STUDY THE EFFECTS OF OLIVE LEAF EXTRACT ON PHYSIO-CHEMICAL AND SENSORY TRAITS OF MUTTON MEAT AT LOW TEMPERATURE

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ABSTRACT

Received at: 19/7/2014

Accepted: 27/9/2014

This study was conducted to evaluate the use of olive leaf extract in two concentrations (2, 4%) by immersion and spray to study physic-chemical quality of mutton lamb storedat low 4 ºC for seven days. For that, an Olive leaf extract (OLE) solution was prepared at the concentration of 2% and 4% weight/volume (W/V). Meat sample were dipped and sprayed with pre-chilled olive leaf extract up to 24 hr (T2: 2% spraying, T3: 2% immersion, T4: 4% spraying, T5: 4%immersion). The samples were then stored under refrigerated conditions (4.0°C). For water holding capacity, after 7 days of storage, T1 and T2 differs significantly among other treatments, but T3,T4 and T5 showed that no differences between them, the highest percentage of water holding capacity (WHC) recorded in T5 (39.950%) while the lowest percentage recorded in T1(37.00%).At 7 days of storage, T1 differs significantly (P≤0.05) among T3, T4 and T5, while T2, T3, T4 and T5 results showed no differences between them, the highest percentage of cooking loss recorded in T1 (45.855%) while the lowest percentage recorded in T4 (42.605%). After 7 days of storage, results showed that T1 differ significantly ($p \leq 0.05$) among T2, T3, T4 and T5, while T2, T3, T4 and T5 not differs significantly between them. The highest value recorded in T1 which recorded (2.115 malonaldehyde/ kg) while the lowest value recorded in T5 (0.893 malonaldehyde/ kg). After 7 days of storage in refrigeration, results showed that no significant differences (P>0.05) among treatments. In conclusion, olive leaf extract can be used as natural antioxidants, which may have implications for meat processors, that face quality optimization problems for their products.

Key words: *Olive leaf Extract, Mutton meat, Low temperature.*

INTRODUCTION

Mutton meat proved 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 were needed for good health throughout life (Lawrie, 2002). It was difficult to ensure the safety of meat supply to the consumers who many times buy meat which could not ensure protection from the effects of attentional danger of inferior quality meat as a result of exposure of meat and meat products to the changes in physico-chemical and microbial characteristics occurring during slaughtering processes, processing, handling packaging and storage of various stages of marketing (Arain *et al.*, 2010).

The olive was the fruit of an evergreen olive tree that grews in the temperate climate of the Mediterranean region (Soni *et al.*, 2006). Olive was one of the most importion fruit trees in these regional countries, where they cover eight million hectare, counted as 98% of the world crop (Pereira *et al.*, 2007). Historical records indicated that the olive tree was first cultivated in ancient Crete as early as 3500 BC. It has been used as a representation of abundance, peace and glory. Its leafy branches were used to crown rules of vast lands and champions of either friendly games or bloody wars (Soni *et al.*, 2006).

Low cost phenolic extracts could be obtained from commercially available olive mill waster water (OMWW) to be used as altermatives to synthetic antioxidants as butylatedhydroxyanisole (BHA) and

butylatedhydroxy toluene (BHT). Furthermore, hydroxytyrosol derived from OMWW can be used to stabilize edible oils (Fki *et al.*, 2005).

Hayes *et al.* (2010) reported that OLE at a concentration of 100 and 200 ug / g muscle had consistently lower levels of lipid oxidation compared to control in both aerobic and modified atmosphere pack conditions.

Therefore, the objectives of this study was to evaluate the use of olive leaf extract in two concentration (2, 4%) and in two methods (immersion and spray) on some physic-chemical quality of red lamb meat under refrigeration for seven days.

MATERIALS and METHODS

Fresh meat lamb purchased from a local market. Olive leaf extract (OLE) solutions prepared at the concentration of 2% and 4% v/v. Meat sample were dipped and sprayed with pre-chilled SL up to 24 hr (T2: 2% spraying, T3: 2% immersion, T4: 4% spraying, T5: 4%immersion). The samples were then stored under refrigerated conditions (4.0°C).

Four replicate samples taken for chemical analyseson 0, 2, 5and7days of the storage time.

