

Preparation of antioxidant active films based on chitosan: diffusivity study of α -tocopherol into food simulants

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Abstract New active films based on chitosan and polycaprolactone blends and containing α -tocopherol were designed for food packaging applications. Mechanical properties, stability against temperature and swelling degree in 50 % ethanol (v/v) were evaluated. Migration kinetics of α -tocopherol from the developed films into butter and food simulants [50 % ethanol (v/v), 95 % ethanol (v/v), and isooctane] at different temperatures were studied. α -Tocopherol was quantified in the food simulants by means of high performance liquid chromatography with diode-array detection at 292 nm. The proposed method exhibited a good sensitivity with a limit of detection of 0.1 mg/L. The kinetics release of α -tocopherol was characterized by determining the partition and the diffusion coefficients by using a mathematical modeling based on Fick's Second Law. The diffusion coefficients obtained ranged between 1.03×10^{-13} and 2.24×10^{-12} cm²/s for 95 % ethanol (v/v) at 4 and 20 °C, respectively. Developed films maintained the antioxidant activity for more than 20 days.

Keywords Active food packaging · Chitosan · α -Tocopherol · Migration kinetics · Partition and diffusion coefficients

Introduction

Foods with high fat content are susceptible to lipid oxidation; this process involves different reactions resulting in the formation of undesirable odors that make the product unacceptable by the consumers (Granda-Restrepo et al. 2009; Gargiulo et al. 2013). In the food industry, direct addition of antioxidant agents into the products is a common practice aimed to prevent the quality deterioration caused by the oxidative rancidity. Nevertheless, this approach presents some drawbacks such as that large amounts of the antioxidants are needed, in some cases there are established maximum permitted levels by food legislation, and moreover at high concentrations they can act as pro-oxidants (Gargiulo et al. 2013). In past years the antioxidant active packaging appeared as an innovative technology that may supply a sustained delivery of the active compound, maintaining an effective concentration over the time (Zhu et al. 2012). In these systems the antioxidant is incorporated into the polymeric matrix intended to be in contact with the food product (Zhu et al. 2013; Heirlings et al. 2004; Siró et al. 2006). Both synthetic and natural antioxidants have been widely used in food packaging applications (Siró et al. 2006; Soto-Valdez et al. 2011; Barbosa-Pereira et al. 2013).

Current trends are focused on the use of natural antioxidants due to consumers' preference (Zhu et al. 2013). Among them, α -tocopherol is commonly used, not only as the active substance but also as an additive for the polymer (Gargiulo et al. 2013; Granda-Restrepo et al.

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2009; Manzanarez-López et al. 2011). α -Tocopherol has been demonstrated to be effective in slowing lipid oxidation (Blanco-Fernandez et al. 2013; Martins et al. 2012). Granda-Restrepo et al. (2009) evaluated the usefulness of an active multilayer film composed by high density polyethylene (HDPE), ethylene vinyl alcohol (EVOH) and low density polyethylene (LDPE) containing α -tocopherol to protect whole milk powder against lipid rancidity. In general, lower concentrations of volatile compounds (pentanal, hexanal, heptanal) were recorded for samples stored with the active system; better efficiency being showed at relatively high temperatures (30–40 °C). Graciano-Verdugo et al. (2010) evaluated the stability of corn oil (by means of the determination of hexanal content) once packaged in LDPE bags additivated with α -tocopherol and stored under specific time–temperature conditions. The films enriched with the antioxidant proved to be effective in retarding the lipid oxidation. Torrieri et al. (2011) proposed the combination of modified atmosphere and antioxidant active packaging additivated with α -tocopherol to extend the shelf-life fresh bluefin tuna fillets. With the proposed system, low grade of oxidation was achieved after 18 days stored at 3 °C.

The use of biodegradable polymers for the development of food packaging materials is also receiving increased attention by the scientific community and the industry because of environmental concerns. Different polymeric matrices have been investigated for this purpose, including chitosan, poly(lactic acid) (PLA), poly- ϵ -caprolactone (PCL), and so on (Manzanarez-López et al. 2011; Siracusa et al. 2008; Lago et al. 2014). Chitosan, a linear polysaccharide of (1–4)-linked 2-amino-deoxy- β -D-glucan, is obtained by partial deacetylation of chitin. Biodegradability, biocompatibility, antimicrobial activity, non-toxicity and versatile physicochemical properties make chitosan useful in a variety of fields, such as cosmetics, biomedicine, pharmaceutical technology and food packaging (Lago et al. 2014). Edible and gas-permeable film packaging can be prepared by a cast/solvent evaporation method, just dissolving chitosan in acid solutions followed by solvent evaporation (Blanco-Fernandez et al. 2013; Aider 2010; No et al. 2007). However, the use of a plasticizer is normally required to achieve good mechanical properties (Rodríguez-Nuñez et al. 2014). Another relevant limitation of chitosan films is their high hydrophilicity, which provokes changes in their physical aspect due to sorption of humidity from the environment (Yang et al. 2012). To overcome this limitation, blends with hydrophobic polymers and multilayer films have been prepared (Suyatma et al. 2004). For example, chitosan/PCL films have been tested for active food packaging and also in the context of tissue engineering and drug delivery (Joseph et al. 2011; Cooper et al.

