



## Potential application of essential oil of *Lavandulastoechas* in poultry meat during refrigerated storage

Jazia Sriti<sup>\*</sup>, Mouna Boulares<sup>2</sup>, Youkabed Zarroug<sup>3</sup>, Rym Essid<sup>1</sup>, Nadia Fares<sup>1</sup>, Selim Jallouli<sup>1</sup>, Saida Djebbi<sup>1</sup>, Nidhal Salem<sup>1</sup>

<sup>1</sup>Laboratory of Bioactive Substances, Centre of Biotechnology of Borj-Cedria, Hammam-Lif, Tunisia.

<sup>2</sup>Research unit, Bio-Preservation and Valorization of Agricultural Products, Higher Institute of Food Industries of Tunisia, El Khadhra City, Tunisia.

<sup>3</sup>Field Crops Laboratory, National Agronomic Research Institute of Tunisia, University of Carthage, Ariana, Tunisia.

### ARTICLE INFO

#### Article history:

Received 09 Jan. 2021  
Received in revised form  
28 Feb. 2021  
Accepted 22 Mar. 2021

#### Keywords:

*Lavandula stoechas*;  
Essential oil;  
Cytotoxicity;  
Poultry meat;  
Sensorial analysis;  
Preservation

### ABSTRACT

The inhibitory effects of essential oil (EO) on bacteria development give them an important role in the fields of the food industry as an additive in food packaging. This study was aimed to identify the effect of *Lavandula stoechas* EO at different concentrations on chicken fillets quality during refrigerated storage. Antimicrobial and antioxidant activities showed that EO extracted has an important antibacterial activity and antiradical potential. In chicken fillets, the lavender EO (100 and 200 ppm) reduced their oxidation and microbial proliferation during refrigerated storage and with no cytotoxicity effect towards murine macrophage cells. During the storage period, the values of pH, dry matter, acidity and cooking loss of treated fillets were lower than that of the control. The statistical analyses proved greatly significant variations of color between the control and the treated by LEO, during storage. The sensory analysis selected an improvement effect in the organoleptic quality of the chicken meat when it was supplemented by 100 ppm of LEO. The statistical analysis of the microbiological characteristics clearly discriminated the control and those treated with LEO ( $p < 0.05$ ). The effectiveness of LEO was proved by its incorporation as a natural food preservative and the improvement of the shelf life of poultry meat products by about 3 days.

**Citation:** Sriti J, Boulares M, Zarroug Y, Essid R, Fares N, Jallouli S, Djebbi S, Salem N. **Potential application of essential oil of *Lavandulastoechas* in poultry meat during refrigerated storage.** J food safe & hyg 2021; 7(1):11-26

### 1. Introduction

The Agri-food sector is considered one of the sectors that directly affect the way life of humans. The food industry must be able to provide food that is likely to ensure the nutritional satisfaction of the consume (1).

The first demand of the consumer was the safety of the product put on sale without any adverse health effects. Thus, the storage of the raw material is very important in seasonal industries. The storage of finished products depends on their nature, the process of conservation and the method of packaging; bulk or packaged.

<sup>\*</sup>Corresponding author. Tel.: +216 79325855  
E-mail address: [jazia.sriti@cbbc.mrt.tn](mailto:jazia.sriti@cbbc.mrt.tn).



Meat is the product of the transformation of muscle after the sacrifice of the animal. Because of its high water content and high nutritional value proteins, this food is essential indispensable in a balanced diet (2). However, these same reasons make it a very favorable niche for the development of microorganisms. Slaughter is the main source of contamination, 80 to 90% of the micro flora found in meat comes from slaughterhouses. However, apply of these antimicrobials is partial since of the residual adverse actions such as chicken skin discoloration, consumer awareness, and corrosiveness to equipment, cost, or limited effectiveness (3). Chicken meat is known as a good source of protein for the reason that it is delicious, easily-cooked and affordable. Though, growth of spoilage bacteria and lipid oxidation may occur in chicken meat which has been disagreed by the poultry industry (4). For some decades, consumers have been turning to natural products. This interest is manifested by a growing demand for biological products that are active and devoid of any harmful effects. Special interest is reserved for the antioxidant and antimicrobial effects of aromatic and medicinal plants, their EO as well as their active compounds in the food industry. Today, the use of EOs in food preservation without processing proves a relevant choice because it helps to control the microbial flora and preserve the food from oxidation phenomena. Lavender oil (*Lavandula stoechas*) is selected for its antibacterial, antifungal, carminative, antifatulence, antiholic, sedative and antidepressive activities (5). Lavender oil is widely used in traditional medicine for their relief cough, neuralgia, insomnia bath and compress (6). Though, many researches confirmed the anti-glycemic

and antidiabetic effect of essential of *L. stoechas* in vivo (7). The goal of this study was to screen the effect of lavender EO addition on the physical, chemical and microbiological properties of poultry meat during refrigerated storage.

## 2. Materials and Methods

### 2.1. Plant material

Aerial parts of *L. stoechas* were collected from Nabeul Governorate at vegetative stage (17 November 2018). Steam distillation was used to extract essential oil from the plant material as described earlier (8).

