (C16:0) peak area that did not undergo oxidation and was therefore used as the inner standard. This approach allowed us to reveal relative content of each FAME in the original sample. This was used in comparative analysis, as well as in all samples with and without (control) EOs after 1, 2, and 4 months of autooxidation. The inhibition of FAME autooxidation was calculated as the ratio of the levels of each FAME in the EO-containing samples to their levels in the original FAME solution and was expressed in percentages. The efficiency of the AO properties of the EO positively correlated with the level of unoxidized FAME remaining in the sample. The results are shown in Table 2. It is noteworthy that the air-derived highly active oxygen radicals used in our experimental system interacted with EOs and FAMEs at different rates. If the reaction rate of oxygen radicals with active oil components was higher than that of their reaction with FAMEs, the oxidation degree was lower than in the control sample in the absence of AOs. In the presence of polyunsaturated fatty acids, some EO components may fail to interact with oxygen radicals. A total absence of FAME oxidation inhibition may be observed in such systems. In some cases the oxidation degree of the substrate may exceed this parameter in the control experiment. This is the case when the EO components interact with oxygen radicals to form new active radicals, which, together with oxygen radicals, oxidize polyunsaturated fatty acids. We observed such effects in several EOs. However, it should be noted that these effects were insignificant, and their values did not exceed the level of experimental error. Therefore, the inhibition of FAME oxidation is due to the competitive reactions with oxygen radicals.

Our study showed that methyloleate was oxidized by 4%, linoleate was oxidized by 10%, and linolenoate was oxidized by 12% in one month under experimental conditions. All EOs inhibited methyloleate oxidation and preserved it by 87–99%. The EOs also effectively inhibited the oxidation of linoleic and linolenic acid esters for one month.

In two months, methyloleate in the control sample was oxidized by 25%, linoleate was oxidized by 40%, and linolenoate was oxidized by 60% (Table 2). All EOs inhibited oleate oxidation. However, some oils demonstrated an increase in linoleate and linolenoate oxidation in the presence of EOs as compared with the

Table 2. Antiradical activity of EOs and inhibition of polyunsaturated fatty acid autooxidation in the presence of 4% EOs with respect to linseed oil for 2 and 4 months

Spice from which the EO was isolated	AE, 10^{-4} L/g s	Concentration of methyl oleate, %, with respect to the original level*		Concentration of methyl linoleate, %, with respect to the original level*		Concentration of methyl linolenoate, %, with respect to the original level*	
		2 months	4 months	2 months	4 months	2 months	4 months
Control	–	75	64	60	21	40	8
Clove bud	522.0	93	90	91	85	86	82
Cinnamon leaves	467.0	88	87	90	84	85	81
Oregano	30.0	86	84	84	76	76	60
Thyme	25.3	87	75	75	36	75	20
Savory	24.2	88	74	76	35	78	21
Nutmeg	22.4	82	72	60	24	39	11
Mace	18.5	84	76	75	46	61	24
Tea tree	18.0	82	75	71	32	55	15
Cinnamon bark	16.2	80	66	57	22	38	10
Juniper	8,2	76	65	55	22	34	8
Cardamom	8.2	79	70	55	23	36	9
Ginger	4.1	79	66	56	21	36	8
Celery	1.0	79	79	61	29	42	8
Lemon	0.9	87	78	80	39	67	17
Coriander	0.4	79	77	66	55	60	38
Caraway	0.2	82	68	54	21	34	6

* The standard deviation of three measurements did not exceed 5% (relative).

control sample. These included the oils of cinnamon bark, cardamom, ginger, juniper, and caraway. Notably, this effect did not exceed 5% for either ester. In these oils and their components, the reaction rate was lower than that of the reaction between oxygen radicals and FAMEs; the EO-derived radicals worked as additional oxidative agents.