Analysis:
Water holding capacity (WHC) was measured according to Wardlaw *et al*. (1973), A meat sample (8 g) and 12 ml of 0.6 M NaCl solution were put into a tube. The tubes were placed into a water bath (5 °C) for 15 min. Then, the tubes were centrifuged at 4100 rpm (5°C) for 15 min. The tubes were poured into a volumetric cylinder in order to collect the separated fluid. The WHC was calculated using the volume of separated fluid (ml)/ 100g meat.

Cooking loss was measured according to Cyril *et al*. (1996), Twenty gram of lamb meat samples were placed in open aluminum boxes and cooked for 15 minute in the oven, pre-heated to 200 C°, after cooking, the samples were dried with a paper towel (cooled for 30 min to 15 C°). Total cooking loss was estimated on each sample as percentage ratio between cooked and raw weight.

Thiobarbituric acid (TBA) value analysis was analyzed according to Tarladgis *et al*. (1960) as adopted by Witte *et al*. (1970), TBA values were expressed as mg malonaldehyde/ kg, A twenty gram of meat was blended with 50 ml of the extracting solution containing 20% Trichloroacitic acid (TCA) in 2 M phosphoric acid. The sample was diluted to 100 ml with distilled water and homogenized by shaking. 50 ml portion was filtered through whatman No. 1 filter paper, 5 ml of filtrate was transferred to the test tube followed by 5 ml Thiobarbituric acid (0.005 M in distilled water). The tube was stoppered and the solution mixed by inversion and kept in the dark place for 15-17 hour at room temperature. The resulting color was measured at 530 nm using UV spectrophotometer (Shimdzu, Japan). TBA values were calculated by multiplying absorbance value of sample by 5.2, the TBA values were calculated as mg MDA /kg meat.

FFA was analyzed as method described by Egan *et al*. (1981), A 100 gm of homogenized with 250 ml of chloroform, blend the mixture for 2-3 min and filter it immediately through a large filter paper. Then re-filter it through a paper containing a small amount of anhydrous sodium sulphate, twenty five ml of 95% ethanol neutralized with drops of 0.1 N NaOH after adding phenolphthalein. The solution was added to 25 ml of the filtered above and the mixture tittered with 0.1 N NaOH until the pink colour persists for 15 seconds. The F.F.A. calculates as oleic acid as percentage of the sample.

Statistical analysis:
The SAS program (SAS, 1989) for Windows was used to study factors examined (treatment and period) in traits. Duncan multiple ranges used to significantly compare between means ($p< 0.05$) (Steel *et al.*, 1996).

RESULTS

Water holding capacity (WHC)
The results of water holding capacity (WHC) percentage were showed in table 1, results showed there were no significant differences between among treatment at 0 day of treatments. After 2 days of treatment, T1(control) differ significantly ($P\leq0.05$) among T3(2% immersion), T4 (4% spray) and T5 (4% immersion), also T2 (2% spray) differ significantly among T4 and T5, but T3,T4 and T5 results showed no differences between them, the highest percentage of WHC recorded in T1 (49.100%) while the lowest percentage recorded in T5 (47.050%).

T1 differ significantly among T2,T3,T4 and T5 at 5 days of storage at refrigeration,T2 differ significantly among T3 and T5, also results showed that T3, T4 and T5 no differences between them the highest percentage of WHC recorded in T5(43.400%) while the lowest percentage recorded in T1 (39.950%).

After 7 day of storage, T1 and T2 differ significantly among other treatments, but T3,T4 and T5 showed that no differences between them, the highest percentage of WHC recorded in T5(39.950%) while the lowest percentage recorded in T1(37.00%).

When compared WHC percentages of same treatment in different periods, results of all treatments showed that percentage of WHC at day 7 differ significantly among WHC percentage at day 5,2 and 0.

Cooking loss (CL)

For cooking loss (CL) percentage (table, 2), at the same period, results showed there were no significant differences (P>0.05) between treatments before treated (0 day) with olive leaf extract. After2 days of storage, results showed that T3 was significantly (P≤0.05) different than T5 and T4 treatments, also T4 differ significantly among T2 and T5, meat samples were treated with 2% of olive leaf extract by immersion (T3) had higher CL percentage (40.720%) while the lowest percentage of CL recorded in T4 (35.290%). After 5 day, results showed there were no significant differences (P>0.05) in CL among treatments. At 7 days of storage, T1 differ significantly (P≤0.05) among T3, T4 and T5, while T2, T3, T4 and T5 results showed no differences between them, the highest percentage of cooking loss recorded in T1 (45.855%) while the lowest percentage recorded in T4 (42.605%). When compared cooking loss percentages of same treatment in different period (table, 2), results showed there were significant differences in CL percentage for all treatments.

