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Edible Chitosan/Acetylated Monoglyceride Films for Prolonged Release of Vitamin E and Antioxidant Activity

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ABSTRACT: α-Tocopherol-loaded chitosan films for pharmaceutical applications and food packaging purposes were designed to attain prolonged antioxidant activity. The films were prepared according to an emulsification/casting-solvent process from dispersions containing chitosan at 1.0 and 1.5% (w/w), combining five different proportions of α-tocopherol (ranging from 0 to 40% (w/w) with respect to chitosan) and two acetylated monoglyceride (AM) : α-tocopherol weight ratios (1 : 1 or 4 : 1). AM is a safe, edible, fully hydrogenated palm-based oil that provided adequate distribution of α-tocopherol in the films. Humidity at equilibrium, puncture strength and puncture deformation decreased as the content in α-tocopherol increased; no relevant effect of cross-linking in a glutaraldehyde atmosphere was observed. Degree of swelling and α-tocopherol release were tested in hydroalcoholic medium that mimic the polarity of the skin and certain foods. The films behaved as superabsorbent and sustained the release for more than 5 days; after 20 days, the antioxidant activity of α-tocopherol in the release medium was in some cases still as higher as that of a freshly prepared solution. The greater the content in α-tocopherol, the slower the release in percentage was. AM exerted a protective effect on α-tocopherol, but when incorporated at too high proportion made the films to have an oily aspect. In sum, the chitosan films showed suitable features to be used as components of α-tocopherol sustained release formulations and long-term antioxidant packaging materials. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 626–635, 2013

KEYWORDS: packaging; films; biomedical applications; alpha-tocopherol; controlled release; antioxidant film; acetylated monoglyceride

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INTRODUCTION

Natural polysaccharides are attractive materials owing to their versatile physic-chemical features, biocompatibility, biodegradability in the environment, and the many sources in nature that can be exploited to obtain them in a renewable way.[1–3] Polysaccharides also represent a relevant proportion of the by-products of the fishery and the agriculture activity.[4] Thus, the search of new applications for these materials may give them an added value and help to reduce the accumulation of unexploited wastes and discards (the so-called "aquatic processing wastes"), contributing to a sustainable growth.[5,6] Chitin is the second most abundant natural polysaccharide, and together with its derivative chitosan, has found a world-wide market as food additive, nutraceutical and water floculant,[7] and a promising potential as safe auxiliary substance in drug delivery and tissue engineering.[8,9] The properties of chitosan dispersions and networks and the possibilities of modulation by cross-linking with suitable agents have been extensively evaluated.[10,11]

The interest in edible/erodible food packaging materials is gaining increasing attention in order to avoid accumulation of plastic waste in the ecosystems.[12] Chitosan combines excellent filmogenicity,[13,14] with a wide antimicrobial activity, being very attractive to prepare active antimicrobial edible/erodible films.[15,16] As microbial spoilage and oxidation are the two major problems affecting food quality,[17,18] combinations of chitosan with antioxidant substances have already received some attention. For example, chitosan films containing green tea extract have been shown useful as active packaging for shelf life extension of pork sausages.[18] To fully exploit the biodegradability of chitosan, the use of chemical cross-linkers and synthetic grafts that lead to permanent covalent bonds should be minimized.[19,20] Edible chitosan films can be obtained applying the solvent-cast approach from acid solutions, although the incorporation of plasticizers at large proportions is required to communicate flexibility for processing and handling.[21] Sustained release of active substances can be achieved tuning the diffusional path by cross-linking and/or forming complexes with the chitosan amino and hydroxyl groups, as described for a variety of molecules such as antimicrobials or antioxidants.[22–24]

The aim of this work was to prepare chitosan films containing α-tocopherol with adequate mechanical features and sustained

release of the antioxidant agent, which may be useful in both food and biomedical fields. α-Tocopherol is the most abundant and active component of vitamin E.[25] In the food technology field, α-tocopherol (E-307 food code) is widely used as preservative and its prolonged release from the package could extend the stability and improve the sensory properties of some foods, as recently proved for fruits.[24,26,27] Conversely, α-tocopherol is orally administered for the management of vitamin E-deficiency related disorders, such as malabsorption syndromes, cystic fibrosis or vision disorders, and even tumors and inflammatory processes.[25] Topical application prevents/treats chronic damage induced by UVB and photocarcinogenesis.[28,29] Chitosan microspheres have been shown to sustain vitamin E release on the skin for 6 h, providing anti-ageing effects.[30] Formation of complexes through chemical interactions between the —NH_2 groups of chitosan and the —OH groups of α-tocopherol[23] could contribute to a prolonged release. To carry out the work, chitosan films were prepared by adding mixtures of α-tocopherol and acetylated monoglyceride (AM) to chitosan solutions containing glycerol as plasticizer, and then applying an emulsification/solvent-cast method. The films were characterized regarding their ability to incorporate hydroalcoholic medium, to sustain the release of α-tocopherol and to maintain its antioxidant capacity. Differently from previous recent works aimed to short term (hours) release of vitamin E and that evaluated the antioxidant effect just after the preparation of the formulations or when treated for few hours in an organic medium,[31] a purpose of the present work is to attain sustained release for several days in an hydroalcoholic medium that mimic the polarity of skin and certain foods,[32] and then to confirm that the antioxidant activity still persists. AM from edible, fully hydrogenated palm based oil was chosen as the emulsifier for the homogeneous distribution of α-tocopherol in the films,[22] since it is safer than other surfactant agents and can simultaneously act as stabilizer of vitamin E.[33] We also evaluated the effect of the cross-linking of the films in a glutaraldehyde vapor environment on their texture and performance as vitamin E controlled release systems. Although the cross-linking may compromise the edibility, it might be useful to tune the properties of the films for other applications.

EXPERIMENTAL

Materials

Chitosan [chitosan 222, Mw 359150 (s.d. 11569) Da][34] was from George S. Daras (Marseille, France), DL-α–tocopherol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma Aldrich (St. Louis, MO), glutaraldehyde from Merck (Darmstadt, Germany), glycerol, methanol absolute and ethanol absolute from Panreac (Barcelona, Spain), and lactic acid (90%, w/w) from Prolabo VWR (Fontenay-Sous Bois, France). AM (Grindsted® Acetem 70-00 Kosher) was kindly supplied by Danisco (Danisco Cultor España, S.A.). Purified water (resistivity above 18.2 MΩ cm^{-1}) was obtained by reverse osmosis (MilliQ®, Millipore, Madrid, Spain). All the other reagents were of analytical grade.

