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ORIGINAL ARTICLE

Essential oils as antibacterial agents against food-borne pathogens: Are they really as useful as they are claimed to be?

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Abstract Most studies evaluating the use of essential oils (EO) as antibacterial agents focus mainly on minimal inhibitory concentrations (MIC) rather than minimal bactericidal concentrations (MBC). In this work, we compared MICs and MBCs of EO from condiment plants commonly used in Mediterranean Europe, namely *Origanum vulgare*, *Salvia lavandulaefolia*, *Salvia officinalis*, *Salvia sclarea* and *Rosmarinus officinalis*, aiming to evaluate their application as disinfecting agents in minimally processed produce. Outbreaks-related pathogens such as *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Yarrowia lipolytica* were used. Results showed that all EO were able to reduce bacterial growth in all bacterial strains tested, particularly *O. vulgare*. However, fewer EO exhibited bactericidal activities, and were only effective against one or two bacterial strains, hence eliminating the possibility to use them as broad range disinfectants. Furthermore, the necessary concentrations were too high for food application. Hence, our work suggests the need to evaluate MBC rather than MIC and questions EO usefulness in controlling undesired microorganisms. Overall, and despite the large volume of data published on EO, results obtained were not very encouraging for a realistic application on produce and question the viability of EOs as disinfecting agents in food.

Keywords Disinfection · Essential oils · Food safety · Pathogens · Vegetables · Spoilage

Introduction

Foodborne diseases of microbiological origin constitute a major food safety concern, posing an ever growing problem on public health. One of the main factors aiding to this situation is the high consumption of "ready to eat" minimally processed (MP) fresh-cut fruit and vegetables (Rojas-Graü et al. 2011), because they are naturally contaminated with microorganisms (Karagözlü et al. 2011). Currently, outbreaks attributed to the consumption of MP produce have been dramatically increasing around the world, involving thousands of people, many of which end up dying (Callejón et al. 2015). Several studies have isolated pathogens from MP lettuce including *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, *Shigella* spp. and *Campylobacter* spp. (Karagözlü et al. 2011; Santos 2009; Santos et al. 2012). In Portugal, Santos (2009) detected *Enterobacteriaceae* at a level of 5.44 log cfu g^{-1} in MP salads, were the genera *Erwinia* spp., *Pantoa* spp., *Enterobacter* spp., *Klebsiella* spp. were present. In the same work, *Citrobacter freundii*, *Leclercia adecarboxylata* and *Hafnia alvei* were also identified, as well as *Aeromonas* and *Pseudomonas*. It is important to note that MP vegetables are foodstuffs which have not gone through any step to ensure the absence of any health risk associated with its consumption, since they were not subjected to treatments to ensure safe levels of pathogens,

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spores or toxins. Thus, disinfection is a critical step on food safety warranty in MP produce and needs to be seriously addressed.

At the industrial level, in most countries, disinfection with chlorine-based products is the only step which allows pathogen destruction and promotes food safety (Gil et al. 2009). However, there is a growing concern about the environmental and health risks associated to chlorine agent's conversion into carcinogenic toxic derivatives, such as trihalomethanes and chloramines, which already face restrictions in their uses (Ölmez and Kretzschmar 2009). On the other hand, the increasing demand for "natural" and "environmentally friendly" products has led to a need to replace chemical disinfectants and additives in the food industry, aiming for new food preservatives, which may be effective and safe, whilst reducing microbial loads and avoiding food allergies and/or intolerances.

Essential oils (EOs) are liquid aromatic products extracted from aromatic plants (*Lamiaceae*), which are soluble in lipids and in organic solvents. The benefits of OEs for therapeutic purposes have been suggested since immemorial times, but it was only in recent years that studies have arisen reporting the EO-induced inhibition of pathogenic bacteria and the shelf-life increase of processed food products (Burt 2004; Kotzekidou et al. 2008; Oussalah et al. 2006; Rožman and Jeršek 2009), as well as their use in edible coatings in fresh produce and fruits (Azevedo et al. 2014; De Martino et al. 2009; Guerreiro et al. 2015).

