Biodegradable Poly(butylene adipate-*co*-terephthalate) Films Incorporated with Nisin: Characterization and Effectiveness against *Listeria innocua*

LUIS BASTARRACHEA, SUMEET DHAWAN, SHYAM S. SABLANI, JAE-HYUNG MAH, DONG-HYUN KANG, JINWEN ZHANG, AND JUMING TANG

ABSTRACT: Biodegradable poly(butylene adipate-*co*-terephthalate) (PBAT) films incorporated with nisin were prepared with concentrations of 0, 1000, 3000, and 5000 international units per cm^2 (IU/cm^2). All the films with nisin inhibited *Listeria innocua*, and generated inhibition zones with diameters ranging from 14 to 17 mm. The water vapor permeability and oxygen permeability after the addition of nisin ranged from 3.05 to 3.61 × 10^{11} g m m^{-2} s^{-1} Pa^{-1} and from 4.80 × 10^7 to 11.26 × 10^7 mL·m·m^{-2}·d^{-1}·Pa^{-1}, respectively. The elongation at break (ε_b) was not altered by the incorporation of nisin ($P > 0.05$). Significant effect was found for the elastic modulus (E) and the tensile strength (σ_s) ($P < 0.05$). The glass transition and melting temperatures with the presence of nisin ranged from −36.3 to −36.6 °C and from 122.5 to 124.2 °C, respectively. The thermal transition parameters such as the crystallization and melting enthalpies and crystallization temperature were influenced significantly ($P < 0.05$) by incorporation of nisin into films. The X-ray diffraction patterns exhibited decreasing levels of intensity (counts) as the concentration of nisin increased in a range of 2θ from 8° to 35°. Formation of holes and pores was observed from the environmental scanning electron microscopy images in the films containing nisin, suggesting interaction between PBAT and nisin.

Keywords: crystallinity, ESEM, oxygen permeability, PBAT, tensile properties, thermal analysis, water vapor permeability, X-ray diffraction

Introduction

Currently, about 41% of the total plastic production is utilized for packaging of commodities, of which about 47% of the packaging is used for packaging of foods. The most commonly used plastic packaging materials are polypropylene, polyethylene, polyvinyl-chloride, polystyrene, polyethylene terephthalate, and nylon. These plastics are produced using fossil fuels and are nonbiodegradable, which means that the wastes will remain in the nature for hundreds of years or longer. Hence there is a need to develop biodegradable materials as alternatives for food packaging (Ray and Bousmina 2005). Several efforts have been made in the development of environmental friendly alternatives, led by the necessity of reducing municipal waste. A considerable variety of applications can be found for such materials, as plastics and surfactants (De Graaf and Kolster 1998). The application of biodegradable materials for food packaging has been very limited due to poor barrier properties against gases and water vapor, and their weak mechanical properties (Sorrentino and others 2007). The utilization of biodegradable materials has to be considered (in despite of the limitations they might have) due to the deleterious effect the commonly used plastics generate (Cutter 2006).

Poly(butylene adipate-*co*-terephthalate) (PBAT) is an aliphatic–aromatic copolyester, which is completely biodegradable. Its chemical structure is presented in Figure 1 (Chivrac and others 2006); the "x" unit (butylene adipate) represents 57% of its composition and the "y" unit (butylene terephtalate) occupies the rest of the structure. PBAT is synthesized by melt polycondensation and melt transesterification of poly(butylene adipate) and poly(butylene terephtalate) (Chivrac and others 2006). PBAT can degrade in a few weeks once it gets in contact with the environment through the intervention of natural enzymes; its degradation takes place by lipases from *Pseudomona cepacia* and *Candida cylindracea* (Herrera and others 2002). This polymer can be extruded to fabricate films and coatings (Jiang and others 2006). So far PBAT has been utilized for fabrication of agricultural films, film lamination for rigid food packaging, and lawn waste bags (Herrera and others 2002). To the best of our knowledge, PBAT has not been tested for its applicability as an antimicrobial packaging material.

According to Suppakul and others (2003), the main goal of antimicrobial packaging systems is to increase the shelf life of foods by extending the lag phase of the microorganisms and then inhibit their growth. Food packages with antimicrobial activity are made by direct incorporation of the active substance in the packaging matrix, by surface modification of the packaging material, or by coating. Two different types of antimicrobial films are found: those that present migration of the active substance to the food and those that do not exhibit migration of such active substance but have the ability to inhibit microbial growth by direct contact with the food

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surface. Currently, in addition to environmental friendly packaging materials, consumers demand more natural products with the use of a lower quantity of additives. The incorporation of antimicrobial agents to the biodegradable materials used for food packaging represents an advantage because those substances are not directly added to the food product, which allows the release of low levels of preservatives to the product's matrix.

The growth of microorganisms is the main cause of food spoilage, resulting in a diminished quality, reduced shelf life, and potential risks against health. In the food industry, the prevention of food spoilage by microorganisms is a vital issue, which is highly related to profit increment because an extended shelf life leads to increased market coverage. The antimicrobial packaging systems present several advantages as a complement to the existing processing technologies against food spoilage. They can enhance the effectiveness of conventional packaging systems with high barriers against gases and water vapor against microorganisms (Han 2005).

Nisin is a peptide produced by *Lactococcus lactis*. It is able to inhibit the growth of Gram-positive bacteria, the microbial wall synthesis, and the outgrowth of spores, without imparting adverse effects to the human health (Rydlo and others 2006; Sanjurjo and others 2006). In the United States, it is the 1st antimicrobial bacteriocin with the status of generally recognized as safe (GRAS) and it is approved by the Food and Drug Administration (FDA) to be used in processed cheese (Sanjurjo and others 2006). Nisin is a positively charged molecule with hydrophobic sections; it is able to bind to the negatively charged sites of the phosphate groups in the cell membranes. The hydrophobic section of the nisin peptide inserts into the membrane, leading to formation of pores. The resulting formation of pores can take place in 2 different ways: the molecule may orient perpendicularly to the cell membrane forming an ion channel that crosses the cell wall, or once a minimum required number of nisin molecules gets in contact with the cell membrane, they are able to form a wedge. This provokes a leakage of internal cell materials, making bacteria lose their capability to reproduce (Cleveland and others 2001). The amount of nisin in a system is usually expressed in international units (IU); 1 g of pure nisin represents 40×10^6 IU (Ray 1992).

