THE ANTIMICROBIAL EFFECT OF ESSENTIAL OILS AGAINST *LISTERIA MONOCYTOGENES* IN *SOUS VIDE* COOK-CHILL BEEF DURING STORAGE

ANA R. GOUVEIA[1], MÁRCIO ALVES[1], JOSÉ M. M. M. de ALMEIDA[2,3], FILIPE MONTEIRO-SILVA[3], GERARDO GONZÁLEZ-AGUILAR[3], JOSÉ A. SILVA[1] and CRISTINA SARAIVA[1,4]

[1]Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, CECAV, University of Trás-os-Montes e Alto Douro, Bl. Lab, B1.03, Vila Real 5001-801, Portugal
[2]Department of Physics, School of Sciences and Technology, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
[3]Centre for Applied Photonics, INESC TEC, Faculty of Sciences of University of Porto, Porto, Portugal

[4]Corresponding author.
TEL: +351259350659;
FAX: +351259350480;
EMAIL: crisarai@utad.pt

Received for Publication February 5, 2016
Accepted for Publication April 10, 2016

doi:10.1111/jfpp.13066

ABSTRACT

Sous vide cook-chill (SVCC) is characterized by vacuum-packaging raw or partially prepared foods before pasteurization, followed by rapid chilling and storage below 3C. The application of essential oils (EOs) to food products is a suitable strategy to control pathogens and to extend their shelf life by reducing microbial levels and oxidative processes. The aim of this study was to evaluate the efficacy of *Rosmarinus officinalis* L. (rosemary) and *Thymus vulgaris* L. (thyme) EOs against *Listeria monocytogenes* ATCC 679, inoculated in beef processed by SVCC stored at 2 and 8C for 1, 2, 3, 7, 14, 21 and 28 days. The composition of EOs was identified by gas chromatography-mass spectrometry analysis. The main compounds identified in rosemary EO were eucalyptol (13.05%), camphor (8.93%), verbenone (8.58%), endo-borneol (7.87%) and α-pinene (6.78%) and in thyme EO were linalool (18.18%), thymol (7.48%), limonene (6.49%), endo-borneol (5.86%) and terpinen-4-ol (5.66%). Using the minimum inhibitory concentration (MIC) method, *L. monocytogenes* was inhibited at 3.9 μL/mL to thyme EO and at 62.5 μL/mL to rosemary EO. Beef samples of M. *longissimus thoracis et lumborum* were packaged in bags, inoculated and one of each EO was added at MIC values. Bags were vacuum-sealed and samples were processed at 55C/65 min for 3 $\log_{10}$ CFU/g reduction. A reduction of the counts of *L. monocytogenes* was observed in all samples at 2C. At 8C counts of *L. monocytogenes* were almost similar in control samples and those with thyme EO with an increase of the microbial counts since day 7. Inversely, counts of *L. monocytogenes* in beef samples with rosemary EO stored at 2 and 8C decreased about 2 $\log_{10}$ CFU. These results support the possibility of using rosemary EO as natural preservative due to its antimicrobial effect against *L. monocytogenes*. Also, our results confirm that an adequate chilling storage is essential to guarantee the safety of SVCC product regarding *L. monocytogenes* to avoid foodborne outbreaks.

PRACTICAL APPLICATIONS

Results support the possibility of addition of EO of rosemary as a natural preservative to reduce *L. monocytogenes* counts. Also, an adequate chilling storage for maintaining this pathogen at acceptable levels is of paramount importance in view of preventing food borne diseases.

INTRODUCTION

Sous vide cook-chill (SVCC) is a food processing technology characterized by vacuum-packaging raw or partially prepared foods before pasteurization, followed by rapid chilling and cold storage below 3C (Baldwin 2012; Hansen and Knochel 2001; Nyati 2000). This mild processing keeps the nutritional

quality of the food (Bolton *et al*. 2000). However, there is a risk associated to this technology: pasteurization might not reduce pathogenic spores to a safe level (Baldwin 2012). To ensure the food safety, pasteurization must prevent the survival of pathogenic bacteria which are able to grow under anaerobic conditions and chilled storage (Sheard and Rodger 1995). Comparatively with traditional cooking methods, this technology has several advantages: the vacuum packaging reduces the oxidative and deteriorative processes and enhances the shelf life of the products, which can be as long as 42 days (Hyytia-Treess *et al*. 2000). In addition, increased tenderness, high flavor profile and retained color and nutrients are advantages of this method (Vaudagna *et al*. 2002).

Listeria monocytogenes has become an increasingly important foodborne-associated pathogen (Sergelidis and Abrahim 2009). This microorganism is one of main microbiological hazard associated with sous vide processing due to its ability of growing at low temperatures, heat resistance compared with other microorganisms, ubiquitous nature and severity of disease (Ghandhi and Chikindas 2007).

Chemical food preservatives have been used as an effective method to control spoilage and pathogenic bacteria. Nowadays, there is a consumer preference for healthy foodstuff without addition of chemical preservatives. Thus, the usage of natural antimicrobial substances as essential oils (EOs) has gained the interests of food industry to meet the consumers' preferences. Essential oils (EOs) are aromatic oily liquids produced from plant material, like leaves, seeds, flowers, roots and twigs (Burt 2004). The growth and survival of *L. monocytogenes* can be controlled by addition of an herb extract as an ingredient in meat (Mytle *et al*. 2006; Djenane *et al*. 2011).

