Electrospun ultra-fine cellulose acetate fibrous mats containing tannic acid-Fe³⁺ complexes

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Abstract

Cellulose acetate (CA) fibrous mats with improved mechanical and antioxidant properties were produced by a simple, scalable and cost-effective electrospinning method. Fibers loaded with small amounts of TA-Fe³⁺ complexes showed an increase in tensile strength of ~117% when compared to that of neat CA and were more resistant than those loaded with TA alone. The water uptake of the fibers increased upon TA or TA-Fe³⁺ incorporation while their thermal behavior was only slightly affected. Fibrous mats loaded with TA-Fe³⁺ showed comparable antioxidant activity with that of CA/TA mats, and a much slower TA release. These results suggest that TA-Fe³⁺ complexes can be incorporated into electrospun CA fibers to improve their mechanical properties and antioxidant activity which may be of interest for the development of active packaging that can extend the shelf life of perishable foods.

Keywords: Electrospinning, Tannic Acid, Ferric ions, Complexation, Antioxidant, Mechanical properties

1. Introduction

Lipid oxidation is the main process involved in the degradation of food systems with atmospheric oxygen. It proceeds via a series of free-radical chain reactions that can lead to the development of odors and off-flavors, changes in color and texture, destruction of valuable nutrients, or even generation of toxic compounds (Addis, 1986; Kanner & Rosenthal, 1992).

The addition of natural antioxidants and the design of suitable vacuum modified or active packaging are the current major strategies to improve the oxidative stability of food systems (Shahidi & Zhong, 2010; Yam, Takhistov & Miltz, 2005). Active packaging can be defined as a system in which the package and the environment interact to extend shelf life or to give some properties to the food that could not be obtained otherwise (Miltz, Passy & Mannheim, 1995; Yam et al., 2005). Antioxidants have been incorporated into polymer cast films (de Abreu, Losada, Maroto & Cruz, 2010; López-de-Dicastillo, Gómez-Estaca, Catalá, Gavara & Hernández-Muñoz, 2012) and electrospun nanofibers (Aytac, Kusku, Durgun & Uyar, 2016a, b) to produce active food packaging, avoiding common problems associated with the direct addition of these compounds into food products (e.g. unstable flavor, need to introduce the antioxidant during standard industrial processes) (Gómez-Estaca, López-de-Dicastillo, Hernández-Muñoz, Catalá & Gavara, 2014; Van Long, Joly & Dantigny, 2016).

Compared with cast films, electrospun nanofibers exhibit extremely high surface area-to-volume ratio and high sensitivity to changes in surrounding atmosphere which

allows a better protection and controlled release of active molecules (Fernandez, Torres-Giner & Lagaron, 2009; Kriegel, Arrechi, Kit, McClements & Weiss, 2008). Electrospinning has been recognized as a simple and cost-effective process to obtain nanofibers containing active compounds among nanofiber producing methods. Cellulose acetate (CA) is the acetate ester of cellulose, the most abundant polymer in nature. CA is often used for the preparation of active packaging films and electrospun fibers due to its excellent hydrophobicity, water insolubility and thermal stability (Wongsasulak, Patapeejumruswong, Weiss, Supaphol & Yoovidhya, 2010) . However, the encapsulation of active compounds in CA nanofibers is complicated due to (1) the toxicity of most solvents used to dissolve CA, (2) the need of special post – drawing processes like thermal heating to overcome the weak mechanical properties, or (3) the need for reinforcing agents (Kriegel et al., 2008; Wu, 2014). Thus, only few studies have focused on possible food-grade applications of CA using electrospinning.

Tannic acid (TA), a hydrolysable tannin, is a naturally occurring plant polyphenol compound ubiquitously distributed in the plant kingdom along with other condensed tannins. Because of the ability to scavenge free radicals by donating hydrogen atom or an electron, TA exhibits strong antioxidant, antibacterial, antimicrobial and anticarcinogenic properties (Liu et al., 2014; Xu, Weng, Gilkerson, Materon & Lozano, 2015). These functional properties make TA an attractive compound for the production of active food packaging films (Peña, De la Caba, Eceiza, Ruseckaite & Mondragon, 2010; Pyla, Kim, Silva & Jung, 2010) and electrospun fibers (Kiechel & Schauer, 2013; Thitiwongsawet, Wichapeng & Waiyaroj, 2013). TA has high binding

affinity with biochemically important metals, such as iron and copper, and with many polymers (Kraal, Jansen, Nierop & Verstraten, 2006; Lopes, Schulman & Hermes-Lima, 1999). Recently, stable TA-Fe³⁺ complexes were prepared in water and used to coat various substrates (Ejima et al., 2013; Rahim et al., 2014; Rahim et al., 2015). It was found that one ferric ion could gradually attach to one, two and three galloyl groups of TA to form respectively, mono-, bi- and tri-complexes when changing the solution pH, yielding free-standing films after pH adjustment and substrate dissolution. Due to the reversible/dynamic nature of cross-link the complexation occurs almost immediately upon mixing TA and Fe³⁺ in water without the need for any special equipment or procedure, and the components are inexpensive. For these reasons, TA-Fe³⁺ complexes have been considered for many applications including drug delivery and catalysis (Guo et al., 2014).