Nisin is the antimicrobial most widely used in the development of active packaging films. It is used alone or incorporated with other antimicrobial substances. Its small molecular size permits the production of films that release the peptide once it is in contact with a liquid or solid food. Nisin is generally incorporated into coatings with acid compounds. One possible reason of the frequent selection of nisin is its regulatory status as a food additive and its ability to inhibit the growth of *Listeria monocytogenes* (Joerger 2007). According to Jin and others (2009), *L. monocytogenes* is of special concern among the vulnerable population. USDA's Food Safety Inspection Service states that *L. monocytogenes* has to be absent in ready-to-eat foods. This bacterium can survive or even grow under refrigeration conditions in acidic products and this is one of the reasons that make *L. monocytogenes* an adequate target microorganism if a specific pathogenic bacterium is not defined in a selected product. According to Warriner and Namvar (2009), *Listeria innocua* and *L. monocytogenes* phenotypes are closely related; even though the presence of *L. innocua* in foods does not represent a hazard, *L. innocua* is very similar to *L. monocytogenes* and therefore can be studied as if it was the pathogenic microorganism (Giraffa and others 1995). According to Rodríguez and others (2006), a surrogate is a nonpathogenic microorganism that shows similar kinetic and inactivation characteristics to the pathogenic target microorganisms normally found in foods. They also share similar behavior when exposed to the similar environmental conditions (oxygen concentration, temperature, pH, and so on), and similar genetic stability. The surrogates are useful to perform experiments in situations in which it is not possible to work with the pathogenic target microorganisms. Some experiments are performed in the food production facilities, pilot plants, or laboratories in which it is not possible to compromise with the safety of the workers. Previous studies revealed that the behavior of *L. innocua* under different conditions of pH and water activity is similar to the behavior of *L. monocytogenes*. *L. innocua* adequately represent as a surrogate of the pathogenic bacteria *L. monocytogenes*. *L. innocua* can be used as a biological indicator of *L. monocytogenes* (Rodríguez and others 2006).

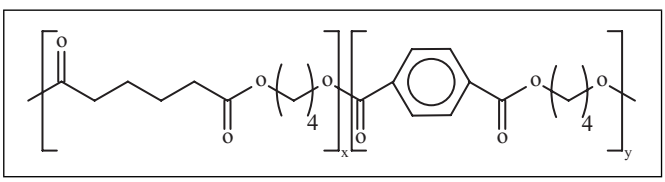

Figure 1—Chemical structure of PBAT (Chivrac and others 2006).

Several studies have reported the thermal and tensile properties (Someya and others 2005; Chivrac and others 2006; Chivrac and others 2007; Ludvik and others 2007; Rhim 2007a; Iwakura and others 2008) and water vapor barrier properties (Rhim 2007a) of PBAT films. These properties are important to characterize plastics for their use in food packaging. The information is important not only for the potential applications in specific foods but also for consideration in selecting the processing conditions and determining the transportation requirements (Robertson 1993). The morphological properties can be valuable in explaining the interaction between antimicrobials and the polymer matrices, and the variations in thermal and mechanical properties after incorporating antimicrobials into polymeric films (Linssen and others 2003; Chivrac and others 2006). The incorporation of antimicrobials can modify the water sorption behavior of the polymers, which may alter the gas barrier and mechanical properties of films. Previous studies have investigated this phenomenon through the construction of water sorption isotherms (Stenhouse and others 1996; Guiga and others 2008).

Two casting methods are available for the production of antimicrobial films. The 1st involves extrusion, and the other is solution casting. In the latter method, selection of the adequate solvent is important to achieve maximum effectiveness of the selected antimicrobial due to their possible interactions (Han 2003). Among the bacteriocins, nisin shows the highest affinity to chloroform, mainly because of the hydrophobic behavior both compounds share. Chloroform has been used to recover nisin from media with nisin-producing culture (Burianek and Yousef 2000).

Thus, the objectives of this study are to (1) develop PBAT films incorporated with different levels of nisin using the solution casting method, (2) study their effectiveness against *L. innocua*, and (3) characterize mechanical, gas barrier, thermal, and morphological properties of the selected materials.

Materials and Methods

Film preparation

The antimicrobial films were prepared by solution casting method. Casting plates were fabricated using aluminum angles of $2.54 \times 0.16 \times 121.9$ cm and polytetrafluoroethylene (PTFE) Teflon sheets of $14 \times 21.6 \times 0.64$ cm (McMasterr-Carr, Chicago, Ill., U.S.A.). To fabricate a casting plate, a frame was prepared using the

aluminum angles and it was attached to the PTFE sheet with epoxy resin (Figure 2). The final PTFE exposed area in the plates was approximately 258.06 cm^2. PBAT resin (F BX 7011) with a density of 1.25 g/cm^3 was obtained from BASF Corp. (Florham Park, N.J., U.S.A.).

A 5% (weight of PBAT/volume of chloroform) solution of PBAT and chloroform was prepared dissolving a predetermined amount of PBAT resin in chloroform (Mallinckrodt Baker, Inc., Phillipsburg, N.J., U.S.A.) to obtain a 50-μm-thick film. Once the resin was completely dissolved, the solution was poured into a casting plate and left overnight in a hood, to permit the evaporation of chloroform. After the complete evaporation of chloroform, the film was removed from the casting plate using a knife to cut the edges and then peeled off.

To fabricate films with nisin, the procedure included the incorporation of Nisaplin powder (Danisco Specialities, Aplin & Barrett Ltd., U.K.), with a minimum nisin content of 1000 IU/mg, after the polymer was dissolved in chloroform. A predetermined quantity of Nisaplin was added to the PBAT/chloroform solutions in glass containers. The closed glass containers with the solutions were subjected to 1 h of ultrasonication using a Tabletop Ultrasonic Cleaner FS-30H (Fisher Scientific, Pittsburgh, Pa., U.S.A.). This step was necessary for uniform dispersion of the Nisaplin powder in the PBAT/chloroform solution (Rhim 2007b). The solutions were then poured onto the casting plates, and left overnight for the evaporation of chloroform. Once the films were ready, they were removed from the casting plates as described earlier. The procedure was done to obtain films with nisin concentrations of 1000, 3000, and 5000 IU/cm^2. All the films were stored in a refrigerator at 4 °C in sealed bags before being subjected to the different experiments performed in this study. The films' thickness was measured to the nearest 0.00254 mm with a micrometer (Micrometer 97231-61, Fred V. Fowler Co., Inc., Newton, Mass., U.S.A.). The thickness was measured at 10 randomly selected points of a 258.06 cm^2 film. The thickness determination was performed 3 times (3 replicates).

Inhibition zone assay

L. innocua ATCC 51742, ATCC 33090, and SEA 15C19 were obtained from the School of Food Science of Washington State Univ. (Pullman). The stock cultures were maintained in tryptic soy broth (TSB: Difco, Becton Dickinson, Sparks, Md., U.S.A.) containing 15% glycerol at −20 °C. One loopful of stock was inoculated onto modified Oxford agar (MOX: Difco, Becton Dickinson, Sparks, Md., U.S.A.), incubated at 37 °C during 24 h and finally stored under refrigeration at 4 °C until use. A single colony of each *L. innocua* strain was transferred with a sterile loop into 9 mL of TSB plus 1% yeast extract (TSBYE: Difco, Becton Dickinson) and grown for 24 h at 37 °C. The inhibition zone determination was carried out according to the procedure described by Tramer and Fowler (1964): a semisoft agar prepared with TSBYE plus 0.7% agar (Difco, Becton Dickinson) and 1% Tween 20 (Fischer Scientific, Fair Lawn, N.J., U.S.A.) was autoclaved and cooled to 48 °C. A volume of 100 mL of the semisoft agar medium was seeded with 1 mL of a cocktail prepared by mixing equal culture volumes of the 3 *L. innocua* strains to have an approximate concentration of 10^6 CFU/mL of culture mix. The detergent Tween 20 was used to improve the diffusion of nisin through the agar medium. About 4 mL of the seeded semisoft agar were poured over petri dishes with tryptic soy agar (TSA: Difco, Becton Dickinson). Once the semisoft agars had solidified, film discs with a dia. of 13 mm were cut using a sterile cork borer from films prepared using selected nisin concentrations of 0, 1000, 3000, and 5000 IU/cm^2. The film discs were placed over the semisoft agars using sterile tweezers. The plates containing the films were kept at 4 °C for 24 h and then incubated at 37 °C for another 24 h. The refrigeration step was important to enhance the release of nisin before the growth of the bacteria so that larger inhibition zones were possible to observe (Neetoo and others 2007). After the incubation, the diameters of inhibition zone were measured to the nearest 0.01 mm with an electron digital caliper (Fisher Scientific, Pittsburgh, Pa., U.S.A.). Four measurements were done in the inhibition zone of each plate (45° apart). This experiment was performed 3 times (3 replicates).

