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Essential oils as additives in biodegradable films and coatings for active food packaging

Lorena Atarés*[a] and Amparo Chiralt [a]

[a] Institute of Food Engineering for Development, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

Abstract

Background
Petroleum derivate plastics represent a serious environmental problem, which is why alternative sustainable solutions must be found. To this aim, recent research has focused on the development of edible/biodegradable packaging for food products. The implementation of this novel packaging requires analyzing thoroughly the effect of the ingredients used on the most relevant properties of the material.
Scope and approach
Essential oils represent an interesting ingredient for biodegradable food packaging, mainly due to their natural origin and their functional (antioxidant/antimicrobial) properties, allowing for obtaining active materials aiming to extend shelf-life and add value to the product. However, their inclusion in edible/biodegradable films for food packaging may imply some impact on several properties of the system (such as optical, tensile…), affecting in turn the consumer acceptability. Before the increasing research on biodegradable materials for food packaging, and the growing interest on natural food additives, this paper aims to review the latest findings on how essential oils impact the most relevant properties of edible films and coatings, namely microstructural, physical (tensile, barrier, optical), antioxidant and antimicrobial.
Key findings and conclusions
Essential oil incorporation affects the continuity of the polymer matrix, leading to physical changes depending on the specific polymer-oil components interactions. Generally, the film structure is weakened by the oil addition, whereas the water barrier properties are improved and the transparency is reduced. Essential oils may provide the films with antioxidant and/or antimicrobial properties. The oil composition and the specific interactions with the polymer determine its effectiveness as an active ingredient.

Keywords

Biodegradable films, edible films, edible coatings, food packaging, essential oils

1. Introduction

Conventional plastics are derived from petroleum, which entails serious environmental concerns. Biodegradable films and coatings represent an interesting alternative to conventional plastic materials, which is why several biopolymers have been exploited to develop materials for eco-friendly food packaging (Azeredo, 2009). Edible films are thin layers of edible materials, which once formed can be placed on - or between- food components, whereas an edible coating is formed as a coating on a food product. The most common materials for the formulation of edible/biodegradable films and coatings are polysaccharides, proteins and lipids, and the combination of these allows for producing blends of improved characteristics (Fabra et al. 2009).

Aiming to the reduction of the use of chemical additives in food industry, growing interest has risen recently on the use of natural food additives with antimicrobial and antioxidant properties that do not have any negative effects on the human health (Alves Silva et al., 2013). Essential oils (EOs) extracted from plants and spices exhibit antimicrobial and antioxidant properties (Viuda-Martos et al., 2010), which makes them interesting additives in food industry. In addition, most of them are classified as *Generally Recognized as Safe* (GRAS) (Ruiz-Navajas et al., 2013). However, their use as food preservatives is often limited due to their strong flavor. In

*corresponding author: loathue@tal.upv.es

order to avoid this problem, EOs can be incorporated into edible films (Ruiz-Navajas et al., 2013).

In the recent years, EOs have been extensively studied as additives in edible/biodegradable emulsified films and coatings. Due to their lipidic nature, they are expected to help reduce the water vapour permeability of hydrophilic films. Moreover, they have proved to have some impact on other film properties (tensile, optical, structural…), as well as providing antioxidant and/or antimicrobial effects. This paper reviews the role of EOs as additives in edible/biodegradable films and coatings for food packaging, namely their effect on the (1) microstructural and physical properties, (2) antioxidant power and (3) antimicrobial capacity of the films. Recent studies dealing with the characterization and/or application of edible films and coatings incorporated with EOs have been systematically classified, and their main conclusions summarized, in order to give an overview on the state of the art.

2. Effect of EO incorporation on the film microstructure

Table 1 shows a representative selection of recent studies (2005-2015), giving an overview of the tests usually performed when dealing with the effect of EOs addition on the structure and physical properties of edible films.

The qualitative observation of the components arrangement into the film structure is often accomplished using Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy. SEM has been repeatedly utilized to investigate the structure of edible films incorporated with EOs, often in comparison to the film with no lipid.

Contrarily to food packaging materials based on non-polar conventional plastics, edible biodegradable films are usually based on hydrophilic materials such as proteins or polysaccharides, and films or coatings are mainly obtained by casting of the film forming aqueous dispersions and subsequent drying. The incorporation of essential oils in the film forming dispersion is carried out by applying emulsification or homogenization techniques, where fine emulsions of essential oils are obtained containing polymer at the continuous aqueous phase. In dried films, lipid droplets remain embedded into the polymer matrix, as can be observed by microscopic techniques.

It is normally observed that the final microstructure of the films is influenced by the structural arrangement of the components in the film forming dispersion. Moreover, their development during the drying period plays an important role, given that destabilization phenomena such as droplet flocculation, coalescence and creaming can occur. In line with water evaporation, the concentration of the oil dispersed phase increases, thus promoting droplet flocculation rate. Subsequently, coalescence and creaming lead the droplets to the forming film surface, where compounds evaporation occurs together with water (steam distillation). In fact, losses of EOs were reported depending on the polymer forming film (Sanchez et al. 2011a, b). Factors promoting the emulsion stability, such as the polymer adsorption at the droplet surface and viscosity increase of the continuous phase, contribute to limit the losses of EOs and affect the final film microstructure. So, polymer-EOs interactions greatly affect the emulsion stability and final microstructure of the film.

Nevertheless, not only the EO nature, but also the homogenization technique determine the mechanisms affecting the microstructural changes in the film. In this sense, relevant structural differences have been observed as the EO droplet size was reduced (Bonilla et al., 2012b). Apparently, droplet reduction favoured the intimate EO incorporation into the polymer matrix, giving rise to increased interactions between the polymer and the oils, a less cohesive polymer matrix and scarcely identifiable oil droplets.

Sánchez-González et al. (2009) obtained hydroxyproplymethylcellulose (HPMC) films incorporated with tea tree EO and observed discontinuities (lipid droplets) homogeneously distributed across the film since little creaming took place due to the high viscosity of the continuous phase. The droplets became more numerous with increased oil concentration. Atarés et al. (2011) formulated HPMC films with different antioxidant agents, including ginger EO. The EO caused discontinuities (EO droplets), giving rise to a more open structure and

thicker films, as compared to films with no EO. The ginger oil droplets could also be observed by SEM on the surface of the films. SEM was also useful in the microstructural studies of edible films based on EOs nanoemulsions, since it gave some insights on nanodroplets organization along the biopolymer matrix (Acevedo-Fani et al, 2015). These authors generally found an increase in the film surface coarseness when EOs (thyme, lemongrass and sage) were incorporated into alginate films.

Not only the oil proportion, but also its composition may determine the final structure of the films. Atarés et al. (2010b) elaborated sodium caseinate films incorporated with little amounts of cinnamon or ginger EOs. It was found that cinnamon oil stayed homogenously distributed in the protein matrix, whereas ginger oil droplets were observed. These authors concluded that the structural differences linked to the oil type are determined by the different behaviour of both EO droplets during drying, and result from the complex interactions taking place between the lipid, the protein and the solvent.

