

EFFECT OF ETHANOLIC EXTRACT OF THYME AND OLIVE LEAVE IN QUALITY CHARACTERISTIC OF LAMB PATTIES DURING STORAGE

Hozan. M. Anwar⁽¹⁾

Researcher

I. A. Baker⁽²⁾

Assist. Prof.

⁽¹⁾Animal production department, Technical Institute of Amedi, Duhok Polytechnic University, Kurdistan Region, Iraq (Hozanmzori060@gmail.com)

⁽²⁾Animal Production Dept., College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region, Iraq (Ibrahim.aswad@uod.ac)

ABSTRACT

The aim of this experiment was to evaluate the effect of adding different levels of each of olive leaf extract (OLE) and thyme leaf extract (TLE) and their combination in some physicochemical, microbial, and sensory characteristic of lamb patties stored either at 4c° for 12 days or at -18c° for 120 days. Results revealed that there are a steady rise ($p<0.01$) in oxidative rancidity, microbial count and free fatty acid in control and treated samples with increasing time in both storage periods. Also, a significant ($p<0.01$) reduction in TBA, microbial count and free fatty acid was observed in samples treated with OLE, TLE and their combination. Moreover, it was found that addition of 1% OLE and 1% OLE +0.05%TLE are more effective against formation of TBA and microbial count, respectively. Sensory characteristics were highest in patties treated with 0.05% TLE and the lowest was noticed in samples treated with 1% OLE.

Keywords: lamb meat, antimicrobial, antioxidant, leaf extracts.

انور وبكر

مجلة العلوم الزراعية العراقية -2022: 53: (1) 219-229

تأثير مستخلص الايثانول من اوراق الزعتر و اوراق الزيتون في الصفات النوعية لاقراص لحم الحملان خلال الخزن

ابراهيم اسود بكر

هوزان مسعود انور

استاذ مساعد

باحث

المستخلص

تهدف هذه التجربة تقييم تأثير اضافة نسب مختلفة من كل من مستخلص اوراق الزيتون والزعتر و خلطانها في بعض الصفات الفيزيوكيميائية و الحمل الميكروبي و الصفات الحسية لاقراص لحم الحملان والمخزونة بالتبريد (4م°) لمدة 12 يوم و كذلك بالتجميد (-18م°) لمدة 120 يوما. اشارت النتائج بوجود زيادة مغنوية في التزنخ التأكسدي والعدد البكتري و الاحماض الدهنية الحرة في اقرص اللحم غير المعاملة و كذا المعاملة يتقدم فترة الخزن بالتبريد والتجميد. كما لوحظ انخفاض في العدد البكتري وقيم TBA والاحماض الدهنية الحرة في اقرص اللحم المعاملة مقارنة بالنماذج غير المعاملة (السيطرة). كما وجد بان اضافة 1% من مستخلص اوراق الزيتون و 1% من مستخلص اوراق الزيتون +0.05% من مستخلص اوراق الزعتر كانا اكثر فعالية ضد التزنخ التأكسدي والعدد البكتري على التوالي. كما بلغ أعلى تقويم حسي لدى اضافة 0.05% من مستخلص اوراق الزعتر و ادناه في اقرص اللحم المعاملة ب 1% من مستخلص اوراق الزيتون.

الكلمات المفتاحية: لحم الحملان, مضادات الاكسدة و البكتريا , مستخلصات الأوراق

INTRODUCTION

It has been well known that deterioration of meat products is mainly caused by lipid oxidation and microbial spoilage. However, the use of antioxidants and antimicrobials provides an effective way for preservation of meat products against spoilage. Nowadays, an increased interest has been focused towards plant-based extract as a source of phenolic antioxidant, antimicrobial, antiviral and anti-inflammatory (13, 45). It has been reported that adding olive leaf extract (OLE) at a rate of 1, 2 and 3%, led to retarded significantly ($p < 0.01$) oxidative rancidity of lamb patties stored at 4c° for 12 days by 52.3, 36.5 and 26.17%, respectively as compared with control. Total plate count, psychrophilic count and coliform bacteria decreased significantly ($p < 0.01$) with the addition of this extract (11). Similarly Al-Rimawi, et al (3), demonstrated the activity of Oleuropein and OLE extract as a natural antioxidant to retarded oxidation of hamburger compared with control samples. Moreover, Robiel, et al., (54), noted that individual addition of OLE with or without Tannic acid to beef patties affected positively color, lipid stability, microbiological loads and protein degradations compared with control. Moreover, working on broiler meat it was indicated that addition of OLE resulted in reduced microbial growth successfully, and maintained the chemical quality and sensory attribution of the product (20, 28). It has been known that thyme have antioxidant capacity due to its content of flavonoids (42), as well as have antimicrobial activity (39, 51). Also, it has been indicated that adding thyme extract at a rate of 0, 250, 500 and 1000 ppm to lamb and chicken patties stored at 4c° for 12 days resulted in inhibiting microbial load and delayed oxidation particularly at a rate of 500 PPM as compared to control (12). Similarly, El Adab, et al., (22) and Sharma, et al., (58) and Huang, et al., (31), demonstrated that addition of thyme essential oil to poultry sausages prove to be an effective in delaying lipid oxidation and inhibiting microbial growth. The objective of this experiment was to study the effect of adding olive leaf extract (1%) and thyme leaf extract (0.5%) and their combination on some physico-chemical, microbial, and sensory characteristic of lamb

meat patties during chilling storage at (4c°) for 12 days and freezing (-18c°) for 120 days. The above concentration was chosen depending on previous studies (11, 12).

MATERIALS AND METHODS

Preparation of thyme and olive leaf extract

Thyme vulgaricus leaves were obtained from the Doski area of Duhok, and olive leaves were obtained from the University of Duhok's College of Agricultural Science in the Kurdistan region of Iraq. They were collected in the spring, washed, and dried at room temperature for 10 days before being ground into powder using a machine grinder. One hundred grams each of crushed leaves were extracted in a closed conical flask with 1000 ml of 70 percent (v/v) aqueous ethanol and 30 percent distilled water, using a magnetic stirrer without a heat plate for 24 hours at room temperature in the dark. The residue was removed three times using the same process then the extract was filtered through clean cheese cloth. The residual solution was dried and evaporated in a vacuum oven at 40c° before being frozen (11,12).

