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Antioxidant α-tocopherol/γ-cyclodextrin–inclusion complex encapsulated poly(lactic acid) electrospun nanofibrous web for food packaging

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ABSTRACT: α-Tocopherol (α-TC) and α-TC/cyclodextrin (CD)–inclusion complex (IC) incorporated electrospun poly(lactic acid) (PLA) nanofibers (NF) were developed via electrospinning (PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF). The release of α-TC into 95% ethanol (fatty food simulant) was much greater from PLA/α-TC/γ-CD–IC–NF than from PLA/α-TC–NF because of the solubility increase in α-TC; this was confirmed by a phase-solubility diagram. 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging assay shows that PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF had 97% antioxidant activities; this value was expected to be high enough to inhibit lipid oxidation. PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF were tested directly on beef with the thiobarbituric acid reactive substance (TBARS) method, and the nanofibers displayed a lower TBARS content than the unpackaged meat sample. Thus, active packaging significantly enhanced the oxidative stability of the meat samples at 4 °C. In conclusion, PLA/α-TC/γ-CD–IC–NF was shown to be promising as an active food-packaging material for prolonging the shelf life of foods. © 2017 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2017**, *134*, 44858.

KEYWORDS: fibers; packaging; porous materials; supramolecular structures

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INTRODUCTION

Oxidative reactions are a great problem for both natural and processed food products containing lipids.[1] Once the food is deteriorated by oxidation, its quality is reduced because of the release of off-odors and off-flavors, color and texture changes, and nutrition losses, and also, the food shelf life is reduced.[1,2] Therefore, antioxidant (AO) agents have been used to prevent lipid oxidation in food products.[1,2] One of the most innovative strategies for this is the incorporation of AO agents into packaging materials because of the sustained release ability of AO agents during storage as well.[1,2]

α-Tocopherol [α-TC; Figure 1(a)] is the main component of vitamin E, and it finds broad application in drug delivery and wound dressings.[3,4] The main limitation of α-TC is its poor solubility in water, and it is also sensitive to oxygen, light, alkali pH, and traces of transition-metal ions.[5] Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucopyranose units, and the most widely used CD types are α-CD, β-CD, and γ-CD with six, seven, and eight glucopyranose units, respectively.[6,7] The size of γ-CD [Figure 1(b,c)] is bigger, its solubility is higher, and its bioavailability is more pronounced than those of two other native CDs (α-CD and β-CD). It was declared that it has no side effects on the absorption of nutrients in food products and nutraceutical applications.[8] CDs have attracted much interest recently in the elimination of the drawbacks of α-TC by complexation. Therefore, CD-inclusion complexes (ICs) of α-TC were used to enhance the solubility[9,10] and protect food products against oxidation.[11] Moreover, CD–ICs of α-TC were incorporated into polymeric films to reduce the diffusion rate of α-TC,[12] retard the oxidation of packaged food during the storage period,[13] and prolong the shelf life of food products.[14]

Poly(lactic acid) (PLA) is a type of aliphatic polyester that is widely used in biological applications because of its biocompatible and biodegradable nature.[15,16] In addition to these advantages, its carbon dioxide, oxygen, and water permeability and light-barrier properties make it an ideal candidate for packaging

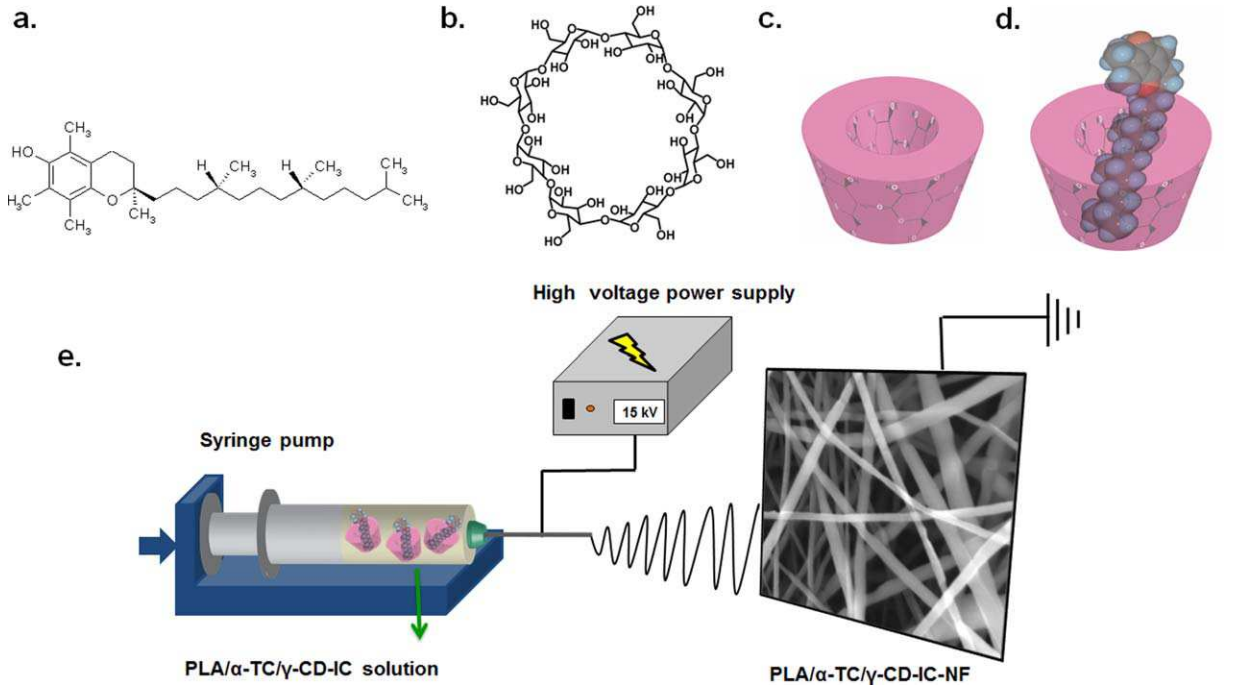

Figure 1. Chemical structures of (a) α-TC and (b) γ-CD, schematic representations of (c) γ-CD and (d) α-TC/γ-CD–IC, and (e) electrospinning of nanofibers from a PLA/α-TC/γ-CD–IC solution. [Color figure can be viewed at wileyonlinelibrary.com]

applications.[17] Furthermore, it has also some other advantages, including the possibility of being produced by a renewable source (corn),[18] its consumption of high amounts of carbon dioxide during production, and its recyclability.[17] Electrospinning is a commonly used method for producing nanofibers mostly from polymers. Electrospun nanofibers are advantageous in terms of their high surface-to-volume ratio and porous structure.[19]

