Journal homepage www.jzs.uos.edu.krd
Journal of Zankoi Sulaimani
Part-A- (Pure and Applied Sciences)

Effect of Olive Leaf Extract on Physio-Chemical and Microbiological Traits of Local Fresh Fish Fillets During Refrigeration Storage

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Article info

Original: 14 Jan. 2015
Revised: 28 Jan. 2015
Accepted: 10 Mar. 2015
Published online:
20 Sep. 2015

Key Words:
Olive leaf extract
Physio-Chemical
microbiological traits
fish fillets

Abstract

This research was performed to evaluate the activity of olive leaf (OLE) as an antioxidant and antimicrobial on some traits of fresh fish, Olive leaf extract (OLE) solutions will prepare at the concentration of 2% and 4% (v/v). Fish sample is divided into five treatment included T1 is considered a control without treatment, while, other fish sample are treated with different concentration of olive leaf extract by using sprays and immersion methods, T2: 2% spraying, T3: 2% immersion, T4: 4% spraying, T5: 4 % immersion). The samples were then stored under refrigerated conditions (4.0°C) for 0, 2, 5and 7 days until analysis. The result were recorded a high water holding capacity in T5 (39.955%), while the lower (WHC) percentage T1 (36.750%) After 7 days of storage. In the end periods storage, recorded a high cooking loss (CL) percentage in T1 (50.325%) while lower CL percentage recorded in T4 (43.865%). T1 gave a high TBA value (5.050 mg MDA/ kg) while, T5 recorded a lower TBA value (2.065 mg MDA/ kg) after 7 days of storage. FFA percentages, at 7 days of storage, the highest percentage recorded in T1 (1.435%), while the lowest value recorded in T4 and T5 (0.999 and 0.997 % respectively). The results observed that T1 recorded a higher TPC and psychrophilic count, which were T5, which were (2.25×10^5 CFU/g) and (2.775×10^3 CFU/g) respectively, while, fish sample were treated with T5 gave a lower TPC and psychrophilic count, which were (1.065×10^5 CFU/g) and (1.434×10^3 CFU/g) respectively. It can be concluded that the positive effect of olive leaf extract proves much more effective as a source of natural antioxidant and antibacterial, which may have uses for solves quality problems in their products.

Introduction

Meat can deteriorate in many ways, one of the common factor is lipid oxidation, resulting in a variety of break – down products such as malonadehyde and cholesterol oxidation products, which produce discoloration and off – flavor development, accelerated by extending of frozen storage period, texture defects, that impairs adverse effects on the overall meat quality (Gray et al., 1996, McCarthy et al., 2001; Brewer, 2007). Growing awareness and concern on the safety and quality of meat leads to numerous developments in meat preservation as can be observed via lipid oxidation and microbial spoilage (Leo & Fidel, 2010). Consumers concerns regarding safety and toxicity of commercial antioxidants have pressed the food industry to find natural sources of phenolic antioxidant and antimicrobial as replacements (Lee and Lee, 2010; Beal et al., 2011). Olive leaves have antioxidants and antimicrobial properties due to their phenolic compounds, particularly, oleuropein, tyrosol and hydroxy tyrosol (McDonald et al., 2001; Pereira et al.,

2007).

Therefore, the main objectives of this study are designed to investigate the effect of the different concentrations and methods of olive leaves extraction on some of physio-chemical and microbial quality on meat slices of local fresh fish (*Caprinus caprio*) during cold storage at 4^{o}C for 7 days.

Material and methods:

Fresh common carp fish (*Caprinus caprio*) was purchased after being harvested, from a local market in Sulaimania province. The fish will be headed, eviscerated, washed and immediately transported to the laboratory in boxes containing enough slurry ice. After fish was filleted manually, the muscular parts of the trucks were selected for further studies.

