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**Antimicrobial and Antioxidant Effect of Essential Oil  
in Refrigerated Beef Burger**

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# **Antimicrobial and Antioxidant Effect of Essential Oil in Refrigerated Beef Burger**

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## **Dedication**

This work is dedicated with sincere gratitude to my dear parents and to my beloved wife Jumana abo Rayyan, who have been constant source of support and encouragement during the challenges of graduate studies and life.

## Declaration

I declare that this thesis is done in fulfillment of the degree of Master in Applied and Industrial Technology, is the outcome of my personal research, except where otherwise acknowledged, and this thesis has not been submitted for a higher degree to any other university or institution.

Salah Aldeen Nassar Mohammad Zamel

Signed: The image shows a handwritten signature in blue ink. The signature is stylized and appears to read 'Zamel' followed by 'Nassar Aldeen' in smaller script below it.

Date: 30/08/2022

## **Acknowledgments**

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## **Abstract**

Microbial growth and oxidation are considered to be the major causes of beef burger deterioration. Because of that, synthetic antimicrobial and antioxidants are used to prevent or delay the microbial growth and oxidation process. Unfortunately, these substances are not safe and may cause harmful impacts on human health, which arose the need to search for natural alternatives in the food industry. In this study, essential oils of clove, thyme, and rosemary serve as natural preservatives to increase the shelf life of refrigerated beef burger up to 7 days without using synthetic antimicrobial and antioxidant agents.

Essential oils were added to beef burger at different concentrations for each essential oil (1 and 1.5% v/w). In addition, two positive control samples for antimicrobial and antioxidant agent were treated with 2% potassium lactate (PL) and 100 ppm Butylated hydroxytoluene (BHT) respectively. All samples were kept at 4°C for 7 days (study period). Measurement of the antimicrobial activity of essential oils was performed by testing total plate count, total coliforms, and *Staphylococcus aureus*. The oxidation stability test was carried out by thiobarbituric acid reactive substances (TBARS) test, and expressed as mg of malonaldehyde/ kg beef burger, in addition to that, the pH of each sample was examined.

Essential oils added to beef burger showed strong antimicrobial effects against the examined microbes. The obtained results revealed that, clove essential oil (CEO) added at concentration 1.5% reduced the microbial activity of *Staphylococcus aureus* by 54% compared with negative control sample, also, there is a significant difference

between it and all treated samples. In addition, results indicated that CEO at both concentrations 1% and 1.5% had the most effect on reducing microbial growth of total coliform (TC) compared with other stored samples. Total plate count results showed that essential oils at all different concentrations used in this study led to reduction in microbial growth in the burger samples without any significant differences between these essential oils (EOs).

Regarding the chemical indicators, CEO at concentrations of 1% and 1.5% were the most effective and retard oxidation by 24% and 35%, respectively compared with negative control sample. Moreover, Thyme essential oil (TEO) at a concentration of 1% and 1.5%, reduced the percentage of oxidation by 14% and 31%, respectively compared with the negative control sample.

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## List of Acronyms

µl: Microliter

ml: Milliliter

kg: Kilogram

gm: Gram

min: Minute

h: hour

°C: Degrees Celsius

ppm: Parts per million

P.L: Potassium lactate

BHT: Butylated hydroxytoluene

EOs: Essential Oils

REO: Rosemary Essential Oil

TEO: Thyme Essential Oil

CEO: Clove Essential Oil

RLE: Rosemary Lyophilized Extract

V/V: Volume/Volume

V/W: Volume/Weight

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

*E. coli.*: *Escherichia coli*

*S. aureus.*: *Staphylococcus aureus*

TPC: Total plate count

TBA: Thiobarbituric acid

TBARs: Thiobarbituric acid-reactive substances

MDA: Malondialdehyde

ROS: Reactive oxygen species

cfu: Colony forming units

SD: Standard Deviation

Con.: Concentration

# **Chapter One: Introduction**

## **1.1 Background**

Foods are produced typically from plants or animals and include a lot of important nutrients such as: (fats, proteins, vitamins, minerals, and carbohydrates). Food is consumed by an organism and digested by the organism cells to provide energy, sustain life, or stimulate growth (Abdulmumeen et al., 2012). A lot of food products, require protection from deterioration during production, storage, and distribution in order to maintain desired shelf-life (Rasooli, 2007).

Nowadays, mostly all food products contain food preservatives. The trends are to retain the natural characteristics of food, extend its shelf life and prevent natural ageing and discoloration that might occurred during food preparation steps (Sharma, 2015). Food preservation refers to the process of keeping foods safe and undamaged (Amit et al., 2017). It is accomplished by creating an unfavorable environment for microbiological spoilage and retarding chemical oxidation (Vaclavik et al., 2021). Preservation methods include techniques such as reducing accessible water (water activity), controlling temperature (low or high) and adding preservatives (Tapia et al., 2020).

It is well known that food products can be spoiled by chemicals, microbes, and enzymes found in the food itself. In addition, food deterioration can occur during food transportation, in terms of loss or decrease in morphological attraction and decrease in food nutritional value (Ahmad et al., 2021).

Synthetic methods of food maintenance can be achieved through nuclear radiation, vacuum packing, and hypobaric packing. Today, specific synthetic chemical substances are used as food preservatives. These Preservatives, are considered primarily effective for an extended time span and pausing or postponing the increase of bacteria, quell the chemical reactions when food is exposed to either oxygen or heat (Zaccheo et al., 2016). However, Food preservatives also prohibit the loss of some major amino acids, and vitamins, and promote the flavors and colors of food (Sharma, 2015).

The ultimate strategy that is used to stretch fresh meat lifetime is the use of antimicrobial and antioxidant additives of synthetic origin, which are being questioned due to an increasing consumer demand for natural, healthy and safe preservatives (Yu et al., 2021). It was reported that, essential oils (EOs) exhibit their antimicrobial activity against food-borne pathogens by interfering with and destabilizing the phospholipid bilayer of the cell membrane, the enzyme systems and the genetic material of bacteria (Burt, 2004; Kim et al., 1995). Therefore, the use of natural preservation methods is very important, and hence this research will study the use of essential oils in preserving beef burgers.

Many medicinal and aromatic plants have been discovered for their essential oils over the past several decades. However, essential oils are complex volatile compounds, which are naturally synthesized in different parts of the plant during secondary metabolism (Akthar et al., 2014). Essential oils have great potential in the biomedical field since they effectively break down a number of pathogens caused by bacteria, fungi, and viruses (Swamy et al., 2016). The presence of aldehydes, phenols, terpenes

and other antibacterial compounds makes essential oils effective against a wide range of pathogens (Swamy et al., 2016).

Meat and meat products such as beef burgers can be damaged by microbes or by fat oxidation. Therefore, it is a significant thing to conserve meat products from spoilage by adding preservatives, especially natural preservatives, to prolong their shelf life and improve their properties (Alsaqali et al., 2016; Aminzare et al., 2016). Lipid oxidation is considered to be the main cause of meat spoilage and meat products due to its high fat content. These damages lead to a loss of some nutritional value, repulsive taste and texture, and loss of water retention (Aminzare et al., 2019)

In recent years, and due to the increasing of consumer awareness towards synthetic preservatives, interest in essential oils and their applications in food preservation has increased (Chouhan et al. events, 2017). To elongate shelf life in the refrigerator, this is the most common method used to preserve fresh meat and meat products. Many synthetic additives have been used for many years (Liu et al., 1992)

Synthetic additives can be correlated with the carcinogenic and toxic properties of preserved foods. Accordingly, the growing consumer interest in healthier meat and meat products and the demand for natural food additives has led the researchers to consider natural alternatives to the food additive (Mariutti et al., 2011). Natural additives are expected to improve meat quality without leaving any remains in the product or in the environment (Simitzis et al., 2008). Essential oils are aromatic and volatile oil extracts obtained from parts of medicinal and aromatic plants, including flowers, roots, twigs, leaves, seeds, buds, bark and pods. They are stored in secretory

cells, ducts or glandular trichomes, but their qualitative and quantitative composition determines their antimicrobial and antioxidant capacity (Taban et al., 2021).

There is a global need to look for conservation alternatives that cover the same antimicrobial properties and compatibility with food. Intoxication, cancer, and other degenerative disorders are linked to the usage of synthetic preservatives and chemical additives (Laranjo et al., 2017). As a result, public concern has grown, as the desire to eat healthier products that use natural preservatives and chemicals rather than synthetic ones (Laranjo et al., 2017).

Several plant-derived EOs can be effectively used in meat and meat products as natural alternatives to synthetic food additives, particularly as effective antimicrobial agents. Moreover, phenolic compounds, such as carvacrol, eugenol, and thymol, are mainly responsible for the antimicrobial activity of EOs to increase the permeability of cell membranes and leading to loss of cellular constituents (Liu et al., 2013). Therefore, it is possible to use these compounds in preserving meat, since meat and meat products are highly prone to microbial deterioration because they are rich in essential nutrients and perishable in nature and if they are not properly preserved, public health problems may occur (Aminzare et al., 2016).

Traditional methods of preservation usually aim to exclude air, moisture, and microorganisms, or to provide environments in which organisms that might cause spoilage cannot survive. Nevertheless, food preservation can be done by boiling, freezing, pasteurizing, dehydrating, smoking, pickling (Sharma, 2015). Such

traditional methods, are efficiently decreasing the growth of bacteria in preserved food (Sharma, 2015).

## **1.2 Essential oils**

Essential oils (EOs) are mixtures of natural, volatile and aromatic compounds extracted from plants (Valdivieso-Ugarte et al., 2019). EOs are generally known as safe (GRAS), so they can be seen as a substitute to extending the shelf life of highly perishable food products by preventing the growth of pathogens in food and inhibiting lipid oxidation (Angane et al., 2022; Shaltout & Koura, 2017). Due to the antibacterial properties of EOs against pathogens caused by bacteria, fungi and viruses, many medicinal plants have been examined and tested then used for their essential oils worldwide (Akthar et al., 2014). Essential oils are considered very distinctive when it comes to fighting against microorganisms due to the existence of mass numbers of alkaloids, phenolic compounds, terpene-derived compounds and other antibacterial compounds. (Akthar et al., 2014; Chouhan et al., 2017). Therefore, essential oils can be used as an alternative against pathogenic microorganisms and have antioxidant effects (Valdivieso-Ugarte et al., 2019).

Cinnamon, cloves, thyme, marjoram and rosemary have strong inhibitory effects on various types of bacteria (Ouattara et al., 1997). Essential oils extracted from several medicinal plants (Table 1.1) have also been reported to have antibacterial effects against foodborne pathogens due to the presence of phenolic compounds such as carvacrol, eugenol and thymol (Gray et al., 1996).

Clove essential oil has many biological activities, however, 22 essential oil components obtained from clove buds have been identified, and eugenol is the major component (76.23%). The essential oil showed strong antibacterial activity against *Staphylococcus aureus* (Xu et al., 2016). In terms of mode of action, essential oils have the ability to interact first with cell walls and membranes. On the one hand, they destroy cell walls and membranes, then lose important intracellular materials, which causes the death of the cell (Xu et al. 2016). In addition, the essential oil penetrates the cytoplasmic membrane or penetrates into the interior of the cell after the cell structure is destroyed, and then inhibits the normal synthesis of DNA and proteins necessary for the growth of bacteria (Xu et al., 2016).

**Table 1. 1 Major components of selected EOs that exhibit antibacterial properties**

Common name of EO	Latin name of plant source	Major components
Clove	<i>Syzygium aromaticum</i>	Eugenol, 123Eugenyl acetate
Rosemary	<i>Rosmarinus officinalis</i>	$\alpha$ -pinene Bornyl acetate Camphor 1,8-cineole
Thyme	<i>Thymus vulgaris</i>	Thymol, Carvacrol, $\gamma$ -terpinene, p-cymene, Linalool

Adapted from (Burt, 2004).

