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Functional Role of Essential Oils as Antimicrobial and Antioxidat Agents in Food Industry: A Review

***Doaa Al-Refaie* [1], *Ghadeer F. Mehyar* [1*] and *Mohammad Shahein*[1]**

[1]Department of Nutrition and Food Technology, Faculty of Agriculture, The University of Jordan, Amman, Jordan

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ABSTRACT

Essential oils (EOs) possess both antimicrobial and antioxidant activities in food systems. Variations in EOs effectiveness were dictated by their components, effective concentrations, intrinsic factors of food composition as well as extrinsic factors such as storage temperature. The antimicrobial and antioxidant activities of EOs are a result of the presence of phenolic components at high concentrations. EOs could have better effectiveness than single component because these constitutes could act additively or even synergistically in EOs. EOs have antimicrobial activity against wide range of microorganisms and their mode of action is related to disintegration of cellular membrane integrity followed by inactivation of other microbial cells components. The antioxidant mode of action for EOs is related to neutralization free radicals and peroxide decomposition in particularly when tested in meat, dairy, fruits and vegetables. The high effectiveness of EOs indicates that they could replace the synthetic food additives. This scientific review summarizes the most recent studies about effectiveness of EOs as antimicrobial and antioxidant agents to be used in food industry.

Keywords: Essential Oils, Antioxidants, Antimicrobial agents, Phenolic Compounds, food industry.

INTRODUCTION

Advanced food processing and preservation technologies lead to large improvements in the quality and safety of produced foods. However, controlling foodborne illness, and consumers' demand for minimally processed, natural and organic foods are increasing (Kozelová et al., 2011). In spite of such high demand, there is still employment of synthetic additives in food industry such as nitrite, sorbate and benzoate. This could be related to the lack of knowledge about benefits and usability of the natural additives (Nazir et al., 2017). The emerging of antibiotic and stress-resistant bacteria species within food systems made the problem more complex and prove the importance of enhancing food spoilage and illness control methods (Bhargava et al., 2011; Pisoschi et al., 2018). These conditions intensity the need for evolving of novel preservation methods for replacing the present techniques.

Functional food ingredients are defined as constituents that have to impact health in addition to its fundamental nutritional value. They can be obtained from different natural sources such as primary, novel sources of plants, animals microorganisms as well as inorganic raw materials (Ribeiro et al., 2021; Nielsen et al., 2021).

* Corresponding author.E-mail: g.mehyar@ju.edu.jo

Among these ingredients are EOs which are extracted from plants, herbs and spices and proved to have antimicrobial, antioxidant activities or other functional properties in addition to provide pleasurable flavor to different foods (Cherif et al., 2019). Additionally, EOs are defined as ethereal or volatile aromatic oily liquids acquired from different plant parts (roots, flowers, buds, leaves, twigs, seeds, fruits, bark, and wood) and have fundamental functional role (Golmohammadi et al., 2018).

Herbs and spices constitute EOs and have long been used as flavor enhancers in addition to their antimicrobial and medical uses. EOs and their constituents have been known to have other properties including insecticidal, antiviral, antimitotic, antioxidant and antiparasitic properties (Molapour et al., 2020; Liang et al., 2020; Khan et al., 2019; Kokoska et al., 2019; Sari et al., 2018). Antioxidant property is a common characteristic in different EOs such as those extracted from cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme (Herman et al., 2019; Bhavaniramya et al., 2019). This review focuses on using EOs as functional food ingredients, their mechanism of action as antimicrobial and antioxidant agents and the applications in different food industries.

Functional Components of EOs

Plants produce different types of functional EOs which are either existing as plant constituent or produced as a result of plant injury or invasion by insects or plant pathogens. It has been reported that the structure and composition of the produced EOs in a certain plant vary by the harvesting season, geographical area and plant part (Kayode et al., 2018; Paibon et al., 2011; Demuner et al., 2011). Ben-Hsouna et al., (2017) reported that there are twenty-one different functional components identified in the EO of Citrus Limon, whereas more than 60 components were identified in litsea, oregano, marjoram, thymus EOs (Ebani et al., 2016). Eighty-four compounds were identified in Nigella sativa EO. P-cymene, limonene and a-pinene, were the major compounds isolated and showed powerful bactericidal activity (Harzallah et al., 2011). Chemically, EOs are made up of more than a hundred hydrocarbon compounds and classified into esters, alcohols, terpenes, phenols, ethers, aldehydes, esters, lactones, coumarins, oxides and ketones (Vergis et al., 2015; Rashid el al., 2013). However, these compounds are further categorized into groups, according to their chemical structures, to terpenes, terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropens and "others" (Hyldgaard et al., 2012). These groups are derived from various precursors of the plant's primary metabolism and are produced over separate metabolic pathways such as mevalonic acid pathway (Başer and Demirci, 2007).

Some components could present in one or several EOs, for example, cinnamon oil contains three substantial constituents including eugenol, linalool and trans-cinnamaldehyde. Linalool composed of about 82 % of the total cinnamon EO whereas eugenol was also isolated as one of the main components from clove and basil EOs (Bhavaniramya et al., 2019; Marchese et al., 2017). The chemical structures of some EOs' components are shown in Figure 1.

