JOURNAL OF Applied Polymer SCIENCE

Special Issue: Bio-based Packaging

Guest Editors: José M. Lagarón, Amparo López-Rubio, and María José Fabra
Institute of Agrochemistry and Food Technology of the Spanish Council for Scientific Research

EDITORIAL

REVIEWS

RESEARCH ARTICLES

JOURNAL OF Applied Polymer SCIENCE

Special Issue: Bio-based Packaging

Guest Editors: José M. Lagarón, Amparo López-Rubio, and María José Fabra
Institute of Agrochemistry and Food Technology of the Spanish Council for Scientific Research

Impact of fermentation residues on the thermal, structural, and rheological properties of polyhydroxy(butyrate-*co*-valerate) produced from cheese whey and olive oil mill wastewater
L. Hilliou, D. Machado, C. S. S. Oliveira, A. R. Gouveia, M. A. M. Reis, S. Campanari, M. Villano and M. Majone, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42818

Synergistic effect of lactic acid oligomers and laminar graphene sheets on the barrier properties of polylactide nanocomposites obtained by the in situ polymerization pre-incorporation method
J. Ambrosio-Martín, A. López-Rubio, M. J. Fabra, M. A. López-Manchado, A. Sorrentino, G. Gorrasi and J. M. Lagarón, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42661

Antibacterial poly(lactic acid) (PLA) films grafted with electrospun PLA/allyl isothiocyanate fibers for food packaging
H. H. Kara, F. Xiao, M. Sarker, T. Z. Jin, A. M. M. Sousa, C.-K. Liu, P. M. Tomasula and L. Liu, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42475

Poly(L-lactide)/ZnO nanocomposites as efficient UV-shielding coatings for packaging applications
E. Lizundia, L. Ruiz-Rubio, J. L. Vilas and L. M. León, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42426

Effect of electron beam irradiation on the properties of polylactic acid/montmorillonite nanocomposites for food packaging applications
M. Salvatore, A. Marra, D. Duraccio, S. Shayanfar, S. D. Pillai, S. Cimmino and C. Silvestre, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42219

Preparation and characterization of linear and star-shaped poly L-lactide blends
M. B. Khajeheian and A. Rosling, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42231

Mechanical properties of biodegradable polylactide/poly(ether-block-amide)/thermoplastic starch blends: Effect of the crosslinking of starch
L. Zhou, G. Zhao and W. Jiang, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42297

Interaction and quantification of thymol in active PLA-based materials containing natural fibers
I. S. M. A. Tawakkal, M. J. Cran and S. W. Bigger, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42160

Graphene-modified poly(lactic acid) for packaging: Material formulation, processing, and performance
M. Barletta, M. Puopolo, V. Tagliaferri and S. Vesco, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42252

Edible films based on chia flour: Development and characterization
M. Dick, C. H. Pagno, T. M. H. Costa, A. Gomaa, M. Subirade, A. De O. Rios and S. H. Flóres, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42455

Influence of citric acid on the properties and stability of starch-polycaprolactone based films
R. Ortega-Toro, S. Collazo-Bigliardi, P. Talens and A. Chiralt, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42220

Bionanocomposites based on polysaccharides and fibrous clays for packaging applications
A. C. S. Alcântara, M. Darder, P. Aranda, A. Ayral and E. Ruiz-Hitzky, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42362

Hybrid carrageenan-based formulations for edible film preparation: Benchmarking with kappa carrageenan
F. D. S. Larotonda, M. D. Torres, M. P. Gonçalves, A. M. Sereno and L. Hilliou, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42263

JOURNAL OF Applied Polymer SCIENCE

Special Issue: Bio-based Packaging

Guest Editors: José M. Lagarón, Amparo López-Rubio, and María José Fabra
Institute of Agrochemistry and Food Technology of the Spanish Council for Scientific Research

Structural and mechanical properties of clay nanocomposite foams based on cellulose for the food packaging industry
S. Ahmadzadeh, J. Keramat, A. Nasirpour, N. Hamdami, T. Behzad, L. Aranda, M. Vilasi and S. Desobry, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42079

Mechanically strong nanocomposite films based on highly filled carboxymethyl cellulose with graphene oxide
M. El Achaby, N. El Miri, A. Snik, M. Zahouily, K. Abdelouahdi, A. Fihri, A. Barakat and A. Solhy, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42356

Production and characterization of microfibrillated cellulose-reinforced thermoplastic starch composites
L. Lendvai, J. Karger-Kocsis, Á. Kmetty and S. X. Drakopoulos, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42397

Development of bioplastics based on agricultural side-stream products: Film extrusion of *Crambe abyssinica*/wheat gluten blends for packaging purposes
H. Rasel, T. Johansson, M. Gällstedt, W. Newson, E. Johansson and M. Hedenqvist, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42442

Influence of plasticizers on the mechanical and barrier properties of cast biopolymer films
V. Jost and C. Stramm, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42513

The effect of oxidized ferulic acid on physicochemical properties of bitter vetch (*Vicia ervilia*) protein-based films
A. Arabestani, M. Kadivar, M. Shahedi, S. A. H. Goli and R. Porta, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42894

Effect of hydrochloric acid on the properties of biodegradable packaging materials of carboxymethylcellulose/poly(vinyl alcohol) blends
M. D. H. Rashid, M. D. S. Rahaman, S. E. Kabir and M. A. Khan, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42870

Journal of Applied Polymer Science

Antibacterial poly(lactic acid) (PLA) films grafted with electrospun PLA/allyl isothiocyanate fibers for food packaging

Hasan H. Kara,[1,3] Fugang Xiao,[2,3] Majher Sarker,[3] Tony Z. Jin,[4] Ana M. M. Sousa,[3] Cheng-Kung Liu,[5] Peggy M. Tomasula,[3] LinShu Liu[3]

[1]Department of Food Engineering, Bayburt University, Bayburt 69100, Turkey

[2]School of Food and Bioengineering, Xu Chang University, Xuchang 461000, China

[3]Dairy and Functional Foods Research Unit, Eastern Regional Research Center, Agricultural Research Service, US Department of Agriculture, Wyndmoor 19038, Pennsylvania

[4]Residue Chemistry and Predictive Microbiology Research Unit, Eastern Regional Research Center, Agricultural Research Service, US Department of Agriculture, Wyndmoor 19038, Pennsylvania

