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## Antioxidant and Antimicrobial Activities of Some Essential Oils Incorporated in Selected Meat and Meat Products during Refrigerated Storage: A review

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**Abstract**

Meat and meat products are recognized as excellent sources of nutrients for humans. It is a rich source of lipids, proteins, minerals, and vitamins. They provide a favorable environment for microbial growth. Lipid oxidation and microbial growth are the two main causes of meat deterioration. To preserve meat and meat products consumers nowadays demand nutritious foods that do not include artificial preservatives. Essential oils (EOs) attracted the interest of the food industry to satisfy consumers' demands. EOs are utilized in meat and meat products applications because they are generally recognized as safe (GRAS) as compared with synthetic antioxidants and antimicrobial materials. They are aromatic oily liquids produced from different parts of plants such as seeds, leaves, flowers, and roots and are recognized to inhibit both lipid oxidation as well as the growth and survival of spoilage and pathogenic microorganisms. Recently, applications of different types of EOs were introduced in meat and meat products. This article provides a recent overview of the application of selected essential oils and their effects on lipid oxidation and microbial growth of some meat and meat products to improve their quality during refrigeration storage.

*Keywords*: Essential oils; meat; lipid oxidation; Antimicrobial; Antioxidant

## 1. Introduction

Meat is recognized as a nutritive food which could be chosen as a good source of animal protein for many people all over the world. Meat and meat products present favorable conditions for microbial growth and lipid oxidation as a source of high-water activity, nutrients, and low levels of acidity [1]. It has a short shelf life of a few days at a refrigerated temperature (0-5°C) due to microbial spoilage, including pathogenic and non-pathogenic microorganisms and lipid oxidation [2]. Lipid oxidation of meat and meat products can vary greatly and could range from color losses, extensive flavor changes, and structural damage on proteins, which leads to loss of freshness [3], limits the shelf-life [4], and discourages repeat purchases by consumers. Oxidation is a well-known non-microbial cause of quality loss in meat. Oxidative stress occurs due to uneven generation of free radicals Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which trigger oxidative and/or nitrosamine stress and damage of macromolecules, including the lipid and protein fractions. Essential oils extracted from different types of plants serve as a safe alternative to synthetic antioxidants and antimicrobials and preserve meat and meat products and by struggling with the food spoilage organisms and borne pathogens, inhibiting lipid oxidation and thus extending the shelf life of the meat and meat products [5]. Essential oils are rich sources of oxygenated monoterpenes such as menthol, α-terpineol, citronellal, and linalool), phenolic terpenoids such as carvacrol and thymol, and terpenic hydrocarbons such as myrcene and α-pinene [6], which are chemical compounds with antioxidant activity which can be used as a natural alternative for preserving meat products [7]. Recently, several research studies were conducted using EOs with the objective of improving the quality of different food stuffs [8,9] including meat and meat products using different types of essential oils [9-16].

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This review article summarizes adequate information from the recent literature and mainly focuses on the application of selected essential oils as natural agents to improve the quality and shelf life of meat and meat products.

