INVESTIGATION ON ANTIMICROBIAL AND ANTIOXIDANT EFFECT OF OLIVE LEAF EXTRACT IN KARADI SHEEP MEAT DURING STORAGE

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ABSTRACT

The purpose of this investigation was to study the effects of adding olive leaf extract (OLE) on the oxidative rancidity and microbial growth in lamb patties. Lamb patties were blended with 1%, 2% and 3% olive leaf extract and untreated (control) group and kept in refrigerator at 4°C for12 days. The results revealed that lipid oxidation of lamb patties started to increase after four days of refrigeration and increase rapidly to reach their maximum after 12 days of storage. Extract of olive leaf apparently retarded significantly (P<0.01) oxidative rancidity of lamb patties by 52.3, 36.5 and 26.1%for treated samples with1, 2, 3% of OLE respectively compared to control. Total plate count, psychrophilic count and coliform decreased significantly (P<0.01) with the addition of extracts during storage. It was concluded that adding (OLE) led to retarded oxidative rancidity and microbial growth during storage (4°C) of lamb patties for 12 days.

Keywords: Olive leaf extract, Lipid Oxidation, Microbial Count, Lamb Patties

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دراسة تأثير اضافة المستخلص الكحولي لاوراق الزيتون في النمو الميكروبي و التزنخ التأكسدى في لحوم الاغنام الكرادية خلال الخزن

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مدرس

قسم الثروة الحيوانية– كلية الزراعة– جامعة دهوك

المستخلص

هدفت هذه التجربة دراسة تأثير اضافة المستخلص الكحولي اوراق الزيتون كمضادات لأكسدة الدهون و النمو الميكروبي في اقراص لحم الحملان. تم اضافة مستخلص الى اقراص اللحم و بمعدل (3,2,1%) في حين تركت المعاملة الرابعة بدون اضافة (سيطرة) و ثم حفظها فى الثلاجة (4° م) لمدة (12) يوما.اشارت النتائج بزيادة التزنخ التأكسدى لاقراص اللحم بعد مرور أربعة أيام من الخزن تبعها زيادة مطردة لتصل أقصاها بعد 12 يوم من الخزن. كما أتضح بان اضافة المستخلص قد أدى الى فرق معنوي(P<0.01) للتزنخ وبنسبة(52.3 ,36.5 ,26.1%) للاقراص المعاملة ب 3,2,1% من المستخلص على التوالي مقارنة بمعاملة السيطرة. كما تبين بان مجموع العد البكتيري والبكتيريا المحبة للبرودة والكوليفورم قد انخفض معنويا (P<0.01) بأضافة المستخلص خلال الخزن. ويمكن الاستنتاج بان اضافة المستخلص الكحولي لاوراق الزيتون يعمل على تثبيط التزنخ التأكسدى والنمو الميكروبي خلال الخزن لمدة 12يوما.

الكلمات المفتاحية: أكسدة الدهون، النمو الميكروبي، أقراص اللحم.

Introduction

Deterioration of meat products is mainly caused by lipid oxidation and microbial spoilage. The use of antioxidants and antimicrobials provides an effective way for preservation of meat products against lipid oxidation and microbial spoilage. An increased interest has been directed towards plant-based extracts as a source of phenolic antioxidants (Skerget et al. 2005) and antimicrobials. It was known that olive leaf extract (OLE) is a phenolic compound and is characterized to have several properties including antioxidant, antimicrobial, antiviral and anti-inflammatory (Bouaziz, et al.2008 and Micol, et al.2005) .It has been found that adding 2% and 3% OLE to beef cubes stored at 4 c for 9 days has a inhibitory beneficial effect on microbial load, total viable and coliform counts .Also, samples treated with 2% OLE delayed the oxidation deterioration compared to other treatedsamples (Aytul,2010).similarly**,** working with bovine and porcine muscle**,** Hayes et al (2009),found that olive leaf extracts exhibit antioxidant activity and increasing color stability in both muscles. The purpose of this study was to establish the optimum concentration of OLE as a natural antioxidant and/or antimicrobial agents to be added to Karadi lamb patties in order to diminish oxidative and microb - iological deterioration**.**

Materials and methods Extraction of olive Leaf

Olive leaves used in this work were obtained from Faculty of Agriculture and Forestry, University of Dohuk. Leaves were washed followed by drying dried at room temperature. Then the leaves were ground by blender. One hundred gm of the powder was extracted with 1000 ml of 70% (v/v) aqueous ethanol in a closed conical flask for 24 hr at room temperature in the dark. The extract was filtered through cheese cloth and the residue was re-extracted three times using the same solvent. The combined filtrate was evaporated in a vacuum oven at 40c°. The obtained aqueous extract was frozen until use.High performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) was used to detect the active compound of the extract.

