CROATICA CHEMICA ACTA
CCACAA **79** (4) 545–552 (2006)

ISSN-0011-1643
CCA-3123
Original Scientific Paper

Chemical Composition and Antioxidant Activity of Essential Oils of Twelve Spice Plants

Olivera Politeo,* Mila Jukić, and Mladen Miloš

Faculty of Chemical Technology, Department of Biochemistry and Food Chemistry, University of Split, Teslina 10/V, 21000 Split, Croatia

RECEIVED AUGUST 31, 2005; REVISED MARCH 2, 2006; ACCEPTED MARCH 16, 2006

Chemical compositions and related total antioxidant capacities of twelve spice essential oils were analyzed. To enable a comparison of their relative antioxidant potentials, essential oils were extracted by hydrodistillation from selected spice plants and their chemical compositions were determined by the GC-MS system on two fused-silica capillary columns of different polarity. Antioxidant effectiveness was examined by four different methods: the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, determination of ferric reducing antioxidant power (FRAP), determination of antioxidant activity with thiobarbituric acid reactive species (TBARS) and automatic determination of the oxidative stability of fat (RANCIMAT). Based on their antioxidant capacity, twelve spice essential oils can be sorted in descending order: Clove (*Syzygium aromaticum* L.) > Basil (*Ocimum basilicum* L.) > Laurel (*Laurus nobilis* L.) > Coriander (*Coriandrum sativum* L.) > Nutmeg (*Myristica fragrans* Houtt.) > Black Pepper (*Piper nigrum* L.) > Everlast (*Helichrysum italicum* G. (Roth) Don) > Mint (*Mentha piperita* L.) > Marjoram (*Marjorana hortensis* Moench.) > Cinnamon (*Cinnamomum zeylanicum* Nees) > Sage (*Salvia officinalis* L.) > Fennel (*Foeniculum vulgare* Muller).

Keywords
spice plants
essential oils
chemical composition
GC-MS
antioxidant activity

INTRODUCTION

About ten years ago, Aruoma[1] and then Halliwell[2] described the experimental strategies for optimization of nutritional antioxidant intake in humans. The antioxidant properties of many aromatic herbs are reported to be effective in this role.[3–5] Apart from their use as aroma additives in food, essential oils from aromatic spice plants have a potential to be used in small amounts in fat-containing food systems to prevent or delay some chemical deteriorations occurring during the storage of these products.

Antioxidant activities of aroma extracts obtained from spices have been investigated in various model systems.[6–8] Shahidi *et al.*[9] reported that the antioxidant effect of aromatic plants is due to the presence of hydroxyl groups in their phenolic compounds. Lagouri *et al.*[10] studied the antioxidant activity of essential oils and they found that oregano essential oil, rich in thymol and carvacrol, has a considerable antioxidant effect on the process of lard oxidation. In our previous works,[11–13] all »phenolic« type essential oils, containing thymol and carvacrol as major components, exhibited strong antioxidant activity.

As a part of an investigation of natural antioxidants from spice plants, we report in this paper a study of the antioxidant activities associated with the chemical com-

* Author to whom correspondence should be addressed. (E-mail: olivera@ktf-split.hr)

position of essential oils without significant amounts of thymol and carvacrol, isolated from twelve different spice plants. Our aim is to find out if they can be potent antioxidants like the »phenolic« type essential oils described above and to estimate which of their constituents could be active in this role.

For this purpose, the screening of antioxidant power was performed *in vitro* by four different methods: the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, determination of ferric reducing antioxidant power (FRAP), determination of antioxidant activity with thiobarbituric acid reactive species (TBARS) and automatic determination of the oxidative stability of fat (RANCIMAT).

EXPERIMENTAL

Plant Material

Twelve spices: Clove, *Syzygium aromaticum* L. (Myrtaceae); Basil, *Ocimum basilicum* L. (Lamiaceae); Laurel, *Laurus nobilis* L. (Lauraceae); Coriander, *Coriandrum sativum* L. (Apiaceae); Nutmeg, *Myristica fragrans* Houtt. (Myristicaceae); Black Pepper, *Piper nigrum* L. (Piperaceae); Everlast, *Helichrysum italicum* G. (Roth) Don (Compositae); Mint, *Mentha piperita* L. (Lamiaceae); Marjoram, *Marjorana hortensis* Moench. (Lamiaceae); Cinnamon, *Cinnamomum zeylanicum* Nees (Lauraceae); Sage, *Salvia officinalis* L. (Lamiaceae) and Fennel, *Foeniculum vulgare* Muller (Apiaceae) were purchased from a local market in Split, Croatia. Plant materials consisted of flower buds (clove), leaves (basil, laurel, mint, marjoram, sage), fruits (coriander, nutmeg, black pepper, fennel), stem bark (cinnamon) and flowered tops (everlast). Voucher specimens of spice plant materials are deposited in the Department of Biochemistry and Food Chemistry, Faculty of Chemical Technology, Split, Croatia.