## 2. Effect of selected EOs on lipid oxidation

The most common form of chemical deterioration in meat and meat products is lipid oxidation of unsaturated fatty acids in meat, which is a major cause of meat deterioration. It is a process in which polyunsaturated fatty acids react with reactive oxygen species, resulting in a series of secondary reactions that in turn lead to the breakdown of lipids and the development of oxidative rancidity. Lipid oxidation (LO) is a major cause of the deterioration of fatty tissues in meats and directly affects meat commercial value and products [5]. LO in meat and meat products is defined as a chain reaction of free radicals and consists of three stages: (1) initiation, (2) propagation, and (3) termination. LO in meat can occur in three different phases: (1) at the pre-slaughter stage (live animal), (2) during slaughter when the muscle is being converted into meat, and (3) after slaughter (meat processing and storage). After the animal is slaughtered, the antioxidant mechanisms present in animal muscles collapse, while the biochemical changes that occur during the conversion of muscle to meat favor oxidation [17]. The drop in pH facilitates the oxidation of muscle components because H+ can promote myoglobin's redox cycle and its pro-oxidant effects. Besides the drop in pH, other postmortem biochemical changes, such as changes in cellular compartmentalization and the release of free catalytic iron and oxidizing enzymes, also contribute to promoting LO [18]. LO affects the texture, color, aroma, and flavor of the meat, leading to rancidity. Desire is responsible for unacceptable taste and off-flavors, which are major reasons for consumer rejection and reduce the nutritional value, organoleptic properties, and shelf life of meat [19]. The development of LO in meat can be prevented by providing an active antioxidant in the diet [17]. Synthetic antioxidants (SA) such as butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBH), and butylated hydroxyanisole (BHA) have been widely used in several countries around the world, but there is a negative impression on the use of the SA due to their possible toxicological and carcinogenic effects [20]. Therefore, synthetic antioxidants have been increasingly replaced by natural antioxidants by the meat processing industry and are preferred due to recent changes in consumer demand [21]. Origanum Syriacum essential oil prevents the development of rancidity, reduces unpleasant odors and gives the meat a pleasant taste. These properties drew researchers' attention to meat preservation technology. Studies have reported that Origanum syriacum L. has a very powerful antioxidant effect due to the high content of thyme and carvacrol in its composition [22, 23]. The effects of adding Origanum syriacum L. essential oil (EO) at different concentrations on lipid and protein oxidation were studied in chicken meat and compared to those of synthetic commercial meat preservatives [24]. Skinless chicken breast, chicken thigh and ground deboned chicken meat samples were treated with 0 ppm (control), 100 ppm, 150 ppm, 250 ppm, and 300 ppm Origanum syriacum L. Essential Oil. The treated meat samples were cooked and analyzed for LO (TBARS levels) and protein oxidation (carbonyl levels) on days 0, 4 and 7 of storage. All additives demonstrated significant lipid and protein antioxidant effects ($p<0.5$) compared to the control treatment during storage. The addition of Origanum syriacum L. Essential oil at the chosen concentration was found to be very effective in improving storage stability, comparable to that obtained with the control (untreated) meat sample. Earlier, Avila-Ramos et al. [25] investigated the antioxidant effects of oregano essential oil and vitamin E in the supplemented broiler chicken diet. The oregano essential oil and vitamin E were incorporated into a diet of corn-soybean meal at a diet concentration of 100 mg/kg combined with vitamin E at concentrations of 10 or 100 mg for 42 days. The results showed that feeding the broilers the meal containing oregano essential oil in combination with vitamin E improved the oxidative stability of the broiler meat stored at 40 °C for 9 days and resulted in a significant ($P<0.05$) shelf life. However, it was found that the dietary supplementation of 100 mg of vitamin E in broiler feed combined with the oregano essential oil is more effective in maintaining the LO stability of meat compared to the corn-soybean meal diet alone, which produced meat that developed LO. The effects of adding lettuce, marjoram, and cumin essential oils at a concentration of 2% on the LO of minced meat during refrigerated storage were investigated [26]. They have been shown to reduce LO due to the antioxidant activities of essential oils, thus improving the quality of the minced meat during refrigerated storage. At the end of the storage period (12 days), the result of the LO showed that the largest increase in the LO value (1.75 mg malonaldehyde/kg) was recorded for the control sample and the lowest value of 0.72 mg malonaldehyde/kg was observed for the sample treated with the 2% cumin essential oil, followed by 0.77 and 0.89 malonaldehyde/kg, which were observed in the meat treated with marjoram essential oil and lettuce essential oil, respectively.

Similar results and good agreement were reported for beef meat treated with a mixture of 1:1 (0.5ml rosemary + 0.5ml oregano) and kept in modified atmosphere packs at 4ºC for 9 days. The essential oil treated samples resulted in a lower LO at day 9 of storage time than that of the control. The value was 0.38 mg of malonaldehyde/kg in the treated meat and 0.72 mg malonaldehyde/kg on day 9 of the storage time.

