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Antimicrobial Packaging Materials from Poly(Lactic Acid) Incorporated with Pectin-Nisaplin® Microparticles

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Abstract. The thermostability of nisin and Nisaplin® was investigated in the presence and absence of pectin. By mixing with pectin, both nisin and Nisaplin® were able to inhibit microbial growth, even after heating to 433 K. In contrast, without pectin, the nisin was totally inactivated after heat treatment. Nisin and pectin in solution were complexed, as shown by atomic force microscopic analysis. Probably, the complex formation between the polysaccharide and the polypeptide improves the nisin thermostability; however, a detailed mechanism remains to be resolved. Thin membranes were prepared by co-extrusion of poly(lactic acid) and microparticles of pectin-Nisaplin®. Despite the high extrusion temperatures, the presence of pectin protected the biological activity of nisin and the resultant membranes were antimicrobial and could be used as an inner layer of multi-layer packaging materials for active packaging.

Keywords: pectin, nisin, active packaging, poly(lactic acid), biodegradable, acoustic emission.

1. Introduction

Research and development of packaging materials and packaging technology have shown a clear and irreversible trend toward "green" and active packaging [1-3]. New studies on packaging materials have emphasized the use of biobased materials. The examples include agricultural fibers, products and byproducts from agricultural processing such as cellulose, starch, and pectin, polymers were synthesized using renewable biobased monomers, such as poly(lactic acid) (PLA), polyhydroxybutyrate, and Ecoflex®, as well as proteins from animal tissues, soybean flour, and corn. Those materials are biodegradable, and their final degradation products are nontoxic and environmentally friendly [4]. Substances with antimicrobial activity can be incorporated into packaging materials *via* physical and/or chemical approaches. These approaches have inspired the development of active packaging materials, which not only provide a physical barrier that passively protects contents from environmental influences, but also offer chemical and biological means to actively moderate changes occurring on the surfaces or in the headspaces of packaged materials [1, 5]. Biologically active substances used for this purpose include bacteriocins, lipids, enzymes, and some volatiles extracted from plant tissues [6-10].

Among biobased materials used for packaging purpose, PLA has received special attention. PLA is similar to conventional petrochemical plastics in its physical characteristics [5]. Thin membranes of PLA are good water vapor barriers, and have relatively low gas transmittance. To date, PLA has shown the highest potential for a commercial major-scale production of biobased packaging materials. PLA has been used for producing loose-fill packaging, compost bags, and food packaging materials. PLA with incorporated bioactive substances has been effectively used for meat, juice, and milk packaging [11-15].

Composite films prepared from PLA, pectin, and nisin were antimicrobially active as the films suppressed the growth of *Lactobacillus plantarum*. In that study, PLA and pectin were co-extruded into films, where the fillers and the added pectin aggregates formed a rough surface. Nisaplin® was then loaded onto the surfaces by a diffusion method [16]. Nisaplin® was able to retain its antimicrobial activity. However, the production process was quite complicated and might be difficult to scale up.

In the present study, we explored a new approach to produce PLA membranes loaded with Nisaplin®. A mixture of pectin and Nisaplin® was first prepared by co-dissolution followed by freeze-drying and milling. The particles of the dried mixture were then co-extruded with PLA into thin membranes. The membranes were evaluated for structural and mechanical properties, and antimicrobial activity.

2. Experimental

2.1. Materials

PLA was obtained from Dow Cargill (Minneapolis, MN), grounded to particles with the size less than 300 µm, and stored in a sealed water-proof bag at 277 K. The weight-average and number-average molecular weights of PLA were 148,000 and 110,000 Dalton, respectively. The glass transition temperature (*Tg*) of the PLA was around 333 K. Pectin and Nisaplin® were purchased from Danisco Cultor USA (New Century, KS). Nisin was a gift from Prof. K. Yam of Rutgers, the State University of New Jersey (New Brunswick, NJ).

Pathogenic bacteria ***L. monocytogenes*** Scott A 724 used for stock cultures were obtained from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center. The stock cultures were maintained at 193 K in Brain Heart Infusion (BHI) broth (Difco Laboratory, Detroit, MI). Working cultures of ***L. monocytogenes*** were maintained on BHI agar at 277 K and were sub-cultured bi-weekly and grown aerobically at 310 K in BHI broth. Prior to inoculation of product, cultures were grown in BHI broth at 310 K for 16–18 h.

2.2. Sample Preparation

2.2.1. Pectin-Nisaplin® and pectin-nisin preparations

A pectin-Nisaplin® complex was prepared by co-dissolving pectin and Nisaplin® (9:1, w/w) in deionized water (D.I. water) at the concentration of 10 g/L under constant stirring. The slurry was freeze-dried, and then ground to small particles. The fractions with the size of ~30 µm were collected and stored dry at 277 K. The milling, sizing, and collecting were conducted at the temperature < 203 K at Air Products and Chemicals, Inc. (Allentown, PA).

A pectin-Nisaplin® complex was also prepared in glass tubes to form a thin film coated on the inner surface of the tubes. Briefly, 0.9 g pectin and 0.1 g Nisaplin® were dissolved in 20 ml D.I. water in a heavy-duty glass tube that was pre-sterilized. The tube with the resultant gel was dried under vacuum at room temperature for 24 h followed by freeze-drying for additional 8 h. The tubes coated with a pectin-nisin complex were also prepared by the same method using pectin (10 mg/ml, pH 7.5) and nisin (10 mg/ml, pH 3.2) solutions at the volume ratio of 360:1 (polysaccharide:polypeptide).

