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# Effect of Removing the Bitter Taste of Olive Leaves on Its Antioxidant and Antibacterial Properties in Some Food Products

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OLIVE leaves extracts constituents are known for their health benefits against some diseases such as: blood pressure, atherosclerosis, diabetes, cancer, as well as antiinflammatory, antioxidant and antimicrobial properties. In the present study olive leaves were treated in order to reduce or eliminate the bitter taste. The treated olive leaves were sensory evaluated as a tea drink. Also the dry ethanol extract of these treated leaves (OLE) were used in preparation of meat sausage as antioxidant and antimicrobial alternative of BHT or BHA. The results indicated that all treated sample of olive leaves tea recorded mean score of overall acceptability ranged from 7.06 to 7.59 (between Like Moderately and Like Very Much) compared to the ordinary green tea sample. OLE of sample No.2 (which treated with 3% citric acid) was achieved the highest percentage of DPPH (38.57%) and phenolic extract yield (15.35%). Therefore, it was used in the preparation of meat sausages with different concentrations. Sausage samples treated with OLE showed the lowest TBA value and total plate count compared to control group during 12 days of storage at 4±0.50 °C. The results showed that also, OLE3 group (400mg) appears to be the best in sensory evaluation as there are either no significant differences or a significant increase in all characteristics compared to other all groups in all periods of storage.

**Keywords:** Olive leaves, Antioxidant, Sausage.

## Introduction

The olive tree (*Olea europaea* L.) has been cultivated in the Mediterranean for more than thousand years. The positive effect on health of its fruits and oil are well known. Also, the leaves of this tree have been used for medical purposes and were introduced recently into the pharmacopoeia especially in southern Europe. They are known as a folk remedy for hypertension and diabetes **(**Schefflera et al*.,* 2008).

Olive leaves, an agricultural waste resulted in during the harvesting or fabrication process of olive fruits, contained considerable biophenols as the other parts of olive trees. Those of leaves contribute to the color and taste of the fruits depending on the nature and concentration. Their quantity also differs between several textures **(**Sahin and Bilgin, 2017**)**.

Olive leaves contain different groups of constituents, such as iridoids, polyphenols, flavones and carbohydrates. Polyphenols are the most abundant antioxidant in our diets; clinical studies have suggested association between the consumption of polyphenol-rich foods or beverages and the prevention of many diseases. Polyphenols are considered reducing agents and together with other dietary reducing agents to protect the body tissues against oxidative stress, various diseases, inflammation and cardiovascular diseases (Scalbert and williamsont, 2000).

Toxicity studies show that olive leaves extracts are generally reliable and do not show any toxic

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effects even at high doses. Results of a study showed that supplementation of olive leaves extract in male and female rats at a single dose of 2,000 mg/kg (acute toxicity) and 100, 200, and 400 mg/kg doses (sub-acute toxicity) given for 28 days did not result in any toxicity. The water soluble extract of olive leaves was also, given at the doses of 360, 600, and 1,000 mg/kg/day for 90 days and did not cause any mortality and toxicity, (Acar-Tek and Ağagündüz, 2020).

Olive Leaves Tea may be an alternative to green tea without the caffeine and with the increased health benefits. Green tea already has a reduced amount of caffeine when compared to coffee or black tea. But, olive leaves infusion don't contain any caffeine. This tea is made from dried olive leaves or olive tree bark (Basuny and Arafat, 2018). The polyphenolic compounds extracted from leaves and olive fruits are excellent antimicrobial and antioxidant agents. The most abundant phenolic component is oleuropein which gives the bitter taste to olive and olive oil. Olive leaves extracts has been associated with health benefits and preservation of food rich in unsaturated fats **(**Al-Rimawi et al., 2017).

Oleuropein concentration is sharply declines when fruits begin to mature. Thus olive oil which is pressed from mature fruits contains very small amounts of Oleuropein (Malik and Bradford, 2008). In contrast, this concentration in olive leaves may reach up 60-90 mg/g of dry matter (Bahloul et al., 2008). Oleuropein is likely to decompose into hydroxytyrosol and elenolic acid under the action of light, acid, base or high temperature. In the enzymatic process, the content of Oleuropein, in olive leaves extract and enzyme are key factors that affect the yield of hydroxytyrosol (Jiao Yuan et al., 2015).

There are a large number of chemical substances that possess antioxidant activity in meat products, but only a few can be used in food products. This effectively minimizes rancidity, retards lipid oxidation, without any damage to the sensory or nutritional properties and resulting in maintaining quality and shelf-life of the products (Al-Rimawi et al., 2017). Meat sausage is a popular meat product enjoyed by millions of consumers. However, an increased concern about its shelf life and nutritional quality has led the food industry to develop new meat product formulations **(**Feiner, 2006 and Baka et al., 2015). Consequently olive leaves which are considered a cheap and available source of natural antioxidants could be used as alternatives to synthetic antioxidants as butylatedhydroxyanisole (BHA) and butylatedhydroxy toluene (BHT) in processing of meat products. But the bitter taste of these phenolic compounds may be inhibits their uses.

Therefore, the objective of this study was to get rid of the bitter taste of the olive leaves firstly. Then the possibility of accepting it as an alternative to green tea. As well as evaluation the antioxidants potential of the phenolic compounds of olive leaves after removing the bitter taste. Also extraction of their phenolic compounds and used it as antioxidants & antimicrobial in meat sausage processing.

