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Korrespondenzadresse:
suncicat@uns.ac.rs

[1]) Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia; [2]) University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia

Antibacterial activity of essential oils against *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes*

Antibakterielle Aktivität ätherischer Öle gegen Escherichia coli, Salmonella enterica und Listeria monocytogenes

Sunčica Kocić-Tanackov[1]), Nevena Blagojev[1]), Irena Suturović[1]), Gordana Dimić[1]), Jelena Pejin[1]), Vladimir Tomović[1]), Branislav Šojić[1]), Jovo Savanović[1]), Snežana Kravić[1]), Neđeljko Karabasil[2])

Summary

Antimicrobial potential of six essential oils (EOs) (basil, ginger, hyssop, caraway, juniper, and sage) against three food-borne bacterial pathogens, commonly found as meat product contaminants (*Escherichia coli, Salmonella enterica* and *Listeria monocytogenes*), using disc diffusion and broth microdilution methods, was evaluated. The EOs composition was determined by gas chromatography-mass spectrometry (GC-MS) technique. Dominant compounds in analyzed EOs were: estragole (in basil EO), cis-pinocamphone (in hyssop EO), α-pinene (in juniper EO), α-thujone (in sage EO), carvone (in caraway EO) and curcumene (in ginger EO). Basil EO inhibited growth of all tested bacteria (disc diffusion method). Tested concentrations of ginger EO lacked bactericidal activity. Only basil EO showed inhibitory effect on *L. monocytogenes* growth. Compared to all tested EOs, caraway EO had the highest antibacterial activity on *E. coli* and *S. enterica.*

Minimal inhibitory concentration (MIC) of basil and sage EOs was 56.8 µl/mL for all tested bacteria. Hyssop, caraway, and juniper EOs were inhibitory at concentration of 113.6 µl/mL on all tested bacterial species. MIC of ginger EO was 113.6 µL/mL for *E. coli* and *L. monocytogenes* and 227.3 µL/mL for *S. enterica.* Minimal bactericidal concentration (MBC) of basil and sage EOs was 113.6 µl/mL for all investigated bacteria. MBC of hyssop, caraway, and juniper EOs was 227.3 µl/mL for all investigation bacteria. MBC of ginger EO was 227.3 µL/mL for *E. coli* and *L. monocytogenes* and 454.5 µL/mL for *S. enterica.* Tested EOs have a great potential as natural antibacterial preservative in food.

Keywords: essential oils, antibacterial activity, disc diffusion, broth microdilution

Zusammenfassung

Unter Anwendung von Plattendiffusions- und Mikrodilutionstests wurde in dieser Studie das antimikrobielle Potenzial von sechs ätherischen Ölen (Basilikum, Ingwer, Ysop, Kümmel, Wacholder und Salbei) gegen drei häufig vorkommende lebensmittelassozierte Pathogene (*Escherichia coli, Salmonella enterica* und *Listeria monocytogenes*) untersucht. Als Hauptkomponenten in den ätherischen Ölen (ÄÖ) wurden bestimmt: Estragol (Basilikum), cis-Pinocamphone (Ysop), α-Pinen (Wacholder), α-Thujone (Salbei), Carvon (Kümmel) und Curcumen (Ingwer). Die Ergebnisse zeigen, dass das ÄÖ aus Basilikum das Wachstum aller getesteten Mikroorganismen inhibierte. In den angewandten Konzentrationen zeigte hingegen das gewonnene ÄÖ aus Ingwer keine antimikrobielle Wirkung. Auf *L. monocytogenes* wirkte ausschließlich das Basilikumöl wachstumshemmend. Das ÄÖ aus Kümmel war wirksamer gegenüber *E. coli* und *S. enterica* im Vergleich zu den anderen ätherischen Ölen.

Die minimale Hemmkonzentration (MHK) der ÄÖ aus Basilikum und Salbei betrug 56,8 µl/ml bei allen untersuchten Bakterien. Bei Ysop, Kümmel und Wacholder betrug die minimale Hemmkonzentration (MHK) 113,6 µl/ml bei allen Bakterien. Die MHK bei Ingwer betrug 113,6 µl/ml für *E. coli* und *L. monocytogenes* und 227,3 µl/ml für *S. enterica.* Die minimale bakterizide Konzentration (MBK) aus den Ölen von Basilikum und Salbei betrug 113,6 µl/ml für alle untersuchten Bakterien. Die MBK von Kümmel und Wacholder war 227,3 µl/ml für alle Bakterien. Die MBK von Ingwer war 227,3 µl/ml für *E. coli* und *L. monocytogenes* und 454,5 µl/ml für *S. enterica.* Die untersuchten ätherischen Öle zeigen ein großes Potenzial, als natürliche antibakterielle Konservierungsmittel in der Nahrung Verwendung zu finden.

Schlüsselwörter: ätherische Öle, antibakterielle Aktivität, Plattendiffusionstest, Mikrodilutionstest

Introduction

Food spoilage and alimentary diseases caused by harmful food-borne pathogens are growing public health problem worldwide. Multiple preservation techniques in the manufacturing and storage of food products are required for successful control of food pathogens. These techniques usually include application of diverse additives, mainly synthetic chemicals. Negative public perception of synthesized food antimicrobials, awareness of their side effects and toxic properties, as well as the emergence of bacterial antibiotic resistance have generated interest in the usage of more naturally occurring compounds. Thus, discovery and development of novel antimicrobials from natural sources became of the uttermost importance (Aumeeruddy-Elalfi et al., 2015). To be recognized as natural antimicrobials, these compounds should be directly derived from biological systems without alteration or modification (Calo et al., 2015). From this point, many medicinal and aromatic plants, including diverse herbs, spices, fruits, and vegetables, have attracted attention of scientists as natural sources of natural agents that could be safer than synthetic sources (Kocić-Tanackov et al., 2014; 2015; Kapoor et al., 2014; Nikolić et al., 2014; Sánchez-Ortega et al., 2014; Sarikurkcu et al., 2015).

Aromatic plants are generally considered as the most important source of natural antimicrobial compounds. Nowadays, more than 25 000 plants have been described for the diversity of the contained bio-active compounds (Calvo et al., 2011). In recent years, many plants have been examined for their effectiveness as food safety and preservation applicants, and have received attention as health promoters (Čabarkapa et al., 2013; Kapoor et al., 2014; Kocić-Tanackov et al., 2014; Šojić et al., 2015).