In this study, an IC of α-TC and γ-CD was synthesized [α-TC/γ-CD–IC; Figure 1(d)] and then added to a PLA solution to produce nanofibers (NF) by electrospinning [PLA/α-TC/γ-CD–IC–NF; Figure 1(e)]. The phase-solubility diagram showed that the solubility of α-TC was improved after its encapsulation within γ-CD. The characterization of α-TC/γ-CD–IC was done by X-ray diffraction (XRD), thermogravimetric analysis (TGA), and ^{1}H-NMR techniques. The morphological characterization of PLA/α-TC–NF, which was produced as a reference sample, and PLA/α-TC/γ-CD–IC–NF were performed by scanning electron microscopy (SEM). The *in vitro* release of α-TC from PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF was determined in 95% ethanol by high-performance liquid chromatography (HPLC). The AO activities of PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF were determined with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method. Finally, PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF were applied to raw beef samples to investigate their prevention of lipid oxidation via a thiobarbituric acid reactive substance (TBARS) assay.

EXPERIMENTAL

Materials

PLA, a commercial polylactide resin identified as an Ingeo biopolymer, was donated by NatureWorks, LLC, Co. [poly (DL-lactic acid), PDLLA, product code 6252D]. α-TC (≥96%; Sigma-Aldrich), γ-CD (Wacker Chemie AG, Germany), thiobarbituric acid (≥98%, Sigma-Aldrich), methanol (extrapure, Sigma-Aldrich), ethanol (99.8%, Sigma-Aldrich), dichloromethane (DCM; extrapure; Sigma-Aldrich), *N*,*N*-dimethylformamide (DMF, ≥ 99%, Sigma-Aldrich), trichloroacetic acid (99.5%, VWR), deuterated dimethyl sulfoxide (minimum deuteration degree = 99.8% for NMR spectroscopy, Merck), and DPPH (Sigma-Aldrich) were purchased and used as received without any further purification. Distilled deionized water was supplied by Millipore Milli-Q ultrapure water system.

Preparation of the IC

The formation of solid α-TC/γ-CD–IC at a 1:1 molar ratio was done according to a freeze-drying method. Initially, γ-CD was dissolved in an aqueous solution; then, α-TC was added to this solution while the solution was stirred. After the solution was mixed overnight, it was frozen at −80 °C for 24 h. Afterward, the mixture was kept in lyophilizer for 48 h.

Preparation of the Solutions

α-TC and α-TC/γ-CD–IC-encapsulated PLA nanofibers were obtained through electrospinning (PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF). α-TC (5% w/w with respect to the polymer) was dissolved in a DCM–DMF (7:3) solvent system at room temperature (RT). Then, 10% w/v PLA was added, and the solution was stirred for 2 h before the electrospinning process. To produce PLA/α-TC/γ-CD–IC–NF, α-TC/γ-CD–IC (5% α-TC w/w with respect to the polymer) was dispersed in DCM–DMF (7:3) at RT. Then, 10% w/v PLA was added to the α-TC/γ-CD–IC solution (PLA/α-TC/γ-CD–IC), and the resulting solution was stirred for 2 h before electrospinning. The vials were covered with aluminum foil during the stirring period to prevent

Table I. Properties of the Solutions Used for Electrospinning and the Morphological Characteristics of the Resulting Nanofibers

Solution	PLA (% w/v)[a]	γ-CD (% w/w)[b]	α-TC (% w/w)[b]	Viscosity (Pa s)	Conductivity (μS/cm)	AFD (nm)
PLA	10	—	—	1.42	1.6	395 ± 120
PLA/α-TC	10	—	5	0.17	1.1	555 ± 205
PLA/α-TC/γ-CD-IC	10	15	5	0.06	1.1	430 ± 170

[a] With respect to the solvent (7:3 DCM/DMF).
[b] With respect to the polymer (PLA).

degradation by light. As another reference sample, we also electrospun a 10% w/v PLA solution in DCM–DMF (7:3). Table I summarizes the compositions of the PLA, PLA/α-TC, and PLA/α-TC/γ-CD–IC solutions.

Electrospinning

The PLA, PLA/α-TC, and PLA/α-TC/γ-CD–IC solutions in 3-mL plastic syringes (needle *i.d.* = 0.8 mm) were loaded horizontally on the syringe pump (KD Scientific, KDS101), and the solutions were fed through the collector at a 1 mL/h rate. A voltage of 15 kV was applied from a high-voltage power supply (AU Series, Matsusada Precision, Inc.). Metal covered with a piece of aluminum foil was used as a collector. The distance between the needle tip and the collector was 10 cm. The experiments were performed at 25 °C and 18% relative humidity

Characterization and Measurements

A phase-solubility study was carried out in an aqueous solution according to a method previously reported by Higuchi and Connors.[20] Excess amounts of α-TC were added to 10-mL aqueous solutions containing increasing amounts of γ-CD (ranging from 0 to 30 m*M*). When equilibrium was achieved after 12 h of stirring at RT, the suspensions were filtered through a 0.45-μm membrane filter. The α-TC concentration was determined spectrophotometrically at 292 nm (Varian, Cary 100). The experiments were performed in triplicate.