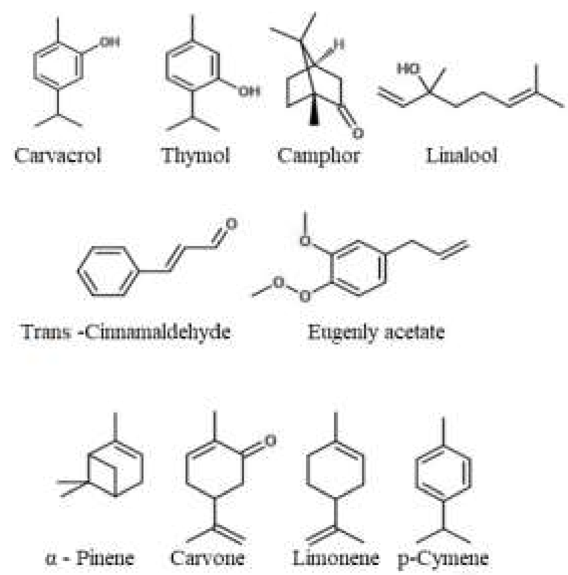

Figure 1: Chemical structures of selected functional components isolated from EOs (source: Tahlan, 2014).

The structure of each EO has a significant impact on its properties. It has been reported that the presence of the aromatic or benzene ring and the side chains determine the extent of antimicrobial activity of these components (Figure 1). For instance, components that possess a higher content of phenolic compounds like thymol, euganol, and carvacrol showed higher antimicrobial and antioxidant activities than other compounds (Tahlan, 2014; Said et al., 2016). It was reported that their mechanism against microbial growth is comparable to those of hydroxyl group, though the position of this hydroxyl group does not have an effect on the level of antimicrobial activity. For the case of non-phenolic compounds, the existence and type of alkyl group affects their antimicrobial properties (Juliano et al., 2000; Ultee et al., 2002).

Terpenes and Terpinoids

Terpenes are hydrocarbons produced by joining several isoprene units (C5H8) and they constitute most diverse class of secondary metabolites and up-to-date over 55,000 members were isolated. Their major group comprises of monoterpens, diterpenes, triterpenes, tetraterpenes as well as hemiterpenes and sesquiterpenes. (Jafri et al., 2019). The monoterpenes compounds are widely used in different industries including fragrances and flavor in cosmetics, drug, perfumes and food (Toauibia, 2015). They are composed by merging two basic-isoprene units and represent the primary components of EOs (Chouhan et al., 2017). Synthesis and production of terpens begun through an acetyl–CoA and execute through the pathway of mevalonic acid (Hyldgaard et al., 2012). These compounds can be arranged into cyclic structures via the action of cyclases. There are various examples of common terpenes including thymol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, geraniol limonene, p-cymene, pinene and terpinene (Stephane et al., 2020).

Enzymatic biochemical modifications of terpenes lead to add oxygen molecules and move or remove methyl groups resulted in the formation of terpenoids (Chouhan et al., 2017). Terpinoids are divided into phenols, aldehydes, alcohols, ketones, esters and epoides (Chouhan et al., 2017). Sesquiterpenoid compounds are formed by the condensation of three isoprene unit and they are found in various forms such as linear, monocyclic, bicyclic, and tricyclic structures. Generally, sesquiterpenoids are very toxic, but some are used as antimicrobials, antifungals, insecticides and antibiotics (Stephane et al., 2020).

It was reported that terpens (Monoterpene hydrocarbons, Oxygenated monoterpenes) are the major constituents (> 85%) of four different EOs (Litsea, oregano, marjoram, thymus), followed by Sesquiterpenes hydrocarbons and Oxygenated sesquiterpenes (< 5%), whereas it was found in another study that sesquiterpenes and oxygenated sesquiterpenes compounds are the predominant components (61.2%) in EO of Eucalyptus globulus fruit (Ebani et al., 2016; Said et al., 2016). However, variations in composition of terpenes in EOs is related to plant species and other environmental factors (Stephane et al., 2020).

Phenylpropenes

In plants, phenylpropenes are aromatic compounds that are synthesized from the amino acid precursor phenylalanine and tyrosine. They collected together in a family of various groups of organic compounds called phenylpropanoids (Anupama et al., 2019; Jafri et al., 2019). Small amount of phenylpropenes' EO have been deeply elucidated and studied. Phenylpropenes that have been studied is eugenol, isoeuganol, vanillin, safrole, cinnamaldehyde, and buteuganol (Jafri et al., 2019). Although phenylpropenes constitute a relatively small part of the essential oils, they play a vital role in flavor industry such as eugenol is responsible for the aroma of cloves (Vergis et al., 2015). The aromatic ring of phenylpropens may contain hydroxy, methoxy and methylene dioxy groups; while the propyl side chain may carry hydroxyl or carboxyl group (Martínez-Ávila et al., 2021).

Methods of Extraction and Isolation EOs

EOs are originally extracted from various plant components such as leaves, fruit, bark, root, balsam, flowers, seeds, fruits and buds (Kayode et al., 2018). Organic solvents such as ethanol, methanol, n-hexane, petroleum ether, toluene, chloroform and water, as well as other solvents are usually used for extraction (Filly et al., 2016; Martínez-Ávila et al., 2021). Steam distillation is the most common commercial method employed for EOs separation while expression and enfleurage are used for extracting essential oils from the most sensitive flowers by using pressing and partitioning in fat phase, respectively (Vergis el a., 2015). Other extraction methods employed are solvent extraction, freeze drying, hydrolyzation, rotary evaporation, florasols extraction, super critical CO2-extraction, hydrodistillation and gas chromatography (Herman et al., 2019; Conde-Hernández et al., 2016). Figure 2 summarize the most common extraction methods of EOs.

