BIODEGRADABLE POLY(BUTYLENE ADIPATE-*CO*-TEREPHTALATE) FILM INCORPORATED WITH NISIN: CHARACTERIZATION, EFFECTIVENESS AGAINST *LISTERIA INNOCUA*, AND NISIN RELEASE KINETICS

By

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To the Faculty of Washington State University:

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Abstract

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A biodegradable plastic film, Poly(butylene adipate-*co*-terephthalate) (PBAT) was incorporated with nisin in different concentrations: 0, 1000, 3000, and 5000 IU cm^{-2}. The effectiveness to inhibit *Listeria innocua* was evaluated, as well as the tensile, barrier, and thermal properties. The results showed that nisin incorporated PBAT films are able to inhibit *Listeria innocua*. No significant effect ($P > 0.05$) was found in the gas barrier properties (oxygen permeability and water vapor permeability) after the addition of nisin into PBAT. The tensile properties elastic modulus (E) and tensile strength (σ_s) were significantly affected ($P < 0.05$), whereas the elongation at break (ε_b) didn't exhibit significant difference after the addition of nisin ($P > 0.05$). The glass transition temperature (T_g) and melting Temperature (T_m) were not significantly affected after the incorporation of nisin ($P > 0.05$), whereas the crystallization temperature (T_c), the crystallinity (χ), and the enthalpies of melting and crystallization (ΔH_m and ΔH_c, respectively) were significantly affected ($P < 0.05$). The reduction in crystallinity was also evident in the *X*-Ray diffraction patterns. Formation of holes and pores was observed in the

PBAT films after the addition of nisin, as it was possible to observe through the Environmental Scanning Electron Microscopy (ESEM) images.

The release kinetics of nisin from PBAT film was evaluated at 5.6, 22 and 40 ºC. The diffusion coefficient (*D*) and partition coefficient (*K*) were calculated. The release of nisin followed Fickian law. The temperature dependence of *D* and *K* was modeled with the Arrhenius equation. The release of nisin exhibited agreement with the Weibull model with upward concavity and the temperature dependence of the scale parameter *b* was described with the Arrhenius equation.

Keywords: Arrhenius equation, barrier properties, Fick's law, tensile properties, thermal properties, *X*-Ray diffraction, ESEM, Weibull.

NOMENCLATURE

α	Mass ratio between the amount of nisin in the sterile distilled water solution and in the film at equilibrium
ε_b	Elongation at break (%)
ε	Strain
θ	Angle for *X*-ray diffraction (degrees)
ΔH_c	Enthalpy of crystallization (J g^{-1})
ΔH_m	Enthalpy of melting (J g^{-1})
$\Delta H_{m_{100}}$	Enthalpy of melting in 100% crystalline form for PBAT
Δp	Water vapor partial pressure difference between film underside and cabinet (Pa)
σ	Stress (Pa)
σ_s	Tensile strength (MPa)
χ	Crystallinity (%)
A_F	Film's exposed area (cm^2)
a_w	Water activity
b	Weibull's scale factor (h^{-1})
b_0	Weibull's scale factor (h^{-1}) at infinite absolute temperature
C_F	Nisin concentration in the film (mg cm^{-3})
$C_{F,0}$	Nisin concentration in the film at time cero (mg cm^{-3})
$C_{F,\infty}$	Nisin concentration in the film at equilibrium (mg cm^{-3})
$C_{S,\infty}$	Nisin concentration in the sterile distilled water solution at equilibrium (mg cm^{-3})
D	Diffusion coefficient of nisin through the film (cm^2 s^{-1})
D_0	Diffusion coefficient of nisin through the film at infinite absolute temperature (cm^2 s^{-1})
D_{wa}	Diffusion coefficient of water vapor in air (m^2 s^{-1})
E	Elastic modulus (MPa)
E_a	Activation energy (J mol^{-1})
ESEM	Environmental Scanning Microscopy
K	Partition coefficient
K_0	Partition coefficient at infinite absolute temperature
l	Half of the films' thickness (cm)
L_0	Initial length of the film (m)
L_t	Length of the film at time *t* (m)
$M_{F,0}$	Amount of nisin in the film at time zero (mg)
$M_{F,t}$	Amount of nisin in the film at time *t* (mg)
$M_{F,\infty}$	Amount of nisin in the film at equilibrium (mg)
$M_{S,t}$	Amount of nisin in the sterile distilled water solution at time *t* (mg)
$M_{S,\infty}$	Amount of nisin in the sterile distilled water solution at equilibirum (mg)
n	Weibull's shape factor
OP	Oxygen Permeability (mL m m^{-2} day^{-1} Pa^{-1})
WVP	Water Vapor Permeability (g m m^{-2} s^{-1} Pa^{-1})

NOMENCLATURE

WVTR	Water Vapor Transmission Rate (g h^{-1} m^{-2})
P	Atmospheric pressure (Pa)
PBAT	Poly(butylene adipate-*co*-terephthalate)
p_T	Water vapor pressure at the testing temperature (Pa)
PTFE	Polytetrafluoroethylene
q_n	Root of $\tan q_n = -\alpha q_n$
R	Gas law constant (8.314 J mol^{-1} K^{-1}),
RH	Relative humidity (%)
RMSE	Root mean square error
R^2	Coefficient of determination
t	Time (h)
T	Temperature (ºC or K)
T_{abs}	Absolute temperature (K)
T_c	Crystallization temperature (ºC)
T_g	Glass transition temperature (ºC)
T_m	Melting temperature (ºC)
V_F	Films' volume (cm^3)
V_S	Volume of the sterile distilled water solution (cm^3)
x	Position in the film (cm)
z	Air gap in film underside (m)

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DEDICATION

I would like to dedicate this thesis work to my dear grandfather, Carlos Enrique Gutiérrez Gasca, who passed away during my first semester in Washington State University.

Querido abuelo, I will never forget your goodness, your affection, your patience, and your knowledge. I feel thankful for having had a grandfather like you. I also feel thankful because the last time I saw you, you were happy and smiling. You showed me how even the most difficult circumstances and adversities in life are blessings that can make us strong and wise. You were, like very few people, the sum of a number of human values.

He who corrects an arrogant man earns insult; and he who reproves a wicked man incurs opprobrium. Reprove not an arrogant man, let he hate you; reprove a wise man, and he will love you. Instruct a wise man, and he becomes still wiser; teach a just man, and he advances in learning.

- Proverbs 9:7-9

THESIS OUTLINE

This thesis is organized into four chapters. Chapter 1 presents an introduction and literature review related to antimicrobial food packaging.

Chapter 2 relates to the effectiveness of nisin-incorporated PBAT films to inhibit *Listeria innocua*, and to the measurement of the gas barrier, tensile and thermal properties of such films. *X*-Ray diffraction and ESEM analysis of nisin-incorporated PBAT films is presented as well. Chapter 2 was submitted for publication to the Journal of Food Science, authored by Luis Bastarrachea, Sumeet Dhawan, Shyam S. Sablani, Jae-Hyung Kang, Dong-Hyun Kang, Jinwen Zhang, and Juming Tang.

Chapter 3 relates to the release kinetics of nisin from PBAT films into water, using Fick's law and Weibull model approaches. Chapter 3 was submitted for publication to Journal of Food Engineering authored by Luis Bastarrachea, Sumeet Dhawan, Shyam Sablani, and Joseph Powers.

Chapter 4 provides a summary of the work done and recommendations for future work.

CHAPTER 1

Introduction

Antimicrobial packaging: an overview

Antimicrobial packaging refers to the food packaging systems that have the property of killing or inhibiting spoilage and pathogenic microorganisms (Han 2003). The packaging incorporated with antimicrobials helps extend the shelf life of foods by prolonging the lag period of microorganisms, thereby diminishing their growth and their number. Antimicrobial packaging is intended to act against microorganisms and enhance the functions of conventional food packaging, which are (1) shelf life extension, (2) maintenance of quality, and (3) safety assurance.

The contamination of refrigerated foods takes place predominantly at surface of food. The application of food-packaging materials with antimicrobial substances incorporated may represent a useful mean to control the microbial contamination of foods. The increasing preference for more natural products (with fewer preservatives) has generated important interest for the antimicrobial food packaging concepts (Vermeiren and others 2002). Nevertheless, the application of antimicrobial food packaging has not been very successful, mainly due to concerns about their applicability and safety (Day 2003), even though a considerable number of examples can be found for antimicrobial technologies, especially for medical and household applications (Vermeiren and others 2002). The commercially available antimicrobial materials contain mainly silver (Ag) and triclosan (2, 4, 4'-trichloro-2'-hydroxydiphenylether) as the antimicrobial agents. For the first case, Ag cations bind with groups rich in electrons (containing sulphur, nitrogen, or oxygen), which can be found in the DNA chain, for example. Such binding blocks are vital

biological processes for microbial survival and reproduction (Vermerien and others 2002). For the second case, it is thought that triclosan kills bacteria by altering the synthesis of lipids which form part of the cell wall (Sanches-Silva and others 2005). The companies that currently offer antimicrobial materials containing Ag are DuPont (USA), Milliken Co. (USA), Surfacine Development Company (USA), and Ishizuka Glass Co. (Japan). The ones that offer antimicrobial materials containing triclosan are Sanitized AG (Switzerland), Microban Products (UK), and Thomson Research Associates (Canada). The applications of the materials such companies commercialize are not directly related to foods. In The USA, two food packaging films with antimicrobial activity are available (Microgarde, Bernard Technologies, Chicago, IL), both containing chlorinated compounds and are effective once they get in direct contact with food stuffs (Vermeiren and others 2002). In Japan, silver containing films have been commercialized for food packaging (Quintavalla and Vicini 2002).

A wide range of antimicrobial substances have been tested in laboratories for their potential applications in the antimicrobial food packaging. These antimicrobial substances include organic acids (benzoic acid, sorbates), enzymes (lysozyme, glucose oxidase), bacteriocins (nisin, pediocin), fungicides (benomyl, imazalil), polymers (predominantly chitosan), natural extracts, antibiotics, and so on. Each antimicrobial substance exhibits a unique mechanism of action. This fact makes them specific for a particular range of foods and microorganisms. Some antimicrobials are able to block or inhibit metabolic and reproductive processes. Other antimicrobials can modify the cell wall conformation leading to the loss of vital internal materials and adaptability in the medium. This fact may represent a disadvantage for the antimicrobial food packaging systems. A film with antimicrobial activity will have limited

applicability for certain food products. From a commercial point of view, this may create a problem since the market and potential applications would be reduced (Han 2003; Han 2005).

The films with antimicrobial activity can be divided into two groups: (1) films from which migration of the antimicrobial takes place into the food and (2) films that don't release the antimicrobial substance and are able to inhibit microbial growth in the food surface (Suppakul and others 2003). In the first case, a preservative is found either within the matrix or on the surface of the food packaging material. The corresponding substance can be released completely or a specific amount on the food surface to perform its biocide action. Figure 1 shows a schematic representations for the first case in which (A) represents a packaging system in which the antimicrobial agent is incorporated in a single layer and is liberated gradually to the food matrix, (B) represents the same concept but with an inner layer, which can be useful to control the release of the antimicrobial compound, and (C) represents another option for the releasing antimicrobial packaging systems, which consists of a layer of food packaging material which is coated with a formulation that contains the antimicrobial substance (Han 2003; Quintavalla and Vicini 2002). For the second case, as it is shown in the scheme (D) of Figure 1, the antimicrobial activity is executed just once the microorganisms get in contact with the surface of the packaging material (Han 2003; Quintavalla and Vicini 2002). For both types of antimicrobial concepts, direct contact with the food is necessary, which makes these technologies a suitable option for foods which are vacuum-packaged such as cheese, meat, fish, or poultry (Vermeiren and others 2002).

Several studies have investigated the effectiveness of antimicrobial films against microbial growth. Nevertheless, some attempts to produce films with antimicrobial activity have failed, since there are many factors involved with their effectiveness to suppress the microbial

growth. The interaction of the antimicrobial agent with the corresponding packaging material may bring an adverse effect in the release of such agent, or the film production procedure can diminish the activity of the antimicrobial agent to levels that make it ineffective for its purpose. The processing operations involved during manufacturing of packaging film, such as extrusion, printing, drying, or lamination, may significantly affect the activity of the antimicrobial compounds, due to phenomena such as degradation and evaporation (in case of volatile antimicrobial substances) (Suppakul and others 2003). On the other hand, it is also important to consider the activity of the antimicrobial substance once it gets in contact with the food matrix. The interaction between the antimicrobial substance and the food components may be strong enough for the antimicrobial agent to become ineffective against the microorganisms it is intended to suppress. This may happen even once the effectiveness of the antimicrobial packaging material has been tested and confirmed in *in-vitro* conditions (Vermerien and others 2002).

Polymers commonly used for packaging of food: relevant properties

Table 1 shows a summary of some of the commonly used polymers intended for packaging of food. The packaging materials exhibit a range of properties, which makes them suitable for specific food applications. The densities, melting points and glass transition temperatures of several polymeric structures are presented in Table 2. These properties are important to characterize plastics for their use in food packaging and are able to provide important information about the possible uses for different foods, as well as about some criteria that have to be taken into account during their processing (Robertson 1993).

Knowledge of thermal and physical properties of polymeric structures is important for evaluating the quality and applicability of films intended for food packaging. The tensile properties generally evaluated are elastic modulus, tensile strength, and elongation at break. The elastic modulus is the force per unit area necessary to increase the length of a film sample to a specific extent. The tensile strength is the force per unit area applied when the film is broken and the elongation at break is the percentage of change in the film length when the film is finally broken after applying a certain level of force (Roff and Scott 1971). The information obtained from the measurement of these properties is useful to get an idea about the potential uses of the packaging materials. It is possible to compare brittle and strong materials with flexible or soft. The test commonly utilized for such purpose is the one from the American Society of Testing and Materials (ASTM), method D882 (Robertson 1993). Table 3 shows the information regarding tensile properties for selected food packaging at specific levels of relative humidity (RH) and temperature (*T*) (Lee and Kim 1997; Roff and Scott 1971).

Other important group of properties of polymeric films is permeability with respect to oxygen and water vapor. These are important parameters to consider when deciding film for packaging of a specific food product. All plastics are permeable to certain degree to oxygen and water vapor (Mullan and McDowell 2003). Table 4 shows the values of water vapor and oxygen permeability for selected films used for food packaging applications (Massey 2004; Piringer 2000).

Properties of antimicrobial food packaging films

The properties of the food packaging films are influenced after the incorporation of an antimicrobial substance. The level of influence depends on the type of film material, the film

preparation procedure, and on the antimicrobial agent utilized. A significant change can be obtained in the tensile properties. Table 5 exhibits some studies in which the tensile properties were evaluated in polymeric films after incorporating the antimicrobials. The significant changes in the characteristics of the films can be expected, since the incorporation of antimicrobial agents in a food also lead to the changes in some important properties (Han 2003).

According to Han and Floros (1997) a significant effect in the tensile properties is not expected when the molecular weight of the antimicrobial molecule is smaller compared to the packaging material. In such a case, the incorporation of the antimicrobial should not alter the conformation of the packaging material's polymer structure, thereby keeping its tensile properties. However, it is possible that even small quantities of the corresponding antimicrobials are able to change the tensile properties (Table 5), if they interact with the packaging material's matrix (Linjaroen and others 2003; Pires and others 2008; Türe and others 2009).

The changes in gas barrier properties of polymeric films containing antimicrobials have not been evaluated extensively. A possible reason for a lack of such studies could be that during the development of antimicrobials films, the effectiveness of antimicrobials against pathogenic microorganisms is determined and optimized before other properties of the films are evaluated. Tables 6 and 7 show the gas barrier properties the films incorporated with antimicrobials. For these properties, it can also be observed that the incorporation of antimicrobials can generate a significant effect, which doesn't seem to imply always that the incorporation of antimicrobials will have an adverse effect. For the case of barrier properties against oxygen, Table 6 shows the incorporation of antimicrobials may even improve the gas barrier properties. Suppakul and others (2006) suggest that this may be caused by an increase in the hydrophobicity of the system, which leads to a lower permeability to water vapor, for example. According to Robertson (1993)

the transmission of gases through a packaging material can take place through two mechanisms: pore effect and solubility-diffusion effect. In the first case, the gases cross the material passing through small pinholes or ruptures present in the structure. In the second case, the concentration difference between the two sides of the packaging material and the solubility of such gases in the corresponding material determines the level of transmission. It can be assumed that the incorporation of antimicrobials is able to affect the structure of the food packaging films, thereby affecting the permeability of gases due to creation of pinholes in the packaging structure or by changing the solubility of the gases in it. It can also be observed that a common generalization cannot be done and the final effect depends on the type of antimicrobial agent incorporated and polymeric structure.