Although they have been the subject of many works, most studies on EO focus on MIC determinations, rather than MBCs. However, the latter are much more important in food safety and industrial-scale sanitizers, where the complete elimination of food-borne pathogens from processed fruits and vegetables is required. A PubMed search for research in antibacterial activities of EOs applied to food products, such as meat, fresh fruit and lettuce, shows approximately 600 papers, all with MIC determinations; however, MBC determinations were performed in less than 10 reports. As MBCs are usually higher than MICs, it is important to take this into consideration when selecting EO for this purpose, because of their strong odour and potential toxicity as it was demonstrated for more than 200 nl/mL of *Salvia officinalis* L. (Lima et al. 2004). Under this context, the objective of this study was to evaluate EOs from plants normally used as condiments in Mediterranean Europe (*Origanum vulgare*, *Salvia lavandulaefolia*, *Salvia officinalis*, *Salvia sclarea* and *Rosmarinus officinalis*), and test their antibacterial activity against the most significant pathogenic and food-spoilage microorganisms that have been identified in outbreaks linked to MP salads (Santos et al. 2012), namely *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Yarrowia lipolytica*. Both MICs and MBCs were determined and compared, aiming to design a possible EO-based disinfection/sanitation realistic strategy to use in MP vegetables.

Materials and methods

Bacterial strains and preparation of cultures

The strains used in this assay were *Listeria monocytogenes* NCTC 11994 (serotype 4b), *L. monocytogenes* CP6 (PFGE type 11), *L. monocytogenes* M12 (PFGE type 3), *Pseudomonas aeruginosa* P2, *P. aeruginosa* P6, *Yarrowia lipolytica* CBS 6659, *Y. lipolytica* ISA 1668 and *Y. lipolytica* ISA 1708. For bacterial growth, trypticase soy agar medium (Biokar Diagnostics, Beauvais, France) was used, supplemented with 0.6% (w/v) yeast extract (TSA-YE) (Oxoid, Hampshire, United Kingdom), incubated at 37 ± 1 °C during 24 ± 2 h. Yeast strains were cultivated on glucose yeast peptone agar (GYP-A): 5 g L^{-1} yeast extract (Oxoid, Hampshire, United Kingdom), 5 g L^{-1} meat peptic peptone (Biokar Diagnostics, Beauvais, France), supplemented with 2 g L^{-1} glucose (COPAM, Portugal), and 20 g L^{-1} agar–agar (Dário Correia, Portugal). Incubation was performed at 25 ± 1 °C during 48 ± 2 h. Serial dilutions of cultures were prepared using Ringer Solution (Biokar Diagnostics, Beauvais, France) for inoculum evaluations.

For MIC assays, trypticase soy broth (Biokar Diagnostics, Beauvais, France), supplemented with 6 g L^{-1} yeast extract (Oxoid, Hampshire, United Kingdom) containing 0.8% (v/v) Tween 80 (TSB-YE-T) (Difco, Becton, Dickinson and Company, Sparks, United States of America) was used for bacteria and glucose yeast peptone broth (as referred above without agar) also containing 0.8% (v/v) Tween 80 (GYP-T) for yeasts.

Essential oils

EOs derived from the following plants were used: *O. vulgare*, *S. lavandulaefolia*, *S. officinalis*, *S. sclarea* and *R. officinalis.* The concentrated extracts were provided by the company Polarome International, United States of America and were produced by distillation.

Minimum inhibitory concentration and minimum bactericidal concentration determinations

The determination of both MIC and MBC were performed as previous described, with modification for the media used (Bouhdid et al. 2010; Cosentino et al. 1999). Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the oils and their components were determined using a broth microdilution method.

Table 1 MIC determinations of strains and essential oils tested

Microorganisms	Essential oil source				
	Origanum vulgare	*Salvia sclarea*	*Salvia lavandulaefolia*	*Salvia officinalis*	*Rosmarinus officinalis*
L. monocytogenes NCTC 11994	0.03[a]	225.0[b]	28.13[c]	112.5[d]	28.13[c]
L. monocytogenes CP6	0.06[a]	225.0[b]	14.06[c]	450.0[d]	14.06[c]
L. monocytogenes M12	0.03[a]	225.0[b]	28.13[c]	450.0[d]	7.03[e]
P. aeruginosa P2	0.06[a]	900.0[b]	112.5[c]	225.0[d]	28.13[e]
P. aeruginosa P6	0.06[a]	225.0[b]	112.5[c]	225.0[b]	450.0[d]
Y. lipolytica ISA 1668	0.11[a]	225.0[b]	7.03[c]	28.13[d]	28.13[d]
Y. lipolytica ISA 1708	1.76[a]	112.5[b]	3.52[c]	28.13[d]	56.30[e]
Y. lipolytica CBS 6659	0.06[a]	11.25[b]	112.5[c]	7.03[d]	14.06[b]