The structure of the films greatly influences the physical properties of these. Benavides et al. (2012) formulated alginate films with oregano EO in variable proportions, and observed a loose texture and a sponge-like structure in these films. This structure transformed into layered with increasing EO content, accompanied by an improvement in the water barrier properties. Ojagh et al. (2010) studied the incorporation of cinnamon EO into chitosan films, and observed sheet-like structures stacked in compact layers when the oil was added. The films with this lipid showed improved water barrier properties, higher tensile strength and lower stretchability.

Atomic Force Microscopy (AFM) allows quantifying the films roughness, hence characterizing the films surface topography. Shojaee Aliabadi et al. (2013) used this technique to investigate the surface morphology of κ-carrageenan films containing *Satureja hortensis* EO, and found that this oil not only altered the internal structure but also increased the films surface roughness.

3. Effect of EO incorporation on the physical properties of edible films

3.1. Tensile properties

The tensile properties of films are normally studied through tensile tests (ASTM D882, 2001), gradually extending the film at a given rate upon breakage, and registering the strength versus time or distance. The most used tensile parameters are the elastic modulus (EM), directly linked to the film rigidity, tensile strength (TS) as a measure of film strength, and percentage elongation at break (%E) linked to the film stretchability upon breakage. These properties are important characteristics for packaging material (Krochta & Johnston, 1997).

The tensile properties of films is determined by a wide array of factors, such as the microstructural characteristics of the material (Krochta & Johnston, 1997). Cuq et al. (1995) stated that the tensile properties of edible protein films are dependent on film constituents, their relative proportions and preparation conditions. The effect of lipid addition on the tensile properties of protein films depends on both the lipid characteristics and its capacity to interact with the protein matrix (Pires et al., 2011).

The studies published on the effect of lipid addition in the tensile properties of edible films have revealed diverse effects. Many works have reported a decrease of TS of protein films caused by lipid addition, both with lipids other than EOs (Gontard et al., 1994; Chen, 1995; Shellhammer and Krochta, 1997; Tanaka et al., 2001; Bertan et al., 2005) and with EOs (Tongnuanchan et al., 2012; Ahmad et al., 2012; Altiok et al., 2010; Pires et al., 2011; Zinoviadou et al., 2009). As regards to films based on polysaccharides, a decrease in TS caused by EO addition has been observed repeatedly (Sánchez-González et al., 2009; Atarés et al., 2011; Sánchez-González et al., 2011a; Moradi et al., 2012; Sánchez-González et al., 2010; Zivanovic et al., 2005; Pranoto et al., 2005; Benavides et al., 2012; Norajit et al., 2010; Shojaee-Aliabadi et al., 2013). Lipid incorporation into the film matrix induces a heterogeneous film structure featuring discontinuities (Zinoviadou et al., 2009). This affects the tensile properties of the films, depending on the characteristics of the lipid added. The strength reduction could be primarily explained by the partial replacement of stronger polymer-polymer interactions by weaker polymer-oil interactions in the film network (Shojaee-Aliabadi et al., 2013). Bonilla et al. (2012b) studied chitosan films

incorporated with basil or thyme EOs, and found that the induced close contact polymer-oil caused by high pressure homogenization gave rise to more fragile films of increased stretchability, due the weakening effect on the chitosan matrix where chain interaction forces are reduced. Tongnuanchan et al. (2012) observed that citrus oil incorporation resulted in TS reduction of fish skin gelatin edible films, which was attributed to the lowered interaction between gelatin molecules. Additionally, an increased extensibility was observed. It was concluded that the addition of lipid or oil in protein or polysaccharide-based films may hinder polymer chain-to-chain interactions and provide flexible domains within the film (Limpisophon et al., 2010). Hosseini et al. (2015) concluded that the destabilization phenomena taking place during film drying could be related with the tensile strength reduction in fish gelatin-chitosan films incorporated with *origanum vulgare* EO.

The TS decrease may be accompanied by either an increase or a decrease of %E. In some studies, such as Pranoto et al. (2005) the EO proportion determines its effect on elongation at break. Shojaee-Aliabadi et al. (2013) observed an increase of κ-carrageenan film stretchability caused by *Satureja hortensis* EO addition, which has a plasticizing effect even at small concentration. Zinoviadou et al. (2009) incorporated oregano EO into whey protein films, and observed an increase in elongation properties at an oil concentration up to 1%. This was explained by the development of a heterogeneous film structure featuring discontinuities, caused by oil incorporation, thus affecting the film stretching ability. Contrarily, Moradi et al. (2012) observed a reduction in chitosan film stretchability caused by the incorporation of *Zataria multiflora* EO, which was attributed to an increase in pore sizes of the films creating possible rupture points.

On the other hand, some works reported the increase of TS due to the incorporation of lipids in the films. Atarés et al. (2010a) observed this behavior in soy protein isolate films with cinnamon EO, and Ojagh et al (2010), who incorporated the same oil into chitosan films. This strengthening effect may be caused by the induced rearrangement of the polymer network caused by the EO. Apparently, some compounds in EO might be able to cross-link chain, thereby improving the tensile properties (Tongnuanchan et al., 2012). EOs consist of numerous chemical compounds with different properties, which could be able to interact with the polymer matrix differently. Phenolic compounds, present in high concentrations in EOs (Burt, 2004) are able to react with more than one protein site, leading to protein cross-links (Haslam, 1989). Strong interaction between cinnamon EO and the polymer matrix has been observed in chitosan films, where a cross linking effect caused a significant increase of tensile strength and a decrease in the elongation at break (Ojagh et al., 2010). Pires et al. (2013) elaborated hake protein films incorporated with several EOs, and explained their findings by the specific oil-protein interactions. For instance, the higher resistance of films with citronella oil may result from the protein crosslinking promoted by β-citronellal. On the other hand, the presence of Δ^3-carene in citronella oil (22.5%), an apolar monoterpene, may be responsible for the increase of the elasticity of films with this oil added.

Some other studies report the non-significant effect of EO addition on the tensile properties of the films. Atarés et al. (2010b) observed no significant effect of cinnamon EO incorporation on the tensile properties of sodium caseinate films, although the same oil at the same proportions had caused some TS increase in soy protein films (Atarés et al. 2010a). Pires et al. (2013) found that TS of hake protein films was not significantly affected by the incorporation of a variety of EOs.

In conclusion, the effect of EO addition on the tensile properties of edible films is rather variable and depends on the specific interactions between the oil components and the polymer matrix. Typically, some weakening effect is caused, mainly attributed to the heterogeneous biphasic structure caused by lipid addition, although some studies have reported the opposite effect. Unlike pure lipid components, EOs have a complex composition, which complicates to predict their effect.

