JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antibacterial and Antioxidant Activities of Essential Oils Isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L.

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Antilisterial activities of *Thymbra capitata* and *Origanum vulgare* essential oils were tested against 41 strains of *Listeria monocytogenes.* The oil of *T. capitata* was mainly constituted by one component, carvacrol (79%), whereas for *O. vulgare* three components constituted 70% of the oil, namely, thymol (33%), γ-terpinene (26%), and *p*-cymene (11%). *T. capitata* essential oil had a significantly higher antilisterial activity in comparison to *O. vulgare* oil and chloramphenicol. No significant differences in *L. monocytogenes* susceptibilities to the essential oils tested were registered. The minimum inhibitory concentration values of *T. capitata* essential oil and of carvacrol were quite similar, ranging between 0.05 and 0.2 μL/mL. Antioxidant activity was also tested, the essential oil of *T. capitata* showing significantly higher antioxidant activity than that of *O. vulgare*. Use of *T. capitata* and *O. vulgare* essential oils can constitute a powerful tool in the control of *L. monocytogenes* in food and other industries.

KEYWORDS: ***Listeria monocytogenes*; *Thymbra capitata* L. (Cav.); *Origanum vulgare* L.; essential oils; antibacterial activity; antioxidant activity**

INTRODUCTION

Addition of spices to food is common in the Mediterranean population mainly due to the pleasant flavor and additionally to the increase in food shelf-life time. Investigations concerning the antimicrobial and antioxidant activities of either the plant, their extracts, and/or essential oils have been conducted in the search for new compounds to replace synthetic ones, because the consumption of "green" products has suffered a tremendous demand (*1*, *2*).

The concern for food safety has increased significantly in recent years; in particular, apprehension has grown about foodborne microorganisms, such as *Escherichia coli* 0157:H7, *Salmonella* spp., and *Listeria monocytogenes* (*3*).

L. monocytogenes is the etiological agent of human and animal listeriosis. In the late 1980s this disease was recognized as foodborne. The consumption of food contaminated with the bacterium, such as meat, fish, vegetables, and dairy products, especially cheese, can constitute a serious health risk to humans, achieving a high mortality rate, especially in "risk groups" that include healthy pregnant women, fetuses and newborn infants, the elderly, and immunocompromised individuals (*4*). Foodborne illness is a serious health problem around the world. Recently, in the United States, the Centers for Disease Control estimated that 76 million people get sick each year, of which ~300 000 are hospitalized and 5000 die as a result of foodborne disease (*5*). *L. monocytogenes* is considered to be a robust bacterium, the ability to grow at refrigeration temperatures and in the presence of high salt content, iron deficiency, and acid contributing mostly to the foodborne transmission. In addition, the importance of this foodborne pathogen is reinforced by the ability to adapt when exposed to sublethal conditions during food processing and gain tolerance to more severe stress conditions, which this bacterium may encounter either during postprocessing conditions or during host invasion (*6*−*9*). Acquisition of antimicrobial resistance by foodborne pathogens will compromise human drug treatments. In reality, the existence of antimicrobial-resistant variants among several food pathogens such as *Salmonella enterica*, *E. coli*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *L. monocytogenes* has already been registered (*10*).

The antimicrobial properties of essential oils were stated long ago, and in the past decades intensive studies were carried out indicating the possibility of their use in the control of foodborne pathogens, contributing to a future reduction in the employment

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10.1021/jf0510079 CCC: $30.25
Published on Web 09/20/2005

of antibiotics (*11*−*16*). Several studies have shown the antimicrobial efficacy of the essential oils of *Origanum vulgare* and *Thymbra capitata* or their major oil components against diverse microorganisms (*17*−*19*).

O. vulgare L. and *T. capitata* L. (Cav.) are two aromatic plants spread throughout southern Portugal. *T. capitata* is used in some Portuguese traditional meat dishes, particularly in rabbit meat, whereas *O. vulgare* is preferentially used in tomato salads, fish food, and "escargot".

The majority of the studies conducted to determine the antibacterial activity of essential oils use a limited number of bacterial strains, and laboratory strains are frequently selected. Consequently, the obtained results may not truthfully indicate either the susceptibility of the bacterial strains isolated from food and other sources or the potential activity of the selected essential oils.

The present study was carried out employing a significant number of strains of one of the most important foodborne pathogens, the bacterium *Listeria monocytogenes*, for the determination of the antibacterial activity of the *T. capitata* and *O. vulgare* essential oils. Another goal of the present study was the evaluation of the antioxidant activity of these essential oils as an important factor to reduce aging, cardiovascular diseases, and cancer development. The obtained data will allow the future application of the most promising essential oil in food systems associating two activities that will contribute enormously to improve food safety and quality.