Results presented reflect the average of three different replicates, expressed in μg mL^{-1} (w/v) of oil in growth media. A different letter in the same row represents significant differences ($P < 0.001$) between the different tested EO, within the same microbial strain

Overnight broth cultures for each microbial strain, prepared in TSB or GYP, were prepared and cell numbers were evaluated by OD_{600} readings and respective calibration curves. Serial doubling dilutions of each oil or component were performed in a 96-well microtiter plate (Orange Scientific, Belgium) over the range of EO concentrations ranging from 900 to 0.03 μg mL^{-1}. Briefly, 50 μL of medium was added to each well, then, concentrated EO samples were added to the first well and serially diluted 1:2 to each adjacent well, up to 10 dilutions. Then 50 μL of the overnight broth cultures for each microbial strain were added to each well after being adjusted so that the final concentration in each well following inoculation was approximately 5.0×10^5 cfu mL^{-1} (as evaluated by OD_{600} readings and the previously obtained calibration curves). After incubation periods 100 μL of each tube was spread on agar media plates for determining the number of surviving organisms. Positive and negative growth controls were included in every test. The plates were incubated for 24 ± 2 h at 37 ± 1 °C for bacteria and for 48 ± 2 h at 25 ± 1 °C for yeasts. After incubation, absorbance was read at 600 nm using a Microplate Reader Mode 680 (BioRad, Hemel Hampstead, United Kingdom). After incubation periods of 24 or 48 h, 100 μL of each tube was spread on TSA-YE-T for bacteria and on GYP-T for yeasts, for determining the number of surviving organisms. The range of concentrations were selected until reaching a minimal value for 10% inhibition. The MIC was found as the lowest concentration which resulted in a significant decrease in inoculum viability (10%).

Once all MICs were determined, the EO with higher activities were selected and tested, magnifying 1–18 times their MIC values for MBC evaluation, by the macro dilution method using 20 mL of the same media, 1 mL of inoculum and under the assay incubations described above. For the MBC we upped the concentrations until we had a 99.9% or more of the initial inoculum killed. Three replicates for each EO were performed, and at least two assays were done for each one.

Statistical analysis

All experiments were performed in triplicate at least in two independent assays, and the data are expressed as the mean ± standard deviation. Statistical analysis was performed with SigmaPlot software (version 12.5) for comparing different treatments, using one-way analysis of variance (ANOVA). Statistical differences with P value less than 0.05 where considered statistically significant.

Table 2 MBC determinations of the strains and the essential oils tested

Microbial strains	Essential oil source		
	Origanum vulgare	*Salvia lavandulaefolia*	*Rosmarinus officinalis*
L. monocytogenes NCTC 11994	7.2[a]	56.2[b]	56.2[b]
P. aeruginosa P2	12[a]	NT	56.2[b]
Y. lipolytica ISA 1708	3.52[a]	63.36[b]	> 25

Results presented reflect the average of three different replicates each expressed in μg mL^{-1} (w/v) of oil in growth media. A different letter in the same row represents significant differences ($P < 0.001$) between the different tested EO, within the same microbial strain

NT not tested, because MIC values were too high

Table 3 MIC and MBC values of essential oils or their components tested in vitro against food borne pathogens in several recent studies

