

## Shelf life extension of refrigerated minced meat using some essential oils

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### Abstract:

The recent approach in food technology focus on finding not only safe and natural biocide, but also being an alternative for the chemical preservatives. Essential oils (EOs) have the ability to act as bactericidal, antitoxigenic, virucidal, fungicidal, antiparasitic besides being flavouring agents. So, it considers a promising new concern to fulfill this purpose. The present study was designed to express the shelf life of minced meat incorporated with lettuce oil 3%, cumin oil 2 % and marjoram oil 2% throughout a refrigerated period at 4 °C and to screen the bioactivity of the aforementioned essential oils with its different concentrations. The count of the total Enterobacteriaceae in the group treated with Marjoram oil 2% was  $4.57 \pm 0.25$ ,  $4.90 \pm 0.96$ ,  $5.53 \pm 0.87$ ,  $5.79 \pm 0.42$  and  $6.02 \pm 0.34$  log<sub>10</sub> cfu/g at zero day, and 3rd, 5th, 7th and 9<sup>th</sup> day, respectively, with regarding to lettuce oil 3% the total Enterobacteriaceae count was  $4.69 \pm 0.30$ ,  $5.06 \pm 0.70$ ,  $5.62 \pm 0.37$ ,  $5.82 \pm 0.02$  and  $6.08 \pm 0.62$  log<sub>10</sub> cfu/g at zero day, and 3rd, 5th, 7th and 9<sup>th</sup> day, respectively. While the total Enterobacteriaceae count of cumin oil 2% was  $4.62 \pm 0.51$ ,  $5.04 \pm 0.89$ ,  $5.60 \pm 0.30$ ,  $5.81 \pm 0.25$ ,  $6.05 \pm 0.25$  log<sub>10</sub> cfu/g at zero day, and 3rd, 5th, 7th and 9<sup>th</sup> day, respectively. In this study the antibacterial effect of lettuce oil 3%, cumin oil 2 % and marjoram oil 2% was recorded and proved with varying degree of potency leading to extent the shelf life of the minced meat. So, they could be utilized effectively as bioactive agents.

**Keywords:** essential oil, lettuce oil, cumin oil, marjoram oil, minced meat, refrigeration.

### Introduction:

Meat has a unique nutrient value. In addition to, supplying a worthy mentioned quality animal proteins, essential amino acids, fatty acids, minerals and vitamins (Singh *et al.*, 2013). So, meat consumption is increased linearly at wide scale all over the world. Although, chemical preservatives used in food industry had some benefits. They also had some downsides with a hazardous cumulative effect. So, the trend aimed to lessen using them and this initiated the researchers to scope for natural antimicrobial agents. These agents could control the microbial burden, eliminate the pathogens, improving the shelf life of food (Tajkarimi *et al.*, 2010) as well as overcome the drawbacks of the chemical preservatives.

Essential oils (EOs) are aromatic oily liquids derived by various methods from all the plant parts. They exhibit antiviral, antibacterial, antimycotic, antitoxigenic, antiparasitic and insecticidal properties. So, EOs used in food preservation to prevent bacterial and fungal growth as they can act against wide variety of Gram-positive and Gram-negative pathogens (**Burt, 2004**).

Among several EOs that may be valuable as antimicrobial agent, lettuce oil (*Lactuca sativa L.*) which is one of the most commonly consumed vegetables and an interesting source of antioxidant such as carotenoids (b-carotene and lutein), vitamins E and C, fiber, as well as phenolics (**Nicolle et al., 2004**) which thought to have a vital role in prevention of diseases through the prevention of low-density lipoprotein oxidation (**Scalbert and Williamson, 2000**). Moreover, lettuce oil is considered a good source of poly unsaturated fatty acids (PUSFAs) as it represents the major fraction (61.6%) of its components. Also, it is a worth trend in dealing with diabetes to decline the consequent diabetic complications especially in combination with insulin (**Eleiwa et al., 2007**).

Cumin (*Cuminum cyminum*) is one of the most vastly used spice. Cumin seeds are used as popular aromatic herbs and culinary spices. In addition to, it is considered a natural antioxidant. So, cumin oil is recommended to be used in food industry (**Allahghadri et al., 2010**). Also, it used in traditional and veterinary medicine as a stimulant, a carminative, an astringent, indigestion, flatulence and diarrhea (**Ani et al., 2006**).