Diffusion of antimicrobial substances through food packaging materials

During the evaluation and characterization of antimicrobial food packaging films, it is important to study the diffusion antimicrobial substances through the packaging films. This information helps determine how likely the studied packaging film can hold the antimicrobial substances and to release them when in contact with the food (Min and Krochta 2007). According to Han (2005) the study of the release of the antimicrobial components from the packaging materials is fundamental since it is necessary to make sure that the liberation will be held in such a way that permits the elimination of microorganisms. The release should not be slower than the microbial growth.

An important factor to be considered is the solubility of the antimicrobial substance in selected foods. If the solubility is very high, the release might take place rapidly, quickly decreasing the antimicrobial concentration on the food’s surface. On the other hand, if the

solubility of the antimicrobial component is low, the antimicrobial may accumulate on the food's surface and then be transported slowly through the food matrix. Both scenarios relate with the diffusion coefficient (*D*) of the antimicrobial through the food, being higher for the first case. The appearance of any of the phenomena described is also dependant on the value of *D* the corresponding antimicrobial has in the packaging material. The diffusion characteristics of antimicrobial can be useful in determination of the amount necessary to maintain the concentration levels above the minimum inhibitory concentration (Han 2005).

Chemical and physical factors are related to the diffusion of antimicrobial substances through packaging materials. The diffusion may be influenced by the presence of hydrogen bonds, ionic bonds, ionic osmosis, hydrophobic interactions, electrostatic interactions, and so on. The configuration of the films' matrix and its implications, like the presence of a tortuous and porous medium can also influence the diffusion phenomenon (Min and Krochta 2007).

Several studies have been performed to evaluate the diffusion of antimicrobial substances in food packaging films (Gemili and others 2009; Han and Floros 1998; Kim and others 2002; Redl and others 1996; Teerakarn and others 2002; Zactiti and Kieckbush 2009). The Fick's second law is considered to model the release behavior, and depending on the particular testing conditions, different analytical solutions are applied to calculate the value of *D*. Table 8 exhibits the analytical solutions for the Fick's second law utilized in previous works to calculate the value of *D* at a specific temperature (*T*). These studies were performed at different temperatures to characterize the behavior of *D* as a function of that parameter. Generally, the value of *D* increases with temperature, thereby raising the release of the antimicrobial. This can be advantageous since the proliferation of microorganisms increases as the temperature gets higher as well, which could be controlled if the release of the antimicrobial substance also increases.

The study of the release kinetics of antimicrobials and the application of mathematical models to fit the data obtained can be a useful tool to make predictions in food packaging systems with antimicrobial activity (Min and Krochta 2007).

Commonly, the *D* of antimicrobials through the film matrix is lower than in the foods. This may lead to a shelf life prolongation since small amounts of the antimicrobial would be transferred to the food, diminishing the number of microorganisms. This could also imply the utilization of smaller quantities of antimicrobials and, a consequent reduction in the production costs, as well as an increment in the profits due to an extended shelf life (Min and Krochta 2007).

Han and Floros (1998) studied the migration of potassium sorbate through LDPE, PET, PP and HDPE (Table 8). In that study, the films were placed in the middle of a cell divided into two chambers, one of the chambers containing a solution with a known concentration of potassium sorbate. The diffusion of potassium sorbate was studied by analyzing the concentration of potassium sorbate from the other chamber. In several studies involving commonly used synthetic films, the method of incorporation of the antimicrobial substance has been by coating the film with a solution containing the antimicrobial (Grower and others 2004; Leung and others 2002), while in others it has been possible to incorporate directly the substance in the film matrix (Siragusa and others 1999). This suggests that it is not always possible to incorporate directly in the films' matrix the studied antimicrobial. If that's the case, it would be necessary to take into account the coating layer rather than the plastic material if the diffusion is to be determined.

Biodegradable food packaging materials

Currently, about 25% of the utilization of plastics belongs to the food packaging area (Jayasekara 2005). The use of plastics for food packaging applications has gained an important level of popularity. These plastics have been demonstrated to be more efficient than other packaging materials and have characteristics that make them more suitable for food packaging, like their tensile, optical, and barrier properties against gases. Nevertheless, the waste that plastic food packaging materials generate represents a serious environmental issue. For most of the cases, plastic materials cannot be degraded by the action of microorganisms, which leads to accumulation. In The United States, synthetic polymers represent 8% of the municipal waste, and even though plastic materials don't bring a danger due to toxicity for humans, they increase the cost of landfill. On the other hand, plastic materials can also bring important damage to the ecosystems. Some animals die due to ingestion of the residues of plastics. Approximately, one million tons of plastics accumulate in the ocean every year. Some strategies are available to reduce this problem. Among them, it is possible to mention incineration, recycling, usage reduction, and the use of biodegradable plastics. The latter strategy has gained popularity and important advances have been achieved in the development of biodegradable plastics (Jayasekara 2005). However, the number of biodegradable plastics used for food packaging applications is very limited (Siracusa and others 2008).

According to Jayasekara and others (2005), a biodegradable polymer is the one that is able to be subjected to significant alterations in its chemical structure under specific environmental conditions. The overall molecular weight is reduced since the main polymeric chain is broken into smaller molecules. In the food packaging area, it is possible to find some applications in which biodegradable plastics are used, as for example disposable cutlery,

drinking cups, plates, overwraps, and lamination films. Table 9 shows some examples of biodegradable materials suitable for food packaging.

Limited research work has been conducted with biodegradable food packaging materials incorporating antimicrobials. Polylactic acid (PLA) has been probably the only synthetic biodegradable food packaging material that has been tested extensively for food packaging applications. Jin and Zhang (2008) tested the efficacy of PLA films incorporated with nisin against *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enteritidis* in liquid foods and in culture media, being able to achieve reductions of the respective microorganisms in the range of 2–4 logarithmic cycles. Jin and others (2009) incorporated nisin and pectin in PLA to assess its efficacy against *L. monocytogenes*. The addition of pectin was done in order to obtain a better incorporation of nisin into the film matrix. It was possible to reduce the *L. monocytogenes* number in a range of 2.1–3.7 logarithmic units under *in vitro* conditions. However, the tensile properties were reduced after the incorporation of pectin and nisin. The possible reason of the selection of PLA in this kind of research is its properties. As it was mentioned previously, in addition to its biodegradability, it has shown to have a versatile behavior that makes it suitable for food packaging applications.

Objectives

The overall objective of the present research work is to develop a biodegradable film with antimicrobial activity. The specific objectives are:

1. Develop an antimicrobial packaging film made from Poly(butylene adipate-*co*-terephtalate) (PBAT) incorporated with nisin.
2. Evaluate effectiveness of antimicrobial PBAT film against *Listeria innocua*.

3. Characterize tensile , thermal, and gas barrier properties of PBAT films incorporated with nisin.
4. Evaluate the diffusion and release kinetics of nisin from the PBAT film.

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Table 1 – Commonly used food packaging materials.

Food packaging material	General characteristics
Low density polyethylene (LDPE)	It is a very versatile plastic. It is also relatively inert from a chemical point of view and insoluble in all solvents at room temperature. Even though its permeability against water vapor is low, many organic vapors and other oils are able to pass easily through its matrix. On the other hand, its permeability to oxygen is high, so it is not an option in products where oxidation may take place (Paine and Paine 1983). However, the barrier properties of this plastic increase with density, and the density can be set by controlling the production process conditions. The thickness of the films generally used is in the range of 30 μm (Kirwan and Strawbridge 2003).
High density polyethylene (HDPE)	It is used where a high strength and a low permeability to gases are necessary. It presents a low permeability to water vapor and a middle level of stiffness. On the other hand, it does not count with a good appearance and is difficult to manipulate due to its lack of cutability and machinability (Massey 2004). In the form of films, it may be used to wrap sugar confectionery products (Kirwan and Strawbridge 2003), as well as in snack foods and dairy products (Massey 2004). The thickness of the films used in the industry ranges from 10 to 125 μm (Massey 2004).
Polypropylene (PP)	It is a plastic that can be applied in several foods either as a film or as a rigid package. Since it has a high melting point, it is possible to use where heat is going to be applied in microwaveable products, hot filling, and so on. Films made from this polymer are smooth and have a good appearance. Nevertheless, it loses flexibility and becomes brittle at freezing temperatures, which does not make it suitable for frozen goods. It is chemically inert and a good barrier against water vapor. In the form of films, it can be used in the following products: biscuits, snack products, chocolate and sugar confectionery, frozen foods, tea, coffee, cheese, and so on (Kirwan and Strawbridge 2003). The thickness of the films made from this polymer is in the range of 10 μm to 2500 μm (Massey 2004).

Table 1 (Continued) – Commonly used food packaging materials.

Food packaging material	General characteristics
Polyethylene terephthalate (PET)	This plastic results from the reaction between ethylene glycol and terephtalic acid and their polymerization. It possesses a high melting point, which makes it ideal for products in which heat treatment (sterilization, for example) is required once they are packaged, and for microwaveable products. It maintains its flexibility even at very low temperatures. On the other hand, presents relatively poor barrier properties to water vapor and oxygen. The thicknesses of the films made from this polymer range from less than 12 μm to approximately 200 μm. In the form of films, it can be used in liquids packaged in bags, coffee packaged under vacuum conditions, and snack foods, as long as it is laminated with EVA to improve its barrier properties (Kirwan and Strawbridge 2003).
Polystyrene (PS)	Films made from this material exhibit high clarity, stiffness, and low barrier properties against water vapor and gases, which makes it suitable for products that present respiration (Kirwan and Strawbridge 2003).
Polyvinyl Alcohol (PVOH)	This plastic exhibits a high barrier property to oxygen. One interesting property of this material is its biodegradability. It can be degraded by a big number of microorganisms. Its tensile properties are very versatile and depend on the manufacture process (Massey 2004).
Ethylene vinyl acetate copolymer (EVA)	It is a plastic similar to polyethylene in several aspects. It is commonly used blended with PE. An increase in the vinyl acetate component represents an increase in the wrapping properties, since toughness and elasticity are improved (Kirwan and Strawbridge 2003). In general, a high content of vinyl acetate results in an increase in the gas permeability and a decrease in stiffness. The films thickness ranges from 25 to 50 μm. It can be used in the coextruded or laminated form to package meat and poultry (Massey 2004).

Table 1 (Continued) – Commonly used food packaging materials.

Food packaging material	General characteristics
Polyvinyl chloride (PVC)	In the unplasticised form, this plastic is hard and brittle. Its properties can be improved by the incorporation of different additives and by modification of certain parameters during the production process. Nevertheless, it is necessary to choose carefully such additives since they may be easily released to the food matrix. Due to its high stretch and cling, it is commonly used to wrap fresh products. Its barrier properties against water vapor and other gases depend on the amount of plasticizer incorporated in the production process. These barrier properties decrease as the concentration of the plasticizer agent increases. This condition provides PVC flexibility to be used in other food products where it is important to have high barrier properties to water, like in the case of meat (Kirwan and Strawbridge 2003).
Polyvinylidene chloride (PVDC)	This material represents a good barrier against water vapor and other gases. Due to its flexibility and ability to contract, it can be used in products subjected to heat treatments to ensure a tight wrapping (for example, poultry). Other products in which it can be applied as packaging films include cured meats, cheese, snack foods, tea, coffee and confectionery (Kirwan and Strawbridge 2003). Films thickness may be in the range of 25 μm (Massey 2004).
Polyamides (PA)	It is a group of plastics formed by several types of nylon, depending on the number of carbon atoms in the monomer. They possess similar mechanical properties to the ones PET presents, and therefore may have the same applications. Nylon 6 and 66 are the ones most frequently encountered in food packaging applications. They can be blended with other polymers (for example, PE, PET, EVA, EVOH, etc.) to produce films (Kirwan and Strawbridge 2003). In general, for food applications they exhibit good durability at low temperatures, thermal stability, and a high barrier to oxygen, but a poor barrier property to water vapor. The thickness of the films ranges from 15 to 50 μm, and the applications depend on the type of nylon. They may be encountered in meat, cheese, snack foods, condiments, shredded cheese, coffee, and so on (Massey 2004).

Table 1 (Continued) – Commonly used food packaging materials.

Food packaging material	General characteristics
Ethylene-vinyl alcohol co-polymer (EVOH)	This plastic exhibits excellent barrier properties against oxygen. It can be used in multilayer structures and is commonly produced through extrusion technology (Lee and Kim 1997). Its properties depend on the ethylene/vinyl alcohol ratio. An ethylene content of 38% presents good mechanical properties and good permeability against oxygen (Stenhouse and others 1996).

Table 2 – Density, melting point, and glass transition temperature of some common plastic films used in food packaging (Brandsch and Piringer 2000; Crosby 1981; Lee and Kim 1997; Roff and Scott 1971).

Plastic	Density (g cm^{-3})	Melting point (ºC)	Glass transition temperature (ºC)
LDPE	0.915–0.935	120	-30 ± 15
HDPE	0.945–0.964	137	-30 ± 15
PP	0.90–0.91	168	-17 ± 5
PET	1.37	256–260	67–81
PS	1.04–1.12	250	80–100
PVOH	1.19–1.27	200–260	70–85
EVA	0.91–0.97		
PVC	1.39–1.43	180	80–100
PVDC	1.67–1.71	170–175	-17.5
PA	1.05–1.14	185–260	37–70

Table 3 – Tensile properties of some common plastic films used in food packaging (Lee and Kim 1997; Roff and Scott 1971; Stenhouse and others 1996).

Plastic	Tensile strength			Elastic modulus			Elongation at break		
	Tensile strength (MPa)	*T* (ºC)	RH (%)	Elastic modulus (GPa)	*T* (ºC)	RH (%)	Elongation at break (%)	*T* (ºC)	RH (%)
LDPE	7–25	20–25	65	0.15–0.34	20–25	65	300–900	20–25	65
HDPE	19–31	20–25	65	0.98	20–25	65	20–50	20–25	65
PP	27–98	20–25	65	1.18	20–25	65	200–1000	20–25	65
PET	157–177			3.5			70	20–25	65
PS	31–49	20–25	65	2.7–3.4	20–25	65	2–3	20–25	65
PVOH	39–118			2.9	20–25	65	225	20–25	65
EVA	6–19	20–25	65				230–560	20–25	65
PVC	42–55	20–25	65	2.8	20–25	65	20–180	20–25	65
PVDC	49–98	20–25	65	0.2–0.6	20–25	65	10–40	20–25	65
PA	49–69	20–25	65	0.7–0.98	20–25	65	200–300	20–25	65
EVOH	55-65			2.0-2.3			100-225		

Table 4 – Oxygen and water vapor permeability of some common plastic films used in packaging (Lange and Wyser 2003; Massey 2004; Piringer 2000).

Plastic	Oxygen permeability (OP)			Water Vapor Permeability (WVP)		
	$OP \times 10^{7}$ (mL m m^{-2} day^{-1} Pa^{-1})	*T* (ºC)	RH (%)	$WVP \times 10^{14}$ (g m m^{-2} s^{-1} Pa^{-1})	*T* (ºC)	RH (%)
LDPE	44.756	25		6.673–8.704	38	100
HDPE	7.127	25		1.741–3.482	38	90
PP	4.936–9.869	23	50	2.321–4.642	23	85
PET	0.098–0.494	23	50	5.803–22.921	23	85
PS	9.869–14.805	23	50	11.315–45.552	23	85
PVOH	0.003	23	0	342.652	23	85
EVA	21.220	23		6.673–17.118		
PVC	0.198–0.790	23	50	18.279	38	90
PVDC	0.001–0.030	23	50	1.161	23	65
PA	0.010–0.098	30	60	5.803–114.314	23	65

Table 5 – Tensile properties of polymeric films incorporated with antimicrobials.