EO	MIC (w/v)	MBC (w/v)	Microbial strains tested	References
Rosmarinus officinalis	4.4 mg/mL	4.4 mg/mL	*Escherichia coli*	Mathlouthi et al. (2015)
	8.8 mg/mL	NA	*Salmonella* Indiana	
	8.8 mg/mL	NA	*Listeria innocua*	
	NA	NA	*Staphylococcus aureus*	
	NA	NA	*Bacillus subtilis*	
	10.0 mg/mL	10.0 mg/mL	*Clostridium perfringens*	Radaelli et al. (2016)
	25 mg/mL	50 mg/mL	*Salmonella* Typhimurium ATCC 1408	Miladi et al. (2016)
	25 mg/mL	50 mg/mL	*Salmonella* Typhimurium LT2 DT104	
	12.5 mg/mL	25 mg/mL	*Salmonella* spp. (S1: 6554)[a]	
	12.5 mg/mL	25 mg/mL	*Salmonella* spp. (S2: 6877)[a]	
	12.5 mg/mL	50 mg/mL	*Salmonella* spp. (S3: 6907)[a]	
	25 mg/mL	50 mg/mL	*Salmonella* spp. (S4: 7215)[a]	
	25 mg/mL	50 mg/mL	*Salmonella* spp. (S5: 7466)[a]	
	12.5 mg/mL	25 mg/mL	*Salmonella* spp. (S6: 7643)[a]	
	12.5 mg/mL	50 mg/mL	*Salmonella* spp. (S7: 7945)[a]	
	12.5 mg/mL	25 mg/mL	*Salmonella* spp. (S8: 9487)[a]	
	12.5 mg/mL	25 mg/mL	*Salmonella* spp. (S9: 9340)[a]	
	12.5 mg/mL	25 mg/mL	*Salmonella* spp. (S10: 9681)[a]	
	12.5 mg/mL	25 mg/mL	*Salmonella* spp. (S11: 9812)[a]	
	25 mg/mL	50 mg/mL	*Salmonella* spp. (S12: 9983)[a]	
Origanum sp.	0.9 mg/mL	1.12 mg/mL	*Escherichia coli*	Mathlouthi et al. (2015)
	0.9 mg/mL	1.12 mg/mL	*Salmonella* Indiana	
	0.9 mg/mL	1.12 mg/mL	*Listeria innocua*	
	0.9 mg/mL	1.12 mg/mL	*Staphylococcus aureus*	
	2.25 mg/mL	2.25 mg/mL	*Bacillus subtilis*	
Origanum majorana	5.0 mg/mL	5.0 mg/mL	*Clostridium perfringens*	Radaelli et al. (2016)
Origanum vulgare ecotype F	50 μg/mL	50 μg/mL	*Bacillus cereus* ATCC 11778	De Martino et al. (2009)
	50 μg/mL	50 μg/mL	*Bacillus subtilis* ATCC 6633	
	50 μg/mL	50 μg/mL	*Staphylococcus aureus* ATCC 2592	
	50 μg/mL	100 μg/mL	*Streptococcus faecalis* ATTC 29212	
	50 μg/mL	100 μg/mL	*Escherichia coli*ATCC 25922	
	100 μg/mL	100 μg/mL	*Proteus mirabilis* ATCC 25933	
	100 μg/mL	> 100 μg/mL	*Proteus vulgaris* ATCC 13315	
	> 100 μg/mL	> 100 μg/mL	*Pseudomonas aeruginosa* ATCC 27853	
	100 μg/mL	100 μg/mL	*Salmonella* Typhi Ty2 ATCC 19430	
Origanum vulgare ecotype S	50 μg/mL	50 μg/mL	*Bacillus cereus* ATCC 11778	
	50 μg/mL	100 μg/mL	*Bacillus subtilis* ATCC 6633	
	50 μg/mL	50 μg/mL	*Staphylococcus aureus* ATCC 2592	
	50 μg/mL	100 μg/mL	*Streptococcus faecalis* ATTC 29212	
	100 μg/mL	100 μg/mL	*Escherichia coli*ATCC 25922	
	100 μg/mL	100 μg/mL	*Proteus mirabilis* ATCC 25933	
	100 μg/mL	100 μg/mL	*Proteus vulgaris* ATCC 13315	
	> 100 μg/mL	> 100 μg/mL	*Pseudomonas aeruginosa* ATCC 27853	
	100 μg/mL	100 μg/mL	*Salmonella* Typhi Ty2 ATCC 19430	