[5]Biobased and Other Animal Coproducts Research Unit, Eastern Regional Research Center, Agricultural Research Service, US Department of Agriculture, Wyndmoor 19038, Pennsylvania

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Correspondence to: L. Liu (E-mail: Linshu.liu@ars.usda.gov)

ABSTRACT: Poly(lactic acid) (PLA) fibers of submicron sizes encapsulating allyl isothiocyanate (AITC) (P*f*A) were made and electrospun onto the surfaces of PLA films. SEM examination confirmed the fusion of the two phases to form a bilayered fiber-grafted film after the film underwent air blowing and water washing to remove the nongrafted fibers. The fiber-grafted PLA films (P*f*A-*g*-film) retain the mechanical properties of PLA. The release of AITC from the fibers was temperature dependent. At temperatures lower than 4°C, the incorporated AITC remained within the fibers without losing activity; at room temperature, AITC released in a sustained manner over weeks. The release of AITC was also dependent on its initial concentration in the PLA electrospinning solution; samples with more AITC incorporated showed a higher release rate. P*f*A-*g*-films significantly inhibited the growth of *Listeria innocua* and *Escherichia coli* k12 when tested on package foods. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42475.

KEYWORDS: electrospinning; fibers; films; packaging; polyesters

Received 18 March 2015; accepted 6 May 2015
DOI: 10.1002/app.42475

INTRODUCTION

There is a growing interest in active packaging. The demand for active and intelligent packaging in the US is projected to reach $3.5 billion in 2017, according to a new report.[1] Different from conventional packaging materials and technologies that only provide a physical barrier to passively protect enclosed items from tampering or contamination from physical, chemical, and biological sources, active packaging is an implement that actively interferes with the biochemical or chemical processes in the headspaces or on the surfaces of enclosed items, going beyond environmental segregation. Perishable commodities, such as fruits, vegetables, meats, sea food, and so on, were noted as the largest market for active packaging. The application of active packaging for fresh or processed foods offers several advantages that include prolonging their shelf-life, enhancing safety and sensory properties, while maintaining the quality of the products, and reducing losses from unsaleable products in the supply chain.

Natural bioactives—such as nisin, thymol, and allyl isothiocyanate (AITC)—have been examined for food preservation; these substances are generally recognized as safe (GRAS) by the U.S. Food and Drug Administration. (For AITC, see 21 CFR 172.515). The natural antimicrobial compound AITC is a colorless oil with a volatile and pungent character. It was originally extracted from mustard, radish, horseradish, or wasabi.[2] AITC can also be produced commercially by the reaction of allyl chloride and potassium thiocyanate that ensures the purity, quality, and availability of the compound, and allows a low production cost that extends its applications.[3] AITC has been demonstrated as an antibacterial and antifungal in the food industry. As for

making active packaging materials, AITC used alone or in combination with modified atmosphere packaging has demonstrated effectiveness in inhibiting bacterial growth in solid food models, cheeses, and chicken meat.[4–6] In these studies, packaging materials, in the form of films or sheets, were used as vectors for the preservative's retention. AITC was mechanically dispersed in polymer solutions, the mixtures were then fabricated into films or sheets by extrusion blowing or casting methods. Biopolymers that have been investigated for AITC incorporation included soy protein isolate, chitosan, and cyclodextrin, or poly(lactic acid) (PLA), or cyclodextrin/PLA composites.[4–7] The incorporation efficiency is limited due to the oily nature of AITC that is not compatible with major food packaging materials; also some of the included AITC loses activity during processing.[8]

PLA has been extensively studied for food packaging applications. PLA is a biomass-oriented polyester that can be synthesized either from lactic acid or by ring-opening polymerization from its dimer, lactide. Films or sheets from PLA possess relatively strong mechanical properties suitable for food packaging. The monomer, lactic acid, obtained from the fermentation of corn starch, is also the final degradation product of PLA, and considered as nontoxic and environmentally benign. Because of these advantages, PLA has received increasing attention by the food packaging industry.[9,10] Incorporated bioactives were released from the PLA at a predetermined rate driven by passive diffusion; parameters influencing the release profile were the concentration and nature of the bioactives, the surface areas, and the three-dimensional structure of scaffold, or the thickness of the film or sheet, and other physical properties of the polymers, such as crystallinity, molecular weight, and molecular weight distribution, and so on. In most cases, the release of incorporated active agents was characterized with an initial burst of the agents, with a large portion of the agents unable to release from the PLA formulations.[10,11]

Besides film forming, PLA is suitable for electrospinning fibers of nano or submicron sizes. The successful production of PLA fibers with AITC and other bioactives incorporated has been reported.[12–15] The preincorporated active substances release at faster rates from PLA fibers than PLA films or sheets, and the release is more sensitive to environmental stimuli, such as temperature and humidity, which can be attributed to the huge surface areas that per unit PLA fibers provide, in comparison with the same amount of PLA in films or sheets. For packaging purposes, fibers or fibrous mats thus obtained are used with another polymeric material that provides the physical barrier and weight-bearing properties.

In this research, a new method to fabricate PLA films grafted with electrospun PLA/AITC fibers was described. The physical and antimicrobial properties of the bilayered films were evaluated for food packaging applications.

EXPERIMENTAL

Materials

PLA pellets (product No. 4060D) with a weight-average molecular weight of 148 kDa and a number-average molecular weight of 110 kDa were obtained from NatureWorks LLC (Minnetonka, MN). Dichloromethane (DCM), ethyl acetate (EA), and hexane were purchased from Fisher Scientific (Fair Lawn, NJ). AITC (98%, $d = 0.94$ g/mL) was purchased from Sigma Aldrich (St. Louis, MO), and stored at 4–7°C for use. *Listeria innocua* (ATCC 51742) and *Escherichia coli* K12 (ATCC 23716) were obtained from the American Type Culture Collection (Manassas, VA., USA.). Frozen stock cultures of each strain were cultured independently in 30 mL Tryptic Soy Broth (BBL/Difco Laboratories, Sparks, MD, USA) in sterile 50 mL conical tubes at 37°C for 18 h.