Film	Antimicrobial	Antimicrobial incorporation method	Tensile properties without the antimicrobial substance			Tensile properties with the antimicrobial substance			Source
			Elastic modulus (KPa)	Tensile strength (KPa)	Elongation at break (%)	Elastic modulus (KPa)	Tensile strength (KPa)	Elongation at break (%)	
PE/PA/PE	Nisin	Solution coating with HPMC (Hydroxypropil methylcellulose)	110*		271*	420*		130*	Guiga and others 2008
Multilayer polyethylene film	Silver nano-particles (0.6% w)	Lamination and extrusion	27000		460	24000		445	Sánchez-Valdez and others 2009
Multilayer polyethylene film	Silver nano-particles (0.6% w)	Blending through sonication and solution casting method	27000		460	25500		495	Sánchez-Valdez and others 2009
Multilayer polyethylene film	Silver nano-particles (0.6% w/w)	Spraying	27000		460	26000		480	Sánchez-Valdez and others 2009
Cellulose derivative polymer	Natamycin formulation (8% w/w)	Blending and solution casting method		0.09*	4.54*		0.0573*	1.69*	Pires and others 2008
Cellulose derivative polymer	Nisin formulation (50% w/w)	Blending and solution casting method		0.09*	4.54*		0.0260*	1.03*	Pires and others 2008
Cellulose derivative polymer	Natamycin and Nisin formulation (8% and 50% w/w, respectively)	Blending and solution casting method		0.09*	4.54*		0.0113*	0.72*	Pires and others 2008
Vinyliden chloride copolymer	Sorbic acid (1.5% w/v)	Blending and solution casting method		34032.52*	11.9		20753.22*	16.2	Limjaroen and others 2003
Vinyliden chloride copolymer	Sorbic acid (2 % w/v)	Blending and solution casting method		34032.52*	11.9		20580.85*	15.4	Limjaroen and others 2003

*Significant difference ($P < 0.05$) in the corresponding property between the films with and without antimicrobial.

Table 5 (Continued) – Tensile properties of polymeric films incorporated with antimicrobials.

Film	Antimicrobial	Antimicrobial incorporation method	Tensile properties without the antimicrobial substance			Tensile properties with the antimicrobial substance			Source
			Elastic modulus (KPa)	Tensile strength (KPa)	Elongation at break (%)	Elastic modulus (KPa)	Tensile strength (KPa)	Elongation at break (%)	
Vinyliden chloride copolymer	Sorbic acid (3% w/v)	Blending and solution casting method		34032.5*	11.9		19594.90*	16.7	Limjaroen and others 2003
Vinyliden chloride copolymer	Potassium sorbate (2% w/v)	Blending and solution casting method		34032.5*	11.9		13251.72*	12.7	Limjaroen and others 2003
Vinyliden chloride copolymer	Potassium sorbate (3% w/v)	Blending and solution casting method		34032.5*	11.9		8673.60*	13.9	Limjaroen and others 2003
Vinyliden chloride copolymer	Nisin (1% w/v)	Blending and solution casting		34032.5*	11.9		7 191.23*	11.1	Limjaroen and others 2003
Vinyliden chloride copolymer	Nisin (2% w/v)	Blending and solution casting		34032.5*	11.9		6563.81*	10	Limjaroen and others 2003
Vinyliden chloride copolymer	Nisin (2.5% w/v)	Blending and solution casting		34032.5*	11.9		5488.23	11	Limjaroen and others 2003
Methyl cellulose	Natamycin (2 mg/10 g of film forming solution)	Blending and solution casting	313230	36630	73.98	380730	37170	60.45	Türe and others 2009
Methyl cellulose	Natamycin (20 mg/10 g of film forming solution)	Blending and solution casting	313230*	36630*	73.98	299900*	22590*	56.76	Türe and others 2009

*Significant difference ($P < 0.05$) in the corresponding property between the films with and without antimicrobial.

Table 6 – Oxygen permeability (OP) and oxygen transmission rate (OTR) of polymeric films incorporated with antimicrobials.

Film	Antimicrobial	Antimicrobial incorporation method	OP × 10^7 before the incorporation of the antimicrobial (mL m m^{-2} day^{-1} Pa^{-1})	OP × 10^7 after the incorporation of the antimicrobial (mL m m^{-2} day^{-1} Pa^{-1})	OTR × 10^3 before the incorporation of the antimicrobial (mL m^{-2} day^{-1})	OTR × 10^3 after the incorporation of the antimicrobial (mL m^{-2} day^{-1})	*T* (ºC)	RH (%)	Source
Vinyliden chloride copolymer	Sorbic acid (1.5% w/v)	Blending and solution casting method	5260.3	3306.2			23	0	Limjaroen and others 2003
Vinyliden chloride copolymer	Sorbic acid (2 % w/v)	Blending and solution casting method	5260.3	5664.9			23	0	Limjaroen and others 2003
Vinyliden chloride copolymer	Sorbic acid (3 % w/v)	Blending and solution casting method	5260.3	8507.3			23	0	Limjaroen and others 2003
Vinyliden chloride copolymer	Potassium sorbate (2% w/v)	Blending and solution casting method	5260.3*	444.1*			23	0	Limjaroen and others 2003
Vinyliden chloride copolymer	Potassium sorbate (3% w/v)	Blending and solution casting method	5260.3*	> 453984*			23	0	Limjaroen and others 2003
LDPE	Linaool (1% w/w)	Extrusion			9.2*	6.1*	23	0	Suppakul and others 2006
LDPE	Methylchavicol (1% w/w)	Extrusion			9.2*	4.7*	23	0	Suppakul and others 2006

*Significant difference ($P < 0.05$) in the corresponding property between the films with and without antimicrobial.

Table 7 – Water vapor permeability (WVP) and water vapor transmission rate (WVTR) of polymeric films incorporated with antimicrobials.

Film	Antimicrobial	Antimicrobial incorporation method	WVP × 10^{14} before the incorporation of the antimicrobial (mL m m^{-2} s^{-1} Pa^{-1})	WVP × 10^{14} after the incorporation of the antimicrobial (mL m m^{-2} s^{-1} Pa^{-1})	WVTR before the incorporation of antimicrobial (g m^{-2} day^{-1})	WVTR after the incorporation of antimicrobial (g m^{-2} day^{-1})	*T* (ºC)	RH (%)	Source
Vinyliden chloride copolymer	Sorbic acid (1.5% w/v)	Blending and solution casting method	110.3*	308.7*			37.8	90	Limjaroen and others 2003
Vinyliden chloride copolymer	Sorbic acid (2 % w/v)	Blending and solution casting method	110.3*	330.8*			37.8	90	Limjaroen and others 2003
Vinyliden chloride copolymer	Sorbic acid (3 % w/v)	Blending and solution casting method	110.3*	441.0*			37.8	90	Limjaroen and others 2003
Vinyliden chloride copolymer	Potassium sorbate (2% w/v)	Blending and solution casting method	110.3*	815.9*			37.8	90	Limjaroen and others 2003
Vinyliden chloride copolymer	Potassium sorbate (3% w/v)	Blending and solution casting method	110.3*	837.9*			37.8	90	Limjaroen and others 2003
LDPE	Linaool (1% w/w)	Extrusion			13.7*	10.5*	38	90	Suppakul and others 2006
LDPE	Methylchavicol (1% w/w)	Extrusion			13.7*	5.2*	38	90	Suppakul and others 2006

*Significant difference ($P < 0.05$) in the corresponding property between the films with and without antimicrobial.

Table 8 – Values of *D* obtained in previous studies using different analytical solutions of Fick's second law.

Film	Antimicrobial substance	Fick's second law analytical solution utilized	$D \times 10^{12}$ (cm^2 s^{-1})	T (ºC)	Reference
LDPE	Potassium sorbate		18300	25	Han and Floros (1998)
PET (biaxially oriented)	Potassium sorbate	$\frac{C(x,t)}{C_1} = \left(1 - \frac{x}{l}\right) + \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{C_1}{n}\sin\left(\frac{n\pi x}{l}\right)e^{\left(-\frac{Dn^2\pi^2 t}{l^2}\right)}$	0.543	25	Han and Floros (1998)
PP	Potassium sorbate		0.465	25	Han and Floros (1998)
HDPE	Potassium sorbate		0.426	25	Han and Floros (1998)
Cellulose acetate	Lysozyme	$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty}\frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2}e^{\left(-\frac{Dq_n^2 t}{l^2}\right)}$	150–2330	4	Gemili and others 2009
Sodium alginate	Potassium sorbate		232000–318000	25	Zactiti and Kieckbush (2009)
Wheat gluten	Sorbic acid		31000	4	Redl and others 1996
Wheat gluten with beeswax	Sorbic acid		41000	10	Redl and others 1996
Wheat gluten with distilled acetylated monoglyrerides	Sorbic acid		75000	20	Redl and others 1996
Wheat gluten	Sorbic acid		22000	4	Redl and others 1996
Wheat gluten with beeswax	Sorbic acid	$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty}\frac{8}{(2n+1)^2\pi^2}e^{\left(-\frac{D(2n+1)^2\pi^2 t}{l^2}\right)}$	30000	10	Redl and others 1996
Wheat gluten with distilled acetylated monoglyrerides	Sorbic acid		56000	20	Redl and others 1996
Wheat gluten	Sorbic acid		16000	4	Redl and others 1996
Wheat gluten with beeswax	Sorbic acid		22000	10	Redl and others 1996
Wheat gluten with distilled acetylated monoglyrerides	Sorbic acid		32000	20	Redl and others 1996

C: concentration of the antimicrobial substance at time t and position x through the film.
C_1: initial concentration of the antimicrobial substance in the film.
M_t: released amount of the antimicrobial substance at time t.
M_∞: released amount of the antimicrobial substance at equilibrium.
l: film's thickness.
α: ratio between the volumes of the solution and the film.
q_n: positive root of $\tan q_n = -\alpha q_n$.

Table 8 (Continued) – Values of *D* obtained in previous studies using different analytical solutions of Fick's second law.

Film	Antimicrobial substance	Fick's second law analytical solution utilized	$D \times 10^{12}$ (cm^2 s^{-1})	T (ºC)	Reference
Cast corn zein	Nisin	$\frac{M_t}{M_0} = 1 - 4\left(\frac{Dt}{h^2}\right)^{\frac{1}{2}}\left\{\pi^{-\frac{1}{2}} + 2\sum_{n=1}^{\infty}(-1)^n ierfc\,\frac{nh}{\sqrt{Dt}}\right\}$	7	5	Teerakarn and others 2002
Cast corn zein	Nisin		77	25	Teerakarn and others 2002
Cast corn zein	Nisin		310	35	Teerakarn and others 2002
Cast corn zein	Nisin		640	45	Teerakarn and others 2002
Wheat gluten	Sorbic acid		31000	4	Redl and others 1996
Wheat gluten with beeswax	Sorbic acid		41000	10	Redl and others 1996
Wheat gluten with distilled acetylated monoglyrerides	Sorbic acid		75000	20	Redl and others 1996
Wheat gluten	Sorbic acid	$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{h^2}\right)^{\frac{1}{2}}\left\{\pi^{\frac{1}{2}} + 2\sum_{n=1}^{\infty}(-1)^n ierfc\,\frac{nh}{\sqrt{Dt}}\right\}$	22000	4	Redl and others 1996
Wheat gluten with beeswax	Sorbic acid		30000	10	Redl and others 1996
Wheat gluten with distilled acetylated monoglyrerides	Sorbic acid		56000	20	Redl and others 1996
Wheat gluten	Sorbic acid	$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi h^2}\right)^{\frac{1}{2}}$	16000	4	Redl and others 1996
Wheat gluten with beeswax	Sorbic acid		22000	10	Redl and others 1996
Wheat gluten with distilled acetylated monoglyrerides	Sorbic acid		32000	20	Redl and others 1996
Acrylic polymer	Nisin	$\frac{M_t}{M_\infty} = \frac{2}{h}\left(\frac{Dt}{\pi^2}\right)^{\frac{1}{2}}$	4	10	Kim and others 2002
Vinyl-acetate ethylene co-polymer	Nisin		9	10	Kim and others 2002

M_0: initial amount of antimicrobial in the film.
M_t: released amount of the antimicrobial substance at time *t*.
M_∞: released amount of the antimicrobial substance at equilibrium.
h: film's thickness.
ierfc: associated function of the mathematical error function (*erfc*).

Table 9 – Biodegradable materials for food packaging.

Material	General characteristics
Polyesters	These materials can be extracted directly from proteins, lipids, polysaccharides, and so on. They can also be produced through commonly used polymerization processes using bio-based monomers (Siracusa and others 2008). Examples of biodegradable polyesters are polylactide, poly-(ε-caprolactone), polybutylene succinate (PBSU), polybutylene succinate adipate copolymer (PBSU-AD), polyethylene succinate (PESU), polyethylene succinate copolymer (PESU-AD), and polyglycolide. Due to their low level of production, the cost of these materials may be high compared with the commonly used synthetic packaging materials (Jayasekara and others 2005).
Aliphatic-aromatic copolymers	They consist of a combination between PET and aliphatic polyesters. Their applications can be found as eating utensils and bottles. Their main disadvantage is their high cost in comparison to synthetic plastics and the fact that, even though they can be totally biodegradable, their production process implies the utilization of important amounts of non-degradable resources, and the consequent generation of wastes (Siracusa and others 2008).
Aliphatic polyesters	Materials similar to PE and PP, but with diminished thermal and tensile properties. They are produced by polycondensation of glycol and aliphatic dicarboxylic acid (Siracussa and others 2008).
Polylactide aliphatic copolymer (CPLA)	Mixture between different aliphatic polyesters. They may present properties of softness (like PP) or hardness (like PS) depending on the proportion of the mixture of aliphatic polyesters. They can be incinerated without producing toxic gases. After 12 months in contact with the environment, they are already degraded.

Table 9 (Continued) – Biodegradable materials for food packaging.

Material	General characteristics
Polycaprolactone	This material shows to be a good barrier property against water, oils, and some solvents. It results from the polymerization of non-degradable materials like crude oil. It can be used in food applications when mixed with starch. Presents a melting point of about 59 ºC (Siracusa and others 2008).
Poly(lactic acid) (PLA)	Material with good properties for food packaging applications. Its raw materials come from the fermentation of corn, sugar feedstock, and so on. Its properties depend on the proportion of its copolymers, poly(L-lactic acid) and poly(D-lactic acid), which can convert it in a brittle crystalline plastic or in an amorphous flexible one. Commonly, for food applications, the proportion of the D-lactic acid component is higher, which makes it have properties similar to the one PE exhibits (Siracusa and others 2008).
Polyhydroxyalkanoates (PHA)	Packaging films with good properties for food packaging applications are possible to produce when combining these materials with starch. They come from the microbial fermentation of sugars and lipids, giving a wide range of melting points (40–180 ºC). One common example is polyhydroxybutyrate (PHB), which possesses similar properties to the one PP presents, but with higher stiffness. However, the cost of these materials uses to be high. They are able to degrade in 5–6 weeks (Siracusa and others 2008).

Table 9 (Continued) – Biodegradable materials for food packaging.

Material	General characteristics
Starch-based polymers	It is possible to blend commonly used polymers for food packaging applications with different percentages of starch (10–90 %) (Siracusa and others 2008). Starch has been the most commonly used source in the development of biodegradable food packaging materials, due to its low cost and high availability (Guilbert and Gontard 2005). It is also possible to chemically modify starch, by changing the proportion of its main components (amylose and amylopectin). The higher the proportion of amylose, the lower the flexibility. On the other hand, a high amount of amylopectin affects in general the tensile properties. It is also possible to add chemical agents due to esterification, which leads to changes in the physical properties as well (Jayasekara 2005).

