

Influence of melt processing on biodegradable nisin-PBAT films intended for active food packaging applications

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ABSTRACT: Biodegradable poly(butylene adipate-*co*-terephthalate) (PBAT) films incorporated with different levels of the antimicrobial peptide nisin were developed by melt processing. Structural, morphological, thermal, mechanical, and antimicrobial properties of the films were determined. The X-ray diffraction patterns exhibited decreasing levels of intensity at 2θ values as the concentration of nisin increased. Scanning electron microscopy showed a heterogeneous morphology when higher amounts of nisin were incorporated. The antimicrobial films tested presented no significant differences in the melting temperature (123–125°C), and the crystallization temperature ranged from 69 to 75°C. The addition of nisin caused no significant modification in tensile strength values. However, results of Young's modulus and deformation at break differed significantly among samples. Active films demonstrated inhibition against the Gram-positive bacterium *Listeria monocytogenes*. These results demonstrated that PBAT/nisin films produced by melt processing present a great potential for use as active food packaging materials aiming enhanced food safety. © 2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 2016, 133, 43212.

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INTRODUCTION

Polymeric materials have dominated the world market mainly by their low cost, excellent thermal and mechanical properties, as well as the diversity of applications. However, most petroleum-derived polymers are poorly biodegradable and thus cause a significant environmental impact. Biodegradable polymers represent a very interesting alternative to the replacement of synthetic plastic packaging materials,^{1–3} especially for use in short-term packaging.⁴ Within the family of biodegradable polymers, poly(butylene adipate-*co*-terephthalate) (PBAT) is a promising alternative due to its excellent properties for film extrusion and coatings.^{5,6} PBAT is an aliphatic aromatic copolyester prepared by polycondensation reaction of 1,4-butanediol with both adipic and terephthalic acids.^{7,8} This polymer exhibits good thermal and mechanical properties, which can be compared with those of low-density polyethylene (LDPE) concerning its tensile properties.^{9,10} PBAT has a high elongation at break,^{11,12} in some cases higher than 600%, and presents lower weight temperatures during extrusion (120–150°C).^{13,14} Further-

more, it is a polymer totally free of heavy metals and allowed for food packaging applications by the Food and Drug Administration (FDA).^{15,16} PBAT is completely biodegradable and can be decomposed in a few weeks once it gets in contact with the environment through the intervention of natural enzymes.^{17,18}

In this context, the utilization of PBAT incorporating antimicrobials for food packaging may be an attractive alternative to enhance food safety, through an eco-friendly approach.^{5,19} Antimicrobial packaging is a promising category of active packaging presenting an embedded and/or immobilized antimicrobial agent, which is able to eliminate or inhibit spoilage and/or pathogenic microorganisms. An advantage on the use of packages containing antimicrobial agents is the diffusion of these compounds to the surface of the packaging in a controlled manner.²⁰ According to EU Regulation 450/2009, these materials can extend the shelf life of packaged foods and are allowed to incorporate components specifically designed to release substances into or onto the food.²¹ These packages are also known as active packaging offering several function not found in conventional packaging systems.^{21–23}

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Nisin is a natural antimicrobial compound that has been used in the development of active packaging. This bacteriocin is produced by *Lactococcus lactis*, a bacterium commonly present in milk, showing activity against a broad range of Gram-positive bacteria.^{24,25} Nisin is recognized as a safe additive by the FDA and is approved as a natural antimicrobial agent for food used in more than 50 countries.^{24,26} In addition, it is a heat stable antimicrobial peptide, non-toxic, sensitive to digestive proteases, and does not contribute to off-flavor.²⁷ Its small molecular size allows the production of films that release the peptide once it is in contact with a liquid or solid food.¹⁷ Studies have shown that nisin has a dual mode of action, by inhibiting cell wall synthesis and formation membrane pores causing a rapid cell death.^{26,28} Consequently, nisin can induce cell autolysis and inhibit the outgrowth of bacterial spores.^{28–30} The incorporation of this bacteriocin into packaging materials can control microbial contamination by inactivating the target bacteria by contact or by reducing the growth rate and maximum population.²⁷

Nowadays, studies about nisin incorporation into PBAT matrix are developed using the casting method.^{5,17} However, the use of melt processing has not been reported for the preparation of active PBAT films incorporating bacteriocins, such as nisin. Furthermore, the use of common processing equipment to prepare these films makes it possible to produce larger amounts in a single step without using solvents, having economic implications and most importantly, positive environmental consequence. In this sense, the objective of the present study was to develop PBAT films containing different amounts of nisin by melt processing. The interest of this study comprises active and functional packaging for use in food. For this purpose, the influence of the extrusion process on antimicrobial activity of PBAT films, their mechanical, thermal, and morphological properties were investigated.

EXPERIMENTAL

Materials

PBAT (Ecoflex[®] F Blend C1200), was supplied by BASF Corporation (Florham Park, NJ). PBAT density is 1.27 g cm⁻³ at 23°C, melt flow index (MFI) is 13 g 10⁻¹ min at 190°C 2.16 kg. A commercial nisin (Nisaplin[®]) was purchased from Danisco Brasil Ltda (Cotia, Brazil). According to the manufacturer, the formulation contains denatured milk solids and NaCl as fillers, and 2.5% pure nisin.

Sample Preparation

The polymer used in this study was previously dried at 60°C for 24 h before use. The samples were prepared using different nisin contents (up to 3 wt %) prepared by melt processing using a co-rotation twin-screw extruder Haake H-25, model Rheomix PTW 16/25, $L/D = 25$, matrix with $L/D = 3$. The temperature profile (feed to die) was 130–140°C, with a speed of 150 rpm and a constant feed rate of 10 g min⁻¹. The lowest possible processing temperature was chosen in order to avoid any thermal degradation of either the antimicrobial additive or the polymer, considering that PBAT melts around 116°C,^{5,31,32} whereas the temperature at which nisin retains its bioactivity is 120°C.³³ After processed, the samples were granulated in a Sagec SG-35 as described by Zehetmeyer *et al.*³⁴ The granular materi-

als were injected using a Battenfeld Plus 350, with a profile of temperature 135–140°C and mold temperature of 40°C, according to ASTM D 4101-55b, as type I specimens ASTM D 638.

