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Antimicrobial and antioxidant activities of commercially available essential oils and their oleoresins

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Abstract

The objectives of this study were to evaluate and compare *in vitro* antibacterial and antioxidant activities of commercially available oregano, rosemary, sage and thyme essential oils (EOs) against their corresponding oleoresins (ORs) for potential application in food packaging systems. Thyme EO showed the best antimicrobial activity against *Staphylococcus aureus, Bacillus cereus*, *Escherichia coli* and *Pseudomonas fluorescens* (MIC values ranging 0.4±0.1 - 8.7±2.3 mg/mL). Overall, the antimicrobial efficacy of thyme and oregano EOs was found to be higher than that observed for their ORs. Additionally, these ORs did not exhibit better antioxidant properties when compared with their EOs, providing 90% lower antiradical activity and without significant differences in reducing power. The results suggested that commercially available thyme and oregano EOs could be used effectively as hurdle against food-borne pathogens and spoilage bacteria, as well as in terms of preventing lipid oxidation in foods.

Keyworks: *essential oils; oleoresins; antimicrobial agents; antioxidant activity; active packaging*

Introduction

Food spoilage microorganisms can result in a steady reduction in product quality. Food-borne bacterial pathogens are considered of great concern in terms of public health. Consequently, microbial contamination of food can result in food safety risks and a reduction in product quality and shelf-life; all of this leading to significant economic losses incurred by the food and beverage industries.

Essential oils (obtained by distillation; EOs) and their extracts (oleoresins produced from extraction with solvents; ORs) are natural phytochemicals possessing unique properties which can be exploited within various food processing and food packaging (active systems) applications (1). In terms of the ever growing restrictions around the use of synthetic food preservatives, there is a significant requirement for the availability of natural food extracts which possess pleasant sensory properties and appropriate preservative action (both in terms of biological and chemical control) and which are acceptable to the retailing market and the consumer (2). An important number of plant extracts, which might find application in foods, have been commonly consumed by humans without health adverse effects (3).

The antimicrobial compounds present in spices and herbs are mostly contained within the EO fraction. Therefore, EOs and ORs produced using different solvents have been shown to have activity against bacteria, fungi and viruses, thereby demonstrating their hurdle capacity during food manufacture (1). Oxidation of lipids in foods leads to rancidity, product shelf-life reduction and presents a unique category of public health concerns; namely, the presence of free-radicals and other chemical-based undesirables in foods. Metabolites originated from oxidized lipids are known to undesirably influence human health and every effort should be employed to decrease human interaction with these substances (4). Consequently, natural antioxidant sources are continuously being sought to counteract oxidation reactions in foods and to replace synthetic forms.

Limited research has been conducted to date which compares the antimicrobial and antioxidant activities of EOs and ORs using assessment methodologies that are directly comparable (5, 6, 7).

The objectives of this study were to examine the *in vitro* antimicrobial activity of commercially available EOs and ORs of oregano, rosemary, sage and thyme against common bacterial food spoilage and food pathogen strains of *Staphylococcus aureus, Bacillus cereus*, *Escherichia coli* and *Pseudomonas fluorescens,* and to determine their antioxidant activity.

Materials and Methods

Essential oils and oleoresins supply

The EOs of oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) were obtained by steam distillation and their oleoresins extracted using hexane in accordance with internal quality control protocols of the supplier. The EOs and their ORs, considered flavouring preparation as defined by Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods, were provided by National Food Ingredients LTD (Limerick, Ireland). Commercial specifications of the natural products are showed in Tables 1 and 2. The EOs and ORs were stored at 4 ºC in their commercial screw-cap aluminium containers until required for use.