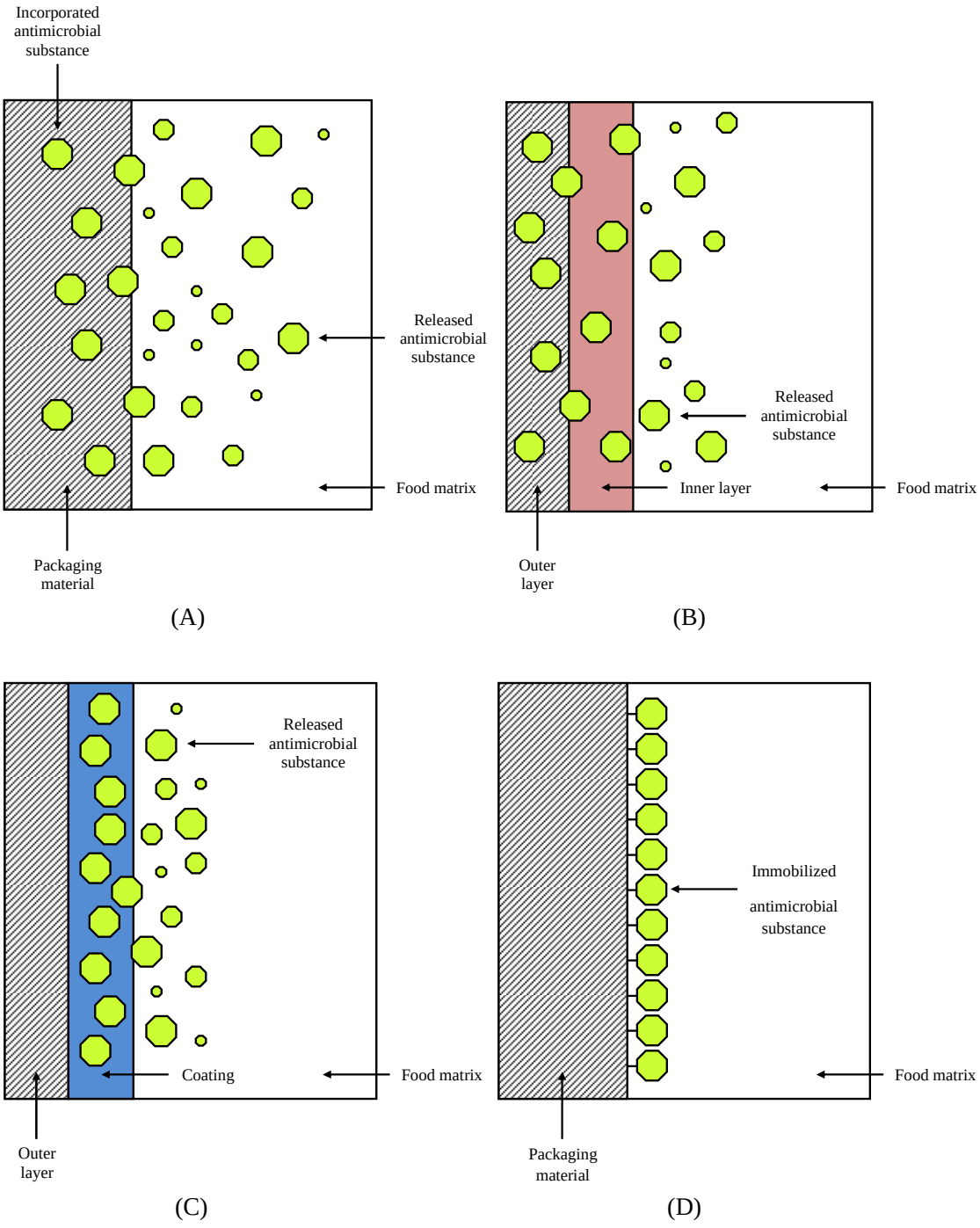

Figure 1 – Antimicrobial food packaging systems. Adapted from Han (2003) and Quintavalla and Vicini (2002).

CHAPTER 2

Biodegradable Poly(butylene adipate-*co*-terephthalate) film incorporated with nisin: characterization and effectiveness against *Listeria innocua*

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 (WVP) and oxygen permeability (OP) 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} day^{-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 ºC and from 122.5 to 124.2 ºC, respectively. The thermal transition parameters such as the crystallization and melting enthalpies and crystallization temperature were influenced significantly ($P < 0.05$) by the 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 35º. Formation of holes and pores was observed from the Environmental Scanning Electron Microscopy (ESEM) images in the films containing nisin, suggesting interaction between PBAT and nisin.

Keywords: PBAT, nisin, *L. innocua*, WVP, OP, tensile properties, thermal analysis, crystallinity, *X*-ray diffraction, ESEM.

Nomenclature

ε_b	Elongation at break (%)
ε	Strain
θ	Angle for *X*-ray diffraction (degrees)
ΔH_c	Enthalpy of crystallization (J g^{-1})
ΔH_m	Enthalpy of melting (J g^{-1})
$\Delta H_{m_{100}}$	Enthalpy of melting in 100% crystalline form for PBAT
Δp	Water vapor partial pressure difference between film underside and cabinet (Pa)
σ	Stress (Pa)
σ_s	Tensile strength (MPa)
χ	Crystallinity (%)
a_w	Water activity
D_{wa}	Diffusion coefficient of water vapor in air (m^2 s^{-1})
E	Elastic modulus (MPa)
ESEM	Environmental Scanning Microscopy
L_0	Initial length of the film (m)
L_t	Length of the film at time *t* (m)
OP	Oxygen Permeability (mL m m^{-2} day^{-1} Pa^{-1})
WVP	Water Vapor Permeability (g m m^{-2} s^{-1} Pa^{-1})
WVTR	Water Vapor Transmission Rate (g h^{-1} m^{-2})
P	Atmospheric pressure (Pa)
PBAT	Poly(butylene adipate-*co*-terephthalate)
p_T	Water vapor pressure at the testing temperature (Pa)
PTFE	Polytetrafluoroethylene
R	Gas law constant (8.314 J mol^{-1} K^{-1}),
RH	Relative humidity (%)
R^2	Coefficient of determination
T	Temperature (ºC or K)
T_c	Crystallization temperature (ºC)
T_g	Glass transition temperature (ºC)
T_m	Melting temperature (ºC)
z	Air gap in film underside (m)

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 non-biodegradable, which means that the wastes will remain in the environment 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, driven 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). It has a molecular weight of 48000 g mol^{-1} (Chivrac and others 2006). PBAT can degrade in a few weeks once it gets in contact with the soil 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 (mulching), 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 for foods.

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 either 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 allow migration of the active substance to the food and those which do not exhibit migration of such active substance but have the ability to inhibit microbial growth by direct contact with the food 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 since 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 since 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 human health (Rydlo and others 2006; Sanjurjo and others 2006). In The United States, it is the first antimicrobial bacteriocin with the status of GRAS (generally recognized as safe) 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 two 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. Generally, the most vulnerable groups include pregnant women, elderly, and people with immune deficiency (Warriner and Namwar 2009). 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 doesn't 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 non-pathogenic microorganism that shows similar kinetic and inactivation characteristics to the ones the pathogenic target microorganisms present. They also share similar behavior when exposed to the same 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 the safety of the workers. Previous studies have shown that the behavior of *L. innocua* under different conditions of pH and water activity is similar to the behavior of *L. monocytogenes* and then it represents and adequate surrogate of the pathogenic bacteria. *L. innocua* can be used as a biological indicator of *L. monocytogenes* (Rodríguez and others 2006).

Several studies have reported the thermal and tensile properties (Chivrac and others 2006; Chivrac and others 2007; Iwakura and others 2008; Ludvik and others 2007; Rhim 2007a;

Someya and others 2005) and water vapor barrier properties (Rhim 2007a) of PBAT films. These properties are important to characterize plastics for its use in food packaging. The information is important not only for the potential applications in specific foods but also for consideration in selecting processing conditions and determining transportation requirements (Robertson 1993). 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 moisture sorption isotherms (Guiga and others 2008; Stenhouse and others 1996).

Two casting methods are available for the production of antimicrobial films. The first 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 the present work are to (1) develop PBAT films with different levels of nisin incorporated using the solution casting method, (2) study their effectiveness against *L. innocua*, and (3) characterize mechanical, gas barrier, and thermal properties of the selected materials.

Materials and Methods

Films preparation

The antimicrobial films were prepared by solution casting method. Casting plates were fabricated using aluminum angles of 2.54 × 0.16 × 121.9 cm and polytetrafluoroethylene (PTFE) Teflon sheets of 14 × 21.6 × 0.64 cm (McMasterr-Carr, Chicago, IL). 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 Corporation (Florham Park, NJ).

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, NJ) 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., UK), with a minimum nisin content of 1000 IU mg^{-1}, 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 at room temperature using a Tabletop Ultrasonic Cleaner FS-30H (Fisher Scientific, Pittsburgh, PA). 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 at room temperature. Once the films were ready, they were removed from the casting plates as described above. 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 ^{o}C in sealed bags before being subjected to the different experiments performed in this work. The films' thickness was measured to the nearest 0.00254 mm with a micrometer (Micrometer 97231-61, Fred V. Fowler Co., Inc., Newton, MA). The thickness was measured at 10 randomly selected points of a 258.06 cm^2 film. The thickness determination was performed in triplicate.

Inhibition zone assay

L. innocua ATCC 51742, ATCC 33090 and SEA 15C19 were obtained from the School of Food Science of Washington State University (Pullman). The stock cultures were maintained in Tryptic Soy Broth (TSB: Difco, Becton Dickinson, Sparks, MD) containing 15% glycerol at -20 ºC. One loopful of stock was inoculated onto Modified Oxford Agar (MOX: Difco, Becton Dickinson, Sparks, MD), incubated at 37 ºC during 24 h and finally stored under refrigeration at 4 ^{o}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, Sparks, MD) and grown for 24 h at 37 ºC. 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, Sparks, MD) and 1% Tween 20 (Fischer Scientific, Fair Law, NJ) was autoclaved and cooled to 48 ºC. 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 three *L. innocua* strains to have an approximate concentration of 10^6 CFU mL^{-1} 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, Sparks, MD). Once the semisoft agars had solidified, film discs with a diameter 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 ^{o}C for 24 h and then incubated at 37 ^{o}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). Four measurements were done in the inhibition zone of each plate (45^{o} apart). Three replicates were performed for this experiment.

Water sorption isotherms

The water isotherms of PBAT films prepared using selected nisin concentrations were determined through the isopiestic methods (Sablani and others 2009). Film strips of 2 × 8.5 cm were kept for at least two weeks at room temperature 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, NJ) except for CH_3COOK and K_2CO_3, which were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). After a minimum of two weeks under equilibration, the water content of the films

was determined with a thermogravimetric analyzer TGA/SDTA 851 e (Mettler-Toledo, Columbus, OH) with a heating rate of 20 °C min^{-1} to achieve a temperature of 115 ºC. The samples were held for 20 min at 115 ºC. The PBAT film samples tested were in the range of 10–20 mg, taken from the original film strips. The experiments were performed in triplicate. The water contents in dry basis (DB) 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 diameters of 5.0 and 9.0 cm (respectively) were fabricated using acrylic sheets (McMasterr-Carr, Chicago, IL) (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, NJ) maintained at 25 ºC and at a RH of 0% achieved with anhydrous calcium sulfate (Drierite, W. A. Hammond Drierite Co. Ltd., Xenia, OH). 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 h^{-1} 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:

$$\mathrm{WVP} = \mathrm{WVTR}\frac{L}{\Delta p} \quad (1)$$

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

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

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 mol^{-1} 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 ºC), equal to 2.5×10^{-5} m^2 s^{-1}(Çengel 2006). The experiment was performed in triplicate, with two film samples per replicate.

The OP was determined with a MOCON OX-TRAN® 2/21 (Modern Controls, Inc., Minneapolis, MN) at 0% RH and 23 ^{o}C following the ASTM method D3985-95 (ASTM 1995). The test was performed in triplicate, with two 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 a RH of approximately 50% (achieved with a supersaturated solution of $Mg(NO_3)_2$ (Fisher Scientific, Fair

Law, NJ)) and at approximately 23 ºC. A screw-driven universal testing machine (Instron 4466, Instron Corporation, Norwood, MA) 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^{-1} 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 (with five 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):

$$E = \frac{\sigma}{\varepsilon} \quad (3)$$

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

$$\varepsilon = \frac{L_t - L_o}{L_o} \quad (4)$$

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 (DSC, Q2000, TA Instruments, New Castle, DE) was used to determine the thermal properties of the PBAT films. Samples of 5–10 mg were first equilibrated at 25 ºC for 1 min, then cooled down from 25 to -70 ºC at a cooling rate of -5 ºC min^{-1} and then heated to 200 ºC at a heating rate of 10 ºC min^{-1}. The samples were held at 200 ºC for 1 min and cooled down back to 25 ºC. 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; Chivrac and others 2007). The degree of crystallinity (χ) was calculated from the following formula:

$$\chi = \frac{\Delta H_m}{\Delta H_{m_{100}}} \times 100 \qquad (5)$$

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^{-1} (Chivrac and others 2006). The determination of the thermal properties was conducted in triplicate for every nisin concentration.

X-ray diffraction

Films with dimensions of 3 × 3 cm were examined in a scanning range from 8 to 35º (2θ), with a step of 0.05º, 3 s each, using 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 Company (Field Emmision Instruments), Hillsboro, OR). 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 the present study. The general linear model (GLM) 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 (HSD) test ($\alpha = 0.05$). For the case of the moisture sorption isotherms, a complete randomized factorial design with two independent factors (a_w and nisin concentration) was performed. The analysis was conducted with the software SAS version 9.1 (SAS Inst. Inc., Cary, NC).

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 the present 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 100–5000 IU mL^{-1}). In the present 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 by the film samples as the level of a_w (RH) increased (Figure 7). For the hydrophobic PBAT films at the selected nisin concentrations, there

was no significant difference between 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. Which 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 moisture 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 × 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.

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 Equation 1. In the present study, however, the overall changes observed in the film thickness as the nisin concentration increased did not show any significant effect on the transmission rates ($P > 0.05$) and on the permeability values (WVP and OP).

Tensile properties

Figure 8 shows the tensile curves for the nisin-incorporated PBAT films. 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 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 cristallinity in the films with nisin was not dramatically big. However, cristallinity might not be the only factor 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 non-uniform 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 ºC and from 122.5 to 124.2 ºC, 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 ºC 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 usually crystallize above the glass transition temperature, since 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 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 the present study, the incorporation of the antimicrobial nisin did not significantly affect the glass transition and melting temperatures of PBAT polymer (Figures 9 and 10).

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) since both terms are related as shown by Equation 5. The exothermic peaks observed for the selected nisin concentrations are presented in Figure 11. Similar trends were reported in previous works with PBAT (Chivrac and others 2006; Chivrac and others 2007; Iwakura and others 2008; Someya and others 2005).

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 12 shows the *X*-ray diffraction patterns for the selected PBAT films evaluated in the present 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 five diffraction peaks were observed at 2θ values ranging from 16 to 25º. The five diffraction peaks observed in PBAT films with 0 IU cm^{-2} corresponded to 586 counts (16.1º), 827 counts (17.45º), 802 counts (20.25º), 729 counts (23º), and 491 (24.85º). 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 31.8º 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, since no remarkable peaks were observed. The decrease in the intensity after the incorporation of nisin into PBAT confirms the decrease in cristallinity (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 (ESEM)

Figure 13 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 becomes more porous.

Conclusions

PBAT films containing nisin may represent a good option for active food packaging since 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 mechanical properties.

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

Nisin concentration (IU cm^{-2})	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 of 3 replicates. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

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 × 10^{7} (mL m^{-1} m^{-2} day^{-1} Pa^{-1})	WVP × 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 of 3 replicates. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

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 of 3 replicates. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

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

Nisin concentration (IU cm^{-2})	T_c (ºC)	T_g (ºC)	T_m (ºC)	ΔH_c (J g^{-1})	ΔH_m (J g^{-1})	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 of 3 replicates. Treatments followed by the same letter within the same column are not significantly different ($P > 0.05$).