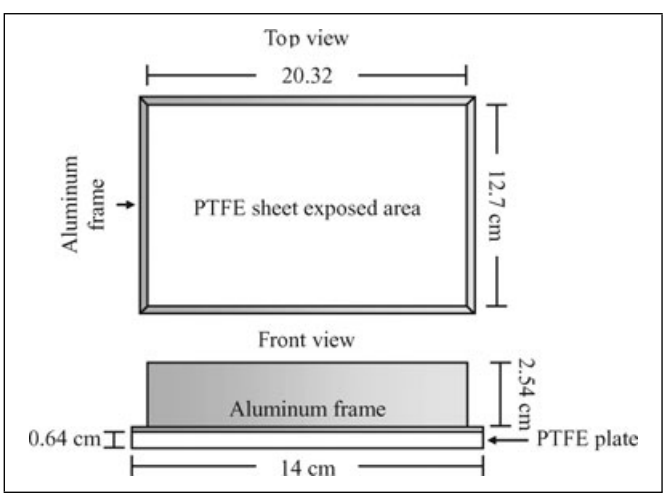

Figure 2—Scheme of a casting plate.

Water sorption isotherms

The water isotherms of PBAT films prepared using selected nisin concentrations were determined using the isopiestic methods (Sablani and others 2009). Film strips of 2 × 8.5 cm were kept for at least 2 wk in hermetic glass jars with supersaturated salt solutions of lithium chloride (LiCl, 11% RH), potassium acetate (CH_3COOK, 23% RH), magnesium chloride ($MgCl_2$, 33% RH), potassium carbonate (K_2CO_3, 43% RH), magnesium nitrate ($Mg(NO_3)_2$, 53% RH), sodium nitrite ($NaNO_2$, 64% RH), sodium chloride (NaCl, 75% RH), and potassium chloride (KCl, 84% RH). All the salts were from Fisher Scientific (Fair Law, N.J., U.S.A.) except for CH_3COOK and K_2CO_3, which were obtained from Sigma-Aldrich, Inc. (St. Louis, Mo., U.S.A.). After a minimum of 2 wk under equilibration, the water content of the films was determined with a thermogravimetric analyzer TGA/SDTA 851 e (Mettler-Toledo, Columbus, Ohio, U.S.A.) with a heating rate of 20 °C/min to achieve a temperature of 115 °C. The samples were held for 20 min at 115 °C. The PBAT film samples tested were in the range of 10 to 20 mg, taken from the original film strips. The experiments were performed 3 times (3 replicates). The water contents in dry basis of the selected films were plotted against water activity (a_w), and different models of water sorption isotherms (Iglesias and Chirife 1982) were evaluated to fit the experimental data.

Water vapor permeability (WVP) and oxygen permeability (OP)

The gravimetric modified cup method from the American Society of Testing and Materials (ASTM) method E96-92 (McHugh and others 1993) with some modifications was employed to determine the WVP of PBAT films. Test cups with internal and external dia. of 5.0 and 9.0 cm (respectively) were fabricated using acrylic sheets (McMasterr-Carr) (Figure 3). The height of the lower portion for holding the water was 1.2 cm. A volume of 6 mL of distilled water was placed in the test cups and films were mounted leaving a 0.9 cm air gap between the film and the water surface. The cups were placed in a Dry Keeper™ Non-electric Dessicator cabinet

(Scienceware, Pequannock, N.J., U.S.A.) maintained at 25 °C and at an RH of 0% achieved with anhydrous calcium sulfate (Drierite, W. A. Hammond Drierite Co. Ltd., Xenia, Ohio, U.S.A.). The water loss from each cup was determined every 12 h during a period of 24 h. The water vapor transmission rate (WVTR) in $g \cdot h^{-1} \cdot m^{-2}$ was calculated from the slope of the straight curve of water loss against time divided by the film's exposed area (0.002 m^2). The WVP was calculated using

$$\mathrm{WVP} = \mathrm{WVTR}\frac{L}{\Delta p}, \tag{1}$$

where L is the film thickness in m and Δp is the water vapor partial pressure difference in Pa between the 2 sides of the film (underneath the film and cabinet). The value of Δp was obtained from (Thirathumthavorn and Charoenrein 2007)

$$\Delta p = P - (P - p_T)e^{\left(\frac{WVTR \times R \times T \times z}{D_{wa} \times P}\right)}, \tag{2}$$

where P is the atmospheric pressure in Pa, p_T is the water vapor pressure at the testing temperature in Pa, R is the gas law constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$), T is the testing temperature in K, z is the air gap between the film and the water surface, and D_{wa} is the water diffusivity in air at the testing temperature (25 °C), equal to 2.5×10^{-5} $m^2 \cdot s^{-1}$ (Çengel 2006). The experiment was performed 3 times (3 replicates), with 2 film samples per replicate.

The OP was determined with an MOCON OX-TRAN® 2/21 (Modern Controls, Inc., Minneapolis, Minn., U.S.A.) at 0% RH and 23 °C following the ASTM method D3985-95 (ASTM 1995). The test was performed 3 times (3 replicates), with 2 samples per replicate.

Tensile properties

The ASTM method D882-02 (ASTM 2002) was followed for the determination of elongation at break (ε_b), elastic modulus (E), and tensile strength (σ_s). Film strips from the different nisin concentrations (1 × 10 cm) were conditioned in a closed cabinet for 48 h at an RH of approximately 50% (achieved with a supersaturated solution of $Mg(NO_3)_2$ [Fisher Scientific, Fair Lawn, N.J., U.S.A.]) and at approximately 23 °C. A screw-driven universal testing machine (Instron 4466, Instron Corp., Norwood, Ma., U.S.A.) with a 10 kN electronic load cell and mechanical grips were used for this experiment. An initial grip separation of 5 cm and a rate of grip separation equal to 50 cm/min were applied. Films' deflection (strain) was measured with an extensometer MTS 634.12E-24 and the data (load and extension) were obtained by computer. The experiments were performed in triplicate (3 replicates, with 5 film strips per replicate) for films with the selected concentrations of nisin. The elastic modulus is the force per unit area necessary to increase the length of a film sample to a certain proportion, and is defined by (Roff and Scott 1971)

$$E = \frac{\sigma}{\varepsilon}, \tag{3}$$

where E is the elastic modulus in MPa, σ is the stress in MPa, and ε is the strain, defined by

$$\varepsilon = \frac{L_t - L_o}{L_o} \tag{4}$$

where L_t is the length of the deformed film at time t and L_o is the original length of the film, both quantities in m. The value of E can be calculated from the slope of the straight portion of the curve obtained by plotting σ against ε. The values of σ at the corresponding values of ε are calculated by dividing the load by the cross-sectional area of the sample films. From the same curve of σ versus ε it is possible to obtain the value of σ_s, where the stress achieves a maximum value before the film breaks. The value of ε_b was calculated by multiplying ε (where $\sigma = \sigma_s$) by 100 and expressed as percentage.

