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Anti-Listerial Activity of Oregano and Cinnamon Essential Oils in Vacuum Packed Ground Ovine Meat during Refrigerated Storage

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ABSTRACT

This work aimed to study the antibacterial activity of various Essential Oils (EOs) against food borne pathogens as well as the effect of their incorporation at different concentrations on vacuum-packed ground ovine meat, experimentally inoculated with *Listeria monocytogenes*, during 12 days of storage at 4°C. In summary, pathogenic bacteria, particularly *L. monocytogenes, Salmonella enteritidis* and *Escherichia coli*, showed high sensitivity towards citrus, rosemary, thyme, cinnamon and oregano EOs due to their richness in bioactive compounds. The Minimum Inhibitory Concentrations (MICs) of various EOs against *L. monocytogenes* and *E. coli* were about 0.5% for oregano and thymus EOs and 0.7% for cinnamon EO. Besides, addition of EOs at different concentrations resulted in the improvement of biochemical and microbiological qualities of ground vacuum packed sheep meat, during refrigerated storage. High concentrations of oregano (1%) and cinnamon (1.4%) EOs had the most efficient antilisterial activities compared to the control and other meat samples. The treatment of ovine meat with oregano or cinnamon EOs preserved a better content of proteins, a high ratio of PUFAs and a favorable balance between w-6 and w-3 PUFA, resulting in the production of healthier meat.

Keywords: Food preservatives, Meat quality, Sheep meat preservation, Vacuum packaging.

INTRODUCTION

Sheep meat has high cultural and ritual value (Prache *et al*., 2022). For consumers, sheep meat is within food traditions in many countries and is considered as food with particular sensory attributes (Prache *et al*., 2022). Also, ovine meat is a good source of highly digestible essential amino acids and valuable vitamins and micronutrients (Prache *et al*., 2022). However, as well as meat products, it may be contaminated by several pathogens including *E. coli, Salmonella* spp., *Clostridium* spp. and *L. monocytogenes*) (Yousefi *et al*., 2020; Prache *et al*., 2022). Food poisoning caused by pathogens, mainly *L. monocytogenes,* leads to serious illness and 30% rate of mortality among patients (Cho *et al*., 2020). This foodborne pathogenic bacteria is able to grow in minimal nutrients, at temperatures between 1 and 45°C (Boulares *et al*., 2017) and it can grow in a variety of foods (Yousefi *et al*., 2020). For this reason, many techniques are used for quality preservation and safety of meat products such as super-chilling, high pressure, and addition of chemical preservatives. This later strategy has some drawbacks like the neurotoxic or carcinogenic effect (Agrimonti *et al*., 2019). In this regard, naturally occurring compounds like Essential Oils (EOs) have significant potential as food preservatives due to their notable antimicrobial activity against different food pathogens and spoilage microorganisms (Boulares *et al*., 2018; Agrimonti *et al*., 2019). EOs are natural volatile aromatic oils extracted from various parts of plants, and known to be rich in natural bioactive

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compounds (Yousefi *et al*., 2020). They have received "Generally Recognized As Safe (GRAS)" status and are commercially used as natural preservatives (Cho *et al*., 2020) in various food products like fruits, meats, fish, and processed foodstuffs (Abdollahzadeh *et al*., 2014; Boulares *et al*., 2018). In meat industry, there is an increased attention on natural plant extracts for their potential antioxidant, antimicrobial, and flavoring activities (Yousefi *et al*., 2020). Moreover, the intrinsic properties of foods as well as the extrinsic determinants (temperature, vacuum packaging, targeted germ) can influence bacterial sensitivity (Abdollahzadeh *et al*., 2014). Besides, large quantities of EOs are applied to improve the antimicrobial activity (Yousefi *et al*., 2020) but can adversely alter the sensorial properties of final products (Yu *et al*., 2021). Thus, many approaches suggested the incorporation of EOs to edible films, their combination with new packaging methods (Yousefi *et al*., 2020) or with other compounds (Boulares *et al*., 2018; Yu *et al*., 2021)

In this context, the purpose of the present study was: (1) To determine the antibacterial effect of lemon, rosemary, oregano, cinnamon and thyme EOs against selected Gram-positive and Gram-negative food contaminants, and (2) To evaluate the effect of oregano and cinnamon EOs on microbiological and biochemical quality of ground ovine meat during vacuum storage at 4 °C in order to confirm their antagonistic activity against *L. monocytogenes* in food matrix.

MATERIALS AND METHODS

Antimicrobial Activities of Essential Oils

Essential Oils

The EOs of lemon (*Citrus limonum*) (57.05% of limonene) and rosemary (*Rosmarinus officinalis*) (45.97% of 1,8-cineol) were produced by the laboratory of medicinal plants LPM CERINA (Nabeul, Tunisia).

The EOs of oregano (*Origanum compactum*) (46.56% of carvacrol), cinnamon (*Cinnamomum verum*) (79.82% of cinnamaldehyde) and thyme (*Thymus zygis*) (62.08% of thymol) were purchased from a pharmacy in Manouba, Tunisia. All samples were stored at 4°C before use.

