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Antioxidant potential of olive leaf extract and oleuropein in fresh and frozen Hamburger

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Antioxidant potential of olive leaf extract and oleuropein in fresh and frozen Hamburger

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This Thesis is dedicated to memory of my father,

and to my wonderful mother,

Who have raised me to be the person I am today.

Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledges, and that this thesis (or any part of the same) has not been submitted for the higher degree to any other university or institute.

Signed……

Mohammad Sadi Tarawa

Date 21/5/2016

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Abstract

Oxidation is one of the major reasons that cause hamburger deterioration. Antioxidants are used to prevent or delay oxidation process. Chemical antioxidants are used usually in meat industry; Sodium Erythorbate is an example of such antioxidants. These chemical preservatives 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. In this study natural antioxidants (Oleuropein and OLE) were used in fresh and frozen hamburger and compared to Sodium Erythorbate which is used widely in frozen hamburger. Thiobarbituric acid-reactive substances (TBARS) spectrophotometric method was used for determination of oxidation products of hamburger samples (expressed as mg of Malonodialdehyde (MDA) per Kg of hamburger sample). Results of this study showed that Oleuropein can be used as natural antioxidants with similar affects as synthetic antioxidants (e.g. Sodium Erythorbate), where statistical analyses showed that there is no significant differences between the amounts of oxidation products of hamburger samples treated with Oleuropein and those treated with Sodium Erythorbate, indicating that Oleuropein works as an antioxidant as Sodium Erythorbate. This study showed also that 0.5% of Oleuropein or Sodium Erythorbate is the best concentration to be used in frozen hamburger while most meat companies use 1.5% of Sodium Erythorbate as antioxidant for frozen hamburger. Regarding fresh hamburger samples, results proved the activity of Oleuropein and OLE as natural antioxidant where they retarded oxidation of hamburger compared to control samples (without antioxidants) and results showed that 0.5% of Oleuropein and 1.5% of OLE is the best concentration to be used in fresh hamburger samples.

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Abbreviations

OLE	Olive leave extract
TBARS	Thiobarbituric acid-reactive substances
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
HCL	Hydrochloric acid
MDA	Malondialdehyde

Chapter One

Introduction

1.Introduction

The problem of food preservation has grown more complex as new food products are frequently being introduced to the market, requiring longer shelf life and greater assurance of protection from microbial and chemical deterioration. Consumers are becoming more aware of their food choices and tend to consume natural foods or minimally processed foods as healthier alternatives (Sullivan, 2011). Uses of preservatives as a chemical process for food preservation is widely used. Preservatives are natural or synthetic substances that are added to food items, cosmetics and pharmaceuticals in order to increase their shelf life and maintain their quality and safety by inhibiting, retarding or arresting their oxidation, fermentation, acidification, microbial contamination (Anand et al., 2013). Artificial preservatives are cheap chemical substances that are effective and stable, however they have certain disadvantages including possible toxicological 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 (Hamid et al., 2010) .

1.1. Meat

Meat is defined as the flesh of animals used as food. The term fresh meat includes meat from recently processed animals as well as vacuum-packed meat or meat packed in controlled-atmospheric gases, which has not undergone any treatment other than chilling to ensure preservation. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens, in addition to other physico-chemical deteriorations.

Meat contains a number of unsaturated fatty acids, which, by virtue of their double bonds, are prone to oxidation. Oxidation ultimately results in breakdown products which produce off-odors and off-flavors (rancid, warmed-over, cardboard, 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 (Brewer, 2008).

1.2. Meat preservation

The processes used in meat preservation are principally concerned with inhibiting microbial spoilage, although other methods of preservation are sought to minimize other deteriorative changes such as color and oxidative changes.

A number of interrelated factors influence the shelf life and keeping quality of meat, specifically holding temperature, atmospheric oxygen (O_2), endogenous enzymes, moisture (dehydration), light and, most importantly, micro-organisms. All of these factors, either alone or in combination, can result in detrimental changes in the color, odor, texture and flavor of meat (Faustmann & Cassens, 1990). Although deterioration of meat can occur in the absence of micro-organisms such as proteolysis, lipolysis and oxidation. Traditionally, methods of meat preservation may be grouped into three broad categories based on control by temperature, by moisture and, more directly, by inhibitory processes . (Lawrie & Ledward, 2006).

The most investigated new preservation technologies for fresh meat are non-thermal inactivation technologies such as high hydrostatic pressure (HHP), new packaging systems such as modified atmosphere packaging (MAP) and active packaging (AP), and natural

antimicrobial compounds and biopreservation. All these alternative technologies are mild process, energy saving, environmentally friendly and guarantee natural appearance.

Plants such as fruits, vegetables, herbs, spices and teas are major sources of natural preservatives such as antioxidants, where a large diversity of phenolic compounds are present. Olive tree (Olea Eurepaea) belongs to the family of oleacea. Olive leaves are by- products from olive trees cultivation procedures and from olive oil mills. 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 leave extracts has been associated with health benefits and preservation of food rich in unsaturated fats (Sikora et al., 2008).

Chapter Two

Literature review

2. Literature review

2.1. Oxidation

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 (Wsowicz et al., 2004).

Oxidation, leading to rancidity and deleterious changes in foods caused by lipid oxidation include 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 much variation exists in their kind and levels present. 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 (Wsowicz et al., 2004).

In living cell Oxidation metabolism result in the formation of free radical (Antolovich at al., 2002; Pourmorad et al., 2006), which are defined as high energy atoms with unpaired electron (Madhavi et al., 1996).

Although they are unwanted metabolic by-products, they are continuously released by aerobic metabolisms (Mantle et al., 2000). Free radicals can also be produced by light energy, photochemical smog, tobacco products, polyunsaturated fats (as in deep fried foods), alcohol,

radiation, physical stress that leads depletion of immune system antioxidants, modification of proteins caused by gene expression changes (Pourmorad et al., 2006). Figure l. summarizes the possible causes and effects of free radicals on living cells.

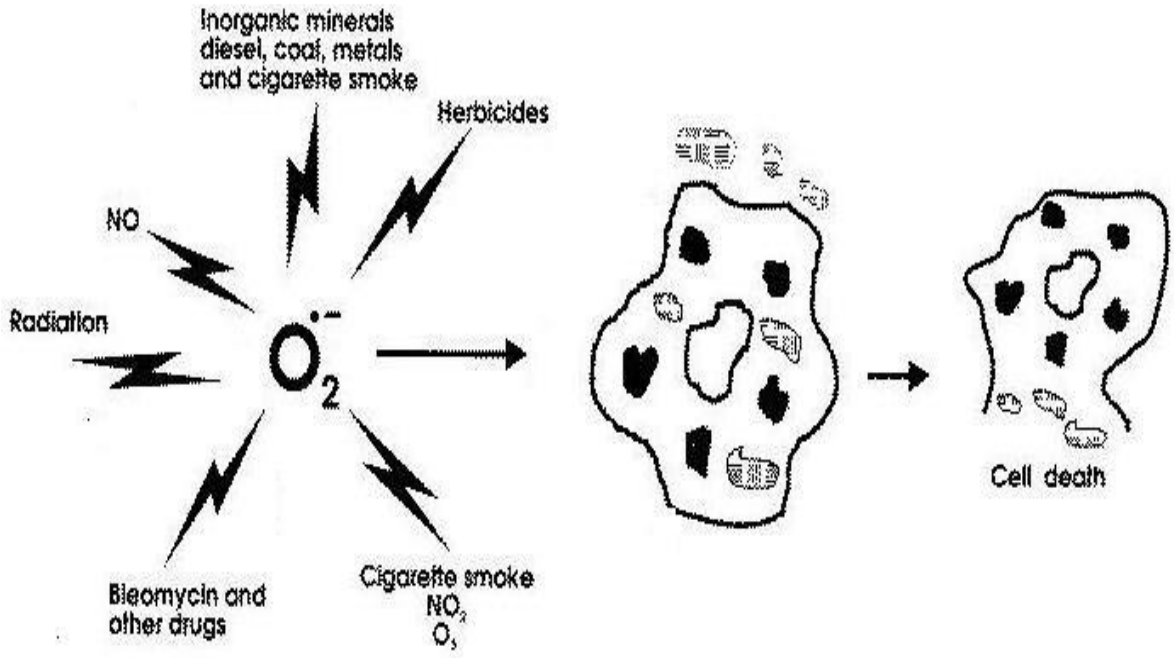


Figure 1: Causes and effects of free radicals on living cell

Source: (Pourmorad et al., 2006).

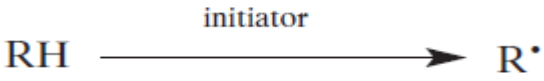
Free radicals, as they are unstable, have a tendency of being stabilized in a way of reducing their energy level by transferring their excess electron to nearby substances. As an example, when they are formed within the body, they attack nearby tissues by oxidizing membrane lipids, cellular proteins, and DNA, which lead to complete shutdown of cellular activities such as respiration and terminates the cell. Furthermore, the interaction of oxygen free radicals with members of lipid portion of body leads to formation of new radicals such as hydroperoxides, superoxide, and hydroxyl radicals, whose type may interact with biological systems in a citotoxic manner (Benavente-Garcia et al., 2000).

2.1.1. Mechanism of lipid oxidation

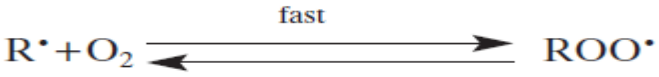
They are three different mechanisms of lipid oxidation, which yielding different oxidation products: a free radical mechanism, photo-oxidation and process related to lipoxygenase activity.

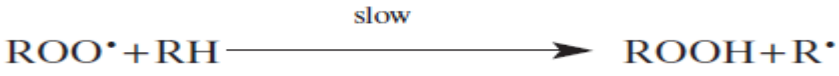
Autoxidation is a spontaneous reaction of molecular oxygen with lipids, leading to oxidative deterioration. It proceeds by a free radical chain mechanism involving three steps (Snyder et al., 1985; Hamilton et al., 1997, Gordon, 2001):

(1) Initiation step : homolytic hydrogen atom abstraction from a methylene group that leads to alkyl radical (R•) formation;



(2) Propagation step : formation of peroxy radicals (ROO•) able to react with unsaturated fatty acids and form hydroperoxides (ROOH);





(3) Termination step : formation of non-radical products by interaction of R• and ROO•.

Where: R• – fatty acid radical; ROOH – fatty acid hydroperoxide; ROO• – peroxy radical.

Initiation stage causes very little changes in lipids, after that lipid deterioration is fast and off-flavours become noticeable. Hydrogen abstraction from unsaturated fatty acids becomes selective for the most weakly bound hydrogen. The ease of hydroperoxidation depends on the number of double bonds present (Snyder et al., 1985). In monoeic acids, the most labile are hydrogen atoms on the carbon atoms adjacent to the double bond. In polyunsaturated acids, the most susceptible to abstraction of hydrogen are methylene groups between two double bonds. The radicals formed are not stable and the abstraction is followed by electron rearrangement to form conjugated dienes (Belitz, Grosch and Schieberle, 2009). Termination can also occur by antioxidants that interrupt the free radical chain reaction. Hydroperoxides, the primary oxidation products, are unstable and easily decompose involving monomolecular or bimolecular reactions (Belitz, Grosch and Schieberle, 2009). Decomposition products – peroxy and alkoxyl radicals – are highly reactive and may act as initiators of autoxidation. Decomposition of hydroperoxides produces non-volatile monomeric compounds, including di- and tri oxygenated esters derived from the corresponding keto-, hydroxy-, hydroperoxy- and epoxide esters (Snyder et al., 1985). Monohydroxyperoxides of unsaturated fatty esters are also precursors of volatile decomposition products (pentane, heptane, octane, pentanal, hexanal, heptanal, octanal, decanal and others) (Gordon, 2001). Unsaturated aldehydes and ketones undergo autoxidation, producing volatile compounds (dimers, oligomers, hydroperoxy epoxides hydroperoxy epidioxides and dihydroperoxides, Those secondary products decompose the same way as monohydroxyperoxides to produce similar volatile compounds. Hydroperoxides formed at the initial stage of autoxidation are non-volatile, odorless and relatively unstable compounds. They decompose to form volatile aromatic

compounds, which are perceived as off-flavors and as a warning that food is no longer edible (Gordon, 2001).

Another mechanism of oxidation occurs in the presence of sensitizer and UV-light. Photo-oxidation pathway is an alternative route leading to the formation of hydroperoxides instead of the free radical mechanism. Excitation of unsaturated fatty acid or oxygen may occur in the presence of light and a sensitizer. There are two types of photo-oxidation (Gordon, 2001): an electron or a hydrogen atom transfers between an excited triplet sensitizer and a substrate (PUFA), producing free radicals or radical ions; and triplet oxygen ($^3O_2$) can be excited by light to singlet oxygen ($^1O_2$), which reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide. The third mechanism of oxidation is based on lipoxygenase activity. Lipoxygenase is an enzyme which is a very important source of hydroperoxides. Lipoxygenase produces similar flavor volatiles to those produced during autoxidation. A molecule of lipoxygenase contains an iron atom, which is in high spin state Fe (II) and must be oxidized to Fe (III) by fatty acid hydroperoxides or hydrogen peroxide. The active enzyme abstracts a hydrogen atom from the methylene group of a polyunsaturated fatty acid with the iron being reduced to Fe (II) (Gordon, 2001). A conjugated diene system is formed, followed by reaction with oxygen. Peroxyl radical and finally hydroperoxide are generated. The second type of enzyme reacts with an esterified substrate, before the release of fatty acids by lipase, additionally ketodiene fatty acids are formed (Belitz, Grosch and Schieberle, 2009).

2.2. Antioxidants

Antioxidants may be defined as substances which can, when present at low concentrations compared to that of oxidizable substrates, significantly delay or inhibit oxidization of those substrates (Antolovich et al., 2002). Antioxidants are substances that protect cells from the damage caused by unstable molecules known as free radicals (Hamid et al., 2010). Antioxidants act by different mechanism, including control of oxidation substrates (lipids and oxygen), control of prooxidants, and inactivation of free radicals. They are classified according to the mechanism of action into two groups:

(1) Primary antioxidants (chain-breaking antioxidants) are free radical acceptors. As they act as hydrogen donors they are able to scavenge lipid radicals, e.g

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$$

Antioxidant radicals are stable due to delocalization of the unpaired electron around a phenol ring and cannot easily react with fatty acids. They are able to terminate radical chain process by reacting with radicals, e.g. (Reische et al., 2002):

$$ROO^{\bullet} + A^{\bullet} \rightarrow ROOA$$

The most important primary antioxidants are: tocopherols, BHT, BHA, and PG.

