

# Biodegradable Composites from Polyester and Sugar Beet Pulp with Antimicrobial Coating for Food Packaging

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**ABSTRACT:** Totally biodegradable, double-layered antimicrobial composite films were introduced for food packaging. The substrate layers of the sheets were prepared from poly(lactic acid) (PLA) and sugar beet pulp (SBP) or poly(butylene adipate-co-terephthalate) (PBAT) and SBP by a twin-screw extruder. The active layer consisting of antimicrobial in either PLA or chitosan was formed on the surfaces of the substrates by solution coating followed by solvent evaporation. Air-coupled ultrasonic evaluation and microscopic examination showed a homogeneous structure for the composite sheets. Mechanical analysis and acoustic

emission demonstrated the double-layered composites possessing appropriate tensile strength and fracture characteristics for the use as light weight-bearing materials. The release of Nisaplin<sup>®</sup> or allyl isothiocyanate (AIT) effectively suppressed the growth of *Listeria monocytogenes* and *Salmonella* cells, respectively; the co-release of Nisaplin<sup>®</sup> and AIT played a synergistic effect on inhibition of *L. monocytogenes*. © 2012 Wiley Periodicals, Inc. <sup>†</sup> J Appl Polym Sci 000: 000–000, 2012

**Key words:** biodegradable; biopolymers; drug delivery systems; composites; extrusion

## INTRODUCTION

“Green packaging” and “active packaging” are frequently used phrases in the literature of the food packaging community.<sup>1,2</sup> The former refers to the use of environmentally friendly packaging materials; the latter refers to the application of technologies that can interfere with biochemical processes occurring in the headspace or on the surface of packaged foods. This can be achieved either by releasing preincorporated bioactives to the surrounding area, or by absorbing harmful substances from it. The selection of adequate materials and technologies for food preservation is based on their capability to maintain food quality, and their impact on the economy and environment. “Green packaging” and “active packaging” represent an irreversible trend in the production of advanced packaging materials. Along this direction, a series of

composite materials designed for food packaging have been developed,<sup>3–8</sup> including those presented by our laboratory.<sup>9–12</sup> These composites consist of biodegradable poly(lactic acid) (PLA) or poly(butylene adipate-co-terephthalate) (PBAT) as a matrix phase, and an agricultural byproduct of sugar beet pulp (SBP) as a filler phase. Antimicrobial actives can be incorporated into the composites during or post material processing. The resultant composites showed excellent activity against Gram-positive, Gram-negative, or Gram-positive/negative bacteria.<sup>7–12</sup> PLA is obtained from ring-opening polymerization of lactide or direct polymerization from lactic acid, a fermentation product of plant polysaccharides. PLA brings in enormous advantages, such as processability with currently existing instrumentation for conventional thermoplastics, flexibility in thermal and mechanical properties, and nontoxicity of its final degradation products,<sup>9,10</sup> although its gas barrier properties remain to be improved<sup>1</sup>. Currently, the annual production of PLA is around 300,000 tons worldwide, and it has become a major research focus for green packaging and active packaging.<sup>11–14</sup> However, the application of PLA as food packaging material is restricted due to its relatively higher cost of production in comparison with fossil fuel derived thermoplastics. PBAT, known by the trade name of Ecoflex<sup>®</sup>, is a globally certified biodegradable thermoplastic, derived from fossil fuels. It is ideal for disposable packaging as it decomposes in compost.

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However, as the price of crude oil increases, the use of PBAT also faces a challenge.

Recently, we converted SBP to a thermoplastic-like material (TSBP), and then coextruded it with either PLA or PBAT to obtain novel biodegradable composites,<sup>15,16</sup> PLA/TSBP, and PBAT/TSBP. The composites thus prepared can retain the mechanical properties of their parent plastics, even at higher SBP contents up to at least 50% by weight. The U.S. beet sugar industry generates 1.5 million dry tons of SBP annually. It is now mainly sold as low priced livestock feed. The use of SBP to manufacture TSBP containing composites represents a new approach to effectively reduce the cost of making totally biodegradable thermoplastics.

In this research, price-competitive, active packaging materials from PLA/TSBP and PBAT/TSBP were prepared by loading onto their surfaces two well known bioactives, the polypeptide nisin (in the commercial formulation of Nisaplin<sup>®</sup>), and a plant essential oil of allyl isothiocyanate (AIT). Both AIT and Nisaplin<sup>®</sup> have been approved by FDA for the use in food industries. The composite films thus prepared are intended to be further processed as food containers. The structural and mechanical properties of the composite films, as well as their antimicrobial activities were investigated.

## EXPERIMENTAL

### Materials

PLA with a weight-average molecular weight of 148 kDa and a number-average molecular weight of 110 kDa (product No. 4060D) was obtained from Nature Works LLC (Minnetonka, MN). PBAT (Ecoflex F BX7011) was obtained from BASF (Florham Park, NJ), having a density of 1.26 g/cm<sup>3</sup>, a weight-average molecular weight of 145 kDa and polydispersity of 2.4 (GPC analysis). Dichloromethane (DCM) was purchased from Fisher Scientific (Barrington, IL). Chitosan (low molecular weight) and AIT were purchased from Sigma Aldrich (St. Louis, MO). Nisaplin<sup>®</sup> containing 2.5% nisin was from Danisco Cultor USA (New Century, KS). Acetic acid was purchased from Mallinckrodt (St. Louis, MO). Polymeric diphenyl methane diisocyanate (pMDI) was from Bayer Material Science, LLC (Pittsburgh, PA). All chemicals were used as received.

*Listeria monocytogenes* and *Salmonella* Stanley were obtained from the culture collection of the U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center. Bacterial culture media, tryptic soy broth (TSB) and brain heart infusion broth (BHIB) were from Difco (Becton, Dickinson, and company; Sparks, MD). The strains were maintained in TSB at 2°C and transferred bimonthly.

### Preparation of composite films

Composite thermoplastics, PLA/TSBP and PBAT/TSBP, were prepared by converting SBP to TSBP fol-

lowed by co-extrusion with PLA or PBAT.<sup>15,16</sup> Briefly, SBP was first mixed with glycerol and water (5 : 1 : 1; w/w) by blending the three parts in a kitchen mixer, and stored in sealed plastic bags at room temperature for at least 8 h. This formulated SBP was then extruded to obtain thermoplastic SBP (TSBP) using a corotating twin-screw extruder (Leistritz ZSE-18). The diameter of the screw was 18 mm with a length-to-diameter ratio ( $L/D$ ) of 40. The extruder has seven individual heating zones and an adapter/die section. The zone temperatures were set from 80°C (feed zone) to 90°C (die) and the screw speed was 100 rpm.

Composite films, PLA/TSBP and PBAT/TSBP (50/50; w/w), were prepared using the same extruder with the addition of 2% pMDI (w/w). The zone temperatures were set at 120-130-130-130-130-, and 130°C from feeding zone to die for PBAT/TSBP; and 120-175-180-180-180-180-, and 170°C from feeding zone to die for PLA/TSBP; the screw speed was 100 rpm. The moisture was vented at the sixth heating zone of the barrel. The extruded films were cool down to 45°C on a floor standing 3-roll haul-off system (Thermo Fisher Scientific, England).