Isolation of Essential Oils

A hundred grams of dried plant material was subjected to three-hours of hydrodistillation using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and stored under nitrogen in sealed vials at –18 °C until required.

The chemicals and all applied solvents were of pro analysis purity and were purchased from Fluka Chemie, Buchs, Switzerland.

Gas Chromatography-Mass Spectrometry

Analyses of volatile compounds were run on a Hewlett – Packard GC-MS system (GC 5890 series II; MSD 5971A, Hewlett Packard, Vienna, Austria). Two columns of different polarity were used: a HP-101 column (Methyl silicone fluid, Hewlett Packard; 25 m × 0.2 mm i.d., film thickness 0.2 μm) and a HP-20M column (Carbowax, Hewlett Packard; 50 m × 0.2 mm i.d., film thickness 0.2 μm). Oven temperature was programmed as follows: isothermal at 70 °C for 4 min, then increased to 180 °C, at a rate of 4 °C min^{-1} and subsequently held isothermal for 15 min (for HP-20M column); isothermal at 70 °C for 2 min, then increased to 200 °C, at a rate of 3 °C min^{-1} and held isothermal for 15 min (for HP-101 column). The carrier gas was helium (1 mL/min). The injection port temperature was 250 °C and the detector temperature was 280 °C. Ionization of sample components was performed in the EI mode (70 eV). A volume of 1 μL was injected.

The linear retention indices for all compounds were determined by co-injection of the sample with a solution containing a homologous series of C_8-C_{22} *n*-alkanes.[14] The individual constituents were identified by their retention indices identical to the compounds known from literature data,[15] and also by comparing their mass spectra with spectra of either the known compounds or with those stored in the Wiley mass spectral database (Hewlett Packard, Vienna, Austria).

Choice of the Method for Determination of Antioxidant Activities

As previously described, antioxidant activity assessment requires use of different methods.[16,17] Like in numerous studies, [8,18–23] DPPH, FRAP, TBARS and RANCIMAT can be cited as relatively simple methods that can be used to measure the antioxidant potential of essential oils. The DPPH method is sensitive and requires little sample material.[24] The TBARS method is also sensitive and achieves reproducible results. The FRAP method is fast, easy to handle, with highly reproducible results.[25] Although the RANCIMAT technique has been questioned,[26] this procedure is commonly used in the food industry and governmental analytical laboratories.[27]

Determination of Antioxidant Activity with the 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

The antioxidant activity of volatile compounds was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH.[28] A methanolic stock solution (50 μL) of the essential oils (concentrations of stock solutions were 50, 20, 10 and 5 g/L) was put into a cuvette, and 2 mL of 6×10^{-5} mol L^{-1} methanolic solution of DPPH was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined with a Perkin-Elmer spectrophotometer after 1 h for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the DPPH radical without antioxidant, *i.e.* the control, was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution.[24] Percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen & Duh:[29]

$$\% \text{ inhibition} = ((A_{C(o)} - A_{A(t)}) / A_{C(o)}) \times 100$$

where $A_{C(o)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 1 h.

Determination of Ferric Reducing Antioxidant Power (FRAP Assay)

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain[30] as a measure of »antioxidant power«. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from the colorless oxidized Fe^{III} form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100–1000 μmol/L) of $FeSO_4 \cdot 7H_2O$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the antioxidant tested was calculated with reference to the reaction signal given by an Fe^{2+} solution of known concentration, this representing a one-electron exchange reaction. The results were corrected for dilution and expressed in μmol Fe^{II}/L. The sample to be analyzed was first adequately diluted to fit within the linearity range.