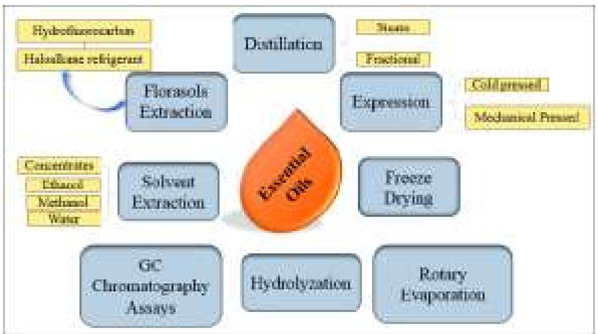

Figure 2: Most common methods for extracting EOs (source: Herman et al., 2019).

Steam distillation is a process of extracting compounds that are not broken down and decomposed at elevated temperatures. This method is based on distilling these compounds within a stream introduced inside the fresh plant materials. The steam loaded with volatile compounds is then separated and distillated to produce a mixture of hydrophobic compounds. The ratio of the extracted EOs by steam distillation is up to 93 %, while the residual EOs (7 %) can be further extracted by other methods (Herman et al., 2019). Essential oil of lavender was extracted by steam distillation and found to contain different intact compounds including lavandulol, linalool, linalyl acetate, 8-cineol, lavandulyl acetate, β-ocimene, terpinen, fenchone, viridiflorol and camphor. Among these compounds, linalool was the most active antimicrobial compound (Zuzarte et al., 2012).

Florasols extraction is a solvent based process but utilizes a new type of benign gaseous solvents. This method is used for the extraction of aromatic oils and biologically active components from botanical materials. The extraction process is based on selectivity of the solvent to volatile plant compounds and produces a clear oil free of waxes. There is no thermal degradation of the products in this method since it occurs at or below ambient temperatures (Patel et al., 2011). Expression method, also known as cold pressing, refers to any physical process whereas the glands of EO in the peel are crushed or broken by physical stresses to release the oil. It is used only in the citrus oils production (Handa, 2008). Freeze drying method is used to produce high nutritional and valuable products. The absence of liquid water in this method (since water is converted into small ice crystals before being separated by sublimation) and its low temperature resulted in deactivation most of the microbiological reactions and gives a final product of excellent quality (Antal et al., 2014). On the other hand, gas chromatography had a high effectiveness in extracting EOs from edible plants leaves in term of purity of the extracted fractions comparing to other methods (Constanza, et al., 2015).

Hydrolyzation is a method of extraction that encompasses the immersion of plant materials completely in water, followed by boiling. The advantage of this method is preserving the content of extracted oils to a specific limit since the surrounding water prevent the extracted oils from overheating because it acts as a heat barrier (Tongnuanchan and Benjakul, 2014). Furthermore, this method of extraction provides the least impact on the extracted oil properties. For example, it was found that solvent extraction and hydrolyzation methods produced oils with better organoleptic properties than that produced by steam distillation. That was related to differences in EOs composition produced by the different extraction methods. Previous studies showed that to ensure and maintain the EOs organoleptic properties to a higher degree, low pressure with liquid carbon dioxide extraction methods could be used (Conde-Hernández et al., 2016; Patel et al., 2011). In a comparative study it was found that EO of rosemary can be extracted by supercritical CO2-extraction, hydrodistillation, and steam distillation. The highest yield and antioxidant activity was obtained with the CO2-supercritical extraction followed by steam distillation, and finally by hydrodistillation. The antioxidant activity of oil obtained by supercritical extraction was approximately 14 times more than that obtained in oils of hydrodistillation or steam distillation, whereas the number of compounds identified were higher in stream distillation than other methods (Conde-Hernández et al., 2016). EOs of herbs extracted by hexane shown to have higher antimicrobial activity than similar extractions by steam distillation (Paibon, 2011). Indeed, the cost of extraction is to be considered, it is reported that all extraction methods, except steam distillation, are very expensive, therefore steam distillation method is the most commonly used at the commercial scale (Filly, 2016).