2011; Sarasam and Madihally 2005; El-Kamel et al. 2007). Commonly, the films are prepared by blending both components in the presence of organic solvents, such chloroform, to solubilize PCL (Olabarrieta et al. 2001).

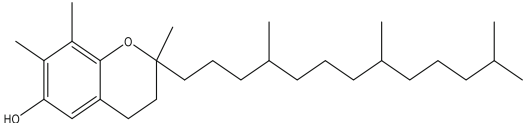
The aim of the present study was to develop films based on chitosan/PCL enriched with α -tocopherol to be used as antioxidant active food packaging avoiding the use of organic solvents. Film properties were evaluated in order to assess their suitability as food packaging. The kinetics of migration of α -tocopherol from the active films to three food simulants (50 % ethanol (v/v), 95 % ethanol (v/v) and isooctane) at different temperatures were evaluated. The partition and diffusion coefficients were calculated using a mathematical modeling based on the Fick's Second Law. Antioxidant activity was determined for prepared films.

Materials and methods

Reagents, standards solutions and food samples

Chitosan Food Grade (359,150 Da; deacetylation degree 76.2 %) from shrimp shells was supplied by George S. Daras (Marseille, France). Acetylated monoglyceride (Grindsted[®] Acetem 70-00 Kosher) was from Danisco (Grindsted, Denmark). All chemicals were of analytical grade. Methanol (MeOH, gradient grade for liquid chromatography and hypergrade for LC–MS), ethanol (EtOH, absolute for analysis) and isooctane were obtained from Merck (Darmstadt, Germany). Water used for all solutions was obtained from a Milli-Q water purification system (Millipore; Bedford, MA, USA). Butter was kindly supplied by Feiraco (Spain). This butter is characterized by a higher content in polyunsaturated fatty acids particularly in Omega-3 fatty acids and in conjugated linoleic acid (CLA) compared with a conventional butter. These differential characteristics are due to the milk used in its elaboration, which is obtained from cows' balanced diet. Poly- ϵ -caprolactone (PCL, 45,000 Da), glacial acetic acid and α -tocopherol (CAS No. 59-02-9) were supplied by Sigma-Aldrich (Steinheim, Germany). The chemical structure and physicochemical characteristics of α -tocopherol are shown in Table 1. Data was acquired from Scifinder[®] database. A stock solution of α -tocopherol of known concentration (1000 μ g/mL) was prepared in MeOH. From this stock solution an intermediate solution of 100 μ g/mL was prepared. Then, working solutions were made by dilution with MeOH to obtain the calibration standard solutions of α -tocopherol. The concentration of these solutions ranged from 0.05 to 10 μ g/mL. Solutions were stored at 4 °C and protected from light.

Table 1 Chemical structure and physico-chemical properties of α -tocopherol

Chemical structure	Physico-chemical properties
	Molecular formula: C ₂₆ H ₄₄ O ₂ Molecular weight (MW): 430.712 Melting point (BP): 3 °C Log P (octanol–water): 12.18

Active films preparation

Chitosan (1.2 % w/w) was dissolved in acetic acid 23 % (w/v) under stirring. Ultrasound was applied for few minutes and the solution was stored overnight at 4 °C for complete bubbles removal. Then, glycerol was added as plasticizer to the chitosan solution (25 % w/w respect to chitosan). Meanwhile, a mixture of α -tocopherol and acetylated monoglyceride melted at 60 °C (1:1 weight ratio) was prepared and protected from the light. Film forming solutions were prepared by mixing aliquots of chitosan–glycerol solution (33.3 g) preheated at 60 °C with 6.7 mL of PCL solution in glacial acetic acid (0.006 or 0.012 g/mL) and melted acetylated monoglyceride (0.08 g) or α -tocopherol/acetylated monoglyceride mixture (0.16 g). Six different film compositions were obtained (Table 2).

The film forming solutions were homogenized over 2 min (HeidolphDixax 600, Schwabach, Germany) and then ultrasound was applied over 5 min to remove bubbles. 16 g of each mixture were poured on polyethylene Petri dishes (81 mm in diameter), which were exposed to heating at 37 °C for 72 h for solvent removal and, consequently, film formation.