Films thus obtained had thicknesses of 0.62 mm and 0.87 mm for PLA/TSBP and PBAT/TSBP, respectively. The films were cut to rectangular shapes, 115 mm × 230 mm for airborne ultrasonic evaluation, 5 mm × 30 mm for the dynamic mechanical analysis, 10 mm × 100 mm for acoustic emission and mechanical property analysis; and a circular shape 50 mm in diameter for the antimicrobial tests. Appropriate sized samples were cut out for microscopic examination. Specimens loaded with bioactives were also cut out and tested. The visual images of film specimens were recorded with a Nikon D1x (Tokyo Japan) camera equipped with a Nikon 100-mm macro lens.

### Loading bioactives on the composite films

Nisaplin<sup>®</sup> and AIT were loaded on PLA/TSBP or PBAT/TSBP either by dipping the composite films in the bioactive formulations (Table I) or casting the formulations on the surfaces of the composite films. The films were then placed in a chemical hood for 4 h to allow the solvents to evaporate. Films thus dried were stored in zipped plastic bags and used within 3 days. The amount of nisin and AIT loaded on the film surfaces of was 0.51 mg/cm<sup>2</sup> and 20.4 μL/cm<sup>2</sup>, respectively, found by measuring the weight change of each individual film and the concentration of actives in the correlative formulations.

### Microscopic imaging

Microscopic examination was carried out by scanning electron microscopy (SEM) and confocal laser

**TABLE I**  
**Composition of Bioactive Coating Solutions**

Solvent, 10 mL	PLA (mg)	Chitosan (mg)	AIT ( $\mu$ L)	Nisaplin <sup>®</sup> (mg)
Dichloromethane	200	0	0	0
	200	0	200	0
	200	0	0	200
	200	0	200	200
1% Acetic acid	0	200	0	0
	0	200	200	0
	0	200	0	200
	0	200	200	200

scanning microscopy (CLSM) as described previously.<sup>12</sup> All specimens were freeze-dried prior to microscopic examination. For SEM examination, samples were coated with a thin layer of gold in a sputter coating apparatus (Edwards High Vacuum, Wilmington, MA), then examined with a Quanta 200 FEG SEM (FEI, Hillsboro, OR) in the high-vacuum/secondary electron imaging mode. Digital images were collected at 50 $\times$ , 500 $\times$ , and 1000 $\times$ .

For CLSM examination, film samples were glued to microscope slides (1  $\times$  3 in.) and placed in the sample stage of an IRBE optical microscope with a 10 $\times$  lens integrated with a laser scanning microscope (Leica Microsystems, Exton, PA). The parameters for the image acquisition were set for confocal reflection (633 nm) and confocal fluorescence (excitation 412, emission 475 nm) in two channels.

### Airborne ultrasonic inspection

The airborne ultrasonic (AU) inspection was performed on a custom-made AU tester that was manufactured by NDT Automation (Princeton Junction, NJ).<sup>17,18</sup> The experimental setup consists of two ultrasonic transducers having a central frequency of 200 kHz, a transmitter with a 50 mm active area, which transmits ultrasonic pulses with a tone burst through a power amplifier, and a receiver with a 25 mm active area connected to a preamplifier. The receiver detects and records the numbers and intensity of pulsing waves that penetrated through the film. The transducers were mounted on a X-Y scanner controlled by a PC using software UTWIN version E1.81 (NDT Automation; Princeton Junction, NJ) that allows the transducer array to be moved over the entire surface of a specimen. The UTWIN software is used to analyze the ultrasonic quantities and their distribution in an image and to create a classifier capable of assessing the structure of a film specimen. The amplitudes of the transmitted airborne signals at every point on the film surface were measured, color-coded, and mapped into an image file for each specimen. These color-coded amplitude maps are called "C-scan."

### Dynamic mechanical analysis

Dynamic mechanical analysis (DMA) was performed on a TA Instruments Q800 Dynamic Mechanical Analyzer.<sup>20</sup> The storage modulus ( $E'$ ), loss modulus ( $E''$ ), and loss tangent (delta tangent) were measured as a function of temperature. A nominal strain of 0.1% was used with an applied frequency of 1.50 Hz. Each sample was equilibrated in the sample chamber under dry nitrogen at  $-50^{\circ}\text{C}$  for at least 2 min prior to the test. The operation temperature was increased at a heating rate of  $5^{\circ}\text{C}/\text{min}$ ; data were collected from  $-50^{\circ}\text{C}$  to  $100^{\circ}\text{C}$  and analyzed with the TA Instruments Universal Analysis 2000 software (version 4.5A).

### Mechanical test and acoustic emission analysis

The PLA/TSBP and PBAT/TSBP films with and without antimicrobial coating were tested for their mechanical properties, tensile strength, modulus, elongation, and fracture energy according to the methods reported previously.<sup>19</sup> The tensile strength is defined as the maximum stress to fracture of the composite samples. Modulus is a physical property representing the stiffness of a composite. It is measured by determination of the slope of a line tangent to the initial stress-strain curve. Fracture energy, also called toughness, is determined by the measurement of the energy needed to fracture the specimens and is the area under the stress-strain curve. All samples were stored in desiccators at  $23^{\circ}\text{C}$  and 50% relative humidity overnight, and tested under the same conditions. A mechanical property tester (MTS Insight 5) and Testworks 4 data acquisition software (MTS Systems, Minneapolis, MN) were used throughout this test. The gauge length was 50 mm; the strain rate (crosshead speed) was set at 5 mm/min.

Acoustic emission (AE) measurements tests were performed simultaneously with mechanical property examination.<sup>19</sup> A small piezoelectric transducer was clipped against the samples. This transducer resonated at 150 kHz (Model R15, Physical Acoustics, Princeton, NJ) and was 10 mm in diameter. AE signals captured by this transducer when the mechanical property tester stretched the samples were processed with an AE WIN analyzer (Physical Acoustics). The AE WIN was connected to a PC base with enhanced graphing and data acquisition software with all the features and options of the SPARTAN 2000.

### Inoculum preparation and antimicrobial test

Foodborne pathogenic Gram-positive *L. monocytogenes* Scott A and Gram-negative *Salmonella* Stanley H0558 were selected for use in this study. Prior to



inoculum preparation, *S. Stanley* (in TSB) and *L. monocytogenes* (in BHIB) were grown aerobically at 37°C for 16–18 h.

Composite films loaded with and without Nisaplin® or AIT were tested using a liquid release method as described previously.<sup>12,21</sup> Each film sample was cut to 10 pieces and exposed to UV light (Germicidal ultraviolet irradiator, Peoria, AZ) for 5 min at 8 mW/cm<sup>2</sup> to eliminate possible microbial contamination. Five pieces of each film sample (9.8 cm<sup>2</sup> of total coated surface areas) were placed in a glass tube containing 10 mL of BHIB or TSB inoculated with overnight cultures of *L. monocytogenes* or *S. Stanley* with cell concentrations of about  $1 \times 10^4$  CFU/mL. The test tubes were held at room temperature and shaken at 100 rpm. Inoculated medium, 1.0 mL, was sampled from each tube at time points of 0, 24, and 48 h. Samples were serially diluted with sterile 0.1% peptone water, and surface plated onto BHI agar or TSB agar (100  $\mu$ L per plate and two plates per dilution). All plates were incubated at 37°C and colony forming units (CFU) were counted after 24 h. Inoculated medium without film sample served as negative controls and inoculated medium with noncoated film sample served as positive controls.

