Role of olive leaves extract as antioxidant and antimicrobial in quality preservation Karadi sheep meats during frozen storage

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Abstract

The objective of the present study was to investigate the effect of natural extract of olive leaves on the quality meat properties in sheep meat, meat cubes were prepared from lean steaks of leg cuts from Karadi sheep carcasses, After that excess fat and connective tissue were trimmed from meat, meat cubes were treated with 1%, 3% and 5% olive leaves extract (OLE) and 0.01% BHT and untreated control treatment stored at -18^{o}C for 60 days and evaluated for chemical and microbial attributes at 0, 30 and 60 days of frozen storage. Results obtained can be summarized as follows:

1. It appears that meat samples treated with 3% or 5% OLE had lower TBA values (1.32 and 1.61 mg MDA/ kg meat) respectively, and followed the BHT and 1% OLE treatments, which were 1.98 and 2.23 mg MDA/ kg meat respectively, and followed the control treatment (3.66 mg MDA/ kg meat) after 60 days of frozen storage.
2. It seems that addition of olive leaves extract at concentrations of 3% or 5% OLE to sheep meat samples retarded metmyoglobin formation by 32.12 and 32.61% respectively as compared with control treatment (51.30%), 1% OLE (47.83%) and BHT treatment (36.49%) after 60 days of frozen storage.
3. It is observed that meat samples treated with 3% or 5% OLE were more effective as a natural antioxidant and antibacterial than synthetic antioxidant (BHT) and 1% OLE treatment in retarding total volatile nitrogen (TVN) formation after 60 days of frozen storage as compared with control treatment.
4. It seems that meat samples treated with 3% or 5% OLE had inhibitor effects for total plate count. Psychrotrophic bacteria of sheep meat after 60 days of frozen storage in comparison to other treatments.

It can be concluded that the treated sheep meat with olive leaf extract at concentrations 3% or 5% were more effective as antioxidant and antimicrobial in retarding of lipid oxidation and total volatile nitrogen formation, stabilizing of meat colour and meat proteins and inhibition of microbial growth of sheep meat as compared with synthetic antioxidant (BHT) and 1% OLE control treatment during frozen storage for 60 days.

دور مستخلص اوراق الزيتون كمضاد أكسدة ومضاد مايكروبي في حفظ نوعية لحوم الاغنام الكرادية المخزونة بالتجميد

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الخلاصة

تهدف الدراسة الحالية لبحث تأثير المستخلص الطبيعي لأوراق الزيتون في خواص اللحم النوعية في لحوم الأغنام الكرادية، اذ تم تحضير مكعبات من شرائح اللحم الخالص لقطعيات الفخذ من ذبائح الأغنام الكرادية، بعد

إزالة الدهن الخارجي والأنسجة الرابطة من اللحم بعد ذلك تمت معاملة مكعبات اللحم مع تراكيز مختلفة من مستخلص أوراق الزيتون 1، 3 و15% ومضاد أكسدة صناعي (BHT) بتركيز 0.01% ومعاملة مقارنة. خزنت عينات اللحم المعاملة وغير المعاملة في التجميد في درجة حرارة – 18 م° لمدة 60 يوماً لتقدير الصفات الكيميائية والمايكروبية في مدد خزنية 0، 30، 60 يوماً من الخزن المجمد. يمكن تلخيص النتائج كما يلي:

1. أظهرت عينات اللحم المعاملة مع 3% أو 5% من مستخلص أوراق الزيتون أوطأ قيم TBA (1.32 و1.61 ملغم مالون الديهايد/ كغم لحم) على التوالي وتبعها المعاملات BHT و1% من مستخلص أوراق الزيتون (1.98 و2.23 ملغم مالون الديهايد/ كغم لحم) على التوالي ثم تلتها معاملة المقارنة (3.66 ملغم مالون الديهايد/ كغم لحم) بعد 60 يوماً من الخزن المجمد.
2. لوحظ ان إضافة مستخلص أوراق الزيتون بتراكيز 3% أو 5% إلى عينات لحم الغنم أعاقت تكوين الميت مايوكلوبين بمقدار 32.12 و32.61% على التوالي مقارنة مع معاملة المقارنة (51.30%) ومعاملة 1% من مستخلص أوراق الزيتون (47.83%) ومعاملة BHT (36.49%) بعد 60 يوماً من الخزن المجمد.
3. لوحظ بأن معاملة عينات اللحم بمستخلص أوراق الزيتون بتراكيز 3% أو 5% كانت أكثر كفاءة كمضادات أكسدة ومايكروبية طبيعية من مضاد الأكسدة الصناعي (BHT) ومعاملة 1% من مستخلص أوراق الزيتون في أعاقة تكوين النتروجين الكلي المتطاير بعد 60 يوماً من الخزن المجمد مقارنة مع معاملة المقارنة.
4. لوحظ بأن عينات اللحم المعاملة مع 3% أو 5% من مستخلص أوراق الزيتون أظهرت تأثيرات مثبطة للعدد الكلي للبكتيريا والبكتيريا المحبة للبرودة للحم الغنم المعامل بعد 60 يوماً من الخزن المجمد مقارنة مع المعاملات الأخرى.