2.2.2. Composite preparation

Composites of PLA/pectin, and PLA/pectin-Nisaplin® were prepared by extrusion according to the procedure described previously [16]. A Werner-Pfleiderer ZSK30 corotating twin-screw extruder equipped with a two-hole (4 mm in diameter) die plate was used in sample preparation. The barrel of the extruder comprised 14 sections with L/D ratio of 44:1. The temperature profile of the eight heating zones of the barrel was maintained at 408 K (zone 1), 463 K (zone 2), 450 K (zones 3-8), and approximately 428 K at the exit. PLA was fed into the barrel section 1 with a gravimetric feeder (Model 3000, Accurate Inc., Whitewater, WI). For composite preparation, pectin or pectin-Nisaplin® microparticles were fed into the barrel section 7 with a loss-in-weight feeder, where the particles were mixed with melted PLA. In all cases, the total feed rate was approximately 75 g/min, the screw speed was 130 rpm, and the residence time was about 2.5 min. The pressure and torque at the die were allowed to stabilize between formulations before sample collection. Strands were pelletized by a laboratory pelletizer (Killion Extruder, Inc., Cedar Grove, NJ). Strands and pellets of PLA were also prepared by the same procedure.

Thin membranes of PLA, PLA/pectin, and PLA/pectin-Nisaplin® were prepared from their corresponding pellets with a Brabender single-screw extruder (C.W. Brabender, South Hackensack, NJ) with four temperature zones (423, 443, 443 and 423 K). Formulations were extruded with a hangar-type die at 423 K. The thickness of the resultant membranes was measured by a micrometer (Ames, Waltham, MA).

The membranes thus obtained were cut into tensile bars (16.42x1.91 cm, LxW) for destructive mechanical test according to ASTM D 638-99, strips (38.1×7.0 mm, L×W) for non-destructive dynamic mechanical analysis, and discs (1.6 cm in diameter) for the evaluation of antimicrobial activity. The bars, strips and discs were also examined by microscopy. All samples were stored in a desiccator over dry $CaCl_2$ at ~293 K.

2.3. Sample Characterizations

2.3.1. Microscopy

Scanning electron microscopy (SEM) was used to characterize the structural features of the extruded samples, such as filler distribution in the matrix phase and microstructure on the fracture surfaces. Topographical images were obtained using a Quanta 200 FEG microscope (FEI, Hillsboro, OR) operating in high-vacuum/secondary electron-imaging mode. Samples were mounted on a specimen stub and sputtered with a thin layer of gold prior to examination. Images were collected at magnifications of 500X, 2,500X, and 25,000X.

Atomic force microscopy (AFM) was used for the characterization of pectin, nisin, and pectin/nisin complexes at a concentration of 5 μg/ml. Samples, about 2 μl of each, were spotted on freshly cleaved mica, air-dried, and imaged in a model Nanoscope IIIa scanning probe microscope with TESP cantilevers (Veeco/Digital Instruments, Santa Barbara, CA) operated in the intermittent contact and height modes.

2.3.2. Dynamic mechanical analysis (DMA)

Small deformation dynamic mechanical analysis was performed by a DMA RSA II Solids Analyzer (Rheometric Scientific, Piscataway, NJ). The samples were equilibrated in the sample chamber under dry nitrogen at 173 K prior to test. The temperature was increased at the heating rate of 10 K /min. The data were collected to determine storage modulus (*E'*), loss modulus (*E"*), and lost tangent (δ) from 173 to 473 K and analyzed by Rheometric Scientific Orchestrator software, version 6.5.7. The sample length between the jaws was 23 mm; a nominal strain of 0.1 % was used, and the applied testing frequency was 10 rad/s.

2.3.3. Measurement of mechanical properties and acoustic emission

The mechanical property measurements were performed by an upgraded Instron tester (Model 1122, Instron Corp., Norwood, MA) [17]. Experimental settings are: at 294 K and 65 % relative humidity; gauge length of 102 mm; crosshead speed at 50 mm/min. To determine tensile strength, Young's modulus, elongation at break, and toughness, the data were collected and analyzed by Testworks 4 data acquisition software (MTS Systems Corp., Minneapolis, MN) throughout this study. For performance of the cyclic test, a sample was stretched to 2 % strain and then was released back to 0 % strain. Upon the sample reaching 0 % strain, it was stretched again. A total of five cycles was tested. The peak stress was recorded for each cycle.

Acoustic emission (AE) measurements were performed simultaneously with the tensile tests. When samples are stretched, structural rearrangements occur accompanied by internal stress redistribution and as a result, sound waves are produced. The production and propagation of the sound waves can be detected and used as a probe to characterize the structural properties of the sample and to determine the deformation mechanism caused by the external force. A piezoelectric transducer (model R15, Physical Acoustics Corp., Princeton, NJ) was mounted against the sample. The transducer was small (10 mm in diameter), light (~2 g in weight), and resonated at 150 kHz, which allowed it to filter out ambient noise, and collected only the frequencies of interest. AE signals emanating from the transducer when the Instron stretched the samples were processed by an upgraded LOCAN-AT AE analyzer (Physical Acoustics Corp.), which was connected to a PC host with enhanced graphics and data acquisition software, APARTAN 2000, with all features and options.

2.3.4. Antimicrobial activity assay

Specimens on discs of PLA, PLA/pectin, and PLA/pectin-Nisaplin®, as well as tubes coated with pectin-Nisaplin® and pectin-nisin were placed in an incubator that was pre-heated at 433 K for 10 min, and then immediately placed in a container cooled with ice-water. When the temperature reached ~203 K, the specimens were placed on the bench to be cooled. When the temperature reached room temperature, the specimens were placed in a desiccator over $CaCl_2$.

