ANTIOXIDANT CAPACITY OF GLYCOSIDICALLY BOUND AND FREE VOLATILES FROM SELECTED SPICE PLANTS IN TWO LIPID MODEL SYSTEMS

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Lipid peroxidation is one of the major factors that cause deterioration during the storage and processing of foods [1]. Today there is growing interest in the studies of natural additives as potent antioxidants. Many spices and herbs are found to be potent sources of natural antioxidants [2–8]. There is also a growing interest in studying antioxidant capacity of glycosidically bound volatile compounds [9]. In our previously researches we evaluated the free radical scavenging ability and ferric reducing capacity of volatile aglycones liberated after enzymatic hydrolysis from, in Croatia cuisine, the most used spice plants: basil (*Ocimum basilicum* L.), laurel (*Laurus nobilis* L.), and clove (*Syzygium aromaticum* L. Merr et Parry) [10, 11]. But for real estimation of the ability to retard lipid peroxidation it was necessary to use real lipid media. For this reason, the aim of this paper is to evaluate the antioxidant capacity of glycosidically bound compounds from selected spice plants using two lipid model systems and to compare the obtained results with those for total (essential oil) and fractionated mix of free volatiles (CH and CHO fractions) as well as for frequently used commercial antioxidants (BHT, BHA, α-tocopherol, vitamin C) and pure eugenol.

TABLE 1. Chemical Composition of Free Volatile Aglycones Isolated from Basil, Laurel, and Clove, %

Compound	Basil	Laurel	Clove	Compound	Basil	Laurel	Clove
2-Heptanol	-	-	0.8	2-Hexenyl butanoate*	-	0.3	-
Hex-2-en-4-in-1-ol	0.2	-	-	3,7-Dimethyl-1,5-octadiene-3,7-diol*	2.4	-	-
3-Hexen-1-ol	1.4	-	-	4-Methyl benzyl alcohol	-	0.1	-
Acetic acid	-	0.4	-	2,2′-Oxybis-diacetate ethanol	1.7	-	-
1-Octen-3-ol	0.5	-	-	Phenylpropyl alcohol	-	0.2	-
Benzaldehyde	-	0.2	-	4-Ethyl benzyl alcohol	-	0.3	-
Linalool	0.8	-	-	2-Hexenyl butanoate*	-	0.3	-
Hotrienol	0.3	-	-	3,7-Dimethyl-1,5-octadiene-3,7-diol*	-	0.7	-
Butanoic acid	-	1.9	-	Eugenol	44.0	0.3	80.5
Lavandulol	0.7	-	-	Cinnamyl alcohol	-	0.3	-
α-Terpineol	0.4	-	-	Benzoic acid	-	1.7	0.3
2-Butenoic acid*	-	0.3	-	Chavicol	29.5	1.1	2.3
3,7-Dimethyl-1,5-octadiene-3,7-diol*	0.4	-	-	Phytol	0.3	-	-
Benzyl acetate	-	0.5	-	Vanillin	2.9	8.0	7.2
2-Butenoic acid	0.1	8.1	-	*exo*-2-Hydroxycineole	-	0.5	-
2-Hydroxymethyl benzoate	0.2	-	-	1-Cyclohexene-1-methanol	-	0.8	-
Methyl ester of 2-hydroxybenzoic acid	-	-	0.9	4-Hydroxy-3,5-dimethoxybenzaldehyde	-	1.4	-
Nerol	0.8	-	-	3-Hydroxy-β-damascone	0.4	-	-
α-Methyl benzyl alcohol	-	-	0.4	4-Hydroxy-3,5-dimethoxybenzaldehyde	0.2	-	-
m-Cresol	-	-	Tr.	Methyl ester of 4-hydroxybenzenebutanoic acid	-	-	0.9
Geraniol	0.4	-	-	4-Hydroxy 3-methoxy benzeneacetic acid	-	-	Tr.
Benzyl alcohol	5.7	63.4	1.1	Methyl ester of hexadecanoic acid	-	-	0.1
2-Phenyl ethanol	2.7	0.3	Tr.	Hexadecanoic acid	0.3	-	0.9
cis-1,3.3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-ol	-	0.9	-	Total	96.3	92.0	95.4

-: not identified; *correct isomer (*E* or *Z*) is not identified; identification is performed by MS; Tr.: trace.

