

Poly(lactic acid) Membranes Containing Bacteriocins and EDTA for Inhibition of the Surface Growth of Gram-Negative Bacteria

LinShu Liu,¹ Tony Jin,² David R. Coffin,¹ Cheng-Kung Liu,³ Kevin B. Hicks¹

¹Crop Conversion Science and Engineering Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, Pennsylvania 19038

²Food Safety Intervention Technology Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, Pennsylvania 19038

³Fat, Oils and Animal Co-products Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, Pennsylvania 19038

Received 14 August 2009; accepted 4 October 2009

DOI 10.1002/app.31550

Published online 18 March 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Films containing antibacterial reagents, ethylenediamine-tetraacetic acid disodium salt (EDTA) and Nisaplin[®], were produced by coextrusion with poly(lactic acid) in the presence of a pharmaceutical grade glycerol triacetate. The incorporation of EDTA-Nisaplin[®] particles resulted in a heterogeneous biphasic structure, as revealed by scanning electronic microscopy, confocal laser microscopy, and acoustic emission tests. The inclusion of glycerol triacetate reduced the Young's modulus and tensile strength, while enhancing the flexibility and the toughness of the resulting blends. The inclusion of the plasticizer

also allowed the extrusion to occur at a temperature as low as 120°C to maintain the biological activity of Nisaplin[®], which in combination with EDTA, plays a synergistic effect on suppression of the growth of the Gram-negative bacteria, *E. coli* O157:H7. The films thus obtained show potential as packaging materials with a wide spectrum of antimicrobial activity. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 117: 486–492, 2010

Key words: poly(lactic acid); bacteriocin; synergistic activity; active packaging; extrusion

INTRODUCTION

Poly(lactic acid) (PLA) is under intensive research and development for replacing petroleum-derived thermoplastics for use as engineering materials. PLA possesses good mechanical properties comparable to some petroleum-derived thermoplastics and can be fabricated into various physical forms using equipment that is used for the processing of these thermoplastics. PLA is derived from 100% renewable resources such as corn or sugar beets. PLA degrades to nontoxic compounds in landfills or under commercial composting conditions. It has been used in the production of goods for food service use, household use, and other applications with light load-bearing requirements. PLA is also a promising packaging material, because it is resistant to oil, is a good water vapor barrier, and has acceptable gas transmittance levels.^{1–3}

Recently, we have developed a new method⁴ to incorporate nisin, a short-chain, heat-sensitive antimicrobial polypeptide, into PLA to form active films

for fresh food packaging. To avoid the loss of antimicrobial activity, the PLA was first blended with its monomers or dimers at 160°C to form a melt, and following this step, the operating temperature was reduced to 120°C. Because of the presence of the plasticizers, the PLA remained in a melt state at 120°C for a certain time period. Nisin was then added to the melt and the blending was continued. The resultant films were active in suppressing the growth of the pathogenic bacteria *L. monocytogenes* and other Gram-positive bacteria. In this study, as part of our continuing research, we improved the operating conditions by using glycerol triacetate (GTA) as a plasticizer and inclusion of EDTA into the Nisaplin[®] formulation. The resultant films from these blends were investigated for structural, mechanical, and biological properties. This study is aimed at evaluating the potential of PLA-based films as new antimicrobial packaging materials that can inhibit the growth of a wide spectrum of bacteria ranging from Gram-positive to Gram-negative.

Correspondence to: L. Liu (linshu.liu@ars.usda.gov).

EXPERIMENTAL

Materials

Poly(lactic acid) (PLA) with a weight-average molecular weight of 135 KDa and polydispersity index of

1.28 was obtained from NatureWorks (Minnetonka, MN). The PLA pellets were ground into microparticles with a size of about 300 μm . Glycerol triacetate (GTA) and ethylenediamine-tetraacetic acid, disodium salt (EDTA) were purchased from Sigma-Aldrich (Milwaukee, WI). Nisaplin[®] was purchased from Danisco Cultor USA (New Century, KS).