Plant EOs are mainly responsible for antimicrobial activity of an aromatic plant (Tajkarimi et al., 2010). Chemically, EOs are complex mixtures of terpenoides, monoterpenes, sesquiterpenes and possibly diterpenes with different groups of aliphatic constituents, such as hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones (Fisher and Phillips, 2006; Bakkali et al., 2008).

EOs have been used in food for centuries, since they have a pleasant odor and sometimes a distinctive taste, in addition to their antimicrobial properties which make them suitable alternatives for antibiotics.

In the present study, six commercially available essential oils were tested as potentially suitable as additives in meat industry, and evaluateasd well as their antimicrobial potential against three food-borne pathogens, commonly found contaminants of meat products.

Materials and Methods

Essential oils

Six EOs (Herba d.o.o., Belgrade, Serbia) were used in this study: basil *(Ocimum basilicum),* ginger *(Zingiber officinalis),* hyssop *(Hyssopus officinalis),* caraway (*Carum carvi* L.), juniper *(Juniperus communis)* and sage *(Salvia officinalis).* These EOs have been selected based on their specific aroma which, can potentially enhance the flavor of meat products.

Determination of chemical composition of essential oils

The EOs composition was determined by gas chromatography-mass spectrometry (GC-MS) technique. GC-MS analyses were carried out using an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7890B coupled with a 5977A mass selective detector with a HP-5MS Agilent Technologies column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The GC oven temperature was programmed from 70 °C (2 min), to 220 °C at the rate of 4 °C/min and kept under isothermal conditions for 10 min. The injector was maintained at 250 °C. Helium was used as carrier gas at a constant flow rate of 1.0 mL/min. The mass spectrometer operated in the electron ionization mode. Data acquisition was carried out in the scan mode (range 50–450 amu), solvent delay time was 2 min. The oil samples were dissolved in hexane, and 1 µl aliquots were injected in split mode with split ratio of 1:20. The identity of the components of EOs was determined by comparison of their retention indices and mass spectra with literature data (Adams, 2007; Davies, 1990) and the mass spectra databases (Wiley 10th & NIST 2011 MS Library). Retention indices (RI) were determined relative to the retention times of series of n-alkanes with linear interpolation. The percentages of determined compounds were calculated by the area normalization method, without considering response factors. The component percentages were calculated as mean values from duplicate GC-MS analyses of all tested samples.

Strains and preparation of bacterial cell suspension

Antibacterial activity of selected EOs was evaluated on: *Escherichia coli,* ATCC 25922, Lot 335-69-1, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (group D) ATCC 13076, Lot 345-93 and *Listeria monocytogenes,* ATCC 19111; Lot 277-28, obtained from American Type Culture Collection. These cultures were maintained on Mueller-Hinton agar (MHA) (Merck, Darmstadt, Germany) slants at 4 °C and subcultured weekly onto fresh slants.

Twenty-four hours old bacterial cultures grown on Mueller-Hinton agar were used for preparation of the bacterial cell suspension tests. The cells were harvested with sterile loop into 10 mL of 0.85 % sterile saline. The cell suspension was adjusted with saline to give a final concentration of 1×10^8 CFU/mL using McFarland Standard (bioMérieux SA, Marcy I'Etoile, France) and McFarland densitometer (Biosan SIA, Riga, Latvia).

Disc diffusion method

Disc diffusion method was adapted from Clinical and Laboratory Standards Institute (CLSI) guidelines (Institute, C.a.L.S., Document M02-A11, Wayne) for the preliminary examination of the EOs antibacterial activity. Fifteen mL of Mueller-Hinton agar was poured into 90-mm Petri dishes and left to solidify and dry. A sterile swab was immersed in the culture suspension and the entire surface of a Muller-Hinton agar was inoculated. Plates were left for 15 min to allow any excess surface moisture absorbsion before applying the discs. The 6-mm test discs (HiMedia Laboratories Pvt. Ltd., Mumbai, India) were impregnated with 5, 10, and 20 µL of undiluted EOs and placed onto inoculated Muller–Hinton plates. Discs without EOs served as negative controls. Inverted plates were then incubated at 37±1 °C for 24 h. The diameters of inhibition zones

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including diameter of the EO impregnated discs were measured in millimeters. The presence or absence of an absolute inhibition zone was used as a criteria for the definition of strain sensitivity to different EOs. According to Moreira et al. (2005) and Jorgensen and Ferraro (2009), this sensitivity is classified by the diameter of the inhibition zone as follows: not sensitive (–) for diameter less than 8 mm; sensitive (+) for diameter 9–14 mm; very sensitive (++) for diameter 15–19 mm, and extremely sensitive (+++) for diameter larger than 20 mm. The tests were performed in duplicates for each EO.

Broth microdilution method

Minimal inhibitory concentration (MIC) and minimal bactericidalconcentration (MBC) of the EOs were determined by broth microdilution method according to CLSI guidelines (Institute, C.a.L.S., Document M07-A9, Wayne), with some modifications. Briefly, 100 µL of Muller-Hinton broth was pipetted into each well of a sterile 96-well microtiter plate (Greiner Bio-One GmbH, Kremsmünster, Austria). After that, in the first column of wells in microtiter plate 100 µL of each EOs was transferred. After mixing by pipetting, 100 µL of the mixture was transferred to the next column of wells in a process of 1:1 serial dilution until the column number 12 (ranging from 0.22 to 454.54 µL/mL). One hundred µL of the mixture from the last well was discarded. As the final step, each well was inoculated with 10 µL of the tested microorganism suspension (10^8 cfu/mL).

The same tests were simultaneously performed with the control sample (Muller-Hinton broth + bacterial suspension) and sterility control (Muller-Hinton broth + EO).

Incubation of microtiter plates was performed at 37 °C for 24 h. After incubation, wells that contained clear broth suspension were used for inoculation of previously dried Muller-Hinton agar plates (24 h for 37 °C).

The MIC was determined as the lowest EOs concentration that inhibited the growth in the well (clear broth suspension), but still showed slightly visible growth on the plate. MBC was determined at the EOs concentration that inhibited the growth in the well and showed no visible growth on the plate (the presence of ≤2 cfu per plate is acceptable). All tests were performed in duplicates for each EO.