3.2. Barrier properties

The films ability to retard moisture loss from the product is an important characteristic that affects product quality (Pranoto et al., 2005), and hence the water barrier properties of a film or

coating material should be taken into account when applying onto a moist product. The water vapour permeability (WVP) of edible films is normally quantified through a gravimetric method (ASTM E96-95), often modified for application of hydrophilic materials (McHugh et al., 1993; Gennadios et al., 1994).

The water vapour transfer processes in films depend on the hydrophilic-hydrophobic ratio of the film constituents (Hernandez, 1994). Hence, the incorporation of hydrophobic EO into hydrophilic polymer matrices may result in the improvement of their water vapour barrier properties. This trend was observed by Sánchez-González et al. (2009) in HPMC films with tea tree EO, by Pires et al. (2013) in hake protein films with different EOs (thyme was the most effective, coherently with Pires et al., (2011)) and also by Atarés et al (2010b), who incorporated cinnamon and ginger EOs into sodium caseinate films. Other studies have reported the same effect (Sánchez-González et al., 2009; Atarés et al., 2011; Ojagh et al., 2010; Moradi et al., 2012; Tongnuanchan et al., 2012; Pires et al., 2011, 2013; Benavides et al., 2012; Shojaee-Aliabadi et al., 2013; Zivanovic et al., 2005). However, being complex mixtures of numerous chemical compounds, the hydrophobicity of EOs is a variable feature, thus affecting their efficacy as water vapour permeability depressors. Ahmad et al. (2012) elaborated gelatin films with bergamot or lemongrass EOs and noted that they affected the water permeability differently, which was attributed to their differing properties.

Apart from WVP reduction, other effects of EO addition on the water barrier properties of edible films have been found in literature. Oregano EO did not affect the WVP of whey protein films (Zinoviadou et al., 2009). Teixeira et al. (2014) found that clove EO reduced the WVP of fish protein films, whereas garlic and oreganum EOs did not induce significant changes. Pruneda et al. (2008) observed the increase of soy based films WVP after oregano EO addition. Pranoto et al. (2005) observed that garlic oil addition greatly increased the WVP of alginate edible films. Probably garlic oil contributed to extend intermolecular interactions of the structural matrix in alginate film, therefore enhancing moisture passing through the edible film. Atarés et al. (2010a) found that the addition of small proportions of cinnamon and ginger EOs resulted in a reduction in the water vapour barrier properties of soy protein films. This was attributed to the low oil proportion and the lipid discontinuities not being relevant to increase the tortuosity factor for transfer of water molecules, which is responsible for the reduction of WVP (Perez-Gago & Krochta, 2001). Ahmad et al. (2012) studied gelatin films incorporated with bergamot or lemongrass EOs, and found some slight WVP increase when bergamot oil was added. This was attributed to the bergamot oil distributing irregularly in the film, leading to heterogeneous film structure. These studies, among others, demonstrate that it cannot be assumed that WVP of edible is reduced simply by adding a hydrophobic component in the formulation, since the impact of the lipid addition on the microstructure of the emulsified film is a determining factor in water barrier efficiency. In turn, the microstructure of the emulsified film is affected by other factors, such as the physical state of the EO and its distribution into the polymer matrix.

The physical state of the lipid may have an important impact on WVP. Ginger EO incorporated in HPMC films was most effective as WVP depressor at low temperature (Atarés et al., 2011). The liquid state of ginger oil could favour molecular mobility at high temperature, thus promoting the transport of water molecules through the emulsified films. Similar results were observed by Atarés et al. (2010b) in sodium caseinate films.

Moreover, the lipid distribution into the polymer matrix may also determine its efficacy as WVP depressor. The smaller the lipid droplets, the more homogeneously distributed it is in the film matrix and the lower the WVP (Debeaufort & Voilley, 1995; Karbowiak et al., 2007; McHugh & Krochta, 1994; Perez-Gago & Krochta, 2001). Atarés et al. (2010a) tested the efficacy of cinnamon and ginger EOs as WVP depressors in soy protein films, and found that cinnamon EO, being homogeneously integrated in the protein network, was more effective than ginger EO at the same oil: protein ratio. In fact, the difficulties in integrating the lipid in the hydrophilic network may cause matrix disruptions and create void spaces at the protein–lipid interface.

The oxygen permeability of edible films is measured much more rarely than WVP (table 1). This property is greatly affected by relative humidity and temperature at which film equilibrates since diffusion depending properties are dependent on molecular mobility, which increases when the film moisture content or temperature increase (Miller & Krochta, 1997). OP is an important

feature to be taken into account when aiming to formulate edible films and coatings to reduce lipid oxidation. In general, it is expected that EO addition helps increase oxygen permeability due to its hydrophobic character. Atarés et al. (2011) formulated HPMC films with ginger EO and found a significant oxygen permeability increase. In spite of this, HPMC-ginger EO coatings exhibited some antioxidant activity, which suggests that oxygen permeability is not the only factor determining the antioxidant capacity of edible films, as will be pointed out below.

3.3. Optical properties

Optical properties (colour, transparency and gloss) of films and coatings may have a great impact on the food product appearance and hence on the consumer acceptability (Sivarooban et al., 2008; Kunte et al., 1997).

3.3.1. Colour

The colour of edible films incorporated with EOs is directly influenced by the type and concentration of the oil added (Du et al., 2009). Moradi et al. (2012) elaborated films with chitosan, *Zataria multiflora Boiss* essential oil (ZEO) and/or grape seed extract (GSE). Whereas the incorporation of ZEO did not affect the colour, the incorporated GSE films had a brownish color. Pires et al. (2013) formulated hake protein films with a variety of EOs, and found that only films with tarragon and coriander EOs were significantly more yellow than the control films. The type of EO determined the colour changes in the studies performed by Ahmad et al. (2012) and Tongnuanchan et al. (2012) on gelatin films. No significant effect on the films colour was found by Pranoto et al. (2005), who incorporated garlic EO into alginate films. The same happened in the studies of Pires et al. (2011) on thyme EO- hake protein films, and Atarés et al., (2011) on ginger EO.

Other EOs tend to have a strong effect on colour, which is the case for cinnamon. Ojagh et al. (2010) formulated chitosan films with cinnamon EO, and found that the slight yellowness of chitosan was increased by this EO. Atarés et al. (2010 a,b) elaborated films with cinnamon or ginger EOs and, even at low concentration, cinnamon EO affected the colour to a higher extent than ginger, increasing the chroma and reducing the whiteness index as the oil proportion was increased. Siripatrawan & Harte (2010) incorporated green tea extract into chitosan films, and found that its incorporation led to significant colour changes as the extract concentrations increased. Sánchez González et al. (2009) tested the incorporation of tea tree EO into HPMC films and found a significant increase of the whiteness index when this oil was added. This effect was attributed to the increase in diffuse reflectance provoked by light scattering in the lipid droplets (the higher the intensity of light scattering, the higher the whiteness index).