Olive leaf extract (OLE) extracts was prepared at the concentration of 2% and 4% v/v. T1 (fish fillets without any treatment), while fillets were immered and sprayed with olive leaf extract at different concentrations (T2: 2% spraying, T3: 2% immersion, T4: 4% spraying, T5: 4 % immersion). The samples were then stored under refrigerated conditions (4.0°C) for 0, 2, 5and 7 days of the refrigeration storage.

Physio- chemical analysis:

Water holding capacity (WHC) was measured according to Wardlaw *et al*., (1973), About 8 g of fish flesh was mixed with 12 mL of NaCl (0.6 M) in a test tube and incubated at 5 C for 15 min. The test tubes were then centrifuged at 4100 rpm for 15 min. The WHC (mL/100g meat sample) was calculated in the resulting supernatants.

Cooking loss was measured according to Cyril *et al*. (1996), twenty gram of fish flesh 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 20 gram of fish flesh was prepared by mixing about 20 g of fish flesh in 50 mL of extraction solution containing 20% TCA previously prepared in 2 M phosphoric acid. Filtered through whatman No. 1 filter paper; 5 ml of filtrate was transferred to the test tube followed by addition of 5 mL of Thiobarbituric acid (0.005 M in distilled water) and kept in 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.

Free fatty acids (FFA) was analyzed according to the method described by Egan *et al*., (1981), Briefly, about 100 gm of fish flesh was homogenized and mixed with about 250 mL of chloroform for 2-3 min. The mixture then filtered through filter paper containing anhydrous sodium sulphate, (25 ml of 95% ethanol neutralized with drops of 0.1 N NaOH after adding phenolphthalein), This solution was added to 25 ml of the filtered above and the mixture tittered with 0.1 N NaOH until the pink color persists for 15 seconds. The FFA calculates as oleic acid as percentage of the sample.

Microbiological tests:-

Sample preparation

A serial dilution (10^{-1}-10^{-7}) was made by adding about 20 gm of fish flesh to 225 mL of Butterfiled phosphate solution.

Total plate count (AOAC 966.23 C, 1995**)**

The dilutions prepared before was used, 1ml from the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} etc., dilutions were poured separately into each of two petri-dishes. Additional dilutions plates were used when expecting higher bacterial levels. The agar was allowed to harden and one series of duplicate plates were placed in a 35 ±1ºC incubator for 48 hours. A colony counter has been used to count colonies on the duplicate plates in a suitable

range (30-300 colonies per plate). Average of the counts was obtained from duplicated plates, multiplied by the dilution factor and reported this number as the aerobic plate count (Standard plate count) per gram at the incubation temperature used.

Psychrophilic bacterial count (APHA, 1992)

Plate count agar was used; 1ml of the series dilutions that were prepared earlier was used, then the plates were incubated at 5-7ºC for 10 days. The same procedure for counting was used as in Total Plate count.

Psychrotrophic bacterial count (AOAC, 1995)

The same protocol of pour plate procedure had been used with the exception of the incubation temperature; the cultured plates had been incubated at 20ºC for 4-5 days.

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 ($p \leq 0.05$) (Steel *et al.*, 1996).

Results and discussion:

Water holding capacity (WHC)

The results of water holding capacity (WHC) percentage were shown in table I, there were no significant differences among treatments (T1, T2, T3, T4) except T5 differed. After 2 days, the results showed that T5 (4% emersion) differed significantly ($P \leq 0.01$) among T1(control), T3(2% emersion) and T4(4% spray) while there were no significant difference between T5 and T2(2% spray), the highest percentage of WHC recorded with T5(47.00%) while the lowest percentage recorded with T1 (45.75%). T1 differed significantly with T2, T3, T4 and T5 at days 5 of refrigeration storage, also results showed that T2, T3, T4 and T5 showed no variation with other treatments, the highest percentage of WHC recorded in T5 (44.350%) while the lowest percentage recorded in T1 (40.900%). After 7 days of refrigeration storage, T1 differed significantly with other treatments, but T2, T3, T4 and T5 showed that no differences among them, the highest percentage of WHC recorded in T5 (39.955%) while the lowest percentage recorded in T1 (36.750%). When compared WHC values of the same treatment in different periods, results of all treatments showed that percentage of WHC at day 7 differed significantly among WHC values at day 5, 2 and 0.