Thymus vulgaris L. (thyme) is an aromatic plant of the Lamiaceae family (Solomakos *et al*. 2008) and its EO showed antibacterial activity as observed by different authors (Solomakos *et al*. 2008). Thymol has been referred as the main antibacterial compound of the *T. vulgaris* EO (Govaris *et al*. 2011; Pesavento *et al*. 2015) and usually comprises over 50% of its chemical composition (Rota *et al*. 2008).

Rosmarinus officinalis L. (Rosemary) is recognized by its antioxidative and antimicrobial activities (Ojeda-Sana *et al*. 2013), being eucalyptol its main compound which is known for having activity against bacterial membrane (Van Vuuren and Vijoed 2007).

The present study was performed in order to evaluate the efficacy of these EOs against *L. monocytogenes*, inoculated in beef processed by SVCC technology and stored at 2 and 8C.

MATERIAL AND METHODS

Essential Oils

Essential Oils Extraction. Fresh aerial parts of *Rosmarinus officinalis* L. (rosemary) and *Thymus vulgaris* L. (thyme) were collected from University of Trás-os-Montes e Alto Douro (UTAD), Vila Real. Specimens were identified. Plants were dried until stable weight and submitted to hydrodistillation during 3 h using a Clevenger type apparatus as described by Rohani *et al*. (2011). Afterward, the EOs were recovered by separating the water present in the essential oil and stored at −20C and in the dark.

Volatile Composition EOs by Gas Chromatography–Mass Spectrometry and Gas Chromatography–Thermal Conductivity Detection Analysis. Analysis was carried out using a Thermo Scientific TRACE 1300 gas chromatograph coupled to a ISQ Series Single Quadrupole MS (Thermo Fisher Scientific, Inc.). Separation of analytes was performed with a Thermo Scientific TG-5MS column (60 m × 0.25 mm × 0.25 μm). The oven temperature program was as follows: initial temperature of 60C held for 2 min, increasing to 280C at a rate of 10.00C/min and held for 5 min. Samples and standards were freshly prepared prior to analysis using n-Hexane (Merck) in 1.0% (v/v) and 0.2% (v/v) concentrations, respectively, and the volume injected was 1.0 μL using an autosampler. The injector was set to split mode (1:5), operating at 250C and 165 kPa. The mass spectrometer transfer line and ion source temperatures were set to 280 and 250C, respectively, with the last operating under electron impact mode (70 eV, mass scan range 30–400 amu).

Analysis of the same samples was also carried out using a Shimadzu GC-2010 Plus (Shimadzu Corp., Japan). Separation of analytes was performed with a Zebron ZB-5 column (30 m × 0.25 mm × 0.25 μm) using a similar oven temperature program and injection/injector parameters except for carrier gas flow which was set to 82.5 kPa. The detector temperature and current was programmed to 300C and 75 mA, respectively, with a make-up flow of 5.0 mL/min. All analytical separations were performed using helium 99.999% as carrier gas.

Identification of analytes was performed by comparison of the Kovats and linear retention indices, using NIST/EPA/NIH mass spectral library (2011) and other libraries, namely, Pherobase and by comparison of authentic standards.

Determination of Minimum Inhibitory Concentration (MIC) of EOs. *Listeria monocytogenes* ATCC 679 was used in this study. The culture was stored at −20C in brain–heart infusion broth (BHI) (Biokar Diagnostics BK015HA) supplemented with 25% glycerol (v/v) (Panreac). After subcultured two times in BHI, a 12 h culture was diluted with isotonic saline (0.9%) and serial decimal dilutions were performed to compare the turbidity of the suspension with the McFarland standard until achievement of a final Ca of approximately 5 log CFU/mL.

The method used to determine the minimum inhibitory concentration (MIC) was based on Sarker *et al.* (2007). Geometric and successive dilutions of each EO were performed in a sterile 96-well microtiter plate (adapted from Karaman *et al.* 2003). A dilution series of the EOs was obtained using BHI. The positive control was obtained with 100 μL BHI and 20 μL of the bacterial suspension, while the negative control was completed with 100 μL BHI. Lastly, 20 μL of the aqueous solution resazurin (prepared by dissolving 135 mg in 40 mL sterile distilled water) was added to the wells. The microplates were covered with parafilm and incubated for 24h at 37C. For each EO, the highest dilution with no visible growth was considered the MIC.

Antimicrobial Activity of OEs in Beef

Inoculum Preparation. *Listeria monocytogenes* strain ATCC 679 was grown in tryptone soya broth (Oxoid, Hampshire, UK) at 30C for 18 h to achieve a viable cell population of 8 $\log_{10}$ CFU/mL. The culture was then transferred to a sterile centrifuge bottle and centrifuged at 10,000 × g for 10 min at 4C. The supernatant was decanted and the pellet suspended in sterile 0.1% peptone solution. The washing step was repeated twice. Bacterial cell count was determined by optical density (OD) method at 600 nm, a population of 1×10^8 CFU/mL. Serial (10-fold) dilutions in 0.9% isotonic saline were performed in duplicate to yield approximately 1×10^6 CFU/mL. To verify the number of viable *L. monocytogenes* in the suspension, dilutions were spread on Oxford Agar (Biokar Diagnostics BK110) supplemented with Oxford selective supplement (CCCFA) (Biokar Diagnostics BS003) in duplicate and incubated for 48 h at 37C.