MATERIALS AND METHODS

Materials. The aerial dried flowering parts of *O. vulgare* L. were acquired in the local market, whereas a collective population of *T. capitata* L. (Cav.) was collected in Nave do Barão (Algarve, Portugal) at the flowering phase. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were purchased from Fluka (Steinheim, Germany). α-Tocopherol and chloride potassium were purchased from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) was purchased from Acros (Geel, Belgium). 2,2′-Azobis(2-amidinopropane) dihydrochloride (ABAP) was purchased from Aldrich (Steinheim, Germany). Sodium dodecyl sulfate and acetic acid were purchased from Riedel-de Haën (Seelze, Germany). Linoleic acid was purchased from Sigma-Aldrich (Steinheim, Germany). The culture medium tryptic soy agar (TSA) was purchased from Oxoid (Basingstoke, Hampshire, U.K.). All of the components of the defined medium Trivett and Meyer (TM) were purchased from Sigma-Aldrich.

Isolation of Essential Oils. The oils were isolated from dried material by hydrodistillation, for 4 h, using a Clevenger-type apparatus (*20*). The oil yields of *T. capitata* and *O. vulgare* were 2.9 and 1.8% (v/w), respectively.

Gas Chromatography (GC). GC analyses were performed using a Perkin-Elmer 8700 gas chromatograph equipped with two flame ionization detectors (FIDs), a data handling system, and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness = 0.25 μm; J&W Scientific Inc., Rancho Cordova, CA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness = 0.15 μm; J&W Scientific Inc.). Oven temperature was programmed from 45 to 175 °C, at 3 °C/min, subsequently at 15 °C/min to 300 °C, and then held isothermal for 10 min; injector and detector temperatures were 280 and 290 °C, respectively; and carrier gas, hydrogen, was adjusted to a linear velocity of 30 cm/s. The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.2 μL of a pentane−oil solution. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, without using correction factors.

Gas Chromatograph−Mass Spectrometry (GC-MS). The GC-MS unit consisted of a Carlo Erba 6000 Vega gas chromatograph, equipped with a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness = 0.25μm; J&W Scientific Inc.), and interfaced with a Finnigan MAT 800 ion trap detector (ITD; software version 4.1). Oven temperature was as above; transfer line temperature was 280 °C; ion trap temperature was 220 °C; carrier gas, helium, was adjusted to a linear velocity of 30 cm/s; split ratio was 1:40; ionization energy was 70 eV; ionization current was 60 μA; scan range was 40−300 u; and scan time was 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9−C_{17} *n*-alkanes, and GC-MS spectra with corresponding data of components of reference oils (RO), laboratory-synthesized components (LSC), and commercially available standards (CAS) from a homemade library: RO1, *Thymus caespititius* oils; RO2, *Cinnamomum zeylanicum* oil; RO3, *Achillea milefollium* oils; CAS1, Extrasynthese (Cymit Química, S.L., Barcelona, Spain); CAS2, Sigma-Aldrich (Steinheim, Germany); CAS3, Fluka (Steinheim, Germany); CAS4, Riedel-de Haën (Seelze, Germany).

Antilisterial Activity Determination. The strains of *L. monocytogenes* used in this study are listed in **Table 1**. Strains were kept at −70 °C and maintained in TSA at 4 °C during the study. The cultures were recovered from freezing by growth in tryptic soy broth (TSB) and prior to the assay were first grown in a TSA plate for 24 h at 30 °C; from this plate a loop was used to inoculate 10 mL of TSB for ∼2 h in order to reach the exponential phase. The antibacterial activity was determined by agar diffusion method, and 0.1 mL of the previously

Table 1. Description of *L. monocytogenes* Strains

L. monocytogenes strain	serotype	origin	source
EGD	1/2a	clinical	Trudeau Institute, New York
NCTC 7973	1/2a	guinea pig, lymph nodes	England
Scott A		related to Mexican-style cheese outbreak	37
C679	nd[a]	Portuguese cheese	INETI-DTIA[b]
C680	nd	Portuguese cheese	INETI-DTIA
C681	4b	Portuguese cheese	INETI-DTIA
C682	nd	Portuguese cheese	INETI-DTIA
C683	nd	Portuguese cheese	INETI-DTIA
C684	nd	Portuguese cheese	INETI-DTIA
C685	nd	Portuguese cheese	INETI-DTIA
C687	nd	Portuguese cheese	INETI-DTIA
C688	nd	Portuguese cheese	INETI-DTIA
C713	nd	Portuguese cheese	INETI-DTIA
C736	nd	Portuguese cheese	INETI-DTIA
C737	nd	Portuguese cheese	INETI-DTIA
C739	nd	Portuguese cheese	INETI-DTIA
C759	nd	Portuguese cheese	INETI-DTIA
C760	nd	Portuguese cheese	INETI-DTIA
C778	nd	Portuguese cheese	INETI-DTIA
C779	nd	Portuguese cheese	INETI-DTIA
C782	nd	Portuguese cheese	INETI-DTIA
C830	nd	Portuguese turkey meat	INETI-DTIA
C882	4b	Portuguese cheese	INETI-DTIA
C895	1/2a	Portuguese cheese	INETI-DTIA
C897	1/2a	Portuguese cheese	INETI-DTIA
C958	nd	Portuguese cheese	INETI-DTIA
C969	nd	Portuguese cheese	INETI-DTIA
C996	nd	processed meat	INETI-DTIA
C997	nd	processed meat	INETI-DTIA
C1003	nd	pork meat	INETI-DTIA
C1006	nd	pork meat	INETI-DTIA
C1046	nd	hamburger	INETI-DTIA
C1048	nd	Portuguese cheese	INETI-DTIA
C1049	nd	hamburger	INETI-DTIA
C1050	nd	cow's milk	INETI-DTIA
C1051	nd	cow's milk	INETI-DTIA
C1052	nd	cow's milk	INETI-DTIA
C1053	nd	processed meat	INETI-DTIA
G14	nd	cheese factory environment	INETI-DTIA
SLU2157	1/2a	clinical	38
SLU 1922	4b	clinical	39