3.3.2. Transparency

The film transparency has been measured using a variety of methods. Tongnuanchan et al. (2012) measured the light transmittance of gelatin films at 200-800 nm, according to Shiku et al. (2004). Additionally, the “transparency value” was calculated by dividing the absorbance at 600 nm by the film thickness, according to Han & Floros (1997). The greater “transparency value” represents the lower transparency of film, which is why some other authors call this index “opacity index” (Gómez Estaca et al., 2009b). It was found that EO incorporation caused a film transparency reduction. The oils addition resulted in an important decrease of light transmission, possibly due to the light scattering at the interface of EO droplets imbedded in the film matrix. Consequently, an increase in opaqueness of films containing EOs was observed, which was affected by the type of EO. The same trend was observed by other authors (Siripatrawan & Harte, 2010; Pires et al., 2013; Norajit et al., 2010; Hosseini et al., 2009) using the same measurement method.

Apart from the “transparency value”, Pires et al. (2011) calculated the opacity (%) of hake protein-thyme EO films by obtaining the reflectance measurements of each sample with a black backing and with a white backing. Percentage opacity was calculated as:

$$\text{opacity}(\%) = \frac{Y_{\text{black backing}}}{Y_{\text{white backing}}} 100 \qquad \text{equation 1}$$

where Y is the tristimulus value Y. As the thyme oil proportion was increased in the films, the "transparency value", increased. However, the opacity (%) was not affected by the oil proportion, hence the authors considered that the changes in the "transparency value" may be related to the variable film thickness. All films obtained in this study were clear enough to be used as see-through packaging or coating materials.

In other studies, the theory of Kubelka-Munk has been used to study the translucency of EO incorporated edible films. This method consists of obtaining the surface reflectance spectra of the films using a spectrocolorimeter, both on a white and a black background. The film transparency is then evaluated through the internal transmittance (0-1, theoretical range) by appliying the Kubelka-Munk theory for multiple scattering to the reflection data (Hutchings, 1999). An increase in the internal transmittance (T_i) can be assumed as an increase in transparency. Sánchez-González et al. (2011a) used this method to study the optical properties of HPMC or chitosan films incorporated with diverse EOs. It was found that T_i values were significantly lower in films incorporating the highest amounts of EO. The composite films were more opaque than pure CH and HPMC films. This phenomenon is related with the light scattering provoked by lipid droplets (with a different refractive index) distributed throughout the film network. The transparency reduction caused by EO addition was also observed by Sánchez-González et al. (2009) when working with tea tree incorporated HPMC films. Atarés et al. (2011) also observed that HPMC films with a small proportion of ginger oil were more opaque than pure HPMC films.

3.3.3. Gloss

According to norm ASTM D523 (1999), gloss can be defined as the ratio of the luminous flux reflected from a specimen to the luminous flux reflected from a standard surface under the same geometric conditions. The surface morphology reached during the film drying determines the final gloss of the films (Sánchez-González et al., 2011a). EO incorporation in a polymer matrix normally produces an increase in heterogeneity and a surface roughness increase, which is normally linked to a gloss decrease (Ward & Nussinovitch, 1996). This effect is explained by the migration of oil droplets or aggregates to the film surface during drying, decreasing the specular reflectance in the air-film interface, and thus contributing to reduced gloss. This trend has been repeatedly observed in studies such as Sánchez-González et al. (2009, 2011a) on chitosan and HPMC films with bergamot, lemon or tea tree EOs, and Shojaee-Aliabadi et al. (2013), who incorporated *Satureja hortensis* extract into carragenaan films.

Probably, the amount of oil incorporated determines its effect on the films gloss. Atarés et al. (2010a) measured the gloss of soy protein films incorporated with little amounts of cinnamon or ginger EOs. All the films were described as only slightly glossy and no particular effect of the presence, type or proportion of oil was observed.

4. Effect of EO incorporation on the antioxidant properties of edible films and coatings

Oxidation is one of the most important ways of food degradation (Viuda-Martos et al., 2011). It takes place during the processing and storage of food products, and affects both their sensory and nutritional properties. Lipid oxidation is responsible for rancid odours and flavours, a decrease in nutritional quality and the formation of potentially toxic compounds (Yanishlieva et al., 2006). Due to the bad image of chemical additives, there has been growing interest and research on natural antioxidants without negative effects on the human health (Alves Silva et al., 2013).

Because of their chemical composition (rich in biologically active compounds such as terpenoids and phenolic acids), it has been long recognized that EOs have antioxidant properties (Ruiz Navajas et al., 2013; Alves Silva et al., 2013). The antioxidant action of EO incorporated into edible films and coatings was reviewed by Bonilla et al. (2012a). Briefly, two mechanisms take part in this action: promotion of the oxygen barrier capacity since antioxidants can act as oxygen scavengers (Bonilla et al. 2013) and specific antioxidant action when compounds diffuse to the coated product. Table 2 shows some recent studies dealing with the effect of EO addition on the *in vitro* antioxidant properties of edible films, quantified using a

variety of methods. Previously, the film extract is obtained by merely dissolving it in an appropriate solvent or through solidification, grinding and solution.

The antioxidant capacity of edible films is normally correlated with its available phenolic compound, which can be quantified using the Folin-Ciocalteau method. This method is based on the reaction between the film extract and Folin-Ciocalteau reagent, and the absorbance at 765 nm. A calibration curve is obtained using gallic acid, and the results are expressed as milligrams of gallic acid equivalent (GAE) per milliliter of extract. Oussalah et al. (2004) used this method to test the release of phenolic compounds over storage of milk protein-EOs films, and the migration was found to be correlated with the EOs chemical composition. Shojaee-Aliabadi et al. (2013) used this method and observed how the total phenolic content increased as the EO proportion in the film became higher.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was one of the earliest synthetic radicals used to test the activity of phenolic antioxidants. The disappearance of the DPPH radical absorption at 515nm by the action of antioxidants is measured spectrophotometrically until the absorbance remains constant (10 min to 6 hours). The 'antiradical efficiency' is quantified with the amount of antioxidant required for a 50% decrease in initial DPPH radical concentration and the time required to reach the steady state DPPH radical concentration. This assay is limited because DPPH radicals interact with other radicals (alkyl), and the time response curve to reach the steady state is not linear with different ratios of antioxidant/DPPH (Frankel & Meyer, 2000). Siripatrawan & Harte (2010) formulated chitosan films incorporated with green tea extract, and used DPPH test. They found that pure chitosan films showed some antioxidant capacity, and this increased with increasing green tea extract concentration. Dashipour et al. (2015) used the same method and corroborated that the *in vitro* antioxidant activity of carboxymethilcellulose – *Zataria multiflora* EO films increased proportionally with EO content. Norajit et al. (2010) used this method to test the antioxidant capacity of alginate films incorporated with ginseng extract, and found that all films containing the antioxidant had a significantly higher activity than those of pure alginate.