Blend preparation

PLA, plasticized PLA, and their blends with EDTA-Nisaplin[®] in the form of strands or thin films were prepared using a HAAKE MiniLab II Rheomex CTW5 bench-top twin-screw extruder (ThermoFisher Scientific, Newington, NH) as reported previously.⁴ The extruder employs two corotating conical screws (109.5 mm in length, and 5 and 14 mm in diameter at the die and rear ends, respectively) and has a bypass channel which allows materials to be either recycled or directly extruded through the use of a "cycle/flush" valve. To determine the flow behavior of samples, the back channel is constructed as a rheological slit capillary with two pressure sensors. The rheological measurements, torque and ΔP , are a function of both composition and operating temperature, reflecting the resistance of the polymers to screw rotation and the pressure drop along the slit capillary. The higher the value, the more elastic the samples are. The extruder is connected with and operated by a computer equipped with operation/analysis software (Polylab Monitor Software, version 4.17).

PLA and a designated amount of plasticizers, 5–30% of the total weight, were mixed mechanically in a glass vial. A fraction of the mixture was first added to the extruder using a pneumatic feeder. The extruder was operated at 160°C at the beginning, with the unit set to recycle mode. After the PLA was melted, the barrel temperature was then set to 120°C. When this temperature was reached, EDTA-Nisaplin[®] (5 wt % of total mass) was added and the extrusion was continued. For film preparation, a slit die (5 mm in width and 0.5 mm in thickness) was attached to the exit.

Measurements of properties

Microscopic analysis

Scanning electron microscopy (SEM) and confocal laser microscopy (CLM) were used for morphology examination. For SEM, samples were vacuum-dried for 48 h before investigation. The dried samples (5 \times 7 mm) were mounted onto specimen stubs and sputter coated with a thin layer of gold. SEM images of topographical features of the membrane were collected using a Quanta 200 FEG environmental scanning electron microscope (FEI Co.,

Hillsboro, OR) operated in the high vacuum/secondary electron imaging mode at an acceleration voltage of 10 kV. For CLM, images were obtained by confocal fluorescence for Nisaplin[®] (ex/em, 488/530 nm) and confocal reflection for PLA (633 nm) in two separate channels using an IRBE optical microscope with a 10 \times lens integrated with a Model TCS-SP laser scanning confocal microscope (Leica Microsystems, Exton, PA). The distribution of Nisaplin[®] and PLA was visualized in sets of optical sections extending from the surface to deep (\sim 30 μm) within the blends.

Thermal analysis

Differential scanning calorimetry (DSC) was performed on extruded samples. The samples were crimp sealed in 40 μL stainless steel pans. All samples were first scanned from 20 to 200°C at a heating rate of 10°C/min, then held at 200°C for 20 min, cooled to 20°C at 10°C/min, kept at 20°C for 10 min, and heated to 200°C again. Tests were run in triplicate for each sample, and the results were averaged. Data on glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), and melting enthalpy (ΔH_m) of the samples were collected. Crystallinity, X_c , was calculated, according to the equation as follows:

$$X_c = (\Delta H_m / \Delta H_m^0) \times (100/w)$$

where ΔH_m designates the measured enthalpy of melting, and ΔH_m^0 is the enthalpy for 100% crystalline polymer. For, PLA, $\Delta H_m^0 = 93.6$ J/g. The letter w represents the mass fraction of PLA in the blends.^{3,5}

Dynamic mechanical analysis

Dynamic mechanical analysis was performed using a Rheometrics RSA II analyzer (Piscataway, NJ). Each sample was equilibrated in the sample chamber under nitrogen at -100°C before running the test. Temperature was increased at a heating rate of 10°C/min. Storage modulus (E') and loss modulus (E'') were measured as a function of temperature. Data were collected from -100 to 100°C and analyzed using Rheometric Scientific Orchestrator software, version 6.5.7. A nominal strain of 0.1% was used with an applied frequency of 10 rad/s (1.59 Hz). The average data were calculated based on three specimens for each sample.

Mechanical testing and acoustic emission

All samples were conditioned at 23°C and 50% relative humidity for 7 days before testing. Specimens

were then characterized for tensile strength, stiffness, and toughness by the use of an upgraded Instron mechanical property tester, model 1122 (Instron Corp., Norwood, MA) with a gauge length of 60 mm and a strain rate of 50 mm/min. The Testworks 3.1 data station and analysis software (MTS Systems Corp., Minneapolis, MN) were used throughout this work. Tests for each sample were performed five times to obtain an average value.

