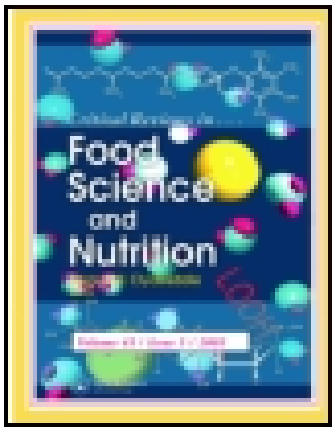

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Oregano Essential Oil as an Antimicrobial and Antioxidant Additive in Food Products

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Food consumers and industries urged the need of natural alternatives to assure food safety and quality. As a response, the use of natural compounds from herbs and spices is an alternative to synthetic additives associated with toxic problems. This review discusses the antimicrobial and antioxidant activity of oregano essential oil (OEO) and its potential as a food additive. Oregano is a plant that has been used as a food seasoning since ancient times. The common name of oregano is given to several species: Origanum (family: Lamiaceae) and Lippia (family: Verbenaceae), amongst others. The main compounds identified in the different OEOs are carvacrol and thymol, which are responsible for the characteristic odor, antimicrobial and antioxidant activity; however, their content may vary according to the species, harvesting season

and geographical sources. These substances as antibacterial agents make the cell membrane permeable due to its impregnation in the hydrophobic domains, this effect is higher against gram-positive bacteria. In addition, the OEO has antioxidant properties effective in retarding the process of lipid peroxidation in fatty foods, and scavenging free radicals. In this perspective, the present review analyzes and discusses the state of the art about the actual and potential uses of OEO as an antimicrobial and antioxidant food additives.

Keywords carvacrol, thymol, natural products, food safety and quality.

INTRODUCTION

Global interest in preservation of food systems has recently increased because of the greater economic costs of deterioration and poisoning caused by oxidation and microbial pathogens (Bajpai et al., 2009). In addition, the food industries have paid attention to this perception, adopting the natural alternatives to assure food safety and quality. This resulted in a continuous search for effective natural antioxidant and antimicrobial compounds that preserve food quality and safety without detriment of its sensorial attributes (Ayala-Zavala et al., 2009). Oregano essential oil (OEO) has been found to be amongst the most effective antimicrobial and antioxidant natural agents (Benavides et al., 2012). In view of the published data on the antimicrobial efficacy of essential oil (EO), the following general ranking (in order of decreasing antibacterial activity) can be made: oregano / clove / coriander / cinnamon > thyme > mint > rosemary > mustard > cilantro / sage (Sara, 2004). Recent studies have also shown that OEO is effective as antioxidant and flavoring agent with the properties of functional food or nutraceutical products (Loizzo et al., 2009), retarding lipid oxidation (Handl et al., 2008); which makes oregano a source of EO with potentially extensive use in the food industry.

Worldwide there is a large number of species that are designated by the name of oregano, most of them belong to the genera *Origanum* (family: *Lamiaceae*) and *Lippia* (family: *Verbenaceae*) (Martínez-Rocha et al., 2008; Amadio et al., 2011). There are different types of oregano from the genus *Origanum: O. vulgare* L, *O. viride* and *O. virens* representing the Mediterranean region. In Mexico, a plant complex consisting of aromatic woody-based herbs or

shrubs from various genera of the families: *Lamiaceae* (*Calamintha, Hedeoma, Hyptis, Mesosphaerum, Monarda, Origanum, Plectranthus* and *Poliomintha*), *Verbenaceae* (*Lantana* and *Lippia*), *Asteraceae* (*Brickellia*) and *Fabaceae* (*Dalea*) are regarded as oregano (Martínez-Rocha et al., 2008). The most popular Mexican oreganos are *Lippia graveolens* Kunth (Syn: *L. berlandieri* Schauer) and *Poliomintha longiflora* A. Gray (Rivero-Cruz et al., 2011). Regarding that there are many types of oregano, it is important to analyze their chemical composition and bioactive properties.

Carvacrol and thymol are the main antimicrobial and antioxidant *monoterpene* phenolic compounds that constitute about 78ï 85% of OEO (Govaris et al., 2010). The antimicrobial activity of these compounds is attributed to their lipophilic character that makes them more attractive to the cell membrane structures. Consequently, their presence cause membrane expansion, increases fluidity and permeability, disturbs embedded proteins, inhibits respiration, and alters ion transport processes (Cristani et al., 2007). These compounds act as antioxidant agents quenching free radicals by donating hydrogen atoms or electrons, retarding lipid oxidation (Sánchez-Escalante et al., 2003; Choe and Min, 2006). In addition to the antimicrobial and antioxidant properties of OEO, carvacrol and thymol provide the characteristic flavor and odor.

The bioactive components of oregano are beneficial as flavoring or seasoning agents in some of the most accepted cuisines around the world. Oregano is traditionally used in Italian, Greek and Mexican dishes. Flowering tops are used in beers and ales, and fresh and dried leaves can be added to soups, casseroles, sauces, stew, stuffing, eggs, olives, teas, tomato-based dishes and strong-flavored foods like chili and pizza (America, 2005). Reflecting on all the culinary uses

and functional properties of oregano, this review analyzes and discusses the antimicrobial and antioxidant activity of OEO and its potential as a food additive.

CULINARY USES OF OREGANO

Oregano has been cultivated since ancient times, thanks to its herbal and therapeutic properties and is currently added to various food commercial preparations mainly as flavoring ingredient (Tibaldi et al., 2011). *Origanum* species have been cultivated in Egypt for over 3000 years; the ancient Greeks used it since classical times and has been grown in England since the 13th century. Some of the earliest records of *Origanum* uses dated back to 1600-1200 B.C., when images of the plants were inscribed on tablets by the Hittites of Asia Minor/Syria (America, 2005). Nowadays there are various types of oregano present worldwide; these belong to the family *Lamiaceae* which comprises about 260 genera and 7000 species (Cosge et al., 2009). The genus *Origanum* is one of the economically important plants of this family and most of the reports are related to *O. vulgare*, which is found mainly on the Mediterranean coast and Eurasia. Besides is the Central-South American or Mexican oregano that belongs to the genus *Lippia* (*L. graveolens*) (Vernin et al., 2001; Avila-Sosa et al., 2010; Mechergui et al., 2010).

Most of the so-called oregano plants share some major volatile compounds, responsible of the characteristic odor and flavor. *O. vulgare* subsp. *hirtum* has the classic pungent, hot and spicy oregano flavor (HSA, 2005). *O. majorana* has a subtle flavor is both sweet and spicy and can be used in most dishes. In combination with bay, garlic, rosemary, sage, savory and thyme, oregano add zest to pasta, roast beef, lamb or pork, stuffing, sauces, salads and egg dishes (Belsinger and

Richter, 2005). Mexican oregano is characterized by a strong flavor and a high yield of EO, is widely used in Mexican and North American dishes, alcoholic beverages, baked goods, meat and meat products, condiments, relishes, milk products, processed vegetables, and snack foods (Mechergui et al., 2010). *O. vulgare* contains an EO whose composition is dominated by monoterpenes and sesquiterpenes, which are responsible for the aroma and flavor of oregano (Crocoll et al., 2010). Considering the diversity of oregano plants and culinary uses based on their aroma properties is evidently that their chemical composition also varies.