Film characterization

Film thickness was measured for three different films in three different places at room temperature and environmental humidity using a digital gauge. Mechanical properties were determined using a texturometer (TA-TX Plus Texture Analyzer, Stable Micro System Ltd., Surrey, United Kingdom) according to ASTM D6241-04. Films

were fixed between a support rig and an upper plate with a 1 cm in diameter hole. A spherical ball probe (P/5S, 5 mm in diameter) descended at 1 mm/s to the film. The mechanical properties were assessed determining the puncture strength (PS) and the puncture deformation (PD) (Gontard et al. 1993; Sobral et al. 2001; Blanco-Fernandez et al. 2013) as specified in Eqs. 1 and 2, respectively.

$$PS = FM/T \quad (1)$$

$$PD = \left[(D^2 + l_0^2)^{0.5} - l_0 \right] / l_0 \quad (2)$$

where FM is the maximum force applied before film rupture, T the film thickness, D the distance covered by the probe since it contacts with the film until the film breaks, and l_0 the orifice radio of the rig support.

Film swelling degree was measured by immersing dried pieces of ca. 50 mg (W_0 ; 15 × 15 mm) in 20 mL of ethanol: water 50:50 (v/v) mixture. After 8 days, samples were removed, wiped, weighed (W_t) and placed again in the swelling medium. The study was performed in triplicate at 4 and 37 °C. The swelling degree was estimated as follows:

$$Q (\%) = (W_t - W_0) / W_0 \times 100. \quad (3)$$

Migration test

Food simulants

Active films containing α -tocopherol were placed in 125 mL amber flasks in contact with 100 mL of the selected simulants and substitutes (50 % EtOH (v/v), 95 % EtOH (v/v), and isooctane). These simulants were selected

Table 2 Films composition (in mg/g dried film) and mechanical properties

Film code	Chitosan	Glycerol	α -tocopherol	AM	PCL	Thickness (mm)	PS (N/mm)	PD (%)
CHT0PCL0	689.6	172.4	0	138.1	0	0.050 (0.012)	732 (88)	16.21 (1.88)
CHT0PCL10	644.8	161.2	0	129.1	64.9	0.055 (0.015)	595 (186)	11.80 (0.71)
CHT0PCL20	605.5	151.4	0	121.2	121.8	0.051 (0.004)	362 (64)	6.49 (0.30)
CHT3PCL0	605.9	151.5	121.3	121.3	0	0.043 (0.013)	439 (64)	13.81 (1.67)
CHT3PCL10	571.1	142.8	114.3	114.3	57.5	0.053 (0.015)	457 (84)	15.73 (3.76)
CHT3PCL20	540.1	135.0	108.1	108.1	108.7	0.051 (0.010)	122 (42)	8.74 (1.28)

PS puncture strength, PD puncture deformation

considering that the antioxidant films may be intended to use with lipid foodstuff. All assays were done by triplicate.

Aliquots of 0.5 mL were removed at selected time intervals. In the case of the isoctane, the solvent was evaporated under nitrogen vapor stream, and then the residue was dissolved in 0.5 mL of MeOH. After that, all samples were filtered through a 0.45 μm PTFE membrane filter (Advanted, Toyo Roshi Kaisha, Ltd., Japan).

Food samples

The antioxidant active films were cut in circles of 42 mm of diameter and each of them were introduced in one migration cell filled with approximately 40 g of butter. This way only one of the faces of the film was in contact with the food. All migration cells were stored at 4 °C and at each selected time samples were withdrawn. The polymeric film was carefully cleaned and after that, extracted. Due to the complexity of the food matrices, in order to simplify the analysis, the amount of the α -tocopherol released to the food samples was indirectly calculated as the difference between the initial amount and the amounts at selected time intervals in the polymeric films.

Extraction of α -tocopherol from active films

The antioxidant was extracted from the films as follows. Approximately 10 cm² were placed in a 125 mL amber flask containing 100 mL of MeOH, then the flask was hermetically closed and immediately stored at 40 °C during 24 h. After that, an aliquot was taken, filtered through a 0.45 μm PTFE membrane filter (Advanted, Toyo Roshi Kaisha, Ltd., Japan) and analyzed by HPLC. To verify that the extraction had been complete, the film was cleaned with MeOH to remove any remaining migrant and was subjected to a second extraction under the same conditions.

Chromatography

HPLC–DAD

The HPLC system (Hewlett Packard, Waldbronn, Germany) consisted of a quaternary pump, a degassing device, an autosampler, a column thermostating system, and a diode-array detector (DAD). Agilent Chem-Station for LC and LC/MS systems software was used. Operating conditions were as follows: the chromatographic separation was performed on a Kromasil ODS C18 (25 \times 0.32 cm, 5 μm particle size) column thermostatted at 35 °C and using methanol as mobile phase. The flow rate was set at 0.5 mL/min and the injection volume was 20 μL . Detection was performed at 292 nm.

