

## EFFECT OF OREGANO ESSENTIAL OIL AND MODIFIED ATMOSPHERE PACKAGING ON *Listeria monocytogenes* OF MINCED MEAT STORED AT 5 °C.

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### ABSTRACT

Fresh minced meat was artificially contaminated with *Listeria monocytogenes* and subjected to different treatments; 0.25% oregano essential oil (OEO); 0.5% OEO; 1% OEO; modified atmosphere packaging (MAP); combination of 0.25% OEO+ MAP; 0.50% OEO+ MAP and finally 1% OEO+ MAP to study the effect of these treatments on *Listeria monocytogenes* and on shelf life of minced meat stored at 5°C. The initial contamination level of *Listeria monocytogenes* on minced meat was 3.64 log<sub>10</sub> cfu/g. It was seemed that a reduction of 0.36, 0.95 and 1.52 log<sub>10</sub> CFU/g units were obtained by the treatments with 0.25% OEO, 0.50% OEO and 1%OEO, respectively. This antimicrobial effect, increased with increasing concentration of OEO. On day 4 there was a significant difference (P<0.05) for the number of *Listeria monocytogenes* between the control and all the treatments. After 17 days of storage at 5 °C the log<sub>10</sub> CFU/g of *Listeria monocytogenes* on minced meat treated with MAP+1%OEO was still lower by 2.97 units as compared with the initial number. Results revealed that the total viable count; *Enterobacteriaceae* and *Listeria monocytogenes* were inhibited by all treatments used as compared with the untreated sample. Sensory evaluation revealed that the presence of essential oil contributed to the maintenance of visual appearance of minced meat for long time. The addition of 0.25; 0.5 and 1% oregano essential oil (OEO) to samples packed in MAP affected positively the odour and the colour of the minced meat. Microbiological changes supported the sensory results. Minced meat treated with 0.5% oregano essential (OEO); 1% OEO; Modified Atmosphere Packaging (MAP); 0.25% OEO + MAP ; 0.5% OEO+ MAP and 1% OEO + MAP showed a shelf life at 5°C of 5, 6,11,12, 14 and 17 days respectively. This signifies a prolongation of shelf life at 5°C of 1, 2, 7, 8, 10 and 13 days respectively, as compared with control samples. The antimicrobial effect increased with increasing concentration of OEO. The best results were obtained by treating minced meat with 1% OEO combined with MAP (85% CO<sub>2</sub>+10% O<sub>2</sub>). Apparently, a synergistic activity between the oregano essential oil (OEO) and the modified atmosphere packaging (MAP) is involved.

### INTRODUCTION

*Listeria monocytogenes* is a foodborne pathogen of great concern to food industry and public health (WHO, 1988; Frederiksen, 1991; McCarthy, 1997; Gary *et al.*, 2001). Several outbreaks and sporadic cases of *Listeriosis* have been associated with a variety of foods including meat, fruit, vegetables and dairy products (Silliker, 1986; Griffiths, 1989; Bell and Kyriakides, 1998). Individuals particularly at risk include pregnant women, newborns or infants, and immunocompromised persons. It is important to note that the mortality rate within these groups is 30% (Carpenter and Harrison, 1989; Golden *et al.*, 1990). *Listeria monocytogenes* has been isolated frequently from beef meat,

poultry and meat products (Genigeorgis, *et al.*, 1989; Uyttendaele, *et al.*, 1999; Petersen and Madsen, 2000; Scanga, *et al.*, 2000; Fantelli and Stephan, 2001). Growth on such meats during refrigerated storage could increase the risk. This development has stimulated the interest of researchers and the food industry in determining the potential of the organism to contaminate various foods and survive processing procedures (WHO, 1988; Frederiksen, 1991).

Microbial growth in fresh meats is the main factor associated with quality deterioration spoilage and economic loss (Lambert *et al.*, 1991). Psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of fresh meats (Lambert *et al.*, 1991). One way to retard microbial growth is through antimicrobial agents.

Essential oils are regarded as natural alternatives of chemical preservatives. In addition, their use in foods meets the current demands of consumers for mildly processed or natural products. The antimicrobial properties of essential oils are well documented (Conner, 1993; Beuchat, 1994; Helander *et al.*, 1998; Aligiannis *et al.*, 2001). However, more information on the use of oregano essential oil in modified atmosphere packaging of meats is needed (Panagiotis and George, 2002).

Modified Atmosphere packaging (MAP) and refrigerated meat products have increasing need for quality improvement and extending the shelf life (Genigeorgis, 1985; Lambert *et al.*, 1991; Panagiotis and George, 2002). Developments in packaging materials and techniques over the last 20 years have made the use of modified atmospheres at the retail level possible (Panagiotis and George, 2002).