(2) Secondary antioxidants, they act through various mechanisms, as: reducers and chelators of metals (e.g. citric acid, phosphoric acid, EDTA); oxygen scavengers and reducing agents (e.g. ascorbic acid, ascorbyl palmitate, sulfites); singlet oxygen quenchers (carotenoids) (Reische et al., 2002); and substances are able to recover primary antioxidants (ascorbic acid).

Also antioxidant can classified to:

(A) Synthetic antioxidant, these are phenolic compounds that perform by capturing free radicals and stopping the chain reactions, the compound include (Hurrell, 2003):

i. Butylated hydroxyl anisole (BHA).

ii. Butylated hydroxyrotoluene (BHT).

iii. Propyl gallate (PG) and metal chelating agent (EDTA).

iv. Tertiary butyl hydroquinone (TBHQ).

v. Nordihydro guaretic acid (NDGA).

Synthetic antioxidants are relatively inexpensive, but their safety has been questioned.

(B) Natural antioxidant, extracted from plants can be used as alternatives to the synthetic antioxidants generally recognized as safe and have varying activity on lipid oxidation compared to synthetic antioxidant (Wojciak et al., 2011 , Dai et al., 2010). They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structures and include the following (Hurrell, 2003):

1. Minerals antioxidants: These are cofactor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, zinc and manganese.
2. Vitamins antioxidants: It is needed for most body metabolic functions. They include- vitamin C, vitamin E, vitamin B.

3. Phytochemicals: These are phenolic compounds that are neither vitamins nor minerals. These include:
    a. Flavonoids: These are phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers and bark their colors.
    b. Catechins: are the most active antioxidants in green and black tea.
    c. Carotenoids: are fat soluble color in fruits and vegetables. Beta carotene, which is rich in carrot and converted to vitamin A when the body lacks enough of the vitamin.

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 (Silva et al., 2006).

Table 1: The most frequently encountered phenolics in plants

Antioxidant class	Examples of substances
Phenolic acids	
Hydroxybenzoic acid series	Vanillic acid
Hydroxycinnamic acid series	Ferulic acid, chlorogenic acid
Flavonoids	Quercetin, catechin, rutin
Anthocyanins	Delphinidin
Tannins	Procyanidin, ellagic acid, tannic acid
Lignans	Sesamol
Stilbenes	Resveratrol
Coumarines	*ortho*-Coumarine
Essential oils	*S*-Carvone

Source: (Pokorny, 2007)

Phenolic content of plants may be different in different growing stages, as an example, Aloe vera shows equal or higher antioxidant and radical scavenging activity compared to BHT and α-tocopherol, as the extracts of Aloe vera of different growth periods vary in phenolic content (Hu et al., 2003). Phenolic acids can be divided into two groups as hydroxybenzoic acid and hydroxycinnamic acids both of which have single-ring structure (Manach et al., 2008).

A) Hydroxybenzoic acid: Gallic acid and ellagic acid can be classified as members of this group. This acid type can be found in onions, black radish and several red fruits such as berries at very low concentrations. Teas are also available sources of gallic acid.

B) Hydroxycinnamic acid: Caffeic acid, ferulic acid, p-coumaric acid and sinapic acids are examples of this group. They show heat sensitive properties. This type of acids can be obtained from kiwi, apple, blueberry, cherry and plum.

Flavanoids are the most common group of plant phenolics. They include several hydroxyl groups within their basic two ring carbon skeleton. Commonly they have (C6-C3-C6)

carbon structure consisting of two benzene rings linked by an oxygen containing heterocycle as shown in Figure 2,3 (Pietta, 2000).

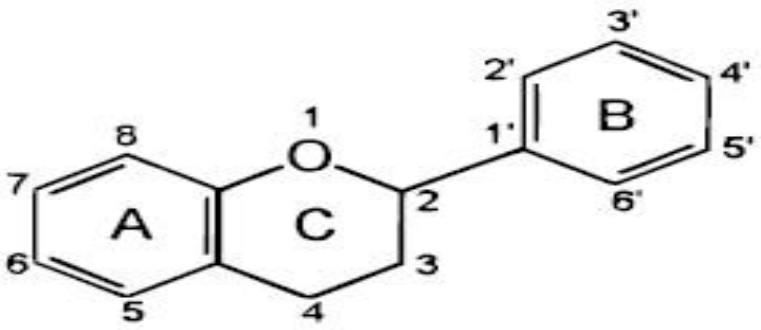


Figure 2: Basic flavanoid structure

Source:(Pietta, 2000).

Flavanoids can be divided into two fraction anthocyanins and anthoxanthins. Anthocyanins may have color pigments such as red, blue or purple. Anthoxanthins are rather colorless, white or yellowish (Manach et al., 2008). They can be sub grouped as flavones, flovonols, flavanols and isoflavanols as shown in Figure ٣.

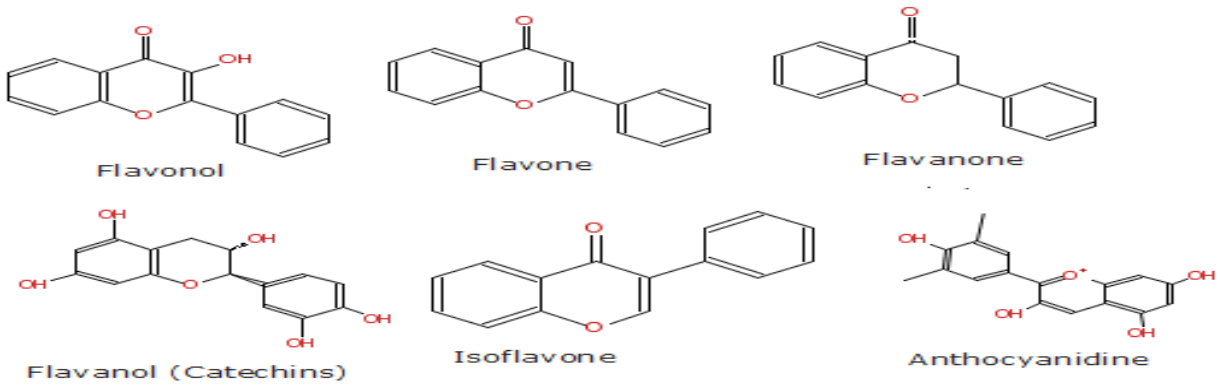


Figure 3: Chemical structures of flavonoids

Source: (Lakhanpal and Rai, 2007)

Flavones are the phenolic groups containing one carboxyl group. Flavones mainly consist of apigenin and luteolin. Olives, parsley and celery are the most important edible sources (Manach et al., 2008).

Flavonols are the most common type of flavanoids. Quercetin, kaempferol and myricetin are three most widely distributed flavonols. Onion, kale, apple, red wine and tea are the most common sources of this group (King and Young, 1999; Manach et al., 2008).

Flavanols can be found in both the monomer form as catechins and the polymer form as proanthociyanidins. Monomer forms can be found in apricots while polymer form can be obtained from red wine, grape seed, black tea and chocolate (Manach et al ., 2008).

Isoflavones are the flavanoids showing structural similarities to estrogens although they are not steroids. This similarity causes isoflavone to bind estrogen receptors at some point (Manach et al., 2008). They are specific for legumes such as soybean. The most well-known isoflavones are genistein and daidzein (King and Young, 1999). They are sensitive to heat and mostly hydrolyzed into glycosides during soymilk production (Manach et al., 2008).

Tannins can be divided into two major classes; Hydrolyzable Tannins and Condensed Tannins:

a) Hydrolizable tannins contain a central core of polyhydric alcohol such as glucose and hydroxyl groups which are partially or completely esterified by gallic acid (gallotannins) or ellagic acid (ellagitannins). Acid, base or certain enzyme hydrolysis causes the break of ester bonds so that basic components are exposed again.
b) Condensed Tanninsare essentially oligomeric derivatives of flovonols (flavan-3-ols, flavan- 3,4-ols or both), such as catechin and epicatechin. They are more complex than

hydrolyzabl tannins. Condensed tannins can be found in fruits, vegetables, cocoa, red wine and legume family (Manach et al., 2008).

2.3. Oleuropein

Leaves from olive tree, are rich in biophenols (BPs), such as Oleuropein, verbascoside, ligstroside, tyrosol or hydroxytyrosol. These compounds have shown several biological activities such as antioxidant and anti microbial, and consequently can be used in food application (Malik et al., 2006).

Oleuropein is the most abundant phenolic compound in olive leaves and fruits and is responsible for the characteristic bitterness of olive fruit. Health benefits of this compound have been extensively investigated, It has been reported that oleuropein, and related compounds such as tyrosol, verbascoside, ligustroside, 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. 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 (Wsowicz et al., 2004).

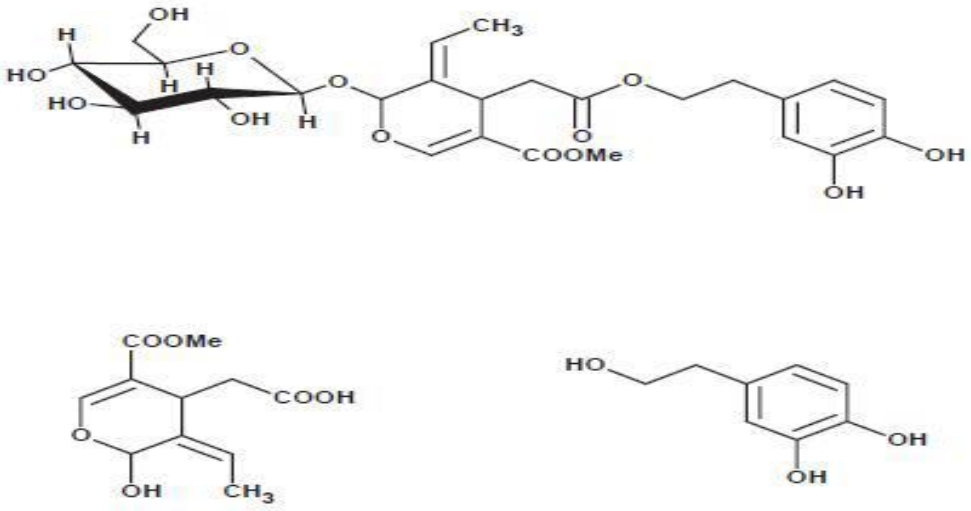


Figure 4:: Structure of Oleuropein and its components

Source: (Al-Azzawie et al., 2006)

The phenolic groups in olive leaf extract, their examples and relative amounts contained within OLE are presented in Table 2. The molecule structure of most commonly encountered ones are given in Figure 5.

Group Name	Example Compound	% Amount in OLE
Oleuropeosides	Oleuropein	24.54
	Verbascoside	1.11
Flavones	Luteolin-7-glucoside	1.38
	Apigenin-7-glucoside	1.37
	Diosmetin-7-glucoside	0.54
	Luteolin	0.21
	Diosmetin	0.05
Flavonols	Rutin	0.05
Flavan-3-ols	Catechin	0.04
Substitued Phenols	Tyrosol	0.71
	Hydroxytyrosol	1.46
	Vanilin	0.05
	Vannilic acid	0.63
	Caffeic acid	0.34

Table2: The phenolic groups in OLE, their examples and relative amounts in OLE

Source:(Benavente-Garcia et al., 2000).

Garcia et al. (2000) reported the sequence of the antioxidant capacity of the flavanoids in olive leaf extract as; rutin > catechin ≈ luteolin > OL ≈ hydroxytyrosol > diosmetin > caffeic acid > verbascoside > oleuropein > luteolin-7-glucoside ≈ vanillic acid ≈ diosmetin-7-glucoside > apigenin-7-glucoside > tyrosol > vanillin.

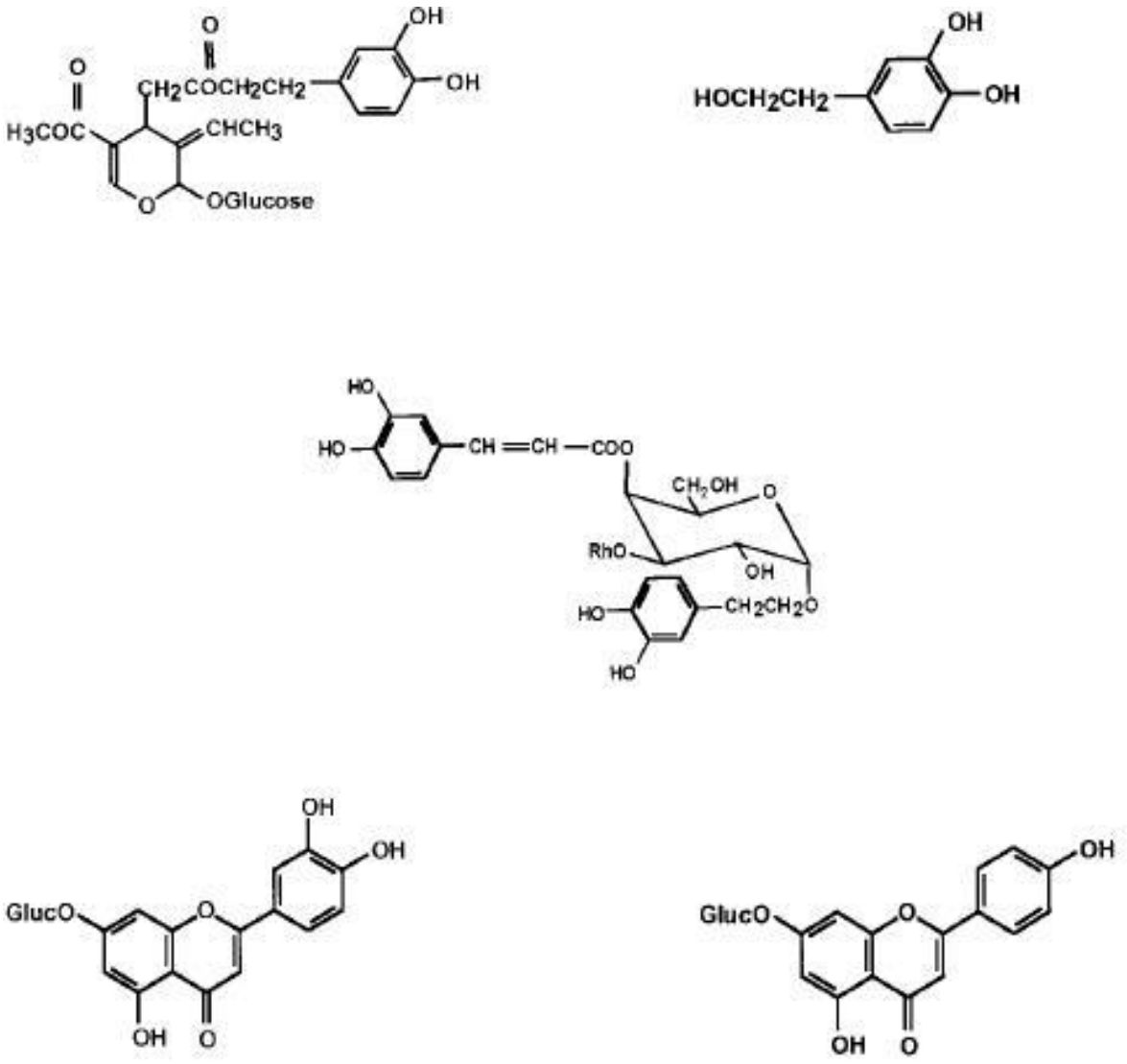


Figure 5: Molecular Structure of Phenolics Obtained from OLE: Oleuropein, Hydroxytyrosol, Verbascoside, Luteolin-7-glucoside and Apigenin-7-glucoside

Source: (Benavente-Garcia et al., 2000).