It is noteworthy that the oxidation rate of polyunsaturated fatty acids in the hexane solution of FAMEs was much higher than that in linseed oil under similar conditions. The inhibition of FAME oxidation significantly depended on the EO composition and acid structure (Table 2). The control sample was characterized by the oxidation of 36% of the oleic acid in 4 months. The EOs of clove bud, cinnamon leaves, and oregano preserved 90, 87, and 84% of the oleic acid, respectively, after the same period of oxidation. The EOs from coriander, lemon, savory, tea tree, cardamom, and nutmeg preserved 70% of the methyloleate. Other EOs demonstrated low AO efficiency with respect to this ester.

Esters of linoleic and linolenic acids were less resistant to oxidation. In 4 months 79% of the linoleate and 92% of the linolenoate was oxidized (Table 2). The most effective AOs for these FAMEs were the EOs of clove bud, cinnamon leaves, and oregano. In the presence of these EOs, the amount of linoleate after 4 months of autooxidation was four times higher than in the control sample, and the level of linolenoate was 7–8 times higher than that in the control experiment (Table 2). Moreover, even after 6 months of autooxidation, the samples supplemented with the EOs from clove bud and cinnamon leaves preserved 80–85% of the methyl linoleate and 80% of the methyl linolenoate, whereas the control sample preserved only 16 and 3% of these esters, respectively.

The main component of EOs from clove bud and cinnamon leaves was eugenol, while those in oregano EO were carvacrol and thymol (Table 1). These substances belong to the group of phenols and are known to possess high AO and antiradical activities [4, 9, 10]. Owing to the presence of phenols, the EOs of clove bud, cinnamon leaves, and oregano effectively protected diene and triene acids against oxidation. Notably, oregano EO was shown to be an effective inhibitor of autooxidation of a polyunsaturated fatty acid ester mixture isolated from the mouse brain: arachidonic, docosapentaenoic, and docosahexaenoic acid esters. In the control experiment, the esters of these acids underwent full oxidation in 4 months, while the addition of 10% oregano EO preserved them by about 60% [10]. In our study oregano EO preserved 76% of the linoleate and 60% of the linolenoate after 4 months of autooxidation. In 6 months of autooxidation, it saved 67 and 49% of these esters, respectively.

Table 1 shows that carvacrol and thymol were the main components of the EOs obtained not only from oregano but also thyme and savory. However, the two latter oils demonstrated poorer AO activities than the oregano oil. After 4 months autooxidation preserved only 36% of the methyl linoleate and 20% of the methyl linolenoate (Table 2). Carvacrol and thymol are isomeric phenols carrying methyl and isopropyl substitutes. The similarity of their structure results in similarity of their biological activities, including the AO activity, which were confirmed both in vitro and in vivo [9, 11, 12]. However, insignificant differences in the structures of isomers led to quantitative differences in their activity. For example, quantitative characteristics of AO activity are affected by the level and ratio of thymol and carvacrol in an EO. In the oregano EO, the total content of two phenolic compounds was maximal (67.51%), while it was 47.47% in thyme EO and 49.71% in savory EO (Table 1). However, the carvacrol : thymol ratio was different. In the oregano oil it was 15 : 1, while those from thyme and savory it was 1 : 19 and 1.8 : 1 respectively. Our study of the AO properties of EOs showed that the antiradical efficiency of these three EOs decreased from 30.0×10^{-4} L/g s to 24.2×10^{-4} L/g s. (Table 2). The rate of the first fast stage of the reaction between the EO components and the free diphenylpicryl hydrazyl (DPPH) radical was maximal for the oregano EO and minimal for the savory EO [9]. However, in the FAME system, the difference in the inhibition of polyunsaturated fatty acid oxidation was much higher than the difference in the antiradical activity obtained in the system with DPPH-radical. Indeed, 4 months of autooxidation in the presence of oregano EO provided preservation of three times more linolenic acid than was preserved in the presence of EOs obtained from thyme and savory (Table 2). It appears that the mechanisms and kinetic characteristics of the reaction of carvacrol and thymol in the model system with DPPH-radical [9] depend to a lesser degree on the ratio of phenols and monoterpenes than in a system with highly reactive oxygen radicals (in the FAME solution). Similar effects of the composition and component ratio on the oxidation inhibition capacity of polyunsaturated fatty acids was shown for the EOs of mace and nutmeg (Table 2). The activity of the mace EO was higher than that of those obtained from nutmeg, though the AO activity of these EOs differed insignificantly (Table 2). It appears that it was only the higher concentration of γ-terpenene and methyl eugenol found in the mace EO that caused the higher efficiency of the latter.