Preparation of meat Patties

Ribs of meat were cut from the carcasses of Karadi lambs slaughtered at about ten months of age, obtained from Duhok's primary butchery shops. The samples were moved to the laboratory under full sterilization conditions. The fat and connective tissues were removed from samples after 24 hours storage at 4c° . The meat lambs' muscles were cut into small pieces and minced through a 0.8 cm plate in meat grinder. Minced meat were divided into five treatment groups (5kg). The first sample was left untreated (control), while the others were blended with 1 % Olive leaf extract (T1), 0.05 % Thyme leaves extract (T2), 1 % Olive leaf extract, plus 0.05% Thyme leaf extract (T3), and 0.5 % Olive leaf extract plus 0.025 % Thyme leaves extract (T4). Patties (100g) were developed using a meat former (10 cm wide and 1 cm thick) and placed on plastic foam meat trays, wrapped in polyethylene film, and stored for 12 days at 4c° or at -18c° for 120 days to evaluate physico-chemical, microbial count, and sensory characteristic.

Lipid oxidation indicater in meat Measurement of Thiobarbituric acid(TBA)

Lipid oxidation as Thiobarbituric acid(TBA) was determined by spectrophotometer (6400 – JENWAY, UK) following the method of Witte,et al.,(67). To determine the TBA values, the color was measured at 530 nm. The TBA value was determined and multiplying the absorbance by 5.2 factors and expressing it as mg MDA/kg meat:

$$\text{TBA (mgMDA/kgmeat)} = A \times 5.2$$

Measurement of myoglobin (Mb)

The concentration of myoglobin in the minced meat was determined as described by Krzywicki, (40), and the absorbance was measured with a spectrophotometer at (700 and 525 nM) (6400 JENWAY. UK). The following formula was used to measure myoglobin concentration:

$$(\text{A525-A700}) \times \text{dilution} \quad \text{F.}$$

$$\text{Myoglobin (mg/gm meat)} = \frac{\text{F.}}{\text{Wt. of sample}}$$

Free fatty acids (FFA)

Free fatty acids (FFA) were used to determined according to Egan, et al., (20), the following formula was used:

$$0.282 \times \text{ml NaOH} \times 0.1\text{N NaOH}$$

$$\text{FFA \%} = \frac{\text{Weight of sample (g)}}{\text{Weight of sample (g)}}$$

Microbial Count

Microbial count was determined as recommended by the American Public Health. Association for food stuff examination (APHA), (6) .Total plate count (TPC) was determined on nutrient agar medium, and the plates of different dilutions were incubated at 37 c° for 48 h. The average number of colonies per countable plate as well as the total number of colonies per gram (CFU/g) was determined. Psychrophilic bacteria (PSY) were determined on nutrient agar medium, and the plates, and the inoculated plates were inoculated at 7c° for 10 days.

Sensory Evaluation

The investigated samples were evaluated using a panel test according to Cross, et al., (15).

Statistical Analysis

Statistical analysis of the data was carried out using GLM to estimate Best Linear unbiased effects (56), of the main effects (treatment, period and their interaction). on studied traits

Duncan, (18), was performed to detect significant differences among means of treatment combination (treatments X period).

RESULTS AND DISCUSSION

TBA: TBA values routinely used as an index of lipid oxidation in meat products in store and the rancid flavor is initially detected in meat products when TBA values reach to 0.9 (19). The TBA values for the lamb patties treated with different levels of OLE and TLE and their combination during storage at 4 c° for 12 days are given in Table (1). It seems from the table that TBA values for treated samples were significantly ($p < 0.01$) lower than that recorded for control sample. During storage at 4 c° lipid oxidation in the control started to increase at day 1 from 0.944 mg MDA/Kg meat to 2.556 mg MDA/Kg meat at day 12 of storage. while treated meat samples apparently retarded oxidative rancidity during storage by 86.9,83.3,79.4 and 86.8 % for T1, T2, T3 and T4, respectively as compared to untreated control patties. In the present study, the TBA values for lamb patties treated with different concentration of TLE, OLE and untreated (control) during storage at -18 c° up to 120 days are given in Table (2). During storage, lipid oxidation of patties increased from 0.736 (Days 1) to reach 2.547 mg MDA/kg meat on 120 days of storage. while the maximum values for lamb patties treated with thyme and OLE extracts were 0.614mg MDA/kg meat at the end of storage period, thus treatment resulted retarded in oxidation by 86.5,82.6,75.8 and 78.2% , in T1,T2,T3 and T4 ,respectively .Therefore , it seems that OLE, TLE and their combination added to meat patties are effective in retardation of oxidative rancidity during storage at 4 c° for 12 days as well as during storage at -18 c° for a period of 120 days . While such effect of OLE may be attributed to the hydroxyl group (hydroxyl tyrosol , gallic acid ,trycol ,vanilic acid ,ferulic acid and cinnaric acid) within the phenolic structure constituent present on OLE (43),as well as Olive leaves have substantial amounts of bioactive compounds (oleuropein, verbascoside, luteolin-7-Oglucoside,apigenin-7O-glucoside-hydroxy ,tyrosol,and tyrosol) in antioxidant properties, which have been associated with their strong characteristics as preservatives (25,38).The presence of