يمكن الاستنتاج بأن معاملة لحم الغنم بمستخلص أوراق الزيتون بتراكيز 3% أو 5% كانت أكثر كفاءة كمضادات أكسدة ومضادات مايكروبية طبيعية في أعاقة أكسدة الدهون وتكوين النتروجين الكلي المتطاير وثباتية لون وبروتين اللحم مع تثبيط النمو المايكروبي للحم الغنم مقارنة مع مضاد الأكسدة الصناعي (BHT) ومستخلص أوراق الزيتون بتركيز 1% خلال الخزن المجمد لمدة 60 يوماً.

Introduction

Sheep meat proves to be an excellent source of high biological value protein, vitamins B-complex, minerals such as iron, copper, zinc and phosphorus, also a source of a long-chain omega-3 polyunsaturated fatty acids, that are needed for good health throughout life (1). It is difficult to ensure the safety of meat supply to the consumers who many times buy meat which cannot ensure protection from the effects of patentional danger of inferior quality meat as a result of exposure of meat and meat products to the changes in physico-chemical and microbials characteristics occurring during slaughtering processes, processing, handling packaging and storage of various stages of marketing (2). The microbial growth, color changes and oxidative rancidity are the major factors affecting the quality and acceptability of meat and meat products in consumers purchase decisions and expections of satifaction, because they are presumed to be indicators of meat quality and frishness (3, 4, 5). Lipid oxidation is a major cause of meat deterioration, resulting in a variety of break- down products such as malonadehyde and cholesterol products, which produce discoloration, drip losses, off- odours and off-flavor development, texture defects, as well as that are adverse effects on the overall of meat quality (6, 7). Consumers concerns regarding safety and toxicity of synthetic antioxidants increased interest has been directed towards plants based extracts as a source of phenolic antioxidants and antimicrobial (8, 9, 10, 11). Generally, olive leaves have antioxidants and antimicrobials properties due to their phenolic compounds,

particularly, oleuropein, tyrosol and hydroxyl tyrosol (12, 13). Therefore, the main objectives of this study are designed.

1. To investigate the effect of the different concentrations of olive leaf extract and synthetic antioxidant on some of physico-chemical and sensory properties of karadi sheep meat steaks during frozen storage at -18^{o}C for 60 days.
2. To evaluate the effectiveness of olive leaf extract as a source of natural antioxidant and antimicrobial agent to provide protection against oxidative rancidity, colour loss and microbial growth in meat steaks of Karadi sheep during frozen storage at -18^{o}C for 60 days.

Materials and Methods

The present experiment was carried out at the central laboratory of college of Agriculture, University of Baghdad and Faculty of Agricultural Sciences-University of Sulaimania.

