

## Influence of $\alpha$ -tocopherol on physicochemical properties of chitosan-based films

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### ABSTRACT

Chitosan has been exploited as a material for the development of edible films, and additionally can be used as a carrier of functional compounds such as  $\alpha$ -tocopherol. The aim of this work was to evaluate the effects of the incorporation of  $\alpha$ -tocopherol in chitosan-based films. FTIR and thermal analyses were performed and showed that the incorporation of  $\alpha$ -tocopherol affects the chemical structure of chitosan-based films with the establishment of new chemical bonds and the decrease of crystallinity. Results also showed that the increase of  $\alpha$ -tocopherol concentration promotes a decrease of water content (from 12.6 to 11.4%) of the films. The addition of  $\alpha$ -tocopherol to chitosan films leads to a significant reduction ( $p < 0.05$ ) of tensile strength from 34.06 to 16.24 MPa, and elongation-at-break from 53.84 to 23.12%. Film opacity values (ranging from 4.74 to 7.83%) increased when  $\alpha$ -tocopherol was incorporated into the film. Antioxidant capacity of chitosan-based films was evaluated and was enhanced when  $\alpha$ -tocopherol was present in the film matrix. Results showed that  $\alpha$ -tocopherol can be successfully added to the chitosan films enhancing the final quality and shelf-life extension of food products.

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### 1. Introduction

The environmental impact of non-biodegradable plastic material wastes is of increasing global concern. There is an urgent need to develop renewable and environmentally friendly bio-based polymeric materials (Khwaldia, Arab-Tehrany, & Desobry, 2010). Edible films can help solve the waste disposal problem by partially replacing non-biodegradable plastics. They can also act as a barrier to external influences such as water vapor, oil and oxygen, as a vehicle for functional compounds, and at the same time reducing e.g. bruising and breakage and thus preserving/improving food integrity.

Chitosan is a natural polymer obtained by deacetylation of chitin, and when compared with other polysaccharides, chitosan has several advantages such as biocompatibility, biodegradability and no toxicity, while also presenting functional properties as bacteriostatic and fungistatic (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Kumar, 2000). The cationic character of chitosan offers an opportunity to establish electrostatic interactions with other compounds. Due to these characteristics, chitosan has been widely used for the production of edible films (Aider, 2010; Rivero, García, & Pinnotti, 2010; Ziani, Oses, Coma, & Maté, 2008). Chitosan films present good barrier properties when compared with other polymers such

as methylcellulose and corn starch (Debeaufort & Voilley, 2009; García, Pinotti, Martino, & Zaritzky, 2009). Also, mechanical properties of chitosan films can be improved e.g. by the addition of plasticizers (Yoshida, Junior, & Franco, 2009); however, the presence of such compounds can affect the structure of chitosan films.

Recently, edible films and coatings appeared as an efficient vehicle for functional ingredients such as antioxidants, antimicrobials, nutrients, and flavors (Lin & Zhao, 2007; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009). Antioxidants can be incorporated into edible films in order to enhance food stability, functionality and safety and to control the oxidation of fatty components and pigments, contributing to food quality preservation (Lee, 2005). The incorporation of an antioxidant into chitosan-based films is an interesting way of extending chitosan-based films' functional properties. In fact, multifunctional packaging systems containing active substances have a high potential for commercial food packaging applications. Consumers prefer foods with improved safety; this is particularly relevant in the case of fresh and minimally processed food using this packaging system (Lee, 2005).

Lipid oxidation is one of the major processes occurring in food systems and has strong effects on development of off-flavors, color loss, bad odors and harmful compounds. The prevention of oxidation by addition of antioxidants is highly desirable in preserving fatty acids from oxidative deterioration. Vitamin E is an important antioxidant that can help reduce risk of diseases such as cardiovascular diseases and it is considered a GRAS compound (Strandberg & Albertsson, 2006). The lipophilic compound  $\alpha$ -tocopherol is the

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most abundant and biologically active form of vitamin E (Bramley et al., 2000) and is widely used in the pharmaceutical, food and cosmetic industries. Edible oils originating from plants, such as palm oil, sunflower oil, usually contain a large amount of  $\alpha$ -tocopherol (Kamal-Eldin & Andersson, 1997). Due to its antioxidant properties and availability,  $\alpha$ -tocopherol was chosen for this study. This natural antioxidant has been used in synthetic packaging materials such as low-density polyethylene to protect the packaged food from oxidative degradation (Koontz et al., 2010; Wessling, Nielsen, Leufvén, & Jägerstad, 1999). Therefore, chitosan films containing an active antioxidant, such as  $\alpha$ -tocopherol, can be used as an active packaging system on food surface. In order to develop effective chitosan edible films with antioxidant activity, their physical, transport and mechanical properties should be assessed.

This work was carried out to understand the potential changes in transport, mechanical and thermal properties of chitosan-based films when  $\alpha$ -tocopherol was incorporated. The *in vitro* antioxidant capacity of the films with and without  $\alpha$ -tocopherol was also tested.

## 2. Material and methods

### 2.1. Material

$\alpha$ -Tocopherol with 98% purity was supplied by Sigma–Aldrich (St. Louis, MO, USA). Chitosan was obtained from Golden-Shell Biochemical Co. Ltd. (China) with a degree of deacetylation of 95%. Tween 80 (Acros Organics, Belgium);  $\iota$ (-)-lactic acid 90% (Acros Organics, Belgium); and distilled water were used to prepare the film-forming solutions. DPPH radical scavenging assay was performed using 2,2-diphenyl-1-picrylhydrazyl (Sigma, Germany) and methanol P.A. (Riedel-de Haën, Germany).

### 2.2. Preparation of edible films

Chitosan-based films were prepared according to Souza et al. (2010) with some modifications. Film-forming solutions of chitosan were prepared dissolving 1.5% (w/v) chitosan in a 1% (v/v) lactic acid solution. For complete dissolution of chitosan, this solution was left for 8 h under agitation, using a magnetic stirrer, at room temperature (20 °C). Tween 80 (0.1% w/v) was added to the solution as a surfactant and as an emulsifier to assist  $\alpha$ -tocopherol dissolution in film-forming solutions. The solution was stirred for 20 min at 40 °C for homogenization.