Preparation of PLA Films and PLA/AITC Solutions

PLA films were cast from PLA/DCM (5%, w/v) solution that was obtained by stirring PLA pellets in DCM in closed Erlenmeyer flasks overnight. Five milliliters of the PLA solution were loaded on a glass plate using a K-101 Control Coater apparatus, spread over with a spiral wound meter bar (RK Print-Coat Instruments Ltd, Royston, UK) at the speed setting of #3 as described in the operation menu by the provider. The glass plates with the spread solutions were placed in a hood for $\sim$ 15 min to allow the organic solution to evaporate. The films were then peeled off from the supports, measured for thickness and weight, and stored in a desiccator. PLA/AITC solutions were prepared by the addition of the appropriate volumes of AITC to PLA/DCM (5.0%, w/v) to achieve 5, 10, 15, or 25 wt % AITC in relation to the PLA content. The mixtures were stirred for about 20 min prior to electrospinning.

PLA/AITC Fibers Electrospun-Grafted to PLA Films

PLA fibers containing AITC (P*f*A) were electrospun-grafted onto the surfaces of PLA films (P*f*A-*g*-film) in one step by the electrospinning technique using the NaBond NEU-01 (NaBond Technologies Co., Limited, China) with a tubeless spinneret attached inside the chamber and connected to an external syringe pump (model TCI-IV; Veryark Technology, Guangxi, China). This type of spinneret allows the polymer solution to be run inside a closed chamber with a proper vapor-exhaust vapor system. A piece of the PLA film (20 × 5 cm) was placed on aluminum foil covering a drum collector. The schematic representation of the electrospinning setup used to perform the experiments can be found elsewhere.[16]

To ensure the homogeneous distribution of the fibers on the film surface, after several preliminary trials, the electrospinning conditions were chosen as follows: flow rate, 0.5 mL/h; voltage, 20 kV; distance from the tip of the spinneret to the collector, 8 cm; spinneret operating at a constant X-axial sliding, while the drum motor speed was set to zero.

All experiments were performed in triplicate and carried out at room temperature.

Characterization of PLA/AITC Fibers-Grafted PLA Films

The P*f*A-*g*-films thus obtained were measured for thickness, and characterized for morphology, evidence of fiber grafting, mechanical properties, encapsulation, and release of AITC.

The thickness of the films was measured at five different points using a Mitutoyo absolute thickness gage (Mitutoyo Corp., Kanagawa, Japan) with a ±0.001 mm precision. Reported values are the mean of the five measurements. The graft of fibers to

the surfaces of the films was evaluated by blowing the film with an air stream at the speed of 1.35 m/s from 20.0 cm distance for 3.0 min. The graft of fibers to the surfaces of the films was also evaluated by water washing; briefly, a film (5 × 5 cm) was sandwiched within 2 pieces of metal plates with a square window (4 × 4 cm) in the middle of the top plate, then placed in a 1.5 L beaker containing 800 mL D.I. water, and stirred at 280 rpm for 3.0 min using a Hei-Torque overhead stirrer (RZR 2041; Heidolph Instrument, Schwabach, Germany); the tip of the turbine stirrer was set 10.0 cm above the films. The washed films were air-dried followed by drying in a desiccator over anhydrous calcium sulfate until no further weight change was recorded in three subsequent measurements, using an analytical balance.

For SEM examination, specimens of the films were mounted with adhesive to specimen stubs, and the edges were painted with colloidal silver adhesive. The specimens were then sputtered with a thin layer of gold and examined in the high-vacuum/secondary electron imaging mode of a Quanta 200 FEG scanning electron microscope (SEM, FEI, Hillsboro, OR). Digital images were collected at 500, 2500, and 5000×.

Mechanical property measurements performed on the films included tensile strength, tensile modulus, maximal elongation, and fracture energy. These properties were measured using an upgraded Instron mechanical property tester, model 1122, equipped with Testworks 4 data acquisition software (MTS Systems Corp., Minneapolis, MN). Samples of P*f*A-*g*-films and PLA films were cut to strips of 5 × 45 mm in size and tested at the following settings: grip length (clamp distance), 25 mm; strain rate (crosshead speed), 50 mm/min. Each test was performed in triplicate and averages were taken.

GC was used to determine the stability and release profile of AITC from the grafted PLA films under various conditions, in terms of temperature and time periods. All samples were analyzed using an SRI 8610C gas chromatograph (SRI Instruments, Torrance, CA) equipped with a flame ionization detector and an MXT-1 column (15 m × 0.53 mm × 5 μm film thickness; RESTEK, Bellefonte, PA). The detector was operated at 250°C. Helium was used as carrier gas to give a 4.0 mL/min column flow and the oven temperature was programmed at 115°C isothermal.

For the AITC standard graph, 1 μL of liquid AITC was injected to a 70 mL air-tight gas jar with a rubber septum (Fisher Scientific, Fair Lawn, NJ), and converted to the gas form by heating the whole jar at 80°C for 15 min with stirring. A fraction of the gas (1–40 μL) thus generated was taken and injected into the GC to obtain a number of spots of different concentrations (0.0132–0.528 μg) of AITC to develop the standard line.

To check the degradation of AITC in glass vials, 2 μL of liquid AITC was added to each 2 mL by volume six air tight vials. The vials were heated at 80°C for 15 min with stirring. Gas (250 μL) from the head space was injected into the GC from each vial at six different times (1–90 h). GC area was recorded for each time.

To quantify the AITC content in P*f*A-*g*-films, a piece of film with known amount of fiber (W_f, mg) was dissolved in a centrifuge tube with 3 mL of EA to obtain a clear solution. Hexane was slowly added (1.5 mL) with stirring to precipitate PLA. The tube was centrifuged for 15 min and 1 mL of sample was taken from the top of the solution. The solution was filtered with a 0.2 μm syringe filter and stored in a GC vial. A 0.2 μL sample was injected into the GC and the amount of AITC (W_g, μg) was recorded, the AITC content in the PLA film was then calculated, according to $\% = (15W_g/W_f) \times 100$.