The reducing power method has been described by several authors (Gulcin et al., 2003; Oyaizu 1988). The film powder is mixed with potassium ferric cyanide solution and trichloroacetic acid is added. After centrifugation, the supernatant is mixed with ferric chloride solution and the absorbance is measured at 700nm. The results are normally expressed as mmol ascorbic acid per g of film, and high absorbance indicates high the reducing powder. Pires et al. (2011) used this method and observed how the addition of thyme oil increased the reducing power of hake protein films. Pires et al. (2013) used this test to compare the reducing power of hake protein films incorporated with different EOs, and concluded that coriander was the most effective. Moradi et al. (2012) used this method to conclude that the addition of *Zataria multiflora* EO to chitosan films could yield higher antioxidant activity, which was significantly increased with higher EO concentration.

The ferric-reducing antioxidant power (FRAP) assay measures directly the ability of antioxidants to reduce a ferric tripyridyltriazine complex to the ferrous complex at low pH. The resulting blue colour measured spectrophotometrically at 593nm is taken as linearly related to the total reducing capacity of electron-donating antioxidants (Frankel & Meyer, 2000). This method was used by Ruiz-Navajas et al. (2013) to test the antioxidant capacity of chitosan – thyme oil edible films, revealing that the antioxidant capacity occurred in a concentration dependent manner. Gómez-Estaca et al. (2009a) used FRAP assay to test the antioxidant capacity of gelatin films with oregano or rosemary EOs, and found that, accordingly to the response of pure EOs, those films including oregano were more active than those containing rosemary. This was also coherent with the higher phenol content in oregano EO.

Ferrous ion chelating activity (FIC) assay measures how effectively the compounds in the antioxidant sample can compete with ferrozine for ferrous ion (Ruiz-Navajas et al. 2013; Carter, 1971). The antioxidant causes the inhibition of the Fe^{+2}-ferrozine complex, which is spectrophotometrically quantified at 562nm. Ruiz-Navajas et al. (2013) used this method and found that chitosan films showed great chelating activity, which was increased by thyme oil addition in a concentration-dependent manner.

The original Trolox Equivalent Antioxidant Capacity (TEAC) method was modified by Re et al. (1999), and this modification has been very frequently used to test the antioxidant capacity of a wide variety of samples. In this method, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid), a vitamin E analogue, is used as an antioxidant standard. The substrate is ABTS (2,20-azinobis(3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt), a colourless compound which is initially oxidized to ABTS radical cation, a blue chromophore. This solution is then diluted with ethanol to an absorbance at 734 nm of 0.70 (± 0.02) and mixed with the antioxidant sample. The absorbance reduction is then registered over a 6 minutes period and compared to a calibration curve obtained with trolox solutions. Tongnuanchan et al. (2012) used this test to evaluate the antioxidant capacity of gelatin films containing citrus EOs, and found that lemon was significantly more effective than the rest, which was coherent with the FRAP results. Gómez-Estaca et al. (2009a) tested the antioxidant capacity of gelatin films with oregano or rosemary EOs using the TEAC method, and found that the films containing oregano EO were more active than those containing rosemary. This was in agreement with the results of TEAC performed on pure extracts.

5. Effect of EO incorporation on the antibacterial properties of edible films and coatings

The growth of spoilage microorganisms and food-borne pathogens is one of the most important causes for food degradation (Viuda-Martos et al., 2011). The presence of spoilage microorganisms in food can accelerate the lipid oxidation and other oxidation processes, or can produce changes in the organoleptic properties of the foods (Saggiorato et al., 2012). Food-borne pathogens are directly responsible for certain illnesses in the human organism, or can be indirectly responsible due to the production of toxins (Saggiorato et al., 2012).

Antimicrobial packaging could reduce food losses and increase the shelf-life of food products (Zhang et al., 2015). EOs extracted from plants and spices are rich sources of bioactive compounds (terpenoids, phenolic compounds…) which have been recognized as antimicrobial agents (Ruiz Navajas et al., 2013; Burt, 2004). The EOs affect microbial cells by various antimicrobial mechanisms, including attacking the phospholipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material of bacteria, and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids (Arques et al., 2008; Burt et al., 2007).

Most of the reported studies deal with the antibacterial action of EOs. However, their antifungal activity has also been reported (Saggiorato et al., 2012; Avila-Sosa et al., 2012, Perdones et al., 2012 and 2014; Alves Silva et al., 2013; Roselló et al., 2015).

The antibacterial activity of EOs can be tested *in vitro* using a variety of methods, as reviewed by Burt (2004). The most frequently used have been referred to as disk diffusion method, agar wells method, agar dilution method and broth dilution method. Gómez Estaca et al. (2010) utilized the agar wells method to qualitatively test the antibacterial activity of eight EOs on a wide range of bacteria. In order to test the *in vitro* antimicrobial activity of garlic oil, Pranoto et al. (2005) mixed dilute oil with nutrient broth and bacterial cultures of *E. coli, Salmonella typhimurium*, *S. aureus* and *B. cereus*. After observing that garlic oil inhibited growth of all bacteria tested, it was incorporated into edible films.

Since the use of EOs as food preservatives is limited by strong flavouring, inclusion into edible films represents an interesting alternative. However, since the EO forms part of the chemical structure of the film and interacts with the polymer and the plasticizer, the diffusion of the antimicrobial compounds into the product may be reduced (Ruiz-Navajas et al., 2013). The release of antimicrobial agents from the edible films depends on many factors, including electrostatic interactions between the antimicrobial agent and the polymer chains, osmosis, structural changes induced by the presence of the antimicrobial, and environmental conditions (Avila-Sosa et al., 2012). In spite of this, it has been stated that, in comparison with direct application, smaller amounts of antimicrobial agents would be needed when edible films are used as carriers in order to achieve a specific food shelf life due to a gradual release on food surfaces (Ponce et al. 2008).

Table 3 shows some recent studies dealing with the effect of EO addition on the antimicrobial activity of edible films. Screening of antibacterial activity of edible films is often done by the disk diffusion method, in which a film disk is laid on top of an inoculated agar plate. Ruiz-Navajas et al. (2013) used it to test the antibacterial activity of chitosan-thyme EO edible films, to find that they exhibited excellent *in vitro* antibacterial activity against a variety of Gram-positive and Gram-negative bacteria. Benavides et al. (2012) used the same method and found that alginate-oregano EO films were significantly more effective against Gram-positive than against Gram-negative bacteria. Seydim & Sarikus (2006) incorporated oregano, rosemary and garlic EOs into whey protein films, and found that the most effective antimicrobial films were those incorporated with oregano, whereas the least effective were those including rosemary oil. The same method has been used in other recent studies (Sánchez-Aldana et al., 2015;, Jouki et al., 2014; Aliheidari et al., 2013; Emiroğlu et al., 2010; Iturriaga et al., 2012; Pires et al., 2013).