## Materials and Methods

*A-Removing of the bitter taste from olive leaves*

The fresh olive leaves samples were collected (in late January) from the planted trees in the farm of Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. After cleaning and washing with tap water, the leaves were divided into 7 parts as shown in figure (1).

1- T1: the first part was soaking in 3% sucrose solution.

2- T2: the second part was soaking in 3% citric acid solution.

3-T3: the third part was soaking in 3% sodium carbonate solution.

The previous three parts were treated for 72 h then washing three times by tap water and dried at 60 °C.

4- T4: the fourth part was minced and incubated at 40 °C with maintain of their moisture content for 5 h then dried at 60 °C.

5- T5: the fifth part was dried at room temperature and in shade without any other treatments as a control.

6- T6: the sixth part were minced and dried at 60 °C.

7- T7: the seventh part were minced and incubated at 40 °C with maintain of their moisture content for 5 hr then dried at room temperature and in shade.

All previous drying operations were carried out until constant weight, then grinded, sifted and stored at 5°C in tight packs.

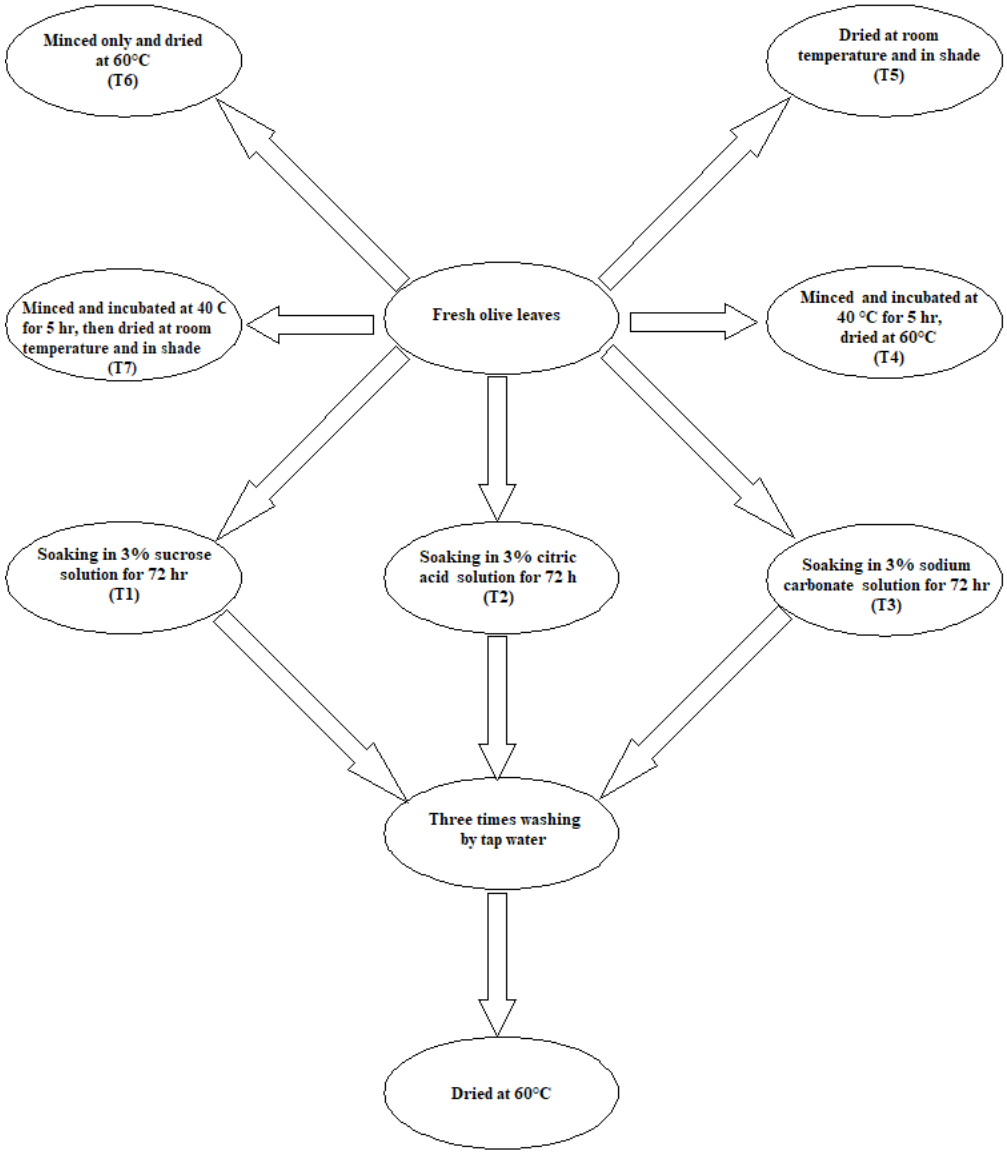


**Fig. 1. Treatments of removing the bitter taste from olive leaves.**

*B-Nutritional uses of treated olive leaves*

*1- Olive leaves tea preparation*

Infusion tea was prepared by immersing 2 gm from the previous treatments of olive leaves in 200 mL of boiling water in a glass jar. The residue was removed through filtration after 5 minutes, (Kodama et al., 2010). Green tea from local market was used for comparison.

*2- Olive leaves extraction (OLE)*

Method of Baker (2014) was used with some modification as follow:

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 whatman number one filter paper using Buchner funnel and the residue was re-extracted three times using the same solvent. The combined filtrate was evaporated in a rotary evaporator (at 40 C°) to minimize the volume, then drying in a vacuum oven at 40 °C to dryness and kept at -20 °C.