Results showed an inverse relationship between water holding capacity and cooking loss as showed in table II, which showed increase in WHC percentage and decrease in CL percentage in same treatments at same periods.

Measuring the ratio of WHC and cooking loss could be good parameters to detect the quality of meat via denaturation of protein (Skipnes et al. 2007). It may possible be due to the role of olive leaves extract with concentration 2 and 4% as natural antioxidant may protect fish tissues from lipid oxidation and protein denaturation, this might allow to bind water and increasing water holding capacity and less drip which led to increase ability of meat tissues to retain water and decreasing moisture loss during storage and cooking (Arora, 2000). Offer and Trinick, (1983) were 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. Increase in pH value might be due to the large number of hydrophilic sites on meat protein, resulting in a more binding of water molecules through hydrogen and ionic bonding to the hydrophilic sites of polypeptides (Hamm, 1977).

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

Treatment			Storage periods (days)			
			0	2	5	7
control		T1	50.250±0.071 a A	45.750 ± 0.495 a B	40.900 ± 1.131 a C	36.750 ± 0.495 a D
2%	spray	T2	50.00 ± 0.141 a A	45.750 ± 0.778 ab A	43.800 ± 0.424 b C	39.00 ± 0.283 b D
	Emersion	T3	49.850 ± 0.495 a A	45. 900 ± 0.566 a B	43.700 ± 0.283 b C	39.300 ± 0.566 b D
4%	spray	T4	50.850 ± 0.636 a A	46.950 ± 0.707 a B	43.950 ± 0.212 b C	39.800 ± 0.141 b D
	Emersion	T5	48.700 ± 0.566 b A	47.00 ± 0.283 b B	44.350 ± 0.212 b C	39.955 ± 0.078 b D

-Means having different lower-case at the same column and upper-case at the same row are significantly varied at ($p \leq 0.01$).

Cooking loss:

Table II showed that there were significant variations in cooking loss among treatments at zero time, while after 2 days variations were observed among treatments. At 5 days, the treatments, showed that T1 (control) differed significantly with T2 (2% spray) and T4 (4% spray) while not differed with T3 (2% emersion) and T5 (4% emersion), the highest cooking loss percentage recorded in T1 (47.745%) while the lowest percentage recorded in T2(39.310%). At 7 days of storage, results showed that T1 (control) differed significantly with T4(4% spray) while not differed with T2, T3 and T5. T4 didn't differ significantly with T2, T3 and T5. The high cooking loss percentage was recorded in T1 (50.325%) while the lowest percentage recorded in T4 (43.865%). Results of cooking loss in the present investigation, study showed that there was an increase in all treatments as storage periods proceeded, and cooking loss percentage was higher in T1(control) than other treatments, It seems that the more storage time the more cooking loss of the samples (Wang, 2000; Al-Haju, 2005. Gorge, 2000 was reviewed that several factors could affect the loss of weight through losing 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 element.

Table II: Effects of different concentrations of olive leaf extract on cooking loss (CL) percentages of fresh fish slices during storage at 4°C for 7 days. (Mean ± S.D.)

Treat			Storage periods (days)			
			0	2	5	7
control		T1	32.470± 0.410 a A	38.060 ± 0.976 a A	47.745 ± 1.520 a B	50.325 ± 1.365 a B
2%	spray	T2	33.625 ± 0.912 a A	37.305 ± 3.033 a AB	39.310 ± 2.956 b B	45.500 ± 8.556 ab C
	Emersion	T3	33.540 ± 0.000 a A	37.595 ± 1.478 a A	43.345 ± 0.742 ab B	49.010 ± 0.311 a b C
4%	spray	T4	32.905 ± 0.375 a A	37.325 ± 0.445 a AB	39.375 ± 0.530 b BC	43.865 ± 1.308 b C
	Emersion	T5	33.330 ± 0.325 a A	39.495 ± 0.06 a B	42.400 ± 1.683 ab BC	46.365 ± 3.104 ab C

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.01$)

Thiobarbituric acids values:

Table III, shows results of TBA values, there were no significant differences among all treatments at zero time and day 2 of storage periods.