### 2.2. GC-MS analysis

The analysis of EO was conducted using Agilent GC 7890A gas chromatograph equipped with a 5975C mass spectrometer and a HP-5MS (30 m x 0.25 mm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25  $\mu$ m f.t). The following oven temperature was used: 1 min at 40°C, then up to 100°C with the rate of 8°C/min, held for 5 min. then heated to 200°C at a rate of 10°C/min, and then kept constant at 200°C for 3 min finally it rised to 300°C at a rate of 12°C/min, transfer line temperature was 250°C. Helium (carrier gas) flow rate was 1 mL/min; the injector split ratio was 100:1; the mass range and electron impact was 50-550 m/z and 70 eV, respectively.

### 2.3. Cytotoxicity assay

Cytotoxicity of EO was determined in murine macrophage cell line Raw 264.7, preserved in RPMI-1640 medium added with 10% fetal bovine serum (FBS) (9). Cell viability was estimated after incubation with 0.1% trypan blue by counting the stained cells under light microscope. Macrophages were applied on a 96

multi-well plate at 105 cells/well, allowed to adhere overnight at 37°C under 5% CO<sub>2</sub>; after replacing the medium by a fresh one containing 10 µL of a serial dilution of EOs, cells were incubated for 24 h at 37°C and viability was estimated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test.

## 2.4. Biological activity

### 2.4.1. DPPH assay

In microtubes, 0.5 mL of EO at different concentrations was mixed with 3 mL of a DPPH solution (0.004% in methanol). The mixture was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm. The concentration of oil that could scavenge 50% of the DPPH radicals (IC<sub>50</sub>) was calculated according to the method of Ceylan et al. (10).

### 2.4.2. Ferric-reducing power (FRAP) assay

This method consists of mixing of EO (1000 µL) at different concentrations with phosphate buffer (1250 µL, 0.2 mol/L, pH 6.6) and potassium ferricyanide (1250 µL, 1%). The resulting mixture was incubated for 20 min at 50°C. Then, 1250 µL of trichloroacetic acid (10%) was added to stop the reaction. Finally, a volume of 1250 µL of the supernatant was added to 1250 µL of H<sub>2</sub>O and 250 µL of a 0.1% solution of ferric chloride. Absorbance was measured at 700 nm, referring to a positive control which was ascorbic acid. The results are expressed in effective concentration (EC<sub>50</sub>, µg/mL), EO corresponding to an absorbance equal to 0.5 (11).

### 2.4.3. Disk diffusion assay

The antibacterial activity of LEO was performed using well diffusion method (12). Bacterial strains were grown respectively on Luria-Bertani (LB) broth at 37°C for 18-24 h and were inoculated into Mueller-Hinton (MH) agar. Petri dishes containing WB (Winge broth) medium were aseptically inoculated with a suspension of 10<sup>8</sup> CFU (colonies forming unit)/mL of *Candida albicans*. The turbidity was adjusted to 0.5 McFarland to yield approximately 10<sup>8</sup> CFU/mL with a densimat. After 18-24 h of incubation at 37°C for, the antibacterial activity was determined by measuring the zone of growth inhibition (IZ) around wells. Gentamicin and amphotericin B (10 µg/disc) was used as antimicrobial standards.

### 2.4.4. The minimal inhibitory concentration

The minimal inhibitory concentration (MIC) was determined by microdilution method as described by De Lima Marques et al. (13). Subsequent serial decimal dilutions were performed on sterile 96-well microplates. Each EO dilution was placed in contact with a microbial in oculum in exponential growth phase in MH or WB medium. The initial microbial concentration was adjusted to 5×10<sup>5</sup> CFU/ mL for bacterial strains and 5×10<sup>4</sup> CFU/mL for yeasts with sterile saline.

## 2.5. Microbiological and physicochemical quality of chicken fillets

### 2.5.1. Samples preparation

Two Kilograms of fresh chicken fillets were provided by a Tunisian poultry industry; El MAZRAA. Skinless and boneless chicken breast fillets with an average

weight of 150 g were transferred to the laboratory at 1h post-preparation in refrigeration temperature. Upon arrival and under aseptic conditions, two concentrations of LEO (LEO<sub>1</sub>: 100 ppm, LEO<sub>2</sub>: 200 ppm) were incorporated and well distributed in chicken fillets. Finally, chicken breast fillets (CBF) samples were immediately sampled (day 0), placed in polystyrene trays, covered under aerobic packaging with films in order to have an additional physical separation to the surrounding environment and cold stored at 4°C for 9 days (14).

#### 2.5.2. Proximate analysis

The CBF samples were analyzed for proximate composition: moisture content was determined by air-drying of a portion of minced fillet at 105°C for 24 h (method 950.46), and dry matter according to the official methods of the Association of Official Analytical Chemists (15).

##### 2.5.2.1. PH and total acidity

The method of Boulares et al. (16) was used to assess the value of pH. Total acidity was assessed as described by Al-Masri et al. (17).