The objective of these investigations was to evaluate the effect of the treatment with different concentrations of oregano essential oil (OEO) or/and modified atmosphere packaging (MAP) on *Listeria monocytogenes* and on the shelf life of minced meat stored at 5 °C.

## **MATERIALS AND METHODS**

**Minced beef:** Minced beef (14 Kg) of normal pH (pH 5.74) was Obtained from a local processing plant (Al-Hassa, Saudi Arabia) and transported under refrigeration to the laboratory of college of Agricultural and Food Sciences., King Faisal University of Saudi Arabia, within an hour. The compositional analysis of minced meat was determined.

**Table (1). Composition\* of minced beef meat %.**

<b>Content</b>	<b>Water</b>	<b>Protein</b>	<b>Lipid</b>	<b>Ash</b>
Percentage	67.55 (0.565)	18.32 (0.420)	13.25 (0.318)	0.88 (0.078)

\* The values stated refer to three samples, with SD in brackets.

**Extraction of essential oil:** Five hundred grams of dried oregano (*Origanum vulgare*) was purchased from El-Kusari, a local retail spice market (Al-Hassa, Saudi Arabia) and placed in a 2-L flask and 1L of distilled water was added. A continuous steam distillation extraction was performed for 3h and the oil was collected and stored at 4 °C (Skandamis and Nychas, 2000).

***Listeria monocytogenes*:** *Listeria monocytogenes* ATCC 7644 obtained from Oxoid Limited UK (Quality control organisms) was used in the study. Stock cultures were maintained at 4 °C on tryptose phosphate agar (Oxoid CM283) slants and were transferred monthly. Intermediate cultures were prepared by inoculating a loopful from the slant into liquid brain heart infusion (BHI) (Oxoid CM 225) which was then incubated aerobically at 37 °C for 24 h. One drop of this BHI culture was transferred into a second tube of sterile BHI, which was again incubated for 24 h at 37 °C. One loop of this culture was streaked onto plate count agar (PCA) (Oxoid CM 325) and incubated for 24 h at 37 °C. A colony of the PCA culture was transferred into 100 ml of sterile BHI and incubated for 24 h at 37 °C (Zeitoun and Debevere 1991). The working culture was prepared by diluting 10 ml of BHI culture in sterile 90 ml BHI until  $10^{-3}$  and used as inocula.

**Artificial contamination of the minced meat:** One hundred and forty ml of the working culture of *Listeria monocytogenes* were added to 14 Kg minced meat and mixed aseptically. After this artificial contamination, the meat was kept at 5 °C for 2 h to drain and to allow the attachment of the *Listeria monocytogenes* cells on the meat. The contamination level was  $3.64 \log_{10}$  cfu/g, determined on Oxford medium (Oxoid CM 856). The inoculated minced beef was divided into four batches of 3.5 Kg. Each batch (3.5 Kg) was further mixed with the appropriate volume of oregano essential oil (OEO) (final concentration 0 (control), 0.25, 0.50 and 1.00 % v/w). After treatment with oregano essential oil (OEO), each batch (3.5Kg) was divided to two groups (a bout 1.75 Kg each) (i) first group was stored aerobically and packed (60 g/bag) in Sidamil plastic bags (permeability : 6ml O<sub>2</sub> /m<sup>2</sup> /24 h, 15 ml CO<sub>2</sub> /m<sup>2</sup> /24 h, 2ml N<sub>2</sub> /m<sup>2</sup> /24 h, at 1 atmosphere and 23 °C) and stored at 5 °C. (ii) Second group was packed (60 g/bag) under modified atmosphere in Sidamil plastic bags and stored at 5°C.

**Gas packaging:** Minced meat (second group) was packed in the above plastic bags, the bags were filed with a gas mixture, 85%CO<sub>2</sub> + 15% O<sub>2</sub>.

**Analysis:** Samples were analyzed for microbial contents, sensory qualities and pH after 0, 2, 4, 5, 6, 8, 11, 12, 14, 16, and 17 days of storage at 5 °C.

**Microbiological analysis:** At each sampling time, three Samples were aseptically taken by means of 25 g of minced meat (each) and added to sterile physiological saline supplemented by 0.1% peptone (225ml), and homogenized in a stomacher (Lab Blender 400, Seward Medical, London) for 60 s at room temperature. From this homogenate decimal dilutions were made in duplicate in sterile physiological saline containing 0.1% peptone. The Oxford medium (Oxoid CM 856) was used for selective enumeration of *Listeria monocytogenes* (Curtis *et al.*, 1989; Zeitoun and Debevere, 1991). The plates were incubated aerobically at 37 °C for 48 h. Typical *Listeria monocytogenes* colonies were 2-3 mm in diameter, black with a black halo and sunken center (Curtis *et al.*, 1989). Total viable count (TVC) were determined in plate count agar (PCA; Oxoid CM 325), incubated at 25 °C for 72 h (Panagiotis and George, 2002). *Enterobacteriaceae* were determined as colony forming units on Violet Red Bile Glucose Agar (VRBG) (Oxoid CM 485), overlaid with the same medium and incubated at 37 °C for 24 h. (Zeitoun *et al.*, 1994; Panagiotis and George, 2002).