Low cost phenolic extracts could be obtained from commercially available olive mill waste water (OMWW) to be used as alternatives to synthetic antioxidants as BHA and BHT. Furthermore, hydroxytyrosol derived from OMWW can be used to stabilize edible oils, (Fki et al., 2005), (Hayes et al., 2009), reported that, muscle had consistently lower levels of lipid

oxidation compared to control in both aerobic and modified atmosphere pack conditions, when OLE were used at a concentration of 100 and 200μg/g.

In addition to its antioxidant properties, phenolic compounds within olive leaf extract have shown antimicrobial activities against several microorganisms including; E. coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus, Salmonella typhi and Vibrio parahaemolyticu (Markin et al., 2003). Furthermore, OLE affects macrophage function and modulates inflammatory response; those may contribute to the activity against infectious agents (Lee-Huang et al., 2003). Although the individual phenolic compounds in olive leaf extract may show strong in vitro activities, the antioxidant and antimicrobial activities of combined phenolics showed similar or better effects than the individual phenolics (Lee&Lee, 2010). (Aytul, 2010) reported the effect of Oleuropein as antioxidant in refrigerated stored beef cubes treated with 1%, 2%, and 3% OLE for 9 days, level of oxidation product in control sample higher than treated samples during refrigerated storage, and the best result against lipid oxidation was obtained from beef cubes treated with 2% OLE after 9 days of cold storage.

2.3 . Oxidation impact on sensory properties of food and its implications on health

Oxidation of lipids leads to losses in sensory quality of food (Eriksson, 1978). That is the case of fat and fat-containing foods, which undergo oxidation. Lipid oxidation results in off-flavors and odors indicating poor-quality products (Coppin & Pike, 2001).

Oxidation of fatty acids in food results in the formation of volatile compounds among which many have an unpleasant odor and are responsible for flavor problems in food industry (Grosch, 1982). As a result of fatty acids autoxidation, initially odorless monohydroperoxides are formed, which eventually break down into mainly volatile products. This group comprises aldehydes, ketones, alcohols, acids, hydrocarbons, furanones, and lactones (Grosch, 1982). Due to the low odor threshold of the majority of these compounds, the presence of volatile hydroperoxide degradation products even at low concentration impairs the sensory properties of oils or fat-containing products.

Possibilities of human exposition to oxidized fats in the diet, deep fat frying, pre-cooked frozen and chilled foods and powdered foods have been considered (Kubow, 1990). However, there is also an assumption that in most cases oxidized fats are rendered unpalatable because of the deterioration in flavor and appearance long before the changes have appreciably reduced nutritive value or created toxicity (Ziemlanski et al., 1991). The susceptibility to oxidation is increased by increasing polyunsaturated fatty acids in food. Thus one nutritional effect of oxidation is to reduce the essential fatty acid content of edible fats. More serious nutritional problem of lipid oxidation is affected by interactions of lipid oxidation products with other food components, mainly with vitamins and proteins. Several studies have demonstrated effects of feeding lipid oxidation products to experimental animals that may be interpreted as due to oxidative damage. The observed symptoms of the administration of oils

and fats subjected to oxidation are elevated liver and kidney weights, cellular damage in various organs, altered fatty acid composition of tissue lipids, cardiac fibrotic lesions, and hepatic bile duct lesions (Kubow, 1990; Eder, 1999). Studies on the possible pathological significance of lipid oxidation products were concerned on the effect of lipid peroxides, secondary products, especially malondialdehyde and cholesterol oxidation products. Malondialdehyde is a bifunctional aldehyde, its very reactive compound in cross-linking reactions with DNA and proteins (Addis, 1986). The toxicity of oxidized cholesterols has been demonstrated in several studies. The oxysterols are absorbed from the intestinal tract and are transported in the blood to arterial deposition sites at rates similar to cholesterol (Kubow, 1990). There is considerable evidence that some cholesterol oxidation products are powerful atherogenic agents in vivo and in vitro. They have also cytotoxic and mutagenic properties (Osada et al., 1998).

Dietary lipid peroxides participate in the development of cancer in humans. It was demonstrated a strong reaction between lipid peroxides and DNA (Addis, 1986). Low molecular products of decomposition of fatty acid peroxides are absorbed into the circulatory system and incorporated into the liver or have access to other body tissues (Kubow, 1990).

Mixture product of lipid oxidation can occur in daily diets. For this reason foods should be protected to minimize their concentration in foods and eliminate their deleterious effects. Thus the use of natural antioxidants has been gaining considerable importance (Johnson, 2001).

2.5. Methods for the measurement of lipid oxidation

There are several chemical and physical methods to assess the quality of fats and fat-containing foods. Peroxide value (PV) is probably the mostly used one in which concentration of peroxides (hydroperoxides) is determined as a measure of the extent of oxidation. Because of the unstable and intermediate nature of peroxides and their sensitivity to temperature, the PV is an approximate indicator of the state of oxidation but particularly in the early stage of oxidation it serves as a good tool for the measurement of a degree of oxidation. The iodometric determination of PV proposed by Lea is the most common (Gray, 1985). The TBA (thiobarbituric acid) test is one of the most commonly used method and is based on the measurement of the absorbance of TBA-malonaldehyde complex at 532–535 nm. Malonaldehyde is a three carbon dialdehyde being one of intermediates formed in the oxidation of lipids. The objections to this method point out that depending on the aldehyde type peaks at different absorbance maxima are observed and TBA can react with other compounds, not being a part of the lipid oxidation system yielding also a red pigment. Total volatile carbonyl compounds – a measure which is related with off-flavor – can be also measured utilizing a formation of orange colored hydrazones in the reaction of carbonyls with 2,4-dinitrophenylhydrazine and utilized as an indicator of oxidation process. Also anisidine value is used for the assessment of a degree of oxidation, its conjunction with peroxide value (as Totox) and physical methods – measured conjugated dienes (at 234 nm) and trienes (at 268 nm) which can serve as a relative measurement of oxidation, or fluorescence based on the reaction compounds with a structure of N,N-disubstituted 1-amino- -3-iminoprene with peroxidising lipids (Angelo, 1996).

3. Problems statement

Lipid oxidation during storage of frozen and fresh hamburger leads to change in color, off - flavor, rancidity, and loss of nutritional value. The short shelf life of fresh hamburger, and formation of oxidation products affects the quality of hamburger. Also the use the chemical preservatives to protect hamburger has adverse effects on health.

4. Objectives

1. Retard lipid oxidation of frozen hamburger by adding natural antioxidant (oleuropein).
2. Compare natural antioxidant (Oleuropein) with synthetic antioxidant (Sodium Erythorbate) during the storage time of frozen hamburger.
3. Determining the optimum concentration of the Oleuropein and Sodium Erythorbate to retard oxidation in frozen hamburger.
4. Retard lipid oxidation of fresh hamburger by adding natural antioxidant (Oleuropein and Olive leaves extract).
5. Determining the optimum concentration of Oleuropein and olive leave extract to retard oxidation in fresh hamburger.
6. To produce natural meat product (fresh and frozen hamburger) without addition of chemical preservatives or additives.

Chapter Three

Materials and methods

5. Material and methods

5.1. Materials

The Chemicals used to determine the oxidative stability thiobarbituric acid-reactive substances (TBARS) are thiobarbituric acid (TBA), trichloroacetic acid (TCA), and Hydrochloric acid (HCl).

Sodium Erythorbate was used as synthetic antioxidant, Oleuropein and Olive leaves extract were used as natural antioxidant. Ethanol was used to extract olive leaves.

5.1.2. Methods

5.1.3. Extraction olive leaves

10 grams of olive leaves dried were placed in a soxhlet thimble in a Soxhlet apparatus and were extracted with 200 ml of 80% ethanol for 2 hours 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 hours. The concentrated extracts were stored in a refrigerator at 4°C until use(Yateem et al., 2014).

5.1.4. Concentration of antioxidant

Oleuropein was obtained from Hunan Kang Biotech company- China, three concentration from Oleuropein (0.25 %, 0.5%, 0.75%), and three concentration from Sodium Erythorbate (0.25 %, 0.5%, 0.75%) were prepared to be used.

5.1.5. Preparation of Meat Samples

5.1.5.1. Frozen hamburger

The packed vacuum frozen boneless beef was obtained from Siniora Meat Co. (Jerusalem-Palestine). The meat was thawed until zero temperature at the core, then the plastic cover removed and the meat was broken down with a mixer machine (disc 4.5 mm). Fat was minced by using mincer machine (disc 1 mm), and it was added to meat with the other components including spices (salt, pepper) and onions. Then the mixture was homogenized by mixing it for 3 minutes. The mixture was divided into seven batches: control and treated samples. six treated samples are mixed with 0.25 %, 0.5%, 0.75% Oleuropein (w/v) and 0.25 %, 0.5%, 0.75% Sodium Erythorbate (w/v). Then the samples are cooled to -1 C° and formed, finally the samples underwent shock freezing and stored in freezer at -12 C° for six months.

5.1.5.2. Fresh hamburger

The packed vacuum frozen boneless beef was obtained from Saniora Meat Co. (Jerusalem-Palestine). The meat was thawed until zero temperature at the core, then the plastic cover removed and the meat was broken down with a mixer machine (disc 4.5 mm). Fat was minced by using mincer machine (disc 1 mm), and it was added to meat with the other components including spices (salt, pepper) and onions. The mixture was divided into seven batches: control and treated samples. Six treated samples are mixed with 0.5 %, 1%, 1.5% Oleuropein (w/v) and 0.5 %, 1%, 1.5% OLE. Then the samples were cooled to -1 °C and formed, finally the samples were stored in refrigerant at 4 °C for 20 days .

5.1.6. Oxidative Stability of frozen and fresh hamburger

For the determination of oxidative stability of refrigerated (fresh) and frozen stored hamburger, thiobarbituric acid-reactive substances (TBARS) assay was performed with a slightly modified method of Aytul, 2010. For analysis, 5 g sample was placed into a beaker containing 50 mL of 0.38 % TBA and 15 % TCA prepared in 0.25 N HCl solution. The sample was homogenized at 10000 rpm for 3 min and three 15 mL aliquots obtained from homogenate was heated for 45 min in boiling water bath to develop a pink color and then cooled in tap water. The boiled samples were then clarified by centrifugation at 5000 rpm for 10 min and their absorbance was measured at 532 nm by using a spectrophotometer. Higher values of absorbance indicate higher oxidation of hamburger samples. Average of three absorbance values will be used to determine the oxidative stability of stored samples (Aytul,2010 ; Bekhit et al., 2003)

5.1.7. Data Analysis

For fresh and frozen hamburger Oneway ANOVA used to study the effect of different concentration added to hamburger. Then the independent sample t- test between each two concentration was used.

The Null Hypothesis for the ANOVA stated that: there is NO statistical significant difference between the means (averages) of the oxidation among the different concentrations and storage time for both fresh and frozen hamburger.

While the Alternative Hypothesis stated that: there is a statistical significant difference between the means (averages) of the oxidation among the different concentrations at different time of storage for both fresh and frozen hamburger.

The analysis is conducted for the different concentration of Oleuropein, OLE, and Sodium Erythorbate separately, according to type of produced hamburger.

In addition, simple linear regression was used to study the relation between the concentrations and time for Oleuropein, OLE, and Sodium Erythorbate.

5.1.8. Calculations (calibration curve)

Absorbance of different concentrations (1-15 ppm) of malondialdehyde (MDA) was used for determination of the amounts of oxidation products (as mg of MDA per Kg of hamburger sample) in Hamburger samples. The calibration curve was found to be linear ($A= 0.1035C + 0.0436$ with r^2 of 0.9965), see Appendix 1.

6. Methodology

The design of the study has been divided into two parts:

The first part: Preparation of three batches of frozen hamburger, the first as control, the second batch was treated with three concentrations of Oleuropein, and the third batch was treated with Sodium Erythorbate at the same concentrations.

The second part: Preparation three batches of fresh hamburger , the first as control, the second batch was treated with three concentrations of Oleuropein, and third batch was treated with OLE at the same concentration .

Chapter four

Results and Discussion

7. Results and discussion

7.1. TBARS method

The TBARS Method used in this study was according to Aytul,2010, Bekhit et al., 2003 but slightly modified due to difference in type of the sample, Aytul determined the oxidation stability of chicken breast sample but in this study the oxidative stability of both hamburger samples fresh and frozen was determined.

The modified procedure:

- A solution containing 0.38% TBA and 15% TCA in 0.25NHCL solution was prepared.
- 5g of sample was placed into beaker.
- 50ml of 0.38% TBA and 15% TCA in 0.25NHCL solution was added to the beaker.
- Sample was homogenized at 1000rpm for 3 minutes.
- 15ml aliquots obtained from homogenate was heated for 45 min in boiling water bath at 90 C°.
- Pink color was developed.
- The solution was cooled in tap water.
- The solution was centrifuged at 5000 rpm for 10 minutes.
- Absorbance was measured at 532nm by using spectrophotometer.
- Three samples were analyzed and the results were expressed as average of three samples ± SD (SD: standard deviation).

  Blank was made exactly as in the sample preparation but TBA was not used in the process.

In the modified TBARS method, 5g of sample were used compared to Aytul, 2010, where 2.5g were used. Additionally, the boiling time was increased from 10 min to 45 min to allow appearance of pink color, this is due to the industrial process of hamburger making such as freezing or refrigeration leading longer reaction time of TBA with MDA formed by oxidation, and because hamburger is an emulsion formed from meat and fat.