Heat-treated specimens were tested for antimicrobial activity. For specimens coated on glass test tubes, each tube was filled with 9.0 ml BHI broth and inoculated with 1 ml *L. monocytogenes* (approximately $1 \cdot 10^5$ cells) that was pre-cultured overnight. The tubes were shaken at 150 rpm at 297 K. Aliquots containing 1 ml of incubation solution were sampled at predetermined time points and diluted in series with sterile phosphate buffer (Hardy Diagnostics, Santa Maria, CA). The diluted test solutions were pour-plated onto BHI agar. The plates were incubated at 310 K for 24 h. For specimen discs, 3 pieces from the disc of each sample were immersed in a glass tube with 9.0 ml of BHIB, and then tested by the same procedures described above.

Specimens without heat-treatment and inoculant free medium were used as controls. All tests were performed in triplicate.

3. Results and Discussion

3.1. Structural Analysis

Membranes of pure PLA and PLA/pectin-Nisaplin® composites had completely different appearances to the naked eye. The PLA membranes were clear and colorless; while the composites were brown, opaque, and appeared to have particulates present. To the touch, the PLA was smooth, while the composite membranes were rough. Although both membranes were fabricated under the same conditions, the PLA membranes had a thickness of about 0.2 mm, the composites containing pectin and Nisaplin® were typically 0.65 mm in thickness, approximately three times the thickness of the PLA only samples. As revealed by confocal laser scanning microscopy in our previous study, the increase in the thickness of composite membranes could be attributed to the formation of two distinct layers that mainly consisted of discontinuous particulates on both sides of the membranes [16].

3.1.1. AFM images of the pectin-nisin complexes

Fig. 1 illustrates the topographical structure and arrangement of soluble pectin, nisin and pectin-nisin mixture, following spreading and air-drying on freshly cleaved mica. Pectin (5 µg/ml) was complex, oriented network of thin strands (Fig. 1a). The height of strands measured 0.5–1 nm when analyzed individually by section analysis and less, around 0.3–0.5 nm by particle analysis of whole fields (2.5 µm^2). Nisin solution (5 µg/ml) were composed of a dispersion of circular particles or short, linear aggregates of circular particles (Fig. 1b), ranging from 0.3–1 nm in height from particle analysis and around 1 nm in width, after accounting for tip broadening. A mixture of soluble pectin and nisin (5 and 1.25 µg/ml, respectively) consisted of a loose network or strands, decorated with circular particles (Fig. 1c). Whole fields (2.5 µm^2) of pectin-nisin mixtures had mean particle heights around 0.38 nm (SD = 0.15) and section analysis through selected circular particles superimposed on strands was as high as 1.22 nm, whereas strand heights without particles were 0.5–0.7 nm.

Nisaplin® contains several components besides nisin, creating additional complexity for microscopic analysis. Therefore, the structure of the mixture of pectin and Nisaplin® was not a subject of AFM study. Since nisin is the only active component of Nisaplin®, it could be reasonably believed that the pectin-nisin complexes were also formed when mixing the Nisaplin® with pectin.

3.1.2. Microscopic images of PLA/pectin-Nisaplin®

SEM images of PLA and PLA/pectin-Nisaplin® membranes are shown in Fig. 2. The PLA membranes displayed a homogeneous sub-structure and relatively uniform, smooth morphology. The composite membranes were heterogeneous, showing a craggy surface. Furthermore, there were evenly distributed pectin-Nisaplin® microparticles in the composite membranes. The size and distribution of the microparticles in the membrane showed no change in comparison with that seen prior to feeding into the extruder with the PLA (Fig. 3). The introduction of pectin-Nisaplin® also created a porous structure. Pores were embedded in the PLA matrix phase (Figs. 2b and 2d). Most pores were adjacent to the pectin-Nisaplin® particles. Some pores were interconnected. Possibly, some heat sensitive components in Nisaplin® might have degraded and "evaporated" at higher temperature and under higher pressure during extrusion, therefore, pores were created and particles were segregated. That result is different from our previous study, where the particles were extruded with PLA together, pectin particles aggregated to each other, resulting in an increase in particle size [16].

3.2. Antimicrobial Activity of Pectin-Nisaplin® and Pectin-Nisin

The effects of heat-treatment on the antimicrobial activity of Nisaplin® and nisin, as well as their complexes with pectin were examined by measuring their capability to suppress the growth of *L. monocytogenes* on agar. As shown in Fig. 4, the application of non-heated Nisaplin® specimens to bacteria-containing BHI broth achieved 3.5 and 7.5 log reduction of *L. monocytogenes* growth on the agar at the beginning of the test and after incubation for 24 h, respectively. The colony forming units of *L. monocytogenes* counted for the samples applied with heat-pretreated Nisaplin® were similar to those recorded for the control specimens over the whole testing period, indicating that the heat-treatment destroyed the antimicrobial activity of Nisaplin®. However, the heat treatment did not affect the antimicrobial activity of pectin-Nisaplin® complex. There was no difference in the number of *L. monocytogenes* determined between samples applied with heat-treated pectin-Nisaplin® and non-heat-treated Nisaplin®.