Results and Discussion

Chemical composition of essential oils

As shown in Table 1 main constituent of basil EO was estragole (82.96 %) and this oil according to Lawrence (1992) and Grayer et al. (1996) can be defined as "estragol chemotype".

Carvon (72.11 %) and limonene (23.03 %) were the dominant compounds of caraway EO. Curcumene (20.09 %), zingiberene (13.87 %), β-sesquiphellandrene (9.94 %), and β-bisabolene (9.72 %) were compounds determined at highest percentage in ginger EO. α-Pinene (26.14 %), sabinene (6.35 %), terrpinen-4-ol (6.32 %), β-cymene (4.41 %), δ-cadinene (4.0 %), limonene (4.02 %), β-myrcene (3.92 %), and β-elemene (3.62 %) were principal constituents of juniper EO. α-Thujone (22.40 %), camphor (18.85 %), and 1,8-cineol (14.63 %) were prevalent constituents of sage EO. cis-Pinocamphone (40.27 %) and trans-pinocamphone (16.67 %) dominated in hyssop EO (Table 1).

EOs chemical composition depends on geographical origin of the plant, ways, and harvesting time, drying method, method of oil extraction, as well as the plant parts used for EOs extraction (Suppakul et al., 2003; Daferera et al., 2003; Burt, 2004; Rasooli, 2008; Bakkali et al., 2008). These are the main reasons of great composition diversity of the same EOs in the literature. Similar results to those obtained in this study were obtained by Angioni et al. (2003), Pepeljnjak et al. (2005), Glišić et al. (2007) for juniper EO; Bozin et al. (2007), Porte et al. (2013) for sage EO, Pripdeevech et al. (2010), Beatović et al. (2015), Avetisyan et al. (2017) for basil EO; Sedláková et al. (2003), Meshkatalsadat et al. (2012), Rasooli and Allameh (2016) for caraway EO; Mitić and Đorđević (2000), Kizil (2010), Zawiślak (2013) for hyssop EO; Kizhakkayil and Sasikumar (2012), Nampoothiri et al. (2012), Kamaliroosta et al. (2013) for ginger EO.

Generaly, biological activity was attributed to the main constituents of EOs (Bakkali et al., 2008), although in some papers was indicated that EOs have a greater antimicrobial activity than just mixtures of their major antimicrobial components (Gill et al., 2002; Mourey and Canillac, 2002), suggesting that they owe their enhanced activity to the synergism of major and minor compounds (Burt, 2004; Tajkarimi et al., 2010).

Because of the great number of constituents, EOs seem to have no cpecific cellular targets (Carson et al., 2002; Bakkali et al., 2008). Several mechanisms have been proposed to explain EOs antimicrobial activity (Bakkali et al., 2008), but mostly it is attributed to their phenolic nature. Phenolic compounds disrupt the cell membrane resulting in the inhibition of the functional properties of the cell and eventually leakage of the internal contents of the cell; they alter microbial cell permeability, damage cytoplasmic membranes, interfere with cellular energy (ATP) generation system, and disrupt proton-motive force (Bakkali et al., 2008; Bajpai et al., 2012). EOs and their components are hydrophobic, which results in their diverse effectiveness against Gram-positive and Gram-negative bacteria. In the most papers it is indicated that EOs have higher activity against Gram-positive bacteria (Burt, 2004; Kim et al., 2011) due the differences in cell wall structure between Gram-positive and Gram-negative bacteria (Ratledge and Wilkinson, 1988). It was considered that lipopolysaccharide layer in Gram-negative cell wall blocks the penetration of hydrophobic compounds of EOs (Vaara, 1992). On the contrary, in was also indicated that EOs have higher activity against Gram-negative bacteria (Deans and Ritchie, 1987; Stecchini et al., 1993; Tassou et al., 1995; Hao et al., 1998; Wan et al., 1998). These findings are the consequence of differences in EOs composition (Burt, 2004).

Essential oils antimicrobial activity

According to the results of disk diffusion method presented in Table 2, tested EOs significantly differ in their activity against tested bacterial strains.

Ginger EO expressed no antibacterial activity and no inhibition zone was observed for any of the tested bacteria. *L. monocytogenes* has been reported as the most resistant species compared to *E. coli* and *S. enterica,* since only basil EOs demonstrated antimicrobial activity against *L. monocytogenes.* Basil EO was more effective against *E. coli* and *S. enterica,* with visible inhibition zones ranging from 13–17 mm and 11–17 mm, respectively. The highest antibacterial activity against *E. coli* and *S. enterica* was detected for caraway EO, with inhibition zones ranging from

