LWT - Food Science and Technology 113 (2019) 108290

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Antioxidant starch-based films with encapsulated eugenol. Application to sunflower oil preservation.

Emma Talón*, María Vargas, Amparo Chiralt, Chelo González-Martínez

Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universitat Politècnica de València, Valencia, Spain

ARTICLE INFO

Keywords:
Antioxidant
Release
Barrier properties
Tensile and optical properties
Sunflower oil

ABSTRACT

Starch films containing eugenol, which was added to the film-forming dispersion in free form or encapsulated with different wall materials (whey protein or lecithin), were obtained by casting. The physical and the antioxidant properties of the films, the release kinetics of eugenol in different food simulants and their performance at preventing sunflower oil oxidation during storage were evaluated. Encapsulated eugenol modified the film microstructure, yielding less stretchable films with reduced water affinity, transparency and oxygen permeability as compared to films formulated with non-encapsulated eugenol. The addition of eugenol microcapsules containing oleic acid promoted the eugenol retention in the starch matrix during film formation and thus, these films exhibited the greatest antioxidant activity. Films developed with encapsulated eugenol powder containing lecithin and oleic acid were highly effective at preventing sunflower oil oxidation even throughout 53 days of storage at 30 °C, maintaining low and almost constant values of peroxide index, conjugated dienes and trienes in comparison with the control samples.

1. Introduction

Current research efforts have been focused on the use of natural and biodegradable packaging materials, which could also contain active compounds, such as antioxidants and antimicrobial agents that provide them with several functional properties non-existent in conventional packaging systems (Carrizo, Taborda, Nerín, & Bosetti, 2016).

The encapsulation of the active compounds in different wall systems can diminish the active losses during processing of packaging materials containing these compounds. Likewise, encapsulation can modulate the release kinetics of the active into the product (Valencia-Sullca et al., 2016). Of the different encapsulation techniques, microencapsulation by spray-drying represents a common and interesting option since it is a fast process in which the temperatures reached are relatively low and thus, very convenient for the purposes of the microencapsulation of thermosensitive compounds (Barrow, Wang, Adhikari, & Liu, 2013). This method could also contribute to improving the retention of volatile compounds in the final encapsulating powder (Jafari, He, & Bhandari, 2007).

The choice of the wall materials for the encapsulation of active ingredients is critical in the production of microparticles by spray-drying, since they greatly influence the properties of the emulsion before drying and the retention of the active compound and the life of the product in powdered form after drying (Botrel, de Barros Fernandes, Borges, & Yoshida, 2014). Whey protein (WP) and lecithin (L) are capable of successfully encapsulating active compounds and stabilizing oil-in-water emulsions (Bae & Lee, 2008). When developing micro and nanocapsules by means of spray-drying techniques, WP and L are often combined with carbohydrates like maltodextrins, since they increase oxidative stability due to the formation of a dry crust around the droplets during drying (Kagami et al., 2003). The addition of oleic acid in the capsule formulation can also increase the retention and stability of the hydrophobic active compounds (Pokharkar, Shekhawat, Dhapte, & Mandpe, 2011).

The incorporation of different free or encapsulated active compounds into films can have an impact on their physical properties, which, in turn, can affect their functionality as packaging material (Sánchez-González, Arab-Tehrany, Cháfer, González-Martínez, & Chiralt, 2015). The oxygen barrier properties of the packaging can directly affect the quality and shelf-life of the food product, since a high barrier capacity and potential oxygen scavenging ability could greatly reduce the oxidation reactions (Bonilla, Talón, Atarés, Vargas, & Chiralt, 2013). In turn, the functional properties of the active compound could be affected by the release kinetics towards the food system (Sánchez-González, Cháfer, González-Martínez, Chiralt, & Desobry, 2011a). These parameters have been little explored and are dependent

* Corresponding author.
E-mail address: emtalar@etsid.upv.es (E. Talón).

https://doi.org/10.1016/j.lwt.2019.108290
Received 9 January 2019; Received in revised form 17 June 2019; Accepted 19 June 2019
Available online 20 June 2019

on several factors, such as the food product characteristics and film structure.

Sunflower oil is rich in unsaturated fatty acids, mainly oleic and linoleic. Regular sunflower oil contains 69% linoleic acid, 20% oleic acid and 11% saturated fatty acids (Khan, Choudhary, Pandey, Khan, & Thomas, 2015), although several varieties of sunflower oil are produced containing high linoleic, high stearic (~18%) and high oleic (~70%) acids (Anushree, André, Guillaume, & Frédéric, 2017). The highly reactive nature of the unsaturated fatty acids is associated with their oxidation (rancidity), which can modify the organoleptic properties of the oil, causing losses in nutritional value and quality (Kucuk & Caner, 2005) and affecting its shelf life.

Starch-based films possess high oxygen barrier capacity (Jiménez, Fabra, Talens, & Chiralt, 2012a) and could be a low-cost biodegradable alternative for oil packaging, mainly for flexible unitary dose packs. The appropriate OP of an edible film or coating depends on its application. For example, when the edible film is used on high fat foods, the oxygen permeability must be very low. On the other hand, the functional properties of these films can be enhanced by incorporating natural antioxidant compounds which can act as oxygen scavenging agents (Bonilla et al., 2013).

Eugenol is a natural phenolic compound abundant in clove (90%) and cinnamon (60%) essential oils with interesting antimicrobial and antioxidant properties (Chatterjee & Bhattacharjee, 2013; Kamatou, Vermaak, & Viljoen, 2012). Eugenol has been widely used in pharmaceuticals, cosmetics and food products and, recently, in active packaging for food preservation (Sanla-Ead, Jangchud, Chonhenchob & Suppakul, 2012). This natural compound could be incorporated into the formulation of bioactive films to be used for food preservation, leading to an antioxidant effect on the surface of the product, thus preventing the occurrence of some oxidative reactions. However, it is difficult to handle due to the limited water solubility and it is susceptible to a loss of functionality during its handling or processing (Choi, Soottitantawat, Nuchuchua, Min, & Ruktanonchai, 2009).

The aim of this study was to evaluate the physical properties, the release kinetics in different food simulants and the antioxidant performance for the sunflower oil preservation of corn starch-based films containing eugenol. The latter was added to the film-forming dispersions in free form or microencapsulated (powder) with different wall-materials (whey protein or lecithin).

2. Materials and methods

2.1. Raw materials

To obtain the microencapsulated eugenol, lecithin (L) lipoid S45 (Batch 574510, Lipoid, Germany), whey protein isolate (WP) 90S (Batch 131848) and maltodextrin (MD) Kyrosan E18 (Batch 02157372) from Haarla (Tampere, Finland), Eugenol (E) (Batch STBD6235V, Sigma-Aldrich, Madrid, Spain) and purified oleic acid (OA) (Batch 15C030505, VWR Chemicals Germany) were used.

Corn starch (S) (Batch RMA12, Roquette Laisa SA, Benifaió, Spain) and glycerol (Panreac Química SLU, Castellar del Vallès, Barcelona, Spain) were used to prepare the films.

2,2-Diphenyl-1-pikryl-hydrazyl (DPPH) and Folin-Ciocalteu, from Sigma-Aldrich (Madrid, Spain) and glacial acetic acid, absolute ethanol, methanol, sodium carbonate (Na_2CO_3), magnesium nitrate ($Mg(NO_3)_2$) and phosphorus pentoxide (P_2O_5), provided by Panreac Química SLU (Barcelona, Spain) were used to carry out the different analyses.

2.2. Preparation of the microcapsules

Microencapsulated eugenol was obtained as described in a previous study (Talón, 2018), by spray-drying. Briefly, 43% (w/w) of WP:MD or L:MD solids (ratio 1:42) and 3% (w/w) of eugenol (total eugenol amount: 65.22 mg Eu/g powder) with and without 7% (w/w) of OA (total eugenol amount: 56.60 mg E/g powder) were dissolved. All the dispersions were homogenized with a rotor-stator (Ultra-Turrax T 25 Basic, IKA Werke GmbH and Co. KG, Germany) at 11,000 rpm for 6 min, and afterwards were microfluidized three times with a high-pressure homogenizer (Microfluidics M-110Y, Newton, Massachusetts, USA) at 103.4 MPa. The different microencapsulated eugenol powders were obtained by using a spray-drier (Mobile Minor TM spray-drier, GEA Niro, GEA Process Engineering A/S, Søborg, Denmark) with a rotary atomiser with an inlet air temperature of 180 °C. The outlet temperature was kept at 80 ± 2 °C by controlling the feed rate.

