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Biopolymers carrying essential oils, or their compounds, for food antimicrobial packaging

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Abstract: Biodegradable antimicrobial materials for food packaging applications are in great demand by the food industry and society alike for the purposes of extending food shelf life, thus reducing the environmental impacts associated with synthetic plastics. Among the natural and non-toxic active compounds available, essential oils and their major components have been widely studied due to their antioxidant and antimicrobial properties together with their Generally Recognized as Safe status. In this review, the antimicrobial and antioxidant properties of several essential oils and their major compounds are summarized, as well as their action when included in different biopolymer-based matrices. Both the method of incorporating active ingredients into the biopolymer matrix and the yield of such processes as a function of the technique used (casting methods or thermoplastic processing) are also assessed. The effect of active compounds on the film's functional properties is reviewed, as well as the effective release of the active ingredients into different food systems and food simulants, as affected by polymer-active interactions and the nature of the food. Finally, the antimicrobial action of some of these active compounds (embedded in different biopolymer matrices) is also discussed both in *in vitro* studies and in antimicrobial tests performed using foods of different composition.

Keywords: Biopolymer, essential oil, antimicrobials, controlled release, food shelf life, mechanical properties, barrier properties.

1. INTRODUCTION

One of present-day society's challenges is to develop biodegradable active materials for food packaging applications in order to improve the shelf-life of food and, at the same time, reduce the environmental impacts associated with synthetic plastics. Biopolymers from different origins can be used for food packaging applications or food coating purposes, but the functional properties of biopolymer-based materials in terms of their mechanical and barrier properties need to be adapted to food requirements. To this end, numerous studies have been carried out applying different strategies to reduce the drawbacks of using biopolymers for packaging purposes. Polar biopolymers, such as starch, chitosan, cellulose derivatives or proteins, exhibited high water sensitivity and the water plasticization effects lead to a worsening of the mechanical and barrier properties. On the other hand, more hydrophobic matrices, such as biodegradable polyesters, exhibited brittleness and great oxygen permeability, which in turn limit their ability to control the oxidation reactions responsible for food deterioration. Of the different strategies used to adapt these materials to food packaging requirements, polymer blending with or without compatibilizers [1, 2], extrusion reaction using a catalyst to favour crosslinking [3] or multilayer films combining materials with complementary barrier properties, have been analysed [4].

Likewise, the incorporation of antimicrobial or/and antioxidant compounds into biopolymer-based materials is a good approach to obtain active films, with more competitive properties, useful to extend the shelf life of foodstuffs. The release kinetics of actives into different food systems need to be evaluated in order to analyze the effectiveness of these materials for food preservation, adapting them to specific target applications thus helping to enhance food quality and safety. Of the available natural and non-toxic active compounds, essential oils and their major components have been widely studied, due to their antioxidant and antimicrobial properties together with their Generally Recognized as Safe status.

In this review, the antimicrobial properties of several essential oils and their major compounds are summarized, as well as their action when included in different biopolymer-based matrices. Both the method of incorporating active ingredients into the biopolymer matrix and the yield of such processes, depending on the technique used (casting methods or thermoplastic processing), are also discussed. Likewise, the effect of active compounds on the film's functional properties is reviewed, as well as the factors affecting the effective release of the active ingredients into different food systems or food simulants, depending on the polymer-active interactions and the nature of the food. Finally, the antimicrobial action of some of these active compounds, embedded in different biopolymer matrices, when applied to foods of different composition is also discussed and compared with the results obtained in *in vitro* studies.

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2. ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS OR THEIR COMPOUNDS

The antimicrobial properties of plant ESs have been widely studied. Both EOs and several of their constituents represent a natural alternative to chemical food preservatives. EOs are complex in composition, being a

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source of bioactive compounds, such as terpenoids and phenolic compounds. It has long been recognized that EOs exhibit antioxidant [5, 6] and antimicrobial properties. Several antimicrobial mechanisms have been described to explain the action of EOs against microorganisms, namely membrane destabilization and disruption [7, 8]; the inhibition of membrane localized metabolic events [9, 10]; damage in the membrane proteins [11]; the depletion of proton motive force [12]; and the leakage of cytoplasmic constituents, metabolites and ions [13, 14]. Table 1 gives an overview of the current state of the art in this topic, showing some of the most recent and relevant studies dealing with the antimicrobial action of EOs and their major compounds. These studies reinforce the potential use of EOs and their major components as food preservatives due to their capacity to inhibit the growth of a wide variety of pathogenic and/or food-spoiling microorganisms.

Moreover, a synergistic effect between EOs and their major components has recurrently been observed in different studies. Ouedrhiri et al. [28] found that the antibacterial capacity of an EOs mixture (*Origanum compactum, Origanum majorana* and *Thymus serpyllum*) was greater than that of each essential oil, especially against *S. Aureus* and *E. Coli*. Ye et al. [33] concluded that cinnamaldehyde and carvacrol exhibit a high degree of antibacterial activity and display synergistic antimicrobial activity for most of the bacteria tested (Table 1).

Nonetheless, the concentrations of EOs or their individual constituents required to inhibit bacteria in foods are frequently higher than what is organoleptically acceptable [35, 36]. Moreover, the highly volatile nature of these compounds, along with their great sensitivity to oxidation, which makes them prone to deterioration, limits their free-form application onto the surface of food systems. In an attempt to avoid this, what has been studied is the incorporation of EOs into the formulation of films and coatings. Table 2 summarizes recent studies of the antimicrobial activity of biopolymer films into which either EOs or their individual major compounds have been incorporated. A wide variety of EOs has been tested in different carrying biopolymers, and the *in vitro* studies, using the agar diffusion method, have proven their efficiency as antimicrobials. They mainly act against different bacteria, although they are also reported to exhibit antifungal activity [5, 50, 58, 61].

There have been fewer studies into real food systems, although several authors report an effective or limited antimicrobial action, depending on the kind of food matrix. For instance, Guerreiro et al. [52] incorporated eugenol and/or citral into alginate or pectine films, and proved their antimicrobial activity when applied to raspberries. However, Higueras et al. [62] could not find any notable antilisterial action of carvacrol on chicken breast, despite its proven effectiveness in *in vitro* studies. Interactions of active compounds and microorganisms were greatly affected by food components [63], as described below.