Thiobarbituric acid value:

Effects of different concentration of sodium lactate (SL) olive leaf extract on Thiobarbituric acid values (TBA) of fresh meat sample during storage at 4^{o}C for 7 days are presented at table (3).

Results showed there were no significant (P>0.05) differences among treatments at 0 day.

After 2 days of storage, results showed that T1 significant differences ($p \leq 0.05$) among T5,T2 and T3. Results of T4 significantly differ among T3, T2 and T1, the highest value recorded in T1 (0.593 malonaldehyde/ kg) while the lowest value recorded (0.375 malonaldehyde/ kg).

At 5 day of storage at refrigeration, results showed that T5 differ significantly ($p \leq 0.05$) among T1 and T2. T1 results not differ among T2,T3 and T4, the highest value recorded in T2 (0.732 malonaldehyde/ kg) while the lowest value recorded in T5 (0.560 malonaldehyde/ kg).

After 7 day of storage, results showed that T1 differ significantly ($p \leq 0.05$) among T2, T3, T4 and T5, while T2, T3, T4 and T5 not differ significantly between them. The highest value recorded in T1 which recorded (2.115 malonaldehyde/ kg) while the lowest value recorded in T5 (0.893 malonaldehyde/ kg).

When compared TBA values of same treatment in different periods, results showed that TBA values increase as storage period increase, results of 7 days of storage recorded highest value as compared to other periods.

Free fatty acids value (FFA)

When compared among treatments at the same periods, results of free fatty acids (FFA) percentage are showed (Table, 4) no significant differences (P>0.05) among treatments at 0 day (before treatment with olive leaf extract).

After 2 day of storage, results showed that T1 differ significantly among other treatment, while there were no significant differences among other treatments, the highest value recorded in T1 (0.605 %) as compared to lowest value of FFA which recorded in T5 (0.280 %).

After 5 days of storage, results showed that T1 differ significantly among other treatment, while there were no significant differences among other treatments, the highest value recorded in T1 (0.861%) while the lowest value recorded in T5(0.445 %).

After 7 days of storage in refrigeration, results showed that no significant differences (P>0.05) among treatments. When compared FFA values of same treatment in different periods, results showed that FFA values increase as storage period increase, results of 7 days of storage recorded highest value as compared to other periods.

Table 1: Effect of different concentrations of olive leaf extract on water holding capacity (WHC) percentages of fresh meat sample during storage at 4°C for 7 days. (mean ± S.D.)

Treat			Storage periods (days)			
			0	2	5	7
Control		T1	49.655±0.502 aA	49.100± 0.141 aA	39.950± 0.354 a B	37.00± 0.141 a C
2%	spray	T2	49.350± 0.495 aA	48.750± 0.778 ab A	41.850± 0.919 b B	38.800± 0.424 b C
	Emersion	T3	49.350± 0.212 aA	48.050± 0.354 bc B	43.050± 0.212 c C	39.450± 0.071 bc D
4%	spray	T4	49.300± 0.566 aA	47.600±0.707 c B	42.450± 0.495 bc C	39.750± 0.212 bc D
	Emersion	T5	48.700± 0.0.707 aA	47.050± 0.212 c B	43.400± 0.141 c C	39.950±0.212 c D

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.05$).

Table 2: Effect of different concentrations of olive leaf extract on cooking loss (CL) percentages of fresh meat sample during storage at 4°C for 7 days. (mean ± S.D.)

Treat			Storage periods (days)			
			0	2	5	7
Control		T1	32.620± 0.113 aA	38.365± 1.973 ac B	40.215± 1.082 a B	45.885± 0.615 a C
2%	spray	T2	32.160± 1.471 aA	39.525± 1.534 ac B	40.840± 2.418 a BC	43.565± 0.021 ab C
	Emersion	T3	32.695± 0.276 aA	40.720± 0.849 a B	41.860± 0.820 a B	42.960± 0.269 b B
4%	spray	T4	33.060± 0.679 aA	35.290± 1.824 b A	40.420± 1.838 a B	42.605± 0.106 b B
	Emersion	T5	33.195± 0.643 aA	37.810 ± 1.315 c A	42.405± 1.450 a C	42.780±0.283 b C

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.05$).