[a] Not determined. [b] Instituto Nacional de Engenharia e Tecnologia Industrial, Departamento de Tecnologia das Indústrias Alimentares.

obtained exponential phase culture was used to inoculate the TSA plates. Sterile filter paper disks of 6 mm were distributed on the agar surface containing 3 μL of the isolated essential oils or 2 μL of carvacrol standard. Sterile water and the antibiotic chloramphenicol (30 μg/disk) were used as control. Inhibition zones were determined after an incubation period of 24 h at 30 °C.

The minimum inhibitory concentration (MIC; the minimum concentration that causes a reduction of growth) for the *T. capitata* essential oil and carvacrol standard was determined using a microdilution method. Concentrations of the essential oil of *T. capitata* and carvacrol ranging from 0 to 0.35 μL/mL with increments of 0.05 were tested. Each *L. monocytogenes* strain was grown overnight in a defined medium TM (*21*) [TM composition per liter: K_2HPO_4, 8.50 g; $NaH_2PO_4 \cdot H_2O$, 1.50 g; NH_4Cl, 0.50 g; $MgSO_4 \cdot 7H_2O$, 0.41 g; $FeCl_3 \cdot 6H_2O$, 0.048 g; NaOH, 0.24 g; nitrilotriacetic acid, 0.48 g; L-cysteine hydrochloride, 100 mg; L-leucine, 100 mg; DL-isoleucine, 200 mg; DL-valine, 200 mg; DL-methionine, 200 mg; L-arginine hydrochloride, 200 mg; L-histidine hydrochloride, 200 mg; riboflavin, 1 000 μg; thiamine hydrochloride, 1000 μg; D-biotin, 100 μg; α-lipoic acid, 1 μg; glucose, 0.2% (w/v)], collected and washed in Ringer solution, and finally resuspended in TM medium at appropriate concentration. The defined medium TM was chosen for the MIC determination because it is one of the most suitable to test the behavior of *L. monocytogenes* strains to several stresses (*5*, *22*). Each well of a microplate (Greiner Labortechnick, Frichenhausen, Germany) was filled with 180 μL of the defined medium with the appropriate essential oil or standard concentration. The inoculation of the wells was done by using a volume of 10−20 μL of the previously prepared culture. Negative control was constituted by the culture in TM with no essential oil. Three replicates for each *L. monocytogenes* strain were used. The growth was followed by spectophotometry ($A_{655\ nm}$) using a microplate reader (Benchmark, Bio-Rad, Hemel Hempstead, U.K.).

The MIC value was determined from the growth curves. The minimum concentration that did not show listerial growth was considered to be the minimum bactericidal concentration. The purity of the culture was determined by growing the culture in TSA medium and observed by Henry illumination.

Data on susceptibility of *L. monocytognes* strains to the essential oils tested were analyzed by analysis of variance (ANOVA), and in postanalysis the multiple-comparison Tuckey test was used.

Antioxidant Activity Measurement. *TBARS Assay.* Two sets of experiments based on a modified thiobarbituric acid (TBA) reactive species assay (TBARS) were used to measure the antioxidant ability of the sample (essential oils or tested substances): without (1) and with (2) a lipid peroxidation inducer. In both cases egg yolk homogenate was used as lipid-rich media obtained as described by Dorman et al. (*23*); that is, an aliquot of yolk material was made up to a concentration of 10% (w/v) in KCl (1.15%, w/v). The yolk was then homogenized for 30 s followed by ultrasonication for a further 5 min. For set 1 of the TBARS assay, 500 μL of the homogenate and 100 μL of sample, solubilized in methanol, were added to a test tube and made up to 1 mL with distilled water, followed by the addition of 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% (w/v) TBA in 1.1% (w/v) sodium dodecyl sulfate (SDS). Each essential oil and tested substance was assayed in the concentrations of 160, 320, 640, 800, and 1000 mg/L. This mixture was stirred in a vortex and heated at 95 °C for 60 min. After cooling, at room temperature, 5 mL of butan-1-ol was added to each tube, stirred, and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm using a Shimadzu 160-UV spectrophotometer. All of the values are expressed as antioxidant index (AI%), whereby the control is completely peroxidized and each oil and tested substance demonstrated a comparative percentage of antioxidant protection. The AI% was calculated using the following formula: $(1 - t/c) \times 100$, c being the absorbance value of the fully oxidized control and t the absorbance of the tested sample (*24*). For set 2 of the TBARS assay, 50 μL of ABAP (0.07 M) was added to induce lipid peroxidation, soon after the addition of sample, the remaining procedure being as reported above.