79 **Table 1.- Product specification of the commercial essential oils used.**

Product	Description	Appearence	Physical constants at 20°C	GC[(1)] analysis	Legislative status
Thyme oil red	The volatile oil obtained by steam distillation of common thyme (*Thymus vulgaris* L.)	Red/brown liquid	D[(2)]=0.9150 - 0.9350 RI[(3)]= 1.490 - 1.505 OR[(4)]= -5.0 - 1.0 deg	Conforms to an approved standard Total phenols by GC: 45- 60% area	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 100%
Rosemary oil	The volatile oil obtained by steam distillation of the twigs and flowering tops of the rosemary plant (*Rosemarinus officinalis* L.)	Pale yellow liquid	D=0.8950 - 0.9200 RI=1.464 - 1.476 OR=-5.0 - 10.0 deg	Conforms to an approved standard	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 100%
Sage oil dalmatian	The volatile oil obtained by steam distillation of the dried leaves of Dalmatian sage (*Salvia officinalis* L.)	Pale amber liquid	D=0.9100 - 0.9300 RI=1.465 - 1.475 OR=2.0 - 29.0 deg	Conforms to an approved standard Thujones by GC: 27-33% area	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 100% Contain Thujone (Natural extracts): 33%
Origanum oil	The volatile oil obtained by steam distillation of dried origanum herb (*Origanum vulgare L.)*	Yellow/ brown liquid	D= 0.9300 - 0.9640 RI= 1.502 - 1.510 OR= -5.0 - 5.0 deg	Conforms to an approved standard Total phenols by GC: 60-75% area	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 100%

80 (1) GC, gas chromatography.
81 (2) D, density.
82 (3) RI, refractive index.
83 (4) OR, optical rotation.

84 **Table 2.- Product specification of the commercial oleoresins used.**

Product	Description	Appearence	Volatile oil content (v/w %)	Solvent residue	GC[(1)] analysis	Legislative status
Thyme oleoresin	A natural flavouring obtained by hexane extraction & distillation of thyme (*Thymus vulgaris* L.)	Dark green viscous liquid	20.00 - 27.00	Hexane residues from extraction: ≤25 ppm	Conforms to an approved standard	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 58-62%
Rosemary oleoresin	A natural flavouring obtained by hexane extraction & distillation of rosemary herb (*Rosemarinus officinalis* L.)	Green liquid	≤0.10	Hexane residues from extraction: ≤25 ppm	N.P. [(2)]	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 27%
Sage oleoresin	A natural flavouring obtained by hexane extraction & distillation of sage herb (*Salvia officinalis* L.)	Dark green paste	5.00 - 10.00	Hexane residues from extraction: ≤ 25 ppm	Conforms to an approved standard Thujones by GC: 0.1 - 1 % area	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 50% Contains Thujone (Natural extracts): 1%
Oregano oleoresin	A natural product obtained by hexane extraction & distillation of oregano (*Origanum* spp.) standardised for volatile oil content and flavour.	Green/ brown liquid	18.00 - 25.00	N.P	Conforms to an approved standard	Flavouring preparation as defined by Regulation (EC) N° 1334/2008 Contains flavouring preparations: 46.8%

85 **(1)** GC, gas chromatography.
86 **(2)** N.P., not provided.

Bacterial cultures

The antimicrobial activity of the EOs and ORs were evaluated against both Gram-positive and Gram-negative bacteria relevant to food spoilage and food safety. Bacteria used in the present study were; *S. aureus* (NCIMB 13062), *B. cereus* (NCIMB 9373), *E. coli* (NCIMB 9132) and *P. fluorescens* (NCIMB 9046).

Bacterial cultures were grown, and assisted through shaking (170 rpm) using a shaker table, in Mueller-Hinton broth (MH; Merck, Darmstadt, Germany) at 37 ºC (*S. aureus* and *E. coli*) or 30 ºC (*P. fluorescens* and *B. cereus*) for 18 hr. These overnight cultures were diluted as required using sterile MH broth to give a working concentration in the range of $5x10^5$-$1x10^6$ cfu/mL. The whole experiment was repeated three times from broths of new bacterial cultures.

Disc agar diffusion method

The antimicrobial activity of the studied EOs and ORs were qualitatively assessed using the disc agar diffusion method following the recommendations of the Clinical and Laboratory Standards Institute (8). A volume of 100 µL of the diluted bacterial cultures was thoroughly spread onto MH agar. Subsequently, sterile filter paper discs (6 mm in diameter; Whatman International Ltd, Maidstone, UK) were aseptically impregnated with 15 µL serially diluted emulsions (0.25-25 mg/mL) of each EO or OR. Emulsions were formed by emulsifying the EOs or the ORs with a sterile mixture consisting 10% dimethyl sulphoxide (DMSO; Sigma Aldrich Ireland Ltd, Dublin, Ireland) and 0.5% Tween-20 solution (Sigma Aldrich), and homogenised using sterile "Ultra Turrax Tube Dispenser" equipment (IKA, Staufen, Germany). The discs, impregnated with EO or OR, were transferred to the bacterial seeded agar plates. Discs containing 10 µg of streptomycin (Oxoid, Basingstoke, UK) and 15 µL of DMSO-Tween-20 mixture were used as positive and negative controls, respectively. The seeded agar plates containing the discs were stored at 4 ºC for 2 h before incubation at 37 ºC (*S. aureus* and *E. coli*) or 30 ºC (*P. fluorescens* and *B. cereus*). Inhibition halos surrounding discs containing EOs, ORs or antibiotic, after an incubation period of 18 h, were measured using a digital calliper.