CHEMICAL COMPONENTS PRESENT IN OEO

The composition of EOs of the same species depends mainly on the harvesting season and geographical sources (**Table 1**) (Arana-Sánchez et al., 2010). The main components of *Lippia palmeri* S. Wats EOs from Alamos and Puerto del Oregano (Sonora, Mexico) were p-cymene (22.37 and 14.25%), thymol (21.39 and 15.11%), *γ*-terpinene (6.69 and 4.23%), iso-aromandrene (16.7 and 0.62%), respectively (Ortega-Nieblas et al., 2011). The EOs from *O. vulgare* L. sp. *glandulosum* (Desf.) Ietswaart collected from three localities of north Tunisia (Nefza, Bargou and Krib) presented as main components: *p*-cymene (36, 40 and 46%), thymol (32, 39 and 18%), *γ*-terpinene (24, 12 and 16%), and carvacrol (2, 2 and 15%), respectively (Mechergui et al., 2010). The EOs from *O. applii* (criollo) and *O. majoricum* (mendocino), from La Consulta, Mendoza, Argentina, showed thymol (33.8 and 12.9%) and carvacrol (both <0.1 %) contents (Amadio et al., 2011). The two major components of *L. graveolens* from El Sauce, La Union, El Salvador, collected in mid-November and at the end of December were carvacrol (71 and 34.6%) and thymol (5ï 7%), respectively (Vernin et al., 2001).

On the other side, Turkish oregano (*O. onites* L.) was analyzed from leaves harvested during the months of June to September. The maximum EO yield appeared in the middle of July and the main components were carvacrol, thymol, *γ*-terpinene, *p*-cymene, *α*-terpinene and *α*-pinene; carvacrol content was the highest in the July harvest (Ozkan et al., 2010). The carvacrol content in EO from *O. onites* varied monthly (47.41-73.65%) and the highest values were obtained in the flowering period of May (Yaldiz et al., 2005). Clearly, the season of growth, geographical location affected the qualitative and quantitative composition of OEO, this information must be contemplated to obtain the higher benefits of this oil.

The most consistently reported compounds are the monoterpene phenolic compounds thymol and carvacrol that have antioxidant and antimicrobial activities. Their biosynthesis proceeds from the methyl-erythritol-phosphate (MEP) pathway is located in the plastids and the initial substrates are glyceraldehyde-3-phosphate (GA3P) and pyruvate **(Figure 1)**. Geranyl diphosphate (GPP) is the precursor for all monoterpenes. DMAPP (dimethylallyl diphosphate) is the backbone to which different numbers of the isomer IPP (isopentenyl diphosphate) are added to form GPP and farnesyl diphosphate (FPP). Afterward, the linear carbon skeletons of GPP and FPP are converted to the basic terpene skeletons by terpene synthases, a widespread class of enzymes responsible for the huge structural diversity of monoterpenes, including carvacrol and thymol, and sesquiterpenes (Tholl, 2006; Crocoll et al., 2010).

OEO AS AN ANTIOXIDANT ADDITIVE IN FOODS

The antioxidant effect of OEO is attributed to their major components, carvacrol and thymol, and it is the result of various possible mechanisms: free-radical scavenging activity, transition-

metal-chelating activity, and/or singlet-oxygen-quenching capacity (Shan et al., 2005). Carvacrol and thymol can donate hydrogen atoms to free radicals and convert them to more stable nonradical products **(Figure 2)**. The major hydrogen-donating antioxidants are monohydroxy or polyhydroxy phenolic compounds with various aromatic ring substitutions, whose reduction potential is lower than that of a free radical, and can donate hydrogen to that radical, unless the reaction is kinetically unfavorable (Choe and Min, 2006).

The antioxidant activity of OEO differed according to the localities and harvest season. The concentration of EO from *O. vulgare* L. sp. *glandulosum* collected from three localities of north Tunisia (Krib, Bargou and Nefza) that provided 50% inhibition (IC_{50}) of the DPPH·radical (2,2-diphenyl-1-picrylhydrazyl free radical) ranged from 59-80 mg/L; and the total phenolic content, varied from 9.37-17.70 mg of gallic acid equivalents/g (Mechergui et al., 2010). The total phenolic content of extracts of *L. graveolens* from different areas in Mexico (Guanajuato, Puebla and Queretaro) ranged from 211.8-270.2 mg/g, flavonoids from 136.1-200.1 mg/g, the acetylene reduction activity and trolox equivalent antioxidant capacity (TEAC) were similar to the values of gallic acid (Martínez-Rocha et al., 2008). EO from Turkish *O. onites* had the highest reducing/antioxidant capacity in June (31.02 mg Ascorbic Acid Equivalents/g and 116.74 mg/L DPPH IC_{50}) and the lowest in September (28.96 mg AAE/g and 123.75 mg/L) (Ozkan et al., 2010). Besides biological variability, it should also be considered the inter-lab variation on the estimation of antioxidant capacity.

The antioxidant properties of oregano and their major components have been proved to be effective in retarding the process of lipid peroxidation in fatty foods (Lagouri et al., 2010). The rates of increment of the peroxide value and conjugated dienes in cottonseed oil, during

frying of potato chips, were depressed when oregano was added, with the protective action of oregano extract 2 g/L being considerably greater than that of ground oregano 2g/L (Houhoula et al., 2004). Yanishlieva et al. (1999) established that thymol and carvacrol applied at different concentrations (0.02, 0.05, 0.10 and 0.20%) differed in the mechanism of their oxidant inhibitory action, which depended on the character of the lipid medium, being thymol a better antioxidant in triacylglycerols of sunflower oil than in triacylglycerols of lard. In the same study thymol was a better antioxidant in lipids than carvacrol; this fact was attributed to the greater steric hindrance of the phenolic group in thymol than in carvacrol.

Milk protein-based edible films containing 1.0% (w/v) oregano applied on beef muscle slices stabilized lipid oxidation (Oussalah et al., 2004). Fried salted peanuts added with OEO 20 g/kg and olive oil showed higher oxidative stability during storage, evaluated by the peroxide and conjugated diene values (Olmedo et al., 2009). In addition, the combinations of oregano extract 4412 ppm and olive oil 1126 ppm. in fresh potatoes resulted in a reduction of acrylamide content, up to 49%, after heating (Kotsiou et al., 2010). The successful combination of OEO, thyme oil pepper oil and clove oil (all EO from 0.05 to 1%) has resulted in a patent claimed to stabilize polyunsaturated fatty acids (Cloughley and Horrobin, 1996).

In food animal the addition of OEO may improve the dietary value and lead to a better oxidative stability and longer shelf-life of fat, beef, eggs, and turkey (Franz et al., 2010; Botsoglou et al., 2003). OEO (100 mg/kg of feed) added to the diet of chickens exerted an antioxidant effect in the animal tissues; the pattern of fatty acids of the abdominal fat was altered, showing higher content of linolenic fatty acid (Avila-Ramos et al., 2012). The addition of oregano (5-10%) and carrot leaf (5-10%) to pasta formulations effectively enhanced the

chemical and nutritional parameters, increasing the omega-3 fatty acid content and the antioxidant properties (Boroski et al., 2011). Furthermore, it has been seen that the addition of carvacrol (200 mg/L headspace) increases the antioxidant compounds in ôDukeô blueberries reducing decay (Wang et al., 2008). Chinese bayberries were treated with carvacrol (1 εL/L), reducing fruit decay and increased total phenolic, anthocyanin, and individual flavonoid compounds (Jin et al., 2012). Cooked beef patties added with oregano (10%) were subjected to an *in vitro* digestion procedure, and the resulting micelles isolated from the digested meats significantly increased cellular reduced glutathione (GSH) content and prevented its H_2O_2-induced depletion in human intestinal Caco-2 cells (Ryan et al., 2009).

Few descriptive reports have been published about the pharmacological and toxic effects of the OEO and its major constituents. Carvacrol inhibited the growth of myoblast cells after activation of mutated N-ras oncogene, suggesting the possibility that carvacrol may find application in cancer therapy (Zeytinoglu et al., 2003). Chromosomal aberration assay of primary hepatocytes, isolated from control or carvacrol-watered rats did not testify any genotoxic activity of carvacrol (Slamenova et al., 2011). Carvacrol has been proposed as a potent anti-cancer compound with an IC_{50} of 50 mg/L at 48 h inducing growth inhibition in human cervical cancer cells (Mehdi et al., 2011). Ipek et al. (2003) observed that carvacrol did not increase the formation of sister chromatid exchange indicating its potential for use as an antigenotoxic agent in mammalian cells. Thymol and carvacrol stimulated responses against cognitive deficits induced by amyloid beta or scopolamine and acute toxicities in rats (Azizi et al., 2012). Although there have been reports about the toxicity of the OEO constituents the calculated median lethal doses of thymol (565.7 mg/kg) and carvacrol (471.2 mg/kg) were found to be much higher than

their therapeutic doses (thymol 0.5 mg/kg, carvacrol 1 mg/kg). Although the number of studies on genotoxic effects of OEO constituents is still limited, the available data discussed above reinforce the view that, in addition to the health related benefits, the use of this oil does not pose a high risk to consumers.