Micellar Model System. The samples were analyzed according to the method already described by some authors (*25*, *26*) with slight modifications. Fifteen milliliters of the micellar suspension of linoleic acid was prepared with 0.1 M solution of SDS set in aqueous 0.01 M phosphate buffer (pH 7.4) and linoleic acid to a concentration of 0.026 M. The micellar suspension was stirred in a reactor at 50 °C. After equilibration for 10 min, 75 μL of 0.07 M ABAP in water was added, and peroxidation was monitored at 234 nm. After 20 min, 75 μL of the sample (32 mg/mL) dissolved in methanol was added, and the kinetics was monitored for the following 20 min. The buffered SDS solution was used as a blank. The ratio between the slope of the linear plot of absorbance versus time after and before the addition of the sample gave the antioxidant index (AI%): $AI\% = [1 - (S_{inh}/S_{ABAP})] \times 100$, where S_{inh} and S_{ABAP} are the slopes after and before the addition of the sample solution, respectively.

The analytical values of the antioxidant activity measurement represent means of four replicates. Analysis of variance was performed by ANOVA procedures (SPSS 10.0 for Windows). Data obtained were subjected to one-way analysis of variance and Tuckey's test analysis.

RESULTS

The chemical compositions of the essential oils of *T. capitata* and *O. vulgare* are listed in **Table 2** in order of the components' elution on a DB-1 column. Oxygen-containing monoterpenes dominated the essential oil of *T. capitata* (81%), carvacrol (79%) being the most representative component of this group. Monoterpene hydrocarbons (48%) and oxygen-containing monoterpenes (38%) constituted the most important groups of *O. vulgare* oil. γ-Terpinene (26%), *p*-cymene (11%), and thymol (33%) were the dominant components of those groups.

The results of the determination of the antilisterial activity of the *T. capitata* and *O. vulgare* essential oils are summarized in **Table 3**. Essential oils of both *T. capitata* and *O. vulgare* exhibit anti-listeria activity. The conducted one-way between-groups analysis of variance indicated that the essential oil of *T. capitata* had a significantly higher activity ($P < 0.05$) against the 41 strains of *L. monocytogenes* used than the essential oil of *O. vulgare* and the antibiotic (chloramphenicol). Moreover, the anti-listeria activity of *O. vulgare* essential oil was similar ($P > 0.05$) to that of the antibiotic. No differences ($P > 0.05$) in susceptibility to the essential oils between the 41 strains of *L. monocytogenes* were registered.

For the determination of MIC of the essential oil of *T. capitata*, 12 *L. monocytogenes* strains were selected. As it was not possible to distinguish the *L. monocytogenes* strains according to their susceptibility to tested essential oils, 5 *L. monocytogenes* strains (C681, C830, C882, C895, and C897) that were previously tested for their tolerance to several stresses, namely, acid and salt (*6*), were included. Concentrations ranging from 0.05 to 0.35 μL/mL were used. The minimum concentration that caused a decline in growth was considered to be the MIC value. The results are summarized in **Table 4**. The MIC value varied between 0.05 and 0.20 μL/mL, and the minimum bactericidal concentration (MBC, the minimum concentration at which no recovery of viable cells was registered) for this essential oil was 0.30 μL/mL for all *L. monocytogenes* strains tested.

The antilisterial activity of carvacrol, the main component of the *T. capitata* essential oil, was determined by disk diffusion technique and by the determination of the MIC value. Five *L. monocytogenes* strains were selected for this determination. The results are summarized in **Table 5**. The activity of carvacrol determined by disk diffusion assay was higher ($P < 0.05$) when compared to that of the antibiotic chloramphenicol (**Table 5**). The *L. monocytogenes* strains used were equally susceptible ($P > 0.05$) to carvacrol. The MIC for carvacrol ranged from 0.05 to 0.15 μL/mL (**Table 5**). The MBC was 0.25 μL/mL for all *L. monocytogenes* strains tested. These values were slightly inferior

Table 2. Percentage Composition of the Essential Oils Isolated by Hydrodistillation from the Aerial Parts of *T. capitata* and *O. vulgare*[a]