3. INCORPORATION METHODS OF EOs OR THEIR COMPOUNDS INTO POLYMER MATRICES

In order to develop biodegradable antimicrobial packaging systems incorporating EOs, the processability of the biopolymers and of the active ingredients should be taken into account. Casting methods or thermoplastic processing could be used to incorporate active compounds into the polymer matrices to obtain active biomaterials for packaging applications. Nevertheless, the former method has scarce industrial applicability because of the need to evaporate great amounts of solvent. In the case of aqueous systems, this is energetically non-sustainable and for organic solvents, the fact that they are toxic and dangerous to handle compromises their application. So, this kind of active compound incorporation is restricted to coating the surface of thermo-processed films with a thin layer of the active solution and carrier. Then, smaller amounts of solvent need to be evaporated, thus enhancing the process feasibility. However, the solvent casting method has been widely used to obtain biopolymer active matrices that incorporate essential oils or their compounds, at a laboratory level, for the purposes of obtaining the functional properties of the biopolymer matrices and their potential use as antimicrobial/antioxidant materials [57, 64, 65]. Casting methods involve solving the biopolymer in an appropriate medium and adding the active ingredient under continuous stirring conditions. When using aqueous media and hydrophilic biopolymers, the solvent-polymer-EO mixture forms immiscible systems (emulsions) and requires a high degree of homogenization to achieve a good dispersion with small droplets. Rotor-stator homogenizers, sonication [66] or microfluidization [65] has been used to this end. The latter can increase the stability of the film-forming dispersion by reducing its particle size. Finally, to obtain the active matrices, the dispersion containing the active ingredients is cast in a levelled plate and is allowed to dry under controlled conditions.

The casting/solvent evaporation method exhibits some advantages for the incorporation of active ingredients, but also significant drawbacks, depending on the nature of the polymer (polar or non-polar), its solubility in different solvents and the chemical affinity between the polymer, EO compounds and solvent. Hydrophobic non-polar biopolymers, such as biodegradable polyesters (like polylactic acid: PLA) have to be solved in non-polar organic solvents, such as dichloromethane (DCM) or chloroform, which require special precautions during film processing and the use of a fume hood. In these cases, the EO compounds gain solubility and true solutions of the macromolecules and active compounds can be obtained.

Notable differences in films' microstructure and losses of EO can be found in films obtained by casting method, depending on the polymer and solvent polarity, and aqueous and non-polar systems can be differentiated. Figure 1 shows the cross section of a chitosan film where droplets of the non-miscible cinnamon EO are embedded in the polymer matrix, introducing discontinuities which affect both the mechanical behaviour and mass transfer phenomenon through the film. On the other hand, essential oil compounds encapsulated in lecithin liposomes produced a laminar structure in chitosan films in line with the restructuration (phase transition) of the lecithin during the film drying step

Table 1: Recent studies on the antimicrobial action of essential oils and their major compounds. Minimal inhibitory concentration (MIC) ranges have been included.

EO	Microorganisms tested	MIC	Reference
Myrcia ovata Cambessedes	*Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Enterecoccus faecalis, Serratia marcescens, Escherichia coli, Salmonella enteritidis*	0.78-400 μl/ml	[15]
Etlingera fimbriobracteata	*Bacillus subtilis, Bacillus spizizenii., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Saccharomyces cerevisiae*	0.15-625 μg/ml	[16]
Bergamot-mint (Mentha citrata)	*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, Pseudomonas aerugenosa*	125->1000 μg/ml	[17]
Cinnamon bark	*L. monocytogenes, Salmonella enterica and E. coli O157:H7*	313-625ppm	[18]
Thyme		313-625ppm	[18]
Eucalyptus globulus	*S. aureus, B. subtilis, L. innocua, E. coli, P. aerugenosa*	3-5 mg/ml	[19]
Thymus	*Penicillium digitatum, Penicillium italicum and Geotrichum citri-aurantii*	<500->4000 μg/ml	[20]
Mustard	*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Escherichia coli OI57:H7, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida, Pectobacterium carotovorum, Salmonella enterica subsp. enterica*	12.5-200 μg/ml	[21]
Cinnamon		200-400 μg/ml	[21]
Origanum vulgare	*Staphylococcus aureus, Escherichia coli, Sarcina lutea, Bacillus cereus, Pseudomonas aeruginosa, Salmonella typhimurium, Enterococcus faecalis, Candida albicans*	85->512 μg/ml	[22]
Mentha x piperita L.	*Streptococcus pyogenes, Streptococcus mutans, Lactobacillus acidophilus, Streptococcus salivarius, Streptococcus sangunis, Enterecoccus feacalis, Pseudomonas aeruginosa, Staphylococcus aureus*	238-1125 μg/ml	[23]
Mentha pulegium L.		593-1192 μg/ml	[23]
Lavandula angustifolia Mill.		160-2333 μg/ml	[23]
Satureja montana L.		23-125 μg/ml	[23]
Salvia lavandulifolia Vahl		2901208 μg/ml	[23]
Thymus numidicus	*Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa*	0.117-0.469 mg/ml	[24]
Salvia officinalis L.,		3.7-15 mg/ml	[24]
Lime	*E. coli, S. typhinurium, B. cereus, S. aureus, L. monocytogenes*	750-1500 mg/l	[25]
Cinnamon	*L. monocytogenes, E. coli, Ps. Fluorescens, L. plantarum, L. sakei*	250-500ppm	[26]
Satureja montana L.	*Salmonella*	0.39-0.78 mg/ml	[27] a
Thymus vulgaris L		0.78-1.56 mg/ml	[27]
Rosmarinus officinalis L.		12.5-25 mg/ml	[27]
Origanum compactum	*Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*	0.031->4 %(v/v)	[28]
Origanum majorana		0.125-2%(v/v)	[28]
Thymus serpyllum		0.125->4%(v/v)	[28]
Rosmarinus	*C. perfringens*	10 mg/ml	[29]

***officinalis* L. (rosemary),**			
***Mentha* × *piperita* L. var. Piperita (peppermint)**		10 mg/ml	[29]
***Origanum majorana*L. (marjoram),**		5 mg/ml	[29]
***Ocimum basilicum* L. (basil)**		5 mg/ml	[29]
***Thymus vulgaris* L. (thyme)**		1.25 mg/ml	[29]
***Pimpinella anisum* L. (anise)**		10 mg/ml	[29]
Carvacrol, eugenol, β-resorcylic acid, transcinnamaldehyde, thymol and vanillin	*E. coli O157:H7*	-	[30]
Eugenol	Coliforms, *Staphylococcus spp.*	-	[31]
Eugenol	*E. coli, K. pneumoniae*	249–999μg/ml 0.063-0.999μg/ml	[32]
Cinnamaldehyde		122-245μg/ml	[32]
Cinnamaldehyde	*E. coli S. aureus Y. regensburgei S. intermedius K. kristinae L. garvieae S. sanguinis E. cloacae S. haemolyticus A. hydrophila S. enteritidis*	0.16-0.63 mg/ml	[33]
Carvacrol		0.16-0.31 mg/ml	[33]
Thymol	*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Vibrio alginolyticus, Vibrio alginolyticus, Salmonella typhimurium, Salmonella typhimurium, 12 Salmonella enteritidis* strains	32-128 μg/ml	[34]
Carvacrol		64-512 μg/ml	[34]
Eugenol	*L. monocytogenes, Salmonella enterica, E. coli O157:H7*	625 ppm	[18]

Table 2: Recent studies on the antimicrobial action of essential oils and their major components embedded in biopolymer matrices. In vitro test (IVT) in culture media or in different food systems have been included.