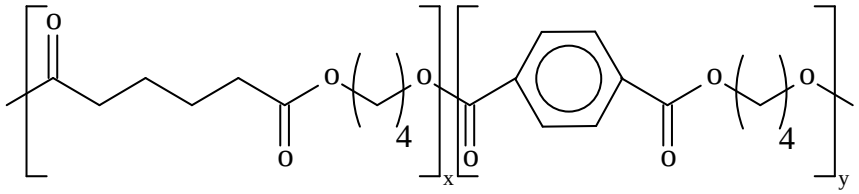

Figure 1 – Chemical structure of PBAT (Chivrac and others 2006).

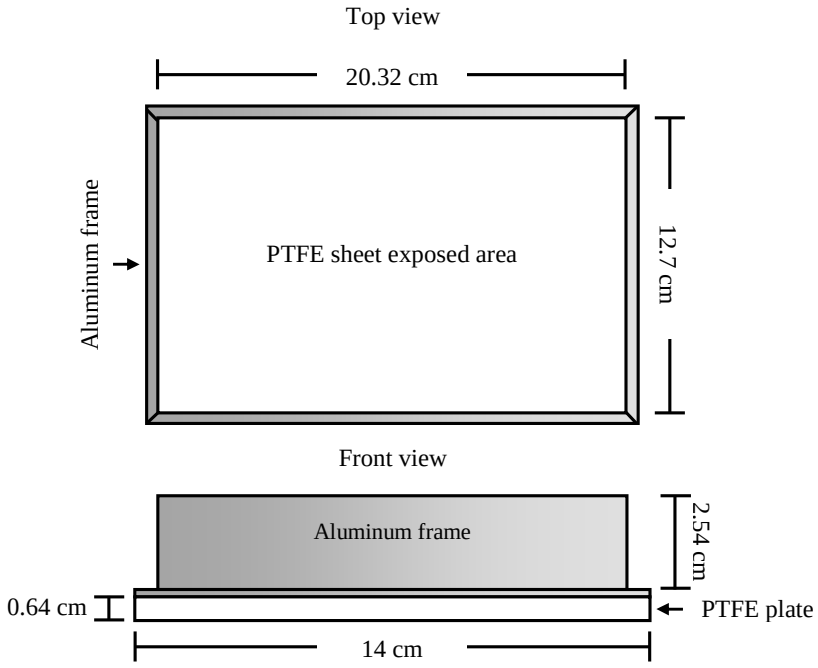

Figure 2 – Scheme of a casting plate.

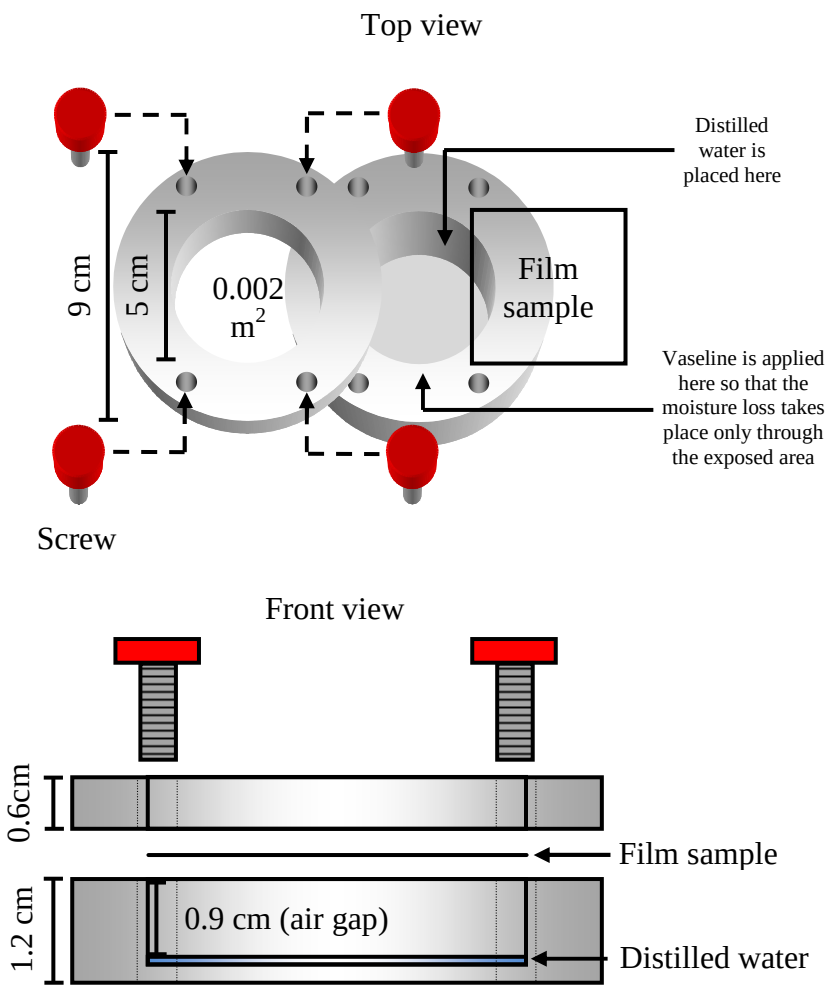

Figure 3 – Scheme of a test cup used for the determination of WVP.

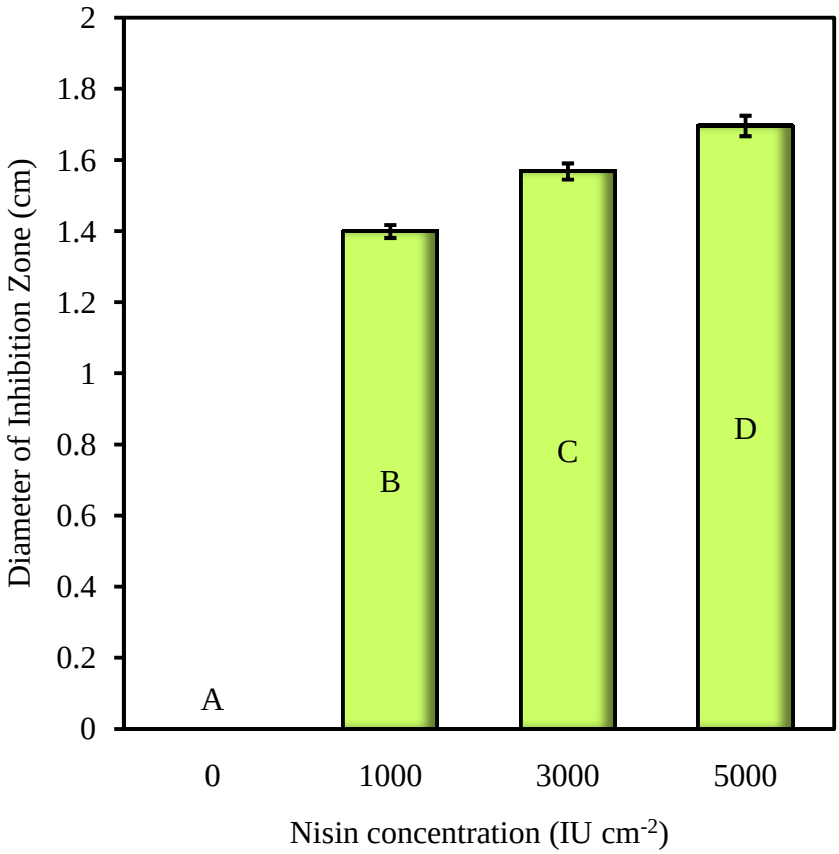

Figure 4 – Diameters of inhibition zone. Error bars represent ± 1 standard deviation of 3 replicates. Treatments with the same letter are not significantly different (*P* > 0.05).

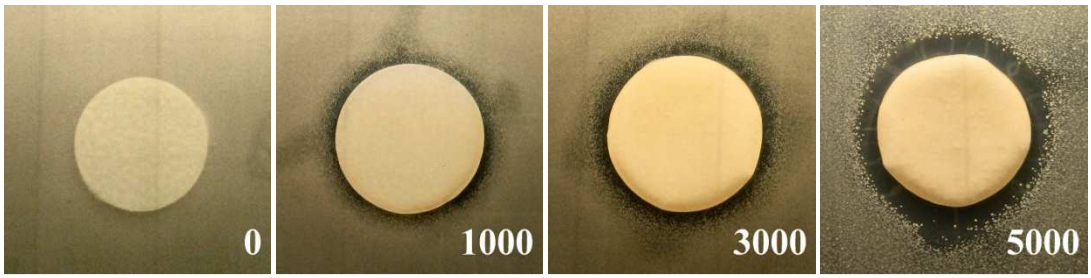

Figure 5 – Pictures showing the inhibition zones under the nisin concentrations evaluated in IU cm^{-2}.

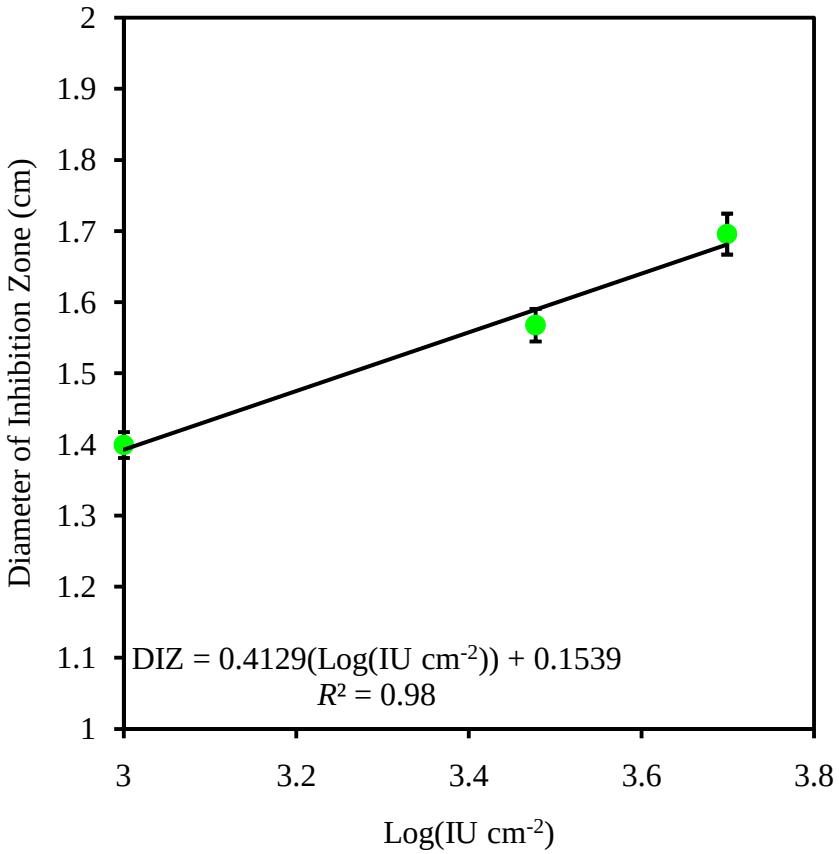

Figure 6 – Relation found between the diameter of inhibition zone (DIZ) and the logarithm of the nisin concentration, including regression line (from 1000 to 5000 in IU cm^{-2}). Error bars represent standard deviation of 3 replicates.

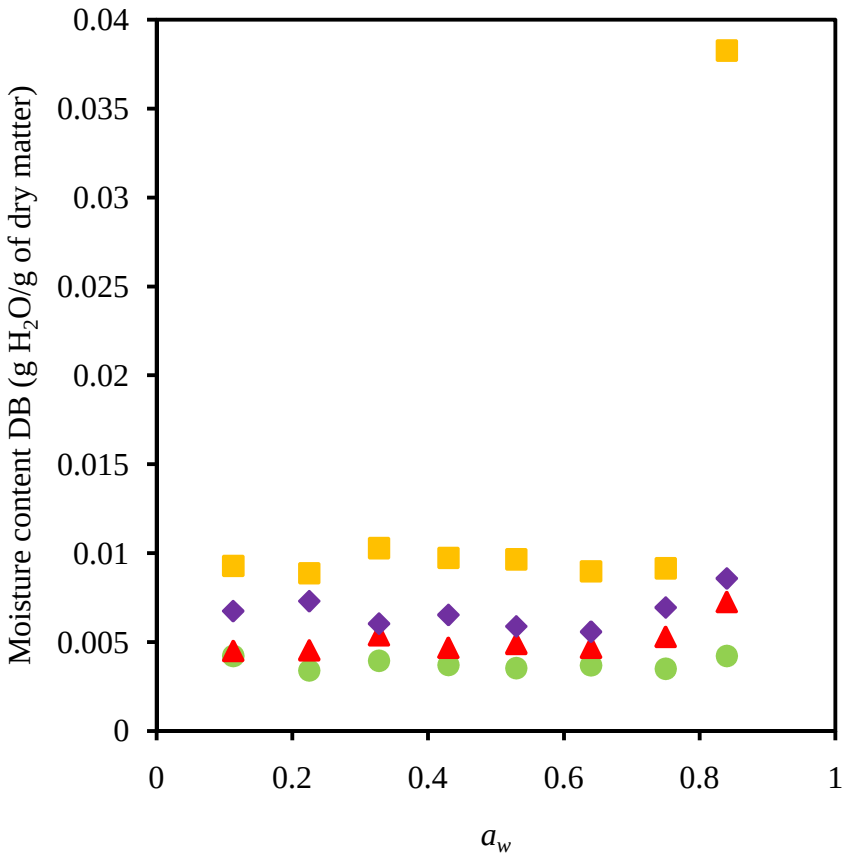

Figure 7 – Moisture content (DB) at equilibrium under different levels of a_w for the different nisin concentrations: 0 IU cm^{-2} (●), 1000 IU cm^{-2} (▲), 3000 IU cm^{-2} (♦), and 5000 IU cm^{-2} (■).

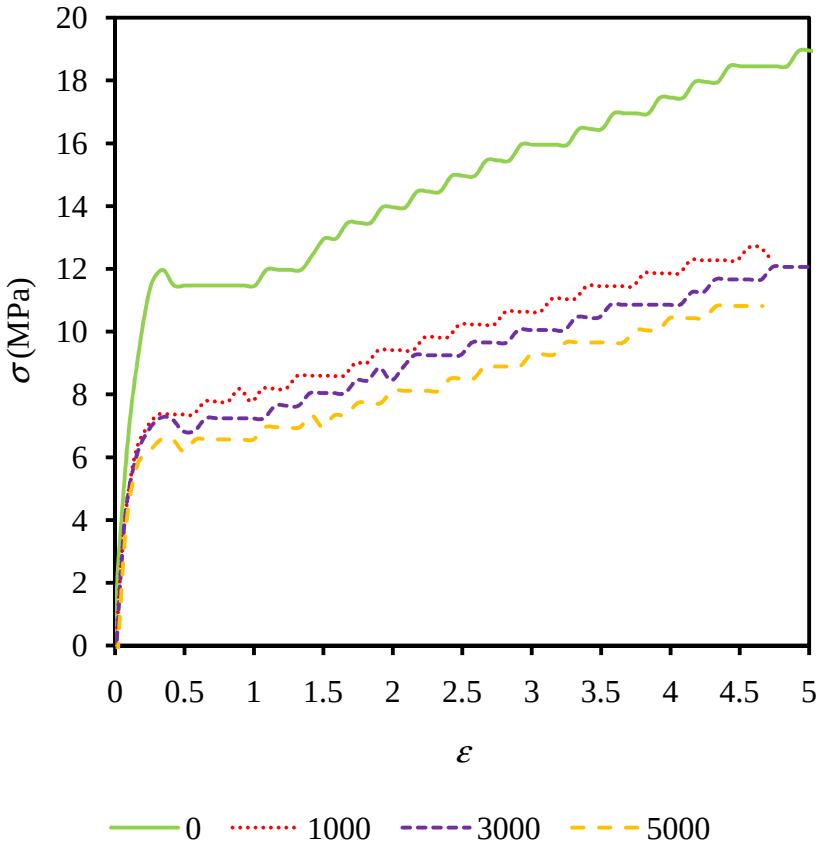

Figure 8 – Tensile curves for the different nisin concentrations in IU cm^{-2}.

