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Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils

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Abstract

Essential oils extracted by hydrodistillation from *Cuminum cyminum* and *Rosmarinus officinalis* were characterized by means of GC and GC–MS. *C. cyminum* and *R. officinalis* contained α-pinene (29.1%, 14.9%), 1,8-cineole (17.9%, 7.43%) and linalool (10.4%, 14.9%), respectively, as the major compounds. *C. cyminum* oil exhibited stronger antimicrobial activity than did *R. officinalis* oil against *E. coli, S. aureus* and *L. monocytogenes*. Complete death time on exposure to *Cuminum cyminum* L. and *Rosmarinus officinalis* L. oils were 20 and 25 min 180 and 240 min and 90 and 120 min for *E. coli*, *S. aureus* and *L. monocytogenes*, respectively. Radical-scavenging and antioxidant properties were tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and the β-carotene bleaching test. These properties were compared to those of *Thymus x-porlock* essential oil, used as a reference ingredient. The radical scavenging performance of the rosemary oil was better than that of *C. cyminum*. Results from the antioxidant test were better than those provided by the radical-scavenging activity. *C. cyminum* and *R. officinalis* essential oils may be considered as potent agents in food preservation.

Keywords: *Cuminum cyminum*; *Rosmarinus officinalis*; Essential oil; *E. coli*; *S. aureus*; *L. monocytogenes*; Antioxidant; Radical scavenging; Antimicrobial

1. Introduction

The exploration of naturally-occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives (Gould, 1995). Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics (Essawi & Srour, 2000). To prolong the shelf-life of fruits and vegetables, the growth of microbial populations must be controlled and several post-harvest processes, such as washing and removal of damaged tissues, are employed to reduce initial high counts. It is well known that clean sanitation is essential in keeping the microbial population to a minimum, because storage life is shorter with high initial microbial loads (Bolin, Stafford, King, & Huxsoll, 1977). Nychas (1995) reported antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic and onion against both bacteria and fungi. Phenolic components, present in essential oils, have been known to possess antimicrobial activity and some are classified as generally recognized as safe (GRAS) substances and therefore could be used to prevent post-harvest growth of native and contaminant bacteria (Kabara, 1991; Singh, Singh, Bhunia, & Simmon, 2001). Oxidation of lipids, which occurs during raw material

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storage, processing, heat-treatment and further storage of final products, is one of the basic processes causing rancidity of food products, leading to their deterioration. Due to undesirable influences of oxidized lipids on the human organism, it seems to be essential to decrease contact with products of lipid oxidation in food (Karpinska, Browski, & Danowska-Oziewics, 2001). In order to prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. But, according to toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have already been documented. For example, these substances can show carcinogenic effects in living organisms (Ames, 1983; Baardseth, 1989). From this point of view, governmental authorities and consumers are concerned about the safety of their food and about the potential effects of synthetic additives on health (Reische, Lillard, & Eintenmiller, 1998). The present study deals with the chemical composition, antibacterial, antioxidative and radical-scavenging properties of the essential oils of *Cuminum cyminum* and *Rosmarinus officinalis* L. obtained by steam-distillation. The rationale for this work was to test the possibility of creating a protective atmosphere by using natural compounds that could extend the shelf-life of foodstuffs while minimizing organoleptic alterations.

2. Materials and methods

2.1. General

The major equipment types used were a Clevenger apparatus, GC (9-A-Shimadzu), GC/MS (Varian-3400), microbial culture media (Merck), Shimadzu UV-2501PC spectrophotometer. Chemicals were of analytical grade.

2.2. Plant materials

The plants (*Cuminum cyminum* and *Rosmarinus officinalis*) were collected from the National Botanical Garden of Iran during May–June 2005.