Sample Preparation, Inoculation and Thermal Inactivation. *Longissimus thoracis et lumborum* (LTL) muscles were obtained from four Portuguese bulls (9–11 months; 90–150 kg carcass weight) and were excised from the carcasses, between the sixth thoracic and the fourth lumbar vertebra, at 72 h post mortem. Muscles were directly collected from a butchery of Maronesa beef (an autochtonous breed) and transported within 10 min to the laboratory, in portable coolers. The pH was measured directly in the muscle using a combined glass electrode with a pH meter (Crison Instruments, Spain). Only muscles whose pH were below or equal to 5.8 were used. Muscles were cut into pieces of approximately 200 g and two samples of each piece were immediately (72 h post mortem) investigated for the presence of *L. monocytogenes* according to ISO 11290-1. If at least one positive sample from each muscle was detected in one meat cut, all piece cuts of the whole muscle were totally excluded from the inoculation experiments. Then, beef cuts were vacuum-packaged and kept at −80C until beginning the experiment. Meat was allowed to defrost for two hours at 2C prior to the experiments and subsequently prepared by removing a layer of ∼1 cm from the meat surface and aseptically cutting in small meat pieces (0.5 cm thick, surface 2.5 × 2.5 cm) of, sensibly, 10 g each.

Beef samples were inoculated using a microsyringe with 0.1 mL aliquots of a 1×10^8 CFU/mL *Listeria monocytogenes* ATCC 679 suspension in 17 × 15 cm vacuum pouches (Combitherm, HAFRI). The additions of EOs (mL OE/g of meat) were done at MIC values. The individual packages were vacuum-sealed using a SAMMIC V-420 SGA machine.

Beef samples were placed in water-bath pre-adjusted to 55C. The core temperature was monitored continuously using thermocouples connected and inserted into noninoculated control samples. Samples were processed at 55C until 21 min and 39 s (time required to inactivate 90% population-D value, data not published). After that, samples were immediately immersed in ice water at 3C during 10 min, and afterward the products were stored at 2 and 8°C, for 1, 2, 3, 7, 14, 21 and 28 days.

Bacterial Enumeration. At each sampling time, samples (10 g) were added with sterile buffered peptone water (0.1% w/v) and then homogenized for 30 s at room temperature. Subsequent dilutions were obtained (1:10) in sterile peptone water (0.1% w/v). Samples dilutions were cultured in oxford agar (Biokar Diagnostics BK110) supplemented with oxford selective supplement (Biokar Diagnostics BS003) in duplicate at 37C for 48 h.

Statistical Analysis. For all experiments the assays were carried out in triplicate. Experimental results were expressed as means and standard deviations. The statistical analysis of the *L. monocytogenes* counts after SVCC was carried out by ANOVA. The differences ($P < 0.05$) between the mean values were determined using the Tukey-Kramer test, using Statistica 12.

RESULTS AND DISCUSSION

Volatile Composition of the EOs

Table 1 summarizes the results of volatile composition of thyme and rosemary EOs. In order to simplify the analysis of the results, only compounds with more than 0.5% were selected. Thirty-six and 44 compounds representing 87.17 and 87.53% of the total OE of rosemary and thyme EOs, respectively, were determined. Rosemary EO was characterized by a high proportion of oxygenated monoterpenes (59.49%), such as eucalyptol (13.05%), camphor (8.93%), verbenone (8.58%) and endo-borneol (7.87%). Several authors reported similar or different values – for instance – Boutekedjiret *et al.* (1999), Wang *et al.* (2008) and Guetat *et al.* (2014) reported eucalyptol as the main chemical compound of rosemary EO, with 16.27, 27.23 and 26.13%,

TABLE 1. CHEMICAL COMPOSITION (%) AND PRINCIPAL CHEMICAL CLASSES (%) OF *ROSMARINUS OFFICINALIS* L AND *THYMUS VULGARIS* L. ESSENTIAL OILS (EO)

Volatile compounds	KI	RT	Rosmarinus officinalis L.	Thymus vulgaris L.
NI		8.25	–	0.72
Tricyclene	930	8.88	–	–
α-thujene	935	8.89	–	0.60
α-pinene	940	9.20	6.78	0.80
Camphene	952	9.42	398	0.88
1-Octen-3-ol	959	9.76	–	4.62
Octan-3-one	967	9.92	–	1.41
β-pinene	979	9.96	3.14	3.56
Myrcene	991	10.03	3.02	–
Octanal	1.001	10.24	–	0.65
D-3-carene	1.012	10.55	0.81	–
α-terpinene	1.019	10.64	0.50	–
p-cymene	1.025	10.79	–	4.91
D-limonene	1.030	10.91	2.38	6.49
Eucalyptol	1.033	10.99	13.05	0.62
γ-terpinene	1.063	11.42	0.93	4.39
Cis-p-Mentha-8-en-1-ol	1.076	11.61	–	0.88
α-terpinolene	1.089	11.99	0.78	0.68
Linalool	1.098	12.09	3.37	18.18
Eucarvone		12.71	0.53	–
Camphor	1.145	13.17	8.93	1.55
Pinocamphone	1.157	13.42	–	–
Endo-borneol	1.169	13.54	7.87	5.86
4-terpineol	1.179	13.67	3.50	5.66
p-cimen-8-ol	1.186	13.76	0.54	–
α-terpineol	1.191	13.88	3.10	–
Decanal	1.195	13.91	–	1.42
Myrtenol	1.200	13.99	–	–
Isoborneol	1.209	14.08	1.87	–
Verbenone	1.218	14.32	8.58	–
Nerol	1.242	14.81	–	0.74
Geraniol	1.258	15.19	–	0.89
Thymol	1.290	15.38	–	7.48
Bornyl acetate	1.291	15.45	5.95	–
p-thymol	1.294	15.57	–	2.27
Trans-pinocarvyl acetate	1.297	15.62	0.60	–
Caryophyllene	1.427	17.72	2.67	2.39
Caryophyllene oxide	1.609	20.08	0.49	2.72
t-canidol	1.638	20.67	–	1.20
Eicosane	2.000	24.36	–	0.50
Heneicosane	2.100	25.45	–	0.69
Heptacosane	2.700	27.80	–	0.78
Grouped compounds	–	–	–	–
Oxygenated monoterpenes (%)	–	–	58.49	46.96
Monoterpene hydrocarbons (%)	–	–	23.04	22.61
Oxygenated sesquiterpenes (%)	–	–	0.49	4.22
Sesquiterpene hydrocarbons (%)	–	–	2.91	2.74
Aliphatic constituents (%)	–	–	0.45	2.25
Non-isoprenoid compounds (%)	–	–	–	6.27
Other derivates (%)	–	–	1.79	2.48
Total identified (%)	–	–	87.17	87.53