Preparation of meat patties

Sample of meat (*longissimus dorsi* muscle) were obtained from yearling karadi lamb carcasses. The samples were transferred directly to the laboratory under complete aseptic conditions without undue delay. After chilling 24 hr at (4c°), the connective tissues and fat were trimmed off from the meat sample. The meat samples were cut into pieces, and minced using meat grinder. Minced meat(1kg each) were divided into four treatment ,the first untreated (control) and the remaining samples were blended 1,2,and 3% OLE, respectively. Patties (100g) was formed using a meat former, and placed on plastic foam meat trays, wrapped with polyethylene film and stored for 12 days at 4°C to evaluate TBA, microbial count and sensory attributes at days 1, 4, 8 and 12 of storage.

Analytical Methods

Lipid oxidation as thiobarbituric acid (TBA) was determined by spectrophotometer (6400 – JENWAY, UK) following the method of Witte et al, (1970).

Microbial Count

The method suggested by American Public Health Association for determination microbial count (APHA, 1992).

Sensory Evaluation

The investigated samples were evaluated using a panel test according to Cross et al. (1978).

Statistical Analysis

Statistical Analysis of the data was carried out using GLM to estimate Best Linear unbiased effects (SAS, 2002) of the main effects on studied traits.(Duncan, 1955) was performed to detect significant differences among means of treatment combination (treatments X period).

Results and Discussion

The active compounds found in olive leaf extract are Cinammic acid, Ferulic acid ,Tyrosol, Hydroxyle tyrosol ,Gallic acid ,Vanallic acid ,Coumaric acid (Table 1) Similarly ,it was found that the most active antioxidative constituent of olive leaf extract are phenolic (Erdohan and Turhan,2011; Benavente-Garcia,et al.,2000)

Table 1.The active compounds of olive leaf extract(OLE)

The active compounds Of OLE	Retention time of standard (min)	Retention time of sample(min)	Concentration mg/100gm olive leaf extract
Cinammic acid	1.11	1.17	0.157
Ferulic acid	1.194	1.194	0.148
Tyrosol	3.11	3.098	0.470
Hydroxyletyrosol	4.01	4.015	0.263
Gallic acid	5.18	5.19	0.179
Vanallic acid	6.35	6.36	0.120
Coumaric acid	7.19	7.19	0.150

TBA

The TBA values for the lamb patties containing different levels of OLE during storage at 4°C for 12 days is presented in Table (2).Result revealed that TBA values for treated samples were significantly (P<0.01) lower than that recorded for control samples. During storage at 4°C lipid oxidation of patties in the control batch started to increase from 0. 324 mg MDA/kg meat (Day one) to 0.904 mg MDA/kg meat (Day 4) and increased rapidly at 12days of storage to reach 2.41 mg MDA/kg meat. While the meat patties contained extract of OLE apparently retarded oxidative rancidity during storage at 4 °c by 52.3, 36.5 and 26.1% for treated samples with 1,2,3% OLE, respectively as compared to control (Table 2). Therefore it was noticed from TBA values demonstrated that the level of 1% OLE extract added to meat patties was more effective in comparison with other concentrations (2% and 3% OLE). Such effect of OLE could be attributed to the hydroxyl groups (hydroxytyrosol, Gallic acid ,tyrosol ,vanillic acid ,ferulic acid and cinnamic acid) within the phenolic structures of constituents present in crude of OLE. (McDonald et al., 2001). Similarly, Aytul(2010) and Aytul, et al (2008) found that OLE could delay the oxidation of deboned beef cubes meat.