**3. Effect of selected EOs on microbial growth**

Poor hygiene practices in meat slaughterhouses can lead to meat contamination with spoilage as well as pathogenic bacteria that cause serious risks to human health [26]. Additionally, the complete elimination of bacteria from meat processing environments is considered a difficult task because bacteria can attach to meat contact surfaces and form biofilms where they can survive even after disinfection and cleaning [28]. Meat and meat products are common sources of foodborne pathogens such as *salmonella*, *Escherichia coli*, *listeria*, *campylobacter* and *staphylococcus aureus*, which could cause a serious health risk when consumed [29]. To prevent microbial contamination and growth of this livestock diet, essential oils from different sources could be used [30]. Thyme, clove, cinnamon, oregano, allspice, rosemary, ginger, lemon balm, basil, coriander, savory, marjoram, basil, and fennel essential oils have potential antimicrobial effects when used in meat and meat products [30]. Sage essential oil has also been shown to have a potent inhibitory effect against gram-positive bacteria in meat products [32]. Garlic, cumin, black pepper, and oregano essential oils have also been reported to reduce the growth of spoilage bacteria [33]. Moreover, pathogens such as E. coli, L. monocytogenes, and Salmonella spp. inhibit when incorporated into meat and meat products [33]. Oregano essential oil has been used in combination with modified atmosphere packaging (MAP) as a barrier to extend the shelf life of fresh duck meat [35]. The results showed that the application of chitosan coating alone inhibited the growth of microorganisms and prevented LO throughout storage but the storage stability was further improved by incorporating cinnamon or oregano essential oils in the coating material, which lowered ($P < 0.05$) values for total viable count (TVC) of *Enterobacteriaceae* and the shelf-life of the treated meat was prolonged by at least 7 days compared to that of the control. The longer shelf life of meat treated with EOs was observed compared to the same MAP alone. The effectiveness of cinnamon essential oil (*Cinnamomum zeylanicum*) in concentrations of 0.5%, 1% and 1.5% and of thyme essential oil (Thymus vulgaris) in concentrations of 1%, 1.5% and 2% for their ability to control pathogenic bacteria in beef burger. The shelf life of ground beef stored at 2°C for a storage period of 12 days was studied [36]. Chicken breast meat was treated with thyme and caraway essential oils at a concentration of 1% v/w for each EO and stored at 4°C for 16 days [37]. The treated meat samples were evaluated on days 0, 4, 8, 12 and 16 of storage. The result showed that the number of Lactobacillus spp. ranged from 1.35 log CFU.g-1 to 3.04 log CFU.g-1 at day 4 in the control group, which was stored in air. Alsaiqali *et al*. [38] evaluated the antibacterial activity of cumin, thyme, and parsley essential oils at concentrations of 0.3, 0.6, and 1.2% against Salmonella enterica, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa in inoculated beef burgers aged 4 days that had been stored for a long time at 4°C. They found that high concentrations (0.6 and 1.2% of the essential oils) compared to the low concentration (0.3%) had the strongest inhibitory effect on most bacteria during the beefburger's storage period. This result may suggest the application of these essential oils in the burger at concentrations of 0.6% and 1.2% to improve the shelf life of the burger. According to the study by Saad et al. [39] they studied the effects of cinnamon (*Cinnamomum zeylanicum*), garlic (Allium sativum) and thyme (Thymus vulgaris) essential oils at concentrations of 1% to determine their effect on the inhibition of pathogenic microorganisms (E. coli and Staphylococcus aureus) in minced meat stored at 4ºC for 5 days. The results obtained showed that the treated ground beef samples showed significant ($p<0.5$) decreases in E. coli and S. aureus levels. S. aureus and E. coli levels decreased from 6.98 log cfu/g to 2.42 log cfu/g, 3.00 and 2.95 cfu/g for cinnamon, garlic, and thyme treated ground beef essential oils by day 4 of