Determination of Antioxidant Activity with Thiobarbituric Acid Reactive Species (TBARS Assay)

Modified thiobarbituric acid reactive species (TBARS) assay[20] was used to measure the potential antioxidant capacity using egg yolk homogenates as lipid rich media. Briefly, 0.5 mL of 10 % (w/v) homogenate and 0.1 mL of sample solutions to be tested were added to a test tube and made up to 1.0 mL with distilled water. 0.05 mL of 2,2'-azobis (2-amidinopropane) dihydrochloride solution (0.07 mol L^{-1}) in water was added to induce lipid peroxidation. 1.5 mL of 20 % acetic acid (pH = 3.5) and 1.5 mL 0.8 % (w/v) thiobarbituric acid in 1.1 % (w/v) sodium dodecyl sulphate solution was added and the resulting mixture was vortexed, and then heated at 95 °C for 60 min. After cooling, 5.0 mL of butan-1-ol was added to each tube, then extensively vortexed and centrifuged at 1200 g for 10 min. Absorbance of the organic upper layer was measured using a spectrophotometer (PerkinElmer Lambda EZ 201, Roma, Italia) set at 532 nm. All the values were based on the percentage antioxidant index (AI %):

$$\text{AI \%} = (1 - A_T/A_C) \times 100$$

where A_C is the absorbance value of the fully oxidized control and A_T is the absorbance of the test sample.

Determination of Oxidative Stability of Fat (RANCIMAT)

A Rancimat 743 (Metrohm, Switzerland) was used to determine the antioxidant lipid activity of volatile compounds contained in the essential oils of the spice plants. The Rancimat worked on the following principle: A solution of different concentrations of antioxidant (100 μL) was added to the lard (2.5 g) giving a final concentration of 0.20 %, 0.08 %, 0.04 % or 0.02 % of antioxidant in the reacting system. The lard with and without addition of antioxidant was heated at 110 °C and an airflow of 20 L/h was constantly blown into the mixture.

The antioxidant activity index (AAI) was calculated from the measured induction times, according to the following formula by Forster *et al.*[31]

AAI = Induction time of lard with antioxidant / Induction time of pure lard

RESULTS AND DISCUSSION

Chemical Composition of Essential Oils

The analyses were successful without previous fractionation of essential oils. Except for laurel (93.0 %), more than 95 percent of constituents were identified in all other essential oil samples. The results of these analyses are presented in Table I as a relative peak area of each constituent. It seems that there were no similarities among chemical compositions of the studied essential oils. Some oils have very simple chemical composition. For example, the clove, coriander and fennel essential oils were composed of only five, eight and seven compounds, respectively. On the other side, some oils were very complex. The everlast, nutmeg and, laurel essential oils were composed of 37, 24 and 22 compounds, respectively. Other essential oils had fewer than 20 identified compounds. In some of the essential oils, the main constituents accounted for more than 90 % of total oil, *e.g.,* cinnamon (*trans*-cinnamaldehyde 94.0 %), coriander (linalool 92.0 %) and clove oils (eugenol 91.2 %). In fennel essential oil, the content of *trans*-anethol was 77.6 %; in black pepper, the content of caryophyllene was 57.6 %, and in sage essential oil, the content of thujone was 56.5 %. In other essential oils, the main compounds accounted for less than 50 % of total oil. The main compounds of these last ones were the following: estragole (24.7 %) and linalool (23.5 %) in basil oil; neomenthol (44.1 %) and isomenthone (30.9 %) in mint oil; 1.8-cineole (34.9 %) and linalool (13.5 %) in laurel oil; terpinen-4-ol (40.8 %), γ-terpinene (16.3 %) and α-terpinene (11.0 %) in marjoram oil; α-cedrene (18.3 %), α-pinene (11.3 %) and 2-methylcyclohexyl-pentanoate (10.5 %) in everlast oil, and sabinene (25.4 %), α-pinene (15.8 %), myristicine (14.8 %) and β-pinene (13.4 %) in nutmeg oil.