Table 2. Changes in thiobarbituric acid values (MAD mg/kg meat) of lamb patties during storage for 12 days at 4°C

Treatment	Period (day)			
	1	4	8	12
Control	0.324±0.03 j	0.904 ± 0.06 g	1.130 ± 0.02 f	2.41 ± 0.05 a
1% OLE	0.413±0.04 ji	0.948 ± 0.02 g	0.967 ± 0.00 g	1.15 ± 0.00 f
2% OLE	0.437±0.03 i	0.978 ± 0.16 g	1.100 ± 0.00 f	1.53 ± 1.53 c
3% OLE	0.574±0.02 h	1.309 ± 0.02 e	1.410 ± 0.02 d	1.78 ± 0.00 b

Means bearing similar letters within each column or row are not differ significantly (p<0.01) otherwise ,they differ significantly (p<0.01) .C=control, OLE=olive leaf extract

Microbial changes

The effects of different concentration of OLE to lamb patties stored at 4°C for 12 days on total plate count (TPC), psychrophillic bacterial count (Psy) and coliform bacteria demonstrated in Table (3).Result revered that the TPC count decreased significantly with the addition of OLE during the storage.A remarkable increase (P<0.01) was noticed in (TPC) throughout storage, especially in the control from 0.85 to 100×10^5, as compared with treated groups with 1% (0.35 to 6×10^5),2% (0.2 to 7×10^5),3% (0.4to12×10^5)CFU/g.OLE samples at days 1 and 12 days. Similarly, Aytul (2010)and Aytul, et al(2008), indicated that using 2% and 3% OLE had inhibitory influence in controlling the microbial load (TPC and Coliform count) of beef cubes stored at 4ºC for 9 days.Result given in Table(3) showed that addition of OLE resulted in a significantly (P<0.01) decrease of PSY as compared to the control sample.This reduction was amounted to 92.7,91.4 and 87.1% for treated sample with 1,2 and 3%OLE, respectively as compared to the untreated sample. Coliform counts of the prepared lamb patties containing different concentration of the tested OLE during storage are shown in Tables(3). It was observed that total coliform count increased significantly (P<0.01) with the advances storage period. However, bacteria in treated group grow much slower (P<0.01)

than did the bacteria of control group indicating antibacterial effects of OLE on lamb patties. Similarly, Aliabadi, *et al*.,(2012) reported that using olive leaves had beneficial effect in controlling the microbial infections

Table 3 Effect of olive leaf extracts on change in total plate count, psychrophilic bacteria count and coliform count of lamb patties stored for 12 days at 4°C.

Treatment1	TPC×105			
	Period (day)			
	1	4	8	12
Control	0.85 ± 0.80 f	1.75 ± 0.14 ef	53.0 ± 1.73 b	100 ± 5.77 a
1% OLE	0.35 ± 0.02 f	1.25 ± 0.14 ef	3.30 ± 0.05 edf	6.00 ± 0.57 ed
2% OLE	0.20 ± 0.00 f	0.30 ± 0.05 f	1.05 ± 0.02 ef	7.00 ± 0.57 d
3% OLE	0.40 ± 0.00 f	0.40 ± 0.05 f	1.05 ± 0.02 ef	12.0 ± 0.57 c
	PSY×105			
Control	0.55 ± 0.08 e	1.25 ± 0.14 e	42.0 ± 1.15 b	70.0 ± 2.30 a
1% OLE	0.15 ± 0.02 e	0.85 ± 0.08 e	2.40 ± 0.23 e	5.10 ± 0.05 d
2% OLE	0.10 ± 0.00 e	0.15 ± 0.02 e	0.80 ± 0.05 e	6.00 ± 0.57 d
3% OLE	0.20 ± 0.00 e	0.25 ± 0.02 e	0.80 ± 0.05 e	9.00 ± 0.57 c
	Coliform×102			
Control	0.080 ± 0.010 f	0.150 ± 0.01 f	3.90 ± 0.05 d	45.0 ± 0.57 a
1% OLE	0.075 ± 0.002 f	0.166 ± 0.00 f	2.80 ± 0.11 e	14.8 ± 0.69 c
2% OLE	0.035 ± 0.002 f	0.060 ± 0.00 f	0.25 ± 0.02 f	3.15 ± 0.60 ed
3% OLE	0.080 ± 0.010 f	0.170 ± 0.02 f	0.70 ± 0.17 f	27.0 ± 0.57 b

Means bearing similar letters within each column or row are not differ significantly (p<0.01) otherwise ,they differ significantly (p<0.01) .C=control, OLE=olive leaf extract

Table 4 Change in sensory attributes of patties an affected by adding OLE during storage at 4°C for 12 days.