phenolics and antioxidant activity in olive leaf extract has been reported by Stamatopoulos, et al., (63). The effect of thyme, on the other hand, could be due to scavenger nature of flavonoids and phenolic content (4, 36, 57, 61, 62). The capacity of EOs to act as antioxidants is due to the fact that phenols are chain-breaking antioxidants. They donate an H-atom from the phenolic hydroxyl group to peroxy radicals (ROO) which are responsible for the propagation of the oxidative radical chain). Therefore, they can slow down the peroxidation of unsaturated lipids (52). These results were similar to those obtained by Kassem, et al., (36), who mentioned that TBA values of minced meat were obtained over 9 days of storage at 4°C for control showed a rapid increasing with increasing storage period, TBA values of thyme treated sample on day 0 were significantly lower ($p < 0.05$) than those of control samples (12, 37). The

antioxidant action of thyme has been shown in vitro (14), from its application in animal products (30, 41), as well as veal (59), pork (35), and lamb in particular (48). These results were in agreement with Medina, et al., (44, 66, 10). Also, it was indicated that the amount of oxidative products for control hamburger samples and treated ones with OLE (0.25%, 0.5%, and 0.75%) increases with storage time from day 1 to six months (3, 23, 55). Furthermore, since MDA values are considered as indicators of rancidity in fat product and Verme, and Sahoo, (65), demonstrated that MDA concentration between 1.0 and 2.0 mg/kg as threshold value for rancidity, therefore, the lamb patties treated with OLE, Thyme and their combination extracts in current work would not deceive consumer up to 12 days of storage due to the antioxidant activity OLE and Thyme.

Table 1. Effect of thyme leaf extracts (TLE) and Olive leaf extracts (OLE) on changes in MDA (mg malondialdehyde / kg meat) values of lamb meat during storage at 4 °C for 12 days

Treatment	Period days			
	1	4	8	12
C	0.944±0.004 d	1.452±0.006 c	1.778±0.017 b	2.556±0.004 a
T1	0.150±0.007 l	0.260±0.014 j	0.297±0.001 i	0.335±0.011h
T2	0.193±0.001 k	0.292±0.001 i	0.353±0.002 hg	0.476±0.017 f
T3	0.235±0.028 j	0.377±0.009 g	0.481±0.009 f	0.526±0.005 e
T4	0.164±0.004 lk	0.332±0.003 h	0.384±0.003 g	0.469±0.012 f

For each trait, means with different letters within each column and each row differed significantly ($p < 0.01$). C= control T1=1%OLE T2= 0.05%OLE T3= 1%OLE,0.05%OLE T4= 0.5%OLE,0.025%OLE

Table 2. Effect of thyme leaf extracts (TLE) and Olive leaf extracts (OLE) on changes in MDA (mg malondialdehyde / kg meat) values of lamb meat during storage at -18 °C for 120 days

Treatment	Period days				
	1	30	60	90	120
C	0.736±0.055 e	0.991±0.018 d	1.166±0.016 c	2.310±0.017 b	2.547±0.036 a
T1	0.122±0.006 q	0.206±0.009 po	0.230±0.015 no	0.273±0.012 nm	0.344±0.014 kl
T2	0.157±0.001pq	0.230±0.016 no	0.269±0.018 nm	0.410±0.003ij	0.441±0.003 ih
T3	0.202±0.007 po	0.290±0.007 lm	0.384±0.008 kj	0.469±0.024 h	0.614±0.019 f
T4	0.133±0.001 q	0.231±0.003 no	0.329±0.009 l	0.420±0.008 ihj	0.554±0.016 g

For each trait, means with different letters within each column and each row differed significantly ($p < 0.01$). C= control T1=1%OLE T2= 0.05%OLE T3= 1%OLE,0.05%OLE T4= 0.5%OLE,0.025%OLE

Free Fatty acid (F.F.A)

It is known that presence of free fatty acid resulted from decomposition of lipid in frozen meat (29). The initial values of F.F.A in the current investigation ranged between 0.545 to 0.883 for all treated and control samples. A significant ($p < 0.01$) rise with advancing period of storage was recorded to reach maximum values of 1.375, 1.128, 1.090, 1.158 and 1.174 for control, T1, T2, T3 and T4, respectively at the end of 12 days of storage at 4 °C (Table 3). Such increase in FFA (lipolysis) is a result of

enzymatic hydrolysis of esterified lipid (33). The connection between lipolysis and lipid oxidation rancidity in that free polyunsaturated fatty acid are oxidized more rapidly than esterified lipid (7). Also, result revealed that addition of OLE, Thyme and their combination reduced significantly ($p < 0.01$) F.F.A as compared with control untreated patties particularly at the end of storage of 12 days. This reduction was amounted to 17.9, 20.7, 14.9 and 14.6% for T1, T2, T3 and T4, respectively. Similarly,

Hama, (28), noticed that addition of OLE to broiler breast resulted in reduction FFA during storage for 7 days as compared with control.

Table 3. Effect of thyme leaf extracts (TLE) and Olive leaf extracts (OLE) on changes on free fatty acids (FFA) % of lamb meat during storage at 4 c° for 12 days

Treatment	Period days			
	1	4	8	12
C	0.883±0.037 ed	0.951± 0.059 cd	1.099±0.016 cb	1.375± 0.133 a
T1	0.770±0.049 edf	0.789±0.032 edf	0.827± 0.019 ed	1.128±0 cb
T2	0.545±0.019 g	0.638±0.037 gf	0.751±0.049 ef	1.090±0.049 cb
T3	0.789±0.065 edf	0.847±0.033 ed	1.061± 0.005cb	1.158±0.004 b
T4	0.864±0.018 ed	0.879±0.016 ed	0.954±0.036 cd	1.174±0.012 b

For each trait, means with different letters within each column and each row differed significantly ($p<0.01$).C= control T1=1%OLE T2= 0.05%TLE T3= 1%OLE,0.05%TLE T4= 0.5%OLE,0.025%TLE

Myoglobin concentration

It is known that color stability is very important to retail shelf life because consumer will not buy dark or discoloured meat (26). Furthermore, changes in meat color are attributed to oxidation of red oxymyoglobin in metamyoglobin, which give meat an unattractive brown color (50). Results related

to the effect of treatment on Mb are demonstrated in Table (4). It seems from the Table (4) that color values decreased significantly ($p<0.01$) over storage period .This reduction in color was amounted to 43.9,50.9,41.0,54.0 and 49.7 % for contro,T1,T2,T3 and T4 ,respectively