TABLE 1: *Chemical composition of essential oils.*

No.	Compounds	RI[a]	Ginger (%)	Juniper (%)	Sage (%)	Hyssop (%)	Basil (%)	Caraway (%)
1.	Hexanal	853	0.22	–	–	–	–	–
2.	cis-Salvene	874	–	–	0.35	–	–	–
3.	3-Carene	916	0.16	–	–	–	–	–
4.	α-Thujene	919	–	1.89	–	–	–	–
5.	α-Pinene	925	2.22	26.14	5.66	0.68	0.28	–
6.	Camphene	936	6.57	0.34	6.22	–	–	–
7.	Sabinene	953	–	6.35	–	1.72	0.16	–
8.	β-Pinene	957	0.23	2.27	1.34	9.65	0.43	–
9.	Methylheptenone	961	0.40	–	–	–	–	–
10.	β-Myrcene	966	0.52	3.92	0.37	0.71	–	–
11.	β-Cymene	997	0.16	4.41	1.56	0.61	–	–
12.	Limonene	1001	–	4.02	–	1.42	0.23	23.03
13.	β-Thujene	1003	5.05	–	–	–	–	–
14.	1,8-Cineol	1005	2.02	–	14.63	–	4.13	–
15.	Fenchone	1059	–	–	–	–	0.24	–
16.	Linalool	1069	0.21	–	–	1.27	0.66	–
17.	Fenchol	1081	–	–	–	–	0.20	–
18.	α-Thujone	1086	–	–	22.40	–	–	–
19.	β-Thujone	1090	–	–	4.36	0.47	–	–
20.	trans-Pinocarveol	1114	–	0.35	–	0.80	–	–
21.	Camphor	1123	–	–	18.85	–	0.50	–
22.	trans -Pinocamphone	1139	–	–	0.84	16.67	–	–
23.	Borneol	1144	0.93	–	5.27	–	–	–
24.	Terrpinen-4-ol	1156	–	6.32	0.99	–	–	–
25.	cis-Pinocamphone	1160	–	–	–	40.26	–	–
26.	p-Cymene-8-ol	1163	–	0.56	–	–	–	–
27.	α-Terpineol	1169	0.46	0.93	0.15	0.34	–	–
28.	Myrtenol	1177	–	0.38	–	2.64	–	–
29.	Estragole	1186	–	–	0.25	–	82.96	–
30.	Verbenone	1190	–	0.30	–	–	–	–
31.	Fenchyl acetate	1207	–	–	–	–	0.26	–
32.	trans-Carveol	1218	–	–	–	–	–	0.35
33.	Cumaldehyde (Cumal)	1224	–	–	–	0.38	–	–
34.	Carvone	1238	–	0.71	–	–	–	72.11
35.	4-Anisaldehyde	1241	–	–	–	–	0.37	–
36.	Perillaldehyde	1267	–	–	–	–	–	0.22
37.	Borneol acetate	1276	–	0.60	2.15	–	0.18	–
38.	2-Undecanone	1282	0.22	–	–	–	–	–
39.	γ-Terpinene	1288	–	–	–	0.61	–	–
40.	Carvacrol	1291	–	–	1.16	–	–	–
41.	Myrtenyl acetate	1319	–	–	–	0.36	–	–
42.	δ-Elemene	1332	0.18	–	–	–	–	–
43.	α-Cubebene	1346	–	1.36	–	–	–	–
44.	Cyclosativene	1363	0.45	–	–	–	–	–
45.	Copaene	1375	0.80	1.33	0.27	–	–	–
46.	Geranyl acetate	1380	0.15	–	–	–	–	–
47.	β-Bourbonene	1385	–	–	–	2.26	–	–
48.	β-Elemene	1393	1.33	3.62	–	–	0.16	–
49.	Isoledene	1400	–	0.47	–	–	–	–
50.	Methyleugenol	1404	–	–	–	0.43	0.85	–
51.	Caryophyllene	1423	–	2.67	1.67	0.53	–	–
52.	β-cubebene	1432	–	0.24	–	–	–	–
53.	γ-Elemene	1436	0.60	1.32	–	–	–	–
54.	α-Bergamotene	1439	–	–	–	–	2.39	–
55.	α-Humulene	1459	–	3.01	3.01	–	–	–
56.	β-Farnesene	1475	0.78	–	–	–	0.19	–
57.	Aromadendrene	1466	0.38	–	–	2.44	–	–
58.	β-Guajen	1477	0.80	–	–	–	–	–
59.	γ-Muurolene	1482	–	1.71	0.23	–	–	–
60.	Germacrene	1488	–	2.03	–	–	–	–
61.	β-Selinene	1491	–	0.94	–	–	–	–
62.	Curcumene	1495	20.09	–	–	–	–	–
63.	α-Selinene	1502	–	1.19	–	–	–	–
64.	α-Muurolene	1505	–	1.30	–	–	–	–
65.	Zingiberene	1509	13.87	–	–	–	–	–
66.	γ-Cadinene	1518	1.70	1.64	–	0.46	0.64	–
67.	β-Bisabolene	1523	9.72	–	–	–	–	–
68.	Myristicine	1527	–	–	0.31	–	–	–
69.	Calamenene	1530	–	–	0.20	–	–	–
70.	δ-Cadinene	1532	–	4.00	–	–	–	–
71.	α-Panasinsen	1536	0.47	0.25	–	–	–	–
72.	β-Sesquiphellandrene	1537	9.94	–	–	–	–	–
73.	γ-Bisabolene	1541	0.23	–	–	–	–	–
74.	Eudesma-3,7(11)-diene	1549	–	0.35	–	–	–	–
75.	Elemol	1557	0.51	–	–	2.27	–	–
76.	Germacrene B	1566	0.20	1.33	–	–	–	–
77.	Nerolidol	1570	0.46	–	–	–	–	–
78.	β-Thujaplicin	1577	–	–	–	–	3.35	–
79.	Spathulenol	1586	–	0.98	–	3.67	–	–
80.	Caryophylene oxide	1592	–	1.16	0.87	3.57	–	0.30
81.	Ledol	1600	–	–	1.41	–	–	–
82.	Humulene epoxide	1617	–	0.88	–	0.77	–	–
83.	Zingiberenol	1620	0.60	–	–	–	–	–
84.	Cedrene	1636	0.38	–	–	–	–	–
85.	tau-Cadinol	1647	–	2.03	–	–	–	–
86.	β-Eudesmol	1656	0.16	–	–	0.34	–	–
87.	α-Cadinol	1660	–	1.30	–	–	–	–
88.	Damascone	1789	1.60	–	–	–	–	–
89.	Nerylacetone	1804	2.80	–	–	–	–	–
90.	Curcuhydroquinone	1946	0.60	–	–	–	–	–
91.	Epimanool	2008	–	–	0.26	–	–	–

[a]: Retention index relative to n-alkanes on HP-5MS column

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TABLE 2: *Antimicrobial activity of the essential oils against E. coli ATCC 25922, S. enterica subsp. enterica serovar Enteretidis (group D) ATCC 13076, and L. monocytogenes ATCC 19111 by disc diffusion method.*

Essential oil	Diameter of inhibition zone (mm)								
	E. coli			*S. enterica*			*L. monocytogenes*		
	5 µL	10 µL	20 µL	5 µL	10 µL	20 µL	5 µL	10 µL	20 µL
Basil	13±0*	14±0	17±0	11±1.4	14.5±0.7	17±1.4	0	10.5±0.7	12.5±0.7
Ginger	0	0	0	0	0	0	0	0	0
Hyssop	0	0	11±1.4	0	9.5±0.7	11.5±0.7	0	0	0
Caraway	0	13.5±0.7	28±4.2	10.5±0.7	19.25±4.6	24.5±3.5	0	0	0
Juniper	9.5±0.7	11.5±0.7	11.5±0.7	0	0	8±0	0	0	0
Sage	11.5±0.7	14±1.4	27±1.4	8±0	10.5±0.7	13.5±0.7	0	0	0

*: Standard deviation for two measurements

13.5–28 mm and 10.5–24.5 mm, respectively. Caraway EO expressed no antimicrobial activity on *L. monocytogenes.* Considering the antimicrobial potential against *E. coli,* caraway was the most effective, followed by sage, basil, juniper, and hyssop, while against *S. enterica* it was followed by basil, sage, hyssop and juniper EOs.