- **Collection of olive leaves:** Olive leaves were collected from gardens of college of Agriculture, University of Baghdad. They were collected on jane and properly prepared for drying process in the day they were collected. The leaves were washed to impurities and dried in the air-oven at 40° C for 3days and then ground to pass a 2mm screen to ensure the plant powders.
- **Preparation of olive leaves extract:** 20 gram of each dried olive leaves powder was extracted with 400ml of 70% (v/v) ethanol for 2 hr at 40° C by using shaker. The samples were centrifuged at 5000 rpm for 15 min., ethanol was evaporated by a rotary evaporation. The remaining aqueous solution was dried in air-oven at 40°C and the percent (w/w) extraction yields of plant materials were calculated. The crude extracts were kept in refrigerator in glass bottle until use in the formulation (8).
- **Preparation of meat samples:** The leg cuts were removed for Karadi lamb carcasses, which were slaughtered at about more than one year old. These cuts were obtained from local primary butchery in Kurdistan region, then carried to the laboratory in cold chain, after 24 hr. of chilled storage at 4°C. The leg cuts were separated into lean meat,fat and bone. The external fat and heavy connective tissues were trimmed off from lean meat streaks of leg cuts. The outer surfaces of streak were removed to avoid possible contamination before cutting into approximately 6×2×2 cm cubes.
- **Experimental treatments:** The meat cubes were randomly divided into five batches (2 kg each batch) as control and treated samples: control samples were immersed into distilled water and three treatments were immersed into solutions containing 1%, 3% and 5% olive leaf extract (OLE) (w/v), in 1:1 ratio (meat: distilled water), the fourth treatment was immersed into 95% ethanol solution containing 0.01% butylated hydroxyl toluene (BHT) (w/v) in 1:1 ratio (meat: ethanol solution) for 20 hr at 4°C. After treatment, the samples were drained and divided into portions. The portions were placed on plastic foam meat trays, packed into polyethylene film and wrapped aluminum foil and kept in a freezer at -18° C for 60 days, and evaluated for physical, chemical, microbial and sensory evaluation at 0, 30 and 60 days of storage time.
- **Lipid Oxidation measurement in meat:**
- **Thiobarbituric acid (TBA) value:** The TBA values were determined according to the method described by Witte *et al*., (14). Twenty grams of the meat were blended with 50 ml of cold solution containing 20% trichloroacetic acid in 2 M Phosphoric acid. The resulting slurry was then transferred quantitatively to a 100 ml volumetric flask with distilled water and homogenized by shaking. A 50 ml portion was filtered through whatman NO.1 filter paper. 5 ml of filtrate was transferred to a test tube

followed by 5 ml of fresh thiobarbituric acid (0.005 M in distilled water). The blank prepared by mixture 5 ml of distilled water with 5 ml of TBA. The tubes were stopped and the solution was mixed and kept in the dark for 15-17 hr at room temperature to develop the color reaction. the resulting color was measured in spectrophotometer (Model 11 4050, LKB Biochrom, UVlUIS, Germany) at 530 nm. The TBA value was expressed as mg malonaldehyde (MDA)/ kg meat, which was calculated by multiplying the absorbance by 5.2 factor as follows.

$$\text{TBA value (mg MDA/kg meat)} = A530 \times 5.2$$

- **Determination of percent metmyoglobin (Met-Mb):** The percent metmyoglobin (Met-Mb) formation of each treatment was measured at 0, 30 and 60 days of frozen storage according to the modified procedure of Lee *et al*., (15). Meat sample (5g) was blended with 25 ml ice-cold phosphate buffer (PH 6.8, 40 Mm) for 10 s in a magnetic stirrer. The mixture was allowed to stand for 30min at 4° C (Model T.J6 Beckman, England). The supernatant was further clarified by filtration through What man NO.1 filter paper. The absorbance of filtrate was read at 700, 572 and 525nm with UV-VIS spectrophotometer (Model UV-2100 U, Shimadzu co. Koyto, Japan). The percent metmyoglobin (Met-Mb) was determined using the formula by Krzywicki (16). $\text{Met-Mb (\%)} = ((1.395-(A575-A700 / (A525-A700)) \times 100$

A: Absorbance (nm).

- **Total Volatile Nitrogen (T.V.N):** Total Volatile nitrogen was determined according to described method of Egan *et al*.,(17). A 100 g of the meat sample was mixed with 300 ml of 5% trichloroacetic acid (TCA) in the blender, the mixture was filtered through Whatman NO.1 filter paper, 5 ml of the filtrate were transferred to macro-kjeldahl distillation apparatus, then 5 ml of 0.2 M of NAOH were added to the distillation, which was carried, and the distillate was collected in 15% of 4% boric acid. The distillate was titrated with 0.01N of HCL, using methyl red-bromocresol green as indicator. The blank carring out using 5ml of 5% TCA instead of the meat sample. The TVN was estimated as follows.

$$\text{T.V.N (mg N/100g meat)} = \frac{\text{ml of 0.01N HCL} \times 14(300+M/100) \times 100}{500}$$

