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Review article

Incorporation of essential oils with antibiotic properties in edible packaging films

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ABSTRACT

Over the last few years, the studies have been conducted to develop and to apply edible films from different agricultural commodities. A wide range of materials are used to produce edible films including polysaccharides, proteins, lipids and their blends; these materials are known as a supporter factor for various additives. In selecting an antimicrobial method, the beneficial effects against the target microorganism and their interactions with polymer matrix is essential. Generally, the active components of plant extracts inhibit microorganism's growth through disruption of the cytoplasmic membrane, disturbance the proton motive force and electron flow. Plant-derived essential oils (EOs) usually have a relatively high vapor pressure and are capable of reaching microbial pathogens through the liquid and the gas phase. Bioactivity of EOs in the vapor phase makes them useful as possible fumigants for stored commodity protection. Edible films containing plant-derived volatile EOs provide new ways to enhance microbial safety and shelf-life of foods. The main objective of this article is to evaluate the antimicrobial impact of plant extracts and essential oil used in edible films, based on the advantages and disadvantages of them.

Keywords: Edible film, Plant extract, Essential oil, Antimicrobial, Food packaging

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1. Introduction

Edible films are thin layers which could be used directly in foods or as a separate layer between food components as barrier to gases, vapors, oils and moisture (Bourtoom, 2008; Du et al., 2011). Edible films have been developed to be a substitute for the synthetic plastic food packaging (Bourtoom, 2008; Dhanapal et al., 2012). The most commonly applied natural polymers include: polysaccharides (starch, chitosan, alginate, and gums), proteins (collagen, soybean and gluten proteins, and milk proteins), and fats (bee wax, carnauba wax, fatty acids, and glycerol) (Kraśniewska et al., 2012; Krochta et al., 1994). Natural additives from plant extracts, essential oil, active compounds, plant-based products, and vitamins could improve the antioxidant and antimicrobial activity of edible films (Campos et al., 2011; Silva-Weiss et al., 2013). Extraction and steam distillation are the most common procedures to obtain plant extract and essential oil, respectively (Wihodo et al., 2013). Food additives with antimicrobial activities are suitable candidates to overcome cross-contamination problem, they could be integrated into edible films and coatings (Krochta et al., 1994).

Antimicrobial compounds are either natural or synthetic. Antimicrobials agents with functional groups include: organic acids, chitosan, some polypeptides as nisin, lacto-peroxidase system, plant extracts and essential oils (Campos et al., 2011; Kraśniewska et al., 2012). Various studies on antimicrobial edible films as an alternative food packaging method have been conducted, because of consumer demands and environmental problems (Krochta et al., 1994; Sani et al., 2017; Teixeira et al., 2014).

Essential oils (EOs) are complex combinations of lipophilic ingredients with biological properties approved by foods and drugs administration (FDA) as "generally recognized as safe" (GRAS). The antimicrobial activities of essential oils and plant extracts have been long known in edible films. The antimicrobial activity of EOs (Espina et al., 2011) is influenced by raw plant material (genotype and, part of the plant), harvest time, geographical and ecological conditions (Settanni et al., 2012)and extraction method (Burt,

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2004). Plants extracts and essential oils are rich in bioactive compounds such as terpenoids, alkaloids, polypeptides and phenolic particles; they have high antimicrobial potential against a wide range of microorganisms. It should be noted that the plant extracts and essential oils have antioxidant activity and food flavoring properties(Silva-Weiss et al., 2013).

There are some limitations regarding the direct application of EOs in food preservation process including; strong aroma, potential toxicity and the extraction costs. Reducing the doses of food matrix is a significant factor for Eos (Sanchez-Gonzalez et al., 2011). Good examples of such plants are oregano, thyme, salvia, parsley, clove, coriander, garlic and onion, which contain aromatic phenolic compounds, such as Carvacrol, Eugenol and Thymol (Du et al., 2011). The mechanism of EO with antimicrobial activities related to the destruction of cell wall, cytoplasmic membrane of bacteria and fungi, then cytoplasmic leakage, next prevention of the synthesis of DNA, RNA, proteins and polysaccharides in microorganisms. The antibacterial activity of plant extracts is determined by the amount of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) as well as disk diffusion methods (Tongnuanchan et al., 2012). In fact, MIC has the lowest concentration of antimicrobial agents which inhibits the growth of microorganism. Recent studies have demonstrated that Oregano, Thyme, Cinnamon and Clove are more active against strains of *Escherichia coli* (*E. coli*) (Krochta et al., 1994). In this article, we have evaluated several properties of essential oils according to the above advantages and the main objective of this article was to evaluate the antimicrobial activity of plant extracts and essential oil in edible films.