### 1.3 Microbial deterioration of meat and meat products

There is a wide variety of meat products including cured meats, patties, nuggets, and meatballs which considered as excellent source of essential nutrients with high-quality proteins, fat, and minerals (Aminzare et al., 2019). Meat spoilage results from the development of off-odor known as putrefaction. Microbial growth, alongside with

biochemical and enzymatic degradation, are commonly responsible for the spoilage of meat and meat products (Devlieghere et al., 2004). In fact, bacterial contamination is one of the major contributors to fresh meat quality deterioration, as these products are highly susceptible to microbial contamination if not properly stored and handled.

#### **1.4 Mechanism of essential oil as antimicrobial**

Some researches demonstrated that the antimicrobial effects of the essential oils act by causing structural and functional damages to the bacterial cell membrane (Goñi et al., 2009). It has been shown that plant compounds affect microbial cells through various antimicrobial mechanisms, including attacking the phospholipid bilayer of the cell membrane, disrupting enzyme systems, and affecting the genetic material of bacteria (Viuda-Martos et al., 2011). Although the antibacterial properties of essential oils and their constituents have been previously reviewed, the mechanism of action has not been studied in detail (Lambert et al., 2001).

Given the large number of different classes of compounds existing in EOs, it is known that their antibacterial activity is not due to a specific mechanism and has a multiple targets within the cell (Lambert et al., 2001).

An important characteristic of essential oils and their components is the hydrophobicity, which enables them to partition with the lipids present in the cell membrane of bacteria and mitochondria, rendering them more permeable by disturbing the cell structures (Chouhan et al., 2017). Subsequent leaks of ions and other cell contents can occur. Some leakage from bacterial cells is tolerable without the loss of viability, while massive loss of cell contents or leakage of important

molecules and ions is fatal (Denyer, 1991). There is some evidence that tea tree oil can induce cell death with *E. coli* (Cox et al., 1998; Cox et al., 2000)

In general, EOs, which have the strongest antibacterial properties against food-derived pathogens, contain a high proportion of phenolic compounds such as carvacrol, eugenol, and thymol (Farag et al., 1989). Therefore, it seems reasonable that their mechanism of action is similar to that of other phenols. This is commonly thought as cytoplasmic membrane disruption, Proton Motive Force (PMF) disruption, electron flow, active transport, and cell content coagulation (Mohammed & Al-Bayati, 2009).

The action and antibacterial activity of individual E.O components method is influenced by their chemical structure. The importance of the presence of hydroxyl groups in phenolic compounds such as carvacrol and thymol has been confirmed. The relative position of the hydroxyl groups on the phenolic ring does not appear to have a significant effect on the level of antibacterial activity. It appears to be comparable (Lambert et al., 2001; Ultee et al., 2002). However, a study conducted by (Dorman & Deans, 2000) found that carvacrol and thymol have different effects on Gram-positive and Gram-negative bacteria.

The phenolic ring is important due to the shortage of activity of menthol in comparison with carvacrol (Ultee et al., 2002). The antibacterial activity is stimulated when an acetate is added to the molecule. On the other hand, the geranyl acetate has shown more activity against various types of gram-positive and negative kinds than geraniol (Dorman & Deans, 2000). In the case of non-phenolic components of EOs, the kind of alkyl group has been discovered to have an effect on EOs activity. For

example, limonene (1-methyl-4-(1-methylethenyl) - cyclohexene) is more active than p-cymene (Dorman & Deans, 2000).

The mechanism of action of EOs has not been studied in detail (Lambert et al., 2001). Given the large number of different groups of chemical compounds found in EOs, it is very likely that although bacterial cells have multiple targets, their antibacterial activity is not due to any specific mechanism. Another important parameter for the action of food preservatives is their ability to lower pH level. The pH of both *E. coli* and *Salmonella* has been shown to be lowered by the action of mustard EOs (Turgis et al., 2009).

In fact, the mechanisms of action of the EOs include cell wall degradation, cytoplasmic membrane damage, cytoplasmic coagulation, and membrane proteins damage, increased permeability leading to leakage of cell contents, decreased proton motive force and intracellular ATP pool. Aside from decreased ATP synthesis, increased hydrolysis, increased membrane permeability and decreased membrane potential due to increased membrane permeability (Nazzaro et al., 2013; Pellegrini et al., 2017).

### **1.5 Mechanisms of Lipid Oxidation**

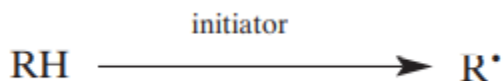
Lipid oxidation is a major cause of deterioration in meat and meat products due to their high fat content and high water activity leading to loss of nutritional value, unpleasant flavor, texture, and water holding capacity (Aminzare et al., 2019; García-Lomillo et al., 2017).

Oxidation of lipids is one of the most serious problems that shortens the shelf life of meat and meat products. Antioxidants are used to regenerate free radicals, thereby slowing the oxidation of lipids, delaying the development of off-flavors, and improving color stability. The use of synthetic antioxidants to reduce oxidative damage can be considered a disadvantage to consumers. In addition, recent growing consumer understanding of these dangers has replaced synthetic antioxidants with naturally occurring bioactive compounds. Plant materials are a rich source of bioactive phenolic compounds. Therefore, they can be effective alternatives to synthetic antioxidants. Aminzare et al., (2019) used natural antioxidant compounds such as herbs, spices, fruits, plant essential oils and extracts in meat and meat products to improve their quality and shelf-life.

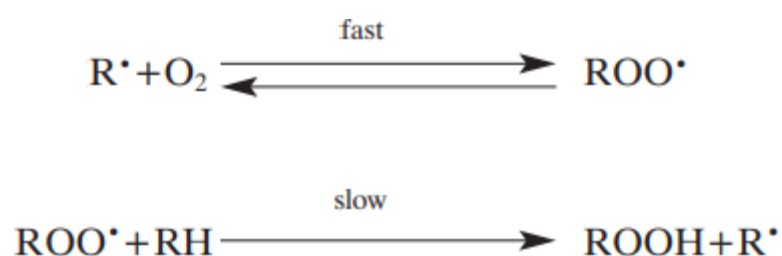
Lipid oxidation can be described as the oxygen-dependent oxidative degradation of saturated and unsaturated fatty acids. This fatty acid modification is primarily carried out by an autocatalytic free radical mechanism called autoxidation, which consists of three phases (Kumar et al., 2015).

Undesired chemical reactions can take place among which lipid oxidation is the most unfavorable and can negatively affect the colour of the meat product (Youssef, 2014). Lipid oxidation has been recognized through three different mechanisms (free radical mechanisms, photo-oxidation, and processes associated with lipoxygenase activity) that produce different oxidation products. Auto-oxidation is the spontaneous reaction of molecular oxygen with lipids, leading to oxidative degradation. It proceeds through a free radical chain mechanism that involves three steps:

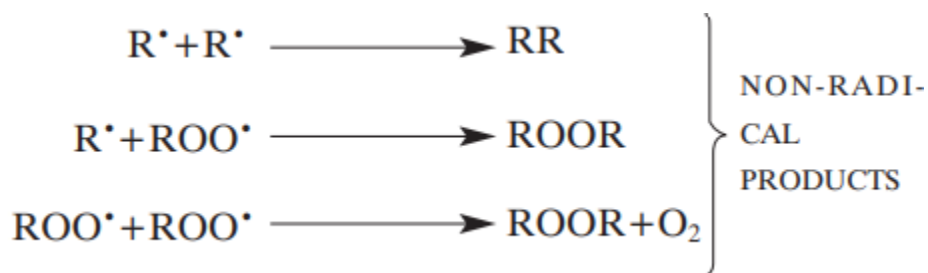
1. Initiation step – homolytic hydrogen atom abstraction from a methylene group that leads to alkyl radical (R•) formation.



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 (Shahidi & Wanasundara, 2002).

## 1.6 Antioxidant

The term "antioxidant" means any substance that can delay or prevent the oxidation of oxidizable substrates. Various factors, such as oxygen, metal ions, moisture, heat, and

the presence of light can accelerate the oxidation of lipids. Therefore, to prevent or delay the oxidation of sensitive foods, oxygen and metal catalysts must be removed to keep foods cool and protected from light (Wang et al., 2021). Antioxidants inhibit chain reactions, remove reactive oxygen species (ROS), interfere with self-oxidation chain reactions, quench O<sub>2</sub> radicals, prevent peroxide formation, and adhere to catalysts such as metal ions. By doing so, it inhibits lipid peroxidation. Antioxidant properties have been proposed for a variety of substances (Aminzare et al., 2019).

Free radicals and the production of reactive oxygen species (ROS) are both inhibited by natural antioxidants (Ouslimani et al., 2005). Phenolic compounds are the substances that contribute the most to the antioxidant activity in different medicinal plants essential oil (Fokou et al., 2020). Their antioxidant action is attributed to their molecular structure, in particular, the number and positions of hydroxyl groups, and the substitutions of aromatic rings, and to their ability to eliminate free radicals through the donation of hydrogen atoms (Soethe et al., 2022).

### **1.7 Consequences of Lipid Oxidation on Meat and Meat Products quality**

For lipid oxidation of meat and meat products, there are several elements that affect it including high levels of polyunsaturated fatty acids (PUFAs) as components of membrane phospholipids, lack of antioxidants, high concentrations of antioxidants and reactive radicals, high levels of salts, the presence of (NaCl) and free molecules Oxygen, which is usually introduced into the meat mixture during processing. Researchers have demonstrated that salts can reduce the activity of glutathione peroxidase, superoxide dismutase, and catalase (Jiang & Xiong, 2016).

Oxidative spoilage of meat and meat products leads to off-flavour development, discoloration, formation of toxic compounds, loss of nutrients, loss of drips, and shortened shelf life (Aminzare et al., 2019; Contini et al., 2014; Fallowo et al., 2014). Under normal physiological conditions, oxygen molecules release a series of reactions leading to the formation of free radicals. During metabolic reactions, small amounts of consumed oxygen are converted to reactive oxygen species (ROS). Free radicals, especially ROS and reactive nitrogen species (RNS), which can interact with fatty acids, nucleic acids, and proteins, are involved as intermediates in several homeostatic processes (Moylan et al., 2014).

Oxidative stress is a problem caused by an imbalance between ROS (reactive oxygen species) and antioxidant production. This can damage muscle organelles, cells, and tissues. Myofibril proteins can be affected by ROS during storage, affecting meat quality and damaging cellular structures. ROS have been shown to decrease collagen production in muscle tissue, resulting in a firmer meat texture and reduced collagen solubility (Archile-Contreras & Purslow, 2011; Falowo et al., 2014).

### **1.8 Consequences of Lipid Oxidation on Human Health.**

Although a major concern for lipid oxidation has been evolved on the effects on sensory and functional qualities of meat. On consequence, lipid oxidation also produces free radicals and toxic compounds that can cause diseases, or have negative impacts on the health of humans who consume the products. Many primary and secondary by-products of lipid oxidation such as 4-hydroxynonenal, malonaldehyde are known as potential carcinogens (Csala et al., 2015).