2.3. Preparation of the films

Firstly, an aqueous starch dispersion 3% (w/w) was heated at 95 °C for 30 min, in order to gelatinize the starch, shaking every 5 min. Thereafter, the dispersion was cooled for 10 min in an ice bath and glycerol was added in a starch:glycerol ratio (w/w) of 1.5:0.5. The formulations were homogenized with a rotor-stator (Yellow Line DL 25 Basic, IKA, Janke and Kunkel, Germany) at 13,500 rpm for 3 min and degassed by means of a vacuum pump. Free Eugenol (E) or microencapsulated eugenol powder were then added to obtain the film-forming dispersions (FFDs) by using a rotor-stator (13,500 rpm, 3 min) and by magnetic stirring for 5 min, respectively, with the starch eugenol ratio shown in Table 1 (about 0.017–0.029 g E/g total solids). FFDs were poured into Teflon plates by keeping the total amount of starch and glycerol constant (11 mg/cm^2) in the plate. After drying at controlled relative humidity and temperature (53% RH and 25 °C), the films were conditioned in a desiccator at 25 °C and 53% RH, using an oversaturated $Mg(NO_3)_2$ solution.

2.4. Characterization of the films

2.4.1. Microstructure, moisture content, solubility and thickness

The microstructure of previously dehydrated film samples was observed by Field emission scanning electron microscopy (FESEM) (JEOL, model JSM-5410, Japan). The samples were cryofractured by immersion in liquid nitrogen in order to observe the cross-section surfaces, mounted on the sample holder using the double-sided carbon tape, coated with platinum and observed using an accelerating voltage of 2 kV.

The water content (g H_2O/100 g dry matter) of the different conditioned films was determined by placing them into a vacuum oven (TEM-T vacuum. J.P. Selecta, S.A., Barcelona, Spain) at 60 °C for 48 h and later on, in a desiccator with phosphorus pentoxide until reaching constant weight. Three replicates were considered per formulation.

For the solubility test, pieces of dry samples were transferred to a mesh of known weight and distilled water was added in a film-water ratio of 1:50 for 7 days at 20 °C. The meshes with the samples were placed in an oven (J.P. Selecta, S.A., Barcelona, Spain) at 60 °C for 48 h and subsequently, were transferred to a desiccator with phosphorus pentoxide until constant weight was reached. The assay was performed in triplicate and the results were expressed as g of solubilized film/

Table 1
Nominal mass fraction (g/g total solids) of the different components of the films, including capsule compounds, and theoretical starch:eugenol (S:E) ratio.

Film	S[a]	Gly[a]	WP[a]	LE[a]	MD[a]	E[a]	OA[a]	S:E
S	0.750	0.250	-	-	-	-	-	
S-E	0.728	0.243	-	-	-	0.029	-	1:0.040
S-E-WP	0.500	0.167	0.007	-	0.305	0.021	-	1:0.040
S-EOA-WP	0.500	0.167	0.006	-	0.266	0.017	0.044	1:0.034
S-E-L	0.500	0.167	-	0.007	0.305	0.021	-	1:0.040
S-EOA-L	0.500	0.167	-	0.006	0.265	0.018	0.044	1:0.036

[a] S: Starch, Gly: Glycerol, E: Eugenol, WP: Whey Protein Isolate, OA: Oleic acid, LE: Lecithin.

100 g film.

The film thickness was measured at sixteen random points on three samples of all the formulations conditioned at 25 °C and 53% RH by means of a digital electronic micrometer with an accuracy of 0.001 mm (Palmer model COMECTA, Barcelona).

2.4.2. Barrier, tensile and optical properties

Water vapour permeability (WVP) was determined gravimetrically following a modification of the ASTM E96-95 gravimetric method (1995), at 25 °C and a RH gradient of 53–100%, using Payne permeability cups (Talón et al., 2017). The side of the films in contact with air during film drying was exposed to the atmosphere at the lowest RH (53%). The cups were weighed periodically using an analytical balance (ME36S, Sartorius, Germany; ± 0.00001 g) at intervals of 1.5 h for 24 h after the steady state had been reached. The slope of the weight loss versus time was plotted and the WVP was calculated according to Bonilla et al. (2013).

Oxygen permeability (OP) was determined by following the ASTM Standard Method D3985-05 (2010). Three 50 cm^2 replicates of each formulation were measured by using the Ox-Tran equipment (Model 1/50, Mocon, Minneapolis, USA) at 25 °C and 53% of RH. Oxygen permeability was calculated by dividing the oxygen transmission rate (OTR) by the difference in oxygen partial pressure between the two sides of the film and multiplying by the film thickness.

The mechanical behavior of the films was analyzed using a Universal Testing Machine (Stable Micro System TA-XT plus, Haslemere, England) following ASTM standard method D882 (2001). The thickness of eight pre-conditioned film pieces of 25 mm × 100 mm was measured at six random points by means of an electronic digital micrometer (Comecta S.A., Barcelona, Spain), were mounted in the film's extension grip and stretched at a rate of 50 mm min^{-1} until breaking. Force-distance curves were obtained and transformed into stress-strain curves. The tensile properties were analysed in terms of elastic modulus (EM), tensile strength (TS) and percentage of elongation at break (%E).

Optical properties were determined in triplicate by measuring the reflection spectrum of the samples from 400 to 700 nm of wavelength using a MINOLTA spectrocolorimeter (model CM-3600d, Minolta CO., Tokyo, Japan). The transparency was measured by means of the internal transmittance (T_i), applying the Kubelka-Munk theory of the multiple dispersion of reflection spectrum (Hutchings, 1999) given the reflection spectra of both black and white backgrounds. Measurements were taken on the side of film which was in contact with air during film drying. CIEL*a*b* colour coordinates and chromatic parameters (chroma and hue) were obtained from the reflectance of an infinitely thick layer of the material by considering illuminant D65 and observer 10°.

2.4.3. Quantification of eugenol in the films and antioxidant activity

The antioxidant activity of the films was carried out by using two different methodologies: 2,2-diphenyl-1-pikryl-hydrazyl (DPPH) (Brand-Williams, Cuvelier, & Berset, 1995) and Folin-Ciocalteu (Stojanovic et al., 2012). Since eugenol is a phenolic compound, it is also possible to relate its antioxidant power to the total amount of the compound.

The film samples (1.5 g) were dispersed in 100 mL of methanol and kept under stirring for 15 days for the purposes of taking the antioxidant activity measurements. After that, the samples were homogenised by using the rotor-stator (Yellow Line DL 25 Basic, IKA, Janke and Kunkel, Germany).

The antioxidant capacity was determined by means of DPPH by mixing between 0.15 and 1.05 mL (0.15 ml volume increments) of different appropriately diluted samples with a methanol solution of $DPPH^{\cdot}$ (with an absorbance of 0.70 ± 0.02 (λ = 515 nm), as described by Talón et al. (2017). The samples were kept in the dark in covered cuvettes for 4 h, when the reaction stabilized (Bortolomeazzi, Sebastianutto, Toniolo, & Pizzariello, 2007). The absorbance measurements (λ = 515 nm) were taken in a spectrophotometer (Evolution 201 VisibleUV, ThermoScientific, Germany). The assay was performed in triplicate and the $DPPH^{\cdot}$ concentration (mM) in the reaction medium was calculated from the calibration curve determined by linear regression. The percentage of the remaining $DPPH^{\cdot}$ (% $DPPH^{\cdot}_{rem}$) was calculated following equation (1).

$$\%[DPPH]_{rem} = \frac{[DPPH^{\bullet}]_{t=4h}}{[DPPH^{\bullet}]_{t=0}} \cdot 100 \quad (1)$$

where, the $DPPH^{\cdot}_{t=4h}$ is the concentration of DPPH after 4 h and $DPPH^{\cdot}_{t=0}$ is the concentration at the start of the reaction.

From these values, the EC_{50} (Efficient Concentration) parameter was determined, which indicates the antioxidant concentration necessary to reduce 50% of initial DPPH. To perform the Folin-Ciocalteu analyses, 100 μm of appropriately diluted samples was mixed with 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of Na_2CO_3 and was completed with distilled water until 10 mL. After 2 h of reaction, a spectrophotometer (Evolution 201 VisibleUV, ThermoScientific, Germany) was used to measure the absorbance of the samples at 765 nm, in triplicate. Pure eugenol was used as a standard and the results were expressed as mg·L^{-1} of eugenol.

