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Use of different methods for testing antioxidative activity of oregano essential oil

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Abstract

The antioxidant properties of the essential oil from oregano in relation to its chemical composition were examined. The antioxidant activity was investigated with three different methods: the β-carotene bleaching (BCB) test, the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the thiobarbituric acid reactive species (TBARS) assay. It was found that the total essential oil, its fraction as well as its pure constituents have a significant antioxidant effect when tested by each method, respectively. Generally the antioxidant activity of the oregano essential oil is less effective than the ascorbic acid, but comparable with the α-tocopherol and the synthetic antioxidant butylated hydroxytoluene (BHT). The synergy among minor oxygen containing compounds was suggested as possible factor, which influenced the antioxidant power of the oregano essential oil. The antioxidant concentrations influenced its antioxidant power, too.
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1. Introduction

The most widely used synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA, propyl galate PG and tertiary butyl hydroquinone TBHQ) have been suspected to cause or promote negative health effects (Barlow, 1990; Branen, 1975; Chan, 1987; Namiki, 1990; Pokorny, 1991). For this reason there is a growing interest in studies of natural additives as potential antioxidants. Many sources of antioxidants of plant origin have been studied in recent years. Among these the antioxidant properties of many aromatic plants and spices have shown to be effective in retarding the process of lipid peroxidation in oils and fatty foods and have gained the interest of many research groups.

Numerous types of antioxidants with varied activities were identified. Their antioxidant effect was due to the presence of hydroxyl groups in their chemical structure (Shahidi, 2000; Shahidi, Janitha, & Wanasundara, 1992; Vekiari, Oreopoulou, Tzia, & Thomopoulos, 1993). Several non-volatile compounds such as carnosol, quercetine, caffeic acid and rosmarinic acid are well known to be good scavengers of free radicals, but some volatile compounds from essential oils possess also the potential as natural agents for food preservation. A number of studies on antioxidant activities of essential oils from various aromatic plants reported that the oregano essential oil, rich in thymol and carvacrol, has a considerable antioxidant effect on the process of the lard oxidation (Lagouri, Blekas, Tsimidou, Kokkini, & Boskou, 1993; Tsimidou & Boskou, 1994). Yanishlieva and Marinova (1995) examined the antioxidant activity of hexane extracts of oregano grown in Bulgaria, as well as the mechanism of action of pure thymol and carvacrol (Yanishlieva, Marinova, Gordon, & Raneva, 1999). In zour previous work (Milos, Mastelic, Jerkovic, & Katalinic, 2000) we presented the chemical composition and the antioxidant effect of oregano glycosidically bound volatiles on the lard oxidation process. Recent publications (Abdalla & Roozen, 1999, 2001; Bendini, Gallina Toschi, & Lercker, 2002; Cervato, Carabelli, Gervasio, Cittera, Cazzola, & Cestaro, 2000; Damechki, Sotiropoulou, &

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Tsimidou, 2001; Martinez-Tome, Jimenez, Ruggieri, Frega, Strabbioli, & Murcia, 2001; Vichi, Zitterl-Eglseer, Jugi, & Fraz, 2001) showed antioxidative activities of oregano.

The aim of the present study was to examine the antioxidant properties of oregano essential oil by using three different methods, namely, the β-carotene bleaching (BCB) test, the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the thiobarbituric acid reactive species (TBARS) assay.

2. Experimental

2.1. Materials

Oregano (*Origanum vulgare* L., ssp. *hirtum*) was collected in central Dalmatia in October 2001. The plant material consisted of flowered tops and stalks (15–20 cm). Air-drying of oregano was performed in a shady place at room temperature for 10 days. The plant material was used for the isolation of the essential oil, immediately after drying. A hundred grams of the dried plant material was subjected to a 3-h hydrodistillation using a modified Clevenger-type apparatus. The body of the modified Clevenger-type apparatus consists of two concentric tubes. The inner tube is graduated and filled with water and a known amount of *n*-pentane, whose role is to retain the essential oil and to separate it from the water. Also, instead of a condenser of the "cold-finger" type an Allihn type condenser is used. The obtained essential oil was dried over anhydrous sodium sulfate and stored under nitrogen in sealed vial at −20 °C until required. The voucher specimens of oregano plant material and essential oil are deposited in the Department of Biochemistry and Food Chemistry, Faculty of Chemical Technology, Split, Croatia.