The crystalline structure of the powder of γ-CD and α-TC/γ-CD–IC were determined by XRD (PANalytical X'Pert powder diffractometer) with the application of Cu Kα radiation in a 2θ range of 5–30°. Because α-TC is a liquid compound at RT, XRD analysis was not run for the pure α-TC.

The thermal stabilities of α-TC, γ-CD, and α-TC/γ-CD–IC was investigated via TGA (TA Q500). The measurements were performed under a nitrogen atmosphere, and the samples were heated up to 500 °C at a constant heating rate of 20 °C/min.

The ^{1}H-NMR spectra were recorded on a Bruker DPX-400 at 400 MHz. To determine the molar ratio of α-TC/γ-CD–IC, 20 mg/mL of α-TC, γ-CD, and α-TC/γ-CD–IC were dissolved in deuterated dimethyl sulfoxide. The integration of the chemical shifts (δs) given in parts per million was calculated with Mestrenova software.

The viscosity of the PLA, PLA/α-TC, and PLA/α-TC/γ-CD–IC solutions were analyzed at RT via an Anton Paar Physica MCR 301 rheometer equipped with a cone–plate accessory (spindle type CP40-2) at a constant shear rate of 100 1/s, and the conductivity of the solutions was measured with an Inolab 720-WTW at RT.

The morphological characterization of PLA–NF, PLA/α-TC–NF, and PLA/α-TC/γ-CD–IC–NF was performed with SEM (FEI-Quanta 200 FEG). The samples were stuck on metal stubs with double-sided adhesive copper tape and then coated with 5-nm Au/Pd (PECS-682). The calculation of the average fiber diameter (AFD) of each nanofiber was made from the SEM micrographs taken. The diameters of at least 100 fibers were measured for each sample, and the average and standard deviation values were reported.

To determine the α-TC release from PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF, 20 mg of the nanofibers were individually immersed in 30 mL of 95% ethanol, and the solutions were stirred at RT for 6 h. A volume of 0.5 mL of sample solution was withdrawn at specified time intervals, and was refilled with an equal amount of fresh medium. Then, the amount of α-TC was decided by HPLC (Agilent, 1200 series) equipped with a Variable Wavelength Detector (VWD) UV detector. A C18 column (Agilent, column dimensions = 4.6 × 150 mm, particle size = 5 μm.) operating at 1 mL/min with a 98:2 v/v methanol–water eluent was used for separation. The detection was accomplished at 292 nm. The calibration curve was obtained to convert area values to concentration. The experiments were performed in triplicate, and the results are given as the average plus or minus the standard deviation.

AO tests for PLA–NF, PLA/α-TC–NF, and PLA/α-TC/γ-CD–IC–NF were performed according to a DPPH radical-scavenging assay. For this purpose, PLA–NF; PLA/α-TC–NF, and PLA/α-TC/γ-CD–IC–NF with equivalent amounts of α-TC were immersed in 3 mL of a 10^{-4} *M* DPPH solution prepared in methanol, and then, the mixtures were kept in the dark at RT for 15 min. Last, the absorbance of the solutions was measured by ultraviolet–visible spectroscopy (Varian, Cary 100) at 517 nm. To calculate the AO activity (%), the absorbance of DPPH was defined as 100%, and the AO activity (%) was calculated on the basis of the following equation:

$$\text{Antioxidant activity } (\%) = \left(A_{\text{control}} - A_{\text{sample}}\right) \Big/ A_{\text{control}} \times 100 \qquad (1)$$

where A_{control} and A_{sample} are the absorbance values of the control DPPH solution and DPPH solution with nanofibers, respectively. The experiments were carried out in triplicate, and the results are given as average plus or minus standard deviation values.

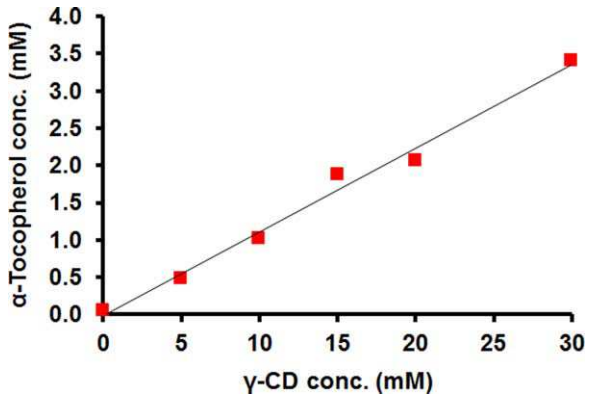

Figure 2. Phase-solubility diagram of the α-TC/γ-CD system in water. [Color figure can be viewed at wileyonlinelibrary.com]

The oxidative stability was evaluated by changes in the TBARSs. Lipid oxidation was analyzed in raw beef samples (control), which were purchased from the local market, and raw beef samples packaged with PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF were put into polyethylene zip bags and subjected to refrigerated storage at 4 °C for 4, 7, 10, and 21 days. At certain time intervals, the meat samples were taken out, and TBARS were calculated on the basis of the method of UNisa *et al.*[21] The procedure was as follows: 5 g of the meat sample was homogenized in 35 mL of 7.5% trichloroacetic acid. The homogenized sample was centrifuged (3000*g*, 2 min), and 5 mL of the supernatant was mixed with 5 mL of 20 m*M* thiobarbituric acid. Finally, the solution was mixed and kept in the dark for 20 h at 24 °C. The pink color that formed was measured spectrophotometrically (UV spectrophotometer, Shimadzu, Japan) at 532 nm. The results were expressed as milligrams of malonaldehyde (MDA) per kilogram of the sample. TBARS determinations for each sample were performed in triplicate. A Student's *t* test was applied for data comparison. Statistical analyses were done with Minitab Version 13.2 software (Minitab, Inc.) at a 0.05 level of probability.