Thermal analysis

A differential scanning calorimeter (Q2000, TA Instruments, New Castle, Del., U.S.A.) was used to determine the thermal properties of the PBAT films. Samples of 5 to 10 mg were 1st equilibrated at 25 °C for 1 min, then cooled down from 25 to −70 °C at a cooling rate of −5 °C/min and then heated to 200 °C at a heating rate of 10 °C/min. The samples were held at 200 °C for 1 min and cooled down back to 25 °C. The melting temperature (T_m) was considered as the minimum point of the melting peaks. The glass transition temperature (T_g) was taken at the maximum point of heat flow where a change in the specific heat takes place. The melting enthalpy (ΔH_m) was measured from the area of the melting peaks with the instrument's software. The crystallization temperature (T_c) was considered as the maximum point of the exothermic peak and the enthalpy of crystallization (ΔH_c) was determined from its area with the instrument's software (Chivrac and others 2006, 2007). The degree of crystallinity (χ) was calculated from the following formula:

$$\chi = \frac{\Delta H_m}{\Delta H_{m_{100}}} \times 100, \tag{5}$$

where ΔH_m is the melting enthalpy of the samples and $\Delta H_{m_{100}}$ is the melting enthalpy of PBAT in 100% crystalline form, which corresponds to a value of 114 J/g (Chivrac and others 2006). The determination of the thermal properties was conducted 3 times (3 replicates) for every nisin concentration.

X-ray diffraction

Films with dimensions of 3 × 3 cm were examined in a scanning range from 8° to 35° (2θ), with a step of 0.05°, 3 s each, using

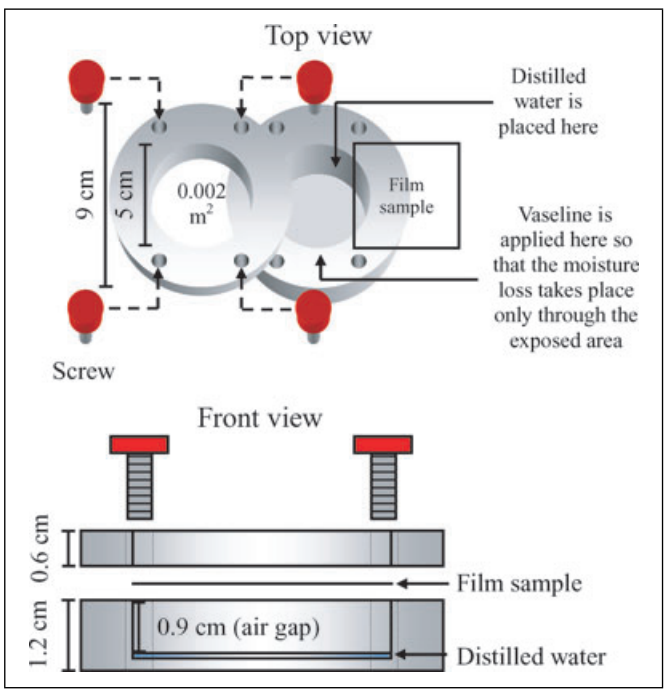

Figure 3—Scheme of a test cup used for the determination of WVP.

a D-500 powder X-ray diffractometer (Siemens, Bruker, Karlsruhe, Germany). The instruments' copper target tube was set at 35 kV and 30 mA, with a wavelength of 1.5 Å. The test was performed at room temperature.

Environmental Scanning Electron Microscopy (ESEM)

The films were analyzed through a FE SEM Quanta 200F (FEI Co. [Field Emission Instruments], Hillsboro, Oreg., U.S.A.). Film samples (approximately 6 × 6 mm) of every nisin concentration were subjected to analysis at an accelerating voltage of 30 kV.

Data analysis

A complete randomized design was applied in this study. The general linear model was utilized to analyze the data and significant differences ($P < \alpha$) were determined in the different properties tested between the nisin concentrations through the Tukey's honestly significant difference test ($\alpha = 0.05$). For the case of the water sorption isotherms, a complete randomized factorial design with 2 independent factors (a_w and nisin concentration) was performed. The analysis was conducted with the software SAS version 9.1 (SAS Inst. Inc., Cary, N.C., U.S.A.).

Table 1 — Diameters of inhibition zone and film thickness for the selected nisin concentrations.

Nisin concentration (IU/cm²)	Diameter of inhibition zone (cm)	Film thickness (μm)
0	0 A	47.9 ± 4.35 A
1000	1.40 ± 0.02 B	58.4 ± 1.21 B
3000	1.57 ± 0.02 C	59.4 ± 1.65 B
5000	1.70 ± 0.03 D	61.8 ± 2.91 B

Values are means ± 1 standard deviation. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

Results and Discussion

Inhibitory zone assay

An increasing zone of inhibition was observed with increasing concentration of nisin in the PBAT films (Table 1). The diameters of inhibition zone ranged from 14 to 17 mm as nisin concentration increased from 1000 to 5000 IU/cm^2. The difference in the diameter of inhibition zone between each nisin concentration was significant ($P < 0.05$) (Figure 4). Similar size of inhibition zones were obtained by Neetoo and others (2007) with films containing nisin concentrations of 1000 IU/cm^2. Dos Santos and others (2008) observed that the inhibition zones generated by nisin-loaded films in agar media containing *L. monocytogenes* had the same diameter as the corresponding film discs, suggesting that inhibition zone was generated only due to the surface of the film in direct contact with agar media. Nevertheless, in this study, diameters of inhibition zone were larger than the diameters of the corresponding PBAT film discs, and no inhibition was exhibited by the films with 0 IU/cm^2 (Figure 5). According to the work of Friedmann and Beach (1950) it is possible to obtain a linear relation between the diameter of inhibition zone in solid media and the logarithm of the nisin concentration applied into the medium (in the range of 100 to 5000 IU/mL). In this study, similar behavior (Figure 6) was observed by plotting the diameter of inhibition zone against the logarithm of the nisin concentrations in the films, and the regression analysis brought a coefficient of determination (R^2) equal to 0.98 (from 1000 to 5000 IU/cm^2).