### Statistical analysis

Antimicrobial experiments were conducted in duplicate. Data were analyzed using analysis of variance with SAS version 9.2 software (SAS Institute; Cary, NC). Duncan's multiple range testing was used to determine the significant differences of mean values. Significance was defined at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

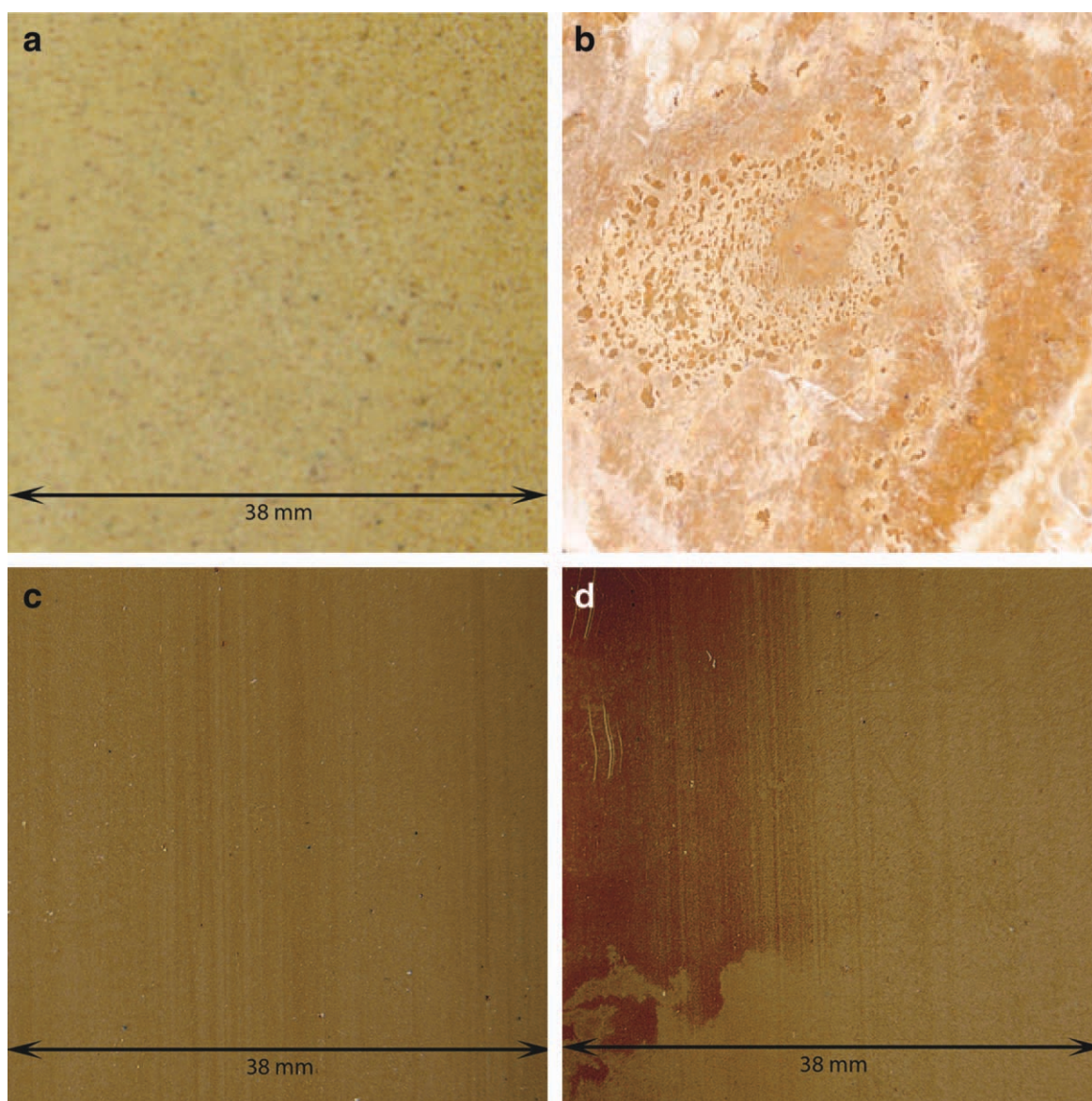
### Morphology and airborne ultrasonic inspection

Both PBAT/TSBP and PLA/TSBP were smooth to the touch, with the former being more flexible than the later. PLA/TSBP and PBAT/TSBP and their correlative coating composites were first examined visually for appearance; this was followed by a set of nondestructive tests for structural characterization. These included airborne ultrasonic inspection, scanning electron microscopy, confocal laser scanning microscopy, and dynamic mechanical analysis. The appearances of the composites are shown in Figure 1. Both PLA/TSBP [Fig. 1(a)] and PBAT/TSBP [Fig. 1(c)] were flat and opaque. No individual particles could be distinguished in the matrix phase of PBAT, although individual particles were barely enough to view in the PLA composites. These visual characteristics were very different from what we had found with extruded PLA/pectin films, where

individual pectin particles could be clearly seen.<sup>12</sup> In this study, the SBP was converted to a thermoplastic-like material TSBP prior to coextrusion with polyesters; the TSBP is much more miscible with PLA or PBAT than the nonpretreated SBP or pectin. The inclusion of pMDI further enhanced intersurface adhesion between the two phases.<sup>22</sup> For PLA/TSBP coated with the suspension of Nisaplin® in PLA [Fig. 1(b)] or PBAT/TSBP coated with the chitosan in diluted acetic acid, crinkled surfaces were observed after coating, where isolated small light-yellow spots scattered throughout the surfaces [Fig. 1(d)]. Supposedly, those spots were Nisaplin® and chitosan precipitations that were formed, when the aqueous phase was removed. This could be attributed to the hydrophobic/hydrophilic heterogenic surface property of this composite. The hydrophilic Nisaplin® and chitosan prefers to dwell on the relatively more hydrophilic areas. Furthermore, the chitosan coating failed to form a continuous layer adhered to PLA/TSBP films, but easily form a good coating on the PBAT/TSBP films.

The C-scan images from the AU inspection showed a "wave" pattern with vortices for both composites (Fig. 2). In other words, viewed with AU inspection, the composites' surfaces are no longer smooth. The distortion is fairly regular. This elastic phenomenon, normally called melt fracture, is often seen for polymeric extrudates having a larger size in width (or diameter) than the die, when a polymer is extruded at the speed above the critical flow rate (however, the surface may become smooth again as the shear stress is further increased).<sup>23,24</sup> For filler-matrix type composites, one can imagine that the shear stress of fluids around the fillers is different from that away from the fillers; the former is dependent on the interfacial properties; the latter is inversely proportional to the weight average molecular weight of the polymer.<sup>25</sup> In this case, the mobility of the fillers was different from the matrices at temperatures over the melting point of PLA or PBAT, and caused "slipping" of melted fluids around the particles,<sup>26</sup> thus resulting in a surface roughness as revealed by AU inspection.