The present study assesses the feasibility of using TA-Fe³⁺ complexes to produce electrospun CA fibrous mats with improved functionality for food applications. The resultant fibrous mats were characterized for morphology and fiber diameter size by scanning electron microscopy (SEM) and for thermal properties by differential scanning calorimetry (DSC). Other properties such as mechanical and antioxidant as well as the swelling and release behaviors were also measured and compared with those of neat CA and of CA fibrous mats loaded with TA alone.

2. Materials and Methods

2.1. Materials

Cellulose acetate (CA, Mw=30,000 Da, acetyl content=39.8%), tannic acid (TA, C₇₆H₅₂O₄₆), iron (III) chloride hexahydrate (FeCl₃•H₂O), food-grade acetic acid, 2', 2'-azinobis (3-ethylbenzothiazine-6-sulfonic acid) (ABTS), magnesium nitrate hexahydrate (Mg(NO₃)₂•6H₂O), potassium persulfate (K₂S₂O₈), disodium hydrogen phosphate (Na₂HPO₄•12H₂O), sodium dihydrogen phosphate dehydrate (NaH₂PO₄•2H₂O), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used throughout the study. Millipore water with a resistivity of 18.2 MΩ. cm was used in all experiments.

2.2. Methods

2.2.1. Preparation and characterization of the CA, CA/TA and CA/TA-Fe³⁺ solutions

CA was dissolved in 70% w/w acetic acid/water at a polymer-to-solvent concentration of 19% w/w in a covered 500 mL plastic beaker. The mixture was continuously stirred at room temperature for 3h to ensure complete polymer dissolution. For the CA/TA solutions, an amount of 1wt% or 2wt% TA (based on the weight of CA powder) was dissolved in the obtained CA solution for 2 h at room temperature, under constant stirring (700 rpm/min). The CA/TA-Fe³⁺ solutions were prepared the same way but by

also adding FeCl_3 at a TA- Fe^{3+} mass ratio of 4:1 (Ejima et al., 2013). The solutions were then kept overnight in the refrigerator to eliminate air bubbles. The CA/TA solutions thus prepared were light brown while the CA/TA- Fe^{3+} solutions were dark green suggesting the preferential formation of TA- Fe^{3+} mono-complexes (Ejima et al., 2013).

The electric conductivities of the prepared solutions were measured using a conductivity meter (IQ270G; Scientific Instruments, Loveland, CO) while the surface tensions were determined using a Fisher ScientificTM Surface Tension Apparatus (ring diameter, 1.905 cm; Fisher Scientific, Suwanee, GA). The measurements were conducted at room temperature and each sample was examined three times, and an average was taken.

2.2.2. *Electrospinning*

The fibrous mats were prepared by electrospinning technique using a NaBond NEU-PRO unit (NaBond Technologies Co., Limited, China). A tubeless spinneret (NaBond Technologies Co., Limited, China) connected to an external syringe pump (Veryark, model TCI-IV) was attached inside a cabinet housing a grounded drum collector and with forced air convection (Sousa et al., 2015). A solution volume of 3 mL was loaded onto a 5 mL plastic syringe with an attached metallic needle (outer diameter $\times$ length = 0.9 mm $\times$ 60 mm and inner diameter = 0.560 mm) placed in the spinneret. The fibrous mats were produced at room temperature (22-25 °C) under

different process parameters within the range of applied voltage 20.5-22.5 kV, of distance tip-to-collector 12.5-13.5 cm and of feed rate 0.5-2.0 mL/h. The mats were collected onto the drum rotating at 40 rpm/min and covered with non-sticky aluminum foil. The CA mats thus obtained were dried in vacuum overnight at room temperature and then stored at controlled relative humidity (53%) for three days before being tested.

2.3. Characterization of fibers

2.3.1. Scanning electron microscopy (SEM)

The morphology and size of the electrospun fibers were investigated in a Quanta 200 FEG scanning electron microscope (SEM, FEI, Hillsboro, OR) following a previous procedure (Sousa et al., 2015). The samples were coated with a thin film of gold after being mounted with adhesive on specimen stubs, and the edge painted with colloidal silver adhesive. The images were acquired in the high-vacuum/secondary electron imaging mode SEM using accelerating voltages close to 10 kV and working distances in the range 10.1-11.4 mm. The average fiber diameters were determined using the image analysis software XT Document (FEI Corp, Hillsboro, OR). Mean values of fiber diameter sizes (FDS) and respective standard deviations were obtained after measuring a significant number of fiber diameters in the SEM images shown in Fig. 2.

