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Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil

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Abstract

The present paper examines the chemical composition and antioxidant capacity of free volatile aglycones from basil compared to their essential oil. The comparison of chemical composition of volatile aglycones with the chemical composition of essential oil reveals four common compounds: eugenol, chavicol, linalool and α-terpineol. For the evaluation of the mentioned antioxidant capacities, two different methods were performed: the 2,2′-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH) and ferric reducing/antioxidant power assay (FRAP). DPPH method shows that free volatile aglycones possess good antioxidant properties comparable with that of the essential oil and well-known antioxidant butylated hydroxytoluene (BHT), but less than pure eugenol. The results obtained by FRAP method show that these compounds are some less effective antioxidants than essential oil and BHT.
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Keywords: *Ocimum basilicum* L.; Volatile aglycones; Essential oil; Chemical composition; GC–MS; Antioxidant capacity; DPPH; FRAP

1. Introduction

The antioxidants are an increasingly important ingredient in food processing. Their traditional role involves, as their name suggests, inhibiting the development of oxidative rancidity in fat-based foods, particularly meat and dairy products, and fried foods. The most widely used synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA) are very effective in their role as antioxidants. However, their use in food products has been failing off due to their instability, as well as due to a suspected action as promoters of carcinogenesis (Namiki, 1990). For this reason, there is a growing interest in the studies of natural healthy (non toxic) additives as potential antioxidants (Baratta et al., 1998; Tomaino et al., 2005).

Essential oil from aromatic and medicinal plants has been known to possess biological activity, notably antibacterial, antifungal and antioxidant properties (Baratta et al., 1998; Baratta, Dorman, & Deans, 1998).

Ocimum basilicum L. (Lamiaceae), respectively, named basil, is an aromatic herb that has been used traditionally as a medicinal herb in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunctions (Simon, Morales, Phippen, Vieira, & Hao, 1999). It has a long history as culinary herb, thanks to its foliage adding a distinctive flavor to many foods. It is also a source of aroma compounds and essential oils containing biologically active constituents that possess insecticidal (Deshpande & Tipnis, 1997), nematicidal (Chaterje, Sukul, Laskal, & Ghoshmajumdar, 1982), fungistatic (Reuveni, Fleisher, & Putievsky, 1984) and antimicrobial properties (Wannissorn, Jarikasem, Siriwangchai, & Thubthimthed, 2005).

Together with the essential oil, there is a growing interest for the study of glycosidically bound volatile compounds. These compounds have been extensively studied

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in case of grapes and wines (Gunata, Bayonove, Baumes, & Cordonnier, 1985), fruits (Schwab, Mahr, & Schreier, 1989) and aromatic plants (Stahl-Biskup, Intert, Holthuijzen, Stengle, & Schulz, 1993). To our knowledge, only one study was published about the chemical composition of volatile aglycones from basil (Lang & Hörster, 1977). The antioxidant capacity of basil essential oil has been studied several times (Baratta et al., 1998; Lee, Umano, Shibamoto, & Lee, 2005; Tomaino et al., 2005), but the antioxidant properties of glycosidically bound volatile compounds from basil have not been studied to date. The aim of this paper is to isolate and identify these compounds as well as to determine their antioxidant capacity. This is a part of our investigation project, dealing with antioxidant capacity of glycosidically bound volatile compounds from aromatic plants (Milos, Mastelic, & Jerkovic, 2000; Radonic & Milos, 2003).

2. Materials and methods

2.1. Materials

Dried and chopped basil leaves (Kotanyi spice company, Austria) were purchased from a local market in Split, Croatia. All of the applied chemicals were of pro analysis purity and were purchased from Fluka Chemie (Buchs, Switzerland).

2.2. Isolation of essential oil

The 100 g of plant material and 500 ml of water have been placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistillation for 3 h. The obtained essential oil was separated, dried over anhydrous sodium sulphate and stored under argon in a sealed vial, at −20 °C before usage. The voucher specimen of the basil leaves is deposited in the Laboratory of Biochemistry and Food Chemistry, Faculty of Chemical Technology, Split, Croatia.