Free radicals can harm a variety of biological components, including nucleic acids, lipids, and proteins thus disrupting the usual redox balance and causing increase in oxidative stress. Diabetes mellitus, neurodegenerative disorders (Parkinson's disease, Alzheimer's disease), cardiovascular diseases (atherosclerosis and hypertension), respiratory diseases (asthma), cataract development, rheumatoid arthritis, and various cancers have all been linked to free radical-induced oxidative stress (colorectal, prostate, breast, lung, bladder cancers) (Phaniendra et al., 2015). Although, excessive amounts of reactive oxygen species are directly or indirectly involved in various diseases such as inflammatory diseases, cancer, diabetes, autism, Alzheimer's disease, Parkinson's disease, atherosclerosis, heart failure, fatty liver, chronic fatigue syndrome, obesity, and depression (Huang & Ahn, 2019)

### **1.9 Chemical preservatives in meat products.**

Some food additives and preservatives have been shown to have the following side effects in human and animal models: Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). (BHT) and (BHA) are synthetic monocyclic phenolic compounds that are widely used in many food formulations as food preservatives due to their antioxidant properties (Parke & Lewis, 1992). BHA and BHT are suspected of causing health risks such as hyperactivity in children, lung, liver and kidney damage, and most importantly cancer (Ukwo et al., 2022). Studies have shown that BHA and BHT are carcinogenic at high doses and induce squamous cell carcinoma of the posterior gastric region of rodents at concentrations above 3000 ppm, but 250 mg / kg of BHT. Increases naturally occurring neoplasms and tumor-promoting activity (Williams et al., 1999).

BHA can chemically induce gastric tumors in rats, and BHA and BHT act as Chemopreventive agents at low doses, but can be carcinogenic at high doses (Inetianbor et al., 2015)

### **1.10 Natural Antioxidant in Meat and Meat Products**

Natural antioxidants are ubiquitous in food and medicinal plants. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerotic and anti-cancer effects (Nikhade et al., 2019). Appropriate evaluation of antioxidants from food and medicinal plants is important for investigating potential antioxidant sources and facilitating their application in functional foods, pharmaceuticals, and food additives (Xu et al., 2017).

Natural antioxidants contain active ingredients that enhance the antioxidant capacity of meat products. The antioxidant activity of these ingredients is most often due to phenolic acids, flavonoids and essential oils (Tomović et al., 2017). The most important source of natural antioxidants comes from the daily intake of vegetables and fruits. But antioxidants from other plants and agricultural waste should not be ignored (Anwar et al., 2018).

### **1.11 Plant Polyphenols.**

Polyphenols are natural compounds synthesized exclusively by plants with chemical features related to phenolic substances and eliciting strong antioxidants properties (Singla et al., 2019). In the last two decades, there has been more interest in the

potential health benefits (Fig. 1.1) of dietary polyphenols as antioxidant (Ganesan & Xu, 2017).

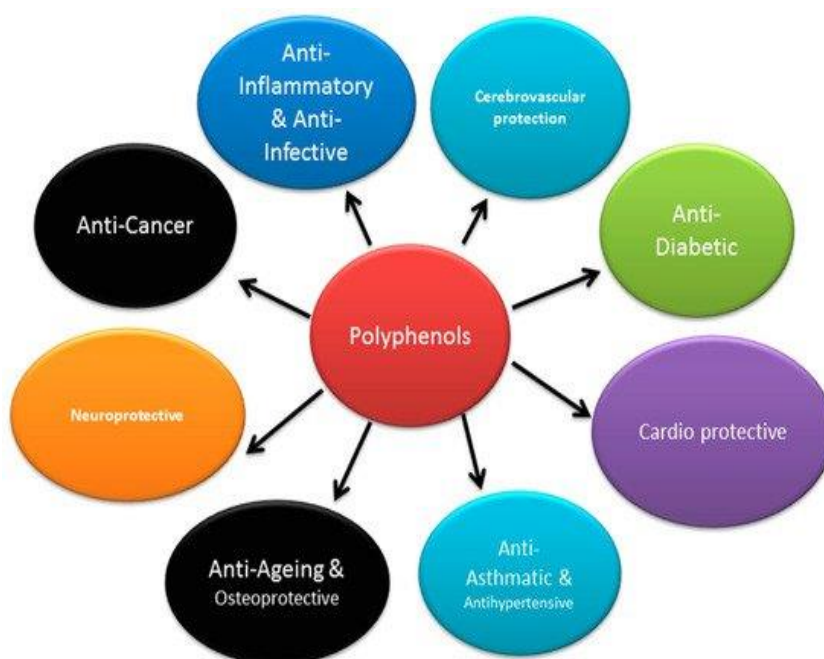


Figure 1. 1 Health benefits of polyphenols

Epidemiological studies strongly suggest that, long term consumption of diets rich in plant polyphenols offer protection against the development of certain cancers, lung damage, pancreatitis, cardiovascular diseases, gastrointestinal problems, type 2 diabetes, osteoporosis, and neurodegenerative diseases (Cory et al., 2018; Pandey & Rizvi, 2009).

The exact mode of antibacterial action of phenolic compounds is still unknown, but it is generally accepted that they have a variety of cellular sites of action. Antibacterial phenols can be added directly to fresh food formulations or incorporated into food contact materials and released into the direct environment of fresh foods (Bouarab Chibane et al., 2019).

### **1.12 Problem statement**

Refrigerated beef burgers have short shelf life due to the processing methods used, their properties, chemical composition, and the potential for mutual contamination. In addition, the oxidation of lipids during storage of refrigerated beef burgers leads to discoloration, rancidification, offensive odors and reduced nutritional value. The short shelf life of fresh beef burgers and the formation of oxidation products affect the quality of beef burgers. Moreover, the use of chemical preservatives to protect beef burgers from spoilage has a negative impact on human health.

### **1.13 Hypothesis**

1. Essential oils of rosemary, thyme, and clove have antimicrobial activity on the growth of the beef burger pathogenic and spoilage bacteria.
2. Antimicrobial active compounds of essential oils could substitute potassium lactate as antimicrobial agent.
3. Antioxidant active compounds of essential oils could substitute butylated hydroxytoluene (BHT) as antioxidant agent.
4. The shelf life of beef burger could be extended using EOs of different aromatic herbs as natural preservatives.

## **1.14 Objectives**

The objectives of this study were:

1. To measure the antimicrobial activity of essential oils of thyme, clove, and rosemary on most common bacteria found in beef burger.
2. To determine the optimum concentration of essential oil which reduces microbial growth or retard oxidation in refrigerated beef burger.
3. To substitute the use of synthetic preservatives by natural preservatives from essential oils.
4. To increase the shelf life of beef burger from four days to seven days by inhibit microbial growth and retard lipid oxidation by adding natural preservatives (essential oil).
5. To compare the effect of natural and synthetic preservatives during the storage time of refrigerated beef burger.
6. To produce natural meat product (refrigerated beef burger) without addition of chemical preservatives.

## **Chapter Two: Literature Review**

### **2.1 Antimicrobial activity of essential oils in meat and meat products.**

Many microbes are beneficial, but some can cause illness or even death after eating contaminated food (Abebe et al., 2020). Several preservation methods have been developed to ensure the microbial safety of food, as well as its nutritional value and sensory characteristics (Quinto et al., 2019). Those methods sometimes have undesired effects on the nutritional and/or organoleptic aspects of food, the use of natural antimicrobial food preservatives could ensure the safety and quality of food being an alternative to other systems of preservation such as chemical or thermal ones.

Intoxication, cancer, and other degenerative disorders are linked to the usage of synthetic preservatives and chemical additives. As a result, public concern has grown, as has the desire to eat healthier products that use natural preservatives rather than chemicals and synthetic preservatives. This necessitates the search for conservation alternatives with similar antibacterial characteristics and food compatibility (Laranjo et al., 2017).

Essential oils and their components have become increasingly popular as present antimicrobial and antioxidant agents. Many recent studies are conducted to estimate the effect of EOs obtained from sources like oregano, rosemary, thyme, sage, basil, turmeric, coriander, ginger, garlic, nutmeg, clove, mace, savory, and fennel, so as to enhance the sensory qualities and extend the shelf life of meat and meat products

(Goulas & Kontominas, 2007; Jayasena & Jo, 2013). Generally, essential oils can consist of more than 60 individual organic components with low molecular weights and large differences in antimicrobial and antioxidative activities (Burt, 2004).

The antimicrobial effect of thyme essential oil at supplementation levels of 0.3%, 0.6%, and 0.9% against *Escherichia coli* O157:H7 has been examined in minced beef meat by Solomakos et al. (2008). However, the results showed that, EO at 0.3% possessed a weak antibacterial activity against the pathogen. On the other hand, treatment of minced beef meat with EO at 0.6% showed an inhibitory activity against *E. coli* O157:H7 during storage at 10 °C, but not at 4 °C. The same study indicated that treatment of minced beef meat at 0.9% showed unacceptable organoleptic properties in minced meat.

Sheeladevi and Ramanathan (2012) investigated the antibacterial activity of plant essential oils against five types of food-borne bacteria. Antimicrobial activity of essential oils of cinnamon, clove, oregano, rosemary and thyme against *Campylobacter sp.*, *Listeria sp.*, *Yersinia sp.*, *Salmonella sp.* and *Pseudomonas sp.* has been studied. Most essential oils have shown high antibacterial activity against all bacteria in food. Among the essential oils studied, clove, cinnamon, and thyme were better able to inhibit all five types of bacteria in food.

Selim (2011) tested the antimicrobial activity of clove and thyme essential oils with concentrations of 0.5% and 1%, against *Escherichia coli* O157:H7 in minced beef meat stored at 7°C for 14 days, he showed that, thyme was active against bacteria, but clove didn't show any effect.

Alsaqali et al. (2016) assess the antibacterial activity of three different concentrations (0.3, 0.6, and 1.2%) of thyme essential oils against *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aeruginosa*, and *E. coli*. Bacterial species were artificially inoculated into beef burgers stored at  $4 \pm 1$  °C for 4 days. The results showed that high concentrations of thyme EO (0.6 and 1.2%) had a better reduction effect on bacterial counts compared with low concentrations (0.3%).

Most of the essential oils are regarded as safe, and the use of essential oils and their component as natural preservatives in food reduces the count of yeast cells, in addition, essential oils prevent or reduce the growth of molds (Krisch et al., 2011). Also, Plant-derived EOs have shown remarkable antimicrobial potency against spoilage and pathogenic microorganisms in meat and meat products (Jayasena & Jo, 2013).

Laranjo et al. (2017) found that, the use of essential oils in food preservation has an antimicrobial activity against a wide range of microorganisms, which generally attributed to the phenolic compounds owned by EOs. Moreover Kassem et al. (2011b) studied the improvement of the quality and stability of the burgers by adding thyme EO at different concentrations (0.02, 0.04, and 0.06%) which was stored at a temperature of 5 °C. The results revealed that, samples treated with 0.04 and 0.06% of thyme EO revealed significant reduction of microbial load after 12 days of storage as compared with the control samples. In addition, thyme essential oil at a concentration of 0.06% improves sensory quality of treated samples.

The antibacterial effects of thyme EO supplemented at 0.3, 0.6, and 0.9% levels against *E. coli* O157:H7 were studied in ground beef by Solomakos et al. (2008b), treatment of ground beef sample with thyme EO (0.6%) inhibited the growth of *E. coli* O157:H7 during storage at 10 °C . In addition, Marino et al. (2001) reported that clove, rosemary and thyme essential oils had a high inhibitory effect, especially against gram-positive bacteria.

Shaltout and Koura (2017) studied the effectiveness of thyme oil on microbial growth at different concentrations (0.5%, 1.5%, 2%) as a natural preservative to increase the shelf life of minced meat stored at 2°C for 12 days. The results showed that, the treated samples induced a decrease in microbial growth compared with the control sample. Moreover, thyme oil added at 2% concentration showed the best value compared to other EOs used in this study.

Fратиanni et al., 2010 evaluated the effect of thyme essential oil on the stored fresh chicken breast meat for 3 weeks at 4°C. They found that thyme essential oil reduced the microflora which is naturally present in meat. They noticed that the total microbial content was also reduced by 50% compared to the control sample. In addition, a clear effect on the growth of lactic acid bacteria was noted, while thyme essential oil effectively inhibited the growth of *Escherichia coli*.