Antioxidant Activity of Essential Oils

Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other

TABLE I. Percentage compositions of twelve essential oils

No.	Compound	RI[(a)] HP-101 / HP-20M	Peak area / %											
			Clove	Coriander	Basil	Mint	Black pepper	Laurel	Marjoram	Everlast	Nutmeg	Fennel	Cinnamon	Sage
1	Salvene	825 / –	–	–	–	–	–	–	–	–	–	–	–	0.5
2	α-Thujene	930 / 1032	–	–	–	–	–	–	2.2	–	1.8	–	–	–
3	α-Pinene	936 / 1038	–	1.1	–	2.2	3.3	1.9	–	11.3	15.8	0.2	–	4.5
4	Camphene	954 / 1060	–	–	–	–	–	–	–	–	–	–	–	2.8
5	Sabinene	975 / 1092	–	–	–	–	9.5	1.2	3.6	–	25.4	–	–	–
6	β-Pinene	972 / 1102	–	–	0.2	0.9	–	–	–	0.6	13.4	–	–	1.5
7	Δ^3-Carene	1009 / 1131	–	–	–	–	1.3	0.4	–	–	–	–	–	–
8	Myrcene	981 / 1148	–	–	–	–	0.8	–	0.8	0.6	2.0	–	–	0.5
9	α-Phellandrene	978 / 1161	–	–	–	–	0.4	–	0.8	–	1.0	–	–	–
10	α-Terpinene	996 / 1163	–	–	–	0.3	1.4	0.3	11.0	0.4	2.0	–	–	–
11	1,8-Cineole	1027 / 1179	–	–	3.5	3.8	–	34.9	–	–	–	–	–	14.1
12	Limonene	1023 / 1183	–	–	0.2	–	8.8	0.9	0.3	4.6	3.4	0.6	–	–
13	β-Phellandrene	1001 / 1187	–	–	–	–	–	–	3.4	–	1.7	–	–	–
14	γ-Terpinene	1049 / 1231	–	1.6	–	0.7	0.6	0.8	16.3	0.8	3.9	–	–	–
15	*p*-Cymene	1020 / 1247	–	0.8	–	–	0.2	0.2	1.5	0.4	0.7	–	–	–
16	α-Terpinolene	1083 / 1260	–	–	–	–	1.3	0.2	2.8	0.1	1.0	–	–	–
17	(*Z*)-2-methyl-2-butene acid	1028 / 1272	–	–	–	–	–	–	–	0.4	–	–	–	–
18	Dodecane	1190 / 1329	–	–	–	–	–	–	–	0.2	–	–	–	–
19	Fenchone	1062 / 1365	–	–	–	–	–	–	–	–	–	12.4	–	–
20	(*E*)-2-methyl-2-butene acid	1128 / 1373	–	–	–	–	–	–	–	1.6	–	–	–	–
21	4-methyl anisole	– / 1392	–	–	–	–	–	–	–	0.2	–	–	–	–
22	Thujone	1104 / 1396	–	–	–	–	–	–	–	–	–	–	–	56.5
23	*trans*-Sabinene hydrate	– / 1431	–	–	–	–	–	1.1	–	–	–	–	–	–
24	Isomenthone	1029 / 1452	–	–	–	30.9	–	–	–	–	–	–	–	–
25	α-Copaene	1365 / 1466	–	–	–	–	0.5	–	–	4.2	0.4	–	0.9	–
26	γ-Elemene	1482 / 1469	–	–	–	–	2.4	–	0.2	–	–	–	–	–
27	Camphor	1122 / 1482	–	1.4	0.6	–	–	–	–	–	–	0.3	–	5.7
28	β-Bourbonene	1354 / –	–		0.1	–	–	–	–	–	–	–	–	–
29	Linalool	1085 / 1507	–	92.0	23.5	–	–	13.5	3.7	0.7	0.3	–	–	–
30	Menth-8-ene	– / 1530	–	–	–	3.7	–	–	–	–	–	–	–	–
31	Bornyl acetate	1266 / 1550	–	–	0.4	–	–	0.3	–	–	0.2	–	0.3	0.3
32	Neoisomenthol	1145 / 1556	–	–	–	3.6	–	–	–	–	–	–	–	–
33	Terpinen-4-ol	1162 / 1561	–	–	–	–	–	2.4	40.8	–	6.2	–	–	t
34	β-Elemene	1364 / –	–	–	0.6	–	–	–	–		–	–	–	–
35	Dihydrocarvone	1169 / –		–	–	–	–	–	0.4	–	–	–	–	–
36	Caryophyllene	1395 / 1585	1.2	–	0.6	2.6	57.6	2.1	1.3	6.7	–	–	0.7	0.9
37	Germacrene B	1400 / 1606	–	–	–	–	3.4	–	–	–	–	–	–	–
38	Neomenthol	1153 / 1612	–	–	–	44.1	–	–	–	–	–	–	–	–
39	Alloaroma–dendrene	1447 / 1613	–	–	0.2	–	–	–	–	–	–	–	–	0.2
40	*trans*-Pino–carveole	– / 1614	–	–	–	–	–	–	–	0.1	–	–	–	–
41	α-Terpineol	1295 / 1624	–	0.2	1.2	0.3	–	0.3	6.1	0.5	0.4	–	–	0.2
42	α-Humulene	1430 / 1638	0.1	–	0.4	0.2	2.6	–	0.3	–	–	–	0.6	6.9
43	Fenchol	– / 1646	–	–	–	1.0	–	–	–	–	–	–	–	–

(cont.)