Where: M = moisture content

- **Microbiological analysis:** Microbiological examination of total plate count (TPC) and Psychotrophic bacteria count (PSY) and total coliform count (TCC) were carried out by American Public Health Association (18).
- **Serial dilution**: 11 g of each sample was homogenized with 99 ml of 0.1% sterile peptone water in a seward Stomacher (Waring-torringtion ct. USA) for 2 min to a 1:9 dilution (W/V). One ml of the original homogenate was transferred serially into test tubes containing 9 ml of 0.1% sterile peptone water to final dilution of 10-7. Plate pour method was used in the following tests.
- **Total plate count (TPC):** One ml from each dilution was plated in duplicates, using nutrient agar medium. The inoculated plates were incubated at 37° C for 4 hrs.
- **Psychrotrophic bacteria count (PSY):** Serial dilutions were prepared as above, using nutrient agar medium for plating. The inoculated plates were incubated at 7°C for 10 days.
- **Statistical analysis:** The data were analyzed using Statistical Analysis System (19). General Linear Model (GLM) procedure was used to evaluate treatment and storage period as fixed effects. Duncan's multiple range test (20) was used to determine significant differences among means within each factor on all studied traits.

Results and Discussions

- **Lipid oxidation in meat samples:** Changes in thiobarbituric acid (TBA) values of control and treated meat samples stored at -18^{o}C for 60 days are given in Fig. (1). The results showed that treatments and storage period had significant effects on TBA values ($P<0.05$). During storage at -18^{o}C, TBA values in control samples started to increase from 0.68 (day 0) to 1.36 (day 30) and increased rapidly after 60 days of storage at -18^{o}C to reach 3.66 mg malonaldehyde (MDA)/ kg meat. While, meat samples treated with 3% and 5% OLE had lower TBA values (1.32 and 1.61 mg/ MDA/kg meat) respectively, followed by synthetic antioxidant (BHT) and 1% Ole treatments, which were 1.98 and 2.23 mg MDA/kg meat respectively after 60 days of frozen storage compared with control samples (Fig. 1). The TBA values in all treatments increased during frozen storage due to the lipid oxidation. Although treatment with 5% OLE resulted in retarding oxidative process of meat samples by 57%, the best result against lipid oxidation was obtained from meat samples treated with 3% OLE, this treatment retarded lipid oxidation by 66% after 60 days of frozen storage in comparison with control samples and other treatments. It was observed that meat samples treated with 3% and 5% OLE kept TBA values under consumption level of 2 mg MDA/kg meat after 60 days of frozen storage. TBA values are considered as an index of lipid oxidation in meat products during storage (21). Therefore, TBA values in these treatments consideration are accepted as good quality. Verme and Sahoo (22) indicated that if the TBA value increased more than 2 mg MDA/kg meat as a threshold value for oxidative rancidity in meat products during storage, While control samples and 1% OLE treatment exceeded TBA value more than 2 mg MDA/kg meat in 60 days during storage. This result may be attributed to the amount of hydroxyl groups within the phenolic structures of constituents present in olive leaf extract mainly oleuropein and hydroxyl tyrosol. It is assumed that inhibition of lipid oxidation and hydrogen donor ability is enhanced with the increasing amount of hydroxyl groups (12). Phenolic compounds possessing at least two hydroxyl groups are considered as iron binding and reducing properties (23). The amount of oxidation in meat samples treated with 1% OLE was considerably higher than that meat samples treated with 3% and 5% OLE throughout the frozen storage. Higher level of oxidation in 1% OLE samples may be explained by the considerably lower concentration of antioxidant material within samples. Phenolics in 1% OLE may be enough to neutralize metal ions to some point. However, it may also reduce ions such as Fe (III) to their most active pro-oxidative state as Fe (II) (23). This consideration indicated that meat samples treated with 3% and 5% OLE treatments gave better results than 1% OLE treatment in oxidative stability of lamb meat steaks during frozen storage. In the current study, data of TBA values indicated that meat samples with olive leaf extract showed a good antioxidant properties. Skerget *et al*.,(8) and Lee and Lee (10) reported that phenolic compounds within olive leaf extract have antioxidative activity against lipid oxidation. Also, Gok and Bor (24) demonstrated that direct addition of olive leaf extract at concentrations 500 and 1000 ppm to beef meatballs stored at 4^{o}C for 12 days resulted in a significant decrease in TBA values than the control samples. They showed that higher antioxidant activities were recorded with addition of higher amount of antioxidant substances. Similarly, Hayes *et al*., (25) reported lower TBA values on the beef patties with 200 ug/ g olive leaf extract. Strong protective effect of phenolic compounds of olive leaf extract against lipid oxidation in meat products was indicated by Paiva- Martins *et al*., (26). Moreover, the polyphenolic extracts are excellent electron and proton donors. These substances could cause an inhibition of

the chain reactions during lipid oxidation (27). It can be concluded that the positive effect of olive leaf extract proves much more effective as a source natural antioxidant than synthetic antioxidant (BHT) in suppressing lipid oxidation and extended shelf- life of meat products during frozen storage, which may have implications for meat processors, that face quality optimization problems for their products.