Table 3 continued

EO	MIC (w/v)	MBC (w/v)	Microbial strains tested	References
Origanum vulgare ecotype SG	50 μg/mL	100 μg/mL	*Bacillus cereus* ATCC 11778	
	50 μg/mL	100 μg/mL	*Bacillus subtilis* ATCC 6633	
	100 μg/mL	100 μg/mL	*Staphylococcus aureus* ATCC 2592	
	100 μg/mL	100 μg/mL	*Streptococcus faecalis* ATTC 29212	
	100 μg/mL	100 μg/mL	*Escherichia coli*ATCC 25922	
	> 100 μg/mL	> 100 μg/mL	*Proteus mirabilis* ATCC 25933	
	> 100 μg/mL	> 100 μg/mL	*Proteus vulgaris* ATCC 13315	
	> 100 μg/mL	> 100 μg/mL	*Pseudomonas aeruginosa* ATCC 27853	
	> 100 μg/mL	> 100 μg/mL	*Salmonella* Typhi Ty2 ATCC 19430	
Origanum compactum	0.5 μL/mL	0.5 μL/mL	*Listeria monocytogenes* NCTC 11994	Mith et al. (2014)
	0.25 μL/mL	0.25 μL/mL	*Listeria monocytogenes* S0580	
Origanum compactum	0.5 μL/mL	0.5 μL/mL	*Salmonella* Typhimurium ATCC 14028	
	0.25 μL/mL	0.5 μL/mL	*Salmonella* Typhimurium S0584	
	0.25 μL/mL	0.5 μL/mL	*Escherichia coli* O157:H7 ATCC 35150	
	0.5 μL/mL	0.5 μL/mL	*Escherichia coli* O157:H7 S0575	
Origanum heracleoticum	0.25 μL/mL	0.25 μL/mL	*Listeria monocytogenes* NCTC 11994	
	0.25 μL/mL	0.25 μL/mL	*Listeria monocytogenes* S0580	
	0.125 μL/mL	0.125 μL/mL	*Salmonella* Typhimurium ATCC 14028	
	0.25 μL/mL	0.25 μL/mL	*Salmonella* Typhimurium S0584	
	0.25 μL/mL	0.25 μL/mL	*Escherichia coli* O157:H7 ATCC 35150	
	0.25 μL/mL	0.25 μL/mL	*Escherichia coli* O157:H7 S0575	
Mentha×piperita	10.0 mg/mL	10.0 mg/mL	*Clostridium perfringens*	Radaelli et al. (2016)
Ocimum basilicum	5.0 mg/mL	5.0 mg/mL		
Pimpinella anisum	10.0 mg/mL	20.0 mg/mL		
Thymus vulgaris	1.25 mg/mL	1.25 mg/mL		
	1.56 mg/mL	3.12 mg/mL	*Salmonella* Typhimurium ATCC 1408	Miladi et al. (2016)
	1.56 mg/mL	1.56 mg/mL	*Salmonella* Typhimurium LT2 DT104	
			S. strains isolated from food	
	1.56 mg/mL	3.12 mg/mL	*Salmonella* spp. (S1: 6554)	
	1.56 mg/mL	1.56 mg/mL	*Salmonella* spp. (S2: 6877)	
	1.56 mg/mL	1.56 mg/mL	*Salmonella* spp. (S3: 6907)	
	1.56 mg/mL	3.12 mg/mL	*Salmonella* spp. (S4: 7215)	
	1.56 mg/mL	1.56 mg/mL	*Salmonella* spp. (S5: 7466)	
	1.56 mg/mL	1.56 mg/mL	*Salmonella* spp. (S6: 7643)	
	0.78 mg/mL	1.56 mg/mL	*Salmonella* spp. (S7: 7945)	
	1.56 mg/mL	1.56 mg/mL	*Salmonella* spp. (S8: 9487)	
	0.78 mg/mL	1.56 mg/mL	*Salmonella* spp. (S9: 9340)	
	1.56 mg/mL	1.56 mg/mL	*Salmonella* spp. (S10: 9681)	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S11: 9812)	
	1.56 mg/mL	1.56 mg/mL	*Salmonella* spp. (S12: 9983)	
Thymus vulgaris thymoliferum	0.5 μL/mL	0.5 μL/mL	*Listeria monocytogenes* NCTC 11994	Mith et al. (2014)
	0.25 μL/mL	0.25 μL/mL	*Listeria monocytogenes* S0580	
	0.25 μL/mL	0.5 μL/mL	*Salmonella* Typhimurium ATCC 14028	
	0.25 μL/mL	0.5 μL/mL	*Salmonella* Typhimurium S0584	
	0.25 μL/mL	0.25 μL/mL	*Escherichia coli* O157:H7 ATCC 35150	
	0.25 μL/mL	0.5 μL/mL	*Escherichia coli* O157:H7 S0575	