Marjoram (*Origanum majorana L.*) essential oil belonging to the family Lamiaceae that possesses a wide inhibitory spectrum against a panel of gram-negative bacteria, gram-positive bacteria and yeasts (**Mohamed and Mansour, 2012**). Besides, it is commercially used as a spice and is traditionally used to treat asthma, indigestion, headache, rheumatism, dizziness, gastrointestinal disorder and migraine (**Abdel-Massih and Abraham, 2014**).

Therefore, the aim of the present study is to assess the antimicrobial activity of lettuce oil, cumin oil and marjoram oil with different concentrations on the shelf life of the minced meat during refrigeration.

## 2. Materials & methods:

### Essential oils and Raw materials:

The three EOs used in this study; lettuce oil (*Lactuca sativa L.*), cumin oil (*Cuminum cyminum L.*) and marjoram oil (*Origanum majorana L.*) were kindly obtained from the squeezing and extraction of natural oils unit in the National Research Centre, Dokki, Giza.

**Preparation of sample:** A total of 1 kg of fresh minced beef was procured from local butcher shop on the day of slaughter from Zagazig City, Sharqia province, Egypt, transferred to the meat technology laboratory of the Food Control Department at Zagazig University in refrigerated containers for determination of pH, color & odour scores, aerobic plate count (APC), total Enterobacteriaceae count (TEC).

The samples were prepared according to the technique recommended by **APHA (2001)**. A total of 1 kg of fresh minced beef was divided into 4 groups (250 g of each). The 1<sup>st</sup> group was the control group (untreated).

The 2<sup>nd</sup> group was treated by adding 7.5 ml lettuce oil (%v/g) to achieve 3% concentration.

The 3<sup>rd</sup> group was treated by adding 5 ml cumin oil (%v/g) to obtain 2% concentration.

The 4<sup>th</sup> group was treated by adding 5 ml marjoram oil (%v/g) to get 2% concentration.

Each group was packed in polyethylene bag, labeled and stored in the refrigerator at 4°C. The sample was taken every alternative day (0, 3, 5, 7 and 9) and analyzed for physicochemical, sensory and bacteriological aspect. The experiment was conducted in triplicate.

### **1- Sensory examinations:**

Color and odour scores evaluation was performed by a panel of seven judges consisting of faculty and postgraduate students of faculty of Vet. Med. Sci., Zagazig Univ. A 5-point descriptive scale (**Sahoo and Anjaneyulu, 1997**) was used where (1) very undesirable, (2) moderately undesirable, (3) moderately desirable, (4) desirable and (5) very desirable, for color. While, for odour (1) very unpleasant, (2) moderately unpleasant, (3) moderately pleasant, (4) pleasant and (5) very pleasant.

### **2-Keeping quality tests:**

#### **1. Determination of pH (Pearson, 2006):**

In a blender, approximately 10 g of the sample were blended in 10ml of neutralized distilled water. The homogenate was left at room temperature for 10 minutes with continuous shaking. The pH value was determined by using an electrical pH meter (Bye model 6020, USA). Calibration of pH meter by using two buffer solutions of exactly known pH (alkaline pH 7.01, acidic pH 4.01). Therefore, pH electrode was washed with neutralized water and then introduced into the homogenate after the temperature correction system was adjusted.

#### **2. Determination of Total Volatile Nitrogen (TVN):**

The technique applied for determination of total volatile nitrogen (TVN) was recommended by **Food and Agriculture Organization "FAO" (1980)** as follows:

In a clean dry beaker, 10g of the sample were added to 30 ml of distilled water and thoroughly mixed by a blender for 2 minutes. Thus, 2 drops of 0.02 M HCl were added to bring the pH value to 5.2. The homogenate was slowly heated to 70°C and then cooled to room temperature and filtered.

Accurately, the outer ring was filled with 2ml of the sample extract and 1ml of saturated potassium carbonate (KCO<sub>3</sub>). The Conway unit was rotated as gently as possible and the dish was covered and incubated at 36°C for 2 hours, HCl in the inner ring was titrated against 0.01M NaOH by using methyl red indicator (T<sub>1</sub> ml).

$$\text{TVN}/100\text{g} = 26.88 \times (2 - T_1)$$

Where,

T<sub>1</sub> = volume of NaOH consumed in the titration.