Water sorption isotherms

The films did not exhibit substantial water sorption as the level of a_w (RH) increased (Figure 7). For the PBAT films at the selected nisin concentrations, there was no significant difference between

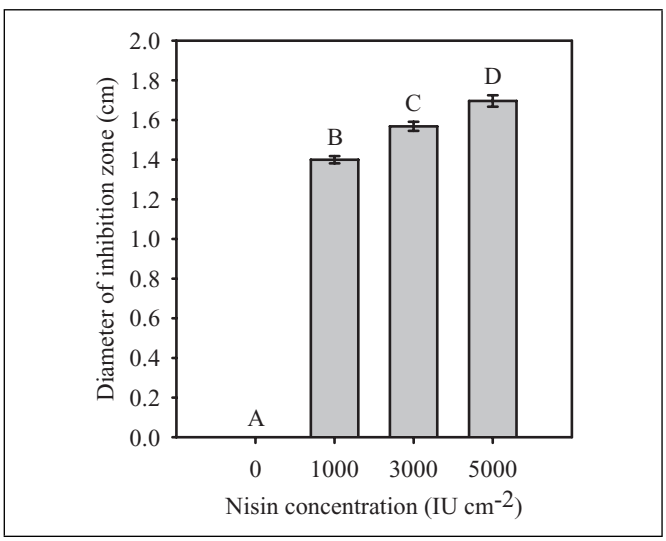

Figure 4 — Diameters of inhibition zone. Error bars represent ±1 standard deviation. Treatments with the same letter are not significantly different ($P > 0.05$).

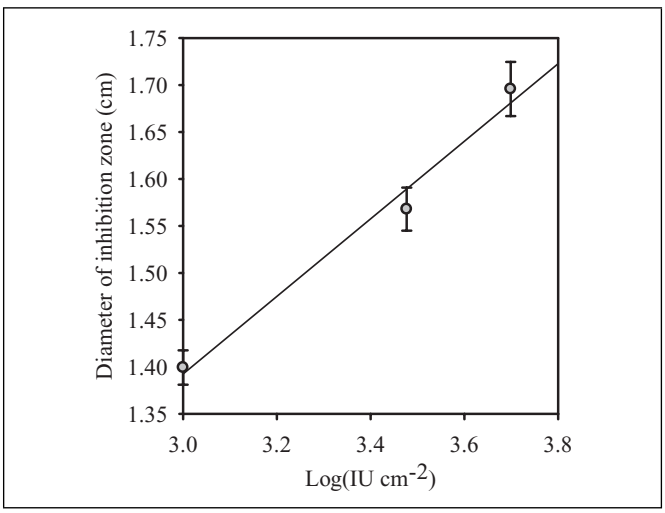

Figure 6 — Relation found between the diameter of inhibition zone (DIZ) and the logarithm of the nisin concentration, including regression line (from 1000 to 5000 in IU/cm²).

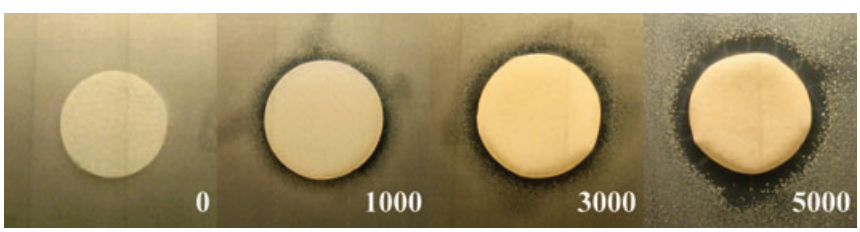

Figure 5 — Pictures showing the inhibition zones under the nisin concentrations evaluated in IU/cm².

the amount of water adsorbed with increasing a_w from 0.11 to 0.75 ($P > 0.05$) except at the nisin concentration of 5000 IU/cm^2, where there was a significant increase ($P < 0.05$) in the equilibrium water content at a_w equal to 0.84. This is possibly due to the hydrophobic nature of PBAT polymer. According to Adebayo and others (2008), the presence of acyl groups (–CO–) in plastics may substantially reduce their capacity to retain water. Contrary to what happens with hydroxyl groups (–OH), the acyl groups are not able to build hydrogen bonds with water molecules. The presence of acyl groups is a characteristic of the PBAT molecule (Figure 1). Thus, it can be assumed that the hydrophobicity of PBAT was not influenced by the incorporation of nisin. PBAT did not show changes in its water sorption characteristics under a wide range of RH. This explains why it was not possible to fit the experimental water sorption data obtained for the selected nisin concentrations with commonly used models of water sorption isotherms for food and other materials.

Water vapor permeability (WVP) and oxygen permeability (OP)

The values of WVP ranged from 3.05 to 3.61 $\times$ 10^{11} g·m·m^{-2}·s^{-1}·Pa^{-1} (Table 2), and no significant difference in WVP was observed with increasing concentration of nisin from 0 to 5000 IU/cm^2 ($P > 0.05$). Comparable results of WVP were obtained by Rhim (2007a) for PBAT films. The WVP values of PBAT films are 50 to 100 times higher than those of low-density polyethylene and polypropylene films (Rhim 2007a). One of the drawbacks of the PBAT films is that they have poor water vapor barrier properties. However, WVP of PBAT films was not influenced by the incorporation of nisin at the selected concentrations.

Table 2—Oxygen permeability (OP) and water vapor permeability (WVP) values of the PBAT films with the different nisin concentrations.

Nisin concentration (IU/cm^2)	OP $\times$ 10^7 (mL·m^{-1}·m^{-2}·d^{-1}·Pa^{-1})	WVP $\times$ 10^{11} (g·m^{-1}·m^{-2}·s^{-1}·Pa^{-1})
0	4.80 ± 0.94 A	3.04 ± 0.26 A
1000	10.7 ± 4.91 A	3.49 ± 0.33 A
3000	7.54 ± 6.64 A	3.40 ± 0.31 A
5000	11.3 ± 6.47 A	3.61 ± 0.73 A

Values are means ± 1 standard deviation. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

The OP of PBAT films was not affected with increasing concentration of nisin from 0 to 5000 IU/cm^2 ($P > 0.05$) (Table 2). A high variability in OP was found in the films containing nisin. Possible interactions between nisin and PBAT may increase the free volume, thus increasing the ability of oxygen to diffuse through the film matrix (McHugh and Krochta 1994; Sablani and others 2009). The incorporation of nisin significantly affected ($P < 0.05$) the films' thickness (Table 1); however, the level of nisin in PBAT films did not significantly influenced the film thickness ($P > 0.05$). The barrier properties of the PBAT films are affected by the film thickness as the transmission rates of gases are negatively related with film thickness as shown in Eq. (1). In this study, however, the overall changes observed in the film thickness as the nisin concentration increased did not show any significant effects on the transmission rates ($P > 0.05$).