In order to evaluate whether the activity of the EOs or ORs was bacteriostatic or bactericidal, a piece of agar (6 mm in diameter) from the inhibition zones were aseptically transferred to tubes containing sterile Tryptone-Soya broth enriched with 0.5% yeast extract (Merck). These broths were incubated at 37 ºC (*S. aureus* and *E. coli*) and 30 ºC (*P. fluorescens* and *B. cereus*) for up to 4 days. Tubes with no growth (no turbidity observed) were considered to have a bactericidal effect.

Minimum inhibitory concentration (broth dilution method)

The antimicrobial activity of EOs and their ORs was evaluated using a modified broth dilution method (7) and following the recommendations of the CLSI (9). Serially-diluted aliquots of each natural substance emulsified in DMSO-Tween 20 (0.1 mL), as described above and ranging from 0.25 to 25.00 mg/mL, were added into microtubes containing 0.9 mL bacterial culture. In parallel, a growth control (without the presence of EOs or ORs) and a sterility control (without bacterial culture) were prepared. All microtubes were incubated under the same conditions as employed in the disc diffusion experiment. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of substance producing no bacterial pellet (inhibited growth).

In vitro evaluation of the antioxidant properties

Different concentrations of each EO or OR were prepared in methanol ranging from 200 µL/mL to 0.5 µL/mL. Absorbance was spectrophotometrically measured using a Cary 300 UV-Visible Spectrophotometer.

For the scavenging and reducing activities, a negative control, without the sample, and a positive control, with Butylated hydroxyanisole (BHA) solution (0.25-10 mg/mL) instead of the sample, was used. A negative control, with all components but not including the sample, and a positive control with $Na_2EDTA\ 2H_2O$ (0.001-0.100 mg/mL) were carried out for all determinations of chelating activity.

Chelating effect

The chelating power of EOs and ORs was spectrophotometrically determined following the method described by Decker and Welch (10) by measuring the competition with ferrozine for ferrous ion. Briefly, the reaction mixture contained 1 mL of different concentration dilutions of EO or OR samples in methanol, 1 mL $FeSO_4$ (0.125 mM) and 1 mL Ferrozine (0.3125 mM). The reaction mixture was vortexed and left standing for 10 min in the dark and the absorbance measured at 562 nm against a blank (methanol). For all ORs, the spectrophotometric measurements were carried out after centrifugation at 9630g for 5 min at room temperature (Beckman model J2-21).

The Fe^{2+}-chelating activity was calculated as percentage of inhibition (I) of the complex by the following equation: $I\ (\%) = (A_c - A_n)/A_c \times 100$, where A_c and A_n are absorbances for control and dilution samples, respectively. The concentration of EOs or ORs providing 50% inhibition of iron-ferrozine complex (IC_{50}) was calculated by graphing the percentage of inhibition against EO or OR concentration. The lower the IC_{50} value, the better the substance in terms of radical chelation.

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The free radical activity of the EOs or ORs was determined using DPPH as a free radical according to Brand-Williams et al. (11) with minor changes. For each substance, volumes of 50 µL of EOs or ORs of different concentration dilutions in methanol were added to 5 mL of a 0.1 mM methanol DPPH solution. The mixture was left to stand for 30 min in the dark at room temperature before reading their decrease in absorbance at 517 nm against a blank (methanol). All determinations were carried out in triplicate. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the following equation: $RSA\ (\%) = (A_{DPPH} - A_n)/A_{DPPH} \times 100$, where A_{DPPH} is the absorbance of the DPPH solution and A_n is the absorbance of the solution when the EO or OR sample were added at a particular concentration. The EO or OR concentration providing 50% of radical scavenging activity (IC_{50}) was calculated from the graph of RSA percentage against EO or OR concentration. In the DPPH assay, the lower the IC_{50} value, the better the ability to scavenge radicals.