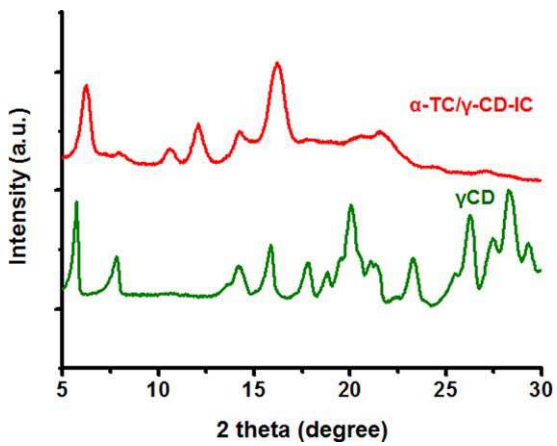

Figure 3. XRD patterns of γ-CD and α-TC/γ-CD–IC. [Color figure can be viewed at wileyonlinelibrary.com]

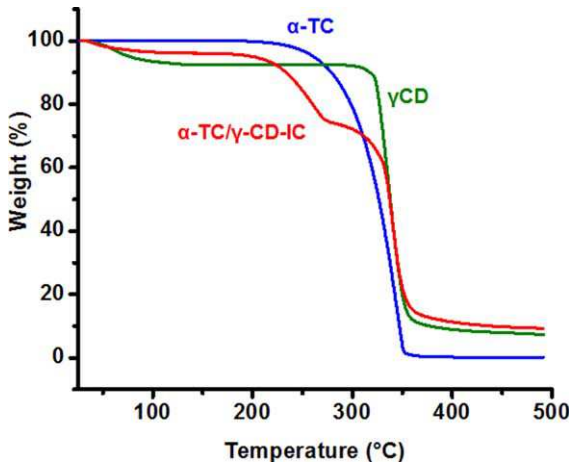

Figure 4. TGA curves of α-TC, γ-CD, and α-TC/γ-CD–IC. [Color figure can be viewed at wileyonlinelibrary.com]

RESULTS AND DISCUSSION

Phase-Solubility Studies

The phase-solubility profile of the α-TC/γ-CD system is given in Figure 2. As shown in the diagram, the solubility of α-TC increased from 0 to 30 m*M* of γ-CD. The linear increment in the α-TC solubility in water with increasing γ-CD concentration showed that the α-TC/γ-CD system possessed an A_L (linear)-type character. The A_L-type diagram also suggested the formation of a 1:1 complex between α-TC and γ-CD.

Crystalline Structure of the IC

XRD patterns of γ-CD and α-TC/γ-CD–IC are shown in Figure 3. Because α-TC had a liquid nature at RT, we could not run XRD for this molecule. It is a known fact that the cage-type crystal packing structure of native CDs turns into a channel-type structure once an IC is formed with a guest molecule.[22] As shown in the graph, the cage-type crystalline peaks of γ-CD disappeared in α-TC/γ-CD–IC, and a characteristic major peak ($2\theta = 6°$) of hexagonal channel-type packing of γ-CD was observed. Therefore, the formation of the IC between α-TC and γ-CD was successfully achieved.

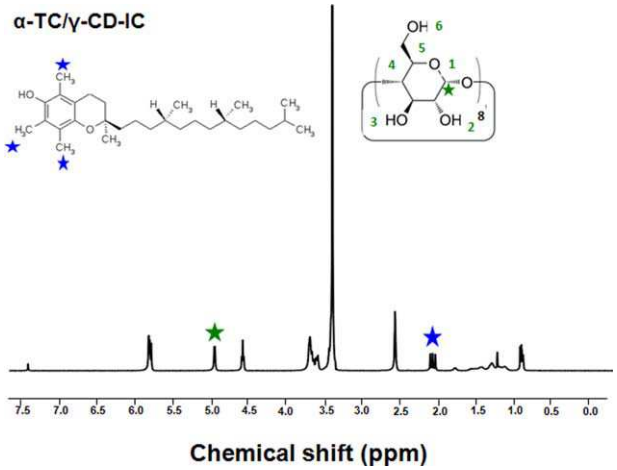

Figure 5. ^{1}H-NMR spectrum of α-TC/γ-CD–IC. [Color figure can be viewed at wileyonlinelibrary.com]