The oregano essential oil (0.5 g) was fractionated on a silica gel (30–60 μm, Mallinckrodt Baker B.V., Deventer, The Netherlands) column (length 20 cm; i.d. 2 cm). Pentane (50 ml) was used to obtain a fraction, which contained only nonpolar hydrocarbons (CH fraction), and diethyl ether (50 ml) was used to obtain a fraction of polar (oxygen containing, CHO fraction) compounds. These fractions were concentrated to 0.5 ml and subjected to thin layer chromatography (TLC) on silica gel plates in order to check results of the column chromatography separation. Different solvents were used as a mobile phase: *n*-hexane for CH fraction and *n*-hexane:ethyl acetate 85:15 (v/v) for CHO fraction. Two percent vanillin-sulphuric acid was used as a detection reagent. The fractions obtained by column chromatography were also subjected to GC/MS analysis (GC/MS conditions as described in Section 2.2) and good separation results were confirmed.

In order to obtain a fraction of phenolic compounds, 1 g of the essential oil was dissolved in 5 ml pentane and extracted with sodium hydroxide solution (20%) in water. In this manner, phenolic compounds were removed from the pentane layer. The aqueous phase, containing dissolved phenolic compounds sodium salts, was neutralized with hydrochloric acid solution (10%) in water. Finally, isolation of the phenolic compounds was performed by extraction with pentane (5 × 5ml). The effectiveness of this separation method was tested by TLC on silica gel plates (mobile phase: *n*-hexane:ethyl acetate 85:15 v/v) and a purity of the phenolic compounds fraction was confirmed. These separation results were confirmed by GC/MS analysis too (GC/MS conditions as described in Section 2.2).

Butylated hydroxytoluene, α-tocopherol, 2,2′-diphenyl-1-picrylhydrazyl, thiobarbituric acid, β-carotene, ascorbic acid and all of the applied solvents were of pro analysis purity and were purchased from Fluka Chemie, Buchs, Switzerland. Anhydrous sodium sulfate was obtained from Merck, Darmstadt, Germany.

2.2. GC–MS

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). The fused-silica HP-20 M polyethylene glycol column (50 m × 0.2 mm, 0.2 μm thickness, Hewlett-Packard, Vienna, Austria) was directly coupled to the mass spectrometer. The carrier gas was helium (1 ml/min). The program used was 4 min isothermal at 70 °C, then 4 °C/min to 180 °C and 10 min isothermal. 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).

The linear retention indices for all the compounds were determined by co-injection of the sample 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 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).

2.3. Determination of antioxidant activity with the β-carotene bleaching (BCB) test

Antioxidant activity of the oregano volatile compounds was determined according to slightly modified version of the β-carotene bleaching method (Pratt, 1980). The β-carotene (0.1 mg) was added to a boiling flask together with linoleic acid (20 mg) and Tween 40 (100 mg), all dissolved in chloroform. After evaporation

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to dryness, under vacuum at 50 °C by a rotary evaporator, oxygenated distilled water (50 ml) was added and the mixture was emulsified for 1 min in a sonificator to form emulsion A. 200 μl of ethanolic stock solution of each antioxidant (concentrations of stock solutions were 0.1, 0.5, 1.0, 2.0, 3.0 and 4.0 g/l) was mixed with 5 ml of emulsion A in open-capped cuvettes. A control, without antioxidant, consisting of 200 μl of ethanol and 5 ml of emulsion A was prepared. A second emulsion (B) consisting of 20 mg of linoleic acid, 100 mg of Tween 40 and 50 ml of oxygenated water was also prepared. Ethanol (200 μl), to which 5 ml of emulsion B was added, was used to zero the spectrophotometer. Readings of all samples were taken immediately ($t=0$) and at 15 min intervals for 120 min on a Perkin-Elmer Lambda EZ 201 spectrophotometer at 470 nm. The cuvettes were thermostated at 50C between measurements. All determinations were performed in duplicate. The percentage inhibition was calculated from the data with the slightly modified formula (Mallet, Cerati, Ucciani, Gamisana, & Gruber, 1994):

$$\% \text{ inhibition} = \left[\left(A_{A(120)} - A_{C(120)}\right)/\left(A_{C(0)} - A_{C(120)}\right)\right] \times 100$$

where $A_{A(120)}$ is the absorbance of the antioxidant at $t=120$ min, $A_{C(120)}$ is the absorbance of the control at $t=120$ min, and $A_{C(0)}$ is the absorbance of the control at $t=0$ min.