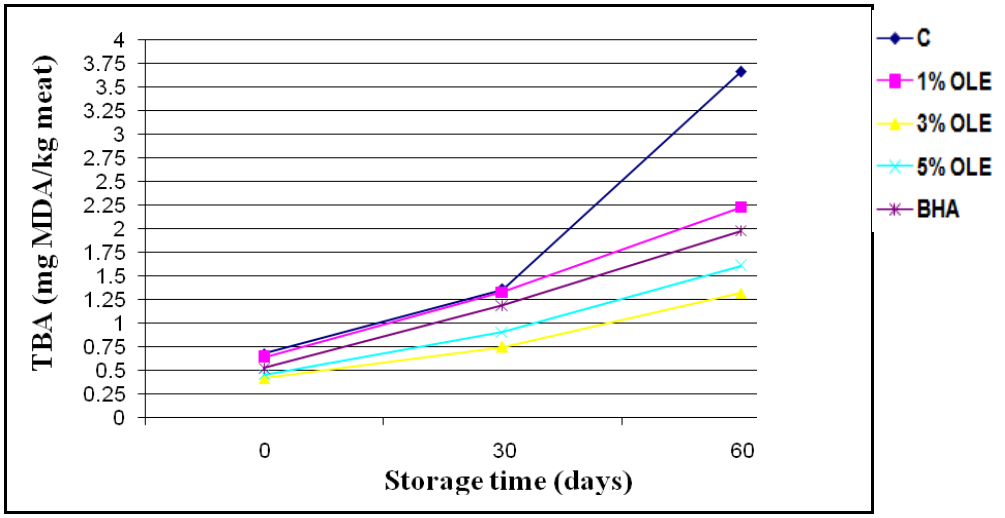

Fig. (1) Changes in thiobarbituric acid (TBA) values (mg MDA/kg meat) of sheep meat steaks treated with different concentrations of olive leaf extract and synthetic antioxidant (BHT) during storage at -18^{o}C for 60 days

- **Metmyoglobin formation:** Changes in metmyoglobin (Met-Mb) formation for meat samples treated with different concentrations of olive leaf extract (OLE) and synthetic antioxidant (BHT) stored at -18^{o}C for 60 days are presented in Fig. (2). The treatment and storage period had significant effects on metmyoglobin (Met-Mb) percentages ($P<0.05$). The result in Fig. (2) showed that the percentages of Met-Mb in control samples at the initial of the storage period was 34.69% then increased ($P<0.05$) rapidly to 51.30% after 60 days of the frozen storage. In control samples myoglobin oxidation quickly turns oxymyoglobin to metmyoglobin producing an undesirable brown colour as the storage progresses. This result confirms the previous studies, which have postulated that in meat and meat products pigment oxidation during storage (28, 29). Genot *et al*., (30) concluded that O2 can initiate lipid oxidation, leading to the formation of pro-oxidant substances capable of reacting with oxymyoglobin and resulting in Metmyoglobin formation. Also, Renerre *et al*., (31) reported that the susceptibility of myoglobin to autoxidation is the main factor in explaining colour stability in meat and meat products during storage. On the other hand, addition of olive leaf extract at concentrations 3% and 5% OLE to meat samples retarded ($P<0.05$) metmyoglobin formation from 25.19 to 32.12% and from 25.88 to 32.61% from day 0 to day 60 of the frozen storage respectively as compared with control samples and other treatments. It was observed that metmyoglobin percentage in meat samples treated with synthetic antioxidant (BHT) declined to 36.49% at the end of the frozen storage period (Fig. 2). While, meat samples treated with 1% OLE had the highest of metmyoglobin percentage was 47.83% after 60 days of the frozen storage as compared with treated samples. Probably, this increased in metmyoglobin formation for 1% OLE treatment associated with the lowest concentration of antioxidant material within samples. Phenolics compound in 1% OLE treatment may not be enough to retard myoglobin oxidation and reducing metal iron as pro-oxidant substance (23). In general, all