2.3. Oil extraction and analysis

The plant materials were steam-distilled for 90 min in full glass apparatus. The oils were isolated using a Clevenger-type apparatus. The extraction was carried out for 2 h after a 4-h maceration in 500 ml of water. The oils were stored in dark glass bottles in a freezer until they were used. 1/2, 1/4 and 1/8 dilutions of the oils were made with dimethylsulphoxide (DMSO). These dilutions were used in antibacterial analysis. Undiluted oil was taken as dilution 1. GC analysis was performed by a GC (9-A-Shimadzu) gas chromatograph equipped with a flame ionization detector. Quantitation was carried out on Euro Chrom 2000 from KNAUER by the area normalization method. The analysis was carried out using a DB-5 fused-silica column (30 m × 0.25 mm, film thickness 0.25 μm) using a temperature programme of 40–250 °C at a rate of 4 °C/min, injector temperature 250 °C, detector temperature 265 °C, carrier gas was helium (99.99%). The GC/MS unit consisted of Varian-3400 gas chromatograph coupled to a Saturn II ion trap detector. The column was the same as of the GC under the same conditions as stated above. The constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with the literature data.

2.4. Oil dilution solvent

Bacterial strains were streaked on Mueller Hinton agar plates using sterile cotton swabs. Five microlitres of dimethylsulphoxide (DMSO), loaded on sterile blank disks, were placed on the agar plates and were incubated at 37 °C for 24 h. There was no antibacterial activity on the plates and hence DMSO was selected as a safe diluting agent for the oil. Five microlitres from each oil dilution, followed by sterilization, using a 0.45 μm membrane filter, were added to sterile blank discs. The solvent also served as control.

2.5. Microbial strain and growth media

E. coli (ATCC 25922), *S. aureus* (ATCC 25923) and *L. monocytogenes* (PTCC 1298) were employed in the study. Nutrient agar was used to maintain *E. coli* and *S. aureus*. Blood agar was used to maintain *L. monocytogenes*. Bacterial suspensions were made in brain heart infusion (BHI) broth to a concentration of approximately 10^8 cfu/ml. Subsequent dilutions were made from the above suspension, which were then used in the tests.

2.6. Oil sterility test

In order to ensure sterility of the oils, geometric dilutions, ranging from 0.036 to 72.0 mg/ml of the essential oil, were prepared in a 96-well microtitre plate, including one growth control (BHI + Tween 80) and one sterility control (BHI + Tween 80 + test oil). Plates were incubated under normal atmospheric conditions, at 37 °C for 24 h. The contaminating bacterial growth, if at all, was indicated by the presence of a white "pellet" on the well bottom.

2.7. Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oils in question. Briefly, 0.1 ml from 10^8 cfu/ml bacterial suspension was spread on the Mueller Hinton Agar (MHA) plates. Filter paper discs (6 mm in diameter) were impregnated with 5 μl of the undiluted oil and were placed on the inoculated plates. These plates, after remaining at

4 °C for 2 h, were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

2.8. Determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations

All tests were performed in brain heart infusion (BHI) broth supplemented with Tween 80 detergent (final concentration of 0.5% (v/v). Test strains were suspended in BHI broth to give a final density of 10^7 cfu/ml and these were confirmed by viable counts. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were assessed according to our modified procedure (Rasooli & Mirmostafa, 2003). MIC was determined by a broth dilution method in test tubes as follows: 40 μl from each of various dilutions of the oils were added to 5 ml of brain heart infusion (BHI) both in tubes containing 10^7 cfu/ml of live bacterial cells. The tubes were then incubated on an incubator shaker to evenly disperse the oil throughout the broth in tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as the MIC. Cell suspensions (0.1 ml) from the tubes showing no growth were subcultured on BHI agar plates in triplicate to determine if the inhibition was reversible or permanent. MBC was determined as the highest dilution (lowest concentration) at which no growth occurred on the plates.