Film Preparation

The PBAT/nisin films were produced in planar sheet extruder (AX Plásticos, model AX 16:26 Chill Roll). The granular material was placed directly into the hopper of the extruder with screw speed of 65 rpm, a temperature profile (feed to die) of 135–145°C and torque of 40–50 Nm. Films of pure PBAT were also prepared and used as a reference material. A total of seven measurements were performed on each film to obtain an average value of thickness that was about 0.07 mm.

Sample Characterization

Fourier Transformation Infrared Spectroscopy. Infrared spectra of pure and processed PBAT and also PBAT/nisin films were obtained with a Fourier transformation infrared (FTIR) Varian 640 IR Thermo Scientific spectrometer in attenuated total reflectance (ATR) mode with a diamond crystal collecting 32 scans. The spectral region investigated was 500–3500 cm⁻¹, with a spectral resolution of 4 cm⁻¹.

Morphological Properties. The morphology of nisin was examined by transmission electron microscopy (TEM) using a microscope JEOL model JEM-1200 Ex II, operated at an accelerating voltage of 80 kV. A solution with nisin and acetone was prepared and placed in ultrasound for 30 min to complete dissolution, a drop of this solution was dripped onto 300 mesh Cu grids.

The morphology of the cross-section was observed by scanning electron microscopy (SEM), using an electron microscope JEOL model JSM-5800 operated at 10 kV. Samples were cut directly from the films, and to obtain fracture faces, PBAT/nisin and pure PBAT film were cooled in liquid nitrogen, and then broken and glued onto a sample holder. All samples were sputter coated with a thin layer of gold prior to analysis in order to increase their electrical conductivity.

Thermogravimetric Analysis. The thermal stability evaluation was carried out using a thermogravimetric analyzer model QA 50 (TA Instruments). All thermogravimetric analyses were performed on 10 mg samples, heated from 25 to 800°C at the rate 20°C min⁻¹ under nitrogen atmosphere (50 mL min⁻¹). The inorganic contents (wt %) of each sample was assessed by thermogravimetric analysis (TGA) as the combustion residue left at 600°C.

Differential Scanning Calorimetry. The thermal behaviour of PBAT and PBAT/nisin films were determined using a differential scanning calorimetry (DSC) Thermal Analyst Q20 (TA Instruments) in a dry nitrogen atmosphere (50 mL min⁻¹). The analyses were performed on 7.0 ± 3.0 mg samples, heated from ambient temperature to 180°C and kept for 2 min to erase the thermal history. The samples were then cooled down to -50°C and heated again until 200°C. All runs were conducted with a heating rate of 20°C min⁻¹. The crystallinity degree was determined using $\Delta H_m^0 = 114 \text{ J g}^{-1}$ for PBAT.^{35,36}

X-ray Diffraction. X-ray diffraction (XRD) measurements were performed using a Siemens D-500 diffractometer. Nisin powder, PBAT, and PBAT/nisin films were scanned in the reflection mode using an incident CuK_α radiation ($\lambda = 1.54 \text{ \AA}$), at a step width of $0.05^\circ \text{ s}^{-1}$ from $2\theta = 1^\circ$ to 35° .

Dynamic Mechanical Analysis. The samples were studied using dynamic mechanical analysis (DMA) Q800 (TA Instruments), using single cantilever geometry. The injection-molded samples were heated from -50°C to 100°C . The heating rate was fixed at 3°C min^{-1} and the frequency was set to 1 Hz for all samples.

Mechanical Tests. Tensile tests were carried out using specimens in the form of tie type I and size according to ASTM D-638 standard (3.2 mm thick), in a universal test machine Emic trials, DL 10,000, at a speed of 50 mm min^{-1} and base length of 50 mm. The samples were acclimatized for 24 h at $23 \pm 2^\circ\text{C}$ with humidity $50\% \pm 5$ before analysis. The module was determined using the traction extensometric with deformation up to 25 mm. Eight samples for each formulation were tested. The deformation at break (ϵ), Young's modulus (E), and tensile strength (σ) were determined.

Antimicrobial Properties. The antimicrobial activity of PBAT/nisin films, as well as PBAT films without nisin (control) was evaluated using the inhibition zone assay in agar plates.²⁷ Film pieces with 2 cm^2 were cut and placed on Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) agar plates. Each film sample was exposed to UV light for 15 min each side to eliminate possible microbial contamination. Then, 10 mL of the indicator strain *Listeria monocytogenes* ATCC 7644 (10^7 CFU mL^{-1}) was inoculated in BHI soft agar (7.5 g L^{-1}), which was poured onto plates. *Staphylococcus aureus* ATCC 25923, *Clostridium perfringens* ATCC 3624, and *Bacillus cereus* ATCC 9634 were also tested. Petri dishes were stored at 4°C during 12 h to initiate nisin desorption and after incubated at 37°C for 24 h. The antimicrobial activity is evidenced by clear zones (no microorganism growth or survival) surrounding film pieces.

All bacteria were stored at -20°C in 20% (vol/vol) glycerol and propagated twice on BHI broth before use. All determinations were carried out in triplicate ($n = 3$).