2.5. Kinetics of eugenol release

Three food simulants of differing polarities were used to perform the release rate of eugenol from the different films according to the Commission regulation (EU) 10/2011 (14 January 2011): ethanol 10% (v/v) (ET10%), ethanol 20% (v/v) (ET20%) and ethanol 50% (v/v) (ET50%). Pieces of films from each formulation with controlled weight were cut into small parts and placed into vials containing 100 mL of each food simulant. Release studies were carried out in triplicate for 16 days at 22 °C under stirring conditions. Successively, the samples were taken after different film-solvent contact times and the absorbance was measured at 282 nm where eugenol absorbance shows a maximum (Pramod, Ansari, & Ali, 2013) by means of a spectrophotometer (Evolution 201 VisibleUV, ThermoScientific, Germany). The absorbance measurements were related to the corresponding standard calibration curve, expressed as mg of eugenol per gram of film.

To predict the release kinetics, Peleg's model (Peleg, 1988), described by equation (2), was applied to the experimental data:

$$M_t = M_0 + \frac{t}{k_1 + k_2 t} \quad (2)$$

where M_t is eugenol content at time t, M_0 is the initial eugenol content, k_1 is the kinetic constant of the model that is inversely related to the mass transfer rate at the beginning of the process and k_2 is a constant of the model that is related to the asymptotic value, which can be related to the equilibrium value ($1/M_\infty$, where M_∞ is the amount of active compound released at equilibrium).

2.6. Antioxidant performance of the films in sunflower oil

The films that showed the greatest antioxidant activity and adequate mechanical behavior were considered as potential packaging materials for sunflower oil. To evaluate the antioxidant performance, film samples (area of 11 cm × 6 cm) containing 10 mL of commercial sunflower oil were thermosealed with a vacuum packing machine (SAECO Vacio Press Elite, Barcelona, Spain) to form bags, as described by Galarza, Costa, de Oliveira Rios, and Flôres (2017). The protective effect of films in delaying sunflower oil oxidation was evaluated and compared to that of low density polyethylene (PE) and pure corn starch films (S). As control, an open glass Petri dish containing 10 mL of sunflower oil was considered. All the samples were stored at 30 °C and 53 %RH and exposed to fluorescent light at an intensity of 1000–1500 lux (measured by using a digital Luxometer; model RS Pro ILM1332A,

RS Components, Madrid, Spain). The oxidative stability of sunflower oil was measured in terms of the peroxide value (PV) and diene and triene content after 0, 4, 7, 11, 14 and 18 days of storage. Furthermore, the long-term effectiveness of S and S-EOA-L films at preventing oil oxidation was also measured after 53 days of storage, in terms of PV and diene and triene content.

In order to determine the PV of the samples, the titrimetric method was employed by using an automatic titrator (Titrando, Metrohm Ion Analysis, Switzerland). To this end, 1 g of oil was dissolved in 10 mL of solvent (glacial acetic acid:1-decanol volume ratio of 3:2, containing 10–15 mg L^{-1} of iodine) and mixed with 200 μL of an oversaturated KI solution. The mixture was thoroughly sacked and kept in a dark place for 1 min. Then, 50 mL of distilled water was added, and the solution was titrated with 0.01M or 0.001M $Na_2S_2O_3$, depending on the PV predicted. A blank control sample, without sunflower oil, was also prepared by following the same procedure described. All the analyses were performed in triplicate.

The conjugated dienes and trienes were determined by means of a spectrophotometric method, according to European Regulation EC2568/91 European Union Commission (1991). The absorbance of the appropriately diluted samples in isooctane was measured at wavelengths of 232 nm and 268 nm to determine the conjugated dienes and trienes, respectively (spectrophotometer Evolution 201 VisibleUV, ThermoScientific, Germany).

2.7. Statistical analysis

The results were submitted to an analysis of variance (ANOVA) using Statgraphics Centurion XVI software (Manugistics Corp., Rockville, Md.). Fisher's least significant difference (LSD) procedure was used at the 95% confidence level.

3. Results and discussion

3.1. Microstructure

The microstructure of the films was studied to observe potential differences in the arrangement of the different components, to evaluate the effects on the film microstructure of the active compound incorporation, and for a better understanding of the films' physical properties. The microstructure of the cross-section of the films is shown in Fig. 1. As can be observed, the presence of free eugenol (S-E) did not notably affect the microstructure of the starch matrix. This could be due to the loss of eugenol during the film drying process and the good integration of the remaining eugenol within the polymer matrix.

When eugenol was incorporated as microencapsulated powder, the film microstructure was modified, which reveals the partial disintegration of capsules and the incorporation of the capsule material to the starch matrix. This was mainly observed for capsules with L (S-E-L and S-EOA-L), where a certain level of phase separation was observed. In films containing capsules with OA, some structural discontinuities appeared, in accordance with the lack of miscibility of the released OA in the starch phase (Fabra, Talens, & Chiralt, 2009). In these films, small voids produced by OA droplets when they evaporated under vacuum conditions in the FESEM equipment were observed. This coincides with the observations of Jiménez, Fabra, Talens, and Chiralt (2012b) in starch films containing fatty acids. In films containing L, the effect of OA addition was less marked, probably due to the formation of OA-L micellar associations which reached a better dispersion in the starch matrix.

3.2. Physical properties of the films

3.2.1. Moisture content, solubility and thickness

The moisture content, solubility, thickness and barrier, mechanical and optical properties of films are shown in Table 2. The thickness of the films significantly increased with the addition of the encapsulated material, especially when it contained OA in the formulation.

The highest equilibrium moisture content (EMC) of the films stored at 53% RH and 25 °C was observed for S and S-E films due to their higher mass fraction of starch and glycerol (Table 1). Glycerol contains hydroxyl groups capable of interacting with water by means of hydrogen bonds, providing greater affinity for water in the films (Farahnaky, Saberi, & Majzoobi, 2013). The incorporation of microencapsulated eugenol significantly decreased the EMC of the films, possibly due to the reduced starch and glycerol content (Table 1). As expected, EMC was reduced significantly in films containing OA (S-EOA-WP and S-EOA-L) ($p < 0.05$) in line with the more hydrophobic nature of this compound (Sánchez-González, Chiralt, González-Martínez, & Cháfer, 2011b).

As concerns water solubility, the lowest values were obtained in S-E and S formulations. The incorporation of encapsulated eugenol produced a significant ($p < 0.05$) increase in solubility, consistent with the soluble character of the wall materials, especially MD. S-EOA-WP and S-EOA-L films showed intermediate values due to the presence of OA, which implied an increase in the lipid fraction that is not water soluble.

3.2.2. Barrier properties

It is widely recognized that the biopolymer films offer a barrier to gases and the differences in permeability values are ascribable to the nature of the film components and the film microstructure (e.g. increase in density, pore formation, presence, channelling and packing pattern of the lipids in the lipid-biopolymer films). The increase in oxygen solubility in the films when their water content rose has also been reported (Srinivasa, Ramesh, & Tharanathan, 2007; Valenzuela, Abugoch, & Tapia, 2013). In Table 2, the oxygen barrier properties of the films are shown. The incorporation of free eugenol did not significantly affect the OP values of pure starch films, which could be due to the low amount of eugenol retained in the films. However, incorporating encapsulated eugenol significantly ($p < 0.05$) decreased the OP values of the films. This may be attributed to the lower water content of the films and to the oxygen scavenging property of eugenol. Bonilla et al. (2013) obtained similar results when adding lipids with antioxidant properties to hydrocolloid-based films. When the lipid shows antioxidant activity, a chemical oxygen blocking effect produced by the lipid could be responsible for the reported improvement in the oxygen barrier properties. In the same way, Jiménez, Fabra, Talens, and Chiralt (2013) observed a similar reduction in the OP when incorporating tocopherol into starch-sodium caseinate films. This effect was less noticeable in those formulations incorporating OA, in agreement with both the liquid state of the oil as well as its more hydrophobic nature, which facilitates oxygen transport in the matrix (Atarés, Pérez-Masiá, & Chiralt, 2011).