Reducing power

The reducing power of the EO or OR was measured following the procedure described by Oyaizu (12). Briefly, different concentrations of EOs and their ORs (1 mL) were mixed with 0.2 M phosphate buffer (pH 6.6; 2.5 mL) and potassium ferricyanide (1%; 2.5 mL). The mixture was incubated at 50 ºC for 20 min and trichloroacetic acid (10%, 2.5 mL) was added and the mixture centrifuged at 800g for 10 min. An aliquot of upper clear solution (2.5 mL) was taken and transferred to a tube with 0.5 mL $FeCl_3$ (1%) and 2.5 mL distilled water, and the absorbance was measured at 700 nm. From measurements taken from three independent experiments, the mean absorbance at 700 nm was calculated. According to Le et al. (13), the reducing power was defined as the concentration of EO or OR (µL/mL) that produced 0.5 absorbance units (linear regression method).

Statistical analysis

Data were collected from three independet experiments and each measurement was carried out in duplicate (three experiments x two samples). All determined data were statistically analysed (means and standard deviations) and the differences among mean values were compared through “Student t-test” (Statistica release 7.1, Statsoft Inc., USA).

Results and discussion

Antimicrobial activity

The antimicrobial activity associated with the EOs and their related ORs studied is displayed in Table 3. Based on data from the screening method employing disc agar diffusion, the highest inhibition zones were found for thyme EO and the effects of the inhibition were determined to be bactericidal. In this case, the observed inhibition zones against Gram-negative bacteria (*E. coli*, 27.37±4.49 mm, and *P. fluorescens*, 15.56±1.39 mm) and Gram-positive bacteria (*S. aureus*, 49.58±0.85 mm, and *B. cereus* , 53.03±0.16 mm) were found to be different ($p<0.05$) when compared to experimental controls. This effect (bactericidal) was also shown for oregano EO. Considering the MIC values determined from the four EOs, thyme also induced the strongest growth inhibition against the selected spoilage and pathogenic test microorganisms. Both *S. aureus* and *B. cereus* were inhibited with minimum concentrations of 5.0±0.1 and 0.4±0.1 mg thyme EO/mL broth, respectively, whereas the calculated MIC for *E. coli* and *P. fluorescens* was 8.7±2.3 and 3.1±1.3 mg thyme EO/mL broth, respectively. The latter values were not significantly different to those determined when testing oregano EO against these same Gram-negative bacteria (8.8±2.5 and 3.8±1.4 mg oregano EO/mL broth, respectively). Compared to the antimicrobial activity of thyme and oregano EOs, sage and rosemary EOs generally produced lower antimicrobial effects, with both having higher MIC values and reduced cellular injury. From the growth inhibition data generated, through the use of the broth dilution method, no significant differences ($p>0.05$) in susceptibility were determined between Gram-positive and Gram-negative bacteria, with the exception of that determined when thyme EO was assessed.

In vitro studies have reported that EOs inhibited both spoilage and pathogenic bacteria at concentrations between 0.2 and 10.0 mg/mL (14). This wide variability is not only caused by the type of substance being assessed and selected microorganism chosen for evaluation but by other factors that can affect the final determination such as the method used to obtain the EO, the growth phase of the microorganism in question and the culture media chosen for microbial growth. The determined antimicrobial activities of the EOs tested in this study (ranging 0.4-8.7 mg EO/mL broth) compared well with concentrations reported previously (1, 14, 15, 16, 17, 18). Moreover, it is assumed that Gram-negative microorganisms are slightly less susceptible than Gram-positive bacteria to EOs, but data presented in the scientific literature vary widely (1, 14, 15). In this study, we determined that susceptibility was dependent upon the test method utilised and the type of EO being assessed. Hence, comparing MIC values for Gram-positive and Gram-negative bacteria for all EOs assessed did not show a clear trend since *B. cereus* and *P. fluorescens* were determined to be the most sensitive bacteria. However, inhibition halos were larger ($p<0.05$) against Gram-positive bacteria than those observed for the studied Gram-negative microorganisms.