Films containing  $\alpha$ -tocopherol were prepared exactly as the control films however, after Tween 80 addition,  $\alpha$ -tocopherol was added to chitosan solution. Two different concentrations (0.1 and 0.2% (w/v)) were chosen based on preliminary experiments (data not shown) where it was determined that for  $\alpha$ -tocopherol concentrations above 0.2% the film would be too “greasy” on the surface. The obtained suspensions were homogenized with an Ultra-Turrax homogenizer (T 25, Ika-Werke, Germany) in 2 cycles, each of 2 min duration at 10,000 rpm.

To prepare the films, 30 mL of each solution were cast onto 90 mm Petri dishes and placed in an oven at 35 °C for 16 h; they were subsequently stored at 20 °C and 54% RH, in a desiccator containing a saturated solution of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , until further use.

### 2.3. Fourier-transform infrared (FTIR) spectroscopy

The IR spectra of the films were determined with an FT-IR spectrometer (Perkin–Elmer 16 PC spectrometer, Boston, USA), using Attenuated Total Reflectance (ATR) mode. Each spectrum results from 16 scans at 4  $\text{cm}^{-1}$  resolution for a spectral range from 400–4000  $\text{cm}^{-1}$ . All the readings were performed at room temperature (20 °C). In the case of overlapping peaks, deconvolution was

performed to calculate the contribution of the individual peaks using Peakfit software version 4.12 (SYSTAT Software, Richmond, CA). Deconvolution was used to estimate the area related to the specific vibration of the desired peak. Spectra of films were deconvoluted with a smoothing filter of 20%. Each spectrum was baseline corrected and the absorbance was normalized between 0 and 1.

### 2.4. Thermal analyses

The thermal stability and degradation profile of all chitosan film samples was assessed by thermogravimetric analysis (TGA) with a Shimadzu TGA 50 according to (Casariego et al., 2009). Film samples (approximately 5 mg) were put in high pressure stainless steel pans. An empty pan of the same type as the sample pan was used as a reference. Samples were heated at a constant rate of 10 °C  $\text{min}^{-1}$  from 20 to 580 °C in nitrogen atmosphere.

Differential scanning calorimetry (DSC) measurements were performed with a Shimadzu DSC-50 (Shimadzu Corporation, Kyoto, Japan) calibrated with Indium as standard. Ca. 10 mg of the sample was placed in aluminum DSC pans (Al crimp Pan C.201-52943). The measurements were performed between 20 and 250 °C at a heating rate of 10 °C  $\text{min}^{-1}$  under a nitrogen atmosphere. Data were analyzed using TASYs software (Shimadzu Corporation, Kyoto, Japan).

### 2.5. Thickness

The film thickness was determined with a digital micrometer (No. 293-5, Mitutoyo, Japan). Five thickness measurements were randomly taken on each testing sample. The mean values were used to calculate water vapor permeability (WVP) and tensile strength (TS).

### 2.6. Solubility and moisture content

The film solubility in water was determined according to the method reported elsewhere (Casariego et al., 2009). The measurement of solubility of the films was determined as the percentage of soluble material after 24 h of immersion in water. To determine the moisture content of films about 50 mg of film were dried at 105 °C during 24 h. The weight loss of the sample was determined, and the moisture content was calculated as the percentage of water removed from system. Three replicates were obtained for each sample.

### 2.7. Water vapor permeability (WVP)

The measurement of WVP was performed gravimetrically based on ASTM E96-92 method (Guillard, Broyart, Bonazzi, Guilbert, & Gontard, 2003; McHugh, Avena-Bustillos, & Krochta, 1993). The water transferred through the film and adsorbed by the desiccant (silica gel) was determined from weight loss of the permeation cell (cup containing distilled water at 100% RH;  $2.337 \times 10^3$  Pa vapor pressure at 20 °C). The slope of weight loss versus time was obtained by linear regression. The measurement (WVP) of the films was determined as follows:

$$\text{WVP} = (\text{WVTR} \times L) / \Delta P \quad (1)$$

where WVTR is the water vapor transmission rate ( $\text{g m}^{-2} \text{s}^{-1}$ ) through the film,  $L$  is the mean film thickness (m), and  $\Delta P$  is the partial water vapor pressure difference (Pa) across the two sides of the film. For each type of film, WVP measurements were replicated three times.

### 2.8. Tensile strength (TS) and elongation-at-break (EB)

Mechanical properties were measured with an Instron Universal Testing Machine (Model 4500, Instron Corporation) following the

guidelines of ASTM Standard Method D 882-91. The grip separation and crosshead speed were defined at 30 mm and 5 mm min<sup>-1</sup>, respectively. *TS* was expressed in MPa and *EB* was expressed in percentage (%). *TS* and *EB* tests were replicated at least five times for each film.

### 2.9. Color, opacity and light transmittance of the films

The color of the films was determined with a Minolta colorimeter (CR 400; Minolta, Japan). A white color plate was used as a standard for calibration and as a background for color measurements of the films. *L*<sup>\*</sup>, *a*<sup>\*</sup>, *b*<sup>\*</sup> values of each film were evaluated by reflectance measurements (Rao, Kanatt, Chawla, & Sharma, 2010). The opacity of the samples was determined according to the Hunterlab method, as the relationship between the opacity of each sample on a black standard and the opacity of each sample on a white standard (Casariego et al., 2009).

The light transmission characteristics of the films were measured using a UV–Vis spectrophotometer Jasco V-560 (Tokyo, Japan). The percentages of light transmission were determined over the range 250–700 nm for each chitosan-based film (Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2008). The measurements were repeated three times for each film.

### 2.10. DPPH radical scavenging assay

The radical scavenging activity of chitosan-based films was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Byun, Kim, and Whiteside (2010). Briefly, approximately 100 mg of film was placed in a flask containing 2 mL of methanol and was stirred for 3 h at room temperature (20 °C). The supernatant obtained was analyzed for DPPH radical scavenging activity. 2 mL of methanolic solution of DPPH (0.06 mM) was mixed with 500 µL of supernatant. The control was obtained using 500 µL of methanol without the presence of the film. The mixture was vortexed vigorously and left for 30 min at room temperature in the dark. The remaining DPPH was determined by absorbance at 517 nm using a UV spectrometer (Varian-UV–Vis Spectrophotometer, Germany). The radical scavenging activity (RSA) of the chitosan films was calculated according to the equation:

$$\%RSA = 100 \times (1 - A_{\text{sample}}/A_{\text{control}}) \quad (2)$$

where *A*<sub>sample</sub> represents the absorbance of the sample solution and *A*<sub>control</sub> represents the absorbance of DPPH solution without the addition of the film. All experiments were carried out in triplicate. The antioxidant capacity of chitosan-based films was expressed in % DPPH radical scavenging activity/100 mg of film.