IP[b]	component	RI[c]	*T. capitata*	*O. vulgare*
RO1, RO2	α-thujene	924	0.6	1.4
CAS1, RO1	α-pinene	930	0.5	0.7
CAS1, RO1	camphene	938	0.1	0.2
CAS1, RO1,	sabinene	958	0.2	0.1
CAS2	octen-3-ol	961		0.4
CAS1, RO1, RO2	β-pinene	963	0.1	0.2
CAS1, RO1, RO2	myrcene	975	1.4	2.0
CAS2, RO1, RO2	α-phellandrene	995	0.1	0.3
CAS2, RO1, RO2	δ-3-carene	1000	0.1	
CAS2, RO1, RO2	α-terpinene	1002	1.0	3.4
CAS2, RO1, RO2	***p*-cymene**	1003	6.7	**10.7**
CAS2, RO1, RO2	1,8-cineole	1005	0.2	
CAS3, RO1, RO2	limonene	1009	0.2	0.6
CAS3, RO1, RO2	*cis*-β-ocimene	1017		2.5
CAS3, RO1, RO2	*trans*-β-ocimene	1027	0.1	0.3
CAS2, RO1, RO2	**-γterpinene**	1035	3.0	**25.9**
CAS3	*trans*-sabinene hydrate	1037	0.2	0.2
CAS2, RO1, RO2	terpinolene	1064	0.1	0.1
CAS2	linalool	1074	1.0	0.4
CAS2	borneol	1134	0.2	0.3
CAS2, RO1, RO2	terpinen-4-ol	1148	0.8	0.1
CAS3, RO1, RO2	α-terpineol	1159	t[d]	0.1
RO1	methylthymol	1208		1.1
RO1	methylcarvacrol	1224		2.5
CAS4, RO1	**thymol**	1275		**32.6**
CAS3, RO1	**carvacrol**	1286	**78.6**	0.3
CAS2, RO3	eugenol	1327	0.1	
RO1	carvacrol acetate	1348	0.3	
CAS2, RO1, RO2	β-caryophyllene	1414	2.2	4.5
CAS3, RO1, RO2	α-humulene	1447	0.1	0.7
RO2	γ-muurolene	1469		0.8
RO1, RO2	germacrene D	1474		0.7
RO1, RO2	β-bisabolene	1495		2.0
RO2	γ-cadinene	1500	0.1	0.3
RO2	calamenene	1505		0.5
RO 2	δ-cadinene	1505		1.1
RO1, RO1	elemol	1530	0.1	
RO1, RO1	spathulenol	1551		0.1
CAS3, RO1, RO2	caryophyllene oxide	1561	0.2	0.4
RO2	α-cadinol	1626		0.2

[a] Quantification was performed by the normalization method. Peak area percent standard deviation ≤ 0.5. For quantification details see Materials and Methods. [b] Identification procedure. All components were identified on the basis of a homemade library created with reference oils (RO), laboratory-synthesized components (LSC), and commercial available standards (CAS). RO1, *Thymus caespititius* oils; RO2, *Achillea milefollium* oils; RO3, *Cinnamomum zeylanicum* oil; CAS1, Extrasynthese (Cymit Química, S.L.); CAS2, Sigma-Aldrich; CAS3, Fluka; CAS4, Riedel-de Haën. [c] Retention index relative to C_9–C_{17} *n*-alkanes on the DB-1 column. [d] Trace (<0.05%).

Table 3. Antilisterial Activity (Inhibition Zone, Including Diameter of the Disk, 6 mm) of the Oils from *T. capitata* and *O. vulgare*[a]

