





Review

Essential Oils and Their Application on Active Packaging Systems: A Review

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Abstract: The food industry is continuously evolving through the application of innovative tools and ingredients towards more effective, safe, natural and ecofriendly solutions to satisfy the demands of the costumers. In this context, natural sources (i.e., leaves, seeds, peels or unused pulp) can entail a valuable source of compounds, such as essential oils (EOs), with recognized antioxidant and antimicrobial properties that can be used as natural additives in packaging applications. The current trend is the incorporation of EOs into diverse kinds of biodegradable materials, such as edible films, thus developing active packaging systems with improved preservation properties that can offer benefits to both the food and packaging industry by reducing food waste and improving the management of packaging waste. EOs may be added into the packaging material as free or encapsulated molecules, where, especially this last option, has been revealed as very promising. The addition of these lipophilic compounds provides to the end-product various bioactivities of interest, which can eventually extend the shelf-life of the product by preventing food spoilage. Pairing biodegradable packaging with EOs extracted from natural agro-industrial by-products can lead to a more sustainable food industry. Recent knowledge and advances on this issue will be reviewed in the present work.

Keywords: food waste; packaging; circular economy; essential oils; preservation



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1. Introduction

Packaging is essential for protecting food products from the environment and is intended to ensure food safety at the same time that industrial and consumer requirements are satisfied [1]. Researchers have investigated new advances on packaging systems, leading to the development of active packaging. This is one of the most promising fields in the packaging sector, which aims to prolong shelf life, ensure food quality and safety and improve product appearance [2]. Active packaging materials are characterized by incorporating components with biological properties that are slowly released into the food product [3]. According to the Regulation 1935/2004/EC and the Regulation 450/2009/EC, active materials in which the active packing is included are referred to as “materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food” [4,5]. Several examples of active packaging systems include oxygen and carbon dioxide scavengers, but also emitters of flavor, aroma and other compounds. Among these molecules, those that have biological properties are considered target compounds, since they can enhance the shelf-life of the food products [6,7].

Alternatively, numerous studies have demonstrated that plants and other natural sources such as leaves, seeds, peels or unused pulp from food industry, may be a valu-

able and efficient source of compounds such as polyphenols, flavonoids, tocopherols, pigments or essential oils (EOs) with biological properties [7–9]. All these molecules have been reported as potential candidates to be included in active packaging systems. In particular, EOs are well known for their bioactive molecules, volatiles and antioxidant and antimicrobial properties but their use has been limited as a consequence of their strong flavor [7].

EOs are secondary metabolites synthesized by plants. As many other secondary metabolites, they possess different bioactivities that have been employed for centuries by utilizing the whole plant or by the application of the EOs previously extracted. Traditionally, they have been used mostly for pharmacological, medicinal, aromatic or cosmetic purposes. However, from the 19th century, their application has been wider in the nutritional field [10]. Many different EOs have been characterized, even though the chemical profile depends on the species, the collection area and season or the extraction solvents and techniques. Their physical properties prompt their use in the food industry since they contain volatile aroma compounds, they are commonly liquid and colorless at room temperature, they are lipophilic and they can create emulsions with hydrophilic solvents. Further, they possess several bioactivities, such as antimicrobial, antifungal, antioxidant, antiviral, antiparasitic or insecticidal. Thus, these compounds have been used in the food industry as aroma and flavor ingredients, but also for preservative purposes [11–14]. The possibility of developing active packaging systems containing natural bioactive compounds such as EOs is a promising alternative since it can reduce food safety risks associated with chemically synthesized additives [1].

Currently, EOs application in active food packaging are strongly linked to their incorporation into biodegradable films in combination with another polysaccharide-protein- or lipid-based edible film [15]. Another recent approach is the development of composite films or multicomponent films to take advantage of their main beneficial properties [16].

Hence, the incorporation of EOs is increasingly common, because they can enhance antimicrobial and antioxidant activity, among others, or reduce water vapor permeability [17]. Particularly, when producing biofilms, hydrophilic matrices are usually used (for increasing water vapor permeability), formed by protein or polysaccharides polymers that serve as a base on which to incorporate other substances such as lipid components [18]. Ideally, for this purpose, the chosen materials should be of low viscosity, with high hygroscopicity and emulsifying capacity, low reactivity, low cost and with no effect on organoleptic properties of the processed food [19,20]. Specifically, lipophilic substances, such as EOs, incorporated into hydrophilic materials is performed by the application of emulsification or homogenization [21]. Thus, the association of EOs and biodegradable films is gaining more attention, especially by the active food packaging industry.

Therefore, the objective of this manuscript is to review the potential of using EOs in active packaging to extend food shelf life, preservation and other properties and also, to provide an overview of current trends in their use in packaging. Briefly, the methodology applied restricted bibliography to the last 5–10 years and the search of information followed the final structure of the manuscript using different key words such as “EOs”, “active packaging” or “food applications”.

2. Application of Essential Oils in Food Preservation and Packaging Sector

Most of the permitted food additives are applied for their preservation properties, attributed to their recognized bioactivities. Additives with antimicrobial properties can control food spoilage and/or prevent contamination by foodborne pathogens, including acetic, malic, lactic, benzoic, sorbic and propionic acids, potassium and calcium acetates, carbon dioxide, benzoates, sorbates, propionates, nitrites, nitrates or parabens. The most used additives that prevent food browning, caused by chemical or enzymatic reactions, are sulfites. Even though all these compounds are permitted to be used in the food industry, the current trend is replacing chemically synthesized compounds for natural ones. In this sense, the use of EOs is considered as an alternative to the use of synthetic additives [3,22].