## **2.2 Antioxidants activity of essential oils in Meat and Meat Products**

Antioxidants are substances that help slow the oxidation of molecules by inhibiting the ongoing formation of free radicals (Fennema, 1996). It can bind to metals and remove species that initiate or sustain oxidation. Antioxidants can also quench high-

energy oxygen species and prevent peroxide formation. Finally, antioxidants break down lipid peroxides and improve the stability of both meat color and flavor (Brewer, 2008).

In the food industry, especially meat industry, researchers are in continuous search of natural solutions for minimization of oxidative rancidity and extension of the shelf-life of meat products as alternatives of synthetic antioxidants such as BHT and BHA (Horbańczuk et al., 2019; Naveena et al., 2008).

Natural antioxidants are commonly found in foods and herbal medicines. These natural antioxidants, especially polyphenols and carotenoids, have many biological effects, including anti-inflammatory, anti-aging, anti-atherosclerotic, and anti-cancer. Proper evaluation of antioxidants in food and herbal medicine is essential to explore potential sources of antioxidants and promote their application in functional foods, pharmaceuticals and functional foods (Xu et al., 2017).

Antioxidants have been used in the food processing industries as means of preventing oxidation, improving taste, aroma, and colour. Although synthetic antioxidants such as butylated hydroxytoluene (BHT) can have adverse effects on human health, they are still widely used in the food processing industries (Admassu & Kebede, 2019). As a result, essential oils of plant origin and spices have become well known as a natural substitute for compounds of synthetic origin. (Cardoso-Ugarte & Sosa-Morales, 2021).

Youssef (2014), compared the effects of adding rosemary and clove extracts with BHA/BHT, on lipid oxidation of fresh beef burger during refrigerated storage at  $4\pm 1^{\circ}\text{C}$  for 15 days. He observed a significant reduction in TBARS values for all treated samples compared with control sample. The study also showed that, TBARS values were significantly low in rosemary extract and clove extract than BHA/BHT treated samples. According to these results, rosemary and clove extracts could be used in place of synthetic antioxidants. In another study carried by Laranjo et al. (2017), they found that the use of essential oils in food preservation have antioxidant activity, generally attributing this to the phenolic compounds owned by EOs.

The antioxidant activity of rosemary lyophilized extract (RLE) was estimated in chicken burger stored at  $4^{\circ}\text{C}$  for 21 days. The RLE showed strong antioxidant capacity and inhibited 48.29% of lipid oxidation in comparison to the control with low production of malonaldehyde (Pereira et al., 2017).

Alsaqali et al. (2016), evaluated the activity of antioxidant effect of thyme essential oil at different concentrations (0.3, 0.6 and 1.2 %) in beef burger stored at  $4\pm 1^{\circ}\text{C}$  for 4 days. The results indicated that, high concentration of thyme oil (1.2 %) reduced the values of thiobarbituric acid (TBA) compared to the control sample. Additionally, Kassem et al. (2011b) studied the improvement of the quality and stability of refrigerated burgers by using thyme EO at different concentrations (0.02, 0.04, and 0.06%). They found that, the storage time significantly affected the thiobarbituric acid (TBA) values, as treated samples with thyme EO showed lower values of TBA compared with the control samples.

The effectiveness of thyme essential oil at different concentrations (0.5, 1.5, and 2%) as natural antioxidants was evaluated in a study conducted by Shaltout and Koura (2017) on a minced meat stored at 2°C for 12 days. The obtained results showed that thyme oil reduced the rate of oxidation thus increasing the shelf life of the product.

Zahid et al. (2019) compared the effects amongst butylated hydroxytoluene (BHT) and clove extract (CE), as antioxidants on the oxidative stability in fresh beef patties during refrigerated storage. The study showed that addition of BHT and CE to patties significantly restrained lipid oxidation respect to the control sample. Furthermore, CE as a natural antioxidant evinced its efficiency in term of oxidative stability of fresh beef patties. The same study concluded that CE could substitute the use of BHT when making beef patties during storage.

## **Chapter Three: Materials and Methods**

### **3.1 Materials:**

#### **3.1.1 Essential oils**

Different Essential oils (analytical grade) were purchased from Al-shams Company (Nablus, Palestine).

These essential oils are:

1. Thyme oil (*Thymus vulgaris*)
2. Clove oil (*Syzygium aromaticum*)
3. Rosemary oil (*Rosmarinus officinalis*)

#### **3.1.2 Chemicals and microbiological media**

All chemicals used in this investigation were of analytical reagent grade. Thiobarbituric acid, trichloroacetic acid, Hydrochloric acid, Butylated hydroxytoluene, and Potassium lactate were purchased from Sigma Aldrich (Germany). While microbiological media including Plate Count Agar, Violet Red Bile Agar, Baird Parker Agar, and Eosin Methylene Blue were also purchased from (Oxoid Company).

### **3.2 Methods:**

#### **3.2.1 Preparation of beef burger samples**

The packed vacuum frozen boneless beef was obtained from Siniora Food Industries Company (Jerusalem-Palestine). Meat was thawed until it reached - 5°C degree at its core, then the plastic cover was removed, and the meat was minced into sizes of 4

mm. Fat ingredient, was minced by the aid of mincer machine into 4 mm and mixed in with the other ingredients. The mixture was then mixed and homogenized for about four minutes. After that, beef burger was divided into nine Formulas (1.5 kg each): one formula remained as negative control (without antimicrobial or antioxidant agents) and the rest eight were treated and prepared as follows:

Formula 2- added with antioxidant positive control (100 ppm Butylated hydroxytoluene).

Formula 3- added with antimicrobial positive control (2 % potassium lactate).

Formula 4- added with 1% rosemary essential oil (REO).

Formula 5- added with 1.5 % REO.

Formula 6- added with 1 % thyme essential oil (TEO).

Formula 7- added with 1.5 % TEO.

Formula 8- added with 1 % clove essential oil (CEO).

Formula 9 -added with 1.5 % CEO.

The samples then chilled to 0°C, shaped into patties (90g per patty), packed in plastic bags, and closed tightly before being stored in the refrigerator at 4°C for 7 days (study period).



**Figure 3. 1 Preparation of beef burger patties.**

### **3.2.2 Microbial analysis**

The microbial analysis performed was total plate counts (TPC), total coliform (TC), and *Staphylococcus aureus*. Peptone water and Plate Count Agar were sterilized by autoclaving at 121°C for 15 min. Violet Red Bile Agar (VRBA) used for determination of coliform counts was sterilized by boiling. *S. aureus* analysis was performed using Baird-Parker Agar. The media used were prepared and sterilized according to the guidelines given by the manufacturers.

#### **3.2.2.1 Total plate count determination**

Briefly, 10 g beef burger sample was added into 90 ml peptone water then thoroughly homogenized using stomacher, the prepared sample at  $10^{-1}$  was used for all microbial determination (TPC, TC and *Staphylococcus aureus*). Appropriate decimal dilution up to  $10^{-7}$  of samples were pour-plated with 15 ml of PCA in triplicate, the poured

plates were allowed to solidify and incubated in inverted positions at 37 °C for 36 h (Adam & Abugroun, 2010). Colony counts for microbiological tests were made using colony counter (Gerber, Colony Star 8500).

### **3.2.2.2 Total Coliform count**

From the same prepared sample for TPC, appropriate decimal dilutions ( $10^{-1}$ - $10^{-4}$ ) of beef burger samples were pour-plated on 15 ml VRBA. The medium was allowed to solidify before incubating at 37 °C for 24 h. Typical dark red colonies were considered as coliform colonies (Firstenberg-Eden et al., 2004).

### **3.2.2.3 Staphylococcus aureus count**

Appropriate decimal dilutions ( $10^{-1}$ - $10^{-4}$ ) of beef burger samples were pour-plated on 15 ml Baird Parker Agar. The medium was allowed to solidify before incubating at 37 °C for 24 h. At the end of the incubation period, the colonies were counted and their numbers were determined (Shahbazi & Shavisi, 2018).

For microbial assay, colony counts between 25 and 250 were used for calculating the number of colony-forming units (CFU) per gram of sample according to the formula, colony count =  $n * 1/V * 1/d$  (Shewail et al., 2018). Where n is the number of colonies counted per plate, V is the volume of inoculum in each plate (ml), and d is the dilution factor used to determine the colony count. The average number of the countable colonies after the incubation time of the triplicate plates was used for the calculations.

### **3.2.3 Chemical analysis**

A thiobarbituric acid reactive substances (TBARS) assay was performed to determine the oxidative stability of refrigerated beef burgers as described by Aytul (2010). For analysis, 5 g of sample was placed in a beaker containing 50 ml of 0.38% TBA and 15% trichloroacetic acid prepared in 0.25N HCl solution. The sample was homogenized (IKA T-25 Ultra Turrax Homogenizer) at 10,000 rpm for 3 min., and three 15 ml aliquots from the homogenate were heated in a boiling water bath for 1 hour to develop a pink color and cooled in tap water. The boiled samples were then clarified by centrifugation (Labnet International C0226R) at 5000 rpm for 10 min., and absorbance was measured at 532 nm using a spectrophotometer (Jenway 74 Series Spectrophotometer). Higher oxidation of the beef burger sample indicates higher absorbance. An average of three absorbance values were used to determine the oxidative stability of the refrigerated samples (Aytul, 2010; Bekhit et al., 2003). On the calibration curve, the absorbance of malondialdehyde (MDA) at various concentrations (1-15 ppm) was used to determine the amount of oxidation products (mg MDA / kg beef burger sample). The pH measurement of beef samples was determined using a pH Meter (Hanna HI 2210) according to Ouerfelli et al. (2019).

### **3.3 Data analysis**

The data were expressed as mean  $\pm$  SD, statistical analysis was performed with one-way analysis of variance (ANOVA), followed by LSD's fisher test and letters of mean separation. A p value of less than 0.05 ( $p < 0.05$ ) was considered statistically significant. The statistical analysis was performed using the Sigma Stat software (version 2.03).

## **Chapter Four: Results**

### **4.1 Antimicrobial Activities of essential oils on Beef burger patties**

#### **4.1.1 Effect of essential oils on total plate count of beef burger patties**

All burger samples that were kept for 7 days at 4°C showed an increase in bacterial growth from the first day until the seventh day. However, negative control sample showed the numerous developments of bacterial growth compared to treated samples which showed a very clear decrease in microbial growth during the preservation period.

Similar effect of each of essential oils against microbial growth (Table 4.1) was observed as follows, TEO at a concentration of 1% and 1.5% led to a reduction in microbial growth by 69% and 71%, respectively. As for REO, at a concentration of 1% and 1.5%, it reduced microbial growth by 70% and 65%, respectively. In consequence, CEO at a concentration of 1% and 1.5% had its effect on reducing microbial growth by 69% and 63%, respectively, compared to the negative control, and the results also showed that the burger sample with potassium lactate had the lowest microbial growth at seven days. The results (Table 4.1) summarized that all essential oils used in this study led to a reduction in microbial growth in the burger samples, without any significant differences between the concentrations of these oils and without any significant difference according to the potassium lactate.