Treatment	Flavor			
	Period (day)			
	1	4	8	12
Control	4.00 ± 0.57 abc	5.00 ± 0.00 a	4.66 ± 0.33 ab	3.66 ± 0.33 bc
1% OLE	3.66 ± 0.33 bc	5.00 ± 0.00 a	3.66 ± 0.33 bc	3.33 ± 0.33 c
2% OLE	3.00 ± 0.57 c	3.33 ± 0.33 c	3.00 ± 0.57 c	1.66 ± 0.33 d
3% OLE	3.00 ± 0.57 c	3.00 ± 0.00 c	3.00 ± 0.57 c	1.66 ± 0.33 d
	Color			
Control	3.00 ± 0.00 bc	5.00 ± 0.00 a	3.00 ± 0.57 bc	2.33 ± 0.33 cd
1% OLE	2.66 ± 0.66 cd	4.00 ± 0.00 ab	3.00 ± 0.57 bc	2.66 ± 0.33 cd
2% OLE	2.33 ± 0.33 cd	4.33 ± 0.33 a	2.00 ± 0.57 cd	1.33 ± 0.33 d
3% OLE	1.66 ± 0.33 cd	4.00 ± 0.57 ab	2.00 ± 0.00 cd	1.33 ± 0.33 d
	Tenderness			
Control	5.00 ± 0.00 a	4.33 ± 0.33 ab	3.66 ± 0.33 bcd	4.00 ± 0.00 bc
1% OLE	4.33 ± 0.33 ab	4.00 ± 0.00 bc	4.00 ± 0.00 bc	3.66 ± 0.33bcd
2% OLE	4.00 ± 0.00 bc	4.00 ± 0.00 bc	3.33 ± 0.33 cd	3.33 ± 0.33 cd
3% OLE	4.00 ± 0.00 bc	4.00 ± 0.00 bc	3.33 ± 0.33 cd	3.00 ± 0.00 d
	Juiciness			
Control	5.00 ± 0.00 a	4.33 ± 0.33 ab	3.66 ± 0.33 bcd	4.00 ± 0.00 bc
1% OLE	4.33 ± 0.33 ab	4.00 ± 0.00 bc	4.00 ± 0.00 bc	3.66 ± 0.33bcd
2% OLE	4.00 ± 0.00 bc	4.00 ± 0.00 bc	3.33 ± 0.33 cd	3.33 ± 0.33 cd
3% OLE	4.00 ± 0.00 bc	4.00 ± 0.00 bc	3.33 ± 0.33 cd	3.00 ± 0.00 d
	Acceptance			
Control	4.66 ± 0.57 a	4.66 ± 0.33 a	3.66 ± 0.33 bcd	3.00 ± 0.00 def
1% OLE	4.33 ± 0.33 ab	4.00 ± 0.00 abc	3.33 ± 0.33 cde	3.66 ± 0.33bcd
2% OLE	3.00 ± 0.00 def	3.33 ± 0.33 cde	1.66 ± 0.33 g	1.66 ± 0.33 g
3% OLE	2.33 ± 0.33 gf	2.66 ± 0.33 ef	1.66 ± 0.33 g	1.66 ± 0.33 g

Sensory Sensory characteristics of cooked patties as affected by different concentration of OLE are presented in Table (4).Result of analysis of variance revealed that added 1% OLE to lamb patties had significantly better overall acceptability compared to untreated or other treated groups**.**Means bearing similar letters within each column or row are not differ significantly ($p<0.01$) otherwise ,they differ significantly ($p<0.01$). C=control, OLE =olive leaf extract.

Conclusion From the results obtained ,it can be conclude that OLE is an effective natural antioxidant and antibacterial, and may have a functional application in the develop of healthy meat products.

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