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TABLE 2. Chemical Composition of Basil, Laurel and Clove Essential Oil and Its Fractions

Compound	Basil			Laurel			Clove		
	oil	CHO	CH	oil	CHO	CH	oil	CHO	CH
α-Pinene	-	-	-	2.4	-	19.0	-	-	-
Sabinene	-	-	-	7.0	-	12.0	-	-	-
β-Pinene	-	-	1.9	-	-	-	-	-	-
Δ^3-Carene	-	-	-	-	-	3.5	-	-	-
α-Terpinene	-	-	-	-	-	2.8	-	-	-
Limonene	-	-	1.8	-	-	8.7	-	-	-
1,8-Cineole	4.2	4.3	-	45.4	40.0	-	-	-	-
γ-Terpinene	-	-	-	0.8	-	8.0	-	-	-
β-Ocimene	-	-	-	0.2	-	-	-	-	-
p-Cymene	-	-	-	0.4	-	2.3	-	-	-
α-Terpinolene	-	-	-	0.3	-	2.1	-	-	-
trans-Sabinene hydrate	-	-	-	-	1.2	-	-	-	-
Camphor	0.6	0.5	-	-	-	-	-	-	-
Linalool	25.1	30.6	-	8.5	14.8	-	-	-	-
Bornyl-acetate	0.4	0.5	-	0.3	0.4	-	-	-	-
Terpinen-4-ol	0.7	0.7	-	-	2.7	-	-	-	-
β-Bourbonene	-	-	1.0	-	-	-	-	-	-
β-Elemene	-	-	5.1	-	-	-	-	-	-
α-Bergamotene	4.3	-	34.0	-	-	-	-	-	-
trans-Caryophyllene	0.6	-	5.1	4.1	-	21.3	0.2	-	88.7
Alloaromadendrene	0.3	-	2.0	-	-	-	-	-	-
Estragole	24.9	23.2	-	-	-	-	-	-	-
α-Terpineol	1.3	1.1	-	1.5	2.1	-	-	-	-
Carvone	0.7	0.4	-	-	-	-	-	-	-
α-Humulene	0.4	-	3.6	-	-	2.0	-	-	10.0
Germacrene D	0.4	-	4.4	-	-	-	-	-	-
α-Terpinyl acetate	-	-	-	9.1	11.9	-	-	-	-
β-Cubebene	-	-	7.6	-	-	-	-	-	-
Neryl-acetate	-	-	-	-	0.3	-	-	-	-
α-Amorphene	1.9	-	14.5	-	-	-	-	-	-
cis-Calamenene	0.2	-	2.3	-	-	-	-	-	-
γ-Cadinene	0.3	-	2.9	-	-	-	-	-	-
β-Farnesene	-	-	2.4	-	-	-	-	-	-
δ-Cadinene	-	-	1.5	-	-	1.7	-	-	1.3
α-Farnesene	-	-	-	0.7	-	8.2	-	-	-
α-Bisabolene	-	-	1.8	-	-	-	-	-	-
cis-Methyl cinnamate*	1.9	1.7	-	-	-	-	-	-	-
Caryophyllene-oxide	-	-	-	1.7	2.6	-	-	-	-
Methyl-eugenol	3.8	3.3	-	10.0	15.4	-	-	-	-
trans-Methyl cinnamate*	15.8	15.3	-	-	-	-	-	-	-
Spathulenol	-	0.8	-	-	-	-	-	-	-
Eugenol	5.7	6.3	-	2.6	3.9	-	92.7	92.5	-
cis-Isoelemicine	-	-	-	0.6	-	3.4	-	-	-
Aromadendrene	-	-	-	0.7	-	4.3	-	-	-
Carvacrol	-	Tr.	-	-	-	-	-	-	-
α-Cadinol	5.8	7.6	-	-	-	-	-	-	-
Toreiol	-	0.2	-	-	-	-	-	-	-
Eugenyl acetate	-	-	-	-	-	-	7.1	7.5	-
Chavicol	0.3	0.7	-	-	-	-	-		-
Total	99.6	97.2	91.9	96.3	95.3	99.3	100.0	100.0	100.0

-: not identified; *correct isomer (*E* or *Z*) is not identified; identification is performed by MS; Tr.: trace.

TABLE 3. The Antioxidant Capacity of Glycosidically Bound and Free Volatiles from Basil, Laurel, and Clove Tested by the Rancimat Method