Antimicrobial	**Carrying biopolymer**	**Concentration (g/g polymer)**	**Microorganisms**	**In vitro test (IVT) /food application**	**Reference**
Oregano	Alginate	0-1/1	*E. coli, S. Enteritidis, S. aureus, L. monocytogenes*	IVT: agar diffusion	[37]
Thyme, sage, lemongrass	Alginate	1/3	*E coli*	IVT: E. coli inactivation	[38]
Eucalyptus globulus	Chitosan	0-4/2	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia Candida albicans, Candida parapsilosis*	IVT: agar diffusion	[39]
Cinnamon	Chitosan	0-1/1	*E. coli O157:H7, S. enterica, L. monocytogenes*	IVT: agar diffusion and liquid medium	[18]
Maqui Berry (Aristotelia chilensis)	Chitosan	0-1/2	*Listeria innocua, Serratia marcescens, Aeromonas hydrophila, Achromobacter denitrificans, Alcaligenes faecalis, Pseudomonas fluorescens, Citrobacter freundii and Shewanella putrefaciens CECT 5346*	IVT: agar diffusion	[40]
Oregano bergamot	HPMC	0-2/4	*Escherichia coli*	IVT	[41]
Thyme	Chitosan	0.25-1:1	*S. marcenscens, A. hydrophila, A. faecalis , A. denitrificans, L. innocua*	IVT: agar diffusion	[6]
Lime	Pectic	-	*Escherichia coli O157:H7,*	IVT: agar diffusion	[25]

	extract		*Salmonella Typhimurium , Staphylococcusaureus, Bacillus cereus and Listeria monocytogenes*		
Cinnamon	Chitosan	0.4-2/2	*L. monocytogenes, E. coli, L. plantarum, L. sakei, P. fluorescens*	IVT: agar diffusion	[26]
Oregano	Mucilage	0-2/35	*L. monocytogenes, Salmonella typhimurium, Bacillus cereus, Yersinia enterocolitica, Pseudomonas aeruginosa, S. aureus, E. coli, E. coli O157:H7, Shewanella putrefaciens,and Vibrio cholera*	IVT: agar diffusion	[42]
Matricaria recutita	Sodium caseinate	1/5	*Listeria monocytogenes, S. aureus and E. coli O157:H7*	IVT: agar diffusion	[43]
Clove, fennel, cypress lavender, thyme herb-of-the-cross, pine and rosemary	Gelatin alone or with chitosan	0.75/1	*P. fluorescens, L. acidophilus, L. innocua, E. coli.*	IVT agar diffusion and application on fish	[44]
Citronella, coriander, tarragon, thyme	Hake protein	0.25/1	*B. thermosphacta, E. coli, L. innocua, L. monocytogenes, P. putida, S. typhimurium, S. putrefaciens*	IVT: agar diffusion	[45]
Oregano, bergamot	HPMC	0-0.1/1	*E. coli*	Application on plums	[41]
Thyme, oregano	Soy protein	0-5/5	*S. aureus, E.coli, P. aeruginosa, L. plantarum, total viable, lactic acid bacteria, Staphilococcus spp, coliforms, Pseudomonas spp*	IVT: agar diffusion and application on beef patties	[46]
Lemon	Chitosan	3/1	*Botrytis cinerea*	IVT and application on strawberry	[47]
Bergamot	HPMC, chitosan	2/1	Total aerobic mesophilic microorganisms, yeast and mould	Application on grapes	[48]
Bergamot, lemon, tea tree	HPMC, chitosan	0-3/1	*E. coli, L. monocytogenes, S. aureus*	IVT	[49]
Bergamot	Chitosan	0-3/1	*P. italicum*	IVT	[50]
Tea tree	Chitosan	0-2/1	*Penicillium italicum, L. monocytogenes*	IVT	[51]
Citral and eugenol	Alginate and pectin	0.05-0.15/1	Moulds and yeast, aerobic mesophilic	Application on raspberry	[52]
Cinnamaldehyde	PLA and PCL	9/1	Mesophilic and psychrophilic bacteria	Application on mushrooms	[53]
Thymol	Starch	-	*Listeria monocytogenes, Salmonella Typhimurium*	Application on cantaloupe juice	[54]
Cinnamaldehyde	PLA	0.02/0.8	*E. coli, Bacillus cereus*	IVT	[55]
Mandarin, lemon, sweet orange, hybrid of Oroval clementine x Tarocco orange	Chitosan, methylcellulose	1/2	*Listeria monocytogenes*	IVT	[56]
Chitosan	Basil, thyme	0-0.5/1	*Aspergillus niger, B. cinerea, R. stolonifer*	IVT	[57]
Chitosan	Cinnamon leaf	0-0.5/1	*Aspergillus niger,Botrytis cinerea, Rhizopus stolonifer*	IVT and application on strawberry	[58]

Starch-gelatin blend	Oregano, clove, cinnamon bark	0-0.25/1	*Colletotrichum gloesporoides, Fusarium oxysporum f.sp. gladiolo*	IVT	[59]
Chitosan	Eugenol, cinnamon leaf	0.476/1	*Escherichia coli*	IVT	[60]

[60]. During this step, the water loss induced the lamellar structure of the polar lipids, which alternates with the polymer layers giving rise to a high barrier material containing EO compounds inside lipid layers. On the contrary, a smooth fracture can be appreciated in PHBV films containing eugenol, due to its good miscibility with the polymer. In this way, eugenol plasticized the matrix and also affected the tensile and barrier properties due to the weakening effect on the polymer network. In semi crystalline matrices, these compounds can also affect the degree of crystallinity [67].

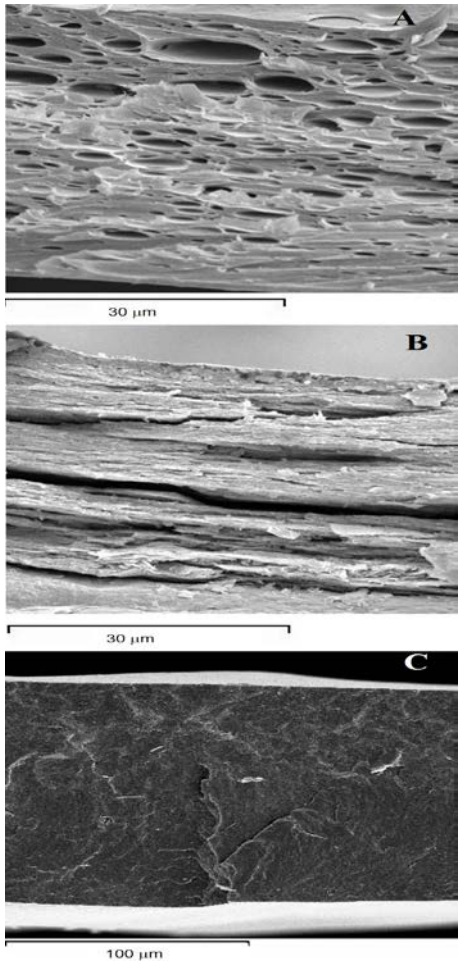

Figure 1. The film microstructure of different biopolymers containing EOs, or some of their compounds, obtained by different techniques. A: chitosan-cinnamon leaf EO film, obtained by casting. B: chitosan-lecithin encapsulated eugenol film, obtained by casting. C: PHBV-Eugenol, obtained by compression moulding.