Beside the disk diffusion method, during further investigation microdilution method was used. Results of EOs MIC and MBC are presented in Figures 1–3.

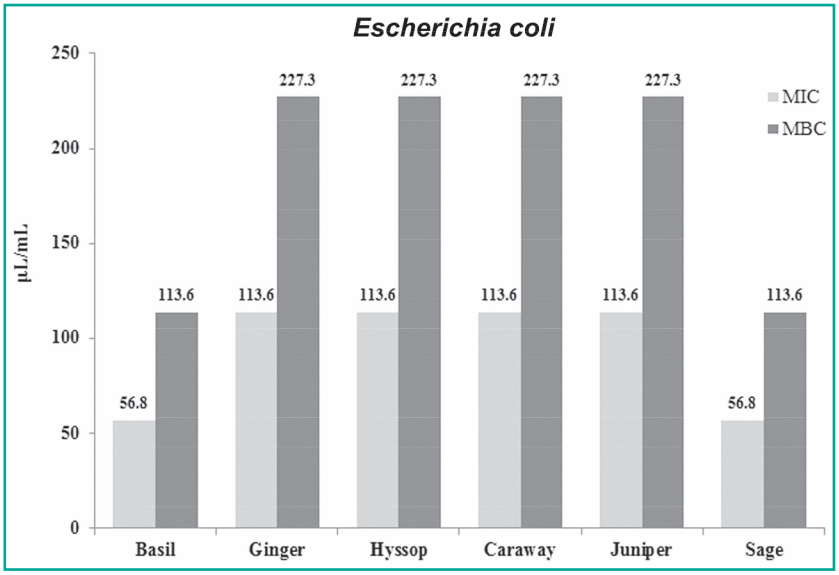

FIGURE 1: *Essential oils minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for E. coli ATCC 25922.*

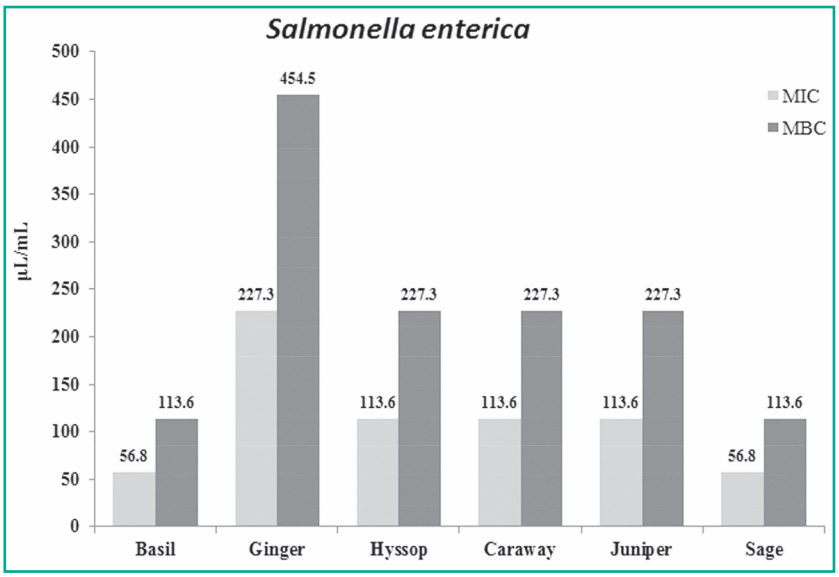

FIGURE 2: *Essential oils minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for S. enterica subsp. enterica serovar Enteretidis (group D) ATCC 13076.*

Results of microdilution method indicate that all tested EOs expressed antimicrobial activity against all tested bacteria, at the concentration range of 56.8–454.5 µL/mL. MIC and MBC obtained for each EO against *E. coli* are identical to those against *L. monocytogenes.* Basil and sage EOs were more effective against these two species, with MIC/MBC 56.8/113.6 µL/mL. Ginger, hyssop, caraway, and juniper EOs exhibited similar activity against these two species, with slightly higher MIC/MBC: 113.6/227.3 µL/mL. Five of six EOs demonstrated the same activity against *S. enterica,* while ginger was less effective and at higher concentrations of the oil *Salmonella* cells were inhibited, with MIC/MBC: 227.3/454.5 µL/mL.

Published data on this subject are very difficult to compare. The chemical composition of EOs and extracts as before mentioned vary with the botanical source and age of the plant, condition of the plant (dried or fresh), location, altitude, climate and environmental conditions (Suppakul et al., 2003; Daferera et al., 2003). Besides this, the selection of tested microorganisms, the way of exposure of the microorganisms to EOs and the method of antimicrobial activity evaluation vary among the different publications. As a result of this diversity of experimental conditions, in literature we have plenty of seemingly contradictory results. For example, Tarek et al. (2014) reported that caraway EO has very low MIC values against some Gram-positive and Gram-negative bacteria (≤ 1 µL/mL), using disc diffusion method. However, Dussauit and Lacroix (2014) examined several EOs intended for food application, with oil concentrations up to 5000 µL/mL. They obtained MIC value of caraway EO against *E. coli, S. enterica* and *L. monocytogenes* ≥ 5000 µL/mL, which is around 40 times higher concentration than those used in this research.