In active packaging, the packaging materials can incorporate components with biological properties destined to be slowly released into the food [3] (Figure 1). The use of EOs in appropriate amounts may improve the water vapor barrier property and also provide antioxidant and antimicrobial activity to the packaging films.

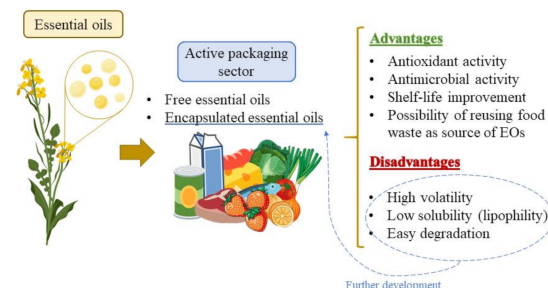








Figure 1. Essential oils (EOs) application strategies in the food active packaging sector: advantages and disadvantages.

In fact, the antimicrobial properties of EOs (together with antioxidant activity) have been analyzed in several studies where they have been found to be efficient against a wide range of food-borne pathogens [23,24]. Particularly, the antimicrobial effects of EOs are frequently associated with their hydrophobic/lipophilic character that allows them to permeate through membranes and their layers [25]. Table 1 presents some of the most studied compounds detected in different EOs (terpenes, terpenoids, phenols, esters, aromatic compounds, aldehydes, lactones, ketones are within these molecules) which have been demonstrated to have antimicrobial effects [10]. These antimicrobial attributes have prompted their use in active packaging to preserve food quality and extend the shelf-life of the final product [26].

Table 1. Different classes of compounds present in EOs with reported antimicrobial activity, characteristics and examples of some of them. Based on [26–30].

Chemical Class	Characteristics	Examples	Example of Chemical Structure
Hydrocarbons	- Their basic structures are 5-carbon-based units (isoprenes) - They act as major compounds (90%)	Citronellol, limonene, α -pinene, camphor, myrcene, E- β -Ocimene	 (Limonene)
Phenols	- They include phenolic terpenoids - They are aromatic components among the most reactive	Carvacrol, eugenol, thymol, chavicol	 (Thymol)
Esters	- Pleasant smell	Eugenol acetate, geranyl acetate, linalyl acetate, bornyl acetate	 (Eugenol acetate)
Alcohols	- Pleasant aromas and no reported contraindications	Linalool, menthol, borneol, santalol, nerool, citronellol, generaniol, terpineol, pinocarveol	 (Linalool)
Volatile compounds	- They include aromatic compounds	<i>p</i> -cymene, γ -terpinene, camphene, etc.	 (<i>p</i> -cymene)
Aldehydes	- They are unstable and are oxidized easily - Derived from spices and fruits (aromatic compounds)	Benzaldehyde, citronellal, cinnamaldehyde, myrtenal, citral, citronellal	 (Citronellal)

Regarding natural antioxidants, most of them are phenolic compounds, which prevent oxidation through the scavenging of free superoxide and hydroxyl radicals. The mechanism of action of this reaction consists on a proton donation by the phenolic compound inducing their own oxidation, and then get stabilized their polarity by electron dislocation [31,32]. An example of this reaction is shown with carvacrol (a component highly present in EOs) as an antioxidant molecule (Figure 2).

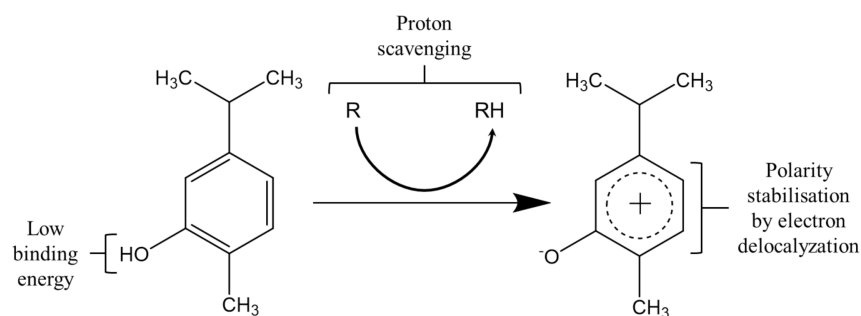


Figure 2. Carvacrol proton donation. This reaction results in the antioxidant bioactivity of phenolic compounds.

In vitro antioxidant capacity may be measured by determining the oxidation rate of several chemicals, like DPPH (2,2'-diphenyl-1-picrylhydrazyl), TBARS (2-thiobarbituric acid substances) or AAPH (2,2'-azobis(2-methylpropionamide) dihydrochloride) when exposed to potential antioxidants [33]. Regarding food oxidation spoilage, TBARS is a useful indicator to evaluate lipid oxidation, while other methods may include determination of metmyoglobin formation percentage in meats and free fatty acids content, which indicates the polyunsaturated fatty acids (PUFAs) oxidation degree [34,35]. Running these tests could provide a wider spectrum of information of the activities of the compounds of interest and how the oxidation of food products takes place.

While research often focuses on isolated phenolic compounds as potential additives (as they are well-known, potent antioxidants and generally account for low toxicity), these same compounds are present at proper concentrations in EOs extracted from plants and thus can be used to provide extra properties to biodegradable materials such as biofilms [36,37]. EOs do not only have the specific phenolic compounds of each species, but also valuable volatile and aromatic compounds [28].

In active packaging applications, EOs have been applied in different ways, free and encapsulated, both in non-degradable and biodegradable materials.