On the other hand, using aqueous solvents leads to significant losses of the EO compounds due to a steam distillation effect at surface level during the film drying step. Throughout this step, EO droplets flocculate, coalesce and cream to the top of the drying film to a different extent, where EO compounds volatilize together with water. In this way, particular constituents of the EO are lost depending on their relative amount in the oil. In an aqueous solvent, the major compounds can lose over 50% of the EO added to the film-forming dispersion, depending on the emulsion stability and the total lipid creaming during film drying. Smaller droplets, higher surface particle charge and the interfacial adsorption of amphiphilic molecules, such as proteins or surfactants, enhanced emulsion stability and reduced the losses of EO during film drying [57]. Micro and nanoencapsulation of EO compounds before film preparation could be a way to minimize this problem and improve the effectiveness of active packaging containing essential oils. In this sense, the use of liposomes or nanoliposomes, which can act as carrier systems of a wide range of compounds, represent an interesting alternative [68]. Cyclodextrins have also been very effective at entrapping ES compounds when they are incorporated into the polymer matrices. A high ratio of carvacrol could be adsorbed in chitosan films containing cyclodextrins, depending on their glycerol and moisture content, when the films are immersed in the compound (the so-called *nanosponges*) [69].

Table 3 shows some examples of different methods used to incorporate essential oils into biopolymer matrices in order to develop antimicrobial packaging concepts. Materials produced by thermoplastic techniques that are used industrially for the purposes of processing synthetic plastics are usually dry mixed and afterwards extruded or melt blended to obtain thermoformable packaging products.

In the first step, the biopolymer is blended with the plasticizing additives and the active ingredients by using a hot mixer. The mixing temperature depends on the melting point of the biopolymer. Blending temperatures of 155ºC and 80ºC have been used for PLA and polycapralactone (PCL), respectively [83]. In the second step, also performed at the mixing temperature and at high pressure, a hot-plate hydraulic press can be used to obtain films or samples that are suitable for extrusion. EO compounds can also be incorporated in different zones of the screw in an extruder after polymer melting. Recently, Requena et al. [67] have shown that the use of spraying to incorporate EOs at the interface of two thermoplastic films and the subsequent thermo-compression of the films, could be an appropriate strategy with which to improve the process of obtaining active films containing EO and reduce the loss of active compounds.

Table 3. Incorporation methods of essential oils (EO) or EO compounds into biopolymer matrices.

Method	EO/Compound	Biopolymer	Reference
1.CASTING METHODS			
Dissolution in DCM	Cinnamon EO	PLA	[70]
Dissolution in chloroform	Oregano EO	PLA	[71]
	Cinnamaldehyde	PLA/PCL	[53]
	Oregano EO	PLA-CNC	[72]
	Cinnamaldehyde, allyl isothiocyanate,	PCL	[73]
	Eugenol	PHB	[74]
Dissolution in ethanol	Thymol	Zein	[75]
Emulsification in distilled water	Cinnamon EO	Agar/Alginate	[76]
Encapsulation with cyclodextrin and water dispersion	Eugenol and carvacrol	WPI	[77]
Encapsulation with soy oil and alginate and dispersion in aqueous medium	Lemongrass EO	Sodium alginate	[78]
Encapsulation in soy lecithin liposomes and dispersion in 1% aqueous acetic acid	Cinnamon leaf EO, eugenol	Chitosan	[60]
Dispersion in 1% aqueous acetic acid	Bergamot, oregano, basil, clove, EO, carvacrol	Chitosan	[48, 58, 65, 79]
2. THERMOPLASTIC PROCESSING			
Melt blending and compression moulding	Thyme EO	Wheat gluten	[80]
Melt blending and extrusion.	Thymol	PBS	[81]
Melt blending and compression moulding	Thymol	PLA	[82]
Spraying between thermocompressed layers plus thermo- compression	Oregano EO, carvacrol, clove EO, eugenol	PHBV	[67]

During thermal processing, a part of the EO compounds can evaporate or decompose, depending on the thermal conditions and their respective volatility and thermostability. The encapsulation of essential oils with compounds, such as cyclodextrins, can protect active ingredients against evaporation and thermal degradation, thus minimizing their loss. Cyclodextrins, which are starch derivatives with a ringed structure, are able to form inclusion complexes with volatile compounds that are held tight within the molecular structure of the cyclodextrins [84]. Loaded cyclodextrins with trans-2-hexenal were included in PLA matrices by extrusion in order to obtain antimicrobial sheets, which exhibited antifungal activity against several food spoilage fungi, despite the significant losses of antimicrobials occurring during thermal processing [85]. Likewise, Wen et al [86] incorporated cinnamon essential oil/beta cyclodextrin inclusion complex into PLA nanofibers via electrospinning technique. The thermal stability of cinnamon essential oil in the PLA matrix was significantly improved due to strong interactions between beta cyclodextrin and the essential oil. Moreover, the electrospun nanofilm showed antimicrobial activity against both Gram-positive and Gram-negative bacteria, and prolonged the shelf-life of pork meat stored at 25ºC.

4. IMPACT OF EOs ON THE FILM FUNCTIONAL PROPERTIES.

The EO compounds in the polymer matrices have different effects on the functional properties of the materials used for packaging purposes. The discontinuities in the matrix (droplets) or the weakening of chain interactions can provoke changes in the mechanical resistance and barrier capacity of the materials, depending on the film's microstructure and the interactions between compounds and biopolymer.

4.1. Barrier properties

Being mainly hydrophobic mixtures/compounds, EOs may be expected to improve the water barrier properties of biopolymer films, which are frequently based on hydrophilic materials, such as carbohydrates and proteins. This trend has been observed in several studies, as reported in Table 4. However, the opposite trend has also been repeatedly reported (Table 4), which suggests that the hydrophobic-hydrophilic balance is not the only factor to be taken into account when aiming to explain the effect of EOs on the barrier properties of biopolymer films. Essential oil incorporation in hydrophilic film matrices, such as polysaccharides or proteins, implies the occurrence of a dispersed phase disrupting the matrix homogeneity, and

giving rise to an emulsified film. In fact, the water vapour transfer phenomena through the biopolymer films is strongly dependent on the droplet size distribution in heterogeneous matrices. This, in turn, is mainly determined by the initial droplet size of the film forming aqueous dispersion, under determined homogenization conditions, and the emulsion destabilization phenomena taking place during the film drying step. Other factors, such as the physical state of the lipid and the interactions with the matrix, also play an important role. Fine lipid droplets, homogenously distributed in the film matrix, are most effective as water permeability reducers [94], since the high tortuosity factor in the continuous phase creates a great resistance to mass transfer. However, interactions of the EO compounds with the polymer chains may also limit chain aggregation in the film, leading to more open polymer matrices with less mass transfer resistance [65, 95].