AU is an ideal inspection method for large items, such as pipes and containers, or biopolymers of leather and hide, etc.<sup>17</sup> AU inspection involves pulsing ultrasonic signals at the materials and measuring the penetrated signal amplitude. With the use of the UTWIN software, the ultrasonic energy penetrating through the composite films was measured, color-coded, and mapped (Fig. 3). A dark blue color indicates low penetration of the tone burst through the materials, a dark red color refers to a large signal transmitted, other colors in the spectrum ranging from red to blue point out the degree of penetration (Fig. 3). As shown in Figure 2, both the PLA/TSBP

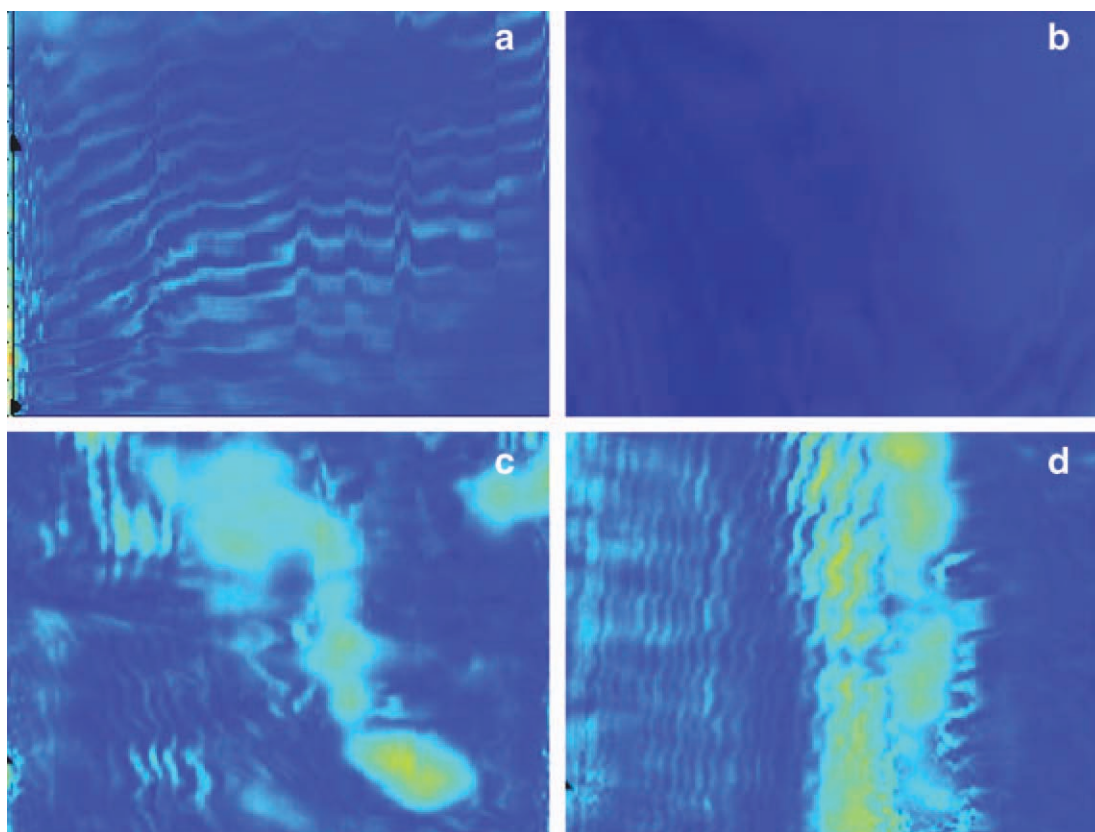


**Figure 1** Photographs of PLA/TSBP (a), PLA/TSBP film coated with Nisaplin® dispersed in PLA/dichloromethane (b), PBAT/TSBP (c), and PBAT/TSBP coated with chitosan in diluted acetic acid solution (d). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

(a) and PBAT/TSBP (b) display low amplitude of ultrasonic signals emanating from the materials, being colored blue and light blue. However, coating the composite films with PLA/DCM (c) or chitosan/acetic acid solutions (d) enhances signal penetration (yellow color indicates high amplitudes). This indicates some changes in their physical properties occurred when coating was carried out. We have traced the weight change of samples before and after coating and during the drying. It was found that DCM evaporated from the films to the air in less than 4 h, while more than 80% water from the diluted acetic acid was still remained (data not shown). TSBP is a hydrophilic component, and it may have absorbed the aqueous solution and

“kicked out” entrapped air. Since air has much low acoustic impedance; while water is a fairly good match for most commonly used materials, including biopolymers. More energy lost when the sound has to move between a plastic and air than passing a solid–liquid surface or a wet-biopolymer. This may contribute to the increase of ultrasonic signals detected after coating with chitosan in dilute acetic acid solution. For the cases using DCM as solvent, some resident PLA may be dissolved and thus its crystallinity would be reduced when it reprecipitated under the present experimental conditions. The velocity of an ultrasonic wave is proportionally increases at a direct ratio of the elastic constant and at an inverse ratio with the density of the material<sup>27</sup>;



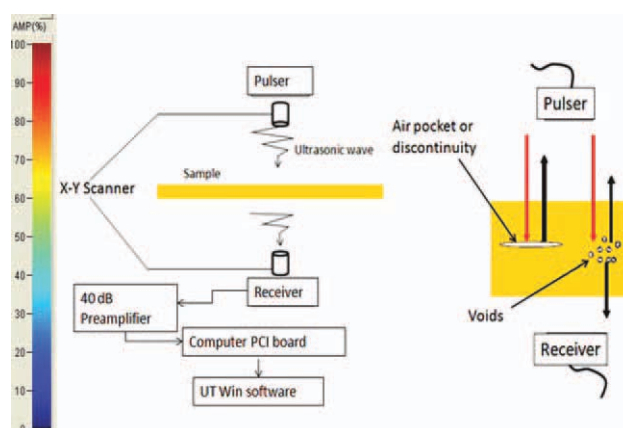


**Figure 2** C-scan of PLA/TSBP (a), PBAT/TSBP (b), PLA/TSBP coated with PLA and nisaplin in dichloromethane (c), and PBAT/TSBP coated with chitosan in diluted acetic acid (d). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