	diameter of inhibition zone (mm)		
strain	*T. capitata*	*O. vulgare*	chloramphenicol
C 679	33.75 ± 1.77a	36.25 ± 1.77b	20.00 ± 0.00b
C 680	25.25 ± 1.77a	19.00 ± 4.24b	23.00 ± 1.41b
C 681	22.50 ± 2.12a	27.50 ± 3.50b	24.00 ± 1.40b
C 682	28.50 ± 0.71a	19.50 ± 0.71b	23.50 ± 0.71b
C 683	23.50 ± 0.71a	19.00 ± 1.41b	22.75 ± 2.47b
C 684	28.25 ± 2.47a	23.10 ± 4.38b	24.00 ± 1.41b
C 685	29.50 ± 0.71a	24.50 ± 0.71b	22.50 ± 2.12b
C 687	27.25 ± 0.36a	17.50 ± 2.12b	23.75 ± 1.06b
C 688	34.75 ± 3.18a	31.00 ± 5.66b	23.75 ± 1.06b
C 713	29.50 ± 6.36a	28.50 ± 2.12b	23.50 ± 0.71b
C 736	33.00 ± 2.83a	22.00 ± 1.41b	22.50 ± 0.00b
C 737	29.50 ± 3.54a	18.25 ± 2.48b	21.75 ± 0.36b
C 739	30.00 ± 0.00a	35.00 ± 0.00b	22.00 ± 1.41b
C 759	34.50 ± 0.70a	32.00 ± 4.20b	21.50 ± 2.10b
C 760	30.25 ± 3.18a	28.25 ± 4.60b	23.75 ± 2.47b
C 778	29.50 ± 1.41a	21.50 ± 4.95b	24.50 ± 2.12b
C 779	28.75 ± 1.77a	29.50 ± 0.70b	20.50 ± 0.70
C 782	19.00 ± 2.83a	16.75 ± 3.18b	23.50 ± 0.71b
C 830	30.50 ± 0.71a	31.75 ± 0.35b	22.75 ± 0.35b
C 882	18.00 ± 4.24a	19.00 ± 1.41b	23.00 ± 4.24b
C 895	29.50 ± 4.24a	28.75 ± 3,18b	25.25 ± 3.18b
C 897	18.50 ± 2.12a	25.00 ± 7.07b	25.00 ± 0.00
C 958	30.75 ± 1.06a	21.00 ± 3.53b	24.75 ± 1.77b
C 969	32.50 ± 0.71a	20.50 ± 2.12b	22.50 ± 2.12b
C 996	30.00 ± 1.41a	23.50 ± 2.12b	24.75 ± 0.35b
C 997	26.75 ± 0.35a	16.75 ± 2.47b	23.25 ± 1.06
C 1003	29.75 ± 3.18a	20.50 ± 0.71b	21.25 ± 0.35b
C 1006	30.50 ± 2.12a	25.50 ± 2.83b	24.25 ± 1.77b
C 1046	28.00 ± 1.41a	27.00 ± 0.70b	21.25 ± 0.35b
C 1048	28.25 ± 0.71a	23.25 ± 1.06b	26.25 ± 1.77b
C 1049	31.50 ± 3.53a	26.25 ± 3.89b	24.75 ± 1.06b
C 1050	29.50 ± 2.12a	24.25 ± 4.60b	23.25 ± 1.77b
C 1051	21.00 ± 1.41a	19.75 ± 3.18b	24.75 ± 1.06b
C 1052	31.25 ± 1.77a	26.25 ± 1.77b	21.25 ± 3.18b
C 1053	28.75 ± 1.77a	23.50 ± 4.95b	22.25 ± 1.76b
G 14	22.50 ± 3.53a	20.00 ± 0.00b	24.00 ± 1.41b
SLU 1922	30.00 ± 1.41a	28.50 ± 1.41b	21.25 ± 1.77b
SLU 2157	31.00 ± 0.00a	28.50 ± 4.24b	19.25 ± 1.06b
NCTC 7973	21.00 ± 1.41a	21.50 ± 2.12b	25.00 ± 0.00b
Scott A	23.50 ± 2.12a	22.00 ± 1.41b	23.50 ± 2.12b

[a] Data are the mean of two independent experiments ± standard deviation. Data with different letters are significantly different ($P < 0.05$)

Table 4. Minimum Inhibitory Concentration of the Essential Oil of *T. capitata* for Each of the Selected *L. monocytogenes* Strains[a]

L. monocytogenes strain	MIC (μL/mL)
NCTC7973	0.20
ScottA	0.20
C681	0.05
C759	0.15
C782	0.10
C830	0.20
C882	0.05
C895	0.15
C897	0.20
G14	0.15
SLU1922	0.05
SLU2157	0.05

[a] Data are the mean of three independent experiments.

to the MIC (0.20 μL/mL) and MBC (0.30μL/mL) of the essential oil of *T. capitata* (**Table 4**).

Tables 6 and **7** show the antioxidant indices (percent) of the essential oils of *T. capitata* and *O. vulgare* as well as those of BHT, BHA, and α-tocopherol, in different concentrations, using TBARS assay in the absence or in the presence of the radical inducer ABAP, respectively. In the absence of the radical inducer, the essential oils showed antioxidant activity mainly at higher concentrations, ranging from 83 to 87% from 640 to 1000 mg/L (**Table 6**). For concentrations ≥640 mg/L no significant differences were observed in the antioxidant indices of *T. capitata* and *O. vulgare* oils and BHT; that is, their activities were comparable. α-Tocopherol and BHA were less effective than the essential oils tested at least in the concentration range of 320−800 mg/L for BHA and 1000 mg/L for α-tocopherol.

It is noteworthy that in the presence of ABAP, α-tocopherol and BHA showed higher antioxidant capacity, regardless of the concentrations tested, than without the radical inducer. In the case of BHT the differences were not as evident as those observed for α-tocopherol and BHA. The antioxidant index of *T. capitata* oil was not affected when ABAP was added, in contrast to that observed for *O. vulgare* oil. In the latter case, the presence of ABAP determined a decrease of the antioxidant index.

Table 5. Activity of Carvacrol (Inhibition Zone, Including Diameter of the Disk, 6 mm) and Minimum Inhibitory Concentration for Each *L. monocytogenes* Strain

L. monocytogenes strain	inhibition zone[a] (mm)		carvacrol MIC[b] (μL/mL)
	carvacrol	chloramphenicol	
C830	40.00 ± 5.66a	27.00 ± 0.35b	0.10
C882	36.00 ± 2.83a	24.00 ± 1.41b	0.05
C897	44.00 ± 10.96a	29.00 ± 0.35b	0.10
NCTC7973	38.00 ± 2.47a	26.00 ± 0.35b	0.10
Scott A	37.00 ± 0.71a	25.00 ± 1.77b	0.15

[a] Data are the mean of two independent experiments ± standard deviation. Data with different letters are significantly different ($P < 0.05$). [b] Data are the mean of three independent experiments.