After 5 days of storage, results showed that T1 differed significantly among T2, T3, T4 and T5, and T2, T3, T4 and T5 didn't differ with other, the highest TBA values recorded in T1(1.735 malonaldehyde/ kg) while the lowest value recorded in T5 (1.100 malonaldehyde/ kg). At the 7 days of storage, results showed that T1 differed significantly among other treatments, the highest TBA value recorded in T1 (5.050 malonaldehyde/ kg) while the lowest value recorded in T5 (2.065 malonaldehyde/ kg). 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, 2001). Lee and Lee (2010) reported that phenolic compounds within olive leaves extract have ant oxidative activity against lipid oxidation. Also, Gok and Bor (2012) demonstrated that direct addition of olive leaves extract at concentrations 500 and 1000 ppm to beef meatballs stored at 4°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. Extract of olive leaves was reported to be a good source of phenolic compounds and used as an electron and proton donors (Djenane et al., 2002). On the other hand, it's used to protect meat samples against lipid oxidation (Paiva-Martins et al., 2007).

Table III: Effects of different concentrations of olive leaf extract on Thiobarbituric acid values (mg malonaldehyde/kg) of fresh fish slices during storage at 4°C for 7 days. (Mean ± S.D.).

treat			Storage periods (days) 0	2	5	7
2%	control	T1	0.420± 0.051 a A	0.816 ± 0.001 a A	1.735 ± 0.163 a B	5.050 ± 0.778 a C
	spray	T2	0.420 ± 0.048 a A	0.712 ± 0.067 a A	1.272 ± 0.071 b B	2.275 ± 0.064 b C
	Emersion	T3	0.423 ± 0.028 a A	0.0751 ± 0.006 a A	1.232 ± 0.014 b B	2.130 ± 0.014 bc C
4%	spray	T4	0.415 ± 0.028 a A	0.736 ± 0.008 a A	1.200 ± 0.071 b B	2.240 ± 0.283 b C
	Emersion	T5	0.424 ± 0.033 a A	0.654 ± 0.062 a AB	1.100 ± 0.014 b B	2.065 ± 0.078 bc C

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.01$).

Free fatty acids (FFA):

The results of FFA are shown revealed no significances variation among all treatments at zero day (Table, IV), After the 2 days of storage, results showed that T3 (2% emersion) and T5 (4% emersion) differed significantly with T1, T2 and T3, which didn't differ with other, also T1, T2 and T3 didn't differ significantly with other, the highest FFA value recorded in T1, T2 and T4 (0.590, 0.565 and 0.545 % respectively). After 5 days of storage, results showed T1 differed significantly with other treatments, also T3 differed significantly with T2, T4 and T5, the highest FFA percentage recorded in T1 (0.968%) while the lowest percentage recorded in T3(0.706%). At 7 days of storage, results showed that T1 differed significantly with T3, T4 and T5, other treatments didn't differ with other, the highest percentage recorded in T1(1.435%) while the lowest value recorded in T4 and T5 (0.999 and 0.997 % respectively. The highest FFA value recorded in T1 (1.025%) and the lowest value was recorded in T5 (0.955%). Rodriguez et al., 2006 mentioned that FFA correlate with lipid oxidation since it has a pro-oxidant activity, however it doesn't relate with the effect of quality of the products. Therefore, the higher value of FFA is possibly due to the action of lipolytic enzymes on lipid from higher bacterial load leading to increase in the release of free fatty acids, which contribute positively to the generation of undesirable aroma and flavor (Al-Sherick, 2005).

