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# Antioxidant, sensory and antibacterial activities of some essential oils in beef kofta

**[1]Elham Hosny Mohamed, [2]Reham Amin Abd El Aziz, [3] Marionette Nassif Zaghloul**

[1] *Veterinarian*

[2]*Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University*

[3]*Food Hygiene Dept., Animal Health Research Institute, Benha branch*

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**ABSTRACT**

This study investigated the antioxidant, antibacterial and sensory effects of lemongrass (*Cymbopogon Citrates*), orange (*Citrus Sinensis*) and thyme *(Thymus vulgaris*) oils at concentrations (0.5 and 1%) on beef Kofta at 4 °C for 10 days storage period. All essential oils used had considerable effects on pH, Thiobarbituric Acid Reactive substance (TBARs) and Total Volatile Basic Nitrogen (TVBN) values as well as aerobic plate count (APC), Coliform count, *Staphylococci* count and *Staphylococcus aureus* count. Lemongrass, orange and thyme oils at the two concentrations improved sensory properties of kofta. Lemongrass oil in two concentrations (0.5 and 1%) gave the best results as antibacterial and antioxidant, and also gave good organoleptic properties, but lemongrass oil 0.5% was more accepted. Orange oil at 0.5% showed the lowest results as antibacterial and antioxidant with accepted organoleptic properties. On other hand orange oil 1% is more effective as antibacterial and antioxidant and demonstrated more enhancements of sensory attributes. Thyme oil in the two concentrations (0.5 and 1%) was effective as antibacterial and antioxidant. Thyme oil at concentration 1% had unacceptable organoleptic properties in kofta, only thyme oil 0.5% can be used. So, we suggested that lemongrass oil 0.5%, thyme oil 0.5% and orange oil 1% can be applied to kofta as natural preservatives.

## 1. INTRODUCTION

Meatball (Kofta) is derived from the word "kufte" which is a Persian word and its basic raw material is ground beef. Hundred kinds of meatballs can be produced and consumed by adding various materials into meat and using different kinds of cooking techniques (Kundakç et al., 2009). During slaughter, pathogenic bacteria may contaminate the carcass and subsequently distribute via cut meat or raw meat materials intended for further processing into meat products (Borch, 2002). Meat as a food has a complex physical structure and chemical composition that is very susceptible to oxidation (Rather et al., 2016). However, to maintain the safety and prolong the shelf-life of meat and meat products, the meat industry uses synthetic preservatives that have been widely used to control the lipid oxidation and to eliminate bacteria (Ballester-Costa et al., 2017). The use of these synthetic preservatives could cause health problems for consumers over a long-term period (Alves-Silva et al., 2013).

Awareness of consumer changed towards (GRAS) and green natural preservatives. Essential oils, that exhibit antimicrobial activity, and also possess antioxidant properties, can be considered as healthy ingredients for meat products.

These aromatic oily liquids can reduce the incidence of food-borne diseases and retard lipid oxidation (Burt, 2004).

Lemongrass (*Cymbopogan Citratus*) is an aromatic plant belonging to family Poaceae (Naik et al., 2010). Lemongrass has medical functions and safe as well antifungal activity, make its oil potential multi-functional food additive (Abd-El Fattah et al., 2010).

Citrus essential oils as orange have been found to inhibit Gram-positive and Gram-negative bacteria as well as yeasts, molds and food poisoning bacteria (Chun-Lin et al., 2013).

Thyme (*Thymus vulgaris*), an aromatic plant of the Labiateae family, is the most important compounds of thyme EO are the phenols thymol (44-60%) and carvacrol (2.2-4.2%), which constitutes the major and more active constituents (Di Pasqua et al., 2005).

The objective of the present study was to investigate the antioxidant as well as the antibacterial effectiveness of three essential oils (lemongrass, orange and thyme oils) at various concentrations (0.5 and 1%) on sensory properties (color, appearance, odor, texture, taste and over all acceptability), chemical measurement (pH, TBA and TVBN) and bacterial growth (aerobic plate count, *enterobacteriaceae* count, coliform count, *Staphylococcus* count and Staphylococcus aureus count) of beef kofta during refrigerated storage (4 °C).

## 2. MATERIAL AND METHODS

*2.1. Collection of samples*

Accurately, 4.5 kg of frozen beef were purchased from butcher shops in El-Sharkia governorate, Egypt and was directly minced and packed in sterile polyethylene bags.

Ingredients used in preparing beef Kofta samples were 70% meat, 12% fat, 9% flour, 2.1% common salt, 1.2% onion, 1% garlic powder and spices mixture 1.2% (black pepper,

* Corresponding author:Reham Amin Abd El Aziz ,Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University

cumin, ginger powder, nutmeg & turmeric powder). The ready-made herbal oils of lemongrass (*Cymbopogon Citrates*), orange (*Citrus Sinensis*) and thyme *(Thymus vulgaris*) used in this study at pure state, were purchased from National Research Center, Dokki, Cairo, Egypt.

*2.2.Preparation of beef Kofta*

Beef meatballs were prepared by mixing the ingredients (minced meat, flour, common salt, onion, garlic powder and spices mixture) in a blender to obtain meat dough. The meat were divided into two groups, the first group (200 gm) was kept untreated (control), and the second group (treated group) was divided into 6 subgroups (200 gm of each) and were mixed with lemongrass at concentrations of (L1) 0.5%, (L2) 1%, orange oil at concentrations (O1) 0.5%, (O2) 1% and thyme oil at concentrations (T1) 0.5% and (T2) 1%.