Nisin Migration Test. Migration of nisin from the films was tested into three simulant solutions³⁷: distilled water, 0.9% (wt/vol) NaCl containing 5% (vol/vol) Tween 20 (Merck, Darmstadt, Germany), and a 3% (vol/vol) acetic acid solution (Dinamica Ltda, Diadema, Brazil). Each film sample (0.30 g) was immersed into 3 mL of simulant solutions and shaken at 150 rpm at 25°C . Aliquots were removed after 4, 24, and 48 h for determination of antimicrobial activity onto BHI agar plates previously inoculated with a swab submerged in the indicator strain suspension (*L. monocytogenes* ATCC 7644), which corresponded to approximately (10^7 CFU mL^{-1}). The reciprocal value of the highest dilution that produced an inhibition zone was taken as the activity unit (AU) per milliliter.³⁸

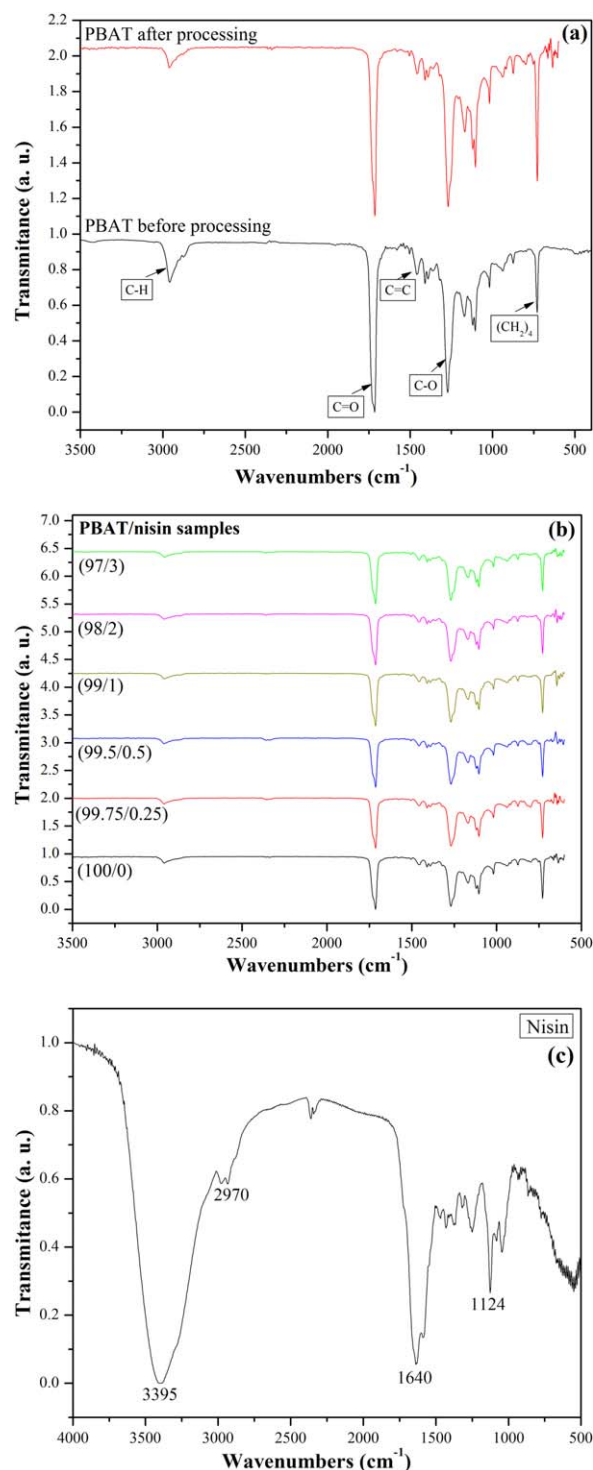


Figure 1. (a) FTIR spectra of pure PBAT before and after processing. (b) FTIR spectra of PBAT/nisin films. (c) FTIR spectra of nisin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Infrared Spectra

The FTIR spectra reveal that PBAT before and after processing exhibit the same characteristic peaks [Figure 1(a)], showing that processing parameters did not affect the chemical structure of

the polymer. Many degradation mechanisms of PBAT can occur depending on the processing conditions,^{36,39} for example, very high temperatures and very long residence time of the material within the extruder can lead to hydrolysis of the ester linkage, a water-based degradation mechanism that randomly takes place in the polymer.³⁵ It was reported that no significant chain modification of PBAT would be expected by processing up to 200°C,¹⁴ and the degradation is more pronounced in the temperature range of 270–360°C.⁴⁰ Al-Itry *et al.*³⁵ developed PLA/PBAT blends using a temperature range from 140 to 180°C, a very different processing temperature that was employed in this work. Even using such high temperatures to process the PBAT, they identified the same characteristic FTIR peaks, without any change in PBAT structure, indicating its high thermal stability and processability.³⁹

PBAT before and after the processing showed a sharp peak at 726 cm⁻¹, which is associated with the vibrations of adjacent methylene (—CH₂—) groups of the polymer backbone. A peak in the region of 1266 cm⁻¹ is assigned to the stretching of the C—O groups from ester bonds. The most intense of all the peaks appears in the region of 1710 cm⁻¹, a typical peak of the carbonyl (C=O) groups in the ester linkage. A peak around 3000 cm⁻¹ corresponding to C—H stretching of aliphatic and aromatic portions was also identified. Similar PBAT spectra were reported by Brandelero *et al.*,⁴¹ which determined the presence of a peak in the region of 1708 cm⁻¹ due to the stretching of the carbonyl groups, and a stretching between 1020 and 880 cm⁻¹ due to the presence of the substituted benzene ring.

The FTIR spectra of PBAT and PBAT/nisin films [Figure 1(b)] were quite similar to pure PBAT before processing [Figure 1(a)], showing no significant changes in the molecular structure of the samples during processing, and also no changes were observed with nisin addition. The physical incorporation of nisin in the polymer matrix did not alter the chemical structure of PBAT once there were no changes on peaks intensity or shifts, even for samples with higher amount of nisin (3 wt %). A similar behavior was reported by Scaffaro *et al.*⁴² where films of poly(ethylene-co-vinyl acetate) with two commercial formulations of nisin showed similar FTIR spectra to that obtained for the neat matrix. The authors also claim that no specific peaks could be associated to the presence of nisin in the polymer matrix. In general, the most important assignment observed in the FTIR spectra for PBAT/nisin films was the presence of the signal at 1720 cm⁻¹ due to carbonyl groups in the ester linkage [Figure 1(b)]. The peak in the region of 1200 cm⁻¹ is assigned to the stretching of the C—O groups from ester bonds.