NI, not identified; KI, Kovats index; RT: retention time.

respectively. However, Arnold *et al.* (1997), Salido *et al.* (2003) and Bernardes *et al.* (2010) reported that camphor as the most abundant compound.

The volatile compounds of thyme EO were characterized by 46.96% of oxygenated monoterpenes, mostly represented by linalool (18.18%), thymol (7.48%), endo-borneol (5.86%)

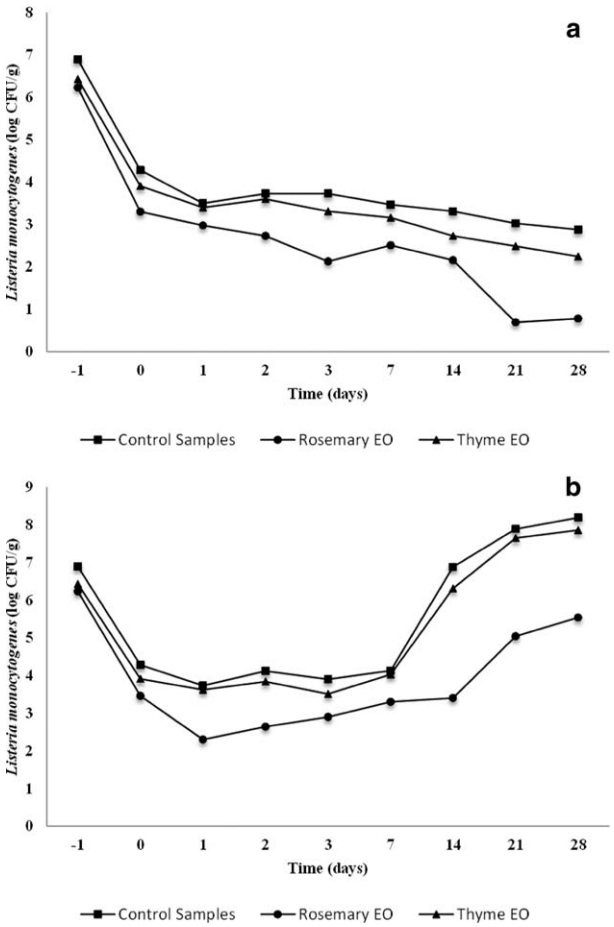

FIG. 1. BEHAVIOR OF *L. MONOCYTOGENES* IN PRESENCE AND ABSENCE OF ESSENTIAL OIL (EO) AT 2C (A) AND 8C (B) (−1) time, mean population of *L. monocytogenes* (log CFU/g) inoculated.

and terpinen-4-ol (5.66%). Thyme species have a higher polymorphic variation and as such, it was described that *Thymus vulgaris* has a chemical polymorphism with six different chemotypes divided into two main groups. These groups are the phenolic chemotype represented by thymol and carvacrol among others. The second chemotype group is composed by nonphenolic compounds such as linalool, geraniol, α-terpineol and trans-thujan-4-ol/terpinen-4-ol. In the current study, the chemical profile of thyme EO belonged to the linalool chemotype. Similar results were reported by Torras *et al.* (2007) and Ballester-Costa *et al.* (2013), although with higher percentages (36.32–60.55% and 44.00%, respectively). However, the chemical profile of thyme EO used in the present work differs from the others studies that reported thymol as the main chemical compound (Grosso *et al.* 2010; Govaris *et al.*, 2011; Hudaib *et al.* 2002; Nezhadali *et al.* 2014).

The differences in the chemical composition of thyme and rosemary EOs when compared with other studies can be attributed to the different origin of the collected samples associated to climatic effects (Gachkar *et al.* 2007), harvesting season and geographical environment (Nezhadali *et al.* 2014). This can be attributed to the differences in temperatures, humidity, duration of solar exposition and intensity occurred in different countries or regions (Gachkar *et al.* 2007; Govaris *et al.* 2011; Nezhadali *et al.* 2014).