### 2.11. Statistical analysis

Statistical analysis was performed with software SigmaPlot 11.0 for Windows. One-way analysis of variance and Tukey's multiple comparisons test were performed to determine the significant differences. Significance was defined at a level of *p* < 0.05.

## 3. Results and discussion

### 3.1. Fourier-transform infrared (FTIR) spectroscopy

The effect of  $\alpha$ -tocopherol in chitosan films was evaluated by FTIR analyses; when compounds are mixed, physical bonds and chemical interactions are reflected by changes in characteristic spectral peaks.

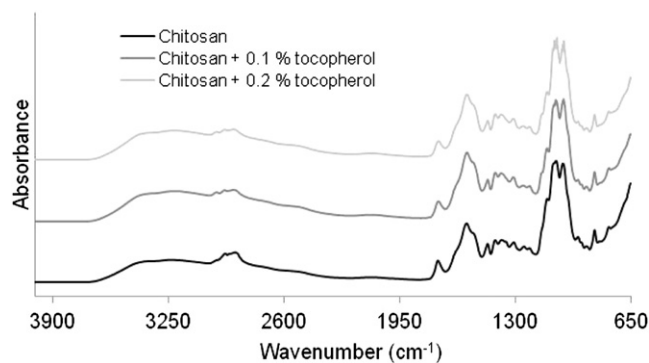


Fig. 1. FTIR spectra of the chitosan films for increasing  $\alpha$ -tocopherol concentrations.

Fig. 1 shows FTIR spectra of chitosan (CH) films containing 0, 0.1 and 0.2% of  $\alpha$ -tocopherol (TOC). The broad band ranging between 3100 and 3500 cm<sup>-1</sup> is attributed to O–H stretching vibration that overlaps the N–H stretching vibration in the same region. The broad band between 2800 and 3100 cm<sup>-1</sup> is attributed to C–H stretching vibration. The peak at 1557 cm<sup>-1</sup> was due to the amide bending vibration (N–H), and the peak at 1733 cm<sup>-1</sup> is characteristic of the presence of a carbonyl group (C=O) in the film matrix (Pawlak & Mucha, 2003; Xu, Kim, Hanna, & Nag, 2005; Ziani et al., 2008).

The deconvolution of the FTIR spectra was performed for two area bands: 800–1600 cm<sup>-1</sup> and 2600–3600 cm<sup>-1</sup>, the corresponding peaks and areas are presented in Tables 1 and 2, respectively.

By deconvolution of the area band between 800 and 1600 cm<sup>-1</sup>, seven bands were observed for chitosan films with and without  $\alpha$ -tocopherol (Table 1 and Fig. 2). When  $\alpha$ -tocopherol is added to chitosan films a new peak (ca. 940 cm<sup>-1</sup>) appears that can be related with the C–O vibrations of the alkoxy group (C–O–R) (Ning, Jitugao, Xiaofei, & Ying, 2007).

Results showed a shift of the peak at 1026 cm<sup>-1</sup> to 1019 and 1020 cm<sup>-1</sup> for 0.1 and 0.2% of  $\alpha$ -tocopherol, respectively, ascribed to C–O stretching of hydroxyl and ether bonds (Bu et al., 2010). Those bonds results from the presence of  $\alpha$ -tocopherol in the chitosan-based film matrix. A shift of the peak at 1088.7 cm<sup>-1</sup> to peaks at 1074.8 and 1076.7 cm<sup>-1</sup> was also observed with the increase of  $\alpha$ -tocopherol concentrations of 0.1% and 0.2%, respectively. This shift has been related to the C–O–H bending (Jamróz et al., 2007). Both shifts (at 1026 and 1088.7 cm<sup>-1</sup>) may originate from a modification of the level of hydration of C–O–H groups, as previously suggested by Kačuráková and Mathlouthi (1996), which correlates well with the lower moisture content observed in chitosan films containing  $\alpha$ -tocopherol as described below in Section 3.3. Moisture content and solubility.

Table 1

List of FTIR peaks and fractional areas of bands between 800 and 1600 cm<sup>-1</sup> for chitosan (CH) films for increasing  $\alpha$ -tocopherol (TOC) concentrations.

1.5% CH		1.5% CH–0.1% TOC		1.5% CH–0.2% TOC	
Peak (cm <sup>-1</sup> )	Area (%)	Peak (cm <sup>-1</sup> )	Area (%)	Peak (cm <sup>-1</sup> )	Area (%)
849.4 ± 12.5	0.8 ± 0.6	842.5 ± 0.1	5.7 ± 0.2	843.0 ± 0.1	5.7 ± 0.1
1026.6 ± 2.7	34.6 ± 0.5	941.9 ± 0.3	4.1 ± 0.1	940.3 ± 0.7	4.3 ± 0.1
1088.7 ± 4.8	36.4 ± 1.0	1019.4 ± 0.2	29.5 ± 0.4	1020.1 ± 0.8	28.8 ± 0.8
1310.0 ± 5.0	3.4 ± 0.0	1074.8 ± 0.1	30.3 ± 0.8	1076.6 ± 1.0	29.3 ± 0.6
1382.7 ± 1.6	6.4 ± 0.4	1126.9 ± 0.6	8.9 ± 0.3	1131.0 ± 0.6	7.8 ± 0.2
1447.1 ± 1.4	2.5 ± 0.1	1245.0 ± 0.4	0.7 ± 0.1	1241.9 ± 0.0	1.5 ± 0.0
		1319.1 ± 0.4	3.1 ± 0.0	1316.9 ± 0.1	3.6 ± 0.0
		1387.6 ± 0.3	5.4 ± 0.0	1384.8 ± 0.0	6.7 ± 0.0

**Table 2**

List of FTIR peaks and fractional areas of bands between 2600 and 3600  $\text{cm}^{-1}$  for chitosan (CH) films for increasing  $\alpha$ -tocopherol (TOC) concentrations.