### 3. Determination of Thiobarbituric Acid Number (TBA):

The method adopted for estimation of TBA by **Pikul et al. (1989)** was applied as follow:

TBA test which depends on determination of malonaldehyde (MD) as an end product of lipid peroxidation. The extent of oxidative rancidity is normally reported as TBA number or values and expressed as milligrams of malonaldehyde equivalents per kilogram of the samples.

Actually, 10 grams of the sample were blended with 50 ml of distilled water in warring blender for 2 minutes. The mixture was transferred quantitatively into Kjeldahl flask by washing with an additional 47.5 ml of distilled water. However, 2.5 ml of HCL were added to 125 ug/g fat and then a small amount of stones was placed to prevent pumping. The flask was heated at the highest heat obtainable in the Kjeldahl distillation apparatus.

Approximately, 10 minutes after the boiling were required to collect 50 ml of the distillate. Therefore, 5 ml of the distillate were mixed to 5 ml of TBA reagent in 50 ml glass stoppered tube. The contents were mixed and immersed in a boiling water bath for 35 minutes.

A distilled water TBA reagent blank was prepared and treated like samples. After heating, cooling under tape water for 10 minutes was applied. The optical density of sample against the blank was measured by using Spectrophotometer (UNICAM969AA Spectronic, USA) at a wave length of 538 nm.

$$\text{TBA value} = R \times 7.8 \text{ (mg malonaldehyde /Kg)}$$

Where,

R = Reading of sample against blank

### **3-Bacteriological analyses**

#### *Preparation of samples for bacteriological examination:*

Minced meat samples were prepared for microbiological analysis in accordance with ISO 6887-1(2003). For the Aerobic plate count (Baumgart & Firnhaber, 1986); One ml of each previously prepared serial dilution was carefully transferred into separate, duplicate, appropriately marked Petri dishes, and thoroughly mixed with about 15 ml of previously melted and adjusted ( $45 \pm 1^{\circ}\text{C}$ ) plate count agar (Oxoid, CM325). After solidification, the inoculated plates as well as control one were inverted and incubated promptly for  $48 \pm 2$  h at  $37^{\circ}\text{C}$ . The countable plates with 30-300 colonies were recorded and the total colony count per cm<sup>2</sup> was calculated. However, for the enumeration of *Enterobacteriaceae* (ICMSF, 1978); 0.1 ml from the original and the subsequent prepared dilutions were spread on surface of Petri dish in duplicate plate containing Violet red bile glucose agar (VRBGA), and incubated at  $37^{\circ}\text{C}$  for 24 hours. All large purple colonies were counted and the average number of *Enterobacteriaceae* per gram of sample was calculated and recorded.

### **3. Results and Discussion:**

**Table (1): Effect of different natural preservatives on colour and odour scores of minced meat stored at  $4^{\circ}\text{C}$ .**

Keeping quality	Fit for human consumption	Border line	Decomposed
Control (untreated samples )	0 - 3 <sup>rd</sup> day	3 -5 <sup>th</sup> day	6 <sup>th</sup> day
samples treated with lettuce oil 3 %	0 -5 <sup>th</sup> day	5 -7 <sup>th</sup> day	8 <sup>th</sup> day
Samples treated with Marjoram 2 %	0 -5 <sup>th</sup> day	7 -8 <sup>th</sup> day	9 <sup>th</sup> day
Samples treated with Cumin oil 2%	0 -5 <sup>th</sup> day	7 -9 <sup>th</sup> day	10 <sup>th</sup> day

**Tables (2) Effect of different natural preservatives on pH on minced meat stored at  $4^{\circ}\text{C}$**

Treatments	Refrigerated storage ( Days )				
	Day 0	Day 3	Day 5	Day 7	Day 9
Control	5.68	6.73	6.96	7.42	7.79
Lettuce oil 3%	5.66	5.90	6.11	6.25	6.34
marjoram oil 2%	5.65	5.79	5.94	6.03	6.18
Cumin oil 2%	5.62	5.73	5.85	5.96	6.07