Microbial Strains

The antibacterial activity of the tested EOs was evaluated against a number of Gram-positive and Gram-negative bacteria including (i) Pathogens: *Pseudomonas aeruginosa* ATCC 2134, *Salmonella enteritidis* DMB 560, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6835, *Listeria monocytogenes* ATCC 19115; (ii) Psychrotrophic bacteria: *Pseudomonas fluorescens* DMS 50090, *Pseudomonas putida* DSM 291, *Aeromonas hydrophila* ATCC 7966, (iii) Lactic Acid Bacteria (LAB) : *Lactobacillus plantarum* ATCC 14917, *Lactococcus lactis* KF147, *Leuconostoc mesenteroides* ATCC 8293, *Lactobacillus paracasei* ATCC 25302 and *Carnobacterium piscicola* AT 71101238000999. Working cultures were stored at -20°C and when necessary, grown overnight in nutrient broth (Biolife, Milan, Italy) at appropriate temperature.

Disc Diffusion Method

The antibacterial activity of various EOs against the target bacteria was evaluated using the disc diffusion method by measuring the diameter of the inhibition zones in mm observed around the paper dishes (6 mm in diameter) impregnated with EOs on Mueller Hinton Agar (Biokar) inoculated with bacterial suspension at 10^8 CFU mL^{-1} (El Adab *et al*., 2016). The paper dishes were impregnated with 10, 20, 30, 40 and 50 µL of each tested pure EO. After incubation at appropriate temperature for 24 hours, the bacterial sensitivity toward EOs was classified from not sensitive to extremely sensitive according to Ponce *et al*. (2003).

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Minimal Inhibitory Concentration (MIC)

The microdilution method was used to determine the MICs of oregano, thyme and cinnamon EOs against *L. monocytogenes* and *E. coli* showing the highest sensitivity in disc diffusion method. This analysis was carried out in Mueller Hinton Broth (Biokar) supplemented with different concentrations of EOs (0 to 4%, v/v) and Tween 80 (0.5%, v/v) to ensure EOs solubility. All bacterial inoculums in exponential growth adjusted at 10^8 CFU mL^{-1} were added to each EO dilution emulsified in Tween 80 and, then, incubated at 37°C for 24 hours. The MICs were determined based on the measurement of the bacterial growth after incubation of 100 µL of the inoculums on the solid culture mediums suitable for each bacteria. The MIC is defined as the lowest EO concentration able to produce a reduction of 90% in the bacterial growth.

Meat Quality Assessment during Storage

Meat Preparation, Treatment, and Storage

Thigh muscle from sheep meat was purchased from a butcher shop at Tunis at 1 h post-slaughter and kept at refrigeration temperature throughout transportation to the laboratory. Under sterile conditions, fresh sheep meat was grounded and inoculated with 4 log CFU/g of *L. monocytogenes* (Abdollahzadeh *et al*., 2014) before being homogenized during 2 min in a stomacher and, then, divided into batches. One batch was served as control (L). The remaining inoculated batches were treated with oregano EO at concentrations of 0.7 (O1L) and 1.4 (O2L) (v/w) mL 100 g^{-1} and cinnamon EO at concentrations of 0.5 (C1L) and 1 (C2L) (v/w) mL 100 g^{-1}. After that, samples were homogenized in a stomacher to ensure uniform distribution of added compounds. Obtained samples were further sampled (day 0), vacuum packed into polystyrene, keeping boxes and cold stored at 4°C for up to 12 days (Govaris *et al*., 2010).

Sampling for pH, proteins as well as microbiological analysis was evaluated on days 0, 3, 6, 9 and 12. Free fatty acids determination was performed at the beginning and the end of storage period.

Microbiological Analysis

Mesophilic Aerobic Plate Count (MAPC) and Psychrotrophic Bacterial Count (PBC) were enumerated on Plate Count Agar (Oxoïd, Ltd., Basingstoke, England) after incubation at 30°C for 72 hours and 7°C for 10 days, respectively. Lactic Acid Bacteria (LAB) were counted using de Man, Rogosa, and Sharpe agar medium (Biokar) after incubation of plates at 37°C for 48 hours. Coliforms were enumerated using desoxycholate gelose and incubated at 30°C for 24 hours. *L. monocytogenes* was counted using Palcam agar (Biokar) and incubated at 37°C for 48 hours (Boulares *et al*., 2017).

pH Measurement

pH measurement was determined at room temperature, on different homogenized ground sheep meat samples in distilled water (1:10 w/v) using a pH-meter (WTW portable pH-meter pH 315i/SET. Wissenschaftlich) (Boulares *et al*., 2018).

Proteins Determination

Nitrogen content was determined using Kjeldhal method according to ISO 937 (1978). Crude protein was calculated by multiplying the nitrogen content by the conversion factor 6.25 as specified in Commission Regulation (EC) No 543/2008.

Fatty Acids Composition

The extraction of lipid fraction and Fatty Acids (FAs) content were determined according to Boulares *et al*. (2017).

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Chloroform/methanol (2:1 v/v) solution was used for the extraction of lipids. Then, fatty acids content was performed based on gas chromatography injection of methyl esters of fatty acids stored at –40°C. The proportion of each fatty acid was expressed as a percentage of the total fatty acids.

Statistical Analysis

Results of descriptive analyses were presented as mean and Standard Deviation (SD). The obtained results were performed in triplicates, in two individual replications. A one-way Analysis Of Variance (ANOVA) in SPSS software version 20 (IBM Corp 2011) was used. Duncan's test was performed at a significance level of 5% to highlight significant differences among the samples and during storage time.