2.1. Free EOs Combined with Packaging Materials

Free EOs have been combined with different biodegradable materials, such as paper or edible films. Cardboard and paper have been traditionally used as packaging material but new packaging systems such as active cardboard tray coated with emulsions including encapsulated EOs are continuously being developed. These are widely used as food and vegetables packaging material (with waterproof layers) and are considered as an alternative to the high use of plastics by the packaging sector [38,39]. However, due to the water vapor permeability of these materials, it is convenient and necessary to apply a layer of some material that makes them resistant to the presence of water, vapors and gases. EOs are an attractive alternative for the creation of such films, due to their waterproofing properties and biological activities, turning packaging into an active material and increasing its value. For example, eugenol (a common component of EOs) was linked to cellulose by polycarboxylic acid to create a paper-based active packaging. This new material was used to pack wheat flour and other grain foods and prevented water absorption similarly to typical packaging paper and preserved the mechanical properties of the grain products, although ductility and tensile strength were reduced. Besides, it significantly provided insecticide

and insectifuge capacities, which extended the product shelf life without compromising its original flavor, taste and odor [40].

Regarding edible films, EOs are added into a hydrophilic matrix resulting in an aqueous dispersion. In this way, the lipid droplets become embedded in the matrix once the film is subjected to a drying process causing structural reorganizations of the hydrophilic components [41,42]. For instance, in a study performed on a chitosan-based film, as the formed dispersion loses water through drying, the concentration of lipid particles increases, which also increases the risk of emulsion phases separation, flocculation or loss of EOs. In particular, the stability of the film microstructure can be improved by application of microfluidization which entails a reduction of the droplet size and the viscosity of the aqueous phase, also resulting in an increase of the ζ -potential, which indicates that the emulsion can be stable against aggregation events [43]. Few studies have displayed the advantages of using EOs as an additional ingredient directly added to the edible films or biodegradable trays to enhance their packaging properties. *Syzygium aromaticum* EOs were incorporated into two kinds of edible films, one elaborated with gelatin and other with a mixture of gelatin and chitosan. Both materials containing the EOs showed significant anti-bacterial activity, especially against Gram-negative bacteria, when tested in chilled fish samples [44]. Another work used cassava bagasse and polyvinyl alcohol to create biodegradable trays containing EOs from *Origanum vulgare* or *Eugenia caryophyllata* (*Syzygium aromaticum*). The EOs were incorporated into the trays in two ways: directly, by adding them into the mixture of ingredients or by coating the surfaces with EOs. The best results were achieved when trays were coated with the highest amount of EOs, showing inhibitory effects against different bacterial and fungi species [45]. Other biodegradable films containing EOs have been tested in different food matrices such as fruits (i.e., strawberries), prolonging their shelf-life but also preventing or reducing the growth of bacterial colonies and acting as fungicides [46]. Additionally, the active biodegradable films containing encapsulated EOs showed no cytotoxicity while they permitted to maintain the sensorial properties of the food products during storage [47].

2.2. Encapsulation of EOs

Encapsulation is an affordable and ideal technique to protect EOs from limiting factors, including oxidative processes, photodegradation, thermal conditions or high relative humidity. Encapsulation also provides a controlled release of the EO from the capsule which can last up to 45 days [48]. Encapsulation also favors the incorporation of fats in the different matrices utilized for creating packaging materials, since it reduces the negative interactions between the lipid phase and the matrix, whose nature is normally hydrophilic. Therefore, the use of encapsulated lipophilic molecules prevents the alteration of the properties of the final structure of the packaged product [49]. In addition, this technique is useful to prevent changes in the organoleptic properties of the food product caused by EOs and other lipids [39,50].

Basically, capsules can be classified into three large groups according to their size: macrocapsules, microcapsules and nanocapsules. The latter present a diameter between 0.05 and 1 μm , although the most common average measure is between 0.1 and 0.5 μm [51]. The use of capsules with diameters in the nanoscale range is more convenient since the particle size is inversely proportional to the stability, that is, the smaller the diameter of the capsules, the greater the stability during incorporation into the matrices. Likewise, a better control of its availability and release is also achieved, favoring the creation of active packaging systems that prolong food shelf-life. Active packaging can be developed by the direct incorporation of the nanocapsules into the matrix of the packaging material or they can be emulsified in an aqueous base to make a waterproofing barrier to cover the package [22,52–54].

Several studies have assessed the suitability of EOs' encapsulation and different encapsulation materials. For instance, rosemary EO was successfully encapsulated in a benzoic acid–chitosan nanogel that was lately included in the starch–carboxy methyl

cellulose film [55]. Other study used zein to synthesize a film containing microencapsulated rose hip seed EOs and the protective properties of this material were evaluated in bananas and cumquats. Negative controls, in both kinds of fruit, showed discoloration and signs of rotting with storage time, but when packaged in zein-rose hip EO film, spoilage slowed down [56]. Lectin was employed to encapsulate different concentrations of *Thymus zygis* EO (0.25 to 0.5 g/g polymer) to optimize the EO retention. The best approach provides a 45–55% of EO retention which improved the antifungal activity of the film, its water barrier capacity, gloss and resistance [57]. Cyclodextrins, which have been extensively studied in recent years, have been used to encapsulate EOs. A work presented a successful solution for the encapsulation of EOs extracted from coriander by combining them with cyclodextrins. This mixture allowed the encapsulation of the coriander EO, which becomes further stabilized by their inclusion in dextrin-derived nanosponges. This final product permitted the development of a controlled release mechanism stable at high temperature. Thus, the encapsulation of the EO allowed extending the action time of the bioactivities associated to the major biocompounds present in coriander [58]. Another study analyzed the encapsulation efficiency of different concentrations of cinnamon EO (0.5 to 3 g) and β -cyclodextrin (1 to 3 g). Then, an electro spun nanofibrous film was developed using the electrospinning polyvinyl alcohol to which it was added β -cyclodextrin and cinnamon EO. When this film was tested with strawberries showed a prolonged shelf-life of the product while maintaining its sensorial properties during storage. The shelf-life of strawberries was similar to that achieved with common plastic film but the nanofibrous film presented two additional features: it was non-toxic and biodegradable [46]. EOs are a current field of study in the food packaging sector that could compete with the actual materials of food packaging for its enormous versatility and possibilities that need to be more explored.