FTIR spectra of nisin [Figure 1(c)] gives a broad band at 3395 cm⁻¹ due to OH stretching of COOH groups, a peak at 2970 cm⁻¹, which can be attributed to C—H stretching, another peak at 1640 cm⁻¹ attributed to amide group, and a peak at 1124 cm⁻¹ that can be ascribed to OH group. Very similar infrared spectra to nisin, with an additional peak 1527 cm⁻¹ associated to bending of primary amines, have been previously reported.^{29,43}

Morphological Properties

The cross-section images obtained from pure PBAT and PBAT/nisin films are depicted in Figure 2. The nisin formulation and its solution dispersion were also visualized by transmission electron microscopy (TEM) (Supporting Information Figure S1). The morphology of pure PBAT was homogeneous, while nisin agglomerates were visible on the films containing 1, 2, or 3 wt % nisin [Figure 2(c–e)]. As the nisin concentration increased, particles of this antimicrobial substance were observed possibly due to its partial aggregation and migration to the film surface. Meira *et al.*²⁷ reported a similar behavior during the preparation of polypropylene/montmorillonite nanocomposites containing nisin.

Besides, small voids and holes were observed in the films containing 2 or 3 wt % nisin [Figure 2(d,e)]. These holes in the cross-sectional polymer matrix were probably created by the air that adhered on the irregular surfaces of the salt particles. The air was separated from the carriers during extrusion, forming bubbles under pressure by rotation of the twin-screw, and then escaping, leaving void spaces.³³ Crazing, cavitations, shear bending, and shear yielding have been identified as important energy dissipation processes involved in the fracture of toughened polymer systems. Since PBAT has different elastic properties compared with other polymers, PBAT particles can act as stress concentrators under stress.³¹ Li *et al.*⁴⁴ also observed small pores and gaps in their study, and affirm that they were generated during the extrusion process. Liu *et al.*³³ prepared membranes of poly(lactic acid) and nisin and verified that small pores were also created with introduction of nisin. They affirm that some heat-sensitive components in the Nisaplin might have degraded or evaporated at higher temperature and under higher pressure during extrusion, creating pores and segregating particles. Bastarrachea *et al.*¹⁷ observed a very similar morphology with PBAT/nisin films, and report that 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. It is interesting to note that the interaction between PBAT polymer chains and nisin formed the holes as the PBAT molecules were no longer able to build bonds with each other. The morphological characterization can be helpful to explain the interactions between the polymer matrix and the additive and the thermal or mechanical behavior of the resulting composites.

Thermal Properties

Thermogravimetric analyses (TGA) were performed to investigate the thermal stability of pure PBAT and PBAT/nisin films. Table I displays the characteristic temperatures $T_{10\%}$ and $T_{50\%}$ corresponding to the initial decomposition temperature (10% degradation) and the maximum degradation rate temperature, respectively, and also the fraction of non-volatile residue at 600°C. PBAT starts its degradation around 320°C, and ends around 490°C. The maximum decomposition temperature of this polymer can be indicated by the maximum point obtained from the DTG curves (Supporting Information Figure S2), which was 415°C. PBAT/nisin films showed a similar decomposition temperature even at the highest concentration of nisin, this temperature remained constant around 374°C (Table I). The weight loss at 50% ($T_{50\%}$) occurs at the same temperature