2.3. Isolation of glycosidically bound volatile compounds

Upon the addition of internal standard, octyl-β-D-glucopyranoside, 100 g of plant material was extracted with boiling ethyl acetate under reflux for 2 h. After percolation, the extract was concentrated to dryness in a rotating evaporator, under reduced pressure. The residue was dissolved in boiling water and, after cooling, the sediment was removed by filtration. The filtrate was subjected to liquid–solid chromatography in a glass column (150 × 20 mm) containing Amberlite XAD-2 as adsorbent (Gunata et al., 1985) at a rate of 2 ml/min. Sugars, amino acids and proteins were removed by washing with 500 ml of distilled water. The glycosides extract was collected by eluting 100 ml of methanol. The methanolic extract containing the glycosides was concentrated to dryness under reduced pressure and redissolved in 2 ml of citrate-phosphate buffer (0.2 M, pH 5.0). The remaining volatile compounds were removed by liquid–liquid extraction with 4 × 5 ml of *n*-pentane over 24 h. Prior to enzymatic hydrolysis, TLC and GC–MS tested the absence of free volatile compounds. Thin layer chromatography was performed on 0.2 mm precoated silica plates (Kiesegel 60, Merck) with hexane/ethyl acetate (85:15, v/v) as eluent. The volatile compounds were detected using 2% vanillin in concentrated sulfuric acid.

2.4. Enzymatic hydrolysis and extraction of free volatile aglycones

In a typical experiment, β-glucosidase from bitter almonds (10 mg, 5–8 U/mg; Fluka) was added to the glycosidic extract. The enzymatic hydrolysis was realized during 48 h at 37 °C. Occasionally, the mixture was shaken thoroughly by hand. After hydrolysis, the liberated volatile aglycones were extracted from aqueous layer with 4 × 5 ml of *n*-pentane. The combined pentane extract was concentrated to 0.5 ml and 2 μl was injected for GC–MS analysis.

2.5. Gas chromatography–mass spectrometry

The analyses of the 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 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 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 the sample components was performed in the EI mode (70 eV). Injected volume was 1 μl. The linear retention indices for all the compounds were determined by co-injection of the samples with a solution containing the homologous series of C_8–C_{22} *n*-alkanes (Van Den Dool & Kratz, 1963). The individual constituents were identified by their identical retention indices referring to the compounds known from the literature data (Adams, 1995), 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). The aglycone concentrations were calculated from the GC peak areas related to GC peak area of 1-octanol (from the internal standard octyl-β-D-glucopyranoside). Preliminary GC–MS analysis showed the absence of 1-octanol as potential aglycone in plant material.

2.6. Determination of antioxidant activity with 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant capacity of basil essential oil and of the volatile aglycones was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (Brand-Williams, Cuvelier, & Berset, 1995). An ethanolic stock solution (50 ml of the antioxidant (concentrations of stock solutions were 20, 10, 5 and 1 g/l for volatile aglycones (due to limited quantity of the aglycones only few concentration were used) and 50, 30, 20, 10, 5, 1, 0.5, 0.3, 0.2, 0.1, 0.05, 0.03, 0.02, 0.01 g/l for essential oil, BHT and eugenol) was placed in a cuvette, and 1 ml of 0.004% ethanolic solution of DPPH was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined by UV–VIS Perkin–Elmer Lambda EZ 201 spectrophotometer after 2 h for all samples. Ethanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without the 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 (Blois, 1958). All determinations were performed in triplicate. The percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen & Duh (1994):