2.9. Bactericidal kinetics of the oils

Forty microlitres of each oil at the dilution determined by MBC, was added to each 5 ml of brain heart infusion (BHI) broth in tubes containing bacterial suspension of 10^7 cfu/ml and were then incubated at 37 °C in an incubator shaker. Samples (0.1 ml) were taken after 5, 10, 15, 20, 25, 30, 45, 90, 120, 150, 180, 210 and 240 min. The samples were immediately washed with sterile phosphate buffer, pH 7.0, centrifuged at 10000 rpm/1 min, resuspended in the buffer and were then spread-cultured on BHI agar for 24 h at 37 °C. Phosphate buffer was used as diluent when needed. Bactericidal experiments were performed three times. Microbial colonies were counted from triplicates after the incubation period and the mean total number of viable cells per ml was calculated. The mean total number of viable bacteria from bactericidal kinetics experiments at each time interval was converted to $\log_{10}$ viable cells using routine mathematical formulae. The trend of bacterial death was plotted graphically.

2.10. Radical-scavenging capacity of the oils

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of 2,2-diphenylpicrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). Fifty microlitres of 1:5 concentrations of the essential oils in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. Trolox (1 mM) (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The essential oil from *Thymus x-porlock* was used as a natural reference. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated in following way:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate.

2.11. β-Carotene–linoleic acid assay

Antioxidant activity of essential oils was determined using the β-carotene bleaching test (Taga, Miller, & Pratt, 1984). Approximately 10 mg of β-carotene (type I synthetic, Sigma–Aldrich) was dissolved in 10 ml of chloroform. The carotene–chloroform solution, 0.2 ml, was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma–Aldrich) and 200 mg Tween 40 (Sigma–Aldrich). Chloroform was removed using a rotary evaporator at 40 °C for 5 min and, to the residue, 50 ml of distilled water were added, slowly with vigorous agitation, to form an emulsion. Five millilitres of the emulsion were added to a tube containing 0.2 ml of essential oil solution, prepared according to Choi, Song, Ukeda, & Sawamura (2000) and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 10 μl of water instead of essential oils. Butylated hydroxy anisole (BHA; Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as inhibition percentage with reference to the control after 60 min of incubation, using the following equation: $AA = 100(DR_C - DR_S)/DR_C$,where

AA = antioxidant activity,
DR_C = degradation rate of the control = $[\ln(a/b)/60]$,
DR_S = degradation rate in presence of the sample = $[\ln(a/b)/60]$,
a = absorbance at time 0,
b = absorbance at 60 min.

3. Results and discussion

3.1. Chemical composition of the essential oils

The results obtained by GC and GC–MS analyses of the essential oils of *C. cyminum* and *R. officinalis* are

Table 1
Chemical composition of *Cumminum cyminum* essential oil

No.	Compounds	RI	%
1	Isobutyl isobutyrate	892	0.8
2	α-Thujene	922	0.3
3	α-Pinene	931	29.1
4	Sabinene	971	0.6
5	Myrcene	981	0.2
6	δ-3-Carene	998	0.2
7	*p*-Cymene	1013	0.3
8	Limonene	1025	21.5
9	1,8-Cineole	1028	17.9
10	(*E*)-Ocimene	1038	0.1
11	γ-Terpinene	1051	0.6
12	Terpinolene	1082	0.3
13	Linalool	1089	10.4
14	α-Campholenal	1122	0.03
15	*trans*-Pinocarveole	1130	0.07
16	δ-Terpineole	1154	0.09
17	Terpinene-4-ol	1169	0.5
18	α-Terpineole	1180	3.17
19	*trans*-Carveole	1213	0.4
20	*cis*-Carveole	1217	0.07
21	Geraniol	1242	1.1
22	Linalyl acetate	1248	4.8
23	Methyl geranate	1310	0.2
24	α-Terpinyl acetate	1342	1.3
25	Neryl acetate	1351	0.09
26	Methyl eugenol	1369	1.6
27	β-Caryophyllene	1430	0.2
28	α-Humulene	1463	0.2
29	Spathulenol	1562	0.07
30	Caryophylleneb epoxide	1586	0.1
31	Humulene epoxide II	1608	0.08
32	Acetocyclohexane dione (2)	1704	0.4