**Table (3): Effect of different natural preservatives on TVN of minced meat stored at 4 °C**

Treatments	Refrigerated storage ( Days )				
	Day 0	Day 3	Day 5	Day 7	Day 9
Control	2.41	19.03	27.58	33.89	41.38
Lettuce oil 3%	2.29	7.82	11.79	16.15	20.53
Marjoram oil 2%	2.26	6.95	10.02	14.72	18.29
Cumin oil 2%	2.18	6.61	8.99	13.47	17.06

**Table (4): Effect of different natural preservatives on TBA of minced meat stored at 4 °C**

Treatments	Refrigerated storage ( Days )				
	Day 0	Day 3	Day 5	Day 7	Day 9
Control	0.05	0.84	1.09	1.38	1.75
Lettuce oil 3%	0.04	0.29	0.51	0.76	0.89
Marjoram oil 2%	0.04	0.22	0.38	0.63	0.77
Cumin oil 2%	0.03	0.18	0.32	0.55	0.72

**Tables (5): Effect of different natural preservatives on APC of minced meat stored at 4 °C**

Treatments	Refrigerated storage ( Days )				
	Day 0	Day 3	Day 5	Day 7	Day 9
Control	6.19 ± 0.16	6.68 ± 0.32	6.92 ± 0.26	6.94 ± 0.94	7.27 ± 0.94
Lettuce oil 3%	5.86 ± 0.77	6.19 ± 0.11	6.66 ± 0.00	6.79 ± 0.17	6.97 ± 0.18
Cumin oil 2%	5.95 ± 0.22	6.27 ± 0.51	6.52 ± 0.27	6.64 ± 0.15	6.86 ± 0.10
Marjoram oil 2%	5.84 ± 0.42	6.22 ± 0.39	6.46 ± 0.09	6.74 ± 0.02	6.89 ± 0.06



**Table (6): Effect of different natural preservatives on TEC of minced meat stored at 4 °C**

Treatments	Refrigerated storage ( Days )				
	Day 0	Day 3	Day 5	Day 7	Day 9
Control	4.87 ± 0.36	5.24 ± 0.64	5.98 ± 0.36	6.13 ± 0.01	6.46 ± 0.01
Lettuce oil 3%	4.69 ± 0.30	5.06 ± 0.70	5.62 ± 0.37	5.82 ± 0.02	6.08 ± 0.62
Cumin oil 2%	4.62 ± 0.51	5.04 ± 0.89	5.60 ± 0.30	5.81 ± 0.25	6.05 ± 0.25
Marjoram oil 2%	4.57 ± 0.25	4.90 ± 0.96	5.53 ± 0.87	5.79 ± 0.42	6.02 ± 0.34

Organoleptic profile not only determine what we eat, but often allows us to evaluate the quality of the food and some cases, identify unwanted contaminants (Rasooli, 2007).

Sensory evaluation on minced beef along the time of storage after treatment was presented in **table (1)** and indicated that sensory characteristics of minced beef were enhanced by different treatments. It is obvious from results mentioned above in the same **table (1)** that in the beginning of the storage, there wasn't a clear effect between the comparable groups on the colour score especially at zero day. As the period storage advanced, the effect of different EOs could be noticed.

Generally, Cumin oil 2% remained the highest on all the storage interval till the end, approving that Cumin oil 2% has a potent activity in delaying the sensory changes in of the treated minced meat samples and extending its shelf life to 9<sup>th</sup> days and decomposed at 10<sup>th</sup> days, followed by the Lettuce oil 3% then Marjoram oil 2%. The direct addition of EOs to food may alter the sensory characteristics of food (Seydim and Sarikus, 2006).

The pH is considered as one of the most important factors reflecting the meat quality. The permissible limits of pH ranged from 5.2 to 6.6 depending on preslaughter treatment, degree of contamination and storage condition (EO S).

The data recorded in table (7) Revealed that the initial pH values were nearly the same in all groups. On the 3<sup>rd</sup> day the pH values gradually increased during refrigeration storage at 4 °C ;however it still within the permissible limits of E.O.S (2005),by the 5<sup>th</sup> day the control untreated group samples had a higher pH value (6.96)than other groups( 6.11, 5.94 and 5.85) for lettuce oil 3 % , cumin 2 % and

marjoram 2 % respectively. This may due to the action of microbial load which lead to protein proteolysis with appearance of alkyl group. Meanwhile the all treated samples were still within the permissible limits Of (**EOS, 2005**).