Conserving the bioactivities of EOs presents a challenge that needs to be addressed. The concentration of EOs extracts needed to maintain their bioactivities in the food could alter the organoleptic characteristics of the food making it less attractive to the consumer. This makes the use of EOs at industrial scale difficult. Furthermore, the use of encapsulated EOs has shown some physical modifications such as plasticizing effect or tensile and color properties variations [3,59–61].

3. Current Trends on the Application of EOs in the Food Industry

Currently there is a growing interest in the production of active packaging using renewable and environmentally friendly materials [62]. Innovative active packaging has been shown to extend the shelf life of food products and reduce the growth rate of certain microorganisms. These improvements can be achieved through the formation of biocomposite systems based on natural biopolymers or synthetic materials.

Some articles have stated that edible packaging has gained popularity within consumers, since an edible film or coating, apart from prolonging the shelf-life of the food product, can also be consumed together with the food [26]. On the other hand, other studies have highlighted that few cases of films can be really eaten (whereas others can only be more easily degraded) and that consumers will only accept edible films if they feel them as safe [63]. These packaging systems can incorporate components intended to be immobilized at the film, released into food or able to absorb substances responsible for spoilage [64]. Among other things, packaging protects food from dehydration and acts as a gas barrier from the surrounding media. Edible films can serve as carriers of active compounds such as antimicrobials, antioxidants, texture enhancers or key nutrients, among others [65]. Furthermore, these properties can be enhanced by incorporating active substances to the films such as EOs.

EOs can be incorporated in food and beverage packaging systems (edible films) as an additional ingredient directly added to edible films or encapsulated into the edible films. For instance, different amounts of EOs from *Origanum vulgare* and *Eugenia caryophyllata* were incorporated into cassava bagasse–polyvinyl alcohol-based trays. EOs were incorporated into the trays in two ways, directly by adding them into the mixture of ingredients at

proportions 6.5, 8.5 and 10.0% (*w/w*) or by coating the surface with EOs at concentrations 2.5, 5.0 and 7.5 g oil/100 g tray. The best results were achieved when trays were coated with the highest amounts of EOs, especially for *O. vulgare* that displayed total inhibition of molds, yeasts and few Gram-positive and negative bacteria, showing total inhibition or important reduction of the bacterial viability [45].

Hence, EOs exhibit antimicrobial and antioxidant characteristics while they are compatible with several types of food, contributing to the increase of food products shelf life or enhancing their organoleptic properties [10,43]. In the United States, several EOs that have already been used in food packaging have been recognized as safe ingredients and included in the Generally Recognized as Safe (GRAS) category established by the Food and Drug Administration (FDA) [17]. However, it must also be considered that they are chemically unstable and easily oxidized, being sensitive to light, oxygen and temperature changes. In this sense, nanoencapsulation techniques can be used to improve EOs chemical stability and enhance their physicochemical capabilities, thus allowing their use in food packaging [66]. For example, fish oil was encapsulated by emulsion electrospinning into polyvinyl alcohol nanofibers. The encapsulation efficiency for high omega-3 fatty acids ($92.4 \pm 2.3\%$) provided an oil load capacity of $11.3 \pm 0.3\%$ and thus, an even distribution of oil into the fibers and fortification of the food matrix with less amounts of PUFAs [67]. The type and amount of EOs incorporated into the packaging material will provide specific biological activity [68].

Some of the most commonly EOs used in packaging systems have been extracted from jasmine, rosemary, peppermint, cinnamon, oregano, thyme, cumin, eucalyptus, rosewood, clove, tea tree, palmarosa, geranium, lavender, lemongrass, mandarin, bergamot or lemon. With respect to the food matrixes on which packaging systems with EOs have been used, different food products such as fresh meat, butter, fresh octopus, ham and fish can be found [3]. Regarding their major identified components, they belong to the hydrocarbon monoterpenes, such as α -pinene, β -pinene, α -selinene and p-cymene, or to the oxygenated monoterpenes group, such as thymol, carvacrol, geraniol, borneol, eugenol, linalool, terpineol-4-ol, 1,8-cineole, α -terpinyl acetate and camphor [13–15,49]. Another study evaluated variable concentrations of some major hydrocarbon and oxygenated monoterpenes present in EOs. These compounds applied at a concentration of 0.2 $\mu\text{g}/\text{mL}$ and a pH 4.0 showed antibacterial effect against *E. coli* and *L. monocytogenes*. This work underlined that oxygenated monoterpenes exerted more effective antimicrobial effect than hydrocarbon ones. In fact, the efficiency of these molecules was evaluated in orange or apple juice in combination with heat treatments and displayed synergistic lethal effect against *E. coli*. [14].

Thus, the aim of food packaging regarding biodegradable materials in combination with EOs is to perform antioxidant and antimicrobial assays to evaluate the final packaging system in contact with the food matrix to provide robust results. Table 2 summarizes some of the recent assays using films with incorporated EOs for new packaging systems in the food industry.

Table 2. Recent examples of active films containing EOs as main constituents showing beneficial properties for the packaged food product.

Film	EOs	Food	Main Results	Ref.
Gelatin	Orange (<i>Citrus sinensis</i> (L.) Osbeck) EO	Pink shrimp (<i>Parapenaeus longirostris</i>)	Antioxidant and antimicrobial activity Extension of shelf-life of nearly 10 days	[69]
Gelatin-	Oregano EO	Refrigerated Rainbow Trout Fillets	Lowering of total volatile basic nitrogen, peroxide value, thiobarbituric acid and microbial growth	[70]

Table 2. Cont.