*3- Beef sausage preparation*

Beef sausage was prepared according to Heinz and Hautzinger (2007) with some modification**.** Refrigerated beef meat manually cut using band saw, minced and mixed with other ingredients as described in the following Table:

| Ingredients | control | OLE1 | OLE2 | OLE3 | BHA |
|---|---|---|---|---|---|
| Minced meat (%) | 60 | 60 | 60 | 60 | 60 |
| Fat (%) | 15 | 15 | 15 | 15 | 15 |
| Soy protein (%) | 10 | 10 | 10 | 10 | 10 |
| Starch (%). | 4 | 4 | 4 | 4 | 4 |
| Sodium pyrophosphate (%) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Salt (%) | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Garlic (%) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Spices (%) | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 |
| Water (%) | 7 | 7 | 7 | 7 | 7 |
| OLE (mg/kg meat) | - | 200 | 300 | 400 | - |
| BHA (mg/kg meat) | - | - | - | - | 200 |

OLE: dry olive leaves extract, BHA: butylatedhydroxyanisole.

Each group was then stuffed into casing sausage by the filling machine then packaged it in foam dishes then wrapping by polyethylene bags. Immediately, part of the samples was carried out to sensory evaluation, chemical and microbial evaluation at zero time. Remaining samples were kept at 4.0±0.50 °C up to 12 days.

*C- Chemical, antioxidants and antimicrobial analysis:*

*1- Total phenolic and flavonoids compounds contents:*

The total phenolic content (TPC) was determined spectrophotometrically by the Folin-Ciocalteu method as described by Oboh and Imafidon (2018). The results were expressed as mg Gallic Acid Equivalents (GAE) /g of sample weight. The aluminum chloride method was used for the determination of the total flavonoid content of the extracts according to Khatiwora et al. (2010).

*2- Identifation and Determination of phenolic and flavonoids*

A High Performance Liquid Chromatography (HPLC), Agilent, Germany 1200 system equipped with a variable wavelength detector was used to determine phenolic acids and flavonoids according to the method described by Sagdic et al. (2011).

*3- Antioxidants activity*

Free radical scavenging activity of different extracts of the olive leaves were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) according to Shekhar and Anju (2014).

Percentage inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{\text{Absorption of control} - \text{Absorption of test sample}}{\text{Absorption of control}}$$

*4-Thiobarbituric acid number (TBA)*

Thiobarbituric acid (TBA) value was determined according to Pearson (1970).

*5- Total Aerobic plate counts*

Total aerobic plate count (TAPC) was determined using a nutrient agar medium. The count was then calculated as colony-forming unit per gram (cfu/g) of sample as reported by Sharf (1966).

*6- pH values*

The pH value was determined according to Aitken et al. (1962).

*D- Sensory evaluation:*

*1- Sensory evaluation of olive leaves tea*

The seven treatments of olive leaves in addition to green tea were sensory evaluated by a taste panel comprising of 10 staff members at the department of Special Food & Nutrition of Food Technology Research Institute.

The panelists were instructed to score their acceptance for 6 attributes of the infusions tea: color, aroma, taste, aftertaste, astringency and overall acceptability according to Courage (2015).

*Scoring of samples:* The method of Wichchukit and O'Mahony (2014) for hedonic scale was used to evaluate the point scored by the panelists for each sample as follow:

9 = Like Extremely, 8 = Like Very Much, 7 = Like Moderately,

6 = Like Slightly, 5 = neither Like nor Dislike, 4 = Dislike Slightly, 3 = Dislike Moderately, 2 = Dislike Very Much, 1 = Dislike Extremely.

*2-Sensory evaluation of sausage*

Sensory evaluation was determined immediately after meat sausage manufacturing

(zero-time) and after 3, 6, 9 and12 days of storage at 4±0.5 °C. The samples were prepared by cooking in boiling water for 10 min and subjected to member s trained sensory panel to evaluate color, odor, texture and overall acceptability of these formulas according to Suderman et al. (1981).

*E- Statistical analysis*

The obtained data were exposed to analysis of variance followed by multiple comparisons between means (P≤0.05) applying LSD. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS, 1996).

## Results and Discussion

The data in Table 1 show the sensory evaluation of olive leaves tea after treated (remove the bitter taste). Six treated samples in addition to untreated control sample (T5) were evaluated for aroma, color, taste, after taste, astringency and overall acceptability compared to green tea sample. The data showed that a significant difference was found among all treated samples and green tea in aroma, color and taste, while no significant difference in after taste and astringency for treated samples T1, T2, T3 and T4. T5 showed significant difference in all tested attributes compared to green tea and other treatments. This may be due to its high concentration of oleuropen (58.72 mg/g) as shown in Table 3. Tayoub et al. (2012) reported that oleuropen which is the main phenolic compound in olive leaves, is responsible for the bitter taste in olive leave and fruits. Soler-Rivas et al. (2000) reported that processing of olives considerably changes the profile of phenolic compounds and therefore both the organoleptic properties and the antioxidant capacity of the product as compared with the starting material. To evaluate the point scored by the panelists for each treatment A 9-point hedonic scale method was used. For taste, T1 and T7 in the present study being ranked best with a mean score of 6.90 and 7.00 (Like Moderately compared to T5 which being ranked worst with a mean score of 4.40 (Dislike Slightly). All treated samples recorded mean score of overall acceptability ranged from 7.06 to 7.59 (between Like Moderately and Like Very Much) compared to the green tea sample which recorded the highest mean score for all attributes (between Like Very Much and Like Extremely).