Chitosan Deacetylation Degree

The degree of deacetylation was estimated from the carbon/nitrogen ratio (C/N) obtained by elemental analysis (Carlo-Erba 1108 Elemental Analyzer, Fisons Instruments, UK) as follows[35]:

$$DD = 100 - [(C/N - 5.145)/(6.861 - 5.145)] \cdot 100 \quad (1)$$

In this equation, 5.145 and 6.861 represent the C/N ratios in completely N-deacetylated chitosan and in the fully N-acetylated chitin, respectively. DD resulted to be 76.2% for the batch evaluated.

Films Preparation

Chitosan films incorporating α-tocopherol were prepared adapting previously described solvent-cast methods.[22,26,27] Briefly, adequate amounts of chitosan were added under magnetic stirring to 0.6 and 0.8% (w/v) lactic acid aqueous solutions to render, respectively, 1 and 1.5% (w/w) chitosan dispersions. The bubbles were removed applying ultrasound for 5 min and letting the dispersions at rest at 4°C for 12 h. Then, the plasticizer agent glycerol was incorporated to the solutions at 25% (w/w) respect chitosan. Separately, AM and α-tocopherol at 1 : 1 and 4 : 1 weight ratios were blended by melting at 60°C under dark for 2–3 min and, immediately, the blends were mixed at various proportions with the chitosan-glycerol solutions preheated at 60°C (Table I). The systems were homogenized using a Heidolph Diax 600 (Schwabach, Germany) at 9500 min^{-1} for 1.5 min and applying ultrasound for 5 min to remove the bubbles. Finally, 10 g of each mixture were poured on polyethylene Petri dishes (51-mm diameter) and kept for 3 days at 37°C (air oven) for water evaporation. The films obtained were cut in 15 × 15 mm^2 pieces and dried (70°C, 24 h). Some films were cross-linked in glutaraldehyde saturated atmosphere for 15 min inside a hermetically closed chamber with 25% glutaraldehyde aqueous solution at the bottom. Then, the films were transferred to a desiccator and vacuum was applied for 15 min to remove unreacted glutaraldehyde. A code CHxTy_z was used to identify the films, where x indicates the concentration of chitosan in the starting solution, y, content in α-tocopherol, and the subindex z, AM : α-tocopherol weight ratio in the starting blend (namely 1 : 1 or 4 : 1) (Table I).

Films Characterization

Infrared Analysis. FTIR-ATR spectra of chitosan, α-tocopherol, AM, and also of the films were recorded between 4000 and 400 cm^{-1} using a Varian 670IR spectrophotometer (Varian, Santa Clara, CA) fitted with universal ATR sampling accessory of PIKE MIRacle crystal, which is composed of a diamond ATR with a zinc selenide focusing element in direct contact with the diamond.

Thickness and Mechanical Properties. The thickness of each film was measured in three different regions with a digital gauge at ambient temperature and humidity and after being kept at 37°C in air oven for 24 h. The experiments were carried out in triplicate.

Puncture strength (PS) and deformation were estimated at 37°C according to the ASTM D6241-04 method* using a TA-TX Plus Texture Analyzer (Stable Micro Systems, Surrey, UK) fitted with a film support rig with a hole of 1 cm in diameter. The films

*ASTM D6241-04. Standard test method for the static puncture strength of geotextiles and geotextiles-related products using a 50-mm probe. ASTM book of standards 2009, 04.13

Table I. Composition of the Dispersions Used to Prepare Chitosan-Glycerol Films Comprising AM and α-Tocopherol at 1 : 1 or 4 : 1 Weight Ratios as Indicated in the Sub-Indexes in the Codes

Films	Chitosan 1% (w/w) solution (g)	Chitosan 1.5% (w/w) solution (g)	Glycerol (mg)	AM/α-tocopherol (mg/mg)	α-Tocopherol content in the films (mg/g)	Thickness (mm)
$CH1T0_{1:1}$	14.62	–	37.50	60/0	0	0.135 (0.025)
$CH1T1_{1:1}$	14.62	–	37.50	7.5/7.5	25.78	0.072 (0.005)
$CH1T2_{1:1}$	14.62	–	37.50	15/15	49.03	0.090 (0.010)
$CH1T3_{1:1}$	14.62	–	37.50	30/30	89.31	0.133 (0.015)
$CH1T4_{1:1}$	14.62	–	37.50	60/60	151.55	0.103 (0.009)
$CH1.5T0_{1:1}$	–	14.44	56.25	90/0	0	0.156 (0.009)
$CH1.5T1_{1:1}$	–	14.44	56.25	11.2/11.2	26.75	0.140 (0.009)
$CH1.5T2_{1:1}$	–	14.44	56.25	22.5/22.5	50.79	0.192 (0.014)
$CH1.5T3_{1:1}$	–	14.44	56.25	45/45	92.22	0.225 (0.028)
$CH1.5T4_{1:1}$	–	14.44	56.25	90/90	155.72	0.196 (0.020)
$CH1T0_{4:1}$	14.62	–	37.50	150/0	0	0.160 (0.020)
$CH1T1_{4:1}$	14.62	–	37.50	30/7.5	23.93	0.080 (0.008)
$CH1T2_{4:1}$	14.62	–	37.50	60/15	42.75	0.100 (0.015)
$CH1T3_{4:1}$	14.62	–	37.50	120/30	70.43	0.088 (0.005)
$CH1T4_{4:1}$	14.62	–	37.50	240/60	104.18	0.150 (0.020)
$CH1.5T0_{4:1}$	–	14.44	56.25	225/0	0	0.210 (0.003)
$CH1.5T1_{4:1}$	–	14.44	56.25	45/11.2	24.77	0.120 (0.025)
$CH1.5T2_{4:1}$	–	14.44	56.25	90/22.5	44.08	0.126 (0.005)
$CH1.5T3_{4:1}$	–	14.44	56.25	180/45	72.23	0.163 (0.014)
$CH1.5T4_{4:1}$	–	14.44	56.25	360/90	106.14	0.201 (0.012)

Codes ending in 0 refer to formulations containing AM but not α-tocopherol. The thickness of the films ($n = 3$) is also given as mean value and, in parenthesis, standard deviation.