7.2. Oxidation products of fresh Hamburger

Oleuropein was added to fresh hamburger as natural antioxidant in three concentrations (0.5%, 1% and 1.5%). Additionally, olive leaves extract obtained from extraction of olive leaves with ethanol was also used in fresh hamburger at three concentrations (0.5%, 1% and 1.5%). The amounts of oxidation products (expressed as mg MDA per Kg hamburger sample) were determined spectrophotometrically.

7.2.1. Effect of Oleuropein on the oxidation of fresh hamburger

The effectiveness of Oleuropein was different depending on the Oleuropein concentration added. The oxidative stability of hamburger samples was examined by conducting thiobarbituric acid reactive substances (TBARS) assay. Absorbances obtained from these assays at 532 nm, were then used to calculate amount of TBARS. Table 3 shows the amounts of oxidation products of fresh hamburger samples treated with different concentrations of Oleuropein from day 1 to day 21. As can be seen in table 3 and figure 6, the amounts of oxidation products for control hamburger samples and treated ones (with 0.5, 1, and 1.5%) increases with storage time (from day 1 to day 21) indicating that oxidation increases with

time. However, the increase in the oxidation products of control hamburger sample was higher than that for treated samples (0.5, 1, and 1.5%).

The slopes of the plots of oxidation products vs. day (figure 7) show this increase of oxidation by time. In addition, the slope of the plot decreases with increasing concentration of Oleuropein. This confirms the effect of Oleuropein on the oxidation products of fresh hamburger which decreases significantly.

These results were analyzed statistically to test if there is a statistical significant difference between the amounts of oxidation products as storage time increases from day 1 to day 21. Results showed a significant difference ($P > 0.05$), between the amounts of the 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%, see Table 3. The capital letters indicate significant differences between amounts of oxidation products as storage time increases (from day 1 to 21).

Table 3: Oxidation products of fresh hamburger samples treated with different concentrations of Oleuropein (0.5, 1.0, and 1.5%) determined by TBARS spectrophotometric method

Storage time	mg MDA/ kg hamburger			
	Control Sample	0.5%Oleuropein	1%Oleuropein	1.5%Oleuropein
Day1	28.5±0.59 aE	١٧,٢±0.52bE	14.2±1.3 cE	10.9±0.37 dE
Day 7	46.6±3.2 aD	١٧,٤ ±1.4bD	18.1±0.1 bD	14.9±0.1 cD
Day 13	89.6±0.27 aC	48.9±0.28 bC	27.2±0.3 cC	24.6±4.3 dC
Day 17	96.1±0.58 aB	51.6±0.96 bB	34.2±0.2 cB	29.3±1.6 dB
Day 21	107.4±0.27 aA	60.0 ±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).

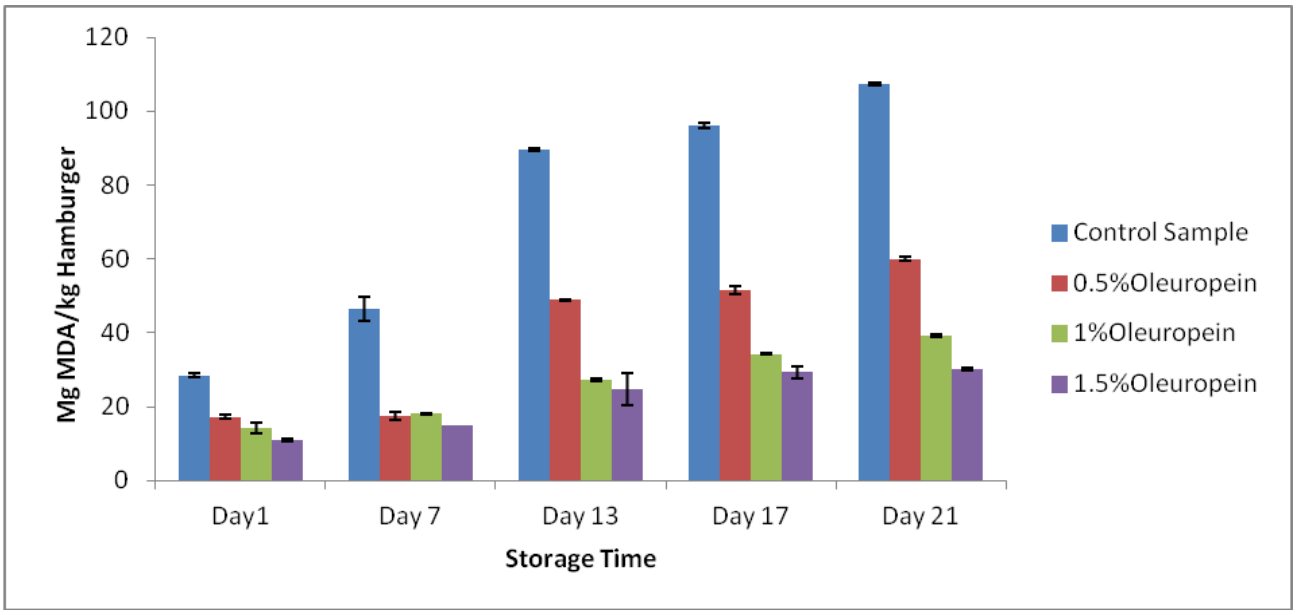


Figure 6: Oxidation products of fresh hamburger samples treated with different concentrations of Oleuropein (0.5, 1.0, and 1.5%) determined by TBARS spectrophotometric method

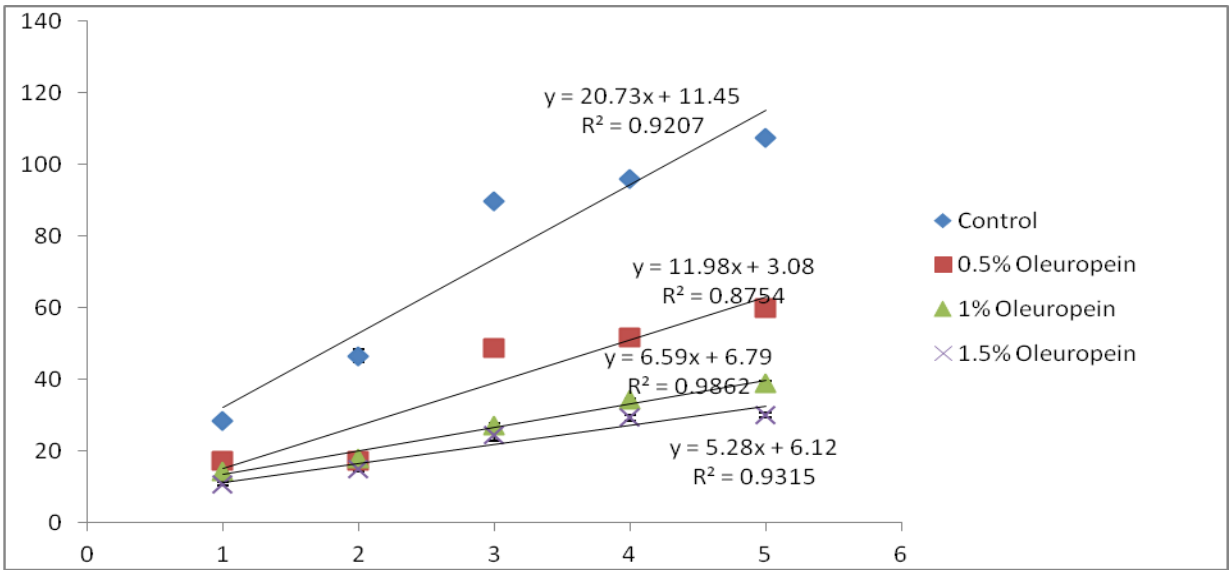


Figure 7: Oxidation products of fresh hamburger samples treated with different concentrations of Oleuropein (0.5, 1.0, and 1.5%) determined by TBARS spectrophotometric method.

7.2.2. Effect of Oleuropein on the shelf life of fresh hamburger

Usually fresh hamburger is consumed within 5-7 days of refrigeration without any preservatives or antioxidant. Comparing the amounts of oxidation products for fresh samples (control with 46 mg/kg) with that for treated hamburger samples (0.5%, 1%, 1.5%) after three weeks (51 mg/kg, 39 mg/kg, and 30 mg/kg), indicates that oxidation products of treated hamburger samples after 3 weeks is similar to oxidation products of fresh product after one week. This result indicates the prolongation of shelf life of fresh hamburger in terms of oxidation products. Also it is interesting to note that the amounts of oxidation products decreases with increasing the amounts of Oleuropein (0.5, 1.0, and 1.5%).

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.

7.2.3. Optimum concentration of Oleuropein in fresh hamburger samples

To determine the best concentration of Oleuropein in fresh hamburger samples for three weeks, statistical analyses were done to determine if there are significant differences between the oxidation products of hamburger treated with different concentrations of Oleuropein from day one to twenty one days (Appendix 2).

At day one, results showed that 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 are 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 day one.

At day 7 and at day 11, a statistical difference between oxidation products for control hamburger samples and treated ones with 0.5, 1.0, and 1.5% Oleuropein, was observed the amounts of oxidation products for control samples are higher than those for treated ones. Regarding the samples treated with Oleuropein, there are no statistical 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 fresh 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, and so the best concentration of Oleuropein is 1.5% 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.

In conclusion, to preserve fresh hamburger samples from oxidation for three weeks, it is recommended to use 1.5% Oleuropein as natural antioxidant.

7.2.4. Effect of olive leaves extract (OLE) on the oxidation of fresh hamburger

The same study was conducted but using OLE at three concentrations as in the case of Oleuropein (0.5, 1.0, and 1.5%). The results are shown in table 4 and figure 8.

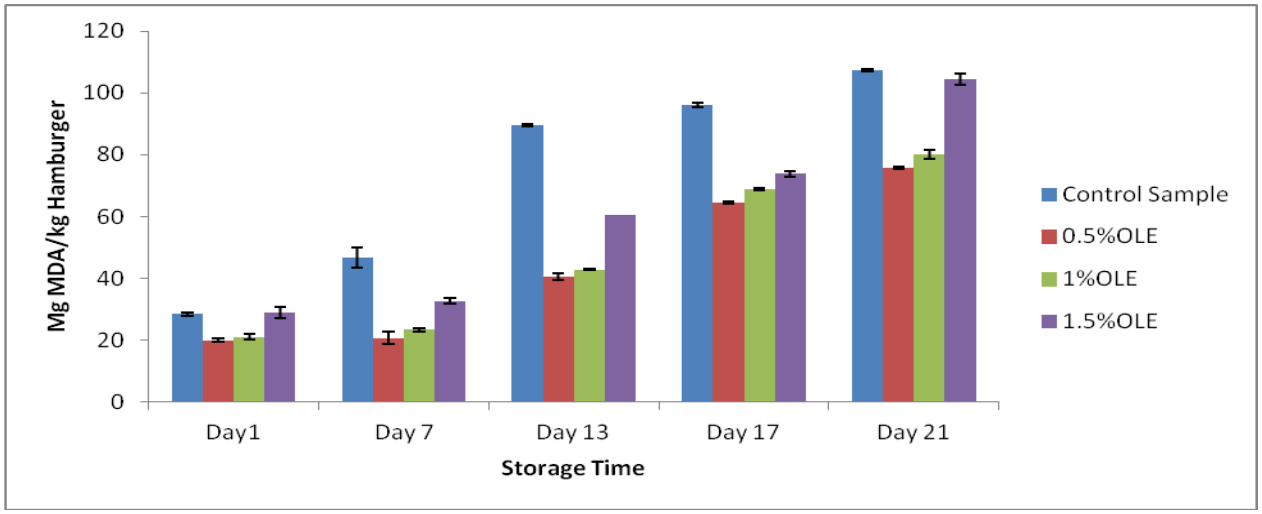


Figure 8: Oxidation products of fresh hamburger samples treated with different concentrations of OLE (0.5, 1.0, and 1.5%) determined by TBARS spectrophotometric method.

Table 4 shows the amounts of oxidation products of fresh hamburger samples treated with different concentrations of OLE from day one to day twenty one. As we can see from table 4 and figure 8, the amounts of oxidation products for control hamburger samples and treated ones (with 0.5, 1, and 1.5%) increases with storage time (from day 1 to day 21) indicating that oxidation increases with time. However, the increase in the oxidation products of control hamburger sample (not treated with Oleuropein) was higher than that for treated samples (0.5, 1.0, and 1.5%).

Table 4: Oxidation products of fresh hamburger samples treated with different concentrations of OLE (0.5, 1.0, and 1.5%) determined by TBARS spectrophotometric method.

Stored time	Result mg MDA/kg hamburger			
	Control Sample	0.5%OLE	1%OLE	1.5%OLE
Day1	28.5±0.59 aE	20.0±0.68dE	21.1±0.75cE	28.9b±1.8E
Day 7	46.6±3.2 aD	20.7±2dD	23.3±0.43cD	32.7±0.96bD
Day 13	89.6±0.27 aC	40.4±1.5dC	42.8±0.2cC	60.5±0.07bC
Day 17	96.1±0.58 aB	64.5±0.53dB	68.9±0.47cB	73.8±0.19bB
Day 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).

The slope of the plots of oxidation products vs. day (figure 9) proves this finding. However and in contrary to the results obtained for Oleuropein, the slope of the plot increases with increasing the concentration of Oleuropein, indicating that 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 doses or in the presence of metal ions (Azam et al., 2004, Watjen et al., 2005). Polyphenols and particularly flavonoids are examples of substances with such dual behavior (Fukumoto & Mazza, 2000 ; Trueba & Sanchez, 2001 ; Sakihama et al., 2002). Phenolic acids have also been reported as pro-oxidants (Fukumoto & Mazza, 2000 ; Maurya & Devasagayam, 2010 ; Sakihama et al., 2002).