Antioxidant evaluation of OEO and its components has contributed to understand the mechanisms of action; however, it is noticed that most of the studies are obviating the complexity of the oxidation reactions in food systems. Antioxidant evaluations mostly consist of radical inhibition processes; although they should also contemplate the inclusion of more real biochemical reactions involved in the oxidation process: e.g. it is important to consider the antioxidant potential of OEO and its components as protective agents of proteins, food membranes, carbohydrates and nucleic acids; as well as the presence of endogenous antioxidant systems; and their participation in total antioxidant capacity. In addition, more studies are needed to understand the inhibition process of oxidative enzymes, related with food quality decay, by applying OEO and/or its components. In the same context, the trend in this area is to understand the role of interactions amongst endogen and exogenous antioxidant compounds in food systems that can caused a synergic or antagonistic effects; nevertheless, few studies have contemplated these evaluations.

Considering the antioxidant properties of OEO, its application to food systems can be contemplated to a wider range of food products, like several edible vegetable oils, sausage, dairy, dressing and seafood. Some of the reviewed studies selected a more technological approach of the addition of OEO precluding the nutritional aspects. However, nowadays the research must include a globalized point where both aspects must be observed, in order to create and promote

functional foods that meet the desired technological characteristics without affecting the nutritional content. In addition, it would be interesting to start the evaluation of these foods in human systems and not only *in vitro* simulations of various human processes, taking into consideration the antioxidant characteristics of the product obtained with the addition of OEO. Considering these future studies, the understanding of the real antioxidant contribution of OEO in food systems can be improved.

OEO AS AN ANTIMICROBIAL ADDITIVE IN FOODS

Carvacrol and thymol are the major antimicrobial compounds found in different OEO **(Table 2)**. It has been found that these agents cause alterations in the fungi hyphal morphology and aggregates, resulting in reduced diameters and lyses (Numpaque et al., 2011). In addition, the chemical modification of these phenolic compounds to various ether and ester derivatives has been reported to result in change in biological activity (Numpaque et al., 2011). In bacteria, the cell membrane is a very important target to the OEO components like terpenoids that could interfere with the phospholipid bilayers membrane (Gumus et al., 2010). Thymol and carvacrol are able to disintegrate the outer membrane of the gram-negative bacteria **(Figure 3)**, releasing the lipopolysaccharide components, and thus increasing the permeability of the adenosine triphosphate in the cytoplasmic membrane, and consequently changing the passive permeability of the cell (Guarda et al., 2011). *p*-cymene is a hydrophobic compound that provokes greater swelling of the cytoplasmic membrane compared to carvacrol (Silva and Fernandes, 2010). Generally, plant EOs are more active against gram-positive bacteria than gram-negative bacteria. Some authors suggest that the outer membrane surrounding the cell wall of gram-negative

bacteria may restrict diffusion of hydrophobic compounds through its lipopolysaccharide covering and the vital functions of the cell (Gutierrez et al., 2009). EO of *Origanum compactum* on *P. aeruginosa* and *Staphylococcus aureus* affected their membrane potential and membrane permeability (Bouhdid et al., 2009). EO of *Origanum acutidens* showed major sensitivity in *Proteus vulgaris* and *Salmonella typhimurium* with respect to other bacteria (Cosge et al., 2009).

Antimicrobial synergism amongst the OEO components has been reported. The synergistic activity between carvacrol (0 to 0.5 mM) and cymene (0 to 0.5 mM) cause a major destabilization of the membrane and a decrease in the membrane potential than the individual components (Ultee et al., 2002). The amphipathicity of thymol and carvacrol can explain their interactions with biomembranes and thus the antimicrobial activity (Cristani et al., 2007). Carvacrol can be accumulated in the cell membrane and its hydrogen-bonding ability and proton-release ability may induce conformational modification of the membrane resulting in the cell death (Ben Arfa et al., 2006). The carvacrol (75mg/kg) ï thymol (62.5 mg/kg) combination had the highest number of synergistic combinations against *L. innocua*, followed by a single thymolï eugenol combination (García-García et al., 2011). For *Pseudomonas aeruginosa*, 96% of the inhibition observed with OEO can be attributed to the additive effect of thymol and carvacrol with the remaining 4% from the other components (Lambert et al., 2001). The combination of EO from *O. vulgare* subsp. *hirtum* at 0.6% with nisin at 500 IU/g showed stronger antimicrobial activity against *S. enteritidis* (Govaris et al., 2010).

Oregano (250 ppm) in combination with thyme (10,000 ppm), lemon balm (25,000 ppm), basil (20,000 ppm), marjoram (5,000 ppm), rosemary (10,000 ppm) or sage (100,000 ppm) had the strongest effect against *Bacillus cereus* (Tajkarimi et al., 2010;Gutierrez et al., 2008).

Carvacrol (0.3, 0.5, 0.8, or 1 mM) increased the synthesis of heat shock protein 60 HSP 60 (GroEL) and inhibited the synthesis of flagellin in *E. coli* O157:H7 (Burt et al., 2007). Crude extract of *O. vulgare* inhibited the growth of trypanosomes in different developmental stages causing epimastigote growth (175 ε g/mL) and induced trypomastigote lysis (115 ε g/mL) (Santoro et al., 2007). The examination of the antimicrobial activity of *O. glandulosum* EO against gram-positive bacteria: *Bacillus subtilis*, *S. aureus, L. monocytogenes*, and gram-negative bacteria: *E. coli*, *Klebsiella pneumo*niae, *P. aeruginosa*, *Citrobacter freundii, Salmonella typhimurium* and yeasts (*Candida albicans* 444 and *C. albicans* 9036) and molds revealed that is more antifungal than antibacterial (Bendahou et al., 2008). Where *E. coli, S. aureus* and *S. thyphimirium* with inhibition zones measured at 24ï 27 mm, 23ï 24 mm and 25ï 26 mm, respectively, *P. aeruginosa* and *K. pneumoniae* were the most resistant strains to the oils and *L. monocytogenes* showed a modest sensibility. The extent of inhibition of the tested microorganism growth was dependent on the concentrations of EO used (ÖZcan and Chalchat, 2009). The MIC of OEO was determined by broth dilution method as 0.05% (v/v) for staphylococci with the exception of *Sthaphylococcus sciuri* (0.8%, v/v) and 0.1% (v/v) for *E. coli*, inhibiting the biofilm formation and eradicated established biofilm at MIC level (Oral et al., 2010). Sub-inhibitory concentrations of the OEO reduced the level of biofilm formation of the tested strains. For *S. aureus* and *Staphylococcus epidermidis* the biofilm inhibitory concentration (0.125ï 0.500%, v/v, for oregano, and 0.031ï 0.125%, v/v, for carvacrol and thymol) and biofilm eradication concentration (0.25ï 1.0 % v/v, for oregano and 0.125ï 0.500%, v/v, for carvacrol and thymol) values were twofold or fourfold greater than the concentration required to inhibit planktonic growth (Nostro et al., 2007).