Pectin-Nisaplin® microparticles were co-extruded with PLA to form a thin membrane. Fig. 5 shows the antimicrobial activity of the resultant composite

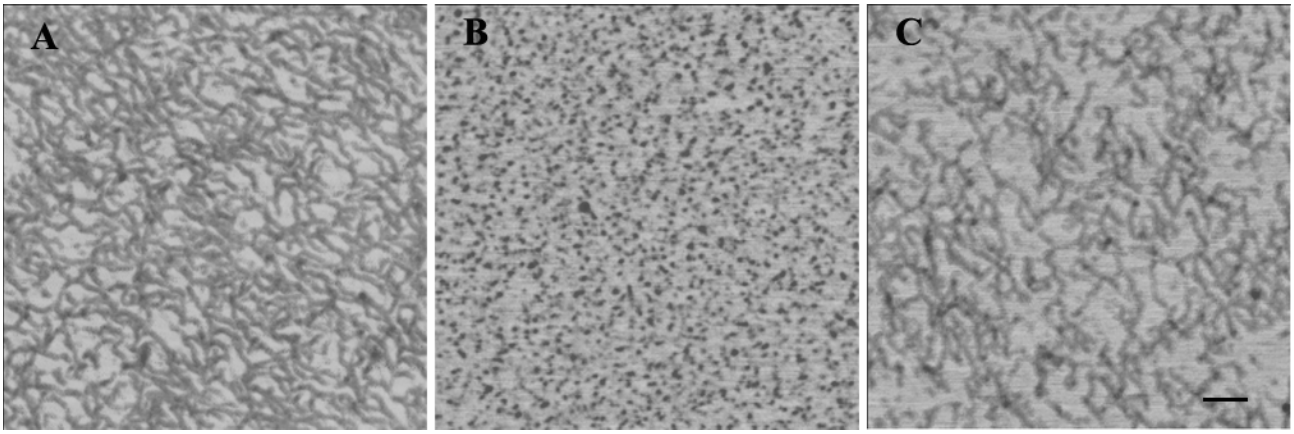

Fig. 1. AFM images of pectin (a), nisin (b) and pectin-nisin complex (c). Bar = 125 nm

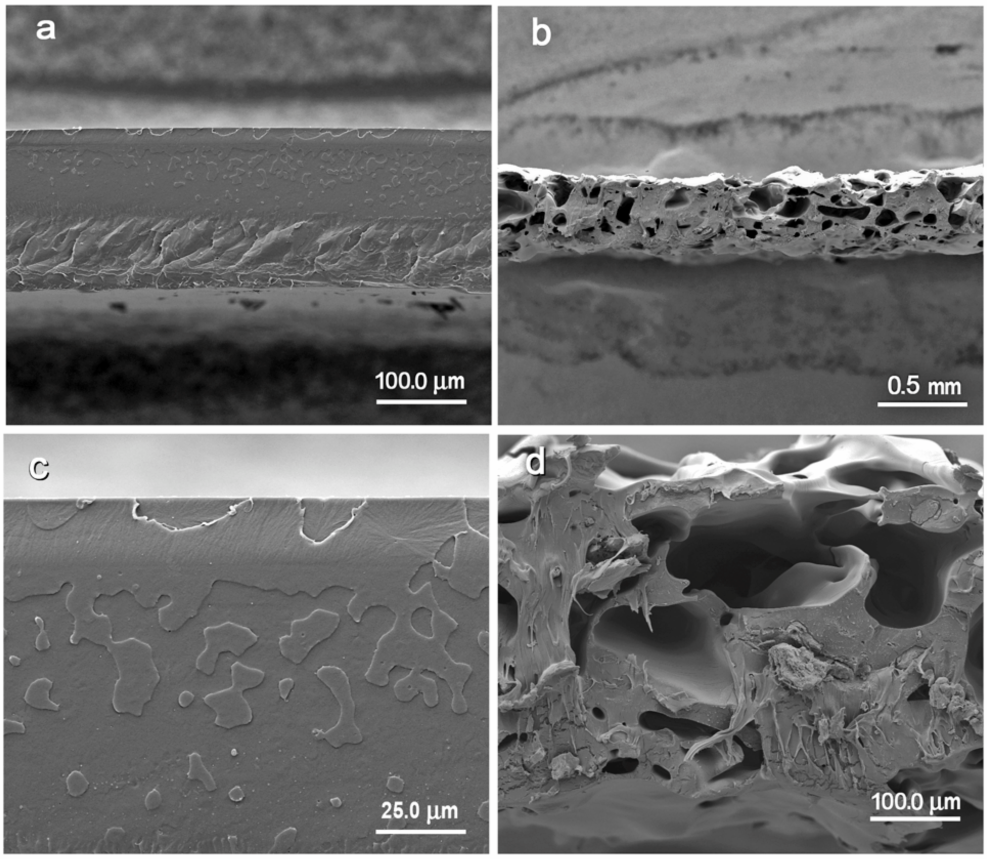

Fig. 2. SEM micrographs of fracture surfaces: PLA (a, c); PLA/pectin-nisaplin® (b, d). Images were captured at low (a, b) and high (c, d) magnifications