Release of AITC from P*f*A-*g* Film

The AITC release kinetics were investigated at three temperature segments: 22 ± 2°C, 2–4°C, and −20 ± 2°C. Briefly, a piece of film (10 ± 0.2 mg) was placed in a 70.0 mL GC vial, immediately sealed with the lid, and incubated in a chamber, which was maintained at one of the three temperatures. At predetermined time periods, 0.2 μL of the gas was taken from the headspace of the film and injected into the GC for AITC measurement; the data thus obtained was used to calculate the amount of AITC released from the film using the standard curve. After each sampling, the vial was purged with dry N_2 gas for 2 min to ensure that no AITC residues in the headspace were left from the previous measurement. The cumulative amount of AITC released was calculated by combining the measured amount of each test with the sum of the values of previous measurements. Three samples were measured for each test.

Antibacterial Activity of P*f*A-*g* Film

The antimicrobial efficacy of the films was tested on real foods: grapes and ready-to-eat (RTE) deli turkey meat. Grapes and RTE meat were purchased at local grocery stores and stored at 4°C until used. Meat samples were placed on a sterilized tray in a biohood and the upper surface of each sample was inoculated with 0.1 mL of *L. innocua* or *E. coli*. The inoculum was then spread evenly over the meat surface using sterile spreaders. Grapes were dipped in *L. innocua* or *E. coli* inoculum for 1 min. After inoculation, the grape and meat samples were kept under the biohood for 2 h to allow bacterial attachment before film treatments. A piece of P*f*A-*g*-films was placed above the petri dish with inoculated food samples inside of a plastic bag then sealed. The bags were stored at 22°C for 40 h and samples were transferred into individual sterile stomacher bags and then hand-massaged in 20 mL of 0.1% peptone water for 1 min. Serial dilutions of the resultant bacterial suspensions were made in 0.1% peptone water and surface-plated (100 μL) onto Palcam agar plates for *Listeria* and TSA for *E. coli*. All plates were incubated at 37°C for 24–48 h. The bacterial populations were enumerated by counting the colony forming units (CFU). Bags without P*f*A-*g*-films served as controls.

Statistical Analysis

All antimicrobial experiments were conducted independently twice using two film samples per treatment, per the experimental design. Duncan's multiple range test was used to determine the significant differences of mean values. Significance was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Morphology and Physical Properties

The PLA films possessed smooth, nonwoven surfaces, and no obvious defects or detrimental structures could be observed by

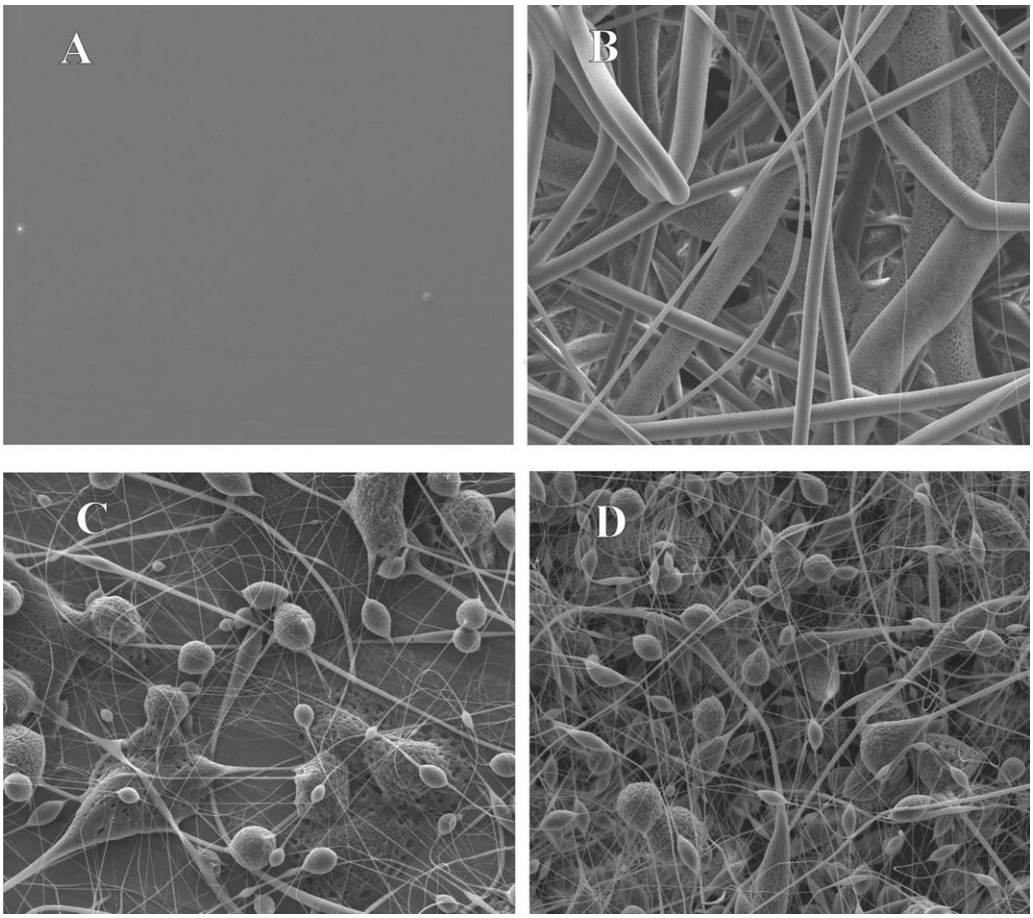

Figure 1. Scanning electron micrographs of (A) PLA Film and (B–D) P*f*A-*g*-films. AITC/PLA in the electrospinning solutions were 5, 15, and 25% for B, C, and D, respectively. Magnification, 2500×.