The addition of essential oils to hydrophilic films usually leads to a reduction in the WVP, as it promotes an increase in both the matrix hydrophobicity and tortuosity factor for mass transport (Acosta et al., 2016). In the present study, no changes in WVP values were observed when eugenol was incorporated into the starch matrix in free form (Table 2). Nevertheless, a significant increase in the WVP was obtained when eugenol was added as WP-based microcapsules, especially when they contained OA. In contrast, eugenol encapsulated in L-based powder did not affect the WVP values, except when it contained OA, when WVP also increased. The water vapor transfer process in films depends both on the hydrophilic/hydrophobic ratio of the film constituents, which affects the solubility of water molecules, as well as on the heterogeneity of the component arrangements (dispersed phases), which determine the prevalent diffusion pathways and tortuosity factor of the matrix. WP is more hydrophilic than L and so will introduce differences in the hydrophilic/hydrophobic nature of the matrix which affect the water solubility (Ahmad, Benjakul, Prodpran, & Agustini, 2012). Likewise, the presence of OA in the capsules leads to a more open matrix, where the starch chains may be hindered in its packing,

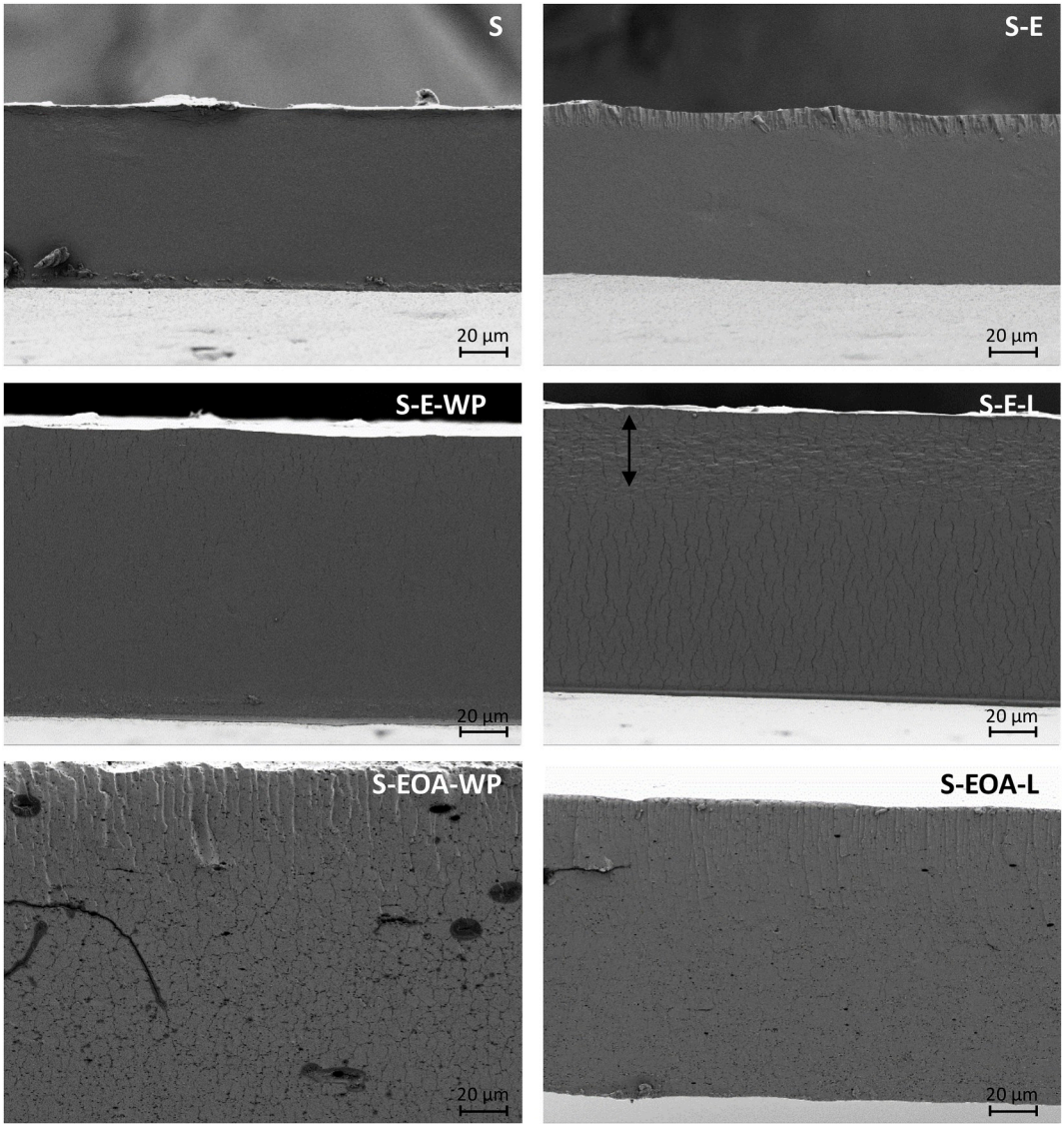

Fig. 1. Field emission scanning electron microscope micrographs of the cross-sections of the films. S: starch; S-E: starch film containing free E; S-E-WP: starch film containing encapsulated E in whey protein; S-EOA-WP: starch film containing encapsulated E in whey protein and OA; S-E-L: starch film containing encapsulated E in lecithin; S-EOA-L: starch film containing encapsulated E in lecithin and OA. The arrow in the S-E-L micrograph points out to the phase separation layer.

thus offering more facilities for mass transport (Kechichian, Ditchfield, Veiga-Santos, & Tadini, 2010).

3.2.3. Mechanical properties

The tensile strength (σ) and the elongation at break (%E) are the maximum stress and elongation that a film can withstand before it breaks up, respectively (Parris & Coffin, 1997). The elastic modulus (EM) is related to the stiffness of the material at low strains. These three mechanical parameters are useful for describing the mechanical properties of the films, and are closely related to its structure (McHugh & Krochta, 1994) and they are shown in Table 2. Pure starch films showed a mechanical behavior similar to that found in previous studies (Talón et al., 2017).

In general, when free or encapsulated eugenol was added to starch films, they became significantly more extensible (greater %E) and less rigid (with lower EM values), with no remarkable differences among films. Lipids (eugenol, lecithin or OA) interfere in the polymer chain association in the films, which implies a reduction in the polymer matrix cohesion forces and favoured the sliding of the chains during film stretching, thus reducing EM and increasing %E values (Bonilla, Atarés, Vargas, & Chiralt, 2012). This more plastic behaviour was more marked in S-E-OA-L films, surely to their greater total lipid content.

3.3. Optical properties

Table 3 shows the lightness, chrome and hue of the films. Starch films containing free eugenol became slightly more yellow (higher h^*_{ab} values) with respect to pure starch films. The incorporation of microencapsulated eugenol significantly reduced the lightness and hue and increased the chrome (towards a more saturated colour), especially in films containing L, in line with the natural colour of L, as can be observed in Fig. 3.

The addition of microencapsulated eugenol to films reduced the T_i values, especially in those films containing oleic acid. Jiménez, Sánchez-González, Desobry, Chiralt, and Tehrany (2014) observed similar behaviour when adding nanoliposomes in corn starch and sodium caseinate films, which exhibited reduced T_i values, mainly at low wavelengths.

3.4. Antioxidant capacity of the films

The EC_{50} and total eugenol content values of the films are shown in

Table 2
Oxygen permeability (OP, $cm^3 \cdot mm \cdot m^{-2} \cdot atm^{-1} \cdot day^{-1}$), water vapor permeability (WVP, $g \cdot mm \cdot KPa^{-1} \cdot h^{-1} \cdot m^{-2}$), equilibrium moisture content (EMC, g $H_2O \cdot 100^{-1}$g dry film), water solubility (WS, g soluble film/100^{-1}g film), thickness (μm), elastic modulus (EM, MPa), tensile strength (σ, MPa) and percentage of elongation (%E) at break of films equilibrated at 25°C-53% RH. Mean values and (standard deviation).