Tensile properties

No significant difference was found for ε_b of PBAT films with increasing concentration of nisin (Table 3). However, the values of E and σ_s of the films without nisin were significantly different ($P < 0.05$) from the films containing nisin. No significant difference ($P > 0.05$) was exhibited for E and σ_s of PBAT films with increasing concentration of nisin. From 1000 to 5000 IU/cm^2, a reduction in the range of 39% to 52% for E (stiffness) and in the range of 29% to 40% for σ_s (firmness) was identified after the incorporation of

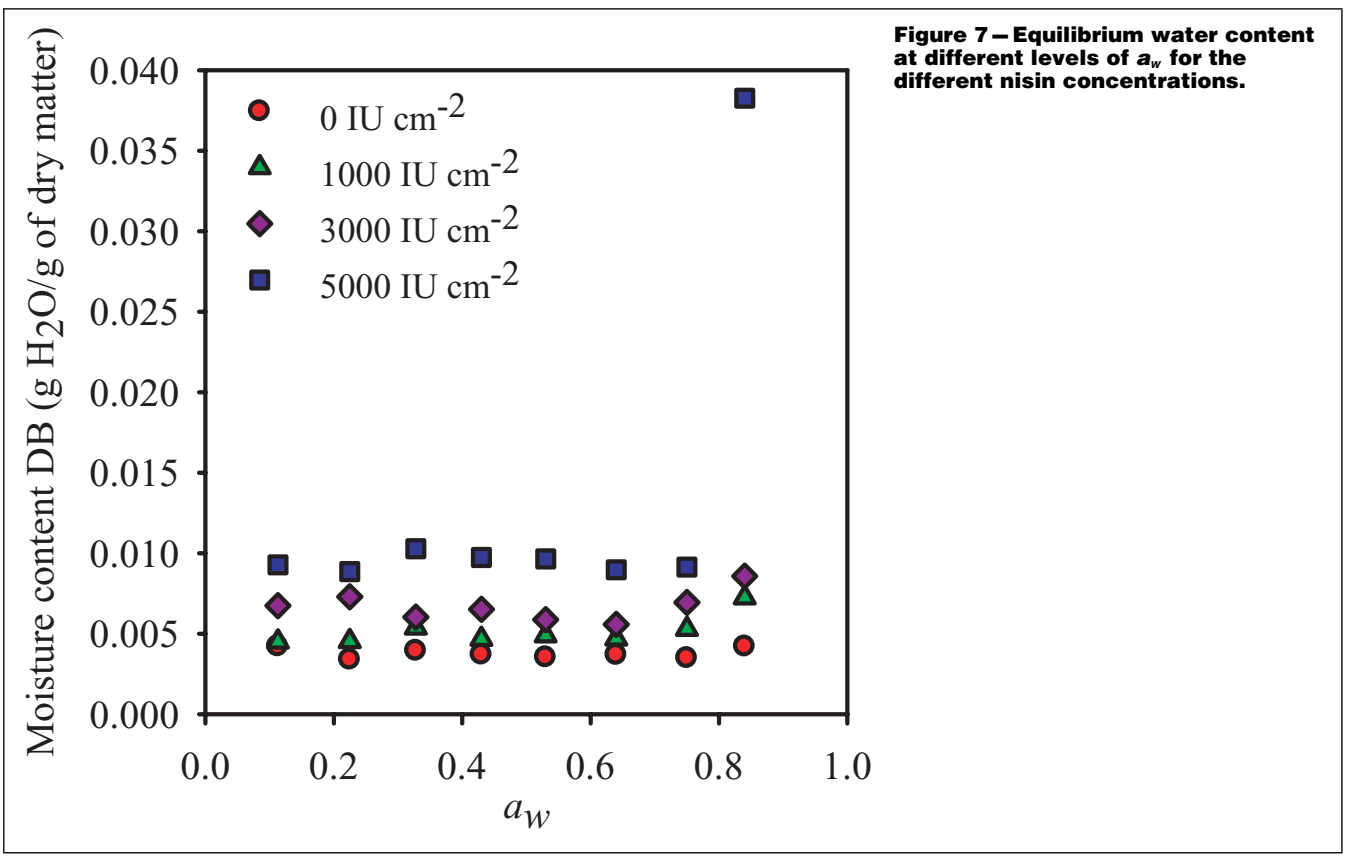

Figure 7—Equilibrium water content at different levels of a_w for the different nisin concentrations.

nisin. The mechanical properties of PBAT films were in the same order of magnitude reported earlier (Chivrac and others 2006; Ludvik and others 2007; Rhim 2007a). As will be explained further, the change in crystallinity in the films with nisin was not dramatically big. However, the factor of crystallinity might not be the only one contributing to the change in the tensile properties; the observed formation of holes and pores in the films with nisin (described further) and the possible lack of uniformity in the films' structure due to the production method (solution casting), may also affect the behavior of these parameters. The formation of cavities in the film matrix and the nonuniform thickness the solution casting method produces may create a weaker structure, which is easier to break, thereby reducing the tensile properties.

Thermal analysis

The glass transition temperature (T_g) and melting temperature (T_m) of PBAT films with nisin concentration between 0 and 5000 IU/cm^2 ranged from −36.6 to −36.3 °C and from 122.5 to 124.2 °C, respectively. Both T_g and T_m of PBAT films were not significantly influenced ($P > 0.05$) by the incorporation of nisin up to 5000 IU/cm^2 (Table 4). The crystallization temperature (T_c) increased from 59.2 to 70.7 °C with increasing concentration of nisin from 0 to 5000 IU/cm^2. However, the film with a nisin concentration of 0 IU/cm^2 was the only significantly different from the films containing nisin ($P < 0.05$), whereas there was no significant difference in T_c between the films containing the different levels of nisin ($P > 0.05$). Crystallization of partially amorphous polymers, unlike low molecular weight materials (salts and sugars), takes place at a slow rate and over a wide range of temperature. The overlapping of polymer chains may not take place completely and some regions will not crystallize due to chain entanglements. Amorphous polymers

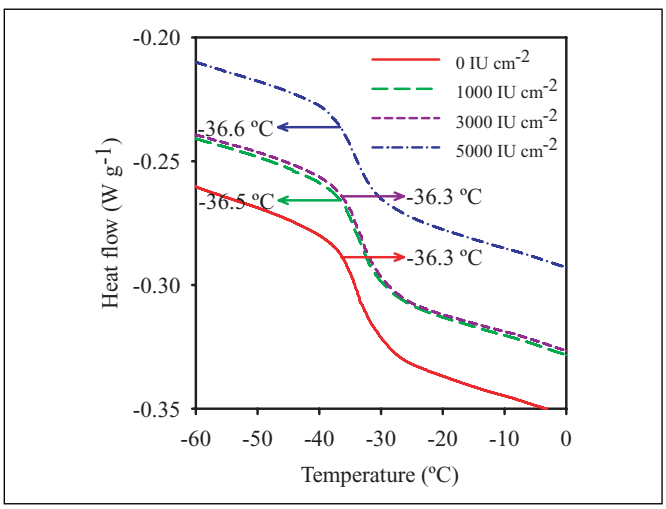

Figure 8—DSC thermogram showing the glass transition for the different nisin concentrations and the corresponding values of T_g.

Table 3—Tensile properties of the PBAT films with the different nisin concentrations: elongation at break (ε_b), elastic modulus (E), and tensile strength (σ_s).