Apart from the agar disk diffusion method, Shojaee-Aliabadi et al. (2013) used the disk volatilization method in order to examine the antimicrobial activities in vapor phase of carrageenan- *Satureja hortensis* EO films. Disks were cut and laid on the inside surface of the upper lid, with no direct contact between it and the bacteria strains. All tested bacteria were more inhibited by direct contact with the antimicrobial films than by the vapors, and a close relationship was found between the results of the direct-contact and vapor-phase tests.

6. Application of active food packaging

In the last years, many research studies have focused on the application of active food packaging on a broad array of real food systems to the aim of studying their antioxidant and antibacterial effect.

The overall efficacy of edible films and coatings as oxidation protectors depends not only on their chemical antioxidant ability but also on their oxygen barrier properties. As commented on above, the incorporation of EOs into a polymer matrix may have important consequences on the final structure of the emulsified film, thus affecting its barrier properties and its antioxidant effectiveness (Bonilla et al. 2012a). In order to test the actual capability of edible films and coatings to improve the shelf life of food products prone to oxidation, model and real food systems have been used, as reported by the studies in table 4. These studies have dealt with the application of potentially antioxidant films or coatings including EOs on a wide variety of food systems, namely meat, fish, fruit, vegetables, oils and nuts.

Sánchez González et al. (2011c) incorporated bergamot EO into HPMC or chitosan edible coatings and tested their efficacy on coated grapes, but no differences caused by coating formulation were observed. Atarés et al. (2010b) used an accelerated rancidity test according to Osés et al. (2008) to evaluate the antioxidant capacity of sodium caseinate films with cinnamon or ginger EOs. Sunflower oil was used as model system, and its peroxide value was quantified over storage time. It was found that all formulations effectively protected the oil against oxidation. Atarés et al. (2011) performed the same test using almond oil protected with HPMC films to test the effect of ginger oil addition. A trend towards a better protection was found in films containing ginger oil, even though this oil caused a significant oxygen permeability increase. The same formulations were applied on roasted almonds as coatings and it was found that almonds stored at intermediate relative humidity were efficiently protected against lipid oxidation due to ginger oil addition.

Oussalah et al. (2004) stored meat samples covered with milk protein films with oregano or pimento EOs. In this case, the incorporation of EOs did not improve the protection against lipid oxidation. This was inconsistent with previous studies, since phenolic compounds have repeatedly proved their antioxidant power (Lee & Anh, 2003, Nam et al., 2003, Chen et al. 1999). However, the ability of oregano oil to inhibit lipid peroxidation was more important than that of pimento oil, which is richest in phenolic content. Gómez-Estaca et al. (2007) elaborated gelatin based edible films, with or without chitosan, and incorporated oregano or rosemary EOs in order to test their effect when applied on cold-smoked sardine. The stability of the product was improved by coating with gelatin based edible films, and films enriched with EOs were able

to slow lipid oxidation. Volpe et al. (2015) proved the efficacy of carrageenan edible films incorporated with lemon EO to increase the shelf life of trout fillets, as compared to the films without EO.

The antimicrobial activity of edible films and coatings incorporated with EOs have also been tested in model and real food systems (Table 5). Sánchez-González et al. (2011c) analyzed the antimicrobial properties of chitosan films with different concentrations of bergamot, lemon and tea tree EOs. Inoculated agar plates were utilized as model food systems and the periodical microbial counts revealed the antimicrobial activity of the films. Sánchez-González et al. (2011c) proved the antimicrobial activity of HPMC or chitosan coatings incorporated with bergamot EO applied on grapes. Similar studies performed on other fruits had similar results, such as Raybaudi-Massilia et al. (2008) on melon and Salvia-Trujillo et al. (2015) on apples. Zinoviadou et al. (2009) formulated whey protein-oregano EO edible films and proved their antimicrobial activity on beef samples over refrigerated storage. Emiroğlu et al. (2010) tested the antimicrobial activity of soy edible films incorporated with thyme and oregano EOs on fresh ground beef patties and found microbial count reductions over refrigerated storage. Wu et al. (2014) proved the antimicrobial activity of gelatin-chitosan films with oregano EO on the storage of carp muscle.

7. Conclusions and future trends

Essential oils have been incorporated into edible/biodegradable films and coatings through emulsification, having an impact on the film structural, physical and bioactive properties. EO addition entails discontinuities in the polymer network, leading to changes in the physical properties (typically some weakening, reduced water permeability and increased opaqueness). Aiming to extend shelf-life and add value to the product, EOs can provide the films and coatings with antioxidant and/or antimicrobial properties, depending both on their composition and the interactions with the polymer matrix. The antioxidant activity depends not only on the specific antioxidant activity of the oil compounds but also on the film's oxygen permeability. The incorporation into edible films can promote the antimicrobial capacity of EOs, and the effectiveness of the edible film against microbial growth will depend on the oil nature and the type of microorganism. More work is necessary in this research area in order to optimize the effectiveness of the bioactive incorporated agents.

In most research works in this area, the incorporation of EO into edible films and coatings has been done through conventional emulsification. The contact between the EO components and the polymer matrix could be improved with nano-emulsification, which would also reduce EO volatilization losses. This technique is emerging as a potential tool in food technology, and it would probably yield EO-polymer nano-emulsions presenting higher resistance to destabilization phenomena than conventional emulsions. Therefore, the close components contact in dry films would probably provide them with improved properties such as increased transparency and reduced water permeability. Recent studies have also proven the improved antimicrobial capacity of EO when encapsulated into nanometric surfactant micelles. Apparently, being trapped into the vesicle of the nanoliposomes, EO can be released slowly, hence improving the antimicrobial activity of the film. Wu et al. (2015) incorporated cinnamon EO nanoliposomes into gelatin films, and found an improvement of the antimicrobial stability along with a decrease in the release rate. Controlled/sustained release is a relevant treat to be attained when incorporating active ingredients into food packaging. In this respect, release studies are becoming more and more important in this area.

EO can easily be incorporated into polymer aqueous solutions to obtain cast films. However, casting is not readily applicable in packaging industry, unlike compression molding or extrusion, in which high temperature is applied to the material. In this sense, few studies are reported (Pelissari et al., 2011; Valderrama & de Rojas, 2012) and the challenge is how to keep the antimicrobial activity of essential oil in the films during the high temperature of the plastic production processes.

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Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *Journal of Food Science*, 70(1), 45-51.