Formulation	OP (cc·mm/(m^2·atm·day)	WVP (g·mm/KPa·h·m^2)	EMC (g H_2O/100g dry film)	Solubility (g soluble film/100g film)
S	0.537 (0.019)^{c}	5.33 (0.08)^{a}	14.2 (0.3)^{d}	24 (4)^{a}
S-E	0.49 (0.02)^{bc}	5.31 (0.19)^{a}	13.3 (0.3)^{c}	22 (8)^{a}
S-E-WP	0.13 (0.03)^{a}	5.79 (0.11)^{b}	10.80 (1.02)^{b}	45 (5)^{b}
S-EOA-WP	0.324 (0.016)^{ab}	5.94 (0.06)^{bc}	9.7 (0.2)^{a}	37 (2)^{b}
S-E-L	0.26 (0.14)^{ab}	5.4 (0.3)^{a}	10.18 (0.19)^{ab}	46 (4)^{b}
S-EOA-L	0.6 (0.3)^{c}	6.12 (0.13)^{c}	9.5 (0.2)^{a}	37 (5)^{b}
Formulation	**Thickness (μm)**	**EM (MPa)**	**σ (MPa)**	**%E**
S	82 (5)^{a}	238 (38)^{c}	6.5 (0.7)^{b}	15 (5)^{a}
S-E	85 (6)^{b}	167 (94)^{b}	6.3 (1.6)^{b}	30 (9)^{b}
S-E-WP	121 (7)^{c}	283 (43)^{c}	8.4 (1.4)^{c}	36 (5)^{bc}
S-EOA-WP	133 (7)^{d}	158 (70)^{ab}	4.3 (0.8)^{a}	33 (9)^{bc}
S-E-L	132 (5)^{d}	109 (25)^{a}	4.4 (1.0)^{a}	40 (8)^{cd}
S-EOA-L	136 (6)^{e}	144 (27)^{ab}	4.9 (0.9)^{a}	45 (11)^{d}

^{abcde} Different letters in the same column indicate significant difference among formulations ($p < 0.05$).

Table 3
Lightness (L*), hue (h_{ab}*), chroma (C_{ab}*) and internal transmittance (T_i) at 460 nm of wavelength values of the film. Average values and standard deviations.

Films	L*	C_{ab}*	h_{ab}*	Ti (460 nm)
S	82.21 (1.05)^{d}	3.3 (0.3)^{a}	102.1 (1.4)^{f}	0.857 (0.003)^{d}
S-E	82.7 (0.3)^{d}	6.367 (0.103)^{b}	93.5 (0.5)^{e}	0.86 (0.00)^{d}
S-E-WP	79.2 (0.4)^{b}	11.9 (0.3)^{c}	87.13 (0.15)^{c}	0.836 (0.003)^{c}
S-EOA-WP	79.9 (0.7)^{c}	13.2 (0.5)^{d}	91.6 (0.4)^{d}	0.806 (0.004)^{b}
S-E-L	78.0 (0.7)^{a}	14.0 (0.3)^{e}	78.6 (0.3)^{a}	0.830 (0.003)^{c}
S-EOA-L	77.36 (0.10)^{a}	18.2 (0.4)^{f}	83.6 (9.3)^{b}	0.751 (0.013)^{a}

^{abcdef} Different letters in the same column indicate a significant difference among formulations ($p < 0.05$).

Table 4. The lower the EC_{50} values, the greater the antioxidant activity of the tested sample. The reaction lasted for 4 h, based on previous studies (Talón, 2018). A similar reaction time was found by Bortolomeazzi et al. (2007) in studies carried out with eugenol and other polyphenols from wood smoke and smoke flavourings used by the food industry.

As can be observed, all the formulations exhibited antioxidant activity (all were capable of reducing 50% of DPPH). The EC_{50} value of pure eugenol was in accordance with the results obtained by Brand-Williams et al. (1995). There were significant differences among the films ($p < 0.05$), which were consistent with the different total eugenol retention in each film (Table 4). Thus, the lowest antioxidant activity (AA) was found in films containing non-encapsulated eugenol (the greatest EC_{50} value), in agreement with the lowest total eugenol content (1.82 mg E/g film). The loss of eugenol during film drying was in the range of the losses reported by Sánchez-González et al. (2011a) during the formation of chitosan films containing essential oils.

When eugenol was previously microencapsulated, the total eugenol content detected in the films significantly increased, and thus the antioxidant activity was also improved (lower EC_{50}). The lowest EC_{50} values (and so, the greatest AA) were found in those formulations incorporating OA, especially in S-EOA-L films (5.6 mg E/g film). Thus, the addition of oleic acid and L as carriers improved the eugenol retention capacity of the polymer matrix and thus the preservation of its antioxidant activity during film formation. This can be explained by the affinity of eugenol to OA, which contributed to the binding of the compound. Monedero et al. (2010) observed similar behaviour when studying the release of n-hexanal from soy protein films with blends of beeswax and OA. Pokharkar et al. (2011) also observed a positive effect of OA on the retention of eugenol in nanostructured gels with mixtures of stearic acid and OA.

3.5. Eugenol release kinetics

Table 5 shows Peleg's model kinetic constants (k_1 and k_2) for the three food simulants. In every case, a good fit of the model was obtained ($R^2 \geq 0.98$) as can be seen in Fig. 2.

It is remarkable that the films initially presented different eugenol contents (Table 4), which were consistent with the values obtained at equilibrium (M_∞) in each case.

The increasing polarity of the simulant affected the % of eugenol released but this effect depended on the formulation used. Thus, in films with free eugenol (S-E), the amount of eugenol released rose together with the polarity of the simulant, this being significantly higher in the case of the ET50% (the least polar solvent), due to the stronger affinity of the free eugenol to this simulant. The opposite trend was observed in OA-based formulations, probably due to the greater affinity of eugenol to OA, which promotes its retention in the oil phase, leading to a slightly smaller release. In general, the release of eugenol in S-E-L and S-E-WP films was not significantly affected by the type of simulant.

Taking these results into account, films incorporating OA were selected because they better preserved the initial eugenol content, leading to a higher amount of eugenol released over time even when the least polar simulant was used (1.6–2.8 mg E/g film). When comparing S-EOA-WP and S-EOA-L films, the latter showed a significantly higher percentage of released eugenol (50%).

The release rate (k_1) usually depended on the film structure and the affinity between the released compound and the surrounding medium. In this study, the greatest differences were found between the eugenol release rate in films containing E in free form and encapsulated. As expected, the encapsulation allowed the initial release rate in the different media to slow down ($p < 0.05$), in comparison with the films incorporating free E. No noticeable differences were found among the different films containing encapsulated E, except for the S-EOA-L formulation which showed the lowest k_1 values.

In every case, the release rate significantly decreased ($p < 0.05$) when the simulant polarity rose, in line with the lower capacity of the medium to hydrate and open the polymer matrix structure.

Table 4
Antioxidant activity of films containing free and microencapsulated eugenol, expressed in terms of EC_{50} values, and their total eugenol content calculated by the Folin-Ciocalteu method.

Sample	EC_{50} (mg film/mg DPPH)	EC_{50} (mg E/mg DPPH)	mg E/g film	Eugenol retention (%)
S-E	117 (12)^{d}	3.8 (0.4)^{d}	1.82 (0.14)^{a}	5.6 (0.4)^{a}
S-E-WP	87 (4)^{c}	2.07 (0.10)^{c}	2.3 (0.5)^{ab}	11 (2)^{ab}
S-EOA-WP	40.9 (1.3)^{a}	0.84 (0.03)^{b}	4.5 (0.3)^{c}	27 (2)^{c}
S-E-L	74 (2)^{b}	1.81 (0.06)^{c}	3.0 (0.8)^{b}	13 (4)^{b}
S-EOA-L	34 (4)^{a}	0.69 (0.08)^{b}	5.6 (0.6)^{d}	31 (3)^{d}
Eugenol	-	0.091 (0.002)^{a}		

^{abcd} Different letters in the same column indicate significant difference among samples ($p < 0.05$).

Table 5
Parameters of Peleg's model: k_1 (min·g film·mg^{-1} eugenol); k_2 (g film·mg^{-1} eugenol); M_∞ ($1/k_2$, mg eugenol released at equilibrium·g^{-1} film), M_∞/M_0 (g eugenol released at equilibrium/initial g eugenol in the film). Mean values and (standard deviation).