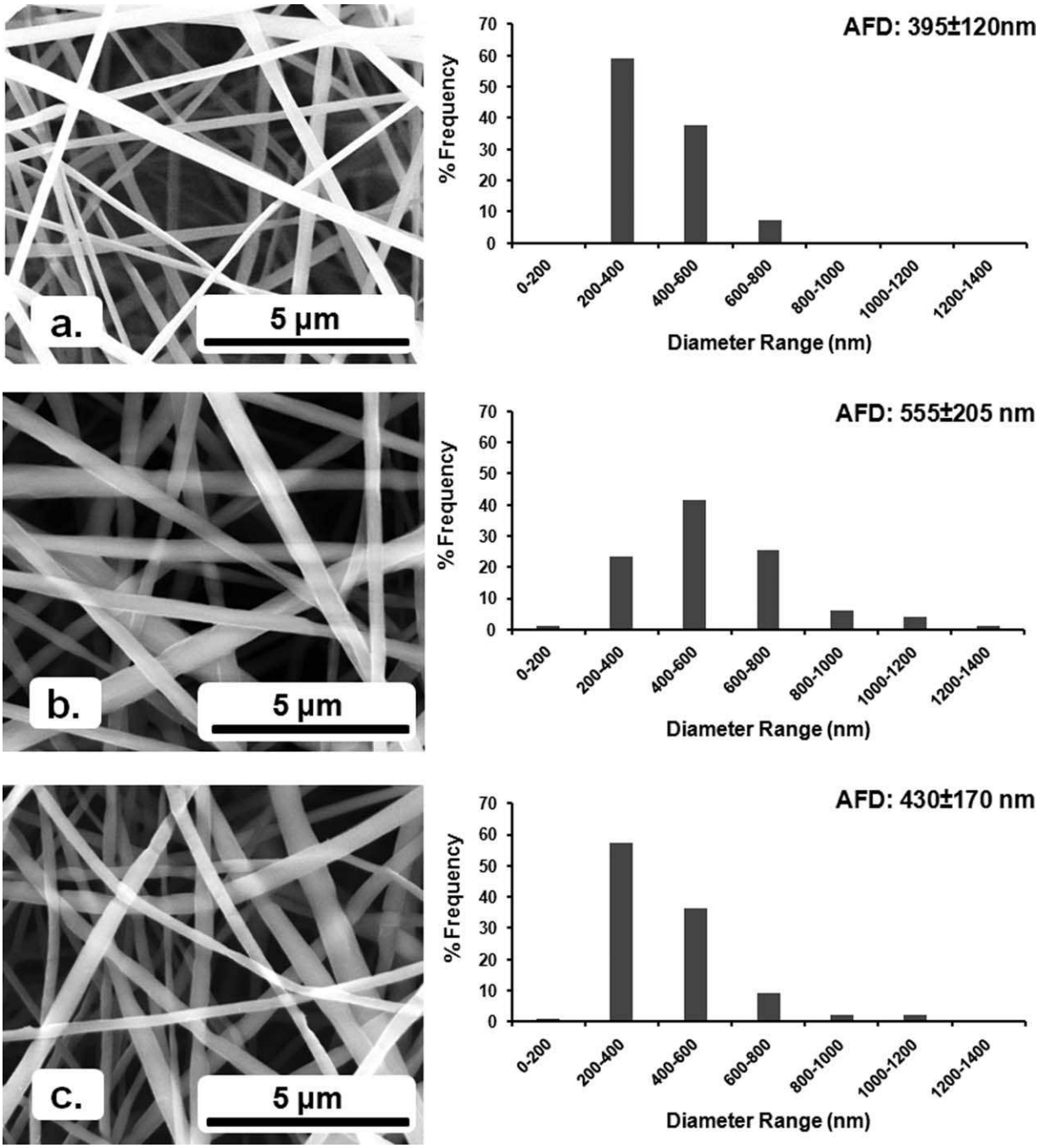

Figure 6. SEM images and fiber diameter distributions with the AFDs of the electrospun nanofibers obtained from solutions of (a) PLA, (b) PLA/α-TC, and (c) PLA/α-TC/γ-CD–IC.

Thermal Analysis of the IC

The thermal stability of α-TC/γ-CD–IC was further investigated by TGA (Figure 4). TGA measurements of α-TC, γ-CD, and the reference were taken. The thermal degradation of α-TC started around 200 °C and continued up to 385 °C. The weight loss of γ-CD occurred in two steps, the first was weight loss, which was below 100 °C and corresponded to water loss, and the second one was above 275 °C and was attributed to the main decomposition of CD. α-TC/γ-CD–IC exhibited three steps of weight losses. These weight losses were ascribed to water loss and the thermal degradations of α-TC and γ-CD, respectively. As shown in the graph, the thermal stability of α-TC did not change by complexation.[16] The amount of α-TC in α-TC/γ-CD–IC, which was determined from the TGA data, was found to be 21.0%, and it was quite close to the theoretical amount used initially (24.9%).

Molar Ratio of the IC

The molar ratio of α-TC/γ-CD–IC was calculated by ^{1}H-NMR, and the spectrum is given in Figure 5. First, ^{1}H-NMR spectra

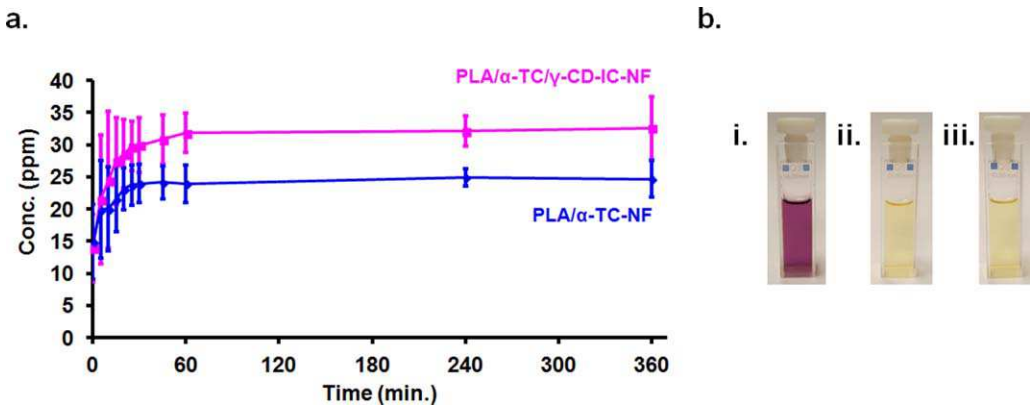

Figure 7. (a) Cumulative release of α-TC from PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF into 95% ethanol ($n = 3$). The error bars in the figure represent the standard deviations. (b) Photographs of the DPPH solutions in which (i) PLA–NF, (ii) PLA/α-TC–NF, and (iii) PLA/α-TC/γ-CD–IC–NF were immersed (after 15 min of reaction). [Color figure can be viewed at wileyonlinelibrary.com]

were recorded for the α-TC and γ-CD molecules to determine the peaks of the each proton. Then, while we calculated the molar ratio, the integration of the peaks at 2.0 and 4.9 ppm were used for α-TC and γ-CD, respectively. As a result, the molar ratio of α-TC to γ-CD was determined to be 0.9:1.0 from the ^{1}H-NMR calculations.