**Table 4. 1 Microbial analysis of total plate count content (cfu/gm) in beef burger during 7 days at different essential oil concentrations.**

Treatments	0 day	4 days	7days
<b>Negative control</b>	1.6E+04±7.8E+02 e	7.1E+04±3.6E+03 b	24E+04 ±2.5E+04 a
<b>P. L 2%</b>	1.6E+04±7.8E+02 e	1.7E+04±1.1E+03 e	5.3E+04±4.9E+03c,b
<b>TEO 1%</b>	1.6E+04±7.8E+02 e	2.3E+04±1.5E+03 e	7.1E+04±1.1E+04 b
<b>TEO 1.5%</b>	1.6E+04±7.8E+02 e	1.8E+04±1.7E+03 e	8.5E+04±1.1E+04 b
<b>REO 1%</b>	1.6E+04±7.8E+02 e	4.9E+04±3.6E+03c,b	7.3E+04±7.0E+03 b
<b>REO 1.5%</b>	1.6E+04±7.8E+02 e	3.5E+04±2.0E+03 d	7.1E+04±3.2E+03 b
<b>CEO 1%</b>	1.6E+04±7.8E+02 e	1.3E+04±1.2E+03 e	9.0E+04±5.6E+03 b
<b>CEO 1.5%</b>	1.6E+04±7.8E+02 e	0.9E+04±4.0E+02 e	7.3E+04±2.5E+03 b

- Small letters (a-e) indicates significant differences in number of total bacterial count among samples during storage time, LSDs= 21860

#### **4.1.2 Effect of essential oil on total coliform count of beef burger patties**

Microbial growth of coliform bacteria in all treated and untreated burger samples, which were kept for seven days at a temperature of 4 °C, increased with the progress of storage up to the seventh day. The highest value appeared in the burger sample containing 1% TEO, while the lowest value was determined in the burger sample containing potassium lactate (Table 4.2).

The results showed that CEO had a clear effect on reduction of microbial growth in both concentrations (1 and 1.5%). Nevertheless, treatment of beef burger with 1.5% CEO showed greater inhibitory action compared with that obtained for beef burger treated with 1% CEO (Table 4.2). A concentration of 1% and 1.5% CEO reduced

microbial growth by 80% and 95% respectively compared with the negative control sample. Moreover, 1% and 1.5% REO, had an effect on reducing the microbial count by 10% and 14%, respectively, compared to the negative control sample. Whereas, thyme essential oil, at a concentration of 1.5%, reducing the total number of coliform bacteria by 13%, compared to the negative control (Table 4.2).

In comparison with the potassium lactate added burger sample, there is no significant difference between PL and CEO at a concentration of 1.5%. Also, there is no significant difference between TEO and REO at the same concentration, while 1% concentration of TEO had no effect on microbial growth. The results indicate that CEO at both concentrations had the most effect on reducing microbial growth compared to the negative control sample and the rest of the other samples.

**Table 4.2 Effect of essential oils (thyme, rosemary, and clove) at concentrations (1% and 1.5%) on total coliform (cfu/gm) in refrigerated beef burger after 7 days of storage.**

<b>Treatment</b>	<b>0 day</b>	<b>4 days</b>	<b>7 days</b>
<b>Negative Control</b>	1.5E+01±1 j	38E+02±265 e	5.5E+03±306 b
<b>P. L 2%</b>	1.5E+01±1 j	3.1E+02±31 j	0.09E+03±10 j
<b>TEO 1%</b>	1.5E+01±1 j	8.8E+02±125 h,i	8.0E+03±0.0 a
<b>TEO 1.5%</b>	1.5E+01±1 j	5.3E+02±61 i,j	4.8E+03±322 d
<b>REO 1%</b>	1.5E+01±1 j	36E+02±153 e	5.0E+03±400 c
<b>REO 1.5%</b>	1.5E+01±1 j	21E+02±252 f	4.8E+03±100 d
<b>CEO 1%</b>	1.5E+01±1 j	3.0E+02±29 j	1.1E+03±58 g,h
<b>CEO 1.5%</b>	1.5E+01±1 j	2.1E+02±21 j	0.27E+03±26 j

- Small letters (a-j) indicates significant differences in number of total bacterial count among samples during storage time, LSDs= 348

#### **4.1.3 Effect of essential oil on *Staphylococcus aureus* count of beef burger patties**

The effect of different essential oil on the *Staphylococcus aureus* during 7 days of storage of refrigerated beef burger at 4 °C was shown in (Table 4.3). The results indicated that, the growth of *Staphylococcus aureus* in all samples increased significantly during the period of storage. However, the highest value was obtained from the beef patties of control sample, and the lowest value was in beef patties treated with 1.5% CEO at seventh days.

It was found that, CEO at a concentration 1% and 1.5% significantly reduced the number of colonies by 15% & 54% respectively after 7 days of storage compared with the negative control sample (Table 4.3). Also, TEO at concentration 1% and 1.5% were significantly reduced the number of colonies by 22% & 35% respectively after 7 days of storage compared with the negative control sample. In addition, the clove at 1.5% concentration significantly reduced the number of colonies by 36% compared with potassium lactate after 7 days of storage.

Thyme essential oil at concentrations 1% and 1.5% didn't significantly affect the *S. aureus* growth compared with potassium lactate. However, REO didn't significantly affect the microbial activity at both concentrations compared with negative control. The results revealed that CEO at concentration 1.5% reduced the microbial activity of *S. aureus* by 54% compared of negative control after 7 days of storage, also, there is significant difference between it and all treated samples. Clove and thyme EOs are both natural substances that are not harmful when consumed in medicine and food products.

**Table 4.3 Effect of essential oils of medicinal plants (thyme, rosemary, and clove) at concentrations 1% and 1.5% on total count of *Staphylococcus aureus* (cfu /gm) in beef burger after 7 days of storage.**

Treatments	0 day	4 days	7 days
<b>Negative Control</b>	1.9E+02±21 f	3.5E+02±32 d,e	6.4E+02±40 a
<b>P. L 2%</b>	1.9E+02±21 f	3.2E+02±30 d,e	4.6E+02±23 c,d
<b>TEO 1%</b>	1.9E+02±21 f	2.8E+02±21 e	5.0E+02±30 b,c
<b>TEO1.5%</b>	1.9E+02±21 f	2.7E+02±25 e	4.2E+02±25 c,d
<b>REO 1%</b>	1.9E+02±21 f	3.1E+02±30 d,e	6.2E+02±105 a,b
<b>REO 1.5%</b>	1.9E+02±21 f	3.8E+02±65 d	5.9E+02±47 a,b
<b>CEO 1%</b>	1.9E+02±21 f	3.1E+02±31 d,e	5.4E+02±57 b,c
<b>CEO 1.5%</b>	1.9E+02±21 f	2.3E+02±31 e,f	2.9E+02±25 e

- Small letters (a-f) indicates significant differences in number of total bacterial count among samples during storage time, LSDs=83

#### **4.2 Antioxidant activity of different essential oils on beef burger patties**

The TBA test has been widely used to measure lipid oxidation in meat and meat products (Hu et al., 2010). Essential oils of thyme, rosemary, and clove were added to refrigerated beef burger as natural antioxidant in two concentrations (1% and 1.5%). The oxidative stability of beef patties was examined by conducting thiobarbituric acid reactive substances (TBARS) assay. Absorbance obtained from these assays at 532 nm, were then used to calculate amount of oxidation by TBARS. Results are given in Table (4.4) as mg of MDA/ kg beef burger.

The initial TBARS value of refrigerated beef burger was determined as 3.27 mg MDA/kg beef burger. Although the level of lipid oxidation was increased in all

samples during the study period it was noticed that treatment with essential oils reduced the lipid oxidation of beef burger by different percentages, the best antioxidant activity was obtained from beef sample treated with 1.5 % CEO.

The results of the oxidation test for beef burger samples kept at a temperature of 4°C for a period of 7 days, in which the amount of MDA/kg was measured using the TBARs method (Table 4.4), showed that, all samples had the oxidation developed, and this was evident by the increase in the amount of MDA compound produced during the study period. However, some essential oils used in this study led to reduce oxidation compared with the negative control sample, whereas, clove essential oil at a concentration of 1% and 1.5% was the most effective in oxidation reduction by 24% and 35%, respectively compared to the negative control sample.

Thyme essential oil, at a concentration 1% and 1.5%, reduced the percentage of oxidation by 14% and 31%, respectively compared with the negative control sample. Compared with BHT, CEO and REO at a concentration of 1.5% were better than BHT by 13% and 7%, respectively. Also, there is no significant difference between BHT and CEO at a concentration of 1%. Results showed that REO at both concentrations did not show any effect on reducing oxidation.

**Table 4.4 Oxidation products of refrigerated beef burger samples treated with different concentrations of essential oils (1%, and 1.5%) determined by TBARS (mg MDA/ kg) method.**

<b>Treatments</b>	<b>0 day</b>	<b>4 days</b>	<b>7 days</b>
<b>Negative control</b>	3.27±0.19 j	12.78±0.15 g	26.34±0.51 b
<b>BHT 100 ppm</b>	3.27±0.19 j	5.37±0.51 i	19.64±1.26 d,e
<b>TEO 1%</b>	3.27±0.19 j	11.82±0.51 g	22.78±1.09 c
<b>TEO 1.5%</b>	3.27±0.19 j	11.40±0.32 g	18.26±0.95 e,f
<b>REO 1%</b>	3.27±0.19 j	12.53±0.63 g	28.52±1.19 a
<b>REO 1.5%</b>	3.27±0.19 j	11.94±0.32 g	28.69±1.12 a
<b>CEO 1%</b>	3.27±0.19 j	6.92±0.44 h	19.98±1.02 d
<b>CEO 1.5%</b>	3.27±0.19 j	5.87±0.19 i	17.17±0.07 f

Small letters (a-j) indicate differences in the amounts of oxidation products for control samples and treated samples (1%, and 1.5%) at each storage time, LSDs=1.58

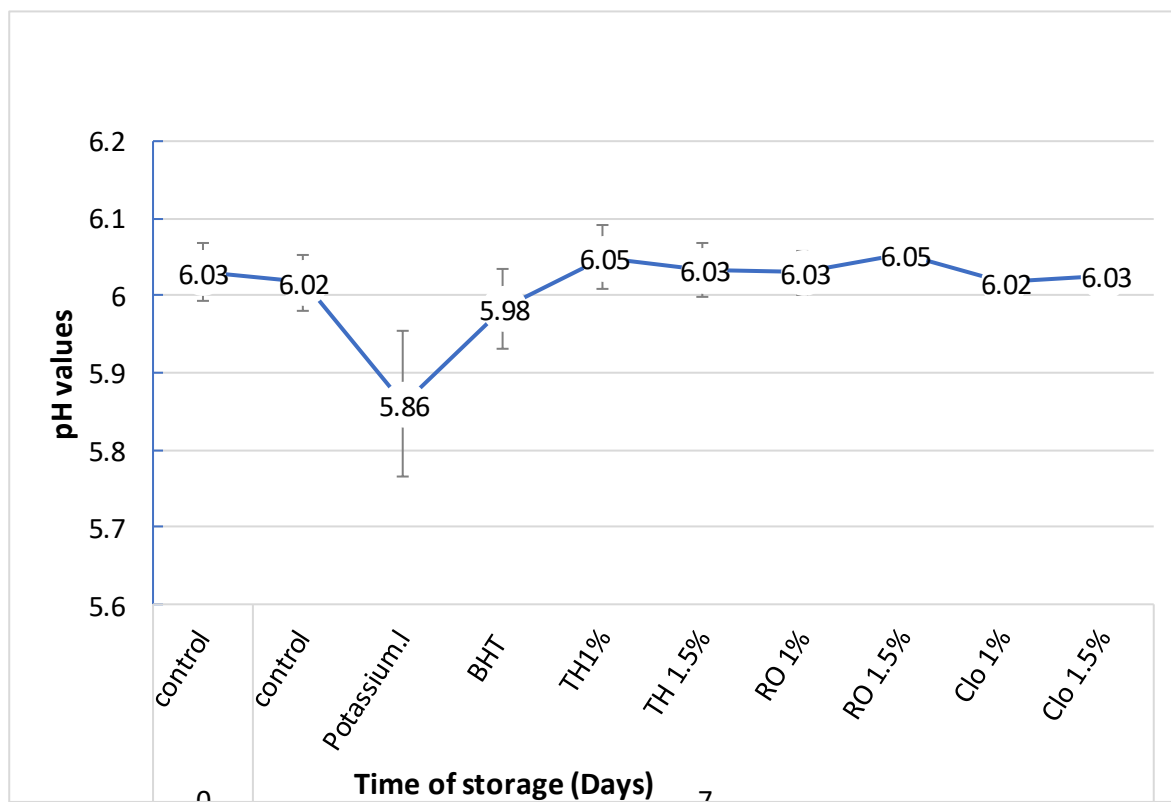
#### **4.3 Changes in pH of beef burger patties.**

Storage time affected all the treatments by changing the pH values during the storage period with a slight effect. Lowest pH was found for the sample with potassium lactate with a pH of 5.86. It was shown in (Table 4.5, and Figure 4.1), that pH of P.L sample changed more on day 7 than other stored samples. Similar results were obtained for BHT treated sample showing a decrease in pH value (5.98) after 6 days of storage. The treated samples with essential oils didn't show significant difference compared with negative control sample.