Table IV: Effects of different concentrations of olive leaf extract on free fatty acid values of fresh fish slices during storage at 4oC for 7 days. (Mean ± S.D.)

Treat			Storage periods (days) 0	2	5	7
2%	control	T1	4.1×10^{2}± 42.426 a A	5.08×10^{3} ± 35.36 a A	6.1×10^{4} ± 141.421 a A	2.9×10^{5}±28284.271 a B
	spray	T2	3.5×10^{2} ± 14.142 a A	4.85×10^{3} ±70.71 a A	5.15×10^{4} ± 919.239 a A	2.125×10^{5}±14849.242 ab B
	Emersion	T3	3.2×10^{2} ± 14.142 a A	4.9×10^{3} ±141.42 a A	5.3×10^{4} ± 282.842 a A	1.3×10^{5} ± 28284.271 ab B
4%	spray	T4	4.25×10^{2} ±21.213 a A	4.4×10^{3} ±848.53 a A	4×10^{4} ± 1414.214 a A	2.25×10^{5}± 84852.814 ab B
	Emersion	T5	5.05×10^{2} ±7.0711 a A	4.05×10^{2} ±70.721 a A	3.515×10^{4} ± 700.036 a A	1.065×10^{5} ± 2121.320 b B

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.01$).

Total plate count:

As shown in table V, there were no significant variations among treatment at 0, 2 and 5 days of storage. After 7 days of storage, the total plate counts showed that were significant differences between T1 and T5, while there no significant variations among T2, T3 and T4, and also no significant differs among T5,T2,T3 and T4, the highest total plate count was recorded in T1(2.25×10^5 CFU/g) and lowest TPC recoded in T5 (1.065×10^5 CFU/g). Spoilage of foods due to bacterial infections has been a major problem for many years and still a great amount of foodstuff become unusable due to these infections worldwide (Negi et al., 2005).In general, a significant decrease was noticed for meat samples treated with olive leaves extract at concentrations (2% or 4% OLE) in their TPC during storage for 7 days. Gok and Bor (2012) found that addition 1000 ppm of olive leaves 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., (2002) concluded that the olive leaves extract had the highest protective activity against microbes, which points to the longest shelf-life of the product examined.

Table V: Effects of different concentrations of olive leaf extract on total plate counts (CFU/gm) of fresh fish slices during storage at 4°C for 7 days. (Mean ± S.D.)

treat			Storage periods (days) 0	2	5	7
control		T1	0.200± 0.000 a A	0.590 ± 0.042 a B	0.968 ± 0.014 a C	1.435 ± 0.007 a D
2%	spray	T2	0.210 ± 0.028 a A	0.565 ± 0.064 a B	0.826 ± 0.021 b C	1.015 ± 0.007 ab D
	Emersion	T3	0.195 ± 0.007 a A	0.380 ± 0.028 b B	0.706 ± 0.006 c C	1.050 ± 0.071 b D
4%	spray	T4	0.195 ± 0.007 a A	0.545 ± 0.049 a B	0.814 ± 0.016 b C	0.999 ± 0.001 bc D
	Emersion	T5	0.205 ± 0.007 a A	0.430 ± 0.028 b B	0.778 ± 0.028 b C	0.997 ± 0.001 bc D

-Means having different lower-case at the same column and upper-case at the same row are significantly different at ($p \leq 0.01$).

Psychrophilic and Psychrotrophic count:

Table VI, showed the results of the psychrophilic count of five treatments, results showed there were no significant variations among treatment at 0, 2 and 5 days of storage.

At 7 days of storage, results showed that T1 was significant variation among T2, T3, T4 and T5, while T2 and T3 were varied insignificantly, also T3 differed significantly with T4 and T5, T4 differs significantly with T5. The highest psychrophilic count recorded in T1 (2.775×10^3 CFU/g) while the lowest count recorded in T5 (1.434×10^3 CFU/g). Gok and Bor (2012) reported that adding 1000 ppm of olive leaves extract to beef meatballs resulted in the highest decrease in aerobic bacteria, Psychrophilic bacteria and Pseudomonas after 10 days of the refrigerated storage than control samples, which explained that the highest amount of olive leaves extract (1000 ppm) had the highest antimicrobial effect, the protective effect of essential oil and ethanolic extract of olive leaves against food Borne and spoilage bacteria.