##### 2.5.2.2. Cooking loss and color measurements

The cooking loss was determined according to Noori et al. (14). The colorimetric parameters L\* (Lightness), a\* (redness) and b\* (yellowness) of CBF were measured according to Ben Abdesslem et al. (18) using a colorimeter (Minolta Chroma Meter, CR-300, Tokyo, Japan).

##### 2.5.2.3. Texture measurement

The texture of control and treated CBF was measured according to Kaewprachu et al. (19).

##### 2.5.2.4. Microbiological analysis

Mesophilic aerobic plate count (MAPC), coliform count (CC), yeasts and moulds were calculated according to method to Boulares et al. (16).

##### 2.5.2.5. TBAR<sub>s</sub>

The lipid oxidation of meat samples was evaluated by the measurement of thiobarbituric acid reactive substances (TBAR<sub>s</sub>) using a method of Noori et al. (14).

#### 2.5.3. Shelflife kinetic study

To expand a predictive representation in order to amount the shelf life of CBF treated with lavender EO, a mathematical model was developed using an Accelerated Shelf Life Test carried out as a function of mesophilic aerobic plate count (MAPC), coliform count (CC) and pH. Chicken fillets were stored at controlled isothermal conditions of 4°C, 14°C and 24°C. Sampling was evaluated in the same time intervals to evaluate the deterioration process during storage time based on variation of storage temperature (4°C: 0, 3, 6 and 9 days; 14°C: 0, 3 and 6 days and 24°C: 0 and 3) (18).

Equation (1) exhibited the kinetic equation, and equation (2) is the Arrhenius equation, which is focusing on the accelerated shelf-life simulation test.

$$\frac{d[A]}{dt} = K [A]^n \quad (\text{Eq 1})$$

Where [A] is the quality factor which can be quantified, such as chemical, physical, microbiological or sensory parameters, k is the reaction rate constant (days<sup>-1</sup>), n is the reaction order and t is the reaction time (days). The order which gave the best regression (R<sup>2</sup>) among the experimental values was chosen as representative.

The effect of temperature ranging from 4°C to 24°C on the rate of MAPC, CC and pH was determined by means of the Arrhenius equation:

$$k = k_0 \exp(-E_a/RT) \quad (\text{Eq 2})$$

Where k: the reaction rate constant, R: the universal gas constant (8.31 J K<sup>-1</sup> mol<sup>-1</sup>), T: the absolute temperature (Kelvin, K), E<sub>a</sub>: the activation energy (J mol<sup>-1</sup>) of the studied action and k<sub>0</sub>: the pre-exponential factor of the frequency factor (20).

This equation allows to define the MAPC, CC and pH indicating the end of the shelf life focused on the danger level. The shelf life of chicken breast fillets can be finally predicted accordingly:

$$\text{SL (Days)} = \frac{\ln [A_L] - \ln [A_0]}{K_{4^\circ\text{C}}} \quad (3)$$

SL is the shelf life expressed as days, A<sub>L</sub> corresponded to the limit values of MAPC, CC and pH of chicken fillets, A<sub>0</sub> is the correspondent values at initial storage time. K and T represents the pseudo zero rate constants at the selected temperature.

#### 2.5.4. Sensory analysis

Sensory analysis was performed by expert panelists of 9 judges from El MAZRAA Industry. Cooked chicken meat samples were coded randomly and served to panelists to evaluate color, odor, taste, after taste, hardness, juiciness and overall acceptability using a 6-point hedonic scale, where: 5 was extremely like and 0 was extremely dislike (ISO13299, 2016).

#### 2.6. Data analysis

All data were subjected in triplicate and the results were expressed as means values ± standard deviations (SD). An analysis of variance (ANOVA) was carried using STATISTICA software. A Duncan test was used to determine the statistical difference of the mean value at 5%.

### 3. Results

#### 3.1. EO composition

EO compounds identified in *L. stoechas* are listed in Table 1. The chemical composition showed the presence of fenchone (57%) and camphor (24%), as the major compound of LEO.

**Table 1.** Essential oil composition of *Lavandula stoechas*

| Compound                    | %                |
|-----------------------------|------------------|
| $\alpha$ -pinene            | 0.61 $\pm$ 0.03  |
| Camphene                    | 1.76 $\pm$ 0.01  |
| p-cymene                    | 0.44 $\pm$ 0.02  |
| D-limonene                  | 0.79 $\pm$ 0.03  |
| 1,8-cineol                  | 0.72 $\pm$ 0.02  |
| cis-Linalool oxide          | 1.19 $\pm$ 0.04  |
| Fenchone                    | 57.97 $\pm$ 2.13 |
| Linalool                    | 1.46 $\pm$ 0.01  |
| Camphor                     | 24.65 $\pm$ 1.02 |
| Borneol                     | 0.48 $\pm$ 0.00  |
| p-cymen-8-ol                | 0.43 $\pm$ 0.00  |
| Estragol                    | 0.63 $\pm$ 0.01  |
| D-carvone                   | 0.50 $\pm$ 0.02  |
| Myrtenyl acetate            | 0.67 $\pm$ 0.03  |
| $\alpha$ -cadinene          | 0.50 $\pm$ 0.01  |
| delta-cadinene              | 0.85 $\pm$ 0.02  |
| cis- $\alpha$ -copaene-8-ol | 0.67 $\pm$ 0.02  |
| Viridiflorol                | 2.99 $\pm$ 0.02  |

The values shown in this table were the average of three replicates and given as mean  $\pm$  SD (n = 3).