Table 4. Effect of thyme leaf extracts (TLE) and Olive leaf extracts (OLE) on changes on Myoglobin (Mb) concentration of lamb meat during storage at 4 c° for 12 days

Treatment	Period days			
	1	4	8	12
C	2.469±0.010 b	1.972±0.032 e	1.484±0.006 h	1.383±0.010 j
T1	2.051± 0.023 d	1.490±0.010 h	1.097±0.007 m	1.005±0.025 n
T2	2.346±0.023 c	1.561±0.004 g	1.471±0.016 ih	0.930±0.044 o
T3	2.764±0.020 a	1.811±0.006 f	1.419±0.003 ij	1.271±0.011 k
T4	2.080±0.044 d	1.158±0.011 l	1.162±0.011 l	1.045±0.004 nm

For each trait, means with different letters within each column and each row differed significantly ($p<0.01$).C= control T1=1%OLE T2= 0.05%TLE T3= 1%OLE,0.05%TLE T4= 0.5%OLE,0.025%TLE

Microbial changes

Microbial qualities of lamb patties was assessed through estimation of total plate count (TPC) and psychrophilic bacteria (PSY) during storage at 4 c° for 12 days and during freezing storage at -18 c° for 120 days .Result given in Table (5) revealed that there was a significant ($p<0.01$) steady rise during storage at 4 c° for 12 days in untreated control patties from their initial value in TPC (8.1 vs 103.2×10^5) and Psy (4.0 vs 84.3×10^5). The results also indicated that addition of OLE and thyme extracts resulted in a significant ($p<0.01$) reduction on all counts of studied bacteria, and the highest reduction was observed in T3 being 71.8% for TPC and 81.6% for Psy. In the current study , result presented in Table (6) revealed that there is a significant ($p<0.01$) steady rise in TPC and Psy count with increasing storage period up to 120 days from their initial value in control of TPC (6.066×10^5) and psy (4.950 vs.

98.95×10^5).Also, the result indicated that addition of OLE and thyme and their combination resulted in a marked significant ($p<0.01$) reduction on counts of both studied bacteria, and particularly the addition 1%. OLE and 0.05 %, TLE T3).It has been demonstrated that the use of natural antimicrobials such as organic acids , essential oils and plant extracts could be considered as a good strategy to inhibit microbial spoilage of meat products (49). The plant extracts and essential oils showed a potential antimicrobial effect through the following mechanisms (a). The phenolic compounds in these extracts and essential oils affect either enzyme activity or cause protein denaturation ,(b) it causes changes in the permeability of microbial cells, and (c) it causes changes in the function of the normal activity of cell membranes such as electron transfer , nutrient exchange , protein synthesis ,nucleic acids and enzymatic activity (5).The antimicrobial capacity of thyme could

be attributed to the activity of phenolic compound present in thyme extract through its effect on cell membrane of the microorganism by causing an increase in the permeability and leakage of the vital intracellular constituents and finally disrupt the cell respiration and microbial system (2). Also, the strong

antimicrobial effect of olive leaves extract are mainly attributed to phenolic compounds including caffeic acid, verbascoside, oleuropein, luteolin 7-glucoside, rutin, apigenin 7-o-glucoside (8,53,64). Similar results have been shown by other workers (1,9,11,12,24,32,36).

Table 5. Total plate count (T.P.C), psychrophilic bacteria count (PSY) as affected by different level of Thyme leaf extract (TLE) and Olive leaves extracts (OLE) on lamb patties stored for 12 days at 4 °C
TPCx10⁵

Treatment	Period days			
	1	4	8	12
C	8.1±0.346 h	52.5±1.44 c	94.8±0.46 b	103.2±0.69 a
T1	3.20±0.17 ji	7.60±0.23 h	10.30±0.63 g	49.20±0.69 d
T2	2.00±0.17 jk	6.90±0.057 h	9.80±0.34 g	48.40±0.46 d
T3	1.00±0.11 k	4.50±0.057 i	7.90±0.86 h	28.00±0.46 e
T4	1.75±0.086 jk	6.90±0.57 h	12.80±0.28 f	48.60±0.80 d

PSY x10⁵

Treatment	Period days			
	1	4	8	12
C	4.00±0.34 hi	27.50±0.46 c	42.70±1.03b	84.30±0.46 a
T1	1.70±0.11 kj	3.25±0.028 I	5.45±0.028 g	24.50±0.17 d
T2	1.40±0.057 kj	3.48±0.011 i	4.80±0 hg	24.40±0.057 d
T3	0.85±0.028 k	1.90±0.057 j	4.60±0.17 hg	15.50±0.17 e
T4	1.25±0.08 kj	3.12±0.011 i	6.50±0.057 f	24.20±0.17 d

For each trait, means with different letters within each column and each row differed significantly ($p < 0.01$). C= control T1=1%OLE T2= 0.05%TLE T3= 1%OLE,0.05%TLE T4= 0.5%OLE,0.025%TLE

Table 6. Total plate count (T.P.C), psychrophilic bacteria count (PSY) as affected by different level of Thyme leaf extract (TLE) and Olive leaves extracts (OLE) on lamb patties stored for 120 days at -18 °C
TPC x10⁵

Treatment	Period days				
	1	30	60	90	120
C	6.066±0.088ml	25.00±1.73 h	65.00±1.15 c	77.00±1.04b	90.86±0.52 a
T1	1.66±0.14 qr	4.30±0.32 mno	11.40±0.30 k	36.90±0.75 f	53.23±0.88d
T2	0.90±0.057 r	2.86±0.14 pqo	7.05±0.32 l	28.16±0.98g	43.50±0 e
T3	0.63±0.12 r	1.76±0.14 qr	3.83±0.011 pno	18.83±0.54 j	35.98±0.33 f
T4	0.96±0.12 r	2.40±0.057pqr	4.743±0.060mn	22.433±0.20i	37.36±0.18 f