2. Antimicrobial compounds in active films

Active, intelligent or smart packaging refers to the packaging systems used with foods, pharmaceuticals and several other types of products. They can be used to extend the shelf life, monitor freshness, display information on quality, improve safety and improve the convenience of food products (Han & Rooney, 2002). To extend the shelf life and to improve the quality and safety of the products, natural compounds such as organic acid, bacteriocins, enzymes, fungicides and spice extracts have been used as food preservatives (Bakri et al., 2005). Also, these components are new strategy of modern food industry and active food packaging systems.

3. Essential oils

EOs are produced in glandular structures of plant cell wall; these materials are known as secondary metabolism. The extracts have been used in foods and drinks for decades and could act as an antimicrobial, antioxidant (Clove and Origanum essential oils) and antifungal agents (Bajpai et al., 2012; Bakri et al., 2005; Burt, 2004; Roby et al., 2013; Teixeira et al., 2013). Nowadays consumers are aware of the hazardous impact of synthetic additives, therefore we have observed an increasing interest for natural components (Choi et al., 2000; Ormancey et al., 2001).

EOs at all concentrations, have powerful flavor and aroma which could affect organoleptic properties of the products in the acceptable threshold (Ismail et al., 2011). Also in pungent and spicy flavored products, the acceptable threshold is more than other components that cause some limitations for EOs application. A suitable solution for this problem is to focus on some characteristic of the components such as antioxidant activity, because they are known as GRAS (Generally Recognized as Safe) by the FDA (Manso et al., 2013). Linalool, thymol, eugenol, carvone, cinnamaldehyde, vanillin, carvacrol, citral and limonene are some non-hazardous example of proved flavor of GRAS (Evandri et al., 2005; Hyldgaard et al., 2012; Mahmoudi et al., 2013). However, these products are confirmed as GRAS, there are some doubts about them, therefore, researcher are conducting further studies to ensure consumers about the safety of these products, for instance Bakkali in 2008 has done a comprehensive study on EOs by focusing on pharmaceutical, health, cosmetic, agricultural and food industries (Bakkali et al., 2008).

4. The main ingredients of essential oils

EOs as natural oils with low molecular weights are typically obtained by distillation and have the characteristic odor of the plant or the other sources (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) from which they are extracted, and according to their origin; climate, soil composition, plant organ, age and vegetative cycle, they have various quality (Bajpai et al., 2012; Bakkali et al., 2008; Husnu et al., 2015). These components have some function for instance, antiviral, antibacterial, anti-mycotic, anti-toxigenic, anti-parasitic, and insecticidal characteristic (Baser et al., 2015). EOs have complex matrix of 20-60 ingredients in different concentration (Bakkali et al., 2008; Burt, 2004). Table 1 shows different EOs with their components. The main purpose of using EOs in food packaging is to create a safe and secure package with longer shelf life and higher qualities. EOs with antimicrobial properties and biosynthetic sources have listed in Fig. 1 (Pichersky et al., 2006).These essential oil consists of aromatic and aliphatic constituents (hydrocarbon ingredients or derivatives of oxygen) including terpenes (monoterpenes (C10), sesquiterpenes (C15), hemiterpenes (C5), diterpenes (C20), triterpenes (C30), tetraterpenes (C40), terpenoids (terpene with oxygen), monoterpenes and sesquiterpenes (Angioni et al., 2006; Jordan et al., 2006; Mejri et al., 2010).

The health benefits of Oregano EO (with high carvacrol) can be attributed to its properties as an antiviral, antibacterial, antifungal, anti-parasitic, antioxidant, anti-inflammatory, digestive, emmenagogue, anti-allergenic, acetylcholine esterase inhibition, radical scavenging effect, white blood cell macrophage stimulant and cardiac depressant (Can Baser 2008; Economou et al., 2011; Ipek et al., 2005; Koparal et al., 2003).