(cont.)

			Peak area / %											
No.	Compound	RI[a] HP-101 / HP-20M	Clove	Coriander	Basil	Mint	Black pepper	Laurel	Marjoram	Everlast	Nutmeg	Fennel	Cinnamon	Sage
44	Estragole	1183 / 1655	–	–	24.7	–	–	–	–	–	–	2.2	–	–
45	γ-Cadinene	1428 / –	–	–	0.4	–	–	–	–	–	–	–	–	–
46	Borneol	1165 / –	–	–	–	–	–	–	–	–	–	–	–	0.7
47	Germacrene D	1442 / 1669	–	–	0.5	1.2	0.3	–	–	–	–	–	–	–
48	Piperitone	1216 / 1673	–	–	–	0.6	–	–	–	–	–	–	–	–
49	α-Cedrene	– / 1674	–	–	–	–	–	–	–	18.3	–	–	–	–
50	α-Muurolene	1506 / 1683	–	–	–	–	–	–	–	0.3	–	–	0.3	–
51	Carvone	– / 1684	–	–	0.8	–	–	–	0.3	–	–	–	–	–
52	Neryl acetate	1345 / 1692	–	–	–	–	–	0.3	–	7.6	–	–	–	–
53	β-Farnesene	1452 / –	–	–	0.3	–	–	–	–	–	–	–	–	–
54	β-Bisabolene	1499 / 1694	–	–	0.2	–	–	–	–	4.6	–	–	–	–
55	β-Selinene	1419 / 1695	–	–	–	–	1.3	–	–	3.4	–	–	–	–
56	Benzenepropanal	1140 / 1709	–	–	–	–	–	–	–	–	–	–	0.3	–
57	Δ-Cadinene	1497 / 1716	t	–	0.2	0.2	0.3	0.2	–	1.9	0.2	–	0.4	–
58	α-Zingiberene	– / 1724	–	–	–	–	–	–	–	1.3	–	–	–	–
59	α-Farnesene	1518 / 1725	–	–	–	0.4	–	0.6	0.3	–	–	–	–	–
60	ar-Curcumene	– / 1747	–	–	–	–	–	–	–	5.1	–	–	–	–
61	Nerol	1223 / 1762	–	–	–	–	–	–	–	1.2	–	–	–	–
62	α-Bergamotene	1414 / 1779	–	–	2.7	–	–	–	–	0.8	0.2	–	–	–
63	Geraniol	– / 1787	–	1.0	–	–	–	–	–	–	–	–	–	–
64	2-Methylcyclohexyl pentanoate	– / 1798	–	–	–	–	–	–	–	10.5	–	–	–	–
65	*trans*-Anethole	1273 / 1809	–	–	0.2	0.1	–	–	–	–	–	77.6	–	–
66	Safrole	– / 1809	–	–	–	–	–	–	–	–	3.3	–	–	–
67	Cresole	– / 1812	–	–	–	–	1.4	–	–	–	–	–	–	–
68	2-Methylcyclohexyl octanoate	– / 1856	–	–	–	–	–	–	–	2.1	–	–	–	–
69	α-Terpinyl acetate	1333 / 1880	–	–	–	–	–	12.2	–	–	–	–	–	–
70	Methyl cinnamate[b]	1281 / 1900	–	–	1.5	–	–	–	–	–	–	–	–	
71	Caryophyllene oxide	– / 1917	–	–	–	–	–	2.3	–	–	–	–	–	–
72	*cis*-Calamenene	1549 / 1927	–	–	0.3	–	–	–	–	–	–	–	0.2	–
73	α-Amorphene	1439 / –	–	–	2.3	–	–	–	–	–	–	–	0.1	–
74	α-Guaiene	1404 / –	–	–	–	–	0.2	–	–	–	–	–	–	–
75	Methyl eugenol	1390 / 1947	–	–	4.1	–	–	13.5	–	–	0.9	–	–	–
76	Anisaldehyde	– / 1947	–	–	–	–	–	–		–	–	0.6	–	–
77	Geranyl propanoate	1421 / 1956	–	–	–	–	–	–	–	0.2	–	–	–	–
78	Nerolidol	1513 / 1996	–	–	–	–	–	–	–	0.2	–	–	–	–
79	*trans*-Cinnam aldehyde	1280 / 1997	–	–	–	–	–	–	–	–	–	–	94.0	–
80	Neryl propionate	1685 / 2017	–	–	–	–	–	–	–	0.2	–	–	–	–
81	Methyl cinnamate[b]	1364 / 2020	–	–	11.1	–	–	–	–	–	–	–	–	–
82	Guaiol	1567 / 2083	–	–	–	0.4	–	–	–	0.3	–	–	–	–

(cont.)