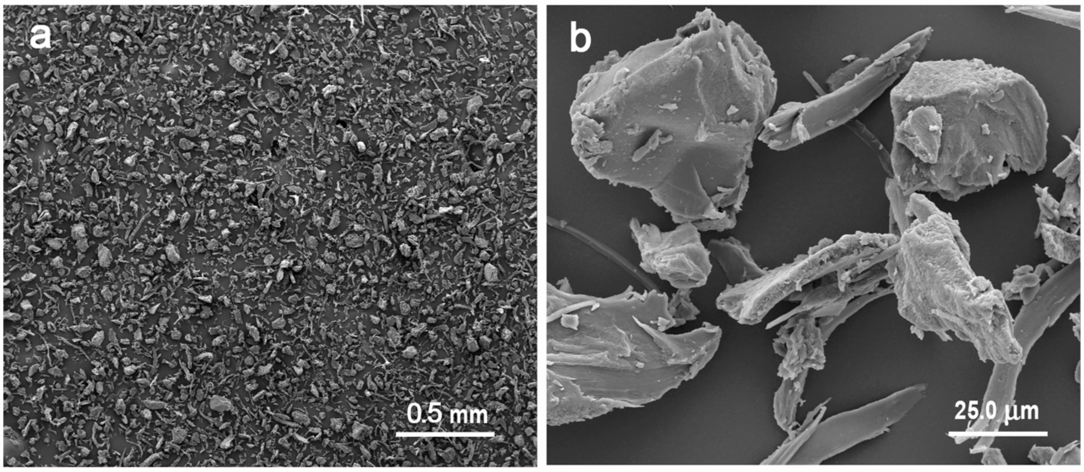

Fig. 3. SEM images of pectin-nisaplin® particles

membranes. A 3.5-log and a 6.5 log reduction in the *Listeria* colony forming units were observed at the beginning of the test and 24 h after the experiment, respectively, in comparison with the control (no membranes added). The membrane from PLA only did not exhibit any antimicrobial activity, which agrees with our previous study [16].

Nisaplin® is a commercial product, which is a mixture of 2.5% nisin and >97 % milk solids and salt added as stabilizers. Nisin is the only active component in Nisaplin®. Therefore, we investigated the antimicrobial activity of nisin and pectin-nisin after heat treatment. As shown in Fig. 6, there was no significant difference in the growth of *L. monocytogenes* among the samples of nisin

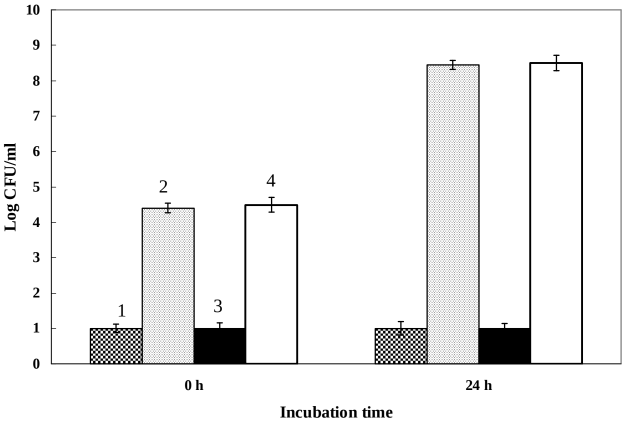

Fig. 4. Effect of heat treatment on antimicrobial activity of Nisaplin® against *L. monocytogenes*: Nisaplin®-not heated (1); Nisaplin®-heated (2); Nisaplin®/pectin-heated (3) and control (4)

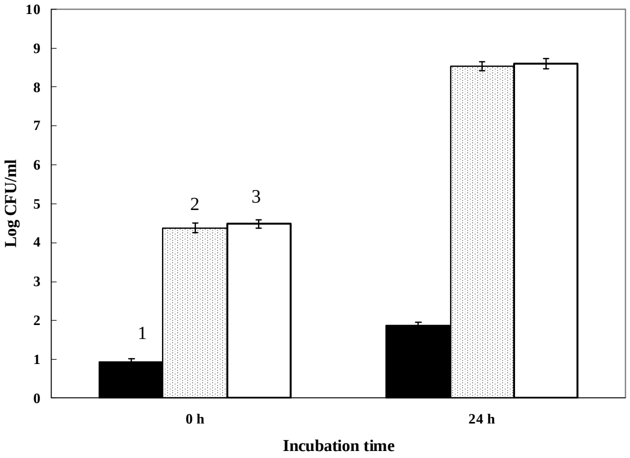

Fig. 5. Antimicrobial activity of pectin-nisaplin® incorporated in PLA thin membrane: PLA/pectin-nisaplin® (1); PLA membrane (2) and control (3)

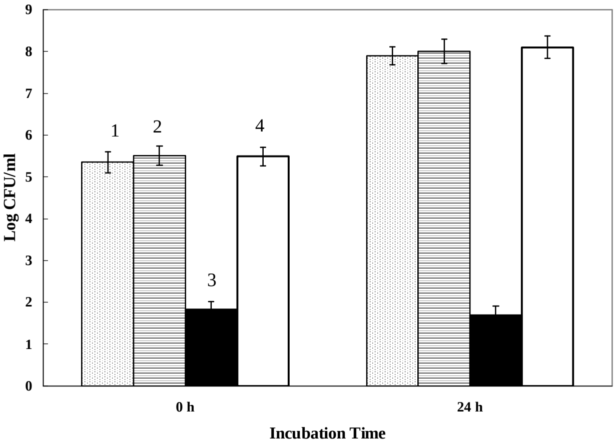

Fig. 6. Effect of heat treatment on antimicrobial activity of nisin against *L. monocytogenes:* heat-treated without pectin (1); pectin alone with heat-treatment (2); heat-treated with pectin (3) and control (4)

treated with heat, pectin, and control (no membrane added). In contrast, the pectin-nisin complex retained strong antimicrobial activity even after heat treatment. For the pectin-nisin treated samples, in comparison with the control, there was more than a 3.5 log and more than a 6 log reduction of *L. monocytogenes* at the beginning of culture and after 24 h, respectively. The results indicated that the mixing of nisin with pectin improved the thermostability of nisin, thus protecting its antimicrobial activity.