So, from the mentioned results, we could clarify that on day zero, cumin oil 2% showed lower pH out of all the samples throughout the storage period. The pH followed an increasing trend throughout the storage period in all treated samples but still within the normal limits. The increase in pH during the storage period might be due to accumulation of metabolites due to growth of Gram-negative bacteria such as *Pseudomonas*, *Moraxella*, *Acinetobacter* etc. (**Kirsch et al., 1952 and McDowell et al., 1986**).

Total volatile nitrogen (TVN) measurement is the traditional chemical mean most widely used for evaluation of the degree of meat spoilage and it should not exceed 20 mg TVN/100g according to **E.S.O (2005)**.

From the results achieved in table (8), it could be noticed that total volatile nitrogen (TVN) of control 2.41, 19.03, 27.58, 33.89 and 41.38 for zero day, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day of the storage period, respectively. But, in case of lettuce oil 3%, TVN value was 2.29, 7.82, 11.79, 6.25, 16.15 and 20.53 at zero day, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day of the refrigerated period, respectively. While, in case of cumin oil 2%, the TVN value was 2.26, 9.95, 10.03, 14.11 and 18.29 at zero day, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day throughout the whole storage, respectively. Meanwhile, using marjoram oil 2% showed TVN values were 2.18, 6.61, 8.99, 13.47 and 17.06 throughout the storage interval.

The mentioned result cleared that TVN mg % values in the examined treated and untreated samples initially measure normal values until the day 5 we notice that the TVN value in control untreated samples (27.58) exceed the permissible limits and the treated samples were still within the permissible limits, by the day 9 the value for lettuce oil treated samples became higher than the permissible limits (20.53).

This increase in TVN might be due to microbial activity at low temperature (**Ibrahim and Desouky, 2009**).

The data recorded in table (4) revealed that the TBA value of the control group was 0.05, 0.84, 1, 09, 6.151.38 and 1.75 for zero day, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day of the storage period, respectively. But, in case of lettuce oil 3%, the TBA value was 0.04, 0.29, 0.51, 0.76 and 0.89 at zero day, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day of the refrigerated period, respectively. While, in case of cumin oil 2%, the TBA value was 0.03, 0, 18, 0.32, 0.52 and 0.72 at zero day, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day throughout the whole storage, respectively. Meanwhile, using marjoram oil 2% showed TBA value as 0.04, 0.22, 0.38, 0.63 and 0.77 throughout the storage interval.

So, from the above mentioned results, we could clarify that The TBA followed an increasing trend throughout the storage period in all the samples. TBA is a good indicator for assessment of meat quality and degree of lipid oxidation (Ndaw et al.,



2008). It has been proposed that a maximum TBA value indicating the good quality of minced meat is 0.9 mg MDA/kg (EOS, 2005).

The data recorded in table (9) revealed that the TBA value of the control group was 0.05, 0.84, 1.09, 6.15 and 1.38 for zero day, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day of the storage period, respectively. But, in case of lettuce oil 3%, the TBA value was 0.04, 0.29, 0.51, 0.76 and 0.89 at zero day, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day of the refrigerated period, respectively. While, in case of cumin oil 2%, the TBA value was 0.03, 0.18, 0.32, 0.52 and 0.72 at zero day, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day throughout the whole storage, respectively. Meanwhile, using marjoram oil 2% showed TBA value as 0.04, 0.22, 0.38, 0.63 and 0.77 throughout the storage interval.

So, from the above mentioned results, we could clarify that The TVN followed an increasing trend throughout the storage period in all the samples. Additionally, the total volatile nitrogen values of all treatments were in the range of permissible level (< 0.9mg MDA /kg) established by Egyptian standard specifications (ESS, 2005).

A Correlation between sensory evaluation and chemical parameters (PH, TVN and TBA) was observed in all treated and control groups suggestion that the use of natural preservatives improve the quality of minced meat to an extent. But studying their effects on bacteriological quality on minced meat as an example for meat products still needed.