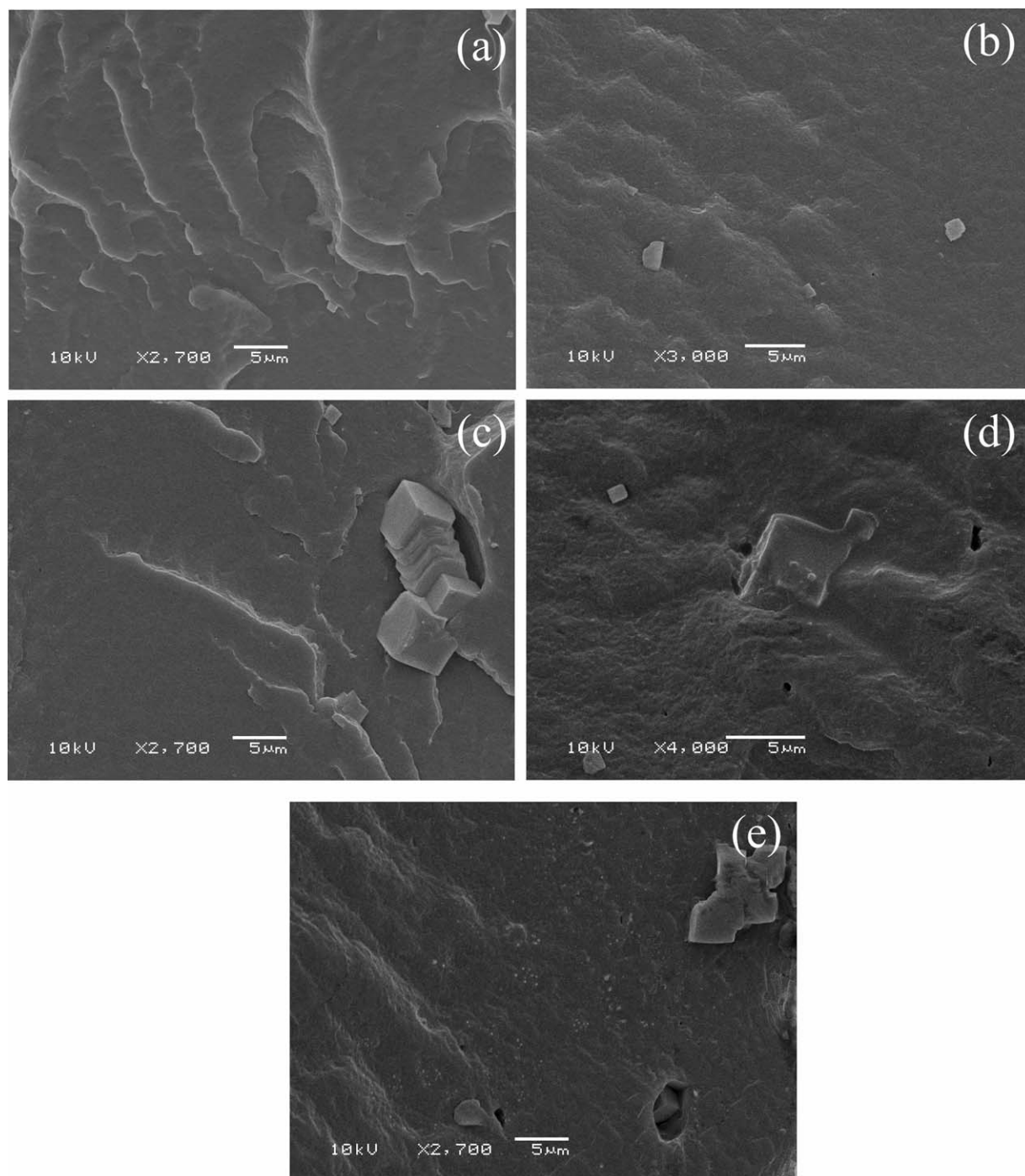


Figure 2. Scanning electron micrographs of pure PBAT and PBAT/nisin films (cross section). (a) 0 wt %, (b) 0.5 wt %, (c) 1 wt %, (d) 2 wt %, and (e) 3 wt % nisin.

for the different films (around 403°C). The TGA patterns of the PBAT/nisin films were quite similar and evinced that increasing concentrations of nisin did not affect thermal stability of the polymer matrix. No significant differences ($P > 0.05$) were observed for weight loss at $T_{10\%}$ and $T_{50\%}$ values.

Other authors observed an increase in the degradation temperature of PBAT/montmorillonite samples containing higher clay contents.³⁶ This suggests that layered silicates enhance the ther-

mal stability of the polymer matrix because they act as a heat barrier. Chen and Yang⁴⁵ prepared nanocomposites of PBAT with montmorillonite nanoparticles via melt blending, and revealed that the incorporation of organoclay also increases the thermal stability of the biopolymer.

The literature also reports that PBAT shows two main stages of degradation. The first one is between 340 and 400°C and may be due to the maximum decomposition of aliphatic copolyester

Table I. TGA and DSC Results of Pure PBAT and PBAT/Nisin Films

Samples	$T_{10\%}$ (°C)	$T_{50\%}$ (°C)	T_g (°C)	T_m (°C)	T_c (°C)	X_c (%)	Residue
(100/0)	379	404	-30	125.4	75.1	10.7	0.14
(99.75/0.25)	373	400	-29.9	125.3	75.2	10.5	0.58
(99.5/0.5)	373	403	-29.6	126.2	74.8	10.5	0.58
(99/1)	374	403	-28.7	123.7	71.6	9.4	1.01
(98/2)	373	402	-28.4	124.4	70.4	8.9	1.72
(97/3)	374	403	-28.3	123.3	69.6	7.9	3.00

adipic acid and 1,4-butanediol. The second one occurs at around 600°C, and it is explained by decomposition of aromatic copolyester terephthalic acid.⁴⁶

The DSC thermograms of PBAT and PBAT/nisin films are shown in Figure 3. This analysis was carried out to study the effect of melt processing on the PBAT/nisin crystallization behavior.

The PBAT films with 0.25 wt % or 0.5 wt % nisin presented temperature of crystallization (T_c) similar to that of pure PBAT [Figure 3(a)]. However, the systems with 1, 2, or 3 wt % nisin showed a slight decrease in T_c values. The T_c decreased from 75.1°C for pure PBAT to 69.6°C in the sample with higher nisin concentration (Table I). The degree of crystallinity (X_c) was 10.7% for pure PBAT film, whereas films with 3 wt % nisin showed 7.9%. In these sense, nisin added to PBAT matrix caused minor changes in the film crystallinity. PBAT is a random copolymer and is difficult to crystallize.³² Bastarrachea *et al.*¹⁷ proposed that crystallization of partially amorphous polymers, unlike low molecular weight materials like sugars and salts, takes place at a slow rate and over a wide range of temperature. Thus, the overlapping of polymer chains may not take place completely and some regions will not crystallize due to chain entanglements. In addition, the presence of some additives, such as antimicrobials, could decrease available space for crystal growth thus reducing its crystallinity and inducing variations in thermal and mechanical properties.^{27,47} The crystal structure of PBAT has been previously studied, indicating that a melt-crystallized PBAT sample contained small crystals with a wide size distribution.¹⁴ The crystalline region of PBAT is composed of butylene terephthalate (BT) units, whereas a noncrystalline region is formed with all butylene adipate (BA) units.

Basic thermal properties are very important for the study of the crystallization behavior of polymeric materials. In this study, the glass transition temperature (T_g) of PBAT remained almost unchanged despite nisin addition. Pure PBAT presented T_g at -30°C, and the values to PBAT/nisin films ranged from -29.9°C to -28.3°C, (samples with 0.25 wt % to 3 wt % nisin). The melting temperature (T_m) of pure PBAT was 125.4°C [Figure 3(b)]. Similar results for PBAT were found by Abdelwahab *et al.*³² that reported T_g and T_m values of -35°C and 116°C, respectively. T_g and T_m are important factors to take into account because they give information about the level of association between polymer chains.¹⁷ The higher the values of T_g and T_m and the stronger the intermolecular bonds between polymer chains, the polymer can be either a supercooled liquid

with high viscosity or a crystalline solid if the room temperature is between T_g and T_m .⁴⁸ The T_g and T_m of PBAT/nisin films did not change with nisin addition and these results can be visualized in Figure 3(b).