1050 Table 1: Recent papers studying the effect of essential oils on films properties. (HPMC: hydroxyproplymethylcellulose; RT: room temperature)

	Matrix	Essential oils	Matrix: oil proportion	T-%RH conditioning	Physical & microstructural tests								Reference
					SEM	AFM	Tensile	WVP	OP	Colour	Opacity	Gloss	
Polysaccharides	Lignocellulose	Cedarwood	1:0-0.2	23ºC-50%	-	-	+	+	-	-	-	-	Shen & Kamdem (2015)
	HPMC	Tea tree	5%:0.5-2%	20ºC-54.4%	+	-	+	+	-	+	+	+	Sánchez-González et al. (2009)
	HPMC	Ginger	1:0.1	25ºC-33/53%	+	-	+	+	+	+	+	+	Atarés et al. (2011)
	HPMC, chitosan	Bergamot, lemon, tea tree	1:0.5-2	20ºC-54.4%	-	-	+	+	-	-	+	+	Sánchez-González et al. (2011a)
	Chitosan	Cinnamon	2%(w/v):0.4-2% (v/v)	25ºC-51%	+	-	+	+	-	+	-	-	Ojagh et al. (2010)
	Chitosan	*Zataria multiflora* Boiss, grape seed	20:0-20	25ºC-52%	-	-	+	+	-	+	-	-	Moradi et al. (2012)
	Chitosan	Bergamot	1:0-3	20ºC-54.4%	-	-	+	+	-	-	+	+	Sánchez-González et al. (2010)
	Chitosan	Green tea extract	2%: 0-20% (w/v)	25ºC-50%	-	-	+	+	-	+	+	-	Siripatrawan & Harte (2010)
	Chitosan	Basil, thyme	1:0.5-1	5ºC-58%	+	-	+	+	-	-	-	+	Bonilla et al. (2012b)
	Alginate	Garlic	1g:0-0.4ml	-	-	-	+	+	-	+	-	-	Pranoto et al. (2005)
	Alginate	Oregano	1:0-1	25ºC-50%	+	-	+	+	-	+	-	-	Benavides et al. (2012)
	Alginate	Ginseng	2%:0.5g/ml	-	+	-	+	+	-	-	+	-	Norajit et al. (2010)
	Alginate	Thyme, lemongrass, sage	3%:1%	25ºC-50%	+	-	+	+	-	+	+	-	Acevedo-Fani et al. (2015)
	κ-carrageenan	*Satureja hortensis*	1% (w/v): 1-3% (v/v)	25ºC-53%	+	+	+	+	-	+	+	+	Shojaee-Aliabadi et al. (2013)
	Fish gelatin and chitosan	*Origanum vulgare*	1.75%:0.4-1.2%	25ºC-50%	+	+	+	+	-	-	+	-	Hosseini et al. (2015)
Proteins	Soy protein	Cinnamon, ginger	1:0.025-0.100	25ºC-33%	-	+	+	+	-	+	+	+	Atarés et al. (2010a)
	Na caseinate	Cinnamon, ginger	1:0.025,0.075	25ºC-33/53%	+	+	+	+	-	+	+	+	Atarés et al. (2010b)
	Fish protein	Clove, garlic, origanum	4mg:1μl	RT-57%	-	-	+	+	-	+	+	-	Teixeira et al. (2014)
	Fish skin gelatin	Bergamot, kaffir lime, lemon, lime	1:0.5	25ºC-50%	+	-	+	+	-	+	+	-	Tongnuanchan et al. (2012)
	Gelatin	Bergamot, lemongrass	3:0-20	25ºC-50%	+	-	+	+	-	+	+	-	Ahmad et al. (2012)
	Gelatin	Oregano, lavender	1:0.04-0.12	25ºC-65%	+	-	+	+	-	+	-	-	Martucci et al. (2015)
	Hake protein	Thyme	1g: 0.025-0.25ml	RT-57%	-	-	+	+	-	+	+	-	Pires et al. (2011)
	Hake protein	Citronella, coriander, tarragon, thyme	1g: 0.25 ml	RT-57%	-	-	+	+	-	+	+	-	Pires et al. (2013)

*corresponding author: loathue@tal.upv.es

Whey protein	Oregano	1:0.1-0.3	25ºC-53%	-	-	+	+	-	-	-	-	Zinoviadou et al. (2009)

Table 2: Recent studies dealing with the effect of essential oil addition on the *in vitro* antioxidant properties of films

Matrix		Essential oils	Matrix: oil proportion	Tests						Reference
				Total phenolics	DPPH	Reducimg power	FRAP	Ferrous ion chelating activity	TEAC	
Polysacharides	Chitosan	*Zataria multiflora* Boiss, grape seed extract	1:0.25-0.5	+	+	+	-	-	-	Moradi et al. (2012)
	Alginate	Ginseng	2%:0.5g/ml	-	+	+	-	-	-	Norajit et al. (2010)
	Chitosan	Thyme	1:0.25-1	+	+	-	+	+	-	Ruíz-Navajas et al. (2013)
	κ-carrageenan	*Satureja hortensis*	1% (w/v): 1-3% (v/v)	+	+	-	-	-	-	Shojaee-Aliabadi et al. (2013)
	Carboxymethyl cellulose	*Zataria multiflora* Boiss	1% (w/v): 1-3% (v/v)	+	+	-	-	-	-	Dashipour et al. (2015)
	Mucilage	Oregano	1%:0-2%	+	+	-	-	-	-	Jouki et al. (2014)
	Chitosan	Green tea extract	2%: 0-20% (w/v)	+	+	-	-	-	-	Siripatrawan & Harte (2010)
Proteins	Hake protein	Thyme	1g:0.025-0.25ml	-	+	+	-	-	-	Pires et al. (2011)
	Hake protein	Citronella, coriander, tarragon, thyme	1g:0.25 ml	-	+	+	-	-	-	Pires et al (2013)
	Fish skin gelatin	Bergamot, kaffir lime, lemon, lime	1:0.5	+	+	-	+	-	+	Tongnuanchan et al. (2012)
	Tuna-skin and bovine-hide gelatin	Oregano, rosemary	1:0.1-5	+	-	-	+	-	+	Gómez-Estaca et al. (2009a)
	Milk protein	Oregano, pimento	5%(w/v):1%(w/v)	+	-	-	-	-	-	Oussalah et al. (2004)

Table 3: Recent studies dealing with the effect of essential oil addition on the antimicrobial activity of edible films (*in vitro* tests)