The data in Table 2 show the total phenolic and flavonoids contents in dried olive leaves samples. It was observed that T1 which treated with 3% sucrose solution showed the highest amount of phenols and flavonoids (13.83 and 15.20) followed by T2 (12.93 and 14.62) which treated with 3% citric acid solution compared to the untreated control T5 ( 22.21 and 15.12 mg/g respectively). T3 which treated with 3% sodium carbonate showed the lowest value of total phenolic compounds (3.17), while T4 showed the lowest value of total flavonoids (2.50 mg/g). The decreasing in total phenolic and flavonoids contents in the treated samples compared to the untreated sample (T5) may be due to removing of some phenolics compounds from the olive leaves by diffusing from the leaves into soaking liquids or their oxidation by the action of some bacteria or decomposition enzymes (Johnson & Mitchell, 2018). The phenolic and flavonoid content of olive leaves varies according to many factors such as climatic conditions, moisture content, age and variety of the plant, agricultural practices and the extraction procedures used (Ghomari et al., 2019). Martinho et al. (2019) found that phenolic compounds in fresh and freeze-dried leaves ranging from 2.09 to 8.44 and 7.72 to 24.65 mg gallic acid equivalents/g leaves, respectively.

**TABLE 1. Sensory evaluation of olive leaves tea.**

| Sample | Aroma | Color | Taste | After taste | Astringency | overall acceptability |
|---|---|---|---|---|---|---|
| T1 | 7.65$^{b}$ | 7.90$^{b}$ | 6.90$^{b}$ | 7.70$^{ab}$ | 7.80$^{ab}$ | 7.59$^{b}$ |
| T2 | 6.50$^{c}$ | 7.70$^{b}$ | 6.30$^{b}$ | 7.40$^{ab}$ | 7.40$^{ab}$ | 7.06$^{b}$ |
| T3 | 7.00$^{bc}$ | 7.80$^{b}$ | 6.70$^{b}$ | 7.40$^{ab}$ | 7.80$^{ab}$ | 7.34$^{b}$ |
| T4 | 7.30$^{b}$ | 7.40$^{bc}$ | 6.15$^{b}$ | 7.40$^{ab}$ | 7.40$^{ab}$ | 7.13$^{b}$ |
| T5 | 6.30$^{c}$ | 6.80$^{c}$ | 4.40$^{c}$ | 5.00$^{c}$ | 5.10$^{c}$ | 5.52$^{c}$ |
| T6 | 7.05$^{bc}$ | 7.35$^{bc}$ | 6.30$^{b}$ | 7.20$^{b}$ | 7.50$^{ab}$ | 7.08$^{b}$ |
| T7 | 7.25$^{b}$ | 7.70$^{b}$ | 7.00$^{b}$ | 7.00$^{b}$ | 6.80$^{b}$ | 7.15$^{b}$ |
| Green tea | 8.55$^{a}$ | 8.65$^{a}$ | 8.55$^{a}$ | 8.70$^{a}$ | 8.70$^{a}$ | 8.63$^{a}$ |
| LSD | 0. 83 | 0. 67 | 0. 85 | 1.37 | 1.56 | 0.90 |

Values followed by the same letter in the same column are not significantly different at P≤ 0.05.

**TABLE 2. Total phenolic and flavonoids contents and antioxidant activity in treated and untreated dried olive leaves samples.**

| Samples | Total phenols (mg/g) | Total flavonoids (mg/g) | DPPH% |
|---|---|---|---|
| T1 | 13.83 | 15.20 | 92.59 |
| T2 | 12.93 | 14.62 | 86.71 |
| T3 | 3.17 | 3.08 | 80.75 |
| T4 | 5.20 | 2.50 | 81.98 |
| T5 | 22.21 | 15.12 | 86.40 |
| T6 | 8.27 | 3.42 | 86.02 |
| T7 | 10.90 | 14.36 | 89.61 |

Furthermore, the free radical scavenging activities of extracts was measured by the ability to scavenge DPPH radical. From the presented results, it could be concluded that all samples were shown to be remarkably good for quenching DPPH. T1 showed the highest activity (92.59%), followed by T7 (89.61%). In this results, it could be observed that the antioxidant activity power of olive leaves (DPPH %) were not correlated with the polyphenols content.

The antioxidant activity of phenolic hydroxyl compounds in the olive leaves extract could be due to the presence of the hydroxyl groups in their structure such as oleuropein, hydroxytyrosol, and luteolin-7-O-glucoside acid. Antioxidant activity usually depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group. The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity. Olive leaves extract seemed to have almost zero iron chelating activity at concentrations of 200–1000 ppm. Some phenolic compounds are able to chelate iron, while others which do not have a galloyl moiety do not, (Hayes et al., 2011).

The identification of the phenolic compounds in olive leaves samples were illustrated in Table (3). Twenty-five phenolic compounds were identified and quantified by HPLC. Oleuropein was the major compound in all samples. T5 showed the highest content of Oleuropein (5872.29) followed by (2694.92 and 353.84 mg/100g) for Salycillic acid and Hespirdin, respectively. Hespirdin also, showed highest levels in treatments 2, 7, 1, 6, 3 and 4 (162.50, 151.86, 129.01, 100.41, 57.62 and 35.24 respectively). Soler-Rivas et al. (2000) reported that, olive tree leaves have a high content of flavonoids especially, the aglycones apigenin, quercetin, kaempferol and hesperitin, which were identified by reversed phase HPLC.