**Table 2.** Antioxidant activities of essential oil of *Lavandula stoechas*

| EO                                       | Synthetic standards         |                               |
|------------------------------------------|-----------------------------|-------------------------------|
|                                          | BHT                         | Ascorbic acid                 |
| DPPH (IC <sub>50</sub> , mg/ml)          | 1.4 $\pm$ 1.21 <sup>a</sup> | 0.046 $\pm$ 0.08 <sup>b</sup> |
| Reducing power(EC <sub>50</sub> , mg/ml) | 4 $\pm$ 1.15 <sup>a</sup>   | -                             |
|                                          |                             | 0.068 $\pm$ 0.06 <sup>b</sup> |

IC<sub>50</sub> value: the effective concentration at which the antioxidant activity was 50%. EC<sub>50</sub> value was obtained by interpolation from linear regression analysis. Each value is expressed as mean SD (n=3). One-way ANOVA followed by Duncan's multiple range test was used.

### Antioxidant activity of LEO

The essays of the anti-radical activity showed an important inhibitive capacity of the radical DPPH (Table 2). LEO has a very higher anti-radical capacity reaching a value of 1.4 mg/mL. The antioxidant capacity of LEO was also evaluated by the ferric reducing ability of plasma (FRAP) assay (Table 2). EO has lower EC<sub>50</sub> values than synthetic standards (EC<sub>50</sub> = 68 µg/mL).

### 3.3. Antimicrobial activity of LEO

The antibacterial activity of LEO was evaluated against nine strains. Only four bacterial strains clearly varied according to the EO tested (Table 3). The highest inhibition diameter was obtained against *Methicillin-resistant Staphylococcus aureus* (MRSA) with IZD of 46 mm. This activity was stronger to selected antibiotics and this higher activity was confirmed by at low concentration equal to 32 µg/mL. *E. faecalis* and *S. aureus* strains also showed significant sensitivity for the two EO tested with an MIC to 1 mg/mL. Regarding the antifungal activity, the two EO tested showed average activity against *Candida albicans* strain with an inhibition diameter equal to 10 mm. This activity was more important with at MIC of 2mg/mL (Table 3).

## 3.2. Preservation of poultry meat using LEO

### 3.2.1. Cytotoxicity power

EO of lavender was evaluated for their cytotoxicity towards murine macrophage cells. According to the MIC values EO displayed no cytotoxicity effect with LC<sub>50</sub> of 235 µg/mL. LEO have a potential application in the food and pharmaceutical industries. For this reason results the use of this plant in application on poultry meat during refrigerated storage.

### 3.2.2. Microbiological and physicochemical quality of chicken fillets

The starting pH value of the CBF increased from 5.5 to an average value of 6.4 for control, after 9 days of storage (Figure.1). During refrigerated storage, we observed a significant ( $p < 0.05$ ) increase of pH for all tested CBF. In all analyzed samples, a progressive increase in the acidity during storage was detected (Figure 1). In fact, the acidity increased in control from an initial value of 1.1 to 1.6 g/kg, after 9 days of storage. Initial recorded cooking loss of control CBF was about 13.2% (Figure 1). At the first day of storage, the weight loss decreased to reach a value of 12.6% with the increase of LEO. The change in the cooking loss of all studied samples was increased significantly ( $p < 0.05$ ) by increasing storage time. During storage period, the variation in TBAR<sub>s</sub> contents was decreased in the CBF incorporated with 200 ppm of LEO and reached 0.05 mg/Kg (Figure 2).

### 3.2.3. Color and texture variation

Changes in the color parameters L\* (lightness), a\* (redness) and b\* (yellowness) of all CBF during refrigerated storage are illustrated in Figure 3. In this study, we noticed that L\* varied considerably during storage after treatment with LEO. For the untreated CBF, the value of L\* increased slightly during storage period. In fact, the color of CBF run clear and this is due to the degradation of the myoglobin contained in chicken meat.