Antimicrobial properties of EOs

Effective components and microbial spectrum

Food industry primarily uses EOs as flavoring agent, as well as a good source of natural antimicrobial compounds. EOs are natural compounds that are extremely complex and composed of mixtures of functional ingredients; not less than 50 individual aromatic compounds (Reyes-Jurado et al., 2015; Ameh et al., (2016). Choe et al., (2011) investigated the inhibitory effects of 45 EOs on eight bacteria (four Gram positive and four Gram negative), two fungi, and one yeast. All oils exhibited inhibition over activity relative to controls. Gram negative bacteria were generally more resistant than Gram positive bacteria to oil treatment. *Pseudomonas aeruginosa* was the most resistant bacteria. Oils that exhibited high antimicrobial properties against bacteria and fungi were cinnamon bark *(Cinnamomum zeylanicum)*, lemon grass *(Cymbopogon flexuosus)*, savory *(Satureja montana)*, Roman chamomile *(Cbamaemelum nobile)*, rose wood *(Aniba rosaeodora)*, spearmint *(Mentha spicata)* and tea tree *(Melaleuca alternifolia)*. It was also reported that some examples of EOs that proved to have effective functional properties are clove, lemon, tea tree, cinnamon , oregano, thyme, mustard, peppermint, lavender, and eucalyptus (Bhavaniramya et al., 2019). EOs have an important role in the preservation of food by prevention growth and biofilm formation of pathogenic and spoilage microorganisms (Jafri et al., 2019). Lavender EOs revealed an antibacterial activity against a wide range of microorganisms including antibiotic resistant bacteria, yeasts, *Cryptococcus neoformans*, *Aspergillus* strains and *Candida* species (Said et al., 2015; Posadzki et al., 2013). EOs extracted from cloves showed an antibacterial activity especially on the multi resistant *Staphylococcus* spp. (Bhavaniramya et al., 2019). Several studies reported that peppermint EOs showed significant antimicrobial activities especially against Staphylococci, and it also exhibited an antifungal activity (Saharkhiz et al., 2012). Furthermore, it was reported that it is hard to predict the resistance of a certain microorganism to a specific EO, since this is determined by environmental factors such as temperature, available nutrients, medium water activity, dissolved oxygen, pH and atmospheric relative humidity and intrinsic factor of food composition (Nazzaro et al., 2013).

The antimicrobial properties of terpenoinds are related to the functional groups exist in the compound. In the case of phenolic terpenoids, it has been found that the presence of a hydroxyl group and delocalized electrons are substantial for their antimicrobial activity (Hyldgaard *et al*., 2012). In the other hand, the antimicrobial activity phenylpropenes depends on the target strains and species of microorganism, the number and kind of substituent on the aromatic ring, and other factors such as temperature and medium selected for microorganism growth (Pauli et al., 2010). It was also reported that the antimicrobial activities of EOs are highly related to the existence of monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives (Ben-Hsouna et al., 2017). Effective EOs components including terpens, terpenoid, ketones and fatty acid esters such as carvacrol, eugenol, cinnamaldehyde, citral, thymol, belongs to the phenolic compounds (Bhavaniramya et al., 2019; Kayode et al., 2018). Generally, terpenes showed varied antimicrobial activity, from effective antimicrobial compounds to weak or no antimicrobial activity such as limonene, α-pinene, β-pinene and α-terpinene (Allenspach et al., 2020). Carvacrol possess the highest activity with less toxicity; for this reason, it is widely used in food industry like drinks and sweets as both preservative and flavoring agent. Ketones components of EOs displays weaker antimicrobial activity than carvacrol whereas the linear hydrocarbons have the least activity therefore rarely used in the food industry (Badawy et al., 2019). Eugenol is an oily liquid that is clear to pale yellow, it is mainly extracted from nutmeg, clove oil, basil, bay leaves, and cinnamon. Several studies have reported the high antimicrobial effectiveness of eugenol as well as its antifungal activity against *Alternaria alternata* and *Curvularia lunata* (Chouhan et al., 2017; Heer et al., 2017). Clove oil is mainly extracted from clove buds and it contains various compounds such as the phenyl propanoids, eugenyl acetate, eugenol, thymol, carvacrol, and cinnamaldehyde as major compounds. From these compounds, eugenol revealed strongest antimicrobial properties due to its activity against synthesis of ergosterol, a specific fungal cell-wall component that acts to maintain cell membrane integrity. Eugenol also can prevent formation of germ tube during spore germination in certain type of yeast like *Candida albicans* (Végh et al., 2012; Chaieb et al., 2007). Moreover, the major compound of *Eucalyptus* plant (contains more than seven hundred botanical species) oil is cineol in addition to other compounds including α-terpineol, α-pinene, cryptone, p-cymene, phellandral, transpinocarveol, cuminal, globulol, aromadendrene, limonene. *Eucalyptus* oil showed a significant effect against food spoilage and pathogenic microorganism and also it is mainly used as flavoring agent (Tyagi and Malik, 2011). Cinnamaldehyde is an organic compound that have been used as flavor- and odor-giving compound. It is a pale yellow viscous liquid produced naturally in the bark, leaf, and root of *Cinnamomum zeylanicum* cinnamon trees. Studies revealed that the cinnamon EOs have antibacterial effect against various pathogens including both Gram negative bacteria such as *E. coli* O157:H7 and *Salmonella typhimurium* and Gram positive bacteria besides its potential inhibitory effects against fungi, and it has antioxidant, antiparasitic and free radical scavenging properties (Pereira et al., 2014). It has been also that cinnamaldehyde possess a strong antimicrobial activity against *Escherichia coli* and *Salmonella typhimurium* (Vergis et al., 2015).

Mode of action

Different compounds of EOs act together additively or synergistically, therefore EOs could have better activity than single component. The antimicrobial properties of EOs cannot be referred to a single mode of action but most of the times to multiple mechanisms of action against microorganisms. However, the exact mechanism of action of EOs in food safety/quality is still not clearly understand and needs extensive investigations. Figure 3 summarizes different mechanisms of EOs against microorganisms. These mechanisms include cell wall destruction, membrane protein damage, coagulation of cytoplasm, damaging cytoplasmic membrane and other mechanisms.