For psychrotrophic counts, as shown in table VI, results had shown there were no significant differences between treatments at 0, 2 and 5 days of storage. At 7 days of storage, results showed that T4 and T5 differed significantly among T1, T2 and T5, T4 and T5 didn't differs with others, also T3, T4 and T5 didn't differ with others. The highest psychrotrophic counts recorded in T2 (2.109×10^3 CFU/g) and lowest recorded in T5 (1.049×10^3 CFU/g). The result had indicated that fish samples treated with 4% emerged OLE had a

highest inhibitory effect against the Psychrotrophic bacteria count during storage for 7 days. Therefore, three percent OLE treatment can be used for controlling the microbial load of fish fillets during 7days of storage at 4°C. Previous reports indicated that olive leaf extract and its phenolic compounds have an inhibitory effect against Pyschrotrophic bacteria (Kubo et al., 1995; Markin et al., 2002). It's been reported that the active compound Oleuropein found in olive leaves had activity against *Lactobacillus plantarum* (Ruiz-Barba et al., 1991). Also, its affecting the growth of Salmonella enteriditis (Tassou & Nychas,1995) and Bacillus cereus (Tassou et al., 1991). Tassou & Nychas, 1994 also reported that Oleuropein inhibited *Staphylococcus aureu*s and its secondary metabolite; the enterotoxin. Combination of both oleuropein and hydroxytyrosol also had inhibitory activity against several pathogenic strains (Bisignano et al., 2001).

Table VI: Effects of different concentrations of olive leaf extract on Psychrophilic and psychrotrophic counts of fresh fish slices during storage at 4oC for 7 days. (Mean ± S.D.)

			Psychrophilic count				Psychrotrophic counts			
Treat			Storage periods (days)							
			0	2	5	7	0	2	5	7
control		T1	37.5± 24.74 a A	104.5 ± 6.36 a A	307 ± 101.82 a A	2.7755×10^3±240.41 a B	17.5± 3.536 a A	120 ± 14.14 a A	283.5 ± 23.335 a A	2.060×10^3 ± 46.669 a B
2%	spray	T2	29.5 ± 6.36 a A	112.5 ± 12.02 a A	267 ± 12.72 a A	2.370×10^3 ± 601.74 b B	24 ± 1.414 a A	102.5 ± 31.82 a A	236 ± 36.770 a A	2.109×10^3 ± 128.693 a B
	Emersion	T3	16.5 ± 9.192 a A	94.5 ± 10.60 a A	224 ± 46.66 a A	2.1255×10^3 ± 21.92 bc B	20.5 ± 3.536 a A	105.5 ± 3.53 a A	233 ± 18.385 a A	1.7715×10^3± 893.076 a B
4%	spray	T4	25 ± 14.14 a A	153 ± 67.88 a A	136.5 ± 4.950 a A	1.889×10^3 ± 486.48 c B	26.5 ± 3.536 a A	102 ± 5.65 a A	217 ± 1.414 a A	1.127×10^3 ± 28.284 b B
	Emersion	T5	25.5 ± 0.707 a A	97.5 ± 17.67 a A	162 ± 32.52 a A	1.4345×10^3 ± 4.94 d B	20 ± 1.414 a A	71.5 ± 21.920 a A	198.5 ± 2.121 a A	1.049×10^3 ± 57.983 b B

Means having different lower-case at the same column and upper-case at the same row is significantly different at ($p \leq 0.01$).

Conclusion:

In summary, the extract of olive leaves shows a very good antioxidant and antibacterial activities and might be applied in meat and meat products in order to support the quality and shelf life of the products.

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