In less polar polymer systems, such as biodegradable polyesters, the EO compounds are more miscible and usually act as plasticizers, thus promoting all of the diffusion dependent phenomena, such as water vapour or gas permeation [67]. The balance of the different effects determines the final influence of EO incorporation on the barrier properties of the biopolymer. In some cases, EO incorporation does not have a significant effect on the barrier properties of the films [41].

In general, the incorporation of EOs into the film matrix has been found to increase gas permeation. This can be mainly attributed to the greater solubility of gas molecules in the matrix, associated with the presence of more hydrophobic compounds. When EOs are incorporated, a lipid dispersed phase occurs in hydrophilic polymers, while hydrophobic matrices become more plasticized. Therefore, an increase in the gas permeability may be expected. As summarized in Table 4, this trend has been reported for several polymer-EO systems. However, as concerns oxygen permeability, films containing EOs or their compounds exhibit reduced values; this has been attributed to their antioxidant activity, which can exert an oxygen scavenging effect in the films [96, 97].

4.2. Mechanical properties

As regards the effect of EO incorporation into biopolymer films, the most frequently reported trend is a reduction in both the Elastic Modulus (EM) and Tensile Strength (TS) (Table 5), respectively associated with film stiffness and strength. The incorporation of EO into the polymer structure leads to the partial replacement of the stronger interactions between the polymer chains by weaker polymer-oil interactions [51, 89], thus allowing for the occurrence of structural discontinuities which reduce the mechanical resistance of the emulsified structure in hydrophilic matrices, where films tend to become less resistant to fracture and less stretchable. In hydrophobic matrices, the better compound compatibility leads to polymer plasticization, reducing both stiffness and strength, but enhancing the film extensibility.

Opposite behaviour can occur for particular EO-polymer blends where specific interactions are given. In fact, several studies have reported a strengthening effect as a result of the cinnamon EO incorporation in soy protein or chitosan matrices [26, 98]. In these cases, EO induces the rearrangement of the polymer network and some compounds provoke chain cross-linking, thereby improving the tensile properties [99]. Consisting of numerous chemical compounds with different molecular structures, EOs could interact with the polymer matrix differently. They also affect the film-water interactions, which also play a key role in mechanical behaviour. Otoni et al. [88] obtained soy protein films with carvacrol or cinnamaldehyde and observed a strengthening effect, which was explained by the reduction in the water adsorption capacity of the films, thus limiting their plasticizing action and making these films stiffer, under determined conditions of relative humidity.

5. RELEASE OF EO FROM THE POLYMER MATRICES INTO FOOD OR FOOD SIMULANTS.

The development of active packaging involves not only the incorporation of antimicrobial and/or antioxidant substances into the materials, but also the controlled release of the active compounds so as to maintain an effective concentration in the foodstuff over a period of time [82, 100]. In this sense, migration studies are required in order to determine the release rate of the actives from the film into the packaged food. In general, these studies are carried out in food simulants, which are liquid systems, which emulate the different hydrophilic or lipophilic nature of real foodstuffs. These have been standardized to find out how the different factors influence the migration process and to compare the behaviour of different active compounds and polymer matrices in distinct types of more or less hydrophilic foods. Aqueous food with neutral or acid pH can be properly simulated with distilled water containing 10-50% ethanol or 3% acetic acid, whereas 95% aqueous ethanol, oil or isooctane can be used to simulate fatty foodstuffs [101, 102].

Three coupled mechanisms are involved in the release mass transfer process: the diffusion of the food components (mainly water) into the polymer matrix, the relaxation of the macromolecular network, associated with the compound diffusion, and the diffusion of the active compound through the polymer. These mechanisms are coupled to a different extent until the thermodynamic equilibrium between film and food system phases is reached. Different models have been used to describe compound release kinetics, such first order kinetics and Fickian [103] or Peppas equations [104, 105]. Parameters that quantify the amount of compound released at equilibrium, and its release rate, are a useful means of understanding the extent to which the release takes place and to predict its effectiveness as an active. As concerns the delivery rate of actives, constant rate values (k)

Table 4: Barrier properties (water vapor -WVP- and oxygen –OP- Permeability are commented) of biopolymer films as affected by the presence of EO.

Matrix	EO	Concentration (g/g polymer)	Main observations on the effect of EO incorporation		Reference
HPMC	Oregano, bergamot	0-0.1/1	WVTR was not significantly affected	OTR increased coherently with EO proportion	[41]
HPMC	Plai, ginger, fingerroot	-	WVP upward trend	OP upward trend	[87]
Soy protein	Carvacrol, cinnamaldehyde	0.67/10	WVP upward trend	-	[88]
Lignocellulose	Cedarwood	0-0.2/1	WVP was reduced	-	[89]
Fish gelatin and chitosan	Origanum vulgare	0.4-1.2%/1.75%	WVP increased coherently with EO proportion	-	[90]
Gelatin	Oregano, lavender	0.04-0.12/1	WVP not affected by oregano, reduced by lavender	-	[91]
Alginate	Thyme, lemongrass, sage	1/3	WVP downward trend or reduction	-	[38]
Whey protein	Almond, walnut	0.5-1/8	WVP was reduced	OP was increased	[92]
Mucilage	Oregano	0-2/35	WVP was increased	OP was increased	[42]
Alginate	Oregano	0-1/1	WVP was reduced	-	[37]
Chitosan	Lemon	3/1	WVP was reduced	-	[47]
HPMC, chitosan	Bergamot, lemon, tea tree	0-2/1	WVP was reduced	-	[64]
Chitosan	Bergamot	0-3/1	WVP was reduced	-	[50]
Chitosan	Tea tree	0-2/1	WVP was reduced	-	[51]
HPMC	Tea tree	0-2/5	WVP was reduced	-	[93]
Chitosan	Basil, thyme	0-0.5/1	WVP was reduced	-	[57]
Chitosan	Cinnamon leaf	0-0.5/1	WVP was increased	OP was increased	[58]
Starch-gelatin blend	Oregano, clove, cinnamon bark	0-0.25/1	WVP was reduced	OP was reduced	[59]
Chitosan	Eugenol, cinnamon leaf	0.476/1	WVP reduced by eugenol	-	[60]

Table 5: Tensile properties (elastic modulus: EM, tensile strength: TS and percentage elongation at breack: %E) of biopolymer films as affected by the presence of EO..