Acoustic emission (AE) analysis was performed simultaneously with the mechanical testing. A small transducer (10 mm in diameter, 20 g in weight; Model R15, Physical Acoustics Corp., Princeton Junction, NJ) was clipped against the sample specimen. The transducer resonates at 150 KHz. As the samples were stretched, the AE signals emanating from this transducer were processed with a Model 1220A preamplifier and an upgraded LOCAN-AT acoustic emission analyzer (Physical Acoustics Corp., Princeton Junction, NJ), which was connected to a PC with enhanced graphing and data acquisition software.

Antimicrobial activity test

The pathogenic bacteria *Escherichia coli* O157:H7 Oklahoma used for stock cultures was obtained from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center.

Each glass test tube, containing 0.18–0.20 g of PLA sample, was filled with 9 mL of Tryptic Soy Broth (TSB) and inoculated with 1 mL of an overnight culture of *Escherichia coli* O157:H7 Oklahoma ($\sim 1 \times 10^3$ cells). The test tubes were shaken at 150 rpm at 22°C. Cell populations (CFU: colony forming unit) were determined at 0, 24, and 48 h. Aliquots containing 1 mL of incubated sample were serially diluted with sterile phosphate buffer (Hardy Diagnostics, Santa Maria, CA), then pour plated onto Tryptic Soy Agar. Plates were incubated at 37°C for 24 h before counting. Film-free inoculated medium served as a control. All tests were performed in duplicate with three membrane specimens used in each replication ($n = 6$). Each data point was expressed as the mean \pm SD. All data were analyzed by analysis of variance using SAS version 9.1 software (SAS Institute, Cary, NC). Duncan's multiple range tests were used to determine the significant difference of mean values. Unless stated otherwise, significance is expressed at the 5% level.

RESULTS AND DISCUSSION

GTA is a well-known chemical that has been used as a food additive, pharmaceutical excipient, and plasticizer in the plastics industry. In this study,

TABLE I
Effect of GTA on the Rheological Behavior of PLA/GTA Blend

% GTA (w/w)	Stage I, at 160°C		Stage II, at 120°C	
	Torque (N cm)	ΔP (bar)	Torque (N cm)	ΔP (bar)
0	49	81	>200	>100
5	40	49	79	75
10	36	42	66	68
20	24	21	42	44
30	11	17	33	27
30 and (5% EDTA + 5% Nisaplin [®])	19	22	47	39

GTA plasticized PLA containing EDTA-Nisaplin[®] particles was extruded for the examination of structural properties and antimicrobial activity.

Table I shows the rheological properties of PLA with different GTA contents under the present processing conditions. For the PLA samples without the addition of GTA, as the operating temperature decreased from 160°C to 120°C, the measured rheological parameter, torque, increased from 49 Ncm to >200 Ncm. Another parameter, ΔP , also increased from 80 bar to >100 bar. Consequently, the physical state of the samples gradually changed from predominately viscous (at 160°C) to highly elastic (at 120°C), and finally became so elastic that extrusion was not possible. With the inclusion of GTA, the PLA/GTA blends exhibited lower values of torque and ΔP even at the lower temperatures. As more GTA was blended with the PLA, the values of the rheological properties of the blends became lower. When GTA content was over 20 wt %, the blends showed appropriate viscoelastic properties at 120°C (torque < 40 Ncm), where the EDTA-Nisaplin[®] was added to the extruder and the extrusion was continued. The addition of solid EDTA-Nisaplin[®] particles to the blends slightly increased the values of torque and ΔP . However, with a higher GTA content, plasticized PLA containing EDTA-Nisaplin[®] could easily be prepared. At the GTA content of 30 wt %, the blends could also be made directly at 110°C, having a ΔP value ranging from 25 to 45 bar.

The organization and microstructure of PLA films and the blend films are shown in Figures 1 and 2. The SEM micrographs show smooth topography for all samples tested [Fig. 1(a–d)]. However, the PLA and plasticized PLA films had smoother surface characters than the films containing EDTA or EDTA-Nisaplin[®]. The inclusion of Nisaplin[®] and EDTA showed an even deposition of the protein/salt particles into the matrix phase and introduced bright marbling characteristics to the blend surfaces. This phenomenon was further evaluated by CLM (Fig. 2),