Table 3: Effects of different concentrations of olive leaf extract on Thiobarbituric acid values of fresh meat sample during storage at 4°C for 7 days. (mean ± S.D.)

Treat			Storage periods (days)			
			0	2	5	7
control		T1	0.251± 0.148 aA	0.593± 0.081 a B	0.713± 0.006 a B	2.115± 0.120 a C
2%	spray	T2	0.228± 0.042 aA	p0.563± 0.028 a B	0.732 ± 0.026 a C	1.025± 0.035 b D
	Emersion	T3	0.309± 0.017 aA	0.569± 0.026 a B	0.673± 0.014 ab B	0.948± 0.055 b C
4%	spray	T4	0.292± 0.018 aA	0.501±0.059 ab A	0.642± 0.059 ab B	0.913± 0.017 b C
	Emersion	T5	0.275± 0.109 aA	0.375 ± 0.031 b A	0.560± 0.033 b B	0.893±0.003 b C

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.05$).

Table 4: Effects of different concentrations of olive leaf extract on free fatty acid values of fresh meat sample during storage at 4°C for 7 days. (mean ± S.D.)

Treat			Storage periods (days)			
			0	2	5	7
control		T1	0.205± 0.000 aA	0.605± 0.148 a B	0.861± 0.070 a C	1.025± 0.021 a D
2%	spray	T2	0.220± 0.028 aA	0.370± 0.099 b B	0.565± 0.064 b C	0.986± 0.008 a C
	Emersion	T3	0.195± 0.007 aA	0.345 ± 0.007 b A	0.595± 0.092 b B	0.973±0.004 ab C
4%	spray	T4	0.195± 0.007 aA	0.310±0.014 b A	0.660± 0.014 b B	0.985± 0.007 a C
	Emersion	T5	0.205± 0.007 aA	0.280± 0.028 b A	0.445± 0.007 c B	0.955± 0.021 ab D

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.05$).

DISCUSSION

The determination of the WHC and cooking loss allows conclusions to be drawn about the degree of denaturation of the proteins and therefore the quality of the meat (Skipnes *et al*., 2007).

The highest percentage of WHC recorded in T5 (4% immersion), followed by T4(4% spray), T3 (2% immersion), T2 (2% spray) while the lowest percentage recorded in T1(control).

It is possible due to the role of olive leaves extract with concentration 2 and 4% as natural antioxidant may enough to bind water and increasing water holding capacity which led to increase ability of meat tissues to retain water and decreasing moisture loss during storage and cooking (Arora, 2000).

Mitsamato *et al.* (1995) found that phenlic compounds in plant extracts stabilized cell integrity and enhanced the ability of meat tissue to retain sarcoplasmic components, which resulted in less drip and more weight retention during storage.

It was observed that meat samples treated with olive leaves extract in low concentration (2%) had lower WHC which might be because of protein lose their buffering capacity as the distance from isoelectric point increases (Offer and Trinick, 1983). Increase in pH value might be due to the large number of hydrophilic sites on meat protein, resulting in more binding of water molecules through hydrogen and ionic bonding to the hydrophilic sites of polypeptides (Hamm, 1977).

Results of cooking loss in our study showed that increase in all treatments after increase of storage, and cooking loss percentage is higher in T1(control) than other treatments, Wang (2000) observed that the cooking loss increased with extending storage period, and AL -Haju (2005) who recorded the increase in cooking loss associated with advancing in storage period. The moisture loss through cooking was mentioned to be associated with weight loss which leads to loss of meat juice or drips, water evaporation, evaporation of volatile materials, some nutritious elements loss, extracting of meat juice due to cooking shrinkage and loss of water soluble nutritional elements (Gorge, 2000).

TBA values are considered as an indicator of lipid oxidation in meat products during storage (Raharjo and Sofos, 1993). Therefore, TBA values in these treatments consideration are accepted as good quality. Verma and Sahoo (2000) 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 exceeded TBA value more than 2 mg MDA/kg meat in 7 days during storage. This result may be attributed to the amount of hydroxyl groups within the phenolic structures of constituents present in olive leaves 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 (McDonald *et al.*, 2001). Phenolic compounds possessing at least two hydroxyl groups are considered as iron binding and reducing properties (Keceli and Gordon, 2002).