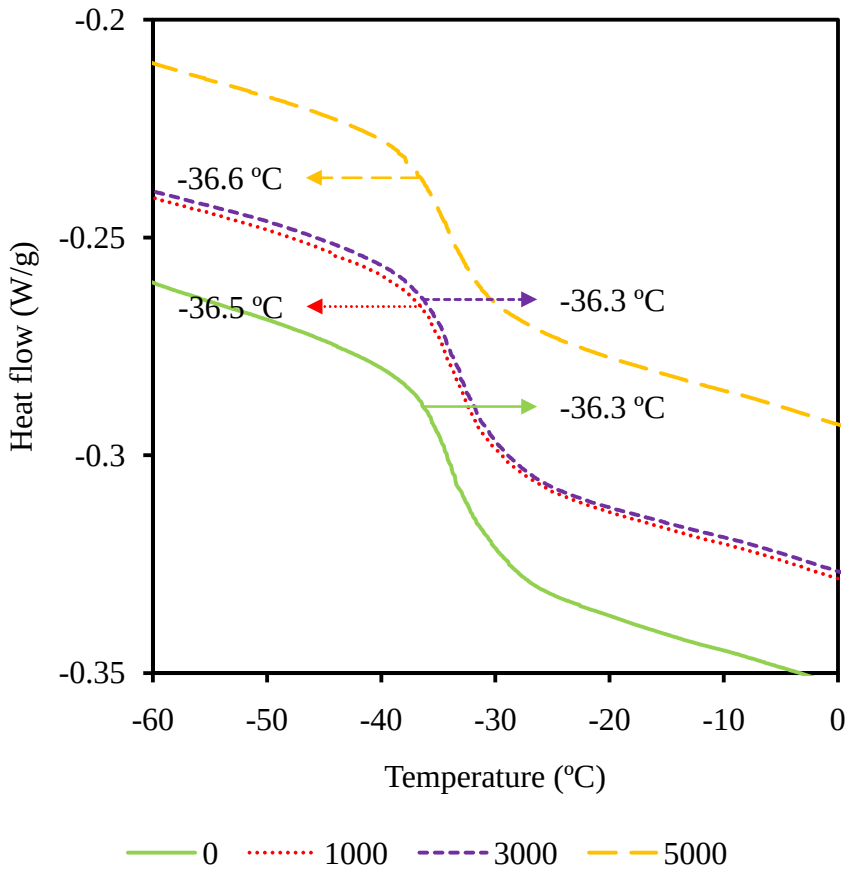

Figure 9 – DSC thermogram showing the glass transition for the different nisin concentrations in IU cm^{-2} and the corresponding values of T_g.

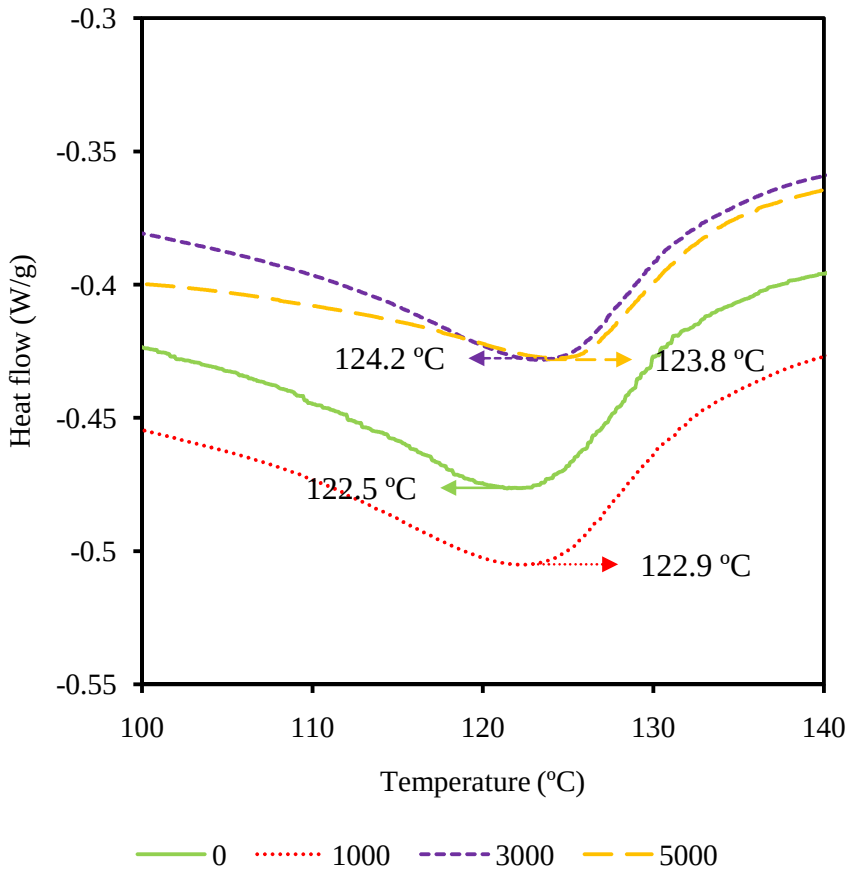

Figure 10 – DSC thermogram showing the melting peaks for the different nisin concentrations in IU cm^{-2} and the corresponding values of T_m.

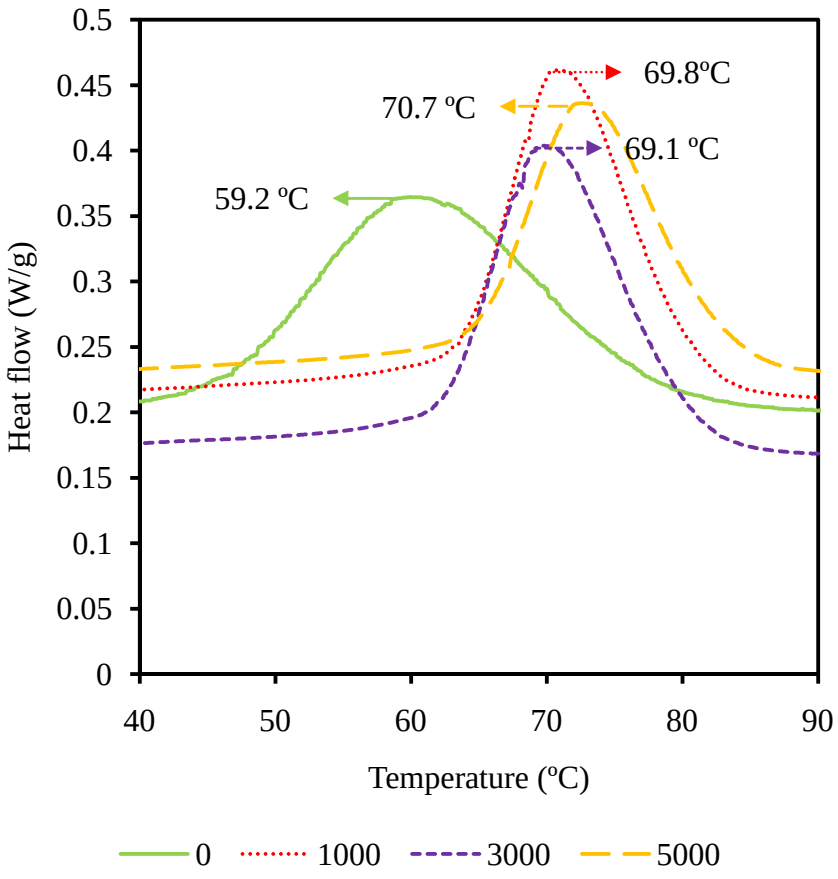

Figure 11 – DSC thermogram showing the exothermic peaks for the different nisin concentrations in IU cm^{-2} and the corresponding values of T_c.

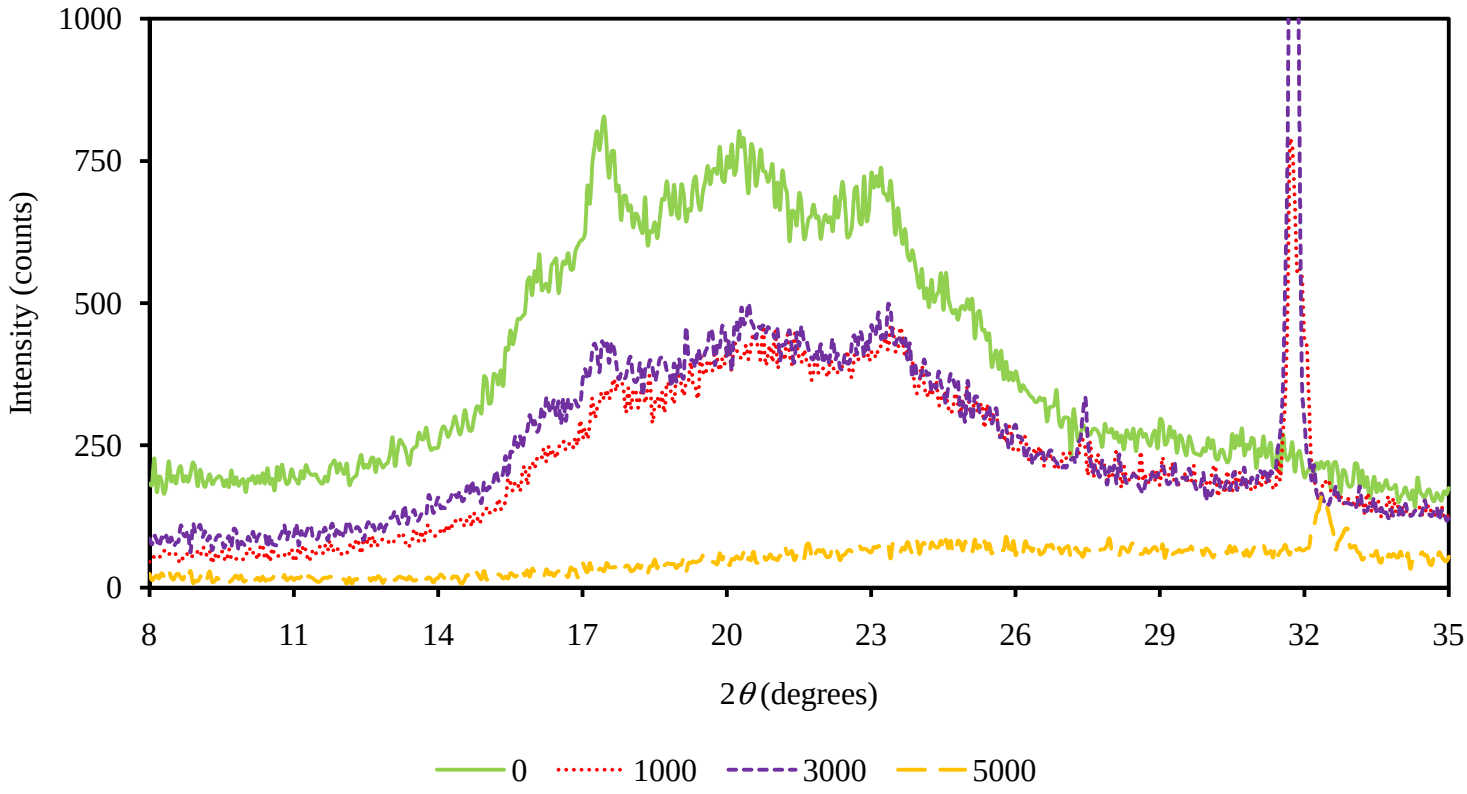

Figure 12 – *X*-ray diffraction patterns for the nisin concentrations in IU cm^{-2}.

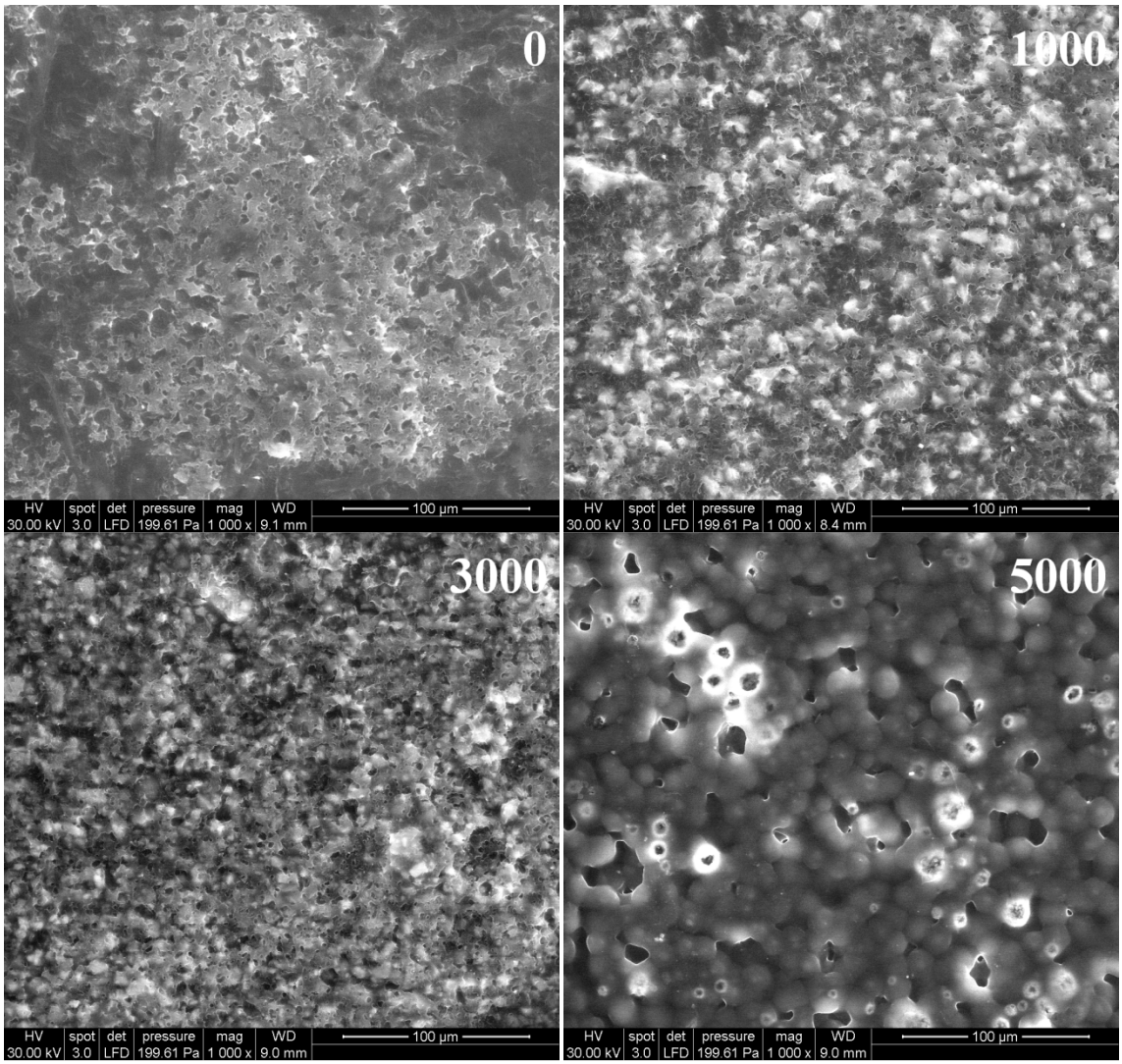

Figure 13 – ESEM images of PBAT with the nisin concentrations IU cm^{-2}.

