

Research Paper

Essential oils of thyme and Rosemary in the control of *Listeria monocytogenes* in raw beef

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Abstract

This study was developed in order to evaluate two alternatives for the control of *Listeria monocytogenes* in raw bovine meat pieces, both based on the use of *Thymus vulgaris* and *Rosmarinus officinalis* essential oils (EOs). The antilisterial activity of different concentrations of the EOs was tested *in vitro* using agar dilution and disk volatilization techniques. In addition, *L. monocytogenes* was inoculated in meat pieces, which were submerged in edible gelatin coatings containing 2% (v/v) EOs or submitted to the vapor of EOs (0.74 $\mu\text{L}\cdot\text{cm}^{-3}$). *L. monocytogenes* was quantified after one, 48 and 96 hours of storage (7 °C). In the *in vitro* tests, the EO of *T. vulgaris* presented higher activity. The two options used (edible gelatin coating and vapor activity), in spite of exercising effects with differentiated behaviors, presented antibacterial activity against *L. monocytogenes* inoculated in raw bovine meat ($p < 0.05$). Greatest antibacterial activity were obtained in the experiment that used edible coatings containing EOs, at 48 hours of storage reductions in bacterial counts between 1.09 and 1.25 Log CFU.g⁻¹ were obtained. In the vapor effect experiment, the EO of *T. vulgaris* caused the highest reduction in the population of bacteria inoculated in raw bovine meat ($p < 0.05$), 0.40 Log CFU.g⁻¹ at 96 hours of storage. This study supplied important information regarding new and promising natural alternatives, based on the concept of active packaging, for the control of *L. monocytogenes* in the meat industry.

Key words: *Thymus vulgaris*, *Rosmarinus officinalis*, antilisterial compounds, active packaging, meat safety.

Introduction

The contamination of ready-to-eat (RTE) foods, especially meat products, with *L. monocytogenes* has been related as a serious public health problem (Sofos, 2008; Mor-Mur and Yuste, 2010). However, another fact that deserves equal attention is the presence of this pathogenic bacterium in raw bovine meat, which has already been reported in some recent studies (Chen *et al.*, 2009; Karakolev, 2009; Pesavento *et al.*, 2010). These facts demonstrate the presence of *L. monocytogenes* in the meat chain and em-

phasize that, besides good manufacturing practices, control alternatives should be development.

Several alternatives are available for the control of microorganisms in the food industry. One of them is the use of active packaging with antimicrobial effects, such as the surface application of coatings and edible films elaborated with proteins, polysaccharides or lipids, and the liberation of volatile compounds in the atmosphere surrounding the food (Devlieghere *et al.*, 2004; Bourtoom, 2008; Ustunol, 2009). This control options are extremely effective in the case of foods with mostly surface microbiological contami-

nation, such as raw bovine meats marketed as steaks or in various sized pieces.

Also, in this context, there is an increasing demand for safe and natural foods without the addition of chemical preservatives, which has been motivating research on the antimicrobial effects of plant derivatives (Dimitrijevic *et al.*, 2007). An example of those substances is the essential oils (EOs), known for presenting biological activity, especially antibacterial, antifungal and antioxidant properties (Burt, 2004; Tajkarimi *et al.*, 2010). The antilisterial activity of different EOs was demonstrated *in vitro* in some recent studies. Oliveira *et al.* (2012) verified that the EOs of *Cinnamomum cassia* (Chinese cinnamon), *Melaleuca alternifolia* (tea tree) and *Cymbopogon flexuosus* (lemongrass) were effective against *L. monocytogenes* ATCC 19117 inoculated in agar and broth culture medium. Andrade *et al.* (2012) and Millezi *et al.* (2012), studying the same strain of *L. monocytogenes*, verified, respectively, that the EOs of *Cymbopogon nardus* (citronella), *Cinnamomum zeylanicum* (cinnamon) and *Zingiber officinale* (ginger), and the EOs of *Thymus vulgaris* (thyme), *Cymbopogon citratus* (lemongrass) and *Laurus nobilis* (bay), presented antilisterial activity.