$$\%\ \text{Inhibition} = ((A_{C(0)} - A_{A(t)})/A_{C(0)}) \times 100$$

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

2.7. Determination of ferric reducing antioxidant power (FRAP assay)

Determination of ferric reducing/antioxidant power FRAP is a simple direct test for measuring of antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but can be used for plant extracts too. The total antioxidant potential of the sample was determined using a ferric reducing ability (FRAP) assay (Benzie & Strain, 1996) as a measure of "antioxidant power". This assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{2+}-tripyridyltriazine compound from colorless oxidized Fe^{3+} form by the action of electron donating antioxidants. The working FRAP reagent was prepared by mixing 10 parts of 300 mmol/l acetate buffer, pH 3.6, with 1 part of 10 mmol/l TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/l hydrochloride acid and with 1 volume of 20 mmol/l ferric chloride. Freshly prepared FRAP reagent (1.5 ml) was warmed to 37 °C and a reagent blank reading was taken at 593 nm (M1 reading). Subsequently, 50 μl of the sample (concentrations of stock solutions were 20, 10, 5 and 1 g/l) and 150 μl of deionized water were added to the FRAP reagent. Final dilution of the sample in the reaction mixture was 1:34. The sample was incubated at 37 °C throughout the monitoring period. The change in absorbance between the final reading (4-min reading) and the M1 reading was selected for the calculation of FRAP values. Standard curve was prepared using different concentrations (0.1–5 mmol/l) of $FeCl_2 \times 4H_2O$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the antioxidant under the test was calculated with reference to the reaction signal given by a Fe^{2+} solution of known concentration. The results were corrected for dilution and expressed in mmol Fe^{2+}/l. All determinations were performed in triplicate.

3. Results and discussion

3.1. Chemical composition of free volatile aglycones compared with essential oil composition

The content of free volatile aglycones in dried plant material was 0.14 mg/g. As shown in Table 1, the GC–MS analysis of the aglycones revealed twenty-three compounds, representing 96.3% of the total aglycone fraction. Aliphatic alcohols and acids, terpene compounds, derivative of phenylpropanes and derivative of norisoprenoid were identified. The main aglycones were phenylpropanoids eugenol (44.0%) and chavicol (29.5%). Other quantitatively important aglycones were benzyl alcohol (5.7%), vanillin (2.9%), 2-phenyl ethanol (2.7%) and not identified isomer of 3,7-dimethyl-1,5-octadiene-3,7-diol (2.4%). The obtained results show only some qualitative similarity with those reported by Lang & Hörster (1977). They found only four compounds in aglycone fraction of morphological undifferentiated callus and cell-suspension cultures with thymol and linalool as major ones. This is probably due to difference in the isolation of glycosides. The aglycones such as aliphatic alcohols, 2-phenylethanol, benzyl alcohol, eugenol, linalool, geraniol, nerol and α-terpineol can, more or less, be considered common in aglycone fraction of Lamiaceae family (Stahl-Biskup et al., 1993) and the eugenol was found to be the main aglycone in most plants of this family (Milos et al., 2000; Radonic & Milos, 2003; Stahl-Biskup et al., 1993).

The total content of the essential oil (yield = 6.20 mg/g), determined by the gravimetric method, is 44 times higher than that of the aglycones. Among 33 compounds identified in basil essential oil, representing 97.0% of the total oil (Table 2), monoterpene compounds, sesquiterpene compounds and derivative of phenylpropanoid were identified. The major compound was monoterpene alcohol linalool (28.6%). The second most important compound was phenylpropanoid estragole with the peak area of 21.7%. Other important compounds were (*E*)-methyl cinnamate (14.3%), α-cadinol (7.1%), eugenol (5.9%), 1,8-cineole (4.0%), methyl eugenol (3.1%) and α-bergamotene (2.2%). This means that this essential oil represents a true methyl cinnamate chemotype (Guenter, 1965). All this supports the assumption that if alcohols and phenols are the main components of the essential oil, the corresponding aglycones can also be detected (Stahl-Biskup & Holthuijzen, 1995). Comparing the chemical composition of the essential oil