**Table 4. 5 pH of refrigerated beef burger samples (controls and treated samples)**

Treatments	0 day	4 days	7 days
Negative control	6.03±0.04 b,c	6.02±0.02 b,c	6.02±0.04 b,c
P.L 2%	6.03±0.04 b,c	5.83±0.03 e	5.86±0.04 d,e
BHT 100ppm	6.03±0.04 b,c	5.97±0.03 c	5.98±0.09 c
TEO 1%	6.03±0.04 b,c	5.94±0.04 c	6.05±0.05 b,c
TEO 1.5%	6.03±0.04 b,c	6.05±0.05 b,c	6.03±0.04 b,c
REO 1%	6.03±0.04 b,c	6.19±0.04 a	6.03±0.04 b,c
REO 1.5%	6.03±0.04 b,c	6.16±0.05 a	6.05±0.03 b,c
CEO 1%	6.03±0.04 b,c	5.98±0.14 c	6.02±0.03 b,c
CEO 1.5%	6.03±0.04 b,c	6.21±0.02 a	6.03±0.02 b,c

Small letters (a-e) indicate differences in the pH values of products for control sample and treated samples (1%, and 1.5%) at each storage time, LSDs=0.07



**Figure 4. 1 pH values of refrigerated beef burger samples at zero and 7 days.**

## Chapter Five: Discussion

### 5.1 Microbial activity

Total plate count, total coliform, and *Staphylococcus aureus* count of the three different essential oils (TEO, REO, and CEO) are given in Tables (4.1-4.3) respectively. Plenty of spices and herbs exert antibacterial influences due to their essential oil fractions. Studies revealed that the essential oils from plants like thyme, sage, rosemary, clove, garlic, and onion show antimicrobial activity against both bacteria and molds (Fu et al., 2007; Sahalie et al., 2018). The antimicrobial activity of the plants varies depending on the composition, structure and functional groups of the oils (Bhavaniramy et al., 2019).

The phenolic compounds present in essential oils are responsible for their antimicrobial activity (Medina et al., 2006; Silva et al., 2012). The mechanism of action of phenolic compounds on bacterial cell has been partially attributed to interact with protein membranes, causing deformation in their structure and interfering with their functionality (Álvarez-Martínez et al., 2020). In addition, Nieto (2020) reported that carvacrol and thymol are capable of inactivating essential enzymes, reacting with cell membranes and altering genetic material.

Essential oils of clove, thyme, and rosemary were shown to possess strong antibacterial activity against *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Conner, 1993). Furthermore, clove and rosemary essential oils showed antibacterial effect against meat spoilage pathogenic bacteria (Gonelimali et al., 2018; Ouattara et al., 1997; Sharafati Chaleshtori et al., 2015). Our results agree

with the findings of Xu et al. (2016), who showed that clove essential oil exhibited strong antibacterial activity against *Staphylococcus aureus*. These results might be attributed to the presence of phenolic compounds such as carvacrol, eugenol and thymol that are able to inhibit bacterial growth (Kim & Wei, 1995; Ojagh et al., 2011).

Radünz et al. (2019) reported that, clove essential oil showed inhibitory effect up to the concentration of 0.304 mg/ml against *S. aureus*, *E. coli*, *L. monocytogenes* and *S. typhimurium* bacteria. Thus, this effect was independent of the characteristics of the microorganism membranes. The action of clove essential oil is ascribed to the presence of eugenol which promotes the rupture of the bacterial cytoplasmic membrane, so increasing its permeability and allowing the extravasation of the ions and the loss of intracellular proteins, causing bacterial death (Devi et al., 2010; Radünz et al., 2019; Sharafati Chaleshtori et al., 2015).

Thyme essential oils exhibit antimicrobial activities against a wide range of pathogenic microorganisms (Cai et al., 2019). Many studies have demonstrated that, carvacrol and thymol possess potent antibacterial agents against both Gram-positive and Gram-negative bacteria (Kachur & Suntres, 2020). Ghaderi-Ghahfarokhi et al. (2016) studied the antimicrobial activity of thyme essential oil and they showed that thyme essential oil had an effect on reducing the microbial growth of *Staphylococcus aureus* bacteria in refrigerated beef burgers.

Treatment of minced beef with 1% TEO inhibited *E. coli* O157:H7 growth during storage at 7°C (Selim, 2011). Similar action of TEO against *E. coli* O157:H7 was previously confirmed in vitro at temperatures higher than that of refrigeration (Burt & Reinders, 2003; Imelouane et al., 2009). It was found that, the major phenolic compound of thyme essential oil (ie, thymol, and carvacrol) are primarily responsible for their significant antimicrobial activity against bacterial growth (Kachur & Suntres, 2020).

Thyme essential oil effectively inhibited the growth of *Escherichia coli* and lactic acid bacteria in chicken breast meat stored at 4°C. This prevents the chicken meat spoilage and extends the shelf life of the fresh product (Fратиanni et al., 2010).

Thymol is the major component of TEO which is structurally similar to carvacrol having hydroxyl group at a distinct position on the phenolic ring (Mousavian et al., 2021). It is evident that the interaction of thymol affects membrane permeability which in turn causes loss of membrane potential, leakage of K<sup>+</sup> ions, and ATP and carboxy fluorescein (Jafri et al., 2019; Zhou et al., 2019). In addition to this, thymol also interacts with periplasmic proteins by hydrophilic and hydrophobic interactions (Jafri et al., 2019).

The rosemary essential oil inhibitory effects on total coliform during the study period shown in (Table 4.2). The inhibitory activity of rosemary essential oil was significantly lower than clove essential oil. Our results agree with the finding of Canillac and Mourey (2001) who reported that rosemary essential oil rich in monoterpenes, are permissive for the growth of *E. coli* and *E. aerogenes*. However,

these bacteria are susceptible to essential oils containing large amounts of phenolic and oxygenated compounds.

## **5.2 Chemical analysis**

Lipids oxidation considered a major cause of deterioration in the quality of muscle foods and can directly affect many quality characteristics such as flavor, color, texture, nutritive value, and safety of the food (Bienkiewicz & Kołakowska, 2003; Gallo et al., 2012; Youssef, 2014). Phenolic compounds are important dietary components and they have antioxidant activity as well as other health benefits. Epidemiological evidence suggests that a diet high in antioxidant fruits and vegetables reduces the risk of many oxidative stress-related diseases, cancers, diabetes, and cardiovascular diseases (Kumar & Goel, 2019). The number and position of the hydroxyl groups in a particular phenolic compound leads to the variation in their antioxidant potential (Balasundram et al., 2006). Furthermore, phenolic acids are found ubiquitously and well documented for other health protective effects like antimicrobial, anticancer, anti-inflammatory, and anti-mutagenic (Kumar & Goel, 2019). The major component of the clove oil includes eugenol, and eugenol acetate whereas the minor constituent includes vanillin, tannins, the constituents like methyl amyl ketone, and methyl salicylate add up to the characteristic pleasant odours (Kumar et al., 2021).

Our findings (Table 4.4) is consistent with Kong et al. (2010), who observed that clove extracts are highly effective antioxidants in cooked pork patties and stabilized red color (myoglobin) during refrigerated storage. The results indicate that an increasing of clove essential concentration from 1% to 1.5% leading to decrease the

oxidation rate of beef burger. These results could be related to the amount of hydroxyl groups within the phenolic structures of constituents present in clove essential oil. It is assumed that inhibition of lipid oxidation and hydrogen donor ability is enhanced with the increasing amount of hydroxyl groups (McDonald et al., 2001).

In this study, the antioxidant activity of clove and thyme EOs at concentration of (1.5%) showed significantly inhibition compared to BHT which agreed with Jirovetz et al. (2006) who investigated that the antioxidant property of phenolic compounds surpass the activity of synthetic antioxidant such as BHT. Moreover, other researchers found that ethanol extract of the clove buds exhibit remarkable scavenging activity, compared with synthetic antioxidants such as butylated hydroxyl toluene (Nassar et al., 2007). Such findings may be attributed to the high antioxidant effect of thyme essential oil, which is related to the scavenger nature of its flavonoids and phenolic content as apigenin, luteolin, thymonin, carvacrol, 1,8-cineole, and thymol (Kassem et al., 2011a). Our results in (Table 4.4), also steady with Ghaderi-Ghahfarokhi et al. (2016) that showed a significant improvement in the reduction of thiobarbituric acid reactive substances (TBARS) amount for thyme essential oil treated burgers in comparison with control samples after 8 days of chilled storage.

The role of the antioxidants is to neutralize the free radicals in biological cells, the free radicals having a negative impact on living organisms. A special role in neutralizing the effects of the oxidative stress related to the presence of free radicals is played by the enzyme called superoxide dismutase (Munteanu & Apetrei, 2021). Hence, the use of natural antioxidants in meat products appears as a good option to reduce the consumption of synthetic additives, since they are widely consumed by the

general population without showing signs of low toxicity, besides carrying out functional activities beneficial to human health.

The effect of natural and synthetic antioxidants on pH values of beef burgers stored at 4 °C for 7 days is presented in (Table 4.5). At zero day, the pH of the controls and all tested samples had the values (6.03). The pH values of all treatments with essential oils were similar therefore, it was not affected by essential oil addition. These results are in agreement with the results obtained by Mohamed and Mansour (2012) which reported that, addition of natural herbal extracts to beef patties did not significantly change the pH values of all formulas after preparation and during storage. However, after the storage period, the pH values of beef burger samples treated with potassium lactate and BHT decreased to 5.86 and 5.98, respectively.

## Chapter Six: Conclusion and Recommendations

In the last few decades, consumers are demanding healthy safe food with least concentration of synthetic food additives and least heat treatment. There are many ongoing studies on the biological properties of essential oils for their possible use as alternatives to synthetic antioxidant such as BHT. EOs which have a wide spectrum of antimicrobial activity are 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, three types of essential oils have been used for detection of their antibacterial activity against total plate count, total coliform, and *S. aureus*. Antioxidant activity of the EOs was also studied using TBARS test.

All EOs exhibit antibacterial activity, clove was amongst the strongest. These activities are mainly attributed to the presence of the active compounds in their compositions. An increase in the clove and thyme essential oils concentrations lead to a decrease in bacterial counts of *S. aureus* and total coliform. All essential oils, clove, thyme, and rosemary have good antibacterial properties on total plate count without significant difference between the two concentrations (1% and 1.5%). This means that there is no point in using a high concentration of oils, thus, the lower concentration (1%) can be used. Although the results clearly indicated that rosemary essential oils showed a reduction in total plate count and total coliform, it showed no effect on growth of *S. aureus*.

The clove and thyme provided the best antioxidant protection against beef burgers deterioration during 7 days of storage at 4°C. Moreover, clove at 1.5% was superior to

BHT in inhibiting the lipid oxidation. However, rosemary essential oil demonstrated a decrease in their antioxidant capacity. In conclusion, the application of essential oils on beef burger reduces the microbial growth and delayed oxidative deterioration, thus can be used as alternatives to synthetic preservatives to increase the shelf life of refrigerated beef burger.