Interesting alternatives to control *L. monocytogenes* are the EOs of *T. vulgaris* and *Rosmarinus officinalis* (rosemary), spices that are used to provide flavor to foods, such as meats and their derivatives. The antilisterial activity of these EOs has already been demonstrated *in vitro* (Pandit and Shelef, 1994; Dimitrijevic *et al.*, 2007; Singh *et al.*, 2003; Solomakos, 2008), as well as in food products, such as pork liver sausage (Pandit and Shelef, 1994), hotdogs (Singh *et al.*, 2003) and minced beef meat (Solomakos *et al.*, 2008).

This study was developed with the intention of evaluating two new alternatives for the control of *L. monocytogenes* in raw bovine meat pieces, both based on the use of *T. vulgaris* and *R. officinalis* EOs as antibacterial agents: the submission to their vapors and the application of edible gelatin coatings.

Material and Methods

Microorganism used, standardization, inoculum preparation and storage

The microorganism used was *L. monocytogenes* ATCC 19117, acquired from the Culture Collection Section of the Medical Biology Division of the Adolfo Lutz Institute (São Paulo, SP, Brazil). For standardization, inoculum preparation and storage were followed the procedures previously described by Oliveira *et al.* (2010a, 2010b).

EOs

EOs of leaves and flowers of *T. vulgaris* and leaves of *R. officinalis* were acquired from Ferquima Indústria e Comércio Ltda. (Vargem Grande Paulista, SP, Brazil).

Chemical composition

A gas chromatography (GC) apparatus coupled to a mass spectrometry (MS) apparatus (GCMS-QP 2010 Plus, Shimadzu) was used. The EOs, diluted in dichloromethane, were injected with a AOC-5000 Auto Injector (Shimadzu). The chromatographic conditions were the following: column oven temperature 40 °C, injection temperature 220 °C, splitless injection mode, sampling time 0.50 min, He carrier gas, pressure 60.2 Kpa, total flow 14 mL.min⁻¹, column flow 1 mL.min⁻¹, linear velocity 36.1 cm.s⁻¹, purge flow 3 mL.min⁻¹, split ratio 10.0, total program time 54.50 min, column Equity-5 (30 m x 0.25 mm x 0.25 µm), ion source temperature 200 °C, interface temperature 250 °C and solvent cut time 4 min. The components were identified by comparing their mass spectra and retention times with those available in the Wiley 8 Library and Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) 1.2 Library. The percentage of each component was obtained by integrating the peak area.

Antilisterial activity of EOs in culture medium

Dilution in agar

EOs were homogenized in Tryptic Soy Agar (TSA) (Himedia®, Mumbai, Maharashtra, India) supplemented with 0.6% (w/v) yeast extract (Himedia®, Mumbai, Maharashtra, India) (TSA-YE) and containing 0.5% (v/v) Tween 80 (Vetec, Duque de Caxias, RJ, Brazil) to obtain the following concentrations: 0.00, 0.03, 0.06, 0.12, 0.25, 0.50, 1.00 and 2.00% (v/v). The culture medium containing EOs (8 mL) was distributed in Petri dishes (50 mm diameter), and three repetitions were conducted. Aliquots (40 µL) of Tryptic Soy Broth (TSB) (Himedia®, Mumbai, Maharashtra, India) containing 8 Log CFU.mL⁻¹ of *L. monocytogenes* were inoculated on the surface of the culture medium and spread with a Drigalski spatula. The incubation was performed at 37 °C/24 hours. The lowest concentrations that resulted in complete inhibition of the bacterial growth were called Minimum Inhibitory Concentrations (MICs) (Oliveira *et al.*, 2012).

Disk volatilization

Filter paper discs (30 mm diameter) were fastened in the center of the lower part of the cover of glass Petri dishes (90 x 16 mm), which contained 20 mL of TSA-YE, with the aid of double-faced adhesive tape. The distance between the surface of the culture medium and the disc was 13 mm. EOs were added to the discs at concentrations of 0.18, 0.36, 0.54 and 0.72 µL.cm⁻³. At the highest concentration, the EO was used pure (60 µL); for the other concentrations, the EO