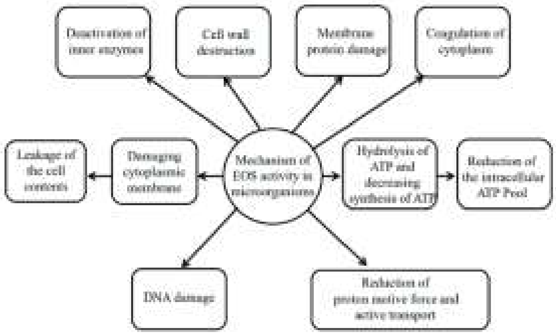

Figure 3: Mechanisms of EOs in microorganisms (modified from Bhavaniramya et al., 2019).

Table 1 presents some types of essential oil and their antimicrobial mechanisms of action against different bacterial strains. The listed EOs are effective against both Gram positive and Gram negative bacteria, although there is some specificity of the antimicrobial action to the type of microorganism. The cytoplasmic membrane appears to be the first target of the most EOs. EOs are hydrophobic compounds which are similar to microbial cellular membranes, therefore EOs quickly transmitted through the microbial cell and mitochondrion membranes causing damages in the structure and increased permeability which led to drain and leakage of ions and other cellular materials such as proteins, nucleic acids and ATP (Churklam et al., 2020; Kang et al., 2019). It also dissipates the ionic gradient across the membrane inducing depolarization of membranes' fatty acids. This effect causes disrupting in Proton Motive Force (PMF), electron flow, active transport and coagulation of cell contents (Vergis et al., 2015). Once entering the bacterial cells, EOs also deactivate the inner enzymes in particularly histidine decarboxylase causes DNA damage and ATP depletion (table 1). These changes cause instant death of the bacterial cells. Regarding the effect of EOs' concentration, it is known that Gram positive bacteria are more susceptible than Gram negative bacteria to the EOs. This could be associated with the presence of lipopolysaccharides outer membrane in the Gram negative bacteria than Gram positive bacteria, which acts as an extra barrier against macromolecules and hydrophobic compounds of EOs. This provides the Gram-negative bacteria a relatively higher tolerance thus needs higher concentrations of EOs than the Gram positive bacteria for effective inhibition (Vergis et al., 2015; Ben-Hsouna et al., 2017).

Table1: Some essential oils and their mechanisms of action as antimicrobial compounds against selected bacterial strains.

EO	Effective components	Bacteria strains	Mechanism of action	Reference
Cinnamon	Eugenol Cinnamaldehyde Linalool	*E. coli* *E. coli* O157:H7 *L. monocytogens* *S. aureus* *S. typhimurium* *C. jejuni* *Candida* spp.	- Disruption of the cell wall/membrane - Production of ROS - DNA damage - Inhibition of enzymatic activity - Exhausting intracellular ATP. - Inhibit H^+-ATPase activity - Induction of oxidative stress response	Marchese et al., 2017 Pereira et al., 2014 Vergis et al., 2015
Clove	Eugenol Thymol, Carvacrol, Cinnamaldehyde	*C. jejuni* *E. coli* *L. monocytogens* *S. aureus*	- Disruption of the cell wall/membrane - Production of ROS - DNA damage	Marchese et al., 2017 Végh et al., 2012 Chaieb et al., 2007

		S. enteritidis *Candida albicans*	- Inhibition of enzymatic activity - Disrupt cell wall synthesis in fungi - Prevent formation of germ tube in yeast.	
Lemon Grass	Neral Geranial	*M. luteus* *B. cereus* *E. coli* *L. innocua* *L. monocytogens* *S. aureus*	- Destabilization of cell wall and desfunctioning	Chao & Young, 2000 Herman et al., 2019
Oregano	Carvacrol P-Cymene	*Y. enterocolitica* *L. monocytogenes* *E. durans* *E. faecium* *E. faecalis* *Candida* species *S. cerevisiae*	- Dissipation of potassium gradient - Depolarization of membrane - Coagulation of cytoplasmic - Lesion of the cell membrane - Reduction of ergosterol	Ebani et al., 2016
Rosemary	Camphor Eucalyptol	*Cl. perfringens*	- Increase in membrane rigidity - Effect on lipid polymorphism	Herman et al., 2019 Conde-Hernández et al., 2016
Thyme	Thymol p-cymene Linalool	*S. enteritidis* *S.typhimurium* *E. coli* *S. aureus* *B. cereus*	- Disintegrate the outer membrane, - Releasing lipopolysaccharides - Increase membrane permeability to ATP	Boskovic et al., 2015 Van Haute et al., 2016
Lavender	Linalool Linalyl acetate	*L. innocua* *P. fluorescens* *Cryptococcus neoformans,* *Aspergillus strains* *Candida species*	- Disrupt bacterial membranes - Inhibit germ tube formation - Inhibit conidial development	Said et al., 2015 Posadzki et al., 2013 Marín et al., 2016 Erland and Mahmoud, 2016
Peppermi nt	Menthol	*Staphylococcus aureus*	- Disrupt cytoplasmic membranes permeability - Leakage of electrolytes	Saharkhiz et al., 2012 Kang et al., 2019
Tea tree	Carvone Terpinen-4-01 r-terpinene	Biofilms of *E. coli O157:H7* *L. monocytogenes* *Salmonella* spp.	- Damage in the outer membranes	Nazzaro et al., 2013 Sadekuzzaman et al., 2017

Studies have shown that each EO component have a particular mode of action although similarities could be found. Cinnamon oil contains three substantial constituents including eugenol, linalool and trans-cinnamaldehyde. Cinnamaldehyde proved to have the antimicrobial activity against E. coli and S. typhimirium by exhausting the levels of intracellular ATP and can also easily access the periplasm and deeper parts of the bacterial cell causing their disfunctioning (Vergis et al., 2015).