(cont.)

No.	Compound	RI[a] HP-101 / HP-20M	Peak area / % Clove	Coriander	Basil	Mint	Black pepper	Laurel	Marjoram	Everlast	Nutmeg	Fennel	Cinnamon	Sage
83	α-Cadinol	– / 2085	–	–	4.4	–	–	–	–	–	–	–	0.2	–
84	Eugenol	1377 / 2098	91.2	0.5	11.6	–	–	3.4	–	–	0.2	–	–	–
85	Eugenyl acetate	– / 2107	7.4	–	–	–	–	–	–	–	–	–	–	–
86	Torreiol	– / 2112	–	–	–	–	–	–	–	0.4	–	–	–	–
87	Thymol	1362 / 2115	–	–	0.2	0.2	–	–	0.2	0.5	–	–	–	–
88	Elemicine	1521 / 2165	–	–	–	–	–	–	–	–	0.3	–	–	–
89	β-Eudesmol	1613 / 2176	–	–	–	–	–	–	–	0.3	–	–	–	–
90	γ-Gurjunene	1616 / –	–	–	–	–	–	–	–	0.2	–	–	–	–
91	Myristicine	1496 / –	–	–	–	–	–	–	–	–	14.8	–	–	–
92	Chavicol	– / c	–	–	0.6	–	–	–	–	–	–	–	–	–
93	Coumarin	c / –	–	–	–	–	–	–	–	–	–	–	0.3	–
Total:			99.9	98.6	97.6	97.4	97.6	93.0	96.3	92.8	99.5	93.9	98.4	95.3

[a] RI, retention indices relative to C_8-C_{22} alkanes on polar HP-20 M and apolar HP-101 columns (sorted according to HP-20 M)
[b] Correct isomer is not identified
[c] Retention times are outside retention times of homologous series of C_8-C_{22} alkanes (identified by MS)
t Peak area < 0.1%
- Not identified

constituents in small quantities or to synergy among them.[32] In this study, the antioxidant activities related to the contents of essential oils of twelve aromatic spice plants belonging to different plant families were determined. The results are summarized in Table II. It was found that the essential oils of all analyzed plants showed very different antioxidant capacities. Stronger activity is indicated by a higher antioxidant index determined by each of the three different methods: DPPH, FRAP and TBARS. In contrast, the RANCIMAT test showed almost the same results for all tested oils. The results from Table II suggest that the essential oils from three spice plants, *i.e.,* clove, basil and laurel, could be used as a potential source of natural antioxidants with possible applications in food systems. The antioxidant activity of clove essential oil is mainly due to the high content of eugenol. The same result was previously indicated by the lipid-malonaldehyde assay.[33]

TABLE II. Antioxidant activity of twelve essential oils using the corresponding concentrations (A = 50 g/L, B = 20 g/L, C = 10 g/L, D = 5 g/L) measured by four different methods: DPPH, FRAP, TBARS and RANCIMAT

	DPPH % inhibition				FRAP mmol / L				TBARS AI %				RANCIMAT AAI
	A	B	C	D	A	B	C	D	A	B	C	D	A
Clove	94	93	93	93	740	440	131	88	65	49	32	22	1.5
Basil	93	93	88	85	78	25	13	7	45	29	26	22	1.1
Laurel	93	89	80	68	22	10	4	2	38	18	4	1	1.1
Coriander	88	69	44	30	12	5	2	<1	39	21	18	9	1.0
Nutmeg	82	56	39	24	11	3	2	<1	67	40	30	24	1.1
Black Pepper	61	37	22	14	11	3	2	1	36	36	27	16	0.9
Everlast	42	20	14	11	7	2	1	<1	45	30	17	17	0.9
Mint	38	20	12	8	<1	<1	<1	<1	36	19	12	5	0.9
Marjoram	29	15	11	9	3	1	<1	<1	70	50	49	26	1.0
Cinnamon	14	9	7	6	<1	<1	<1	<1	–	–	–	–	0.9
Sage	14	6	5	5	2	1	<1	<1	27	10	9	<1	0.9
Fennel	9	7	5	2	<1	<1	<1	<1	54	41	26	13	0.9

Regarding antioxidant activities of basil and laurel essential oils, it seems interesting that they showed good antioxidant activities despite the fact that the major constituents of these oils, *i.e.*, estragol and 1,8-cineole, are not known as potent antioxidants.[34] The antioxidant effectiveness of their essential oils is probably due to a relatively high content of eugenol (11.6 %) and methyl-eugenol in basil oil (4.1 %) and of methyl-eugenol in laurel oil (13.5 %).