Table 3 continued

EO	MIC (w/v)	MBC (w/v)	Microbial strains tested	References
Thymus capitatus	0.5 µL/mL	0.5 µL/mL	*Listeria monocytogenes* NCTC 11994	
	0.5 µL/mL	1 µL/mL	*Listeria monocytogenes* S0580	
	1 µL/mL	1 µL/mL	*Salmonella* Typhimurium ATCC 14028	
	0.5 µL/mL	1.5 µL/mL	*Salmonella* Typhimurium S0584	
	0.5 µL/mL	1 µL/mL	*Escherichia coli* O157:H7 ATCC 35150	
	0.25 µL/mL	0.25 µL/mL	*Escherichia coli* O157:H7 S0575	
Thymus daenensis	4.0 mg/mL	4.0 mg/mL	*Escherichia coli*	Moghimi et al. (2016)
Satureja montana	0.78 mg/mL	0.78 mg/mL	Salmonella typhimurium ATCC 1408	Miladi et al. (2016)
	0.78 mg/mL	0.78 mg/mL	Salmonella typhimurium LT2 DT104	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S1: 6554)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S2: 6877)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S3: 6907)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S4: 7215)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S5: 7466)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S6: 7643)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S7: 7945)[a]	
	0.39 mg/mL	0.78 mg/mL	*Salmonella* spp. (S8: 9487)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S9: 9340)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S10: 9681)[a]	
	0.39 mg/mL	0.39 mg/mL	*Salmonella* spp. (S11: 9812)[a]	
	0.39 mg/mL	0.78 mg/mL	*Salmonella* spp. (S12: 9983)[a]	
Cinnamomum cassia	0.5 µL/mL	0.5 µL/mL	*Listeria monocytogenes* NCTC 11994	Mith et al. (2014)
	0.25 µL/mL	0.25 µL/mL	*Listeria monocytogenes* S0580	
	0.25 µL/mL	1 µL/mL	*Salmonella* Typhimurium ATCC 14028	
	0.25 µL/mL	1 µL/mL	*Salmonella* Typhimurium S0584	
	0.5 µL/mL	1 µL/mL	*Escherichia coli* O157:H7 ATCC 35150	
	0.25 µL/mL	0.25 µL/mL	*Escherichia coli* O157:H7 S0575	
Cinnamomum verum	0.5 µL/mL	0.5 µL/mL	*Listeria monocytogenes* NCTC 11994	Mith et al. (2014)
	0.25 µL/mL	0.5 µL/mL	*Listeria monocytogenes* S0580c	
	0.5 µL/mL	0.5 µL/mL	*Salmonella* Typhimurium ATCC 14028	
	0.5 µL/mL	1 µL/mL	*Salmonella* Typhimurium S0584	
	0.5 µL/mL	0.5 µL/mL	*Escherichia coli* O157:H7 ATCC 35150	
	0.25 µL/mL	0.5 µL/mL	*Escherichia coli* O157:H7 S0575	
Eugenia caryophyllus	1 µL/mL	> 1.5 µL/mL	*Listeria monocytogenes* NCTC 11994	
	1 µL/mL	> 1.5 µL/mL	*Listeria monocytogenes* S0580	
	1 µL/mL	1.5 µL/mL	*Salmonella* Typhimurium ATCC 14028	
	1 µL/mL	1.5 µL/mL	*Salmonella* Typhimurium S0584	
	1 µL/mL	1 µL/mL	*Escherichia coli* O157:H7 ATCC 35150	
	1 µL/mL	1 µL/mL	*Escherichia coli* O157:H7 S0575	