Further studies on the essential oil compounds are needed to link the chemical content with particular functional properties. Extraction of the active ingredients of these oils or other oils and their applications as preservatives or antioxidants on food may give appreciable results, further research is necessary to study if there is a synergistic effect.

## Chapter Seven: References

1. Abdulmumeen, H. A., Risikat, A. N., & Sururah, A. R. (2012). Food: Its preservatives, additives and applications. *International Journal of Chemical and Biochemical Sciences*, 1(2012), 36-47.
2. Abebe, E., Gugsu, G., & Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of tropical medicine*, 2020.
3. Adam, Y., & Abugroun, H. (2010). Effect of Sudanese marketing condition on quality attributes of meat products. *Pakistan Journal of Nutrition*, 9(12), 1149-1156.
4. Ahmad, J., Ali, M. Q., Arif, M. R., & Iftikhar, S. (2021). Review Article on; Traditional and Modern Techniques For Food Preservation. *International Journal of Modern Agriculture*, 10(3), 219-234.
5. Alsaiqali, M., El-Shibiny, A., Adel, M., Abdel-Samie, M., & Ghoneim, S. (2016). Use of some essential oils as antimicrobial agents to control pathogenic bacteria in beef burger. *World Journal of Dairy & Food Sciences*, 11(1), 109-120.
6. Álvarez-Martínez, F. J., Barraji3n-Catal3n, E., Encinar, J. A., Rodr3guez-D3az, J. C., & Micol, V. (2020). Antimicrobial capacity of plant polyphenols against gram-positive bacteria: A comprehensive review. *Current medicinal chemistry*, 27(15), 2576-2606.
7. Aminzare, M., Hashemi, M., Ansarian, E., Bimkar, M., Azar, H. H., Mehrasbi, M. R., Daneshamooz, S., Raeisi, M., Jannat, B., & Afshari, A. (2019). Using natural antioxidants in meat and meat products as preservatives: A review. *Advances in Animal and Veterinary Sciences*, 7(5), 417-426.
8. Aminzare, M., Hashemi, M., Hassanzadazar, H., & Hejazi, J. (2016). The use of herbal extracts and essential oils as a potential antimicrobial in meat and meat products; a review. *Journal of Human, Environment and Health Promotion*, 1(2), 63-74.
9. Amit, S. K., Uddin, M., Rahman, R., Islam, S., & Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture & Food Security*, 6(1), 1-22.
10. Anwar, H., Hussain, G., & Mustafa, I. (2018). Antioxidants from natural sources. *Antioxidants in Foods and its Applications*, 3-28.
11. Archile-Contreras, A., & Purslow, P. (2011). Oxidative stress may affect meat quality by interfering with collagen turnover by muscle fibroblasts. *Food Research International*, 44(2), 582-588.
12. Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food chemistry*, 99(1), 191-203.
13. Bhavaniramy, S., Vishnupriya, S., Al-Aboody, M. S., Vijayakumar, R., & Baskaran, D. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain & oil science and technology*, 2(2), 49-55.
14. Bienkiewicz, G., & Ko3akowska, A. (2003). Effects of lipid oxidation on fish lipids—amylopectin interactions. *European journal of lipid science and technology*, 105(8), 410-418.
15. Brewer, S. (2008). Preserving beef quality with natural antioxidants. *Research knowledge management*, 12, 1-14.

16. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*, 94(3), 223-253.
17. Burt, S. A., & Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against *Escherichia coli* O157: H7. *Letters in applied microbiology*, 36(3), 162-167.
18. Cai, C., Ma, R., Duan, M., & Lu, D. (2019). Preparation and antimicrobial activity of thyme essential oil microcapsules prepared with gum arabic. *RSC advances*, 9(34), 19740-19747.
19. Canillac, N., & Mourey, A. (2001). Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. *Food Microbiology*, 18(3), 261-268.
20. Cardoso-Ugarte, G. A., & Sosa-Morales, M. E. (2021). Essential Oils from Herbs and Spices as Natural Antioxidants: Diversity of Promising Food Applications in the past Decade. *Food Reviews International*, 1-31.
21. Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines*, 4(3), 58.
22. Conner, D. (1993). Naturally occurring compounds. *Antimicrobials in foods*, 441-468.
23. Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The role of polyphenols in human health and food systems: A mini-review. *Frontiers in nutrition*, 5, 87.
24. Csala, M., Kardon, T., Legeza, B., Lizák, B., Mandl, J., Margittai, É., Puskás, F., Száraz, P., Szelényi, P., & Bánhegyi, G. (2015). On the role of 4-hydroxynonenal in health and disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1852(5), 826-838.
25. Devi, K. P., Nisha, S. A., Sakthivel, R., & Pandian, S. K. (2010). Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of ethnopharmacology*, 130(1), 107-115.
26. Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*, 88(2), 308-316.
27. Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. *Food Research International*, 64, 171-181.
28. Firstenberg-Eden, R., Foti, D., McDOUGAL, S., & Beck, S. (2004). Performance comparison of the BioSys Optical Assay and the violet red bile agar method for detecting coliforms in food products. *Journal of food protection*, 67(12), 2760-2766.
29. Fokou, J. B. H., Dongmo, P. M. J., & Boyom, F. F. (2020). Essential oil's chemical composition and pharmacological properties. In *Essential oils-oils of nature*. IntechOpen.
30. Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R., & Nazzaro, F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science*, 75(8), M528-M535.
31. Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., & Efferth, T. (2007). Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy research*, 21(10), 989-994.

32. Gallo, M., Ferracane, R., & Naviglio, D. (2012). Antioxidant addition to prevent lipid and protein oxidation in chicken meat mixed with supercritical extracts of *Echinacea angustifolia*. *The Journal of Supercritical Fluids*, *72*, 198-204.
33. Ganesan, K., & Xu, B. (2017). A critical review on polyphenols and health benefits of black soybeans. *Nutrients*, *9*(5), 455.
34. García-Lomillo, J., Gonzalez-SanJose, M. L., Del Pino-García, R., Ortega-Heras, M., & Muñoz-Rodríguez, P. (2017). Antioxidant effect of seasonings derived from wine pomace on lipid oxidation in refrigerated and frozen beef patties. *LWT*, *77*, 85-91.
35. Ghaderi-Ghahfarokhi, M., Barzegar, M., Sahari, M. A., & Azizi, M. H. (2016). Nanoencapsulation approach to improve antimicrobial and antioxidant activity of thyme essential oil in beef burgers during refrigerated storage. *Food and Bioprocess Technology*, *9*(7), 1187-1201.
36. Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in microbiology*, *9*, 1639.
37. Goñi, P., López, P., Sánchez, C., Gómez-Lus, R., Becerril, R., & Nerín, C. (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food chemistry*, *116*(4), 982-989.
38. Goulas, A. E., & Kontominas, M. G. (2007). Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. *Food chemistry*, *100*(1), 287-296.
39. Gray, J., Gomaa, E., & Buckley, D. (1996). Oxidative quality and shelf life of meats. *Meat science*, *43*, 111-123.
40. Horbańczuk, O. K., Kurek, M. A., Atanasov, A. G., Brnčić, M., & Brnčić, S. R. (2019). The effect of natural antioxidants on quality and shelf life of beef and beef products. *Food technology and biotechnology*, *57*(4), 439.
41. Hu, Y., Jia, J., Qiao, J., Ge, C., & Cao, Z. (2010). ANTIMICROBIAL ACTIVITY OF PU-ERH TEA EXTRACTS IN VITRO AND ITS EFFECTS ON THE PRESERVATION OF COOLED MUTTON. *Journal of Food Safety*, *30*(1), 177-195.
42. Huang, X., & Ahn, D. U. (2019). Lipid oxidation and its implications to meat quality and human health. *Food science and biotechnology*, *28*(5), 1275-1285.
43. Imelouane, B., Amhamdi, H., Wathelet, J.-P., Ankit, M., Khedid, K., & El Bachiri, A. (2009). Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. *Int. J. Agric. Biol*, *11*(2), 205-208.
44. Inetianbor, J., Yakubu, J., & Ezeonu, S. (2015). Effects of food additives and preservatives on man-a review. *Asian Journal of Science and Technology*, *6*(2), 1118-1135.
45. Jafri, H., Ansari, F. A., & Ahmad, I. (2019). Prospects of essential oils in controlling pathogenic biofilm. In *New look to phytomedicine* (pp. 203-236). Elsevier.
46. Jayasena, D. D., & Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science & Technology*, *34*(2), 96-108.

47. Jiang, J., & Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat science*, *120*, 107-117.
48. Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A., & Schmidt, E. (2006). Chemical composition and antioxidant properties of clove leaf essential oil. *Journal of agricultural and food chemistry*, *54*(17), 6303-6307.
49. Kachur, K., & Suntres, Z. (2020). The antibacterial properties of phenolic isomers, carvacrol and thymol. *Critical reviews in food science and nutrition*, *60*(18), 3042-3053.
50. Kassem, G., Atta-Alla, O., & Ali, F. (2011a). Adición de aceite esencial de tomillo y aceite de jojoba para mejorar la calidad de la hamburguesa de vacuno. *Archivos de zootecnia*, *60*(231), 787-795.
51. Kassem, G., Atta-Alla, O., & Ali, F. (2011b). Improving the quality of beef burger by adding thyme essential oil and jojoba oil. *Archivos de zootecnia*, *60*(231), 787-795.
52. Kim, J., Marshall, M., Cornell, J., III, J. P., & Wei, C. (1995). Antibacterial activity of carvacrol, citral, and geraniol against *Salmonella typhimurium* in culture medium and on fish cubes. *Journal of Food Science*, *60*(6), 1364-1368.
53. Kim, M. R., & Wei, C.-i. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of agricultural and food chemistry*, *43*(11), 2839-2845.
54. Kong, B., Zhang, H., & Xiong, Y. L. (2010). Antioxidant activity of spice extracts in a liposome system and in cooked pork patties and the possible mode of action. *Meat science*, *85*(4), 772-778.
55. Krisch, J., Tserennadmid, R., & Vágvölgyi, C. (2011). Essential oils against yeasts and moulds causing food spoilage. *Science against microbial pathogens: Communicating current research and technological advances*, Badajoz, Spain.
56. Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, *24*, e00370.
57. Kumar, V., Mishra, D., Joshi, M. C., Mishra, P., & Tanwar, M. (2021). Herbs and Spices—New Processing Technologies. *Syzygium aromaticum: Medicinal Properties and Phytochemical Screening*. In *Herbs and Spices-New Processing Technologies*. IntechOpen.
58. Kumar, Y., Yadav, D. N., Ahmad, T., & Narsaiah, K. (2015). Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety*, *14*(6), 796-812.
59. Lambert, R., Skandamis, P. N., Coote, P. J., & Nychas, G. J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology*, *91*(3), 453-462.
60. Laranjo, M., Fernandez-Leon, A. M., Potes, M., Agulheiro-Santos, A., & Elias, M. (2017). Use of essential oils in food preservation.
61. Liu, J., Shi, Y., Sun, J., Tang, S., Hu, Y., & Jin, R. (2013). A theoretical study on the interaction between melamine and acrylamide functional monomer in molecularly imprinted polymers. *Food Sci*, *34*, 96-101.
62. Medina, E., De Castro, A., Romero, C., & Brenes, M. (2006). Comparison of the concentrations of phenolic compounds in olive oils and other plant oils:

