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Oleuropein from Olive Leaf Extract as Natural Antioxidant of Frozen Hamburger

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Abstract: Oxidation is one of the major causes of hamburger deterioration. Antioxidants are used to minimize oxidation process. There is a growing interest in the substitution of synthetic food antioxidants by natural ones from vegetable sources. In meat industry, sodium erythorbate is antioxidant that is usually used and is an example of chemical antioxidant. Effect of olive leaf extract rich in oleuropein on the quality of frozen hamburger was investigated. The objective of this study was to evaluate the usage of oleuropein from olive leaf extract as natural antioxidant in frozen hamburger stored at -12 °C compared with sodium erythorbate. Results suggested that olive leaf extracts might be useful to the meat industry as an efficient alternative to synthetic antioxidants by retarding oxidation of hamburger compared with sodium erythorbate 0.5% of oleuropein and 0.5% of sodium erythorbate are the best concentrations to be used in frozen hamburger.

Key words: Oleuropein, olive leaf extract, lipid oxidation, frozen storage, beef burger quality, natural antioxidants.

1. Introduction

Oxidation is one of the most important processes occurring in food systems. It affects many interactions among food constituents, leading to undesirable products. Food lipids are food components that are very susceptible to oxidation processes, therefore oxidation reactions are one of the major sources of deterioration that occurs during manufacturing, storage, distribution and final preparation of foods [1]. Lipid oxidation, leading to rancidity and deleterious changes in foods causes not only loss of flavor or development of off-flavors, but also loss of color, nutrient value, and the accumulation of compounds, which may be detrimental to the health of consumers. Lipid oxidation products are ubiquitous in foods, although levels of these compounds are generally low, the problem of lipid oxidation severely compromises the quality of some food products and limits the shelf-life of others. All foods that contain lipids, even at a very low level (< 1%), are susceptible to oxidation [1].

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 [2], which can precipitate health hazards and economic losses in terms of inferior product quality [3]. 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

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unsaturated fatty acids which, by virtue of their double bonds, are prone to oxidation [4]. The different applied pretreatments in the production of burgers, such as mincing, cooking, and salt addition, can initiate the oxidation process [4] by enhancing the formation of reactive oxygen species. This is a particular problem in pre-cooked, frozen, re-heated meat products; leading to highly vulnerable product to oxidation [4]. 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. 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 [5].

TBARS method (thiobarbituric acid) is one of the most commonly used methods to determine lipid oxidation and assess the quality of fats and fat-containing foods, and is based on the measurement of the absorbance of TBA-malondialdehyde complex at 532-535 nm. Malonodialdehyde is a three carbon dialdehyde being one of intermediates formed in the oxidation of lipids [1].

A considerable number of studies had been done to assess the antioxidant activity of many herbs, spices and their extracts. Natural plants and plant extracts contain large diversity of phenolic compounds [6] that have anti-oxidative effects. 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 [6]. 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.

Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases such as cancer. In the last few years, the identification and development of phenolic compounds or extracts from different plants has become a major area of health- and medical-related research [6, 7]. The polyphenolic compounds extracted from leaves and olive fruits are excellent antimicrobial and antioxidant agents [7]. The most abundant phenolic component is oleuropein (Fig. 1) which gives the bitter taste to olive and olive oil. Olive leave extracts have been associated with health

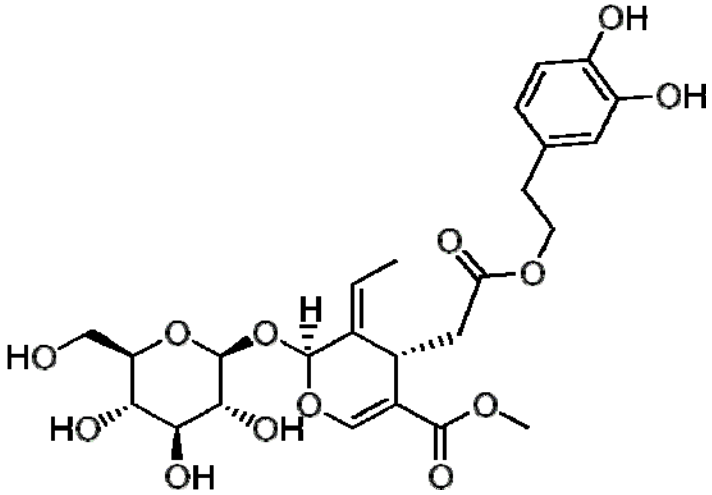

Fig. 1 Structure of oleuropein.