Antimicrobial Screening of EOs

The application of the broth microdilution method is useful to compare the antibacterial effect between EOs and to identify the minimal concentrations of EO that did not allow bacterial replication (Mazzarrino *et al.* 2015). Thyme EO showed a strong antimicrobial activity with a MIC at 3.9 μL/mL. Rosemary EO at 62.5 μL/mL showed bactericidal activity after 24 h of incubation. Other authors obtained different results: regarding to thyme EO, lower concentrations 2.5 μL/mL and 1 μL/mL were reported by Sokovic *et al.* (2010) and Carvalho *et al.* (2015), respectively. However, in these studies, thymol and p-cymene were the major compounds of this EO. Sokovic *et al.* (2010) reported that thymol, as individual component, showed more antibacterial potential than the linalool, the major compound of thyme EO, in this study. Moreover, Mazzarrino *et al.* (2015) showed that MIC for *L. monocytogenes* was high (5 μL/mL); while the MIC value for rosemary was higher (62.5 μL/mL) than the obtained to thyme EO. Pesavento *et al.* (2015) and Barbosa *et al.* (2016) showed a MIC 10-fold lower (5 μL/mL) than obtained in this study. Azeredo *et al.* (2011) also found a lower MIC of 20 μL/mL. The discrepancies between the MIC for each OE and others studies can be related to the different strains of *L. monocytogenes* used, the solubility of the different compounds of the EOs in liquid medium and the composition of EOs that can be affected by several factors (Djenane *et al.* 2011).

Antimicrobial Activity in Beef

The evolution of *L. monocytogenes* counts after SVCC processing is presented in Fig. 1a,b according the storage temperature at 2 and 8C, respectively. As expected, the SVCC process achieved a reduction of *L. monocytogenes* counts about 3 log CFU/g. At 2C, the initial population of 4.28 CFU/g of *L. monocytogenes* (day 0) decreased to 2.88 CFU/g by the end of storage in control samples, however maintaining a similar behavior after day 1. When rosemary EO was added at 1.25% (v/w), a continuous reduction of *L. monocytogenes* populations were observed at day 7. By the end of storage a higher bactericidal effect was observed, with counts under 1 log CFU/g, at day 21 and 28. Comparatively, when thyme EO was added at a concentration 0.078% (v/w), the population of *L. monocytogenes* were kept below 4

TABLE 2. MEANS (±STANDARD DEVIATION) OF BEHAVIOR OF *L. MONOCYTOGENES* BY STORAGE TEMPERATURE (*T*) AND ESSENTIAL OIL (EO) EFFECTS, ACCORDING THE STORAGE TIME

Time (days)	2C	8C	Temp. effect	Control	Rosemary EO	Thyme EO	EO effect	EOxT
0	3.82 ± 0.50	3.87 ± 0.45	n.s.	4.28 ± 0.18^{a}	3.37 ± 0.28^{b}	3.91 ± 0.32^{a}	***	n.s.
1	3.29 ± 0.30	3.45 ± 0.39	n.s.	3.62 ± 0.18^{a}	2.99 ± 0.20^{b}	3.51 ± 0.23^{a}	***	n.s.
2	3.35 ± 0.52	3.53 ± 0.69	n.s.	3.93 ± 0.25^{a}	2.68 ± 0.24^{b}	3.72 ± 0.18^{a}	***	n.s.
3	3.06 ± 0.84	3.43 ± 0.49	n.s.	3.81 ± 0.27^{a}	2.51 ± 0.64^{b}	3.41 ± 0.29^{a}	***	n.s.
7	3.05 ± 0.49^{a}	3.73 ± 0.65^{b}	**	3.80 ± 0.37^{a}	2.77 ± 0.42^{b}	3.60 ± 0.68^{a}	**	n.s.
14	2.73 ± 0.57^{a}	5.53 ± 1.65^{b}	***	5.10 ± 1.97^{a}	2.78 ± 0.79^{b}	4.52 ± 1.97^{c}	***	***
21	2.07 ± 1.13^{a}	6.87 ± 1.57^{b}	***	5.46 ± 2.68^{a}	2.87 ± 2.54^{b}	5.07 ± 2.86^{a}	***	n.s.
28	1.97 ± 1.21^{a}	7.20 ± 1.28^{b}	***	5.54 ± 2.95^{a}	3.16 ± 2.76^{b}	5.05 ± 3.09^{a}	***	n.s.

n.s., nonsignificant.

In each storage time, for each effect, means with different letters differ significantly: $^{**}P < 0.01$, $^{***}P < 0.001$.

log CFU/g at day 7. After this point, lower counts (approximately 2.24 log CFU/g) were observed at day 28.

At abusive temperatures (8C), in control samples, the behavior of *L. monocytogenes* during storage of control samples at 8C was expected until day 7. After this day, an exponential growth of *L. monocytogenes* occurred, reaching a population of 8.19 log CFU/g at day 28. However, the addition of rosemary EO extended the lag phase up to day 14 with a later onset of exponential growth, reaching lower values (5.54 log CFU/g) when compared to control samples. Comparatively, in thyme EO samples, the population of *L. monocytogenes* shows a similar behavior as observed in control samples but with CFU values below 8 log CFU/g counts at day 28.

Table 2 shows the effect of storage temperature and the antimicrobial effect of thyme and rosemary EOs against *L. monocytogenes* in beef samples, according the storage time. As expected, counts of *L. monocytogenes* were lower at 2C after day 3 ($P < 0.001$) comparatively to 8C storage. The addition of rosemary EO at 1.25% (v/w) showed a higher inhibitory effect against *L. monocytogenes* compared with addition of thyme EO and control, in all days of storage, resulting in highly significant differences ($P < 0.001$) for the most of storage days. In contrast, the addiction of thyme EO (0.078% [v/w]) to beef samples was not significant for microbial counts.

