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# Olive Leaf Extract as Natural Antioxidant Additive of Fresh Hamburger Stored at 4°C Running Title: Antioxidants from Olive Leaves in Hamburger

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**Abstract** Oxidation is one of the major problems that cause hamburger deterioration. Antioxidants are used to prevent or delay oxidation process. The chemical preservatives or antioxidants are not safe and have harmful effects to human health. Currently there is a trend to use natural antioxidants in industry since they are considered as safe compared to chemical ones. The objective of this study was to evaluate the usage of olive leaf extract as well as oleuropein as natural antioxidant additives in fresh hamburger stored at 4°C. Results proved the activity of oleuropein and olive leaves extract as natural antioxidants retarded oxidation of hamburger compared to control samples (without antioxidants). 0.5% of oleuropein and 1.5% of olive leaves extract is the best concentration to be used in fresh hamburger.

***Keywords:*** *olive leaf extract, lipid oxidation, chilling storage, beef burger quality, natural antioxidants*

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## 1. Introduction

The quality attributes of meat products deteriorate due to the lipid oxidation during processing and storage. Lipid oxidation is responsible for development of primary and secondary oxidation products, reduction in nutritional quality, as well as changes in flavor [1], which can precipitate health hazards and economic losses in terms of inferior product quality [2]. Lipid oxidation is a rather complex process whereby the unsaturated fatty acid fraction of membrane phospholipids is oxidized, and hydroperoxides are formed which are further susceptible to oxidation or decomposition to secondary oxidation products, such as short-chain aldehydes, ketones, and other oxidized compounds that may adversely affect the overall quality and acceptability of meat and meat products.

Meat contains high number of prooxidants such as, heme groups, and transition metals and also unsaturated fatty acids which, by virtue of their double bonds, are prone to oxidation [3]. Oxidation ultimately results in breakdown products which produce off-odors and off-flavors (rancid, warmed-over, cardboard, and grassy) with consequent decrease in nutritional quality and safety. This is a particular problem in pre-cooked, frozen, re-heated meat products because heat, added salt and processing can initiate the oxidation process [3]. Antioxidants are added to fresh and processed meat and meat products to prevent lipid oxidation, retard development of off-flavors, and improve color stability. In the food industry, they can be divided into natural and synthetic antioxidants. Synthetic antioxidants have been confirmed for their toxicological and carcinogenic effects. Awareness about the harmful effects of these chemicals in food is increasing. Meanwhile, natural preservatives offer greater advantages due to their non-toxic nature along with a wide range of health benefits [4].

Plants such as fruits, vegetables, herbs, spices and teas are major sources of natural bioactive compounds such as antioxidants, where a large diversity of phenolic compounds are present [5]. Antioxidants can prevent lipid peroxidation using the following mechanisms: preventing chain inhibition by scavenging initiating radicals, breaking chain reaction, decomposing peroxides, decreasing localized oxygen concentrations and binding chain initiating catalysts, such as metal ions [5]. There are a large number of chemical substances that possess antioxidant activity, but only a few can be used in food products [5]. This effectively minimizes rancidity, retards lipid oxidation, without any damage to the sensory or nutritional properties, resulting in maintaining quality and shelf-life of meat products.

The polyphenolic compounds extracted from leaves and olive fruits are excellent antimicrobial and antioxidant agents [6]. The most abundant phenolic component is oleuropein which gives the bitter taste to olive and olive oil. Olive leave extracts has been associated with health benefits and preservation of food rich in unsaturated fats

[6]. Leaves from olive tree, are rich in biophenols (BPs), such as oleuropein, verbascoside, ligostroside, tyrosol or hydroxytyrosol [7,8]. These compounds have shown several biological activities such as antioxidant and antimicrobial, and consequently can be used in food application [9]. Oleuropein is the most abundant phenolic compound in olive leaves and fruits and is responsible for the characteristic bitterness of olive fruit [9]. Health benefits of this compound have been extensively investigated. It has been reported that oleuropein, and related compounds such as tyrosol, verbascoside, ligostroside, and demethyoleuropein, act as antioxidants by preventing the formation of free radicals by its ability to chelate metals such as copper and iron, which catalyze free radical generation reactions such as lipid oxidation [10]. In addition it lowers the risk of coronary diseases, several cancers, and could have antimicrobial and antiviral activity. In addition, oleuropein has been reported to repel insects, and protect against pathogens [11].