benefits and preservation of food rich in unsaturated fats [7]. Leaves from olive tree, are rich in biophenols (BPs), such as oleuropein, verbascoside, ligostroside, tyrosol or hydroxytyrosol [8, 9]. These compounds have shown several biological activities such as antioxidant and antimicrobial, and consequently can be used in food application [10]. 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 [11]. In addition it lowers the risk of coronary diseases, several cancers, and could have antimicrobial and antiviral activity [11].

Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and sodium erythorbate 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, the objective of this study was to evaluate the effect of the addition of oleuropein from olive leaves extract (OLE) on lipid peroxidation in bovine hamburger, and to compare it with sodium erythorbate which is used as synthetic additive to frozen hamburger.

2. Materials and Methods

2.1 Materials and Chemicals

Sodium erythorbate, thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrochloric acid, and ethanol were obtained from Sigma-Aldrich company (Sigma-Aldrich, St. Louis, MO, USA).

2.2 Concentrations of Oleuropein and Sodium Erythorbate

Oleuropein was obtained from Hunan Kang Biotech company, China. Three concentrations from oleuropein and sodium erythorbate (0.25%, 0.5%, 0.75%) were prepared to be used.

2.3 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 0.25%, 0.5%, 0.75% oleuropein (w/v) and 0.25%, 0.5%, 0.75% sodium erythorbate. Control sample was used without preservative. Then the samples were cooled to -1 °C and formed, finally the samples were stored in freezer at -12 °C for 6 months.

2.4 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 [14]. Meat sample (5 g) was homogenized with 20 mL tri-chloroacetic acid solution (15% w/v) and then centrifuged at 3,000 g for 10 min. 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 1650 PC, 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 malondialdehyde per kg of hamburger.

2.5 Statistical Analysis

All 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 Frozen Hamburger

Usually frozen hamburger is consumed within 6 months of frozen storage. The effectiveness of oleuropein was found different according to the oleuropein concentration added. Table 1 shows the amounts of oxidation products of frozen hamburger samples treated with different concentrations of oleuropein from day one to six months. The amount of oxidation products for control hamburger samples and treated ones (with 0.25%, 0.5%, and 0.75%) increases with storage time (from day 1 to six months) indicating that oxidation increases with time. This was also shown by Al-Rimawi et al. [15] using olive leaf extracts and oleuropein in fresh hamburger. However, the hamburger sample was higher than that for treated increase in the oxidation products of control samples (0.25%, 0.5%, and 0.75%).

Results showed that, the amount of the oxidation products ($p \leq 0.05$) increases significantly as storage time increases from day one to three months for control hamburger samples as well as for samples treated with 0.25% oleuropein. While from 3 to 6 months of storage, there is no significant difference between the amounts of oxidation products of these hamburger samples (control or those treated with 0.25% oleuropein), which indicates that the amounts of oxidation products after three months become almost constant for hamburger samples (control) or for those treated with 0.25% of oleuropein. For hamburger samples treated with 0.5% or 0.75% oleuropein, the results showed that the amounts of oxidation products increase significantly as storage time increases from day one to four months, while after four months the amounts of oxidation products do not change significantly.

3.2 Optimum Concentration of Oleuropein in Frozen Hamburger

After first day and one week of storage, there is no statistical difference between the amounts of oxidation products of the different oleuropein concentrations and the control, which implies that oxidation of hamburger is not high at this time of storage.

Table 1 Effect of oleuropein on TBARS values of frozen hamburger during storage at -12 °C.