was diluted in ethanol: 0.18 $\mu\text{L}\cdot\text{cm}^{-3}$ (15 μL of EO and 45 μL of ethanol); 0.36 $\mu\text{L}\cdot\text{cm}^{-3}$ (30 μL of EO and 30 μL of ethanol); and 0.54 $\mu\text{L}\cdot\text{cm}^{-3}$ (45 μL of EO and 15 μL of ethanol). A Petri dish with a filter paper disc containing only ethanol (60 μL) and a Petri dish without filter paper disc were also used. TSB-YE, containing 8 Log CFU.mL⁻¹ of *L. monocytogenes*, was inoculated (100 μL) on the culture medium and spread with a Drigalski spatula. The Petri dishes were sealed with Polyvinyl Chloride (PVC) plastic film and incubated (37 °C/24 hours). The diameter of the inhibition zones were measured using a digital caliper rule, acquiring the average of two diametrically opposed measurements. This methodology was adapted from López *et al.* (2005, 2007) and Nedorostova *et al.* (2009).

Antilisterial activity of EOs in raw beef

Acquisition and preparation of beef

The raw bovine meat (*Quadriceps femoris*) was acquired commercially vacuum packed wrapped. To reduce the accompanying microbiota, a layer of approximately 1 cm of meat was removed from the external surface of each piece.

Edible gelatin coating

For bacterial inoculation, meat pieces (5 x 5 x 2.5 cm and, approximately, 50 g) were submerged in TSB, containing 8 Log cfu.mL⁻¹ of *L. monocytogenes*, for ten minutes (Figure 1A) and, subsequently immediately, air-dried for one hour suspended by a support (adapted from 20). For the air-dried step, the meat pieces were fixed to hooks that were fastened to wires, both of stainless steel (Figure 1B).

Two coatings containing EOs were elaborated: CT, for *T. vulgaris* EO; and CR, for *R. officinalis* EO. A coating without the addition of EOs was also used, and was called CW.

In the Table 1 are the edible coatings solutions formulations. To the solutions preparation were followed the procedures described below. The powdered edible gelatin (24 g) was added slowly, under agitation, in the smallest volume of sterile distilled water (50 mL for CT and CR, and 70 mL for CW), called part A. The mixture was microwaved until completely dissolved (1-2 min). The gelatin had a bloom of 240 and was manufactured by Gelco Gelatinas do Brasil Ltda. Fábrica de Conservas for Kraft Foods Brasil S.A. (Pedreira, SP, Brazil). EOs (20 mL), when used, were added to 30 mL of a Tween 80 solution (25% v/v in distilled sterile water) and homogenized by vigorous agitation. The final percentage of EO in CT and CR was 2% (v/v). Finally, all the components mentioned above were added, together with 900 mL of sterile distilled water (part B), to one container and homogenized slightly for the elaboration of the coatings solutions, that presented a final volume of 1000 mL (Figure 1C).

CT and CR contained the highest concentration of EOs that was tested *in vitro* (2% v/v) because previous tests demonstrated that the MICs founded by the agar dilution method did not presented antilisterial effect when used in gelatin coatings applied over raw bovine meat.

For the coatings application, the meat pieces were submerged in the solutions presents in Table 1 for ten seconds and, subsequently immediately, air-dried for ten minutes (adapted from 20) (Figure 1D).

Additionally, the following treatment was elaborated: meat pieces inoculated with *L. monocytogenes* and not submitted to coatings application. This treatment was called WC.

The meat pieces were conditioned in cylindrical polyethylene terephthalate (PET) packaging (85 x 43 mm internal dimensions, 170 mL capacity, transparent) (Figure 1E), that were wrapped in plastic film (PVC) and stored at 7 °C.

Vapor activity

Meat pieces were cut (6 x 6 x 0.6 cm, approximately 30 g) and put in Petri dishes (90 x 16 mm) that contained filter paper discs (30 mm diameter) fastened to the central internal part of the dish covers with the aid of double-faced adhesive tape. For inoculation, that was conducted immediately after placing the meat pieces inside the Petri dishes, 100 μL of peptone water 0.1% (w/v) containing 5 Log CFU.mL⁻¹ of *L. monocytogenes* was added in the center of the surface of the meat pieces with the aid of Drigalski spatulas. Pure EOs (60 μL) were added to the filter paper discs at a concentration of 0.74 $\mu\text{L}\cdot\text{cm}^{-3}$.