SEM [Figure 1(A)]. The films used for grafting electrospun P*f*A fibers were thin; the thickness was only 12 ± 2 μm. Under present experimental conditions, the electrospinning process was hard to carry out with PLA films thicker than 12 μm, because the electrical conductivity of the drum was dramatically reduced by wrapping with a thicker film, and so electrostatic force in drawing the polymer solution from the tip of the spinneret to the PLA film lying on the drum. PLA is electrospinnable, and PLA solution was used here as a carrier polymer for AITC encapsulation. At low AITC content [Figure 1(B)], pure beadless fibers were obtained that were smooth in morphology and large in diameter (0.5–2.5 μm). As the AITC content increased from 5 to 15%, beaded fibers were produced, and the fiber diameter reduced (<100 nm). The surfaces of the beads and fibers turned rough as two separated phases could be observed, one continuous phase of PLA with embedded islands that were thought to be AITC and voids created by AITC escaping during process [Figure 1(C)]. This trend was seen when the AITC content further increased to 25% [Figure 1(D)]. It was also seen in other cases, where electrospun fibers of PLA, poly(ethylene oxide) (PEO), or polyvinyl alcohol (PVA) containing organic or inorganic particles, flavors, and living cells were produced. These polymers also enable nonelectrospinnable polymers, such as alginate or pectin, to form fibers by co-electrospinning.[17–19]

When a mixture of an electrospinnable polymer and a nonelectrospinnable material was subjected to electrospinning, there is a threshold; beyond a certain composition ratio, the system is dominated by nonelectrospinnability; while around the threshold is the transition from fibers to beads. This could be attributed to the changes in solution properties of the carrier polymers. The inclusion of AITC interrupted the intra- and interchain reactions of PLA macromolecules, and the interaction between the PLA and the solvent. Consequently, the electric conductivity, surface tension, and solution viscosity of the mixture are different from that of the pure polymer solution. Once the electrostatic force induced by the high electric field is no longer able to overcome the resistance of the solution drop, it cannot draw the cone-shaped drop of PLA solution to a jet, and then to a fiber toward the opposite electrode, and beads were formed. Another assumption is that phase-separation may occur during electrospinning, resulting in a PLA-rich phase of fibers and an AITC-rich phase of beads.[4]

The grafting of the P*f*A fibers (beadless fibers and beaded fibers) on PLA films was evaluated. No significant weight changes (>1 mg) could be measured on samples that were either blown with air jet or washed with water, in comparison with non-treated samples. However, a slight removal of fibers from the film surfaces were observed by SEM; the fibers found on the

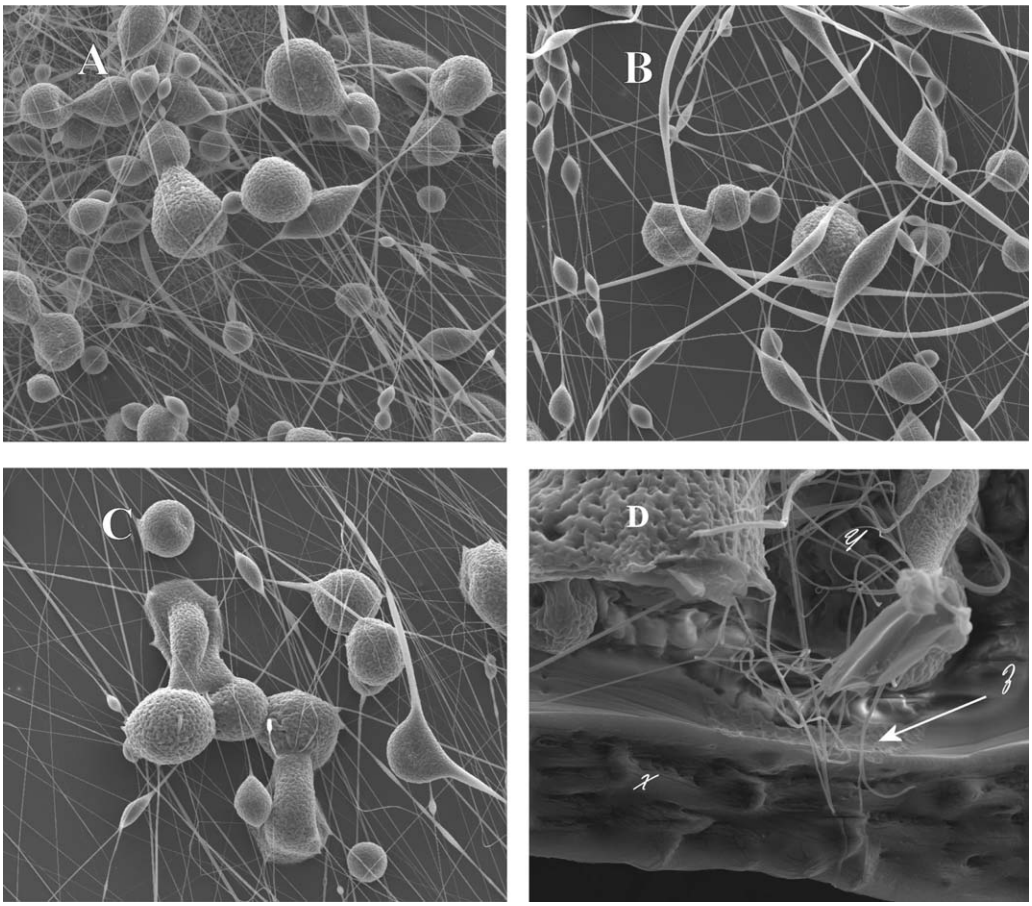

Figure 2. Scanning electron micrographs of P*f*A-*g*-films (AITC/PLA, 15:100; w/w; in the electrospinning solutions). Top view: without treatment (A); treated with air blowing (B) or water washing (C). View of cross-section (D): film layer (X); fibrous mats (Y), and fusion area (Z). Magnification: A–C, 2500×; D, 5000×.

surfaces of air-blown film [Figure 2(B)] and water-washed film [Figure 2(C)] were less dense than those of the nontreated film [Figure 2(A)]. Most electrospun fibers remained firmly on the surfaces of PLA films, regardless whether they included beads or not. That was further confirmed by the cross-section view of the films [Figure 2(D)], where no gaps or clear border lines between the fibers and the films could be detected. Presumably, under the experimental conditions, the solvent did not evaporate completely from the PLA/AITC jet before accelerating to the PLA film on the drum collector; the residual solvent "glued" the fibers and beads onto the film by the dissolution of PLA of the film at the "landing spots" of the PLA fibers. The graft process described here is similar to the grafting procedure used in horticulture—to insert the scion (the PLA fibers) onto the rootstock (the PLA film) to form a new entity.

Nevertheless, a portion of the electrospun P*f*A fibers (and beaded-fibers) were laced with the PLA film together by physical interactions, owing to the same chemical structure and surface interaction from the huge surfaces areas of the submicron fibers. Considering the end application, the films may undergo mechanical fracturing, bending, striking, and collision during packaging and transportation, the firm graft of the electrospun P*f*A fibers to PLA film is an important property.