222 **Table 3.- Antimicrobial activity of the essential oils and the oleoresins obtained from thyme, oregano, sage and rosemary against strains**
223 **of *S. aureus*, *B. cereus*, *E. coli* and *P. fluorescens*.**

	S. aureus			*B.cereus*			*E. coli*			*P. fluorescens*		
	MIC[1]	$Ø_{inh}$[2]	Bacterial effect [3]	MIC	$Ø_{inh}$	Bacterial effect	MIC	$Ø_{inh}$	Bacterial effect	MIC	$Ø_{inh}$	Bacterial effect
Essential oils (EO)												
Thyme EO	5.0±0.0	49.6±0.8	1	0.4±0.1	53.0±0.1	1	8.7±2.3	27.3±4.4	1	3.1±1.3	15.5±1.3	1
Oregano EO	8.3±2.9	46.1±4.5	1	0.8±0.3	43.2±4.3	1	8.8±2.5	27.3±1.0	1	3.8±1.4	13.5±0.3	1
Sage EO	5.6±1.3	20.2±1.6	0	6.7±2.9	20.0±1.5	1	16.7±5.8	8.1±0.6	1	4.4±1.3	6.0±0.0	Nd
Rosemary EO	15.0±5.0	12.5±1.0	0	1.5±0.9	13.5±1.5	1	15.0±5.0	8.4±0.6	0	13.3±2.9	7.1±0.8	0
Oleoresins (OR)												
Thyme OR	18.8±2.5	13.6±2.0	0	9.4±1.3	12.0±1.6	1	23.3±2.9	7.7±0.5	0	20.0±5.0	6.0±0.0	Nd
Oregano OR	4.4±1.3	25.7±3.7	0	3.3±1.4	20.6±3.6	1	12.5±2.9	12.3±1.5	1	5.8±3.8	6.0±0.0	0
Sage OR	18.3±2.9	14.6±0.6	1	7.5±2.5	16.9±0.6	1	>25	6.0±0.0	Nd	>20	6.0±0.0	Nd
Rosemary OR	23.3±2.	11.1±0.7	0	1.8±0.9	14.3±0.6	1	>25	6.0±0.0	Nd	22.5±3.5	6.0±0.0	Nd
Streptomycin	Nd[4]	21.5±0.6	Nd	Nd	25.9±1.4	Nd	Nd	23.4±2.5	Nd	Nd	16.6±0.7	Nd

224 [1] MIC, minimum inhibitory concentration (mg/mL) ± standard deviation.
225 [2] $Ø_{inh}$, zone of inhibition (mm) ± standard deviation, including the disc diameter (6 mm).
226 [3] Bacterial effect: 0, bacteriostatic; 1, bactericidal.
227 [4] Nd, not determined.

Among the four ORs studied, discs impregnated with oregano-derived OR provided the largest inhibitions halos, which were similar or higher than those measured for 10 µg streptomycin discs. Hence, oregano-derived OR demonstrated strong antimicrobial activity against *S. aureus, E. coli*, *B. cereus* and *P. fluorescens* reaching MIC values of 4.4±1.3, 12.5±2.9, 3.3±1.4 and 5.8±3.8 mg oregano OR/mL broth, respectively. Only the activity of rosemary OR extract (1.8±0.9 mg/mL) against *B. cereus* was stronger than that produced by oregano OR. Overall, Gram-negative bacteria were more resistant to ORs, especially *P. fluorescens*.

A clearer trend was observed for ORs than for EOs when compared the antimicrobial activity according to the Gram stain. Gram-positive bacteria were more sensitive to ORs than Gram-negative bacteria by testing both the broth dilution and disc agar diffusion methods. This difference may be explained by the diffusion of volatile compounds, mainly from EOs, within the test media and by their penetration of bacterial cells; this being made more difficult by the presence of the outer cell membrane of Gram-negative microorganisms (1).

In this study, commercial forms of thyme- and oregano-derived EOs showed the best results in terms of their preservative properties. The antibacterial activity associated with these oils has been attributed to the presence of a number of active constituents. It has been proposed that EOs possessing a powerful antibacterial activity, such as those from oregano or thyme, contain a high percentage of carvacrol and/or thymol (14, 19, 20, 21). Thymol and carvacrol cause disruption of the cellular membrane and are related to the strong antimicrobial properties associated with thyme and oregano-derived EOs.