The meat pieces were submitted to EOs only at the concentration of 0.74 $\mu\text{L}\cdot\text{cm}^{-3}$, because it was similar to the highest concentration used in the disc volatilization (0.72 $\mu\text{L}\cdot\text{cm}^{-3}$), and because previous tests demonstrated that the other concentrations (0.18, 0.36 and 0.54 $\mu\text{L}\cdot\text{cm}^{-3}$) that were used *in vitro* did not inhibit the bacterium in the meat.

A treatment composed of meat pieces put in Petri dishes without filter paper discs with EOs was used also. The Petri dishes were sealed with plastic film (PVC) and stored (7 °C).

The treatments were denominated: VT, when the meat was submitted to EO of *T. vulgaris*; VR, when it was submitted to EO of *R. officinalis*; and WV, when no EO was applied.

Quantification of *Listeria monocytogenes*

The analyses were conducted after one, 48 and 96 hours. For the coating evaluation experiment, 25 g of meat was added to 225 mL peptone water. For vapor activity, 10 g was added in 90 mL peptone water. The homogenization was conducted in Stomacher (490 strokes/minute for two minutes). Aliquots of 1 mL were removed, and serial decimal dilutions were performed in 9 mL of peptone water 0.1% (w/v). Surface plating was performed on Oxford agar



Figure 1 - Main stages of the experiment that evaluated the antibacterial activity of edible gelatin coatings containing essential oils (EOs) of *Thymus vulgaris* and *Rosmarinus officinalis* against *Listeria monocytogenes* inoculated in raw beef pieces. (A) *L. monocytogenes* inoculation in raw beef pieces. (B) Drying of the raw beef pieces after the bacterial inoculation step. (C) Recipients containing the edible coating solutions of gelatin, from left to right: solution without the addition of EOs, solution containing *T. vulgaris* EO, solution containing *R. officinalis* EO. (D) Drying of the raw beef pieces after the edible coating application. (E) Raw beef piece with the application of edible gelatin coating containing *T. vulgaris* EO inside a polyethylene terephthalate packaging, after 1 hour storage at 7 °C.

(Himedia®, Mumbai, Maharashtra, India), and the incubation was conducted at 37 °C/24 hours.

Experimental designs and statistical analyses

For the disc volatilization stage, a Completely Randomized Design (CRD) was used with three repetitions. When detected significant differences in the variance analysis, regression testing was performed.

For the coating experiments, a CRD was used, with four repetitions, in a 4 x 3 factorial outline (four applied meat treatments and three storage times). When significant differences in the variance analysis were detected, the Scott-knott test on the qualitative values and regression testing on the quantitative values were performed.

For the determination of vapor activity, a CRD was used, with four repetitions, in a 3 x 3 factorial outline (three applied meat treatments and three storage times). When significant differences in the variance analysis were detected, the Scott-knott test on the qualitative values and regression testing on the quantitative values were performed.

The software Sisvar version 5.3 was used.

Results and Discussion

Chemical composition of EOs

In the EOs of *T. vulgaris* and *R. officinalis*, 13 and 16 constituents were identified, respectively. In *T. vulgaris*, there was 28.07% of the phenylpropanoid thymol, followed

Table 1 - Composition of edible coatings.

Constituents	Edible coatings		
	CT ^a	CR ^b	CW ^c
Edible gelatin powder (g)	24	24	24
Sterile distilled water (part A) (mL)	50	50	70
Tween 80 at 25% (v/v) in sterile distilled water (mL)	30	30	30
Sterile distilled water (part B) (mL)	900	900	900
Essential oil of <i>Thymus vulgaris</i> (mL)	20	NU ^d	NU ^d
Essential oil of <i>Rosmarinus officinalis</i> (mL)	NU ^d	20	NU ^d

^aMeat with edible gelatin coating containing essential oil *Thymus vulgaris*.

^bMeat with edible gelatin coating containing essential oil *Rosmarinus officinalis*. ^cMeat with edible gelatin coating without essential oil. ^dNot used.

by 18.89% of para-cymene and 12.70% of linalool, both monoterpenes. For *R. officinalis*, only monoterpenes were observed as the majority constituents: 1,8-cineole (33.10%), camphor (19.03%), alpha-pinene (11.55%) and beta-pinene (10.27%) (Table 2). Dimitrijevic *et al.* (2007)

similarly verified thymol (17.37%), para-cymene (15.3%) and carvacrol (12.10%) as the majority in the EO of *T. vulgaris*. For *R. officinalis*, they found a prevalence of 1,8-cineole (52.20%) and camphor (10.08%).