**Sensory analysis:** Sensory evaluation of meat samples was performed during storage according to Gill and Jeremiah (1991) by a sensory panel composed of five members (Staff from the laboratory). The same trained persons were used in each evaluation, and all were blinded to which product was being tested. The sensory evaluation was carried out in artificial light and the temperature of packed product was similar to ambient temperature. Special attention was given to the colour and the presence of exudate in the pack prior to opening and the assessment of abnormal odours during the opening of the pack (Panagiotis and George, 2002). Each attribute was scored on a three-point hedonic scale where: 1= acceptable; 2= marginal; and 3= unacceptable. If scores disagreed, the majority verdict was recorded. Assessment was designed to identify spoilage conditions exclusively. Odour characteristic of raw minced beef was regarded as acceptable. Distinct putrid, sweet, sour or cheesy odours were regarded as indicative of spoilage and therefore unacceptable. Bright colours typical of fresh oxygenated minced meat were considered acceptable. A persistent dull appearance, or unusual colour or appearance was considered unacceptable. The time in days before the taste-panel considered the quality to be at the limit of acceptability (score= 2-1) was defined as the sensory shelf life of samples, under packaging conditions and oregano essential oil concentrations.

**Chemical analysis:**

**PH measurement:** At each sampling time, 10 g of minced meat were blended with 90 ml distilled water for 1 min (Trout *et al.*, 1992) and the pH values were measured, using a digital pH meter (Thermo Orion, model 260A).

**Compositional analysis:** Moisture, ash and fat contents were determined according to AOAC (1985). Protein contents were determined according to Egan *et al.* (1981).

**Statistical analysis:** Obtained data were analyzed using analysis of variances two ways (ANOVA) and subjected least significant difference (LSD) at 0.05% level of significant was used to compare the treatment means (Waller and Duncan, 1969). Computations were done using SAS (1996).

## **RESULTS AND DISCUSSION**

Many herbs and spices have been used for centuries to provide distinctive flavours to food but they also exhibit antimicrobial activity. The compounds responsible for antimicrobial activity are often in the essential oil fraction which consists mainly of phenolic compounds (Conner, 1993; Beuchat, 1994; Nychas, 1995). The major antimicrobial components of oregano essential oil are carvacrol, thymol and thyme (Adam *et al.*, 1998; Aligiannis *et al.*, 2001; Valero and Salmeron, 2003). The essential oils are hydrophobic and their primary site of toxicity is the membrane. They accumulate in the lipid bilayer according to a partition coefficient that is specific for the compound applied, leading to disruption of the membrane structure and function (Helander, *et al.* 1998; Juven *et al.* 1994; Sikkema *et al.* 1994). A critical concentration of the essential oils is needed to cause

leakage of cellular constituents (Mendoza-Yepes *et al.* 1997). The effect of the treatment with different concentrations of oregano essential oil (OEO) and Modified Atmosphere packaging (MAP) on the total viable count during storage at 5°C is illustrated in Table 2. The initial number of total viable count was 4.61 log<sub>10</sub> CFU/g (day zero). The use of 0.5% OEO and 1% OEO resulted in a reduction of the total viable count from log<sub>10</sub>= 4.61 to log<sub>10</sub>= 3.75 and log<sub>10</sub>= 2.84, respectively. The antimicrobial effect increased with increasing concentrations of OEO. The data indicated a marked increase in the lag phase for all samples packed in modified atmosphere. After 4 days of storage at 5 °C there was a significant difference (P<0.05) for the number of total viable count between the control and all the treatments. After 8 days of storage at 5°C, the number of total viable count on samples treated with MAP+ 0.5%OEO and MAP+ 1% OEO were still lower than the initial number (day zero). The addition of 0.25; 0.5 and 1% oregano essential oil (OEO) to samples packed in MAP increased the antimicrobial effect. It needs to be stressed that the antimicrobial effects were affected by the concentration of OEO, as well by the MAP. Minced meat treated with 0.5% oregano essential (OEO); 1% OEO; Modified Atmosphere Packaging (MAP); 0.25% OEO + MAP ; 0.5% OEO+ MAP and 1% OEO + MAP showed a shelf life at 5°C of 5, 6, 11, 12, 14 and 17 days respectively. This signifies a prolongation of shelf life at 5°C of 1, 2, 7, 8, 10 and 13 days respectively, as compared with control samples.