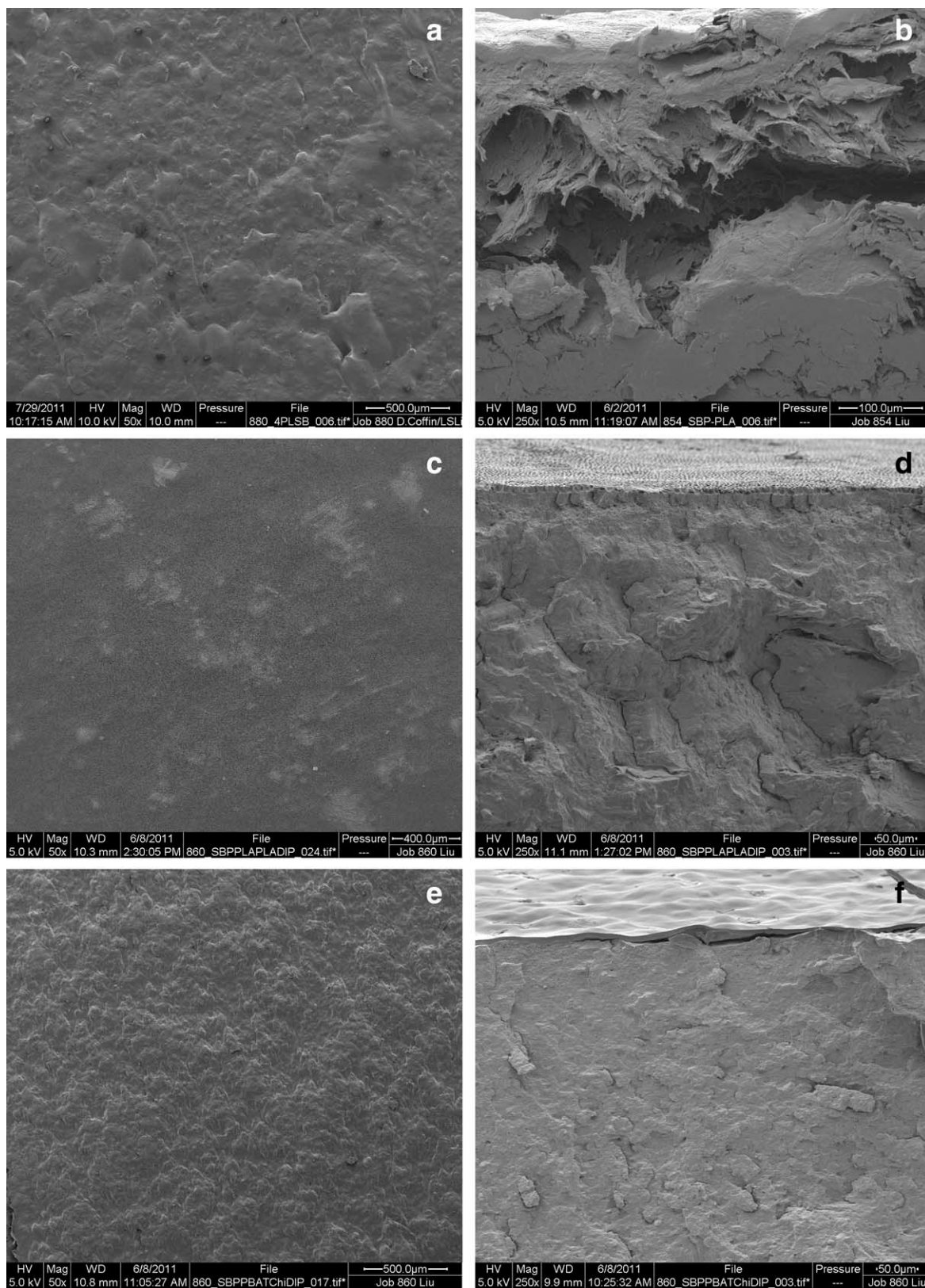
accordingly, more acoustic signals can be captured by the receiver.

SEM images are shown in Figure 4. The thickness of the bioactive coating layers is about 7.5  $\mu\text{m}$ . In contrast to visual observation, the PLA/TSBP composite films showed a rough morphologic character under microscopic observation [Fig. 4(a)]. With higher magnification, one can see TSBP threads stretched in PLA matrix phase. There were also small pores and gaps with entrapped air dispersed under the surface of the films that were generated during extrusion [Fig. 4(b)]. Similar morphology was observed for PBAT/TSBP films. After coating the PLA/TSBP with PLA/DCM, the surfaces of the films became smooth [Fig. 4(c)]. This could be attributed to the dissolution of resident PLA in DCM. Figure 4(d) also showed that the solvent penetrated into the films as much as 10–50  $\mu\text{m}$  in depth. The PLA solution penetrated into voids and gaps, which were occupied by air originally. The solute re-precipitated when the solvent was evaporated, driving entrapped air away. Coating PBAT/TSBP films with chitosan in dilute acetic acid solution resulted in a wrinkled thin layer laying on the surface of the films [Fig. 4(e,f)], being consistent with visual observation.

The results of CLSM examination are shown in Figure 5. Both TSBP particles [Fig. 5(a)] and Nisaplin<sup>®</sup> powders [Fig. 5(b)] have autofluorescence at 412/475 nm (ex/em) due to their polypeptide components; however, the TSBP has a much large particle size than the nisin. The extruded PLA/TSBP composites showed a homogeneous distribution of TSBP (colored green) in PLA matrix phase (colored

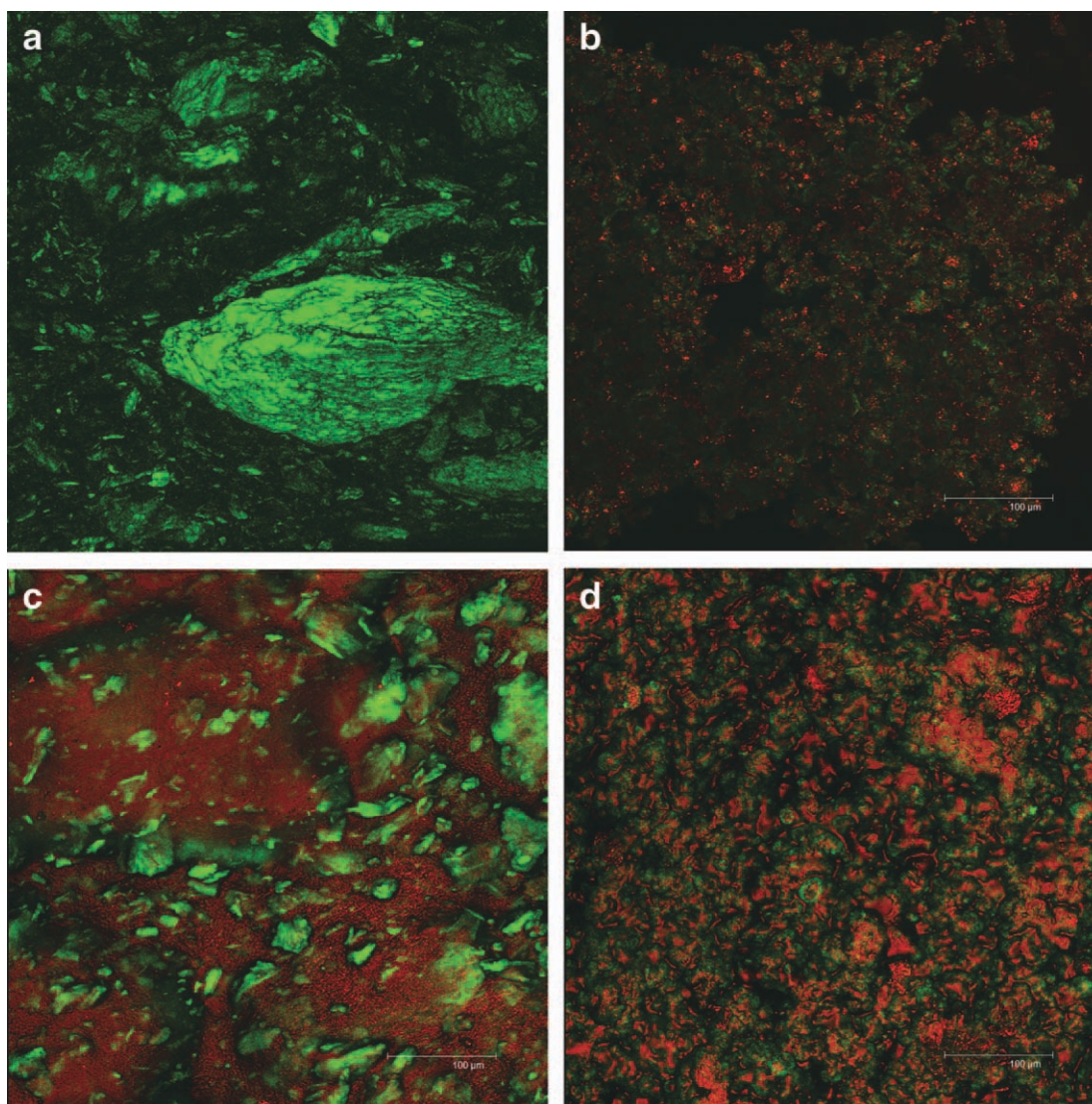


**Figure 3** Experimental setup used for composite film AU evaluation. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