storage. However, S. aureus and E. coli decreased from 10.71 log CFU/g in the control ground beef to 3.52 log CFU/g, 6.06 log CFU/g and 3.52 log CFU/g for cinnamon essential oils, garlic and thyme. S. aureus and E. coli were not detected in the minced meat on day 5 of the storage period. In another study, Hernndez-Ochoa, et al. [40] evaluated the effects of clove and cumin essential oils at concentrations of 750, 1500 and 2250 on five strains of pathogenic bacteria, namely Salmonella, Escherichia coli O157:H7, Campylobacter jejuni, Yersinia enterocolitica, Listeria monocytogenes, Clostridium perfringens, and Toxoplasma Gondi Staphylococcus aureus) using beef scapula pulp. Results showed that clove seed essential oils were effective in inhibiting strains of pathogenic bacteria at concentrations ranging from 750 mg/L to 2250 mg/L. Clove essential oil resulted in a reduction of 3.78 log UFC/g at an application of 2250 and Cumin essential oil at a reduction of 3.78 log UFC/g at an application of 750 L. Recently, Sharma et al. [41] investigated the effects of a sodium caseinate coated with Zakaria multiflora Boiss essential oil at concentrations of 0.5, 1 or 1.5% on the shelf life of fresh veal during 15 days storage at 4°C. Over 10 days of refrigerated storage, the meat had significantly fewer lactic acid bacteria, psychrotrophic bacteria, Enterobacteriaceae and total viable bacteria throughout the storage period compared to the untreated meat (control). The caseinate-coated samples coated with Zakaria multiflora boiss essential oil at concentrations of 1 and 1.5% were found to be significantly more stable ($P<0.05$), hence the coating technique containing the essence becomes urgently recommended to replace synthetic preservatives for meat products. Nehme *et al*. [42] claimed that the use of essential oils in high concentrations as antimicrobial and antioxidant natural agents to improve the safety and shelf life of meat and meat products could be inconsistent with sensory acceptability due to the essential oils' strong odor. They suggested that studies should be conducted on a combination of concentrations of essential oils applied in meat and sensory evaluation to avoid rejection of the meat and meat products. Lin *et al*. [43] investigated the incorporation of thyme essential oil/cyclodextrin polylysine nanoparticles into active packaging containing gelatin nanofibers. The results showed that the essential oils incorporated into the active packaging system significantly enhanced the antimicrobial properties against bacteria such as Campylobacter jejuni. This fact indicates that natural substances such as essential oils play a significant role in the antioxidant activity of the active packaging. Recently, Osaili *et al*. [44] studied the effect of a combination of vacuum packaging and three essential oils including cinnamaldehyde, thymol and carvacrol at concentrations of 1 or 2% on the growth of spoilage-causing microorganisms in marinated camel meat cuts during storage of 4 and 10 hours for 7 days. They found that treating the camel meat with vacuum packaging and the essential oils was an effective means of controlling spoilage microorganisms compared to aerobic packaging of camel meat. However, maximum decreases in spoilage-causing microorganisms were observed under aerobic conditions. The combination treatments resulted in a maximum reduction in the total number of mesophilic plaques, yeasts and molds, mesophilic lactic acid bacteria, Enterobacteriaceae and Pseudomonas spp. were 1.2, 1.4, 2.1, 3.1 and 4.8 log CFU/g, respectively. It was found that the addition of essential oils at a concentration of 2% in the vacuum pack was more effective in retarding meat spoilage. Sotelo-Boyás. *et al*. [45] studied the in vitro antibacterial and antimicrobial activity of thyme essential oil nanoencapsulated in chitosan with an average size of 9.1 nm and found that it had activity against Bacillus cereus, S. aureus, L. monocytogene, Salmonella Typhi , Shigella dysenteriae and E. coli. The antibacterial activity of Thymus daenensis essential oil against E. coli both in nanoemulsion and in free form was reported [46]. They claimed that the nanoemulsion form of Thymus