Synthetic antioxidants, such as butylated hydroxytoluene (BHT), were extensively used to delay, retard, or prevent the lipid oxidation by scavenging chain-carrying peroxyl radicals or suppressing the formation of free radicals. However, because of the concern over the safety of these synthetic compounds, extensive work is being carried out to find novel and naturally occurring compounds to delay the oxidative degradation of lipids, improve quality, and maintain the nutritional value of foods [12,13]. Therefore, this study sought to evaluate whether the addition of olive leaves extract (OLE) and pure oleuropein could retard lipid peroxidation in bovine hamburger.

# 2. Material and Methods

## 2.1. Materials and Chemicals

Olive leaves were obtained from West Bank in April 2015, air dried for 10 days and grinded using normal grinder to get powdered olive leaves. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrochloric acid, sodium erythorbate, and ethanol were from Sigma-Aldrich company (Sigma-Aldrich, St. Louis, MO, USA).

## 2.2. Extraction of Olive Leaves

Ten grams of dried olive leaves were placed in a soxhlet thimble in a Soxhlet apparatus and were extracted with 200 ml of 80% ethanol for 2 h at 60 °C. Then, the extracts were cooled to room temperature, and filtered through a Whatman No.1 filter to separate coarse particles from the solution. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum for 2 h. The concentrated extracts were stored in a refrigerator at 4°C until use [14].

## 2.3. Concentrations of Pure Oleuropein and OLE

Oleuropein was obtained from Hunan Kang Biotech company, China. Three concentrations from Oleuropein and OLE (0.5%, 1.0%, 1.5%) were prepared to be used.

## 2.4. Hamburger Preparation and Experimental Design

The packed vacuum frozen boneless beef were thawed until zero temperature at the core, the meat was broken down with a mixer machine (disc 4.5 mm). Fat was minced by using mincer machine (disc 1 mm), and was added to the meat with the spices (salt, pepper) and onions. Then the mixture was homogenized by mixing it for 3 min.

The mixture was divided into seven batches: control and treated samples. Six treated samples were mixed with 0.5, 1, 1.5% oleuropein (w/v) and 0.5, 1, 1.5% OLE. Control sample was used without preservative. Then the samples were cooled to -1 °C and formed, finally the samples were stored in refrigerant at 4 °C for 20 days.

## 2.5. Determination of Lipid Oxidation

Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS). TBARs values were determined on fat basis according to the slightly modified method of Aytul (2010) [15]. Meat sample (5 g) was homogenized with 20 mL tri-chloroacetic acid solution (15% w/v) and then centrifuged at 3000$g$ for 10min.The supernatant (2 mL) was mixed with 2 mL thiobarbituric acid solution (0.1% w/v in double distillated water) followed by heating in a water bath at 100◦C for 30 min and then cooling to room temperature. Therefore, TBARS were extracted in chilled atmosphere. The absorbance of each extract was measured at 520 nm in a spectrophotometer (spec 1650PC, Shimadzu, Japan). Malondialdehyde (1,1,3,3- tetraethoxypropane) was used to develop the standard curve for TBARS assay. TBARS values were reported as mg of malonaldehyde per kg of hameburger.

## 2.6. Determination of pH of Hamburger Samples

pH of the hamburger samples was determined using HI 2210 pH meter by insertion the pH electrode into the meat sample.

## 2.7. Statistical Analysis

All the measurements were replicated three times and the data are presented as mean ± SD. The effects of natural antioxidant extracts addition were analyzed and the obtained data were subjected to analysis of variance (ANOVA) accompanied with Duncan test using SPSS software (SPSS Inc., Chicago) to identify the significance ($p < 0.05$) between means of treatments.

# 3. Results and Discussion

## 3.1. Effect of Oleuropein on the Oxidation of Fresh Hamburger

Usually fresh hamburger is consumed within 5-7 days of refrigeration (4°C) without any preservatives or antioxidant. The effectiveness of oleuropein was found different according to the oleuropein concentration added. Table 1 shows the amounts of oxidation products of fresh

hamburger samples treated with different concentrations of oleuropein from day 1 to day 21 during storage period (1-21 days). The amounts of oxidation products for control hamburger samples and treated ones (with 0.5, 1, and 1.5%) increases with storage period (from day 1 to day 21) indicating that oxidation increases with time (Table 1). However, the increase in the oxidation products of control hamburger sample was higher than that for treated samples at 0.5, 1, and 1.5% oleuropein.

Comparing the amounts of oxidation products for fresh samples after one week (46 mg/kg) with that for treated hamburger samples after three weeks (51 mg/kg, 39.1 mg/kg, and 30.1 mg/kg for samples treated with 0.5%, 1%, and 1.5% oleuropein, respectively), indicated that oxidation products of treated hamburger samples after 3 weeks was almost similar (in the case of 0.5% oleuropein) or lower (in the case of 1.0% and 1.5%) to oxidation products of fresh product after one week, see Table 1. This result indicates the prolongation of shelf life of fresh hamburger.