Nisin concentration (IU/cm^2)	ε_b (%)	E (MPa)	σ_s (MPa)
0	513 ± 136*A*	47.7 ± 10.7 A	18.7 ± 2.29 A
1000	512 ± 43.8*A*	29.0 ± 3.82 B	13.2 ± 0.70 B
3000	458 ± 12.3*A*	24.9 ± 1.54 B	11.8 ± 0.38 B
5000	448 ± 12.4*A*	22.9 ± 4.28 B	11.1 ± 0.32 B

Values are means ± 1 standard deviation. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

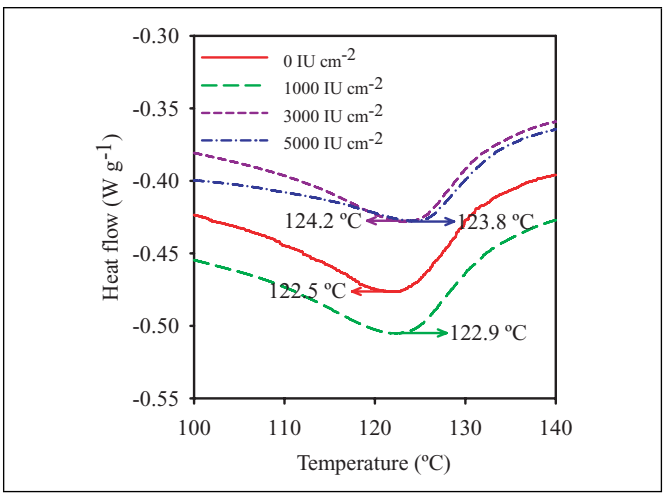

Figure 9—DSC thermogram showing the melting peaks for the different nisin concentrations and the corresponding values of T_m.

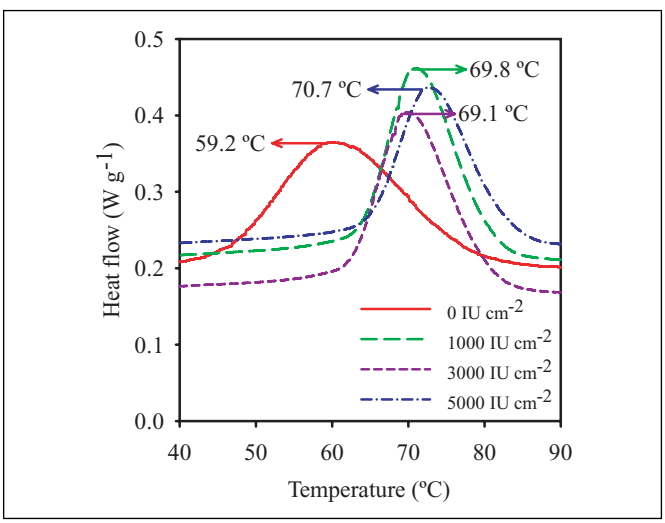

Figure 10—DSC thermogram showing the exothermic peaks for the different nisin concentrations and the corresponding values of T_c.

Table 4—Thermal properties and crystallinity (χ) of the PBAT films with the different nisin concentrations.

Nisin concentration (IU/cm^2)	T_c (°C)	T_g (°C)	T_m (°C)	ΔH_c (J/g)	ΔH_m (J/g)	χ (%)
0	59.2 ± 0.94 A	−36.3 ± 0.21 A	122 ± 0.94 A	19.8 ± 0.42 A	12.5 ± 0.21 A	10.0 ± 0.18 A
1000	69.8 ± 0.94 B	−36.5 ± 0.27 A	123 ± 0.72 A	19.1 ± 0.68 A	12.1 ± 0.32 A	10.6 ± 0.28 A
3000	69.1 ± 0.49 B	−36.3 ± 0.18 A	124 ± 0.48 A	17.9 ± 1.32 AB	8.42 ± 1.00 B	7.38 ± 0.88 B
5000	70.7 ± 1.73 B	−36.6 ± 0.75 A	124 ± 0.92 A	16.4 ± 0.07 B	6.01 ± 0.33 C	5.28 ± 0.29 C

Values are means ± 1 standard deviation. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

usually crystallize above the glass transition temperature, because below T_g there is not enough molecular mobility for polymer chains to interact. If the polymer is heated, its chains will start moving and will find an opportunity to interact with each other, to form a lattice. As mentioned earlier, due to the structural characteristics of polymers, crystallization occurs slowly and over a wide range of temperature. During heating, the polymer will melt if enough heat is applied. Thus, it is common that the T_c and T_m values are substantially separated due to slow crystallization (Groenewoud 2001; Kong and Hay 2003; Menczel and others 2009). T_g and T_m are important factors to take into account because they give information about the level of association between polymer chains. The stronger the intermolecular bonds between polymer chains, the higher the values of T_g and T_m; if the room temperature is between T_g and T_m, the polymer can be either a supercooled liquid with high viscosity or a crystalline solid (Robertson 1993). In this study, the incorporation of the antimicrobial nisin did not significantly affect the glass transition and melting temperatures of PBAT polymer (Figure 8 and 9).

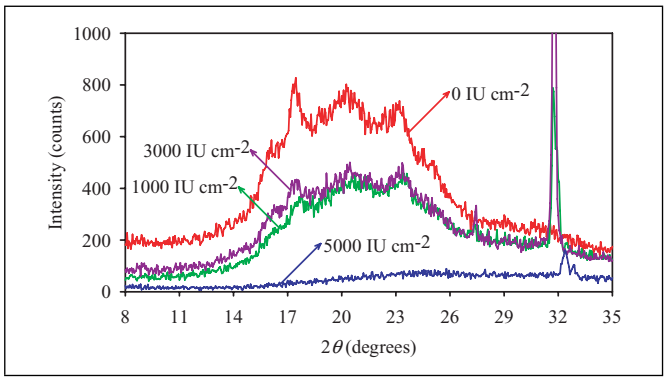

Figure 11—X-ray diffraction patterns for the different nisin concentrations.