Antilisterial activity of EOs in culture medium

Dilution in agar

In the agar dilution technique, the EO of *T. vulgaris* presented higher activity, with a MIC of 0.25% (v/v) against 1.00% (v/v) of *R. officinalis* EO. Several studies founded in the literature also reported the *in vitro* potential of different EOs in the control of *L. monocytogenes*, and, in general, what is observed is that the EOs of *T. vulgaris*, *Cinnamomum* and *Cymbopogon* species stand out in terms of antilisterial activity (Oliveira *et al.*, 2012; Andrade *et al.*, 2012, Millezi *et al.*, 2012). A similar result to the one obtained in this study was founded by Singh *et al.* (2003), that also verified a lower MIC against *L. monocytogenes* for *T. vulgaris* EO in comparison with the value obtained for *R. officinalis*.

Differences in the antibacterial activity among EOs of different plant species are attributed to the chemical com-

Table 2 - Chemical composition of essential oils of *Thymus vulgaris* and *Rosmarinus officinalis*.

Constituents	Essential oils			
	<i>Thymus vulgaris</i>		<i>Rosmarinus officinalis</i>	
	Tr ^a	% ^b	Tr ^a	% ^b
Alpha-pinene	10.267	6.16	10.335	11.55
Camphene	10.797	3.01	10.804	3.11
Beta-pinene	11.854	1.51	11.918	10.27
Myrcene	12.462	2.78	12.462	1.33
Para-cymene	13.854	18.89	-	-
Limonene	13.926	2.61	-	-
1, 8-Cineole	14.008	1.40	14.180	33.10
Gamma-Terpinene	15.071	8.70	15.037	0.81
Linalool	16.683	12.70	16.613	1.51
Camphor	-	-	18.403	19.03
Isoborneol	-	-	19.086	3.34
Terpinen-4-ol	-	-	19.490	0.82
Alpha-terpineol	20.209	3.62	19.985	1.78
Bornyl acetate	-	-	23.349	0.94
Thymol	23.903	28.07	-	-
Carvacrol	24.067	4.63	-	-
Eugenol	-	-	25.757	0.78
Alpha-copaene	-	-	26.422	0.54
Trans (beta)-caryophyllene	-	-	27.924	7.63
Alpha-Humulene	-	-	28.945	1.58
Caryophyllene oxide	32.927	4.01	-	-
Total	-	98.09	-	98.12

^aRetention time. ^bThe percentages represent the integration of the areas of the peaks. The numbers in bold represent the major components.

position and the relative proportions of their individual constituents (Viuda-Martos *et al.*, 2008). Usually, EOs that possess the stronger antibacterial properties contain high concentrations of phenolic compounds, such as carvacrol, eugenol and thymol (Burt, 2004; Dorman and Deans, 2000), the last one founded in the EO of *T. vulgaris* that was used in this study.

Disc volatilization

The higher activity of *T. vulgaris* was again observed. For *T. vulgaris*, the diameters of the inhibition zones formed on the surface of the culture medium below the filter paper disc increased linearly with the increase of EO concentration, varying from 51.46 to 67.50 mm (Figure 2). For the EO of *R. officinalis*, there was no formation of an inhibition area; however, it was possible to observe a discreet reduction of bacterial growth, especially at the highest concentration ($0.72 \mu\text{L}\cdot\text{cm}^{-3}$). The effect of the EO vapor of *T. vulgaris* against *L. monocytogenes* has already been determined *in vitro* by López *et al.* (2007) and Nedorostova *et al.* (2009). Regarding *R. officinalis*, a similar result to that obtained by López *et al.* (2005) was verified, who demonstrated that its vapor did not present activity against *L. monocytogenes* in culture medium.