- correlation with antimicrobial activity. *Journal of agricultural and food chemistry*, 54(14), 4954-4961.
63. Mohamed, H. M., & Mansour, H. A. (2012). Incorporating essential oils of marjoram and rosemary in the formulation of beef patties manufactured with mechanically deboned poultry meat to improve the lipid stability and sensory attributes. *LWT-Food Science and Technology*, 45(1), 79-87.
  64. Mohammed, M. J., & Al-Bayati, F. A. (2009). Isolation and identification of antibacterial compounds from *Thymus kotschyanus* aerial parts and *Dianthus caryophyllus* flower buds. *Phytomedicine*, 16(6-7), 632-637.
  65. Mousavian, D., Mohammadi Nafchi, A., Nouri, L., & Abedinia, A. (2021). Physicomechanical properties, release kinetics, and antimicrobial activity of activated low-density polyethylene and orientated polypropylene films by Thyme essential oil active component. *Journal of Food Measurement and Characterization*, 15(1), 883-891.
  66. Moylan, S., Berk, M., Dean, O. M., Samuni, Y., Williams, L. J., O'Neil, A., Hayley, A. C., Pasco, J. A., Anderson, G., & Jacka, F. N. (2014). Oxidative & nitrosative stress in depression: why so much stress? *Neuroscience & Biobehavioral Reviews*, 45, 46-62.
  67. Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, 22(7), 3380.
  68. Naveena, B., Sen, A., Vaithyanathan, S., Babji, Y., & Kondaiah, N. (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat science*, 80(4), 1304-1308.
  69. Nieto, G. (2020). A review on applications and uses of thymus in the food industry. *Plants*, 9(8), 961.
  70. Ojagh, S., Sahari, M., Rezaei, M., & Hosseini, S. (2011). Applicability of  $\beta$ -carotene and green tea polyphenols as two natural antioxidants in preservation of common kilka (*Clupeonella cultriventris caspia*) with ice. *Int. J. Agric.: Res. Rev*, 1, 174-181.
  71. Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J.-P., & Bégin, A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *International journal of food microbiology*, 37(2-3), 155-162.
  72. Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2(5), 270-278.
  73. Pereira, D., Pinheiro, R. S., Heldt, L. F. S., MOURA, C. d., Bianchin, M., Almeida, J. d. F., REIS, A. S. d., Ribeiro, I. S., Haminiuk, C. W. I., & Carpes, S. T. (2017). Rosemary as natural antioxidant to prevent oxidation in chicken burgers. *Food Science and Technology*, 37, 17-23.
  74. Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian journal of clinical biochemistry*, 30(1), 11-26.
  75. Quinto, E. J., Caro, I., Villalobos-Delgado, L. H., Mateo, J., De-Mateo-Silleras, B., & Redondo-Del-Río, M. P. (2019). Food safety through natural antimicrobials. *Antibiotics*, 8(4), 208.
  76. Radünz, M., da Trindade, M. L. M., Camargo, T. M., Radünz, A. L., Borges, C. D., Gandra, E. A., & Helbig, E. (2019). Antimicrobial and antioxidant

- activity of unencapsulated and encapsulated clove (*Syzygium aromaticum*, L.) essential oil. *Food chemistry*, 276, 180-186.
77. Rasooli, I. (2007). Food preservation—a biopreservative approach. *Food*, 1(2), 111-136.
  78. Sahalie, N. A., Abrha, L. H., & Tolesa, L. D. (2018). Chemical composition and antimicrobial activity of leave extract of *Ocimum lamiifolium* (Damakese) as a treatment for urinary tract infection. *Cogent Chemistry*, 4(1), 1440894.
  79. Selim, S. (2011). Antimicrobial activity of essential oils against Vancomycin-Resistant enterococci (VRE) and *Escherichia coli* O157: H7 in feta soft cheese and minced beef meat. *Brazilian journal of microbiology*, 42(1), 187-196.
  80. Shahbazi, Y., & Shavisi, N. (2018). Chicken meat hamburger preservation using antimicrobial packaging containing cinnamon extract. *Journal of Nutrition, Fasting and Health*, 6(Issue), 7-14.
  81. Shahidi, F., & Wanasundara, U. N. (2002). Methods for measuring oxidative rancidity in fats and oils. *FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER-*, 465-488.
  82. Shaltout, F. A., & Koura, H. A. (2017). Impact of some essential oils on the quality aspect and shelf life of meat. *Benha Veterinary Medical Journal*, 33(2), 351-364.
  83. Sharafati Chaleshtori, R., Rokni, N., Rafieian-Kopaei, M., Deris, F., & Salehi, E. (2015). Antioxidant and antibacterial activity of basil (*Ocimum basilicum* L.) essential oil in beef burger. *Journal of Agricultural Science and Technology*, 17(4), 817-826.
  84. Sharma, S. (2015). Food preservatives and their harmful effects. *International journal of scientific and research publications*, 5(4), 1-2.
  85. Sheeladevi, A., & Ramanathan, N. (2012). Antibacterial activity of plant essential oils against food borne bacteria. *International Journal of Pharmaceutical & Biological Archives*, 3(5), 1106-1109.
  86. Shewail, A., SHALTOU, F., & GERGES, T. (2018). IMPACT OF SOME ORGANIC ACIDS AND THEIR SALTS ON MICROBIAL QUALITY AND SHELF LIFE OF BEEF. *Assiut Veterinary Medical Journal*, 64(159), 164-177.
  87. Silva, N., Barbosa, L., Seito, L., & Fernandes Junior, A. (2012). Antimicrobial activity and phytochemical analysis of crude extracts and essential oils from medicinal plants. *Natural Product Research*, 26(16), 1510-1514.
  88. Singla, R. K., Dubey, A. K., Garg, A., Sharma, R. K., Fiorino, M., Ameen, S. M., Haddad, M. A., & Al-Hiary, M. (2019). Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. In (Vol. 102, pp. 1397-1400): Oxford University Press.
  89. Soethe, C., Steffens, C. A., Hawerth, F. J., Moreira, M. A., do Amarante, C. V. T., & Stanger, M. C. (2022). Quality of 'Baigent' apples as a function of pre-harvest application of aminoethoxyvinylglycine and ethephon stored in controlled atmosphere. *Applied Food Research*, 2(1), 100117.
  90. Solomakos, N., Govaris, A., Koidis, P., & Botsoglou, N. (2008). The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. *Food Microbiology*, 25(1), 120-127.
  91. Tapia, M. S., Alzamora, S. M., & Chirife, J. (2020). Effects of water activity (aw) on microbial stability as a hurdle in food preservation. *Water activity in foods: Fundamentals and applications*, 323-355.

92. Vaclavik, V. A., Christian, E. W., & Campbell, T. (2021). Food preservation. In *Essentials of food science* (pp. 327-346). Springer.
93. Viuda-Martos, M., MA, Fernández-López, J., Abd ElRazik, K., Omer, E., Pérez-Alvarez, J., & Sendra, E. (2011). In vitro antioxidant and antibacterial activities of essential oils obtained from Egyptian aromatic plants. *Food Control*, 22(11), 1715-1722.
94. Wang, J., Han, L., Wang, D., Sun, Y., Huang, J., & Shahidi, F. (2021). Stability and stabilization of omega-3 oils: A review. *Trends in Food Science & Technology*, 118, 17-35.
95. Williams, G. M., Iatropoulos, M., & Whysner, J. (1999). Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food and chemical toxicology*, 37(9-10), 1027-1038.
96. Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J., & Li, H.-B. (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *International journal of molecular sciences*, 18(1), 96.
97. Xu, J.-G., Liu, T., Hu, Q.-P., & Cao, X.-M. (2016). Chemical composition, antibacterial properties and mechanism of action of essential oil from clove buds against *Staphylococcus aureus*. *Molecules*, 21(9), 1194.
98. Youssef, K. (2014). Antioxidant effect of some plant extracts as compared with BHA/BHT on lipid oxidation and some quality properties of fresh beef burgers stored at 4 C. *Suez Canal University journal of food sciences*, 2(1), 9-18.
99. Yu, H. H., Chin, Y.-W., & Paik, H.-D. (2021). Application of Natural Preservatives for Meat and Meat Products against Food-Borne Pathogens and Spoilage Bacteria: A Review. *Foods*, 10(10), 2418.
100. Zaccheo, A., Palmaccio, E., Venable, M., Locarnini-Sciaroni, I., & Parisi, S. (2016). *Food hygiene and applied food microbiology in an anthropological cross cultural perspective*. Springer.
101. Zahid, M. A., Seo, J.-K., Parvin, R., Ko, J., & Yang, H.-S. (2019). Comparison of butylated hydroxytoluene, ascorbic acid, and clove extract as antioxidants in fresh beef patties at refrigerated storage. *Food science of animal resources*, 39(5), 768.
102. Zhou, W., Wang, Z., Mo, H., Zhao, Y., Li, H., Zhang, H., Hu, L., & Zhou, X. (2019). Thymol mediates bactericidal activity against *Staphylococcus aureus* by targeting an aldo-keto reductase and consequent depletion of NADPH. *Journal of agricultural and food chemistry*, 67(30), 8382-8392.

## المخلص

يعتبر كل من الأكسدة والنمو الميكروبي المسبب الرئيسي لتلف البرجر البقري. لذلك يتم استخدام إضافات غذائية صناعية تعمل على منع أو تأخير الأكسدة والنمو الميكروبي المؤديان لتلف المواد الغذائية. ولكن هذه الإضافات الغذائية الصناعية تعتبر غير آمنة ولها أضرار صحية على الإنسان، مما أظهر الحاجة للبحث عن بدائل طبيعية آمنة تعمل كمواد حافظة في الصناعات الغذائية. في هذه الدراسة تم استخدام زيوت أساسية مختلفة من القرنفل والزعر و إكليل الجبل كموا حافظة طبيعية لزيادة مدة الصلاحية لمنتج البرجر البقري لمدة تصل الى 7 أيام دون الحاجة لاستخدام المواد الحافظة الصناعية.

تم إضافة الزيوت العطرية على منتج البرجر البقري بتركيز مختلفة (1 و 1.5% ح/ك)، كذلك هناك عينتان تم معالجتهما بمادة (2%) potassium lactate (100 ppm) butylated hydroxytoluene كموا مضادة ضابطة موجبة للميكروبات والأكسدة على التوالي. كذلك استخدمت احدى العينات بدون أي اضافات كعينة ضابطة سالبة لعمل المقارنات. تم قياس الفعالية التثبيطية الميكروبية للزيوت العطرية بواسطة فحوصات العد البكتيري الهوائي الكلي (total plate count)، *Staphylococcus aureus*، coliforms كذلك تم اجراء فحص تثبيط التأكسد (TBARS) وفحص ال pH.

أظهرت نتائج الدراسة بأن إضافة الزيوت الأساسية أعطت فعالية تثبيطية عالية للميكروبات التي تمت دراستها. إن إضافة زيت القرنفل بتركيز (1.5%) أظهر تثبيط قوي ضد بكتيريا *Staphylococcus aureus* بنسبة 54% مقارنة مع عينة الضبط السالبة كذلك هناك فرق معنوي بينه وبين العينات الأخرى في الدراسة. كما وأظهرت الدراسة بأن زيت القرنفل (1 و 1.5%) كان له تأثير مثبط قوي على بكتيريا الكوليفورم مقارنة مع العينات الأخرى. كذلك العد الكلي للبكتيريا انخفض في جميع عينات البرجر البقري المعالجة بالزيوت الأساسية المختلفة دون أن يكون هناك أي فرق معنوي بين الزيوت المختلفة.

أما بالنسبة للفحوصات الكيميائية، فقد أظهرت النتائج بأن إضافة الزيوت الأساسية للقرنفل بالتراكيز 1% و1.5% كانت الأكثر فعالية مقارنة بالعينات الضابطة السالبة حيث قلت من التأكسد بنسبة تصل إلى 24% و35% على التوالي. وبالنسبة لزيت الزعتر عند التراكيز 1% و1.5% فقد أظهرت النتائج أن نسبة التأكسد قلت بما مقداره 14% و31% على التوالي مقارنة بنتائج العينات الضابطة السالبة.