**Table 3.** Antimicrobial activities of essential oil of *Lavandula stoechas*

|                                                           | Essential oil  |                         | Standard antimicrobial |                |
|-----------------------------------------------------------|----------------|-------------------------|------------------------|----------------|
|                                                           |                |                         | Gentamicin             | Amphotericin B |
| <b>Bacteria</b>                                           | <b>IZ (mm)</b> | <b>MIC (mg/ml)</b>      |                        |                |
| <i>Staphylococcus aureus</i> ATCC 6835                    | 20             | 1±0.00 <sup>b</sup>     | 22                     | -              |
| Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) | 46             | 0.031±0.00 <sup>c</sup> | 25                     | -              |
| <i>Enterococcus faecalis</i> ATCC 29212                   | 22             | 1±0.00 <sup>b</sup>     | 15                     | -              |
| <b>Yeast strain</b>                                       |                |                         |                        |                |
| <i>Candida albicans</i> ATCC 10231                        | 10             | 2±0.00 <sup>a</sup>     | -                      | 20             |

MIC; minimal inhibitory concentration. IZ; inhibition zone diameter. Values were given as mean ± SD (n = 3) and were the average of three replicates. One One-way ANOVA followed by Duncan's multiple range test was used.

At the end of the storage period, the value of L\* decreased slightly and reached 50 for LEO<sub>2</sub>. It can be explained by the fact that the increase of LEO dose contribute to the decrease of the value L\*. The statistical analyses revealed the presence of highly significant changes of color between the control and the treated fillets by LEO, during refrigerated storage.

Concerning the parameter a\*, it varied between (-60) and (+60), indicating respectively the dominance of green and red (Figure 3). This specific color of chicken decreased considerably during storage for all analyzed CBF. We noticed a significant difference (p<0.05) between the control chicken fillets and those treated with LEO.

On the other hand, the increasing doses of LEO caused an increase of the red color of chicken meat when compared to the untreated control. These results showed that LEO has considerably inhibited and reduced the auto-oxidation of myoglobin in the presence of oxygen. For the parameter b\*, it varied from (-60) for blue shades, to (+60) for yellow shades. During

the nine days, it was observed that the color of all analyzed CBF varied and turned towards the dark shade. More, we noted that the loss of the original shade of chicken meat was more pronounced (p<0.05) for the control. In fact, color was changed slightly when chicken fillets were treated with the highest concentration of LEO. This value decreased during storage, highlighting a significant softening of the flesh of the different analyzed samples (Figure 3). Indeed, after 9 days of storage at 4°C, we recorded that the untreated control could not maintain firmness and it became softer (4.68 N). However, adjunction of LEO to CBF showed a distinction when compared to the control sample. In fact, at the end of storage, treated chicken fillets remained firm with hardness about 6.89N for chicken fillets added with the highest LEO concentration.



**Table 4.** Shelf life of chicken breast fillets at different storage temperatures

| Chicken breast fillets   | Parameter | Storage temperature<br>(°C) | Shelf life<br>(Days) |
|--------------------------|-----------|-----------------------------|----------------------|
| <i>Untreated Control</i> | MAPC      | 4                           | 5±0.61               |
|                          |           | 14                          | 3±0.23               |
|                          |           | 24                          | 1±0.03               |
|                          | CC        | 4                           | 6±0.8                |
|                          |           | 14                          | 2±0.01               |
|                          |           | 24                          | 1±0.02               |
|                          | pH        | 4                           | 6±0.76               |
|                          |           | 14                          | 3±0.13               |
|                          |           | 24                          | 2±0.09               |
| <i>Treated with LEO</i>  | MAPC      | 4                           | 7±0.50               |
|                          |           | 14                          | 4±0.21               |
|                          |           | 24                          | 3±0.11               |
|                          | CC        | 4                           | 9±1.30               |
|                          |           | 14                          | 3±0.02               |
|                          |           | 24                          | 2±0.03               |
|                          | pH        | 4                           | 16±1.65              |
|                          |           | 14                          | 4±0.80               |
|                          |           | 24                          | 2±0.20               |

MAPC: Mesophilic aerobic plate count, CC: Coliform count, Values were given as mean ± SD (n=3).

### 3.2.4. Microbiological changes

The variation of microbiological counts of mesophilic aerobic plate counts (MAPC), total coliforms (TC), fecal coliforms (FC) and yeasts and molds (YM) in fresh CBF, during refrigerated storage, are illustrated in Fig. 4. Our result showed that initial MAPC counts in poultry fillets were 3.69 log CFU/g, 3.53 log CFU/g and 3.20 log CFU/g, respectively in the control, LEO<sub>1</sub> and LEO<sub>2</sub>. Thus, the microbial growth presented in control sample was the highest reported initially and during all storage period. We observed that the addition of LEO was associated with a significant effect ( $p < 0.05$ ) on the MAPC when compared to the control. However, the treated fillets with LEO remained consumable until the 9<sup>th</sup> day of storage. Moreover, after the incorporation of LEO, the proliferation of this microorganisms were significantly declined with a reduction rate of 18.36% and 23.46% for LEO<sub>1</sub> and LEO<sub>2</sub>, respectively. The result showed that all treated fillets presented initial loads in TC and FC lower than those recorded for the control with 2.17 log CFU/g and 2 log CFU/g, respectively (Figure 4). These floras proliferated significantly during storage period. On the other hand, their relative charges remained lower than that of the control after the addition of LEO. In fact, the control presented an increase of TC and FC counts which exceeded the upper acceptability limit (3 log CFU/g, JOPF 2000), after 3 days of storage. This result proved that the essential oil has an interesting bacteriostatic activity against FC and TC with respective reduction rates of 10% and 8% for LEO<sub>1</sub> and of 8% and 11.4% for LEO<sub>2</sub>.