Antilisterial activity of EOs in raw beef

Edible gelatin coating

L. monocytogenes population in the meat pieces increased linearly during the storage days independently of the treatment. However, the coatings containing EOs (CT and CR) were effective against *L. monocytogenes* inoculated in raw bovine meat at 48 hours of storage, because their medium average counts were lower than those of the control treatments (CW and WC) ($p < 0.05$). The antilisterial effect of the coatings containing EOs (CT and CR) increased from the first hour of storage until the 48th; however, it decreased after 96 hours (Table 3). This demonstrates that, in spite of the antibacterial substance being gradually liberated from the coating and being able to in-

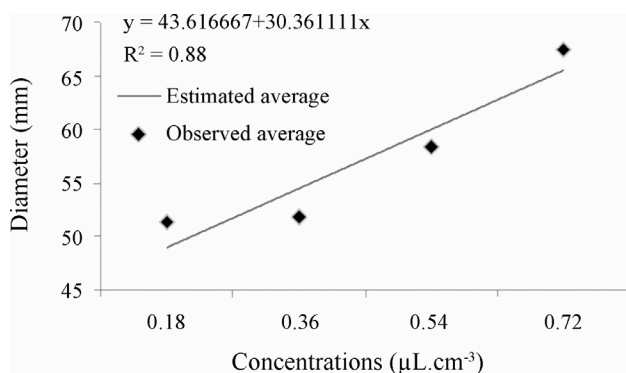


Figure 2 - Effect of the *Thymus vulgaris* essential oil vapor on the formation of *Listeria monocytogenes* inhibition zones on the culture medium surface after incubation at 37 °C for 24 hours.

Table 3 - Effect of the use of different edible gelatin coatings in the counts of *Listeria monocytogenes* in pieces of raw beef stored at 7 °C.

Treatments applied to beef	Bacterial counts (Log cfu.g ⁻¹)		
	1 hour	48 hours	96 hours
CT ^a	4.44 ± 0.32 ^A	5.00 ± 0.22 ^A	5.63 ± 1.01 ^A
CR ^b	4.48 ± 0.59 ^A	5.04 ± 0.22 ^A	5.94 ± 1.04 ^A
WC ^c	5.46 ± 0.72 ^A	6.13 ± 0.66 ^B	5.94 ± 1.17 ^A
CW ^d	5.30 ± 1.22 ^A	6.25 ± 0.42 ^B	6.64 ± 0.21 ^A

The values are de averages of three repetitions ± the standard deviation. Averages followed by the same uppercase letter in the same column do not differ among themselves by the Scott-Knott test, to 5% of probability. ^aMeat with edible coating of gelatin containing essential oil of *Thymus vulgaris*. ^bMeat with edible coating of gelatin containing essential oil of *Rosmarinus officinalis*. ^cMeat without edible gelatin coating. ^dMeat with edible gelatin coating without essential oil.

hibit the bacterial population that grew on the food surface, this effect only increased until 48 hours. Starting from this growth stage, *L. monocytogenes* in the meat was not controlled with the same efficiency. This might have occurred due to the high initial bacterial population, the psychrotrophic characteristic of this species or the presence of an adequate substrate (raw bovine meat) for its development, presenting appropriate intrinsic factors, such as water activity (0.99), pH (approximately 5.6) and nutrient availability (water, proteins, lipids, carbohydrates, soluble non-protein substances and vitamins) (Jay *et al.*, 2005; Lado and Yousef, 2006; Lawrie and Ledward, 2006).

The counts of *L. monocytogenes* in the meats with CT remained slightly lower than in those with CR during the storage days. However, there was no significant difference between the average performance of CT and CR ($p > 0.05$) (Table 3). This fact suggests that the antibacterial activity of EOs in food matrix can differ from its *in vitro* effect, where the EO of *T. vulgaris* was higher.

Even using EO concentrations above the MICs obtained *in vitro* in the elaboration of the coatings, they were not capable of eliminating or completely inhibiting the population of *L. monocytogenes* present in the meat matrix (Table 3). Some authors have demonstrated that the antibacterial effect of EOs can be reduced in the food matrix as a result of the interaction with different food components, which leads to the need for higher concentrations to reduce the bacterial population (Singh *et al.*, 2003; Smith-Palmer *et al.*, 2001). Furthermore, in studies conducted as challenge tests, *L. monocytogenes* was inoculated in the food at concentrations that, most of the time, exceeded the natural contamination levels usually found, which can decrease the effectiveness of the treatments (Neetoo *et al.*, 2010). The microbial growth in naturally contaminated products can be significantly slower (Dalgaard and Jørgensen, 2000), and therefore, the effect of antimicrobial coatings can be more pronounced in fact than in studies where the microorganism was inoculated (Neetoo *et al.*, 2010).