Table 6. Antioxidant Index of the Essential Oils, α-Tocopherol, BHT, and BHA, in Different Concentrations, Using TBARS Assay without ABAP[a]

oil/substance (mg/L)	antioxidant index (%) without ABAP				
	BHT	BHA	α-tocopherol	*T. capitata*	*O. vulgare*
160	86.5 ± 4.5a	61.8 ± 4.5c	74.6 ± 4.5abc	68.0 ± 4.5bc	78.6 ± 4.5ab
320	86.8 ± 3.0a	59.7 ± 3.0c	64.0 ± 3.0c	77.6 ± 3.0b	81.2 ± 3.0ab
640	87.8 ± 2.2a	60.3 ± 2.2c	68.2 ± 2.2b	85.6 ± 2.2a	85.2 ± 2.2a
800	86.0 ± 3.9a	75.6 ± 3.9b	70.2 ± 3.9b	85.8 ± 3.9a	86.6 ± 3.9a
1000	86.3 ± 2.3a	80.0 ± 2.3a	65.2 ± 2.3b	82.1 ± 2.3a	83.2 ± 2.3a

[a] Data are the mean of four replicates ± standard error. In each row, means with different letters are significantly different ($P < 0.05$).

Table 7. Antioxidant Index of the Essential Oils, α-Tocopherol, BHT, and BHA, in Different Concentrations, Using TBARS Assay with ABAP[a]

oil/substance (mg/L)	antioxidant index (%) with ABAP				
	BHT	BHA	α-tocopherol	*T. capitata*	*O. vulgare*
160	82.1 ± 2.1b	89.8 ± 2.1a	94.6 ± 2.1a	73.1 ± 2.1c	53.2 ± 2.1d
320	84.9 ± 3.1b	91.7 ± 3.1a	95.2 ± 3.1a	69.2 ± 3.1c	66.6 ± 3.1c
640	89.6 ± 2.5a	92.7 ± 2.5a	92.5 ± 2.5a	80.0 ± 2.5b	71.7 ± 2.5c
800	88.3 ± 2.3b	97.0 ± 2.3a	95.2 ± 2.3a	83.2 ± 2.3b	61.8 ± 2.3c
1000	90.4 ± 2.9ab	96.4 ± 2.9a	93.2 ± 2.9a	84.1 ± 2.9b	54.9 ± 2.9c

[a] Data are the mean of four replicates ± standard error. In each row, means with different letters are significantly different ($P < 0.05$).

The method of micellar model system revealed also that all samples possessed the ability of preventing the formation of primary hydroperoxydienes components, mainly *T. capitata* oil, because for this oil the antioxidant index percentage was significantly higher (96%) than that obtained with the remaining tested samples (**Table 8**).

Table 8. Antioxidant Index of the Essential Oils, α-Tocopherol, BHT, and BHA at the Concentration of 160 mg/L in the Presence of ABAP, Using the Micellar Model System[a]

oil/substance (mg/L)	antioxidant index (%) with ABAP by the micellar model system				
	BHT	BHA	α-tocopherol	*T. capitata*	*O. vulgare*
160	83.6 ± 4.0ab	84.4 ± 4.0ab	75.7 ± 4.0b	95.7 ± 4.0a	77.0 ± 4.0b

[a] Data are the mean of four replicates ± standard error. Means with different letters are significantly different ($P < 0.05$).

DISCUSSION

The high relative amount of carvacrol detected in the essential oil of *T. capitata* confirms the previous results obtained by Salgueiro for the same species collected in Portugal (*27*, *28*). According to Salgueiro (*27*) this species shows a great chemical homogeneity characterized by high carvacrol relative amounts (60−64%). Concerning *O. vulgare*, a high percentage of thymol was detected, within the range generally described by some authors for this species (*17*); nevertheless, carvacrol was practically absent. However, it is known that O. *vulgare* shows great variability in its essential oil composition not only due to the existence of different subspecies but also due to the influence of numerous factors, mainly environmental and climatic conditions (*29*).

It seems to be recognized that the use of essential oils in food and other supplies tends to increase due to the global world tendency of "green consumerism", which implies the improvement and use of products obtained from vegetable material (*2*).

The essential oils of *T. capitata* and *O. vulgare* possessed significant activity against the different strains of *L. monocytogenes*. However, these antimicrobial activities were more evident for the oils isolated from *T. capitata*. The MIC values for the essential oil of *T. capitata* and its main component carvacrol were very similar, 0.20 and 0.15 μL/mL, respectively. These results suggest that the antilisterial activity of the essential oil of *T. capitata* is due mainly to its content in carvacrol. It becomes clear that there is a relationship between the high activity of the oils and the presence of phenolic compounds: carvacrol for the *Thymbra* oil and thymol for the *Origanum* oil. The best antimicrobial activity observed for the oils from *Thymbra* can be due to the highest percentage of carvacrol (76%), whereas the thymol reached only 33% in the oil from *Origanum*. The MIC values obtained for carvacrol in our study are in agreement with the results obtained in the study of Pol and Smid (*30*).