The coriander and nutmeg essential oils could be interesting antioxidants only if applied at the highest concentration tested. Since their major constituents are not known as antioxidants, it can be suggested that the antioxidant activity of both essential oils is due to their minor constituents.

Essential oils from other examined spices showed very moderate antioxidant capacities. No evaluation of the antioxidant activity of cinnamon essential oil by the TBARS assay was possible, because the main component of oil, *trans*-cinnamaldehyde, strongly interacted with the thiobarbituric acid used in the assay, developing a yellow color.[19]

Further, our study has confirmed that no single testing method is sufficient to estimate the antioxidant activity of essential oils. It was shown that the RANCIMAT test is not appropriate for such investigations, because introducing air into hot measuring systems (fat) during measurement evaporates previously added essential oils and thereby prevents adequate measurements. Results obtained with this method are ambiguous and may guide to incorrect conclusions.

CONCLUSIONS

The study showed that antioxidant activity was related to the chemical composition of the twelve essential oils from spice plants commonly consumed in diet. The results obtained by the use of three different methods (DPPH, FRAP, TBARS) showed that some of these spices can be considered good sources of natural antioxidants. This may be attributed either to high percentage of the main constituents or to synergy among different oil constituents. Because of the conditions used for oxidation (110 °C and airflow of 20 L/h), the results obtained by the RANCIMAT test showed that this test was not appropriate for investigations of volatile compounds. Based on their antioxidant capacity, twelve spice plant essential oils were sorted in descending order: Clove (*Syzygium aromaticum* L.) > Basil (*Ocimum basilicum* L.) > Laurel (*Laurus nobilis* L.) > Coriander (*Coriandrum sativum* L.) > Nutmeg (*Myristica fragrans* Houtt.) > Black Pepper (*Piper nigrum* L.) > Everlast (*Helicrysum italicum* G. (Roth) Don) > Mint (*Mentha piperita* L.) > Marjoram (*Marjorana hortensis* Moench.) > Cinnamon (*Cinnamomum zeylanicum* Nees) > Sage (*Salvia officinalis* L.) > Fennel (*Foeniculum vulgare* Muller).

REFERENCES

1. O. I. Aruoma, *Food Chem. Toxicol.* **32** (1994) 671–683.
2. B. Halliwell, *Free Radical Res.* **25** (1996) 1–32.
3. K. Herrmann, M. Schutt, and H. Muller, *Deut. Lebensm.-Rundsch.* **77** (1981) 134–138.
4. U. Brraco, J. Loliger, and J. Viret, *J. Am. Oil Chem. Soc.* **58** (1981) 686–690.
5. R. E. Kramer, *J. Am. Oil Chem. Soc.* **62** (1985) 111–113.
6. N. Nakatami, *Antioxidants from spices and herbs*, in: F. Shahidi (Ed.), *Natural antioxidants: chemistry, health effects and applications,* AOAC Press, Champaign, Illinois, 1997, pp. 64–75.
7. L. Trojakova, Z. Reblova, H. T. T. Nguyen, and J. Pokorny, *J. Food Lipids* **8** (2001) 1–13.
8. M. Puertas-Mejia, S. Hillebrand, E. Stashenko, and P. Winterhalter, *Flavour Frag. J.* **17** (2002) 380-384.
9. F. Shahidi, P. K. Janitha, and P. D. Wanasundara, *Crit. Rev. Food Sci. Nutr.* **32** (1992) 67–103.
10. V. Lagouri, G. Blekas, M. Tsimidou, S. Kokkini, and D. Boskou, *Z. Lebensm. Unters. For.* **197** (1993) 20–23.
11. M. Miloš, J. Mastelić, and I. Jerković, *Food Chem*. **71** (2000) 79–83.
12. A. Radonić and M. Miloš, *Free Radical Res.* **37** (2003) 673–679.
13. T. Kulišić, A. Radonić, V. Katalinić, and M. Miloš, *Food Chem.* **85** (2004) 633–640.
14. H. Van Den Dool and P. D. Kratz, *J. Chromatogr.* **11** (1963) 463–471.
15. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography and Mass Spectroscopy*, Carol Stream, IL, Allured Publ. 1995.
16. E. N. Frenkel, S. W. Huang, J. Kanner, and J. B. German, *J. Agric. Food Chem.* **42** (1994) 1054–1059.
17. I. I. Koleva, T. A. van Beek, J. P. H. Linssen, A. de Groot, and L. N. Evstatieva, *Phytochem. Anal.* **13** (2002) 8–17.
18. M. T. Baratta, H. J. D. Dorman, and S. G. Deans, *J. Essent. Oil Res.* **10** (1998) 618–627.
19. M. T. Baratta, H. J. D. Dorman, S. G. Deans, A. C. Figueiredo, J. G. Barosso, and G. Ruberto, *Flavour Frag. J.* **13** (1998) 235–244.
20. G. Ruberto, M. T. Baratta, S. G. Deans, and H. J. D. Dorman, *Planta Med.* **66** (2000) 687–693.
21. H. J. D. Dorman, A. C. Figueiredo, J. G. Barroso, and S. G. Deans, *Flavour Frag. J.* **15** (2000) 12–16.
22. G. Ruberto, M. T. Baratta, M. Sari, and M. Kaâbeche, *Flavour Frag. J.* **17** (2002) 251–254.
23. A. Tomaino, F. Cimino, V. Zimbalatti, V. Venuti, V. Sulfaro, A. De Pasquale, and A. Saija, *Food Chem.* **89** (2005) 549–554.
24. M. S. Blois, *Nature* **181** (1958) 1199–1200.
25. S. Llesuy, P. Evelson, A. M. Campos, and E. A. Lissi, *Biol. Res.* **34** (2001) 51–73.
26. E. N. Frenkel, *Trends Food Sci. Technol.* **4** (1993) 220–225.
27. I. Pajero, F. Viladomat, J. Bastida, A. Rosas-Romero, G. Saavedra, M. A. Murcia, A. M. Jiménez, and C. Codina, *Life Sci.* **73** (2003) 1667–1681.