Storage period	mg MDA/kg hamburger			
	Control	0.25% oleuropein	0.5% oleuropein	0.75% oleuropein
First day	20.6 ± 3.62 aE	17.9 ± 1.63 aE	20.4 ± 2.98 aF	17.3 ± 2.1 aF
One week	29.6 ± 1.49 aD	26.8 ± 1.32 aD	26.5 ± 1.2 aE	26.4 ± 2.4 aE
One month	158.7 ± 12.7 aC	117 ± 23.7 cC	100.1 ± 4.02 dD	132.3 ± 23.9 bD
Two months	263.9 ± 1.69 aB	178.2 ± 1.4 bB	165.9 ± 1.6 cC	199.4 ± 1.99 dC
Three months	266.2 ± 0.33 aA	258.3 ± 0.4 bA	243.3 ± 1.1 cB	247.2 ± 0.27 dB
Four months	266.6 ± 0.48 aA	255.3 ± 0.22 bA	247.1 ± 0.42 cA	253.9 ± 5.99 dA
Five months	266.8 ± 1.08 aA	256.2 ± 1.2 bA	247.5 ± 0.52 cA	256.3 ± 4.8 dA
Six months	267.9 ± 0.06 aA	258.8 ± 0.58 bA	248.2 ± 0.5 cA	255.1 ± 3.5 dA

Small letters indicate differences in the amounts of oxidation products for control sample and treated ones (0.25%, 0.5%, and 0.75%) at each storage time.

Capital letters indicate significant differences between amounts of oxidation products as storage time increases (from day 1 to six months).

After one month as well as two, three months of storage, results showed that there is statistical difference between the amounts of oxidation products of control hamburger samples and those treated with oleuropein (0.25%, 0.5%, and 0.75%), indicating that the amount of oxidation products of control hamburger samples is significantly higher than those treated with oleuropein. Regarding the best concentration of oleuropein in frozen hamburger at one month of storage, it was found that 0.5% of oleuropein is the best concentration as it gave lowest amounts of oxidation products. Regarding 0.25% concentration, it protected hamburger from oxidation but its concentration is not enough, while 0.75% showed higher oxidation products compared to 0.25% or 0.5%. This trend may be explained by the fact that antioxidants at high concentrations can work as prooxidants which induce oxidation.

After four, five, and six months there is statistical difference between different concentrations, and the lowest oxidation occurs when using 0.5% oleuropein. In conclusion, to protect frozen hamburger samples from oxidation for six months, it is recommended to use 0.5% oleuropein as natural antioxidant.

3.3 Effect of Sodium Erythorbate on the Oxidation of Frozen Hamburger

Table 2 shows the effect of addition of sodium erythorbate (0.25%, 0.5% and 0.75%) on the lipid oxidation of frozen hamburger stored at -12 °C. The amount of oxidation products ($p < 0.05$) for control hamburger samples as well as for those treated with 0.25% sodium erythorbate increases from day one to two months, but after two months the amounts of oxidation products do not change significantly as storage time increases (for month two to six). For those treated with 0.5% and 0.75% sodium erythorbate, the amount of oxidation products increases significantly from day one to six months of storage. These results can be explained, by the low concentration of sodium erythorbate, which is not sufficient to chelate all the catalyst present in meat products, which may be Heme groups from myoglobin and copper and zinc present in meat tissues.

3.4 Optimum Concentration of Sodium Erythorbate in Frozen Hamburger

After first day and one week, there are no statistical differences between each pair of concentration and control, which implies that oxidation is not high at this early stage of storage and antioxidant is not highly needed at this stage. After one month, 2 and 3 months of storage, results showed that there are statistical differences between the different concentration used, and the least oxidation rate occurs when using 0.5% sodium erythorbate. At 4, 5, and 6 months of storage, there is statistical difference between the amounts of oxidation products of each pair of concentration and the control, except between 0.25% and 0.75%, and the least oxidation products occur when using 0.5% sodium erythorbate.