RESULTS AND DISCUSSION

Antibacterial Effect of Essential Oils

Antibacterial Activity on Laboratory Medium

The results of the inhibitory effect of five tested EOs (citrus, rosemary, thyme, cinnamon and oregano) on the growth of the target strains are shown in Table 1. While LAB were resistant to almost all tested EOs (except of the oregano, possessing mild antibacterial effect), pathogenic and psychrotrophic bacteria showed different sensitivity to the tested EOs. The inhibition zones demonstrated that most of the tested bacteria were resistant to the action of citrus EO with diameters not exceeding 10 mm. The antibacterial activity of citrus EO essentially against *L. monocytogenes, S. enteritidis* and *P. fluorescens* was assigned to its volatile compounds such as monoterpenes (limonene), sesquiterpenes and oxygenated derivates (Bhavaniramya *et al*., 2019).

Moreover, rosemary EO showed an intense antimicrobial effect against pathogens and psychrotrophic spoilage bacteria, particularly *S. aureus* and *E. coli*, which were the most sensitive. This inhibitory effect was attributed to the major compounds of rosemary EO mainly 1,8-cineole, α-pinene and camphor (Bhavaniramya *et al*., 2019).

Concerning thyme EO, it had a strong antibacterial activity against all tested strains with the exception of *P. aeruginosa, P. putida* and *P. fluorescens,* which had been shown to be the least sensitive (Table 1). These results were in agreement with those found by Bhavaniramya *et al*. (2019) who reported that *P. aeruginosa* had an intrinsic resistance to a wide range of antimicrobial compounds owing to the nature of its outer membrane. The highest antibacterial inhibition zone was noted for *S. aureus*. These results confirmed the findings established in the literature reporting the strong antibacterial activity of the active components of thyme EO such as thymol, carvacrol and γ-terpinene (Yu *et al*., 2021).

On the other hand, *A. hydrophila*, *S. aureus*, *L. monocytogenes*, *E. coli* and *S. enteritidis* showed high sensitivity to cinnamon EO, which could be related to the presence of aromatic phenolic compounds like eugenol and cinnamaldehyde (Bhavaniramya *et al.,* 2019).

When studying the effect of oregano EO, the most sensitive strains were *A. hydrophila, S. aureus, E. coli* and *L. monocytogenes* (Table 1), which was in agreement with the study of Bhavaniramya *et al*. (2019). These findings highlighted the strong inhibitory effect of oregano EO due to the presence of carvacrol, thymol, γ-terpinene and p-cymene (Yu *et al*., 2021), being capable to increase the bacterial membrane permeability or destabilizing the cell membrane leading to the leakage of intracellular compounds and interruption of protein synthesis (Yu *et al*., 2021).

These results confirmed that the sensitivity of the indicator strains depended on the nature and the concentration of the major bioactive components present in EOs as well as the nature of the cell wall. In fact, the outer membrane rich in phospholipids, which notably affect the sensitivity, is an integral part of the cell wall of Gram-negative bacteria only (Burt, 2004).

Thus, according to their antibacterial activity, the tested EOs can be classified as follows: Lemon< Rosemary< Cinnamon< Thyme< Oregano.

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Table 1. Inhibition zone diameters (mm) generated by essential oils.

	Inhibition zone diameter (mm)																									
	Doses of citrus essential oil (µl)					Doses of rosemary essential oil (µl)					Doses of thyme essential oil (µl)					Doses of cinnamon essential oil (µl)					Doses of oregano essential oil (µl)				
	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50
Pathogenic bacteria																									
Staphylococcus aureus ATCC 6835	-	7	7	8	8	14	15	18	19	19	16	31	32	35	37	23	22	28	28	33	29	33	35	37	40
Listeria monocytogenes ATCC 19115	7	8	9	9	9	8	9	11	12	14	14	25	29	30	32	13	15	25	26	30	26	28	30	34	36
Escherichia coli ATCC 25922	-	-	6	7	8	13	15	15	17	19	15	27	29	32	35	12	14	22	25	28	28	30	33	35	38
Salmonella enteritidis DMB 560	7	7	9	10	10	10	11	13	13	15	18	18	20	25	27	12	14	23	25	29	23	26	28	30	33
Pseudomonas aeruginosa ATCC 2134	7	7	8	8	8	9	11	12	12	13	9	10	12	13	14	12	13	14	16	18	17	18	19	20	22
Lactic acid bacteria																									
Lactobacillus plantarum ATCC 14917	-	-	7	8	8	6	7	7	8	8	9	10	10	11	11	7	8	9	9	10	9	10	11	11	12
Lactococcus lactis KF147	6	6	7	7	8	8	9	9	9	9	7	8	8	9	10	7	8	8	8	9	9	9	10	12	13
Leuconostoc mesenteroides ATCC 8293	6	7	7	8	8	7	8	8	9	9	7	8	9	9	11	8	8	9	10	11	10	10	11	12	14
Lactobacillus paracasei ATCC 25302	7	8	7	7	8	7	8	9	9	9	8	8	9	9	10	7	8	9	9	11	10	10	11	12	12
Carnobacterium piscicola AT71101238000999	7	8	8	8	8	7	7	9	9	9	9	9	9	10	10	7	8	8	9	10	8	10	11	12	12
Psychrotrophic bacteria																									
Aeromonas hydrophila ATCC 7966	-	7	7	8	8	9	11	11	14	15	15	17	21	22	25	16	18	20	21	24	21	23	27	29	32
Pseudomonas putida DSM 291	7	7	8	8	8	10	12	13	15	15	9	11	12	12	13	12	13	17	21	22	18	19	20	22	24
Pseudomonas fluorescens DMS 50090	8	8	9	9	10	9	10	10	11	13	10	11	13	13	14	13	14	17	18	21	16	17	19	20	21