Catechol was the minor phenolic compounds in treatments T1, T2, T3, T4 and T6. Some phenolic compounds were not detected, such as benzoic acid, in T3, vanillic acid in T3, T4 and T6, caffeic acid in T7 and Naringeninin T6. These results are mostly consistent with many other studies (Rimawi & Salim 2016; Ghomari et al., 2019 and Abdel-Aziz et al., 2020), especially about agreement that Oleuropein is the greatest ingredient in olive leaves. Oleuropein, as previously mentioned, is responsible for the bitter taste in olive leaves and decreased in all treated samples in proportions ranging from 87.89 to 98.28 %. This may be due to either coming out with the soaking solution or hydrolysis by some enzymes (β-glucosidase) or bacteria (*Lactobacillus plantarum*) naturally present on the leaves or in the solution,(Ghabbour et al., 2011). Soler-Rivas et al. (2000) reported that processing of olives considerably changes the profile of phenolic compounds. Oleuropein can be decomposed into hydroxytyrosol and elenolic acid by different factors such as air, light, acid, base, oxidants, metal ions, high temperatures, etc. (Jiao Yuan et al., 2015). In the current study 3-Hydroxytyrosol has been identified while elenolic acid standard was not detectable.

One of the main objectives of the present study was to evaluate the antioxidants potential of the phenolic compounds of olive leaves after removing the bitter taste. The data in Table 2 showed that T1 and T2 maintained of the highest values of phenolic and flavonoids compounds, therefore these samples were selected to extract the phenolic compounds which will be used as antioxidant and antimicrobial in meat sausage preparation.

Table 4 showed the phenolic yield and DPPH value of these extracts, it's clear that T1 gave the highest yield of phenolic compound (17.98%). Followed by T2 (15.35 %).

**TABLE 3. Phenolic and Flavonoids fractions in treated and untreated dryer olive leaves samples (mg/100g).**

| Component | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|
| Pyrogallol | 33.68 | 30.36 | 26.51 | 26.52 | 34.38 | 36.46 | 23.77 |
| Gallic | 3.62 | 2.84 | 1.28 | 0.53 | 1.33 | 0.90 | 1.43 |
| 3-H. Tyrosol | 12.90 | 6.78 | 4.74 | 2.03 | 11.41 | 5.28 | 2.99 |
| Catechol | 0.55 | 0.45 | 0.96 | 0.20 | 1.75 | 0.26 | 1.35 |
| 4aminobenzoic | 0.89 | 2.42 | 1.44 | 0.59 | 4.83 | 3.34 | 0.89 |
| Catechein | 28.60 | 33.93 | 8.04 | 3.52 | 87.35 | 33.32 | 32.81 |
| Chlorogenic | 4.81 | 5.82 | 34.02 | 11.89 | 27.86 | 27.09 | 26.87 |
| Benzoic | 6.07 | 11.07 | nd | 5.65 | 54.79 | 8.96 | 6.29 |
| Vanillic | 7.82 | 11.90 | nd | nd | 38.91 | nd | 5.60 |
| Caffeic | 2.88 | 2.68 | 5.39 | 4.30 | 6.91 | 5.76 | nd |
| Caffeine | 4.58 | 7.39 | 2.46 | 2.54 | 10.91 | 2.84 | 7.54 |
| Ferulic | 7.53 | 15.53 | 3.48 | 3.01 | 32.82 | 12.19 | 16.46 |
| Salycillic | 24.40 | 39.10 | 11.15 | 7.87 | 2694.92 | 51.55 | 63.06 |
| Oleuropen | 339.14 | 305.65 | 178.44 | 100.87 | 5872.29 | 209.71 | 710.65 |
| Ellagic | 42.90 | 54.72 | 38.39 | 22.85 | 87.42 | 48.49 | 66.14 |
| Coumarin | 7.29 | 16.38 | 6.59 | 1.54 | 20.01 | 11.05 | 10.60 |
| Hespirdin | 129.01 | 162.50 | 57.62 | 35.24 | 353.84 | 100.41 | 151.86 |
| Rosmarinic | 16.41 | 29.21 | 7.64 | 3.94 | 113.51 | 16.96 | 21.67 |
| Rutin | 20.62 | 30.75 | 9.53 | 5.32 | 184.01 | 8.60 | 18.46 |
| Quercetrin | 47.33 | 74.79 | 29.90 | 38.58 | 304.68 | 24.80 | 65.98 |
| Naringin | 10.53 | 13.34 | 6.70 | 3.39 | 64.63 | 21.68 | 14.22 |
| Naringenin | 8.13 | 23.56 | 0.55 | 7.32 | 24.41 | nd | 20.72 |
| Quercetin | 30.20 | 25.37 | 11.48 | 11.08 | 26.01 | 15.69 | 11.77 |
| Kampferol | 6.27 | 7.16 | 5.49 | 3.02 | 10.01 | 8.14 | 7.07 |
| Apigenin | 6.76 | 8.78 | 4.26 | 6.64 | 11.01 | 7.23 | 5.91 |

nd: not detected

**TABLE 4. Yield of phenolic compounds and antioxidant activity of some olive leaves extracts (OLE) compared to butylatedhydroxyanisole (BHA).**

| Sample No. | Phenolic yield (%) | DPPH (%) |
|---|---|---|
| T1 | 17.98 | 10.32 |
| T2 | 15.35 | 38.57 |
| BHA | - | 59.97 |

The antioxidants potential of these extracts relative to BHA showed that T2 which treated with 3% citric acid achieved the highest percentage of DPPH (38.57%) and was closest to the BHA value (59.97%). The highest DPPH and lowest phenolic compounds values were in T2, compared to T1 may be due to its carotenoid antioxidants effect. Ibraheem & Abou-Zaidm (2015) reported that carotenoids content of olive variety was increased when socked in citric acid solution, where the highest increasing rate was recorded for 3% citric acid treatment, while, lye treatment had the lowest increasing rate.