were fixed into the support rig with an upper plate to avoid the slippage. A stainless steel spherical ball probe (P/5S) of 5 mm of diameter descended at 1 mm s^{-1}. The maximum force recorded before rupture normalized by the thickness of the film was used to estimate the PS. The puncture deformation (PD), which is a measure of the capacity of deformability, was estimated assuming homogeneous distribution of the stress along the film as follows:

$$\mathrm{PD} = \Delta l/l_0 = [(D^2 - l_0^2)^{0.5} - l_0]/l_0 \quad (2)$$

where D is the distance that the probe covers since the moment that it contacts with the film until the moment that the film breaks, and l_0 is the initial length of the film, that is, the radius of the orifice of the rig support.[36,37]

Moisture Content. Moisture at equilibrium in 50% RH atmosphere was estimated at 25°C by placing the films in a dessicator with 43.4% sulfuric acid solution at the bottom. The weight of the films was monitored for 1 week and the moisture content over time was determined applying the equation:

$$\text{Moisture } (\%) = [(W_t - W_0) \times 100]/W_0 \quad (3)$$

where W_0 and W_t represent the weight of the dried film and after storage in 50% RH atmosphere.

Swelling Behavior. Dried films of ca. 50 mg were accurately weighed (W_0) and placed into ethanol : water 50 : 50 (v/v) mixture (20 mL) at 20°C. At pre-established time intervals, the films were removed from the swelling medium, the surface was wiped with a cotton paper, and the films were weighed again (W_s) and then returned to the correspondent vial. The experiments were performed in triplicate. The swelling degree was estimated as:

$$Q\ (\%) = [(W_s - W_0) \times 100]/W_0 \quad (4)$$

α-Tocopherol Release. Films (15 × 15 mm^2) were immersed in 20 mL of ethanol : water 50 : 50 (v/v) mixture at 20°C and the absorbance was recorded at 292 nm (Agilent 8453 UV–vis spectrophotometer, Agilent Tecnologies, Germany) during 20 days. The ethanol : water 50 : 50 (v/v) medium provides sink conditions and is the recommended solvent to simulate the polarity of certain foods, such as alcoholic, aqueous and lacteous products.[32] Once the release test was ended, the medium was analyzed regarding the antioxidant activity (see below), and the films were transferred to methanol (20 mL) and kept at 40°C under oscillatory movement (50 rpm) for 24 h protected from the light. The amount of α-tocopherol released was quantified as described above.

Antioxidant Activity. The antioxidant activity was determined applying the free radical DPPH method.[38,39] Aliquots of the

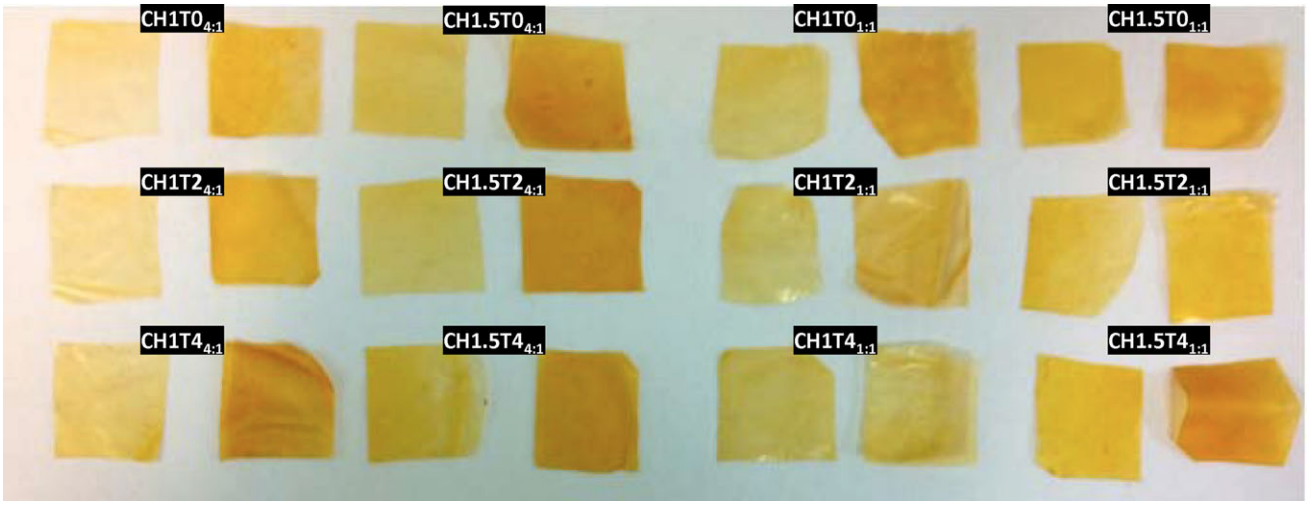

Figure 1. Photographs of chitosan films prepared with AM : α-tocopherol 4 : 1 and 1 : 1 (w/w) ratio. For each film (codes as in Table I), freshly prepared (photo on the left) and cross-linked (photo on the right) specimens are shown. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

release medium where CHxTy$_{4:1}$ films were tested were taken at day 20 and diluted 1.3-fold, twofold, and eightfold (final volume 2 mL) with ethanol : water 50 : 50 mixture. Similarly, aliquots of the CHxTy$_{1:1}$ films release medium were diluted twofold, fourfold, and eightfold (final volume 2 mL). Then, 2 mL of a DPPH solution (30 μg mL^{-1}, in ethanol : water 50 : 50, v/v) were added to each diluted solution and the mixtures were kept protected from the light and the oxygen. After 30 min, the absorbance (A_{sample}) was recorded at 525 nm. A mixture of 2 mL of the DPPH solution and 2 mL of ethanol : water 50 : 50 (v/v), which was prepared and processed as the samples, was used as reference ($A_{reference}$). Control experiments were carried out with freshly prepared α-tocopherol solutions (6.25, 12.5, and 25 μg mL^{-1}) in ethanol : water 50 : 50 (v/v) (2 mL). The antioxidant activity of α-tocopherol in the release medium was calculated in terms of free radical scavenging activity, applying the equation:[40]

$$\%\ \mathrm{ESC} = (1 - A_{sample}/A_{reference}) \times 100 \qquad (5)$$

The % ESC values obtained were then divided by the α-tocopherol concentration in each diluted solution in order to normalize the antioxidant activity.