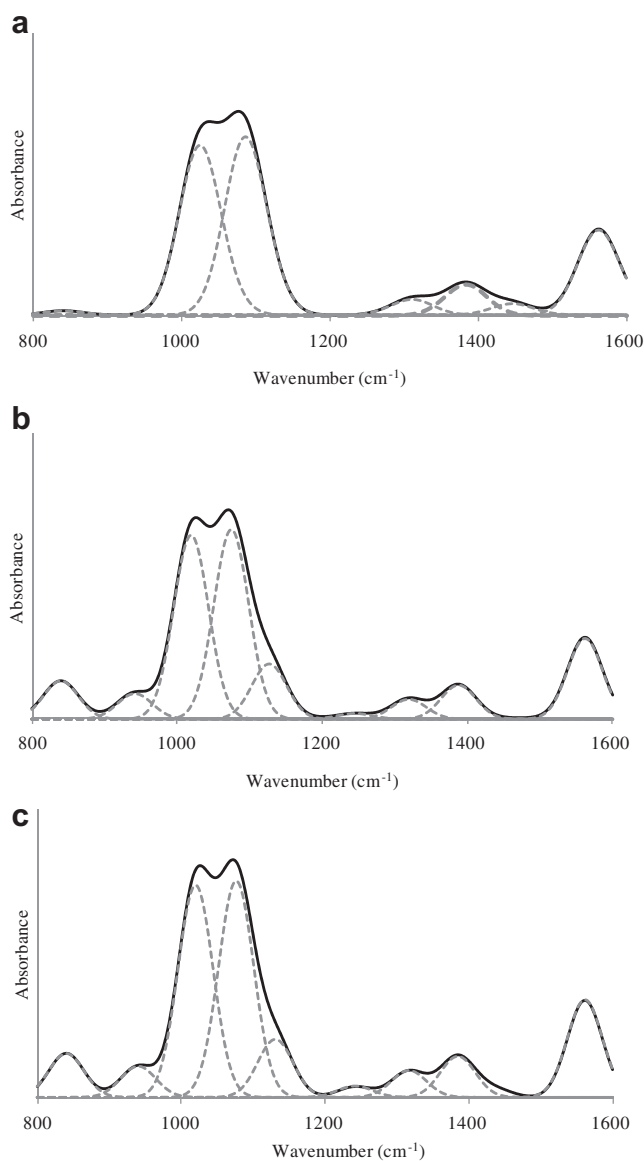
1.5% CH		1.5% CH–0.1% TOC		1.5% CH–0.2% TOC	
Peak ( $\text{cm}^{-1}$ )	Area (%)	Peak ( $\text{cm}^{-1}$ )	Area (%)	Peak ( $\text{cm}^{-1}$ )	Area (%)
2714.4 $\pm$ 1.6	8.5 $\pm$ 0.1	2734.7 $\pm$ 0.2	6.9 $\pm$ 0.0	2734.9 $\pm$ 0.0	6.9 $\pm$ 0.0
2899.2 $\pm$ 0.6	32.2 $\pm$ 1.1	2889.1 $\pm$ 1.6	19.0 $\pm$ 0.2	2890.9 $\pm$ 0.0	19.3 $\pm$ 0.0
3107.5 $\pm$ 1.2	18.9 $\pm$ 0.4	3007.7 $\pm$ 2.0	11.1 $\pm$ 0.3	3009.9 $\pm$ 0.2	10.7 $\pm$ 0.1
3260.3 $\pm$ 1.5	20.9 $\pm$ 0.4	3129.3 $\pm$ 0.7	16.7 $\pm$ 0.0	3130.1 $\pm$ 0.0	16.7 $\pm$ 0.0
3418.8 $\pm$ 1.4	19.4 $\pm$ 0.3	3251.7 $\pm$ 1.3	19.3 $\pm$ 0.1	3253.3 $\pm$ 0.0	19.4 $\pm$ 0.0
		3385.3 $\pm$ 3.4	18.9 $\pm$ 0.5	3389.3 $\pm$ 0.1	19.5 $\pm$ 0.0
		3483.6 $\pm$ 4.2	8.1 $\pm$ 0.5	3488.5 $\pm$ 0.1	7.5 $\pm$ 0.0

Still in the band region between 800 and 1600  $\text{cm}^{-1}$ , a new peak appears at ca. 1245  $\text{cm}^{-1}$  when  $\alpha$ -tocopherol is added, which can be related to the bending vibration of the OH groups from the phenol group present in  $\alpha$ -tocopherol structure (Mart, Sökmen, & Yürük, 2006). Once the phenol group is present only in the  $\alpha$ -tocopherol

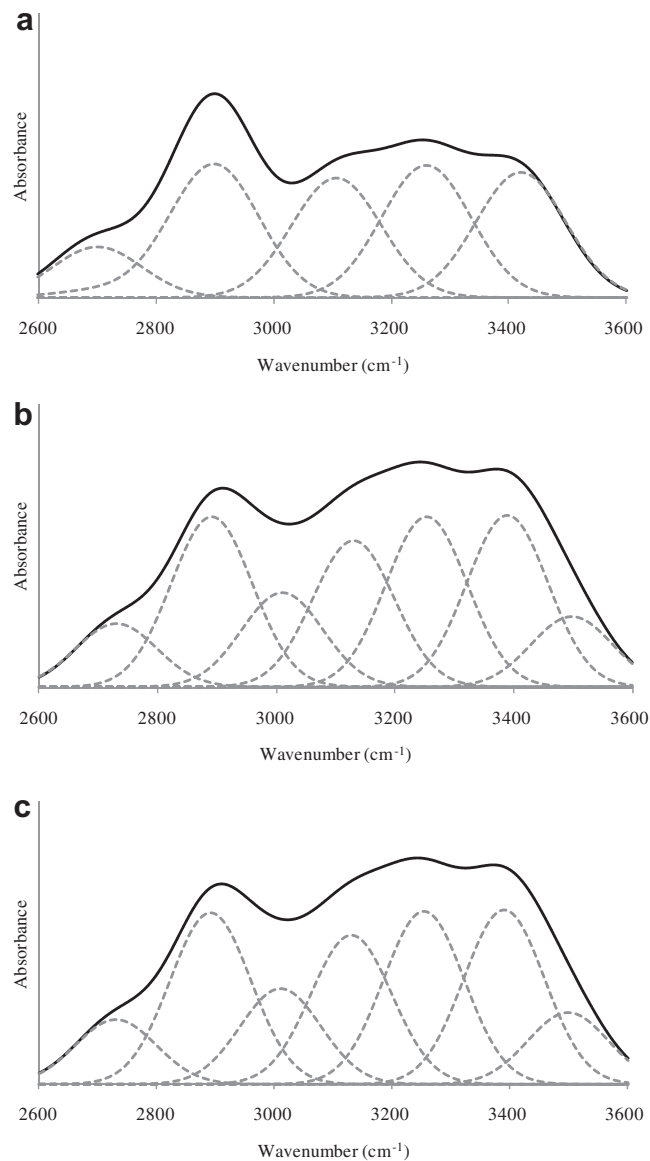
molecules, the presence of this peak is a clear evidence of the incorporation of  $\alpha$ -tocopherol in the film matrix.