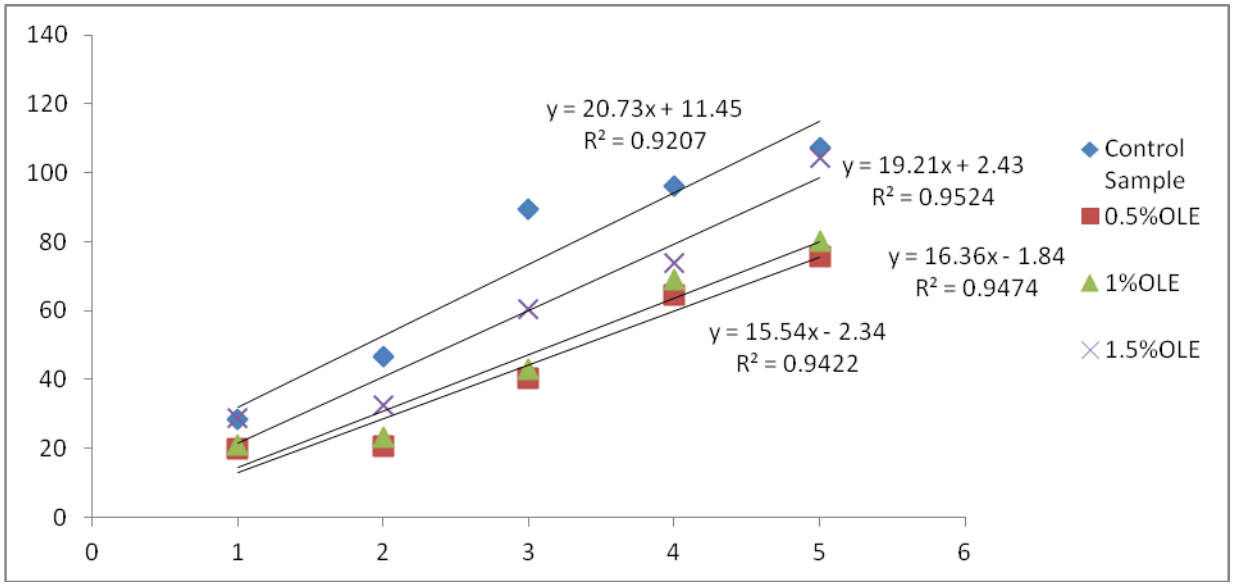


Figure 9: Oxidation products of fresh hamburger samples treated with different concentrations of OLE (0.5, 1.0, and 1.5%) determined by TBARS spectrophotometric method.

These results were analyzed statistically to test if there is a statistical significant difference between the amounts of oxidation products as storage time increases from day 1 to day 21. Results shows that there is a significant difference (P>0.05) between the amounts of the 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%, see Table 4. The capital letters indicate significant differences between amounts of oxidation products as storage time increases (from day 1 to 21).

7.2.5. 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 similar to oxidation products of fresh one after one week which indicates the prolongation of shelf life of fresh hamburger in terms of oxidation products to 2 weeks.

7.2.6. Optimum concentration of OLE in fresh hamburger samples

To determine the best concentration of OLE in fresh hamburger samples for three weeks, statistical analyses were done to determine if there is significant differences between the oxidation products of hamburger treated with different concentrations of OLE from day one to day twenty one, table (Appendix 2).

At day 1, 7, 13, 17, and 21, results showed that there is statistical 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 efficiency of OLE as antioxidant. Additionally there is statistical 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 than Oleuropein because the OLE extract is mixture contained more than one type of phenol content while oleuropein is pure .

7.2.7. Relationship between oxidation and time in fresh hamburger

To study the relationship between oxidation products of fresh hamburger samples and storage time, statistical analysis was conducted using simple linear regression for each concentration and time for both Oleuropein and OLE levels.

Results showed significant strong positive relationship between oxidation products amounts and storage time according to the following expression:

Oxidation = Constant + B*time

The statistical analysis (Appendix 3) shows that the best coefficient for Oleuropein is at 1.5% concentration with value of B 1.03, while the best coefficient for OLE is 0.5% concentration with B value of 2.92, which means that the best concentration of Oleuropein and OLE is 1.5% and 0.5%, respectively in fresh hamburger.

7.2.8. Comparison between Oleuropein and OLE as antioxidant of fresh Hamburger

To compare Oleuropein with OLE in terms of activity as antioxidant, statistical analysis using independent sample t-test is conducted to test the differences between Oleuropein and OLE at each concentration level (Appendix 4).

7.2.8.1. Comparison between samples treated with 0.5% Oleuropein and sample treated with 0.5% OLE

Statistical analyses of the results shown in Figure 10 and table 5 show that there is no significant difference between the means of oxidation for Oleuropein and OLE (the mean of Oleuropein is 38.53 and OLE is 44.54), so OLE and Olueropein can be used, see Appendix 4.

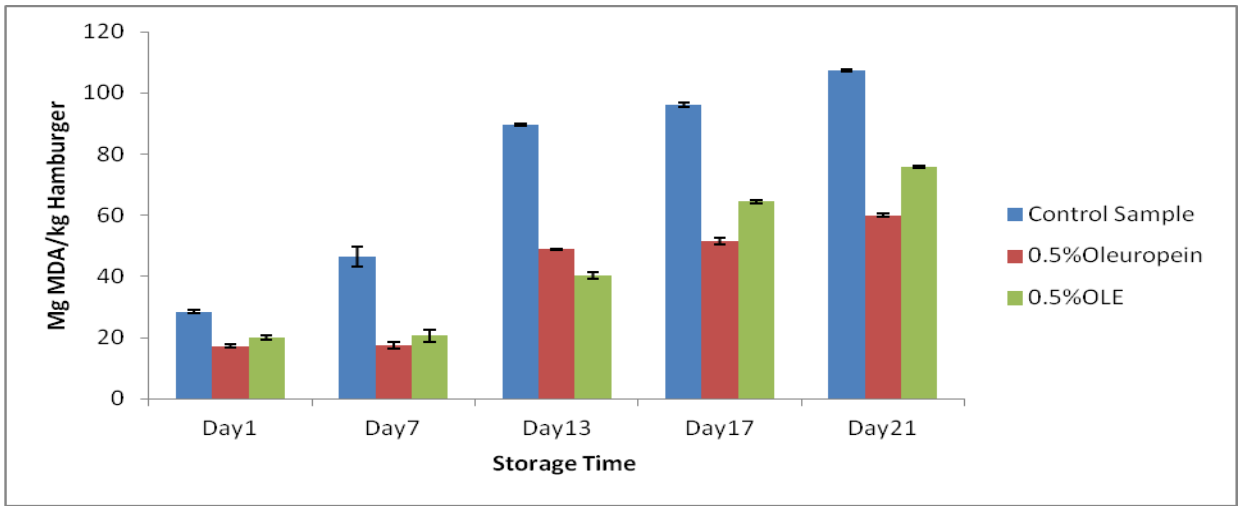


Figure 10: Oxidation products of fresh hamburger samples and treated samples with 0.5% Oleuropein and 0.5% OLE determined by TBARS spectrophotometric method

Table 5: Oxidation products of fresh hamburger samples treated with 0.5% Oleurpein and 0.5% OLE determined by TBARS spectrophotometric method.

Storage time	mg MDA/ kg hamburger		
	Control Sample	0.5%Oleuropein	0.5%OLE
Day1	28.5±0.59 aE	١٧,٢±0.52bE	20.0±0.68dE
Day 7	46.6±3.2 aD	١٧,٤ ±1.4bD	20.7±2dD
Day 13	89.6±0.27 aC	48.9±0.28 bC	40.4±1.5dC
Day 17	96.1±0.58 aB	51.6±0.96 bB	64.5±0.53dB
Day 21	107.4±0.27 aA	60.0 ±0.49bA	75.8±0.36dA

7.2.8.2. Comparison between samples treated with 1% Oleuropein and sample treated with 1% OLE

Statistical analyses of the results shown in Figure 11 and table 6 show that there is significant difference between the means of oxidation for Oleuropein and OLE (the mean of Oleuropein is 27.25 and OLE is 46.75), so Oleuropein is better than OLE at this concentration level, see Appendix 4.

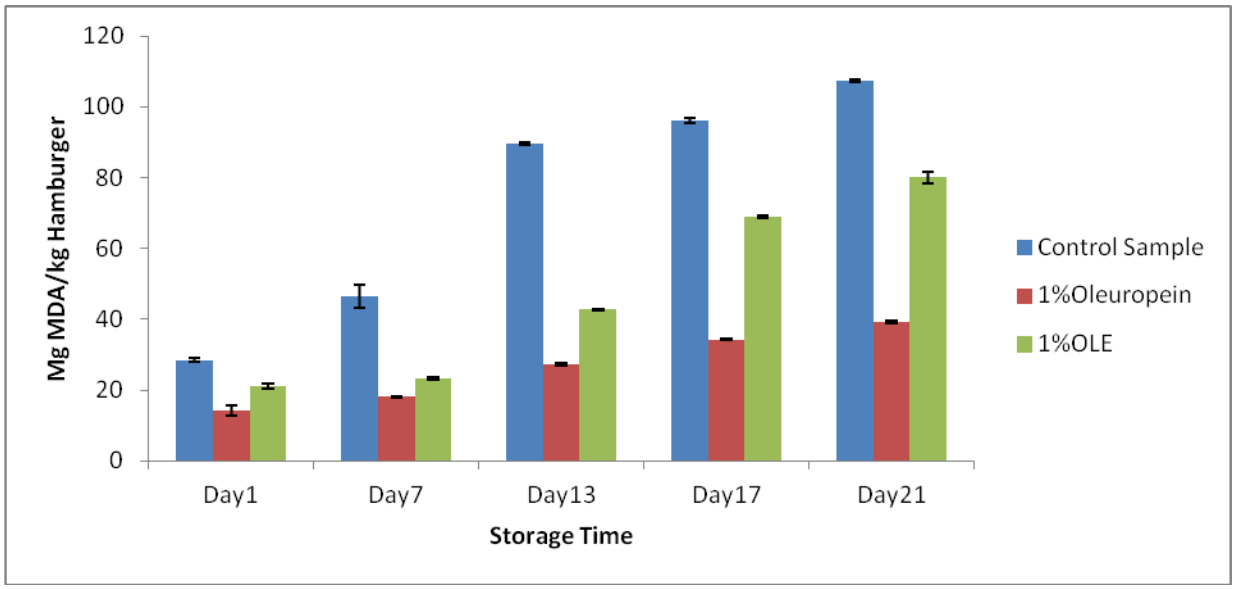


Figure 11:: Oxidation products of fresh hamburger samples and treated samples with 1% Oleuropein and 1% OLE determined by TBARS spectrophotometric method

Table 6: Oxidation products of fresh hamburger samples treated with 1% Oleurpein and 1% OLE determined by TBARS spectrophotometric method.

Storage time	mg MDA/ kg hamburger		
	Control Sample	1%Oleuropein	1%OLE
Day1	28.5±0.59 aE	14.2±1.3 cE	21.1±0.75cE
Day 7	46.6±3.2 aD	18.1±0.1 bD	23.3±0.43cD
Day 13	89.6±0.27 aC	27.2±0.3 cC	42.8±0.2cC
Day 17	96.1±0.58 aB	34.2±0.2 cB	68.9±0.47cB
Day 21	107.4±0.27 aA	39.1±0.3 cA	80.1±1.6cA

7.2.8.3. Comparison between samples treated with 1.5% Oleuropein and sample treated with 1.5% OLE

Statistical analyses of the results shown in Figure 12 and table 7 show that there is significant difference between the means of oxidation for Oleuropein and OLE (the mean of Oleuropein is 21.99 and OLE is 60.08), so Oleuropein is better than OLE at this concentration level, see (Appendix 4).

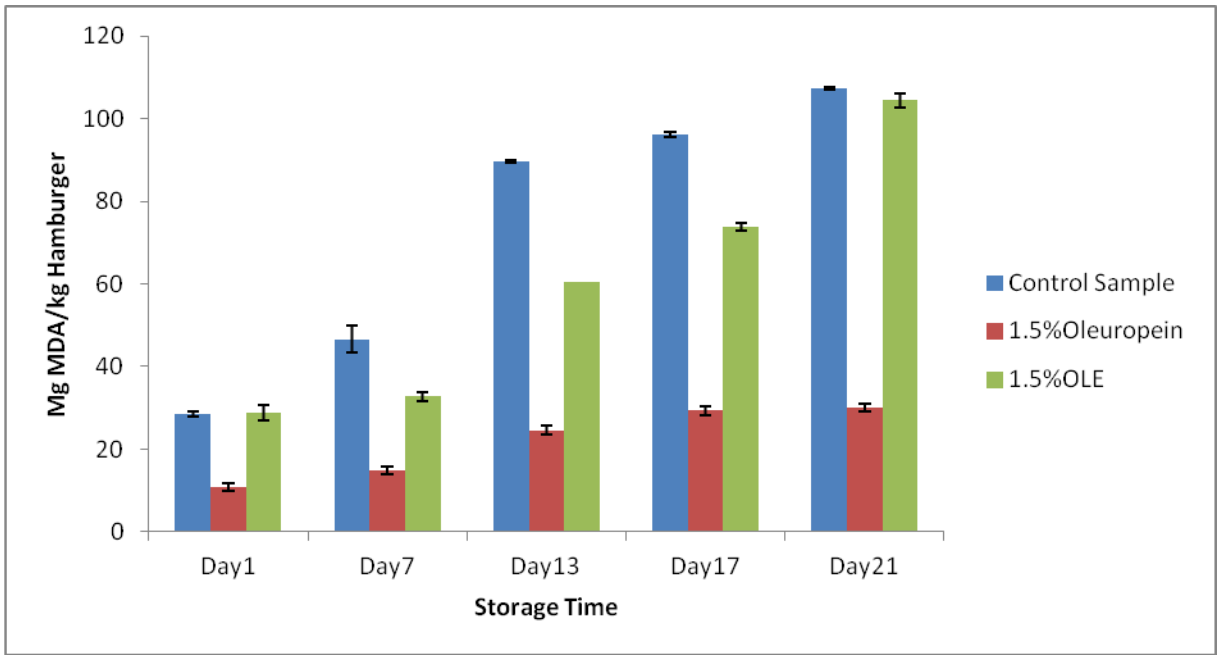


Figure 12: Oxidation products of fresh hamburger samples and treated samples with 1.5% Oleuropein and 1.5% OLE determined by TBARS spectrophotometric method

Table 7: Oxidation products of fresh hamburger samples treated with 1.5% Oleurpein and 1.5% OLE determined by TBARS spectrophotometric method.

Storage time	mg MDA/ kg hamburger		
	Control Sample	1.5%Oleuropein	1.5%OLE
Day1	28.5±0.59 aE	10.9±0.37 dE	28.9b±1.8E
Day 7	46.6±3.2 aD	14.9±0.1 cD	32.7±0.96bD
Day 13	89.6±0.27 aC	24.6±4.3 dC	60.5±0.07bC
Day 17	96.1±0.58 aB	29.3±1.6 dB	73.8±0.19bB
Day 21	107.4±0.27 aA	30.1±0.32 dA	104.4±1.7bA

7.2.9. Effect of addition Oleuropein and OLE on the pH of fresh hamburger samples

Table 8 and (Appendix 5) show that there is no significant difference between the means of pH for the Oleuropein and OLE for each concentration. It observed that there is no change of pH in function of time and hence no significant differences among time for all the concentration for OLE and Oleuropein, which indicates that addition of Oleuropein or OLE to fresh hamburger does not affect the pH of hamburger samples.