Several techniques are being explored to facilitate the addition of OEO as antimicrobial food additive. Antimicrobial effects of chitosan films containing anise, basil, coriander, and oregano EOs (1%, 2%, 3%, and 4%) against *L. monocytogenes* and *E. coli* O157:H7 were similar when applied alone or incorporated in the films; however, the intensity of antimicrobial efficacy was in the following order: oregano >> coriander > basil > anise (Zivanovic et al., 2005). Antimicrobial properties of whey protein isolate based or cellulose-based filter paper films containing oregano and sage EOs (5, 10, 20, 40 and 80 g/kg) were tested against *L. innocua, S. aureus* and *S. enteritidis*, and the highest inhibition zones (aprox. 3750 mm^2) were against *L. innocua* (Royo et al., 2010). Antimicrobial activity was confirmed for both EOs using cellulose-based filter paper (80 g/kg) as supporting matrix, although it was significantly more intense for OEO. Microencapsulation of *L. graveolens* EO using cyclodextrins decreased the minimal bactericidal concentration (0.05%, 0.10% and 0.20% w/v) of the oil against *P. aeruginosa* (Arana-Sánchez et al., 2010). The antimicrobial activity of OEO (2% and 4%), hydrocinnamaldehyde (2% and 4%), cinnamaldehyde (2% and 4%), thymol (2% and 4%), and carvacrol (2% and 4%) incorporated into polypropylene was reported as effective against various gram-negative (*E. coli, Yersinia enterocolitica, P. aeruginosa,* and *S. choleraesuis*), gram-positive bacteria (*L. monocytogenes, S. aureus, B. cereus,* and *Enterococcus faecalis*), yeasts (*C. albicans, Debaryomyces hansenii, Zygosaccharomyces rouxii)*, and molds (*Botrytis cinerae, A. flavus, Eurotium repens, Penicillium roqueforti, P. islandicum, P. commune, P. nalgiovensis*) (Gutierrez et al., 2009)

The OEO and their principal compounds have been applied also as antimicrobials in fruit and vegetables produce. OEO and carvacrol (0.67 and 0.067%) were used to treat apple, mango,

orange, and tomato juices inhibiting the growth of *E. coli* O157:H7 (Friedman et al., 2004). Treatments of apple fruits with EOs from oregano (1% and 10%) showed significant efficacy reducing *Botrytis cinerea* and *Penicillium expansum* growth (Lopez-Reyes et al., 2010). EOs from *O. vulgare* L. and *Rosmarinus officinalis* L. (80 to 0.003 εL/mL) inhibited bacterial microflora associated with minimally processed vegetables (de Azeredo et al., 2011). Nectarine treated with *Aloe vera* in conjunction with thymol (1 mL/L) showed low decay index caused by *Rhizopus stolonifer, B. cinerea* and *Penicillium digitatum* (Navarro et al., 2011). Carvacrol (0.05, 0.2, 0.5 and 1.0 mL/ L) tested in table grapes inhibited the development of *B. cinerea* (Martinez-Romero et al., 2007). Carvacrol and thymol [10, 25, 50, 100, 250 and 500 εL/L of carvacrol, thymol or the mixture of carvacrol:thymol (1:1)] applied in lemon, resulted in low fungal decay induced by *P. digitatum* and *P. italicum* (Perez-Alfonso et al., 2012). OEO (400 ppm) comibinated with thyme (6000 ppm) were evaluated for control of *E. cloacae* using on lettuce (Gutierrez et al., 2009). *Listeria* strains were more sensitive than spoilage bacteria, and oregano and thyme were the most active EOs. The average efficacy of EOs against *Listeria* spp. was in the following order: oregano Óthyme > lemon balm, while the efficacy order of EOs against the spoilage bacteria was: oregano Óthyme > marjoram.

OEO (0.2% v/w) or their principal components have also been used in conjunction with other technologies, such as vacuum-packaging with EDTA (1.5% w/w), lysozyme solution (1.5% w/w) on semi cooked coated chicken meat producing shelf-life extension of 7ï8 days compared to controls (Ntzimani et al., 2010). In other investigation carvacrol (0.3% to 0.5%), caprylic acid (0.25% to 1.0%), and ε-polylysine (0.125% to1.0%) were applied on breaded chicken products showing reduction in *Salmonella* populations (Moschonas et al., 2012). Pectin

edible films that contained carvacrol (0.5%, 1.5% and 3%) and cinnamaldehyde (0.5%, 1.5% and 3%) caused the inactivation of *L. monocytogenes* on ham and bologna (Ravishankar et al., 2012). Dipped carp fillets with carvacrol (0.5%) and thymol (0.5%) delayed bacterial growth and extended their shelf-life (Mahmoud et al., 2004). The studies of the antimicrobial activity of *O. majorana* (1.15, 2.3, 5.75 mg/g) in fresh sausage showed that its addition to fresh sausage exerted a bacteriostatic effect at concentrations lower (1.15 mg/g) than the minimum inhibitory concentration (MIC) (2.3 mg/g) (Busatta et al., 2008). EO of *Origanum syriacum* var. *bevanii* (0.4, 0.8 1.6, 2.4, 3.2 ε g/mL) had a marked antifungal effect against soil contamination with *Sclerotinia sclerotiorum*, both oils reduced sclerotial viability, increasing the number of surviving tomato seedling by 69.8% and 53.3%, respectively (Soylu et al., 2007). In other study, four pathogenic bacteria (*E. coli* O157:H7, *S. enterica*, *B. cereus* and *S. aureus*) were inoculated in a dough made from corn flour with carvacrol (0.5%, 2% and 5%) and all the strains were completely inactivated within 24 h (Ortega-Morente et al., 2010). This information reveals the antimicrobial potential of OEO to be use as a food additive.

Even when several studies showed the antimicrobial efficacy and general mechanisms of OEO and its components a lack of basic knowledge on their possible mode of action still is detected. Some of the research should be focus on evaluating the effect of the OEO and components on the intercellular communication amongst bacterial pathogens (quorum sensing), the effect of these natural compounds on the enzymatic transporters and signal molecules should be also contemplated. Considering the technological uses of this oil a more wide range of food matrices could be treated to take advantage of its antimicrobial efficacy, like fresh meat, seafood, several types of whole and fresh-cut produce. To achieve a more effective treatment of food

products using OEO several technologies must be contemplated: nanoemulsions, nanocapsules, vapors, edible films based in different polymers; conjunction with other preserving technologies like low temperatures, modified atmosphere packaging and irradiation. The generation of this information will profound on the antimicrobial knowledge and effective uses of the OEO on food matrices.

ODOR-FLAVOR PROPERTIES OF OREGANO AND ITS IMPACT IN TREATED FOOD

The odor-flavor properties of OEO have been used to add sensorial appeal to food products. The odor sensory impressions of oregano have been defined as lemony/lemon balm, in addition to fruity and sweet descriptors (Bansleben et al., 2010). Sea bream (*Sparus aurata*) treated with OEO (0.8%v/w) received an odor score significantly higher or similar to control samples (Goulas and Kontominas, 2007). The application of OEO (0.345, 0.69, 1.725, 3.45 mg/g) on sausages showed that the addition of this oil may be a promising route to increase sensorial appeal, in addition to the observed bacteriostatic effect (Busatta et al., 2007). Hydrosols obtained from three Lamiaceae plants (thyme, summer savory and oregano) were effective sanitizers for fresh-cut tomatoes and cucumbers to provide their microbial safety without causing any sensorial defect (Sagdic et al., 2013). Sensory evaluation showed that the addition of oregano EO at 0.6 or 0.9% in minced sheep meat was organoleptically acceptable, and attribute scores were higher for the EO at 0.6 than 0.9% (Govaris et al., 2010). OEO (0.02%) treatment in conjunction with orange dietary fibre (1%) and vacuum packaging conditions increased the shelf-life of bologna sausages, it should be noted that despite the marked aroma of oregano, this was not found unpleasant by the panellists, who valued the samples containing this EO almost equally with the

controls (Viuda-Martos et al., 2010). The sensorial effect of salt, OEO (0.2%) and packaging on fresh rainbow trout fillets during storage at 4 C yielded distinctive and pleasant odor and flavor to trout fillets (Pyrgotous et al., 2010). The effect of oregano (*O. onites*) EO on the extension of shelf life of overwrap packed fresh chicken drumsticks reported that OEO gave a characteristic desirable odor and taste to chicken meat, very compatible to cooked chicken flavor, when its concentration was at 0.1% (Oral et al., 2009). It was evaluated the sensorial effect of vacuum packaging combined with OEO (0.2 % and 0.4%) in fresh Mediterranean octopus, this treatment gave a characteristic, desirable and pleasant odor to the treated samples until days 17 and 23 days of storage (Atrea et al., 2009). Choosing the right combination in aromas between the antimicrobial and antioxidant OEO and the food, the quality, safety, antioxidant status, and flavor could be improved. It is important to consider that new appeals and tastes are always welcome for modern consumers especially when the sources are of natural origin, like OEO. Evidently, more studies considering the sensorial benefits and the combination of flavors with other food products should be tested.