Minimal Inhibitory Concentration (MIC)

The strongest antibacterial activities of oregano, cinnamon and thyme EOs were confirmed using microdilution assay on the two target bacteria; *E. coli* and *L. monocytogenes*

Oregano, cinnamon and thyme EOs, showing the strongest antibacterial activities using disc diffusion assay, were tested in microdilution assay performed on *E. coli* and *L. monocytogenes*. The MIC values are presented in Table 2. Results showed that the MIC of oregano EO (0.5% v/v) against the two pathogens was similar to that (0.43%) determined by Ponce *et al*. (2003).

For thyme EO, the MIC was the same as for *L. monocytogens* and *E. coli* (0.5% v/v). These results were different from those of El Adab *et al*. (2016) reporting MICs of about 0.25 % and 0.03%, respectively, against *E. coli* and *L. monocytogenes*.

In this study, the same MIC values (0.7%) against the two tested strains were found for cinnamon EO. These values were notably higher than that (0.04%) observed by Lv *et al*. (2011) against *E. coli*. This variability could be explained by the chemical composition of the EOs, the interaction between the components and the used extraction method (Ponce *et al*., 2003). Based on disc diffusion method and MICs determinations, for the rest of this study, oregano and cinnamon EOs were selected in order to prove their antilisterial activity on vacuum-packed ground ovine meat during refrigerated storage.

Quality Variation of Ground Ovine Meat during Refrigerated Storage

Previous researches demonstrated that a greater concentration of EOs than in antibacterial assays *in vitro* is needed to achieve the same effect in foods, but the amounts of EOs added should be minimal because large quantities can adversely alter their sensorial properties (Yu *et al*., 2021). Thus, obtained results on MIC determination enabled us to choose two concentrations for oregano EO (0.5 and 1%) and cinnamon EO (0.7 and 1.4%) to incorporate them into ground sheep meat previously inoculated with *L. monocytogenes* and to study their effects on the quality of sheep meat during refrigerated storage.

Microbiological Changes

Changes in the microbial flora of ground ovine meat samples during 12 days of refrigerated vacuum storage are illustrated in Figure 1. Regardless of the treatments, all microbial flora counts increased significantly ($P< 0.05$) throughout the storage period, except the pathogenic bacteria *L. monocytogenes,* which grew significantly ($P< 0.05$) only in the control meat sample from 4.1±0.17 to 5.97±0.2 log CFU/g (Figure 1-a). Contrary, this charge decreased significantly ($P< 0.05$) with the incorporation of oregano and cinnamon EOs. This decrease was improved with the EOs concentrations increase. In fact, final *L. monocytogenes* levels were about 0.82±0.09 and 0.9±0.1 log CFU/g, respectively, for O2L and C2L, which confirm the

Table 2. Minimal Inhibitory Concentrations (MICs) of selected essential oils (% v/v) against two pathogenic bacteria.

Essential oil	*E. coli* ATCC 25922	*L. monocytogenes* ATCC 19115
Origanum compactum	0.5	0.5
Thymus zygis	0.5	0.5
Cinnamomum verum	0.7	0.7