Matrix	EO	Concentration (g/g polymer)	Main observations on the effect of EO incorporation			Reference
			EM	TS	%E	
Soy protein	Carvacrol, cinnamaldehyde	0.67/10	Increased	Increased	Increased	[88
Lignocellulose	Cedarwood	0-0.2/1	Reduced	Reduced	Slightly increased	[89]
Fish gelatin and chitosan	Origanum vulgare	0.4-1.2%/1.75%	Reduced	Reduced	No effect	[90]
Gelatin	Oregano, lavender	0.04-0.12/1	Non reported	Reduced	No effect	[91]
Alginate	Thyme, lemongrass, sage	1/3	Non reported	No effect	No effect/ increased	[38]
Whey protein	Almond, walnut	1/8	No effect	No effect	No effect/ increased	[92]
Mucilage	Oregano	0-2/35	Reduced	Reduced	Increased	[42]
Soy protein isolate	Cinnamon	0.1/1	No effect	Increased	Increased	[98]
	Ginger	0.1/1	Reduced	Reduced	No effect	[98]
Chitosan	Cinnamon	0.1/1	-	Increased	Reduced	[26]
Alginate	Oregano	0-1/1	-	Reduced	Increased	[37]
HPMC,	Bergamot,	0-2/1	Reduced	Reduced	Reduced	[64]

chitosan	lemon, tea tree					
Chitosan	Bergamot	0-3/1	Reduced	Reduced	Reduced	[50]
Chitosan	Tea tree	0-2/1	Reduced	Reduced	Reduced	[51]
HPMC	Tea tree	0-2/5	Reduced	Reduced	No effect	[93]
Chitosan	Basil, thyme	0-0.5/1	Increased	Increased	Reduced	[57]
Chitosan	Cinnamon leaf	0-0.5/1	Reduced	Reduced	Increased	[58]
Starch-gelatin blend	Oregano, clove, cinnamon bark	0-0.25/1	No effect	No effect	No effect	[59]
Chitosan	Eugenol, cinnamon leaf	0.476/1	Reduced	Reduced	No effect	[60]

have been determined by applying first order kinetics equations [82, 106]. Likewise, the diffusion coefficients (D) of compounds have been quantified by fitting Fick's model to the concentration of the active in the food simulant vs. time [75, 81, 82, 107]. The fitting of the Peppas model allows us to obtain information about the coupling of the different mechanisms involved in the process, through the value of the potential coefficient n. Values of n in the range of 0.5 indicate a prevailing Fickian mechanism.

In the case of essential oil compounds embedded in polymer films, there are different factors that influence the compound migration from films to the food system: (i) the physicochemical properties of the migrant substance, such as volatility, polarity and solubility; (ii) the polymer hydrophobicity, which determines the interactions with the EO compounds and the foodstuff; (iii) the composition and properties of the packaged food, mainly the food polarity, and (iv) the storage conditions, such as temperature [62, 100, 107, 108, 109]. All of these factors implied both complex interactions, which make controlled release predictions difficult, and also the necessity for specific studies of a determined packaging material and food system. The use of standardized food simulants can help to compare the migration of different materials, but differences in composition with respect to real foods could lead to divergent results, since particular interactions with different food components may affect migration behaviour and food quality and safety.

As previously commented on, many biopolymers with film forming properties, such as chitosan, starch, modified cellulose, alginate, whey protein or gelatin, are water soluble whereas the EO compounds are non-polar substances [78]. To promote compatibility, what has been analysed is the encapsulation of EO by means of different strategies, affecting the compound release kinetics. Table 6 and 7 summarizes previous studies into the release of actives, encapsulated or not, into different food matrices or simulants. Wu et al. [110] reported a reduction in the release rate of cinnamon EO from fish gelatin films into corn oil as a result of their inclusion in nanoliposomes, which prolongs the activity thus improving the antimicrobial action. In the same way, the encapsulation of carvacrol in β-cyclodextrins reduced the carvacrol release rate from whey protein isolated (WPI) films [77] and cellulose nanocrystal films [111]. However, the release rate of eugenol was not significantly affected by its inclusion in β-cyclodextrin in WPI films, which was attributed to the weak interactions between the compound and cyclodextrins [77]. Nanofillers, such as halloysite nanotubes, were also effective at controlling the release of peppermint EO from pectin films, increasing the EO adsorption capacity of the polymer matrix [106]. Likewise, sepiolite nanofillers slowed down and prolonged the eugenol release from clove EO-gelatin films, through the control of the EO interactions with the polymer matrix [113]. Matiacevich et al. [78] highlighted the effect of the structure of the polymer matrix on the release parameters of lemongrass EO, encapsulated in sodium caseinate microcapsules, when included in alginate films. The more ordered structure of the film greatly slowed down the release rate.

The concentration of the active compound in the film also affects release kinetics. Chen et al. [112] reported an increase in the release rate of nanoemulsified cinnamaldehyde when its concentration rose up to a carbonyl:amino molar ratio of 1 in chitosan films. However, higher contents led to slower delivery rates, which was attributed to microstructural changes that restricted molecular diffusion through the polymer matrix.

The chemical compatibility between the active compound and the foodstuff is a decisive factor in the release of the active agent. Thus, active devices based on chitosan containing β-cyclodextrin-carvacrol complexes, placed in tight packages containing chicken samples, produced carvacrol deliveries of between 95-99% after 9 days of storage, whereas packages without chicken samples only released 35% of the active agent [62]. The great chemical affinity between the carvacrol and the chicken protein promoted carvacrol adsorption in the food, which, while limiting antimicrobial action, affected the sensory properties. Similar effects were observed for Zataria multiflora Boiss EO (ZEO) embedded in chitosan films when applied on mortadella sausages, where a fast and total release of ZEO compounds was observed due to their great affinity with the mortadella fat. Nevertheless, this was mitigated when applied in combination with grape seed extract with higher molecular weight compounds, which interact with ZEO constituents [109]. These observations have also been proven

Table 6. Release kinetics studies for different encapsulated essential oil (EO) compounds from polymer matrices.

Active compound	Encapsulating agent	Polymer	Simulant or food	Main features	Reference
Menthone from peppermint EO	Halloysite nanotubes	Pectine	Ethanol 50% v/v	Encapsulation contributed to the adsorption of actives in the film, thus increasing its release capacity. Films only deliver 4-20 % of the active depending on temperature. Fitted model: first order kinetics.	[106]
Lemongrass EO	Caseinate microcapsules	Sodium alginate	Ethanol 50% v/v	Encapsulated compounds in the film showed a more sustained Fickian release than in the free capsules. Fitted models: Peppas and Weibull equations.	[78]
Cinnamon EO	Nanoliposomes	Fish gelatin	Corn oil	The cinnamon EO inclusion in nanoliposomes allowed for a more controlled release of the actives. No fitted model.	[110]
Carvacrol	β-cyclodextrins	Cellulose nanocrys tals	Water	Encapsulation in β-cyclodextrins allowed for better absorption of the actives in the films while retarding their release. No fitted model.	[111]
Carvacrol or eugenol	β-cyclodextrins	Whey protein	Ethanol 50% v/v	β-cyclodextrin inclusion complexes slowed down the carvacrol release rate, but they did not affect the eugenol release rate. Fitted model: first order kinetics	[77]
Carvacrol	β-cyclodextrins	Chitosan	Chicken fillets	Carvacrol release from chitosan-ciclodextrins films in the package head space was greatly promoted when there was a sample of chicken, due to its adsorption in the sample protein (95-99% compared to 35% without chicken) No fitted model.	[62]
Cinnamaldehyde	Triglyceride-twin 80-nanoemulsions	Chitosan (278 kDa)	Ethanol 10% v/v and ethanol 50% v/v	The release rate of nanoemulsified cinnamaldehyde rose when its concentration increased up to a carbonyl:amino molar ratio of 1 in chitosan films, but higher contents led to slower delivery rates. Ethanol promoted release rate. No fitted model.	[112]

Table 7. Release kinetics studies for different essential oil (EO) compounds from polymer matrices.