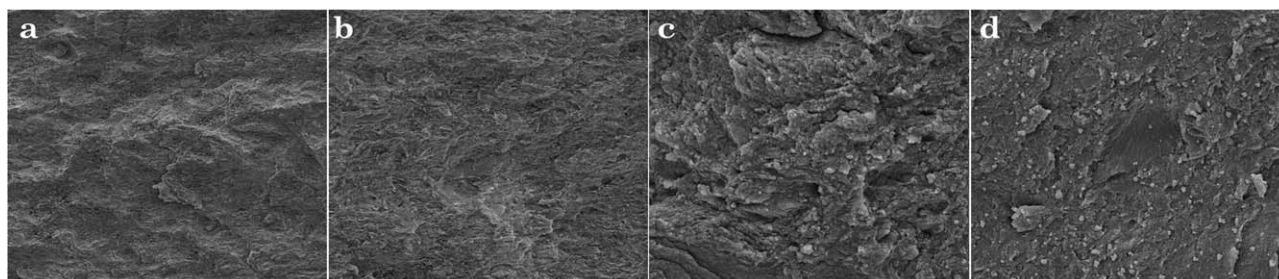


Figure 1 SEM photographs of (a) PLA, (b) plasticized PLA, (c) plasticized PLA with EDTA, and (d) plasticized PLA with EDTA-Nisaplin[®]. Field width, 55 μm .

where the PLA and GTA components were determined by confocal reflection at 633 nm colored red, whereas the protein components were measured by confocal fluorescence at 488/500–550 nm (excitation/emission), colored green. For the blend films, green particles of Nisaplin[®] compositions were evenly distributed within the PLA matrix phase, indicating a well-mixed, integrated structure. The Nisaplin[®] particles were in the size range from 250 nm to 20 μm . These results are consistent with our previous finding, where samples were prepared by the same instrument using a similar procedure.⁴

The thermal properties of PLA and GTA blends with different ratios are shown in Table II. It can be seen that the T_g of all plasticized PLA blends were lower than that of the neat PLA. This was expected, as the primary function of a plasticizer is to lower the T_g . The crystallization and melting temperatures are also shown in Table II. Significant decreases in T_c and T_m can only be seen for the PLA plasticized with greater than 10 wt % GTA. This could be attributed to the high crystallinity of PLA ($X_c = 0.58$) used in this study. Only a large amount of plasticizer could result in the remarkable decrease in both T_c and T_m . For the PLA with 30 wt % GTA, the values for T_c and T_m were 31°C and 50°C, respectively, lower than the neat PLA. As the crystallization took place later upon cooling from the melt, the crystal-

linity of the plasticized PLA with higher GTA contents were also significantly lower than that for neat PLA.

Table III shows the effect of the inclusion of GTA on the thermal properties of PLA blends containing EDTA-Nisaplin[®]. In comparing neat PLA with the PLA containing EDTA-Nisaplin[®], no difference in the T_g could be observed. This indicates that the molecular mobility of the PLA chains was less affected by the inclusion of EDTA-Nisaplin[®] particles. This could be attributed to poor interfacial bonding between the fillers and the matrix. However, the T_g of the plasticized PLA (GTA content, 30 wt %) was increased from 41 to 52°C by the inclusion of EDTA-Nisaplin[®], implying a “bridge effect” of the GTA coated on the particle surfaces that is expected to enhance the surface interaction between the particles and the PLA. The “bridge effect” restricted the flexibility of the PLA macromolecules. The T_c of the unplasticized PLA containing EDTA-Nisaplin[®] was higher than that of the neat PLA ($T_c = 114^\circ\text{C}$ and 110°C for the blends with or without particles, respectively), indicating that the EDTA particles may function as a nucleating reagent when the PLA crystallizes from the melt state. This phenomena was not observed for the plasticized PLA containing EDTA-Nisaplin[®] ($T_c = 79^\circ\text{C}$ and 78°C for plasticized PLA with or without EDTA-Nisaplin[®] particles,