PSY x10⁵

C	4.950±0.31 l	13.30±0.32 j	37.63±1.36 d	50.83±0.63 b	78.95±0.31 a
T1	0.900±0.057qp	2.26±0.14 n	7.23±0.24 k	27.93±0.63 f	46.83±0.38 c
T2	0.83±0.06 qp	2.06±0.088 no	5.40±0.28 l	21.06±0.17 g	38.13±0.088 d
T3	0.33±0.088 q	1.00±0.11 qop	2.69±0.089nm	15.46±0.34 i	28.33±0.16 f
T4	0.56±0.088 q	1.90±0.057nop	3.62±0.034 m	19.83±0.14 h	32.56±0.033 e

For each trait, means with different letters within each column and each row differed significantly ($p < 0.01$). C= control T1=1%OLE T2= 0.05%TLE T3= 1%OLE,0.05%TLE T4= 0.5%OLE,0.025%TLE

Sensory evaluation

Sensory value is considered especially important to any food because it is the ultimate measurement consumers take to accept or reject a product. A spice extract could exhibit an excellent antioxidative capacity, but if it fails sensory test, it will not be accepted (16). The data on sensory evaluation of the lamb patties treated with OLE, thyme, their combination and untreated control stored at

4 °C for 12 days, as well as at - 18 °C for 120 days are given in Tables (7 and 8), respectively. Results of analyses of variance (Table 7) revealed that treatment affected significantly colour ($p < 0.05$) and a highly significant effect ($p < 0.01$) on flavour and aroma, Juiciness, tenderness and overall acceptability. While, a significant effect of period was observed in all studied traits except tenderness ($p > 0.05$). It seems from Table 7

that the highest overall acceptability was observed in patties treated with 0.05% TLE, whereas the lowest score of overall acceptability was noticed in patties treated with 1% OLE and stored at 4°C for 12 days. It appears from Table (8), that treatment affected significantly ($p < 0.01$) flavour and overall acceptability only. Whereas period of storage affected significantly all studied traits except color. The effect of treatment on overall acceptability during freezing was almost the same as in case of patties stored at 4°C for 12 days being the highest score (4.60) was recorded in patties treated with 0.05% Thyme, and the lowest was noticed in patties treated with 1% OLE. It was indicated that treatment had no significant effect ($p > 0.05$) on color and tenderness, whereas the highest flavour and aroma (4.63) and juiciness (3.93) was found in patties treated with 0.05% Thyme, whereas the lowest flavour and aroma (3.06) was noticed in patties treated 1% OLE 0.5% OLE and 0.025% TLE. Similarly, Kassem, et al., (36), found a significant improvement of odour and overall acceptability of beef burger at day 12 due to addition of thyme essential oil, which could be referred to its aromatic effect (46). Also, Abu-Salem, et al., (1), indicated that sensory evaluation was acceptable with good score for luncheon samples treated with thyme and stored at 4°C for 4 months. On the same line, Gahraie, et al., (24), demonstrated

that frozen beef burgers formulated with thyme and cinnamon extracts obtained the highest score ($p < 0.05$), and the antioxidant properties of natural extracts were the main reason for the increased sensory score of natural extracts- incorporated formulation through preventing the formation of oxidation-mediated off-flavour and the off-odours (short-chain aldehydes and ketones) during storage. It was indicated that thyme essential oil could be used as natural antioxidant for inhibitions of industrially chicken nuggets, lipid oxidation, and providing more sensorial acceptability (17). Also, with regard to the addition of OLE it was indicated that addition of 1% OLE to meat patties and minced meat resulted in a significantly better overall acceptability as composed to control untreated samples (11,27,47).

Conclusion

In conclusion addition of OLE and TLE are effective against formation of TBA and microbial count. It seems also that the highest and lowest overall acceptability was noticed samples treated with 0.05% TEL and 1% OLE, respectively. **Acknowledgements**

The authors wish to express their deep thanks to Prof. Dr Jalal E, Alkass for his valuable help in reading the manuscript, and for Mr. Kawa Y, Merkan for help in statistical analysis of the data.

Table 7. Effect of thyme leaf extracts (TLE) and Olive leaf extracts (OLE) on Sensory evaluation of lamb meat during storage at 4°C for 12 days

Factors	Traits				
	Color	Flavor & aroma	Juiciness	Tenderness	Acceptance
Control	3.437±0.157 A	4.250±0.193 A	3.187±0.163 A	4.562±0.128 A	3.875±0.125 A
T1	3.187±0.208 Ba	3.250±0.193 dC	3.125±0.124 A	4.375±0.125 A	2.562±0.157 C
T2	2.937±0.213 bA	4.000±0.223 bA	3.187±0.245 A	4.187±0.163 bA	4.062±0.170 A
T3	2.687±0.150 B	3.000±0.158 D	2.437±0.181 B	3.812±0.227 B	2.752±0.193 cB
T4	3.062±0.170 bA	3.687±0.150 bC	2.812±0.208 bA	3.750±0.193 B	3.187±0.187 B
Period days					
1	3.500±0.135 A	3.600±0.244 A	3.500±0.170 A	3.800±0.155 B	3.100±0.239 A
4	3.250±0.160 bA	3.800±0.155 A	2.500±0.153 B	4.300±0.163 A	3.250±0.160 A
8	2.600±0.152 C	3.450±0.211 A	3.150±0.195 A	4.200±0.171 bA	3.350±0.195 A
12	2.900±0.160 bC	3.700±0.146 A	2.650±0.109 B	4.250±0.160 bA	3.450±0.193 A

For each trait, means with different letters within each column and each row differed significantly ($p < 0.01$). C= control T1=1%OLE T2= 0.05%TLE T3= 1%OLE,0.05%TLE T4= 0.5%OLE,0.025%TLE

Table 8. Effect of thyme leaf extracts (TLE) and Olive leaf extracts (OLE) on Sensory evaluation of lamb meat during storage at -18 c° for 120 days