CONCLUSION

This review found that OEO posses high antimicrobial and antioxidant properties compared to other natural sources, in addition, when combined with other species may have a synergistic effect. We also found that the bioactive properties and composition of OEO can vary considering geographic area, species or collecting season. Irrespective of these variations, carvacrol and thymol are the common volatile compounds responsible for the antimicrobial and antioxidant

properties of the OEO. This oil is generally more active against fungi than bacteria and more effective against Gram-positive than Gram-negative bacteria. It can be suggested that the right combination amongst aroma notes of OEO, antioxidant and antimicrobial activity must be contemplated and evaluated to optimize its use as natural food additive, fulfilling the requirements of consumers of natural preserved and flavorful food.

References

Amadio, C., Medina, R., Dediol, C., Zimmermann, M. É. and Miralles, S. (2011). Oregano essential oil: A potential food additive. *Revista de la Facultad de Ciencias Agrarias* **43**: 237-245.

America, T. H. S. O. (2005). Oregano and marjoram an herb society of america guide to the genus *Origanum*. from http://www.herbsociety.org.

Arana-Sánchez, A., Estarrón-Espinosa, M., Obledo-Vázquez, E. N., Padilla-Camberos, E., Silva-Vázquez, R. and Lugo-Cervantes, E. (2010). Antimicrobial and antioxidant activities of mexican oregano essential oils (*Lippia graveolens* H. B. K.) with different composition when microencapsulated in ɓ-cyclodextrin. *Lett. Appl. Microbiol.* **50**: 585-590.

Atrea, I., Papavergou, A., Amvrosiadis, I. and Savvaidis, I. N. (2009). Combined effect of vacuum-packaging and oregano essential oil on the shelf-life of mediterranean octopus (*Octopus vulgaris*) from the aegean sea stored at 4 degrees C. *Food Microbiol.* **26**: 166-172.

Avila-Ramos, F., Pro-Martinez, A., Sosa-Montes, E., Cuca-Garcia, J. M., Becerril-Perez, C. M., Figueroa-Velasco, J. L. and Narciso-Gaytan, C. (2012). Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. *Poult. Sci.* **91**: 505-511.

Avila-Sosa, R., Gastélum-Franco, M., Camacho-Dávila, A., Torres-Muñoz, J. and Nevárez-Moorillón, G. (2010). Extracts of mexican oregano (*Lippia berlandieri* Schauer) with antioxidant and antimicrobial activity. *Food and Bioprocess Technology* **3**: 434-440.

Ayala-Zavala, J. F., González-Aguilar, G. A. and Del-Toro-Sánchez, L. (2009). Enhancing safety and aroma appealing of fresh-cut fruits and vegetables using the antimicrobial and aromatic power of essential oils. *J. Food Sci.* **74**: R84-R91.

Azizi, Z., Ebrahimi, S., Saadatfar, E., Kamalinejad, M. and Majlessi, N. (2012). Cognitive-enhancing activity of thymol and carvacrol in two rat models of dementia. *Behav. Pharmacol*. **23**: 241-249.

Bajpai, V. K., Al-Reza, S. M., Choi, U. K., Lee, J. H. and Kang, S. C. (2009). Chemical composition, antibacterial and antioxidant activities of leaf essential oil and extracts of *Metasequioa glyptostroboides* miki ex hu. *Food Chem. Toxicol.* **47**: 1876-1883.

Bansleben, A. C., Schellenberg, I., Ulrich, D. and Bansleben, D. (2010). A new and efficient sensory method for a comprehensive assessment of the sensory quality of dried aroma-intensive herbs using oregano as a reference plant. *Flavour Fragrance J.* **25**: 214-218.

Belsinger, S.and Richter, C. (2005). Oregano, Riversong Studios Ltd.

Ben Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N. and Chalier, P. (2006). Antimicrobial activity of carvacrol related to its chemical structure. *Lett. Appl. Microbiol.* **43**: 149-154.

Benavides, S., Villalobos-Carvajal, R. and Reyes, J. E. (2012). Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. *J. Food Eng.* **110**: 232-239.

Bendahou, M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J. M., Bernardini, A. F. and Costa, J. (2008). Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. Essential oil and extract obtained by microwave extraction: Comparison with hydrodistillation. *Food Chem.* **106**: 132-139.

Boroski, M., de Aguiar, A. C., Boeing, J. S., Rotta, E. M., Wibby, C. L., Bonafe, E. G., de Souza, N. E. and Visentainer, J. V. (2011). Enhancement of pasta antioxidant activity with oregano and carrot leaf. *Food Chem.* **125**: 696-700.

Botsoglou, N. A., Govaris, A., Botsoglou, E. N., Grigoropoulou, S. H. and Papageorgiou, G. (2003). Antioxidant activity of dietary oregano essential oil and alpha-tocopheryl acetate supplementation in long-term frozen stored turkey meat. *J Agr Food Chem.* **51**: 2930-2936.

Bouhdid, S., Abrini, J., Zhiri, A., Espuny, M. J. and Manresa, A. (2009). Investigation of functional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. *J. Appl. Microbiol.* **106**: 1558-1568.

Burt, S., (2004). Essential oils: Their antibacterial properties and potential applications in foodsð a review. *Int. J. Food Microbiol.* **94**: 223-253.

Burt, S. A., van der Zee, R., Koets, A. P., de Graaff, A. M., van Knapen, F., Gaastra, W., Haagsman, H. P. and Veldhuizen, E. J. A. (2007). Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in *Escherichia coli* O157 : H7. *Applied Appl. Environ. Microb.* **73**: 4484-4490.

Busatta, C., Mossi, A. J., Rodrigues, M. R. A., Cansian, R. L. and de Oliveira, J. V. (2007). Evaluation of *Origanum vulgare* essential oil as antimicrobial agent in sausage. *Braz. J. Microbiol.* **38**: 610-616.

Busatta, C., Vidal, R. S., Popiolski, A. S., Mossi, A. J., Dariva, C., Rodrigues, M. R. A., Corazza, F. C., Corazza, M. L., Oliveira, J. V. and Cansian, R. L. (2008). Application of

Origanum majorana L. essential oil as an antimicrobial agent in sausage. *Food Microbiol.* **25**: 207-211.

Choe, E. and Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Compr. Rev. Food Sci. F.* **5**: 169-186.

Cloughley, J. B. and Horrobin, D. F. (1996). Stabilisation of polyunsaturates. *Europa Patent Register.* **EP0724618A1**.

de Azeredo, G. A., Stamford, T. L. M., Nunes, P. C., Gomes Neto, N. J., de Oliveira, M. E. G. and de Souza, E. L. (2011). Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. To inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. *Food Res. Int.* **44**: 1541-1548.

Cosge, B., Turker, A., Ipek, A., Gurbuz, B. and Arslan, N. (2009). Chemical compositions and antibacterial activities of the essential oils from aerial parts and corollas of *Origanum acutidens* (Hand.-Mazz.) Ietswaart, an endemic species to turkey. *Molecules* **14**: 1702-1712.

Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., Venuti, V., Bisignano, G., Saija, A. and Trombetta, D. (2007). Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. *J. Agric. Food Chem.* **55**: 6300-6308.

Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., Venuti, V., Bisignano, G., Saija, A. and Trombetta, D. (2007). Interaction of four monoterpenes

contained in essential oils with model membranes: Implications for their antibacterial activity. *J. Agric. Food Chem.* **55**: 6300-6308.