NA no antimicrobial activity, *MIC* minimum inhibitory concentration, *MBC* minimum bactericide concentration

[a] *Salmonella* strains isolated from food

Results and discussion

It is a well-known fact that essential oils (EOs) seem to exhibit large antimicrobial spectra against bacteria, yeasts and molds (Oussalah et al. 2006). In this work, we determined the minimum inhibitory concentrations as well as the minimal bactericidal activities of specific EO from condiment plants used in Mediterranean Europe, namely *O. vulgare*, *S. lavandulaefolia*, *S. officinalis*, *S. sclarea*, and *R. officinalis*. The EO from oregano (*O. vulgare*) is widely used as a flavoring component in pizzas, lasagnas and sauces and can be effective against pathogenic bacteria

(Sahin et al. 2004; Vasudeva and Vasudeva 2015). Sage oil (*S. lavandulaefolia*) is used by the food industry and in pharmaceutical recipes (Pinto et al. 2007). Rosemary EO (*R. officinalis*) has been used in food as a flavoring, antioxidant, antiseptic and preservative to prevent the attack of fungi and other microorganisms (Uçak et al. 2011).

Since our aim was to ascertain possible applications of these EOs as disinfecting agents in minimally processed produce, representative food pathogens identified in recent outbreaks and deterioration microorganisms were selected, particularly *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Yarrowia lipolytica*.

Firstly, antibacterial activities were tested, and the MIC results obtained for all EOs and microorganisms under study are shown in Table 1. Analysis of the data reveal that all EOs were able to reduce bacterial growth, in all strains, but statistically their MIC values varied very significantly among them ($P < 0.001$). Overall, all strains were more sensitive to *O. vulgare* EO ($P < 0.001$), which presented the lowest MIC values, comprised between 0.03 and 1.76 μg mL^{-1} when compared to the other oils where MIC values were much higher (from 20 up to 900 μg mL^{-1}). It was also noted that the majority of bacterial strains studied here was less sensitive to *S. sclarea* ($P < 0.001$), requiring minimal concentrations of 112.5 μg mL^{-1} up to 900 μg mL^{-1}, which would be unrealistic to use. Although using different methods, Souza et al. (2007) also found good inhibitions for similar EOs, with MIC values varying between 0.26 and 1.25 μg mL^{-1} for various food spoilage yeast strains with *O. vulgare* EO, whereas Cleff et al. (2010) found MIC values between 0.21 and 0.50% (v/v) for strains of *Candida* spp. Another work with this EO demonstrated a good antibacterial activity on several strains tested (Dobre et al. 2011). With *R. officinalis* EO, Rožman and Jeršek (2009) also found a good antimicrobial activity against several strains of *Listeria*, as we have found in our work for the strain studied. Gachkar et al. (2007) have studied *R. officinalis* EO action against *Escherichia coli*, *Staphylocvoccus aureus* and *L. monocytogenes* and found a good to moderate activity with all strains as well.

It is somewhat surprising to realize that none of these studies evaluate the bactericidal effect of EO, since they are required for a scaling-up purpose to establish a standard process of disinfection. In this work, we also evaluated MBC values, as shown in Table 2. Only some selected strains and the EOs with better outcomes in Table 1 were selected for this purpose, as follows: *L. monocytogenes* NCTC 11994, *P. aeruginosa* P2 *and Y. lipolytica* ISA 1708, OEs from *O. vulgare*, *S. lavandulaeifolia* and *R. officinalis.*

Results show that although MIC determinations suggested that several EO seemed to be potentially good disinfectants (Table 1), no bactericidal effect was obtained by increasing the volume tested from 150 μL to 20 mL for MBC evaluation in most strains tested, because a too high concentration of EO was required, which, for example in the case of *S. lavandulaeifolia*, would be potentially nephrotoxic and neurotoxic according to previous works (Pinto et al. 2007). Comparing to results obtained with MIC determinations, these results highlight the importance of determining MBC values in addition to MIC values in these type of studies, to effectively evaluate the EO viability as disinfectant under real situations.