samples, met-Mb formation increased with storage progresses (P<0.05). Ours results exhibited that meat samples treated with 3% and 5% OLE and their main phenolic compounds, oleuropein and hydroxytyrosol as a netural antioxidant were more effective in retarding of metmyoglobin formation and lipid oxidation than the control or BHT or 1% OLE samples after 60 days of the frozen storage at -18°C. It is possible due to the ability olive leaf extract at these concentrations as antioxidant by chelating metal ions and free radicals scavenging by reacting with free radical produced from oxidized lipid and decreases the formation of lipid peroxy free radicals and thus lipid oxidation, and that olive leaf extract protects myoglobin from oxidation to metomyoglobin and restricts disruption of cell membranes pf meat from peroxy free radicals, Hence, addition natural antioxidants to meat and meat products directly prevents lipid oxidation in the membranes of meat and indirectly pigment oxidation in the sarcoplasm of meat (32, 24). Similarly, Keokamnerd *et al*., (33) stated that the degree of redness loss was greater for the control samples than it was for the meat with added antioxidant- rich phenolic compounds. The meat colour stability with storage is most likely due to the further meat pH ultimatefrom isoelectric point that provides some protection against oxidation during storage (34). It can be concluded that olive leaf extract proves effective as a natural antioxidant to improve meat color and lipid stability and extended shelf-life of lamb meat steaks during frozen storage.

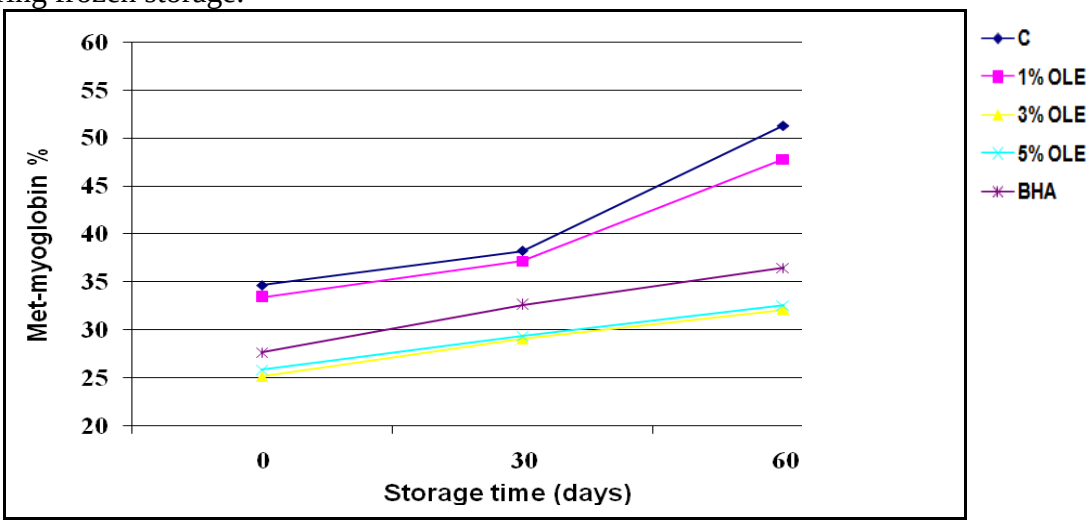

Fig. (2) Effect of different concentration of olive leaf extract and synthetic antioxidant (BHT) on met-myoglobin percentage of sheep meat steaks stored at -18°C for 60 days

- **Total volatile nitrogen (TVN):** The total volatile nitrogen could be used as quality indicator of meat deterioration and to determine of spoilage of meat during storage period (35). Changes in TVN values of control and treated meat samples stored at -18°C for 60 days are shown in Table (1). The results showed that treatment and storage period had significant effects on TVN values (P<0.05). The results in Table (1) indicated that the initial (day 0) of TVN values ranged from 3.51 to 5.35 mg N/ 100 g meat for all treated and untreated meat samples. The results indicated that, as the storage period at -18°C increased, the TVN values increased as shown in Table (1), for all treated meat samples with different rates depending on the nature of treatment, and in particular in control samples reaching the higher TVN value of 17.36 mg N/ 100 g meat after 60 days of frozen storage. While, at the end of the storage period (day 60), meat samples treated with 3% and 5% OLE resulted in significantly lower (P<0.05) TVN values, which were 11.21 and 11.00 mg N/ 100 g

meat respectively, followed by BHT treatment (14.50 mg N/ 100 g meat) then 1% OLE treatment (15.27 mg N/ 100 g meat). When compared to the control, No significant difference between meat samples treated with 3% and 5% OLE at any of the storage period. It was observed from results in Table (1) that meat samples treated with olive leaf extract at concentrations 3% and 5% were more effective as a natural antioxidant and antimicrobial than synthetic antioxidant (BHT) and 1% OLE treatment in retarding TVN formation during storage. This may be due to the role of olive leaf extract as an antioxidation and antimicrobial resulting from their active phenolic compounds and their protective action, which involved in meat protein stability, diminished activity of microbial and proteolytic enzymes during storage (36, 13). In general, TVN values in all meat samples increased as storage period was progresses. This may be attributed to the degradation of protein as a result of the activity of microbial and proteolysis enzymes that contribute to increase accumulation of the free nitrogen groups that might lead to higher TVN values (37). The results confirmed those of Baker (38) and Al-Dhaheri (39), who showed that addition of rosemary extract or water extract of origanum to lamb meat or minced beef resulted in decreasing of TVN values during frozen storage.