RESULTS AND DISCUSSION

Films Preparation

Chitosan films incorporating α-tocopherol and prepared according to an emulsification/casting-solvent process, were semi-transparent with a homogeneous pale yellow color, which indicates a regular distribution of α-tocopherol (Figure 1). Chitosan at two different concentrations and α-tocopherol at five different proportions were used to prepare the films. α-Tocopherol proportions in the films ranged from 5 to 40% (w/w) with respect to chitosan, that is, up to almost twofold greater than the content in vitamin E tested in previously reported chitosan films.[22,31] Furthermore, AM was tested at two different weight ratios with respect to α-tocopherol, namely 1 : 1 and 4 : 1. When AM was used at the previously tested 4 : 1 ratio,[22] the films had an oily aspect even without α-tocopherol, which suggests that the amount of chitosan is not sufficient to sorb all the hydrophobic emulsifier. In fact, in the CH1T4$_{4:1}$ and CH1T4$_{4:1}$ films (Table I) the major component was AM (∼ 50%) and not chitosan (30–32%). Thus, although AM is less oily than other glycerides, another set of films was prepared with AM : α-tocopherol 1 : 1, and the features of both sets compared. In all cases, cross-linking in the glutaraldehyde atmosphere intensified the yellow tonality (Figure 1) and, in some cases, changed the color to orange or brownish-gray due to the reaction of glutaraldehyde with the amine groups of chitosan (Schiff base reaction).

Structural and Mechanical Features

The IR spectra of the chitosan-α-tocopherol films showed the typical peaks of each component (Figure 2); namely, the chitosan peaks at 3290 (—NH_2 and —OH groups), 2872 (—CH_2—), 1648, and 1581 (amine and amide) and 1058 (C—O) cm^{-1},[23] and the α-tocopherol ones at 3467 (hydroxyl groups), 2923, and 2865 (—CH_2— and —CH_3), 1457 (phenyl and methyl groups), 1259 (symmetric vibration of methyl groups), 1083 (phenyl groups), and 917 cm^{-1} (=CH_2 trans).[23] AM exhibits a characteristic peak at 1741 cm^{-1}. The peaks of both α-tocopherol and AM remained without changes after the film formation, but a shift in the band of chitosan from 3290 to nearly 3260 cm^{-1} occurred, which suggest the interaction with the lipid components through hydrogen bonding.[31]

The mean thickness of the films ranged between 70 and 225 μm (Table I) and was not altered by cross-linking with glutaraldehyde. Hardness (PS, Figure 3) and deformability (PD, Figure 4) were evaluated according to the ASTM D6241-04 method (see previous footnote). The films with the highest proportions of AM and α-tocopherol presented the smallest values of PS and PD. This finding is in agreement with the general trend observed for polysaccharide films containing oily substances, since the lipids cannot form continuous and cohesive

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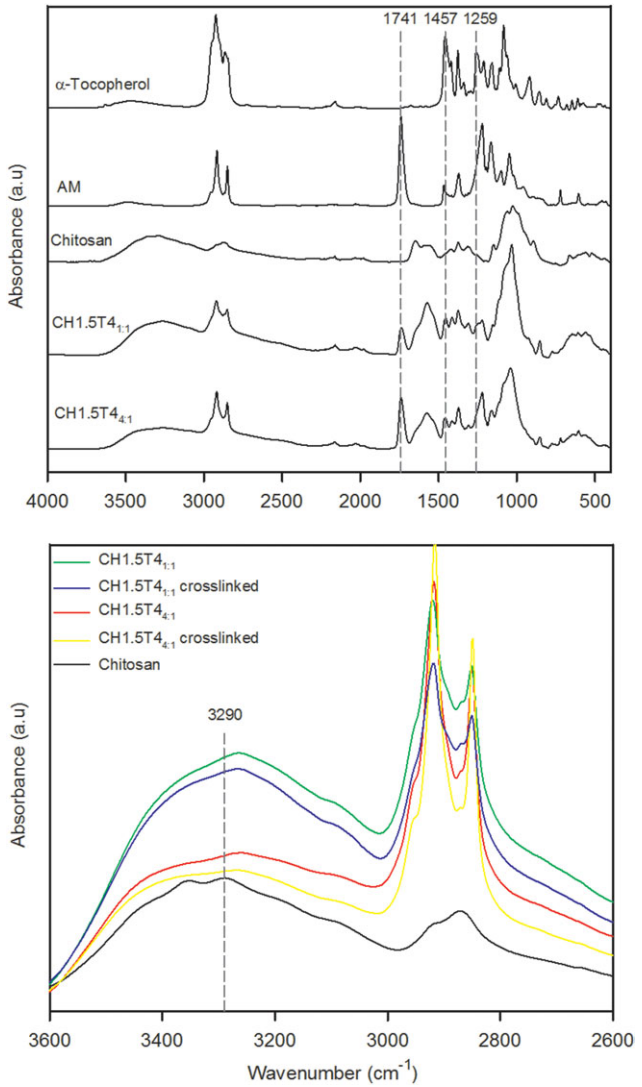

Figure 2. IR spectra of the raw materials and the chitosan films prepared with AM : α-tocopherol 1 : 1 and 4 : 1 weight ratio, before and after cross-linking. Codes as in Table I. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

matrices.[41] It has been pointed out that lipidic globules may interrupt the crystalline structure of the chitosan in the matrix and weaken the intermolecular hydrogen bonds.[22,42] Both PS and PD values recorded for the films prepared with AM : α-tocopherol 4 : 1 weight ratio and low total content in α-tocopherol are in good agreement with those previously reported for other related systems.[22] It should be noticed that the maximum in PS observed for intermediate values of α-tocopherol can be related to the fact that those films contained the lowest amount of AM. That is to say, the films with the extreme α-tocopherol contents are those with the highest content in AM (Table I) and the lowest PS values.

Films with the greatest content in chitosan (namely those prepared from 1.5% chitosan solutions) had higher PS (Figure 3), which is in agreement with the likelihood of forming more organized matrices. For a given AM : α-tocopherol ratio, the non-crosslinked films prepared from 1.5% chitosan solution showed lower values of PD (Figure 4) than those made from 1.0% chitosan, which suggests that the film structure becomes more fragile as it gets more entangled.[43] In general, the cross-linking in glutaraldehyde atmosphere led to a slight decrease in the PS, except for $CH1T2_{1:1}$ and $CH1.5T2_{1:1}$. As expected, the PD of the films prepared from 1% chitosan solutions decreased after cross-linking. No significant effect of cross-linking on the PS and deformability of AM : α-tocopherol 4 : 1 films was observed, suggesting that the main factors that determine the texture features of these films are the contents in AM and α-tocopherol.