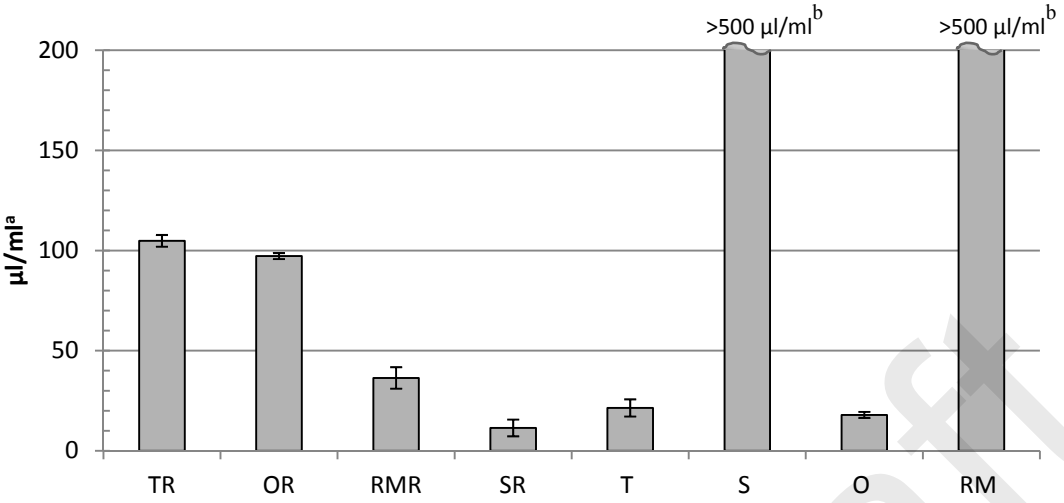
When comparing the antimicrobial activity of EOs and their ORs, ORs possessed a more diminished activity since MIC values were higher (ranging 1.8- >25 mg/mL), inhibition diameters were shorter (ranging 6.0-25.7 mm) and bactericidal effects were only detected for *E. coli* and *B. cereus* (Table 3). Most noteworthy, thyme-derived EO was more effective ($p<0.05$) against all tested bacteria than thyme-derived OR. As an exception, the inhibition of *S. aureus* was stronger with oregano-derived OR than that observed for oregano-derived EO as determined by the significantly ($p<0.05$) lower MIC values. Intrinsic characteristics of this type strain of *S. aureus* might explain this particular antimicrobial activity of oregano- derived OR as compared with their EO.Response to stress factors can widely vary with strain, particularly of *S. aureus* (22). This response would be influenced by the chemical composition of the both oregano-derived oils since their antimicrobial activity depends mainly on carvacrol and thymol concentration (23). Despite many studies have concluded that some plant extracts show stronger antioxidant activity than their essential oils (24, 25, 26, 27), to the best of our knowledge a similar conclusion regarding to their antimicrobial activity has not been established.

Antioxidant activity

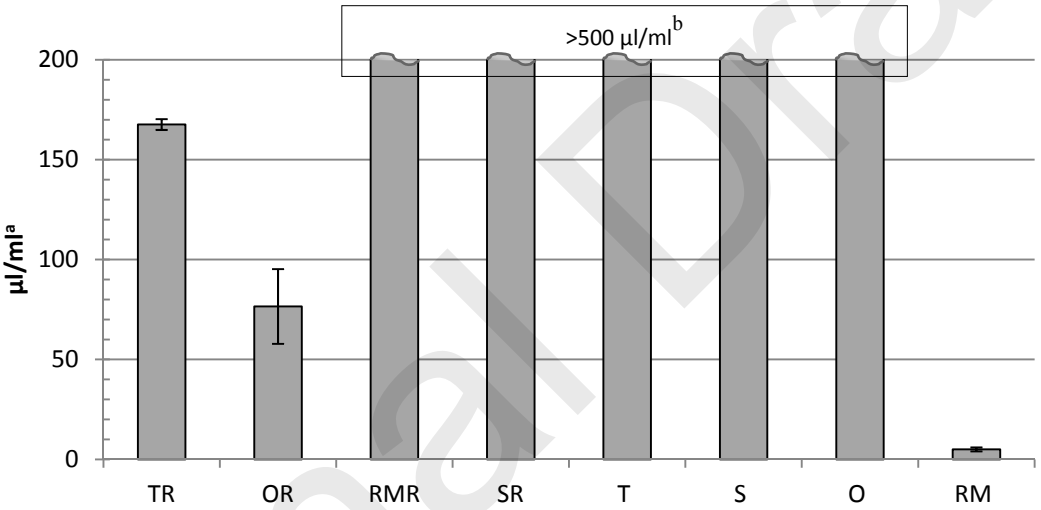
The antioxidant potential of both EOs and ORs assessed in this study varied widely with test method (Figures 1 and 2). Reduction and free radical scavenging activities generally suggest primary antioxidant properties. These methods indicated that the maximum primary antioxidant activity was provided by the EOs derived from thyme and oregano and by the OR derived from sage. The ferric reducing activity, measured by the ability of the tested substance (thyme or oregano) to donate an electron to Fe (III) was significantly ($p<0.05$) stronger than that determined for the remaining EOs and all of the ORs assessed in this study. The concentrations of the four ORs producing 0.5 absorbance units, indicative of reducing activity, were not statistically different from each other and ranged 4.5-9.0 µL OR/mL broth. The reducing power of sage-derived EO (15.9±6.0 µL/mL) was weak and rosemary-derived EO failed to demonstrate this activity in concentrations below 500 µL/mL.