Active compound	Polymer	Simulant or food	Main features	Reference
Carvacrol	Chitosan (400, 180 and 41 kDa)	Olive oil and ethanol 96% v/v	Release rate increased when the chitosan molecular weight rose. In ethanol, carvacrol delivery was faster and more complete than in olive oil. No fitted model.	[100]
***Zataria multiflora* Boiss EO (combined or not with grape seed extract-GSE)**	Chitosan (450 kDa)	Mortadella sausage	Migration of EO compounds to the product was slowed down when combined with GSE. Complete delivery occurred in 6 days for EO without GSE. No fitted model.	[109]
Limonene from Bergamota EO	Chitosan (medium molecular weight)	Water, ethanol 10% v/v, ethanol 50% v/v, ethanol 95% v/v and isooctane	The release kinetics of limonene was promoted when ethanol ratio rose (greater limonene solubility), but the non-polar system implied a very slow delivery rate since it did not provoke swelling of the polymer matrix. Fitted model: Fickian equation.	[107]
Cinnamaldehyde	Agar and sodium	Water	Maximum release (35% of the total content) of	[76]

and eugenol from Cinnamon EO:	alginate		both compounds was reached after 9 h of contact. No fitted model.	
Clove EO	Gelatin-egg white	Water	Sepiolite prolonged eugenol delivery from the films, affecting the interactions between the EO and the polymer matrix. No fitted model.	[113]
Thymol	Zein	Water	Diffusion coefficient (D) of thymol in the films was not affected by its concentration, while maximum delivery was in line with the film's thymol content. Fitted model: Fickian equation.	[75]
Thymol	Poly(lactic acid)	Ethanol 15% v/v and ethanol 95% v/v	Delivery capacity of the films increased as the temperature rose. Release rates were higher in the less polar simulant. Fitted models: First order kinetics and Fickian equation.	[82]
Thymol	Poly(butylene succinate)	Water, acetic acid 3% v/v, ethanol 10% v/v, ethanol 95% v/v and isooctane	Thymol diffusion increased as the solvent polarity decreased. The highest delivery capacity was reached in isooctane. Fitted models: Fickian equation.	[81]
Oregano EO	Poly(lactic acid)-cellulose nanocrystals	Mixed vegetables	Oregano EO was gradually delivered (17% after 14 days), showing two steps with different release rates: from faster to slower.	[72]
Eugenol	Polyhydroxybutyrate	Water, acetic acid 3% v/v, ethanol 50% v/v and *n*-hexane	Maximum release was achieved in the solvent with intermediate polarity (ethanol 50%). No fitted model.	[74]

in food simulants; Petchwattana and Naknaen [81] and Tawakkal et al. [82] analysed the release kinetics of thymol from poly(butylene succinate) and poly(lactic acid) films, respectively, and reported that the maximum release rates were observed with hydrophobic food simulants, such as 95% ethanol and isooctane, due to the greater chemical affinity between thymol and the non-polar solvents. Likewise, Sánchez-González et al. [107] observed a higher release at the equilibrium of limonene from chitosan films as the ethanol percentage increased in the food simulant, in line with the low polarity of limonene. However, no notable delivery of active compounds occurred in isooctane due to the lack of swelling of the polymer matrix, which tightly entraps the active.

Temperature greatly affects the kinetics of active compound delivery from a determined film in a specific medium. Temperature affects the molecular interactions in both film matrices and food systems, and therefore the chemical affinity between the active compounds and the respective substrate can change, provoking different migration behaviour. In general, low temperatures slow down migration kinetics, according to its effect on molecular diffusion, but the equilibrium status can also be modified due to the differences in the involved molecular interactions. In this sense, different authors [106, 110] report a significant effect of temperature on the delivery kinetics of the EO active compounds.

6. FOOD APPLICATIONS OF ACTIVE BIOPOLYMER FILMS CONTAINING EO/COMPOUNDS.

During the last decade, many studies have been carried out on the use of biopolymers as carriers of EOs and their main compounds, with the aim of responding to consumer demand for preservative-free, safer products. Thus, different carbohydrate polymers, such as chitosan, cellulose derivatives, alginates, gelatin, etc., have been used both to incorporate EO and develop film formulations for the purposes of improving the food quality and extending its shelf life. Different plant and animal proteins, such as gelatin, whey, soy and milk proteins, have also been extensively used. Table 8 summarizes several recent studies using these kinds of biopolymers. The use of other biopolymers, such as aliphatic polyesters [127], as carriers of antimicrobials is also being investigated in order to obtain active materials for food packaging (Table 9). Among the most outstanding is polylactic acid (PLA), polycaprolactone (PCL) or the polyhydroxyalkanoates, such as polyhydroxybutyrate (PHB) and polyhydroxybutyrate-co-hydroxivalerate (PHBV) [129]. Among the most effective compounds/EOs may be found carvacrol, thymol, cinnamaldehyde or oregano, cinnamon and clove EOs, attaining the total microbial inhibition of several foodborne pathogens. Some studies are summarized in Tables 8 and 9 and commented on below.

Table 8. Recent studies on applications of active films from food hydrocolloids and EO compounds into food systems.