Food-borne diseases are any illnesses resulting from the consumption of contaminated food, pathogenic bacteria, viruses or parasites, as well as toxins such as poisonous mushrooms and various species of beans that have not been boiled for at least 10 minutes; hence preventing and protecting against this disease is an important issue for consumers. EOs have great inhibitory effects against food-borne spoilage and pathogenic bacterial strains (*Brochothrix thermosphacta*, *Escherichia coli*, *Listeria innocua*, *Listeria monocytogenes*, *Pseudomonas putida*, *Salmonella typhimurium and Shewanella putrefacien*s, the minimum inhibitory concentration for most of the EOs is < 3.0 mg. mL^{-1}. After some research, it has been shown that coriander, origanum, rosemary and thyme essential oils have an inhibitory effect against *L. innocua*. The best method to measure the antibacterial activity of EOs is bacterial count (Teixeira et al., 2013).

Table 1. Main components of several essential.

Common name of EO	Latin name of plant source	Organs of plant	Main components	Reference
Oregano	*Origanum onites*	leaf	Carvacrol, linalool, p-cimen, γ-terpinen, mircen, timol, a -pinen	(Bostancıoglu et al., 2012)
Oregano	*Origanum vulgare*	leaf	o-cimen, Carvacrol	(De Martino et al., 2009)
Thyme	*Thymus vulgaris L.*	leaf	cinnamic acid, a-pigenin, luteolin o-rutinose	(Roby et al., 2013)
Garlic	*Allium sativum*	onion	2-propenyl thioacetonitril, trisulfide methyl 2-propenyl, disulfide di-2-propenyl	(Kırkpınar et al., 2011)
Basil	*Ocimum basilicum*	leaf	linalool, α-cadinol, y-cadinene	(Beric et al., 2008)
Rosemary	*Rosmarinus officinalis*	leaves and bowls flower	Verbenone, borneol, a-pinene, 1,8 cineole, camphor, b-Selinene, a-Selinene	(Sacchetti et al., 2005)
Ginger	*Zingiber officinale*	rhizome	Zingiberone , β-terpineol, p-cineole, camphene	(El-Ghorab et al., 2010)
clove	*Syzygium aromaticum*	flower	Eugenol, Caryophyllene, Eugenol acetate	(Guan et al., 2007)

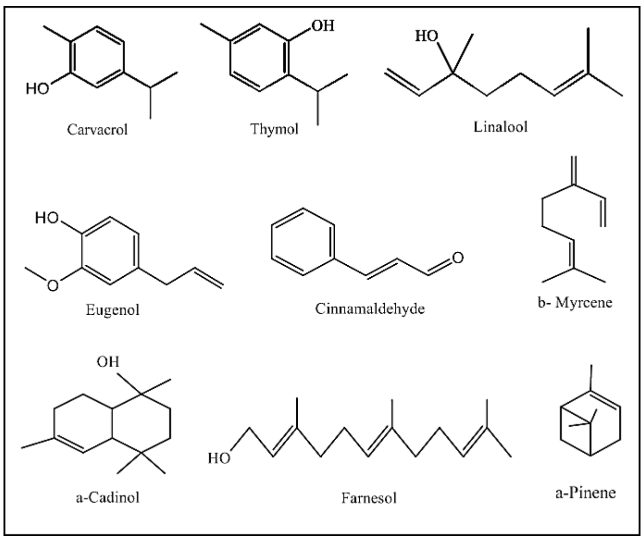

Fig. 1. Selected antimicrobial principles of essential oils (Economou et al., 2011).

5. The problems of applying essential oils

With regard to the mentioned positive effects of essential oils, some studies reported adverse effects of essential oils such as toxic and mutagenic effects (Halicioglu et al., 2011). In most cases, essential oils should not be used undiluted onto the skin, they should only be used undiluted under the care and guidance of a trained medical or aromatherapy practitioner. Undiluted EOs can cause irritation or an allergic reaction in some people. EOs have certain constituents that can make the skin more sensitive to UV which can lead to blistering, skin discoloration or even burning, easier than minor sun exposure. Essential oils can effect on hormones, gut bacteria and other aspects of human health, hence extreme care should be used for women especially in pregnancy period, because there is evidence that essential oils can cross the placenta and get to the baby. The essential oils can affect uterus and extreme care should be taken when using essential oil during pregnancy. Another issue which is not often mentioned is that essential oils should never be stored in plastic containers, especially in concentrated forms. Many essential oils can eat through plastics when undiluted, and even when diluted, they can gradually degrade plastics (Halicioglu et al., 2011; Tisserand et al., 1999). Therefore, they have to be tested before human use. Table 2 shows adverse problems.