Film	EOs	Food	Main Results	Ref.
Gelatin-carboxymethyl cellulose-chitin nanofibers-	<i>Trachyspermum ammi</i> EO (Ajowan)	Refrigerated raw beef	Growth inhibition of <i>Pseudomonas</i> spp., <i>S. aureus</i> , lactic acid bacteria (LAB), molds and yeasts. Stability of the chemical profile, color and sensory properties	[71]
Curdlan-PVA	<i>Thyme</i> EO	Chilled meat	Improvement of antioxidant activity and extension of the shelf life	[72]
Chitosan-cassava starch	<i>Myrcia ovata</i> Cambessedes EOs	Mangaba fruits	Antimicrobial effect against <i>Bacillus cereus</i> , <i>B. subtilis</i> and <i>Serratia marcescens</i>	[73]
Chitosan	Cumin EO nanoemulsion	Refrigerated beef loins	Growth inhibition of mesophilic and psychrophilic bacteria, <i>Enterobacteriaceae</i> and LAB. Antioxidant effects.	[74]
Chitosan	Clove EO	Cooked pork sausages	Microbial growth inhibition, retarded lipid oxidation and shelf-life extension when refrigerated storage	[75]
Chitosan beads	Lavender or red thyme EOs	Strawberry (clamshell)	Antifungal effect against <i>Botrytis cinerea</i> . Maintenance of appearance, color and firmness but odor, flavor and overall acceptability decrease	[76]
Chitosan and whey protein	Garlic EO nanoencapsulated	Vacuum-packed sausages	Retarded lipid oxidation and growth inhibition of main spoilage bacterial	[77]
Whey protein isolate	Oregano and garlic EOs	Kasar cheese slices	Antimicrobial effect against <i>Escherichia coli</i> , <i>Salmonella enteritidis</i> , <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> and <i>Penicillium</i> spp.	[78]
Whey protein isolate-cellulose nanofibers + TiO ₂ nanoparticles	Rosemary EO	Refrigerated lamb meat	Increase of the shelf life and antimicrobial effect against <i>Pseudomonas</i> spp., <i>Enterobacteriaceae</i> , LAB, <i>S. aureus</i> , <i>L. monocytogenes</i> and <i>E. coli</i>	[79]
Pectin	Oregano EO + resveratrol nanoemulsion	Fresh pork loin	Lowering pH effect, color change, retarded lipid and protein oxidation, microbial growth inhibition under high oxygen modified atmosphere packaging	[80]
Sodium alginate	Citral and Eugenol EOs	<i>Arbutus unedo</i> L. Fresh fruit	Improvement of postharvest quality attributes during storage. Reduction of microbial growth	[81]
Sodium caseinate	Ginger EO nanoemulsion	Chicken breast fillets	Growth inhibition of total aerobic psychrophilic bacteria	[82]
Arabic gum- sodium caseinate	Cinnamon or lemongrass EO	Guava fruit	Browning and related enzymes decrease, higher acceptability, antioxidant activity and high content of phenolic compounds	[83]

3.1. Antimicrobial Activity of EOs in Food Systems

To understand the interest on the enhancement of antimicrobial activity of packaging, some facts are needed to be mentioned. First, numerous investigations have been carried out to improve the quality and safety of products during storage without using synthetic additives and opting for other alternatives which are claimed to maintain their nutritional and organoleptic properties while controlling food-borne pathogens. However, packaging is still needed to avoid microbial contamination. The application of EOs to biodegradable materials (edible films) show potential for solving these problems since they could avoid or lower the use of synthetic preservatives [3,66,84]. Table 3 presents EOs with an antimicrobial effect against specific foodborne pathogenic bacterial species (*Campylobacter jejuni*,

Escherichia coli, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*). In a study of Settanni et al., 2012, it was stated that lemon EO was able to inhibit the 42 strains of *L. monocytogenes* with a minimum inhibitory concentration (MIC) of 0.156 $\mu\text{L}/\text{mL}$ and the 35 strains of *S. aureus* with a dose of 0.039 $\mu\text{L}/\text{mL}$ [9]. Another study revealed that the bactericidal activity of cinnamon with respect to *E. coli*, *S. enterica*, *C. jejuni* and *L. monocytogenes*, was more effective than the EOs of oregano and eugenol [85]. A recent study analyzed the antimicrobial activity of EOs obtained from different sources such as *Syzygium aromaticum* L., *Foeniculum vulgare* Miller, *Cupressus sempervirens* L., *Lavandula angustifolia*, *Thymus vulgaris* L., *Verbena officinalis* L., *Pinus sylvestris* and *Rosmarinus officinalis*. Data showed that the most effective EO was the one extracted from *S. aromaticum* L., followed by EOs from *R. officinalis* and *L. angustifolia*, while *P. sylvestris* and *F. vulgare* Miller displayed the poorest antimicrobial activity [44]. In the same experimental work, they analyzed the antibacterial effect of *S. aromaticum* L. EO when incorporated into two kinds of edible films, one based on gelatin and the other based on gelatin-chitosan. The antibacterial activity of the edible films containing *S. aromaticum* L. EO was demonstrated against *E. coli*, *Lactobacillus acidophilus*, *Listeria innocua* and *Pseudomonas fluorescens* [44]. Another study found that an edible film whey protein based with incorporated oregano or clove EOs was able to protect chicken breast fillets against food spoilage microorganisms such as aerobic mesophilic and psychrotrophic bacteria, LAB and *Pseudomonas* spp. [86].