Hydrocolloid	EO compound	Food application	Tested microorganisms	Reference
Chitosan	Carvacrol	Chicken breast	Mesophiles, psychrophiles, *Pseudomonas spp*., enterobacteria, lactic acid bacteria, yeasts and fungi	[62]
Chitosan	Lemon EO	Strawberries	*B. cinerea* and fungi.	[47]
Chitosan	Basil and thyme EO	Pork meat	Total aerobic bacteria, coliforms	[114]
Chitosan	Bergamot EO	Muscatel table grapes	Aerobic mesophilic, yeasts and moulds.	[48]
Chitosan	Green tea extract	Hamburger patties	Total mesophilic, coliform, yeast and mould	[115]
Chitosan-gelatin	Grape seed extract and *Ziziphora clinopodioides* EO.	Minced trout fillets	Total mesophilic and psychrotrophic bacteria, *Pseudomonas spp., P.fluorescens, Shewanella putrefaciens*, lactic acid bacteria, *Enterobacteriaceae* and *Listeria monocytogenes*	[116]
Chitosan-gelatin	Clove EO	Cod fillets	Total bacterial, H_2S producers organisms, luminescent bacteria, *Pseudomonas*, *Enterobacteriaceae* and lactic acid bacteria	[44]
HPMC	Oregano EO	*Formosa* plum	*Escherichia coli*	[41]
Alginate	Palmarosa oil	Fresh-cut melon	Mesophiles, psychrophiles, yeast and mould *Salmonella Enteritidis*	[117]
Sodium alginate	Lemongrass EO	Fresh-cut apples	*Escherichia coli*	[118]
Gelatin	Oregano EO or rosemary or chitosan	Smoked sardine	Total bacteria, H_2S-reducing organisms, luminescent bacteria and *Enterobacteriaceae*	[119]
Gelatin–alginate	Oregano EO	Trout fillets	Total viable, psychrotrophic and lactic acid bacteria, *Pseudomonas spp*. and *Enterobacteriaceae*.	[120]
Pectin	Cinnamon leaf EO	Fresh-cut peaches	*Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureurs*	[121]
Pectin and apple solution	Carvacrol and cinnamaldehyde	Ham and bologna	*L. monocytogenes*	[122]
Gliadin	Cinnamaldehyde	Bread and cheese spread	Moulds and fungi	[123]
Soy protein	Oregano and thyme EO	Beef patties	Total viable, lactic acid bacterial, coliform, *Staphylococccus* and *Pseudomonas*	[46]
Milk protein	Pimento and oregano EO	Beef	*Escherichia coli* and *Pseudomonas spp.*	[124]
WPI	Oregano EO	Beef	Total viable, lactic acid bacteria, *Pseudomonas*	[125]
WPI	Oregano or clove EO	Chicken breast	Total mesophilic, total psycrophilic, lactic acid, *Enterobacteriaceae* and *Pseudomonas*	[126]

Table 9. Recent studies on applications of biodegradable polymer active films with EO compounds into food systems.

Biodegradable polymer	EO compound	Food application	Microorganism tested	Reference
Polylactic acid	Cinnamon EO	Chicken	*Listeria monocytogenes* and *Salmonella typhimurium* (*in vitro* and *in vivo*)	[70]
Polylactic acid	Oregano EO	Rainbow trout	S. aureus, L. monocytogenes, E. coli and *S. Enteritidis* (*in vitro*) Total viable count, psychrotrophilic count, lactic acid bacteria and *Enterobacteruaceae* count (*in vivo*)	[71]

Polylactic acid	Cinnamon EO/β-cyclodextrins	Pork meat	*E. coli* and *S. aureus* (*in vitro*) Viable microbial counts (*in vivo*)	[86]
Polylactic acid and polycaprolactone	Cinnamaldehyde	Button mushroom	Mesophilic and psychrophilic counts	[53]
Poly(lactic acid) and cellulose nanocrystals	Oregano EO	Mixed vegetables	5 strains of *L. monocytogenes* HPB (2569, 2558, 2371, 2812 and 1043)	[72]
Polycaprolactone and metylcellulose	Two blends: (i) organic acids, extract of rosmarinic acid and Asian EO mixture; (ii) organic acids, extract of rosmarinic acid and Italian EO mixture.	Fresh broccoli	*L. monocytogenes*, *S. typhimurium*, *E. coli* and total aerobic microbiota (TAM).	[128]

PLA films with cinnamon EO significantly reduced the bacterial growth in chicken samples inoculated with L. monocytogenes or S. typhimurium [70]. Likewise, PLA-cinnamon EO films doubled the pork's shelf life by maintaining the microbial counts below 1·107 CFU/ml or g or cm2 for 8 days. [86]. Similarly, PLA films with oregano EO have been used to package rainbow trout, thus reducing the total viable counts by half after 12 days of cold storage, keeping these values below the recommended limit. At the same time, the microbial growth of psychrotrophic bacteria, lactic acid bacteria and Enterobacteriaceae was delayed as a result of the oregano EO's antimicrobial activity.

Often, films based on biopolymer blends are used as carriers of these active compounds, since they usually demonstrate better physical properties. In this sense, cinnamaldehyde has been included in PLA-PCL films, which reduced the microbial growth on button mushrooms [53]. Likewise, PCL-methylcellulose films with extract of rosmarinic and Asian or Italian EO mixture inhibit the microbial growth of *E. coli* and *Salmonella Typhimurium* in broccoli, while showed a bacteriostatic effect at 12 and 7 days, respectively [128]. *In vitro* tests with PHB films containing eugenol exhibited antimicrobial activity, with a minimum inhibitory concentration (MIC) of eugenol in the films of 40 and 80 μg eugenol/g PHB for bacteria and fungi, respectively. A total microbial inhibition was observed with a eugenol content of over 200 μg/g PHB [74]. In the same way, Requena et al., [67] reported that PHBV films with carvacrol, eugenol or oregano EO had a fast bactericide effect against E.coli, whereas less, more gradual antimicrobial activity was observed against *L. innocua*.

Despite the remarkable antimicrobial activity of the EO against most foodborne pathogens in in vitro tests, several authors have reported that higher amounts are required to achieve similar results on real foodstuffs. This fact can be explained by the interactions of some food ingredients with the EO compounds, which sequester them, thereby limiting their antimicrobial activity. In general, high contents of proteins or fats have been related with a lower bacterial sensitivity to the EO [130-133], whereas this has not been observed with high carbohydrate contents [131]. In addition, the higher nutrient availability on the food, compared to the culture media, would allow for faster damage repair by bacteria [134]. Thus, despite the proven antimicrobial properties of several EOs in culture media, very low or null antibacterial activity was observed for these EOs against *E. coli* in ready-to-cook chicken [135]; *S. typhimurium* in beef [136]; *Y. enterocolitica* and *L. monocytogenes* in chicken [137]; and *L. monocytogenes* in steak tartare [138]. Likewise, sachets containing carvacrol inside packaged chicken breast fillets did not give rise to an efficient microbial inhibition of fungi, yeast, mesophiles and enterobacteria, because of the headspace carvacrol was adsorbed by the chicken protein matrix, thereby keeping the active compound concentration in the packaging headspace below the MIC [62]. In the same way, edible films from apple puree containing cinnamaldehyde did not exhibit antimicrobial activity against L. monocytogenes in ham, at different active compound concentrations and storage temperatures [122]. Nor did PCL-methylcellulose films containing extract of rosmarinic acid and Italian or Asian EO mixture have any effect on the L. monocytogenes growth in fresh broccoli [128]. Therefore, although the antimicrobial activity of many EOs has been proven in in vitro studies and with some specific foodstuffs, the development of active packaging requires specific studies with a determined food product to ensure antimicrobial effectiveness and food safety.

CONCLUSION

From the point of view of their potential properties, proven in *in vitro* studies, the perspective of using essential oils as antimicrobial or antioxidant components of biopolymer active packaging materials is good. However, their incorporation into polymer matrices leads to changes in the films' functional properties as packaging material and their release is conditioned by the marked interactions with both some food components, as well as with the film matrix. Therefore, a biopolymer film-food system-active compound represents a complex environment, which makes predicting the bioactivity of a compound difficult. Thus, specific studies into a real food need be performed to ensure both the effectiveness of the active against a target microorganism and also the total adsorption of actives in the food matrix, with the corresponding impact on its sensory and quality characteristics.

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