The effect of oregano essential oil and modified atmosphere packaging on pH of the minced meat artificially contaminated with *Listeria monocytogenes* are shown in Table 3. The initial pH value was 5.74. After 4 days of storage at 5 °C, the pH values of minced meat packed in air (control) increased while the pH values of the minced meat packed in modified atmosphere decreased (Tsigarida *et al.*, 2000). The differences were significant (P < 0.05). The decrease in pH is the result of the production of carbonic acid by the dissolved CO<sub>2</sub> in the aqueous phase of the minced meat (Genigeorgis, 1985; Lambert *et al.*, 1991; Panagiotis and George, 2002). Likewise, after 4 days of storage, the pH values of minced meat treated with 0.5% and 1% OEO were significantly lower than the pH values of control samples. The pH values of minced meat treated with MAP and ORE increased at slower rate as compared with the pH value of control.

The assessment of *Enterobacteriaceae* commonly forms part of the microbiological quality monitoring of foods processed for safety (Mossel, 1975; Mossel, 1978; Zeitoun *et al.*, 1994). The Effect of treatment with oregano essential oil (OEO) and modified atmosphere packaging (MAP) on the growth of *Enterobacteriaceae* are illustrated in Table 4. The initial number of *Enterobacteriaceae* was 3.22 log<sub>10</sub> CFU/g. The numbers of *Enterobacteriaceae* were reduced by 0.72 log<sub>10</sub> units for 0.5% OEO and 1.15 log<sub>10</sub> units for 1% OEO. Such reduction would improve the safety of the minced meat.

**Table (2). Effect of oregano essential oil and modified atmosphere packaging on total viable count of minced meat stored at 5°C.**

Treatments	Log CFU of total viable count at n days of storage at 5°C															
	0 day	2 day	4 day	5 day	6 day	8 day	11 day	12 day	14 day	16 day	17 day					
Control (Air)	4.61 <sup>Ca</sup>	5.68 <sup>Ba</sup>	*6.83 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.					
Air +0.25% oregano essential oil	4.55 <sup>Ca</sup>	5.54 <sup>Bb</sup>	*6.61 <sup>Ab</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.					
Air +0.5% oregano essential oil	3.75 <sup>Db</sup>	4.50 <sup>Ccd</sup>	5.73 <sup>Bc</sup>	*6.68 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.					
Air +1% oregano essential oil	2.84 <sup>Ec</sup>	3.34 <sup>Df</sup>	4.15 <sup>Cf</sup>	5.29 <sup>Bb</sup>	*6.53 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.					
MAP (85% CO <sub>2</sub> + 15% O <sub>2</sub> )	4.61 <sup>Fa</sup>	4.55 <sup>Fc</sup>	4.88 <sup>Ed</sup>	5.27 <sup>Db</sup>	5.54 <sup>Cb</sup>	5.92 <sup>Ba</sup>	*6.81 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.					
MAP+0.25% oregano essential oil	4.55 <sup>Fa</sup>	4.43 <sup>Gd</sup>	4.52 <sup>Fe</sup>	4.60 <sup>Ec</sup>	4.98 <sup>Dc</sup>	5.58 <sup>Cb</sup>	6.42 <sup>Bb</sup>	*6.87 <sup>Aa</sup>	n.d.	n.d.	n.d.					
MAP+0.5% oregano essential oil	3.75 <sup>Db</sup>	3.61 <sup>He</sup>	3.70 <sup>Gg</sup>	3.81 <sup>Fd</sup>	3.78 <sup>Ed</sup>	4.27 <sup>Dc</sup>	5.18 <sup>Cc</sup>	5.68 <sup>Bb</sup>	*6.75 <sup>Aa</sup>	n.d.	n.d.					
MAP+1% oregano essential oil	2.84 <sup>C</sup>	2.54 <sup>G</sup>	2.62 <sup>Hh</sup>	2.78 <sup>Ge</sup>	2.74 <sup>Ge</sup>	3.36 <sup>Fd</sup>	4.30 <sup>Ed</sup>	4.87 <sup>Dc</sup>	5.59 <sup>Cb</sup>	6.2 <sup>Bb</sup>	*6.69 <sup>A</sup>					

1- Values with the same superscripts in the same horizontal row (A-I) or vertical column (a-h) are not significantly different (p≥ 0.05).

2- The log colony forming units (C.F.U.) values stated refer to three samples.

3- n.d. = not determined because of spoilage.

4- MAP= 85% CO<sub>2</sub> + 15% O<sub>2</sub>.

5- \* = Typical off odours (spoilage) on the next day.

6-Air= Packed in air in Sidamil plastic bags.

Table (3). Effect of oregano essential oil and modified atmosphere packaging on pH of the minced meat artificially contaminated with *Listeria monocytogenes* and stored at 5°C.