HPLC–MS/MS

An LC–MS/MS system comprising an Accela autosampler, a column oven and Accela 1250 pump fitted with a degasser, coupled to a triple quadrupole mass spectrometer TSQ Quantum Access max controlled by Xcalibur (Thermo Fisher Scientific, San José, CA, USA) was used to confirm the results obtained using the equipment described in former section. MS data were acquired in the positive ion mode employing electrospray ionization (ESI). Mass spectra were monitored in the mass range m/z 200–450. Optimized MS/MS detector settings were: spray voltage 3000 V, vaporizer temperature 340 °C and capillary temperature 350 °C. Nitrogen was used as sheath gas (pressure 35 psi) and as auxiliary gas (pressure 10 arbitrary units). With respect to analytical conditions were the same as in the HPLC–DAD system.

Antioxidant activity

The antioxidant activity of the films was determined by means of the free radical DPPH method (Brand-Williams et al. 1995). Small pieces of dried films (ca. 50 mg, size 15 \times 15 mm) were immersed in 20 mL of 50 % EtOH (v/v) at 37 °C. After 20 days, the medium in contact with the film was used for measuring the antioxidant activity. Samples of the medium (0.5, 1 or 2 mL) were diluted with 50 % EtOH (v/v) to reach a final volume of 2 mL. Then, DPPH (30 $\mu\text{g}/\text{mL}$ in 50 % EtOH (v/v); 2 mL) were added to each aliquot. After 30 min of incubation at room temperature, absorbance at 525 nm was recorded (A_{sample}), and the scavenging percentage (% ESC, Eq. 4) was calculated as follows:

$$\% \text{ ESC} = (1 - A_{\text{sample}}/A_{\text{reference}}) \times 100 \quad (4)$$

$A_{\text{reference}}$ being the absorbance of 2 mL of the DPPH solution mixed with 2 mL of 50 % EtOH (v/v).

The antioxidant activity was given normalizing the scavenging percentage (% ESC) by α -tocopherol concentration in the medium as determined by HPLC (Blanco-Fernandez et al. 2013).

Results and discussion

Films preparation and characterization

Films of chitosan and PCL were prepared from mixtures of chitosan in an aqueous acetic acid solution and PCL in glacial acetic acid, using a solvent/casting method. To avoid the use of toxic organic solvents previously tested to prepare PCL films (Senda et al. 2002), glacial acetic acid was identified as a suitable solvent to obtain PCL solutions



Fig. 1 Appearance of chitosan/PCL films prepared with different contents in PCL and α -tocopherol. Codes as in Table 2

that can be afterwards mixed with aqueous medium (Pok et al. 2010). Acetylated monoglyceride was used as emulsifier agent that helps to disperse α -tocopherol in the polymeric matrix.

The obtained films showed a yellowish color indicative of homogeneous distribution of the antioxidant in their structure (Fig. 1). The thickness of the films after 1 week of storage at room temperature and environmental humidity was approx. 50 μm (Table 2). Strength (PS) and deformability (PD) of the films were determined to gain an insight into their suitability for being used as food packaging material. In general, incorporation of α -tocopherol led to a decrease in the strength of the films (PS, Table 2) which can be related to a less crystalline structure of chitosan as consequence of its mixture with α -tocopherol and acetylated monoglyceride (Martins et al. 2012). PCL also caused a decrease in PS when α -tocopherol was not incorporated. However, when the antioxidant was in the film, the highest PS values were recorded for intermediate PCL levels (CHT3PCL10). A similar dependence was recorded for the deformability (PD) (Table 2).

High hydrophilicity of the polysaccharides is one of the major limitations of polysaccharide-based films, because as soon as they enter into contact with water the films

undergo changes in their physical aspect and properties, which also compromise customer acceptance. Moreover, disintegration of the films could also happen. Incorporation of the hydrophobic PCL to chitosan matrix and the use of glacial acetic acid may be useful tools to prevent undesirable swelling in contact with foods. The swelling of α -tocopherol-containing films was evaluated after immersion in the simulant 50 % EtOH (v/v) for 8 days at 4 and 37 $^{\circ}\text{C}$ (Table 3). Swelling percentages at 37 $^{\circ}\text{C}$ were higher than at 4 $^{\circ}\text{C}$, and no clear effect of PCL was observed on this parameter. However, it should be noticed that the prepared films exhibited remarkably lower swelling than films of chitosan solely previously prepared using lactic acid aqueous solution as dispersing medium, which reached up to 500 % swelling in 50 % EtOH (v/v) (Blanco-Fernandez et al. 2013). Glacial acetic acid may lead to a certain reacylation or cross-linking of chitosan through the unprotonated primary amino group of glucosamine, which results in more hydrophobic films (Lavertu et al. 2012).