2.3.2. Mechanical properties

The tensile strength (*TS*) and elongation-at-break (*EB*) of the CA fibrous mats with and without TA or TA-Fe³⁺ complexes were determined in a texture analyzer (TA-XT 2, Texture Analyzer Technologies Corp., UK) using a load cell of 2 Kg. The tensile grips were lined with pressure-sensitive tape to avoid slippage of the test specimens. To avoid damaging the fibrous mats before testing, a rectangular paper board frame with dimensions 60×60 mm was used to hold and handle the samples. The cut specimens had a rectangular dimension of width × gauge length = 10 mm × 40 mm. The thicknesses of each fiber mat were measured at three different points using a digital micrometer (AMES, 00-0341, WALTHAM, MASS. USA) with a precision of 1 µm, and averaged. A cross head speed of 5 mm/min was used to test, at least, eight film specimens for each fiber mat.

2.3.3. Thermal properties

DSC was performed using a DSC-Q100 differential scanning calorimetry (TA instrument, New Castle, DE). The heating scans were conducted on the pure CA powder and on the electrospun CA, CA/TA and CA/TA-Fe³⁺ fibrous mats at 10 °C/min heating rate in nitrogen.

2.3.4. Swelling behavior

Fibrous mats of neat CA and of CA with and without TA or TA-Fe³⁺ (2cm × 2cm) were weighed (M_d) and placed in a 100 mL glass vial with 70 mL of pH 7.4 phosphate buffer solution. The samples were removed after 48 h, cleaned with soft tissue paper to remove surface water, and reweighed (M). Swelling ratio was estimated from the following equation:

$$\text{Swelling ratio (\%)} = \frac{M - M_d}{M_d} \times 100 \%$$

2.3.5. Antioxidant properties

The antioxidant activity of the fibrous mats was investigated based on inhibition activity of the production of the ABTS radical cation by antioxidants (Awika, Rooney, Wu, Prior & Cisneros-Zevallos, 2003). Briefly, 0.01 g of each fiber mat with and without TA or TA-Fe³⁺ was placed in a 45 mL tube, 5 mL diluted ABTS⁺ was then added and the test tube was vortexed for 10 s in order to fully immerse the fibers in the solution. After stirring at 100 rpm/min for 1h, the solutions were immediately filtered (pore size=0.2 μm) to separate fibers from the solution, in order to prevent the interference of the fibers on adsorption. For all samples, absorbance was recorded immediately at 734 nm using a spectrophotometer (Shimadzu UV-2402, Shimadzu Scientific Instruments, Japan). Trolox® was selected as a standard antioxidant. The

standard curve was linear between 0.25-2 g/L TA and 0.4-3.6 $\mu\text{mol/g}$ Trolox®.

Results are expressed in μmol of Trolox® equivalents (TE) per g of dried fibers.

2.3.6. Release behavior

Pretests recorded the absorption spectra of solutions containing CA fibrous mats loaded with TA or TA-Fe³⁺ complexes between 200 and 800 nm. The maximum absorbance of the fibrous mats loaded with TA was observed at 275 nm (Chang & Juang, 2004) while for the mats loaded with TA-Fe³⁺ complexes was seen at 500 nm (Sungur & Uzar, 2008).

For the release assay measurements, carried out at ambient temperature (22-25 °C), 0.05 g of each fiber mat loaded with TA-Fe³⁺ or TA, were placed in a 45 mL tube containing 10 mL of pH 7.2 phosphate buffer solution, then vortexed for 10 s at 200 rpm in order to fully immerse the fibers in the solution. At designed time points with a 20 minutes interval, the mixtures were filtered through a filter syringe (pore size=0.2 μm), and the absorbance of the solution was measured at 275 nm and 500 nm, separately.

2.3.7. Statistical analysis

Statistical analysis was performed using Statistica 8.0 software (StatSoft, Tulsa, OK, USA). One-way analysis of variance (ANOVA) and Tukey's multiple comparisons

test were performed to determine the significance of differences between each mean value of conductivity, surface tension, TS, EB and antioxidant activity defined for $p < 0.05$. Means of FDS following non-normal distributions were compared using the Mann-Whitney test ($p < 0.05$).