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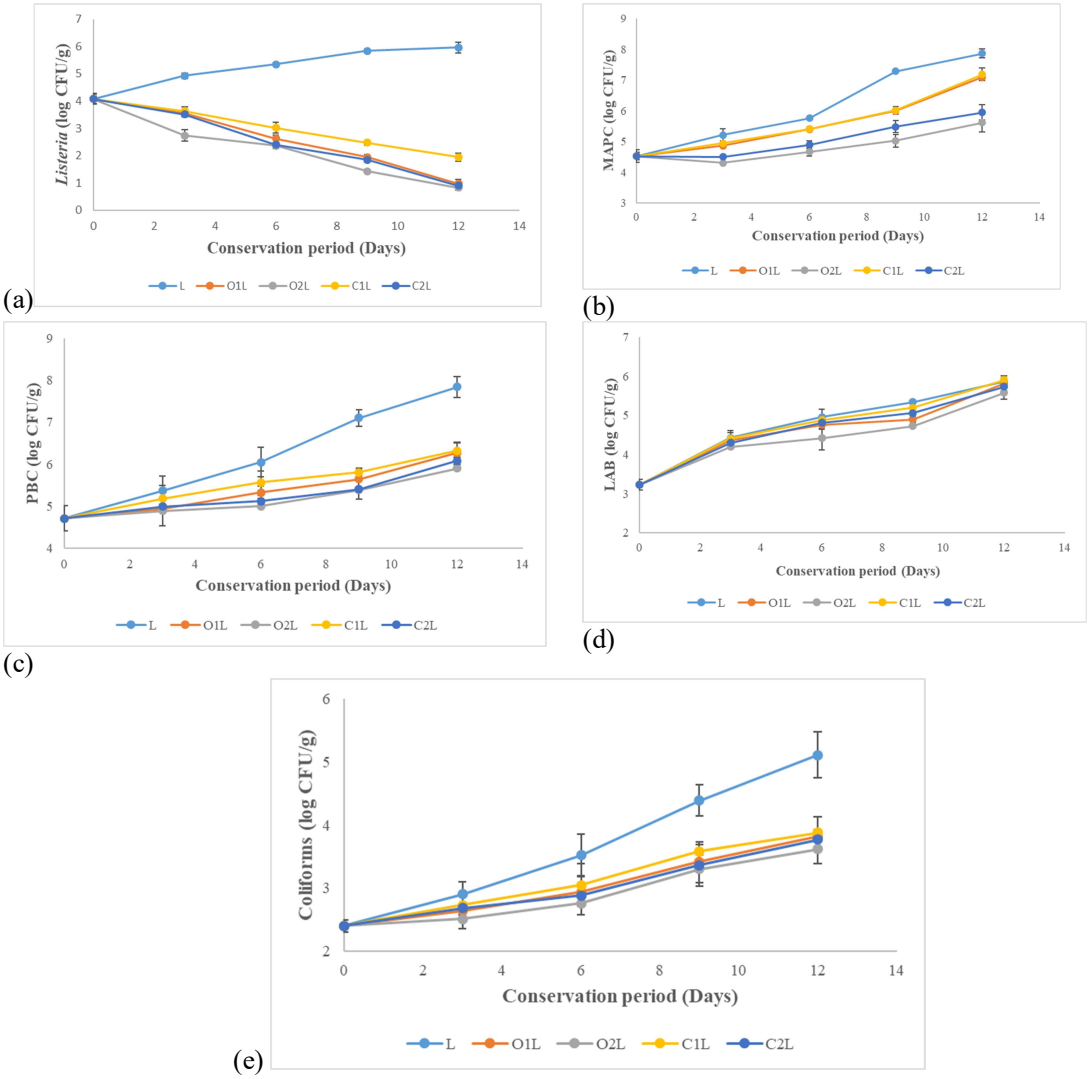

Figure 1.– Microbial counts changes in control and treated vacuum-packed ground ovine meat during refrigerated storage. (a) *Listeria monocytogenes*, (b) Mesophilic aerobic plate count, (c) Psychrotrophic (d) Lactic acid bacteria, and (e) Coliforms bacterial counts. [L: Control ground ovine meat inoculated with *L. monocytogenes*; O1L: Ground ovine meat inoculated with *L. monocytogenes* and treated with pure oregano EO at a concentration of 0.7% (v/w); O2L: Ground ovine meat inoculated with *L. monocytogenes* and treated with pure oregano EO at a concentration of 1.4% (v/w); C1L: Ground ovine meat inoculated with *L. monocytogenes* and treated with pure cinnamon EO at a concentration of 0.5% (v/w); C2L: Ground ovine meat inoculated with *L. monocytogenes* and treated with pure cinnamon EO at a concentration of 1% (v/w)].

antilisterial effect of oregano and cinnamon EOs in meat products, with respective reduction levels reaching 5.15 and 5.07 log CFU/g. This finding was in line with those suggesting that oregano EO inhibited the growth of *L. monocytogenes* by about 4 log CFU/g in vacuum packed meat (Tsigarida *et al*., 2000) as well as *Salmonella enteritidis* in minced sheep meat. More, the incorporation of cinnamon EO in refrigerated ground beef was found to be effective in inhibiting *L. monocytogenes*.

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Concerning literature, observed effect could be ascribed to EOs major active components, such as cinnamaldehyde in cinnamon EO and the two main phenols that constitute about 78–85% of oregano EO, i.e. carvacrol and thymol (Govaris *et al*., 2010; Yu *et al*., 2021). In addition, other minor constituents of the two studied EOs such as the monoterpene hydrocarbons γ-terpinene, p-cymene and linalool, could also contribute to the antibacterial activity (Agrimonti *et al*., 2019) or could be even more effective than the major components. In fact, the synergetic effect of the major and/or minor EOs compounds leads to a strong antibacterial activity by changing the permeability of the bacterial cell wall (Yu *et al*., 2021).

In this study, initial mesophilic aerobic plate counts (MAPC) in ovine meat was 4.52±0.12 log CFU/g showing that it was of acceptable microbial quality (Figure 1-b). The MAPC counts increased significantly ($P< 0.05$) during storage and exceeded the spoilage limit of 7 log CFU/g (Yu *et al*., 2021) for the control, treated meats with the lowest EOs doses, respectively, after the 9th and the 12th days of storage. The addition of the highest EOs concentrations induced the best inhibition, with MAPC counts of about 5.61±0.25 and 5.94±0.3 log CFU/g, at the end of storage for cinnamon and oregano EOs, respectively. These findings were in line with those of Chouliara *et al*. (2007), reporting that 1% of oregano EO inhibited MAPC in chicken meat better than the lowest dose of 0.1%. Furthermore, in respect to untreated control, significant reductions ($P< 0.05$) of about 2.42 and 2.75 log CFU/g were registered at the end of storage for O2L and C2L, respectively. The obtained result was in agreement with the results of Tsigarida *et al*. (2000) and Pateiro *et al*. (2021) showing that a high dose of cinnamon EO (5%) was effective to reduce the growth of MAPC due to its richness in trans-cinnamaldehyde.