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

The antioxidant activity of the oregano volatile compounds was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (Brand-Williams, Cuvelier, & Berset, 1995). A methanolic stock solution (50 μl) of the antioxidant (concentrations of stock solutions were 1.05, 2.0, 4.0, 6.3, 8.4, 10.5, 12.6, 16.8, 21.0, 25.2, 29.4, 33.6, 40.0, 45.0 and 50.0 g/l) was placed in a cuvette, and 2 ml of 6×10^{-5} M methanolic solution of DPPH was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined by Perkin-Elmer spectrophotometer after 1 h for all samples. Methanol was used to zero the spectrophotometer. The 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 (Blois, 1958). All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994):

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

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

2.5. Determination of antioxidant activity with thiobarbituric acid reactive species (TBARS) assay

A modified TBARS assay (Ruberto & Baratta, 2000) was used to measure the potential antioxidant capacity using egg yolk homogenates as lipid rich media. Briefly, 0.5 ml of 10% (w/v) tissue homogenate and 0.1 ml of sample solutions to be tested in methanol (concentrations of stock solutions were 4.0, 20.0 and 40.0 g/l), prepared immediately before use, 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 M) 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 sulfate solution was added and the resulting mixture 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. The absorbance of the organic upper layer was measured using a spectrophotometer (Perkin-Elmer 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.

3. Results and discussion

3.1. Chemical composition of oregano essential oil

The dried oregano flowered tops and stalks yielded 2.9% of essential oil. Determination of the percentage composition of the samples was based on peak area normalization without using correction factors. As shown in Table 1, eleven compounds were identified in the essential oil without fractionation, representing 97.9% of the total oil. The major compounds were phenolic monoterpenes thymol (35.0%) and carvacrol (32.0%). Other important compounds were monoterpenic hydrocarbons γ-terpinene (10.5.%), *p*-cymene (9.1%) and α-terpinene (3.6%). After fractionation, some other compounds were identified which means that more completely analysis was obtained. Similar results were reported for oregano of Greek origin (Vokou, Kokkini, & Bessiere, 1993). The chemical composition of different fractions, were reported in Table 1.

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Seventeen compounds were identified in hydrocarbons (CH) fraction with γ-terpinene (31.0%), *p*-cymene (22.1%), α-terpinene (10.4%) and *trans*-caryophyllene (9.1%) as main components. The fraction with oxygen-containing compounds, which contained four compounds, was represented by thymol (47.3%) and carvacrol (46.4%) as major compounds. The phenolic fraction contained only two compounds, thymol (58.9%) and carvacrol (41.1%).

3.2. Antioxidant activity of oregano essential oil

3.2.1. β-carotene bleaching method

The BCB method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. This fact is used in the antioxidant activity evaluation of the oregano essential oil in comparison with, well known, synthetic and natural antioxidants, namely BHT, ascorbic acid (vitamin C) and α-tocopherol (vitamin E).

Fig. 1. shows the antioxidant activity of the total oregano essential oil in the comparison with those of BHT, α-tocopherol and ascorbic acid. The antioxidant power decreased in the order BHT > α-tocopherol > essential oil > ascorbic acid. BHT and α-tocopherol were the strongest antioxidants. In comparison, the essential oil showed relatively significant antioxidant effect, while ascorbic acid showed no antioxidant activity. Except ascorbic acid, the concentration influences the antioxidant power of each sample. The 50% inhibition were accomplished with $<0.1\ gl^{-1}$ of BHT and α-tocopherol or $2\ gl^{-1}$ of total essential oil, respectively.

Fig. 2 shows the decrease in absorbance of β-carotene in the presence of the total oregano essential oil, as well as its different fractions or single constituents. The control sample without addition of antioxidant oxidized most rapidly and descending bleaching rate were demonstrated in presence of CH > carvacrol > thymol > total essential oil > phenolic fraction > CHO fraction. The CHO fraction was the most potent antioxidant suggesting that the antioxidant activity of oregano essential oil is due to more polar constituents. Among these, the fact that CHO fraction is more effective as antioxidant than phenolic fraction or its pure constituents thymol and carvacrol suggests that the synergy among minor oxygen containing compounds has determining role. On the other hand, the less