Table 2
Chemical composition of *Rosmarinus officinalis* L. essential oil

No.	Compounds	RI	%
1	α-Pinene	934	14.9
2	Camphene	945	3.33
3	3-Octanone	966	1.61
4	Sabinene	972	0.56
5	Myrcene	982	2.07
6	O-Cymene	1013	0.71
7	1,8-Cineole	1024	7.43
8	Linalool	1089	14.9
9	Myrcenol	1104	0.75
10	Camphor	1127	4.97
11	Borneol	1155	3.68
12	Terpinen-4-ol	1166	1.70
13	α-Terpineol	1177	0.83
14	Verbinone	1187	1.94
15	Piperitone	1246	23.7
16	Bornyl acetate	1274	3.08
17	β-Caryophyllene	1424	2.68
18	*cis*-β-Farnesene	1448	1.26
19	Germacrene D	1481	0.52
20	α-Bisabolol	1673	1.01

presented in Tables 1 and 2. Thirty-two and twenty compounds were identified in the essential oils of *C. cyminum* and *R. officinalis*, respectively. As a result of GC and GC–MS analyses, *C. cyminum* and *R. officinalis* contained α-pinene (29.1%, 14.9%), 1,8-cineole (17.9%, 7.43%) and linalool (10.4%, 14.9%), respectively, as the major compounds. In addition limonene (21.5%) was present in *C. cyminum* oil, while piperitone (23.7%) was an additional compound in *R. officinalis* oil. Differences in oil composition of Rosemary have already been reported (Gianni et al., 2005). Our literature survey could not find a report on the chemical composition of *C. cyminum* oil. There were some reports of the presence of alpha-pinene, 1,8-cineole, camphor, verbenone and borneol, constituting about 80% of the total *R. officinalis* oil (Santoyo et al., 2005). The major components, alpha-pinene, borneol, camphene, camphor, verbenone and bornyl-acetate, were also reported to be present in Sardinian *R. officinalis* L. oil (Angioni et al., 2004). Compounds, such as camphene, camphor, verbinone and borneol, reported as the major compounds, were also present in our oil at a total contribution of 13.9% (Table 2). These differences in chemical compositions of oils could be attributed to climatic effects on the plants.

3.2. Antimicrobial activity

As can be seen in Table 3, the essential oils were found to have good to moderate antimicrobial activities against all microorganisms tested. Iacobellis, Lo Cantore, Capasso, & Senatore (2005) reported antibacterial activity of *C. cyminum* L. against Gram-positive and Gram-negative bacterial species. Along with the aforementioned report, our results are in agreement with those of Singh, Kapoor,

Table 3
Determination of MIC, MBC and Disc diffusion assay of the essential oils

Essential oil	Microorganism	Oil dilution				Mean inhibition zone (mm)
		1/8	1/4	1/2	1	
C. cyminum	*E. coli*	S	S	S	S	13.00
	S. aureus	+	S	S	S	10.00
	L. monocytogenes	++	+	S	S	17.67
R. officinalis	*E. coli*	+	S	S	S	16.67
	S. aureus	+++	+	S	S	8.33
	L. monocytogenes	++	+	S	S	16

+ = MIC, S = MBC, ++ = low to medium growth, +++ = medium to good growth.