Structural Properties

The X-ray diffraction showed the crystal structure of PBAT and PBAT/nisin films and the XRD spectra are depicted in Figure 4. The PBAT showed five diffraction peaks of the crystal structure at 16.2°, 17.4°, 20.4°, 23.0°, and 24.8° respectively, at 2θ value. These five characteristic peaks of pure PBAT were also observed for PBAT/nisin samples at the same 2θ values (Figure 4). Nevertheless, small changes in the intensity of the polymer diffraction

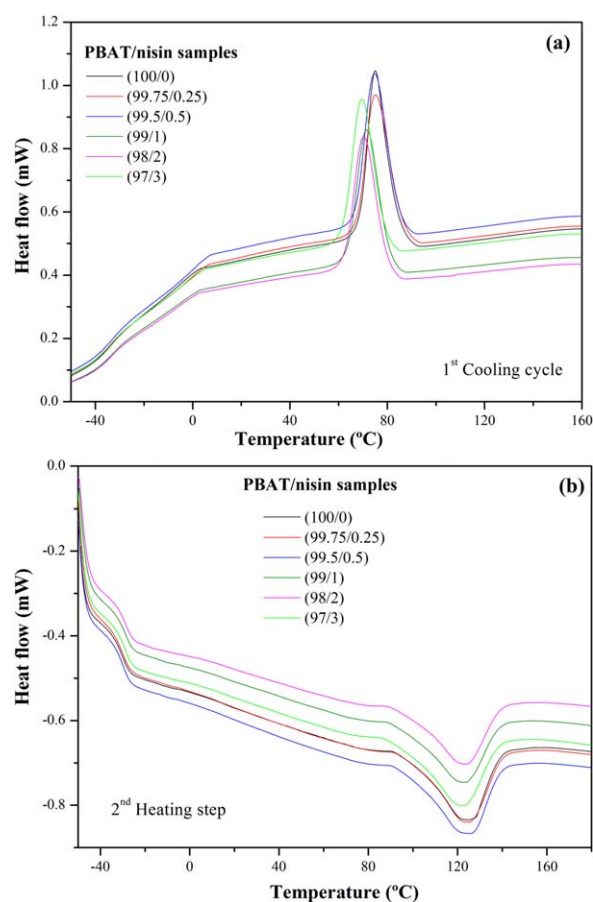


Figure 3. DSC thermograms of pure PBAT and PBAT/nisin films: (a) first cooling cycle and (b) second heating step. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

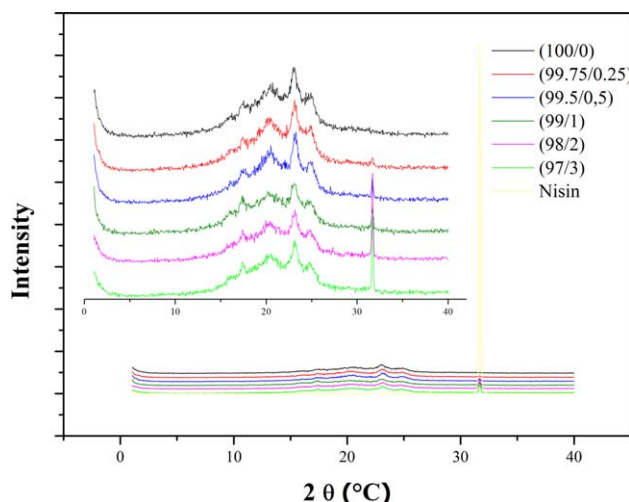


Figure 4. XRD patterns of pure PBAT and PBAT/nisin samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

peak were detected for the PBAT/nisin samples, suggesting that the peak intensity tends to decrease when nisin was added to PBAT matrix (1 wt % to 3 wt % nisin). The decreasing or absence of the diffraction peak indicates the formation of a well dispersed and/or exfoliated structure.¹⁴ The results of the present study agree with those described by Chivrac *et al.*³⁶ for PBAT/OMMT nanobiocomposites, which also observed five characteristic peaks at similar 2θ values. In addition, they observed a decrease of the intensity of the diffraction peaks when clay loading increases, indicating a reduction in the PBAT crystallinity. Similar results were found in the literature,¹⁰ suggesting that the nanofillers likely hinder the crystal growth of PBAT crystallite. When nisin was added to PBAT, crystalline peak intensity decreased by increasing nisin content. Some authors suggested that nisin, as a foreign substance, is able to prevent crystal growth.¹⁷ A similar observation was found by Hosseini *et al.*⁴⁹ by the incorporation of nisin into alginate microparticles, the crystalline peak was broader and amorphous behavior was more evident.

In addition, another remarkable peak can be observed in a range between 31.7° and 31.8° (Figure 4), which belongs to the characteristic diffraction pattern of sodium chloride (NaCl), a component of nisin formulation. This peak begins to be visualized from PBAT/nisin sample with 0.25 wt %, and the intensity

Table II. Crystallinity Degree of PBAT and PBAT/Nisin Samples Calculated by XRD

Sample	Crystallinity by XRD (%)
100/0	27
99.75/0.25	27.4
99.5/0.5	26.4
99/1	22.5
98/2	16.2
97/3	9.2

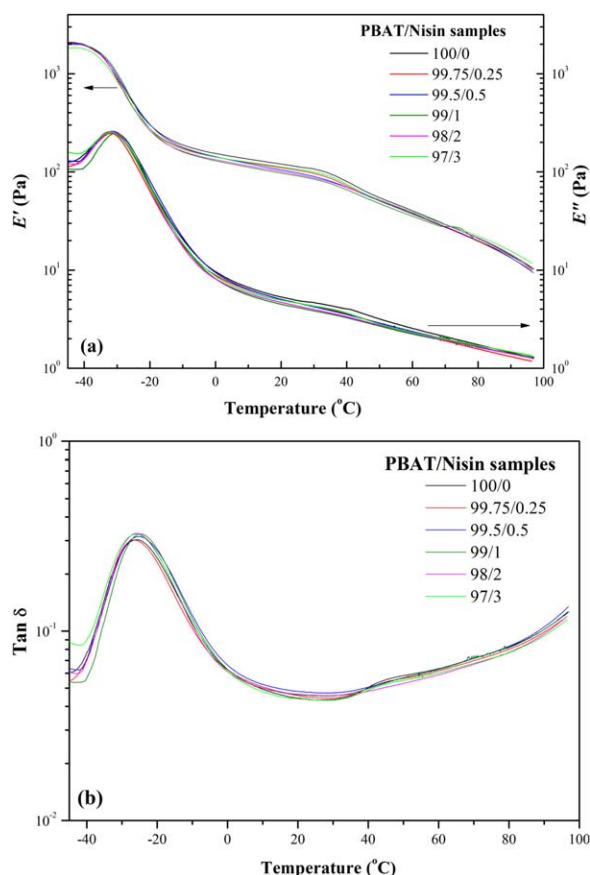


Figure 5. (a) Storage (E') and loss (E'') modulus as function of temperature to pure PBAT and PBAT/nisin films; (b) Tan delta as a function of temperature to pure PBAT and PBAT/nisin samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