CHAPTER 3

Release Kinetics of Nisin from Biodegradable Poly(butylene adipate-*co*-terephthalate) films into Water

Abstract

The release kinetics of nisin from poly(butylene adipate-*co*-terephthalate) (PBAT) to distilled water was studied at 5.6, 22 and 40 ºC. The release kinetics of nisin from PBAT film was described using Fick's second law of diffusion, partition coefficient, and Weibull model. The diffusion coefficients (D) determined were 0.93, 2.29, and 5.78×10^{-10} cm^2/s at 5.6, 22, and 40 ºC, respectively. The partition coefficients (K) calculated were 0.84, 3.89, and 5.2×10^3 at 5.6, 22, and 40 ºC, respectively. The nisin release data at selected temperatures were fitted with the Weibull model ($R^2 > 0.97$) with b and n values ranging from 0.02 to 0.98 and from 0.28 to 0.45, respectively. The temperature dependence of D, K, and Weibull model parameter b was modeled using the Arrhenius equation giving values of activation energy (E_a) of 38.3 KJ mol^{-1} (for D), 38.5 KJ mol^{-1} (for K), and 79.5 KJ mol^{-1} (for b).

Keywords: Activation energy, Arrhenius equation, diffusion coefficient, Fick's second law, partition coefficient, Weibull model

Nomenclature

α	Mass ratio between the amount of nisin in the sterile distilled water solution and in the film at equilibrium
A_F	Film's exposed area (cm^2)
b	Weibull's scale factor (h^{-1})
b_0	Weibull's scale factor (h^{-1}) at infinite absolute temperature
C_F	Nisin concentration in the film (mg cm^{-3})
$C_{F,0}$	Nisin concentration in the film at time cero (mg cm^{-3})
$C_{F,\infty}$	Nisin concentration in the film at equilibrium (mg cm^{-3})
$C_{S,\infty}$	Nisin concentration in the sterile distilled water solution at equilibrium (mg cm^{-3})
D	Diffusion coefficient of nisin through the film (cm^2 s^{-1})
D_0	Diffusion coefficient of nisin through the film at infinite absolute temperature (cm^2 s^{-1})
E_a	Activation energy (J mol^{-1})
K	Partition coefficient
K_0	Partition coefficient at infinite absolute temperature
l	Half of the films' thickness (cm)
$M_{F,0}$	Amount of nisin in the film at time cero (mg)
$M_{F,t}$	Amount of nisin in the film at time t (mg)
$M_{F,\infty}$	Amount of nisin in the film at equilibrium (mg)
$M_{S,t}$	Amount of nisin in the sterile distilled water solution at time t (mg)
$M_{S,\infty}$	Amount of nisin in the sterile distilled water solution at equilibirum (mg)
n	Weibull's shape factor
PBAT	Poly(butylene adipate-*co*-terephthalate)
PTFE	Polytetrafluoroethylene
q_n	Root of $\tan q_n = -\alpha q_n$
R	Gas law constant (8.314 J mol^{-1} K^{-1})
R^2	Coefficient of determination
RMSE	Root mean square error
t	Time (h)
T	Temperature (ºC)
T_{abs}	Absolute temperature (K)
V_F	Films' volume (cm^3)
V_S	Volume of the sterile distilled water solution (cm^3)
x	Position in the film (cm)

Introduction

Packaging plays an important role in extending the shelf life and safety of food products. The functionality of packaging can be enhanced by incorporating active substances. The growth inhibition or elimination of pathogenic or spoilage microorganisms is one of the features that can be added to a food packaging material (Han 2003). Consumer preference for food products with a lower concentration of additives is increasing, and can be partially satisfied by the addition of antimicrobial substances in packaging materials avoiding the direct addition of preservatives into foods. At the same time, the demand for environmental-friendly packaging materials is also growing (Suppakul and others 2003). The replacement of conventional plastics by degradable polymers is of major interest for packaging. However, these biodegradable polymers have not found extensive applications due to their weak mechanical and poor gas barrier properties (Sorrentino and others 2007). Nevertheless, the concept of biodegradable plastics incorporating antimicrobials for packaging of foods is an attractive alternative to enhance food safety while simultaneously protect the environment.

The release kinetics of antimicrobial substances from food packaging materials has not been widely explored in comparison to the release of active substances from drugs or the release of solvents from polymers (Buonocore and others 2003; Galdámez and others 2007). Nevertheless, it is important to know the diffusion rates of an active substance from packaging to the food matrix in order to design an efficient active packaging. An effective antimicrobial packaging system requires controlled release of the active substance to the food matrix. If the release of antimicrobial substance is too slow, the antimicrobial packaging systems will not be effective. The food product may be spoiled as the growth of microorganisms may be faster than the liberation of antimicrobial substance (Han 2003).

Poly(butylene adipate-*co*-terephthalate) (PBAT), an aliphatic-aromatic copolyester, is able to degrade in the environment by the intervention of microbial lipases (Chivrac and others 2006). PBAT has excellent properties for film extrusion and coatings (Jiang and others 2006). It has a water vapor permeability value of approximately 3×10^{11} g m^{-1} m^{-2} s^{-1} Pa^{-1}, a value of oxygen permeability of 4.8×10^{7} mL m^{-1} m^{-2} day^{-1} Pa^{-1}, elongation at break of 513%, elastic modulus of 48 MPa, and a tensile strength of approximately 19 MPa (Bastarrachea and others submitted for publication). The major applications of PBAT have been for agricultural films, lamination for rigid food packaging, and lawn waste bags (Herrera and others 2002). So far, the possible applications of PBAT with antimicrobials incorporated have not been explored.

In studies involving antimicrobial films for food packaging, the bacteriocin nisin has been the main choice because it is a generally recognized as safe (GRAS) additive by the Food and Drug Administration (FDA), and it inhibits the growth of Gram positive pathogens (Joerger 2007). Nisin is able to suppress the synthesis of the microbial cell wall (Sanjurjo and others 2006; Rydlo and others 2006). Nisin's amphipatic behaviour permits it to bind to the microbial cell wall, thereby altering its conformation, which leads to the formation of holes through which the loss of internal materials involved in the reproduction takes place (Cleveland and others 2001). The concentration of nisin is commonly given in International Units (IU) per unit of volume, where 40×10^{6} IU equals 1 g of pure nisin (Ray 1992).

Fick's second law has been commonly used to describe the release kinetics of an antimicrobial from a food packaging material to the food or model food (Buonocore and others 2003; Chung and others 2000; Han and Floros 1998; Kim and others 2002; Redl and others 1996; Teerakarn and others 2002). Generally, an analytical solution of the Fick's second law equation is utilized to determine the value of the diffusion coefficient (*D*). The temperature

dependence of *D* is frequently modeled using the Arrhenius equation. In recent years, the Weibull model which takes into account the non-linear behaviour of several phenomena has been widely used to model kinetics of microbiological destruction and chemical changes (Van Boekel 2008).

Antimicrobial films can be produced by solution casting method. The selection of the solvent used in solution casting method is important as it may decrease the activity of antimicrobial substance when it gets in contact with antimicrobial substances. In industrial applications, nisin is usually recovered from culture media by extraction with chloroform without affecting its activity (Burianek and Yousef 2000).

The objective of the present study was to analyze the release kinetics of nisin from biodegradable PBAT film matrix in distilled water at three selected temperatures. The nisin release from PBAT film was described with the Fick's second law, partition coefficient and Weibull model.

Materials and Methods

Films preparation

The films with nisin incorporated were prepared through solution casting. The film casting plate was fabricated using an aluminum frame and a polytetrafluoroethylene (PTFE) sheet (McMasterr-Carr, Chicago, IL) with dimensions of 14 × 21.6 × 0.64 cm. The aluminum frames of dimensions 2.54 × 0.16 × 121.9 cm were glued with epoxy resin to the PTFE plate allowing an area of 258 cm^2 for fabrication of PBAT film (Figure 1). PBAT resin (F BX 7011, BASF Corporation, Florham Park, NJ) of density of 1.25 g cm^{-3} (Rhim 2007a) was used for fabricating

the films. PBAT resin was mixed with chloroform (Mallinckrodt Baker, Inc., Phillipsburg, NJ) to prepare a solution with a concentration of 5% (weight of PBAT/volume of chloroform). The PBAT/chloroform solution was kept in a closed glass container with a vinyl screw cap. Once the PBAT resin was completely dissolved, Nisaplin (Danisco Specialities, Aplin & Barrett Ltd., UK) with a minimum concentration of 1000 IU of nisin mg^{-1} was incorporated in the PBAT/chloroform solution to have a nisin concentration of approximately 0.245 mg of nisin cm^{-2} (based on the Nisaplin's provider information and on the PTFE plates exposed area). The closed container with the PBAT/chloroform solution incorporated with Nisaplin was then subjected to 1 h of ultrasonic treatment with a Tabletop Ultrasonic Cleaner FS-30H (Fisher Scientific, Pittsburgh, PA). This step was necessary to uniformly disperse the Nisaplin powder in the PBAT/chloroform (Rhim 2007b). After the ultrasonic bath treatment, the solution was poured into the PTFE casting plate and left overnight in a hood allowing the chloroform to completely evaporate. Then, the film was taken from the PTFE casting plate by cutting the edges with a knife and peeling it off. A micrometer (Micrometer 97231-61, Fred V. Fowler Co., Inc., Newton, MA) was used to determine the film thickness. A total of ten measurements were performed on a single film to obtain an average value of thickness. The fabricated films were kept at refrigeration temperature (4 ºC) in sealed bags until being tested.

Procedure for the diffusion test

A diffusion cell consisting a glass container and two aluminum rings was designed to study the release kinetics of nisin from PBAT films to sterile distilled water (Figure 2). The upper ring was attached to the lid of a glass container. The film sample was placed in the lower ring which was then screwed to the upper ring. The internal diameter of the rings was 5 cm and the exposed area

of the film sample was approximately 20.3 cm^2. A hole of 1.5 cm diameter was made in the lid for sampling. The hole was covered with a stopper rubber during the diffusion test (Figures 2 and 3). Sterile distilled water was used to determine the diffusion of nisin. The initial volume of water inside the diffusion cell was 160 mL. In order to obtain a homogenous distribution of nisin inside the diffusion cell, a magnetic stirrer was used to agitate the solution. The diffusion test was performed at three temperatures: 5.6, 22 (room temperature) and 40 ºC. A cold storage room with a controlled temperature was used to perform experiments at 5.6 ºC. For the test run at 40 ºC, the diffusion cell was immersed in a container with water at a temperature of 40 ºC maintained using a hot plate. The temperature of the water inside the container was monitored with a digital thermometer Omega HH23 (Omega Engineering, Stamford, CT) with a type T thermocouple. The initial temperature of distilled water was set to the selected temperatures before the nisin containing PBAT film holder was immersed in the cell. For each temperature, at least 4 replicates of experiment were performed. A volume of 0.5 mL was taken at predetermined time intervals from 0.33 to 1.5 h depending on the test temperature. The change in volume due to sampling was considered while determining the nisin concentration in the diffusion cell. The water samples containing diffused nisin were stored in closed glass vials at 4 ^oC until being analyzed for nisin concentration.

The concentration of nisin diffused in water was determined through the bicinchoninic acid assay (BCA) using a BCA kit from Pierce (Rockford, IL). Bovine serum albumin was used as a standard. A volume of 0.1 mL (taken from the 0.5 mL samples) was mixed with 2 mL of the BCA kit's working reagent and heated in a water bath at 60 ºC for 30 min. The heated samples were immediately cooled in ice and absorbance was measured at 562 nm in an Ultrospec 4000 UV/vis spectrophotometer (Pharmacia Biotech, Cambridge, England).

Diffusion and partition coefficients determination

Fick's second law was considered to test the diffusion mechanism of nisin from PBAT film to distilled water (Crank 1975):

$$\frac{\partial C_F(x,t)}{\partial t} = D\frac{\partial C_F(x,t)}{\partial x^2} \quad (1)$$

where $C_F(x,t)$ is the concentration of nisin in the film at position x and time t (in mg cm^{-3}), and D is the diffusion coefficient of nisin through the PBAT film (in cm^2 s^{-1}). The following assumptions were made while deriving the analytical solution of Equation 1 (Crank, 1975; Chung and others 2000): (1) the initial concentration of nisin in PBAT film is $C_{F,0}$ and it is uniform across the film, (2) the initial concentration of nisin in the sterile distilled water is zero, (3) the water solution is well mixed with no concentration gradient of nisin in water, (4) equilibrium exists at the PBAT film-water interface, the amount of nisin diffused in water is same as amount released from the PBAT film and (5) D is not concentration dependent and is only affected by water temperature. Basing on the previous assumptions, the initial condition is:

$$C_F(x,t) = C_{F,0} \qquad -l < x < l, t = 0 \quad (2)$$

and the boundary conditions are as follows:

$$\frac{\partial C_F(x,t)}{\partial t} = 0 \;\; x = 0, t > 0 \quad (3)$$

$$K\left(\frac{V_S}{A_F}\right)\frac{\partial C_F(x,t)}{\partial t} = \pm D\,\frac{\partial C_F}{\partial x}\;\; x = \pm l, t > 0 \qquad (4)$$

where K is the partition coefficient, equal to the ratio of equilibrium concentration of nisin in the solution ($C_{S,\infty}$) to that in the film sample ($C_{F,\infty}$):

$$K = \frac{C_{S,\infty}}{C_{F,\infty}} \qquad (5)$$

V_S is the volume of the sterile distilled water solution (cm^3), A_F is the exposed area of the film (cm^2), and l is a half of the film's thickness (cm). If we define $C_{S,\infty}$ as $M_{S,\infty}/V_S$ and $C_{F,\infty}$ as $M_{F,\infty}/V_F$, Equation 5 can be rearranged as follows:

$$K = \frac{M_{S,\infty}/V_S}{M_{F,\infty}/V_F} \qquad (6)$$

where $M_{S,\infty}$ and $M_{F,\infty}$ are the amounts of nisin (in mg) in the solution and the film (respectively), and V_F is the volume of the film (in cm^3).

Different analytical solutions are available for Equation 1, and they depend on the conditions to which the flat sheet is subjected (Crank 1975). For a flat sheet in an agitated vessel of limited volume, the analytical solution for Equation 1 is (Crank 1975; Chung and others 2000):

$$\frac{M_{S,t}}{M_{F,0}} = \frac{\alpha}{1-\alpha} - \sum_{n=1}^{\infty} \frac{2\alpha}{1+\alpha+\alpha^2 q_n^2} e^{\left[-\frac{D q_n^2 t}{l^2}\right]} \quad (7)$$

where $M_{S,t}$ is the amount of nisin in the solution at time t, $M_{F,0}$ is the amount of the same component at time 0 in the film, α is the mass ratio between the amount of the studied component in the solution and in the film at equilibrium and is defined as follows:

$$\alpha = \frac{M_{S,\infty}}{M_{F,0} - M_{S,\infty}} = \frac{M_{F,0} - M_{F,\infty}}{M_{F,\infty}} \quad (8)$$

and finally, q_n is the "n" root of $tan\, q_n = -\alpha q_n$. If only the first term of the infinite summation is taken into account (the contribution of following terms is negligible) and logarithm is applied to both terms, Equation 7 becomes (Chung and others 2000):

$$\text{Log}\left[\frac{M_{S,\infty} - M_{S,t}}{M_{F,0}}\right] \approx -D \frac{q_1^2}{2.303 l^2} t + \text{Log}\left[\frac{2\alpha^2}{1+\alpha+\alpha^2 q_1^2}\right] \quad (9)$$

By plotting the logarithm of $(M_{S,\infty} - M_{S,t})/M_{F,0}$ against t, the diffusion coefficient was calculated from the slope of the straight line obtained through linear regression using Microsoft Excel®.

Once the value of D was calculated at each temperature, Equation 7 was used to fit the data. The observed values of $M_{S,t}/M_{F,0}$ were plotted against t and the coefficient of determination (R^2) as well as the root mean square error (RMSE) were calculated through regression analysis (Montgomery 1999) using Microsoft Excel®.

Release kinetics

The kinetic data of nisin release from the PBAT films to distilled water was also characterized using the Weibull model (Van Boekel 2008):

$$\mathrm{Ln}\frac{M_{F,t}}{M_{F,0}} = -(bt)^n \quad (10)$$

where $M_{F,t}$ and $M_{F,0}$ are the amounts of nisin (in mg) in the film at time *t* and 0, respectively, *n* is the shape factor and *b* is the scale parameter. Regression analysis (Montgomery 1999) was performed to determine the values of *b* and *n* with Microsoft Excel®.