Similarly, a significant ($P< 0.05$) increase of Psychrotrophic Bacterial Counts (PBC) was observed in all analyzed meats, during all storage period. At the final day, PBC charges reached 7.84±0.14, 6.27±0.2, 5.90±0.1, 6.32±0.4 and 6.08±0.2 log CFU/g, respectively, for L, O1L, O2L, C1L, and C2L (Figure 1-c).

This result confirmed those obtained by Agrimonti *et al*. (2019) and Govaris *et al*. (2010), reporting that thymol and carvacrol contained in oregano EO were active against *Pseudomonas* spp. in meat sample. Similar observation was noted in the study of Pateiro *et al*. (2021) reporting that the antimicrobial action of cinnamon EO on PBC was attributed to its major component as well as other minor phenolic compounds such as eugenol and linalool able to react with nucleic acids and proteins.

Concerning Lactic Acid Bacteria (LAB) evolution, this flora increased significantly ($P< 0.05$) in all meat samples during their refrigerated vacuum storage. The initial LAB count of about 3.23±0.13 log CFU/g increased for approximately 2.6, 2.35 and 2.5 log CFU/g, respectively, in the control, O2L, and C2L meat samples. Obtained results confirmed the previous observations about the resistance of LAB toward tested Eos, except for the oregano (Figure 1-d).

During storage, coliforms count increased and exceeded 5 log CFU/g in the control meat inoculated by *L. monocytogenes,* after 12 days. However, counts about 3.62±0.23 and 3.77±0.15 log CFU/g were registered, respectively, for O2L and C2L, with no significant difference ($P> 0.05$) between all analyzed meat samples (Figure 1-e).

pH Variation

Assessment of pH values in the control and treated ground ovine meat samples, during 12 days of refrigerated vacuum packed storage, is displayed in Figure 2. In this study, the initial pH value of ground ovine meat was about 6.05±0.01. This value was higher than that found by Govaris *et al*. (2010) (5.27). The pH values decreased by the end of storage at 4°C without any observed significant differences ($P> 0.05$)

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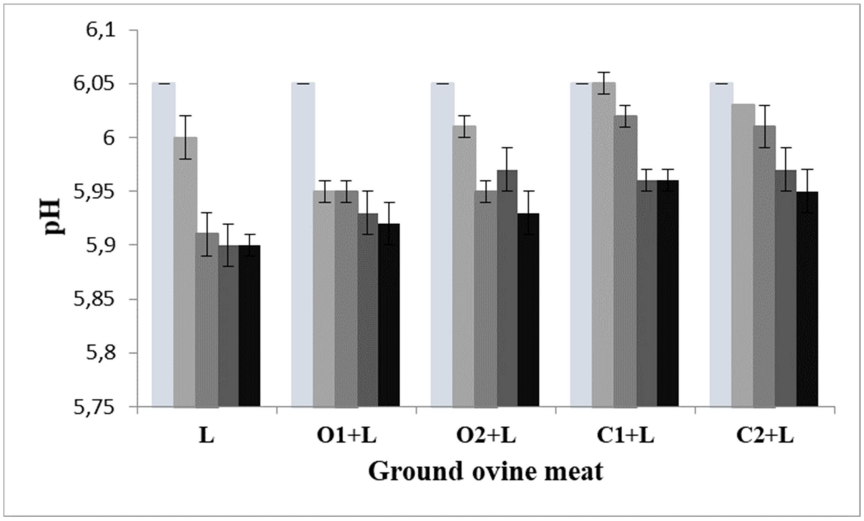

Figure 2. pH changes in the control and treated vacuum-packed ground ovine meat during refrigerated storage [0 (■), 3 (■), 6 (■), 9 (■), and 12 (■) days]. Symbols are defined under Figure 1.

between analyzed samples. The decrease of pH values can be due to the production of lactic acid by LAB in meat as described by Chouliara *et al*. (2007). This pH decrease increased the hydrophobicity of some EOs resulting in a stronger bacteriostatic activity (Yousefi *et al*., 2020) and was in accordance with those of Govaris *et al*. (2010) reporting that good antibacterial activity in the food matrix did not necessarily induce variations in pH values.

Protein Content Variation

At the beginning of storage, the protein content of ground sheep meat was 20.99±1.09% (Table 3). This result was in line with those reported by Govaris *et al*. (2010) (19.8%). For all analyzed meat samples, protein content decreased significantly ($P< 0.05$) during refrigerated storage, to reach about 14±0.61, 14.39±1.47, 15.21±0.4, 15.53±0.78, and 15.87±1.09% at the end of storage, respectively, for the control L, O1L, O2L, C1L and C2L. This result was in agreement with that of Martin-Sanchez *et al*. (2009) suggesting that pH decrease led to proteases and lipases activation enabling slight drop in the protein content. Besides, the increase in added EOs concentrations preserved better protein contents, confirming their strong inhibitory effect on spoilage bacteria i.e. mainly psychrotrophic flora able to produce proteases.