This study showed that thyme EO was less effective than rosemary EO, although it reduced the bacterial counts. This can be related with chemical composition of this EO, in which its concentration of phenolic compounds (i.e., thymol) was lower than observed for rosemary EO. However, these results can be eventually attributed to the lower concentrations used comparatively to those employed in rosemary EO bacteriostatic studies. Pesavento *et al.* (2015) reported higher antimicrobial effect against *L. monocytogenes* with thyme EO at different concentrations in minced meat stored at 4C. These authors found that p-cymene (47.9%) and thymol (43.1%) were the major antimicrobial compounds, in contrast to the results attained in this study, with thyme EO showing lower levels of these compounds, 4.91 and 7.48%, respectively. The compound p-cymene can act as a replacement in the bacteria membrane perturbing it. On the other side, thymol, as other phenolic monoterpenoids having a phenolic ring, can cause structural and functional damages to the cytoplasmic membrane of bacteria (Hyldgaard *et al.* 2012). In our study, linalool was the main chemical compound representing about 18.18% of the total OE composition. *In vitro*, linalool showed a moderated antimicrobial activity; oppositely, it has been reported a higher activity for thymol (Bassolé *et al.* 2010). Consentino *et al.* (1999) referred linalool as the predominant compound of thyme EO in their samples although with lower antimicrobial activity.

Addition of rosemary EO at 1.25% (v/w) was found to be more effective against *L. monocytogenes*. However, no statistical differences were observed by Kahraman *et al.* (2015) with addition of rosemary EO at 0.2% (v/w) in poultry fillets stored at 4C after 7 days. Antimicrobial effect of rosemary EO could be associated to eucalyptol, their main chemical compound. Besides, the presence of other compounds enhanced the results observed by these authors. It has been shown that the oxygen groups existing in eucalyptol can disrupt the cell membrane structure even in subinhibitory concentrations (Sousa *et al.* 2015).

The differences reported by several studies can be explained with the strain of *L. monocytogenes* used and by the main compounds of EOs obtained (Abdollahzadeh *et al.* 2014). On the other hand, it was established that antimicrobial effect of EOs is related with intrinsic factors (proteins and fat) of food, as well as extrinsic factors, including temperature and presence or absence of oxygen (Hayouni *et al.* 2008). Proteins and fat can solubilize phenolic compounds in EOs, reducing their availability for antimicrobial activity. Moreover, the difference between antimicrobial activity *in vitro* and in beef may be associated with EOs interaction with components of the meat (Hayouni *et al.* 2008). Similar

findings were reported by Gill *et al.* (2002), where 0.018% of coriander EO showed significant activity *in vitro*, but similar antibacterial effect was observed at 6% (v/w) in food.

It is important to emphasize the survival of *L. monocytogenes* at refrigeration temperatures and its ability to recover from injury caused by heat treatment, overcoming the antimicrobial activity obtained by EOs addiction. This is a concern with respect to ready-to-eat products and SVCC products that need a minimal processing by consumers. The use of abusive temperatures implied an exponential growth of this bacteria both in control and EO samples. Thus, storage of products at abusive temperatures increases the risk of consumer contamination and occurrence of foodborne outbreaks when *L. monocytogenes* is present.

CONCLUSIONS

The utilization of EO of thyme and rosemary in SVCC improve their safety during storage. Thus, we observed that addition of 0.078% (v/w) of thyme EO displayed a lower antimicrobial effect than rosemary EO at 0.5% (v/w) against *L. monocytogenes*. Since temperature influenced the behavior of *L. monocytogenes*, the storage at 8C showed exponential growth after 7 days in beef samples with thyme EO and after 14 days in beef samples with rosemary EO.

The reduced antimicrobial effect of thyme EO against *L. monocytogenes* can be associated with the *T. vulgaris* chemotype used in the current work that presented low concentrations of thymol.

Also, the differences among the antimicrobial activity of the EOs used may be related to the different concentrations applied in SVCC beef.

This study may be interesting for food processors since our results indicated that addition of rosemary EO may be used as a natural preservative to inhibit the growth of *L. monocytogenes* in SVCC beef and enhance their safety during the storage at adequate chilling temperature.

ACKNOWLEDGMENTS

The authors would like to thank to Ana Leite for her contribution to this research and to CECAV-UTAD. This work is financed by National Funds through the FCT – Fundação para a Ciência e a Tecnologia (Portuguese Foundation for Science and Technology) within UID/EEA/50014/2013 and PEst-OE/AGR/UI0772/2014 projects.

REFERENCES

ABDOLLAHZADEH, E., REZAEI, M. and HOSSEINI, H. 2014. Antibacterial activity of plant essential oils and extracts: The role of thyme essential oil, nisin, and their combination to control *Listeria monocytogenes* inoculated in minced fish meat. Food Control *35*, 177–183.

ARNOLD, N., VALENTINI, G., BELLOMARIA, B. and HOCINE, L. 1997. Comparative study of essential oils from *Rosmarinus eriocalyx* Jordan & fourr from Algeria and *R. officinalis* L. from other countries. J. Essent. Oil Res. *9*, 168–175.

AZEREDO, G., STAMFORD, T., NUNES, P., NETO, N., OLIVEIRA, M. and SOUZA, E. 2011. Combined applications of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochonous microflora associated with minimally processed vegetables. Food Res. Int. *44*, 1541–1548.

BALDWIN, D. 2012. Sous vide cooking: A review. Int. J. Gastr. Food Sci. *1*, 1–30.

BARBOSA, M., MEDEIROS, C., OLIVEIRA, R., GOMES-NETO, J., TAVARES, F., MAGNANI, M. e SOUSA, R. 2016. Efficacy of the combined application of Escherichia coli, Listeria monocytogenes and Salmonella in leaf vegetables. Food Control. *59*, 468–477.