Meat dough was shaped in round balls manually to get raw meat kofta. Kofta samples was packed in polyethylene bag, labeled and stored at 4 °C. All groups were examined periodically every 2 days for sensory, chemically (pH, TBA & TVN) and bacteriologically (APC, Coliform, *Staphylococci* and *staph. aureus*). The experiment was conducted in triplicate for 10 days of storage.

2.3.*Sensory evaluation:* It was carried out according to Fik and Fik (2007).

*2.4.Chemical analysis*

pH of raw beef kofta was measured using electrical pH meter (ACTWA-AD1200-1034678) as described elsewhere (E.S 63/11, 2006).

The extent of lipid oxidation in beef kofta was assessed by measuring 2- thiobarbituric acid reactive substances (TBARS), as described by (E.S 63/9, 2006).

Total volatile basic nitrogen (TVBN) of camel burger was determined according to (E.S 63/10, 2006).

*2.5. Bacteriological analysis*

2.5.1. Preparation of samples following ISO (2017)

Under complete aseptic condition, 25 grams of the examined kofta samples were transferred to aseptic stomacher bag and 225 ml of 0.1% sterile peptone water were aseptically added to the content of bag. Each sample was then homogenized, in the stomacher (Biomereuxsa, France, NO. 42489367) at 2000 rpm for 1-2 minutes, to provide a homogenate from which tenth-fold serial dilutions were prepared.

2.5.2.Bacteriological examination methods

Different prepared beef kofta samples were examined for total aerobic bacterial count according to ISO (2013), while coliform group were determined according to the method described in ISO (2004), and detection of Staphylococci and *Staphylococcus aureus* counts ISO (1999). All previous tests were used to reflect the microbiological quality of the prepared beef kofta samples. The appropriate dilutions were plated onto duplicate plates. Plates were incubated; thin results were expressed as (log cfu/g of samples).

*2.6. Statistical analysis*

The obtained results were statistically evaluated by application of One-way ANOVA test by using SPSS program according to Feldman *et al*. (2003).

## 3. RESULTS

The sensory properties of different treated kofta samples during cold storage (4 °C) were enhanced by using lemongrass, orange and thyme oils compared to the untreated (control) samples at 0 day, 2nd, 4th, 6th, 8th and 10th day of storage period respectively(table 1).

Table 1 Sensory evaluation of the untreated and treated samples of Kofta during cold storage at 4 °C according to Fik and Fik (2007).

| Samples | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | 5 | 4 | 3 | 2 | S | S |
| Lemon grass oil 0.5% | 5 | 5 | 4 | 4 | 2 | 1 |
| Lemon grass oil 1% | 5 | 5 | 5 | 4 | 3 | 2 |
| Orange oil 0.5% | 5 | 4 | 3 | 3 | 1 | S |
| Orange oil 1% | 5 | 5 | 4 | 4 | 2 | 1 |
| Thyme oil 0.5% | 5 | 5 | 4 | 4 | 2 | 1 |
| Thyme oil 1% | 5 | 5 | 4 | 4 | 2 | 1 |

5: Very good. 4: Good. 3: Acceptable. 2: Unacceptable. 1: Bad. S: Spoiled

The differences in pH mean values between various treated and untreated samples were significant during storage at 4 °C. The recorded results indicated decreasing pH values at two concentrations (0.5 and 1%) of treated kofta than control samples during different periods of storage. Also, by increasing oils concentrations leads to more lowering in pH values than values of lower concentrations (Table 2).

The mean values of TBA of control and treated samples, at the two concentrations showed decreasing in TBARs values than control sample especially at 6th, 8th and 10th day of storage period and high concentrations of lemongrass, orange and thyme oils showed decreasing in the TBARs values than lower concentrations (Table 3).

Table 2 Mean pH values of untreated and treated Kofta samples during storage period at 4°C (Mean ± SE)

| | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | $5.42^{a} \pm 0.02$ | $5.75^{a} \pm 0.01$ | $6.82^{a} \pm 0.02$ | $7.15^{a} \pm 0.01$ | $8.67^{a} \pm 0.01$ | $9.76^{a} \pm 0.02$ |
| Lemon grass oil 0.5% | $5.42^{a} \pm 0.02$ | $5.25^{cd} \pm 0.01$ | $5.76^{c} \pm 0.02$ | $6.52^{b} \pm 0.02$ | $7.43^{e} \pm 0.02$ | $8.65^{c} \pm 0.02$ |
| Lemon grass oil 1% | $5.42^{a} \pm 0.02$ | $5.03^{d} \pm 0.03$ | $5.43^{d} \pm 0.01$ | $6.23^{c} \pm 0.02$ | $7.14^{f} \pm 0.02$ | $8.28^{de} \pm 0.02$ |
| Orange 0.5% | $5.42^{a} \pm 0.02$ | $5.66^{ab} \pm 0.02$ | $6.34^{ab} \pm 0.02$ | $6.60^{ab} \pm 0.01$ | $8.35^{b} \pm 0.01$ | $9.41^{b} \pm 0.02$ |
| Orange 1% | $5.42^{a} \pm 0.02$ | $5.52^{abc} \pm 0.02$ | $6.19^{abc} \pm 0.02$ | $6.56^{ab} \pm 0.01$ | $7.60^{de} \pm 0.03$ | $8.07^{e} \pm 0.01$ |
| Thyme 0.5% | $5.42^{a} \pm 0.02$ | $5.44^{bc} \pm 0.01$ | $6.27^{abc} \pm 0.02$ | $6.56^{ab} \pm 0.01$ | $7.86^{c} \pm 0.01$ | $8.53^{cd} \pm 0.02$ |
| Thyme 1% | $5.42^{a} \pm 0.02$ | $5.35^{c} \pm 0.02$ | $6.09^{bc} \pm 0.02$ | $6.53^{b} \pm 0.02$ | $7.00^{cd} \pm 0.01$ | $8.35^{cde} \pm 0.02$ |