Fatty Acids Variation

The variation in FAs composition have an important effect on several nutritional and technological properties of meat like firmness or softness (Prache *et al*., 2022).

The variation of FAs contents of the control and treated ovine meat samples previously inoculated with *L. monocytogenes* and/or incorporated with oregano and cinnamon EOs during 12 days of vacuum packaging storage are presented in Table 4. In this study, the presence of various Saturated (SAFA), Monounsaturated (MUFA) and Polyunsaturated (PUFA) FAs was observed. For the 10 detected FAs, contents were not changed significantly ($P> 0.05$) throughout storage, for all analyzed samples. In addition, during storage period, the highest content was registered for SAFA

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Table 3. Proteins content (%) variations in control and treated vacuum-packed ground ovine meat during refrigerated storage.[a]

	Conservation period (Days)				
Sample[b]	0	3	6	9	12
L	20.99^{aC}±1.09	17.96^{abB}±1.17	15.67^{aAB}±2.15	14.37^{aA}±1.05	14^{aA}±0.61
O1L	20.99^{aCB}±1.09	17.16^{aBA}±±2.4	18.8^{bB}±0.85	15.6^{abA}±1.14	14.39^{abA}±1.47
O2L	20.99^{aC}±1.09	18.24^{abcB}±0.12	16.52^{abBA}±1.9	16.1^{abA}±0.86	15.21^{abA}±0.4
C1L	20.99^{aC}±1.09	20.34^{cC}±0.54	17.72^{abB}±0.64	16.63^{bBA}±0.5	15.53^{abA}±0.78
C2L	20.99^{aC}±1.09	19.57^{bcC}±0.23	17.15^{abB}±0.31	14.24^{aA}±1.54	15.87^{bAB}±1.09

[a] Data are mean±standard deviation. Mean values with different lowercase letters indicate significant differences (P< 0.05) between samples. Mean values with different uppercase letters indicate significant differences (P< 0.05) during storage time. [b] Symbols are defined under Figure 1.

Table 4. Fatty acids content (%) variations in the control and treated vacuum-packed ground ovine meat during refrigerated storage.[a]

		C2L	O2L	L
Fatty acids (%)	Day 0	Day 12	Day 12	Day 12
C14:0	5.22^{B}±0.14	4.38^{aA}±0.17	4.95^{aA}±0.02	4.20^{aA}±0.40
C15:0	0.62^{A}±0.04	0.58^{aA}±0.17	0.69^{aA}±0.04	0.68^{aA}±0.06
C16:0	26.71^{A}±0.27	26.80^{bA}±1.05	26.52^{aA}±0.48	26.70^{bA}±1.19
C16:1 w7	3.06^{A}±0.16	2.93^{aA}±0.13	3.27^{aA}±0.01	2.74^{aA}±1.84
C18:0	15.07^{A}±0.08	14.95^{aA}±0.44	14.61^{aA}±0.06	14.89^{aA}±0.96
C18:1 w9	42.23^{A}±0.22	40.79^{aA}±0.67	40.32^{aA}±0.91	41.70^{aA}±2.26
C18:2 w6	3.85^{B}±0.06	3.67^{aA}±0.07	3.80^{aA}±0.08	3.73^{aA}±0.18
C18:3 w3	0.86^{A}±0.00	0.97^{bB}±0.01	0.84^{abA}±0.08	0.83^{aB}±0.05
C18:4 w3	0.83^{A}±0.15	0.65^{aA}±0.03	0.82^{bA}±0.02	0.84^{cA}±0.01
C20:4 w6	1.34^{A}±0.03	1.33^{bA}±0.04	1.18^{abA}±0.15	1.48^{bA}±0.12
SOMME	99.79^{B}±0.46	97.05^{aA}±0.99	97.00^{aA}±1.60	97.79^{aA}±0.55
∑AGPI	6.88^{B}±0.07	6.63^{bA}±0.07	6.53^{aA}±0.17	6.88^{cA}±0.01
∑AGMI	45.30^{B}±0.38	43.72^{aA}±0.80	43.76^{aA}±0.54	44.44^{bA}±0.42
∑AGS	47.62^{A}±0.02	46.71^{aA}±0.27	46.59^{aA}±0.90	46.47^{aA}±0.11
∑AGPI w-3	1.69^{B}±0.15	1.62^{bA}±0.04	1.61^{aA}±0.09	1.67^{cB}±0.05
∑AGPI w-6	5.19^{A}±0.08	5.01^{aA}±0.03	4.98^{aA}±0.08	5.21^{bB}±0.07
∑w-6/∑w-3	3.07^{A}	3.09^{A}	3.09^{A}	3.12^{A}
AGPI/AGS	0.14^{A}	0.14^{A}	0.15^{A}	0.15^{A}

[a] Data are mean±standard deviation. Mean values with different lowercase letters indicate significant differences (P< 0.05) between samples. Mean values with different uppercase letters indicate significant differences (P< 0.05) during storage time. Symbols are defined under Figure 1.

followed by MUFA and PUFA with no significant differences (P> 0.05) observed between all ground meat samples.