6. Essential oils in food packaging

Active packaging contains polymer films (synthetize and natural) with antioxidant and antimicrobial properties. The antimicrobial activity of carrageenan films with cinnamon and cloves extracts were tested on the mentioned microorganisms and others which were taken from dried salted fish, called as a cocktail microorganism. Cinnamon extract showed better results compared to the clove extract. The study also showed that the films containing Cinnamon extract had no specific effect on the studied microorganisms. In some research, author has evaluated, antimicrobial activity of Clove, Garlic, Cinnamon and finger-root extract against *S. aureus*, *S. epidermidis*, *M. luteus* and *P. aeruginosa*. The experimental results showed that the Cinnamon extract had the topmost inhibitory effect, with MICs of 32.5 to 100 mg/mL, against the growth of *S. aureus*, *S. epidermidis*, *M. luteus and P. aeruginosa*; while Cloves and finger root extracts could only prevent the growth of *S. aureus and S. epidermidis*, and no inhibitory effect was observed in Garlic extract (Poovarodom et al., 2009).

In another study, physical, mechanical and antimicrobial properties of the whey protein-based film, incorporating cinnamon essential oil were examined. Results indicated that the films with Cinnamon essential oil had a significant antibacterial activity against both Gram-positive and gram-negative strains with inhibitory effect on the case study fungi (Bahram et al., 2014).

Origanum vulgare is a common species of *Origanum,* a genus of the mint family, *Lamiaceae,* which could be found in western and south western Eurasia and the Mediterranean region. The antimicrobial activity in *Oregano* is related to high content of phenolic compounds, mainly carvacrol which can cause cell membrane permeability and decompose the outer membrane of gram-negative bacteria (Ponce et al., 2008; Zheng et al., 2009).

Table 2. Adverse problem of essential oils.

EO	Experimental model	Concentration	Results	Reference
Oregano	*Salmonella Typhimurium* (TA 98, TA 100)	93-467 µg	No of mutagenic effect in different concentration of essential oil	(De Martino et al., 2009)
Oregano	soft cheese	0.1-1%	Decreased in Mitotic index, Chromosomal abnormalities and micronuclei	(Hamedo et al., 2009)
Thyme	*Salmonella Typhimurium* (TA 98, TA 100)	93-463 mg/plate	No mutagenic effect	(De Martino et al., 2009)
Thyme	*Salmonella Typhimurium* (TA 97, TA 98)	10-100 µg/ml	No mutagenicity	(Aicha et al., 2008)
Rosemary	*Salmonella Typhimurium* (TA 98)	0.05-2 mg/plate	Protected against bacterial mutagens, NQNO and IQ, ROS-inducing agent, *t*-BOOH and indirect mutagens	(Žegura et al., 2011)
Rosemary	soft cheese	0.1-1%	Decreased in Mitotic index, Chromosomal abnormalities and micronuclei	(Hamedo et al., 2009)
Basil	*Salmonella Typhimurium* (TA 98, TA 100)	96-480 µL/plate	No mutagenic effect	(De Martino et al., 2009)
Basil	*Salmonella Typhimurium* (TA98, TA100, TA102), *E.Coli* (WP2, SY252, IB101)	0-2 µL/plate	No mutagenic effect	(Berić et al., 2008)
Ginger	*Salmonella Typhimurium* (TA98, TA100, TA102)	10-3000 µL/plate	No mutagenicity	(Jeena et al., 2014)

Table 3. Incorporation of plant extracts into edible films.