Table 2 and Fig. 3 show the deconvolution of area band between 2600 and 3600  $\text{cm}^{-1}$  of FTIR spectra for chitosan films. In the range 2700–3000  $\text{cm}^{-1}$ , numerous bands were noted and attributed to the asymmetric and symmetric stretching vibrations of the  $\text{CH}_2$  and  $\text{CH}_3$  (Silva, Rosa, Ferreira, Boas, & Bronze 2009; Xu et al., 2005). When  $\alpha$ -tocopherol is added to chitosan films a shift occurs in the peak at ca. 2714  $\text{cm}^{-1}$  for ca. 2734  $\text{cm}^{-1}$ , with the decrease of the correspondent areas. The peak area at ca. 2899  $\text{cm}^{-1}$  also suffers a change, decreasing from 32% to 19% when  $\alpha$ -tocopherol is added. It may be anticipated that these shifts may be related to changes in  $\text{CH}_2$  and  $\text{CH}_3$  groups vibrations, but at this point there are no scientific evidences available to support this hypothesis.

New peaks at ca. 3007 and 3009  $\text{cm}^{-1}$  were observed for chitosan films with 0.1 and 0.2% of  $\alpha$ -tocopherol, respectively. These peaks are indicators of the increase of *cis* double-bond stretching vibration ( $=\text{CH}$ ) in the film samples (Vlachos et al., 2006) and have been reported as distinctive peaks of  $\alpha$ -tocopherol.



**Fig. 2.** FTIR deconvoluted spectrum in the range 800–1600  $\text{cm}^{-1}$ , corresponding to chitosan films with (a) 0%, (b) 0.1% and (c) 0.2% of  $\alpha$ -tocopherol.



**Fig. 3.** FTIR deconvoluted spectrum in the range 2600–3600  $\text{cm}^{-1}$ , corresponding to chitosan films with (a) 0%, (b) 0.1% and (c) 0.2% of  $\alpha$ -tocopherol.



**Table 3**

Thermal behavior of chitosan (CH) films for increasing  $\alpha$ -tocopherol (TOC) concentrations. The values of the peaks correspond to the values of derivative thermograms obtained by the TGA curve.

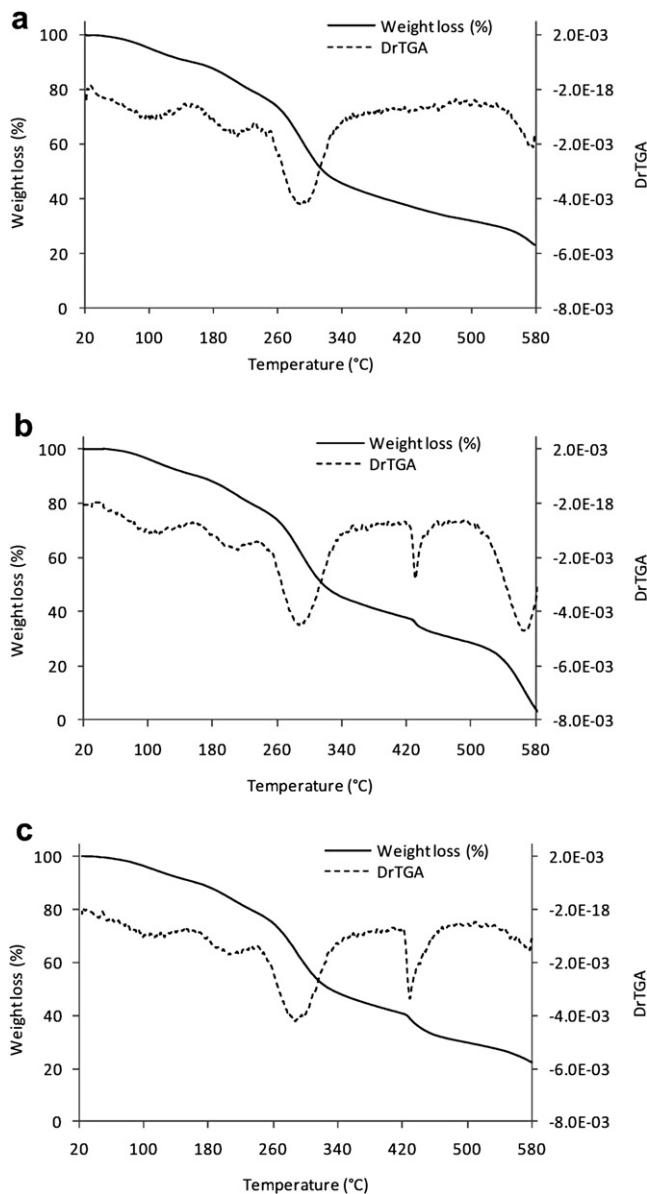
1.5% CH		1.5% CH–0.1% TOC		1.5% CH–0.2% TOC	
Peak temperature (°C)	Weight loss (%)	Peak temperature (°C)	Weight loss (%)	Peak temperature (°C)	Weight loss (%)
101.9 ± 1.9	10.6 ± 0.7	107.4 ± 8.9	8.9 ± 0.2	104.4 ± 19.9	9.5 ± 1.0
202.5 ± 2.7	11.2 ± 0.6	203.4 ± 2.5	12.5 ± 0.6	204.1 ± 5.6	13.1 ± 0.9
291.2 ± 2.8	32.7 ± 1.9	292.7 ± 2.4	32.2 ± 1.9	295.3 ± 2.5	32.5 ± 2.0
		439.1 ± 4.6	6.3 ± 1.3	441.0 ± 2.5	8.6 ± 0.7