Factors	Traits				
	Treatment	Color	Flavor & aroma	Juiciness	Tenderness
Control	3.5±0.212 A	4.23±0.23 BA	3.533±0.215 BA	4.133±0.191 A	3.700±0.227 B
T1	2.833±0.180 A	3.066±0.206 C	3.533±0.191BA	4.00±0.195 A	2.733±0.181 C
T2	3.366±0.231 A	4.633±0.141 A	3.933±0.228 A	4.33±0.186 A	4.600±0.163 A
T3	3.133±0.236 A	3.066±0.153 C	3.600±0.163 BA	4.00±0.195 A	2.933±0.181 C
T4	3.300±0.152 A	3.766±0.145 B	3.400±0.163 B	4.133±0.191 A	3.533±0.165 B
Period days					
1	3.066±0.248 BA	4.200±0.222 A	3.400±0.235 B	4.600±0.130 A	3.600±0.289 A
30	3.200±0.242 BA	3.933±0.181 BA	4.066±0.181 A	4.400±0.190 A	3.866±0.236 A
60	3.333±0.105 BA	3.500±0.248 BC	3.400±0.190 B	4.400±0.163 A	3.633±0.209 A
90	3.666±0.186 A	3.800±0.242 BAC	3.66±0.186 BA	3.666±0.159 C	3.400±0.235BA
120	2.866±0.191 B	3.333±0.251 C	3.466±0.133 B	3.533±0.133 C	3.00±0.239 B

For each trait, means with different letters within each column and each row differed significantly ($p < 0.01$). C= control T1=1%OLE T2= 0.05%TLE T3= 1%OLE,0.05%TLE T4= 0.5%OLE,0.025%TLE

REFERENCES

1. Abu-Salem, F. M. and A.A. Abou-Arab, 2011. "Effect of supplementation of Bambara groundnut (*Vigna subterranean* L.) flour on the quality of biscuits." *African Journal of Food Science* 5, no. 7: 376-383
2. Akthar, M. S., G. Birhanu, and S. Demisse, 2014. Antimicrobial activity of *Piper nigrum* L. and *Cassia didymobotrya* L. leaf extract on selected Food borne pathogens. *Asian Pacific Journal of Tropical Disease*, 4, S911-S919
3. Al-Rimawi, F., M.S. Tarawa, and C. Elama, 2017. Olive Leaf Extract as Natural Antioxidant Additive of Fresh Hamburger Stored at 4 °C Running Title: Antioxidants from Olive Leaves in Hamburger. *American Journal of Food Science and Technology*, Vol. 5, No. 4, 162-166
4. Amarcwicz, R., Z. Egarska, R. Rafalowski, R. B. Pegg, M. Karamac, and A. K. Ska, 2009. Antioxidant activity and free radical – scavenging capacity of ethanolic extracts of Thyme, Oregano, and Marjoram. *Eur. J. Lipid Sei. Tech.*, 111:1111-1117
5. Aminzare, M., M. Hashemi, E. Ansarian, M. Bimkar, H. H. Azar, M. R. Mehrasbi, S. Daneshamooz, M. Raeisi, B. Jannat, and A. Afshari, 2019. Using Natural Antioxidants in Meat and Meat Products as Preservatives: A Review. *Adv. Anim. Vet. Sci.*, 7, 417–426. [CrossRef]
6. APHA, 1992. American Public Health Association. "Compendium of methods for the microbiological examination of Food" 2nd, 3rd (ed), Washington, DC. New York
7. Ashton, I.P. 2002. Understanding Lipid Oxidation in Fish. In : Safety and Quality Issue in Fish Processing (edited by H. Allan Bremner). PP.507 Washington, DC: CRC Press.
8. Aytul, K.K., 2010. Antibacterial and antioxidant activities of olive Leaf extract and its food applications. M.Sc. Thesis, Graduate School of Engineering and Sciences of Izmir Institute of Technology, Izmir, Turkey
9. Aytul, K.K., F. Korel, D.K. Arserim-Uçar, I. Uysal, and O. Bayraktar, 2008. Efficacy of olive leaf extract for enhancing quality of beef cubes, the international congress of meat science and technology, 51, august 10-15, cape town, south Africa
10. Babaie M, N. Yasa, A. Mohammadirad, R. Khorasani, and M. Abdollani, (2007). "On the anti oxidative stress potential of *Zataria multiflora* Boiss (*Avishan shirazi*) in rats," *International Journal of Pharmacology*, 3:510-514
11. Baker, I.A., 2015. Investigation on antimicrobial and antioxidant effect of olive leaf extract in Karadi sheep meat during storage. *Iraqi J. Agric. Sci.*, 46: 1091-1095
12. Baker, I. A., A.D.O. Khalil, and N.H. Khabat, 2015. "Effect of Thyme Leaves Extract on Quality of Lamb and Chicken Meat during Storage." *Science Journal of University of Zakho* 3.2: 205-211