Plant extract	Biopolymer base	Effects of active natural films	References
Thyme	chitosan - starch	Antioxidant activity/ to improve the shelf life of the products sensitive to oxidative processes.	(Talón et al., 2017)
Oregano, rosemary	tuna-skin/bovine-hide gelatin	Increases AOX activity and UV light barrier/ Inhibiting oxidative rancidity	(Gómez-Estaca et al., 2009)
Citrus	fish skin gelatin	Decreases TS and WVP, Increases EB/ incresed radical scavenging activity	(Tongnuanchan et al., 2012)
Clove, garlic, origanum	fish protein	Garlic increased radical scavenging activity/ highest antibacterial activity with clove essential oils/ decreased thickness, water solubility, breaking force and elongation	(Teixeira et al., 2014)
Oregano	Cassava Starch−Chitosan	improved barrier properties/ inhibited the growth of microorganisms	(Pelissari et al., 2009)
Thyme	quince seed mucilage	increase in WVP/ the growth of *Shewanella putrefaciens, Listeria monocytogenes* and *Staphylococcus aureus* was decreased/ Increases AOX activity	(Jouki et al., 2014)

In previous work, the antimicrobial activity of isolated films from whey protein with 1– 4 %w/v oregano, rosemary and garlic essential oils were tested against *Escherichia coli O157:H7, Staphylococcus aureus, Salmonella enteritidis, Listeria monocytogenes* and *Lactobacillus plantarum* bacteria, the purpose of using these films were to cover surfaces of cheese and cured meat. It was determined that addition of 2% oregano essential oils (OEO) to whey protein film had an antimicrobial activity more than Rosemary and garlic extract films, hence this type of packaging increased the shelf-life of products (Seydim et al., 2006).

Others have tested *chitosan* films with the essential oils of *Oregano* and they have observed antimicrobial activity against both *L. monocytogenes* and *E. coli O157:H7*. The films which were prepared with *chitosan* films showed antimicrobial activity, and the highest antimicrobial activity was related to *Chitosan* film with *Oregano* extracts (Jasour et al., 2015; Zivanovic et al., 2005).

In another study, quince seed mucilage films with oregano essential oil were tested for antibacterial and antioxidant properties. Antimicrobial and antioxidant activities have significantly improved with the combination of essential oils. By adding 1.5 to 2% *Oregano* essential oil to quince seed mucilage films, we were able to observe antimicrobial effect against *S. typhimurium, P. aeruginosa, S. aureus, E. coli, S. putrefaciens and Y. enterocolitica,* specially against Gram-positive bacteria (Jouki et al., 2014). The results showed that the application of OEO in edible films were effective on molds growth on strawberries, also it was determined that the addition of 1% oregano essential oil to whey protein isolate of edible films has anti-fungal properties and increases the shelf life of strawberries. The antimicrobial activity of carvacrol and cinnamaldehyde in apple and tomato edible films against *E. coli O157:H7* was investigated and concluded; these films prevented the initial growth of *E. coli O157:H7* after 1 day, but they did not have any impact on reduction of the number of foodborne bacteria after 5 days (Du et al., 2011).

Zinoviadou et al. (2009) studied edible films properties based on whey protein isolate with *Oregano* oil in concentrations of 0.5, 1.0, and 1.5 %w/w. The conclusions approved that moisture absorption was not affected by the addition of *Oregano* oil, although, we have found that by enhancing the essential oil concentration, Young modulus and maximum tensile strength decreased while elongation at break increased. The application of these antimicrobial films reduced the total flora count and *Pseudomonads*, although the growth of *Lactic acid* bacteria has been inhibited significantly.

In particular, the effect of *Origanum vulgare* L. essential oil on physicochemical and antimicrobial properties of bio-based composite edible films has been investigated. An increase in WVP and SW was observed when *Origanum vulgare* L. essential oil was added to the films. According to mechanical properties, OEO decreased tensile strength (TS) and elastic modulus, in addition, OEO was not very effective against Gram-negative bacteria (*Salmonella enteritidis* and *Escherichia coli*),but it had high impact against Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) (Hosseini et al., 2015).

Oregano and lavender essential oils were also incorporated into antimicrobial biogenic gelatin-based films which were used as antioxidant and antimicrobial additives (Martucci et al., 2015) in order to establish the antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus*. Results suggested that the addition of OEO and LE to gelatin films could inhibit the growth of the tested microorganisms. The results indicated an antagonist antimicrobial effect between OEO and LE. Finally, *Oregano*-based films exhibited the most effective antimicrobial and antioxidant properties (Nikoo et al., 2011).