Furthermore, the detected MUFAs in ovine meat that must be provided by diet (Boulares *et al*., 2017) were palmitoleic (C16:1 w-7) and oleic (C18:1 w-9) acids. The richness in C18:1 (42.23±0.22%), known as the major MUFA found in ovine meat, is associated with beneficial effects on the prevention of cancer, autoimmune and inflammatory diseases (Chikwanaha *et al*.,

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2018). Also, linoleic (C18:2 w-6), linolenic (C18:3 w-3), stearidonic (C18:4 w-3) and eicosatetraenoïc (C20:4 w6) acids were the most dominant PUFAs. These findings were partially in agreement with those of Chikwanha *et al*. (2018) reporting that sheep meat is a good source of w-3 PUFA due to the presence of C18:3 leading to the production of long-chain (C20-C22) w-3 PUFA owing the ability to prevent cardiovascular diseases. More, linoleic and linolenic acids in ovine meat can decrease the risk of coronary heart disease and enhance human health (Prache *et al*., 2022; Chikhwanha *et al*., 2018). For arachidonic acid (C20:4 w-6), it plays key roles in reducing inflammation and in the immune function. Besides, C18:2, considered as the major w-6 PUFA, lowers blood LDL cholesterol concentrations. Moreover, it is known that w-6 PUFA reduces the risk of cardiovascular diseases, but balance between w-6 and w-3 PUFAs in diets should be verified. In this study, the ratio ∑w-6/∑w-3 did not differ significantly between control and treated ground meats during storage period. This low ratio being inferior at 4 for all samples reduce risk of mortality of about 70% as reported by Chikhwanha *et al*. (2018). In fact, a single serving of about 100 g of sheep meat was judged sufficient for human wellbeing due to its high ratio of PUFAs and its favorable balance between w-6 and w-3 PUFA.

Concerning SAFAs, they represented approximately 47% of total FAs in all analyzed ground sheep meat. The palmitic (C16:0) and stearic (C18:0) acids were the prominent SAFAs with an interesting low content (< 1%) of pentadecanoic acid (C15:0). These results were in accordance with those of Chikhwanha *et al*. (2018) promoting the consumption of sheep meat when C14:0 and C16:0 contents are low.

This oxidative stability can be attributed to both vacuum package and antioxydant activity of active compounds in oregano and cinnamon EOs such as eugenol, cinnamaldehyde, carvacrol and thymol that play interesting roles in neutralizing free radicals and in prevention of lipid peroxidation, which enhance the nutritional quality of ground meat (Bhavaniramya *et al*., 2019).

CONCLUSIONS

In summary, pathogenic bacteria, particularly *L. monocytogenes* and *S. enteritidis,* showed high sensitivity towards lemon, rosemary, thyme, cinnamon and oregano EOs. Besides, the addition of EOs at different concentrations in vacuum packed ground ovine meat resulted in better biochemical and microbiological qualities. The treatment of sheep meat with oregano and cinnamon EOs preserved a better content of proteins, a high ratio of PUFAs and a favorable balance between w-6 and w-3 PUFA, resulting in the production of healthier meat. Moreover, high concentrations of oregano and cinnamon EOs proved the highly efficient anti-listerial activity compared to the control and other meat samples. Furthermore, this adjunction reduced microbiological charges at the end of the storage period.

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فعالیت ضد لیستیاری (Anti-Listerial) اسانس پونه کوهی (Oregano) و دارچین در بسته بندی خلاء گوشت چرخ کرده گوسفند طی نگهداری در یخچال

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چکیده

هدف این پژوهش بررسی فعالیت ضد باکتریایی اسانس‌های مختلف (EO) علیه پاتوژن‌های ناشی از غذا بود و همچنین تعیین اثر کاربرد آنها در غلظت‌های مختلف بر روی گوشت چرخ‌کرده گوسفندی بسته‌بندی شده در خلاء(که قبلا در آزمایش هایی با لیستریا مونوسیتوژنز تلقیح شده بود) طی ۱۲ روز نگهداری در ۴ درجه سانتی گراد بود. به طور خلاصه، باکتری های بیماری زا، به ویژه *L. monocytogenes*، *Salmonella enteritidis* و *Escherichia coli*، به دلیل غنی بودن از ترکیبات فعال زیستی، حساسیت بالایی نسبت به اسانس‌های مرکبات، رزماری، آویشن، دارچین و پونه کوهی نشان دادند.حدکمینه غلظت‌های بازدارنده (MICs)در اسانس‌های مختلف برعلیه *L. monocytogenes* و *E. coli* حدود ۰.۵ % برای اسانس پونه کوهی و تیموس (thymus) و ۰.۷ % برای اسانس دارچین بود. علاوه بر این، افزودن اسانس در غلظت‌های مختلف منجر به بهبود کیفیت بیوشیمیایی و میکروبیولوژیکی گوشت گوسفندی بسته‌بندی شده در خلاء در طول نگهداری در یخچال شد.غلظتهای بالا در اسانس پونه کوهی (۱%) و دارچین (۱.۴%) کارآمدترین فعالیت ضد لیستی را در مقایسه با شاهد و سایر نمونه‌های گوشت داشتند. تیمار گوشت گوسفندی با اسانس پونه کوهی یا دارچین منجربه حفظ محتوای بهتر پروتئین، نسبت بالای PUFA و تعادل مطلوب بین w-6 و w-3 PUFA گردید و در نتیجه باعث تولید گوشت سالم‌تر شد.

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