**Table 5.** Effect of *Lavandula stoechas* essential oil (LEO) on the sensory criteria of chicken meat

|                     | Control   | LEO, 100<br>ppm | LEO, 200<br>ppm |
|---------------------|-----------|-----------------|-----------------|
| Odor                | 3.25±0.02 | 3.5±0.01        | 2.75±0.00       |
| Surface color       | 3±0.03    | 2.8±0.02        | 2.6±0.01        |
| Inside color        | 3.75±0.04 | 3.75±0.03       | 3.5±0.02        |
| Taste               | 3.5±0.02  | 3.8±0.03        | 2.9±0.01        |
| After taste         | 4±0.04    | 3.9±0.04        | 3.28±0.05       |
| Tenderness          | 4.3±0.04  | 4.25±0.03       | 3.75±0.01       |
| Juiciness           | 4±0.00    | 4±0.00          | 3.75±0.35       |
| Global appreciation | 4±0.00    | 4.1±0.01        | 3.33±0.02       |

### 3.3. Shelf life assessment

In this study, the logarithmic evolution of pH values and microbiological counts of control and treated chicken breast fillets, during storage at different temperatures during 9 days followed the apparent zero and first order kinetics, respectively (Data not shown). The shelf life decreased with the temperature increase from 4°C to 24°C, for all analyzed samples and for all studied criteria. The initial mesophilic aerobic plate count (MAPC) count suggested that the chicken samples were of good quality. However, this count exceeded the recommended limit for control from the 6<sup>th</sup> day of storage (Table 4). This finding indicated that the shelf-life of control was about 5 days and that incorporation of LEO increased the shelf-life of this product to 7 days by maintaining the microbiological count under the upper acceptability limit, over the entire storage period at 4°C. Moreover, LEO extended

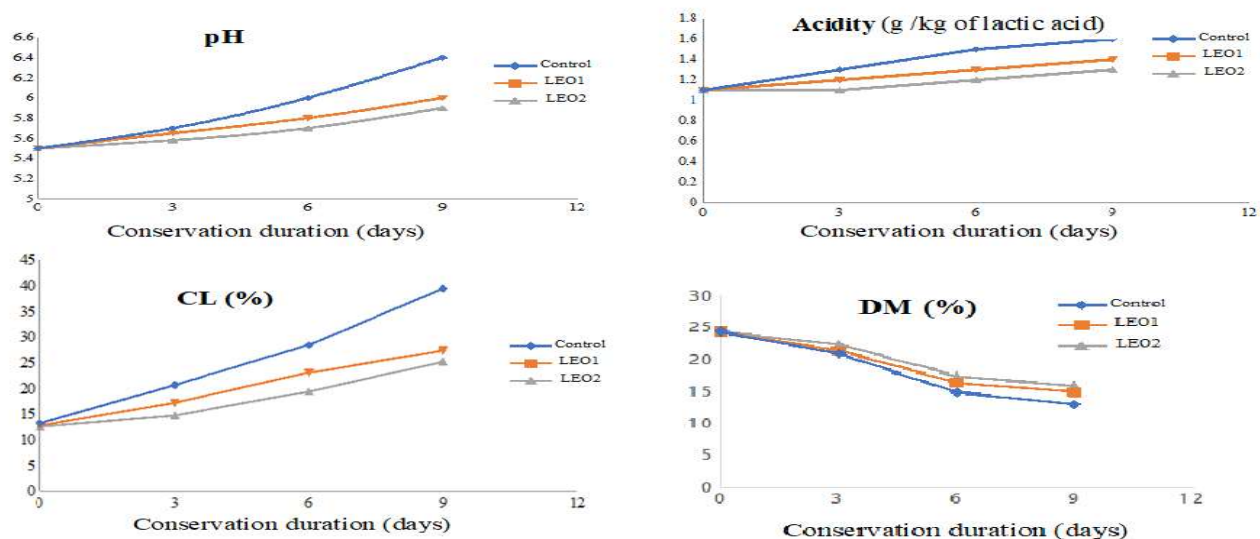
the shelf life of chicken fillets by about 1 and 2 days, for 14°C and 24°C, respectively. In fact, samples stored at 24°C became spoiled and unhealthy for human consumption after 1 and 3 days, respectively for untreated control and treated chicken fillets. These findings confirmed the interesting antimicrobial activity of LEO. The same findings were observed for coliforms counts. In fact, the shelf life of treated chicken fillets was about 9 days comparing to untreated control (6 days). Besides, observed results on pH evolution of chicken fillets, during storage, showed that LEO exerted an inhibitory effect on microbial flora which in turn improved the shelf life of chicken breast fillets at different temperatures (Table 4).

In fact, the shelf life of treated chicken fillets stored at 4°C was about 16 days, while after 6 days the untreated sample was spoiled. Also, untreated fillets stored at 14°C and 24°C spoiled after 3 days and 2 days, respectively.