Crocoll, C., Asbach, J., Novak, J., Gershenzon, J. and Degenhardt, J. (2010). Terpene synthases of oregano (*Origanum vulgare* L.) and their roles in the pathway and regulation of terpene biosynthesis. *Plant Mol. Biol.* **73**: 587-603.

Dimitrijeviĺ, S. I., Mihajlovski, K. R., Antonoviĺ, D. G., Milanoviĺ-Stevanoviĺ, M. R. and Mijin, D. Ǧ. (2007). A study of the synergistic antilisterial effects of a sub-lethal dose of lactic acid and essential oils from *Thymus vulgaris* L.*, Rosmarinus officinalis* L. and *Origanum vulgare* L. *Food Chem.* **104**: 774-782.

Franz, C., Baser, K. H. C. and Windisch, W. (2010). Essential oils and aromatic plants in animal feeding ï a european perspective. A review. *Flavour Fragrance J.* **25**: 327-340.

Friedman, M., Henika, P. R., Levin, C. E. and Mandrell, R. E. (2004). Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157 : H7 and *Salmonella enterica* in apple juice. *J. Agr. Food Chem.* **52**: 6042-6048.

García-García, R., López-Malo, A. and Palou, E. (2011). Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against *Listeria innocua*. *J. Food Sci.* **76**: M95-M100.

Giordani, R., Hadef, Y. and Kaloustian, J. (2008). Compositions and antifungal activities of essential oils of some algerian aromatic plants. *Fitoterapia* **79**: 199-203.

Goulas, A. E. and 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 Chem.* **100**: 287-296.

Govaris, A., Solomakos, N., Pexara, A. and Chatzopoulou, P. S. (2010). The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella enteritidis* in minced sheep meat during refrigerated storage. *Int. J. Food Microbiol.* **137**: 175-180.

Guarda, A., Rubilar, J. F., Miltz, J. and Galotto, M. J. (2011). The antimicrobial activity of microencapsulated thymol and carvacrol. *Int. J. Food Microbiol.* **146**: 144-150.

Gumus, T., Demirci, A., Sagdic, O. and Arici, M. (2010). Inhibition of heat resistant molds: *Aspergillus fumigatus* and *Paecilomyces variotii* by some plant essential oils. *Food Science and Biotechnology* **19**: 1241-1244.

Gutierrez, J., Barry-Ryan, C. and Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int. J. Food Microbiol.* **124**: 91-97.

Gutierrez, J., Barry-Ryan, C. and Bourke, P. (2009). Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiol.* **26**: 142-150.

Gutiérrez, L., Escudero, A., Batlle, R. N. and Nerín, C. (2009). Effect of mixed antimicrobial agents and flavors in active packaging films. *J. Agric. Food Chem.* **57**: 8564-8571.

Handl, S., Hellweg, P., Khol-Parisini, A., Rossmann, B., Thurner, K., Luf, W., Novak, J. and Zentek, J. (2008). Effect of oregano (*O. majorana* and *O. vulgare*) on performance and antioxidative capacity of quails fed a diet rich in ɤ 3 fatty acids. *J. Anim. Physiol. Anim. Nutr.* **92**: 242-245.

Houhoula, D. P., Oreopoulou, V. and Tzia, C. (2004). Antioxidant efficiency of oregano in frying and storage of fried products. *Eur. J. Lipid Sci. Technol.* **106**: 746-751.

HSA (2005). Oregano & marjoram. from http://herbsociety.org/.

Hussain, A. I., Anwar, F., Rasheed, S., Nigam, P. S., Janneh, O. and Sarker, S. D. (2011). Composition, antioxidant and chemotherapeutic properties of the essential oils from two *Origanum* species growing in pakistan. *Revista Brasileira de Farmacognosia* **21**: 943-952.

İşpek, E., Tüylü, B. A. and Zeytinoĵlu, H. (2003). Effects of carvacrol on sister chromatid exchanges in human lymphocyte cultures. *Cytotechnology* **43**: 145-148.

Jin, P., Wu, X., Xu, F., Wang, X. L., Wang, J. and Zheng, Y. H. (2012). Enhancing antioxidant capacity and reducing decay of chinese bayberries by essential oils. *J. Agr. Food Chem.* **60**: 3769-3775.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M. and Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. *Bioresour. Technol.* **99**: 8788-8795.

Kotsiou, K., Tasioula-Margari, M., Kukurová, K. and Ciesarová, Z. (2010). Impact of oregano and virgin olive oil phenolic compounds on acrylamide content in a model system and fresh potatoes. *Food Chem.* **123**: 1149-1155.

Lagouri, V., Bantouna, A. and Stathopoulos, P. (2010). A comparison of the antioxidant activity and phenolic content of nonpolar and polar extracts obtained from four endemic *Lamiaceae* species grown in greece. *J. Food Process. Preserv.* **34**: 872-886.

Lambert, R. J. W., Skandamis, P. N., Coote, P. J. and Nychas, G. J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* **91**: 453-462.

Loizzo, M. R., Menichini, F., Conforti, F., Tundis, R., Bonesi, M., Saab, A. M., Statti, G. A., Cindio, B. d., Houghton, P. J., Menichini, F. and Frega, N. G. (2009). Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. Essential oils. *Food Chem.* **117**: 174-180.

Lopez-Reyes, J. G., Spadaro, D., Gullino, M. L. and Garibaldi, A. (2010). Efficacy of plant essential oils on postharvest control of rot caused by fungi on four cultivars of apples *in vivo*. *Flavour Fragrance J.* **25**: 171-177.

Mahmoud, B. S. M., Yamazaki, K., Miyashita, K., Shik, S. I., Dong-Suk, C. and Suzuki, T. (2004). Bacterial microflora of carp (*Cyprinus carpio*) and its shelf-life extension by essential oil compounds. *Food Microbiol.* **21**: 657-666.

Martínez-Rocha, A., Puga, R., Hernández-Sandoval, L., Loarca-Piña, G. and Mendoza, S. (2008). Antioxidant and antimutagenic activities of mexican oregano (*Lippia graveolens* Kunth). *Plant Food Hum. Nutr.* **63**: 1-5.

Martinez-Romero, D., Guillen, F., Valverde, J. M., Bailen, G., Zapata, P., Serrano, M., Castillo, S. and Valero, D. (2007). Influence of carvacrol on survival of *Botrytis cinerea* inoculated in table grapes. *Int J Food Microbiol.* **115**: 144-148.

Mechergui, K., Coelho, J. A., Serra, M. C., Lamine, S. B., Boukhchina, S. and Khouja, M. L. (2010). Essential oils of *Origanum vulgare* L. sp. *glandulosum* (Desf.) Ietswaart from

Tunisia: Chemical composition and antioxidant activity. *J. Sci. Food Agric.* **90**: 1745-1749.

Mehdi, S. J., Ahmad, A., Irshad, M., Manzoor, N. and Rizvi, M. M. A. (2011). Cytotoxic effect of carvacrol on human cervical cancer cells. *Biology and Medicine* **3**: 307-312.

Moschonas, G., Geornaras, I., Stopforth, J. D., Wach, D., Woerner, D. R., Belk, K. E., Smith, G. C. and Sofos, J. N. (2012). Activity of caprylic acid, carvacrol, epsilon-polylysine and their combinations against salmonella in not-ready-to-eat surface-browned, frozen, breaded chicken products. *J Food Sc.* **77**: M405-M411.

Navarro, D., Diaz-Mula, H. M., Guillen, F., Zapata, P. J., Castillo, S., Serrano, M., Valero, D. and Martinez-Romero, D. (2011). Reduction of nectarine decay caused by *Rhizopus stolonifer*, *Botrytis cinerea* and *Penicillium digitatum* with *Aloe vera* gel alone or with the addition of thymol. *Int. J. Food Microbiol.* **151**: 241-246.