Nisin is a 34-amino acid polypeptide. The presence of lanthionine rings in the peptide chain provides a high level of thermal resistance. Consequently, nisin shows a higher thermostability than most proteins and polypeptides. Furthermore, nisin possesses a high proportion of basic amino acids, giving a net positive charge. It has been suggested that the stability of nisin may also depend on other factors, such as the presence of vicinal chemicals or biopolymers acting as stabilizers [18]. It has been reported that nisin solutions were stable to autoclaving (394 K for 15 min) in the pH range of 3.0–3.5 [19], and at 388 K at pH 2.0 [20]. To date, the highest temperature at which nisin retains its antimicrobial activity has been reported as 413 K [21]. In another study, Nisaplin® was co-extruded with low density polyethylene, a plastic with the melting temperature (T_m) of 393 K. The resultant films inhibited the growth of bacteria on meat [22].

Based on our best knowledge, the present study is the first time that nisin has been reported in a mixture with pectin to remain active after being heated at 433 K for 10 min. This finding, an elevation of the limitation of use temperature, may broaden applications for nisin, because more plastics with the melting point lower or equal to 433 K may be useful in co-extrusion with the polypeptide. Pectin has been used in the stabilization of some protein products [23]. The AFM in this study also showed the existence of complexes formation between pectin and nisin (Fig. 1). The improvement of the peptide thermostability may be attributed to the pectin-nisin complex formation. However, it should be pointed out that the mechanism by which pectin improved the thermostability of nisin is still unclear. By common sense, at temperatures up to 433 K, pectin can be partially (if not totally) degraded and, perhaps, nisin is also degraded. Does nisin retain its structure at 433 K when complexed with pectin, or have interactions occurred between the degradation products of the both compounds? Or, are there other looming possibilities for interesting chemistry? All these questions can not be answered by this preliminary study. More detailed studies from organic chemistry, analytical chemistry, and biochemistry perspectives are required.

For purposes of designing packaging, the films must be capable of resisting considerable mechanical stress without fracture. In the following experiments, PLA/pectin-Nisaplin® membranes were evaluated for their mechanical properties in the context of structural properties and structural changes under stress.

3.3. Dynamic Mechanical Analysis

Two features are immediately obvious from the dynamic mechanical analysis of the membranes (Fig. 7). First, the glass transition temperature (T_g) of the PLA is unchanged by the addition of pectin-Nisaplin® particles. As determined by the break in the storage modulus curve and the peak in the loss modulus plot, the T_g was 335 K. Second, the addition of pectin-Nisaplin® microparticles lowers the storage modulus over the entire range of temperature studied up to the T_g. From 173 K up to the T_g, the storage modulus of the PLA sample was twice as that of the PLA/pectin-Nisaplin® blend. Above the T_g this difference in storage modulus between the two samples was negligible. A similar consistency was also found with the loss modulus and loss tangent curves.

Since the pectin-Nisaplin® particles appear to be present as a discontinuous phase, the results that the modulus of the composites was lower than that of the PLA may indicate poor adhesion between the particles and the matrix phase. Alternatively, two other explanations may be considered. One could be that the presence of the particles in the matrix may lead to an effective cross sectional area of the PLA matrix which is lower than the measured dimensions of the film. Another explanation comes from the mathematical formula used for modulus calculation. As shown by the microscopic analysis, the presence of pectin-Nisaplin® particles created a rough surface on the composite membranes. The discontinuous layer laid on the membrane surfaces resulted in an increase