13. Bouaziz , C.,O. Sharat el dein,E. El -Golli , A.Abid-Essefi, ,C.Brenner, C.Lemaire , andH. Bacha, 2008. Different apoptotic pathwaysinduced by zearalenone. T-2 toxin and ochratoxin A in human hepatoma cells, *Toxicol.* 254 :19-28
14. Burt, S. 2004. Essential oils. Their Antibacterial properties and potential Applications in Foods-A review. *Int. J. Food Microbiol.* 94: 223-253
15. Cross, H.R., R. Moen, and M. Stanfield, 1978. Guide lines for training and testing judges for sensory analysis of meat quality. *Food Technol.*, 32:48-53
16. Dang, N.M.2007. Evaluating stability of restructured pork added with ginger extract by means of chemical and sensory analysis. Faculty of Chemical Engineering, College of Technology, Da Nang University Email:dmnhath@yahoo.com
17. Dashti, H. S., F.A.Scheer, P.F.Jacques, S.Lamon-Fava, and J.M.Ordovás, 2015. Short sleep duration and dietary intake: epidemiologic evidence, mechanisms, and health implications. *Advances in nutrition*, 6(6), 648-659
18. Duncan, D.B., 1955. Multiple Ranges and Multiple test. *Biometric*, 11:16
19. E.O.S., 2005. The Egyptian Organization for Standerdization and Quality Control. Frozen beef burger (1688).
20. Ebeed S.,M. Alaa Eldin , E. El-Manakhly , S. Al-Rashed , H. F. Hetta , J.Philippe,Y. Ramadan , EL.B. Gaber, and A. Eman 2020.Effects of Olive Leaf Extracts As Natural Preservative On Retailed Poultry Meat Quality. *Foods*, 9(8), 1017
21. Egan, H., S.Kirk, and R. Sawyer, 1981. *Fresh food: Pearson's Chemical Analysis of Food*, 8th ed, London Group L+D, New tork
22. El Adab, S.and M. Hassouna, 2015. proteolysis, lipolysis and sensory characteristics of a tunisian dry fermented poultry meat sausage with oregano and thyme essential Oils. *J. Food Saf.*, 36, 19–32. [CrossRef]
23. Elama, C., M.Tarawa, and F. Al-Rimawi, 2017. Oleuropein from olive leaf extract as natural antioxidant of frozen hamburger
24. Gahruie, H. H., H. M. S. Hosseini, H. M. Taghavifard,H.M. Eskandari,T.M. Golmakani, and E. ShadV.2017. lipid oxidation, color changes, and microbiological.quality of frozen beef burgers incorporated with shirazithyme, cinnamon, and rosemary extracts. *Journal of Food Quality*Volume, Article ID 6350156,
25. Giacometti, J.,D.B. Kovačević, , P.Putnik ,D. Gabrić, , T.Bilušić , G.Krešić, and A.R.Jambrak. 2018. Extraction of bioactive compounds and essential oils from mediterranean herbs by conventional and green innovative techniques. A review. *Foodresearchinternational*, 113, 245-262
26. Glitsch, K. 2000. Consumer perceptions of fresh meat quality, cross-national comparison. *British Food Journal.*102(3):177-194
27. Hafez, H. and Abdelrahman, A. M. (2015). Potential protective effect of etanercept and aminoguanidine in methotrexate-induced hepatotoxicity and nephrotoxicity in rats. *European journal of pharmacology*, 768, 1-12.
28. Hama, A.A., 2018. Effect of olive leaves extract on some physical and chemical traits of broiler meat during refrigeration storage. *Journal of Kerbala for Agricultural Sciences*, 5(5):43-52
29. Hardy, R., 1980. Fish Lipids.In :Advance in Fish Science and Technology (edited by J.J. Conell). FarhanNewsBook Ltd.pp.103-111
30. Harpaz, S. ,L. Glatman, V.Drabkin, A.Gelman, 2003. Effects of herbal essential oils used to extend the shelf life of freshwater-reared .Asian Sea Bass Fish (*Lates calcarifer*). *J. Food Prot.*, 66, 410–417. [CrossRef]
31. Huang, Z.,X.Liu, S.Jia, L. Zhang, Y.Luo. 2018. The effect of essential oils on microbial composition and quality of grass carp (*Ctenopharyngodon idellus*) fillets during chilled storage. *Int. J. Food Microbiol.*, 266, 52–59. [CrossRef]
32. Hussein, S.A., M.F. Shahin, and M.R.Masoud, 2015. Effect of using Lemongrass and Thyme on some Beefburger characteristics. *Egyptian Journal of Agricultural Research*, 93(1):133-145
33. Hwang, K. T., and J.M.Regenstein, 1993. Characteristics of mackerel mince lipid hydrolysis. *Journal of Food Science*, 58(1), 79-83
34. Ibrahim, H.M., A.Abou-Arab, and F.M.Abu-Salem, 2011. Antioxidant and

Antimicrobial Effects of some Natural Plant Extracts added to lamb patties during storage. *Grasas. Y Aceites*, 62:139-148

35. Ismail, A.A., M.D. Pierson, 1990. Effect of sodium nitrite and origenum oil on growth and toxin production of *Clostridium botulinum* in TYG broth and ground pork. *J. Food Prot.* 53, 958–960

36. Kassem, G.M., O.A. Atta-Alla, and F.H.M. Ali, 2011. Improving the quality of beef burger by adding thyme essential oil and jojoba Oil. *Archivos de zootecnia*, vol. 60, núm. 231, p. 789

37. Kesavan, R.k., B. Srinivasan, A.S.B. Packirisamy, A.F. Mohammed, S. Kalleary, A. Ganesan, S. Meenatchisundaram and S. Muthusamy 2014. Bio protection and preservation of raw beef meat using pungent aromatic plant substances. *Society of Chemical Industry J. Sci. Food Agric.*, 94: 2456–2463

38. Kiritsakis, K., C. Rodríguez-Pérez, D. Gerasopoulos, and A. Segura-Carretero, 2017. Olive oil enrichment in phenolic compounds during malaxation in the presence of olive leaves or olive mill wastewater extracts. *European Journal of Lipid Science and Technology*, 119(9), 1600425

39. Krisch, J., Z. Pardi, R. Tserennadmid, T. Papp, and C. Vagvölgyi. 2010. Antimicrobial effects of commercial herbs, spices and essential oils in minced pork. *Acta Biologica Szegediensis* 54:131;34

40. Krzywicki, K., 1982. The determination of haem pigments in meat. *Meat Science*, 7(1), 29-36

41. Kykkidou, S., V. Giatrikou, A. Papavergou, M.G. Kontominas, and I.N. Savvaidis, 2009. Effect of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets during storage at 4 C. *Food chemistry*, 115(1), 169-175

42. Lacroix, M., W. Smoragicz, L. Pazdernik, M.I. Kone, and K. Krzystyniak, 1997. radiolysis by natural antioxidants from rose marry and thyme. *Food Res. Int.*, 30:457