3. Results and Discussion

3.1. Morphology, fiber diameter size and physical properties

In the present study, 19 wt% was the minimum CA concentration that could form defect-free fibers (Fig. 1D) which agreed well with the work of Han et al. focusing the electrospinning of CA using the same solvent system (Han, Youk, Min, Kang & Park, 2008). The formation of CA fibers became significant around 17 wt% but most fibers had a beaded morphology (Fig. 1C). Below this concentration, the resultant electrospun mats consisted predominantly of beads with a few incipient fibers (Figs. 1A-B). Thus, 19 wt% was the chosen CA concentration to produce the fibrous mats.

At 19 wt% concentration, the CA solutions led to adequate polymer jets (continuous and free of droplets) and best fiber morphology when spun under different process parameters within predetermined ranges. These differences could be related with the inclusion of TA and TA-Fe³⁺ which affected important solution properties for spinning. While TA did not affect much the conductivity of the CA solution ($\sim 103 \mu\text{S}/\text{cm}$; $p > 0.05$), the addition of TA-Fe³⁺ doubled ($\sim 213 \mu\text{S}/\text{cm}$ for 1%

TA-Fe³⁺; $p<0.05$) or tripled ($\sim 237 \mu\text{S}/\text{cm}$ for 2% TA-Fe³⁺; $p<0.05$) the solution conductivity. In this conductivity range, the increased charge density of the CA solutions with Fe³⁺ resulted in stronger elongation forces imposed on the polymer jets (Son, Youk, Lee & Park, 2004) that could eliminate beads and other defects. No significant differences were seen in the surface tension of CA solutions either loaded with TA-Fe³⁺ or TA, when compared to that of the neat CA (42-45 mN/m; $p>0.05$).

As shown in Fig. 2, the FDS ranged between 150 and 485 nm and followed non-normal distributions ($p<0.05$).

The prepared fibers were much smaller and more uniform in size than those obtained when electrospinning CA in acetone/water (Son et al., 2004) or in a solvent mixture of acetone/DMF/trifluoroethylene (Ma, Kotaki & Ramakrishna, 2005) but were close in size to those prepared in acetone/dimethylacetamide (Suwantong, Opanasopit, Ruktanonchai & Supaphol, 2007). For the same TA concentration, the FDS of CA fibers loaded with TA-Fe³⁺ were not much different than those of fibers loaded with TA alone ($p>0.05$; Fig. 2A vs 2C and Fig. 2B vs 2D). At 1% TA concentration, the loaded fibers (Figs. 2A and 2C) showed similar FDS to that of neat CA ($306\pm 134 \text{ nm}$; $p>0.05$; Fig. 2E) while increasing the loading concentration to 2%, increased the FDS of the loaded fibers ($p<0.05$; Fig. 2A vs 2B for CA/TA-Fe³⁺ and Fig. 2C vs 2D for CA/TA).

The inclusion of TA-Fe³⁺ complexes did not produce obvious defects or

detrimental structures in the CA fibers (Figs. 2A and 2B vs Fig. 2E for the neat CA), and could eliminate a few fiber defects seen in the CA fibers loaded with TA alone (Figs. 2C and 2D). An improvement in fiber morphology was also seen when adding salts to PEO solutions and could occur from the increased net charge density carried by the moving jet (Fong, Chun & Reneker, 1999). When the concentration of TA-Fe³⁺ exceed 2.5%, the solution was electrosprayed onto the drum and no fibers could be collected.

For food packaging purposes, it is important to determine the mechanical properties of the fibrous mats since the materials may undergo various mechanical stresses during packaging, transportation and handling. CA fibers typically exhibit poorer mechanical properties than fibers produced from other related polymers and often require expensive and time consuming post – spinning processes to overcome this limitation (Ma et al., 2005). Thus, it is important to find new and inexpensive ways to improve the mechanical performance of CA fibers.

As shown in Fig. 3A, the addition of either TA-Fe³⁺ or TA had a positive effect on the tensile strength (TS) of the CA fibrous mats prepared with thicknesses between 7 and 9.5 μm .

The TS was significantly higher for the fibrous mats loaded with TA-Fe³⁺ than those loaded with TA alone ($p<0.05$). Fibrous mats loaded with either TA or TA-Fe³⁺ became more resistant as the TA content increased from 1 to 2 wt% ($p<0.05$). The TS of the neat CA was ~2.45 MPa and increased by about 117% upon addition of 2 wt%

TA-Fe³⁺ (~5.34 MPa). This suggests that the TA-Fe³⁺ mono-complexes, preferentially formed at acidic pH (Ejima et al., 2013), bonded with the hydroxyl groups of CA adding more stability to the polymer network than the TA alone. The values of elongation-at-break (EB) ranged between 0.46 and 1.50% and were not significantly affected by the TA or TA-Fe³⁺ incorporation (Fig. 3B). Similar TS and EB values could only be obtained after heat treating the electrospun CA fibers for 1h at 208 °C followed by an alkaline treatment for 24h at room temperature (Ma et al., 2005). In our case, the complexation occurs very rapidly upon mixing TA and Fe³⁺ and no special sample treatment or extra process is needed. Electrospun CA fibers prepared in acetone/dimethylacetamide and loaded with 5-10 wt% curcumin were less resistant (TS~0.14-0.35 MPa) but more flexible, EB~17.5-23.4 % (Suwantong et al., 2007)). For packaging applications, further improvements in mechanical properties can be obtained by using another polymeric material that provides the physical barrier and weight-bearing properties (Kara et al., 2016).