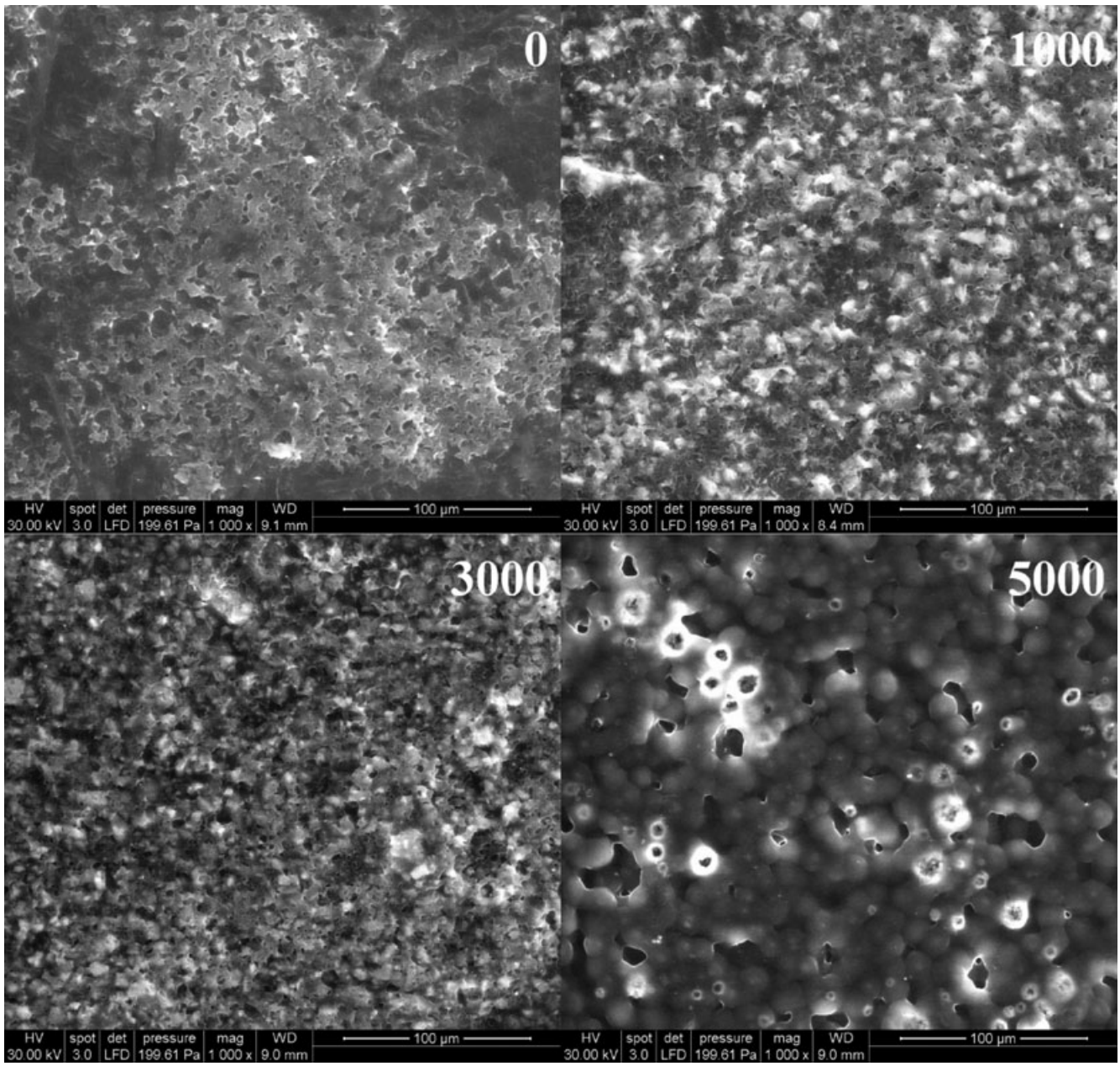

Figure 12—ESEM images of PBAT with the nisin concentrations in IU/cm². The holes and pores can be observed as dark spots.

The value of melting enthalpy (ΔH_m) decreased with increasing concentration of nisin in PBAT films. There was no significant difference ($P > 0.05$) between the ΔH_m of PBAT films with nisin concentration of 0 and 1000 IU/cm^2. However, the values of ΔH_m of the PBAT films decreased significantly with further increase in the nisin concentration to 5000 IU/cm^2 (Table 4). The enthalpy of crystallization (ΔH_c) of PBAT films also decreased with increasing concentration of nisin (Table 4); no significant difference ($P > 0.05$) was observed from 0 to 3000 IU/cm^2, and no significant difference was found from 3000 to 5000 IU/cm^2 ($P > 0.05$). As can be expected, the behavior was the same for the case of crystallinity (χ) because both terms are related as shown by Eq. (5) (Table 4). The exothermic peaks observed for the selected nisin concentrations are presented in Figure 10. Similar trends were reported in previous works with PBAT (Someya and others 2005; Chivrac and others 2006; Chivrac and others 2007; Iwakura and others 2008).

The changes in thermal properties with increasing concentrations of nisin can be used to describe variations in the tensile properties of PBAT films. Walstra (2003) indicated that the presence of foreign molecules in a system decreases the available space for crystal growth thus reducing its crystallinity. It can be inferred that the nisin incorporated in PBAT interacted and obstructed crystal formation. This phenomenon explains the observed decrease in the films' stiffness (E) and firmness (σ_s) as the polymer crystallinity decreased.

X-ray diffraction

Figure 11 shows the X-ray diffraction patterns for the selected PBAT films evaluated in this study. Lower levels of intensity (counts) were observed as the nisin concentration increased, which is in accordance with the reduction in χ shown at higher levels of nisin concentration (the patterns corresponding to 1000 and 3000 IU/cm^2 were almost overlapping). The pattern shown for PBAT without nisin is in agreement with the results obtained by Chivrac and others (2006), in which 5 diffraction peaks were observed at 2θ values ranging from 16° to 25°. The 5 diffraction peaks observed in PBAT films with 0 IU/cm^2 corresponded to 586 counts (16.1°), 827 counts (17.45°), 802 counts (20.25°), 729 counts (23°), and 491 counts (24.85°). It was possible to observe how the intensity decreased at the same values of 2θ in the films with 1000 and 3000 IU/cm^2. At 1000 IU/cm^2, the intensity at the corresponding values of 2θ was 223, 331, 411, 413, and 305. At 3000 IU/cm^2, the intensity at the same values of 2θ was 283, 425, 436, 461, and 302. The peaks observed in the patterns shown by the PBAT films with 1000 and 3000 IU/cm^2 in a range between 31.7° and 31.8° belong to the characteristic diffraction pattern of sodium chloride (NaCl) (Thomas 2010), the main component of Nisaplin. The PBAT films with 5000 IU/cm^2 shows a mainly amorphous behavior, because no remarkable peaks were observed. The decrease in the intensity after the incorporation of nisin into PBAT confirms the decrease in crystallinity (χ) previously observed in the thermal analysis. According to Chivrac and others (2006), this behavior indicates how a foreign substance incorporated in a system is able to block the crystal growth and hence the final crystallinity.

Environmental scanning electron microscopy

Figure 12 shows the images obtained from the ESEM analysis. Small holes and pores were observed in PBAT films containing nisin. According to Linssen and others (2003), the formation of the holes in a polymer matrix is caused by separation of polymer chains, and this phenomenon takes place mainly in amorphous materials. The interaction between nisin and PBAT polymer chains formed the holes as the PBAT molecules were no longer able to build bonds with each other. This observation is also correlated with the barrier and tensile properties. The formation of these holes may increase OP and decrease E and σ_s of the PBAT films as the films become more porous.

Conclusions

PBAT films containing nisin may represent a good option for active food packaging because they inhibited *L. innocua* (which can be expected to react like *L. monocytogenes*). However, some of the PBAT properties were affected after the incorporation of nisin. Significant effect was observed in the tensile properties (E and σ_s), the thermal properties (T_c, ΔH_c, ΔH_m), and in the crystallinity (χ). The gas barrier properties such as WVP and OP were not affected significantly with the incorporation of nisin. Further studies need to be done to identify the possible applications of PBAT films, release kinetics of nisin, and to improve the gas barrier and tensile properties.

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