### Bacteriological profile:

It's important to assess the aerobic plate count, Enterobacteriaceae, Coliforms and fungal counts because they are considered as an indicators for the microbiological quality of the meat products and help in assessing the keeping quality of further processed chicken meat products (Aberle *et al.*, 2001, Kozaèinski *et al.*, 2006 and Cohen *et al.*, 2007).

From the results in table (5) and figure (4) it is evident that the mean count of APC in control group was  $6.19 \pm 0.16$ ,  $6.68 \pm 0.32$ ,  $6.92 \pm 0.26$ ,  $6.94 \pm 0.94$  and  $7.27 \pm 0.94$  log cfu/g at zero 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day, respectively. The mean count of APC in group treated with lettuce oil 3% was  $5.86 \pm 0.77$ ,  $6.19 \pm 0.11$ ,  $6.66 \pm 0.00$ ,  $6.79 \pm 0.17$ ,  $6.97 \pm 0.18$  log cfu/g at zero 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day, respectively. Regarding to cumin oil compared with the same control group, the mean value of minced meat treated with cumin oil 2% was  $5.95 \pm 0.22$ ,  $6.27 \pm 0.51$ ,  $6.52 \pm 0.27$ ,  $6.64 \pm 0.15$  and  $6.86 \pm 0.10$  log cfu/g at zero 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day, respectively. While the mean value of minced meat treated with marjoram 2% was  $5.84 \pm 0.42$ ,  $6.22 \pm 0.39$ ,  $6.46 \pm 0.09$ ,  $6.74 \pm 0.02$  and  $6.86 \pm 0.06$  log cfu/g at zero, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day, respectively.

According to ANOVA analysis, the mean values of APC of all groups of the treatments showed significant difference ( $P < 0.05$ ) and that may be attributed to many causes such as crude extracts held high concentration of active antibacterial

compounds or that crude extracts contain compound that enhanced the antibacterial activity of the effective compounds.

Regarding the results mentioned in the same table (6), it was demonstrated that the initial count of TEC in the control group was  $4.87 \pm 0.36$  at zero day. On subsequent storage intervals, the control group exhibited a higher TEC which valued as  $5.24 \pm 0.64$ ,  $5.98 \pm 0.36$ ,  $6.13 \pm 0.01$  and  $6.46 \pm 0.01$   $\log_{10}$  cfu/g at 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day, respectively. When comparing the result of the control group over the whole period with the other treated groups, it was concluded that marjoram oil 2% has the best efficacy till the end at the 9<sup>th</sup> day. The count of the TEC in the group treated with marjoram oil 2% was  $4.57 \pm 0.25$ ,  $4.90 \pm 0.96$ ,  $5.53 \pm 0.87$ ,  $5.79 \pm 0.42$  and  $6.02 \pm 0.34$   $\log_{10}$  cfu/g at zero day, and 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day, respectively. So, this result confirmed the effectiveness of the marjoram oil. While, cumin oil 2% had considerable effectiveness in decreasing the TEC. Throughout the storage interval, the TEC in this group was  $4.62 \pm 0.51$ ,  $5.04 \pm 0.89$ ,  $5.60 \pm 0.30$ ,  $5.81 \pm 0.25$  and  $6.05 \pm 0.25$   $\log_{10}$  cfu/g at zero day, and 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day, respectively. After that, lettuce oil 3% which has the least potency on the initiate count of TEC during the storage for 9 days. This lesser effect could be attributed to the variation of the content of aromatic compounds and the different component composition.

The complex chemical composition, structure, as well as functional groups of essential oils is responsible for their wide range of antimicrobial activity. (Omidbeygi *et al.*, 2007 and YesilCelik *et al.*, 2007).

Moreover, EOs were more effective at low temperatures due to the higher permeability of the cell membrane and more easily solubility in the lipid membrane (Frangos *et al.*, 2010). The effectivity of cumin oil and marjoram oil against a wide range of gram-negative, in addition to gram-positive bacteria, mould and yeast species had been recorded by (Ağaoğlu *et al.*, 2007, Busatta *et al.*, 2008, De Martino, 2009, Allahghadri *et al.*, 2010, Badee *et al.*, 2013, Sharoba *et al.*, 2015 and El-Shenawy *et al.*, 2015).