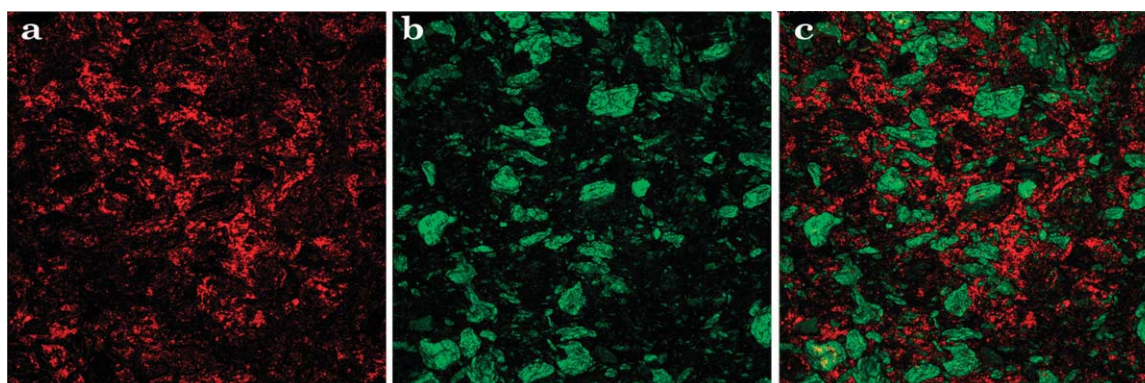


Figure 2 CLM images of plasticized PLA containing EDTA-Nisaplin[®] by (a) confocal reflection for PLA, (b) confocal fluorescence for Nisaplin[®], and (c) confocal reflection/fluorescence in two channels. Field width, 800 μm . [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
Thermal Properties of PLA with Different GTA Contents

% GTA	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
0	58	110	165	54.2	57.9
5	57	108	158	43.6	49.0
10	55	102	152	38.6	45.8
15	49	94	134	32.2	40.5
20	43	82	128	26.2	35.0
30	41	79	115	19.6	29.9

respectively), indicating that the GTA may coat on the surfaces of EDTA particles, thus inhibiting the crystallization of PLA from the particles. The crystallinity, X_c , of the PLA containing EDTA-Nisaplin[®] was slightly increased in comparison with the neat PLA samples. This could be attributed to the increase of the T_c , so that an earlier crystallization may result in a higher degree of crystallinity when the blends were cooled from the melt state. The presence of the plasticizer dramatically reduced the crystallinity of the PLA-EDTA-Nisaplin[®].

The mechanical properties of PLA, plasticized PLA, and plasticized PLA containing EDTA-Nisaplin[®] particles are shown in Table IV. In general, the blend of GTA with PLA resulted in a decrease in Young's modulus and tensile strength, and an increase in the value of breaking elongation of the obtained plasticized polymers. The toughness of the plasticized PLA also increased due to the enhanced flexibility. Furthermore, the inclusion of EDTA and Nisaplin[®] into the blends reduced the mechanical properties of the plasticized blends. The polymeric membranes became less flexible than the plasticized PLA. This is expected and could be attributed to a "filler effect" of the included particles. This supports the DSC findings shown in Table II.

The DMA curves for PLA, PLA/GTA blends, as well as the PLA containing GTA and Nisaplin[®]-EDTA are shown in Figure 3(a) (Storage Modulus, E') and Figure 3(b) (Loss Modulus, E''). The initial storage modulus of PLA showed a flat curve from -100 to about $+55^\circ\text{C}$. It also had a sharp glass transition at about $+55^\circ\text{C}$. Above the T_g , the stress in the PLA sample rapidly decreased to the point that there was essentially no force reading on the instrument. The overall trends for the plasticized PLA curve are similar to that for the plasticized PLA con-

TABLE III
Effect of GTA on the Thermal Properties of PLA Containing Nisaplin[®]-EDTA

% GTA	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
0	58	114	169	54.2	61.0
30	52	78	116	15.8	26.0

TABLE IV
Mechanical Properties of PLA and Its Blends

Composition	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)	Fracture energy (J/cm ³)
100% PLA	40.2 (1.5)	868 (59)	6.2 (0.6)	1.6 (0.2)
30% GTA	7.3 (0.4)	40.1 (2.1)	108.5 (17.7)	5.4 (1.2)
30% GTA + (5% EDTA and 5% Nisaplin [®])	6.6 (0.2)	41.2 (2.5)	62.5 (15.9)	3.4 (1.1)

taining EDTA and Nisaplin[®] particles. Both the plasticized blends had a higher value for the storage modulus than the neat PLA over the temperature range from -100 to -45°C , then showed a continuing gradual decline in E' throughout the remaining temperature range investigated. The T_g values measured for the plasticized samples are similar to those obtained by DSC. In contrast to the neat PLA curve, the storage modulus above the T_g is still significant for the plasticized samples. This result is different from those published by other investigators,³ where 10% of GTA was blended with PLA. This could be attributed to the higher content (30%) of plasticizer in the blends studied here. Furthermore, the decrease in T_g and X_c of the blends may be the reason for the reduction in the E' value for the plasticized PLA and PLA-EDTA-Nisaplin[®] blends in the intermediate temperature range.