Table 1
Chemical composition of free volatile aglycones isolated from basil

No.	Identified compound	Peak area (%)	RI[a] HP-20M	RI[a] HP-101
1	Hex-2-en-4-in-1-ol	0.2	1256	–
2	3-Hexen-1-ol	1.4	1351	838
3	1-Okten-3-ol	0.5	1412	963
4	Linalool	0.8	1510	1084
5	Hotrienol	0.3	1570	–
6	Lavandulol	0.7	1634	1157
7	α-Terpineol	0.4	1650	1172
8	3,7-Dimethyl-1,5-octadiene-3,7-diol[c]	0.4	1676	–
9	2-Butenoic acid	0.1	1709	–
10	2-Hydroxymethyl benzoate	0.2	1715	–
11	Nerol	0.8	1751	1253
12	Geraniol	0.4	1803	1258
13	Benzyl alcohol	5.7	1819	1096
14	2-Phenyl ethanol	2.7	1844	1149
15	3,7-Dimethyl-1,5-octadiene-3,7-diol[c]	2.4	1903	1615
16	2,2′-Oxybis-diacetate ethanol	1.7	1937	1303
17	Eugenol	44.0	2116	1385
18	Chavicol	29.5	–[b]	–
19	Phytol	0.3	–[b]	–
20	Vanillin	2.9	–[b]	1501
21	3-Hydroxy-β-damascone	0.4	–	1627
22	4-Hydroxy-3,5-dimethoxybenzaldehyde	0.2	–	1746
23	Hexadecanoic acid	0.3	–	1972
	Total	96.3		

–, not identified.
[a] Retention indices relative to C_8–C_{22} *n*-alkanes on polar HP-20M and apolar HP-101 column.
[b] Retention times is outside of retention times of homologous series of C_8–C_{22} *n*-alkanes (identified by MS).
[c] Correct isomer (*E* or *Z*) is not identified. Identification is performed by MS.

Table 2
Chemical composition of basil essential oil

No.	Identified compound	Peak area (%)	RI[a] HP-20M	RI[a] HP-101
1	β-Pinene	0.1	–	949
2	Limonene	0.1	1180	1005
3	1,8-Cineole	4.0	1185	1006
4	Camphor	0.5	1477	1109
5	Linalool	28.6	1518	1092
6	Bornyl acetate	0.5	1545	1252
7	Terpinen-4-ol	0.7	1563	1154
8	α-Bergamotene	2.2	1564	1407
9	Caryophyllene	0.3	–	1385
10	Aloaromadendrene	0.1	–	1450
11	Estragole	21.7	1632	1177
12	α-Terpineol	1.0	1653	1176
13	Germacrene D	0.3	1673	1444
14	α-Humulene	0.2	–	1417
15	Carvone	0.4	1685	1207
16	β-Cubebene	0.5	1694	1059
17	β-Burbonene	t	–	1354
18	β-Elemene	0.3	–	1364
19	γ-Cadinene	0.2	1716	1426
20	Calamenene	0.2	–	1483
21	α-Amorphene	1.0	1710	1479
22	β-Farnesene	0.2	–	1452
23	Δ-Cadinene	0.1	1724	1486
24	α-Bisabolene	0.1	–	1506
25	(*Z*)-Methyl cinnamate	1.6	1900	1281
26	Methyl eugenol	3.1	1959	1378
27	(*E*)-Methyl cinnamate	14.3	2019	1364
28	Spatulenol	0.8	2066	–
29	Eugenol	5.9	2105	1368
30	Carvacrol	t	2118	1814
31	α-Cadinol	7.1	2120	1614
32	Torreyol	0.2	2173	–
33	Chavicol	0.7	–	–[b]
	Total	97.0		

–, not identified.
t, trace (<0.1%).
[a] Retention indices relative to C_8–C_{22} *n*-alkanes on polar HP-20M and apolar HP-101 column.
[b] Retention times is outside of retention times of homologous series of C_8–C_{22} *n*-alkanes (identified by MS).

and aglycones, four compounds were established to be common: eugenol, chavicol, linalool and α-terpineol. Our results show moderate qualitative correlation in the chemical composition of essential oil and free volatile aglycones of this plant.