43. McDonald, S., P.D. Prenzler, M. Antolovich, and K. Robards, 2001. phenolic content and antioxidant activity of olive extracts. *Food Chem.* 73: 73-84

44. Medina, E., M. Brenes, C. Romero, A. Garcia, and A. De Castro, 2014. Main

Antimicrobial Compounds in Table Olives. *J. Agric. Food Chem.* 2007, 55, 9817-9823

45. Micol, V., N. Caturla, L. Pérez-Fons, V. Más, L. Pérez, and A. Estepa, 2005. The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). *Antiviral research*, 66(2-3), 129-136

46. Mishra, A. K., and N.K. Dube, 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and environmental microbiology*, 60(4), 1101-1105

47. Moawad, A. A., H. Hotzel, O. Awad, H. Tomaso, H. Neubauer, H.M. Hafez, and H. El-Adawy. 2017. Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. *Gut pathogens*, 9(1), 1-13

48. Moñino, M.I., C. Martínez, J.A. Sotomayor, A. Lafuente, and M.J. Jordán, 2008. Polyphenolic transmission to segureño lamb meat from ewes dietary supplemented with the distillate from rosemary (*Rosmarinus officinalis*) leaves. *J. Agric. Food Chem.*, 56, 3363–3367. [CrossRef]

49. Negi, P. S., 2012. Plant Extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *International journal of food microbiology*, 156(1), 7-17

50. Nerín, C., L. Tovar, D. Djenane, J. Camo, J. Salafranca, J.A. Beltrán, and P. Roncalés, 2006. Stabilization of beef meat by a new active packaging containing natural antioxidants. *Journal of Agricultural and Food Chemistry*, 54(20), 7840-7846

51. Nguefack, J., I. Somda, C.N. Mortensen, and P.H. Amvam Zollo, 2005. Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (*Oryza sativa* L.). *Seed science and technology*, 33(2), 397-407

52. Nieto, G., L. Martínez-Zamora, J.C. Sánchez, G. Ros, and G. Nieto, 2017. Hydroxytyrosol extracts, olive oil and walnuts as functional components in chicken sausages. *J. Sci. Food Agric.*, 97, 3761–3771. [CrossRef]

53. Pereira, A.P., I.C.F.R. Ferreira, F. Marcelino, P. Valentao, P.B. Andrade, R. Seabra L. Estevinho, A. Bento, and J.A.

- Pereira, 2007. Phenolic Compounds and Antimicrobial Activity of Olive (*Olea europea* L. Cv. Corbançosa) Leaves. *Molecules*, 12, 1153-1162
54. Robiel K. M. , A. A. Ibrahim , N. M. Abdelmaguid, W. A. Ibrahim and A. N. Shehata, 2017. Preventive effect of olive leaves extract in combination with tannic acid on the quality losses of the refrigerated ground beef patties. *Asian Journal of Scientific Research* ISSN 1992-1454 DOI: 10.3923/ajsr..215.226
55. Saleh.H. H. and R.H.Khidir , 2013. Role of olive leaves extract as antioxidant and antimicrobial in quality preservation Karadi sheep meats during frozen storage. *Al-Anbar J. Vet. Sci.*, Vol.: 6 No. (2),
56. SAS/STAT., 2002. User Guide for Personal Computers. Release 6.12 SAS. Institute Inc., Cary, NC. U.S.A
57. Senatore, F. , 1996. Influence of harvesting time on yield and composition of the essential oil of thyme growing wild in Campania (south Italy). *J. Agr. Food Chem.*, 44:1327-32
58. Sharma, H., S. K. Mendiratta, R. K. Agarwal, S. Kumar, and A. Soni. 2017. Evaluation of anti-oxidant and anti-microbial activity of various essential oils in fresh chicken sausages. *Journal of Food Science and Technology* 54(2):279;92.
59. Skandamis, P. , K. Koutsoumanis, K. Fasseas,G.J.E.Nychas. 2001. Inhibition of oregano essential oil and EDTA on *Escherichia coli* O157:H7. *Ital. J. Food Sci.*, 13, 65–75
60. Skandamis, P., E.Tsigarida, G.J.E.Nychas, 2002. The effect of oregano essential oil on survival/death of *Salmonella typhimurium* in meat stored at 5 c° under aerobic, VP/MAP conditions. *Food Microbiology*, 19, 97–103
61. Skerget, M. ,P. Kotnik, M. Hadolin, A.R. Hras, and Z. Knez, 2005. Phenols, Pranthocyanidins, flavons and flavonals in sole plant materials and their antioxidant activities . *Food Chemistry* . 89:191-198
62. Stahl-Biskup, E., 1991. The chemical composition of thyme oils. A review of the literature 1960-89. *J. Essent. Oil Res.*, 3:61-82
63. Stamatopoulos, K., A. Chatzilazarou, and E. Katsoyannos, 2014. Optimization of multistage extraction of olive leaves for recovery of phenolic compounds at moderated temperatures and short extraction times. *Foods*, 3(1), 66-81
64. Tahir,L.and N. Khan, 2012.Antibacterial potential of crude leaf, fruit and flower extracts of *Tagetes Minuta L* .*Journal of Public Health and Biological Sciences* 3:74-78.
65. Verme,S.P.,andJ.Sahoo, 2000 .Improving the quality of ground chevon during refrigerated storage by tocopherol acetate preblending .*Meat sci.*, 56:403-413
66. Viuda-Martos, M.,Y. Ruiz-Navajas, J. Fernández-López, and J.A.Pérez-Álvarez, 2009. Effect of adding citrus waste water, thyme and oregano essential oil on the chemical, physical and sensory characteristics of a bologna sausage. *Innov. Food Sci. Emerg. Technol.* 10: 655- 660
67. Witte,V. C., G.F.Krause, and M.E.Baily, 1970.A new extraction method for determiniy 2-7 hiobarbiturie acid values of pork and beef during storage *J. Food Sci.*, 35:582-585