Eugenol is one of the main components of clove, basil, and cinnamon species. Although it inhibits G-ve bacteria by disrupting cell membrane, It has different mechanism in G+ve bacteria in alterations of cell morphology and disruption of cell wall or through production of intracellular reactive oxygen species (ROS) which induces the inhibition of the growth of cell, and damage DNA resulting in cell decomposition and death. Another mechanism is through destructive effect against protease, histidine carboxylase, amylase, and ATPase (Marchese et al., 2017). Eugenol also inhibits production of amylase and protease in Bacillus cereus and causes deterioration of the cell wall of Enterobacter aerogenes, and may cause lysis of cells, and prevention of enzyme action (Vergis et al., 2015). Thymol and carvacrol disintegrate outer membrane of the Gram negative bacteria releasing lipopolysaccharide and increasing permeability of the cytoplasmic membrane to ATP (Vergis et al., 2015). Additionally, p-cymene has a strong affinity for bacterial cell membranes that resulted in membrane expansion, though it does not affect its permeability. It has also an effect on membrane potential and can affect the motility of E. coli cell (Marchese et al., 2017). Moreover, carvone is an important constituent of oregano and tea tree EOs, it causes damage in the outer membranes of the bacterial cells without affecting the cellular ATP pools (Nazzaro et al., 2013).

In addition to antibacterial effect, EOs possess antifungal activity, for example, EO of lemon especially terpenes and the oxygenated terpenes compounds, exhibited a significant antifungal activity against Candida species such as Candida guilliermondii, Candida krusei, Candida parapsilosis, Candida tropicalis Candida albicans and Saccharomyces cerevisiae (Debonne el al., 2018; Ebani et al., 2016). They concluded that EOs can cause extensive lesions of the cell membrane and a reduction of ergosterol, which is a major component present in fungal cell membranes. It was reported that eugenol have the ability to inhibit H+-ATPase activity of Candida spp. or to induce oxidative stress response in C. albicans which stimulate lipid peroxidation of cytoplasmic membranes and cell death (Marchese et al., 2017). The major compounds exist in tea tree EOs are of terpinen-4-ol, γ- terpinene, p-cymene, α-terpinene, 1,8-cineol, α-terpineol, and α-pinene and they have a strong antifungal activity (Benabdelkader et al., 2011).

Antioxidant Properties of EOs

The antioxidant properties of EOs is primarily relying on their chemical structure and compositions. It is measured by free radical scavenging property or so called antioxidant capacity. This method is based on measuring the capacity of the essential oil to scavenge the stable free radical DPPH by donation of hydrogen atom or an electron. Monoterpenes and sesquiterpenes hydrocarbons components including 1,8-cineol, α-pinene, β-pinene, terpinolene, α- and γ-terpinene of EO may act as radical scavenging agents (Ben-Hsouna et al., 2017). In another study it was found that sesquiterpenes and oxygenated sesquiterpenes as predominant compounds of Eucalyptus globulus fruit EO have weak antioxidant activities (Said, 2016). The differences in these results could be related to experimental conditions such as extraction method, medium used, applied temperature and oxygen availability (Lou, 2017). However, the antioxidant effectiveness of EOs is mostly influenced by variety of factors, including their structural properties, , type of oxidation substrate and physical state of the system in addition to the presence of pro-oxidants and synergists (Shahidi and Zhong, 2015).. Efficiency of antioxidants also depends on their concentration and location within the system. The reaction kinetics plays a crucial role in their short-term or long-term protection against oxidation, and involves the speed at which an antioxidant reacts with a selected oxidant, the thermodynamics of the reaction and therefore the extent to which the antioxidant reacts (Zhong and Shahidi, 2015).

Structural families of EOs including terpenoids and phenylpropanoids comprise phenolic compounds and accounted as principal components for several EOs. Generally, phenols have a high antioxidant activity through reactivity with peroxyl radicals and facilitate radical-radical reaction (Amorati et al., 2013). Other terpenoids, non-phenolic EOs components can create alkyl radicals (from terpene hydrocarbon skeleton), which can react rapidly with molecular oxygen to produce peroxyl radicals, that propagates the oxidation chain. Therefore it is reported that non-phenolic terpenoids, particularly unsaturated ones, would autoxidize similarly to unsaturated lipids (Neuenschwander et al., 2010).

Oxygenated monoterpenes such as thymol or carvacrol are the prime chemical compounds, that have the strongest antioxidant activities (Herman et al., 2019). Additionally, it was reported that certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, citronellal, isomenthone, menthone, play a vital role in the antioxidant properties of EOs (Bhavaniramya et al., 2019). EOs extracted from cloves showed a strong antioxidant and free radical scavenging activity when compared to synthetic antioxidant such as butylated hydroxyl toluene (BHT) (Radünz et al., 2019). The oils extracted from the medicinal plants such as cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme show significant antioxidant activities due to the presence of major constituents such as thymol and carvacrol if they are used at effective concentration (Herman et al., 2019).