For the E'' curves, both of the plasticized samples showed similar trends that are significantly different from neat PLA. The most noticeable observation is that a broader E'' peak was recorded for each plasticized blend at the temperature range from -60 to about $+30^\circ\text{C}$. These may result from induced second order transitions. In this temperature range, the E'' values for the plasticized samples were also higher than for neat PLA. Based on these data, it appeared that the inclusion of GTA raises the stiffness of the PLA at lower temperature, whereas reducing the mechanical properties at higher temperature, from about $+30^\circ\text{C}$ to the T_g . These results are considered to be the contribution of the GTA below its T_m of 3°C . At the lower temperature, the coupling of GTA to PLA chains tends to limit the mobility of the macromolecules. These interactions thus seem to lead to an "antiplasticizing" effect in the lower temperature regime.

AE emission was used to probe the deformation of the blends caused by an external force. The deformation of a blend (as it is squeezed, torn, or stretched) is accompanied by a rapid movement, relocation, or breaking of structural elements such as fillers, fibers, matrices, and their interfacial areas. As a result, sound waves are produced that can be detected by an acoustic transducer and converted

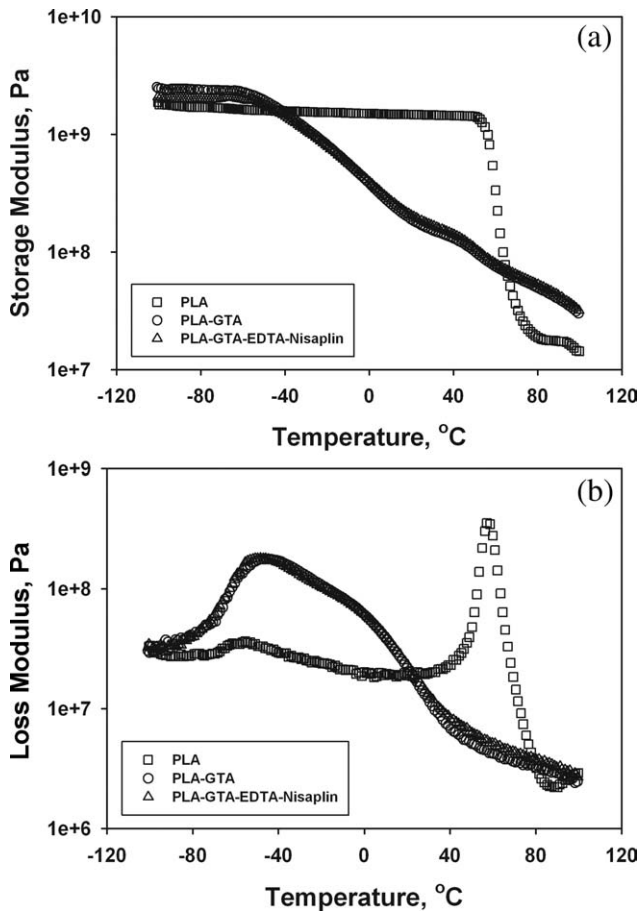


Figure 3 Typical plots of (a) storage modulus and (b) loss modulus as functions of temperature. (□) neat PLA, (○) plasticized PLA, and (△) plasticized PLA containing EDTA-Nisaplin®.

into electronic signals. This basic phenomenon is defined as an acoustic emission event and is translated by an AE analyzer as a “hit.” AE measurements were made simultaneously during tensile stress–strain tests for all sample specimens.