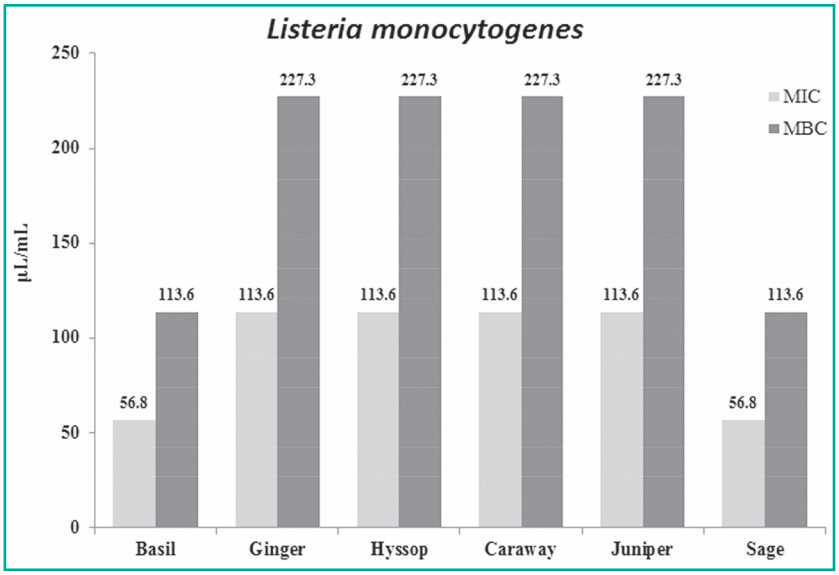

FIGURE 3: *Essential oils minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for L. monocytogenes ATCC 19111.*

Similar diversity in literature data can be noticed for EO basil. Good correlation with results obtained in this study by disc diffusion method was published by Smith-Palmer et al. (1998), Moreira et al. (2005) and Hussain et al. (2008), while De Martino et al. (2009) although at 2 times higher concentration applied, observed smaller zone of inhibition (11 ± 0.4 mm). Considerably higher results of MIC, compared to data obtained in this study were published by Dussauit and Lacroix (2014) and Hussain et al. (2008), ≥ 5000 µL/mL and 1.6–2.6 mg/mL, respectively. Additionally, Dussauit and Lacroix (2014) reported around 80 times higher MIC values for sage EO (≥ 5000 µL/mL), compared to the results obtained in this study (56.8 µL/mL).

Using disk diffusion method, Celikel and Kavas (2008) classified *E. coli* and *L. monocytogenes* as sensitive to very sensitive for sage EO, while accoring to the results obtained in this study *E. coli* could be classified as very to extremely sensitive, and *L. monocytogenes* as not sensitive. Smith-Palmer et al. (1998) obtained wider inhibition zone for *L. monocytogenes* and smaller for *E. coli* and *S. enterica* compared to those in this research. In experiments of De Martino et al. (2009) inhibition zone (13.2 ± 0.6 mm) for *E. coli* was more than two times smaller in comparison to the results obtained in this study, even at two times higher concentration.

Ginger, like all previous EOs, was reported by Dussauit and Lacroix (2014) to exhibit very high MIC (≥ 5000 µL/mL) against all three tested strains. Bellik (2014) reported even higher MIC value of ginger EO against *E. coli* which was 173 840 µL/mL, which is approximately 1500 higher concentration compared to that used in this study.

In this study, ginger EO showed no antimicrobial activity against tested strains applying disc diffusion method, which is in accordance with the results of Sa-Nguanpuang et al. (2011) for *E. coli,* since they didn't observe any inhibition zone either. However, for *S. enteritidis* they observed inhibition zone of 11.8 ± 1.3 mm. Smith-Palmer et al. (1998) used agar well method and obtained diameter of inhibition zone 4.3 mm for all three tested strains, which is contrary to our results.

Hyssop and juniper EOs are less frequently tested than previous ones. Kizil et al. (2010) used agar disc diffusion method, applying 5 and 10 µL of different sort of hyssop EO for antimicrobial activity tested against several bacterial and yeast strains, including *E. coli.* They observed inhibition zone diameter of 20.3 ± 1.8 and 23.3 ± 1.7 mm, for 5 and 10 µL, respectively. For the same volumes applied, in this study no inhibitory effect was observed. De Martino et al. (2009) obtained very similar results for hyssop as they obtained for sage (13.2 ± 0.6 mm, 457 µg/disc), which is significantly lower than that in this study (11 ± 1.4 mm at more than twice lower concentration). Finally, Sharopov et al. (2012) determined MIC of some hyssop EOs by microbroth dilution technique, and for *E. coli* obtained MIC value was 625 µL/mL for all tested hyssop oils, which is approximately 5 times higher than in this research.

According to Pepeljnjak et al. (2005) results, *E. coli* is not sensitive to juniper EO, while in this study and inhibition zone from 9.5 to 11.5 mm was observed. The same authors indicate 8 mm inhibition zone for *S. enteretidis.* Similar inhibition zone was obtained in this research but for 20 µL per disc (they poured 40 µL into 6-mm agar well). Besides, the same authors investigated MIC values of juniper EO against diverse microorganisms, including *S. enteritidis* and they got MIC 70 % v/v. Glišić et al. (2007) also conducted some antimicrobial assays, and they concluded that juniper essential oil showed low antimicrobial activity against all the tested strains, including *E. coli.*

Selection of EOs for application as food ingredient usually starts with evaluation of their antimicrobial potential. However, we should always keep in mind that factors present in complex food matrices such as fat content, proteins, water activity, pH, and enzymes can potentially diminish the efficiancy of EO in real systems. Some researches (Firouzi et al., 2007), indicate that the effective concentration of EO in food is 1–3 % higher than concentration obtained *in vitro.* Unfortunately, this concentration is often higher than what would normally be sensory acceptable, since the production of off-flavor or strong odor limits the use of EOs as food ingredients (Friedly et al., 2009).

Conclusions

According to the results obtained in this study, tested EOs may be a new potential source of natural antimicrobials for food preservation. They exhibit antimicrobial activity against food-borne pathogens *in vitro,* have natural origin less harmful to the people and the environment. Also, they have been considered at low risk for resistance development by pathogenic microorganisms and possess specific flavor that might be appreciated by consumers. Future research should focus on maximum sensory acceptable concentrations, effectiveness of different EOs in various food matrices and synergism between EOs with food components and processing techniques, considering potential commercial application of selected oils in meat products industry.

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Conflict of interest

The authors declare that no conflict of interest among authors.

References

Adams RP (2007): Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing, Coral Stream, IL.

Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally F (2015): Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. Ind Crop Prod 71: 197–204.

Avetisyan A, Markosian A, Petrosyan M, Sahakyan N, Babayan A, Aloyan S, Trchounian A (2017): Chemical composition and some biological activities of the essential oils from basil *Ocimum* different cultivars. BMC Complement Altern Med17 (60), DOI 10.1186/s12906-017-1587-5

Bajpai VK, Baek K-H, Kang SC (2012): Control of *Salmonella* in foods by using a essential oils: a review. Food Res Int 45: 722–734.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008): Biological effects of essential oils – A review. Food Chem Toxicol 46: 446–475.