Morphological Analysis of the Nanofibers

The morphologies of PLA–NF, PLA/α-TC–NF, and PLA/α-TC/γ-CD–IC–NF were investigated by SEM. SEM images and the fiber diameter distributions of the nanofibers are shown in Figure 6. As shown in the images, bead-free nanofibers were successfully obtained at a 10% w/v PLA concentration. The diameters of PLA–NF, PLA/α-TC–NF, and PLA/α-TC/γ-CD–IC–NF were calculated to be 395 ± 120, 555 ± 205, and 430 ± 170 nm, respectively. The slight difference in the AFDs of the nanofibers might have been related to the viscosities and conductivities of the solutions.[23] The solution properties of PLA, PLA/α-TC, and PLA/α-TC/γ-CD–IC and the resulting electrospun nanofibers are shown in Table I. The viscosity and conductivity had reverse effects on the fiber diameter. Both the viscosity and conductivity of the PLA/α-TC solution were lower than those of the PLA solution; so, the AFDs of PLA–NF and PLA/α-TC–NF were not much different from each other. The PLA/α-TC/γ-CD–IC solution exhibited a lower viscosity and conductivity compared to the PLA solution; however, because the viscosity reduction was not as sharp as that in the PLA/α-TC solution, the conductivity reduction was much more dominant, and the AFD of PLA/α-TC/γ-CD–IC–NF was higher than that in PLA–NF.

In Vitro Release Study

The release studies of α-TC from PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF into 95% ethanol (a fatty food simulant) at RT were performed for 6 h, and the amount of α-TC released was evaluated by HPLC [Figure 7(a)]. The release of α-TC from the electrospun nanofibers into different media, including simulated gastric fluid (pH 2), water (pH 6), phosphate buffer, and acetate buffer have been reported previously.[3,24–26] Chen *et al.*[27] and Koontz *et al.*[14] evaluated the release of tocopherol from polymeric films into 95% ethanol and coconut oil. PLA/α-TC/γ-CD–IC–NF released much more α-TC in total than PLA/α-TC–NF; this was probably due to the increment in the solubility, which was shown in the phase-solubility study. However, Koontz *et al.* suggested that much more α-TC was released from α-TC-encapsulated linear low-density polyethylene (LLDPE) films than from α-TC/β-CD–IC films containing LLDPE.[14] The variance in the release of α-TC from our system compared to that in the system of Koontz *et al.* could have been due to the difference in the structure of the materials. Thus, the high surface-area-to-volume ratio of the nanofibers might have differed in relation to their AFDs. Here, the higher AFD of PLA/α-TC/γ-CD–IC–NF might have led to a higher surface-area-to-volume ratio compared to that in PLA/α-TC–NF, and therefore, PLA/α-TC/γ-CD–IC–NF released a much greater amount of α-TC. Furthermore, the hydrophobicity and stability difference between the polymers and α-TC/CD–ICs may have resulted in differences in the release behavior.

AO Activity

The DPPH method is usually used to determine the AO activity of α-TC-encapsulated polymeric films[28–30] and electrospun

Table II. TBARS Values for the Unpackaged Meat Samples and Meat Samples Packaged with Nanofibers

	TBARS (mg of MDA/kg of Meat)			
Sample	Day 4	Day 7	Day 10	Day 21
Control (meat)	0.38 ± 0.07	0.57 ± 0.22	0.81 ± 0.62	1.55 ± 1.04
PLA/α-TC–NF	0.38 ± 0.16	0.59 ± 0.19	0.67 ± 0.39	0.78 ± 0.45
PLA/α-TC/γ-CD–IC–NF	0.28 ± 0.08	0.53 ± 0.25	0.40 ± 0.33	0.78 ± 0.51