For use as packaging materials, the mechanical properties of the P*f*A-*g*-film are of importance. Table I summarized the Young's modulus, tensile strength, maximal elongation, and the toughness of PLA films grafted with and without P*f*A fibers. As shown, the addition of the electrospun fibers seemed to have no effect on the mechanical properties of the PLA films, which have properties similar to those of the many commercially available packaging materials, such as PVC, PS, and low-density PE[20,21] and some of the cellulose derivatives[21–23] such as NatureFlex™ and Cellophane™; e.g., unlike fibers or fibrous mats alone[4–8] that lack suitable mechanical and physical

Table I. Comparison of Mechanical Properties of PLA Films with AITC/PLA Fibers-Grafted PLA Films

	Young' modulus (MPa)	Tensile strength (MPa)	Elongation (%)	Toughness (J/cm^3)
Films before process	2727 ± 761	74.7 ± 5.7	4.2 ± 1.1	2.3 ± 1.1
Films grafted with fibers	2494 ± 138	74.2 ± 5.0	3.6 ± 0.7	1.9 ± 0.3

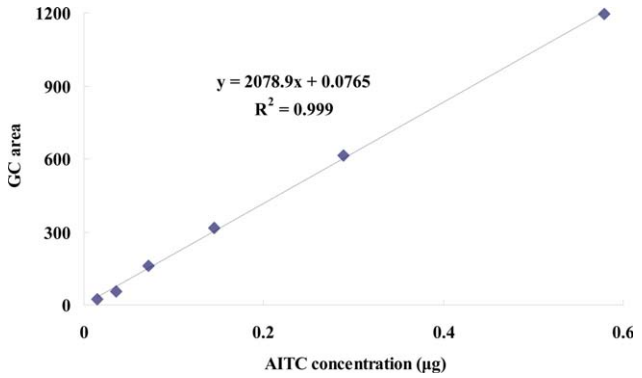

Figure 3. Standard curve of gaseous AITC measured by GC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

functionalities, the P*f*A-*g*-films appear to be promising for food packaging materials.

AITC Encapsulation to and Released from Electrospun AITC/PLA Fibers

We measured the GC peak areas of gaseous AITC samples from 1.0 μL up to 40.0 μL. The AITC standard curve was obtained by plotting the amounts of AITC in the gaseous samples against the correlative GC peak areas (Figure 3). In separate experiments, the AITC gaseous samples were measured after the samples were stored at the temperatures of −20, 4, and 25°C up to 400 h. There were no significant differences in GC measurements that could be detected (data not shown), indicating AITC gaseous samples were stable in the airtight GC vials, and no degradation, nor absorption to the glass walls of the vials occurred. Therefore, in following experiments, AITC measurement was carried out using the same standard curve and the same type of GC vials.

The efficiency of AITC encapsulation is shown in Table II. For the electrospinning solution containing 5% AITC, about 95% of the AITC was incorporated in the resultant fibers. However, the encapsulation rate decreased as the initial concentration of AITC increased. We observed during operation that, for the PLA mixture with higher AITC content, more of the jets flew "horizontally", instead of toward the drum. This can probably be attributed to phase separation of the nonelectrospinnable AITC from PLA. More experiments are needed to verify this hypothesis.

We then investigated the kinetics of the release of volatiles from P*f*A-*g*-films (AITC/PLA, 5% in electrospinning solution) at

Table II. Encapsulation Rate of AITC in Electrospun PLA Fibers

AITC/PLA in PLA solution (%)	AITC found in electrospun fibers (%)	Calculated encapsulation rate (%)
5	4.5 ± 0.2	94.5
10	7.7 ± 0.5	84.7
15	7.8 ± 0.7	59.8
25	9.7 ± 0.4	48.5

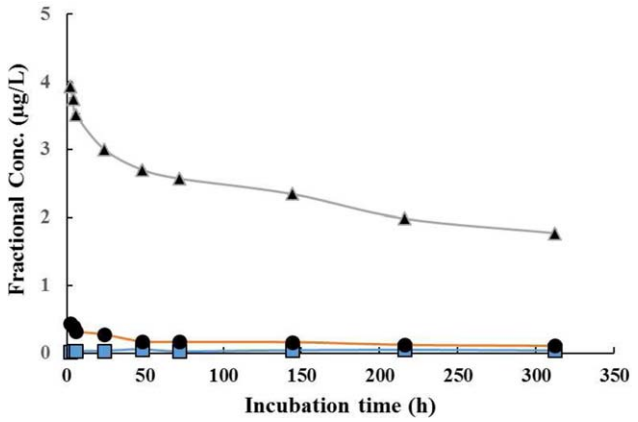

Figure 4. Fractional amount of AITC released from P*f*A-*g*-films (AITC/PLA, 5:100; w/w; in electrospinning solution) at −20°C (square), 4°C (circle), and room temperature (triangle). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

three different temperatures. As shown in Figure 4, for samples incubated at −20°C for 300 h, no AITC release could be detected; for samples incubated at 4°C for the same time period, a negligible amount of AITC was detected; while for samples incubated at room temperature, a sustained release curve was identified, where AITC released at the highest rate at the first three measuring points (up to 6 h) that were followed by a relatively slower rate for two continuous measurements taken at 24 and 48 h, after that AITC released at much slower rates.