28. W. Brand-Williams, M. E. Cuvelier, and C. Berset, *Lebensm. Wiss. Technol.* **28** (1995) 25–30.
29. G. C. Yen and P. D. Duh, *J. Agric. Food Chem.* **42** (1994) 629–632.
30. I. F. F. Benzie and J. J. Strain, *Anal. Biochem.* **239** (1996) 70–76.
31. A. Forster, K. Simon, R. Y. Schmidt, and D. Kaltner, *What is about antioxidative characteristics of hops*, 28th EBC-Congress, Budapest, 2001.
32. A. E. Abdalla and J. P. Roozen, *Food Chem.* **64** (1999) 323–329.
33. K. G. Lee and T. Shibamoto, *Food Chem.* **74** (2001) 443–448.
34. S. J. Lee, K. Umano, T. Shibamoto, and K. G. Lee, *Food Chem.* **91** (2005) 131–137.

SAŽETAK

Kemijski sastav i antioksidacijska aktivnost eteričnih ulja dvanaest začinskih biljaka

Olivera Politeo, Mila Jukić i Mladen Miloš

Analiziran je kemijski sastav i antioksidacijski kapacitet eteričnih ulja dvanaest začinskih biljaka. Kako bi mogli usporediti antioksidacijski potencijal, eterična ulja odabranih začinskih biljaka izolirana su vodenom destilacijom, a njihov kemijski sastav određen je GC-MS sustavom na dvije kolone različite polarnosti. Antioksidacijska aktivnost ispitana je pomoću četiri različite metode: metodom vezivanja slobodnih radikala (DPPH metoda), metodom određivanja sposobnosti redukcije željeza (FRAP metoda), metodom s tiobarbiturnom kiselinom (TBA metoda) i metodom određivanja oksidativne stabilnosti masti (RANCIMAT metoda). Temeljem antioksidacijskog kapaciteta, eterična ulja dvanaest začinskih biljaka mogu se poredati silaznim redom: klinčić (*Syzygium aromaticum* L.) > bosiljak (*Ocimum basilicum* L.) > lovor (*Laurus nobilis* L.) > koriander (*Coriandrum sativum* L.) > oraščić (*Myristica fragrans* Houtt.) > crni papar (*Piper nigrum* L.) > smilje (*Helichrysum italicum* G. (Roth) Don) > menta (*Mentha piperita* L.) > mažuran (*Marjorana hortensis* Moench.) > cimet (*Cinnamomum zeylanicum* Nees) > kadulja (*Salvia officinalis* L.) > komorač (*Foeniculum vulgare* Muller).