The peak at ca. 3418  $\text{cm}^{-1}$ , characteristic of the inter- and intramolecular hydrogen bonding of hydroxyl groups (Cao, Shi, & Chen, 1998) and stretching vibration of secondary amine groups (N–H) (Sun & Wang, 2006), present in chitosan films spectra, shifts to 3385 and 3389  $\text{cm}^{-1}$  with the addition of 0.1 and 0.2% of  $\alpha$ -tocopherol, respectively. In addition to the shift at 3418  $\text{cm}^{-1}$ , new peaks appeared near 3483 and 3488  $\text{cm}^{-1}$  when 0.1 and 0.2% of  $\alpha$ -tocopherol were added to chitosan film, respectively; these peaks are assigned to  $\alpha$ -tocopherol hydrogen bonds (Luo, Zhang, Whent, Yu, & Wang, 2011). These results point out that the main interaction between  $\alpha$ -tocopherol and chitosan would be through hydrogen bonds, as suggested by other authors (Prado et al., 2011).

### 3.2. Thermal analysis

Thermogravimetric analyses determine the changes in weight of the films samples with the increase of the temperature. Table 3 and Fig. 4 show the peak values and the corresponding weight loss for each event, for chitosan films. They present at least three thermal events; however for samples with  $\alpha$ -tocopherol a fourth event was observed. Peak 1 results from the evaporation process; this phenomenon could be related to the hydrophilic nature of the polysaccharide. Peak 2 (around 200 °C) is related to chemisorbed water through hydrogen bonds and the elimination reaction of  $\text{NH}_3$  (Quijada-Garrido, Iglesias-González, Mazón-Arechederra, & Barrales-Rienda, 2007). However, no relevant differences in weight loss were observed between films with or without  $\alpha$ -tocopherol. Peak 3 (around 290 °C) is related with the dehydration, depolymerization and pyrolytic decomposition of the polysaccharide backbone (Zohuriaan & Shokrolahi, 2004). Films containing  $\alpha$ -tocopherol showed a fourth peak (peak 4) associated with  $\alpha$ -tocopherol aromatic structures decomposition at temperatures above 380 °C (Pelissari, Grossmann, Yamashita, & Pineda, 2009). The  $\alpha$ -tocopherol addition had no influence on thermal stability of chitosan films as demonstrated by the small variation in peak temperatures of the above-mentioned thermal events (at ca. 200 and 290 °C).

Differential scanning calorimetry (DSC) analyses were used to measure the enthalpy of melting ( $\Delta h_m$ ) and the peak melting temperature ( $T_m$ ) of the films.  $\Delta h_m$  and  $T_m$  can be associated with the crystallinity of the film samples (Peesan, Supaphol, & Rujiravanit, 2005; Sperling, 2006, chap. 6). Table 4 and Fig. 5 show that the incorporation of  $\alpha$ -tocopherol into chitosan films leads to the decrease  $\Delta h_m$  and to the increase of  $T_m$ . The decrease of  $\Delta h_m$  with the addition of  $\alpha$ -tocopherol can be associated with the decrease of the films crystallinity. It is known that chitosan films are semi-crystalline (Ziani et al., 2008);  $\alpha$ -tocopherol incorporation reduces the relative amount of crystalline structures in the film, leading to a more amorphous structure (Sperling, 2006, chap. 6), which may be due to the interruption of the crystalline structure formation in the chitosan matrix (Park & Zhao, 2004).



**Fig. 4.** TGA and DrTGA curves for chitosan films with (a) 0%, (b) 0.1% and (c) 0.2% of  $\alpha$ -tocopherol.

### 3.3. Moisture content and solubility

Water sensitivity is one of the major problems of polysaccharide-based films, and is evaluated by different methods such as monitoring moisture content, water activity and sorption, solubility, contact angles and through the measurement of the water vapor permeability (Fabra, Talens, & Chiralt, 2010; Ferreira, Nunes, Delgadillo, & Lopes-da-Silva, 2009; Sánchez-González et al., 2009). Solubility of films in water may also provide insight on the behavior of a film in an aqueous environment and is a measure of its water resistance. This is also an important factor that determines biodegradability of films when used as packaging materials (Gnanasambadam, Hettiarachy, & Coleman, 1997). Table 4 shows the solubility and moisture content values obtained for chitosan-based films. The increase of  $\alpha$ -tocopherol concentrations (0–0.2%) leads to a significant decrease ( $p < 0.05$ ) of moisture content values due to the hydrophobic nature of  $\alpha$ -tocopherol. This observation is in agreement with the FTIR results; the change in the peak ascribed

**Table 4**

Values of solubility, moisture content, thickness, water vapor permeability (WVP), enthalpy of melting ( $\Delta h_m$ ) and the peak melting temperature ( $T_m$ ) for chitosan films for increasing  $\alpha$ -tocopherol concentrations.

$\alpha$ -Tocopherol	Solubility (%)	Moisture content (%)	Thickness (mm)	WVP $\times 10^{-11}$ ( $\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$ )	$\Delta h_m$ ( $\text{J g}^{-1}$ )	$T_m$ ( $^{\circ}\text{C}$ )
0%	$31.6 \pm 0.9^a$	$12.6 \pm 0.5^a$	$0.094 \pm 0.002^a$	$6.02 \pm 0.40^a$	59.8	192.4
0.1%	$29.1 \pm 1.5^a$	$11.8 \pm 0.2^{ab}$	$0.115 \pm 0.002^b$	$6.71 \pm 0.27^a$	50.1	194.5
0.2%	$31.5 \pm 2.3^a$	$11.4 \pm 0.2^b$	$0.120 \pm 0.003^b$	$7.38 \pm 0.03^b$	34.6	202.5

<sup>a–b</sup>Different superscripts within the same column indicate significant differences among formulations ( $p < 0.05$ ).

to C–O–H group when  $\alpha$ -tocopherol was added to the film can be related to the amount of the water molecules absorbed in the chitosan film (Kačuráková and Mathlouthi, 1996). However, the chitosan films' solubility was not affected by the incorporation of  $\alpha$ -tocopherol ( $p > 0.05$ ). Ojagh, Rezaei, Razavi, and Hosseini (2010) reported that cinnamon essential oil decreased moisture content and solubility in water of chitosan-based films.