daenensis essential oil had significantly greater inhibitory activity against E. coli when converted to a nanoemulsion form. Thyme/chitosan essential oil nanoparticles at concentrations of 0.05% and 0.1% were investigated for their effect on the microbiological growth of beef burgers fried at a chilled temperature for 8 days [47]. They found that nanoencapsulation of essential oil at these concentrations (0.05% and 0.1%) significantly inhibited microbial growth of total mesophilic counts, S. aureus, Enterobacteriaceae, molds and yeasts more than control burgers and essential thymus burgers. The minimum bactericidal concentration of oregano essential oil nano-emulsions with average droplet diameters of 35 to 55 nm was evaluated against E. coli and S. aureus. They found that the minimum bactericidal concentration did not differ significantly during 90-day storage under refrigerated conditions. This result indicated that the oregano essential oil nano-emulsions did not affect the antibacterial and stability properties of the oregano essential oil nano-emulsions. Edible films obtained from alginate-based nano-emulsions loaded with thyme essential oil showed potent antibacterial activity, reducing the number of bacterial growths by 4.71 logs within 12 h and showing significant anti-E. coli effects [48]. The effects of 1 and 10 min contact times of liposome-encapsulated carvacrol and thymol with an average particle diameter of 270 nm against different strains of Salmonella and S. aureus [49]. Thymol or carvacrol were found to significantly reduce Salmonella aureus. The antimicrobial properties of essential oils are derived from some important bioactive components such as phenolic acids, flavonoids, aldehydes and terpenes, which are present in essential oils. There are many antimicrobial activities of essential oil mechanisms such as: altering the structure of cell membranes and fatty acid profiles, increasing cell permeability, affecting membrane proteins, and inhibiting functional properties of the cell wall [50]. The hypothesis that Gram-positive bacteria are more susceptible to the effect of hydrophobic compounds such as essential oils was proposed [50]. The difference in the susceptibility is attributable to the fact that Gram-positive bacteria have a thick layer of peptidoglycan linked to other hydrophobic molecules such as proteins and teichoic acid. This hydrophobic layer surrounding the Gram-positive bacterial cell may facilitate easy entry of hydrophobic molecules. On the other hand, Gram-negative bacteria have a more complex cell envelope comprising an outer membrane linked to the inner peptidoglycan layer via lipoproteins. The outer membrane contains proteins and lipopolysaccharides, which makes it more resistant to the hydrophobic molecules in EO. Other researchers investigating the antimicrobial activity of EOs showed no notable difference between the MIC values of Gram-positive and Gram-negative bacteria [51-54]. Although it has been hypothesized that the outer membrane is almost impermeable to the hydrophobic compounds, Van de Vel *et al*. [55] argued that some hydrophobic compounds might cross the outer membrane via porin channels. They believe that some EO molecules are more active against Gram-positive bacteria, while others are active against Gram-negative bacteria, but the mechanisms remain unknown. Most studies on the antimicrobial activity of EOs have used E. coli and S. aureus as model microorganisms to represent Gram-negative and Gram-positive bacteria, respectively [56,57]. This could lead to a generalization of results, as not all Gram-negative and Gram-positive bacteria would follow a similar trend as observed in E. coli and S. aureus. Furthermore, the mode of action of EO depends on its chemical profile and the ratio of its active components [58]. The possible mechanisms wherein EOs interfere with bacterial proliferation may involve the following: (1) bacterial outer membrane or phospholipid bilayer disintegration; (2) fatty acid composition change; (3) increase in membrane fluidity resulting in leakage of potassium ions and protons; (4) interference with glucose uptake; and (5) inhibition of enzyme activity or cell lysis.