3.2. Antioxidant capacity of glycosidically bound volatile aglycones compared with that of essential oil

The DPPH method was used to evaluate the antioxidant capacity of basil volatile aglycones and essential oil in comparison with known synthetic antioxidant BHT and pure eugenol. The decrease in absorbance at room temperature was measured every 15 min until the reaction reached steady state or until absorbance declined less than 10%. Typical profiles of the percent inhibition of the DPPH radical by essential oil, volatile aglycones, BHT and pure eugenol in the presence of stock solution concentration of 20 g/l are shown in Fig. 1. The percent inhibition of the DPPH radical as a function of the concentration for essential oil, corresponding mass of eugenol in essential oil and pure eugenol is shown in Fig. 2a, and that for volatile aglycones, corresponding mass of eugenol in volatile aglycones and pure eugenol is shown in Fig. 2b. The decrease in concentration of the aglycones produced reduction in their capacities. The same behavior was observed for the essential oil as well as BHT and pure eugenol. Antioxidant capacities in series of concentrations of each of volatile aglycones, essential oils and pure compounds were used to calculate the effective relative concentration EC_{50}. The amount of sample, necessary to decrease the absorbance of DPPH by 50% (EC_{50}), was calculated graphically (% of inhibition was plotted against the logarithm of antioxidant concentration in reaction system). The data given in Table 3 show that eugenol possess the best radical scavenging capacity ($EC_{50} = 0.096$ g/l). The basil essential oil and known synthetic antioxidant BHT showed similar capacities

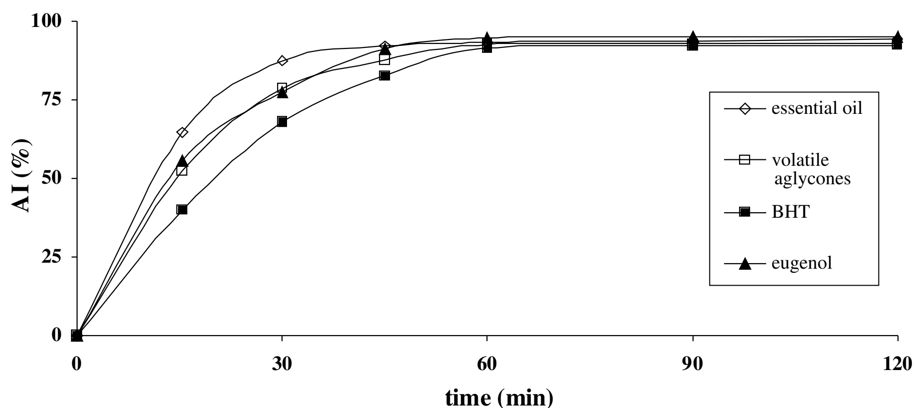

Fig. 1. Typical profile of the percent inhibition of the DPPH radical in the presence of 20 g/l of basil volatile aglycones, basil essential oil, BHT and pure eugenol.