In cumin oil the individual components vary widely depending on the origin and the variety of the raw material, its stage of maturity, the duration and conditions of storage (Georgiev and Stoyanova, 2005, Orav *et al.*, 2004, Singh *et al.*, 2004, Zachariah and Parthasarathy, 2008). So, the antimicrobial activity is due to its main components which include cumaldehyde, cymene, limonene and linalol (AL Juhaimi *et al.*, 2013).

Marjoram oil characterized by a varied pattern in its composition which attributed to several factors including the species, growth stages, origin of herb, climatic and drying conditions (Baatour *et al.*, 2012). These components can penetrate into the interior of the cell and interact with intracellular critical sites of bacterial

activities (Cristani *et al.*, 2007) and inhibit glucosyl transferase enzyme activity, which is responsible for bacteria adhesion to host sites (Tsai *et al.*, 2007).

So, it could authenticate marjoram oil as a bioactive agent of highest grade against TEC even higher than the cumin oil under the same circumstances of the experiment. While, the low activity of the lettuce oil 3% suggests either that the crude extracts held very low amount of active antibacterial substances or the crude extract contained compounds that counteracted the activity of the effective constituents.

#### 4. Conclusion

Marjoram oil, cumin oil and lettuce oil are natural preservatives with a remarkable antibacterial effect. The result of this study confirmed their role during the storage in maintaining the meat quality and extending its shelf life depending on the sensory evaluation and the values of pH, APC and TEC. Therefore, there is a possibility of using them in food as natural preservatives instead of the chemicals without compromising the sensory attributes of the food.

#### 5. References

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## الملخص العربي

### إطالة مدة حفظ اللحوم المفرومة المبردة باستخدام بعض الزيوت الطبيعية

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اتجهت الدراسات الحديثة فى مجال تكنولوجيا الاغذية الى استخدام المواد الحافظة الطبيعية كبديل آمنه للمواد الحافظة الاصطناعية. تمتلك الزيوت الطبيعية القدرة على التأثير على العديد من الميكروبات بالاضافة الى استخدامها كعوامل لتحسين نكهة اللحم لذلك فهي تعتبر وسيلة جيدة تستخدم فى حفظ اللحوم. أجريت هذه الدراسة لاطالة مدة حفظ اللحوم المفرومة باستخدام زيت الخس وزيت البردقوش وزيت الكمون (تركيز ٢%) لدراسة مدى تأثيرها على الحالة الميكروبية للحوم المفرومة بتركيزات مختلفة أثناء التبريد عند (صفر – ٤°م) وكان متوسط قيم العدد الكلى للميكروبات المعوية باستخدام زيت البردقوش ٢% هي  $0.25 \pm 4.57$  و  $0.96 \pm 4.90$  و  $0.87 \pm 5.53$  و  $0.42 \pm 5.79$  و  $0.34 \pm 6.02$  لوغاريتم مستعمرة بكتيرية/جرام فى اليوم الاول والثالث والخامس والسابع والتاسع على التوالي وباستخدام زيت الخس ٣% كان متوسط قيم العدد الكلى للميكروبات المعوية  $0.30 \pm 4.69$  و  $0.70 \pm 5.06$  و  $0.37 \pm 5.62$  و  $0.02 \pm 5.82$  و  $0.62 \pm 6.08$  لوغاريتم مستعمرة بكتيرية/جرام فى اليوم الاول والثالث والخامس والسابع والتاسع على التوالي بينما كان متوسط قيم العدد الكلى للميكروبات المعوية باستخدام زيت الكمون ٢%  $0.01 \pm 4.62$  و  $0.70 \pm 5.05$  و  $0.37 \pm 5.62$  و  $0.02 \pm 5.82$  و  $0.62 \pm 6.08$  لوغاريتم مستعمرة بكتيرية/جرام فى اليوم الاول والثالث والخامس والسابع والتاسع على التوالي. أدى استخدام الزيوت الى تحسين جودة اللحوم المفرومة المبردة بدرجات مختلفة. لذلك توصى الدراسة باضة الزيوت الطبيعية الى اللحوم المفرومة المبردة لما لها من تأثير مثبط على البكتريا وتحسين جودة اللحوم.