#### 3.4. Water vapor permeability (WVP) measurement

The water vapor permeability is the most extensively studied property of edible films mainly because of the importance of water in deteriorative reactions in foods. The presence and increase of  $\alpha$ -tocopherol concentration leads to the increase of WVP values (Table 4). A great number of factors can affect the WVP of chitosan films as e.g. film thickness, water sensitivity and crystallinity.

The increase of  $\alpha$ -tocopherol concentration results in increases of the values of thickness (Table 4), but also can affect the hydrophilic–hydrophobic behavior of the chitosan-based film. Byun et al. (2010) reported that addition of hydrophobic  $\alpha$ -tocopherol to polylactic acid films improves water vapor barrier properties. However, despite the hydrophobic character of  $\alpha$ -tocopherol, WVP values increased when  $\alpha$ -tocopherol was added to chitosan films. This could be due to the effect of  $\alpha$ -tocopherol addition over the cohesion forces of the chitosan network, as observed in the mechanical properties results (Section 3.5.); this would improve water vapor transport through the film matrix. Similar results were found by Bonilla, Atarés, Vargas, and Chiralt (2011) in chitosan-based films containing essential oils (basil and thyme). It was also noticed that the presence of  $\alpha$ -tocopherol originated less crystalline films (Table 4), leading to an increase of WVP. This hypothesis was advanced since it is well known that polymers with high crystallinity usually are less permeable due their ordered structure, and that the mass transfer of a gas in a semi-crystalline polymer is

primarily a function of the amorphous phase (Sperling, 2006, chap. 6; Miller & Krochta, 1997).

#### 3.5. Mechanical properties – TS and EB

Mechanical properties reflect the film's ability to protect the integrity of foods. Fig. 6 shows the effect of the increase of  $\alpha$ -tocopherol concentration on the values of TS and EB of chitosan-based films. Chitosan films present values of 34.06 MPa and 53.85% for TS and EB, respectively. These values are higher than those reported by Ojagh et al. (2010) ( $TS = 10.97$  MPa and  $EB = 24.73\%$ ) and Souza et al. (2010) ( $TS = 13$  MPa and  $EB = 16\%$ ). This could be due to several factors such as chitosan composition and source, plasticizer presence, film preparation and storage (Sánchez-González et al., 2009). It was noted that the  $\alpha$ -tocopherol incorporation significantly decreased the TS and EB of the chitosan films. A similar trend was reported by Jongjareonrak et al. (2008) on fish skin gelatin films incorporated with  $\alpha$ -tocopherol.

In the current work, the addition of hydrophobic  $\alpha$ -tocopherol inside chitosan film induces the development of structural discontinuities, producing a film structure with less chain mobility, and consequently, with less flexibility and resistance to fracture (Sánchez-González et al., 2009). Srinivasa, Ramesh, and Tharanathan (2007) reported that the incorporation of saturated fatty acids into chitosan films decreased TS and EB. Similar behavior was observed when  $\alpha$ -tocopherol was added to chitosan film. Generally, oil addition to polysaccharide films decrease TS and EB values, once lipids are not capable to form continuous and cohesive matrices (Péroval, Debeaufort, Despre, & Voilley, 2002). Also, the water content of the films can affect their mechanical properties. As previously mentioned, the increase of  $\alpha$ -tocopherol concentration on the chitosan films decreased the water content (Table 4). A lower water content is expected to decrease film plasticity, because water also acts like a plasticizer (Rivero et al., 2010).

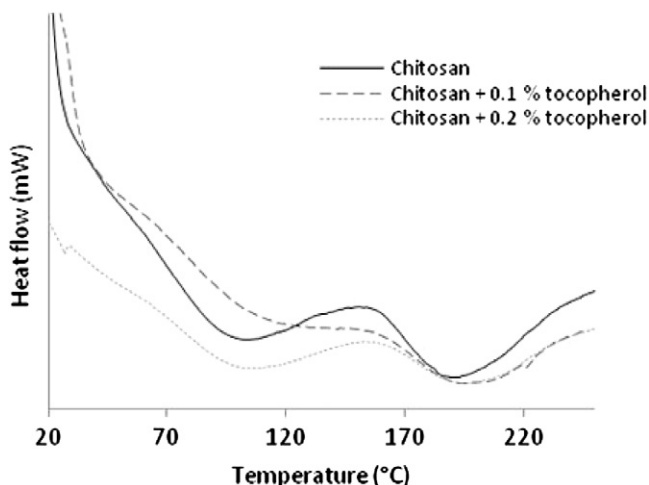


Fig. 5. DSC thermograms of chitosan films with  $\alpha$ -tocopherol.

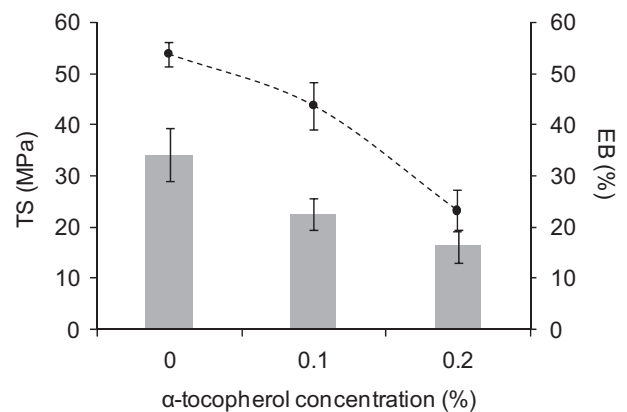


Fig. 6. Tensile strength (TS) and elongation-at-break (EB) of chitosan films for increasing  $\alpha$ -tocopherol concentrations. TS and EB are represented by columns and dots, respectively.

**Table 5**Color parameters,  $L^*$ ,  $a^*$  and  $b^*$ , opacity and DPPH radical scavenging activity of the chitosan films for increasing concentrations of  $\alpha$ -tocopherol.