Method for α -tocopherol quantification

Migration of α -tocopherol from the active films was evaluated using three food simulants. α -Tocopherol released was quantified by means of HPLC–DAD using an isocratic elution mode. Under the set conditions a good resolution was achieved. The linearity of the method was tested by using a series of α -tocopherol standard solutions of known concentrations. The calibration curve was constructed using six concentration levels and the data were fitted to a linear equation ($y = 17.376x - 0.3047$). The linear range was within 0.25–10 mg/L. Each point of the calibration curve was the average of two peak-area measurements. The antioxidant showed a good linearity within the range of concentration studied with a coefficient of determination of 0.9999. The limits of detection and quantification, calculated according the American Chemical Society guidelines (ACS 1980) (defined as a signal three and ten times, respectively, the high of noise level) were 0.10 and 0.25 mg/L, respectively. The repeatabilities within day and between days determined by analyzing ten replicates of the standard and expressed as the percentage of the relative standard deviation [RSD % (n = 10)] were 1.85 and 2.88, respectively.

Identification of α -tocopherol was made by comparison of the retention time and UV spectrum with that of a pure standard solution. Additionally liquid chromatography coupled to mass spectrometry using electrospray ionisation operated in the positive ion mode (HPLC–ESI–MS/MS) was applied in order to confirm the results obtained by HPLC–DAD. The full scan mass spectra exhibited the protonated $[M + H]^+$ molecular ion (mass).

Table 3 Swelling % at 4 and 37 °C after being immersed 8 days in an ethanolic solution (50 %) and % SCV/concentration of each film after 20 days in the swelling medium

Film code	Swelling (%) at 4 °C	Swelling (%) at 37 °C	% SCV/concentration
CHT3PCL0	62.79 (7.86)	91.07 (19.30)	5.172 (2.032)
CHT3PCL10	74.80 (2.08)	92.03 (43.31)	7.072 (2.452)
CHT3PCL20	78.74 (26.02)	89.44 (11.89)	7.223 (1.257)

Migration kinetics of α -tocopherol

Monitoring of the amount of α -tocopherol released and its migration rate to foodstuff is crucial to evaluate the performance of an active film packaging. Blanco-Fernandez et al. (2013) have previously reported on the preparation of chitosan films containing α -tocopherol that underwent a step of cross-linking with glutaraldehyde as a way to provide sustained release. In 50 % EtOH (v/v) medium the films eluted a relevant percentage of α -tocopherol in the first 24 h and then sustained the release for 5 days. The present study avoids the use of the cross-linking agent, but incorporates PCL and glacial acetic acid as a way to slow down the release of the antioxidant agent. The new active films contained 108–121 mg of α -tocopherol per gram of dried film (Table 2). The effect of the materials processing and the formulation composition on the delivery kinetics of α -tocopherol was investigated.

Migration assays were carried out in 50 % EtOH (v/v) at 20 °C using films with and without PLC. In the cases of 95 % EtOH (v/v) and isooctane, the migration tests were performed at 4 and 20 °C and employing only the films without PLC. Results show that PLC addition does not significantly affect diffusion rate of α -tocopherol, probably because the relatively low content in weight of PCL compared to chitosan. According the Commission Regulation (EU) No. 10/2011 (European Commission 2011) the food simulant 50 % EtOH (v/v) is “assigned for foods that have a lipophilic character and are able to extract lipophilic substances; and shall be used for alcoholic foods with an alcohol content of above 20 % and for oil in water emulsions”; 95 % EtOH (v/v) and isooctane are substitutes of the food simulant D2 (vegetable oil). The migration kinetics of α -tocopherol from the active films into 95 % EtOH (v/v) and 50 % EtOH (v/v) at the temperatures studied are shown in Fig. 2. At equilibrium the percentage of α -tocopherol released in 95 % EtOH (v/v) was 58.8 and 96.8 % at 4 and 20 °C, respectively. In the case of 50 % EtOH (v/v) once the equilibrium was attained only 9.6, 4.7 and 6.4 % was released from films with 0, 10, and 20 % PLC, respectively. No migration of α -tocopherol from the active films into isooctane was detected at the quantification level. This behavior can be related to the swelling underwent by the films in contact with ethanol–water

mixture simulants, which leads to changes in polymer conformation that favor the release of the antioxidant. Oppositely, the films did not swell in isooctane. The films were also tested against butter samples; the film was placed in contact with the butter by one side and the assay temperature was set to 4 °C. As in the case of isooctane negligible diffusion was observed which could be explained again by minor conformational changes observed in the films. In the dry state the film structure may be too compact for allowing the antioxidant to migrate outside.

Migration kinetics

Mathematical model

To assess the migration of α -tocopherol from the developed chitosan films into food simulants a mathematical model based on Fick’s Second Law (Eq. 5) was used.

$$\frac{\partial C_p}{\partial t} = D \frac{\partial^2 C_p}{\partial x^2} \quad (5)$$

where C_p is the concentration of the migrant in the polymer (P) at time t (s) and position x , and D is the diffusion coefficient in P (cm^2/s).