Table 1
The composition (area %) of *Origanum vulgare* L. essential oil

No.	Compound	RI[a]	Area%	
			In total oil	In fraction
Hydrocarbons CH fraction (CH)				
1.	α-Thujene	1031	1.4	5.2
2.	β-pinene	1102	–	0.7
3.	Myrcene	1149	–	6.1
4.	α-Terpinene	1161	3.6	10.4
5.	γ-Terpinene	1231	10.5	31.0
6.	*p*-Cymene	1247	9.1	22.1
7.	Terpinolene	1262	–	0.9
8.	Alloocimene[b]	1351	–	0.3
9.	α-Copaene	1466	–	0.4
10.	β-Burbonene	1496	–	0.3
11.	*trans*-Caryophyllene	1578	2.4	9.1
12.	Aromadendrene	1583	–	0.4
13.	α-Humulene	1638	–	1.5
14.	Ledene	1644	–	0.3
15.	β-Bisabolene	1694	1.4	2.0
16.	δ-Cadinene	1729	0.5	3.8
17.	α-Muurolene	1735	–	0.2
				Total 95.8
Oxygen containing compounds fraction (CHO)				
18.	1-Octen-3-ol	1411	1.0	0.8
19.	Borneol	1653	1.0	1.0
20.	Thymol	2115	35.0	47.3
21.	Carvacrol	2140	32.0	46.4
			Total 97.9	Total 95.5
Phenolic fraction				
1.	Thymol	2115	58.9	
2.	Carvacrol	2140	41.1	
				Total 00.0

[a] Retention indices relative to C_8–C_{22} alkanes on polar HP-20M column.
[b] Correct isomer is not identified.

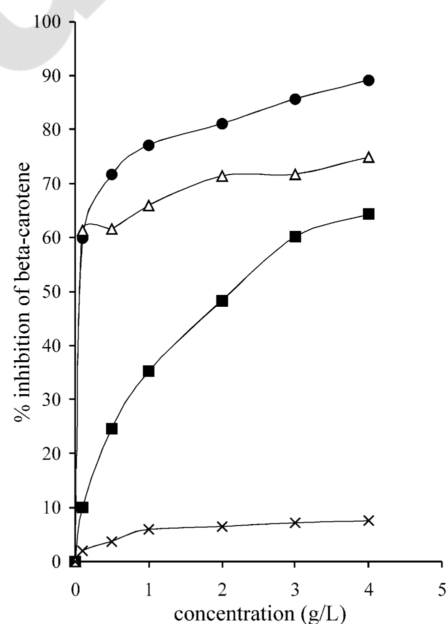

Fig. 1. Antioxidant activity of the oregano total essential oil (■), BHT (•), α-tocopherol (Δ), ascorbic acid (×) as assessed with β-carotene bleaching method.

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effectiveness of total oil in comparison with CHO fraction may be due to smaller concentration of oxygen containing compounds in total oil and because of the presence of the hydrocarbons, which showed the lowest antioxidant activity when isolated as CH fraction.

In spite the fact that polar compound ascorbic acid is well known antioxidant, the β-carotene bleaching test didn't show its antioxidant properties. This interesting phenomenon formulated as the "polar paradox" has been reported earlier (Frankel, Huang, Kanner, & German, 1994; Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002; Porter, 1993). The polar antioxidants remaining in the aqueous phase of the emulsion are more diluted in lipid phase and are thus less effective in protecting the linoleic acid.

3.2.2. DPPH radical scavenging method

Many radical species of different reactivity are formed during a lipid oxidation ($^{\cdot}OH$, $O_2^{\cdot-}$, $L^{\cdot}$, $LOO^{\cdot}$, $LO^{\cdot}$, etc.). Relatively stable organic radical DPPH· has been widely used in the determination of the antioxidant activity of single compounds as well as the different plant extracts (Brand-Williams et al., 1995; Yen & Duh, 1994). The method is based on the reduction of alcoholic DPPH· solutions in the presence of an hydrogen donating antioxidant. DPPH· solutions show a strong absorption band at 517 nm appearing a deep violet colour. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH·, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant (Blois, 1958).