The increase of oxidation products in the fresh (control and treated) hamburger was expected, since the refrigeration temperature (4°C) is not cold enough to retard lipid oxidation, even though the different concentration of oleuropein retard the rate of oxidation, but the oxidation continues during cold storage.

## 3.2. Optimum Concentration of Oleuropein in Fresh Hamburger Samples

At day one, results showed that there is difference between oxidation products for control hamburger samples and treated ones with 0.5, 1.0, and 1.5% oleuropein where the amounts of oxidation products for control samples are higher than those for treated ones. Additionally, there is difference between the amounts of oxidation products of treated hamburger samples themselves where the amounts of oxidation products decreases significantly as the concentration of oleuropein increases in fresh hamburger samples, and so the best concentration of oleuropein is 1.5% at day one.

At day 7, difference between oxidation products for control hamburger samples and treated ones was observed where the amounts of oxidation products for control samples are higher than those for treated ones. Regarding the samples treated with oleuropein, there are no differences between the amounts of oxidation products treated with 0. 5% and 1% oleuropein, but there is difference between the amounts of oxidation products of samples treated with 0.5 or 1.0% and those treated with 1.5% where the amounts of oxidation products of hamburger treated with 1.5% is lower than those treated with 0.5% or 1% indicating that 1.5% is the best concentration at day seven.

At days 13, 17, and 21, and as for day one and seven there is statistical difference between oxidation products for control hamburger samples and treated ones with 0.5, 1.0, and 1.5% oleuropein where the amounts of oxidation products for control samples is higher than those for treated ones. Additionally there is statistical difference between the amounts of oxidation products of treated hamburger samples themselves where the amounts of oxidation products decreases significantly as the concentration of oleuropein increases in fresh hamburger samples, and so the best concentration of oleuropein is 1.5% at days 13, 17, and 21.

## 3.3. Effect of Olive Leaves Extract (OLE) on the Oxidation of Fresh Hamburger

Table 2 shows the effect of addition of OLE (0.5, 1.0 and 1.5%) on the lipid oxidation of fresh hamburger stored at 4°C.

**Table 1. Effect of oleuropein on TBARS values of hamburger during storage at 4°C**

| Storage period (Days) | mg MDA/ kg hamburger | | | |
|---|---|---|---|---|
| | Control | 0.5% oleuropein | 1% oleuropein | 1.5% oleuropein |
| 1 | 28.5±0.59 aE | 17.2±0.52bE | 14.2±1.3 cE | 10.9±0.37 dE |
| 7 | 46.6±3.2 aD | 17.4 ±1.4bD | 18.1±0.1 bD | 14.9±0.1 cD |
| 13 | 89.6±0.27 aC | 46.9±0.28 bC | 27.2±0.3 cC | 24.6±4.3 dC |
| 17 | 96.1±0.58 aB | 48.9±0.96 bB | 34.2±0.2 cB | 29.3±1.6 dB |
| 21 | 107.4±0.27 aA | 51.6 ±0.49bA | 39.1±0.3 cA | 30.1±0.32 dA |

- Small letters indicates differences in the amounts of oxidation products for control sample and treated ones (0.5, 1.0, and 1.5%) at each storage time.
- Capital letters indicate significant differences between amounts of oxidation products as storage time increases (from day 1 to 21).
- Data represent averages of three independent repeats ± standard deviation.

**Table 2. Effect of OLE on TBARS values of hamburger during storage at 4°C.**

| Storage period (Days) | mg MDA/kg hamburger | | | |
|---|---|---|---|---|
| | Control Sample | 0.5% OLE | 1% OLE | 1.5% OLE |
| 1 | 28.5±0.59 aE | 20.0±0.68dE | 21.1±0.75cE | 28.9b±1.8E |
| 7 | 46.6±3.2 aD | 20.7±2dD | 23.3±0.43cD | 32.7±0.96bD |
| 13 | 89.6±0.27 aC | 40.4±1.5dC | 42.8±0.2cC | 60.5±0.07bC |
| 17 | 96.1±0.58 aB | 64.5±0.53dB | 68.9±0.47cB | 73.8±0.19bB |
| 21 | 107.4±0.27 aA | 75.8±0.36dA | 80.1±1.6cA | 104.4±1.7bA |

- Small letters indicates differences in the amounts of oxidation products for control sample and treated ones (0.5, 1.0, and 1.5%) at each storage time.
- Capital letters indicate significant differences between amounts of oxidation products as storage time increases (from day 1 to 21).