An analytical solution of this differential equation that describes the diffusion kinetics was proposed by Crank (1975) and after a small modification can be expressed by the following equation (Simoneau 2010):

$$\frac{m_{F,t}}{A} = C_{P,0} \rho_P d_P \left(\frac{\alpha}{1 + \alpha} \right) \times \left[1 - \sum_{n=1}^{\infty} \frac{2\alpha(1 + \alpha)}{1 + \alpha + \alpha^2 q_n^2} \exp\left(-D_P t \frac{q_n^2}{d_P^2}\right) \right] \quad (6)$$

$$\alpha = \frac{1}{K_{P/F}} \frac{V_F}{V_P} \quad (7)$$

where, $m_{F,t}$ is the mass (μg) of the migrant transferred from P (chitosan-based film) into F (food simulant) after time t ; A is the area of P in contact with F (cm^2); $C_{P,0}$ is the initial concentration of the migrant in P (mg/kg); ρ_P is the density of P (g/cm^3); t is the migration time (s); d_P is the thickness of P (cm); V_P is the volume of P (cm^3); V_F is the volume of F (cm^3); q_n is the positive root of the equation $\tan q_n = -\alpha q_n$; D_P is the diffusion coefficient of the migrant

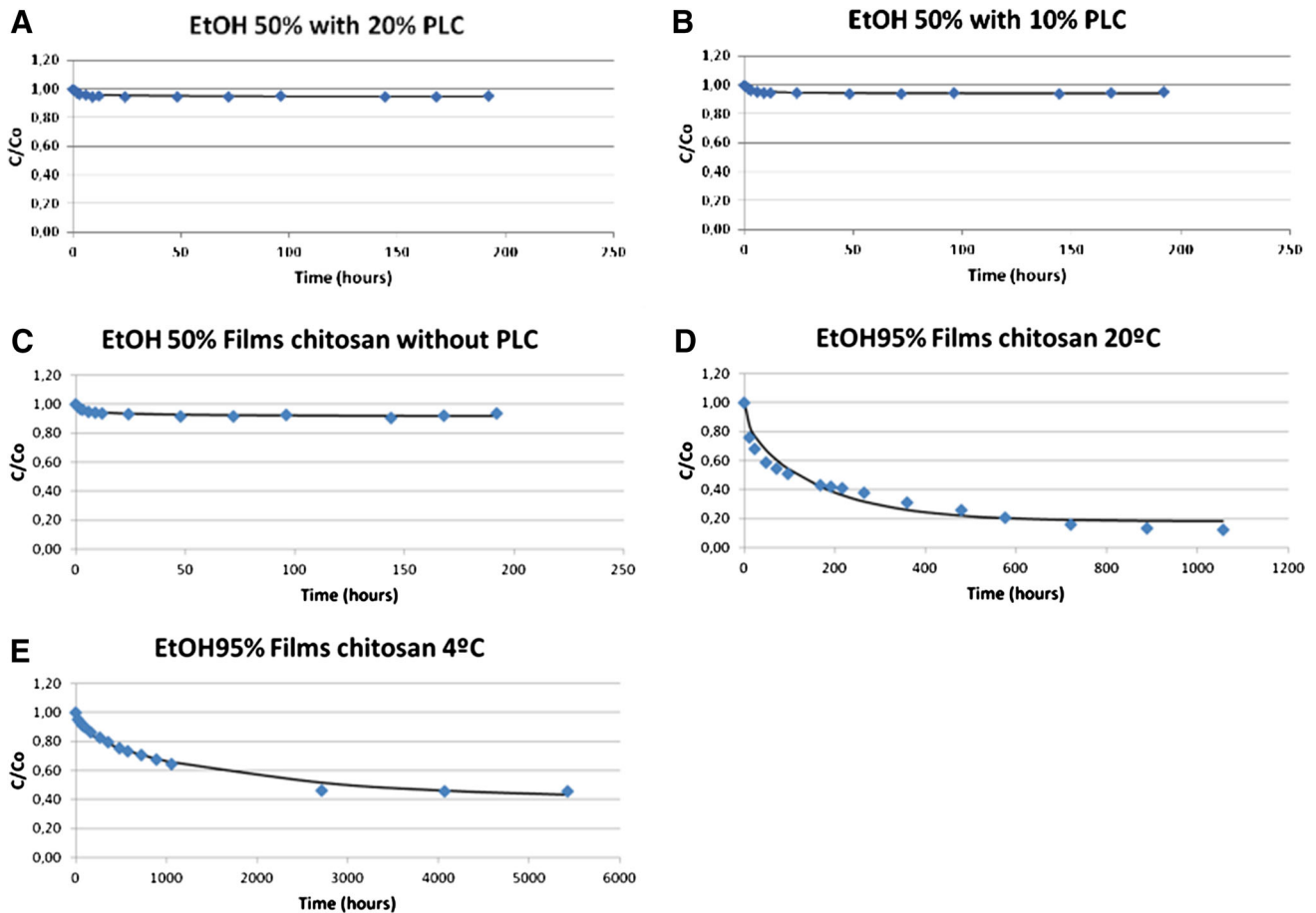


Fig. 2 Diffusion of α -tocopherol from chitosan based films with 20 % of PLC into 50 % ethanol (v/v) food simulant at 20 °C (a); with 10 % of PLC into 50 % ethanol (v/v) food simulant at 20 °C (b);