Values with different letters within the same row differed significantly at ($P < 0.05$)

Table (4) summarized the mean values of TVN, estimating the degree of meat deterioration during the storage period (10 days) at 4 ºC. TVN values increased for all kofta samples with different rates depending on the nature of treatment. The highest rate of increase of TVN values was recorded in control samples. The treatments with 1% lemongrass, thyme and orange oils, respectively, were more effective in delaying the rate of TVN increase during the subsequent cold storage.

The mean values of total aerobic counts (APC) and coliform counts of different untreated and treated beef kofta samples during cold storage were shown in table (5) and (6). The control samples showed the highest APC and coliform counts comparing to others containing lemongrass, orange or thyme oils with two concentrations. High concentrations of oils were more effective in lowering APC and coliform counts than low concentrations.

Tables (7) and (8) showed that Lemongrass, orange and thyme oils in different concentrations have an antimicrobial activity against *Staphylococci* and *Staphylococcus aureus* count in different orders, as lemongrass oil has the highest effect on *Staphylococci* followed by thyme oil then orang oil while effect of oils on *Staph. aureus* in descending order thyme oil > lemongrass oil>orange oil.

## 4. DISCUSSION

Table (1) revealed sensory properties of different treated kofta samples during cold storage (4ºC) were enhanced by using lemongrass, orange and thyme oils compared to the untreated (control) samples at 0 day, 2nd, 4th, 6th, 8th and 10th day of storage period.

Table 3 Thiobarbituric acid reactive substances (TBARs) values of untreated and treated Kofta samples during storage period at 4°C (Mean ± SE).

| | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | $0.02^{a} \pm 0.01$ | $0.31^{a} \pm 0.01$ | $0.56^{a} \pm 0.02$ | $0.93^{a} \pm 0.02$ | $1.27^{a} \pm 0.02$ | $1.44^{a} \pm 0.03$ |
| Lemon grass oil 0.5% | $0.02^{a} \pm 0.01$ | $0.15^{a} \pm 0.02$ | $0.36^{ab} \pm 0.01$ | $0.52^{ab} \pm 0.01$ | $0.67^{b} \pm 0.02$ | $0.75^{d} \pm 0.01$ |
| Lemon grass oil 1% | $0.02^{a} \pm 0.01$ | $0.12^{a} \pm 0.01$ | $0.29^{b} \pm 0.01$ | $0.51^{ab} \pm 0.01$ | $0.61^{c} \pm 0.01$ | $0.66^{e} \pm 0.01$ |
| Orange 0.5% | $0.02^{a} \pm 0.01$ | $0.24^{a} \pm 0.02$ | $0.44^{a} \pm 0.01$ | $0.56^{a} \pm 0.01$ | $0.81^{a} \pm 0.01$ | $0.91^{b} \pm 0.01$ |
| Orange 1% | $0.02^{a} \pm 0.01$ | $0.18^{a} \pm 0.01$ | $0.38^{ab} \pm 0.01$ | $0.53^{ab} \pm 0.01$ | $0.74^{b} \pm 0.01$ | $0.83^{c} \pm 0.02$ |
| Thyme 0.5% | $0.02^{a} \pm 0.01$ | $0.18^{a} \pm 0.01$ | $0.40^{a} \pm 0.01$ | $0.55^{a} \pm 0.01$ | $0.70^{b} \pm 0.02$ | $0.85^{bc} \pm 0.04$ |
| Thyme 1% | $0.02^{a} \pm 0.01$ | $0.14^{a} \pm 0.01$ | $0.34^{ab} \pm 0.02$ | $0.50^{b} \pm 0.02$ | $0.69^{b} \pm 0.02$ | $0.75^{d} \pm 0.02$ |

Values with different letters within the same row differed significantly at P <0.05

Table 4 Total Volatile Basic Nitrogen (TVBN) values of untreated and treated Kofta samples during storage period at 4°C (Mean ± SE).

| | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | $8.49^{a} \pm 0.42$ | $13.53^{a} \pm 0.34$ | $18.66^{a} \pm 0.39$ | $22.64^{a} \pm 0.55$ | $30.30^{a} \pm 0.65$ | $52.06^{a} \pm 0.80$ |
| Lemon grass oil 0.5% | $8.49^{a} \pm 0.42$ | $9.67^{e} \pm 0.25$ | $15.38^{d} \pm 0.34$ | $17.60^{d} \pm 0.25$ | $25.04^{cd} \pm 0.38$ | $45.23^{cd} \pm 0.63$ |
| Lemon grass oil 1% | $8.49^{a} \pm 0.42$ | $7.92^{f} \pm 0.34$ | $9.50^{f} \pm 0.27$ | $13.75^{f} \pm 0.23$ | $22.44^{f} \pm 0.42$ | $35.56^{f} \pm 0.39$ |
| Orange 0.5% | $8.49^{a} \pm 0.42$ | $13.16^{ab} \pm 0.45$ | $17.37^{b} \pm 0.26$ | $20.99^{b} \pm 0.28$ | $26.85^{b} \pm 0.26$ | $49.06^{b} \pm 0.78$ |
| Orange 1% | $8.49^{a} \pm 0.42$ | $11.20^{cd} \pm 0.36$ | $15.63^{cd} \pm 0.32$ | $17.93^{cd} \pm 0.21$ | $24.14^{de} \pm 0.33$ | $44.68^{d} \pm 0.65$ |
| Thyme 0.5% | $8.49^{a} \pm 0.42$ | $12.10^{bc} \pm 0.43$ | $16.41^{c} \pm 0.21$ | $18.52^{c} \pm 0.25$ | $25.70^{bc} \pm 0.22$ | $47.20^{bc} \pm 0.83$ |
| Thyme 1% | $8.49^{a} \pm 0.42$ | $10.42^{de} \pm 0.35$ | $14.25^{e} \pm 0.28$ | $15.50^{e} \pm 0.26$ | $23.37^{ef} \pm 0.34$ | $42.12^{e} \pm 0.57$ |

Values with different letters within the same row differed significantly at P <0.05

Table 5 Changes in aerobic plate count (APC) of untreated and treated Kofta samples during storage period at 4°C (Mean ± SE)

| | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | $4.69 \times 10^{5a} \pm 0.48 \times 10^{5}$ | $5.07 \times 10^{6a} \pm 4.77 \times 10^{6}$ | $3.00 \times 10^{7a} \pm 4.11 \times 10^{6}$ | $3.62 \times 10^{8a} \pm 4.11 \times 10^{7}$ | $1.83 \times 10^{9a} \pm 3.72 \times 10^{8}$ | $3.34 \times 10^{9a} \pm 3.64 \times 10^{8}$ |
| Lemon grass oil 0.5% | $4.69 \times 10^{5a} \pm 0.48 \times 10^{5}$ | $3.54 \times 10^{6b} \pm 4.68 \times 10^{6}$ | $1.75 \times 10^{7bc} \pm 4.68 \times 10^{6}$ | $2.27 \times 10^{8c} \pm 4.65 \times 10^{7}$ | $9.07 \times 10^{8bc} \pm 4.65 \times 10^{7}$ | $1.95 \times 10^{9b} \pm 4.65 \times 10^{8}$ |
| Lemon grass oil 1% | $4.69 \times 10^{5a} \pm 0.48 \times 10^{5}$ | $4.49 \times 10^{5b} \pm 0.47 \times 10^{5}$ | $8.04 \times 10^{6c} \pm 4.69 \times 10^{5}$ | $2.56 \times 10^{7d} \pm 1.04 \times 10^{7}$ | $2.45 \times 10^{8d} \pm 9.75 \times 10^{7}$ | $2.74 \times 10^{8c} \pm 9.75 \times 10^{7}$ |
| Orange 0.5% | $4.69 \times 10^{5a} \pm 0.48 \times 10^{5}$ | $4.74 \times 10^{6a} \pm 4.68 \times 10^{6}$ | $2.71 \times 10^{7a} \pm 4.74 \times 10^{6}$ | $3.36 \times 10^{8ab} \pm 4.50 \times 10^{7}$ | $1.64 \times 10^{9a} \pm 2.81 \times 10^{8}$ | $2.41 \times 10^{9b} \pm 2.69 \times 10^{8}$ |
| Orange 1% | $4.69 \times 10^{5a} \pm 0.48 \times 10^{5}$ | $8.09 \times 10^{5b} \pm 0.48 \times 10^{5}$ | $9.46 \times 10^{6c} \pm 4.85 \times 10^{5}$ | $6.27 \times 10^{7d} \pm 4.13 \times 10^{6}$ | $3.92 \times 10^{8cd} \pm 4.13 \times 10^{7}$ | $7.58 \times 10^{8c} \pm 4.02 \times 10^{7}$ |
| Thyme 0.5% | $4.69 \times 10^{5a} \pm 0.48 \times 10^{5}$ | $4.33 \times 10^{6a} \pm 0.48 \times 10^{5}$ | $2.65 \times 10^{7ab} \pm 3.29 \times 10^{6}$ | $2.52 \times 10^{8bc} \pm 2.89 \times 10^{7}$ | $1.38 \times 10^{9ab} \pm 1.47 \times 10^{8}$ | $2.30 \times 10^{9b} \pm 1.57 \times 10^{8}$ |
| Thyme 1% | $4.69 \times 10^{5a} \pm 0.48 \times 10^{5}$ | $7.60 \times 10^{5b} \pm 0.44 \times 10^{5}$ | $8.16 \times 10^{6c} \pm 4.46 \times 10^{5}$ | $2.95 \times 10^{7d} \pm 4.17 \times 10^{6}$ | $3.48 \times 10^{8cd} \pm 4.01 \times 10^{7}$ | $7.80 \times 10^{8c} \pm 4.07 \times 10^{7}$ |