is higher as nisin concentration increases in the samples. The pattern showed for nisin is in agreement with the literature, which described this characteristic peak for polypropylene/montmorillonite films with 1, 2.5, and 5 wt % nisin.²⁷ All these observations suggest that few changes in the PBAT crystal structure can be expected by the incorporation of nisin into the films. Finally, the XRD spectra of pure PBAT, nisin, and PBAT/nisin samples corroborated with thermal analysis showing that the processing method did not affect the structural properties of PBAT films.

Computer programs for quantifying the degree of crystallinity of the samples were used.^{50,51} Accordingly, the adequacy Lorenz model was used, and the results are presented in Table II. Crystallinity degree of PBAT and PBAT/nisin samples were calculated from XRD data, and these results agree with those presented by the DSC analysis (Table I), where it is possible to verify that the addition of nisin to the polymer matrix slightly reduces the crystallinity.

Dynamic Mechanical Analysis

This analysis allows to measure changes in the mechanical properties of a plastic material, and thus is very sensitive to changes in the temperature and size of thermal transitions.⁴⁴ The dependence of the storage modulus (E'), loss modulus (E'') and $\tan \delta$ as a function of temperature to the pure PBAT and PBAT/nisin films are depicted in Figure 5. It was observed a decrease

Table III. Tensile Properties of Pure PBAT and PBAT/Nisin Samples

Samples	Young's modulus (MPa)	Tensile strength (MPa)	Deformation at break (%)
100/0	48 ± 4 c	7.7 ± 0.3 b	694 ± 77 c
99.75/0.25	53 ± 2 c	8.2 ± 0.1 ab	550 ± 64 c
99.5/0.5	59 ± 2 bc	8.4 ± 0.0 a	465 ± 18 bc
99/1	66 ± 3 ab	8.4 ± 0.1 a	460 ± 35 ab
98/2	67 ± 4 ab	8.3 ± 0.0 ab	446 ± 53 ab
97/3	70 ± 3 a	8.2 ± 0.3 ab	387 ± 33 a

in storage modulus of pure PBAT as a function of temperature [Figure 5(a)]. This is attributed to the enhanced polymer chain mobility with the increase of temperature. Films containing nisin showed little changes in storage modulus and loss modulus when compared to the PBAT without nisin. The storage modulus of pure PBAT starts to decrease at approximately -30°C , which corresponds to the T_g of the polymer confirmed by DSC (Figure 3). This is due to softening of the polymer with increasing temperature. Storage modulus is dependent on the degree of crystallinity above the T_g , whereas below T_g the modulus of crystallinity as well as amorphous phase is almost identical.⁹

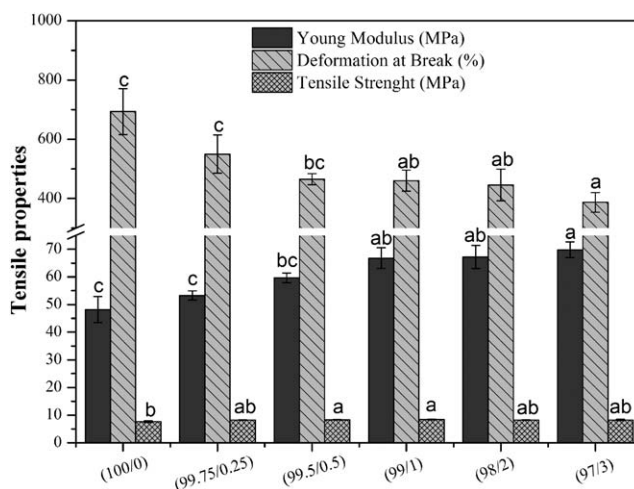
The storage modulus is lower to PBAT due to the rapid phase transition of the amorphous polymer to the elastomeric or rubbery state.⁵² Touati *et al.*⁵³ suggest that the overall reduction in storage modulus indicates a higher mobility of the polymer chains, which is already being attributed to chain degradation, predominantly through β scission. Abdelwahab *et al.*⁵² observed an adverse effect on storage modulus during the preparation of bioblends from polylactide and PBAT with lignin. They reported an increase in the storage modulus and justify that this behavior can be attributed to the enhancement of the interfacial adhesion between the polyester matrix and lignin.

The $\tan \delta$ curves showed two major transitions, the first one, around -20°C , associated to the T_g [Figure 5(b)]. This transition is attributed to the motion of poly(butylene adipate) units, the flexible aliphatic domain. The second one, at 50°C is ascribed to the motion of poly(butylene terephthalate) units, which is a rigid aromatic domain.^{54,55}

Mechanical Properties

The mechanical properties of PBAT and PBAT/nisin samples are shown in Table III. Young's modulus (E), tensile strength (σ) and deformation at break (ϵ) were determined. The mechanical behavior of the samples can be observed in Figure 6. The addition of nisin caused no significant differences in the values of tensile strength when compared to the PBAT film without nisin ($P > 0.05$) (Figure 6). However, this pattern was not observed for Young's modulus and deformation at break ($P < 0.05$). The Young's modulus increased from 48 MPa in the films without nisin to 70 MPa in films with 3 wt % nisin. The deformation at break decreased from 694% in the PBAT films to 387% in the films with 3 wt % nisin (Table III). In this regard, it is found that the addition of nisin changed some of the mechanical properties of the films.