Simulants	Parameters	S-E	S-E-WP	S-EOA-WP	S-E-L	S-EOA-L
ET10%	$\mathbf{k_1}$	45 (27)^{b,1}	11 (3)^{a,1}	10.0 (1.3)^{a,1}	15 (8)^{a,1}	8.6 (0.8)^{a,1}
	$\mathbf{k_2}$	1.09 (0.12)^{e,3}	0.767 (0.017)^{d,23}	0.42 (0.03)^{b,1}	0.66 (0.03)^{c,2}	0.309 (0.015)^{a,1}
	$\mathbf{M_\infty}$	0.9274 (0.1114)^{a,1}	1.30 (0.03)^{b,1}	2.38 (0.17)^{c,3}	1.51 (0.07)^{b,1}	3.24 (0.16)^{d,3}
	$\mathbf{M_\infty/M_0}$[a]	0.51 (0.06)^{a,1}	0.555 (0.012)^{ab,1}	0.53 (0.04)^{a,2}	0.50 (0.02)^{a,1}	0.57 (0.03)^{b,1}
ET20%	$\mathbf{k_1}$	66 (19)^{b,12}	29 (4)^{a,1}	8 (2)^{a,1}	30 (10)^{a,12}	10.4 (1.2)^{a,1}
	$\mathbf{k_2}$	1.025 (0.018)^{d,23}	0.76 (0.05)^{c,2}	0.48 (0.03)^{b,1}	0.56 (0.02)^{b,1}	0.339 (0.016)^{a,1}
	$\mathbf{M_\infty}$	0.976 (0.017)^{a,1}	1.31 (0.08)^{b,1}	2.09 (0.13)^{d,2}	1.80 (0.06)^{c,2}	2.96 (0.14)^{e,12}
	$\mathbf{M_\infty/M_0}$[a]	0.535 (0.009)^{b,1}	0.56 (0.04)^{b,1}	0.46 (0.03)^{a,2}	0.59 (0.02)^{b,2}	0.52 (0.02)^{ab,1}
ET50%	$\mathbf{k_1}$	333 (22)^{d,3}	124 (28)^{c,2}	88 (24)^{b,2}	82 (4)^{b,3}	33 (23)^{a,1}
	$\mathbf{k_2}$	0.82 (0.09)^{c,1}	0.86 (0.04)^{c,3}	0.62 (0.08)^{b,2}	0.62 (0.05)^{b,12}	0.36 (0.05)^{a,1}
	$\mathbf{M_\infty}$	1.24 (0.14)^{a,2}	1.17 (0.06)^{a,1}	1.6 (0.2)^{b,1}	1.62 (0.13)^{b,12}	2.8 (0.4)^{c,1}
	$\mathbf{M_\infty/M_0}$[a]	0.68 (0.07)^{c,2}	0.50 (0.03)^{b,1}	0.36 (0.05)^{a,1}	0.53 (0.04)^{b,12}	0.50 (0.07)^{b,1}

^{abcd} Different letters in the same line indicate a significant difference among formulations ($p < 0.05$).
^{123} Different numbers in the same column indicate a significant difference among food simulants ($p < 0.05$).
[a] Calculated from the total initial eugenol content in the film obtained by the Folin-Ciocalteu method (Table 4).

3.6. Antioxidant performance of the films in preventing sunflower oil oxidation

As commented on above, the S-EOA-L film was selected as a potential food packaging material due to its interesting mechanical properties together with its higher eugenol content and greater antioxidant capacity (Table 4).

Thus, the antioxidant power of S-EOA-L films in protecting sunflower oil was evaluated through the analysis of the evolution of the peroxide index (PV) for 18 days, at 30 °C and 53% RH, with the presence of light to promote oil oxidation. The PV is associated with the presence of peroxides derived from the polyunsaturated fatty acids present in the sample. Hydroperoxides are produced as primary oxidation products that could be derived into secondary products. Thus, the PV shows the initial oxidation stage (Sadeghi, Karami, & Etminan, 2017).

For comparison purposes, a commercial low-density polyethylene packaging (PE), a pure starch film (S, without eugenol) and an open glass Petri dish (Open Control) containing sunflower oil and submitted to the same environmental conditions were also used.

Fig. 3 shows the evolution of PV and conjugated dienes and trienes during storage. The initial PV of sunflower oil was 1.34 mEq O_2/kg, in agreement with other studies (Mohdaly, Sarhan, Mahmoud, Ramadan, & Smetanska, 2010). As observed, different ranges of PV were reached and significant differences among control samples (Open Control and PE) and those packaged using starch-based films were detected ($p < 0.05$). A continuous increase in PV was observed in line with the lengthening of the storage period for every control (S, PE, and Open Control), reaching values of 9.4 mEq/kg, 135.8 mEq/kg and 115.8 mEq/kg, respectively after 18 days of storage. The smallest increase in PV was registered for samples packaged in S and S-EOA-L films. This could be explained by the low OP of these films, with no significant differences in terms of oxygen barrier properties (Table 2). In samples packaged with S-EOA-L films, PV was kept at an average of 3 mEq O_2/kg during the entire storage period, reaching the smallest significant values at storage times longer than 7 days, thus indicating the great antioxidant effect of eugenol in this film. Reis et al. (2015) also observed a reduction in the oxidation ratio in terms of the PV of palm oil packaged in cassava starch films containing antioxidants from yerba mate.

In order to demonstrate the long-term effectiveness of S-EOA-L film packaging at preventing oil oxidation as compared to S films, PV was also measured after 53 days of storage. Samples packaged with S films reached a PV value of 10.69 ± 1.09 mEq/kg, which was above the peroxide limit established by the Codex-Alimentarius (1999) for refined oils (10 mEq/kg). However, samples packaged in S-EOA-L films showed a PV value of around 4.0 ± 0.4 mEq/kg, thus underlining the long-lasting antioxidant effect of S-EOA-L films.

The conjugated dienes and trienes were also measured and the results are shown in Fig. 3B and C. These compounds are formed by the rearrangement of the hydroperoxide double bonds during oxidation (Mohdaly et al., 2010). Thus, conjugated dienes represent the primary degradation products of oil and could be used to confirm the PV content while conjugated trienes are related with the secondary products of the oxidation. The initial contents of conjugated dienes and trienes were 3.81 g/100g and 2.85 g/100g, respectively. Both the samples packaged with PE and the Open Control samples showed an increase in conjugated dienes, which was proportional to the increase in PV. However,

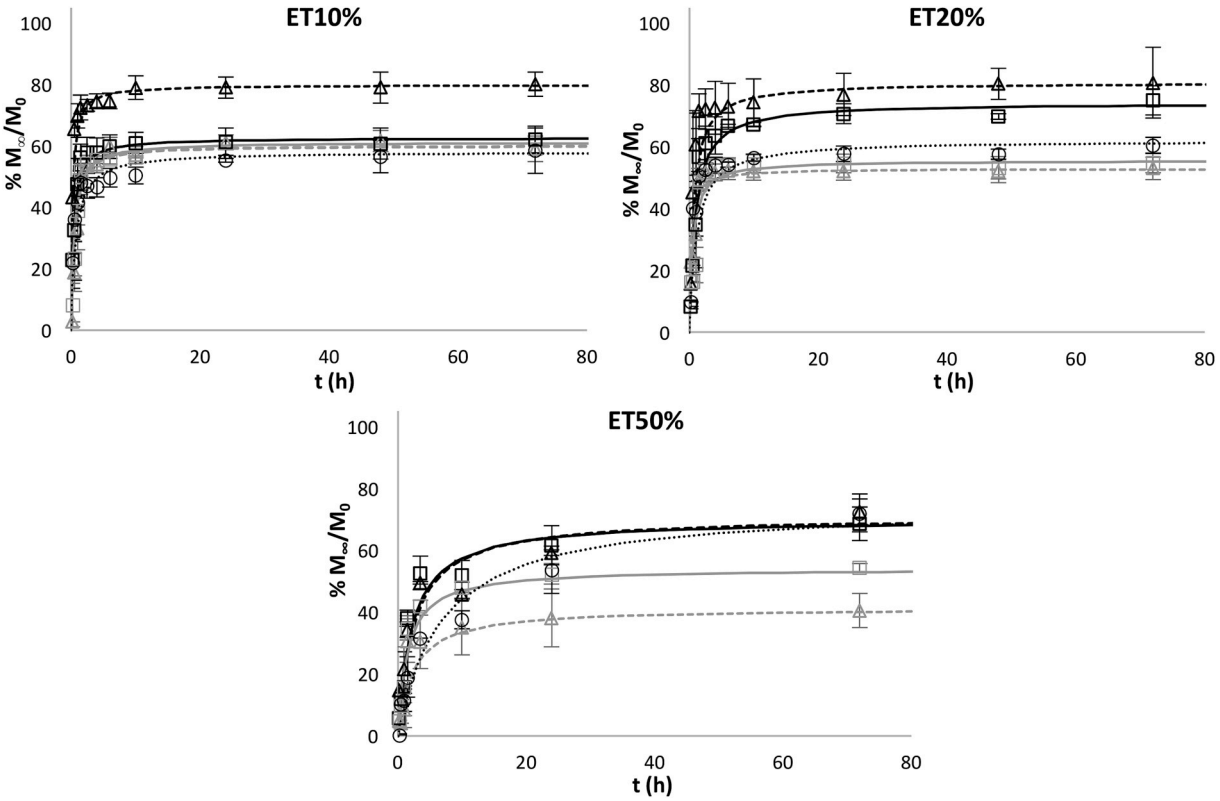

Fig. 2. Percentage of eugenol released with respect to the total eugenol content as a function of time in different solvents: experimental data (△ S-E-WP; △ S-EOA-WP; □ S-E-L; □ S-EOA-L; ○ E-S) and Peleg´s fitted model (- - - S-E-WP - - - S-EOA-WP; — S-E-L; — S-EOA-L; ··· S-E).