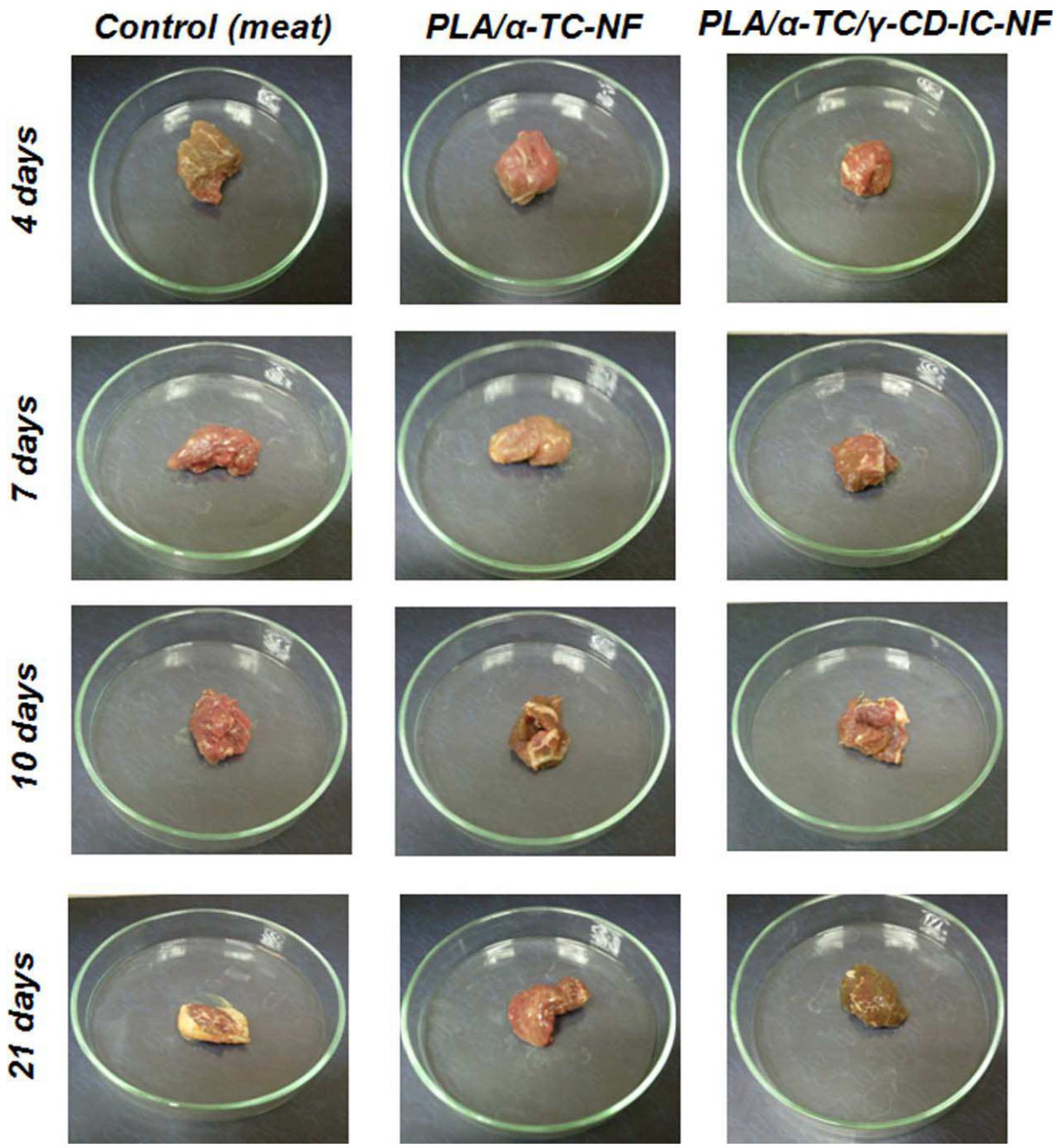

Figure 8. Photographs of the unpackaged meat samples and meat samples packaged with PLA/α-TC–NF or PLA/α-TC/γ-CD–IC–NF during 21 days of storage. [Color figure can be viewed at wileyonlinelibrary.com]

nanofibers.[26,31] It is an indirect method for determining the ability of compounds to scavenge free radicals from complex food systems. The AO activity was determined according to the DPPH radical-scavenging assay, and the absorbance of the solutions were measured via UV–visible spectroscopy. The AO activities of PLA–NF, PLA/α-TC–NF, and PLA/α-TC/γ-CD–IC–NF were 4 ± 0.2, 97 ± 0.2, and $97 \pm 0.1\%$, respectively. However, in the absence of α-TC, PLA–NF exhibited an AO activity because of the high surface area of nanofibers providing more contact with the solution. Despite the high voltage applied during electrospinning process, a quite high AO activity was observed in the α-TC including PLA nanofibers. Additionally, the AO activities of the nanofibers were almost the same. The similar AO activities of the nanofibers could have been due to the high solubility of α-TC in methanol, which favored the release α-TC quickly from the CD cavity and PLA nanofibers. Photographs of PLA–NF, PLA/α-TC–NF, and PLA/α-TC/γ-CD–IC–NF immersed in the DPPH solution at the end of the reaction are shown in Figure 7(b). The color of the solution in which PLA–NF was immersed was light purple, whereas the color of the solutions in which PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF were immersed was yellow. So, we deduced that PLA/α-TC–NF

and PLA/α-TC/γ-CD–IC–NF would be efficient in preventing lipid oxidation.

Lipid Oxidation Analysis (TBARS)

Tocopherols are known to be quite effective compounds for the inhibition of lipid oxidation. Barbosa-Pereira *et al.*[29] reported that an low-density polyethylene (LDPE) film incorporated with a product containing 90% tocopherol homologues (containing 15.5% α-TC) was the most effective film at long-term storage for salmon conservation. Turkey meat stored in packages containing a PE/α-TC film displayed the lowest TBARS values in the study of Pettersen *et al.*[32]

TBARSs are produced through second-stage oxidation, during which peroxides are oxidized to aldehydes and ketones (e.g., MDA). These oxidative reactions are known to be the primary causes of quality loss; they ultimately affect the color, flavor, and nutritional value of foods during storage. The TBARS method has been widely used to determine lipid oxidation in meat products in previous studies.[29,32,33]

The results and photographs of the meat samples are given in Table II and Figure 8, respectively. After 4 days of storage, the TBARS values of the control (unpackaged meat) and PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF packaged meat were 0.38 ± 0.07, 0.38 ± 0.16, and 0.28 ± 0.08 mg of MDA/kg of meat, respectively. Although both of the nanofibers significantly inhibited lipid oxidation, PLA/α-TC/γ-CD–IC–NF displayed a large inhibition of TBARS formation over 10 days of storage compared to PLA/α-TC–NF. The TBARS value of the control reached 1.55 ± 1.04 mg of MDA/kg of meat in 21 days, whereas PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF showed TBARS values of only 0.78 ± 0.45 and 0.78 ± 0.51 mg of MDA/kg of meat, respectively, even after 21 days of storage. So, the control sample suffered a more rapid and intense oxidation than the samples packaged with the nanofibers. Statistical analyses revealed that there was no statistically significant difference between the unpackaged and the meat samples packaged with nanofibers ($p > 0.05$). The short shelf life of the packaged meat was extended with PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF through the retardation of its oxidation. Moreover, slime formation was clearly observed in the control sample at the end of 21 days. Although the release of α-TC from PLA/α-TC/γ-CD–IC–NF was greater, as depicted in Figure 7(a), PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF exhibited almost the same TBARS values, and this could have been related to the complexity of the food systems. However, the release of α-TC was great enough to inhibit lipid oxidation in both PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF. However, in the study of Chen *et al.*,[27] the release of tocopherol was investigated from electrospun ethylene vinyl alcohol (EVOH) and LDPE films into 95% ethanol, and they deduced that the release rate of tocopherol was not acceptable for long-term lipid oxidation inhibition.

CONCLUSIONS

An IC of α-TC and γ-CD was prepared at a 1:1 molar ratio (α-TC/γ-CD–IC). Then, free α-TC and α-TC/γ-CD–IC were encapsulated into a PLA solution to obtain nanofibers by electrospinning (PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF). SEM images showed a bead-free and homogeneous morphology in the nanofibers. The characterization of the complex was done by XRD, TGA, and ^{1}H-NMR. The release of α-TC into 95% ethanol (a fatty food simulant) from the nanofibers was measured via HPLC. Owing to the solubility enhancement shown in the phase-solubility diagram, the released amount of α-TC was much greater from PLA/α-TC/γ-CD–IC–NF when compared to PLA/α-TC–NF. The AO activity of the nanofibers, which was investigated by a DPPH radical-scavenging method, was determined to be 97%. A quite high AO activity of nanofibers is an indication of the potential of nanofibers in the inhibition of lipid oxidation. The TBARS method was used as a direct method to test the potential of the nanofibers as an AO packaging material. PLA/α-TC–NF and PLA/α-TC/γ-CD–IC–NF had lower TBARS values than the unpackaged meat sample. Therefore, PLA/α-TC/γ-CD–IC–NF could be used to prolong the shelf life of meat samples in the food industry.