Pandey, Singh, & Singh (2002) who reported *C. cyminum* oil to be equally good or more effective when compared with standard antibiotics, at a very low concentration. In general, *C. cyminum* oil exhibited stronger activity than did *R. officinalis* oil. The essential oil from *R. officinalis* has been reported to be weakly inhibitory against *E. coli*, *S. aureus* and *L. monocytogenes* as compared to other oils (Lopez, Sanchez, Batle, & Nerin, 2005). In the case of *C. cyminum*, results from the disc diffusion method, and determination of minimal inhibitory and bactericidal concentrations (MIC and MBC), indicate that, *E. coli* is the most sensitive microorganism, with the lowest MBC value (1 μl/ml). Other sensitive microorganisms are *S. aureus* and *L. monocytogenes* with MIC values of 1 μl/ml and 2 μl/ml respectively. Although *L. monocytogenes* required higher oil concentration (2 μl/ml) for complete elimination, it showed a greater zone of inhibition (17.67 mm) on disc diffusion plates. The results obtained from *R. officinalis* oil exhibited the same sequence of sensitivity against microorganisms as a result of exposure to various concentrations of the oil. Zones of inhibition of *E. coli* and *L. monocytogenes*, on exposure to *R. officinalis* oil-rich fractions, were about 17 mm (Santoyo et al., 2005). Our results showed about 16 mm (Table 3) which is very close to the above report. Under equal conditions, the difference in the diameter of zones of inhibition can be attributed to the techniques employed. We have used a minimum amount of whole oil (5 μl) per disc while Santoyo et al. (2005) used oil-rich fractions. In our study, *S. aureus* was least susceptible, while it was the most susceptible in the study conducted by Santoyo et al. (2005). They attributed the antimicrobial property of the essential oil to the presence of alpha-pinene, 1,8-cineole, camphor, verbenone, and borneol, with borneol being the most effective, followed by

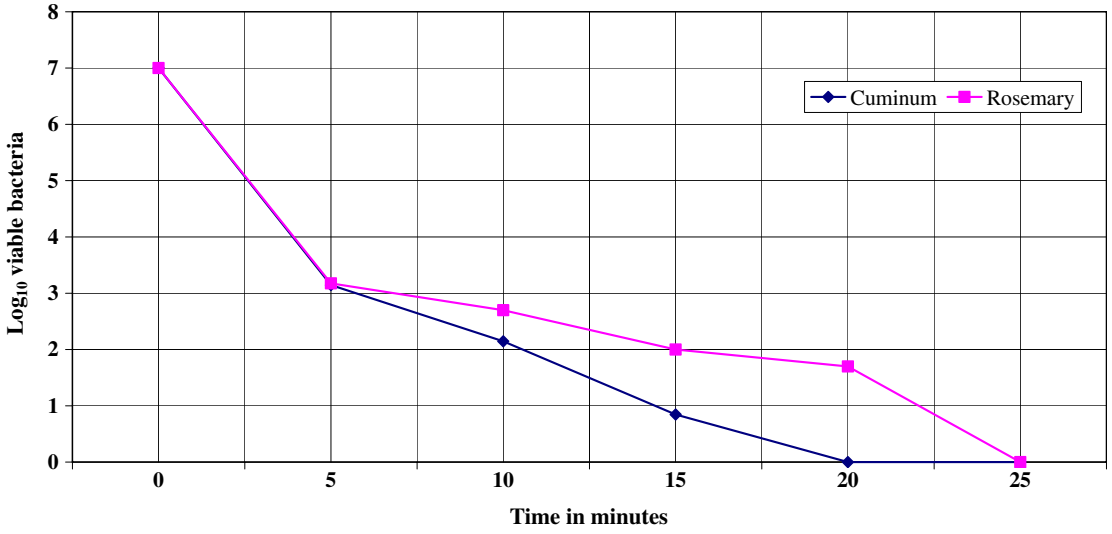

Fig. 1. Kinetics of death of *E. coli* exposed to the MBC levels of essential oils.