Thus, storage at refrigerated condition (4°C) with LEO incorporation preserved a good quality of CBF.

### 3.4. Sensory properties

The sensory evaluation revealed that acceptability of all descriptors decreased significantly ( $p < 0.05$ ) with the incorporation of the highest concentration of LEO in poultry meat in comparison to the other two analyzed samples (Table 5). This could be associated to the initial organoleptic properties of LEO resulting in a sharply decrease of the sensory properties of the treated sample. However, the quality of chicken meat treated with only 100 ppm of LEO was improved in terms of odor, taste and overall acceptability when compared to untreated control. However, no difference was detected in the two tested samples.



**Figure 1.** Evolution of pH, acidity (g/kg of lactic acid), cooking loss (CL, %) and dry mater (DM, %) in untreated (Control) and treated (LEO1: 100 ppm, LEO2: 200 ppm) of poultry meat fillets during storage.

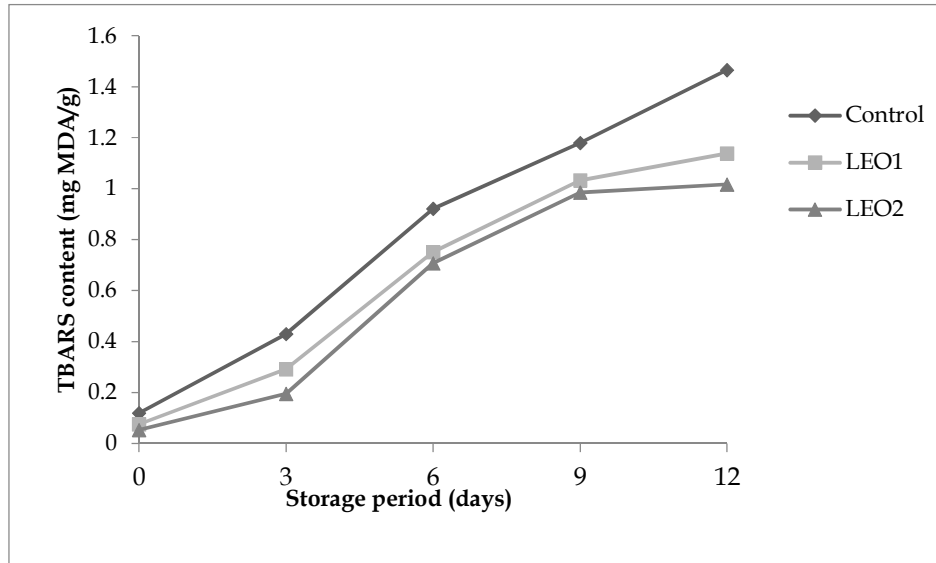


Figure 2. Changes of TBARS values in untreated (control) and treated (LEO1: 100 ppm, LEO2: 200 ppm ) poultry meat fillets during storage

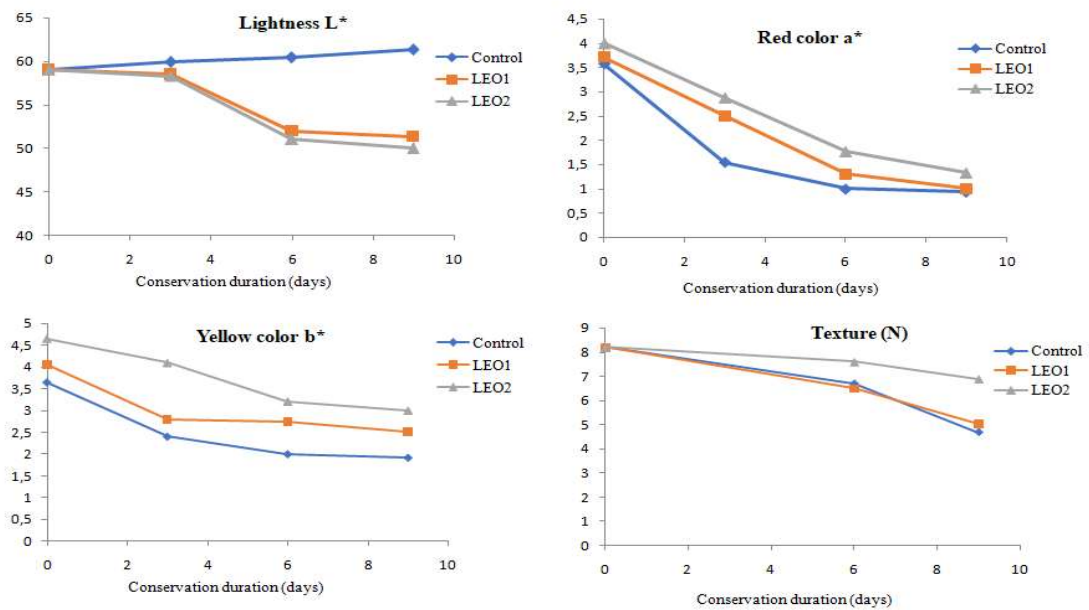


Figure 3. Color and texture (N) evolution in untreated (Control) and treated (LEO1: 100 ppm; LEO2: 200 ppm) poultry meat fillets during storage.