The addition of antimicrobial substances to polymers has been reported as a potential reason for modification of mechanical

**Figure 6.** Mechanical properties of pure PBAT and PBAT/nisin samples.

performance.^{25,47} In this sense, the mechanical properties measured in this research might be affected by the poor dispersion of larger nisin particles, which in turn can cause premature rupture of the film. As a consequence, the values of tensile tests were modified (Table III). Kim *et al.*⁷ also reported a poor interfacial interaction between PBAT and starch. They affirm that the molecular mobility of PBAT could be restricted by starch granules, and it could lead to the decrease of mechanical resistance. As previously discussed, Bastarrachea *et al.*¹⁷ described the formation of holes and pores in films with nisin. In this study, holes and voids were also observed in nisin-containing films [Figure 2(d,e)]. Possibly, the lack of uniformity in the film structure also affected the PBAT/nisin mechanical properties.

Antimicrobial Activity and Nisin Migration

Incorporation of five different nisin concentrations (0.25, 0.5, 1, 2, and 3 wt %) into PBAT matrix resulted in active packaging films with visible zones of inhibition against the indicator strain *L. monocytogenes* (Figure 7). Larger zones of inhibition were verified as the amount of nisin increased in the samples. As

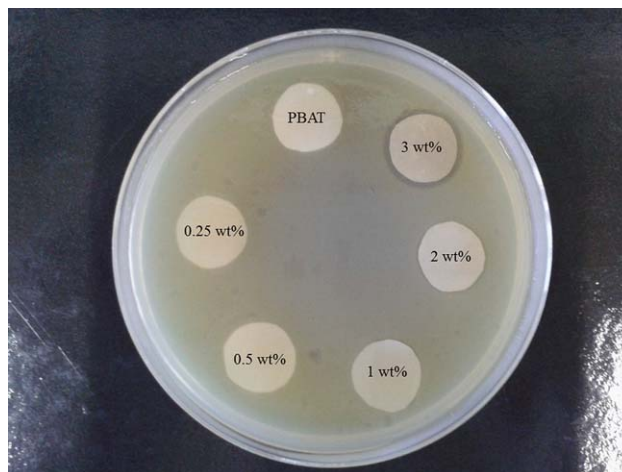
**Figure 7.** Antimicrobial activity of PBAT and PBAT/nisin films tested against *L. monocytogenes* in BHI agar plates. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table IV. Antimicrobial Activity Released from Pure PBAT Film and PBAT/Nisin Films after Periods of Time in Solutions Containing: Distilled Water (A), Tween 20 (B) and Acetic Acid (C)

Samples	Antimicrobial activity (AU mL ⁻¹)								
	4 h			24 h			48 h		
	A	B	C	A	B	C	A	B	C
100/0	0	0	0	0	0	0	0	0	0
99.75/0.25	0	0	0	0	100	0	0	100	0
99.5/0.5	100	100	100	100	100	100	100	100	100
99/1	100	100	100	0	200	0	100	600	100
98/2	100	200	100	100	200	200	200	400	400
97/3	800	400	800	1200	800	400	1600	3200	800

expected, pure PBAT film (without nisin) produced no antimicrobial activity. PBAT/nisin sample with 3 wt % presented the largest inhibition diameter (11.7 mm), while the PBAT samples with 1 wt % and 2 wt % nisin showed smaller inhibition zones (4.0 and 8.3 mm, respectively). Samples with 0.25 wt % and 0.5 wt % nisin may have shown some antimicrobial activity, but this inhibition could not be viewed under the assay conditions. Preliminary tests with *S. aureus*, *C. perfringens*, and *B. cereus* showed that PBAT films containing 3 wt % nisin resulted inhibitory zones around 10 mm against these bacteria (data not shown). These results indicate that nisin can be released from the films prepared by melt intercalation at 140°C and the materials displayed antimicrobial properties against Gram-positive bacteria.

Scaffaro *et al.*²⁵ investigated poly(ethylene-co-vinyl acetate)/nisin films and observed antibacterial activity when 3 wt % nisin was added, but a larger inhibition zone was observed only for 5 wt % nisin. Barbosa *et al.*⁵⁶ examined nisin in cellulose films, and found that the antimicrobial film inhibited *S. aureus*, *L. monocytogenes*, and *B. cereus* strains using the agar diffusion method. Nisin is interesting for incorporation into antimicrobial packaging due to its broad activity against foodborne pathogens.^{26,28} This antimicrobial agent interacts with the external surface of the microorganism and does not have to be internalized to exhibit the antibacterial effect.⁵⁷

Migration of nisin from the active films into simulant solutions was evaluated and the results are shown in Table IV. Antimicrobial activity was not observed for the pure PBAT film (without nisin), used as control. Also, no antimicrobial activity was detected during the first 4 h of immersion of the film with 0.25 wt % nisin. After this time, antimicrobial activity was observed in simulant solutions containing Tween 20 (named as B) and acetic acid (named as C). Samples exhibited best migration in Tween 20 solution: an increase in the antimicrobial activity was observed during the immersion of films with 1 and 2 wt % nisin. Films containing 3 wt % nisin, released 400, 800, and 3200 AU mL⁻¹ in simulant solution B after 4, 24, and 48 h, respectively. All samples showed little activity released when immersed in distilled water (named as A) during the time tested. In solutions of acetic acid (simulant C), films with 2 and 3 wt % nisin released up to 400–800 AU mL⁻¹. As mentioned

by Mauriello *et al.*,⁵⁸ the lower pH may benefit the migration of nisin from the film. The explanation may be that nisin is more soluble at acid pH.

CONCLUSIONS

The results of this study revealed that PBAT films with addition of nisin present antimicrobial properties against food pathogens, even after melt processing of these materials. The processing method did not change the polymer chemical structure. These results pointed for the first time the development of active food packaging material by melt processing using PBAT as a polymeric matrix with an antimicrobial additive. Thus, these data showed that the antimicrobial films obtained in this work present potential for application as active food packaging material.

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