Table 8: pH result of control 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

7.3. Oxidation products of frozen hamburger

Oleuropein (40%) was added to frozen hamburger as natural antioxidant at three concentrations (0.25%, 0.5% and 0.75%). Additionally, Sodium Erythorbate was also used in frozen hamburger at three concentrations (0.25%, 0.5% and 0.75%) as control samples. The amounts of oxidation products (expressed as mg MDA per Kg hamburger sample) were determined spectrophotometrically.

7.3.1. Effect of Oleuropein on the oxidation of frozen hamburger

The effectiveness of Oleuropein was different depending on the concentration added. The oxidative stability of hamburger samples were examined by conducting thiobarbituric acid reactive substances (TBARS) assay. Absorbances, obtained from these assays at 532 nm. Table 9 shows the amounts of oxidation products of frozen hamburger samples treated with different concentrations of Oleuropein from day 1 to 6 months. As we can see from table 9 and figure13, the amounts 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 month) indicating that oxidation increases with time. However, the increase in the oxidation products of control hamburger sample was higher than that for treated samples (0.25, 0.5, and 0.75%).

These results were analyzed statistically to test if there is a statistical significant difference between the amounts of oxidation products as storage time increases from day 1 to 6 months. Results showed that, the amounts of the oxidation products ($P \leq 0.05$) increases significantly as storage time increases from day 1 to 3 months for control hamburger samples, as well as for samples treated with 0.25% Oleuropein, see Table 9. While from 3 to 6 months of storage, there is no significant differences 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 3 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 increase from day 1 to 4 months, while after 4 months the amounts of oxidation products do not change significantly.

Table 9: Oxidation products of frozen hamburger samples treated with different concentrations of Oleuropein (0.25, 0.5, and 0.75%) determined by TBARS spectrophotometric method.

Time	Concentration				Best concentration
	Control	0.25% Oleuropein	0.5% Oleuropein	0.75% Oleuropein	
First day	20.6±3.62aE	17.9±1.63 aE	20.4±2.98 aF	17.3±2.1 aF	No difference
one week	29.6±1.49 aD	26.8±1.32 aD	26.5±1.2 aE	26.4±2.4 aE	No difference
one month	158.7±12.7 aC	117±23.7 cC	100.1±4.02 dD	132.3±23.9 bD	٠,٥%
two months	263.9±1.69 aB	178.2±1.4 bB	165.9±1.6 cC	199.4±1.99 dC	٠,٥%
3 months	266.2±0.33 aA	258.3±0.4 bA	243.3±1.1 cB	247.2±0.27 dB	٠,٥%
4 months	266.6±0.48 aA	255.3±0.22 bA	247.1±0.42 cA	253.9±5.99 dA	٠,٥%
5 months	266.8±1.08 aA	256.2±1.2bA	247.5±0.52 cA	256.3±4.8dA	٠,٥%
6 months	267.9±0.06 aA	258.8±0.58 bA	248.2±0.5 cA	255.1±3.5 dA	٠,٥%

- Small letters indicates 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 month).

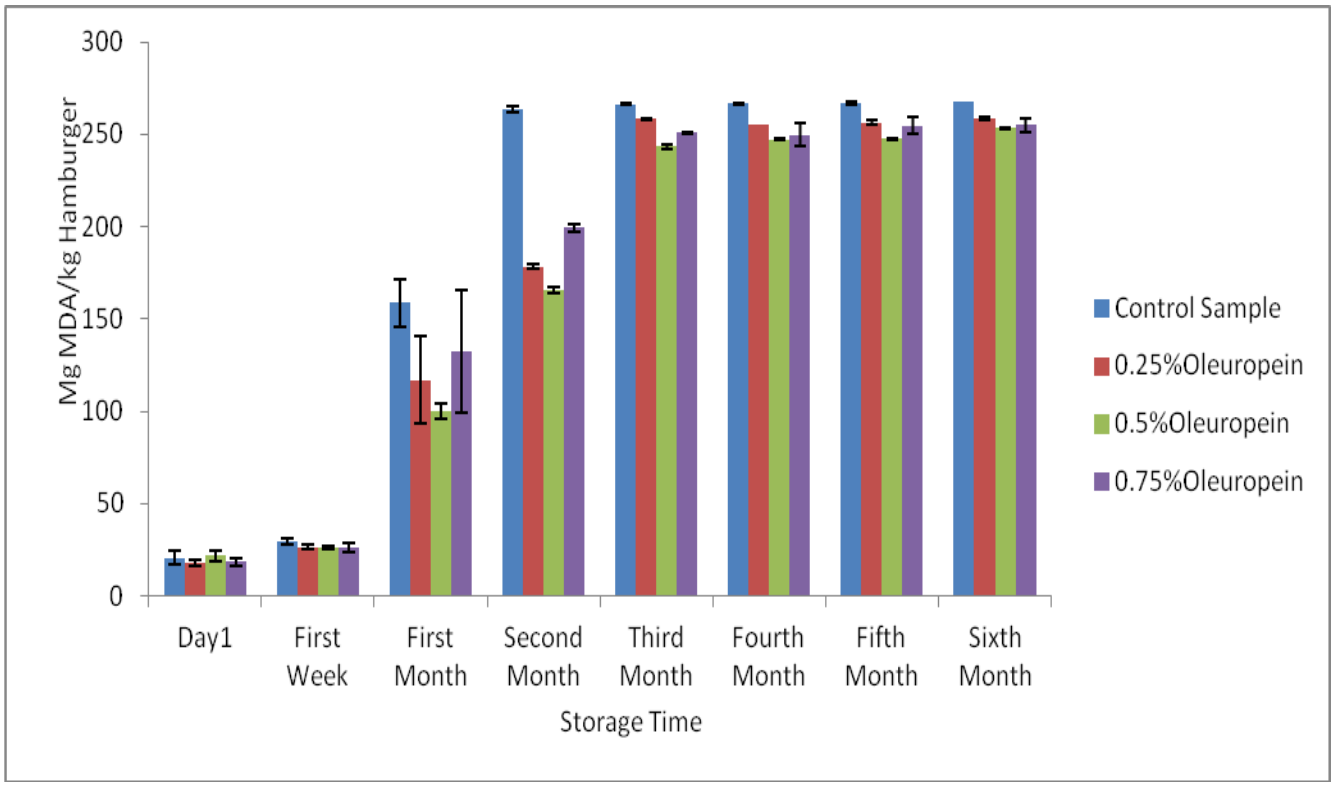


Figure 13: Oxidation products of frozen hamburger samples treated with different concentrations of Oleuropein (0.25, 0.5, and 0.75%) determined by TBARS spectrophotometric method.

7.3.2.Optimum concentration of Oleuropein in frozen hamburger samples

To determine the best concentration of Oleuropein in frozen hamburger samples to be stored for six months, statistical analyses were done to determine if there are significant differences between the amounts of oxidation products of hamburger treated with different concentrations of Oleuropein for each storage period (day 1 to 6 months) (Appendix6) .

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

After one month as well as 2, 3 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 amounts 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 differences between different concentration, 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.

7.3.3. Effect of Oleuropein on the shelf life of frozen hamburger

Usually frozen hamburger is consumed within 6 months of frozen storage. Comparing the amounts of oxidation products for frozen samples, which are 267.9 mg/kg for control sample and 253.3 mg/kg for treated hamburger samples after six month . For those treated with 0.5% it was shown that oxidation products of treated hamburger samples with 0.5% after 6 month is similar to oxidation products of control after two month which indicates the prolongation of shelf life of frozen hamburger in terms of oxidation products has occurred. As it was seen, Oleuropein has antioxidant property, which involve mode of action of polyphenols.

7.3.4. Effect of Sodium Erythorbate on the oxidation of frozen hamburger

The same study was conducted using Sodium Erythorbate at three concentrations as in the case of Oleuropein (0.25, 0.5, and 0.75%). The results are shown in table 10 and figure 14.

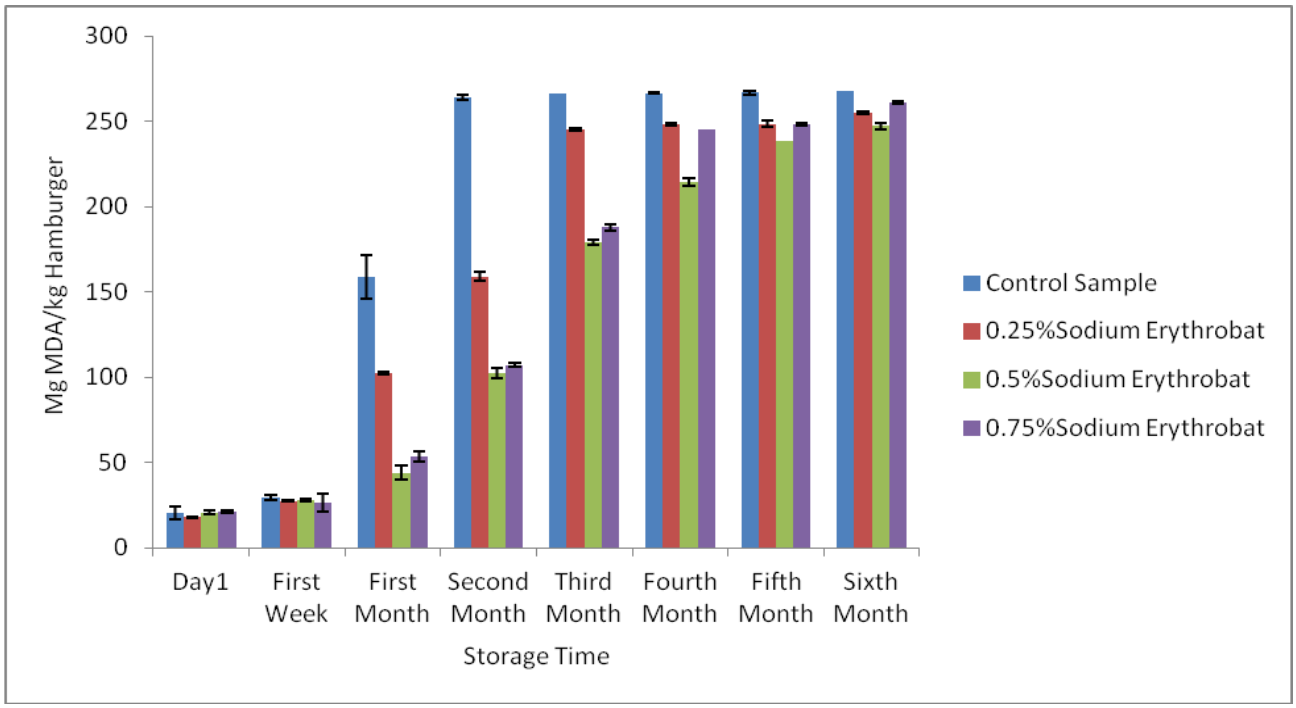


Figure 14: Oxidation products of frozen hamburger samples treated with different concentrations of Sodium Erythrobate (0.25, 0.5, and 0.75%) determined by TBARS spectrophotometric method.

Table 10: Oxidation products of frozen hamburger samples treated with different concentrations of Sodium Erythorbate (0.25, 0.5, and 0.75%) determined by TBARS spectrophotometric method.

Stored time	Result mg MDA/kg hamburger				Best concentration
	Control Sample	0.25% Sodium Erythrobat	0.5% Sodium Erythrobat	0.75% Sodium Erythrobat	
First day	20.6±3.62aE	18±0.44 aE	20.8±1.07aH	21.4±0.88 aH	No difference
Week	29.6±1.49 aD	27.5±0.21 aD	27.8±0.69 aG	29.6±5.4 aG	No difference
one month	158.7±12.7 aC	101.9±0.96 bC	41.9±3.9 dF	51.8±3.04 cF	٠,٥%
two months	263.9±1.69 aB	157.7±2.4 bB	100.7±3.3 dE	106.4±1.2 cE	٠,٥%
3 months	266.2±0.33 aA	245.7±0.7 bA	179.1±1.7 dD	187.9±1.8 cD	٠,٥%
4 months	266.6±0.48 aA	247.9±0.74 bA	214.3±2.1 cC	245.2±0.27 bC	٠,٥%
5 months	266.8±1.08 aA	248.4±1.9 bA	238.4±0.11 cB	247.9±0.74 bB	٠,٥%
6 months	267.9±0.06 aA	254.9±0.64 bA	247.4±1.8 cA	261.2±0.74 bA	٠,٥%

- Small letters indicates 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 month).

Results showed the amount of oxidation products ($P < 0.05$) for control hamburger samples as well as for those treated with 0.25% Sodium Erythorbate, increase from day 1 to 2 months, but after 2 months the amounts of oxidation products do not change significantly as storage time increases (for month 2 to 6). For those treated with 0.5% and 0.75% Sodium Erythorbate, the amounts of oxidation products increases significantly from day 1 to 6 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 myoglobein and copper and zinc present in meat tissues.

7.3.5. Optimum concentration of Sodium Erythorbate in frozen hamburger samples

To determine the best concentration of Sodium Erythorbate in frozen hamburger samples for 6 months, statistical analyses were done to determine if there is significant differences between the oxidation products of hamburger treated with different concentrations of Sodium Erythorbate for each storage period (from day 1 to 6 months) (Appendix6).

After first day and one week, there are no statistical differences between each pair 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 differences between the amounts of oxidation products of each pair concentration and the control, except between 0.25% and 0.75%, and the least oxidation products occurs when using 0.5% Sodium Erythorbate.

7.3.6. Relationship between oxidation and time in frozen hamburger

To study the relationship between oxidation products of frozen hamburger samples and storage time, statistical analysis was conducted using simple linear regression for each concentration and time for both Oleuropein and Sodium Erythorbate levels (Appendix7).

Results, show a significant positive strong relationship between time and oxidation (P-value= 0.000 less than 0.05 level of significant) at the levels of Oleuropein and Sodium Erythorbate which means that oxidation increases with increasing time for each concentration, as in the following relationship:

Oxidation = Constant + B*time

Statistical analysis (Appendices7) shows that there is no better coefficient for Oleuropein or Sodium Erythorbate where the range is between 38.51- 40.86 for all the concentrations, which indicates that Oleuropein is similar to Sodium Erythorbate in terms of antioxidant activity.

7.3.7. Comparison between Oleuropein and Sodium Erythorbate as antioxidant of frozen hamburger

Comparing the result obtained from Oleuropein and Sodium Erythorbate, the best concentration to preserve frozen hamburger for 6 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 were 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 this 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) (Keceli and Gordon, 2002), and there may not be enough antioxidant in the media to neutralize these pro-oxidants.