**Figure 4** SEM images of PLA/TSBP composite films (a and b) and the films coated with 2% Nisaplin<sup>®</sup> suspended in 2% PLA/dichloromethane (c and d); PBAT/TSBP films coated with 2% chitosan in dilute acetic acid aqueous solution (e and f). a, c, and e are the top view of the samples; their width length is 2.7 mm. b and d and f are the view of cross sections of correlative samples; the width length is 0.54 mm for b and 0.32 mm for d and f.





**Figure 5** CLSM images of TSPB particles (a), Nisaplin<sup>®</sup> powders (b), PLA/TSPB composites (c), and PLA/TSPB films coated with Nisaplin<sup>®</sup> in PLA/DCM (d). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

red by confocal reflection at 633 nm) [Fig. 5(c)]. Coating with Nisaplin<sup>®</sup> in PLA/DCM resulted in a “covering” of TSPB images (large green particles) by the Nisaplin<sup>®</sup> consisting of nisin (small green powders) and small salt particles (colored red by confocal reflection) [Fig. 5(d)]. The PLA precipitated from the coating solution cannot be distinguished from the matrix phase by CLSM.

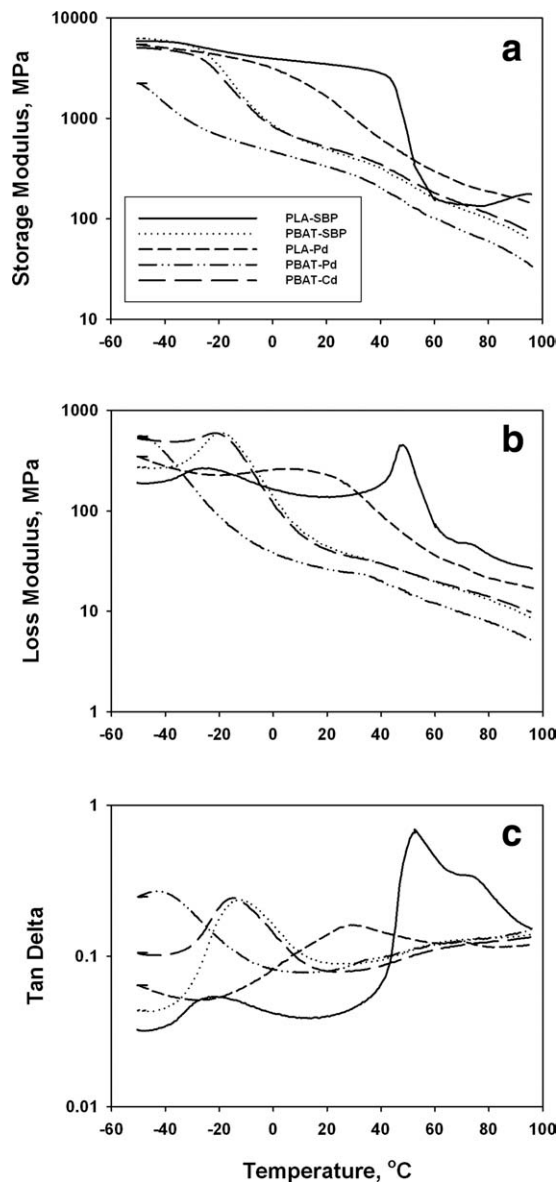
#### Dynamic mechanical analysis

Dynamic mechanical analysis is used to measure changes in the mechanical properties of a plastic material, and thus is very sensitive to changes in the temperature and size of thermal transitions. Using DMA to track changes in thermal transitions that result from changes in composition and processing variables can complement microscopic methods such

as SEM and ultrasound inspection to correlate changes in properties and morphology.<sup>28</sup>

In this study, The PLA/TSPB and PBAT/TSPB films and the films that had been coated with PLA in DCM or chitosan in dilute acetic acid were evaluated by DMA using the conditions described in the experimental section. The results are shown in Figure 6. The decrease in glass transitions are evidenced by a drop in the value of the storage modulus, or by peaks in the loss modulus and loss tangent curves. The PLA/TSPB films showed a fairly sharp transition at about 49°C, while the PBAT/TSPB showed a somewhat broader transition centered at -18°C. This difference between the two types of films was expected, since PLA is a semicrystalline homopolymer, and PBAT is amorphous random copolyester. For the PBAT/TSPB films coated with chitosan in acetic acid, there is very little change in the DMA curve,





**Figure 6** Typical plots of  $E'$  (a),  $E''$  (b) and tangent  $\delta$  (c) as functions of temperature.

although the glass transition appeared to drop slightly to  $-21^{\circ}\text{C}$ . Thus it appears that the chitosan coating has little or no effect on the thermodynamic mechanical properties of the original matrix. There is also a very small peak at about  $40^{\circ}\text{C}$  in the storage modulus curve which was unaffected by the chitosan coating treatment. The effect of coating the films with 2% PLA in DCM is quite different, however. The main transition in the PLA/TSBP films was very significantly broadened, and the temperature of the transition was lowered to about  $19^{\circ}\text{C}$ . The temperature of the transition seen in the PBAT/TSBP films was lowered to at least  $-48^{\circ}\text{C}$ , at or below the minimum temperature tested. Also, the small peak in the storage modulus at  $40^{\circ}\text{C}$  was lowered to  $35^{\circ}\text{C}$ , and seemed to become slightly more prominent.

It was considered that the casting solvents, particularly the DCM, were responsible for the changes seen. It is possible that the interaction of resident PLA with DCM led to changes in the polymer morphology, leading to a less ordered structure, and thereby a lower glass transition temperature. An alternative possibility is that the DCM could have leached some of the glycerol from the TSBP and carried it into the polymer matrices, thus inducing a plasticizing effect on the polymers, leading to a reduced glass transition temperature.

### Mechanical properties and acoustic emission

For use as food containers, the composite films are typically expected to be subjected to mechanical deformation and stretching, and must be capable of resisting considerable stress without fracture. In the present study, the mechanical properties of the composite films were measured in two directions, parallel and perpendicular to the direction of extrusion. The results were summarized in Table II.

In general, the mechanical properties tested in the direction parallel to the direction of extrusion were higher than those obtained in the perpendicular direction. Typically this is due to preferred chain orientation in the direction of processing. From these results, the PBAT/TSBP composite has higher elongation to break, and is tougher than the PLA/TSBP.