The method was used to evaluate the antioxidant properties of the oregano essential oil in comparison with those of known natural and synthetic antioxidants, α-tocopherol, ascorbic acid and BHT (Table 2). The ascorbic acid, the α-tocopherol and the BHT showed the highest radical scavenging activity, while the activity of the oregano essential oil and its fractions or pure constituents were much lower. Our investigation shows that the DPPH method is independent of the substrate polarity. Other authors have already reported that the substrate polarity does not affect DPPH· scavenging activity (Koleva et al., 2002; Pekkarinen, Stockmann, Schwarz, Heinonen, & Hopia, 1999; Yamaguchi, Takamura, Matoba, & Terao, 1998). It was described that radical scavenging abilities of some compounds can be influenced by their different kinetic behaviour (Bondet, Brand-Williams, & Berset, 1997). For slow reacting compounds the influence was attributed to the complex reacting mechanism. In our study, probably, the constituents from oregano essential oil involved one or more secondary reactions, which resulted the slower reduction of DPPH· solutions.

Fig. 3 shows the rate of hydrogen donating ability of the oregano total essential oil, its fractions and pure constituents thymol and carvacrol. Only CH fraction showed very poor radical scavenging. All other investigated antioxidants showed high and almost the same antioxidant effect.

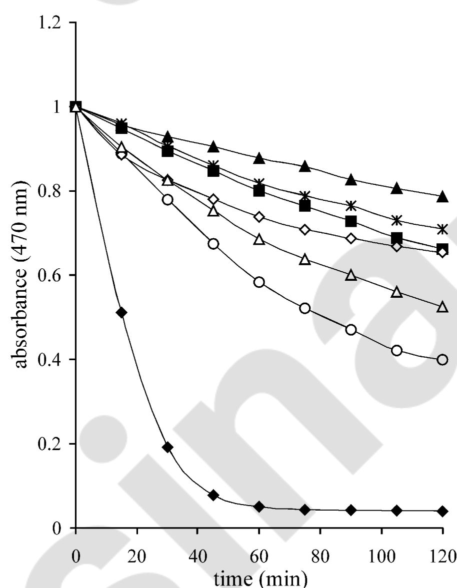

Fig. 2. Rate of β-carotene bleaching in control without antioxidant (◆), oregano essential oil (■), CHO fraction (▲), phenolic compounds fraction (*), CH fraction (○), thymol (◇) and carvacrol (Δ). The concentration of the total essential oil, its fractions and pure compounds was 4 g/l.

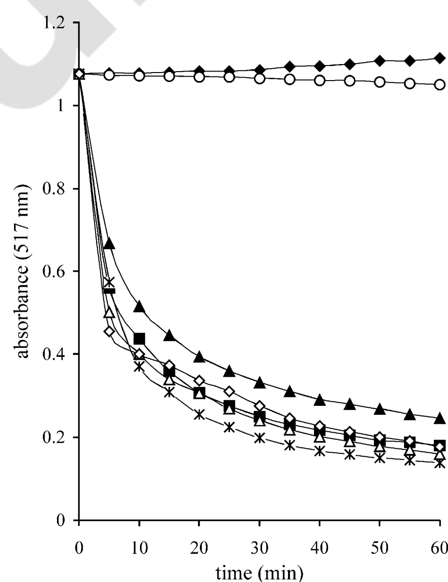

Fig. 3. The reduction of alcoholic DPPH solutions: the rate of hydrogen donating ability of control without antioxidant (◇), oregano essential oil (■), CHO fraction (▲), phenolic compounds fraction (*), CH fraction (○), thymol (◇) and carvacrol (Δ). The concentration of the total essential oil, its fractions and pure compounds was 2.4 g/l.

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Table 2
Radical scavenging of oregano essential oil, its fractions, BHT, tocopherol and ascorbic acid with DPPH

Antioxidant	IC_{50}[a]
Total essential oil	0.5
CH fraction[b]	/
CHO fraction	0.5
Phenolic fraction	0.4
Thymol	0.5
Carvacrol	0.4
BHT	1.8×10^{-2}
α-Tocopherol	8.6×10^{-3}
Ascorbic acid	4.4×10^{-3}

[a] Concentration (g/l) for a 50% inhibition.
[b] CH fraction could not inhibit 50% of the reaction under test conditions.