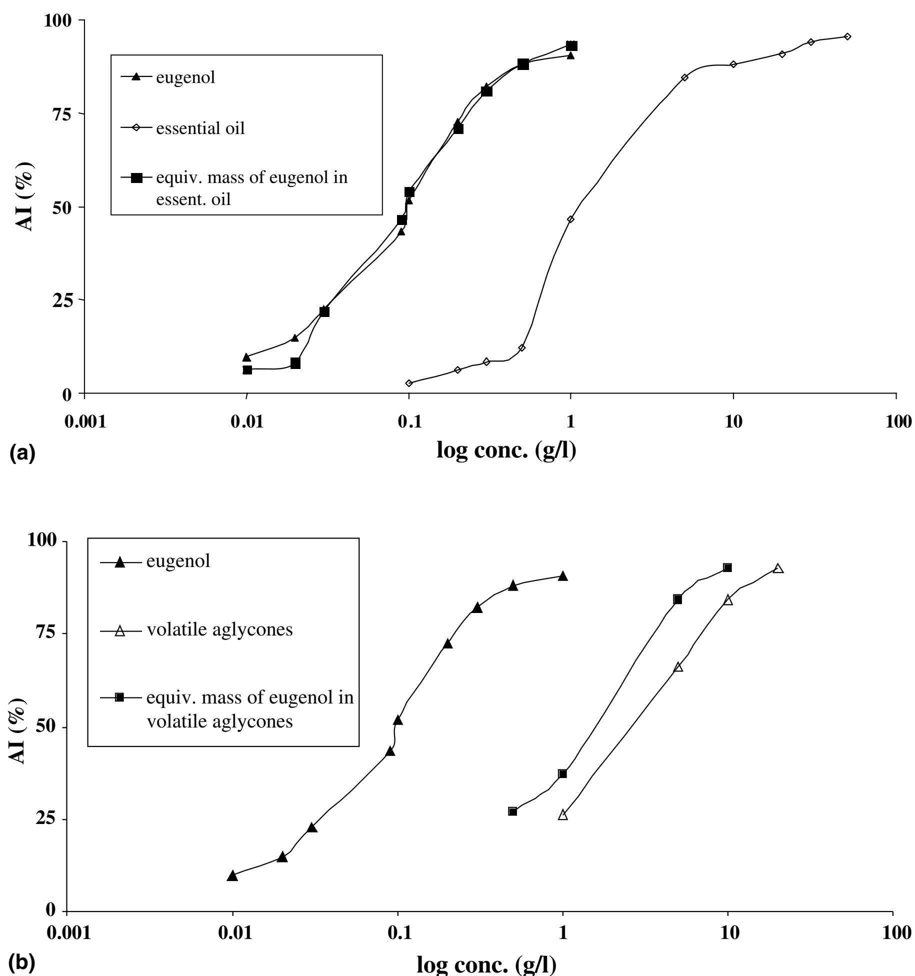

Fig. 2. Antioxidant capacity for total essential oil, corresponding mass of eugenol in essential oil and pure eugenol (a) as well as volatile aglycones, corresponding mass of eugenol in volatile aglycones and pure eugenol (b), measured by DPPH method.

($EC_{50} = 1.378$ g/l and 0.908 g/l), while the capacity of the basil volatile aglycones was significantly lower ($EC_{50} = 3.338$ g/l). The radical scavenging capacity for the corresponding mass of eugenol in essential oil was shown to be comparable to the capacity for the pure eugenol ($EC_{50} = 0.099$ g/l), while EC_{50} for corresponding mass of eugenol in volatile fraction was 1.950 g/l.

FRAP assay is quick and simple to perform, and the reaction is reproducible and linearly related to the molar concentration of the antioxidant present. It is based on comparison

Table 3
Radical scavenging of basil volatile aglycones, basil essential oil, BHT and pure eugenol determined with DPPH method

Antioxidant	EC_{50}[a]
Volatile aglycones	3.338
Essential oil	1.378
BHT	0.908
Eugenol	0.096
Equivalent mass of eugenol in essential oil	0.099
Equivalent mass of eugenol in volatile aglycones	1.950

[a] Concentration (g/l) for a 50% inhibition.

of the total amount of antioxidant to the reducing capacity of the sample. Absorbance changes are linear over a wide concentration range of antioxidant mixture. The reducing power (FRAP) of the liberated volatile aglycones and the essential oil, as well as BHT and pure eugenol, as determined by FRAP assay, is shown in Fig. 3. As in the previous method, decrease in concentration caused a reduction in the reducing power for all of the applied samples. The highest reducing power (FRAP) shows samples of pure eugenol. The reducing power of the volatile aglycones is comparable, but less than the reducing power of essential oil and BHT. A hierarchy in the reducing capacity of samples could be observed as well: eugenol > BHT > basil essential oil > basil volatile aglycones.