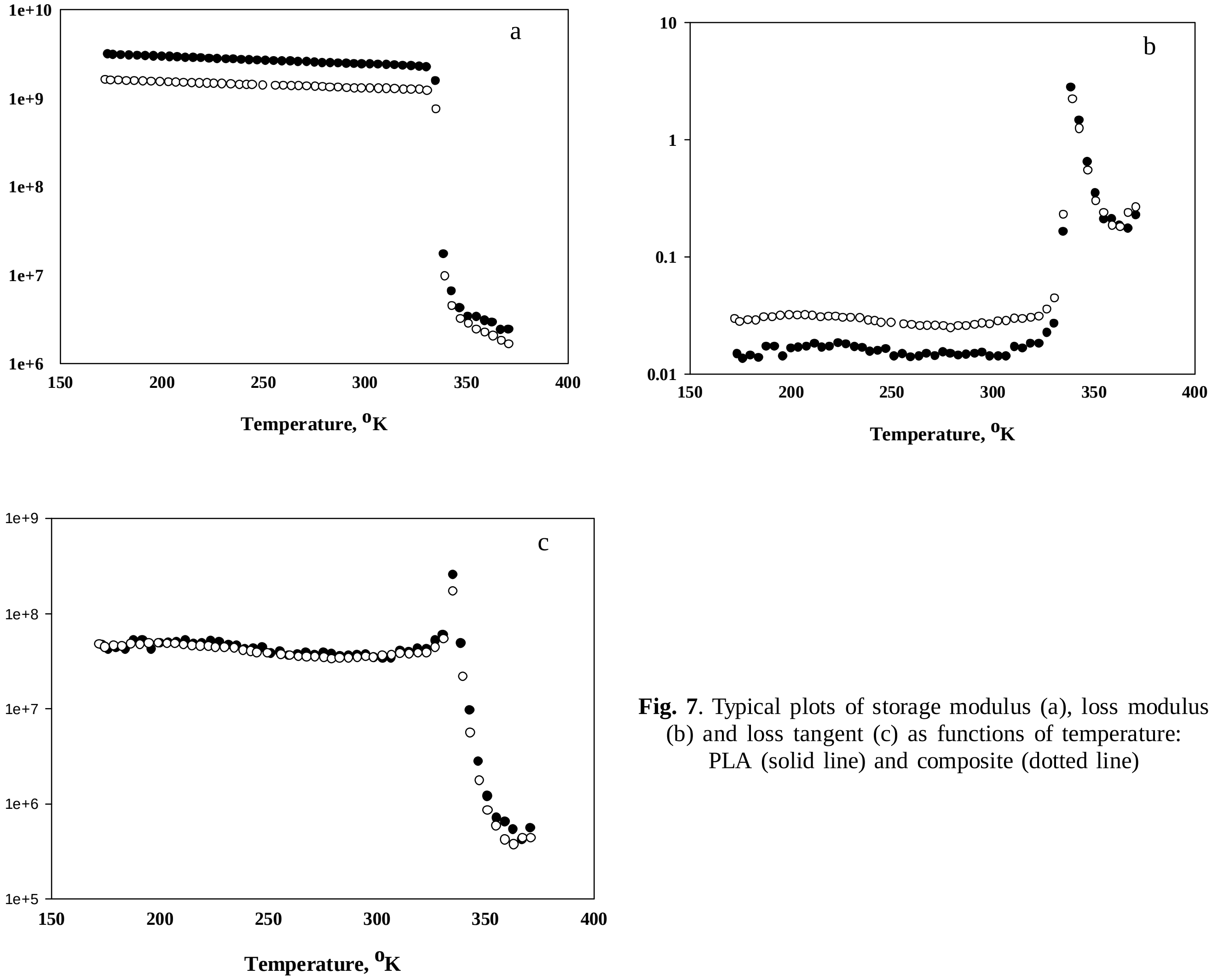

Fig. 7. Typical plots of storage modulus (a), loss modulus (b) and loss tangent (c) as functions of temperature: PLA (solid line) and composite (dotted line)

in the value of measured thickness but does not contribute to the response to external force. Thus, the calculated modulus for the composite membranes may be lower than the actual values.

3.4. Mechanical Testing

Table 1 shows the mechanical properties of PLA and PLA/pectin-Nisaplin® composite membranes. The inclusion of pectin particles reduced the values of stiffness, flexibility, strength, and toughness of the composites, which is consistent with our previous findings [16]. The inclusion of pectin-Nisaplin® resulted in a further decrease in the mechanical properties. The values of the tensile strength, Young's modulus, and fracture energy of the PLA/pectin-Nisaplin® were only 1/2, 3/5, and 1/2 of those for PLA/pectin membranes, respectively. Nisaplin® only contains 2.5 % nisin and is mostly composed of milk solids and salts, which are completely immiscible with PLA. As shown in Fig. 1, the microscopic analysis revealed a porous structure for the composite membranes. Probably, the decrease in mechanical properties could be attributed to the incompatibility between those non-active components of Nisaplin® and the PLA. This was also demonstrated by the cyclic tests. Fig. 8 shows the results of stress as a function of the stretch cycles. In the response to the same strain, the pure PLA membranes (Fig. 8a) display higher stress and little change in the peak stress over the whole testing in comparison with the composite samples (Fig. 8b).

To verify this assumption, we investigated the structural changes by acoustic emission when the samples were submitted to an external force. Fig. 9 shows the relationship of the AE hit patterns with the correlated stress-strain curves. The PLA sample emitted minor sound waves at the beginning of the stretch (which were lower than the threshold, and thus could not be plotted). Even with the increase in both stress and strain, there was no significant AE hit recorded until complete fracture occurred. The specimen displayed a sudden increase at the peak of the stress curve. The only AE hit peak recorded was at the maximum of strain, which correlated to the maximal stress (Fig. 9a). This profile indicates the homogeneous structure of extruded PLA membranes. For composite membranes, a set of discontinuous sound peaks were detected as the samples stretched. Debonding occurred at around 3 MPa, and the highest peak occurred at 20 MPa, where the sample was completely fractured. Between the two acoustic events, the stress-strain curve of the PLA/pectin-Nisaplin® was still showing an increase (Fig. 9b); however, the stretch of the matrix phase and energy consumption was much smaller than that for pure PLA.

Table 1

Mechanical properties of PLA and PLA/Pectin-nisaplin membranes

Samples	Tensile strength, MPa	Elongation at Break, %	Young's modulus, MPa	Toughness J/cm^3
PLA	69.8 ± 3.2	2.74 ± 0.36	3310 ± 276	1.09 ± 0.25
PLA/Pectin	40.2 ± 1.1	1.98 ± 0.07	2598 ± 100	0.35 ± 0.02
PLA/pectin-nisaplin	20.6 ± 5.3	1.83 ± 0.36	1530 ± 309	0.17 ± 0.06

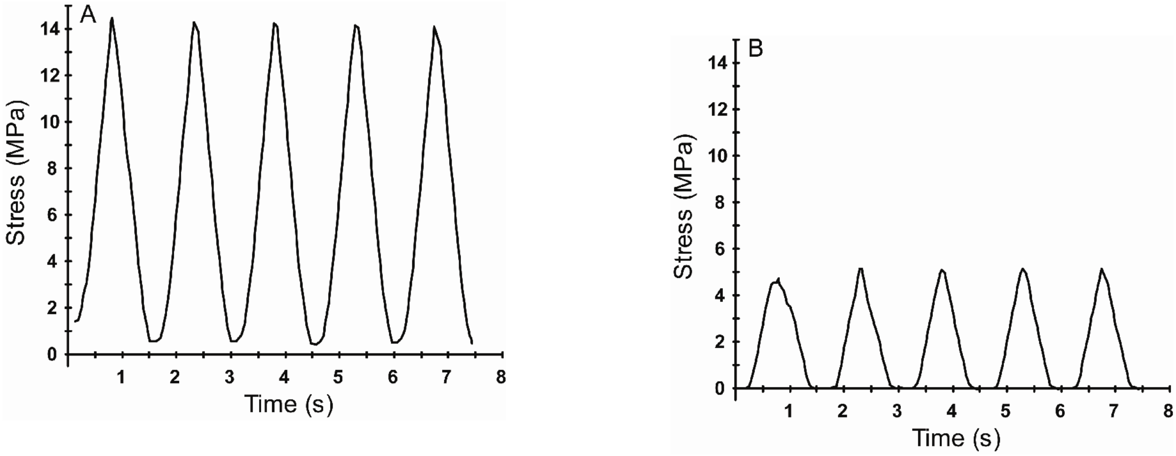

Fig. 8. Time dependent tress curves: PLA (a) and PLA/pectin-Nisaplin® (b) membranes