Treatments	pH of minced meat at n days of storage																
	0 day	2 day	4 day	5 day	6 day	8 day	11 day	12 day	14 day	16 day	17 day						
Control (Air)	5.74 <sup>Cb</sup>	6.24 <sup>Ba</sup>	6.93 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +0.25% oregano essential oil	5.76 <sup>Cab</sup>	6.18 <sup>Bb</sup>	6.87 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +0.5% oregano essential oil	5.78 <sup>Da</sup>	6.00 <sup>Cc</sup>	6.48 <sup>Uc</sup>	6.85 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +1% oregano essential oil	5.79 <sup>Ea</sup>	5.98 <sup>Dc</sup>	6.25 <sup>Ccd</sup>	6.52 <sup>Bb</sup>	6.78 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
MAP (85% CO <sub>2</sub> + 15% O <sub>2</sub> )	5.74 <sup>Cb</sup>	5.52 <sup>De</sup>	5.63 <sup>Di</sup>	5.71 <sup>Cd</sup>	5.78 <sup>Cd</sup>	6.39 <sup>Ba</sup>	6.52 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.						
MAP+0.25% oregano essential oil	5.76 <sup>Cab</sup>	5.48 <sup>Fe</sup>	5.68 <sup>Eel</sup>	5.77 <sup>Ec</sup>	5.84 <sup>Dbc</sup>	5.95 <sup>Cb</sup>	6.34 <sup>Bb</sup>	6.58 <sup>Aa</sup>	n.d.	n.d.	n.d.						
MAP+0.5% oregano essential oil	5.78 <sup>Ea</sup>	5.62 <sup>Gd</sup>	5.69 <sup>Fe</sup>	5.81 <sup>Ec</sup>	5.82 <sup>Lcd</sup>	5.93 <sup>Dd</sup>	6.38 <sup>Cb</sup>	6.55 <sup>Ba</sup>	6.70 <sup>Aa</sup>	n.d.	n.d.						
MAP+1% oregano essential oil	5.79 <sup>Ga</sup>	5.64 <sup>Id</sup>	5.71 <sup>Hb</sup>	5.79 <sup>Gc</sup>	5.88 <sup>Ib</sup>	5.90 <sup>Fb</sup>	6.08 <sup>Ec</sup>	6.17 <sup>Dd</sup>	6.32 <sup>Cb</sup>	6.55 <sup>B</sup>	6.65 <sup>A</sup>						

1. Values with the same superscripts in the same horizontal row (A-H) or vertical column (a-f) are not significantly different (p ≥ 0.05).
2. The pH values stated refer to three samples.
3. n.d. = not determined because of spoilage.
4. MAP= 85% CO<sub>2</sub> + 15% O<sub>2</sub>.
5. Air = Packed in air in Sidamil plastic bags.

Table (4). Effect of treatment with oregano essential oil and modified atmosphere packaging on the growth of *Enterobacteriaceae* on minced meat stored at 5°C.

Treatments	Log CFU of <i>Enterobacteriaceae</i> at n days of storage																
	0 day	2 day	4 day	5 day	6 day	8 day	11 day	12 day	14 day	16 day	17 day						
Control (Air)	3.22 <sup>Ca</sup>	4.25 <sup>Ba</sup>	5.90 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +0.25% oregano essential oil	3.04 <sup>Cb</sup>	3.95 <sup>Bb</sup>	5.68 <sup>Ab</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +0.5% oregano essential oil	2.50 <sup>Dc</sup>	3.25 <sup>Cc</sup>	4.80 <sup>Bc</sup>	5.72 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +1% oregano essential oil	2.07 <sup>Ed</sup>	2.38 <sup>De</sup>	3.04 <sup>Ce</sup>	3.85 <sup>Bb</sup>	5.24 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
MAP (85% CO <sub>2</sub> + 15% O <sub>2</sub> )	3.22 <sup>Ea</sup>	3.15 <sup>Ecd</sup>	3.42 <sup>Dd</sup>	3.55 <sup>Dc</sup>	3.93 <sup>Cb</sup>	4.74 <sup>Ba</sup>	5.52 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.						
MAP+0.25% oregano essential oil	3.04 <sup>Eb</sup>	3.10 <sup>Ecd</sup>	3.08 <sup>Ee</sup>	3.18 <sup>Ed</sup>	3.35 <sup>Dc</sup>	3.94 <sup>Cb</sup>	4.76 <sup>Bb</sup>	5.30 <sup>Aa</sup>	n.d.	n.d.	n.d.						
MAP+0.5% oregano essential oil	2.50 <sup>Ec</sup>	2.42 <sup>Ee</sup>	2.38 <sup>Ef</sup>	2.53 <sup>Ee</sup>	2.64 <sup>Ed</sup>	3.02 <sup>Dc</sup>	3.59 <sup>Cc</sup>	4.18 <sup>Bb</sup>	5.12 <sup>Aa</sup>	n.d.	n.d.						
MAP+1% oregano essential oil	2.07 <sup>Id</sup>	1.88 <sup>Hf</sup>	1.92 <sup>GHg</sup>	2.02 <sup>Gh</sup>	2.10 <sup>Ge</sup>	2.44 <sup>Fd</sup>	3.15 <sup>Ed</sup>	3.66 <sup>Dc</sup>	4.09 <sup>Cb</sup>	4.52 <sup>B</sup>	4.88 <sup>A</sup>						

1. Values with the same superscripts in the same horizontal row (A-I) or vertical column (a-h) are not significantly different ( $p \geq 0.05$ ).