In comparing the uncoated PLA/TSBP films with the films coated with PLA/DCM, a decrease in modulus and tensile strength and a slight increase in elongation for the coated films were seen. This is consistent with the structural changes found in the DMA and microscopy studies, where the dissolution of resident PLA in DCM, and thus a reduction of the PLA's crystallinity, was proposed. For chitosan coated PBAT/TSBP composite films, the rigid chitosan enhanced the tensile strength and initial modulus, while the coated films had lower elongation and toughness.

Table II also shows the mechanical properties of a commercially available Styrofoam, a petroleum-derived material popularly used for manufacturing beverage cups, take-out quick food boxes, and various containers for fresh produce fruits and vegetables and other processed foods. It is clear that the composite films and the films coated with antimicrobial materials have much higher tensile strength, toughness, and elasticity in comparison with Styrofoam.

Although conducting the stress-strain tests, AE measurements were also performed on the same samples. When a sample was stretched, fracture was created and sound waves were also produced and measured. AE measurements indicate the limiting conditions for the end use of a sample by describing

**TABLE II**  
**Mechanical Properties of Composite Films**

Samples	Initial modulus (MPa)		Tensile strength (MPa)		Maximal elongation (%)		Toughness (J/cm <sup>3</sup> )	
	Para <sup>a</sup>	Perp <sup>b</sup>	Para	Perp	Para	Perp	Para	Perp
PLA/SBP	1722 (106)	1516 (50)	21.4 (1.2)	14 (0.5)	11 (2.0) <sup>c</sup>	3.6 (0.8)	1.8 (0.3)	0.4 (0.1)
PBAT/SBP	250 (23)	165 (3)	13 (0.8)	9.7 (0.1)	54 (7)	105 (21)	6.4 (1)	9.2 (2)
PLA/SBP with nisin in PLA/DCM	621 (33)	660 (26)	13 (0.3)	11 (0.7)	12 (2)	5.1 (0.8)	1.4 (0.2)	0.4 (0.1)
PBAT/SBP with chitosan in acetic acid	1472 (110)	1298 (37)	20 (1.7)	13 (0.4)	16 (3.3)	4.8 (0.6)	2.7 (0.8)	0.5 (0.1)
Commercially available styrofoam <sup>d</sup>	3.0 (0.3)		0.1 (0.01)		4.8 (0.7)		0.0 (0.0)	

<sup>a</sup> Para, tested in the direction parallel to extrusion.

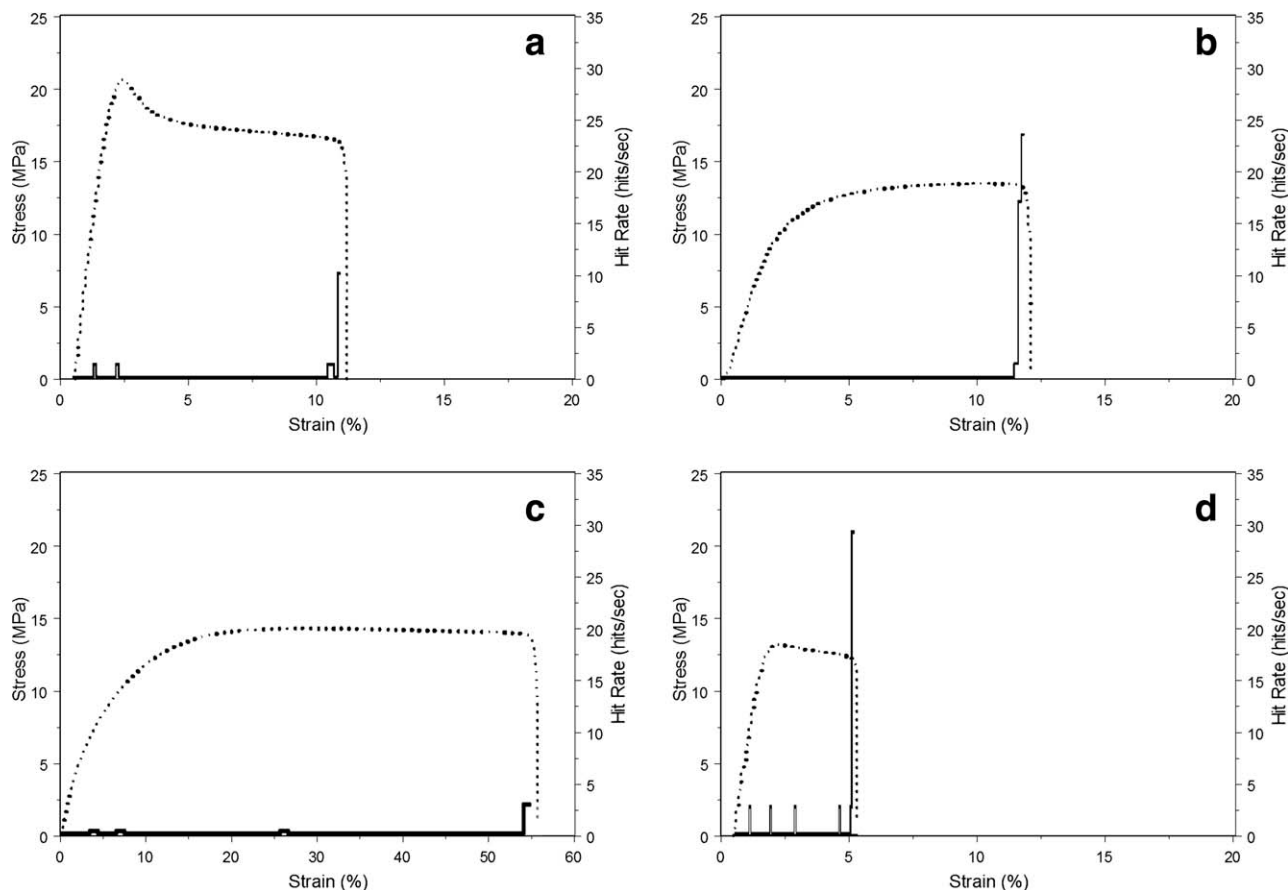
<sup>b</sup> Perp, tested in the direction perpendicular to extrusion.

<sup>c</sup> Number in parenthesis indicates standard deviation.

<sup>d</sup> Arbitrary direction.

its fracture characteristics. Figure 7 displays the stress–strain curves associated with the property change and the corresponding strain-AE hit rate curves due to structure change of an individual sample under external force. The two plots show some similarities and a number of differences. First, there were only a few acoustic signals recorded (for composite PLA/TSBP and PBAT/TSBP, and PBAT/TSBP coated with chitosan in diluted acetic acid) or no signal recorded (for PLA/TSBP coated with PLA

in DCM) while the samples behaved as elastic. Second, all of the samples emitted a sudden big sound peak at the peak stress when the samples were completely fractured, which indicates their uniform structure. Furthermore, there were more acoustic events observed before the peak stress for the chitosan-coated sample (d) than for the others. There were no acoustic signals that could be recorded for the PLA/DCM-treated composite (b), while some signals were detected for the nontreated composite



**Figure 7** Relationship of strain–stress curve (dotted line) and strain-AE hit rate plot (solid line): PLA/TSBP (a), PLA/TSBP coated with PLA in DCM (b), PBAT/TSBP (c), and PBAT/TSBP coated with chitosan in diluted acetic acid (d).