Among the compounds identified in the essential oil and volatile aglycones from basil, eugenol was considered the main contributor of the antioxidant capacity. The antioxidant capacity of eugenol (Dorman, Surai, & Deans, 2000; Nagababu & Lakshmaiah, 1992; Satoh, Ida, Sakagami, Tanaka, & Fusisawa, 1998) has been reported earlier. Surprisingly, in spite of much higher percentage of eugenol in volatile aglycones (44.0%) than in essential oil (5.9%), the essential oil shows higher antioxidant capacity. The comparison of the plots in Fig. 2a shows that the antioxidant capacity of pure eugenol ($EC_{50} = 0.096$ g/l) is more potent than that of the total essential oil ($EC_{50} = 1.378$ g/l). But the comparison of the plot for pure eugenol with the plot corresponding to equivalent mass of eugenol in total essential oil ($EC_{50} = 0.099$ g/l) shows very similar antioxidant capacities for both in all concentration range. This finding clearly suggests that the antioxidant capacity of total essential oil is due only or mainly to the presence of eugenol (5.9%) in its chemical composition and that other constituents do not have significant effect on eugenol capacity. The plots presented in Fig. 2b show that the antioxidant capacity of total aglycones ($EC_{50} = 3.338$ g/l) is also mainly due to the presence of eugenol (44%) in its composition ($EC_{50} = 1.950$ g/l), but it is obvious that other aglycones antagonize antioxidant capacity compared to pure eugenol capacity ($EC_{50} = 0.096$ g/l). It could be explained with smaller antioxidant capacity of volatile aglycones compared to total essential oil. However, the DPPH results show that glycosidically bound basil aglycones possess a good free radical scavenging capacity comparable with basil essential oil and well-known antioxidant butylated hydroxytoluene (BHT), but less than pure eugenol. The results obtained by FRAP method show that these compounds were some less effective antioxidants than essential oil and BHT. The observed differences can be explained by different solvent polarity in the two assays. It is known that substrate polarity does not affect to DPPH scavenging capacity (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002).

Glycosidically bound volatile compounds could be interesting as hidden potential of antioxidant compounds in basil or in other plants. Since volatile compounds can be released from nonvolatile glycoside precursors by enzymatic or chemical pathways during manufacturing process, these compounds can be considered as potential precursors of antioxidant substances in plant material and may contribute to the total antioxidant capacity of plants. This confirms the

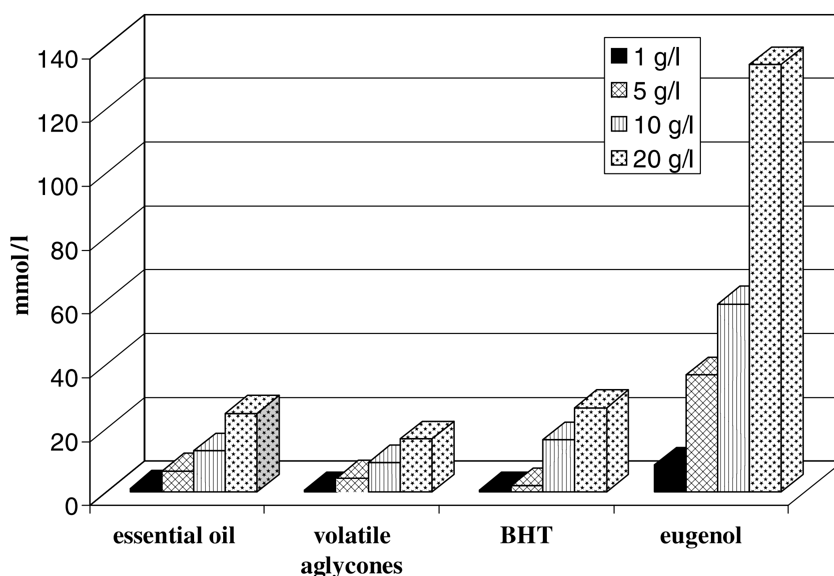

Fig. 3. The reducing power of the basil volatile aglycones, essential oil plus BHT and pure eugenol by using FRAP method.

potential use of herbs and spices in the food industry to increase the shelf life of foodstuffs. The antioxidant properties of glycosidically bound volatile compounds from other plants merit being the objective of future researches.

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References

Adams, R. P. (1995). *Identification of essential oil components by gas chromatography and mass spectroscopy*. Carol Stream, IL: Allured Publ.

Baratta, M. T., Dorman, H. J. D, Deans, S. G., Figueiredo, A. C., Barroso, J. G., & Ruberto, G. (1998). Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal, 13*, 235–244.

Baratta, M. T., Dorman, H. J. D., & Deans, S. G. (1998). Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oil. *Journal of Essential Oil Research, 10*, 618–627.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*, 1199–1200.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie, 28*, 25–30.