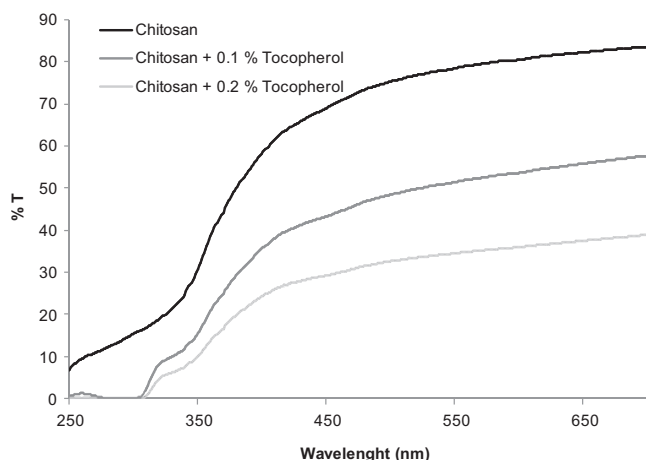
$\alpha$ -Tocopherol	$L^*$	$a^*$	$b^*$	Opacity (%)	DPPH radical scavenging activity/100 mg of film (%)
0%	95.41 $\pm$ 0.47 <sup>a</sup>	3.54 $\pm$ 0.35 <sup>a</sup>	5.94 $\pm$ 1.89 <sup>a</sup>	4.74 $\pm$ 0.49 <sup>a</sup>	10.69 $\pm$ 1.00 <sup>a</sup>
0.1%	92.57 $\pm$ 1.00 <sup>b</sup>	1.66 $\pm$ 0.28 <sup>b</sup>	15.06 $\pm$ 3.04 <sup>b</sup>	6.71 $\pm$ 0.80 <sup>b</sup>	97.42 $\pm$ 0.71 <sup>b</sup>
0.2%	92.34 $\pm$ 0.83 <sup>b</sup>	1.58 $\pm$ 0.29 <sup>b</sup>	15.73 $\pm$ 2.54 <sup>b</sup>	7.83 $\pm$ 0.32 <sup>b</sup>	97.71 $\pm$ 0.22 <sup>b</sup>

<sup>a–b</sup>Different superscripts within the same column indicate significant differences among formulations ( $p < 0.05$ ).

### 3.6. Optical properties – color, opacity and light transmittance of the films

Exposure to visible and ultraviolet light causes oxidative deterioration of packaged foods, leading to nutrient losses, discoloration and off-flavors. Table 5 shows the behavior of the color parameters and opacity of chitosan-based films after incorporation of  $\alpha$ -tocopherol. The incorporation of  $\alpha$ -tocopherol on chitosan-based films leads to a decrease of the  $L^*$  and  $a^*$  parameters, indicating a decrease of the lightness and an increase of the greenness of the film. The increase of  $b^*$  indicates the intensification of the yellowness of the chitosan film. However, there were no significant differences in  $L^*$  between 0.1% and 0.2%  $\alpha$ -tocopherol-chitosan films ( $p > 0.05$ ). This result shows that the addition of  $\alpha$ -tocopherol had a significant effect on  $a^*$  and  $b^*$  parameters of chitosan films. As shown in Table 5, chitosan films without  $\alpha$ -tocopherol presented the lower level of opacity ( $p < 0.05$ ) when compared with films containing  $\alpha$ -tocopherol. These results suggested that the addition of  $\alpha$ -tocopherol decreases the transparency of the chitosan. Siripatrawan and Harte (2010) also observed that aqueous green tea extract incorporated in chitosan films decreases film lightness and increases the opacity.

Chitosan films with and without  $\alpha$ -tocopherol presented different light transmission profiles in visible (350–700 nm) and UV (250–350 nm) ranges (Fig. 7). An increase of  $\alpha$ -tocopherol concentration led to an improvement of the film barrier to UV and visible light. 0.2%  $\alpha$ -tocopherol–chitosan films presented the lowest level of transmittance in the UV range (especially between 250 and 300 nm). As a result, the low level of transmission in the UV range can make  $\alpha$ -tocopherol–chitosan-based films an excellent barrier to prevent UV light-induced lipid oxidation when applied in food systems. Wessling, Nielsen, and Leufvén (2000) also showed that low-density polyethylene (LDPE) film containing  $\alpha$ -tocopherol offered UV protection compared to film without  $\alpha$ -tocopherol.

**Fig. 7.** Light transmission characteristics for chitosan films with  $\alpha$ -tocopherol.

### 3.7. DPPH radical scavenging assay

DPPH radical has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors in order to evaluate the antioxidant activity. DPPH tests were conducted to evaluate whether the  $\alpha$ -tocopherol retained its antioxidant capacity during incorporation in chitosan films. The radical scavenging activity of chitosan films with and without incorporated  $\alpha$ -tocopherol was determined and is presented in Table 5. Chitosan films showed radical scavenging activity of 10.69% on DPPH, which may be mainly attributed to the capacity of residual free amino groups of chitosan to react with free radicals forming stable macromolecular radicals and ammonium groups (Xie, Xu, & Liu, 2001). Films with  $\alpha$ -tocopherol exhibited a higher level of radical scavenging activity with values of 97.42 and 97.71% for 0.1 and 0.2% of  $\alpha$ -tocopherol containing films, respectively. However, no significant difference between the two  $\alpha$ -tocopherol concentrations incorporated in the films was observed. The activity values achieved for  $\alpha$ -tocopherol–chitosan films in this study were of the same order of magnitude as those reported in literature (Byun et al., 2010). According to these authors, the antioxidant activity of polylactic acid film with BHT and polyethylene glycol increased from 14.2% to 90.43% when  $\alpha$ -tocopherol was added to the film.

In the present work, the increased antioxidant activity is due to the effectiveness of the  $\alpha$ -tocopherol incorporated within chitosan films. The interaction between  $\alpha$ -tocopherol and chitosan molecules could influence the total radical scavenging activity of the film, in a similar way as that suggested by Siripatrawan and Harte (2010), who reported that green tea extract incorporation in chitosan films enhanced antioxidant activity possibly due to hydrogen and covalent bonding between polyphenols and chitosan.

## 4. Conclusion

The present work shows that  $\alpha$ -tocopherol can be added to a chitosan film matrix, imparting a good antioxidant activity. This incorporation influenced chemical, mechanical, barrier and color properties of the chitosan films: an increase of  $\alpha$ -tocopherol concentration promoted a decrease of water content and led to an increase of *WVP*, a reduction of *TS* and *EB* values, and an increase of chitosan-based films' opacity. The incorporation of  $\alpha$ -tocopherol may also be used to protect foods from UV light-induced degradation. Therefore, these films can be promising alternatives to synthetic materials and as a vehicle for functional compounds, possibly contributing to food preservation and shelf-life extension.

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