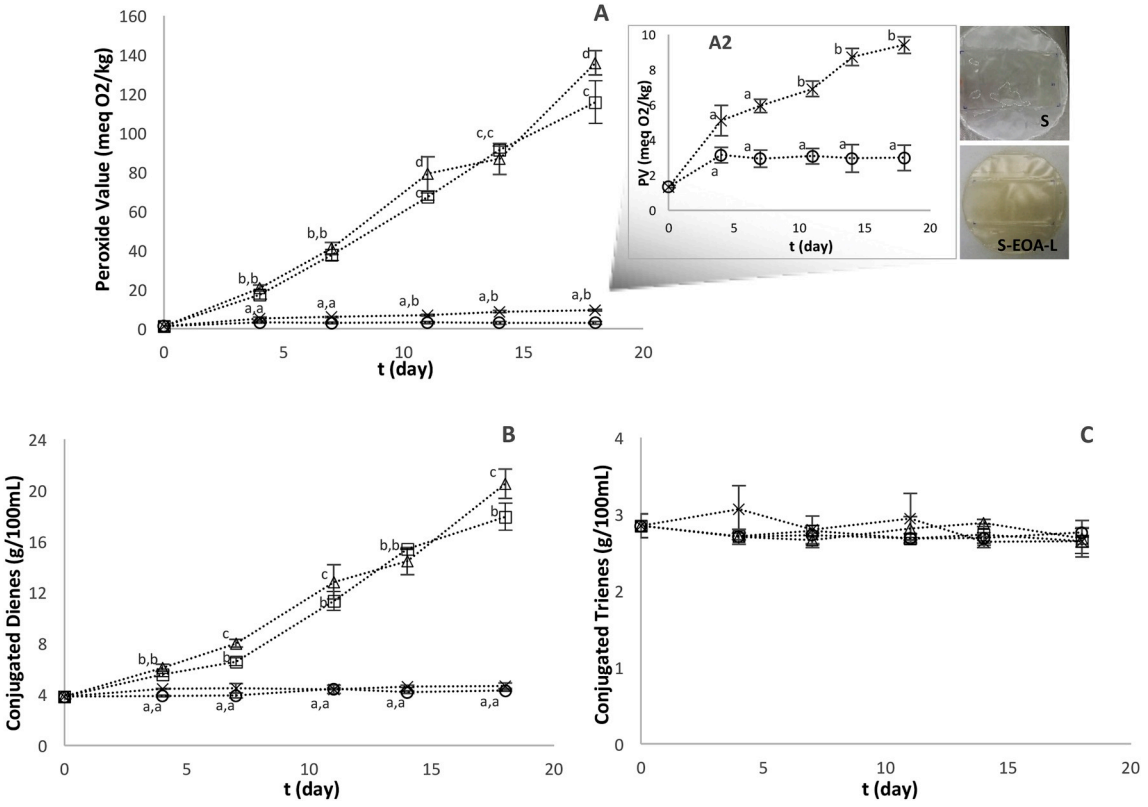

Fig. 3. A) Peroxide Value (meq O_2/kg), B) conjugated dienes and C) conjugated trienes (in g/100g) of sunflower oil packaged with S-EOA-L (○), S (×) and PE (△) films and in a glass Petri dish (□ Open Control). [abc] Different letters in the same time indicate a significant difference among formulations at each time ($p < 0.05$).

both S-EOA-L and S films maintained low values of conjugated dienes during the entire storage period, which indicates that these films prevent the occurrence of primary degradation products. The conjugated trienes remained constant during the whole storage period in the case of every sample, which shows that sunflower oil oxidation process did not reach the second stage of degradation (Galarza et al., 2017).

4. Conclusions

The addition of eugenol encapsulated with lecithin or whey protein in starch-based films modified the microstructure and yielded less resistant films with reduced moisture content, transparency and oxygen permeability as compared to those prepared with free eugenol. The release kinetics of eugenol in different food simulants and their antioxidant effect were influenced by the wall material used for the encapsulation and by the polarity of the solvent. The addition of oleic acid and lecithin promoted the preservation of both the initially added eugenol and the antioxidant activity of the films. Films containing eugenol with lecithin and oleic acid were highly effective at preventing sunflower oil oxidation in accelerated storage conditions.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the financial support provided by the Spanish Ministerio de Educación y Ciencia (Projects AGL2013-42989-R and AGL2016-76699-R). Author Emma Talón thanks the Universitat Politècnica de València for a FPI Grant (99/2011). The authors also thank the services rendered by the Electron Microscopy Service of the UPV.

References

Acosta, S., Chiralt, A., Santamarina, P., Rosello, J., González-Martínez, C., & Cháfer, M. (2016). Antifungal films based on starch-gelatin blend, containing essential oils. *Food Hydrocolloids, 61*, 233–240.

Ahmad, M., Benjakul, S., Prodpran, T., & Agustini, T. W. (2012). Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. *Food Hydrocolloids, 28*(1), 189–199.

Anushree, S., André, M., Guillaume, D., & Frédéric, F. (2017). Stearic sunflower oil as a sustainable and healthy alternative to palm oil. A review. *Agronomy for Sustainable Development, 37*(3), 18.

ASTM (1995). Standard test methods for water vapour transmission of materials. *Standard designations: E96-95. Annual books of ASTM* (pp. 406–413). Philadelphia: ASTM.

ASTM (2001). Standard test method for tensile properties of thin plastic sheeting. *Standard D882. Annual book of American standard testing methods* (pp. 162–170). *Philadelphia*, PA: American Society for Testing and Materials.

ASTM (2010). Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coloumetric sensor. *Designations, 3985–05*.

Atarés, L., Pérez-Masiá, R., & Chiralt, A. (2011). The role of some antioxidants in the HPMC film properties and lipid protection in coated toasted almonds. *Journal of Food Engineering, 104*(4), 649–656.

Bae, E. K., & Lee, S. J. (2008). Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. *Journal of Microencapsulation, 25*(8), 549–560.

Barrow, C. J., Wang, B., Adhikari, B., & Liu, H. (2013). Spray drying and encapsulation of omega-3 oils. *Food Enrichment with Omega-3 Fatty Acids,* 194–225.

Bonilla, J., Atarés, L., Vargas, M., & Chiralt, A. (2012). Effect of essential oils and homogenization conditions on properties of chitosan-based films. *Food Hydrocolloids, 26*(1), 9–16.

Bonilla, J., Talón, E., Atarés, L., Vargas, M., & Chiralt, A. (2013). Effect of the incorporation of antioxidants on physicochemical and antioxidant properties of wheat starch–chitosan films. *Journal of Food Engineering, 118*(3), 271–278.

Bortolomeazzi, R., Sebastianutto, N., Toniolo, R., & Pizzariello, A. (2007). Comparative evaluation of the antioxidant capacity of smoke flavouring phenols by crocin bleaching inhibition, DPPH radical scavenging and oxidation potential. *Food Chemistry, 100*(4), 1481–1489.

Botrel, D. A., de Barros Fernandes, R. V., Borges, S. V., & Yoshida, M. I. (2014). Influence of wall matrix systems on the properties of spray-dried microparticles containing fish oil. *Food Research International, 62*, 344–352.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology, 28*(1), 25–30.

Carrizo, D., Taborda, G., Nerín, C., & Bosetti, O. (2016). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. *Innovative Food Science & Emerging Technologies, 33*, 534–541.