2. The log colony forming units (C.F.U.) values stated refer to three samples.

3. n.d. = not determined because of spoilage.

4. MAP= 85% CO<sub>2</sub> + 15% O<sub>2</sub>.

5. Air = Packed in air in Sidamil plastic bags.



After 4 days of storage at 5°C, a reduction of 1.10, 2.86, 2.48, 2.82, 3.52 and 3.98 log<sub>10</sub> CFU/g units were obtained with the treatments with; 0.5% oregano essential (OEO); 1% OEO; Modified Atmosphere Packaging (MAP); 0.25% OEO + MAP ; 0.5% OEO+ MAP and 1% OEO + MAP respectively as compared with control. A similar trend for total viable count (Table 2) was obtained.

The antimicrobial effect of the oregano essential oil (OEO) and Modified Atmosphere packaging (MAP) against *Listeria monocytogenes* on minced meat stored at 5°C is shown in Table 5. The initial contamination level of *Listeria monocytogenes* on the minced meat was 3.64 log<sub>10</sub> CFU/g (day zero). It was seemed that a reduction of 0.36, 0.95 and 1.52 log<sub>10</sub> CFU/g units were obtained by the treatments with 0.25% OEO, 0.50% OEO and 1%OEO, respectively. This antimicrobial effect, increased with increasing concentration of OEO. On day 4 there was a significant difference ( $P < 0.05$ ) for the number of *Listeria monocytogenes* between the control and all the treatments. No change in the number of *Listeria monocytogenes* on control samples was detected during storage at 5°C for 4 days. Johnson *et al.*, (1988) reported survival but no growth, of two strains of *Listeria monocytogenes* on ground beef held at 5°C. These results are similar to observation in the present study. It should be noted that the use of low-permeability film (Sidamil plastic bags permeability : 6ml O<sub>2</sub>/m<sup>2</sup>/24 h, 15 ml CO<sub>2</sub>/m<sup>2</sup>/24 h, 2ml N<sub>2</sub>/m<sup>2</sup>/24 h, at 1 atmosphere and 23 °C ) in contaminated minced meat stored at 5 °C, controlled the growth of *Listeria monocytogenes* throughout the storage period. Tsigarida *et al.*, (2000) reported that the use of low permeability film in beef fillets stored at 5 °C, controlled the growth of *Listeria monocytogenes* throughout the storage period which in agreement with our results. After 11 days of storage, the number of *Listeria monocytogenes* on minced meat treated with MAP, MAP+ 0.25% OEO, MAP+0.50%OEOand MAP+1% OEO was still lower than the initial number (day zero). After 17 days of storage at 5 °C the log<sub>10</sub> CFU/g of *Listeria monocytogenes* on minced meat treated with MAP+1%OEO was still lower by 2.97 units as compared with the initial number. There seems to be a synergistic effect on the inhibition of *Listeria monocytogenes* between MAP and OEO.

Herbs and spices have been used for many centuries to improve the sensory characteristics and to extend the shelf life of foods (Shahidi *et al.*, 1992). The antioxidant activity of oregano essential oil is well known (Economou *et al.*, 1991; Botsoglou *et al.*, 2003). Carvacrol and thymol, the two major phenols that constitute about 82% of the essential oil, are principally responsible for this activity (Economou *et al.*, 1991; Shahidi *et al.*, 1992; Botsoglou *et al.*, 2003). Lipids oxidation is one of the major problems encountered in meat processing, cooking and refrigerated storage. It affected the quality of the meat due to loss of desirable colour, odour and flavour, and a reduced shelf life (Kanner, 1994; Botsoglou *et al.*, 2003) .The results of sensory tests are presented in Table 6.

Table (5). Effect of oregano essential oil and modified atmosphere packaging on *Listeria monocytogenes* of minced meat stored at 5°C.