Chaterje, A., Sukul, N. C., Laskal, S., & Ghoshmajumdar, S. (1982). Nematicidal principles from two species of Lamiaceae. *Journal of Nematology, 14*, 118–120.

Deshpande, R. S., & Tipnis, H. P. (1997). Insecticidal activity of *Ocimum basilicum* L. *Pesticides, 11*, 1–12.

Dorman, H. J. D., Surai, P., & Deans, S. G. (2000). In vitro antioxidant activity of a number of plant essential oils and phytoconstituents. *Journal of Essential Oil Research, 12*, 241–248.

Guenter, E. (1965). *The essential oil* (Vol. III). New York: Van Nostrand, pp. 399–415.

Gunata, Y. Z., Bayonove, C. L., Baumes, R. L., & Cordonnier, R. E. (1985). The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *Journal of Chromatography, 331*, 83–90.

Koleva, I. I., van Beek, T. A., Linssen, J. P. H., de Groot, A., & Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant capacity: a comparative study on three testing methods. *Phytochemical Analysis, 13*, 8–17.

Lang, E., & Hörster, H. (1977). An Zucker gebundene reguläre Monoterpene Teil II Untersuchungen zur Olbidung und Akkumulation ätherischer Ole in *Ocimum basilicum* Zellkulturen. *Planta Medica, 31*, 112–118.

Lee, S. J., Umano, K., Shibamoto, T., & Lee, K.-G. (2005). Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. *Food Chemistry, 91*, 131–137.

Milos, M., Mastelic, J., & Jerkovic, I. (2000). Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare* L. ssp. *hirtum*). *Food Chemistry, 71*, 79–83.

Nagababu, E., & Lakshmaiah, N. (1992). Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Biochemical Pharmacology, 43*, 2393–2400.

Namiki, M. (1990). Antioxidants/antimutagens in food. *Critical Reviews in Food Science & Nutrition, 29*, 273–300.

Radonic, A., & Milos, M. (2003). Chemical composition and antioxidant test of free and glycosidically bound volatile compounds of savory (*Satureja montana* L. subsp. *montana*) from Croatia. *Nahrung/Food, 47*, 236–237.

Reuveni, R. A., Fleisher, A., & Putievsky, E. (1984). Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes. *Phytopathologische Zeitschrift –Journal of Phytopathology, 110*, 20–22.

Satoh, K., Ida, Y., Sakagami, H., Tanaka, T., & Fusisawa, S. (1998). Effect of antioxidants on radical intensity and cytotoxic activity of eugenol. *Anticancer Research, 18*, 1549–1552.

Schwab, W., Mahr, C., & Schreier, P. (1989). Studies on the enzymatic hydrolysis of aroma components from Carica papaya fruit. *Journal of Agricultural and Food Chemistry, 37*, 1009–1012.

Simon, J. E., Morales, M. R., Phippen, W. B., Vieira, R. F., & Hao, Z. (1999). Perspectives on new corps and new uses. In J. Janick (Ed.), *A source of aroma compounds and a popular culinary and ornamental herb* (pp. 499–505). Alexandria, VA: ASHS Press.

Stahl-Biskup, E., Intert, F., Holthuijzen, J., Stengle, M., & Schulz, G. (1993). Glycosidically bound volatiles. A review 1986–1991. *Flavour and Fragrance Journal, 8*, 61–80.

Stahl-Biskup, E., & Holthuijzen, J. (1995). Essential oil and glycosidically bound volatiles of lemon-scented thyme, *Thymus* × *citriodorus* (Pers.) Schreb. *Flavour and Fragrance Journal, 10*, 225–229.

Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A., et al. (2005). Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chemistry, 89*, 549–554.

Van Den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *Journal of Chromatography, 11*, 463–471.

Wannissorn, B., Jarikasem, S., Siriwangchai, T., & Thubthimthed, S. (2005). Antibacterial properties of essential oils from Thai medicinal plants. *Fitoterapia, 76*, 233–236.

Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free radical and active-oxygen species. *Journal of Agriculture and Food Chemistry, 42*, 629–632.