Figure 1. Scavenging (a) and chelating (b) activity of thyme, oregano, rosemary and sage oleoresins and their corresponding essential oils.

(a)

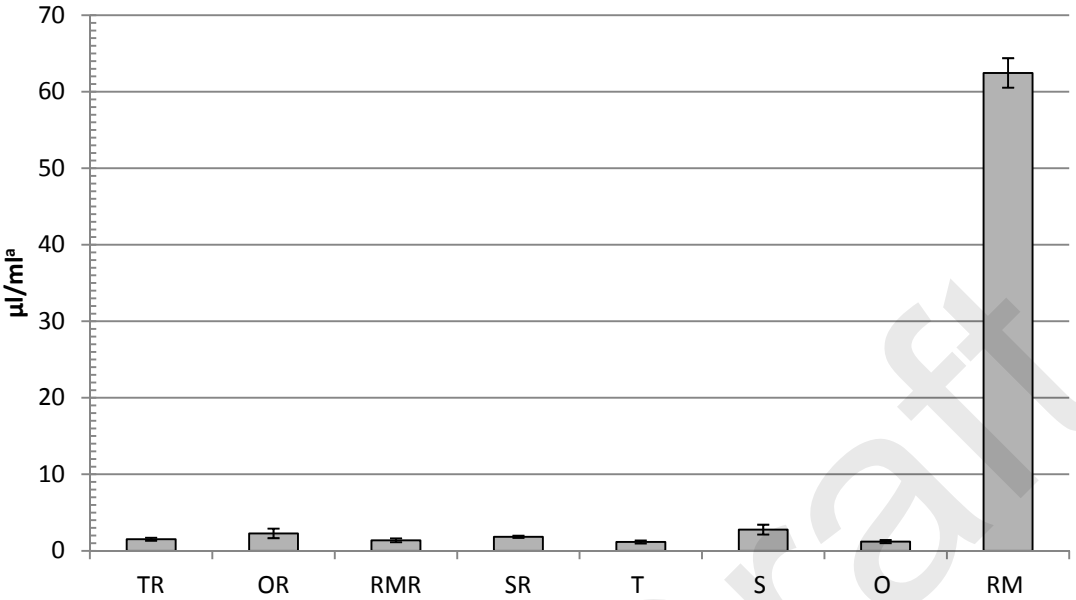


(b)



Thyme (TR), oregano (OR), rosemary (RMR) and sage (SR) oleoresins.
Thyme (T), oregano (O), rosemary (RM) and sage (S) essential oils.
Plotted columns display mean ± standard deviation (n=3).
[a]Concentration of natural substances providing 50% of maximal scavenging/chelating effect (IC_{50}).
[b]Concentrations of 500 µl/ml showed no antioxidant activity.

Figure 2. Reducing activity of thyme, oregano, rosemary and sage oleoresins and their corresponding essential oils.



Thyme (TR), oregano (OR), rosemary (RMR) and sage (SR) oleoresins.
Thyme (T), oregano (O), rosemary (RM) and sage (S) essential oils.
Plotted columns display mean values of duplicate determinations from three independent experiments ± standard deviation.
[a]Concentration of natural substances producing 0.5 absorbance units (λ_{700nm}).