**TABLE III**  
Effectiveness of Antimicrobial Coating on the PLA/TSBP Films Against the Growth of *Listeria monocytogenes* in BHIB at Room Temperature

Composition in coating layer	Cell populations (log CFU/mL)*		
	0 h	24 h	48 h
Control	4.85 a	9.49 a	9.02 a
Bare sheet	4.71 a	9.17 a	9.56 a
PLA	4.66 a	9.22 a	9.63 a
Chitosan	4.03 b	2.87 d	2.58 d
Chitosan and nisaplin <sup>®</sup>	UD	UD	UD
Chitosan and AIT	4.61 a	2.39 e	UD
PLA and nisaplin <sup>®</sup>	2.76 c	4.29 c	3.91 c
PLA and AIT	4.85 a	4.78 b	4.77 b

\* Means within each column that have a common letter are not significantly different ( $P > 0.05$ ); UD, undetectable (<10 CFU/mL).

(a, c). This observation strongly supports the previous finding in the AU evaluation that the DCM dissolved some resident PLA and reduced the void volume.

### Antimicrobial activity

Table III shows the effects of composite films with antimicrobial coating on the cell populations of *L. monocytogenes* in growth medium at room temperature for 48 h. The uncoated samples and the samples coated with PLA had similar growth curves to the control sample indicating the PLA/TSBP film itself and PLA coating are nonactive toward *L. monocytogenes*. The films coated with chitosan or the combination of chitosan and other antimicrobial additives significantly ( $P < 0.05$ ) reduced the cell populations of *Listeria*; and synergistic effects of the combination of chitosan with nisin or AIT were observed. The films coated with the mixture of chitosan and Nisaplin<sup>®</sup> exhibited the greatest anti-*Listeria* activity and reduced the cell populations to undetectable levels (<10 CFU/mL) from 0 h to 48 h. The composite films coated with the mixture of chitosan and AIT also reduced the cells to undetectable levels after 48 h. The inclusion of Nisaplin<sup>®</sup> in the PLA coating layer reduced cell populations by 2 logs initially, although it slightly increased (ca. 1 log CFU/mL) during 48 h of incubation. The inclusion of AIT in PLA inhibited the growth of *Listeria* through 48 h of incubation. Both nisin and AIT, when incorporated into chitosan coatings, displayed significantly ( $P < 0.05$ ) stronger antimicrobial activity than they did in PLA coatings during the 48-h incubation. In addition to the synergistic effect of chitosan and nisin or chitosan and AIT, this may also be due to the faster release of nisin or AIT from chitosan film than from PLA because of the slight

swelling of the chitosan layers that was observed, which should facilitate the release of nisin or AIT into the bacterial medium.

Table IV shows the antimicrobial effect of composite films coated with and without antimicrobial on the cell populations of *Salmonella*. Stanley in growth medium at room temperature for 48 h. Similar to what was seen with *Listeria*, the PLA/TSBP films itself or the films coated with PLA did not show any anti-*Salmonella* activity. However, the films coated with chitosan, or the mixture of chitosan and nisin, or the suspension of nisin in PLA didn't inhibit growth of *Salmonella* as they did to *Listeria*, and there was only ca. a 0.5 log CFU/mL difference between those treatments and controls at 24 h or 48 h. The composite films coated with AIT in chitosan or PLA reduced the *Salmonella* cells to 3.26 and 3.67 log CFU/mL at 48 h, respectively, while the control reached over 9 log CFU/mL. Therefore, AIT exhibited a significantly ( $P < 0.05$ ) stronger suppressing effect on the growth of *Salmonella* than did nisin, suggesting that AIT is a less selective antimicrobial.

Similar results have been observed by other researchers, and nisin has been proven more effective against Gram-positive bacteria than against Gram-negative bacteria because of the differences in cell wall structure.<sup>29</sup> In general, the protective outer membrane surrounding the cytoplasmic membrane and peptidoglycan layer of Gram-negative cells cannot be easily damaged by nisin. The low susceptibility of *Salmonella* Stanley to nisin observed in this study provides further support for the mode of action of nisin on Gram-negative bacteria.

Both nisin and AIT are generally recognized as safe (GRAS) products approved by the U.S. Food and Drug Administration (FDA). Nisin is now used as a biopreservative in 57 countries around the world; AIT has been approved for use as a food preservative in Japan, and as a GRAS flavoring agent in

**TABLE IV**  
Effectiveness of Antimicrobial Coating on the PLA/TSBP Films Against the Growth of *Salmonella* Stanley in TSB at Room Temperature

Composition in coating layer	Cell populations (log CFU/mL) <sup>a</sup>		
	0 h	24 h	48 h
Control	3.87 b	8.99 a	9.14 a
Bare sheet	3.91 ab	8.79 a	9.03 a
PLA	3.85 ab	8.73 a	9.01 a
Chitosan	4.05 ab	8.70 a	8.30 b
Chitosan and nisaplin <sup>®</sup>	4.04 ab	8.60 ab	8.71 ab
Chitosan and AIT	4.01 ab	3.47 c	3.26 d
PLA and nisaplin <sup>®</sup>	3.88 b	8.49 b	8.72 ab
PLA and AIT	4.55 a	3.41 c	3.67 c

<sup>a</sup> Means within each column that have a common letter are not significant ( $P > 0.05$ ).

the United States.<sup>30–32</sup> Some naturally occurring film-forming materials also possess certain antimicrobial activity. A typical example is chitosan, which has demonstrated broad applications in pharmaceutical, cosmetic, and biomedical industries. In some research of food preservation, chitosan functions in both packaging/coating materials and antimicrobial reagent.<sup>6</sup> Incorporating of natural antimicrobials into packaging materials has attracted large source and attention in order to develop active packaging materials that not only provide a physical barrier, but also actively interfere with the biochemical process in the headspace or on the surfaces of packaged foods. It has been shown in many literatures<sup>2</sup> that the direct use of antimicrobial (such as dipping or coating) may dramatically reduce their antimicrobial activities due to their affinity for food particles and inactivation by components in foods. As an additional hurdle to nonthermal processes, antimicrobial packaging can play an important role in reducing the risk of pathogen contamination, as well as in extending the shelf-life of minimally processed foods. The selection of a polymeric substance that can function in both as physical barrier and bioactive depot is critical. In this study, nisin or AIT, and their combination were preincorporated in PLA or chitosan solution, and then coat to a new type of totally biodegradable, light weight-bearing composites, PLA/TSBP and PBAT/TSBP. The resulted double-layer films show stronger antibacterial activities against Gram positive *Listeria* (Table III) or Gram negative *Salmonella* (Table IV) and possess an appropriate tensile strength.

## CONCLUSIONS

Double-layer films with an antimicrobial surface on one side were prepared for food packaging using two biodegradable, light weight-bearing composites as substrate layers: PLA/TSBP and PBAT/TSBP.

With the simple procedure described in this study, the coating caused minor structural changes that decrease the mechanical properties of the resultant films; however, the films still were able to retain a certain significant degree of tensile strength that matches many commercially available food containers made from petroleum-derived thermoplastics. This research explored a new strategy to make active packaging materials for food packaging from the byproducts of agricultural processing. The planning work is to prepare this type of composite films using a multihead extruder to make the films in one step.

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