Benavides et al. (2012) evaluated the effect of the degree of cross-linking and *Oregano* essential oil concentration on antibacterial activity and properties of alginate films. The obtained films shown that, increasing of the degree of cross-linking, cause reduce the water vapor permeability when the concentration of the cross-linking rose. Films incorporated with OEO have affected gram positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) more than gram negative bacteria (*Escherichia coli* and *Salmonella Enteritidis*).

Another useful hydrocolloid which was proposed by Pola et al. (2016) is cellulose acetate in different concentrations of OEO and organophilic montmorillonite clay to control the growth of phytopathogenic fungi. The results indicated that cellulose acetate films showed high antifungal activities against *Alternaria alternata*, *Geotrichum Candidum*, and *Rhizopus Stolonifer*. OEO increased extensibility and decreased WVTR (water vapor transmission rate) of *Cellulose acetate* based films. Similar results were observed regarding the effect of OEO and organophilic montmorillonite on the films thermal resistance.

Ziziphora essential oil in edible starch-chitosan composite films had antimicrobial properties due to the presence of monoterpenes phenols, specially thymol and carvacrol in the essential oil. The results showed that, by increasing the concentration of essential oil, antimicrobial and antioxidant activities have improved as well. Also, the addition of *Thymus kotschyanus* essential oil has changed the color and the transparency of the films (Mehdizadeh et al., 2012).

The properties of edible films based on *chitosan* with *Thymus moroderi* and *Thymus piperella* essential oils were studied by (Ruiz-Navajas et al., 2013), the aim of this research was to determine the antioxidant properties and their effects on the growth inhibition of *aeromonas hydrophila* and *achromobacter denitrificans*; finally the films containing chitosan and *Thymus piperella* essential oils showed more antioxidant activities. Moreover, the *chitosan* films containing *T. piperella* essential oils were more effective against the growth tested strains than chitosan films containing *T. moroderi* essential oils.

Another new antimicrobial films were prepared by incorporating different levels of oregano and *Thyme* essential oils (1, 2, 3, 4 and 5%) into soy protein edible films on fresh ground beef patties (Ruiz-Navajas et al., 2013). Similar results were observed regarding the effect of soy protein edible films with 5% oregano and *Thyme* or a mixture of *Oregano* + *Thyme*. The results showed that films containing *Thyme* and OEO had the highest inhibitory effect against *Pseudomonas aeruginosa* and *Lactobacillus plantarum.*

Dashipour et al. (2015) prepared *Carboxymethyl cellulose* films with 1, 2, and 3% v/v of *Zataria multiflora boiss* essential oil to assess physical properties, and antioxidant and antimicrobial effectiveness against strains of *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* PTCC 1154, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhimurium*. The researchers have found that when the concentration of *Zataria multiflora boiss* essential oil in CMC films was 3% v/v, a higher impact against all the tested strains was observed and also the antioxidant activity improved at higher concentrations of ZEO. Results also showed that the addition of 1% v/v ZEO could increase the mechanical properties (tensile strength, elongation at break) of the films. Films containing ZEO are less transparent compared to films with no EO. While WVP increased, water solubility decreased significantly with incorporation of ZEO.

Antioxidant and antimicrobial activities of edible films based on zein with 2 or 3% *Zataria multiflora boiss* essential oil (ZEO) and 1% monolaurin (ML) were studied by Moradi (Moradi et al., 2016), with the aim of determining antimicrobial effects against *Listeria monocytogenes* and *E. coli* O157: H7 *in vitro* and in minced beef meat. The results indicated that the application of films containing essential oils and ML, could significantly reduce *L. monocytogenes* levels in minced meat during 3 days of storage at 4°C. The antimicrobial films with ZEO and ML were more effective against the growth of *L. monocytogenes*. The growth of E. coli was limited by ZEO, while many studies have proven the antioxidant effects of edible film containing ZEO films. In following of the article we have assessed several properties of essential oils in Table 3. So according to the above advantages, the main objective of this article is to evaluate the antimicrobial activity of plant extracts and essential oils in edible films

The antimicrobial and antioxidant properties of starch edible films containing ginger essential oil have also been studied. They confirmed that the best antibacterial activity of starch edible film was at a concentration of 3% ginger oil; the results also showed that *Ginger's* EO had the most efficient antimicrobial activity against strains of *E. coli* (Miksusanti et al., 2013).