Table 3. EOs and EO components effective against some common food-borne bacteria. Information collected from [9,26,78,79,85,87].

EOs/Components	Bacterial Species				
	CJ	EC	LM	SE	SA
Cinnamaldehyde, thymol, carvacrol, perillaldehyde, eugenol, estragole	+	+	+	+	
Oregano, cinnamon, thyme, bay leaf, allspice, clove bud oils		+	+	+	
Palmarosa oil, salicylaldehyde, geraniol, isoeugenol		+		+	
Ginger root, marigold, jasmine, carrot seeds, celery seeds, mugwort, spikenard, orange bitter oil, benzaldehyde, citral, carvone R, geranyl acetate	+				
Lemongrass oil, citral		+			
Patchouli, gardenia, cedarwood oils	+		+		
Citral, geraniol, carvone S, salicylaldehyde			+		
Marjoram oil, terpineol				+	
Lemon EO			+	+	+
Melissa and lemon oils, terpineol, geraniol, linalool				+	
Rosemary EO		+	+		+
Oregano and garlic EOs		+	+	+	+

CJ: *Campylobacter jejuni*; EC: *Escherichia coli*; LM: *Listeria monocytogenes*; SE: *Salmonella enterica*; SA: *Staphylococcus aureus*; +: active against bacterial species.

Another comparative study analyzed some hydrocarbons and oxygenated monoterpenes commonly present in EOs extracted from eucalyptus, lavender, orange, peppermint, pine and rosemary. Among others, α -pinene, β -pinene and p-cymene, thymol, carvacrol, borneol, linalool, terpineol-4-ol, 1,8-cineole, α -terpinyl acetate and camphor were analyzed in terms of their antimicrobial capacity. They all showed bactericidal effect (0.2 $\mu\text{g}/\text{mL}$ and pH 4.0) against *E. coli* and *L. monocytogenes* and oxygenated monoterpenes were more effective than hydrocarbon monoterpenes [14].

However, the in vitro antimicrobial capacity of EOs is not always maintained when incorporated into in real food products. This is due to the interactive effects of several factors that are characteristic of food. Different factors can modify the interaction between food and the biomolecules embedded in packaging such as the pH, water activity,

additives, salt, fat and protein content, specific of each kind of food product, and also extrinsic determinants, such as the temperature of storage and the composition of the atmosphere [88].

3.2. Antioxidant Activity of EOs in Food Systems

Food spoilage is mainly a result of reactions that take place when food comes in contact with oxygen or through the action of spoilage microorganisms [89]. Oxidative damage is caused by the interactions of reactive oxygen species (ROS) with any compound susceptible to be oxidized and suffer structural changes in the process. Superoxide radicals (O_2^{2-}), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) are classified under the category of ROS [90]. Yet, ROS also exert several undesirable and significant changes in foods such as limiting their shelf-life, lowering nutritional value, loss of color, flavor and odor. All these changes result in, for example, food rancidity [37]. The major parameter responsible for rancidity is the oxidation and peroxidation of fatty acids and other fats present in foods. In packed foods, this process is generally considered to be initiated by change in metals' oxidation state already present in food and/or exposure to light containing ultraviolet (UV) (such as sunlight), which leads to subsequent hydrogen abstraction from fats and in turn, causes successive reactions in adjacent fat molecules [91]. As a side effect of this process, ketones and toxic aldehydes may also be produced [89]. Moisture also plays a determinant role in oxidation, as it may provide ROS derived from the water content of the food product. Additionally, as oxidative reactions take place during time, several molecules (sugars, short chain fatty acids) become more easily available to spoilage organisms and thus, the food surface becomes more favorable to them. In the same way, foods are susceptible to oxidation or enzymatic activity and ROS liberation. Thus, oxidative damage and microbial growth are the most significant causes of food spoilage [3].

One strategy to avoid the interaction between ROS and foods is the exclusion of oxygen using thermoplastic films in packaging [18]. However, this method generates non-degradable packaging waste and many new laws are being proposed to reduce or permanently ban these single-use plastics [92]. New alternatives are being explored to replace the use of synthetic antioxidant compounds. In this context, the incorporation of EOs to films could solve both issues, the reduction of plastic packages and the lowering use of synthetic additives.

The use of hydrophobic oleic films, in combination with other polymers, to coat food products has been demonstrated to significantly delay oxidation [93]. Hence, different vegetable matrices known for their antioxidant properties have been tested as potential new sources of EOs that could be combined with other compounds to develop new active food films. Therefore, EOs of savory spices and herbs, which are known to present high levels of phenolic compounds, are of special interest to be used in active food packaging, since they also add desirable flavor and aromas [94]. Some of the most prominent are oregano, thyme, rosemary, cinnamon or basil, whereas also isolated compounds from varying sources may be included into the packaging film, like α -tocopherol or β -carotene [3,94]. Recent findings are presented in Table 4 where the antioxidant capacity induced by the combination of different films and EOs (with major compounds identified when possible) was assessed in different food matrices.

For instance, in fish skin gelatin films loaded with different citrus EOs, it was found that water vapor permeability was reduced until $2.81 \times 10^{-11} \text{ gm}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, whereas antioxidant capacity was also higher in the films with EOs, showing better results for DPPH, FRAP and ABTS assays. In this particular case, the EOs were mixed with Tween 20 at 25% and then incorporated into film solution at 50% [12,95]. The transfer of EOs from the package is highly influenced by the quantity and distribution of fats present in specific food products. The diffusion of the EOs compounds from packaging is greater towards the lipid matrices. However, foods of high fat content will have an uneven distribution of EOs compounds, since EOs can get trapped in the lipid fraction of the food instead of being distributed uniformly [96]. Likewise, higher lipid content on the part of the packaging

material manages to retain the preservative compounds for a longer time. In these cases, migration occurs more slowly, achieving a gradual, prolonged and efficient diffusion [97].