The same trend was seen for other two P*f*A-*g*-films with AITC/PLA of 10 or 25% in the electrospinning solutions, respectively (Figure 5). The cumulative concentrations of AITC released from each sample can be divided into three zones: at the first 6 h, during the successive 42 h, and the following 400 h; about one-third of the total released AITC was found for each segment. AITC's bactericidal effect is concentration dependent; the higher the concentration used, the higher the antimicrobial activity obtained.[24] It has also been reported that the minimal inhibition concentration for bacteria of gaseous AITC is 30–110

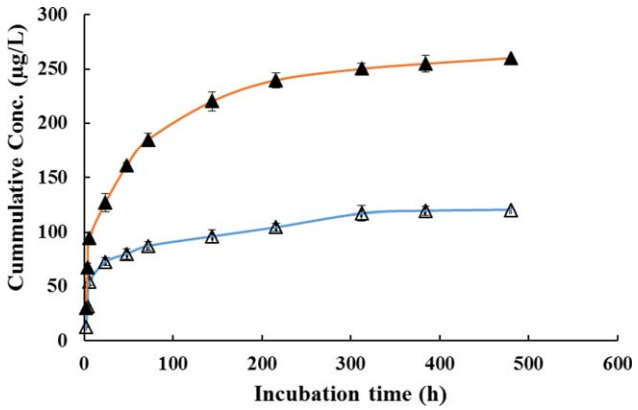

Figure 5. Cumulative amount of AITC released from P*f*A-*g*-films at room temperature. AITC in the fibers: 10% (open triangle) and 25% (solid triangle). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

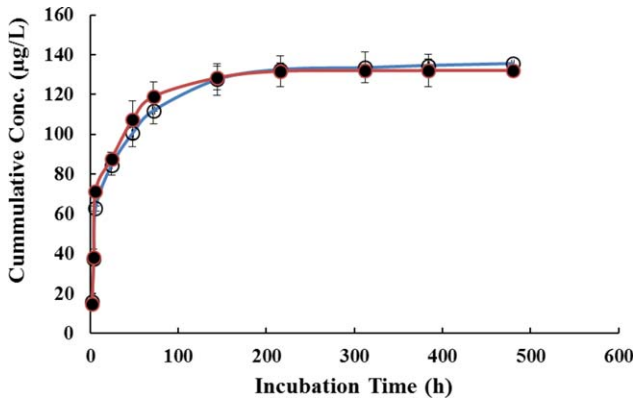

Figure 6. Cumulative amount of AITC released from P*f*A-*g*-films at room temperature after storage at 4°C for 3 weeks (open circle) or a day (solid circle). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

μg/L.[25,26] This requirement could be met by using the P*f*A-*g*-films presented in this study. As shown in Table II and Figure 5, by incubating a piece of grafted film with AITC content higher than 7% (in fiber phase) in a closed system at room temperature, the AITC concentration in the head space reached the minimum value in a few hours, and then continued increasing and was maintained at this value for weeks, which is much longer than that made by film casting method.[10] This could be attributed to the larger surface area owned by the fibers that facilitated the continuous and completed release of incorporated bioactives in this case. In a separate experiment, two samples, both containing 7.8% AITC in the fiber phase, were measured at room temperature for the AITC cumulative release after storage at 4°C for different time periods. As shown in Figure 6, the length of preincubation at 4°C showed no effect on the AITC release profile. It was also found that higher content of AITC in the electrospinning solution correlated with a larger amount of AITC release and a sharper initial release (Figures 4–6). More AITC may accumulate on the surface areas of the fibers that were electrospun from PLA solutions with higher AITC content, in comparison with other electrospinning solutions with lower AITC. An alternative approach to reduce the initial burst of AITC is the pre-encapsulation of the volatile in a second vehicle, such as cyclodextrin prior to electrospinning with PLA, although it adds one step to the process.[4] It is worthwhile noticing that the release of AITC was a function of storage temperature (Figure 4), AITC released from PLA fibers at room temperature, but not at 4°C or lower. At lower temperature, the movement of PLA molecular segments was limited that enhanced the level of chain–chain packing and reduced interstitial spaces among the macromolecular chains. The interstitial space is a measure of fiber-free volume, which determines the mass permeability. Meanwhile, at lower temperature, the movement of the low-molecular-weight AITC was also restricted. These properties should be interesting for the application of the films as active packaging materials for meats, fishes, fruits, and other perishable commodities. Temperatures are controlled at different points for different stages of the supply chain for fresh foods and produce to address the concerns of safety and quality, and the cost. As shown by the present research, AITC encapsulated in PLA fiber is able to maintain its quantity and activity at low temperature. The combination of temperature control and AITC release is expected to further enhance the efficiency of AITC and broaden the utility of the P*f*A-*g*-films in the packaging industries.

Antimicrobial Activity

The antimicrobial activity of the P*f*A-*g*-films on foods was tested using samples prepared from 25% AITC/PLA in electrospinning solutions. The results are shown in Figure 7. Populations of *E. coli* k12 cells on grape without AITC films increased to 3000 CFU/g after 40 h storage at 22°C while the grape sample with P*f*A-*g*-film only had 30 CFU/g. During the same storage period, populations of *E. coli* cells on turkey RTE meat without P*f*A-*g*-film treatment increased to 63,000 CFU/g, but the samples with P*f*A-*g*-film treatment increased to 630 CFU/g. The P*f*A-*g*-films significantly reduced the populations of *E. coli* in grape or meat products [Figure 7(A)]. Similarly, P*f*A-*g*-film significantly inhibited the growth of *L. innocua* on grape and meat samples, as compared 10,000 CFU with 63 CFU for grape and 100,000 CFU with 800 CFU for meat [Figure 7(B)].

Our previous studies demonstrated that AITC coated onto biodegradable composite films exhibited strong antimicrobial activities against food-borne pathogens, such as *Salmonella* and *Listeria monocytogenes*.[10,27] Other studies also showed that AITC is an effective inhibitor of various pathogens in food.[13,20,28,29] This study provides a new approach demonstrating use of electrospun PLA/AITC fibers-grafted PLA films as a new type of

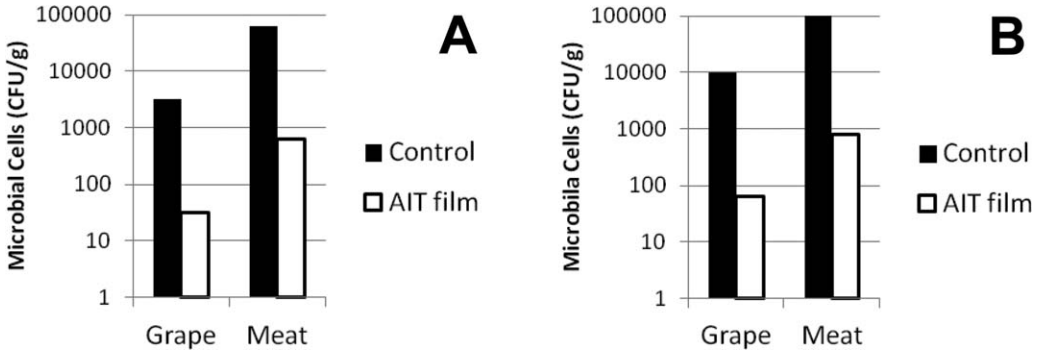

Figure 7. Populations of *E. coli* K12 (A) and *L. innocua* (B) in grape and RTE meat after storage at 22°C for 40 h.

active packaging materials for food packaging via delivering gaseous antimicrobials.