These results are similar to that of Haye et al. (2011) who assessed the Radical scavenging activity of the four natural products compared to synthetic antioxidant BHA using (DPPH) and found that ,the antioxidant efficiency was in the order: ellagic acid > sesamol > BHA > olive leaves extract > lutein. It was also; found that the DPPH radical scavenging activity in terms of antiradical power (ARP) of olive leaves extract and BHA were 28.6 and 55.65 g/L respectively.

Because the highest antioxidant activity (DPPH) of T2 compared to T1 as shown in Table 4, it was used in preparation of meat sausage by different concentration (200, 300 and 400mg OLE/kg meat). Synthetic antioxidant (BHA) was used by 200 mg for comparison.

The date in Table 5 showed the thiobarbituric acid values (TBA) determined as mg malonaldehyde of the prepared meat sausage at zero time and during the storage period. No significant difference was found among all groups in TBA values at zero time, but with progresses storage period, a significant difference among all groups were found. OLE3 group recorded the lowest value of TBA during the period of storage followed by BHA, OLE2, OLE1 and control groups, respectively. This may be due to the highest concentration of the phenolic compounds in OLE3 (400mg). According to Egyptian Organization of Standardization (2005), beef sausage TBA values should not exceed 0.9 mg malonaldehyde/kg (ww).

From the results in Table 5, it could be noticed that control group exceeded this limit after 6 days at 4 °C, 1.227 mg malonaldehyde /kg. While, groups OLE1 and OLE2 were lowered than the control group until 9 and 12 days, where recorded 1.01 and 1.186 mg malonaldehyde /kg, respectively. On the other hand, TBA values after 12 days was below the permitted value in the OLE3 and BHA groups, which were 0.756 and 0.875 mg malonaldehyde / kg, respectively. The results of TBA value (which is considered as an indicator for lipid oxidation) suggested that olive leaves extracts can be successfully used as natural antioxidants.

This finding was in agreement with Baker (2014); Al- Rimawi et al. (2017) and Elama et al. (2017) who reported that usage olive leaves extracts in meat products had consistently lower levels of lipid oxidation compared with control sample.

The pH value is one of very important taste indicator in meat products freshness, where, pH value, due to accumulation of alkaline compounds outcome degradation in protein and this could be considered indicator for meat spoilage. The pH value of various groups of meat sausage during storage at 4 °C for 12 days were presented in Table (6), it could be noticed that the initial pH of control, OLE1, OLE2, OLE3 and BHA groups were 5.75, 5.74, 5.72, 5.69, and 5.73 , respectively. pH of all groups gradually increased during storage, this may be attributed to production of volatile basic components such as total volatile nitrogen and ammonia by meat spoilage bacteria (Lawrie & Ledward, 2006; Osheba, 2013). The highest increment of pH as a result of storage was shown in control group, followed by, OLE1, OLE2, BHA and OLE3, respectively. Such observation was due to the impact of olive leaves extracts which led to the best antioxidant and antimicrobial properties. So, it could restrain microbial growth and inhibit the activity of the endogenous proteases at different degree, which leading to the extension of preservation of meat sausage.

**TABLE 5. Changes in Thiobarbituric acid (TBA) of meat sausage affected by adding olive leaves extracts (OLE) during storage at 4°C for 12 days.**

| Groups | Storage period (Day) | | | | |
|---|---|---|---|---|---|
| | 0-time | 3 | 6 | 9 | 12 |
| Control | 0.381[a] | 0.810[a] | 1.227[a] | 1.750[a] | 2.541[a] |
| OLE1 | 0.381[a] | 0.730[b] | 0.831[b] | 1.01[b] | 1.343[b] |
| OLE 2 | 0.363[a] | 0.460[c] | 0.724[c] | 0.850[c] | 1.186[c] |
| OLE 3 | 0.357[a] | 0.412[d] | 0.596[d] | 0.708[e] | 0.756[e] |
| BHA | 0.358[a] | 0.435[d] | 0.623[d] | 0.760[d] | 0.875[d] |
| LSD | 0.1080 | 0.0247 | 0.0298 | 0.0437 | 0.1130 |

Values followed by the same letter in the same column are not significantly different at P≤ 0.05. OLE 1, 2, 3: contained 200,300,400 mg dry olive leaves extract, BHA: contained 200mg butylatedhydroxyanisole.

**TABLE 6. Changes in pH values of meat sausage an affected by adding olive leaves extracts (OLE) during storage at 4 °C for 12 days.**

| Groups | Storage period (Day) | | | | |
|---|---|---|---|---|---|
| | 0-time | 3 | 6 | 9 | 12 |
| Control | 5.75[a] | 7.21[a] | 7.58[a] | 7.78[a] | 8.16[a] |
| OLE1 | 5.74[a] | 6.12[b] | 7.35[b] | 7.65[b] | 7.88[a] |
| OLE 2 | 5.72[a] | 5.81[c] | 6.36[c] | 6.88[c] | 7.56[c] |
| OLE 3 | 5.69[a] | 5.70[d] | 6.06[e] | 6.43[e] | 6.67[e] |
| BHA | 5.73[a] | 5.75[d] | 6.18[d] | 6.48[d] | 6.97[d] |
| LSD | 0.084 | 0.05 | 0.11 | 0.03 | 0.296 |