without PLC into 50 % ethanol (v/v) food simulant at 20 °C (c); into 95 % ethanol (v/v) food substitute at 20 °C (d); and into 95 % ethanol (v/v) food substitute at 4 °C (e)

in the polymer (cm^2/s); $K_{P/F}$ is the partition coefficient of the migrant between P and F.

the concentration of substance in the chitosan-based film at equilibrium, in $\mu\text{g/g}$; and C_S is the concentration of substance in the simulant at equilibrium, in $\mu\text{g/g}$.

The partition coefficient indicates the ratio between the concentration of the active compound in the polymer and the concentration in the food or food simulant once the equilibrium is reached (Tehrany and Desobry 2004; Sanchez Silva et al. 2007). The partition coefficients were estimated as follows:

The migration kinetics of the active compound from the film into the food simulants is defined by both the partition ($K_{P/F}$) and diffusion (D) coefficients. With respect to partition coefficient, values of $K_{P/F} > 1$ indicate higher concentration of the active compound in the polymeric material than in the food simulant. As shown in Table 4, the partition coefficients ($K_{P/F}$) calculated for the chitosan-based films-50 % EtOH (v/v) system were many times higher than those obtained for the chitosan-based films-95 % EtOH (v/v) system, which are in good agreement

$$K_{P/S} = \frac{C_P}{C_S} \tag{8}$$

where $K_{P/S}$ is the partition coefficient between the chitosan-based film and the food simulant or substitute; C_P is

Table 4 Key parameters and RMSE values obtained for α -tocopherol migration from the chitosan-based films to the food simulants

Films	Simulant	Temperature (°C)	$K_{p/s}$ exp	$K_{p/s}$ est	D (cm^2/s)	RMSE %
w/o PLC	50 % ethanol (v/v)	20	>1000	>1000	1.12×10^{-12}	0.84
10 % PLC	50 % ethanol (v/v)	20	>1000	>1000	1.49×10^{-12}	0.62
20 % PLC	50 % ethanol (v/v)	20	>1000	>1000	1.16×10^{-12}	0.56
w/o PLC	95 % ethanol (v/v)	4	>1000	808.89	1.07×10^{-13}	1.94
		20	490	263.41	2.24×10^{-12}	5.17

with the lower amount of α -tocopherol released (4.7–9.6 %) in the food simulant 50 % EtOH (v/v). In both systems the concentration of the antioxidant was higher in the polymer than in the food simulant; meaning that the antioxidant tends to remain in the polymer.

The rate at which the release of the active compound occurs is given by the diffusion coefficient. The migration process is affected by several factors such as the polymer nature, and the food composition including the fat and alcohol contents, among others (Granda-Restrepo et al. 2009). A faster delivery of α -tocopherol into 95 % EtOH (v/v) compared to 50 % EtOH (v/v) was observed (Table 4), and as might be expected the diffusion coefficients increased with the temperature. With respect to diffusion, no difference was observed between the films with and without PCL. The values recorded for the chitosan-based films are comparable to those described in the literature. Siró et al. (2006) reported a diffusion coefficient of $1.68 \times 10^{-12} \text{ cm}^2/\text{s}$ for α -tocopherol forming complexes with beta-cyclodextrin from LDPE polymer into 95 % EtOH (v/v) at 7 °C, and a value of D one order of magnitude lower ($1.53 \times 10^{-11} \text{ cm}^2/\text{s}$) for the non-complexed form. D values ranging from 6.58×10^{-12} to $4.60 \times 10^{-10} \text{ cm}^2/\text{s}$ were obtained for a LDPE/polypropylene (PP) blend films-95 % EtOH (v/v) system (Zhu et al. 2012). Castro López et al. (2012) evaluated the release of α -tocopherol from polypropylene (PP) film modified with poly(propyleneglycol)-block-poly(ethylene glycol)-block-poly(propyleneglycol) (PPG-PEG-PPG) into the food simulant 50 % EtOH (v/v) at 40 °C and reported values were between 2.4×10^{-12} and $2.0 \times 10^{-11} \text{ cm}^2/\text{s}$. Similar diffusion coefficients were found by Granda-Restrepo et al. (2009) when evaluated the migration of α -tocopherol from a 3-layer active system (an internal layer of LDPE, an intermediate layer of EVOH and an outer layer of HDPE) into whole milk powder. D values at 20, 30 and 40 °C were 2.34×10^{-11} , 3.06×10^{-11} , and $3.14 \times 10^{-11} \text{ cm}^2/\text{s}$, respectively. Heirlings et al. (2004) reported values of the same order of magnitude (1.65×10^{-11} – $4.23 \times 10^{-11} \text{ cm}^2/\text{s}$) from LDPE and ethylene vinyl acetate (EVA) in 95 % EtOH (v/v) when the migration test was performed at 7 °C. Slightly higher values (1.3×10^{-11} – $5.11 \times 10^{-10} \text{ cm}^2/\text{s}$) were found for the diffusion of the antioxidant from LDPE into corn oil (Graciano-Verdugo et al. 2010) and from poly(lactic acid) films into ethanol and vegetable oil (10^{-11} – $10^{-10} \text{ cm}^2/\text{s}$) (Manzanarez-López et al. 2011). Within the same range (7.7×10^{-11} – $1.9 \times 10^{-10} \text{ cm}^2/\text{s}$) were the diffusion coefficients found by Gargiulo et al. (2013) for an active system based on LDPE films with functionalized SBA-15 mesoporous silica. A wide range of diffusion coefficients (4.7×10^{-12} – $2.89 \times 10^{-9} \text{ cm}^2/\text{s}$) was obtained by Hwang et al. (2013), who evaluated the diffusion of α -tocopherol