Values with different letters within the same row differed significantly at P <0.05

Table 6 Changes in coli form count of untreated and treated Kofta samples during storage period at 4°C (Mean ± SE).

| | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | $2.04 \times 10^{3a} \pm 2.28 \times 10^{2}$ | $2.54 \times 10^{4a} \pm 2.28 \times 10^{3}$ | $1.77 \times 10^{5a} \pm 2.28 \times 10^{4}$ | $1.73 \times 10^{6a} \pm 2.25 \times 10^{5}$ | $1.57 \times 10^{7a} \pm 2.25 \times 10^{6}$ | $1.64 \times 10^{8a} \pm 2.23 \times 10^{7}$ |
| Lemon grass oil 0.5% | $2.04 \times 10^{3a} \pm 2.28 \times 10^{2}$ | $1.37 \times 10^{3b} \pm 2.28 \times 10^{2}$ | $1.87 \times 10^{4c} \pm 2.28 \times 10^{3}$ | $1.68 \times 10^{5d} \pm 2.15 \times 10^{4}$ | $1.52 \times 10^{6d} \pm 2.15 \times 10^{5}$ | $1.12 \times 10^{7b} \pm 2.03 \times 10^{6}$ |
| Lemon grass oil 1% | $2.04 \times 10^{3a} \pm 2.28 \times 10^{2}$ | $1.12 \times 10^{3b} \pm 2.28 \times 10^{2}$ | $8.76 \times 10^{3c} \pm 2.28 \times 10^{3}$ | $9.30 \times 10^{4d} \pm 2.25 \times 10^{4}$ | $1.23 \times 10^{6d} \pm 2.18 \times 10^{5}$ | $9.50 \times 10^{6b} \pm 2.15 \times 10^{6}$ |
| Orange 0.5% | $2.04 \times 10^{3a} \pm 2.28 \times 10^{2}$ | $1.42 \times 10^{4a} \pm 2.28 \times 10^{3}$ | $1.67 \times 10^{5ab} \pm 2.28 \times 10^{4}$ | $1.57 \times 10^{6ab} \pm 2.23 \times 10^{5}$ | $1.31 \times 10^{7ab} \pm 2.14 \times 10^{6}$ | $1.57 \times 10^{8a} \pm 2.14 \times 10^{7}$ |
| Orange 1% | $2.04 \times 10^{3a} \pm 2.28 \times 10^{2}$ | $1.21 \times 10^{4a} \pm 2.28 \times 10^{3}$ | $1.38 \times 10^{5ab} \pm 2.28 \times 10^{4}$ | $9.26 \times 10^{5c} \pm 1.70 \times 10^{5}$ | $1.02 \times 10^{7bc} \pm 2.25 \times 10^{6}$ | $1.26 \times 10^{8a} \pm 2.22 \times 10^{7}$ |
| Thyme 0.5% | $2.04 \times 10^{3a} \pm 2.28 \times 10^{2}$ | $1.35 \times 10^{4a} \pm 2.28 \times 10^{3}$ | $1.56 \times 10^{5ab} \pm 2.28 \times 10^{4}$ | $1.20 \times 10^{6bc} \pm 2.18 \times 10^{5}$ | $1.16 \times 10^{7bc} \pm 2.09 \times 10^{6}$ | $1.48 \times 10^{8a} \pm 2.15 \times 10^{7}$ |
| Thyme 1% | $2.04 \times 10^{3a} \pm 2.28 \times 10^{2}$ | $1.03 \times 10^{4a} \pm 2.28 \times 10^{3}$ | $1.12 \times 10^{5b} \pm 2.28 \times 10^{4}$ | $8.93 \times 10^{5c} \pm 1.26 \times 10^{5}$ | $9.40 \times 10^{6c} \pm 2.15 \times 10^{6}$ | $1.04 \times 10^{8a} \pm 2.06 \times 10^{7}$ |

Values with different letters within the same row differed significantly at P <0.05

Table 7 Changes in Staphylococcus count of untreated and treated Kofta samples during storage period at 4°C (Mean ± SE).

| | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | $2.18 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.83 \times 10^{3a} \pm 1.78 \times 10^{2}$ | $1.71 \times 10^{4a} \pm 1.78 \times 10^{3}$ | $2.37 \times 10^{5a} \pm 1.78 \times 10^{4}$ | $1.92 \times 10^{6a} \pm 1.78 \times 10^{5}$ | $1.95 \times 10^{7a} \pm 1.78 \times 10^{6}$ |
| Lemon grass oil 0.5% | $2.18 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.29 \times 10^{2b} \pm 0.18 \times 10^{2}$ | $2.04 \times 10^{3c} \pm 1.76 \times 10^{2}$ | $1.52 \times 10^{4b} \pm 1.76 \times 10^{3}$ | $1.87 \times 10^{5c} \pm 1.76 \times 10^{4}$ | $1.55 \times 10^{6d} \pm 7.83 \times 10^{4}$ |
| Lemon grass oil 1% | $2.18 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.06 \times 10^{2b} \pm 0.17 \times 10^{2}$ | $1.61 \times 10^{3c} \pm 1.83 \times 10^{2}$ | $1.44 \times 10^{4b} \pm 9.17 \times 10^{2}$ | $1.77 \times 10^{5c} \pm 9.17 \times 10^{3}$ | $1.41 \times 10^{6d} \pm 1.76 \times 10^{5}$ |
| Orange 0.5% | $2.18 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.68 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.45 \times 10^{4ab} \pm 1.94 \times 10^{3}$ | $2.26 \times 10^{5b} \pm 2.12 \times 10^{4}$ | $1.74 \times 10^{6a} \pm 1.85 \times 10^{5}$ | $1.84 \times 10^{7ab} \pm 1.85 \times 10^{6}$ |
| Orange 1% | $2.18 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.61 \times 10^{3a} \pm 2.12 \times 10^{2}$ | $1.26 \times 10^{4ab} \pm 2.12 \times 10^{3}$ | $2.20 \times 10^{5b} \pm 1.97 \times 10^{4}$ | $1.20 \times 10^{6b} \pm 2.12 \times 10^{5}$ | $1.41 \times 10^{7bc} \pm 2.12 \times 10^{6}$ |
| Thyme 0.5% | $2.18 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.54 \times 10^{3a} \pm 1.76 \times 10^{2}$ | $1.05 \times 10^{4ab} \pm 1.76 \times 10^{3}$ | $1.68 \times 10^{5b} \pm 1.76 \times 10^{4}$ | $1.13 \times 10^{6b} \pm 1.73 \times 10^{5}$ | $1.02 \times 10^{7c} \pm 1.73 \times 10^{6}$ |
| Thyme 1% | $2.18 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $1.41 \times 10^{3a} \pm 1.94 \times 10^{2}$ | $9.36 \times 10^{3b} \pm 1.94 \times 10^{3}$ | $1.84 \times 10^{4b} \pm 1.76 \times 10^{4}$ | $2.02 \times 10^{5c} \pm 1.76 \times 10^{5}$ | $3.90 \times 10^{6cd} \pm 2.08 \times 10^{6}$ |

Values with different letters within the same row differed significantly at ($P <0.05$)

Table 8 Changes in *Staphylococcus aureus* count of untreated and treated Kofta samples during storage period at 4°C (Mean ± SE).

| | Storage Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Control | $1.47 \times 10^{3a} \pm 1.17 \times 10^{2}$ | $1.41 \times 10^{3a} \pm 1.12 \times 10^{2}$ | $8.04 \times 10^{3a} \pm 9.45 \times 10^{2}$ | $1.58 \times 10^{5a} \pm 1.19 \times 10^{4}$ | $1.27 \times 10^{6a} \pm 1.17 \times 10^{5}$ | $1.03 \times 10^{7a} \pm 9.38 \times 10^{5}$ |
| Lemon grass oil 0.5% | $1.47 \times 10^{3a} \pm 1.17 \times 10^{2}$ | $3.38 \times 10^{2cd} \pm 0.26 \times 10^{2}$ | $4.29 \times 10^{2c} \pm 0.37 \times 10^{2}$ | $3.87 \times 10^{3c} \pm 4.33 \times 10^{2}$ | $1.16 \times 10^{5b} \pm 7.37 \times 10^{3}$ | $8.62 \times 10^{5c} \pm 5.25 \times 10^{4}$ |
| Lemon grass oil 1% | $1.47 \times 10^{3a} \pm 1.17 \times 10^{2}$ | $3.08 \times 10^{2cd} \pm 0.24 \times 10^{2}$ | $3.71 \times 10^{2c} \pm 0.42 \times 10^{2}$ | $3.54 \times 10^{3c} \pm 2.17 \times 10^{2}$ | $1.05 \times 10^{5b} \pm 8.41 \times 10^{3}$ | $7.52 \times 10^{5c} \pm 6.11 \times 10^{4}$ |
| Orange 0.5% | $1.47 \times 10^{3a} \pm 1.17 \times 10^{2}$ | $6.61 \times 10^{2b} \pm 0.52 \times 10^{2}$ | $6.55 \times 10^{3a} \pm 8.74 \times 10^{2}$ | $9.48 \times 10^{4b} \pm 8.45 \times 10^{3}$ | $1.80 \times 10^{5b} \pm 2.78 \times 10^{4}$ | $9.73 \times 10^{6a} \pm 9.69 \times 10^{5}$ |
| Orange 1% | $1.47 \times 10^{3a} \pm 1.17 \times 10^{2}$ | $4.26 \times 10^{2c} \pm 0.34 \times 10^{2}$ | $3.11 \times 10^{3b} \pm 6.59 \times 10^{2}$ | $7.56 \times 10^{4b} \pm 1.05 \times 10^{4}$ | $1.73 \times 10^{5b} \pm 2.91 \times 10^{4}$ | $7.17 \times 10^{6b} \pm 8.13 \times 10^{5}$ |
| Thyme 0.5% | $1.47 \times 10^{3a} \pm 1.17 \times 10^{2}$ | $2.93 \times 10^{2cd} \pm 0.23 \times 10^{2}$ | $3.85 \times 10^{2c} \pm 0.33 \times 10^{2}$ | $3.83 \times 10^{3c} \pm 4.30 \times 10^{2}$ | $1.15 \times 10^{5b} \pm 7.05 \times 10^{3}$ | $8.20 \times 10^{5c} \pm 7.57 \times 10^{4}$ |
| Thyme 1% | $1.47 \times 10^{3a} \pm 1.17 \times 10^{2}$ | $1.91 \times 10^{2d} \pm 0.15 \times 10^{2}$ | $3.28 \times 10^{2c} \pm 0.46 \times 10^{2}$ | $3.51 \times 10^{3c} \pm 2.22 \times 10^{2}$ | $1.04 \times 10^{5b} \pm 8.41 \times 10^{3}$ | $7.25 \times 10^{5c} \pm 4.44 \times 10^{4}$ |