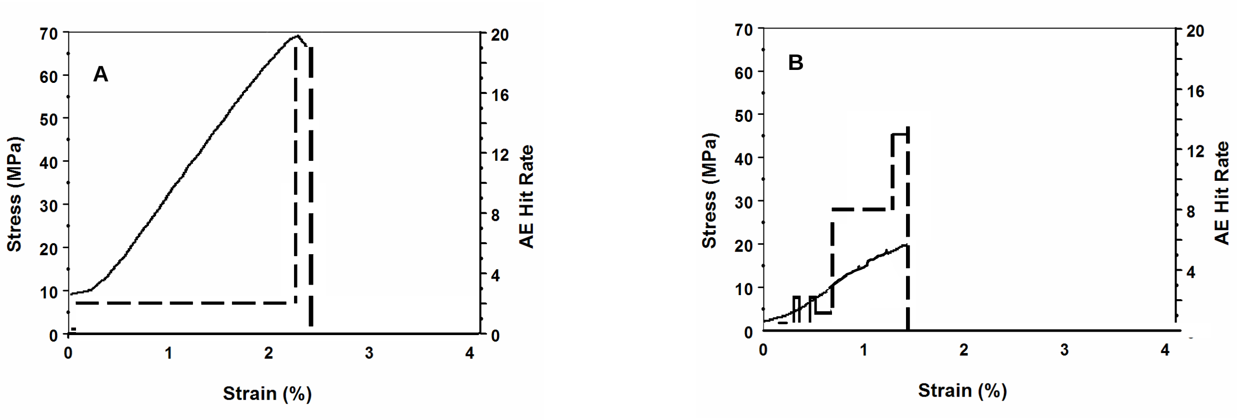

Fig. 9. Relationship of stress-strain curve (solid line) vs. AE patent (broken line) of PLA (a) and PLA/Pectin-nisaplin® composite (b) membrane

Mechanical properties are an important measurement in determining the end use of polymer membranes. For packaging purposes, materials should possess adequate mechanical properties, such as strength, flexibility, and toughness, to fit the requirements of packaged products (weight, size, and shape), transportation, and storage conditions. PLA/pectin-Nisaplin® composite films showed much lower values of tensile strength and toughness in comparison with other packaging materials, such as poly(vinyl chloride) (25 MPa), polystyrene (55 MPa), and blends of soybean protein and carboxy-methylated corncob (29 MPa) [24-26]. PLA/pectin-Nisaplin® alone may not be suitable as a primary packaging material in a single layer. However, it may be suitable as a lining layer of a primary, secondary, or tertiary packaging material, in which the inner layer releases active compounds to suppress microbial growth [27].

4. Conclusions

These preliminary experiments showed that the thermostability of nisin and Nisaplin® could be improved by mixing with pectin. The pectin-nisin and pectin-Nisaplin® complexes were able to suppress the growth of *L monocytogenes*, even after being treated at 433 K during extrusion processing. The pectin-nisin and pectin-Nisaplin® microparticles retained their antimicrobial activity after extrusion with PLA. However, extruded PLA/pectin-Nisaplin® membranes showed reduced mechanical properties, due to the pores created in the PLA phase and poor adhesion between the PLA phase and the particle phase. The extruded PLA/pectin-Nisaplin® membranes may not be suitable for use as a primary packaging material, although they might be used as an active inner layer in multi-layer packaging materials for active packaging.

The mechanism, by which the mixing of pectin with nisin or Nisaplin® improved the thermostability of the polypeptide, remains unclear. Further experiments are planned.

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АНТИМІКРОБНІ ПАКУВАЛЬНІ МАТЕРІАЛИ З ПОЛІ(МОЛОЧНОЇ КИСЛОТИ) ІЗ ВКЛЮЧЕННЯМИ МІКРОЧАСТИНОК ПЕКТИНУ-НИСАПЛИНУ®

Анотація. *Вивчено термостабільність низину та Нисаплину® у присутності та відсутності пектину. Внаслідок змішування низину і Нисаплину® з пектином спостерігається інгібування росту мікробів, навіть при нагріванні до 433 К. Водночас, у відсутності пектину низин повністю дезактивується після термооброблення. Силовим атомним мікроскопічним аналізом встановлено, що низин і пектин у розчині комплексуються. Ймовірно, утворення комплексу між полісахаридами і поліпептидами приводить до підвищення термостабільності низину, незважаючи на те, що механізм цього процесу вимагає ще детального вивчення. Ко-екструзією полімолочної кислоти і мікрочастинок пектин-Нисаплину® були виготовлені тонкі мембрани. Незважаючи на високі температури екструзії, наявність пектину захищає біологічну активність низину і одержані мембрани є антимікробними та можуть використовуватись як внутрішнє покриття багатошарових пакувальних матеріалів.*

Ключові слова: *пектин, низин, упакування, полімолочна кислота, біодеградуючий, акустична емісія.*