The relatedness between antimicrobial activity and phenolic compounds present in some plant essential oils was already observed when using other microorganisms (*24*), in particular *Bacillus cereus* (*31*) and *Helicobacter pylori* (*32*).

Not only did the essential oil of *O. vulgare* show a lower activity in comparison to the *T. capitata* oil, but it is relevant to point out that the achieved activity was similar to that of the antibiotic, indicating the use of oregano essential oil as a powerful tool in the control of this important food pathogen.

Differentiation of *L. monocytogenes* strains by their susceptibility to *T. capitata* or *O. vulgare* essential oils was not recorded. These results are in agreement with the data presented in the work of Mourey and Canillas (*33*). In the study conducted by these researchers different essential oils were used against *L. monocytogenes* strains of serotypes 1/2c and 4b, and no discrimination between the susceptibilities of the two serotypes was evident.

With regard to the antioxidant activity it is accepted that the degree of lipid oxidation should be evaluated by more than one method because it occurs through several steps, originating several types of products. In view of this, in the present work two methods were chosen: (a) the TBARS assay, which measures the extent of lipid degradation by spectrophotometric evaluation, at 532 nm, of the pink pigment produced through reaction of thiobarbituric acid (TBA) with malonaldehyde (MDA), one of the secondary lipid peroxidation products; and (b) the micellar model system, based on the spectrophotometric determination, at 234 nm, of the rate of conjugated diene formation from linoleic acid, that is, the formation of primary components (hydroperoxydienes) of the oxidative process of a lipid. Both *O. vulgare* and *T. capitata* oils demonstrated protective antioxidant ability, being at higher concentrations (640−800 mg/L) as effective as BHT and better than BHA and

α-tocopherol. Only at 1000 mg/L were these two antioxidants able to prevent lipid oxidation as effectively as the essential oils and BHT. The antioxidant activities of the essential oils of *O. vulgare* L. and *T. capitata* were to be expected because their major components were the phenolic compounds thymol and carvacrol, respectively. However, under oxidative process conditions, that is, in the presence of the radical inducer ABAP, the antioxidant ability of the essential of *O. vulgare* decreased and, to a lower extent, so did that of *T. capitata* oil. Some authors (*34*) using the TBARS method with ABAP and testing the antioxidant effectiveness of essential oil components found that carvacrol possessed a higher ability than thymol to prevent oxidation, independently of the concentrations tested (100, 500, and 1000 mg/L). In the assay with ABAP the *O. vulgare* oil was less active as antioxidant agent because antioxidant indices decreased the higher the concentration of the oil was, especially in the scope from 640 to 1000 mg/L. This pattern of behavior was already observed in essential oils of *O. vulgare* and *Artemisia abyssinica* (*35*, *36*), despite their chemical oil compositions. These authors state that several compounds can react with 2-thiobarbituric acid under identical conditions, giving non-malondialdehyde-related colored adducts. Despite the similarity of results found by these authors and those detected by us in the presence of ABAP, the absence of such behavior remains to be explained when in the present work *O. vulgare* oil was tested without ABAP. Beccause with the TBARS assay with ABAP all samples showed, even at the lowest concentration tested, antioxidant effectiveness, the micellar model system was performed at only one concentration (160 mg/L) and in the presence of the radical inducer ABAP. According to the results obtained, it was evident that all samples, including the essential oils, could prevent and reduce the degradation of hydroperoxydienes. Nevertheless, from a comparison of the antioxidant indices (AI%) of the essential oils with both methods, they seem to act preferentially, reducing the formation of hydroperoxydienes, that is, preventing the primary oxidation, because a higher percentage of antioxidant indices was detected by the micellar model system method than by the TBARS method. This was more evident in the *T. capitata* oil mainly constituted by carvacrol, with an antioxidant index of 96%. The presence of available hydrogen atoms from phenol seems to be responsible for the good barrier against the primary oxidative process (*34*).

The imminent increase in the use of essential oils worldwide as antimicrobial agents forces the knowledge on several aspects of their use in food systems, where the efficacy of essential oils may be compromised due to interaction with food components and bacteria such as *L. monocytogenes* and other food pathogens, which possess an adaptation behavior that protects cells against several stress conditions. It is thus crucial to investigate the tolerance response of this bacterium when in the presence of essential oils at sublethal concentrations.

According to the obtained antilisterial and antioxidant activities, *T. capitata* essential oil is the most promising for use in food systems; however, the strong *T. capitata* essential oil aroma may constitute an obstacle to its use. However, a mixture of *O. vulgare* and *T. capitata* essential oils may be considered to overcome this aspect without loss of antibacterial and antioxidant activities.

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Received for review May 2, 2005. Revised manuscript received July 19, 2005. Accepted July 31, 2005.

JF0510079