Results of the swelling degree of CA fiber mats (Fig. 4) show that the fibers absorbed a large amount of water suggesting that water molecules quickly entered the fibrous pores through capillary action (Tungprapa, Jangchud & Supaphol, 2007).

The water uptake in percent of initial fiber mat weight (~270%) changed upon TA or TA-Fe³⁺ incorporation and depended on the amount of added compounds. The fiber mats loaded with either 2 wt% TA-Fe³⁺ or 2 wt% TA showed lower swelling degree (~550-580%) than the equivalents prepared at 1 wt% concentration (~730-750%). For

the same TA concentration, mats loaded with either TA-Fe³⁺ or TA showed similar degree of swelling. Suwantong et al. reported a swelling degree ~370% after CA fiber mats submersion in B/T/M medium at 37 °C (Suwantong et al., 2007), while much higher values (~830% and ~2527%) were reported by other researchers (Tungprapa et al., 2007). In our case, the higher propensity of CA fibers loaded with TA or TA-Fe³⁺ to swell is clearly imparted by the hydrophilic nature of TA. It is believed that the polyphenol acts as a hydrophilic network that promotes the diffusion of water within the hydrophobic CA matrices. After the swelling tests, the CA fibers loaded with TA alone exhibited light brown coloration (e.g. Fig. 4A for 2% loading) while those loaded with TA-Fe³⁺ were light purple (Fig. 4B; 1% loading) or dark purple (Fig. 4C; 2% loading).

Table 1 summarizes the thermal properties obtained from the DSC curves of the CA fibrous mats with and without incorporated TA or TA-Fe³⁺ depicted in Fig. 5. All fibrous mats showed a broad endothermic peak in the range 110-175 °C which was mainly attributed to evaporation of bound water (Ma et al., 2005). This water evaporation peak occurred at 148 °C for the neat CA and sooner (~130 °C) for the other fibrous mats. The CA fibers showed another significant endothermic peak at 223 °C, which was relatively close to the melting temperature (T_m) of CA powder (230 °C; not shown). Electrospinning typically leads to a reduction of the glass transition temperature (T_g) and T_m of the polymers as consequence of the high elongation rates and rapid solidification observed in the process (Pirani, Abushammala & Hashaikeh, 2013; Wen et al., 2016). The addition of 1 wt% or 2 wt%

TA did not affect significantly the T_m of the CA fiber mat nor did the addition of 1 wt% TA-Fe³⁺. The incorporation of 2% TA-Fe³⁺ in turn, decreased the T_m value to 216 °C which could suggest the existence of molecular interactions between the CA and TA-Fe³⁺ complexes. Compared with CA powder (~207 °C; not shown), the neat CA fibers showed a slight decrease in T_g (~197 °C) suggesting that the polymer crystallinity was somehow affected after dissolution in acetic acid/water (70/30) and electrospinning. The same trend was seen in other related studies with CA (Ma et al., 2005) and when electrospinning PLA (Pirani et al., 2013; Wen et al., 2016). Among the loaded samples, the lowest T_g value of 189 °C was seen for the CA fibers loaded with 2 wt% TA-Fe³⁺. The endothermic enthalpies (ΔH_m) decreased from 8.8 J/g for the neat CA to ~7.7-8.0 J/g for the CA fibers loaded with 1 or 2% of TA and then to ~4.6-4.9 J/g for fibers loaded with 1 or 2% TA-Fe³⁺ suggesting that differences in crystallinity became more significant when loading the CA fibers with the complexes.

3.2. Antioxidant activity and TA release from electrospun CA fibrous mats

The kinetics of the TA release from the CA fibrous mats at room temperature is shown in Fig. 6A.