3.5 Comparison between Oleuropein and Sodium Erythorbate as Antioxidant

Comparing the result obtained from oleuropein and sodium erythorbate, the best concentration to preserve frozen hamburger for six months was 0.5% of oleuropein or sodium erythorbate.

The amount of oxidation products in samples treated with 0.25% and 0.75% oleuropein or sodium erythorbate was considerably higher than that of hamburger treated with 0.5% oleuropein or 0.5% sodium erythorbate throughout the frozen storage. Higher level of oxidation at 0.25% oleuropein or 0.25% sodium erythorbate concentration may be explained by the considerably lower concentration of antioxidant material within samples. Phenolics in these samples may be enough to neutralize metal ions to some point. However, it may also reduce ions such as Fe(III) to their most active pro-oxidative state as Fe(II) [16] , and there may not be enough antioxidant in the media to neutralize these pro-oxidants.

Table 2 Effect of sodium erythorbate on TBARS values of frozen hamburger during storage at -12 °C.

Storage period	mg MDA/kg hamburger			
	Control sample	0.25% OLE	0.5% OLE	0.75% OLE
First day	20.6 ± 3.62 aE	18 ± 0.44 aE	20.8 ± 1.07 aH	21.4 ± 0.88 aH
One week	29.6 ± 1.49 aD	27.5 ± 0.21 aD	27.8 ± 0.69 aG	29.6 ± 5.4 aG
One month	158.7 ± 12.7 aC	101.9 ± 0.96 bC	41.9 ± 3.9 dF	51.8 ± 3.04 cF
Two months	263.9 ± 1.69 aB	157.7 ± 2.4 bB	100.7 ± 3.3 dE	106.4 ± 1.2 cE
Three months	266.2 ± 0.33 aA	245.7 ± 0.7 bA	179.1 ± 1.7 dD	187.9 ± 1.8 cD
Four months	266.6 ± 0.48 aA	247.9 ± 0.74 bA	214.3 ± 2.1 cC	245.2 ± 0.27 bC
Five months	266.8 ± 1.08 aA	248.4 ± 1.9 bA	238.4 ± 0.11 cB	247.9 ± 0.74 bB
Six months	267.9 ± 0.06 aA	254.9 ± 0.64 bA	247.4 ± 1.8 cA	261.2 ± 0.74 bA

Small letters indicate differences in the amounts of oxidation products for control sample and treated ones (0.25%, 0.5%, and 0.75%) at each storage time.

Capital letters indicate significant differences between amounts of oxidation products as storage time increases (from day 1 to 6 months).

High level of oxidation at 0.75% oleuropein or sodium erythorbate may be explained by the high concentration of these antioxidants which act as pro-oxidants in which they induce oxidation.

This trend of prooxidant was also observed for ascorbic acid and gallic acid [17]. It is reported that higher concentration of antioxidant may cause production of more reactive substances while reducing metal ions, and may not pace with this rapidity and then end up with higher oxidation levels. This consideration may be the answer for why 0.5% oleuropein and 0.5% sodium erythorbate treatment gave better results than 0.75% oleuropein or sodium erythorbate treatment in oxidative stability of frozen hamburger.

Statistical analyses were also conducted using independent sample *t*-test to test the differences between oleuropein and sodium erythorbate at each concentration level. Statistical analyses showed that there are no significant differences between the means of oxidation for oleuropein and sodium erythorbate at 0.25%, 0.5%, and 0.75% concentrations, indicating that oleuropein can be used as sodium erythorbate which is used widely in meat products as antioxidant.

4. Conclusion

Oleuropein can be used in meat products (frozen hamburger) as an alternative to chemical antioxidants. Comparison between the effect of oleuropein and sodium erythorbate on the rate of oxidation was done with the objective of determining the best concentration to be used. Oleuropein was found to extend shelf life of hamburger samples and delay oxidation as sodium erythorbate. The best concentration of oleuropein and sodium erythorbate used was 0.5%. Oleuropein is effective as natural alternative antioxidant 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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