Antioxidant	Protection factors (PF), g/L			
	1	5	10	20
Aglycone fractions				
Ocimum basilicum L.	1.0	1.0	1.0	1.0
Laurus nobilis L.	1.0	1.0	1.0	1.0
Syzygium aromaticum L. Merill et Perry	1.1	1.3	1.4	2.0
Total essential oils				
Ocimum basilicum L.	0.9	0.9	1.0	1.0
Laurus nobilis L.	1.0	1.0	1.1	1.1
Syzygium aromaticum L. Merill et Perry	1.1	1.1	1.2	1.4
Hydrocarbon CH fraction				
Ocimum basilicum L.	-	-	1.0	-
Laurus nobilis L.	-	-	1.0	-
Syzygium aromaticum L. Merill et Perry	-	-	1.0	-
Oxygen-containing CHO fraction				
Ocimum basilicum L.	1.0	1.0	1.0	1.0
Laurus nobilis L.	1.0	1.0	1.0	1.0
Syzygium aromaticum L. Merill et Perry	1.1	1.1	1.2	1.4
BHT	1.0	1.4	1.6	1.8
BHA	1.4	2.0	2.6	2.9
α-Tocopherol	1.7	2.6	3.0	3.1
Ascorbic acid	1.2	2.1	3.8	3.3
Eugenol	1.1	1.1	1.1	1.4

PF: Induction time of lard with antioxidant/induction time of pure lard.

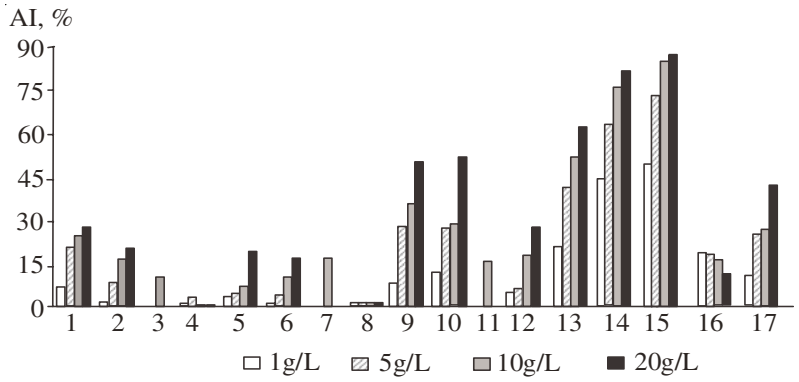

Fig. 1. The antioxidant capacity of glycosidically bound and free volatiles from basil, laurel, and clove tested by the TBARS method: 1 – basil oil, 2 – basil CHO, 3 – basil CH, 4 – basil aglycones, 5 – laurel, 6 – laurel CHO, 7 – laurel CH, 8 – laurel aglycones, 9 – clove, 10 – clove CHO, 11 – clove CH, 12 – clove aglycones, 13 – BHT, 14 – BHA, 15 – tocoferol, 16 – vitamin C, 17 – eugenol.

Selected spices were purchased from a local market. Glycosidically bound volatile compounds from selected spices were extracted with boiling ethyl acetate under reflux for 2 hours. After percolation, the extract was concentrated to dryness and the residue was dissolved in boiling water. The filtrate was subjected to liquid-solid chromatography in a glass column containing Amberlite XAD-2 as adsorbent. The glycosides extract was collected by eluting of methanol.

The methanolic extract containing the glycosides was concentrated to dryness and redissolved in citrate-phosphate buffer. Remaining volatile compounds were removed by liquid–liquid extraction with *n*-pentane.

The enzymatic hydrolysis of glycosidic extract was performed using β-glucosidase. The liberated volatile aglycones were extracted with *n*-pentane.

The major volatile aglycones are phenylpropanoid compounds (Table 1). The main basil volatile aglycones are eugenol (44.0%) and chavicol (29.5%). Of laurel it is benzyl alcohol (63.4%), while the major clove volatile aglycone compound is eugenol (80.5%).

Dried plant materials were also subject to a three-hour hydrodistillation using a Clevenger-type apparatus. The obtained oils were fractionated and two fractions were obtained (CH and CHO fractions). The chemical composition of total and fractionated essential oils from basil, laurel, and clove is shown in Table 2. All compounds are listed in order of their elution from an HP-20M column.

The antioxidant capacity of glycosidically bound and free volatiles from basil, laurel, and clove before and after fractionation as well as for frequently used commercial antioxidants (BHT, BHA, α-tocopherol, vitamin C) and pure eugenol were tested by two lipid model systems: the TBARS method and the Rancimat method (Fig. 1 and Table 3).

Clove volatile compounds, in comparison with other volatile compounds isolated from basil and laurel, were found to be potent antioxidants. The better antioxidant capacity results for all tested volatile mixtures were obtained by using the TBARS method. The extreme conditions of the Rancimat test (100°C and air flow – 20 L/h) probably evaporate volatile compounds and reduce the obtained capacities. Clove volatile compound capacity is comparable with that for widely used commercial antioxidants. If we can account that volatile compounds are released from their nonvolatile glycosidically bound compounds by acid hydrolysis or via chemical pathways during the manufacturing process, the antioxidant capacity of those compounds would contribute to overall clove capacity. That confirms clove′s significant role among spice plants and represents a guideline for replacing commercial antioxidants with natural ones.

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