We observed that for the same time period, the TA release was much slower for the mats loaded with TA-Fe³⁺ than for those loaded with TA alone. For the mats with TA alone, and particularly at 2 wt% TA loading, the TA release occurred in two stages: first, at the highest rate during the first 80 minutes and was followed by a much slower rate for the subsequent measurements. A more sustained release curve was

observed for the CA mats with TA-Fe³⁺ suggesting the higher efficiency of these complexes to release the TA in a more sustained manner. At the end of the test, 70-100% of TA was released from the CA-TA fibers while the fibers loaded with TA-Fe³⁺ only released 13.3% (1% TA loading) and 18.5% (2% TA loading). This partial release of the nominal load may be related with the strong binding affinity between TA and Fe³⁺. Future studies will aim at modifying/adjusting the release tests to better mimic real-life situations and better understand how the complexation of TA affects its release. The controlled release of antioxidants is of great importance to extend the shelflife of food systems prone to lipid oxidation (Gemili, Yemenicioğlu & Altinkaya, 2009). Furthermore, the morphology of CA fibrous mats was preserved after the release experiments (not shown).

The antioxidant activity of the CA fibrous mats was tested by ABTS assays and the results, represented as μmol of Trolox® equivalents (TE) per g of dried fibers, are shown in Fig. 6B. Our results suggest that the TA retained its antioxidant activity after blending and electrospun with CA in the acetic acid/water solvent system. The addition of 1% TA or 1% TA-Fe³⁺ did not increase much the antioxidant properties of the neat CA ($p>0.05$) but a ~41% increase was seen at 2% TA loading ($p<0.05$), suggesting a positive correlation between the amount of loaded TA or TA-Fe³⁺ and the increase in the ABTS radical scavenging capacity.

The antioxidant activity of the fibrous mats loaded with TA-Fe³⁺ was comparable to that of the CA mat loaded with TA alone ($p>0.05$) despite their much lower % of

released TA. This suggests that the TA on the fibers and that released into the solutions contributed for the antioxidant activity which is quantified by the total interactions between the $\text{ABTS}^{\bullet+}$ and TA.

Our results show that TA-Fe^{3+} complexes can be incorporated into electrospun CA fibers to improve their mechanical properties while preserving the TA antioxidant activity. These properties are interesting for the development of active packaging materials that can extend the shelflife of perishable foods. Further improvements in mechanical properties can be obtained by developing multilayered packaging materials, such as those previously reported (Kara et al., 2016). However, methods to enable application of these fibers to foods more sensitive to pH need to be explored. This will be addressed in our next publication.

4. Conclusions

This study provides a new and cost-effective method to produce electrospun CA fibrous mats with antioxidant activity and improved mechanical properties. The simple incorporation of small amounts of TA-Fe^{3+} complexes into the CA fibers leads to similar mechanical improvements than other expensive and more time-consuming methods. Fibrous mats loaded with TA-Fe^{3+} show comparable antioxidant activity with that of CA/TA mats and a more efficient TA release which suggests the potential of these materials for the development of active packaging that can extend the shelf life of perishable foods.

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References.

- Addis, P. (1986). Occurrence of lipid oxidation products in foods. *Food and Chemical Toxicology*, 24(10), 1021-1030.
- Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of agricultural and food chemistry*, 51(23), 6657-6662.
- Aytac, Z., Kusku, S. I., Durgun, E., & Uyar, T. (2016a). Encapsulation of gallic acid/cyclodextrin inclusion complex in electrospun polylactic acid nanofibers: Release behavior and antioxidant activity of gallic acid. *Materials Science and Engineering: C*, 63, 231-239.
- Aytac, Z., Kusku, S. I., Durgun, E., & Uyar, T. (2016b). Quercetin/ β -cyclodextrin inclusion complex embedded nanofibres: Slow release and high solubility. *Food chemistry*, 197, 864-871.
- Chang, M.-Y., & Juang, R.-S. (2004). Adsorption of tannic acid, humic acid, and dyes from water using the composite of chitosan and activated clay. *Journal of Colloid and Interface Science*, 278(1), 18-25.
- de Abreu, D. P., Losada, P. P., Maroto, J., & Cruz, J. (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar* L.). *Food Research International*, 43(5), 1277-1282.
- Ejima, H., Richardson, J. J., Liang, K., Best, J. P., van Koeveden, M. P., Such, G. K., Cui, J., & Caruso, F. (2013). One-step assembly of coordination complexes for versatile film and particle engineering. *Science*, 341(6142), 154-157.
- Fernandez, A., Torres-Giner, S., & Lagaron, J. M. (2009). Novel route to stabilization of bioactive antioxidants by encapsulation in electrospun fibers of zein prolamine. *Food Hydrocolloids*, 23(5), 1427-1432.
- Fong, H., Chun, I., & Reneker, D. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, 40(16), 4585-4592.
- Gemili, S., Yemenicioğlu, A., & Altinkaya, S. A. (2009). Development of cellulose acetate based antimicrobial food packaging materials for controlled release of lysozyme. *Journal of Food Engineering*, 90(4), 453-462.
- Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., &