Figure 4 shows the correlation between the stress–strain curve and strain-AE hit rate pattern of the nonplasticized PLA, and plasticized PLA with and without EDTA-Nisaplin® particles. For those prepared without EDTA-Nisaplin®, regardless of the content of GTA, all samples behaved as linear elastic materials and were characterized with a major peak of AE event [Fig. 4(a,b)]. When the samples were stretched, both the stress and strain increased simultaneously. A series of low intensity AE events were also recorded along with the stretch. However, the sharp peak of AE activity occurred at the maximum elongation at which the samples were stretched to break. These observations are consistent with our previous finding,¹ indicating the homogeneous structures of the PLA and plasticized PLA. For the samples prepared from PLA, GTA, and EDTA-Nisaplin®, the stress–strain profile is similar to that

of the plasticized PLA, whereas the AE-hit curves were totally different. As shown in Figure 4(c), the AE emission has a different feature. AE was emitted at the very beginning of the stretch, followed by a steep increase and then a slower decrease which continued to the end of the process. This character reflects the heterogeneous structure of the blends (Figs. 1 and 2) and indicates the poor adhesion of the particles to the matrix. This results in a peak of AE emission that occurred during the process, at strain levels ranging from 18 to 25% of the original length, not at the end of stretch. This may be attributed to the low particle content (5 wt %) embedded

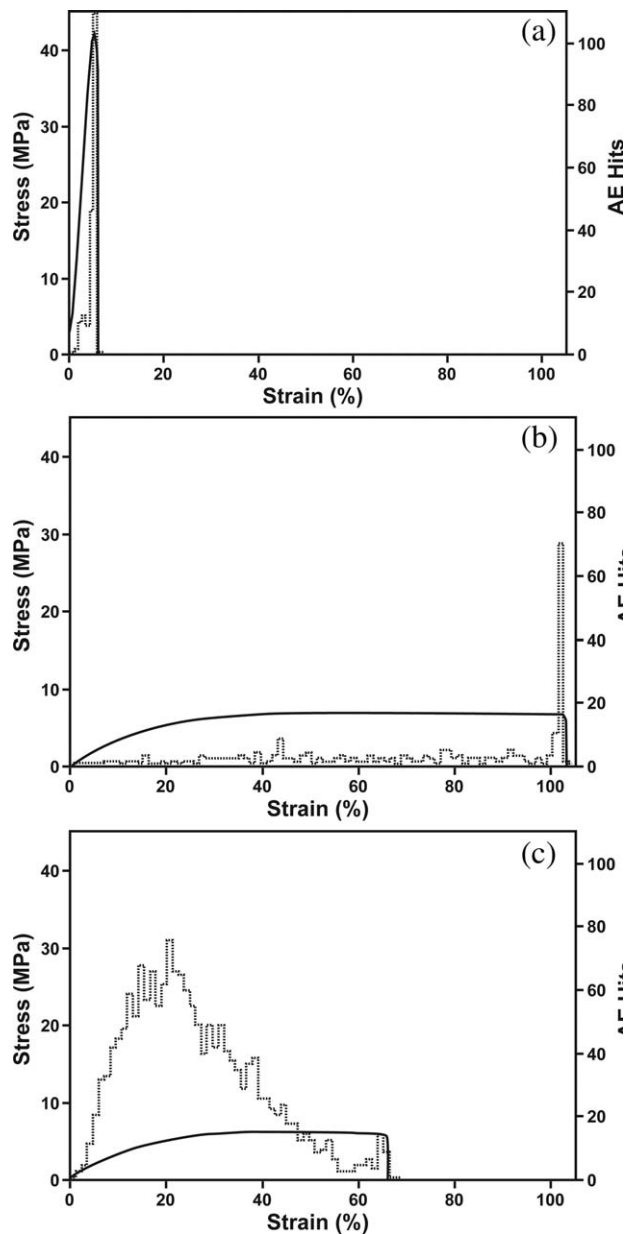


Figure 4 Correlation of stress–strain curve (solid line) and AE emission curve (dotted line). (a) neat PLA, (b) plasticized PLA, and (c) plasticized PLA containing EDTA-Nisaplin®.

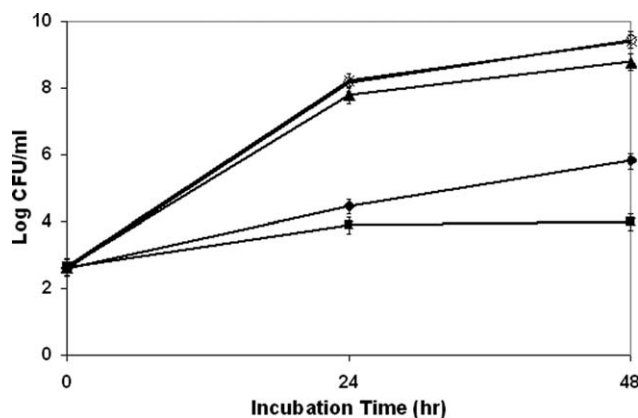


Figure 5 Antimicrobial effect of EDTA-Nisaplin® on PLA membranes (○) control, (x) plasticized PLA membranes, (▲) plasticized PLA with Nisaplin®, (◆) plasticized PLA with EDTA, and (■) plasticized PLA with EDTA-Nisaplin®. Error bars represent the standard deviation of the mean.