Other studies have also verified the antibacterial action of EOs added in films or edible coatings applied in raw fish and raw bovine meat (Lu *et al.*, 2010; Min and Oh, 2009; Ojagh *et al.*, 2010; Oussalah *et al.*, 2004; Zinoviadou *et al.*, 2009), and one of them also used the EO of *T. vulgaris* (Emiroglu *et al.*, 2010). A result similar to what we observed in regard to the control of *L. monocytogenes* was found by Gill *et al.* (2001), who observed that the coating of hams with gelatin gel containing 6% (v/v) *Coriandrum sativum* EO emulsified with lecithin inhibited the growth of this bacterium.

Vapor activity

The population of *L. monocytogenes* exhibited a slight linear decrease during the storage period independently of submission to the EO vapors. The vapor of *T. vulgaris* EO (VT) was effective against *L. monocytogenes* inoculated in raw bovine meat at 96 hours of storage, because their average counts were lower than those of the control treatment (WV) ($p < 0.05$). Notably, the effect of VT against *L. monocytogenes* inoculated in the meat increased with storage time, as shown by the reduction of the counts in relation to WV (Table 4). This result demonstrates that the vapor of this EO was gradually liberated, and it remained active during the entire storage time of the product. The higher antilisterial effect of *T. vulgaris* EO when compared with *R. officinalis* EO verified in the meat inoculation experiment was also observed *in vitro*, where the incubation temperature (37 °C) was more favorable for rapid volatilization of the compounds (Burt *et al.*, 2007).

Skandamis and Nychas (2002) verified the increase in shelf life of raw bovine meat stored in a modified atmosphere together with filter paper squares containing *Origanum vulgare* EO put inside the packaging but not in contact with the meat. Burt *et al.* (2007) verified that carvacrol in the vapor phase was able to reduce or eliminate the population of *Salmonella enterica* serotype Enteritidis inoculated in raw chicken meat. However, no reports were found in the literature regarding the activity of *T. vulgaris* and *R. officinalis* EO vapors against bacteria present in

Table 4 - Effect of the submission to the vapor of the essential oils in the counts of *Listeria monocytogenes* in pieces of raw beef stored at 7 °C.

Treatments applied to beef	Bacterial counts (Log cfu.g ⁻¹)		
	1 hour	48 hours	96 hours
VT ^a	4.51 ± 0.07 ^A	4.30 ± 0.08 ^A	3.88 ± 0.23 ^A
VR ^b	4.34 ± 0.03 ^A	4.20 ± 0.04 ^A	4.15 ± 0.16 ^B
WV ^c	4.60 ± 0.08 ^A	4.47 ± 0.08 ^A	4.28 ± 0.33 ^B

The values are de averages of three repetitions ± the standard deviation. Averages followed by the same uppercase letter in the same column do not differ among themselves by the Scott-Knott test, to 5% of probability. ^aMeat subjected to the vapor of the essential oil of *Thymus vulgaris*. ^bMeat subjected to the vapor of the essential oil of *Rosmarinus officinalis*. ^cMeat not subjected to the vapor of essential oils.

foods or the activity of EO vapors against *L. monocytogenes* inoculated in foods.

Conclusions

The two options used (edible gelatin coating and vapor activity), in spite of exercising effects with differentiated behaviors, presented antibacterial activity against *L. monocytogenes* inoculated in raw bovine meat. However, we emphasize that the greatest reductions were obtained in the experiment that evaluated the antilisterial activity of edible coatings containing EOs.

This study supplied important information regarding new and promising natural alternatives for the control of this pathogenic bacterium in the meat industry. These alternatives are based on the concept of active packaging with the use of EOs of *T. vulgaris* and *R. officinalis* as antibacterial agents.

We conclude that, to prove the real effectiveness and viability of these alternatives studies with naturally contaminated meat must be conducted accompanied by an assessment of the sensory acceptability of these products.

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