from poly(L-lactic acid) (PLLA)/starch blends and PLLA films into ethanol at 13, 23 and 43 °C.

In order to evaluate the effect of the temperature on the diffusion of α -tocopherol from chitosan based films into the food simulant 95 % EtOH (v/v) the E_A was estimated by means of the Arrhenius equation:

$$D = D_0 e^{-\frac{E_A}{RT}} \quad (9)$$

where D is the diffusion coefficient (cm^2/s), D_0 is the preexponential factor (cm^2/s), E_A is the activation energy (kJ/mol), R is the gas constant ($8.314 \times 10^{-3} \text{ kJ/mol K}$), and T is the temperature (K). E_A values were estimated from the slope by representing the logarithm of D versus $1/T$, for the two temperatures evaluated in this work and assuming a linear behavior within this range. The result obtained was 128.2 kJ/mol. A similar value (126.5 kJ/mol) was reported by Graciano-Verdugo et al. (2010) for the diffusion of α -tocopherol from LDPE films containing 20 mg/g of the antioxidant to corn oil. A lower E_A (96.2 kJ/mol) was reported by Manzanarez-López et al. (2011) for the additive from PLLA films to ethanol.

To measure the fit between the experimental and estimated data the root of mean-square error % (RMSE (%)) was calculated using the equation:

$$RMSE(\%) = \frac{1}{M_{P,0}} \sqrt{\frac{1}{n} \sum_{i=1}^n ((M_{F,t})_{\text{exp},i} - (M_{F,t})_{\text{pred},i})^2} \times 100 \quad (10)$$

where n is the number of experimental points per migration/release curve; i is the number of observations; $M_{P,0}$ is the initial amount of the migrant in the polymer (μg).

As can be inferred from the low $RMSE$ (%) values (Table 4), there was a good correlation between the experimental and estimated data. This means that the model can be used to predict the release of α -tocopherol into food simulants.

Antioxidant activity

The antioxidant activity of the films was determined using DPPH assay. Films were immersed in an ethanolic aqueous solution over 20 days, exposed to air (without protective atmosphere), and the antioxidant activity of the tocopherol released was measured recording the DPPH signal decrease at 525 as consequence of its reduction (Brand-Williams et al. 1995; Goupy et al. 1999). The value was then normalized by the α -tocopherol concentration previously determined by HPLC. Although the films showed a decrease in the antioxidant activity when compared with a control of freshly prepared α -tocopherol solution (100 $\mu\text{g/mL}$), which exhibited % SCV/

Concentration of 10.3 ± 1.65 %, the antioxidant activity after 20 days in 50 % EtOH (v/v) was still remarkable (Table 3). Moreover, films prepared with PCL maintained better the antioxidant activity, which suggest a protective effect of PCL against α -tocopherol degradation.

Conclusion

Biodegradable films based on chitosan containing α -tocopherol were prepared to be used as an antioxidant active food packaging. Developed films maintained the antioxidant activity for more than 20 days. The kinetics release of α -tocopherol from the active films into food simulants were evaluated. The key parameters of the diffusion process (partition and diffusion coefficients) were calculated by using a mathematical model based on Fick's second law. The D values obtained were comprised between 1.07×10^{-13} and 2.24×10^{-12} cm^2/s . Overall, the results indicate that a fine balance between the mechanical features and particularly the swelling in contact with food and the migration kinetics of the antioxidant should be attained for the employability of chitosan-based films as antioxidant-eluting food packaging material.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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