Antimicrobial activity of linear low-density polyethylene and low-density polyethylene films contain *Linalool* and *Methyl chavicol*, which stored for one year at ambient temperature against

E. coli, have evaluated. After one year, the amount of additives had decreased, but no difference was observed in antimicrobial activity against *E. coli*. Wrapper are used in food packaging to improve quality, safety and the food shelf life (Burt, 2004).

Gniewosz et al. (2014) studied the effect of basil sweet extract as a natural antimicrobial and antioxidant substance incorporated into a *Pullulan*-based film in order to lengthen the shelf life of apples during refrigeration. Results suggested that *Pullulan* coatings with 24 mg/cm^2 SBE could improve the shelf life of "*Jonagored*" apples. In general, *Pullulan* films with SBE demonstrated low protection against mesophilic bacteria, but good antifungal properties against *R. arrhizus*.

It is reported that the addition of cloves oil to hydroxypropyl methylcellulose films due to phase separation different wrapper and heterogeneity, has a good inhibitory effect on the growth of *E. coli* O175:H7, *S. aureus* and *L. monocytogenes*. The results also showed that films with 1.0, 1.5 and 0.5% concentrations of *clove* oil have inhibited the growth of *E. coli* about 10^5 cfu/mL *E.coli O175:H7*, *S. aureus* and *L.monocytogenes* (Nonsee et al., 2011). In sunflower protein films, the incorporation of the *Clove* essential oil could diminish lipid autoxidation and reduce the growth of total mesophilic microorganisms in cooled sardine patties(Salgado et al., 2013). In another study, Gomez-Estaca et al. (2009) indicated, *clove* essential oil incorporated to fish *Gelatin* edible films, showed the most effective than fish *gelatin /chitosan* edible films with *clove* essential oil on target microorganisms. This could be due to the fact that *gelatin* and *chitosan* are reactive molecules creating ionic and hydrogen bonding which reduces the solubility of films, therefore, the release of the essential oil could reduce antimicrobial activity.

Relatively, Mulla et al. (2017) evaluated the antimicrobial activities of LLDPE films of which the surface had been treated by chromic acid (CA) and clove essential oil, in order to create a great active packaging. The results demonstrated that the antimicrobial activity of *LLDPE*/CA/CLO films against *Salmonella Typhimurium* and *Listeria monocytogenes* in a packed chicken sample for 21 days of refrigerated storage.

Blends of biopolymers could also be used in film formulations. Gomez-Estaca et al. (2010) blended gelatin and chitosan in essential oils such as clove, fennel, cypress, lavender, thyme, herb of-the-cross, pine and rosemary. It was observed that the antibacterial activities were as follows: clove > rosemary > lavender. The antimicrobial activity of clove essential oil has maintained, when it was incorporated into bovine-hide gelatin/chitosan edible films, with no effects on the films matrices. Nevertheless, some differences were observed in water solubility which could determine the release of antimicrobial compounds into food and could affect the antimicrobial activity of the films. gelatin/chitosan/clove films are less soluble compared to gelatin/clove films, then these essential oil could release slowly and keep suitable concentration over a longer period of time. During fish storage, the clove film could delay or prevent the growth of microorganisms and the occurrence of total volatile nitrogen. Therefore, films incorporated with clove essential oil could assure an extended shelf-life for chill-stored fish. Table 3 shows several antioxidant properties of essential oils in edible film.

7. Conclusion

The antimicrobial activity of edible films could be considered as an important factor in maintaining the compounds, approving the quality and increasing the shelf-life of food products without affecting consumer acceptability. Some of the important reasons that led to the formation of antimicrobial films include: stability, low and limited concentrations of products, bioavailability and biocompatibility, and gradual release of antimicrobial compounds. Food safety and quality are affected by chemical interactions between biopolymer packaging materials and foods. There is evidence that natural additives such as plant extracts could change the structure and morphology of edible films, which has a close relationship with the incorporation of plant extracts and physicochemical modifications of edible films. The addition of antimicrobial compounds into biopolymer-based edible films could improve the mechanical, water barrier, and antimicrobial properties. Most foods are perishable and are susceptible to microbial contamination. The use of antimicrobial films has shown to preserve quality and increase the shelf life of various food products.

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