Oregano, thyme, and savory belong to the Lamiaceae family, which includes 3500 species. Among them, there are a number of spicy-aromatic plants, such as basil, mint, balm, catnip, hyssop, rosemary, sage, marjoram, thyme, etc. These plants possess pleasant, intensive flavors and are therefore widely used in cookery. Many of them also possess medical properties known since ancient times. A number of studies confirm the antibacterial and anti-inflammatory effects of these plants and their EOs [3, 4, 12]. The in vivo study of the biological activity of EOs derived from oregano and savory carried out in our laboratory showed that regular consumption of small doses (about 0.3 μg/day) of these oils with food and water increased the lifespan of mice from a line with high cancer risk (the AKR line) by 30%, decreased the risk of leucosis, and increased the average lifespan of healthy Balb-line mice to 120 days (18%). The EOs worked as natural biological AOs, decreased the level of lipid-peroxidation products in the blood, livers, and brains of mice, and increased the resistance of lipids to oxidation. In the absence of exogenous oxidative stress, the uptake of EOs led to modulation of the protective enzymatic system, significantly improved the balance of AO enzymes in liver, positively affected the AO and immune status of mice, and provided resistance to oxidative stress [13–15]. It was found out that brain of extremely elderly mice (24 months old) that took the oregano-derived EO contained high level of polyunsaturated fatty acids, including the extremely important docosahexaenoic acid, whereas its level decreased in the control group by 20% in an age-dependent manner [14]. These effects are due to the biological AO activity of EOs. Being effective biological AOs, they provide prophylactic and therapeutic effects against diseases induced by oxidative stress.

The coriander EO, the main components of which are linalool and γ-terpinene, demonstrated effective inhibition of polyunsaturated fatty acids (Table 1). This EO preserved 38% of the linolenic acid ester after 4 months of oxidation (Table 2). The EOs of lemon and tea tree preserved 15–17% of the methyl linolenoate (Table 2). Notably, these EOs were almost free of phenols. The major AO components of these oils were mono- and sesquiterpene hydrocarbons, mainly α- and γ-terpinenes, caryophyllene, alcohols, such as linalool, and aldehydes, such as citral (Table 1). We previously showed that these substances may compete with FAMEs for oxygen radicals during autooxidation [16–19]. The mechanism of this reaction was described in [20]. Other EOs, including those obtained from celery, cinnamon bark, cardamom, ginger, and caraway, demonstrated poor AO activity in the FAME system. A similar effect of these EOs was observed in the model system of lower aldehyde autooxidation inhibition [2], though their antiradical properties significantly differed from one another in the presence of DPPH-radical (Table 2). This demonstrates that AO activity may not be considered an absolute characteristic of a substance, but as one that depends on the composition of the whole system, as well as on the reaction conditions.

Therefore, our study allowed us to assess the actual ability of EOs to inhibit 4-month-long autooxidation of methyl esters of polyunsaturated fatty acids. Phenols containing EOs isolated from clove bud, cinnamon leaves, and oregano were shown to be the most effective AOs. They preserved 85–90% of the methyl

linoleate and linolenoate in 1–2 months, while these acids underwent 40 and 60% oxidation, respectively, in the control experiment. After 4 months of autooxidation of the control sample, 79% of the methyl linoleate and 92% of the linolenoate was oxidized. In the presence of EOs isolated from clove bud and cinnamon leaves, these FAMEs underwent only 15 and 19% oxidation, respectively. This efficiency of autooxidation inhibition was reached at a 4% concentration of EOs with respect to the fatty acids weight.