Comparing the oils, *O. vulgare* still is the most effective ($P < 0.001$), followed by *R. officinalis*. However, unlike with MIC values, the EOs studied were not effective for all strains when MCB values were considered. In fact MCB were only obtained for *Y. lipolytica* with *O. vulgare* EO (3.52 μg mL^{-1}), *L. monocytogenes* with *R. officinalis* EO (37.5 μg mL^{-1}) and *S. lavandulaefolia* EO (63 μg mL^{-1}). These results point that the bactericidal effect differs among strains and with the EO itself, hence reducing their potential use as broad-range disinfecting agents.

The possibility of using different EO combinations to reach a broader disinfecting effect has been suggested by other authors (Azevedo et al. 2014). This could be of significant importance to the use of EOs in films and other applications such as edible coatings (Guerreiro et al. 2015). Nonetheless, in this work, the high percentages of these EOs was found to induce an unpleasant strong odor, which would limit their use in food products. Frangos et al. (2010) noticed that the presence of salt and oregano oil (0.2% v/w) in cooked trout samples produced a distinct but sensorial acceptable pleasant odor, well received in sensorial analysis, but in contrast to the combined effect of salt and oregano oil at higher concentrations (0.4% v/w) which was found unpleasant to the panelists.

Mejlholm and Dalgaard (2002) corroborate that for many EOs, over 10 μg mL^{-1} are required to extend product shelf-life. Accordingly, to these authors, such high levels often convey a very strong flavor, hence they can only be primarily useful in sauces and products that are mixed with other strongly-flavored food ingredients. Even so, there is a substantial amount of work suggesting the potential use of EOs in food produce. A comparison between MBC and MIC values found in the available research literature in this area is shown in Table 3. All reports concluded the need to use very high levels of EOs to reach MBC values (over 10 μg mL^{-1} and often much more). Such concentrations are likely to induce strong odor, limiting practicality of their use by the food industry. Furthermore, there is the additional risk that, at these levels of concentration, EOs may exhibit toxicity for human consumption as well. Such is the case of sage EO, which is interdicted, when in high concentrations, from beverages in

most European countries because of its high toxicity (Lima et al. 2004).

Furthermore, it has been shown that although EOs may show a good performance in antimicrobial assays performed in vitro, some studies have demonstrated that even greater concentrations of EOs are necessary to obtain similar results in food products (Burt 2004).

So, a basic but fundamental question arises: although they are unequivocally good antibacterial agents, are EO suitable for industrial-scale MP food products? Our results, as well as the results from other reports, suggest that perhaps not, at least, not in the MP produce food context.

If we also consider that the antimicrobial activity displayed by each EO may vary due to several factors like: i) the environmental conditions including soil and climate where the producing plant is grown, ii) the part of the plant extracted, iii) the time of harvest, iv) the age of the plant, v) the extract concentration (Bakkali 2008), and also, vi) the extraction method (Burt, 2004), then the antimicrobial activity reproducibility becomes hard to obtain (Cosentino et al. 1999; Faleiro et al. 2003), which would render EOs even less practical to be used at an industrial scale.

Other authors (Azevedo et al. 2014) have suggested that the use of a single compound instead of the whole mixture comprising each EO, is a better approach. Pirie and Clayson (1964) proposed that EOs can only be primarily useful in sauces and products that are mixed with other food ingredients. On the other hand, some recent studies indicated the possibility of using EOs in synergy with other antimicrobial agents, such as nisin or lysozyme, which could be a possibility for the use of lower concentrations of EOs, thus decreasing the potential toxicity of these compounds (Dehkordi et al. 2008). Therefore, the use of EOs can still be a promising natural and effective way to prevent microorganism proliferation in food products, albeit the need to revaluate their application.

Conclusion

Results presented in this work showed that all the EOs tested presented good antibacterial effects towards an array of pathogenic bacteria associated with food contamination and spoilage. However, when considering the bactericidal effect, only *O. vulgare*, *S. lavandulaefolia* and *R. officinalis* presented a noticeable activity and only against fewer strains. Furthermore, the concentrations required for effective bactericidal activity were too high for the desired purpose of food application because they originated very intense, unpleasant odors. This suggests that although EOs are viewed as good broad-range disinfectants, they might have a limited use as food disinfectants in MP produce. Overall, this work highlights that the application of EOs in foods needs to be further addressed in relation to its practical applicability and efficacy.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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