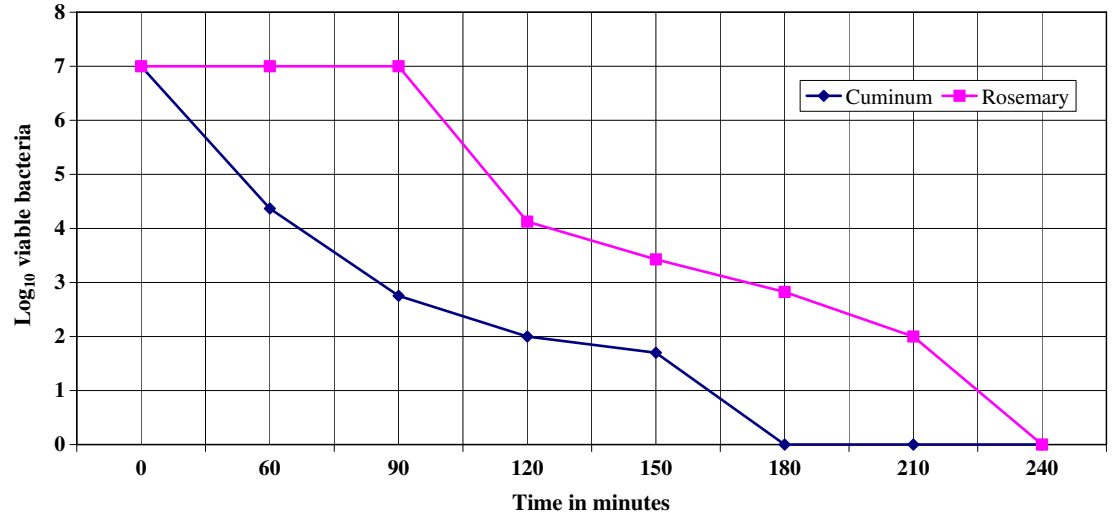

Fig. 2. Kinetics of death of *S. aureus* exposed to MBC levels of essential oils.

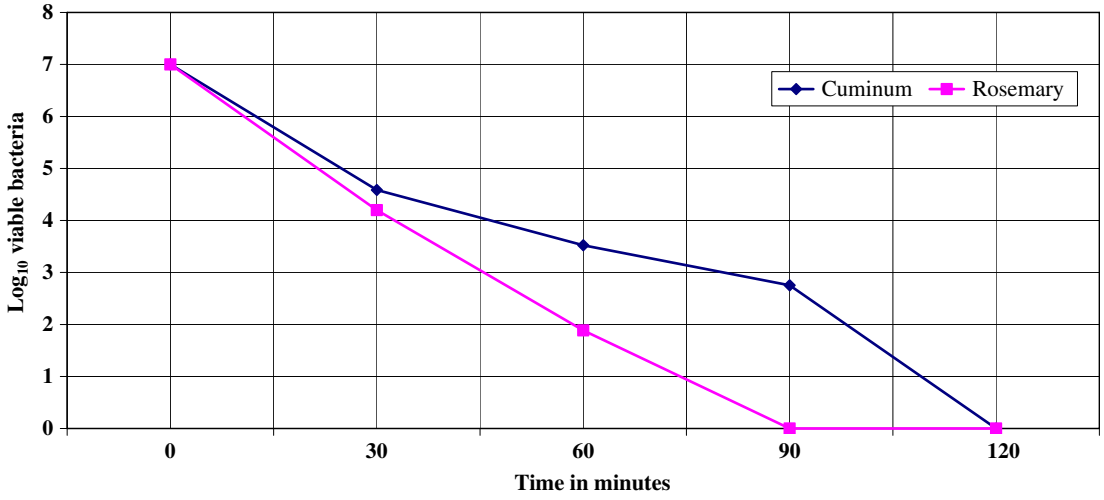

Fig. 3. Kinetics of death of *Listeria monocytogenes* exposed to MBC levels of essential oils.

camphor and verbenone. The quantities of these compounds were very low in our oil. Susceptibility of *E. coli* and *L. monocytogenes* in the present study, suggests that other components of the oils have exerted a antimicrobial effect.