Table (1) Total volatile nitrogen (TVN) values of sheep meat steaks treated with different concentrations of olive leaf extract and synthetic compound (BHT) during storage at -18^{o}C for 60 days. (Mean ± S.E).

Treatment	Total volatile nitrogen (mg N / 100 meat)		
	Storage time (day)		
	0	30	60
C	5.35 ± 0.01 a C	10.56 ± 0.05 a B	17.36 ± 0.02 a A
1% OLE	5.19 ± 0.03 a C	10.50 ± 0.02 a B	15.27 ± 0.02 c A
3% OLE	3.55 ± 0.03 c C	6.29 ± 0.01 c B	11.21 ± 0.01 d A
5% OLE	3.51 ± 0.01 c C	6.09 ± 0.03 c B	11.00 ± 0.02 d A
BHT	4.91 ± 0.01 b C	10.10 ± 0.04 b B	14.50 ± 0.02 b A

Means having different small letters among treatments within same row, which, having different capital letters among storage periods within same column are significant different (P<0.05).

- **Microbial changes:**
- **Total plate count:** Fig. (3) show the effects of different concentrations of olive leaf extract and synthetic antioxidant (BHT) on total plate count (TPC), Psychrotrophic bacteria count (PSY) and total coliform count (TCC) of sheep meat steaks stored at -18^{o}C for 60 days. Results indicated that microbial counts increased (P<0.05) with time during the storage period (Fig. 3). Whereas, at the beginning of the storage total plate count (TPC) of control samples was 4.72 log CFU/g, it increased 2.16 logarithmic unit after 60 days and reached to 6.88 log CFU/g (Figure 3). This value was lower than the spoilage indicator limit of meat and meat products, which is 7.0 log CFU/g (40). At day 60 of frozen storage, higher decrease (P<0.05) in TPC was observed for meat samples treated with 5% OLE (1.85 log CFU/g) and 3%OLE as 1.70 log CFU/g as compared with the control samples. At this day of storage, levels of TPC did not differ (P>0.05) among each of BHT or 1% OLE and control treatment (Fig. 3). In general, a significant decrease was noticed for meat samples treated with olive leaf extract at high concentrations (3% or 5% OLE) in their TPC during storage at -18^{o}C for 60 days. Similar results were reported by Gok and Bor

(24) who found that addition 1000 ppm of olive leaf extract to beef meat balls had the highest antimicrobial effect (P<0.05) against aerobic bacteria count during cold storage for 10 days as compared with the meat samples treated with 500 ppm OLE and untreated meat samples (control). Also, Markin *et al.*, (9) concluded that the olive leaf extract had the highest protective activity against microbes, which points to the longest shelf-life of the product examined.