Table 3
Antioxidant activity of oregano essential oil compounds, tocopherol and BHT measured by thiobarbituric acid method

Antioxdants	AI%[b]		
	100 ppm[a]	500 ppm	1000 ppm
Total essential oil	29.9±8.0	52.9±4.0	64.7±5.2
CH fraction	22.2±1.1	50.6±1.2	60.2±6.7
CHO fraction	26.7±8.0	42.5±4.3	56.7±4.9
Phenolic fraction	33.4±7.1	47.5±4.9	58.6±1.8
Thymol	24.0±2.9	–	41.5±2.2
Carvacrol	24.0±3.7	–	52.8±2.7
α-Tocopherol	72.6±3.6	–	90.0±1.8
BHT	37.5±1.9	–	68.6±1.1

[a] Antioxidants concentration in reacting system.
[b] Antioxidant effectiveness expressed as antioxidant index (as described in Section 2.5). Values represents average of triplicates ±standard deviation.

3.2.3. TBARS

The method, known as thiobarbituric acid reactive species (TBARS) assay, concerns the spectrophotometric measurement of the pink pigment produced through reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) and other secondary lipid peroxidation product. The evaluation of the absorbance at 532 nm gives a measure of the extent of lipid degradation.

The total oregano essential oil, its fractions and pure constituents were examined for their ability to act as radical scavenging agents in comparison among them and with α-tocopherol and BHT. As shown in Table 3, the antioxidant power decreased in the order α-tocopherol > BHT > essential oil. The total essential oil, CHO fraction, pure thymol and carvacrol, as well as hydrocarbon CH fraction exhibited almost the same antioxidant power. It is not unexpectedly since Ruberto and Baratta (2000) showed similar TBA results. They have tested about 100 pure constituents of essential oils and substantially confirmed that monoterpene hydrocarbons γ-terpinene, α-terpinene, *p*-cymene show a very high activity. In our study these compounds are the major components of CH fraction. From results shown in Table 3, it is also obvious that a higher concentrations results in a higher antioxidant effect.

3.2.4. Comparison of the methods

As previously described (Frankel et al., 1994; Koleva et al., 2002) the use of different methods is necessary in antioxidant activity assessment. The presented study shows that no single testing method is sufficient to estimate the antioxidant activity of a studied sample. The combination of three methods, applied in this study, was a good choice to evaluate the antioxidant activity of oregano essential oil and could be recommended for other similar investigations.

The BCB method employs an emulsified lipid, which introduces a number of variables influencing antioxidant activity of examined sample and could be relatively complicate for achieving reproducible results. If this fact is neglected, however, the BCB method can be helpful especially for investigations of lipophilic antioxidants and it is appropriate for the investigation of the antioxidant activity of essential oils. On the other hand if polar compounds (ascorbic acid, rosmarinic acid, caffeic acid etc.) are tested only by the BCB method they would be considered as weak antioxidants. However, the strong antioxidant activity of these compounds can be proven by other testing methods (Koleva et al., 2002).

The DPPH method is faster than BCB method and it can be helpful in investigation of novel antioxidants for a rapid estimation and preliminary information of radical scavenging abilities. The method is sensitive and requires small sample amounts (Blois, 1958). The TBA method is also sensitive and achieves reproducible results. This method is preferable in order to obtain useful data in an environment similar to the real-life situation. Both methods, DPPH and TBA, similarly allow testing of both lipophilic and hydrophilic substances.

The correct estimation of an antioxidant activity of the oregano essential oil required the evaluation of its optimal concentration. Because the specificity and sensitivity are different for each used method, it was impossible to use equal amounts of antioxidants for each test. On the other hand, the effect of using different amounts of antioxidants in the different tests makes it difficult to compare obtained results. However, in spite this fact and independently of the chosen method, multi-concentration measurements provide a more comprehensive picture of oregano essential oil antioxidative activity.

In conclusion, as previously written (Koleva et al., 2002), the antioxidant power depends on the chosen method, on the concentration and on the nature and

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physicochemical properties of studied antioxidants. In present study, it was confirmed that the same antioxidant samples exhibit different antioxidative values depending on the concentration and the measured antioxidant parameter. It is important to achieve a multiple different concentration measurements to avoid the incorrect conclusion in these cases.

Our previous (Milos et al., 2000) presented that the oregano essential oil inhibited hydroperoxide formation and that the CHO fraction showed the highest antioxidative activity. This study, using three different methods confirmed that the oregano essential oil possess remarkable antioxidant properties. The antioxidant effect is due to the presence of thymol and carvacrol, but a possible synergistic effect among oxygen containing compounds can be suggested too. These results indicate that the oregano essential oil could be in use as potential resource of natural antioxidants for food industry so that it is interesting to examine its application as natural antioxidant additive in some final food products.

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