Moisture Uptake and Swelling Behavior

To simulate standard work conditions, dried films were transferred to an environment of controlled humidity (50% RH) and

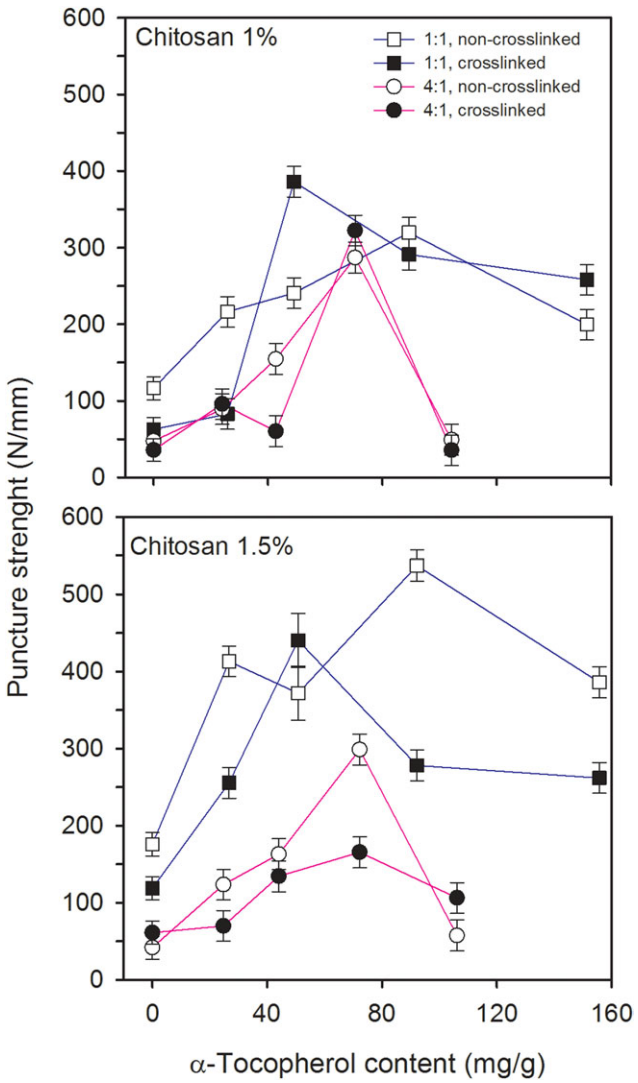

Figure 3. PS estimated as maximum force recorded before the rupture of the films normalized by their thickness according to the ASTM D6241-04 method. The AM : α-tocopherol weight ratio is indicated in the legend. The lines are just a guide for the eye. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

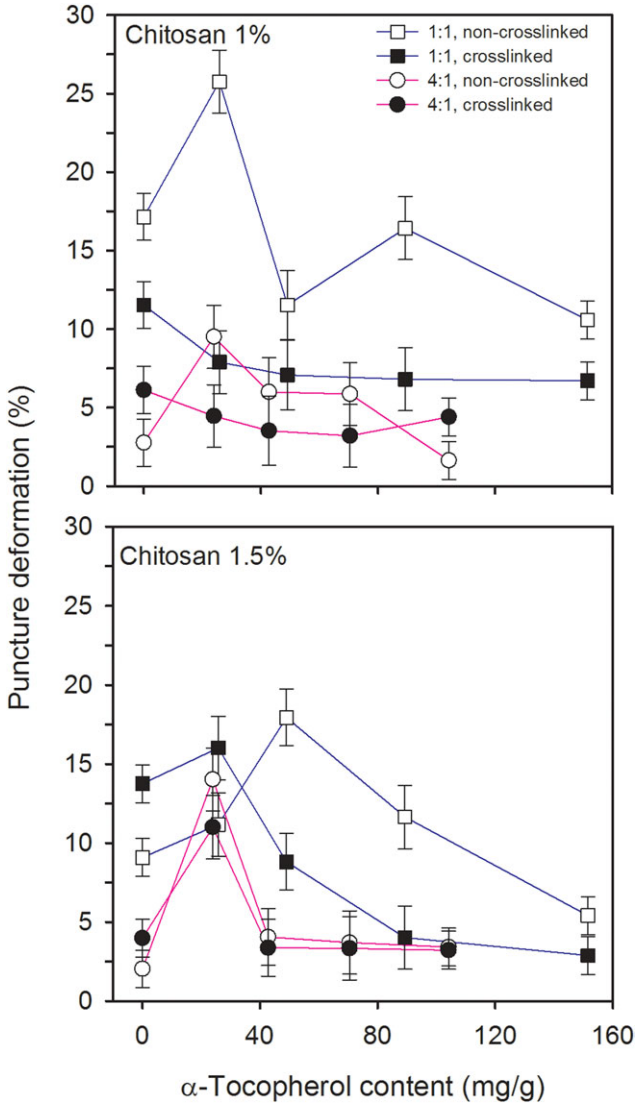

Figure 4. PD of the films determined according to the ASTM D6241-04 method. The AM : α-tocopherol weight ratio is indicated in the legend. The lines are just a guide for the eye. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

temperature (25°C).[22] Films prepared with low contents in AM and α-tocopherol uptook more water from the atmosphere than those prepared with the highest proportions of the vitamin E when the AM : α-tocopherol ratio was 4 : 1 (Figure 5; test *t* significant differences $\alpha < 0.05$ for films containing 23–26 mg α-tocopherol/g *vs.* 100–150 mg α-tocopherol/g). These findings clearly reflect that an increase in the content in lipophilic components makes the films to be more hydrophobic.[44] The influence of α-tocopherol proportion on the moisture uptake became less marked as the concentration of chitosan increased. The cross-linking process led to minor changes in the moisture content.

Swelling of films was evaluated in ethanol : water 50 : 50 (v/v) mixture, which enables sink conditions for α-tocopherol release and simulates the polarity of alcoholic or aqueous foods and also of lacteous products.[32] All the films behaved as superabsorbent, taking up liquid quickly during the first hour of immersion and reaching the equilibrium in few days (Table II). In general, the films prepared with the AM : α-tocopherol 4 : 1 ratio swelled more than those prepared with the 1 : 1 ratio (Table II), which is in good agreement with the weakening effect of AM on the mechanical properties (Figure 3). This means that AM facilitates the entrance of liquid in the chitosan network. Cross-linking made the films to swell less due to restrictions in the mobility of the chains (Table II). After 1 month in ethanol : water 50 : 50 (v/v) all films underwent certain physical disintegration, regardless of the process of cross-linking.