Chatterjee, D., & Bhattacharjee, P. (2013). Comparative evaluation of the antioxidant efficacy of encapsulated and un-encapsulated eugenol-rich clove extracts in soybean oil: Shelf-life and frying stability of soybean oil. *Journal of Food Engineering, 117*(4), 545–550.

Choi, M. J., Soottitantawat, A., Nuchuchua, O., Min, S. G., & Ruktanonchai, U. (2009). Physical and light oxidative properties of eugenol encapsulated by molecular inclusion and emulsion–diffusion method. *Food Research International, 42*(1), 148–156.

Codex-Alimentarius (1999). *Codex-Standards for fats and oils from vegetable sources*, *Vol. 210* p. 1.

Commission regulation10 (2011). EU of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union L Series, 12*, 1–89.

European Union Commission (1991). Regulation EEC/2568/91 on the characteristics of olive and olive pomace oils and their analytical methods. *Off. J. Eur. Communities L, 248*, 1991.

Fabra, M. J., Talens, P., & Chiralt, A. (2009). Microstructure and optical properties of sodium caseinate films containing oleic acid–beeswax mixtures. *Food Hydrocolloids, 23*(3), 676–683.

Farahnaky, A., Saberi, B., & Majzoobi, M. (2013). Effect of glycerol on physical and mechanical properties of wheat starch edible films. *Journal of Texture Studies, 44*(3), 176–186.

Galarza, C., Costa, T. M. H., de Oliveira Rios, A., & Flôres, S. H. (2017). Comparative study on the properties of films based on red rice (Oryza glaberrima) flour and starch. *Food Hydrocolloids, 65*, 96–106.

Hutchings, J. B. (1999). *Food color and appearance* (2nd ed.). Gaithersburg, Maryland, USA: Aspen Publishers, Inc.

Jafari, S. M., He, Y., & Bhandari, B. (2007). Encapsulation of nanoparticles of d-limonene by spray drying: Role of emulsifiers and emulsifying techniques. *Drying Technology, 25*(6), 1069–1079.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012a). Edible and biodegradable starch films: A review. *Food and Bioprocess Technology, 5*(6), 2058–2076.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012b). Effect of re-crystallization on tensile, optical and water vapour barrier properties of corn starch films containing fatty acids. *Food Hydrocolloids, 26*(1), 302–310.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2013). Physical properties and antioxidant capacity of starch–sodium caseinate films containing lipids. *Journal of Food Engineering, 116*(3), 695–702.

Jiménez, A., Sánchez-González, L., Desobry, S., Chiralt, A., & Tehrany, E. A. (2014). Influence of nanoliposomes incorporation on properties of film forming dispersions and films based on corn starch and sodium caseinate. *Food Hydrocolloids, 35*, 159–169.

Kagami, Y., Sugimura, S., Fujishima, N., Matsuda, K., Kometani, T., & Matsumura, Y. (2003). Oxidative stability, structure, and physical characteristics of microcapsules formed by spray drying of fish oil with protein and dextrin wall materials. *Journal of Food Science, 68*(7), 2248–2255.

Kamatou, G. P., Vermaak, I., & Viljoen, A. M. (2012). Eugenol—from the remote maluku islands to the international market place: A review of a remarkable and versatile molecule. *Molecules, 17*(6), 6953–6981.

Kechichian, V., Ditchfield, C., Veiga-Santos, P., & Tadini, C. C. (2010). Natural antimicrobial ingredients incorporated in biodegradable films based on cassava starch. *LWT-Food Science and Technology, 43*(7), 1088–1094.

Khan, S., Choudhary, S., Pandey, A., Khan, M. K., & Thomas, G. (2015). Sunflower oil: Efficient oil source for human consumption. *Emer Life Science Research, 1*, 1–3.

Kucuk, M., & Caner, C. (2005). Effect of packaging materials and storage conditions on sunflower oil quality. *Journal of Food Lipids, 12*(3), 222–231.

McHugh, T. H., & Krochta, J. M. (1994). Sorbitol-vs glycerol-plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry, 42*(4), 841–845.

Mohdaly, A. A. A., Sarhan, M. A., Mahmoud, A., Ramadan, M. F., & Smetanska, I. (2010). Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection. *Food Chemistry, 123*(4), 1019–1026.

Monedero, F. M., Hambleton, A., Talens, P., Debeaufort, F., Chiralt, A., & Voilley, A. (2010). Study of the retention and release of n-hexanal incorporated into soy protein isolate–lipid composite films. *Journal of Food Engineering, 100*(1), 133–138.

Parris, N., & Coffin, D. R. (1997). Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *Journal of Agricultural and Food Chemistry, 45*(5), 1596–1599.

Peleg, M. (1988). An empirical model for the description of moisture sorption curves. *Journal of Food Science, 53*(4), 1216–1217.

Pokharkar, V. B., Shekhawat, P. B., Dhapte, V. V., & Mandpe, L. P. (2011). Development and optimization of eugenol loaded nanostructured lipid carriers for periodontal delivery. *International Journal of Pharmacy and Pharmaceutical Sciences, 3*(4), 138–143.

Pramod, K., Ansari, S. H., & Ali, J. (2013). Development and validation of UV spectrophotometric method for the quantitative estimation of eugenol. *Asian Journal of Pharmaceutical Analysis, 3*(2), 58–61.

Reis, L. C. B., de Souza, C. O., da Silva, J. B. A., Martins, A. C., Nunes, I. L., & Druzian, J. I. (2015). Active biocomposites of cassava starch: The effect of yerba mate extract and mango pulp as antioxidant additives on the properties and the stability of a packaged product. *Food and Bioproducts Processing, 94*, 382–391.

Sadeghi, E., Karami, F., & Etminan, A. (2017). The effect of Ferulago angulata (Schlecht) Boiss essential oil on stabilization of sunflower oil during accelerated storage. *Journal of Food Processing and Preservation, 41*(1).

Sánchez-González, L., Arab-Tehrany, E., Cháfer, M., González-Martínez, C., & Chiralt, A. (2015). Active edible and biodegradable starch films. *Polysaccharides: Bioactivity and Biotechnology,* 717–734.

Sánchez-González, L., Cháfer, M., González-Martínez, C., Chiralt, A., & Desobry, S. (2011a). Study of the release of limonene present in chitosan films enriched with bergamot oil in food simulants. *Journal of Food Engineering, 105*(1), 138–143.

Sánchez-González, L., Chiralt, A., González-Martínez, C., & Cháfer, M. (2011b). Effect of essential oils on properties of film forming emulsions and films based on hydroxypropylmethylcellulose and chitosan. *Journal of Food Engineering, 105*(2), 246–253.

Sanla-Ead, N., Jangchud, A., Chonhenchob, V., & Suppakul, P. (2012). Antimicrobial Activity of cinnamaldehyde and eugenol and their activity after incorporation into cellulose-based packaging films. *Packaging Technology and Science, 25*(1), 7–17.

Srinivasa, P. C., Ramesh, M. N., & Tharanathan, R. N. (2007). Effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films. *Food Hydrocolloids, 21*(7), 1113–1122.

Stojanovic, R., Belscak-Cvitanovic, A., Manojlovic, V., Komes, D., Nedovic, V., & Bugarski, B. (2012). Encapsulation of thyme (*Thymus serpyllum* L.) aqueous extract in calcium alginate beads. *Journal of the Science of Food and Agriculture, 92*(3), 685–696.

Talón, E. (2018). *Incorporation of active components in biopolymer-based films for food use (Doctoral dissertation)*Valencia, Spain: Retrieved from Universitat Politècnica de València. http://hdl.handle.net/10251/101283http://hdl.handle.net/10251/101283.

Talón, E., Trifkovic, K. T., Nedovic, V. A., Bugarski, B. M., Vargas, M., Chiralt, A., et al. (2017). Antioxidant edible films based on chitosan and starch containing polyphenols from thyme extracts. *Carbohydrate Polymers, 157*, 1153–1161.

Valencia-Sullca, C., Jiménez, M., Jiménez, A., Atarés, L., Vargas, M., & Chiralt, A. (2016). Influence of liposome encapsulated essential oils on properties of chitosan films. *Polymer International, 65*(8), 979–987.

Valenzuela, C., Abugoch, L., & Tapia, C. (2013). Quinoa protein–chitosan–sunflower oil edible film: Mechanical, barrier and structural properties. *LWT-Food Science and Technology, 50*(2), 531–537.