Treatments	Log CFU of <i>Listeria monocytogenes</i> at n days of storage at 5°C.																
	0 day	2 day	4 day	5 day	6 day	8 day	11 day	12 day	14 day	16 day	17 day						
Control (Air)	3.64 <sup>Aa</sup>	3.70 <sup>Aa</sup>	3.68 <sup>Aa</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +0.25% oregano essential oil	3.28 <sup>Ab</sup>	3.34 <sup>Ab</sup>	3.37 <sup>Ab</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +0.5% oregano essential oil	2.69 <sup>Ac</sup>	2.62 <sup>Ad</sup>	2.50 <sup>Be</sup>	2.42 <sup>Bc</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
Air +1% oregano essential oil	2.12 <sup>Ad</sup>	2.16 <sup>Al</sup>	2.06 <sup>Ag</sup>	1.94 <sup>Ah</sup>	2.18 <sup>Ac</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.						
MAP (85% CO <sub>2</sub> + 15% O <sub>2</sub> )	3.64 <sup>Aa</sup>	3.42 <sup>Bb</sup>	3.01 <sup>Cc</sup>	3.12 <sup>Ca</sup>	2.96 <sup>Da</sup>	2.54 <sup>Ea</sup>	2.30 <sup>Fa</sup>	n.d.	n.d.	n.d.	n.d.						
MAP+0.25% oregano essential oil	3.28 <sup>Ab</sup>	3.18 <sup>Ac</sup>	2.84 <sup>Bd</sup>	2.76 <sup>Bb</sup>	2.68 <sup>Cb</sup>	2.33 <sup>Db</sup>	2.04 <sup>Eb</sup>	1.98 <sup>Ea</sup>	n.d.	n.d.	n.d.						
MAP+0.5% oregano essential oil	2.69 <sup>Ac</sup>	2.52 <sup>Bb</sup>	2.30 <sup>Ci</sup>	2.28 <sup>Cd</sup>	2.15 <sup>De</sup>	1.98 <sup>Ee</sup>	1.82 <sup>Fc</sup>	1.56 <sup>Gb</sup>	1.34 <sup>Ha</sup>	n.d.	n.d.						
MAP+1% oregano essential oil	2.12 <sup>Ad</sup>	2.07 <sup>Ag</sup>	1.88 <sup>ABh</sup>	1.90 <sup>ABe</sup>	1.74 <sup>BCd</sup>	1.50 <sup>CDg</sup>	1.26 <sup>DEd</sup>	1.31 <sup>DEc</sup>	1.23 <sup>DEd</sup>	1.11 <sup>E</sup>	0.67 <sup>F</sup>						

1. Values with the same superscripts in the same horizontal row (A-H) or vertical column (a-h) are not significantly different ( $p \geq 0.05$ ).
2. The log colony forming units (C.F.U.) values stated refer to three samples.
3. n.d. = not determined because of spoilage.
5. Air = Packed in air in Sidamil plastic bags.

Table (6). Evaluation of sensory quality of minced meat treated with oregano essential oil and modified atmosphere packaging and stored at 5 °C. (A= Colour, B= odour).

A: colour

Treatments	Days of storage at 5°C													
	0 day	2 day	4 day	5 day	6 day	8 day	11 day	12 day	14 day	16 day	17 day			
Control (Air)	1	1	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
Air +0.25% oregano essential oil	1	1	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
Air +0.5% oregano essential oil	1	1	1	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
Air +1% oregano essential oil	1	1	1	1	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
MAP (85% CO <sub>2</sub> + 15% O <sub>2</sub> )	1	1	1	1	1	1	2	n.d.	n.d.	n.d.	n.d.			
MAP+0.25% oregano essential oil	1	1	1	1	1	1	1	1	n.d.	n.d.	n.d.			
MAP+0.5% oregano essential oil	1	1	1	1	1	1	1	1	1	1	n.d.			
MAP+1% oregano essential oil	1	1	1	1	1	1	1	1	1	1	1			

1- n.d. = not determined because of spoilage.

2- MAP= 85% CO<sub>2</sub> + 15% O<sub>2</sub>.

3- Air= Packed in air in Sidamil plastic bags.

4- 1= acceptable 2= marginally acceptable 3= unacceptable

Table (6). Continues B: odour.

Treatments	Days of storage at 5°C													
	0 day	2 day	4 day	5 day	6 day	8 day	11 day	12 day	14 day	16 day	17 day			
Control (Air)	1	1	2*	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
Air +0.25% oregano essential oil	1	1	2*	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
Air +0.5% oregano essential oil	1	1	1	2*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
Air +1% oregano essential oil	1	1	1	1	2*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
MAP (85% CO <sub>2</sub> + 15% O <sub>2</sub> )	1	1	1	1	1	1	2*	n.d.	n.d.	n.d.	n.d.			
MAP+0.25% oregano essential oil	1	1	1	1	1	1	1	2*	n.d.	n.d.	n.d.			
MAP+0.5% oregano essential oil	1	1	1	1	1	1	1	1	2*	n.d.	n.d.			
MAP+1% oregano essential oil	1	1	1	1	1	1	1	1	1	1	2*			

1. n.d. = not determined because of spoilage.