Values followed by the same letter, in the same column, are not significantly different at P≤ 0.05. OLE 1, 2, 3: contained 200, 300, 400 mg dry olive leaves extract, PHA: contained 200mg butylatedhydroxyanisole

Results in Table 7 showed the bacteria total plate count of meat sausage groups. It could be noticed that the control group had the highest level of total plate count bacteria at zero-time ($7.3x10^3$) followed by BHA, OLE1, OLE2 and OLE3 ($7.25x10^3$, $6.2x10^3$, $5.1x10^3$ and $2.1x10^3$ cfu/g, respectively). On the other hand, total plate counts were gradually increased with increasing the storage time, but the control group had the highest values at the same storage period followed by OLE1, OLE2 BHA and OLE3, respectively. This may be due to the phenolic compounds effects of olive leaves extracts which had good antimicrobial properties. It could be also, noticed that OLE3 which contain 400 mg of olive leaves extract/kg meat was the best treatments where it remained without corruption throughout the storage period and recorded $1.7x10^5$ after 12 days of storage, followed by BHA and OLE2 ($8.1x10^5$ and $3.6x10^6$ cfu/g, respectively). Similar results were found that, olive leaves extracts could inhibit the increasing in bacterial growth of meat products during refrigeration storage (Baker, 2014 and Alirezalu et al., 2016). Similarly, Aliabadi et al. (2012) reported that olive leaves extract had beneficial effect in controlling the microbial infections.

The data in Table 8 show the average scores of sensory evaluation of meat sausage groups at zero time, and after 3, 6, 9 and 12 days of storage period at 4 °C. It could be noticed that, no significant difference was found among all groups in all evaluation attributes (color, taste, odor, texture and overall acceptability), at zero time. OLE3 group appears to be the best in sensory evaluation as there is either no significant difference or a significant increase in all characteristics compared to all other groups in any specific period of storage. This may be due to that OLE3 group contained the highest level of olive leaves extracts (400mg/kg). This led to show the best antioxidant and antimicrobial properties and led to reduce interactions oxidation and microbial activity and enzymes contained in meat tissues. Consequently, reduced degrade of the muscle protein led to reduce the quality loss of meat sausage.

This results conforms with the obtained results by Baker (2014) and Alirezalu et al. (2016) who reported that sensory characteristics (color, taste, odor, texture and overall acceptability ) of meat products as affected by different concentrations of olive leaves extracts were significantly better compared to control.

## Conclusion

The olive leaves extract is a major source of polyphenols specially Oleuropein which it is responsible for the unacceptable bitter taste. The bitter taste could be partially reduced or eliminated without much effect on its effectiveness as an antioxidant. Treated olive leaves by 3% citric acid give the best desired results. From the obtained results, it could be concluded that olive leaves extracts after removing the bitter taste could be effective as natural antioxidants and antibacterial. Based on these results, meat sausage incorporated with olive leaves extracts could also, have a significant impact in improving quality properties, sensory evaluation and increasing the shelf life of the product.

**TABLE 7. Effects of olive leaves extracts (OLE) on changes in total plate counts (cfu/g) of meat sausage stored for 12 days at 4°C .**

| Groups | Storage period (Day) | | | | |
|---|---|---|---|---|---|
| | 0-time | 3 | 6 | 9 | 12 |
| Control | $7.3\times10^3$ | $9.2\times10^4$ | $2.2\times10^6$ | - | - |
| OLE1 | $6.2\times10^3$ | $5.8\times10^4$ | $9.8\times10^4$ | $7.5\times10^6$ | - |
| OLE 2 | $5.1\times10^3$ | $2.8\times10^4$ | $6.7\times10^4$ | $8.5\times10^5$ | $3.6\times10^6$ |
| OLE 3 | $2.1\times10^3$ | $2.3\times10^3$ | $5.7\times10^3$ | $1.5\times10^4$ | $1.7\times10^5$ |
| BHA | $7.25\times10^3$ | $6.1\times10^4$ | $9.5\times10^4$ | $3.5\times10^5$ | $8.1\times10^5$ |

OLE 1,2,3: contained 200,300,400 mg dry olive leaves extract, PHA: contained 200mg butylatedhydroxyanisole, (-) Empty cells mean that the sample was corrupted and exit from the test