BASSOLÉ, H., LAMIEN-MEDA, A., BAYALA, B., TIROGO, S., FRANZ, C., VOVAK, J., NEBIÉ, C. and DICKO, H. 2010. Composition and antimicrobial activities of *Lippia* multiflora Moldenke, *Mentha* x *piperita* L. and *Ocimum basilicum* L. essential oils and their major monoterpene alcohols alone and in combinations. Molecules *15*, 7825–7839.

BALLESTER-COSTA, C., SENDRA, E., FERNÁNDEZ-LÓPEZ, J., PÉREZ-ÁLVAREZ, A.J. and VIUDA-MARTOS, M. 2013. Chemical composition and *in vitro* antibacterial properties of essential oils of four *Thymus* species from organic growth. Ind. Crop. Prod. *50*, 304–311.

BERNARDES, W., LUCARINI, R., TOZATTI, M., FLAUZINO, L., SOUZA, M., ANDRADE, M., MARTINS, C., DA SILVA, A. and CUNHA, W. 2010. Antibacterial activity of the essential oil from *Rosmarinus officinalis* and its major components against oral pathogens. Z. Naturforsch. C *65*, 588–593.

BOLTON, D., MCMAHON, C., DOHERTY, A., SHERIDAN, J., MCDOWELL, A., BLAIR, I. and HARRINGTON, D. 2000. Thermal inactivation of *Listeria monocytogenes* and *Yersinia enterocolitica* in minced beef under laboratory conditions and in sous-vide prepared minced and solid beef cooked in a commercial retort. J. Appl. Microbiol. *88*, 626–632.

BOUTEKEDJIRET, C., BELABBES, R., BENTAHAR, F. and BESSIERE, J. 1999. Study of *Rosmarinus officinalis* L. essential oil yield and composition as a function of the plant life cycle. J. Essent. Oil Res. *11*, 238–240.

BURT, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods – A review. Int. J. Food Microbiol. *94*, 223–253.

CARVALHO, R., SOUZA, G., HONÓRIO, V., SOUSA, J., CONCEIÇÃO, M., MAGANANI, M. and SOUZA, E.L. 2015. Comparative inhibitory effects of Thymus vulgaris L., essential oil against Staphylococcus aureus, Listeria monocytogenes and mesophilic starter co-culture in cheese-mimicking models. Food Microbiol. *52*, 59–65.

CONSENTINO, S., TUBEROSO, G., SATTA, M., MASCIA, V., ARZEDI, E. and PALMAS, F. 1999. *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Lett. Appl. Microbiol. *29*, 130–135.

DJENANE, D., YANGUELA, J., MONTAÑÉS, L., DJERBAL, M. and RONCALÉS, P. 2011. Antimicrobial activity of *Pistacia lentiscus* and *Satureja montana* essential oils CECT 935 using laboratory media: Efficacy and synergistic potential in minced beef. Food Control *22*, 1046–1053.

GACHKAR, L., YADEGARI, D., REZAEI, M.B., TAGHIZADEH, M., ASTANEH, S.A. and RASOOLI, I. 2007. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. *102*, 898–904.

GHANDHI, M. and CHIKINDAS, M.L. 2007. Listeria: A foodborne that knows how to survive. Int. J. Food Microbiol. *113*, 1–15.

GUETAT, A., GHAMDI, F. and OSMAN, A. 2014. 1,8-Cineole, α-pinene and verbenone chemotype of essential oil of species *Rosmarinus officinalis* L. from Saudi Arabi. Int. J. Herb. Med. *2*, 68–72.

GILL, O., DELAQUIS, P., RUSSO, P. and HOLLEY, R. 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int. J. Food Microbiol. *73*, 83–92.

GOVARIS, A., BOTSOGLOU, E., SERGELIDIS, D. and CHATZOPOULOU, P.S. 2011. Antibacterial activity of oregano and thyme essential oils against *Listeria monocytogenes* and *Escherichia coli* O157:H7 in feta cheese packaged under modified atmosphere. LWT – Food Sci. Technol. *44*, 1240–1244.

GROSSO, C., FIGUEIREDO, A.C., BURILLO, J., MAINAR, A.M., URIETA, J.S., BARROSO, J.G., COELHO, J.A. and PALAVRA, A.M.F. 2010. Composition and antioxidant activity of *Thymus vulgaris* volatiles: Comparison between supercritical fluid extraction and hydrodistilation. J. Sep. Sci. *33*, 2211–2218.

HANSEN, T.B. and KNOCHEL, S. 2001. Factors influencing resuscitation and growth of heat injured *Listeria monocytogenes* 13-249 in *sous vide* cooked beef. Int. J. Food Microbiol. *63*, 135–147.

HAYOUNI, E., CHARAIEF, I., ABEDRABBA, M., BOUIX, M., LEVEAU, Y., MOHAMMED, H. and HAMDI, M. 2008. Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: their chemical compositions and their preservative effects against Salmonella inoculated in minced beef meat. Int. J. Food Microbiol. *125*, 242–251.

HYLDGAARD, M., MYGIND, T. and MEYER, R. 2012. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Front. Microbiol. *3*, 1–24.

HYYTIA-TREESS, E., SKYTTA, E., MOKKILA, M., KINNUNEM, A., LINDSTROM, M., LAHTEENMAKI, L., AHVENAINEN, R. and KORKEALA, H. 2000. Safety evaluation of *sous vide*-processed products with respect to nonproteolytic *Clostridium botulinum* by use of challenge studies and predictive microbiological models. Appl. Environ. Microbiol. *66*, 223–229.