Skerget *et al.* (2005) and Lee and Lee (2010) reported that phenolic compounds within olive leaves extract have ant oxidative activity against lipid oxidation. Also, Gok and Bar (2012) demonstrated that direct addition of olive leaves 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.

The highest FFA value recorded in T1 (1.025%) and the lowest value recorded in T5 (0.955%).

Accumulation of FFA does not in itself affect quality attributes of the product but have been shown to interrelate with lipid oxidation and have been proposed to have a pro-oxidant effect on lipids (Rodriguez *et al*., 2006). Therefore, the higher value of FFA is possibly due to the action of lipolytic enzymes on lipid from higher bacterial count leading to increase in the release of free fatty acids, which contribute positively to the generation of undesirable aroma and flavor (Al-Sherick, 2005).

CONCLUSION

It can be concluded that the positive effect of olive leaves extract proves much more effective as a source of natural antioxidant, which may have implications for meat processors, that face quality optimization problems for their products.

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دراسة تاثير استخدام مستخلص اوراق الزيتون على بعض الصفات الحسية والفيزيائية والكيميائية على لحوم الحملان المخزونة بدرجة حرارة التبريد

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اجريت هذه الدراسة لغرض تقييم تاثير مستخلص اوراق الزيتون في بعض الخصائص الفيزيائية والكيميائية للحوم الحملان الطازجة والمخزنة في درجة ٤ مئوية لمدة سبعة ايام ، المعاملة الاولى (معاملة السيطرة) وهي اللحوم الغير معاملة، مستخلص اوراق الزيتون حضر بتركيزين ٢و ٤% (حجم/ حجم) ، المعاملة الثانية شملت اللحوم المغمورة بتركيز ٢%، المعاملة الثالثة شملت اللحوم المعاملة برذاذ مستخلص الاوراق تركيز ٢% ، المعاملة الرابعة اللحوم المغمورة بتركيز ٤% ، المعاملة الخامسة اللحوم المعاملة برذاذ مستخلص الاوراق تركيز ٤%، حيث تم معاملتها بهذه التراكيز لمدة اربعة عشرين وبعد ذلك خزنت المعاملات الخمسة بالتبريد (٤ مئوية) لمدة سبعة ايام ، وجرى تقييم بعض الصفات الفيزيائية والكيميائية لهذه الشرائح. نسبة قابلية حمل الماء (WHC)، بعد سبعة ايام من التخزين ، المعاملة ١و٢ اختلفت معنويا عن بقية المعاملات ، بقية المعاملات لم يكن هناك فروقات معنوية بينها ، اعلى نسبة حمل ماء سجلت في المعاملة الخامسة (٣٩.٩٥٠%) واقل نسبة سجلت في المعاملة الاولى (٣٧.٠٠%). نسبة الفقد اثناء الطبخ ، بعد سبعة ايام من الخزن، المعاملة اختلفت معنويا عن المعاملة الثالثة ، الرابعة والخامسة ، ولكن المعاملات الثانية، الثالثة ،الرابعة والخامسة لم تختلف عن بعضها معنويا، اعلى النسب سجلت في المعاملة الاولى (٤٥.٨٥٥%) واقل القيم سجلت في المعاملة الرابعة (٤٢.٦٠٥%). بالنسبة لحامض الثايوباربوتيرك ، المعاملة اختلفت معنويا عن المعاملة الثالثة ، الرابعة والخامسة ، ولكن المعاملات الثانية، الثالثة ،الرابعة والخامسة لم تختلف عن بعضها معنويا بعد سبعة ايام من التخزين، اعلى القيم سجلت في المعاملة الاولى (٢.١١٥ مالونالديهايد/كغم) واقل القيم سجلت في المعاملة الخامسة (٠.٨٩٣ مالونالديهايد/كغم). اما بالنسبة للاحماض الدهنية الحرة ، فلم يوجد اي اختلاف معنوي بين المعاملات بعد سبعة ايام من التخزين بالتبريد. الخلاصة انه يمكن استخدام مستخلص اوراق الزيتون كمضاد اكسدة طبيعي وبالتالي تطبيقه اثناء عمليات تصنيع اللحوم لتلافي مشاكل الجودة المتعلقة بهذه المنتجات.