Values with different letters within the same row differed significantly at $P <0.05$

Natural antioxidants improved both color and flavor stability in meat (Sasse et al., 2009). This improvement may be due to inhibition of lipid oxidation which affects directly the metmyoglobin (Tang et al., 2006). Lemongrass oil 1% produced a noticed strong taste and flavor which may not be accepted for consumers, so lemongrass 0.5% was more accepted. This result agreed with result of Sinha et al. (2014), who mentioned that lemongrass oil is considered safe for human consumption at low concentrations that induced cytotoxicity and genotoxicity at higher concentrations. Orang oil demonstrated enhancement of sensory attributes, with very accepted taste and flavor for two concentrations (0.5% & 1%), also samples containing 1% of Eo, demonstrated higher enhancement than samples containing 0.5%, this finding nearly agreed with that of Hamed- Ahmed (2017). Thyme oil 0.5% demonstrated enhancement of sensory attributes, with accepted taste and flavor, while thyme oil 1% has unacceptable organoleptic properties (colure, odor and taste) in Kofta, this result agreed with that obtained by Solomakos et al. (2008).

The obtained results in table (2) showed that lemongrass oil 1 and 0.5% demonstrated the highest significant ($P<0.05$) lowering effect on pH values than those of control samples followed by thyme oil 1%. This may be due to an activation effect of essential oils as antimicrobial agent causing protein hydrolysis with appearance of alkyl groups. These results agreed with Salem et al. (2010), who recorded that the treated samples with 1.5% and 1% lemongrass oil showed the highest significant ($P<0.05$) pH lowering effect than untreated samples. Also, samples treated with orange oil decreased pH values than pH values of control samples. By increasing concentration of oils to 1%, pH values decreasing than lower concentration of oils 0.5%. On the other hand, Ibrahim et al. (2018) revealed that pH values of raw ground beef (mixed with 1 and 2% orange peel) obviously decreased to be slightly acidic. While, concentration of thyme (1.2%) were significantly low ($p<0.05$) comparing with control samples after 4 days of storage at refrigeration temperature.

As shown in table (3) the present work revealed that treated samples by lemongrass, orange and thyme oils at different concentrations showed decreasing in TBARs values than control sample especially at 6th, 8th and 10th day of storage period. High concentrations showed decreasing in the TBARs values than lower concentrations. Flavonoids present in citrus possess antioxidant activity owing to their free radical scavenging ability (Anagnostopoulou et al., 2006), also thyme has high antioxidant effect related to the scavenger nature of its flavonoids and phenolic contents. These results were similar to that obtained by Zaki et al. (2018), who recorded that control group had the highest TBA value than the other formulated samples. Also, Ibrahim et al. (2018), who mentioned that TBARS values increased over the storage time for all patty samples, control raw patties exhibited higher TBARS values at any given time of chilling as compared to 1 and 2% orange peel formulated patties, during refrigerated storage for 15 days at 4±1 °C. Salem et al. (2010) and Zengin and Baysal (2015) mentioned that TBA values of minced meat were obtained over 9 days of storage at 4 °C and TBA values of control samples, treated samples with thyme oil had significantly lower ($P<0.05$) TBA values than control at each day of testing throughout 6th and 9th days of the storage period.

As reported in table (4) lemongrass, orange and thyme oils usage at concentrations 0.5 and 1% showed decreasing in TVBN values than control samples especially at 6th, 8th and 10th day, also by increasing concentration of oils (1%) was more effective than low concentration (0.5%) in decreasing TVBN values. The significant decrease in TVN values in treated samples might be attributed to antimicrobial effect of those essential oils and to their ability to inhibit the activity of proteolytic enzymes (El-Desouky et al., 2006). These results agree with Zaki et al. (2018), who mentioned that the control sample had higher TVBN than other camel burger containing different levels of lemongrass. TVBN value decreased as the level of lemongrass increased during cold storage at 4 °C for 12 days. Also, Ibrahim et al. (2018) mentioned that during storage period, the TVBN of patties with orange powder was lower than the control sample. The lower TVBN for samples with OP may be due to the effect of the bioactive compounds in citrus peel on microorganisms. And Koura-Hanan (2018) mentioned that treated minced meat with thyme oil at different concentrations showed decreasing in TVBN values than those of control samples and by increasing concentration of