Matrix		Essential oils	Matrix: oil proportion	Microorganisms tested	Reference
Polysaccharides	Chitosan	Thyme	1:0.25-1	*S. marcenscens, A. hydrophila, A. faecalis , A. denitrificans, L. innocua.*	Ruíz-Navajas et al. (2013)
	Chitosan	Bergamot	1:0-3	*B. thermosphacta, E. coli, L. innocua, L. monocytogenes, P. putida, S. typhimurium, S. putrefaciens*	Sánchez González et al. (2010)
	Chitosan	Clove bud, cinnamon, star anise	2%(w/w):2.5-10%	*E. coli, S. aureus, A. oryzae, P. digitatum*	Wang et al. (2011)
	Alginate	Oregano	1:0-1	*E. coli, S. Enteritidis, S. aureus, L. monocytogenes*	Benavides et al. (2012)
	Pectic extract	Lime	0.7-1%:0-1g/l	*Escherichia coli O157:H7, Salmonella Typhimurium , Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes*	Sánchez-Aldana et al. (2015)
	κ-carrageenan	Satureja hortensis	1% (w/v): 1-3% (v/v)	*S. aureus, B. cereus, E. coli, P. aeruginosa, S. typhimurium*	Shojaee-Aliabadi et al. (2013)
	Alginate	Garlic	1g:0-0.4ml	*E. coli, S. typhimurium, S. aureus, B. cereus*	Pranoto et al. (2005)
	Chitosan	Cinnamon	2%(w/v):0.4-2% (v/v)	*L. monocytogenes, E. coli, L. plantarum, L. sakei, P. fluorescens*	Ojagh et al. (2010)
	Chitosan, CMC	Olive, rosemary, capsicum	2% or 0.75%:1%	*L. monocytogenes*	Ponce et al. (2008)
	Mucilage	Oregano	1%:0-2%	*L. monocytogenes, Salmonella typhimurium, Bacillus cereus, Yersinia enterocolitica, Pseudomonas aeruginosa, S. aureus, E. coli, E. coli O157:H7, Shewanella putrefaciens,and Vibrio cholera*	Jouki et al. (2014)
	Metylcellulose	Oregano, thyme	1:0.5	*P. fluorescens*, *A. hydrophila/caviae*, *L. innocua*	Iturriaga et al. (2012)
Proteins	Gelatin,				
	Whey protein	Oregano, Rosemary, garlic	1:0.2-0.8	*L. plantarum, S. enteritidis, E. coli O157:H7 , L. monocytogenes, S. aureus*	Seydim &Sarikus (2006)
	Soy protein	Oregano, thyme	1:0-1	*E. coli*, *S. aureus*, *E.coli* O157:H7, *P. aeruginosa* and *L. plantarum*	Emiroglu et al. (2010)
	Sodium caseinate	Matricaria recutita	5%(w/w):1%(v/v)	*Listeria monocytogenes, S. aureus and E. coli O157:H7*	Aliheari et al. (2013)
	Gelatin alone or with chitosan	Clove	1g/ 0.75 ml	*P. fluorescens, L. acidophilus, L. innocua, E. coli.*	Gomez-Estaca et al. (2010)
	Hake protein	Citronella, coriander, tarragon, thyme	1g/ 0.25 ml	*B. thermosphacta, E. coli, L. innocua, L. monocytogenes, P. putida, S. typhimurium, S. putrefaciens*	Pires et al. (2013)
	Gelatin	Bergamot, lemongrass	3:0-20	*E. coli, S. typhimurium, P. aeruginosa, L. monocytogenes, S. aureus*	Ahmad et al. (2012)

Table 4: Recent studies dealing with the application of essential oil incorporated edible films/coatings on food systems prone to oxidation

Matrix		Essential oils	Matrix: oil proportion	Food system	tests	Reference
Polysacharides	HPMC or chitosan	Bergamot	1:2	Coated cv. Muscatel grapes	- total phenols of grapes - DPPH of grapes	Sánchez-González et al (2011c)
	HPMC	Ginger	1:0.1	Almond oil and coated almonds	-PV of almond oil -PV of coated almonds	Atarés et al. (2011)
	Carrageenan	Lemon	1:1	Trout fillets	Fatty acid profile and lipid quality	Volpe et al. (2015)
	Chitosan, carboxymethyl cellulose	olive, rosemary, onion, capsicum, cranberry, garlic, oreganum	2% or 0.75%:1% 5%:1%	butternut squash	Peroxidase and polyphenoloxidase activities	Ponce et al. (2008)
Proteins	Casein					
	Na caseinate	Cinnamon, Ginger	1: 0.075	Sunflower oil	-PV of sunflower oil	Atarés et al. (2010b)
	Milk protein	Oregano, pimento	5%(w/v):1%(w/v)	Beef muscle	TBA of beef	Oussalah et al.(2004)
	Gelatin (with or without chitosan)	Rosemary, oregano	4%w/v:1.25% or 20%	Smoked sardine	-PV and free fatty acids of muscle -TBA of muscle - total phenol content of muscle - FRAP of the muscle	Gómez- Estaca et al. (2007)

1080 Table 5: Recent studies dealing with the application of essential oil incorporated edible films on the microbial growth in model or real food systems (*in vivo*
1081 tests)
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Matrix		Essential oils	Matrix: oil proportion	Food system	Tests	Reference
Polysaccharides	Chitosan	Bergamot	1:0-3	Agar	Counts of *P italicum*. on inoculated agar	Sánchez González et al. (2010)
	Chitosan, HPMC	Bergamot, lemon, tea tree	1:0.5-3	Agar	Counts of *E.coli*, *L.monocytogenes* and *S.aureus* on inoculated agar	Sánchez-González et al. (2011d)
	Chitosan	Lemon	1:3	Strawberry	Inoculated with *B.cinerea*. % fungal decay	Perdones et al. (2012)
	HPMC, Chitosan	Bergamot	1:2	Grape	Periodic microbial counts of total aerobic mesophilic microorganisms, yeast and mould over storage	Sánchez-González et al. (2011c)
	Alginate	Lemongrass	2% (w/v):0.1-1% (v/v)	Apple	Inoculated with *E.coli*, Counts of psychrophilic bacteria or molds and yeast	Salvia-Trujillo et al. (2015)
	Alginate	Cinnamon, palmarosa and lemongrass	1:0.15-0.35	Melon	Counts of mesophilic, psychrophilic and yeasts and moulds populations in non-inoculated samples. Counts of *S. Enteritidis* in coated inoculated fresh-cut melon	Raybaudi-Massilia et al. (2008)
	Silver carp skin gelatin-chitosan	oregano, cinnamon and anise	1:0.25-1	Carp	Total aerobic count,	Wu et al. (2014)
Protein	Whey protein	Oregano	1:0.1-0.3	Beef	Total viable count, lactic acid bacteria, pseudomonas	Zinoviadou et al. (2009)
	Soy protein	Oregano, thyme	1:0-1	Ground beef patties	*Total viable counts, lactic acid bacterial counts, coliform, Staphylococccus counts, Pseudomonas counts*	Emiroglu et al. (2010)
	Gelatin alone or in combination with chitosan	Oregano rosemary	4%w/v:1.25% or 20%	Sardine	*Total bacteria counts, H_2S-reducing organisms counts, luminescent bacteria counts, Enterobacteriaceae counts*	Gómez Estaca et al. (2007)
	Gelatin alone or in combination with chitosan	Clove	0.75 ml/g biopolymer	Cod	Total bacterial counts, H_2S producers organisms, luminescent bacteria, pseudomonas, enterobacteriaceae, lactic acid bacteria	Gómez-Estaca et al. (2010)

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