- Gavara, R. (2014). Advances in antioxidant active food packaging. *Trends in Food Science & Technology*, 35(1), 42-51.
- Guo, J., Ping, Y., Ejima, H., Alt, K., Meissner, M., Richardson, J. J., Yan, Y., Peter, K., von Elverfeldt, D., Hagemeyer, C. E., & Caruso, F. (2014). Engineering Multifunctional Capsules through the Assembly of Metal–Phenolic Networks. *Angewandte Chemie International Edition*, 53, 5546-5551.
- Han, S. O., Youk, J. H., Min, K. D., Kang, Y. O., & Park, W. H. (2008). Electrospinning of cellulose acetate nanofibers using a mixed solvent of acetic acid/water: Effects of solvent composition on the fiber diameter. *Materials Letters*, 62(4), 759-762.
- Kanner, J., & Rosenthal, I. (1992). An assessment of lipid oxidation in foods (Technical Report). *Pure and applied chemistry*, 64(12), 1959-1964.
- Kara, H. H., Xiao, F., Sarker, M., Jin, T. Z., Sousa, A. M., Liu, C. K., Tomasula, P. M., & Liu, L. (2016). Antibacterial poly (lactic acid)(PLA) films grafted with electrospun PLA/allyl isothiocyanate fibers for food packaging. *Journal of Applied Polymer Science*, 133(2).
- Kiechel, M. A., & Schauer, C. L. (2013). Non-covalent crosslinkers for electrospun chitosan fibers. *Carbohydrate polymers*, 95(1), 123-133.
- Kraal, P., Jansen, B., Nierop, K. G., & Verstraten, J. M. (2006). Copper complexation by tannic acid in aqueous solution. *Chemosphere*, 65(11), 2193-2198.
- Kriegel, C., Arrechi, A., Kit, K., McClements, D. J., & Weiss, J. (2008). Fabrication, functionalization, and application of electrospun biopolymer nanofibers. *Critical Reviews in Food Science and Nutrition*, 48(8), 775-797.
- Liu, F., Kozlovskaya, V., Zavgorodnya, O., Martinez-Lopez, C., Catledge, S., & Kharlampieva, E. (2014). Encapsulation of anticancer drug by hydrogen-bonded multilayers of tannic acid. *Soft matter*, 10(46), 9237-9247.
- Lopes, G. K., Schulman, H. M., & Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1472(1), 142-152.
- López-de-Dicastillo, C., Gómez-Estaca, J., Catalá, R., Gavara, R., & Hernández-Muñoz, P. (2012). Active antioxidant packaging films: development and effect on lipid stability of brined sardines. *Food chemistry*, 131(4), 1376-1384.
- Ma, Z., Kotaki, M., & Ramakrishna, S. (2005). Electrospun cellulose nanofiber as affinity membrane. *Journal of membrane science*, 265(1), 115-123.
- Miltz, J., Passy, N., & Mannheim, C. (1995). Trends and applications of active packaging systems. *SPECIAL PUBLICATION-ROYAL SOCIETY OF CHEMISTRY*, 162(1), 201-201.
- Peña, C., De la Caba, K., Eceiza, A., Ruseckaite, R., & Mondragon, I. (2010). Enhancing water repellence and mechanical properties of gelatin films by tannin addition. *Bioresource technology*, 101(17), 6836-6842.
- Pirani, S., Abushammala, H., & Hashaikeh, R. (2013). Preparation and characterization of electrospun PLA/nanocrystalline cellulose - based composites. *Journal of Applied Polymer Science*, 130(5), 3345-3354.
- Pyla, R., Kim, T.-J., Silva, J. L., & Jung, Y.-S. (2010). Enhanced antimicrobial activity

of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant. *International journal of food microbiology*, 137(2), 154-160.

Rahim, M. A., Ejima, H., Cho, K. L., Kempe, K., Müllner, M., Best, J. P., & Caruso, F. (2014). Coordination-driven multistep assembly of metal–polyphenol films and capsules. *Chemistry of Materials*, 26, 1645–1653.

Rahim, M. A., Kempe, K., Müllner, M., Ejima, H., Ju, Y., Koeverden, M. P. v., Suma, T., Braunger, J. A., Leeming, M. G., & Abrahams, B. F. (2015). Surface-confined amorphous films from metal-coordinated simple phenolic ligands. *Chemistry of Materials*, 27, 5825-5832.

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, 39(11), 4067-4079.

Son, W. K., Youk, J. H., Lee, T. S., & Park, W. H. (2004). Electrospinning of ultrafine cellulose acetate fibers: studies of a new solvent system and deacetylation of ultrafine cellulose acetate fibers. *Journal of Polymer Science Part B: Polymer Physics*, 42(1), 5-11.