Table 2 shows the amounts of oxidation products of fresh hamburger samples treated with different concentrations of OLE from day one to day twenty one. During storage period, the amounts of oxidation products for control hamburger samples and treated ones (with 0.5, 1, and 1.5%) increases with storage period (from day 1 to day 21) indicating that oxidation increases with time (Table 2). However, the increase in the oxidation products of control hamburger sample (without additive) was higher than that for treated samples (0.5, 1.0, and 1.5%).

There is significant difference ($P<0.05$) between amounts of oxidation products as storage time increases from day 1 to day 21 for control, as well as for treated samples with 0. 5%, 1%, and 1.5% (Table 2).

In contrary to the results obtained for oleuropein, higher concentrations of OLE (1 or 1.5%) is not recommended to be used in fresh hamburger samples as higher oxidation products are obtained compared to 0.5%. This may be explained by presence of polyphenol in OLE which have pro-oxidant activity under certain conditions such as at high concentrations or in the presence of metal ions [16,17]. Polyphenols and particularly flavonoids are examples of substances with such dual behavior [18,19]. Phenolic acids have also been reported as pro-oxidants [19,20].

### 3.4. Effect of OLE on the Shelf Life of Fresh Hamburger

Comparing the amounts of oxidation products for fresh samples (control, where the amounts of oxidation products is 46 mg/kg) with that for treated hamburger samples after about two weeks (40.4 mg/kg using 0.5% OLE) indicates that oxidation products of treated hamburger samples after 2 weeks is lower than oxidation products of fresh one after one week.

### 3.5. Optimum Concentration of OLE in Fresh Hamburger Samples

At day 1, 7, 13, 17, and 21, results showed that there is difference between oxidation products for control hamburger samples and treated ones with 0.5, 1.0, and 1.5% OLE where the amounts of oxidation products for control samples are higher than those for treated ones indicating the activity of OLE as antioxidant. Additionally there is difference between the amounts of oxidation products of treated hamburger samples themselves where the amounts of oxidation products increases significantly as the concentration of OLE increases in fresh hamburger samples, and so the best concentration of OLE is 0.5% from day 1 until day 21 of storage.

Comparing this result with that obtained from oleuropein, higher concentration of oleuropein is needed to preserve fresh hamburger samples for three weeks, while 0.5% of OLE is enough for preservation of fresh hamburger samples up to two weeks. This may be attributed to high prooxidant activity of OLE compared to oleuropein.

### 3.6. Effect of addition Oleuropein and OLE on the pH of Fresh Hamburger Samples

pH of control hamburger samples as well as treated samples with oleuropein and OLE was measured from day 1 to 21 to study the effect of addition of oleuropein or OLE on the pH of hamburger, see Table 3. Statistical analysis showed that there is no difference between the pH of control hamburger sample and those treated with oleuropein or OLE indicating that addition of oleuropein or OLE to fresh hamburger does not affect the pH of hamburger.

**Table 3. pH of control hamburger samples and treated samples with Oleuropein and OLE.**

| Type of antioxidant | Storage time | concentration | | | |
|---|---|---|---|---|---|
| | | Control | 0.5% | 1% | 1.5% |
| Oleuropein | Day1 | 5.91 | 5.86 | 5.88 | 5.99 |
| | Day 7 | 5.96 | 5.87 | 5.90 | 5.96 |
| | Day 13 | 5.89 | 5.90 | 5.78 | 5.92 |
| | Day 17 | 5.92 | 5.78 | 5.74 | 5.85 |
| | Day 21 | 5.94 | 5.72 | 5.72 | 5.82 |
| OLE | Day1 | 5.91 | 5.82 | 5.89 | 5.90 |
| | Day 7 | 5.96 | 5.89 | 5.88 | 5.89 |
| | Day 13 | 5.84 | 5.93 | 5.90 | 5.88 |
| | Day 17 | 5.92 | 5.91 | 5.85 | 5.76 |
| | Day 21 | 5.94 | 5.92 | 5.86 | 5.82 |

## 4. Conclusion

The olive leaf extract is a major source of polyphenols which can be used in many types of food such as meat products as an alternative to chemical preservatives and antioxidants. Olive leaves extract and its major phenolic compound oleuropein were used in fresh hamburger as natural antioxidant. Comparison between the effect of oleuropin and OLE on the rate of oxidation was done with the objective of determining the best concentration to be used. Oleuropein and OLE extended the shelf life of hamburger samples and delayed oxidation compared to non-treated sample. The best concentration of oleuropein used was 1.5%, while 0.5% OLE showed the best result. Oleuropein or OLE had no effect on hamburger pH during storage time. Oleuropein and OLE is an effective natural antioxidant, as alternative to chemical antioxidant and further studies must be done so as to study its antimicrobial activities.

## Statement of Competing Interests

The authors have no competing interests.

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