in the matrix phase of the blends. The stretch caused debonding of the particle from the matrix; when most particles were pulled out, the matrix phase was still in a continuous state and was able to sustain some degree of the force.

Antimicrobial activity test

Figure 5 shows the growth curves of *E. coli* O157 in TSB medium during 48 h incubation at 22°C. There was no difference in microbial counts between the plasticized PLA films and the control at 0, 24, and 48 h incubation time, indicating PLA alone did not contribute any antimicrobial activity against *E. coli* O157:H7. PLA membranes with antimicrobials significantly ($P < 0.05$) reduced the growth of *E. coli* O157:H7 during the incubation. Among them, the inclusion of EDTA-Nisaplin® had the greatest effect and films containing Nisaplin® had the least effect on the reduction of *E. coli* O157:H7 populations. Other researchers have indicated that nisin possesses antimicrobial activity against a wide range of Gram-positive bacteria but shows little or no activity against Gram-negative bacteria, such as *E. coli* O157:H7.^{6,7} Our previous study also showed that PLA films containing nisin only reduced less than 1 log CFU/mL of *E. coli* O157:H7 when compared with a control over a 48-h period.⁸ However, our data in this study show that the effect of the inclusion of EDTA-Nisaplin® was significantly ($P < 0.05$) greater than that of each preservative used alone.

Therefore, a synergistic effect clearly played a role in preventing growth of the pathogen. Our results agreed with others using the combination of nisin with EDTA.⁹ A previous study found that 15 mM EDTA was effective against *Escherichia coli* when used in corn zein films with different nisin concentrations.¹⁰

CONCLUSIONS

Based upon these present results and those in our previous study,⁴ the use of plasticized PLA films containing both EDTA and Nisaplin® should be quite effective at preventing growth of representative Gram-positive and Gram-negative bacteria in model systems. Verification of the activity of the present films against Gram positive *Listeria species* is in progress as well as testing of these films in food systems inoculated with both Gram-positive and Gram-negative pathogens. All samples had a smooth morphology, with the inclusion of EDTA and Nisaplin® leading to slightly rougher surfaces. The Nisaplin® was shown to be distributed evenly. Addition of plasticizer resulted in a significant lowering of the T_g , as well as both T_c and T_m , both with and without EDTA-Nisaplin®. Introduction of plasticizer led to expected decreases in tensile strength and modulus, with an accompanying increase in elongation. DMA tests indicated that inclusion of 30% plasticizer eliminated the crystalline melting point for the PLA and also led to slightly higher values for the storage modulus at low temperatures. The structural changes were confirmed by acoustic emission.

References

- Liu, L. S.; Finkenstadt, V. L.; Jin, T.; Fishman, M. L.; Hicks, K. B. *J Appl Polym Sci* 2007, 106, 801.
- Auras, R.; Harte, B.; Selke, S. *Micromol Biosci* 2004, 4, 835.
- Wong, S.; Shanks, R. A.; Hodzic, A. *Polym Eng Sci* 2003, 43, 1566.
- Liu, L. S.; Jin, T.; Coffin, D. R.; Hicks, K. B. *J Agric Food Chem*, submitted.
- Amash, A.; Zugenmaier, P. *Polymer* 2000, 41, 1589.
- Hurst, A. *Adv Appl Microbiol* 1981, 27, 85.
- Ray, B.; Daeschel, M. *Food Biopreservatives of Microbial Origins*; CRC Press, Inc.: Boca Raton, FL, 1992; pp 207–264.
- Jin, T.; Zhang, H. *J Food Sci* 2008, 73, M127.
- Blackburn, P.; Polak, J.; Gusik, S.; Rubino, S. D. PCT/US89/02625; Publication No. W089/12399 (1989).
- Larsen, T. M.S. Thesis, Clemson University, Clemson, SC, 1995.