Beatović D, Krstić-Milošević D, Trifunović S, Šiljegović J, Glamočlija J, Ristić M, Jelačić S (2015): Chemical composition, antioxidant and antimicrobial activities of the essential oils of twelve *Ocimum basilicum* L. cultivars grown in Serbia. Rec Nat Prod 9(1): 62–75.

Bellik Y (2014): Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of *Zingiber officinale* Roscoe. Asian Pac J Trop Dis 4(1): 40–44.

Bozin B, Mimica-Dukic N, Samojlik I, Jovin E (2007): Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., *Lamiaceae*) essential oils. J Agric Food Chem 55: 7879–7885.

Burt S (2004): Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 94: 223–253.

Calo JR, Crandall PG, O'bryan CA, Ricke SC (2015): Essential oils as antimicrobials in food systems – A review. Food Control 54: 111–119.

Calvo MA, Arosemena EL, Shiva C, Adelantado C (2011): Antimicrobial activity of plant natural extracts and essential oils. In: Mendez-Vilas A. (ed.), Science against microbial pathogens: communicating current research and technological advances, Formatex, Badajoz, Spain, 1179–1185.

Carson CF, Mee BJ, Riley TV (2002): Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob Agents Chemother 46: 1914–1920.

Celikel N, Kavas G (2008): Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci 26(3): 174–181.

Clinical and Laboratory Standards Institute (2012): Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Eleventh Edition. Document M02-A11, vol.32, No.1, Wayne, PA, USA.

Clinical and Laboratory Standards Institute (2012): Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Ninth Edition. Document M07-A9, Vol. 32. No.2. Wayne, PA, USA.

Čabarkapa I, Škrinjar M, Blagojev N, Gubić J, Plavšić D, Kokić B, Radusin T (2013): Effect of *Origanum heracleoticum* L. essential oil on marinated chicken meat shelf-life. J Pure Appl Microbiol 7(1): 221–228.

Daferera DJ, Ziogas BN, Polissiou MG (2003): The effectiveness of plant essential oils on the growth of *Botrytis cinerea, Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis.* Crop Protect 22(1): 39–44.

Davies N (1990): Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20 M phases. J Chromatogr A 503: 1–24.

Deans SG, Ritchie G (1987): Antibacterial properties of plant essential oils. Int J Food Microbiol 5: 165–180.

De Martino L, De Feo V, Nazzaro F (2009): Chemical composition and *in vitro* antimicrobial and mutagenic activities of seven *Lamiaceae* essential oils. Molecules 14: 4213–4230.

Dussault D, Vu KD, Lacroix M (2014): *In vitro* evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. Meat Sci 96(1): 514–520.

Firouzi R, Shekarforoush SS, Nazer AHK, Borumand Z, Jooyandeh AR (2007): Effects of essential oils of oregano and nutmeg on growth and survival of *Yersinia enterocolitica* and *Listeria monocytogenes* in barbecued chicken. J Food Protect 70: 2626–2630.

Fisher K, Phillips C (2008): Potential antimicrobial uses of essential oils in food: Is citrus the answer? Trends Food SciTech 19(2): 156–164.

Friedly EC, Crandal PG, Ricke SC, Roman M, O'bryan C, Chalova VI (2009): In vitro antilisterial effects of citrus oil fractions in combination with organic acids. J Food Sci 74: M67–M72.

Grayer RJ, Kite, GC Goldstone FJ, Bryan SE, Paton A, Putievsky E (1996): Intraspecific taxonomy and essential oil chemotypes in Sweet basil, *Ocimum basilicum.* Phytochemistry 43: 1033–1039.

Gill AO, Delaquis P, Russo P, Holley RA (2002): Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int J Food Microbiol 73: 83–92.

Glišić S, Milivojević S, Dimitrijević S, Orlović A, Skala D (2007): Antimicrobial activity of the essential oil and different fractions of *Juniperus communis* L. and a comparison with some commercial antibiotics. J Serb Chem Soc 72(4): 311–320.

Hao YY, Brackett RE, Doyle MP (1998): Efficacy of plant extracts in inhibiting *Aeromonas hydrophila* and *Listeria monocytogenes* in refrigerated cooked poultry. Food Microbiol 15: 367–378.

Hussain AI, Anwar F, Hussain Sherazi ST, Przybylski R (2008): Chemical composition, antioxidant and antimicrobial activities of basil *(Ocimum basilicum)* essential oils depends on seasonal variations. Food Chem 108(3): 986–995.

Jorgensen JH, Ferraro MJ (2009): Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Med Microbiol 49: 1749–1755.

Kamaliroostaa Z, Kamaliroosta L, Elhamiradc AH (2013): Isolation and identification of ginger essential oil. Journal of Food Biosciences and Technology Islamic Azad University 3: 73–80.

Kapoor IPS, Singh B, Singh S, Singh G (2014): Essential oil and oleoresins of black pepper as natural food preservatives for orange juice. J Food Process Preserv 30: 146–152.

Kim S-Y, Kang D-H, Kim J-K, Ha Y-G, Hwang JY, Kim T (2011): Antimicrobial activity of plant extracts against *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fresh lettuce. J Food Sci 76: M41–M46.

Kizhakkayil J, Sasikumar B (2012): Characterization of ginger (*Zingiber officinale* Rosc.) germplasm based on volatile and non-volatile components. Afr J Biotechnol 11(4): 777–786.

Kizil S, Haşimi N, Tolan V, Kilinç E, Karataş H (2010): Chemical composition, antimicrobial and antioxidant activities of hyssop (*Hyssopus officinalis* L.) essential oil. Not Bot Hort Agrobot Cluj 38(3): 99–103.

Kocić-Tanackov SD, Dimić GR, Mojović LV, Pejin JD, Tanackov IJ (2014): Effect of caraway, basil, and oregano extracts and their binary mixtures on fungi in growth medium and on shredded cabbage. LWT – Food Science and Technology 59(1): 426–432.

Kocić-Tanackov S, Dimić G, Mojović Lj, Pejin J, Tanackov I, Djukić-Vuković A (2015): Inhibitory effect of the basil extract on the growth of *Cladosporium cladosporioides, Emericella nidulans* and *Eurotium* species isolated from food. J Food Process Preserv 39: 887–895.