α-Tocopherol *In Vitro* Release

α-Tocopherol release profiles were recorded during 20 days in ethanol : water 50 : 50 (v/v) at ambient temperature

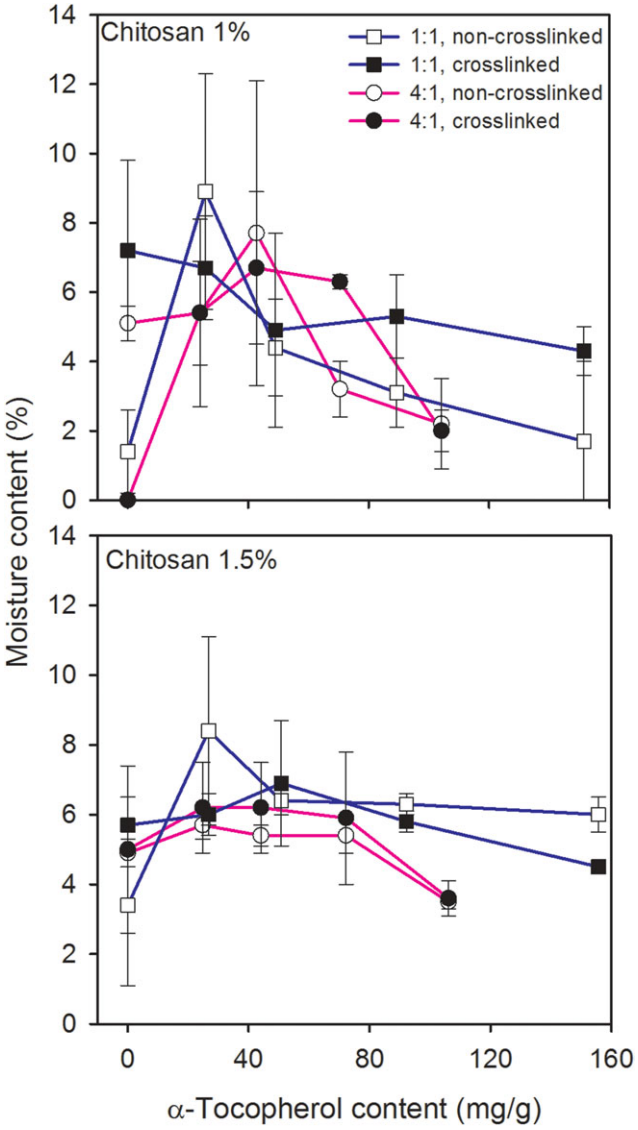

Figure 5. Equilibrium moisture content of films prepared with different AM : α-tocopherol weight ratios and stored at 25°C in an environment of 50% RH. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Swelling Degree (%) of the Films in Ethanol : Water 50 : 50 (v/v) After 20 Days at 20°C

Film	AM : α-tocopherol (1 : 1) non cross-linked	AM : α-tocopherol (1 : 1) cross-linked	AM : α-tocopherol (4 : 1) non-cross-linked	AM : α-tocopherol (4 : 1) cross-linked
CH1T0	535 (78)	327 (13)	914 (58)	796 (111)
CH1T1	449 (68)	255 (22)	1052 (114)	645 (50)
CH1T2	321 (35)	274 (12)	976 (103)	760 (90)
CH1T3	375 (31)	309 (77)	2067 (758)	840 (67)
CH1T4	394 (3)	280 (34)	1531 (96)	1174 (290)
CH1.5T0	442 (120)	320 (22)	3169 (686)	954 (85)
CH1.5T1	314 (24)	294 (4)	2357 (957)	973 (164)
CH1.5T2	383 (32)	231 (35)	3824 (864)	669 (63)
CH1.5T3	430 (78)	293 (71)	1169 (117)	734 (62)
CH1.5T4	350 (9)	297 (74)	622 (38)	512 (37)

Mean values and, in parentheses, standard deviations ($n = 3$).

(Figures 6 and 7). The films released a relevant portion of α-tocopherol in the first 24 h and then efficiently sustained the release over 5 days, although they continued eluting almost 20 days. Those prepared with a greater content in α-tocopherol released more amount of vitamin E per day (Figure 6), but when referred to the total content in α-tocopherol the