**TABLE 8. Changes in sensory evaluation of meat sausage groups during storage at 4 °C for 12 days.**

| Attributes | Period (Day) | Groups | | | | | LSD |
|---|---|---|---|---|---|---|---|
| | | Control | OLE1 | OLE2 | OLE3 | BHA | |
| Color | 0-time | 8.25$^{a}$ | 8.28$^{a}$ | 8.26$^{a}$ | 8.25$^{a}$ | 8.30$^{a}$ | 0.087 |
| | 3 | 6.30$^{c}$ | 7.85$^{b}$ | 8.11$^{ab}$ | 8.20$^{a}$ | 8.25$^{a}$ | 0.27 |
| | 6 | - | 6.25$^{c}$ | 7.65$^{b}$ | 8.15$^{a}$ | 8.18$^{a}$ | 0.26 |
| | 9 | - | - | 7.15$^{b}$ | 8.05$^{a}$ | 8.01$^{a}$ | 0.52 |
| | 12 | - | - | - | 7.95$^{a}$ | 7.91$^{a}$ | 0.49 |
| Taste | 0-time | 8.15$^{a}$ | 8.13$^{a}$ | 8.11$^{a}$ | 8.10$^{a}$ | 8.12$^{a}$ | 0.008 |
| | 3 | 5.80$^{c}$ | 6.86$^{b}$ | 7.65a$^{b}$ | 7.80$^{a}$ | 7.75$^{a}$ | 0.097 |
| | 6 | - | 5.75$^{c}$ | 6.22$^{b}$ | 7.35$^{a}$ | 7.30$^{a}$ | 0.45 |
| | 9 | - | - | 5.35$^{c}$ | 6.92$^{a}$ | 6.88$^{b}$ | 1.208 |
| | 12 | - | - | - | 6.55$^{a}$ | 6.40$^{b}$ | 0.13 |
| Odor | 0-time | 8.18$^{a}$ | 8.21$^{a}$ | 8.21$^{a}$ | 8.22$^{a}$ | 8.20$^{a}$ | 0.10 |
| | 3 | 5.83$^{d}$ | 7.15$^{c}$ | 7.36$^{b}$ | 7.85$^{a}$ | 7.82$^{a}$ | 0.187 |
| | 6 | - | 6.20$^{c}$ | 6.72$^{b}$ | 7.68$^{a}$ | 7.62$^{a}$ | 0.36 |
| | 9 | - | - | 5.38$^{c}$ | 7.01$^{a}$ | 6.85$^{b}$ | 0.120 |
| | 12 | - | - | - | 6.53$^{a}$ | 6.35$^{a}$ | 0.24 |
| Texture | 0-time | 7.75$^{a}$ | 7.80$^{a}$ | 7.83$^{a}$ | 7.85$^{a}$ | 7.82$^{a}$ | 0.15 |
| | 3 | 6.68$^{d}$ | 7.11$^{c}$ | 7.40$^{b}$ | 7.70$^{a}$ | 7.68$^{a}$ | 0.275 |
| | 6 | - | 6.10$^{c}$ | 7.05$^{b}$ | 7.55$^{a}$ | 7.50$^{a}$ | 0.41 |
| | 9 | - | - | 6.90$^{b}$ | 7.28$^{a}$ | 7.20$^{a}$ | 0.294 |
| | 12 | - | - | - | 7.10$^{a}$ | 6.85$^{b}$ | 0.18 |
| Overall Acceptability | 0-time | 8.08$^{a}$ | 8.10$^{a}$ | 8.10$^{a}$ | 8.10$^{a}$ | 8.11$^{a}$ | 0.29 |
| | 3 | 6.283$^{d}$ | 7.253$^{c}$ | 7.630$^{b}$ | 7.887$^{a}$ | 7.875$^{a}$ | 0.11 |
| | 6 | - | 6.07$^{c}$ | 6.91$^{b}$ | 7.68$^{a}$ | 7.65$^{a}$ | 0.63 |
| | 9 | - | - | 6.307$^{b}$ | 7.305$^{a}$ | 7.225$^{a}$ | 0.736 |
| | 12 | - | - | - | 6.92$^{a}$ | 6.73$^{a}$ | 0.40 |

Values followed by the same letter in the same row are not significantly different at P≤ 0.05 OLE 1, 2, 3: contained 200,300,400 mg dry olive leaves extract, BHA: contained 200mg butylatedhydroxyanisole Empty cells (-) mean that the sample was corrupted and exit from the sensory evaluation.

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## تأثير عملية ازالة الطعم المر من أوراق الزيتون على خواصه المضادة للأكسدة والمضاده للميكروبات

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من المعلوم ان مكونات مستخلصات أوراق الزيتون لها تاثير فعال علي بعض الامراض مثل ضغط الدم ،تصلب الشرايين ، والسكري ، والسرطان ، بالإضافة الى فوائد اخرى حيث تعمل كمضادات للالتهابات ومضادات للأكسدة ومضادات للميكروبات.وقد تم في الدراسة الحاليه معامله أوراق الزيتون ببعض المعاملات لتقليل الطعم المر أو التخلص منه حيث تم تقييم أوراق الزيتون المعالجة حسيا كمشروب مماثل للشاي . كما تم استخدام المستخلص الايثانولي المجفف لهذه الأوراق المعالجة (OLE) في اعداد سجق اللحم بديلا عن BHA. وقد أشارت النتائج إلى أن جميع العينات المعالجة والمعدة كشاي أوراق الزيتون سجلت متوسط درجة تقبل عامة تراوحت من ٧,٠٦ إلى ٧,٥٩ مقارنة بعينة الشاي الأخضر. وجد ان العينة رقم ٢ (التي عولجت بحمض الستريك 3%) حققت أعلى قيم نشاط من مضادات الاكسدة DPPH (٣٨,٥٧)٪ والمركبات الفينولية (١٥,٣٥)٪ ولذلك تم استخدامها بتركيزات مختلفة (٢٠٠ .٣٠٠ .٤٠٠مليجرام /كيلوجرام لحوم) في اعداد سجق اللحم. وقد أظهرت عينات السجق المعالجة بـ OLE أقل قيمة من TBA وكذلك العد الكلي للميكروبات مقارنة بمجموعة الكنترول خلال ١٢ يومًا من التخزين على ٤ درجة مئوية. كما أظهرت النتائج ان المجموعة OLE3 (المحتويه علي ٤٠٠مليجرام /كيلوجرام لحوم) تبدو هي الافضل من حيث التقييم الحسي حيث أظهرت عدم وجود فروق معنوية بينها و بين باقي المجموعات او كانت زيادة معنوية لصالحها في جميع الصفات التي تم دراسنها خلال فترة التخزين.