#### 4. Discussion

According to the results, EO of lavender showed two different chemotypes since camphor and fenchone were the major compounds. Our Results were similar with other studies who underlined that fenchone presented to be the main fraction of LEO (21-23). However, other studies have reported that LEO, originating from Turkey, was characterized by a very diverse profile, with a high content of pulegone, menthol and menthone, in parallel with a total absence of fenchone and camphor (24). Indeed, another study reported the presence of  $\alpha$ -thujone, camphor, eucalyptol and fenchone as the major compound of LEO (25). According to Zuzarte et al. (26), LEO could be divided into two chemotypes (fenchone/camphor and fenchone/1,8-cineole) being the fenchone/camphor the mainly usually detected. Concerning the antiradical activity of LEO, the values are less important to those found by Bouyahya et al. (22) which indicated higher activities of Moroccan LEO ( $IC_{50} = 785 \mu\text{g/mL}$ ). On the other hand, Messaoud et al. (27) noted a lower anti-radical activity with  $IC_{50}$  value reaching to  $2321 \mu\text{g/mL}$ . Several studies have revealed that EO have antioxidant properties and thus prevent lipid peroxidation (28). The antioxidant activity of LEO showed a significant anti-free radical effect. This activity was certainly linked to the volatiles compound oil which was dominated by fenchone and camphor. As well as, the power of the EO of lavender to trap the free DPPH radical was in agreement by other studies (21). The antibacterial activity obtained was in concurrence with those mentioned in the documentation (29). The difference in EO dispersion was due to the nature of the bacterial wall. This explains why Gram positive

bacteria were further sensitive to EO than Gram negative bacteria. On the other hand, the antibacterial activity of EO could be specifically attributed to the major volatile compounds, to the synergistic reactions among these constituents and to the effects of minor compounds which can reinforce the antibacterial action (30). The EO of lavender used in this study contained a higher content in camphor which has showed good security against human macrophage cell lines (9,31). According to Boubahya et al. (22), LEO have a potential application in the food processing industries. For this reason results the use of this plant in application on poultry meat during refrigerated storage. In this study, initial pH value was in agreement with that proved by Pires et al. (32). It should be noted that this variation is due to the accumulation of lactic acid and the oxidation of lipids representing the origin of the rancidity of the chicken muscle which influences the organoleptic characteristics of the product (23). Based on the capacity of this EO to reduce the enzymatic hydrolysis causing the rancid taste and the bitter flavor, as well as the increase in free acidity (33), we can deduce that the concentration of 200 ppm was the most effective. Liquid holding capacity in chicken meat, meat and fish are most factors affecting the sensory properties and acceptance of these products as well as for economic reasons such as weight decrease due to water loss particularly during cooking (18). According to Boulares et al. (20), various factors contributed to these properties like pH, temperature, and change in the muscle fibers configuration caused by endogenous enzymes activities. Our finding was in agreement with those of Huff-Lonergan and Lonergan (34) suggesting that the presence of high levels of

antioxidants in the flesh can induce a reduction in proteolytic activity. The total dry matter decreased significantly during refrigerated storage for all the analyzed chicken fillets.

Appearance and color are credited as primordial to guarantee and influential factors in marketability of poultry meat by the consumer (4). This parameter measured the variation of the specific red/pink color of the chicken meat preferred by the consumer and that states the freshness of the product at this tonality (32). These authors detected differences in the color meat from red to brownish and can be explained by the formation and accumulation of myoglobin that derived in a gradual discoloration. This oxidized form of myoglobin lead to more brownish meat which decreased its market value during storage. This result was partially in accordance with that investigated by Pires et al. (32) screening that films containing EO were effective in preserving the red tone and better preventing oxidation of poultry meat. Hardness is the main important quality criterion for fish muscle (19) a soft muscle texture is considered as negative for consumers which demand an appropriate fish value. Considering that the firmness of meat is influenced by the structure of the protein and its water retention capacity, our findings confirmed that the addition LEO reduced protein degradation and thus inhibit the denaturation of myosin and the weakening of myofibrillar network as reported by Xia et al. (35).

## 5. Conclusions

In our study focused on the application of the EO of *L. stoechas* on the quality of chicken breast fillets during storage. Furthermore, these results showed that the addition of EO reduced lipid oxidation during 9 days

of refrigerated storage. Thus, storage at refrigerated condition (4°C) with lavender essential oil incorporation preserved a good quality of chicken breast fillets and increased the shelf-life of this product to 3 days. Indeed, the antifungal activity indicated that essential oil has a significant inhibitory capacity on fungal growth, in comparison with synthetic preservatives. Its use as a natural food preservative appears fully justified.

## Conflict of interest

The authors have no conflict of interest.

## Acknowledgment

This study was supported by Centre of Biotechnology of Borj-Cedria, Tunisia.

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