Essential oils can be employed as effective antioxidants in foods; however, employing natural chemicals can have certain drawbacks, such as a lower antioxidant impact than synthetic compounds and, in some cases, an unpleasant taste and odor when used at effective concentration (Hashemi et al., 2017). The 2.2-diphenyl-1-picrylhydrayl free radical (DPPH), ferric reducing-antioxidant power (FRAP), ß-carotene bleaching essay, ABTS radical scavenging activity, ß-carotene beaching essay and ABTS radical scavenging activity are the most commonly utilized methods for in vitro studies of determining EOs' antioxidant activity (Ahmed, et al., 2019).

Application of EOs in food industry

EOs and their relative compounds are generally recognized as safe (GRAS) for human consumption. Figure 4 illustrates the use and functions of EOs in different industries.

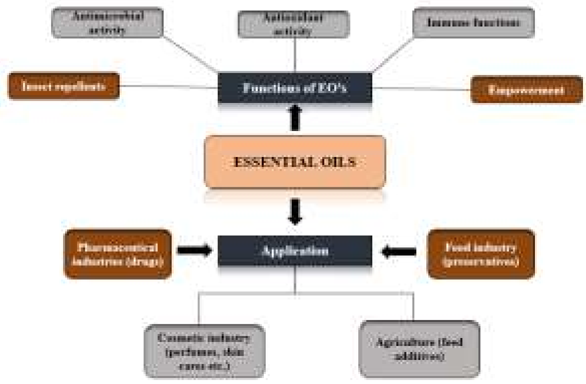

Figure 4: The applications of EOs in different industries (source: Herman et al., 2019).

It is obvious that EOs play a vital role as antimicrobial and antioxidant agents in different industries including food industry (Figure 4). Several studies have explained that EOs possess a strong antimicrobial and antioxidant activity, therefore they can be utilized by the food industry either directly as preservatives or in combination with the food packaging as secondary functional agents (Nazari et al., 2019). The most common EOs used in both food and cosmetic industries is cinnamon oils because of their high preservation effectiveness and being flavoring and aroma agent (Nollet and Rathore, 2017).

For meat applications, several oils proved effective functional agents. At concentrations of 5-20 ml g-1, eugenol, coriander, clove, oregano, and thyme oils were found effective in inhibiting L. monocytogenes, Aeromonas hydrophila, and other autochthonous spoilage flora in meat products, whereas mustard, cilantro, mint and sage oils were less effective or ineffective (Porter and Monu, 2019; Yousefi et al., 2020; Araujo 2019; Skandamis and Nychas, 2001).The efficacy of EO of oregano in a vacuum packed and pasteurized minced (ground) pork product against Clostridium botulinum spores was investigated. Oregano EO concentrations of up to 0.4 µl g-1 were shown to have no significant effect on the number of spores or the rate of growth. However, in the presence of small amount of sodium nitrite, oregano EO delayed the microbial growth. The delay in growth was inversely proportional to the amount of inoculated spores; means the microbial growth was largely reduced at 300 spores g-1 than at 3000 spores g-1 (Delgado-Pando, 2021).

The activity of EOs in meat products appears to be significantly reduced with increasing fat content in the food product (Ji, et al., 2021). For example, mint and cilantro EOs, were ineffective in products with a high fat content, such as pâté and in presence of ham coating made with canola oil (Burt, 2004). This could be related to partitioning of EOs to lipid phase in the product while contaminating microorganisms are usually located in aqueous phase. In another set of studies, it was shown that a high fat content in fish, appears to limit the efficiency of EOs antibacterial activity. For example, oregano oil at 0.5 µl g-1 is more effective on cod fillets than salmon, which is a fatty fish, against the spoilage organism Photobacterium phosphoreum. Even in fatty fish dishes, oregano oil was more effective than mint oil (Mejlholm and Dalgaard, 2002). The use of EO in a coating for shrimps or on the surface of whole fish appears to be efficient in preventing the respective natural spoiling microorganisms (Ozogul et al., 2020).

Meat products are susceptible to the oxidation process and had limited oxidation stability. Aroma, taste, color and product tissue are deteriorated as a result of the oxidation process (Xing et al., 2019). As a result, antioxidants were used to modulate and slow down these oxidation process. Patel (2015) found that plant EOs and allied volatile fractions could be used as multifunctional additives in meat and fish-based food products. Furthermore, EOs are highly sensitive to light, oxidation, and thermal decomposition. Due to probable interactions between the compounds of EOs and food ingredients, the antioxidant and antimicrobial effects of EOs in food systems are lower than that of in vitro which limited the application of EOs as antioxidant and antimicrobial agents in food preservation. As a solution, stabilizing EOs in edible films can help to protect the EOs and may lead to increase their biofunctional effects during the shelf life of food (Fasihi et al., 2019; Mehyar et al., 2018). Active edible coatings containing rosemary and oregano EOs as a natural antimicrobial and antioxidant agents could improve beef stability and organoleptic properties thus have possible application in the food industry (Vital et al., 2016). Encapsulated rosemary oil was found to be more effective than regular rosemary oil against L. monocytogenes in pork liver sausage and it was unclear whether the benefit was due to the encapsulation or the higher percentage level employed (Kaur et al., 2021). Encapsulation of clove essential oil in sodium alginate enhanced antimicrbioal activity against S. aureus and S. Typhimurium but lower antioxidant activity in ground meat products than uncapsulated EO (Radünz, et ai., 2019). Nano-emulsification of Citrus medica var. sarcodactylis EO significantly enhanced its antibacterial, antioxidant and antibiofilm efficiency in tofu than pure essential oil (Lou et al., 2017). Pickering emulsion involves stabilization of O/W or W/O emulsions by solid particles instead of surfactants for incorporation of CEO into the matrix of films. Besides stabilizing effect, Pickering emulsions as carriers to protect antimicrobial compounds from the outside environment effects, especially the oxidation. This method of stabilization enhanced the antioxidant and antimicrobial of CEO incorporated into active packaging and used successfully on sliced wheat bread (Fasihi et al., 2019).