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REFERENCES

1. Koroch, A.R., Juliani, H.R., and Zygadlo, J.A., in *Flavour and Fragrances. Chemistry, Bioprocessing and Sustainability*, Berger, R.G., ed., New York: Springer, 2007, pp. 43–116.
2. Misharina, T.A., Terenina, M.B., and Krikunova, N.I., *Appl. Biochem. Microbiol.*, 2009, vol. 45, no. 6, pp. 710–716.
3. Charles, D.J., *Antioxidant Properties of Spices, Herbs and Other Sources*, New York: Springer, 2013.
4. *Chemistry of Spices*, Parthasarathy, V.A., Chempakam, B., and Zachariah, T.J., Eds., Oxfordshire: CAB Int., 2008, pp. 260–286.
5. Crozier, A., Jaganath, I.B., and Clifford, M.N., *Nat. Prod. Rep.*, vol. 26, pp. 1001–1043.
6. Miller, A.L., *Alt. Med. Rev.*, 1996, vol. 1, pp. 103–110.
7. Misharina, T.A., Alinkina, E.S., Terenina, M.B., Krikunova, N.I., Kiseleva, V.I., Medvedeva, I.B., and Semenova, M.G., *Appl. Biochem. Microbiol.*, 2015, vol. 51, no. 4, pp. 455–461.
8. Ioakimoglou, E., Boyatzis, S., Argitis, P., Fostiridou, A., Papapanagiotou, A., and Yannovits, N., *Chem. Mater.*, 1999, vol. 11, no. 7, pp. 2013–2022.
9. Alinkina, E.S., Misharina, T.A., and Fatkullina, L.D., *Appl. Biochem. Microbiol.*, 2013, vol. 49, no. 1, pp. 73–78.
10. Terenina, M.B., Misharina, T.A., Krikunova, N.I., Alinkina, E.S., Fatkullina, L.D., and Vorob'eva, A.K., *Appl. Biochem. Microbiol.*, 2011, vol. 47, no. 4, pp. 445–449.
11. Ultee, A., Bennink, M.H.J., and Moezelaar, R., *Appl. Environ. Microbiol.*, 2002, vol. 68, no. 4, pp. 1561–1568.
12. Miguel, M.G., *Flavour Fragr. J.*, 2010, vol. 25, pp. 291–312.
13. Burlakova, E.B., Misharina, T.A., Fatkullina, L.D., Terenina, M.B., Krikunova, N.I., Erokhin, V.N., and Vorob'eva, A.K., *Dokl. Biochem. Biophys.*, 2011, vol. 437, pp. 80–83.
14. Burlakova, E.B., Misharina, T.A., Vorob'eva, A.K., Alinkina, E.S., Fatkullina, L.D., Terenina, M.B., and Krikunova, N.I., *Dokl. Biochem. Biophys.*, 2012, vol. 444, pp. 167–170.
15. Alinkina, E.S., Misharina, T.A., Fatkullina, L.D., Nagler, L.G., Kozachenko, A.I., Medvedeva, I.B., Goloshchapov, A.N., and Burlakova, E.B., *Appl. Biochem. Microbiol.*, 2014, vol. 50, no. 1, pp. 88–93.
16. Misharina, T.A., *Appl. Biochem. Microbiol.*, 2001, vol. 37, no. 6, pp. 622–628.
17. Misharina, T.A., Terenina, M.B., Krikunova, N.I., and Medvedeva, I.B., *Appl. Biochem. Microbiol.*, 2010, vol. 46, no. 5, pp. 599–604.
18. Misharina, T.A., *Khim. Rastit. Syr'ya*, 2010, vol. 14, no. 1, pp. 93–98.
19. Misharina, T.A., Terenina, M.B., Krikunova, N.I., and Kalinchenko, M.A., *Khim. Rastit. Syr'ya*, 2010, vol. 14, no. 1, pp. 87–92.
20. Foti, M.C. and Ingold, K.U., *J. Agric. Food Chem.*, 2003, vol. 51, no. 9, pp. 2758–2765.

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