Calculated IC_{50} values from the DPPH assay, as a measurement of the ability of a substance to donate hydrogen to the DPPH radical, for sage (11.4±4.2 µL/mL), oregano (17.9±0.5 µL/mL) and thyme (21.4±4.3 µL/mL) EOs were much lower ($p<0.05$) when compared to all other substances assessed. This results indicated a higher antioxidant activity. Figure 1 shows that all studied ORs had scavenging activity whereas for EOs, sage and rosemary did not show scavenging activity up to a concentration of 500 µL/mg.

The secondary antioxidant properties of the tested oils, generally estimated by assessing chelating activity, was not observed (<500 µL/mL) in all tested substances, with the exception of rosemary-derived EO and oregano- and thyme-derived ORs, whose concentrations, which provided a 50% of the maximum chelating effect (IC_{50}), was determined to be 2.3±0.6 µL/mg, 76.6±18.7 µL/mg and 167.6±2.7 µL/mg, respectively.

Many studies have reported that some plant extracts showed stronger antioxidant activity than their EOs (3, 24, 25, 26, 27, 28). Tanabe et al. (29) assessed the antioxidant activity of 22 herbs extracts, such as oregano, sage, thyme, cinnamon and basil, and observed that lipid oxidation was prevented by all the extracts tested. However, results obtained in this work would indicate that thyme and oregano EOs exhibited a powerful antioxidant activity even higher than their corresponding ORs. This observation was supported in terms of their reducing power, since no significant differences were found, and their antiradical scavenging activity through determined IC_{50} values of thyme and oregano EOs, which were 90% lower when compared to their corresponding ORs. Those EOs significantly inhibited the growth of all tested bacteria. Therefore, it can be concluded that EOs derived from thyme and oregano were more effective in terms of inhibiting bacterial growth and preventing lipid oxidation than thyme- and oregano-derived ORs. This study presents rather unusual results in that EOs

described here are bi-functional in terms of their preservation properties and this phenomenon has rarely been presented in the scientific literature to date. Cao et al. (30) concluded that the essential oil of *Mosla chinensis* possessed both antimicrobial and antioxidant properties. No such trend was observed for sage and rosemary in this study as results indicated that their EOs possessed higher antibacterial activities, whereas their ORs demonstrated greater antioxidant potential. These observations are more in agreement with what has been reported for herbal extracts by other researchers (7, 28). These functional properties of the EOs and ORs may be due to the terpenes and phenolic contents that can act as the principal contributors of the antioxidant and antimicrobial power of the substances tested (31).

Gutierrez et al. (18) recommended that oregano, used alone, or combined with thyme, could be considered as a potential alternative for control of both pathogens and spoilage microbiota in foods. Our results would support this finding, but would suggest that EOs derived from the same herbs could also be used for antioxidant purposes in processed food systems. Negi (3) reported that many plant extracts, mainly essential oils, possess antimicrobial activity against a wide range of bacteria while herbs and spices, mainly with high content of phenolic compounds, are highlighted by their interesting antioxidant activity. The bi-functional roles played by both oregano and thyme EOs highlighted in our study identify them as unusual but relevant alternatives to synthetic ingredients which only possess mono-functional properties.

Conclusions

Overall, the results of this study showed that thyme EOs were the most effective substances against *P. fluorescens*, *E. coli*, *B. cereus* and *S. aureus*, with oregano EO also exhibiting an interesting antimicrobial activity. The studied EOs possessed better antimicrobial properties when compared with their corresponding ORs except for oregano EO acting against *S. aureus* as determinated by the higher MIC value. Additionally, thyme and oregano EOs demonstrated potent antioxidant activity when compared against their respective ORs; possessing antiradical activity to DPPH and ferric reducing power. Among the ORs, only sage and thyme exhibited comparable scavenging and ferrous reducing activities.

Overall, our results suggest that Gram-positive bacteria were more sensitive to ORs than Gram-negative bacteria. However, this trend was not observed when EOs were evaluated.

Hence, thyme and oregano EOs showed great potential for use as highly functional and natural substances, in terms of their potential ability to provide both antioxidant and antimicrobial activities in food processing or food packaging applications.

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