3.3. Bactericidal kinetics of the oils

Figs. 1–3 show reduction times of *E. coli*, *S. aureus* and *L. monocytogenes*, respectively, after exposure to the MBC levels of the oils. Complete death time on exposure to *C. cyminum* L. and *R. officinalis* L. oils were 20 and 25 min, 180 and 240 min and 90 and 120 min for *E. coli*, *S. aureus* and *L. monocytogenes*, respectively. It can be concluded that *E. coli* is the most vulnerable and *S. aureus* is the least vulnerable microorganism, to the oils under study. These values suggest the duration of time required for complete bactericidal effects of the oils.

3.4. Free radical-scavenging capacities of the oils

The DPPH radical-scavenging activities of the essential oils and of references are shown in Fig. 4. *C. cyminum* and *R. officinalis* essential oils notably reduced the concentration of DPPH free radical, with an efficacy lower than that of reference oil *T. x-porlock* (69.3% inhibition) and slightly lower than that of trolox. The performance of the rosemary oil was better than that of *C. cyminum*. Oils with a higher monoterpenic abundance were reported to be almost ineffective. This result is in agreement with the poor performance given by the oils with similar patterns and by single monoterpenic hydrocarbons (Ruberto & Baratta, 2000).

3.5. β-Carotene–linoleic acid assay

The lipid peroxidation inhibitory activities of the essential oils were assessed by the β-carotene bleaching test

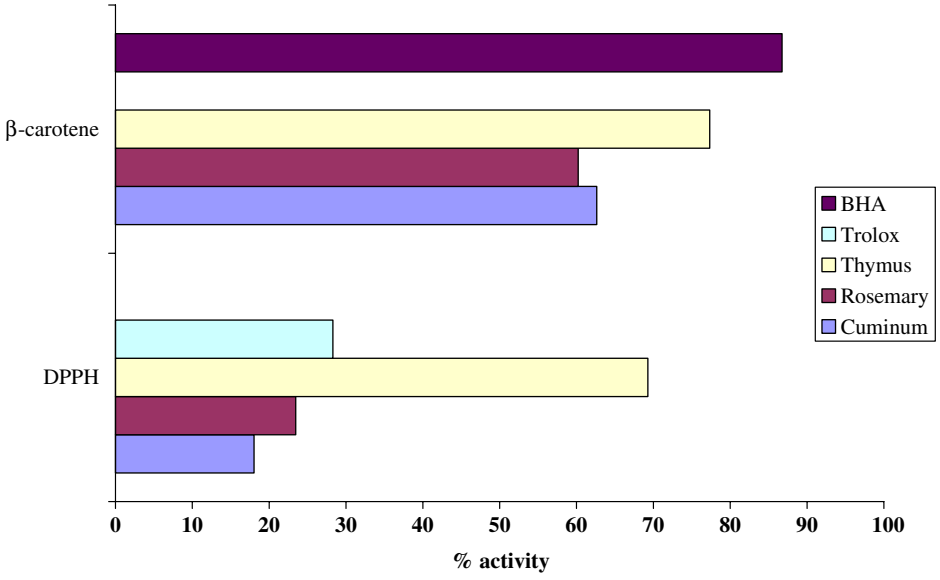

Fig. 4. Free radical-scavenging and antioxidant activities of essential oils in comparison with those of the references.

(Fig. 4). Results of the reference oil (*T. x-porlock*) were almost consistent with data obtained from the DPPH test. Overall results were better than those provided by the radical-scavenging activity. The difference is probably as a consequence of a higher specificity of the assay for lipophilic compounds.

These properties are also very much needed by the food industry in order to find possible alternatives to synthetic preservatives (namely BHT, phenolics). In this context, *C. cyminum* and *R. officinalis* essential oils, gave interesting results, being one of the promising performing extracts in terms of both antimicrobial activity and ability to neutralize free radicals and prevent unsaturated fatty acid oxidation. The results presented here may also contribute to knowledge of the antioxidative and antimicrobial potentials of these species reported elsewhere.

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