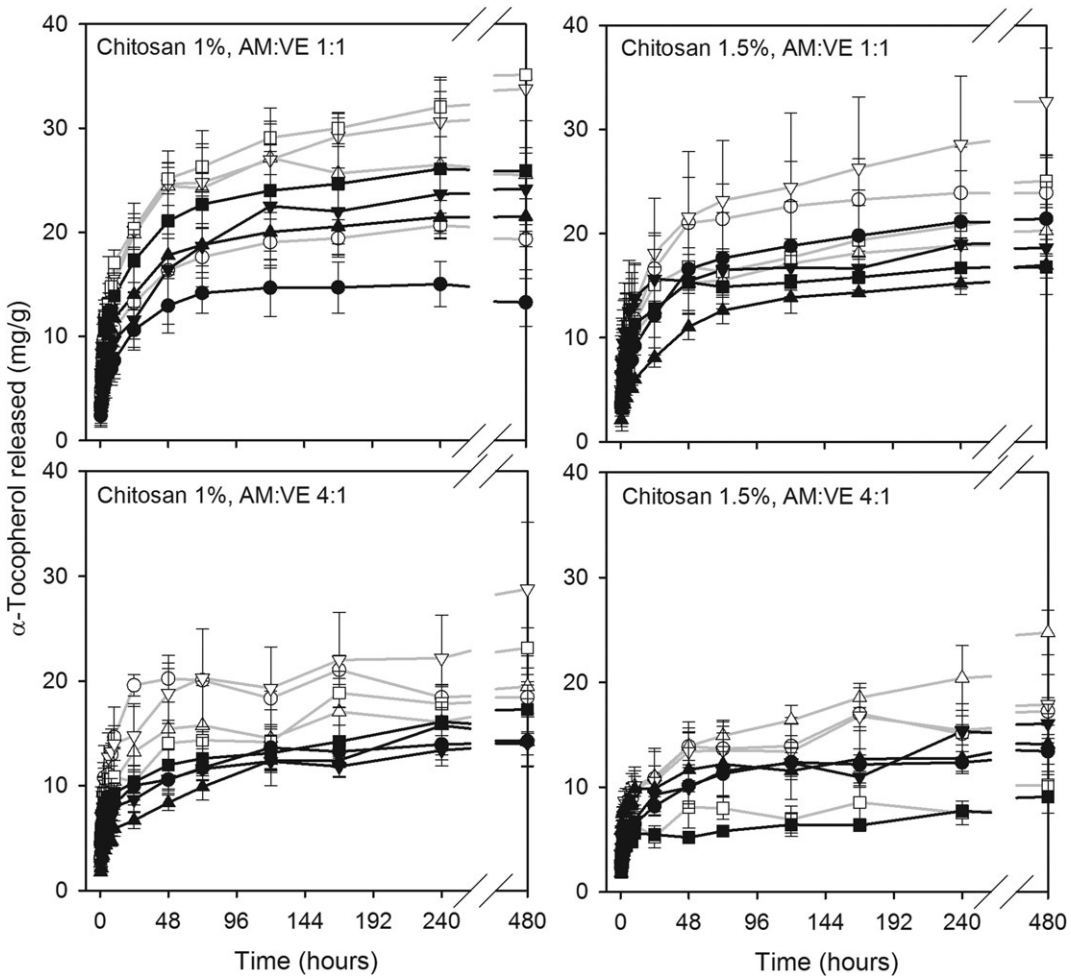

Figure 6. α-Tocopherol release profiles in ethanol : water 50 : 50 (v/v) at 20°C from CH*x*T1 (circles), CH*x*T2 (up triangle), CH*x*T3 (down triangle) and CH*x*T4 (square) non-crosslinked (open symbols, gray lines) and crosslinked (full symbols, black lines) films. *x* represents chitosan concentration used to prepare the films. Codes as in Table I.

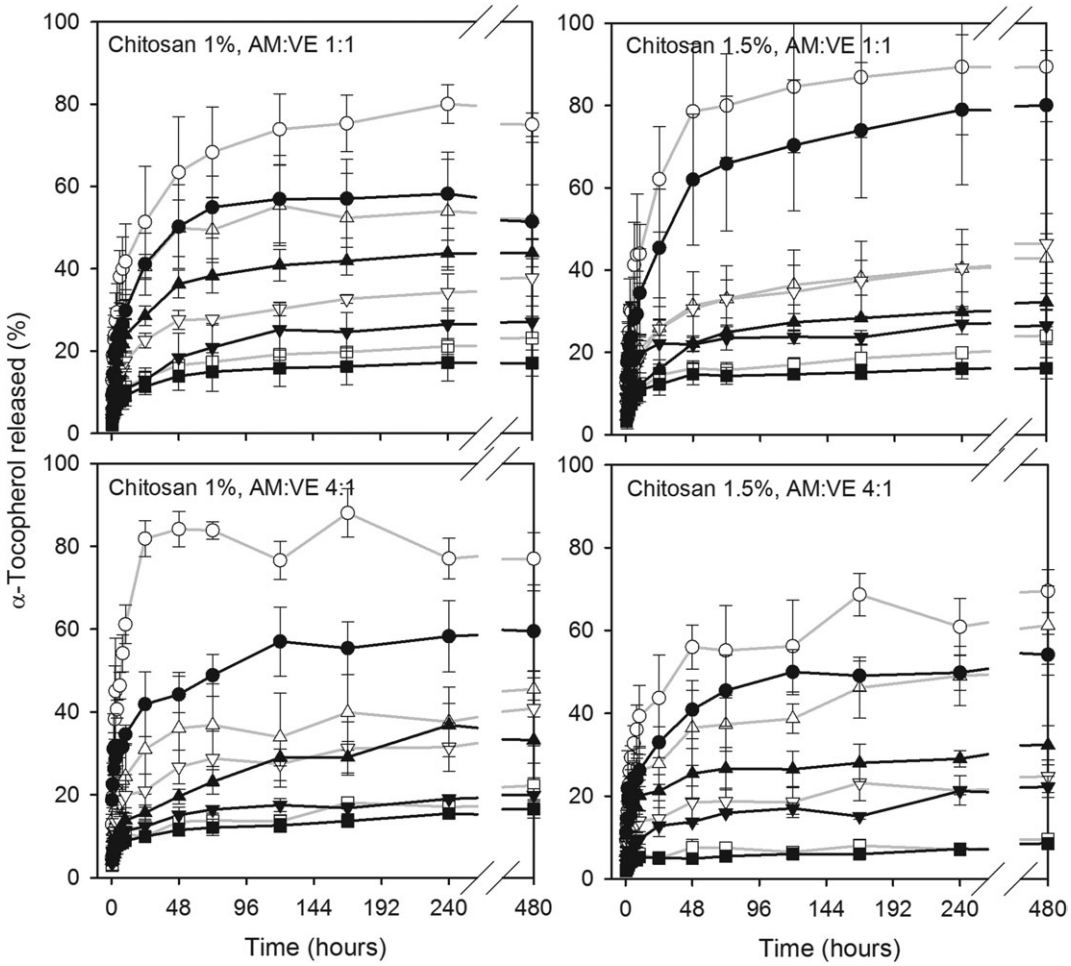

Figure 7. Percentage of α-tocopherol released in ethanol : water 50 : 50 (v/v) at 20°C from CHxT1 (circles), CH*x*T2 (up triangle), CH*x*T3 (down triangle), and CH*x*T4 (square) non-crosslinked (open symbols) and crosslinked (full symbols) films.

percentage released was smaller (Figure 7 and Table III). Crosslinked films (full symbols, black lines in Figure 7) sustained better the release, particularly those containing low proportions of α-tocopherol (AM : α-tocopherol 4 : 1).