2. MAP= 85% CO<sub>2</sub> + 15% O<sub>2</sub>.

3. Air= Packed in air in Sidamil plastic bags.

4. \* = Typical off odours (spoilage) on the next day.

5. 1= acceptable 2= marginally acceptable 3= unacceptable .

It is evident that the volatile compounds of essential oil increased shelf life of minced meat compared with the control with the exception of samples treated with 0.25% oregano essential oil (OEO) and packed in air. The presence of essential oil contributed to the maintenance of visual appearance of minced meat for long time. It was evident that colour remained acceptable for longer period in samples stored under MAP in comparison with samples stored in air. The changes in odour followed closely the changes in bacterial counts Table 2 and 4. The addition of 0.25; 0.5 and 1% oregano essential oil (OEO) to samples packed in MAP affected positively the odour and the colour of the minced meat. According to sensory evaluation, samples treated with 0.5% oregano essential (OEO); 1% OEO; Modified Atmosphere Packaging (MAP); 0.25% OEO + MAP; 0.5% OEO+ MAP and 1% OEO + MAP have a shelf life at 5°C of 5, 6, 11,12, 14 and 17 days respectively. This signifies a prolongation of shelf life at 5°C of 1, 2, 7, 8, 10 and 13 days respectively, as compared with untreated minced meat. This could be explained by the lethal effect of the Oregano essential oil (OEO) and synergistic effect between Modified Atmosphere Packaging (MAP) and Oregano essential oil (OEO).

In conclusion, the effect of oregano essential oil on shelf life and on the inhibition of *Listeria monocytogenes* increases with increasing concentrations of oregano essential oil (OEO). The addition of 0.25; 0.5 and 1% oregano essential oil (OEO) to samples packed in MAP affected positively the odour, colour and the shelf life of the minced meat. The best results were obtained by treating minced meat with 1% OEO combined with MAP (85% CO<sub>2</sub> + 10% O<sub>2</sub>). Apparently, a synergistic activity between the oregano essential oil (OEO) and the modified atmosphere packaging (MAP) is involved.

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### تأثير المعاملة بالزيت العطري العضوي والتعبئة في جو غازي معدل علي *Listeria monocytogenes* في اللحم المفروم المخزن علي 5° م.

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تم إجراء التلقيح الصناعي للحم المفروم الطازج بكتريا *Listeria monocytogenes* بمعدل (3.64 log<sub>10</sub> CFU) لكل جرام من الحم المفروم. وبعد ذلك تم إجراء المعاملات الآتية: المعاملة بالزيت العطري العضوي (المستخلص من أوراق نبات الـ *Origanum vulgare*) عند تركيزات 0.25، 0.50، 1.00%، التعبئة في جو غازي معدل كمعاملة مستقلة. ثم تم المعاملة بتركيزات 0.25، 0.50، 1.00% من الزيت العطري العضوي مع التعبئة في جو غازي معدل وذلك لدراسة تأثير هذه المعاملات علي بكتريا *Listeria monocytogenes* وعلي مدة الصلاحية للحم المفروم المخزن علي درجة حرارة 5° م. أوضحت النتائج أن التأثير المضاد للزيت العطري العضوي علي بكتريا *Listeria monocytogenes* ازداد بزيادة تركيز الزيت. كذلك أظهرت النتائج الانخفاض المعنوي لأعداد بكتريا *Listeria monocytogenes* بواسطة جميع المعاملات بعد أربعة أيام من التخزين إذا ما قورنت بالعينة الغير معاملة. وقد لوحظ أن جميع المعاملات قد أدت إلي إنقاص للعدد الكلي البكتيري والاييتروبيكترياسي إذا ما قورنت بالعينة الغير معاملة. وقد لوحظ أن أعلى تثبيط تم في المعاملة التي طبق فيها 1% من الزيت العطري العضوي مع التعبئة في جو غازي معدل. وقد أظهر التقييم الحسي أن المعاملات بالزيت العطري العضوي قد أدت لتحسين اللون والرائحة. وقد دعمت التغيرات الميكروبيولوجية في اللحم المفروم نتائج التقييم الحسي. وقد تبين أن معاملة اللحم المفروم بالزيت العطري العضوي عند تركيزات 0.5 و 1% والتعبئة في جو غازي معدل كل علي حدة قد أعطي عمر تخزيني 5، 6، 11 يوماً علي التوالي. بينما أعطت المعاملة لكل من (0.25% زيت مع التعبئة في جو غازي معدل) و(0.50% زيت مع التعبئة في جو غازي معدل) و(1% زيت مع التعبئة في جو غازي معدل) عمر تخزيني 12، 14، 17 يوماً، علي التوالي مع أن اللحم المفروم الغير معاملة قد أعطي عمر تخزيني 4 أيام فقط علي درجة 5° م.