Sousa, A. M., Souza, H. K., Uknalis, J., Liu, S.-C., Gonçalves, M. P., & Liu, L. (2015). Electrospinning of agar/PVA aqueous solutions and its relation with rheological properties. *Carbohydrate polymers*, 115, 348-355.

Sungur, Ş., & Uzar, A. (2008). Investigation of complexes tannic acid and myricetin with Fe (III). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 69(1), 225-229.

Suwantong, O., Opanasopit, P., Ruktanonchai, U., & Supaphol, P. (2007). Electrospun cellulose acetate fiber mats containing curcumin and release characteristic of the herbal substance. *Polymer*, 48(26), 7546-7557.

Thitiwongsawet, P., Wichapeng, N., & Waiyaroj, A. (2013). Electrospun Polycaprolactone Fiber Mats as Carriers for Tannic Acid. *Advanced Materials Research* (Vol. 774, pp. 896-900): Trans Tech Publ.

Tungprapa, S., Jangchud, I., & Supaphol, P. (2007). Release characteristics of four model drugs from drug-loaded electrospun cellulose acetate fiber mats. *Polymer*, 48(17), 5030-5041.

Van Long, N. N., Joly, C., & Dantigny, P. (2016). Active packaging with antifungal activities. *International journal of food microbiology*, 220, 73-90.

Wen, P., Zhu, D.-H., Feng, K., Liu, F.-J., Lou, W.-Y., Li, N., Zong, M.-H., & Wu, H. (2016). Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/ β -cyclodextrin inclusion complex for antimicrobial packaging. *Food chemistry*, 196, 996-1004.

Wongsasulak, S., Patapeejumruswong, M., Weiss, J., Supaphol, P., & Yoovidhya, T. (2010). Electrospinning of food-grade nanofibers from cellulose acetate and egg albumen blends. *Journal of Food Engineering*, 98(3), 370-376.

Wu, C.-S. (2014). Mechanical properties, biocompatibility, and biodegradation of cross-linked cellulose acetate-reinforced polyester composites. *Carbohydrate polymers*, 105, 41-48.

Xu, F., Weng, B., Gilkerson, R., Materon, L. A., & Lozano, K. (2015). Development of tannic acid/chitosan/pullulan composite nanofibers from aqueous solution for

potential applications as wound dressing. *Carbohydrate polymers*, 115, 16-24.

Yam, K. L., Takhistov, P. T., & Miltz, J. (2005). Intelligent packaging: concepts and applications. *Journal of food science*, 70(1), R1-R10.

Figure 1. SEM images of electrospun fibers obtained from CA/TA-Fe³⁺ solutions with CA concentration of (A) 13 wt % (B) 15 wt % (C) 17 wt% and (D) 19 wt%. Magnification is 2,500 ×.

Figure 2. SEM images and fiber diameter size (FDS) of electrospun fibers obtained from CA solutions at 19 wt% concentration loaded with: 1 wt% TA-Fe³⁺ (A), 2 wt% TA-Fe³⁺ (B), 1 wt % TA (C) and 2 wt% TA (D). Electrospun fibers of neat CA are shown in (E). The mass ratio of TA-Fe³⁺ in A and B was 4:1. Magnification is 5,000

×

Figure 3. Tensile strength (**A**) and elongation-at-break (**B**) of the neat CA and of the CA fibrous mats loaded with TA and TA-Fe³⁺.

Figure 4. Swelling degree of the neat CA and of the CA fibrous mats loaded with TA and TA-Fe³⁺. Images of the CA fibrous mats loaded with 2 wt % TA (**A**), 1 wt% TA-Fe³⁺ (**B**) and (**C**) 2 wt% TA-Fe³⁺, after the swelling tests.

Figure 5. DSC curves of the neat CA and of CA fibrous mats loaded with TA and TA-Fe³⁺.

Figure 6. TA release (**A**) and antioxidant activity (**B**) of the neat CA and of CA fibrous mats loaded with TA and TA-Fe³⁺. Means with different letters (a, b) are statistically different ($p < 0.05$).

Table 1. Thermal properties (glass transition temperature, T_g ; melting temperature, T_m ; and endothermic enthalpy, ΔH_m) of the neat CA and of the CA fibrous mats loaded with TA and TA-Fe³⁺ obtained from the DSC curves shown in Fig. 5.

Sample	T_g (°C)	T_m (°C)	ΔH_m (J/g)
neat CA	196.7	223.1	8.80
1% TA	192.8	223.4	7.98
2% TA	196.0	222.4	7.72
1% TA-Fe ³⁺	196.5	222.0	4.86
2% TA-Fe ³⁺	189.3	216.3	4.64