To gain an insight into the mechanism of α-tocopherol release and to elucidate the effect of films composition on the release kinetics, the release profiles were fitted to the following equation[45]:

Table III. Kinetic Parameters Obtained by Fitting of eq. (6) to the α-Tocopherol Release Profiles in Ethanol : Water 50 : 50 (v/v)

	AM : α-tocopherol 1 : 1 non-cross-linked		AM : α-tocopherol 1 : 1 cross-linked		AM : α-tocopherol 4 : 1 non cross-linked		AM : α-tocopherol 4 : 1 cross-linked	
Films	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*
CH1T1	17.68 (5.83)	0.36 (0.09)	13.34 (3.35)	0.36 (0.04)	20.50 (2.39)	0.44 (0.04)	22.92 (3.49)	0.18 (0.08)
CH1T2	10.42 (1.26)	0.44 (0.05)	12.03 (2.85)	0.25 (0.05)	12.02 (1.78)	0.24 (0.03)	6.33 (0.31)	0.30 (0.03)
CH1T3	8.45 (1.97)	0.27 (0.03)	4.69 (1.52)	0.33 (0.02)	9.72 (1.58)	0.23 (0.01)	5.6 (1.09)	0.23 (0.04)
CH1T4	4.92 (0.22)	0.28 (0.02)	4.85 (0.37)	0.24 (0.04)	6.14 (1.44)	0.19 (0.04)	4.88 (1.02)	0.22 (0.06)
CH1.5T1	17.28 (0.50)	0.40 (0.07)	16.34 (1.72)	0.33 (0.05)	14.74 (1.74)	0.39 (0.01)	14.83 (2.60)	0.26 (0.04)
CH1.5T2	10.20 (1.73)	0.27 (0.05)	5.97 (0.20)	0.30 (0.01)	12.02 (4.41)	0.27 (0.05)	13.19 (4.60)	0.18 (0.06)
CH1.5T3	9.41 (5.57)	0.30 (0.07)	12.23 (5.40)	0.16 (0.09)	7.60 (0.95)	0.21 (0.02)	4.59 (0.85)	0.27 (0.04)
CH1.5T4	5.06 (0.80)	0.27 (0.03)	6.49 (1.70)	0.18 (0.03)	3.45 (0.65)	0.16 (0.01)	3.45 (0.67)	0.12 (0.04)

Mean values and, in parentheses, standard deviations ($n = 3$).

Table IV. Scavenging Free Radical Activity (%ESC) Normalized to the Concentration of α-Tocopherol in the Release Medium

	AM : α-tocopherol 1 : 1		AM : α-tocopherol 4 : 1	
Films	Non cross-linked	Cross-linked	Non cross-linked	Cross-linked
CH1T1	6.0 (1.8)	6.4 (3.2)	8.8 (1.0)	6.6 (2.1)
CH1T2	3.9 (1,5)	8.1 (2.2)	8.6 (1.2)	7.7 (2.1)
CH1T3	3.5 (1.1)	6.7 (2.1)	9.1 (1.5)	6.8 (1.7)
CH1T4	3.6 (0.5)	5.2 (1.6)	9.2 (1.5)	7.7 (1.4)
CH1.5T1	4.5 (2.0)	6.5 (2.8)	8.6 (0.7)	7.6 (1.4)
CH1.5T2	3.6 (0.8)	6.6 (1.6)	8.5 (1.7)	7.4 (0.8)
CH1.5T3	4.9 (1.5)	6.3 (1.1)	8.1 (1.4)	7.9 (0.8)
CH1.5T4	6.6(1.7)	5.9 (1.2)	9.4 (2.0)	8.9 (1.0)

Mean values and, in parentheses, standard deviations ($n = 3$).

$$M_t/M_\infty = k \cdot t^n \quad (6)$$

where M_t/M_∞ represents the fraction of drug released, k the release rate constant, and n an exponent indicative of the release mechanism. The use of a fix value of 0.5 for the exponent n did not lead to a good fitting; namely, Higuchi equation was not suitable for explaining the release of α-tocopherol. This finding is in agreement with the fact that highly swellable networks do not fit to the assumptions of Higuchi's diffusion model.[46] Particularly, the chitosan films expanded to a large extent when immersed in the hydroalcoholic medium (Table II) and α-tocopherol release could also contribute to create pores. This should lead to an expanded chitosan mesh with the pores filled of release medium, from which α-tocopherol diffuses out the film. This hypothesis is supported by values of exponent $n < 0.5$ (Table III), which have been referred to release processes in which two mechanisms are combined: diffusion through a swollen matrix and through liquid-filled pores.[45] In all cases, eq. (6) fitted well to the release profiles ($r^2 > 0.90$). For a given proportion of chitosan, the release rate constant, k, decreased as the content in α-tocopherol of the films raised, due to the increase in the hydrophobicity of the films caused by the lipidic components. It should be noticed that the developed chitosan films were able to sustain α-tocopherol release for remarkably longer time (more than 1 week) than the few hours release attained so far with chitosan microspheres[30] and with synthetic packaging films.[17]

Antioxidant Activity

The antioxidant activity was evaluated as the ability to take in free radicals, and normalized to the concentration of α-tocopherol in each release medium. The reduction of the free radical DPPH· caused by α-tocopherol was evidenced as a decrease in the absorbance at 525 nm.[38,39] The α-tocopherol reference solution (50 μg mL^{-1}) had a %ESC/concentration value of 10.3 ± 2.5. Samples of the ethanol : water 50 : 50 (v/v) medium at 20 day of the beginning of the release test still exhibited an outstanding antioxidant activity, particularly in the case of the films prepared with AM : α-tocopherol 4 : 1 ratio (Table IV). These latter films had a radical scavenging activity similar to that exhibited by the freshly prepared α-tocopherol solution. The release media from the assay of films prepared with AM : α-tocopherol 1 : 1 ratio were less effective, although they still kept nearly 50% of the antioxidant activity. This finding highlights the protective role of AM against the degradation of the antioxidant over time in non-degassed hydroalcoholic medium.[33] Cross-linking with glutaraldehyde slightly contributed to the stability of α-tocopherol when the AM content was low.

CONCLUSIONS

The chitosan films showed suitable features to be used as components of α-tocopherol sustained release formulations and packaging materials. The texture properties, equilibrium moisture content, and release rate depended on both the total content in α-tocopherol and the AM : α-tocopherol weight ratio. Cross-linking in glutaraldehyde atmosphere enabled a more sustained release and also contributed to maintain the antioxidant activity. α-Tocopherol released from films prepared with AM : α-tocopherol 4 : 1 ratio exhibited a radical scavenging activity after 20 days in non-degassed liquid medium close to that of fresh α-tocopherol. However, such a high content in AM makes the film to have an oily aspect and therefore, a compromise between the maintenance of the antioxidant activity and the adequate aspect of the films should be searched.

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