Nostro, A., Roccaro, A. S., Bisignano, G., Marino, A., Cannatelli, M. A., Pizzimenti, F. C., Cioni, P. L., Procopio, F. and Blanco, A. R. (2007). Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *Journal of Medical Microbiology.* **56**: 519-523.

Numpaque, M. A., Oviedo, L. A., Gil, J. H., Garcia, C. M. and Durango, D. L. (2011). Thymol and carvacrol: Biotransformation and antifungal activity against the plant pathogenic fungi *Colletotrichum acutatum* and *Botryodiplodia theobromae*. *Tropical Plant Pathology* **36**: 3-13.

Ntzimani, A. G., Giatrakou, V. I. and Savvaidis, I. N. (2010). Combined natural antimicrobial treatments (EDTA, lysozyme, rosemary and oregano oil) on semi cooked coated chicken

meat stored in vacuum packages at 4 degrees C: Microbiological and sensory evaluation. *Innov. Food Sco. Emerg.* **11**: 187-196.

Olmedo, R. H., Asensio, C., Nepote, V., Mestrallet, M. G. and Grosso, N. R. (2009). Chemical and sensory stability of fried-salted peanuts flavored with oregano essential oil and olive oil. *J. Sci. Food Agr.* **89**: 2128-2136.

Oral, N., Vatansever, L., Sezer, C., Aydin, B., Guven, A., Gulmez, M., Baser, K. H. C. and Kurkcuoglu, M. (2009). Effect of absorbent pads containing oregano essential oil on the shelf life extension of overwrap packed chicken drumsticks stored at four degrees Celsius. *Poultry Science.* **88**: 1459-1465.

Oral, N. B., Vatansever, L., Aydin, B. D., Sezer, C., Guven, A., Gulmez, M., Baser, K. H. C. and Kurkcuoglu, M. (2010). Effect of oregano essential oil on biofilms formed by staphylococci and *Escherichia coli*. *Kafkas Universitesi Veteriner Fakultesi Dergisi.* **16**: S23-S29.

Ortega-Nieblas, M. M., Robles-Burgueno, M. R., Acedo-Felix, E., Gonzalez-Leon, A., Morales-Trejo, A. and Vazquez-Moreno, L. (2011). Chemical composition and antimicrobial activity of oregano (*Lippia palmeri* S. Wats*)* essential oil. *Rev Fitotec. Mex.* **34**: 11-17.

Ortega Morente, E., Abriouel, H., López, R. L., Ben Omar, N. and Gálvez, A. (2010). Antibacterial activity of carvacrol and 2-nitro-1-propanol against single and mixed populations of foodborne pathogenic bacteria in corn flour dough. *Food Microbiol.* **27**: 274-279.

Oussalah, M., Caillet, S., Salmieri, S., Saucier, L. and Lacroix, M. (2004). Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *J Agr. Food Chem.* **52**: 5598-5605.

ÖZcan, M. M. and Chalchat, J. C. (2009). Chemical composition and antimicrobial properties of the essential oil of *Origanum saccatum* L. *J. Food Saf.* **29**: 617-628.

Ozkan, G., Baydar, H. and Erbas, S. (2010). The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of turkish oregano (*Origanum onites* L.). *J. Sci. Food Agric.* **90**: 205-209.

Perez-Alfonso, C. O., Martinez-Romero, D., Zapata, P. J., Serrano, M., Valero, D. and Castillo, S. (2012). The effects of essential oils carvacrol and thymol on growth of *Penicillium digitatum* and *P. italicum* involved in lemon decay. *Int. J. Food Microbiol.* **158**: 101-106.

Pozzatti, P., Loreto, É. S., Nunes Mario, D. A., Rossato, L., Santurio, J. M. and Alves, S. H. (2010). Activities of essential oils in the inhibition of *Candida albicans* and *Candida dubliniensis* germ tube formation. *Journal of Medical Mycology.* **20**: 185-189.

Pyrgotou, N., Giatrakou, V., Ntzimani, A.and Savvaidis, I. N. (2010). Quality assessment of salted, modified atmosphere packaged rainbow trout under treatment with oregano essential oil. *J. Food Sci.* **75**: M406-M411.

Ravishankar, S., Jaroni, D., Zhu, L., Olsen, C., McHugh, T.and Friedman, M. (2012). Inactivation of *Listeria monocytogenes* on ham and bologna using pectin-based apple, carrot, and hibiscus edible films containing carvacrol and cinnamaldehyde. *Journal of Food Science.* **77**: M377-M382.

Rivero-Cruz, I., Duarte, G., Navarrete, A., Bye, R., Linares, E. and Mata, R. (2011). Chemical composition and antimicrobial and spasmolytic properties of *Poliomintha longiflora* and *Lippia graveolens* essential oils. *J. Food Sci.* **76**: C309-C317.

Rosato, A., Vitali, C., Piarulli, M., Mazzotta, M., Argentieri, M. P. and Mallamaci, R. (2009). *In vitro* synergic efficacy of the combination of nystatin with the essential oils of *Origanum vulgare* and *Pelargonium graveolens* against some *Candida* species. *Phytomedicine* **16**: 972-975.

Royo, M., Fernández-Pan, I. and Maté, J. I. (2010). Antimicrobial effectiveness of oregano and sage essential oils incorporated into whey protein films or cellulose-based filter paper. *J. Sci. Food Agric.* **90**: 1513-1519.

Ryan, E., Aherne, S. A., O'Grady, M. N., McGovern, L., Kerry, J. P. and O'Brien, N. M. (2009). Bioactivity of herb-enriched beef patties. *J. Med. Food.* **12**: 893-901.

Sagdic, O., Ozturk, I. and Tornuk, F. (2013). Inactivation of non-toxigenic and toxigenic *Escherichia coli* O157:H7 inoculated on minimally processed tomatoes and cucumbers: Utilization of hydrosols of *Lamiaceae* spices as natural food sanitizers. *Food Control.* **30**: 7-14.

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J. A. and Roncales, P. (2003). Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties packaged in modified atmosphere. *J. Food Sci.* **68**: 339-344.

Santoro, G., das Graças Cardoso, M., Guimarães, L., Salgado, A., Menna-Barreto, R. and Soares, M. (2007). Effect of oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.)

essential oils on trypanosoma (protozoa: *Kinetoplastida*) growth and ultrastructure. *Parasitol. Res.* **100**: 783-790.

Sari, M., Biondi, D. M., Kaâbeche, M., Mandalari, G., D'Arrigo, M., Bisignano, G., Saija, A., Daquino, C. and Ruberto, G. (2006). Chemical composition, antimicrobial and antioxidant activities of the essential oil of several populations of algerian *Origanum glandulosum* Desf. *Flavour Frag J.* **21**: 890-898.

Shan, B., Cai, Y. Z., Sun, M. and Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agric. Food Chem.* **53**: 7749-7759.

Silva, N. C. C. and Fernandes, A. (2010). Biological properties of medicinal plants: A review of their antimicrobial activity. *J. Venom. Anim. Tox. incl. Trop. Dis.* **16**: 402-413.

Slamenova, D., Horvathova, E., Chalupa, I., Wsolova, L. and Navarova, J. (2011). *Ex vivo* assessment of protective effects of carvacrol against DNA lesions induced in primary rat cells by visible light excited methylene blue (VL plus MB). *Neoplasma* **58**: 14-19.

Soylu, S., Yigitbas, H., Soylu, E. M. and Kurt, ķ. (2007). Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. *J. Appl. Microbiol.* **103**: 1021-1030.

Tajkarimi, M. M., Ibrahim, S. A. and Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control* **21**: 1199-1218.

Tholl, D. (2006). Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr. Opin. Plant Biol.* **9**: 297-304.

Tibaldi, G., Fontana, E. and Nicola, S. (2011). Growing conditions and postharvest management can affect the essential oil of *Origanum vulgare* L. sp. *hirtum* (Link) Ietswaart. *Ind Crop Prod* **34**: 1516-1522.