Thyme was more effective than low concentration in decreasing TVBN values during 12 days of storage at 2 °C.
As shown in table (5) lemongrass, orange and thyme oils usage at concentration 0.5 and 1% remarkably increased APC throughout storage, especially in the control sample at the $8^{th}$ and $10^{th}$ days. In general, a significant decrease was noticed for all treated kofta samples in their APC during the storage period as compared to the control sample. Also, high concentration of oils (1%) was more effective in decreasing this count than lower concentration (0.5%). Antimicrobial activity of different essential oils is directly linked to the presence of phenolic constituents such as eugenol cumin aldehyde, carvone, limonene, linalool and thymol (Najda et al., 2008). Most of these active phenolic compounds are able to inhibit the activity of enzymes involved in the energy regulation and metabolism (Mohammed et al., 2006). These results were similar to results obtained by Zaki et al. (2018) and Salem et al. (2010), who mentioned that the antibacterial effect of lemongrass oil in refrigerated minced beef as decreasing APC than control samples. High concentration of lemongrass (1.5%) was more effective in decreasing APC values than lower concentration (0.5%). Also, the obtained results agreed with those of Hamed-Ahmed (2017) and Ibrahim et al. (2018), who mentioned that 1% orange peel treatments delayed the microbial growth and extended the shelf life of the beef patty up to 12 days. Salem et al. (2010), Elgaafary et al. (2016) and Koura-Hanan (2018) recorded the antibacterial effect of thyme oil in refrigerated minced beef as decreasing APC than control sample. High concentration of thyme oil (1.5 and 2%) was more effective on decreasing APC values than values of low concentration (0.5%).
As shown in table (6) samples treated by different concentrations of lemongrass, orange and thyme oils showed decreasing count of coliform compared to control samples, also high concentration (1%) was more effective in decreasing this count than low concentration (0.5%). The obtained results were similar to that obtained by Salem *et al.* (2010), who mentioned that preserved minced meat at 4 °C during 6 days of storage period. The control samples had the highest counts of coliform compared to other treatment of minced meat with different concentration of lemongrass and thyme (0.5, 1 and 1.5%). Also, the highest concentration of oil recorded more decreasing of coliform count than lower concentration. Citrus essential oils have been found to inhibit Gram-positive and Gram-negative bacteria as well as yeasts, molds and food poisoning bacteria (Chun-Lin et al., 2013).
As shown in table (7) samples treated by different concentrations of lemongrass, orange and thyme oils showed decreasing count of *Staphylococcus* compared to control samples, also high concentration (1%) was more effective in decreasing this count than low concentration (0.5%). This may attribute to Gram-positive bacteria surrounded by a thick peptidoglycan wall not dense enough to resist small antimicrobial molecules, facilitating the access to the cell membrane, and may ease the infiltration of hydrophobic compounds of EOs due to the lipophilic ends of lipoteichoic acid present in cell membrane (Hyldgaard *et al.,* 2012). The obtained results were similar to those obtained by Salem et al. (2010) as treated samples with lemongrass and thyme oils 1.5% respectively showed the lowest *staphylococci* count of different treated and untreated minced beef samples at 4 ºC in order lemongrass oil >thyme oil. The essential oils will result in immediate reduction of bacterial population (Seydim and Sarikus, 2006) and might be more effective against food borne pathogens and spoilage bacteria when applied directly on foods ready to be used, containing a high protein level at acidic pH, as well as, lower levels of fat or carbohydrates (Gutierrez *et al.,* 2008)
As shown in table (8) using lemongrass, orange and thyme oils at concentrations 0.5 and 1% showed decreasing count of *Staph. aureus* compared to control samples. Thyme reported the highest decreasing followed by lemongrass. Carvone, carvacrol, cinnamaldehyde and eugenol showed a significant inhibitory effect against S. aureus (Rahman et al., 2010). The obtained results were similar to those obtained by Salem et al. (2017), who mentioned that essential oils (thyme and lemongrass) have an antimicrobial activity against Methicillin Resistant Staphylococcus aureus (MRSA) in order thyme oil > lemongrass oil. Also, Ramadan et al. (2015), who mentioned that the antibacterial activities of the methanolic and ethanolic orange extract showed antibacterial activity against MRSA.
Moreover, lemongrass, orange and thyme essential oils exhibited strong antibacterial and antioxidant as well as good organoleptic properties. Lemongrass oil in two concentrations (0.5 and 1%) showed the best results as antibacterial and antioxidant, also good organoleptic properties but lemongrass oil 0.5% was more accepted. Orange oil 0.5% showed the lowest results as antibacterial and antioxidant with accepted organoleptic properties. On other hand, orange oil 1% is more effective as antibacterial and antioxidant and demonstrated more enhancements of sensory attributes. Thyme oil in two concentrations (0.5 and 1%) is effective as antibacterial and antioxidant, since thyme oil 1% has unacceptable organoleptic properties in kofta.

## 5. CONCULOSION

It was suggested that lemongrass oil 0.5%, orange oil 1% and thyme oil 0.5% can be applied to kofta as natural preservatives with antioxidants and antimicrobial activities as well as accepted organoleptic properties. Therefore, may be useful in maintaining the meat products quality, extending shelf- life of meat products, preventing economic loss and providing the consumer with food containing natural additives.

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