Lawrence BM (1992): Chemical components of *Labiatae* oils and their exploitation. In: Harley RM, Reynolds T (eds.), Advances in Labiate Science, Royal Botanic Gardens, Kew, UK, 399–436.

Mitić V, Đorđević S (2000): Essential oil composition of *Hyssopus officinalis* L. cultivated in Serbia. Facta Universitatis 2 (2): 105–108.

Meshkatalsadat MH, Salahvarzi S, Aminiradpoor R, Abdollahi A (2012): Identification of essential oil constituents of caraway *(Carum carvi)* using ultrasonic assist with headspace solid phase microextraction (UA-HS-SPME). Dig J Nanomater Biostruct 7 (2): 637–640.

Moreira MR, Ponce AG, Del Valle CE, Roura SI (2005): Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT – Food Science and Technology 38(5): 565–570.

Mourey A, Canillac N (2002): Anti-*Listeria monocytogenes* activity of essential oils components of conifers. Food Control 13: 289–292.

Nampoothiri SV, Venugopalan VV, Joy B, Sreekumar MM, Nirmala Menon A (2012): Comparison of essential oil composition of three ginger cultivars from sub Himalayan region. Asian Pac J Trop Biomed S1347–S1350.

Nikolić M, Jovanović KK, Marković T, Marković D, Gligorijević N, Radulović S, Soković M (2014): Chemical composition, antimicrobial, and cytotoxic properties of five *Lamiaceae* essential oils. Ind Crop Prod 61: 225–232.

Pepeljnjak S, Kosalec I, Kalodjera Z, Blažević N (2005): Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L. *Cupressaceae*). Acta Pharm 55: 417–422.

Porte A, Godoy RLO, Maia-Porte LH (2013): Chemical composition of sage (*Salvia officinalis* L.) essential oil from the Rio de Janeiro State (Brazil). Rev Bras Pl Med Campinas 15(3): 438–441.

Rasooli I (2008): Food preservation – a biopreservative approach. Food 1(2): 111–136.

Rasooli I, Allameh A (2016): Caraway (*Carum carvi* L.) essential oils. In: Preedy, VR (ed.), Essential oils in food preservation, flavor and safety. Academic Press, Elsevier, London, UK, 287–293.

Ratledge C, Wilkinson SG (1988): An overview of microbial lipids. In: Ratledge C, Wilkinson SG (eds.), Microbial Lipids, vol. 1. Academic Press, Elsevier, London, UK, 3–22.

Pripdeevech P, Chumpolsri W, Suttiarporn P, Wongpornchai S (2010): The chemical composition and antioxidant activities of basil from Thailand using retention indices and comprehensive two-dimensional gas chromatography. J Serb Chem Soc 75(11): 1503–1513.

Sa-Nguanpuang K, Kanlayanarat S, Srilaong V, Tanprasert K, Techavuthiporn C (2011): Ginger *(Zingber officinale)* oil as an antimicrobial agent for minimaly processed produce: a case study in shredded green papaya. Int J Agric Biol 13: 895–901.

Sarikurkcu C, Zengin G, Oskay M, Uysal S, Ceylan R, Aktumsek A (2015): Composition, antioxidant, antimicrobial and enzyme inhibition activities of two *Origanum vulgare* subspecies (subsp. *vulgare* and subsp. *hirtum*) essential oils. Ind Crop Prod 70: 178–184.

Sánchez-Ortega I, García-Almendárez BE, Santos-López EM, Amaro-Reyes A, Barboya-Corona JE, Regalado C (2014): Antimicrobial edible films and coatings for meat and meat products preservation. The Sci World J 2014:248935: 1–18.

Sedláková J, Kocourková B, Lojková L, Kubáň V (2003): The essential oil content in caraway species (*Carum carvi* L.). Hort Sci (Prague) 30 (2): 73–79.

Sharopov FS, Kukaniev MA, Thompson RM, Satyal P, Setzer WN (2012): Composition and antimicrobial activity of the essential oil of *Hyssopus seravschanicus* growing wild in Tajikistan. Der Pharma Chem 4(3): 961–966.

Smith-Palmer A, Stewart J, Fyfe L (1998): Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Appl Microbiol 26: 118–122.

Suppakul P, Miltz J, Sonneveld K, Bigger SW (2003): Antimicrobial properties of basil and its possible application in food packaging. J Agr Food Chem 51: 3197–3207.

Stecchini ML, Sarais I, Giavedoni P (1993): Effect of essential oils on *Aeromonas hydrophila* in a culture medium and in cooked pork. J Food Protec 56(5): 406–409.

Šojić B, Tomović V, Kocić-Tanackov S, Škaljac S, Ikonić P, Džinić N, Živković N, Jokanović M, Tasić T, Kravić S (2015): Effect of nutmeg *(Myristica fragrans)* essential oil on the oxidative and microbial stability of cooked sausage during refrigerated storage. Food Control 54: 282–286.

Tajkarimi MM, Ibrahim SA, Cliver DO (2010): Antimicrobial herb and spice compounds in food. Food Control 21(9): 1199–1218.

Tarek N, Hassan HM, Abdelghani SMM, Radwan IA, Hammouda O, El-Gendy AO (2014): Comparative chemical and antimicrobial study of nine essential oils obtained from medicinal plants growing in Egypt. BENI-SEUF Univ J Appl Sci 3(2): 149–156.

Tassou C, Drosinos EH, Nychas GJE (1995): Effects of essential oil from mint *(Mentha piperita)* on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4 °C and 10 °C. J Appl Bacteriol 78: 593–600.

Vaara M (1992): Agents that increase the permeability of the outer membrane. Microbiol Rev 56(3): 395–411.

Wan J, Wilcock A, Coventry MJ (1998): The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens.* J Appl Microbiol 84: 152–158.

Zawiślak G (2013): The chemical composition of essential hyssop oil depending on plant growth stage. Acta Sci Pol Hortorum Cultus 12(3): 161–170.

Address of corresponding author:
Dr. Sunčica Kocić-Tanackov
Department of Food Preservation Engineering
Faculty of Technology
University of Novi Sad
21 000 Novi Sad
Serbia
suncicat@uns.ac.rs