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REFERENCES

1. Tian, F.; Decker, E. A.; Goddard, J. M. *Food Funct.* **2013**, *4*, 669.
2. Gómez-Estaca, J.; López-de-Dicastillo, C.; Hernández-Muñoz, P.; Catalá, R.; Gavara, R. *Trends Food Sci. Technol.* **2014**, *35*, 42.
3. Taepaiboon, P.; Rungsardthong, U.; Supaphol, P. *Eur. J. Pharm. Biopharm.* **2007**, *67*, 387.
4. Sheng, X.; Fan, L.; He, C.; Zhang, K.; Mo, X.; Wang, H. *Int. J. Biol. Macromol.* **2013**, *56*, 49.
5. Brantley, P.; Elmadfa, I.; Kafatos, A.; Kelly, F. J.; Manios, Y.; Roxborough, H. E.; Schuch, W.; Sheehy, P. J. A.; Wagner, K. H. *J. Sci. Food Agric.* **2000**, *80*, 913.
6. Del Valle, E. M. *Process Biochem.* **2004**, *39*, 1033.
7. Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743.
8. Li, Z.; Wang, M.; Wang, F.; Gu, Z.; Du, G.; Wu, J.; Chen, J. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 245.
9. Celik, S. E.; Ozyurek, M.; Guçlu, K.; Apak, R. *React. Funct. Polym.* **2007**, *67*, 1548.
10. Sueishi, Y.; Hori, M.; Inazumi, N. *J. Inclusion Phenom. Macrocyclic Chem.* **2012**, *72*, 467.
11. Koontz, J. L.; Marcy, J. E.; O'Keefe, S. F.; Duncan, S. E. *J. Agric. Food Chem.* **2009**, *57*, 1162.

12. Siró, I.; Fenyvesi, É.; Szente, L.; De Meulenaer, B.; Devlieghere, F.; Orgoványi, J.; Sényi, J.; Barta, J. *Food Additives Contam.* **2006**, *23*, 845.
13. Koontz, J. L.; Marcy, J. E.; O'Keefe, S. F.; Duncan, S. E.; Long, T. E.; Moffitt, R. D. *J. Appl. Polym. Sci.* **2010**, *117*, 2299.
14. Koontz, J. L.; Moffitt, R. D.; Marcy, J. E.; O'Keefe, S. F.; Duncan, S. E.; Long, T. E. *Food Additives Contam.* **2012**, *27*, 1598.
15. Kenawy, E. R.; Bowlin, G. L.; Mansfield, K.; Layman, J.; Simpson, D. G.; Sanders, E. H.; Wnek, G. E. *J. Controlled Release* **2002**, *81*, 57.
16. Badami, A. S.; Kreke, M. R.; Thompson, M. S.; Riffle, J. S.; Goldstein, A. S. *Biomaterials* **2006**, *27*, 596.
17. Auras, R.; Harte, B.; Selke, S. *Macromol. Biosci.* **2004**, *4*, 835.
18. Mehta, R.; Kumar, V.; Bhunia, H.; Upadhyay, S. N. *J. Macromol. Sci. Polym. Rev.* **2005**, *45*, 325.
19. Wendorff, J. H.; Agarwal, S.; Greiner, A. Electrospinning: Materials, Processing, Applications; Wiley: Weinheim, Germany, **2012**.
20. Higuchi, T. K.; Connors, A. *Adv. Anal. Chem. Instrum.* **1965**, *4*, 117.
21. UNisa, I.; Ashwar, B. A.; Shah, A.; Gani, A.; Gani, A.; Masoodi, F. A. *J. Food Sci. Technol.* **2015**, *52*, 7245.
22. Uyar, T.; Hunt, M. A.; Gracz, H. S.; Tonelli, A. E. *Crystal Growth Des.* **2006**, *6*, 1113.
23. Ramakrishna, S.; Fujihara, K.; Teo, W. E.; Lim, T. C.; Ma, Z. An Introduction to Electrospinning Nanofibers; World Scientific: Singapore, **2005**; Vol. *90*.
24. Wongsasulak, S.; Pathumban, S.; Yoovidhya, T. *J. Food Eng.* **2014**, *120*, 110.
25. Fabra, M. J.; López-Rubio, A.; Lagaron, J. M. *Food Hydrocolloids* **2016**, *55*, 11.
26. Aytac, Z.; Uyar, T. *Eur. Polym. J.* **2016**, *79*, 140.
27. Chen, X.; Lee, D. S.; Zhu, X.; Yam, K. L. *J. Agric. Food Chem.* **2012**, *60*, 3492.
28. Byun, Y.; Kim, Y. T.; Whiteside, S. *J. Food Eng.* **2010**, *100*, 239.
29. Barbosa-Pereira, L.; Cruz, J. M.; Sendón, R.; de Quirós, A. R. B.; Ares, A.; Castro-López, M.; Abad, M. J.; Maroto, J.; Paseiro-Losada, P. *Food Control* **2013**, *31*, 236.
30. Jongjareonrak, A.; Benjakul, S.; Visessanguan, W.; Tanaka, M. *Food Hydrocolloids* **2008**, *22*, 449.
31. Munteanu, B. S.; Aytac, Z.; Pricope, G. M.; Uyar, T.; Vasile, C. *J. Nanopart. Res.* **2014**, *16*, 1.
32. Pettersen, M. K.; Mielnik, M. B.; Eie, T.; Skrede, G.; Nilsson, A. *Poultry Sci.* **2004**, *83*, 1240.
33. Camo, J.; Beltrán, J. A.; Roncalés, P. *Meat Sci.* **2008**, *80*, 1086.