## 4. Conclusion

In this review article, the antioxidant and antimicrobial activities of some EOs incorporated into meat and meat products during refrigeration have been highlighted. The incorporation of the EOs approved results in effective results in the quality of the meat and meat products. However, the incorporation of appropriate concentrations of EOs is highly recommended to achieve the benefits of both safety and sensory acceptability of the meat products and at the same time to avoid the rejection of the meat products due to the essential oils' strong odors.

## 5. Conflicts of interest

The authors declared no conflict of interest.

## 6. Acknowledgments

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**Table 1**

Selected essential oils and their concentrations applied in some meat and meat products as antioxidant agents

| S. no. | Essential oil utilized | Method of utilization | Result obtained | Reference |
|---|---|---|---|---|
| 1 | *Citrus Lemon* essential oil | Minced beef was incorporated with lemon essential oil in concentration of 0.0% (control), 0.01%, 0.06%, 0.312% and the treated samples stored at 4 °C for 10 days and analyzed for TBARS. | *Citrus Lemon* essential oil displayed an excellent DPPH scavenging ability with an extract concentration providing 50% inhibition (IC50) of 15.056 µg/ml and a strong β-carotene bleaching inhibition after 120 min of incubation with an IC50 of 40.147 µg/ml. | [59] |
| 2 | *Satureja montana L* essential oil | The essential oil was added at concentrations of 7.8, 15.6, and 31.25 in mortadella-type sausages formulated with different sodium nitrite ($NaNo_2$) levels (0, 100, and 200 mg/kg) and stored at 25°C for 30 days. | Significant effects on lipid oxidation were observed in samples containing *Satureja montana L essential oil* combined with sodium nitrite. The results suggest possible benefits from the combined use of the essential oil and minimal amounts of sodium nitrite in cured meat products. | [59] |
| 3 | *Basil, thyme and tarragon* essential oils. | Basil at concentration of 84.4%, thyme at concentration of 61.6% and tarragon at concentration 76.8% after 48 hours of action and respectively 97.2%, 90.2% and 95.3% after 72 hours of action.. | The beef patties treated with basil essential oil either with or without ascorbic acid had significantly ($p < 0.05$) lower bacterial counts than control from day I of storage onwards. | [60] |
| 4 | Mentha piperita essential oil | The essential oil was incorporated in minced beef at concentrations of 1250, 2500, 2500 and 5000 µg/mL against L.monocytogenes and S.typhimurium. The meat samples were kept at 7 OC for 9 days. | Mentha piperita essential oil showed significant bactericidal and bacteriostatic effects on L. monocytogenes and S. typhimurium and improved the microbiological safety of the minced beef during 9 days of storage period. | [61] |
| 5 | Thymus capitatus essential oil | The essential oil was mixed with the ground beef in concentrations of 0.01, 0.05, 1, and 3% as antimicrobial agents to Control pathogenic and spoilage bacteria in the ground meat. | Thymus capitatus essential oil. Exhibited significant ($p < 0.05$) antiradical activity with an IC50 value of 213.53 µg/ml and this could be attributed to its high content of carvacrol (88.89%). | [62] |

**Table 2**
Selected essential oils and their concentrations applied in some meat and meat products as antimicrobial agents

| S. no. | Essential oil utilized | Method of utilization | Result obtained | Reference |
|---|---|---|---|---|
| 1 | *Zataria multiflora Boiss*. Essential oil | Sodium caseinate (SC) coating enriched with Zataria multiflora Boiss. essential oil at a concentration of 0.5, 1, or 1.5% on the product shelf life of veal meat during storage at $4^0$C for 15 days | The treated samples had markedly less lactic acid bacteria, psychrotrophic bacteria, and Enterobacteriaceae, and total viable counts relative to the control throughout storage. | [41] |
| 2 | *Cinnamomum zeylanicum* essential oil | Cinnamomum zeylanicum essential oil was incorporated in fresh minced beef at concentration of 0.5% , 1% and 1.5% and the treated meat was stored at 20C for 12 days | Significantly ($p < 0.05$) decreases in microbial growth and improvement in the sensory properties were observed compared to the untreated meat (control). Cinnamon oil at concentration 1.5% showed best result. | [36] |
| 3 | *Basil essential oil* | 0.25% of *basil* essential oil + 0.015% (150 ppm) of ascorbic were mixed with and incorporated in beef patties and stored at 1 + I $^{O}$C for 7 days. | The beef patties treated with basil essential oil either with or without ascorbic acid had significantly ($p < 0.05$) lower bacterial counts than control from day I of storage onwards. | [60] |
| 4 | *Mentha piperita* essential oil | The essential oil was incorporated in minced beef at concentrations of 1250, 2500, 2500 and 5000 µg/mL against *L.monocytogenes* and S.typhimurium. The meat samples were kept at 7 $^{O}$C for 9 days. | *Mentha piperita* essential oil showed significant bactericidal and bacteriostatic effects on *L. monocytogenes* and *S. typhimurium* and improved the microbiological safety of the minced beef during 9 days of storage period. | [61] |
| 5 | *Thymus capitatus* essential oil | The essential oil was mixed with the ground beef in concentrations of 0.01, 0.05, 1, and 3% as antimicrobial agents to Control pathogenic and spoilage bacteria in the ground meat. | *Thymus capitatus* essential oil. exhibited significant ($p < 0.05$) antiradical activity with an $IC_{50}$ value of 213.53 µg/ml and this could be attributed to its high content of carvacrol (88.89%). | [63] |
| 6 | *Thyme and rosemary* essential oils | Sous vide of turkey breast meat was treated with the essential oils in concentration 100 µL and inculated with $10^8$ cfu of *listeria monocytogenes* survival. The samples were cooked at 55°C, 60 °C, and 65 °C for 5, 15, 30 and, 60 min. and incubated at 37 °C for 18 h in polyethylene vacuum packs. | The application of temperature and Thyme and rosemary essential oils combinations had a positive effect against the total count of bacteria and especially agains L. monocytogenes which decreased to 2.21 log cfu.g-1 | [64] |