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 induces oxidation. This trend of prooxidant was also observed for ascorbic acid and gallic acid (Yen et al., 2002). 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 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 was also conducted using independent sample t-test to test the differences between Oleuropein and Sodium Erythorbate at each concentration level, see tables (Appendices 8 & 9) Statistical analyses shows 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.

7.3.7.1. Comparison between samples treated with 0.25% Oleuropein and samples treated with 0.25% Sodium Erythorbate

Statistical analysis of the results showed in Table11 and Figure 15 shows that there is no significant difference between the means of oxidation products for Oleuropein and Sodium Erythorbate (the mean of Oleuropein is 171 and Sodium Erythorbate is 162.7), see Appendix 8. Indicating that Oleuropein can be used as Sodium Erythorbate.

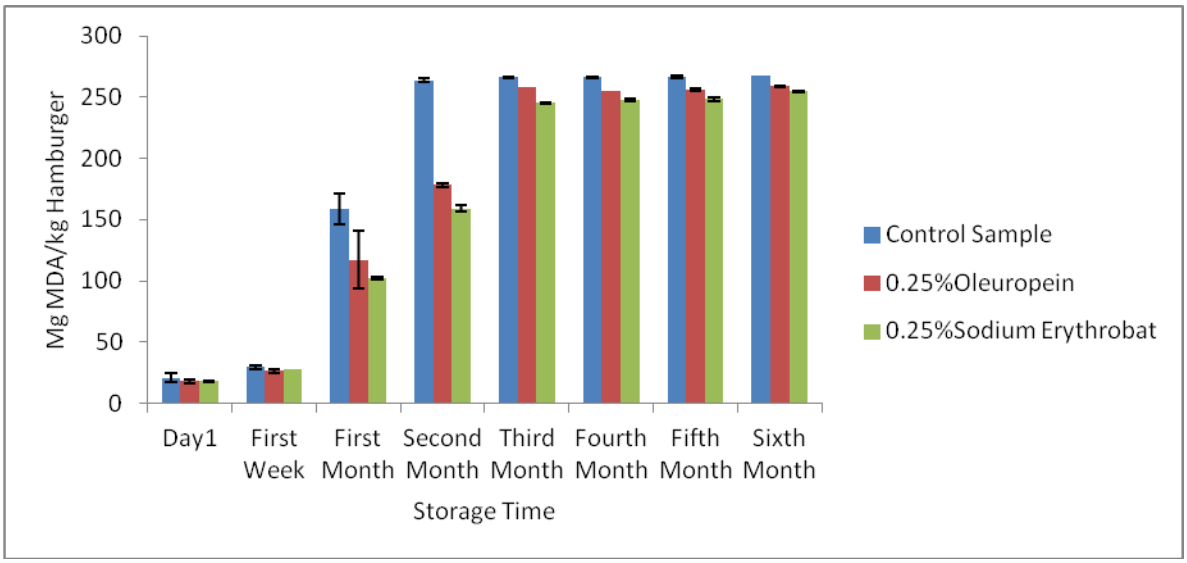


Figure 15: Oxidation products of frozen hamburger samples treated with 0.25% Oleuropein and 0.25% Sodium Erythorbate determined by TBARS spectrophotometric method

Table 11: Oxidation products of frozen hamburger samples treated 0.25% Oleuropein and 0.25% Sodium Erythorbate determined by TBARS spectrophotometric method

Storage time	mg MDA/ kg hamburger		
	Control Sample	0.25% Oleuropein	0.25% Sodium Erythrobat
Day 1	20.6±3.62aE	17.9±1.63 aE	18±0.44 aE
First week	29.6±1.49 aD	26.8±1.32 aD	27.5±0.21 aD
First month	158.7±12.7 aC	117±23.7 cC	101.9±0.96 bC
Second month	263.9±1.69 aB	178.2±1.4 bB	157.7±2.4 bB
Third month	266.2±0.33 aA	258.3±0.4 bA	245.7±0.7 bA
Fourth month	266.6±0.48 aA	255.3±0.22 bA	247.9±0.74 bA
Fifth month	266.8±1.08 aA	256.2±1.2bA	248.4±1.9 bA
Sixth month	267.9±0.06 aA	258.8±0.58 bA	254.9±0.64 bA

7.3.7.2. Comparison between samples treated with 0.5% Oleuropein and samples treated with 0.5% Sodium Erythorbate

Statistical analysis of the results showed in Table12 and Figure 16 shows that there is no significant difference between the means of oxidation products for Oleuropein and Sodium Erythorbate (the mean of Oleuropein is 163 and Sodium Erythorbate is 133), indicating that Oleuropein can be used as Sodium Erythorbate.

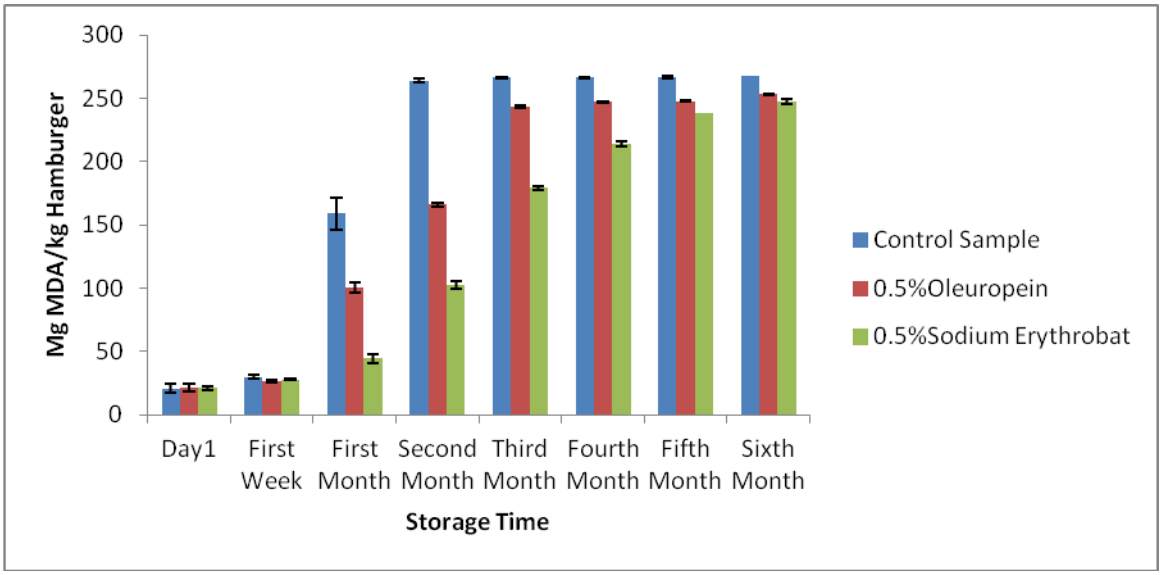


Figure 16:Oxidation products of frozen hamburger samples treated with 0.5% Oleuropein and 0.5% Sodium Erythorbate determined by TBARS spectrophotometric method

Table 12 : Oxidation products of frozen hamburger samples treated 0.5% Oleuropein and 0.5% Sodium Erythorbate determined by TBARS spectrophotometric method.

Storage time	mg MDA/ kg hamburger		
	Control Sample	0. 5% Oleuropein	0. 5% Sodium Erythrobat
Day 1	20.6±3.62aE	20.4±2.98 aF	20.8±1.07aH
First week	29.6±1.49 aD	26.5±1.2 aE	27.8±0.69 aG
First month	158.7±12.7 aC	100.1±4.02 dD	41.9±3.9 dF
Second month	263.9±1.69 aB	165.9±1.6 cC	100.7±3.3 dE
Third month	266.2±0.33 aA	243.3±1.1 cB	179.1±1.7 dD
Fourth month	266.6±0.48 aA	247.1±0.42 cA	214.3±2.1 cC
Fifth month	266.8±1.08 aA	247.5±0.52 cA	238.4±0.11 cB
Sixth month	267.9±0.06 aA	248.2±0.5 cA	247.4±1.8 cA

7.3.7.3. Compare between samples treated with 0.75% Oleuropein and samples treated with 0.75% Sodium Erythorbate

Statistical analysis of the results showed in Table13 and Figure 17 shows that there is no significant difference between the means of oxidation products for Oleuropein and Sodium Erythorbate (the mean of Oleuropein is 173 and Sodium Erythorbate is 143). Indicating that Oleuropein can be used as Sodium Erythorbate.

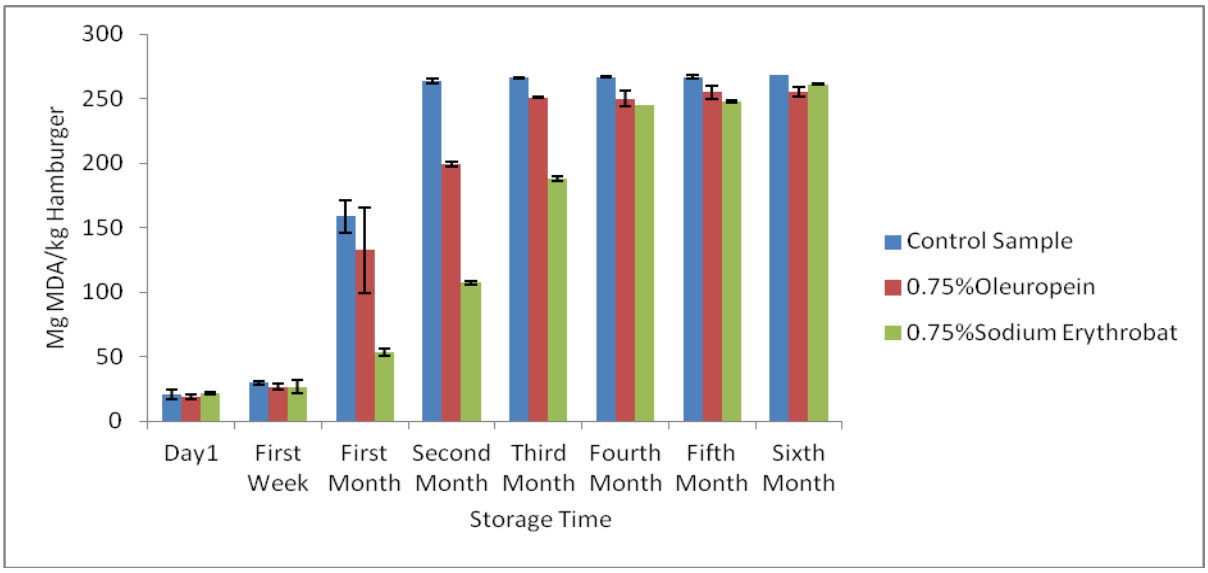


Figure 17: Oxidation products of frozen hamburger samples treated with 0.75% Oleuropein and 0.75% Sodium Erythorbate determined by TBARS spectrophotometric method

Table 12: Oxidation products of frozen hamburger samples treated 0.75% Oleuropein and 0.75% Sodium Erythorbate determined by TBARS spectrophotometric method

Storage time	mg MDA/ kg hamburger		
	Control Sample	0. 75% Oleuropein	0. 75% Sodium Erythrobat
Day 1	20.6±3.62aE	17.3±2.1 aF	21.4±0.88 aH
First week	29.6±1.49 aD	26.4±2.4 aE	29.6±5.4 aG
First month	158.7±12.7 aC	132.3±23.9 bD	51.8±3.04 cF
Second month	263.9±1.69 aB	199.4±1.99 dC	106.4±1.2 cE
Third month	266.2±0.33 aA	247.2±0.27 dB	187.9±1.8 cD
Fourth month	266.6±0.48 aA	253.9±5.99 dA	245.2±0.27 bC
Fifth month	266.8±1.08 aA	256.3±4.8dA	247.9±0.74 bB
Sixth month	267.9±0.06 aA	255.1±3.5 dA	261.2±0.74 bA

Conclusion

8. 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. In this study two types of extracts have been used. Pure oleuropin and oleuropin (OLE) extracted at the laboratories of the Faculty of Science –Al-Quds University. The food product chosen was fresh and frozen hamburger and a 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 and to study also the effect of sodium erythrobate on the shelf of frozen hamburger.

A modified TBARs test was developed due to the industrial process of hamburger making, such as freezing or refrigeration, which leads to longer reaction time of TBA with MDA formed by oxidation, and also because hamburger is an emulsion formed from meat and fat. In this modified TBARS method, 5g of sample and the boiling time was increased from 10 min to 45 min to allow appearance of pink color.

Three concentrations of oleuropein used in frozen hamburger had shown good efficiency in delaying the rate of oxidation compared to the non-treated samples. We can conclude that the best results obtained were using a concentration of 0.5% oleuropein. Sodium erythorbate which is used as a curing agent in processed meat has shown best results with a concentration of 0.5%. The comparison between the use of Oleuropin and sodium erythrobate in frozen hamburger samples has statistically shows no significant difference between the means of oxidative products for Oleuropein and Sodium Erythorbate, which means that oleuropein can be used alternative to chemical preservatives.

In fresh hamburger 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 O.5% OLE showed the best result. Oleuropein can be used as a natural anti-oxidant in fresh hamburger using a lower concentration compared to OLE, this due to the presence of prooxidants in the lab extracted OLE. Oleuropein or OLE had no effect on hamburger PH during storage time, showing that measuring pH is not a suitable method for determination of hamburger deterioration.

Finally From the results obtained ,it can be conclude that oleuropein and OLE is an effective natural antioxidant, as alternative to chemical preservative and further studies must be done so as to study its antimicrobial activities.