HUDAIB, M., SPERONI, E., DI PETRA, A.M. and CAVRINI, V. 2002. GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. J. Pharm. Biomed. Anal. *29*, 691–700.

KAHRAMAN, T., ISSA, G., BINGOL, E., KAHRAMAN, B. and DUMEN, E. 2015. Effect of rosemary essential oil and modified atmosphere packaging (MAP) on meat quality and survival of pathogens in poultry fillets. Braz. J. Microbiol. *46*, 591–599.

KARAMAN, I., SAHIN, F., GULLUCE, M., OGUTÇU, H., SENGUL, M. and ADIGUZEL, A. 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol. *85*, 231–235.

MAZZARRINO, G., PAPARELLA, A., CHAVES-LÓPEZ, C., FABERI, A., SERGI, M., SIGISMONDI, C., COMPAGNONE, D. and SERIO, A. 2015. *Salmonella enterica* and *Listeria monocytogenes* inactivation dynamics after treatment with selected essential oils. Food Control *50*, 794–803.

MYTLE, N., ANDERSON, G.L., DOYLE, M.P. and SMITH, M.A. 2006. Antimicrobial activity of clove (*Syzgium aromaticum*) oil in inhibiting *Listeria monocytogenes* on chicken frankfurters. Food Control *17*, 102–107.

NEZHADALI, A., NABAVI, M., RAJABIAN, M., AKBARPOUR, M., POURALI, P. and AMINI, F. 2014. Chemical variation of leaf essential oil at different stages of plant growth and *in vitro* antibacterial activity of *Thymus vulgaris* Lamiaceae, from Iran. Beni-Suef Uni. J. Appl. Sci. *3*, 87–92.

NYATI, H. 2000. An evaluation of the effect of storage and processing temperatures on the microbiological status of sous vide extended shelf-life products. Food Control *11*, 471–476.

OJEDA-SANA, A.M., BAREN, C.M., ELECHOSA, M.A., JUÁREZ, M.A. and MORENO, S. 2013. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control *31*, 189–195.

PESAVENTO, G., CALONICO, C., BILIA, A., BARNABEI, M., CALESINI, F., ADDONA, R., MENCARELLI, L., CARMAGNINI, L., MARTINO, M. and NOSTRO, A. 2015. Antimicrobial activity of oregano, rosmarinus and thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. Food Control *54*, 188–199.

ROHANI, S., MORADI, M., MEHDIZADEH, T., SAEI-DEHKORDI, S. and GRIFFITHS, M. 2011. The effect of nisin and garlic (*Allium sativum* L.) essential oil separately and in combinations on the growth of *Listeria monocytogenes*. LWT – Food Sci. Technol. *44*, 2260–2265.

ROTA, M.C., HERRERA, A., MARTÍNEZ, R.M., SOTOMAYOR, J.A. and JORDÁN, M.J. 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control *19*, 681–687.

SALIDO, S., ALTAREJOS, J., NOGUERAS, M., SAÁCHEZ, A. and LUQUE, P. 2003. Chemical composition and seasonal variations of rosemary oil from southern Spain. J. Essent. Oil Res. *15*, 10–14.

SARKER, S.D., NAHAR, L. and KUMARASAMY, Y. 2007. Microtitre plate-based antibacterial assay incorporating

resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods *42*, 321–324.

SERGELIDIS, D. and ABRAHIM, A. 2009. Adaptive response of *Listeria monocytogenes* to heat and its impact on food safety. Food Control *20*, 1–10.

SHEARD, M. and RODGER, C. 1995. Optimum heat treatments for "*sous vide*" cook-chill products. Food Control *6*, 53–56.

SOKOVIC, M., GLAMOCILIJA, J., MARIN, P., BRKIC, D. and VAN GRIENSVEN, L. 2010. Antibacterial effects of the essential oil of commonly consumed medicinal herbs using an in vitro model. Molecules *15*, 7532–7546.

SOLOMAKOS, N., GOVARIS, A., KOIDIS, P. and BOTSOGLOU, N. 2008. The antimicrobial effect of thyme essential oil, nisin and their combinations against *Escherichia coli* O157:H7 in minced beef during during refrigerated storage. Meat Sci. *80*, 159–166.

SOUSA, P., OLIVEIRA, Á., FIGUEIREDO, C. and SOUZA, L. 2015. Influence of carvacrol and 1,8-cineole on cell viability, membrane integrity, and morphology of *Aeromonas hydrophilia* cultivated in a vegetable-based broth. J. Food Prot. *78*, 424–429.

TORRAS, J., GRAU, M., LÓPEZ, J., XAVIER, F. and HERAS, C. 2007. Analysis of essential oil from chemotypes of *Thymus vulgaris* in Catalonia. J. Sci. Food Agric. *87*, 2327–2333.

VAN VUUREN, S.F. and VIJOED, A.M. 2007. Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. Flavour Frag. J. *22*, 540–544.

VAUDAGNA, S., SÁNCHEZ, G., INSANI, E., PICALLO, A., GALLINGER, M. and LASTA, J. 2002. *Sous vide* cooked beef muscles: Effects of low temperature-long time (LT-LT) treatments on their quality characteristics and storage stability. Int. J. Food Sci. Technol. *37*, 425–441.

WANG, W., WU, N., ZU, Y. and FU, Y. 2008. Antioxidant activity of *Rosmarius officinalis* L. essential oil compared to its main components. Food Chem. *108*, 1019–1022.