CONCLUSIONS

In this research, AITC and PLA were co-dissolved in DCM and electrospun onto a PLA film, resulting in bead-free or beaded-PLA fibers with AITC encapsulated, depending on the AITC content. The resultant fibers were permanently grafted on the PLA films, were able to retain the quantity and activity of the encapsulated AITC, when stored at the temperatures lower than 4°C for at least 2 weeks as the experiment ended, and release it in a temperature-dependent manner for up to 3 weeks. The released AITC was active in suppressing the growth of *E. coli* K12 and *L. innocua*. Under experimental conditions, the electrospinning/grafting process did not alter the mechanical properties of PLA films. The PLA/AITC fibers-grafted PLA films showed potential for the applications of antimicrobial food packaging.

This research describes a new method to fabricate PLA films grafted with PLA fibers; the substrate film functions as mechanical barrier and the fibrous layer controls the release of encapsulated bioactives. The method can be applied for making other bilayered materials, in which the substrate material and the fiber material share the same good solvent. The electric conductivity of the substrate materials is one of the elements determining the properties and morphology of the fibers and the characteristics of the final products, and remains for continuous research.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Ms. Audrey Thomas-Gahring, Mr. Joseph Uknalis, and Mr. Nicolas Latona for their technical assistance.

REFERENCES

1. U.S. Active & Intelligent Packaging Market. Freedonia. January 2014. http://www.reportlinker.com/p0559165-summary/Active-Intelligent-packaging-Market.html (accessed on February 20, **2015**).
2. Song, L.; Iori, R.; Thornalley, P. J. *J. Sci. Food Agric.* **2006**, *86*, 1271.
3. Romanowski, F.; Klenk, H. In Ullmann's Encyclopedia of Industrial Chemistry; U. Weinheim, etc., Eds.; Wiley-VCH, **2005**.
4. Vega-Lugo, A.-C.; Lim, L.-T. *Innov. Food Sci. Emerg. Technol.* **2011**, *12*, 426.
5. Winther, M.; Nielsen, V. P. *J. Food Protection* **2006**, *69*(10), 2430.
6. Shin, J.; Harte, B. R.; Ryser, E. T.; Selke, S. E. *J. Food Sci.* **2010**, *75*(2), M65.
7. Ko, J. A.; Kim, W. Y.; Park, H. J. *Int. J. Food Microbiol.* **2012**, *153*, 92.
8. Raouche, S.; Mauricio-Iglesias, M.; Peyron, S.; Guillard, V.; Gontard, N. *Innov. Food Sci. Emerg, Technol.* **2011**, *12*(4), 426.
9. Liu, LS.; Jin, T. Z.; Coffin, D. R.; Liu, C.-K.; Hicks, K. B. *J. Appl. Polym. Sci.* **2010**, *117*, 486.
10. Li, W.; Coffin, D. R.; Jin, T. Z.; Latona, N.; Liu, C-K.; Liu, LS. *J. Appl. Polym. Sci.* **2012**, *126*, E362.
11. Jin, T.; Zhang, H. *J. Food Sci.* **2008**, *73*(3), M127.
12. Torres-Giner, S.; Martinez-Abad, A.; Gimeno-Alcañiz, J. V.; Ocio, M. J.; Lagaron, J. M. *Adv. Eng. Mater.* **2012**, *14*(4), B112.
13. Dai, R.; Lim, L-T. *J. Food Sci.* **2014**, *79*, E47.
14. Kim, H.-W.; Lee, H.-H.; Knowles, J. C. *J. Biomed. Mater. Res. Part A.* **2012**, *79A*(3), 643.
15. Chen, J.; Zhou, B.; Li, Q.; Ouyang, J.; Kong, J.; Zhong, W.; Xing, M. M. Q. *Int. J. Nanomed.* **2011**, *6*, 2533.
16. Sousa, A. M. M.; Souza, H. K. S.; Uknalis, J.; Liu, S.-C.; Gonçalves, M. P.; & Liu, L. S. *Carbohyd. Polym.* **2015**, *115*, 348.
17. Ignatova, M.; Starbova, K.; Markova, N.; Manolova, N.; Rashkov, I. *Carbohyd. Res.* **2006**, *341*, 2098.
18. López-Rubio, A.; Sanchez, E.; Wilkanowicz, S.; Sanz, Y.; Lagaron, J. M. *Food Hydrocolloids* **2012**, *28*, 159.
19. Uddin, A. J.; Araki, J.; Fotoh, Y. *Biomacromolecules* **2012**, *12*, 617.
20. Liu, L. S.; Liu, C-K.; Fishman, M. L.; Hicks, K. B. *J. Agr. Food Chem.* **2007**, *55*(6) 2349.
21. Liu, L. S.; Kost, J. In Encyclopedia of Packaging Technology; Yam, K. L., Ed.; John Wiley & Sons, Inc.: New York, **2009**, 110.
22. www.innoviafilms.com (accessed April 15, **2015**).
23. www.canadianbiomassmagazine.ca (accesses April 25, **2015**).
24. Jin, T.; Gurtler, J. *J. Appl. Microbiol.* **2011**, *110*, 704.
25. Delaquis, P. J.; Sholberg, P. L. *J. Food Protection* **1997**, *60*, 943.
26. Isshiki, K.; Tokuoka, K.; Mori, R.; Chiba, S. *Biosci. Biotech. Bioche.* **1992**, *56*(9), 1476.
27. Li, W.; Liu, LS.; Jin, T. *J. Food Protection* **2012**, *75*(12): 2234.
28. Chen, W.; Jin, T.; Gurtler, J. B.; Geveke, D. J.; Fan, X. *Int. J. Food Microbiol.* **2012**, *155*, 165.
29. Kinae, N.; Masuda, H.; Shin, S.; Furugori, M.; Shimoi, K. *BioFactors* **2000**, *13*, 265.