Ultee, A., Bennik, M. H. J. and Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.* **68**: 1561-1568.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J. and Perez-Alvarez, J. A. (2010). Effect of orange dietary fibre, oregano essential oil and packaging conditions on shelf-life of bologna sausages. *Food Control.* **21**: 436-443.

Vernin, G., Lageot, C., Gaydou, E. M. and Parkanyi, C. (2001). Analysis of the essential oil of *Lippia graveolens* HBK from el salvador. *Flavour Fragrance J.* **16**: 219-226.

Yaldiz, G., Sekeroglu, N., Ozguven, M. and Kirpik, M. (2005). Seasonal and diurnal variability of essential oil and its components in *Origanum onites* L. grown in the ecological conditions of cukurova. *Grasas Aceites* **56**: 254-258.

Yanishlieva, N. V., Marinova, E. M., Gordon, M. H. and Raneva, V. G. (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem.* **64**: 59-66.

Wang, C. Y., Wang, S. Y. and Chen, C. (2008). Increasing antioxidant activity and reducing decay of blueberries by essential oils. *J Agr Food Chem* **56**: 3587-3592.

Zivanovic, S., Chi, S.and Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *J. Food Sci.* **70**: M45-M51.

Table 1. Comparation of oregano in differte localities

Type of oregano	Locality	Composition	Reference
L. palmeri	Alamos and Puerto del Oregano (Sonora, Mexico)	*p*-cymene (22.37 and 14.25%), thymol (21.39 and 15.11%), *γ*-terpinene (6.69 and 4.23%), iso-aromandrene (16.7 and 0.62%), respectively.	(Ortega-Nieblas et al., 2011)
O. vulgare L. sp. *glandulosum*	Nefza, Bargou and Krib (North Tunisia)	*p*-cymene (36, 40 and 46%), thymol (32, 39 and 18%), *γ*-terpinene (24, 12 and 16%), and carvacrol (2, 2 and 15%), respectively	(Mechergui et al., 2010)
O. applii (criollo) and *O. majoricum* (mendocino),	La Consulta, Mendoza, Argentina	Thymol (33.8 and 12.9%) and carvacrol (both <0.1 %) contents	(Amadio et al., 2011)
Origanum ehrenbergii Boiss and *O. syriacum L.*	Baskinta Mountain, Lebanese	Thymol (19.6 and 24.7 %), 2-Isopropyl-1-methoxy-4-methylbenzene (14.9 and 7.9 %), carvacrol (6.7 and 17.6%) and p-cymene (16.1	(Loizzo et al., 2009)

		and 8.7%)	
P. longiflora and *L. graveolens*	Sierra de Real de Catorce, San Luis Potosi, Mexico	Carvacrol (18.36 and 13.48 %) and *p*-cymene (14.09 and 7.46%)	(Rivero-Cruz et al., 2011)
Origanum glandulosum Desf.	Bouandas/Bouandas/Sétif and El Guergueria/Hamman El Guergour/Sétif (Algeria)	Carvacrol (28.1 and 72.6%), thymol (44.6 and 18.5 %), *p*-cymene (10.8 and 1.7%)	(Sari et al., 2006)

Table 2. Different types of oregano and its antibacterial activity related to chemical constituents.

Oregano plants	Main constituents	Effective against	Test method	References
*O. vulgar*e	Carvacrol Thymol Cymenol Cymene Ŭ-Pinene	*S. enteritidis* *B. cereus* *E. coli* *L. monocytegenes* *P. aeruginosa* *C. albicans* NRRL 12983 *C. albicans* ATCC 14053 *C. albicans* ATCC 90028 *C. albicans* NRRL 22077 *C. albicans* ATCC 10231 *B. subitilis* *B. pumilis* *S. poona* *S. aureus*	Broth microdilution method Checkerboard method, Diffusion method Resazurin microtitre assays Germ tube inhibition	Govaris et al., 2010; Gutierrez et al., 2008; Dimitrijeviĺ et al., 2007; Rosato et al., 2009; Hussain et al., 2011; Pozzatti et al., 2010

		C. dubliniensis		
O. acutidens	*p*-Cymene Carvacrol	*P. vulgaris* S. *typhimurium* *E. cloacae* *S. aureus* *E. coli* *K. pneumoniae*	Disc diffusion assay	Cosge et al., 2009
O. compactum	Carvacrol Thymol Terpinene *p*-Cymene	*P. aeruginosa* *S. aureu*	Broth microdilution assay	Bouhdid et al., 2009
O. majorana	Terpinen-4-ol ɔ ï Terpinene *cis*-Sabinene-hydrate Thymol	*B. subtilis* *S. flexneri,* *S. aureus* *E. coli* *K.pneumoniae*	Disk diffusion Dilution method and modelling of fungal growth Checkerboard method	Busatta et al., 2008; Giordani et al., 2008; Gutierrez et

	p-Cymene 4-Thujanol	*S.* choleraesius *C. albicans* *B. cereus* *L. monocytegenes* *P. aeruginosa* *B. pumilis* *S. poona*	Resazurin microtitre assays	al., 2008; Hussain et al., 2011
O. acutidens	Carvacrol *p*-Cymene Linalool acetate Borneol ϐ-Caryophyllene	*A. alternata* *F. culmorum* *P. ultimum* *R. solani* *V. dahliae*	Contact assay	Kordali et al., 2008
L. graveolens	Carvacrol *o*-Cymene	*C. albican* *C. dubliniensis*	Germ tube inhibition	Pozzatti et al., 2010

Figure 1. Terpenes biosynthesis. GA-3P, glyceraldehyde-3-phosphate; DOXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; GGPP, geranyl geranyl diphosphate.

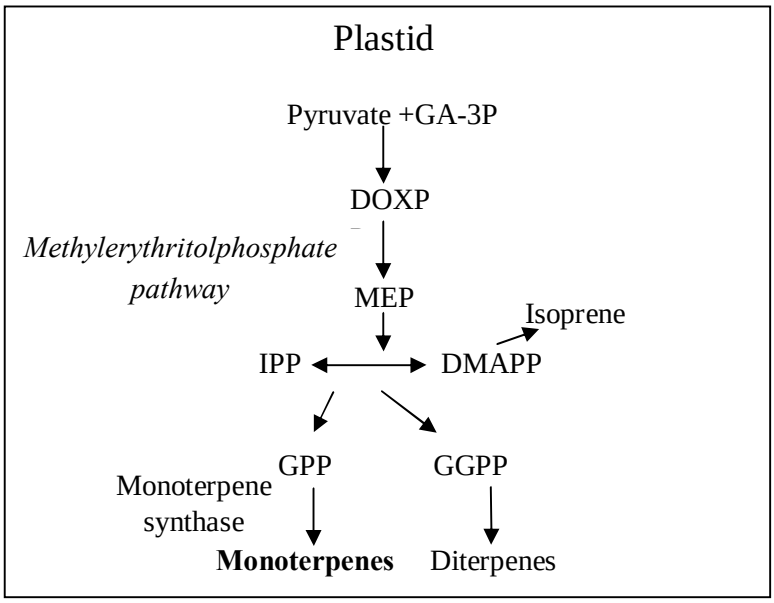

Figure 2. Free radical scavenging mechanism of carvacrol

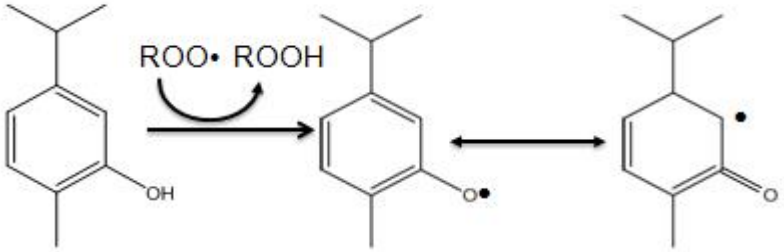

Figure 3. Antibacterial mechanism of thymol and carvacrol disintegrating the outer membrane, releasing cytoplasmatic constituents, and consequently changing the passive permeability of the cell