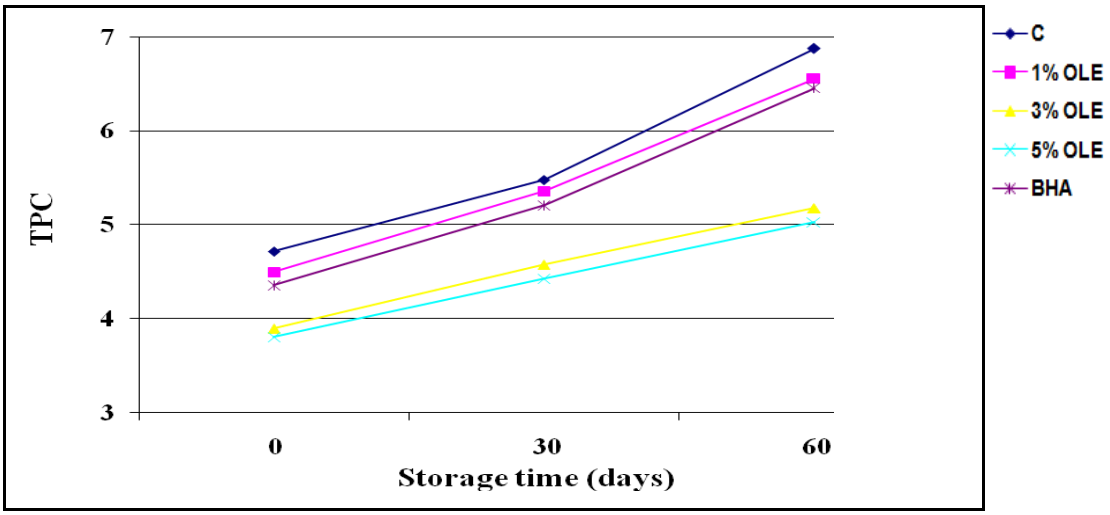

Fig. (3) Effect of different concentration of olive leaf extract and synthetic antioxidant (BHT) on changes in total plate count (TPC) of sheep meat steaks stored at -18°C for 60 days

- **Psychrotropic bacteria count:** The results in Fig. (4) indicated the changes in Psychrotrophic bacteria count (PsY) among treatments during storage at -18°C for 60 days. In control samples results revealed that there was a significant (P<0.05) increase with increasing storage period up to 60 days from their initial values in PsY (3.72 vs 5.62 log CFU/g), this result indicated that PsY value in control samples increased 1.90 logarithmic unit after 60 days of storage period. While, meat samples treated with 3% or 5% OLE showed a little changes from initial values in PsY counts of the same storage period under similar conditions. It was observed that bacterial populations in the treated meat samples with 3% or 5% OLE were lower than that in control by 1.21 and 1.29 log CFU/g respectively for psychrotrophic bacteria counts (PsY) after 60 days of frozen storage (Fig. 4). This result indicated that meat samples treated with 3% or 5% OLE had higher inhibitory effect against Psychrotrophic bacteria count (PsY) during frozen storage for 60 days. Therefore, two percent OLE treatment can be used for controlling the microbial load of sheep steaks during 60 days of storage at -18°C. To take into consideration that Psychrophiles bacteria groups, one of the most of microbes, may cause the spoilage of meat and meat products (41). Previous reports indicated that olive leaf extract and its phenolic compounds have an inhibitory effect against Pyschrotrophic bacteria (9, 42). The results in Fig. (4) showed that the control samples, BHT treatment and 1% OLE treatment presented Psy counts did not have significant differences among other during the whole storage period for 60 days.

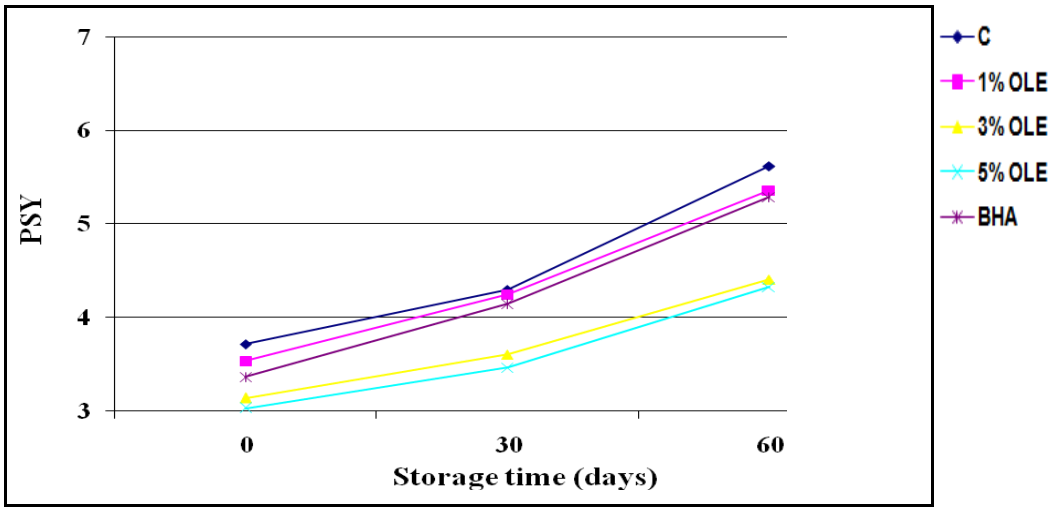

Fig. (4) Effect of different concentration of olive leaf extract and synthetic antioxidant (BHT) on Psychrotrophic bacteria count (PSY) of sheep meat steaks stored at -18°C for 60 days

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