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الإمكانات المضادة للأكسدة لمستخلص ورق الزيتون و Oleuropein في منتجات الهامبرجر الطازجة و المجمدة

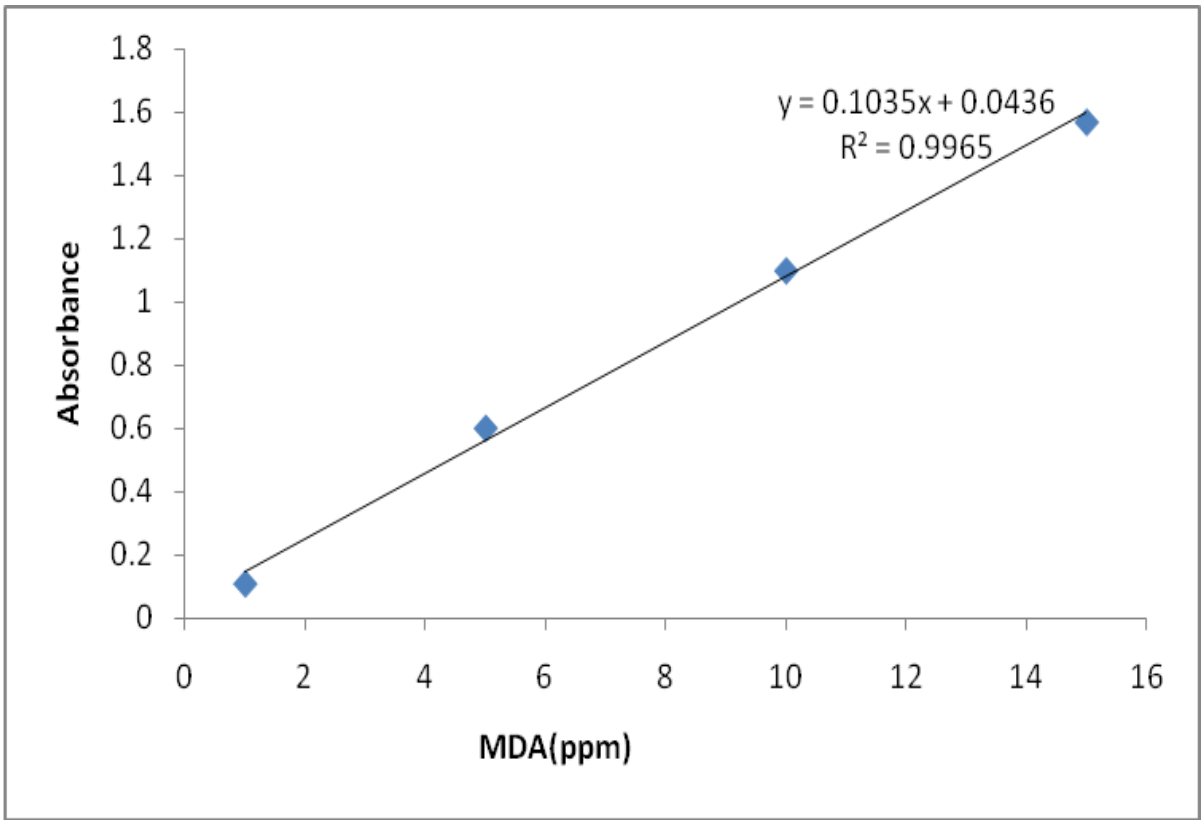
الملخص باللغة العربية:

الأكسدة هي واحد من الأسباب الرئيسية التي تسبب تلف الهمبرغر،ولهذا تستخدم المواد المضادة للأكسدة لمنع أو تأخير عملية الأكسدة. غالبا تستخدم مضادات الأكسدة الكيميائية في صناعة اللحوم مثل إيريثوربات الصوديوم، لكن هذه المواد الحافظة الكيميائية ليست آمنة ولها آثار ضارة على صحة الإنسان.حاليا هناك اتجاه لاستخدام مضادات الأكسدة الطبيعية في الصناعة لأنها تعتبر آمنة بالمقارنة مع المواد الكيميائية. في هذه الدراسة تم استخدام مضادات الأكسدة الطبيعية (مادة الاوليروبين (Oleuropein) ومستخلص ورق الزيتون (OLE)) في الهمبرغر الطازج والمجمد ومقارنتها مع إيريثوربات الصوديوم الذي يستخدم على نطاق واسع في الهمبرغر المجمد. تم استخدام طريقة Thiobarbituric acid–reactive substances (TBARS) spectrophotometric method لتحديد نواتج الأكسدة في عينات الهمبرغر (تم التعبير عنها ب ملغ من ثنائي ألدهيد المالون لكل كيلوغرام من عينة الهمبرغر). وأظهرت نتائج هذه الدراسة أن Oleuropein يمكن استخدامه كمضاد طبيعي للأكسدة على غرار المواد المضادة للاكسدة الاصطناعية (مثل إيريثوربات الصوديوم)، حيث أظهرت التحليلات الإحصائية عدم وجود فروقات ذات دلالة إحصائية بين الكميات الناتجة من عمليات الاكسدة في عينات الهمبرغر المعالجة باستخدام Oleuropein أو باستخدام إيريثوربات الصوديوم .وأظهرت هذه الدراسة أيضا أن أفضل تركيز من Oleuropein أو إيريثوربات الصوديوم هو ٠,٥٪ لاستخدامها في الهمبرغر المجمد بينما تستخدم معظم شركات اللحوم ١,٥٪ من إيريثوربات الصوديوم كمادة مضادة للأكسدة في الهمبرغر المجمد. وفيما يتعلق بعينات الهمبرغرالطازج، أثبتت النتائج فعالية Oleuropein و OLE كمضادات طبيعية للأكسدة حيث أخرت عملية الأكسدة مقارنة مع عينات بدون مضادات أكسدة، وأظهرت النتائج أن ٠,٥٪ من Oleuropein و ١,٥٪ من OLE هو أفضل تركيز لاستخدامها في عينات الهمبرغر الطازج.

Appendices

Appendices:

A 1 : Calibration carve



A 2:Statistical significant difference between the means (averages) of the oxidation among the concentrations (control, 0. 5%, 1% , 1,5%) at each time point

Oneway	F- statistic	sig	control-0.5%	sig	control-1%	sig	control-1.5%	sig	0. 5%-1%	sig	0. 5%-1.5%	sig	1%-1.5%	sig
type = Oleuropein, days = 1.00	297.19	0.00	10.84	0.00	10.10	0.00	17.61	0.00	-0.74	0.62	6.77	0.00	7.51	0.00
type = Oleuropein, days = 5.00	235.46	0.00	32.34	0.00	29.51	0.00	31.74	0.00	-2.83	0.28	-0.60	0.97	2.23	0.46
type = Oleuropein, days = 13.00	554.54	0.00	29.51	0.00	62.35	0.00	64.90	0.00	32.84	0.00	35.39	0.00	2.55	0.54
type = Oleuropein, days = 17.00	3689.32	0.00	47.22	0.00	61.89	0.00	66.85	0.00	14.67	0.00	19.63	0.00	4.96	0.00
type = Oleuropein, days = 21.00	12105.63	0.00	55.80	0.00	68.27	0.00	77.30	0.00	12.47	0.00	21.50	0.00	9.03	0.00
type = OLE, days = 1.00	57.12	0.00	7.37	0.00	8.43	0.00	-0.46	0.95	1.06	0.64	-7.83	0.00	-8.89	0.00
type = OLE, days = 5.00	103.66	0.00	26.00	0.00	23.28	0.00	13.96	0.00	-2.73	0.40	-12.05	0.00	-9.32	0.00
type = OLE, days = 13.00	3391.84	0.00	49.17	0.00	47.15	0.00	29.19	0.00	-2.02	0.03	-19.98	0.00	-17.96	0.00
type = OLE, days = 17.00	370.35	0.00	31.57	0.00	28.45	0.00	22.28	0.00	-3.12	0.07	-9.28	0.00	-6.16	0.00
type = OLE, days = 21.00	564.52	0.00	31.57	0.00	27.31	0.00	3.01	0.06	-4.25	0.01	-28.55	0.00	-24.30	0.00

A 3: Relationship between oxidation and time for fresh hamburger

Regression	R-square	F	sig	constant	t	sig	Days	t	sig
type = Oleuropein, concent = control	0.97	442.51	0.00	27.37	10.42	0.00	4.06	21.04	0.00
type = Oleuropein, concent = 0.50%	0.72	32.72	0.00	14.08	2.76	0.02	2.14	5.72	0.00
type = Oleuropein, concent = 1%	0.94	206.73	0.00	14.41	13.53	0.00	1.13	14.38	0.00
type = Oleuropein, concent = 1.5%	0.93	172.53	0.00	10.24	9.59	0.00	1.03	13.14	0.00
type = OLE, concent = control	0.97	442.51	0.00	27.37	10.42	0.00	4.06	21.04	0.00
type = OLE , concent = 0.50%	0.93	167.31	0.00	11.30	3.69	0.00	2.92	12.93	0.00
type = OLE, concent = 1%	0.94	215.73	0.00	11.23	3.89	0.00	3.12	14.69	0.00
type = OLE, concent = 1.5%	0.94	206.58	0.00	18.57	5.39	0.00	3.64	14.37	0.00

A 4: Comparison between Oleuropein and OLE as antioxidant of fresh Hamburger

T-Test		mean		mean	levene-f	Sig	t	sig
(concentration = 0.5%).	Oleuropein	38.53	OLE	44.54	0.80	0.38	-0.77	0.45
(concentration = 1%).	Oleuropein	27.25	OLE	46.75	25.19	0.00	-2.88	0.01
(concentration = 1.5%).	Oleuropein	21.99	OLE	60.08	14.94	0.00	-4.92	0.00

A 5: t test for pH values

type	T test	Sig
concentration = 0.5	-.785	.451
concentration = 1	-.677	.514
concentration = 1.5	.483	.639

Reggression				
	F	sig	Slope	Sig
concentration = 0.5, type = OLE	1.717	.260a	-.010	.260
concentration = 0.5, type = Oleuropein	.002	.970a	.000	.970
concentration = 1, type = OLE	3.061	.155a	-.012	.155
concentration = 1, type = Oleuropein	.238	.651a	-.006	.651
concentration = 1.5, type = OLE	2.743	.173a	-.011	.173
concentration = 1.5, type = Oleuropein	.866	.405a	-.009	.405
concentration = Control, type = OLE	.087	.783a	-.002	.783
concentration = Control, type = Oleuropein	.087	.783a	-.002	.783

A6. Statistical significant difference between the means (averages) of the oxidation among the concentrations (control, 0. 25%, 0.5% , 0.75%) at each time point

Type	F	sig	control-0.25	sig	control- 0.5	sig	control- 0.75	sig	0.25-0.5	sig	0.25-0.75	sig	0.5-0.75	sig
type = ouleuropein, days1 = first day	1.21	0.37	2.69	0.63	0.21	1.00	3.33	0.47	-2.48	0.68	0.64	0.99	3.12	0.52
type = ouleuropein, days1 = after weak	2.50	0.13	2.80	0.25	3.08	0.19	3.22	0.16	0.28	1.00	0.43	0.99	0.14	1.00
type = ouleuropein, days1 = after month	4.07	0.05	41.74	0.16	58.62	0.04	26.38	0.47	16.88	0.77	-15.36	0.82	-32.24	0.32
type = ouleuropein, days1 = after two month	2006.70	0.00	85.66	0.00	98.03	0.00	64.55	0.00	12.36	0.00	-21.11	0.00	-33.48	0.00
type = ouleuropein, days1 = after 3 month	715.17	0.00	7.97	0.00	22.99	0.00	15.34	0.00	15.02	0.00	7.37	0.00	-7.65	0.00
type = ouleuropein, days1 = after 4 month	24.51	0.00	11.30	0.01	19.45	0.00	16.76	0.00	8.15	0.04	5.46	0.20	-2.69	0.70
type = ouleuropein, days1 = after 5 month	28.37	0.00	10.59	0.00	19.27	0.00	10.56	0.00	8.68	0.01	-0.04	1.00	-8.71	0.01
type = ouleuropein, days1 = after 6 month	38.54	0.00	9.18	0.00	14.67	0.00	12.86	0.00	5.49	0.03	3.68	0.14	-1.81	0.64
type = sodium, days1 = first day	1.82	0.22	2.66	0.40	-0.21	1.00	-0.74	0.96	-2.87	0.34	-3.40	0.22	-0.53	0.99
type = sodium, days1 = after weak	0.50	0.69	2.13	0.79	1.84	0.85	0.00	1.00	-0.28	1.00	-2.13	0.79	-1.84	0.85
type = sodium, days1 = after month	182.90	0.00	56.85	0.00	116.82	0.00	106.90	0.00	59.98	0.00	50.06	0.00	-9.92	0.35
type = sodium, days1 = after two month	3308.33	0.00	106.17	0.00	163.18	0.00	157.47	0.00	57.00	0.00	51.30	0.00	-5.70	0.06
type = sodium, days1 = after 3 month	3213.40	0.00	20.55	0.00	87.15	0.00	78.29	0.00	66.60	0.00	57.75	0.00	-8.86	0.00
type = sodium, days1 = after 4 month	1047.63	0.00	18.67	0.00	52.29	0.00	21.40	0.00	33.62	0.00	2.73	0.08	-30.89	0.00
type = sodium, days1 = after 5 month	302.39	0.00	18.46	0.00	28.38	0.00	18.92	0.00	9.92	0.00	0.46	0.96	-9.46	0.00
type = sodium, days1 = after 6 month	214.14	0.00	13.04	0.00	20.51	0.00	6.73	0.00	7.48	0.00	-6.31	0.00	-13.78	0.00

A7: Relationship between oxidation and time in frozen hamburger

Regression	rsquare	F	sig	Constant	t	sig	days	t	sig
type = ouleuropein, concent = control	0.74	62.70	.000a	18.80	0.76	0.45	38.61	7.92	0.00
type = ouleuropein, concent = o.25	0.85	127.90	.000a	-7.22	-0.41	0.69	39.62	11.31	0.00
type = ouleuropein, concent = o.5	0.88	158.49	.000a	-11.29	-0.73	0.47	38.73	12.59	0.00
type = ouleuropein, concent = o.75	0.83	105.34	.000a	1.03	0.05	0.96	38.31	10.26	0.00
type = sodium, concent = control	0.74	62.70	.000a	18.80	0.76	0.45	38.61	7.92	0.00
type = sodium, concent = o.25	0.88	165.46	.000a	-13.47	-0.88	0.39	39.15	12.86	0.00
type = sodium, concent = o.5	0.94	373.86	.000a	-39.51	-3.93	0.00	38.51	19.34	0.00
type = sodium, concent = o.75	0.94	331.27	.000a	-39.93	-3.52	0.00	40.86	18.20	0.00

A 8: Comparison between Oleuropein and Sodium Erythrobat as antioxidant of frozen Hamburger

T-Test	mean			mean	Levene-F	sig	t	sig
concent = o.25	ouleuropein	171.05	sodium	162.73	0.01	0.91	0.29	0.77
concent = o.5	ouleuropein	163.01	sodium	133.80	0.00	0.96	1.07	0.29
concent = o.75	ouleuropein	173.42	sodium	143.92	0.25	0.62	1.04	0.31

A 9 : Comparison between Oleuropein and sodium Erythrobat as antioxidant of frozen Hamburger

Anova	F	Sig
type = ouleuropein, concent = control	1587.919	0.00
type = ouleuropein, concent = o.25	460.3198	0.00
type = ouleuropein, concent = o.5	7874.139	0.00
type = ouleuropein, concent = o.75	216.078	0.00
type = sodium, concent = control	1587.919	0.00
type = sodium, concent = o.25	20325.37	0.00
type = sodium, concent = o.5	5827.531	0.00
type = sodium, concent = o.75	5716.948	0.00