Table 4. Antioxidant capacity evaluated using different food matrixes by the combination of diverse films and EOs (implicated bioactive compounds identified when possible) through several antioxidant assays.

Food	Film Polymers	Identified Compounds	Source of EOs	Antioxidant Assays	Ref.
Lamb	Polyethylene/polyamide	-	Oregano, rosemary	TBARS, metmyoglobin	[98]
Foal	Polyethylene	r-cymene, d-limonene, camphor, borneol, thymol, carvacrol, [...]	Oregano	TBARS, metmyoglobin	[99]
Beef	Soy protein	-	Oregano, thyme	DPPH, TBARS, Rancimat	[100]
-	Rice starch/fish muscle protein	-	Oregano	DPPH	[101]
Smoked sardine	Gelatin (pigskin)/chitosan	tPC	Oregano, rosemary	FRAP, FFA	[102]
-	Quince seed mucilage	tPC	Oregano	DPPH	[103]
-	Hake muscle protein	Propenyldisulfide, thymol, carvacrol [...]	Oregano, clove, garlic	DPPH	[104]
Pork sausage	-	-	Rosemary	TBARS	[105]
Poultry	Chitosan/montmorillonite	-	Rosemary	TBARS	[106]
Eel	CMC	tPC, rosmarinic acid, isorhamnetin-3-O- hexoside [...]	Rosemary	DPPH	[107]
Swordfish	LDPE/polyamide	Carvacrol, thymol	Rosemary	TBARS [...]	[108]
-	Quince seed mucilage	tPC	Thyme	DPPH	[109]
Chicken sausage	LPDE/nylon	tPC	Thyme, clove	DPPH, TBARS	[110]
Cut peach	Pectin	Eugenol, cinnamaldehyde [...]	Cinnamon	DPPH	[111]
Pork	Chitosan/LDPE	-	Cinnamon	POV, TBARS	[112]
Sweet pepper	Chitosan	-	Cinnamon	TBARS, catalase and peroxidase activity	[113]
Rainbow trout	Gelatin	-	Cinnamon	FFA, TBARS	[114]
Rainbow trout	Chitosan	-	Cinnamon	POV, TBARS	[115]
-	Whey protein	tPC, tFC	Rosemary, cinnamon, basil	ABTS, β -carotene bleaching	[116]
Oat, linoleic acid	HDPE, LDPE, EVA	Sesamol	Sesame	Hexanal retention	[117]
Barley soup	-	DL-Limonene, γ -terpinene, tri-cyclene [...]	Lemon peel	DPPH	[118]
Grapes	Chitosan/glycerol	tPC	Bergamot	DPPH	[119]
-	Chitosan/gelatin	β -carotene	Commercial isolate	DPPH	[120]
Sunflower oil	Cassava starch, LDPE	β -carotene	Carrots	POV	[121]
-	Polyethylene/polypropylene	α -tocopherol	Commercial isolate	DPPH, ABTS	[122]
Salmon	LDPE	α , γ , δ -tocopherols	Commercial isolate	DPPH, TBARS	[123]
-	LDPE/polypropylene	α , γ , δ -tocopherol	Soybean	Conjugated dienes	[124]
Butter	Chitosan/glycerol	α -tocopherol	Commercial isolate	DPPH	[125]

-: Not described; [...]: Others; tPC: total phenolic compounds measured, but not determined; tFC: total flavonoids content; DPPH: 2,2'-Diphenyl-2-picrylhydrazyl-hydrate free radical assay; TBAR: 2-thiobarbituric acid reductive value assay; FRAP: ferric reducing antioxidant power assay; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid assay; FFA: free fatty acid determination; POV: peroxide value; CMC: carboxy-methyl-cellulose; EVA: ethylene vinyl acetate; LDPE: low density polyethylene; HDPE: high density polyethylene.

Oregano EO is well known for its antiseptic properties and may be the most extensively researched herb regarding this field. So, many different studies have been carried out with diverse blends and films in order to test the effectiveness of its preserving properties [126,127]. Research assessing this EO has been conducted on lamb, foal or beef meat,

reporting significant delays in oxidation (2–4 days) compared with control samples. The activity of oregano EO has also been tested in edible blends such as starch, animal protein or cellulosic fibers [101,103]. Moreover, it seems that some blends with just 1% of oregano EO exert antioxidant and antimicrobial activities [127,128]. Similar results have been obtained with thyme. This could be explained because thyme and oregano share thymol and carvacrol as their major phenolic compounds [100]. Thyme EO has been applied for example to beef and swordfish meats [100,108]. Cinnamon EO has also been researched because of its documented bioactive properties, independent of the blend used, and very popular flavor [113]. Cinnamon EO has been tested to delay decomposing and oxidation in fresh fruit and vegetables [46,111,113] and pork or fish meats [112,114,115,129]. In the case of rosemary EO, it has been noted that the phenolic compound rosmarinic acid, a very effective antioxidant, is present at high levels. Hence, its antioxidant potential has been researched upon with several meats [98,105] and fish [102,107]. Notably, rosemary EO has even shown higher antioxidant activity than conventional antioxidants like butylated hydroxytoluene (BHT) [36,105].

Given the evidence supported by these reports, EOs should be considered as adequate antioxidants, which in turn would give added value to the herbs or organic waste from which they can be extracted. However, despite the antioxidant activity of EOs, they must be combined with other materials not to allow oxygen passage [91]. Several biodegradable and/or edible biopolymers like starch films, cellulosic fibers or chitosan are being profiled as suitable alternatives to thermoplastics [130], while animal-derived polymers like gelatin (collagen matrix) or muscle protein are being considered as well [95,131]. Moreover, the conjugation of these EOs with different polymer nanoparticles or formulated in emulsions seems to be a promising choice for coating foods, aside of the packaging film employed [132].