EOs of garlic have been used as a natural food preservative. It was applied in yogurt as food model and revealed as a possible natural food preservative by effectively displaying suitable physicochemical properties (Manso, et al., 2014; Clemente, et al., 2016). In low fat yoghurt, mint oil at 5-20 µl g-1 is beneficial against S. enteritidis. Mint oil, at concentrations of 0.05-5 µl g-1, inhibits the growth of yoghurt starter culture species, but cinnamon, cardamom, and clove oils are far more potent (Mishra et al., 2020).

Vegetables are often low in fat, which may contribute to the positive effective results seen with EOs than other products. A decrease in storage temperature and/or a fall in the pH of the foods appears to boost the antibacterial action of EOs in vegetable dishes. At concentrations of

0.1-10 µl g-1 in washing water, all EOs and their components tested on vegetables appear to be effective against natural spoilage microorganisms and foodborne pathogens (Singh et al., 2002). When employed at 0.15 µl ml-1 in dipping solution, carvacrol and cinnamaldehyde were very successful at lowering the growth of the natural microorganisms on kiwi fruit, but less effective on melon. It is' probable that this effect resulted from pH difference between the fruits; the pH of kiwi fruit was 3.2-3.6, while the pH of the melon was 5.4-5.5 (Roller and Seedhar, 2002).

However, there are various challenges that limit the utilization of EOs in the food industry. Therefore, in vitro laboratory results could not be approved when utilized in large scale food industry. The pH of foods is not stable even within the same food category but determined by many factors such as harvesting stage, and microbial load. The hydrophobic nature of EOs is a limiting factor in terms of application in food industry, but this problem could be overcome by using of emulsifiers and stabilizing agents such as Tween-80, Tween-20 and lecithin (Mishra, 2020). It is believed that low oxygen concentrations in the food product lead to a few oxidative changes to the EOs . Furthermore, a reverse antimicrobial effect was also noticed when compounds are utilized and applied simultaneously in foods. This could be related to disfunctioning of EOs with food components (Shaaban, 2020). Overall, the antimicrobial activity of the essential oil in food systems depends on the interactions between essential oil and food components beside other factors of chemical structure, configuration of the components, and the amount and concentrations used.

Conclusion

Various EOs have antimicrobial and activity against spoilage/ pathogenic microorganisms due to their lipophilic action that make them easily invade the microbial cells. They have also antioxidant activity by their free-radicals scavenging activity. Their modes of action depend on EO's components and type of microorganism but still needs further investigations. The diverse efficacies of the various essential oils are due to the contrasted antimicrobial properties by each single constituent, that could have synergic interactions. Although the antimicrobial property of essential oils is often attributed to their major compounds, interactions between different major and minor constituents could play a vital role. Limited studies about application of EOs in food industry but they have a good potential for broader applications.

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الدور الوظيفي للزيوت العطرية كمضادات للميكروبات والأكسدة في صناعة الأغذية: مراجعة

دعاء الرفاعي[1] وغدير مهيار[1] ومحمد شاهين[1]

[1] قسم التغذية والتصنيع الغذائي، كلية الزراعة، الجامعة الأردنية.

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ملخص

تمتلك الزيوت العطرية نشاطية مضادة لكل من الميكروبات والأكسدة في الأنظمة الغذائية. الاختلافات في فعالية الزيوت العطرية يتم إملاؤها من خلال مكونات هذه الزيوت وتراكيز المواد الفعالة فيها، والعوامل الجوهرية لمكونات الغذاء فضلاً عن العوامل الخارجية مثل درجة حرارة التخزين. إن أنشطة مضادات الميكروبات والأكسدة للزيوت العطرية هي نتيجة لوجود مكونات الفينول فيها وبتركيز عالية. قد يكون للزيوت العطرية فعالية أفضل من أي من مكوناتها لوحدة لان هذه المكونات تعمل بشكل إضافي أو حتى تآزري في الزيوت العطرية. للزيوت العطرية فعالية مضادة لطيف واسع من الكائنات الحية الدقيقة وتكمن فعاليتها بقدرتها على تفكيك الغشاء الخلوي متبوعًا بتعطيل مكونات الخلايا الميكروبية الأخرى. يرتبط أسلوب عمل الزيوت العطرية كمضاد للأكسدة بمعادلة الجذور الحرة وتفكيك مركب البيروكسيد خاصة عند اختبارها في اللحوم والألبان والفواكه والخضروات. إن الكفاءة العالية للزيوت العطرية تُملي احتمالية استخدمها كبدائل للمضافات الغذائية المصنعة. هذه المراجعة العلمية تُلخص آخر دراسات عن كفاءة الزيوت العطرية كعوامل مضادة للميكروبات والأكسدة لاستخدامها في صناعة الاغذية.

الكلمات الدالة: الزيوت العطرية، مضادات الأكسدة، عناصر مضادة للميكروبات، المواد الفينولية، صناعة الأغذية.

* الباحث المعتمد للمراسلة: g.mehyar@ju.edu.jo