3.3. Flavors and Aromas Transference in Active Food Packaging

Flavors and aromas are added to food to enhance their taste and odor, but in the case of active packaging applied with EOs they are not incorporated for enhancing food properties. In fact, the strong and intense odor of EOs might end up transferring some unwanted taste to the packed food when used with preservation purposes. Further, they contain volatile components that are easily evaporated. Hence, the nanoencapsulation of EOs represent a useful technique to mask their odor and flavor and prevent their evaporation. The encapsulation enhances flavor properties and reduces EOs impact on the organoleptic characteristics of the food. It also improves EOs solubility in water-soluble polymers and provides effective releasing properties and better distribution [21]. Thus, nanoencapsulated materials need to have a low reactivity with EOs and no influence on the organoleptic characteristics of the packed food. For instance, eugenol applied to a cellulose material was used to pack grain foods. It preserved the organoleptic properties of the original product while improving the insecticide properties of the package [40]. Cinnamon EO was also included into a polyvinyl alcohol nanofibrous films that allowed masking its intense flavor while providing antibacterial activity against Gram-positive and Gram-negative bacteria. This strategy is effective in extending the shelf-life of quickly perishable fruits, like strawberries [14]. Furthermore, depending on the properties of the compounds or EOs to be nanoencapsulated, different materials can be used. Lately, the use of biopolymers and biocomposites has been addressed as useful materials for their environmentally friendly characteristics: their biocompatibility, their low toxicity and their biodegradability [21].

4. Legislation of EOs in Food

The legal framework that concerns the use of EOs in food involves two approaches, the use of EOs as a food ingredient, that represent an additional nutrient to ingest, or the use of EOs as part of the packaging. In the first scenario, EOs must obey food legislation applied in each region. Depending on the aim of its application, different legislations will apply. EOs will be considered additives when they represent a “substance not normally

consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods" [5]. In the Annex III of the Commission Regulation (EU) No 1130/2011, rosemary extracts are considered as additives. However, no other references to some of the main components of EOs have been found as additives [133]. In the second case, when EOs are treated as flavoring substances, they are considered "products not intended to be consumed as such, which are added to food in order to impart or modify odor and/or taste; or products made or consisting of the following categories: flavoring substances, flavoring preparations, thermal process flavorings, smoke flavorings, flavor precursors or other flavorings or mixtures thereof" [134]. However, in the list of flavoring substances provided by Regulation (EC) No 2232/96, few of the major compounds identified in EOs have been found, such as eugenol, carvacrol, thymol or terpineol, among others [135]. Nevertheless, no limits or indications are presented in this document since it suggests consulting the additional information into EFSA's Compendium of Botanicals. The aim of this compendium is to provide information to improve the safety assessment of botanical products and preparations intended for use in food [136]. Nonetheless, the use of botanicals and derived preparations in food has to comply with the general requirements set out in Regulation (EC) No 178/2002, which lays down the general principles and requirements of food law in the EU [137].

The second scenario includes the treatment of EOs as part of the material intended to contact food. In this case, the developed material has to mainly comply with the Regulation (EC) 1935/2004 that regulates the materials and articles intended to come into contact with food [138] and the Regulation (EC) 2023/2006 that complements the previous one, since it regulates the good manufacturing practices for materials and articles intended to come into contact with food [139]. In the case of United States, the FDA collects in the Code of Federal Regulations a list of EOs, oleoresins and natural extracts for their intended use. Additionally, FDA has recognized some EOs as flavoring agents in food such as linalool, thymol, eugenol, vanillin, carvacrol and limonene [140,141].

5. Conclusions

EOs have been demonstrated to exert different biological activities although antioxidant and antimicrobial properties are the most highlighted. Thus, they could prevent the deterioration caused by oxidation and microbial spoilage in food products. Considering this, they could be incorporated into active packaging materials. In fact, several studies have evaluated the effectiveness of using EOs, as part of the active packaging material, in very different foods matrices. Regarding the different application methods, encapsulation is considered as a suitable technique since it reduces several disadvantages of EOs.

At last, vegetable sources can be considered as reservoirs of bioactive compounds, in some cases EOs. The extraction, obtaining and incorporation of these EOs into new packaging systems such as biodegradable films could be an alternative option in food packaging industry. According to the revised literature, some lines need to be further studied and developed: more studies are necessary to evaluate the effects of adding EOs into new packaging systems regarding their mechanical, organoleptic and biological properties as well as to ensure their safety and lack of side effects for the consumers and the environment. Finally, market studies are strongly recommended before the commercialization of new active packaging materials and the use of EOs to know the acceptance of consumers to these new products.

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Abbreviations

Generic

EO(s)	Essential oil(s)
ROS	Reactive oxygen species
UV	Ultraviolet
FDA	Food and Drug Administration
GRAS	Generally recognized as safe
MIC	Minimum inhibitory concentration
LAB	Lactic Acid Bacteria

Compounds

PUFAs	Polyunsaturated fatty acids
HDPE	High density polyethylene
LDPE	Low density polyethylene
CMC	Carboxymethyl cellulose
BHT	Butylated hydroxytoluene
PLA	Polylactic acid

Techniques

tPC	Total phenolic compounds
tFC	Total flavonoids content
FFA	Free fatty acid determination
POV	Peroxide value
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical assay
TBARS	2-thiobarbituric acid reductive value assay
FRAP	Ferric reducing antioxidant power assay
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid assay

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