Temperature dependence of *D*, *K* and *b*

The temperature dependence of *D*, *K*, and *b* was modeled using the Arrhenius equation. The equation relates the rate constant of a change or reaction as a function of the absolute temperature, and for the studied parameters it is described as:

$$D = D_0 e^{\left[-\frac{E_a}{RT_{\mathrm{abs}}}\right]} \quad (11)$$

$$K = K_0 e^{\left[-\frac{E_a}{RT_{\mathrm{abs}}}\right]} \quad (12)$$

$$b = b_0 e^{\left[-\frac{E_a}{RT_{\mathrm{abs}}}\right]} \quad (13)$$

where E_a is the activation energy (J mol^{-1}), R is the gas constant (8.314 J mol^{-1} K^{-1}), and T_{abs} is absolute temperature (K). The value of E_a relates to the required amount of energy barrier the molecules of the system analyzed have to overcome to be able to react, or the energy required for the studied phenomenon to take place (Van Boekel 2008). By applying natural logarithm to both sides of equal sign in Equations 11, 12, and 13, a linear relation is obtained:

$$\mathrm{Ln}\, D = -\frac{E_a}{R}\left(\frac{1}{T_{\mathrm{abs}}}\right) + \mathrm{Ln}\, D_0 \qquad (14)$$

$$\mathrm{Ln}\, K = -\frac{E_a}{R}\left(\frac{1}{T_{\mathrm{abs}}}\right) + \mathrm{Ln}\, K_0 \qquad (15)$$

$$\mathrm{Ln}\, b = -\frac{E_a}{R}\left(\frac{1}{T_{\mathrm{abs}}}\right) + \mathrm{Ln}\, b_0 \qquad (16)$$

where LnD_0, LnK_0, and Lnb_0 are the intercepts with the vertical axis. The values of the natural logarithm of D, K, and b were plotted against the inverse of the corresponding values of T_{abs} and by linear regression the activation energies were calculated from the slope.

Results and Discussion

Diffusion and partition coefficient determination

The concentration of nisin in the solution contained in the diffusion cell increased with time. The concentration of nisin in solution at equilibrium was higher as the temperature increased. The concentrations of nisin in solution at equilibrium were 15.89, 21.08, and 24.28 × 10^{-3} mg cm^{-3} at 5.6, 22, and 40 ºC, respectively. The exhibited behavior agreed with Equation 7 (Figure 4) and Equation 9 (Figure 5). This suggests that the release of nisin through PBAT is governed by Fickian diffusion and that the assumptions formulated were valid. Nevertheless, a large variability was observed within the replicates performed at every temperature (Figures 4 and 5). A possible reason for this could be the low concentration of nisin in the solution. Even if the solution is well mixed inside the diffusion cell, substantial variability can be obtained in the determination of nisin concentration if such concentration is very low.

The parameters α, D, and K exhibited similar behavior (Table 1). These parameters increased with increasing temperature of solution. Table 2 gives some examples of values of D for nisin through different materials reported in previous studies. It can be observed that the values obtained in the present work are substantially higher in comparison to what has been observed earlier. According to Han and Floros (1998), if the value of D of an antimicrobial substance through a plastic film is high (in the order of 1 × 10^{-8} cm^2 s^{-1}) then the antimicrobial film can be sandwiched in a multilayer structure, which would diminish the release of the active component by interposing barriers to its liberation. Nevertheless, some interaction of nisin can be assumed in the film since the values of α values were not very high. When all the substance contained in the film is released, the value of this parameter tends to infinite. The values of K

also suggest some binding of nisin with the PBAT matrix. According to Chung and others (2000), low values of *K*, in the range of 1×10^{-3}, signify that there is some affinity of the active substance with the film material. According to Franz (2000) the partition coefficient is directly related with the polarities of the migrating substance and the polymer used as packaging material. If both substances are polar, they will exhibit affinity and will interact with each other leading to a low partition coefficient and to a high retention of the migrating substance in the polymer matrix. If the studied substance is much more soluble in the packaging material than in the food ($K << 1$), this implies that at equilibrium just a small percentage of the migrating substance will liberate. According to Neetoo and others (2007), nisin is a molecule with amphipatic nature. It exhibits hydrophobic and hydrophilic behaviors, and may interact with non-polar sites of other molecules (Figure 6). Thus, it can be interpreted that nisin builds bonds with the PBAT polymer chains. On the other hand, the observed values of *D* suggest that the nisin molecules that do not interact strongly enough with PBAT are able to diffuse rapidly. As it was mentioned previously, the observed values of *D* are higher than what has been observed earlier for nisin incorporated in different film materials (Table 2). According to Chivrac and others (2006) the crystallinity of PBAT falls in a range between 8 and 11%. This indicates that it has a predominantly amorphous structure. Additionally, if a foreign substance is able to build bonds with the packaging material matrix, more free volume may be formed in it (McHugh and Krochta 1994). The possible interaction that seems to take place between nisin and PBAT may be forming more free volume in the PBAT film matrix since the PBAT polymer chains are no longer able to interact due to the interference nisin molecules might be creating. Hence, the molecules that do not interact with PBAT chains are able to be released rapidly due to the increased free volume, and that could be a possible reason for the high values of *D* obtained.

Release kinetics

The Weibull's model parameter *b* ranged from 0.02 to 0.98 h^{-1}, and the values for the shape factor (*n*) ranged from 0.28 to 0.45 as temperature increased from 5.6 to 40 ºC (Table 3). The observed trend in nisin release was fitted with the Weibull's model (Figure 7). The value of the shape factor *n* was lower than 1 for all three temperatures, which results in a curve with an upward concavity. The Weibull model has not been widely used to study diffusion phenomena in foods. It has been predominantly applied to characterize changes in food quality parameters and microbial inactivation (Van Boekel 2008). Kong and others (2007) modeled the degradation of thiamin in thermal processed salmon using the Weibull's approach, and the trend exhibited upward concavity at 4 different temperatures. Mateus and others (2007) studied the release kinetics of volatile organic compounds from roasted and ground coffee. The values of the shape factor in this study showed both upward and downward concavity depending on the stripping conditions, which suggests that the changes in substance's behavior may depend on the testing conditions and on the medium in which the substance is released. It is important to notice that, even though the observed data exhibited agreement with Equation 10, the Weibull model may be suitable to characterize the release kinetics only until equilibrium has been achieved. After that point, the value of $M_{F,t}/M_{F,0}$ will not change. It can be seen in Figure 7 that at 5.6 ºC, equilibrium had already been achieved at the last observed value from the previous measurement, and that may be the reason why at the mentioned temperature the value of R^2 was the lowest (less than 0.98).

Temperature dependence of *D*, *K* and *b*

The values of E_a were 38.3 KJ mol^{-1}, 38.5 KJ mol^{-1}, and 79.5 KJ mol^{-1} for *D*, *K* and *b*, respectively (Table 4). The temperature dependence of *D*, *K*, and *b* followed the Arrhenius equation (Figures 8 and 9). The E_a value observed for the case of *D* is comparable with what has been observed in previous works involving the release of an antimicrobial from a film. Redl and others (1996) obtained E_a values in the range of 29.9 to 39.9 KJ/mol for the diffusion of sorbic acid through gluten films. Teerakarn and others (2002) observed a range from 44 to 85 KJ/mol for the diffusion of nisin through protein edible films. The range of E_a may indicate the molecular interactions between the releasing substance and the packaging network. The higher the value of E_a, the stronger such interactions (Redl and others 1996; Teerakarn and others 2002). Han and Floros (1998) obtained an E_a value of 11.8 KJ mol^{-1}, and a value of *D* equal to 1.98×10^{-6} cm^2 s^{-1} for sorbic acid release in low density polyethylene (LDPE). This result suggests how a small E_a can be interpreted as high levels of *D*, since a substantial level of energy is not necessary for the migrating substance to be liberated leading to rapid diffusion.

Above the glass transition temperature (T_g, which corresponds to a value of approximately -36 ºC for PBAT (Bastarrachea and others submitted for publication; Chivrac and others 2006)) the molecular mobility in a system increases with temperature, which leads to an increment in the ability of the material to transport substances through its network. Fluctuations in temperature may create changes in the conformation and crystallinity of the system, thereby blocking or enhancing the liberation of the diffusing substance (Teerakarn and others 2002). This information can be correlated with the effect of temperature on *D*, *K*, and *b*. As the temperature increases, nisin's strength of its interaction interaction with PBAT is reduced and is able to be

released more rapidly. At equilibrium conditions, the higher the temperature, the lower the amount of nisin retained by the PBAT matrix and the faster its liberation.

Conclusions

The release kinetics of nisin from PBAT films to distilled water was described using Fick's second law, partition coefficient and the Weibull model. The temperature dependence of nisin diffusivity, partition coefficient and Weibull equation parameter was modeled using the Arrhenius equation. The diffusion rates of nisin from PBAT films to distilled water were higher in comparison to diffusion of nisin from other films. The nisin incorporated film can be used in a multilayer structure for a more controlled release of the antimicrobial substance.

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Table 1 – Values of α, Diffusion (D), and partition coefficient (K).

T (ºC)	α	$D \times 10^{10}$ (cm^2 s^{-1})	$K \times 10^3$
5.6	1.02 ± 0.2	0.93 ± 0.1	0.84 ± 0.2
22	4.72 ± 6	2.29 ± 1	3.89 ± 5
40	6.32 ± 5	5.78 ± 1	5.20 ± 4

Values are means ± 1 standard deviation.

Table 2 – Values of D obtained for nisin in previous studies from different materials.

Film material	T (ºC)	$D \times 10^{10}$ (cm^2 s^{-1})	Reference
Cast corn zein	5	0.07	Teerakarn and others 2002
Cast corn zein	25	0.77	Teerakarn and others 2002
Cast corn zein	35	3.1	Teerakarn and others 2002
Cast corn zein	45	6.4	Teerakarn and others 2002
Acrylic polymer	10	0.04	Kim and others 2002
Vinyl-acetate ethylene co-polymer	10	0.09	Kim and others 2002
PBAT	5.6	0.93	Present study
PBAT	22	2.29	Present study
PBAT	40	5.78	Present study

Table 3 – Weibull model parameters.

T (ºC)	b (h^{-1})	n
5.6	0.02 ± 0.02	0.28 ± 0.1
22	0.21 ± 0.3	0.42 ± 0.3
40	0.98 ± 0.7	0.45 ± 0.2

Values are means ± 1 standard deviation.

Table 4 – Values of activation energy (E_a), for D, K and b.

Parameter	E_a (KJ mol^{-1})
D	38.325
K	38.476
b	79.473

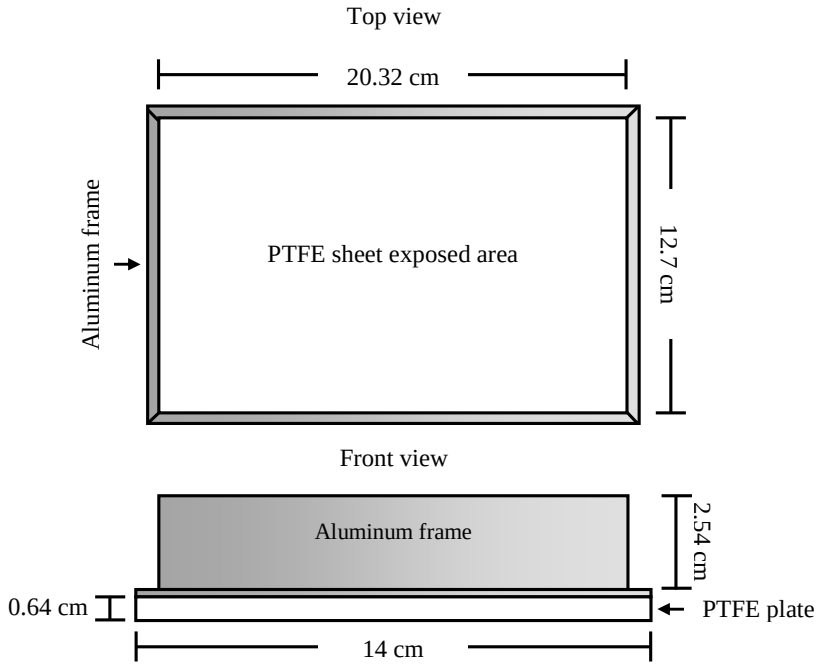

Figure 1 – Scheme of a film casting plate (Bastarrachea and others submitted for publication).

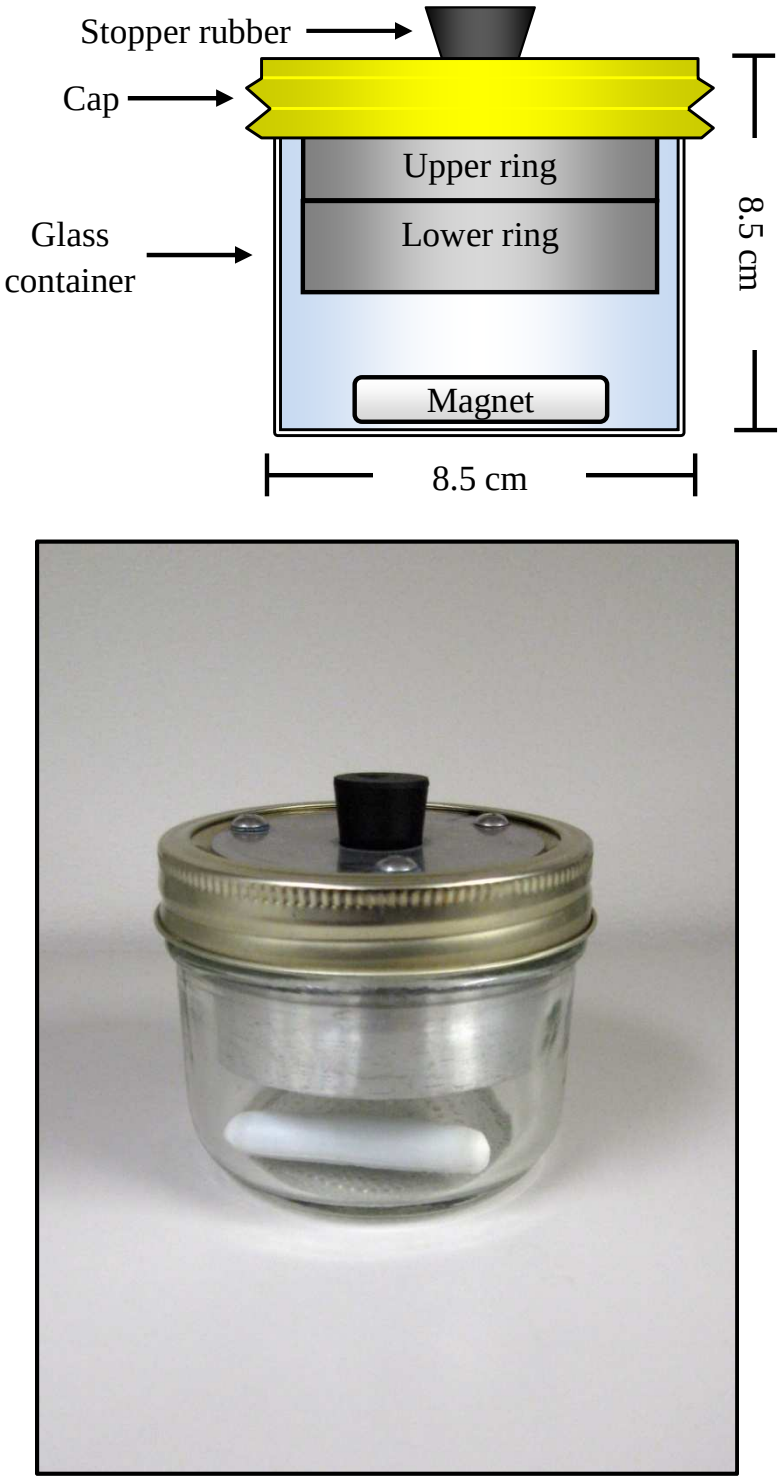

Figure 2 – Scheme of the diffusion cell.

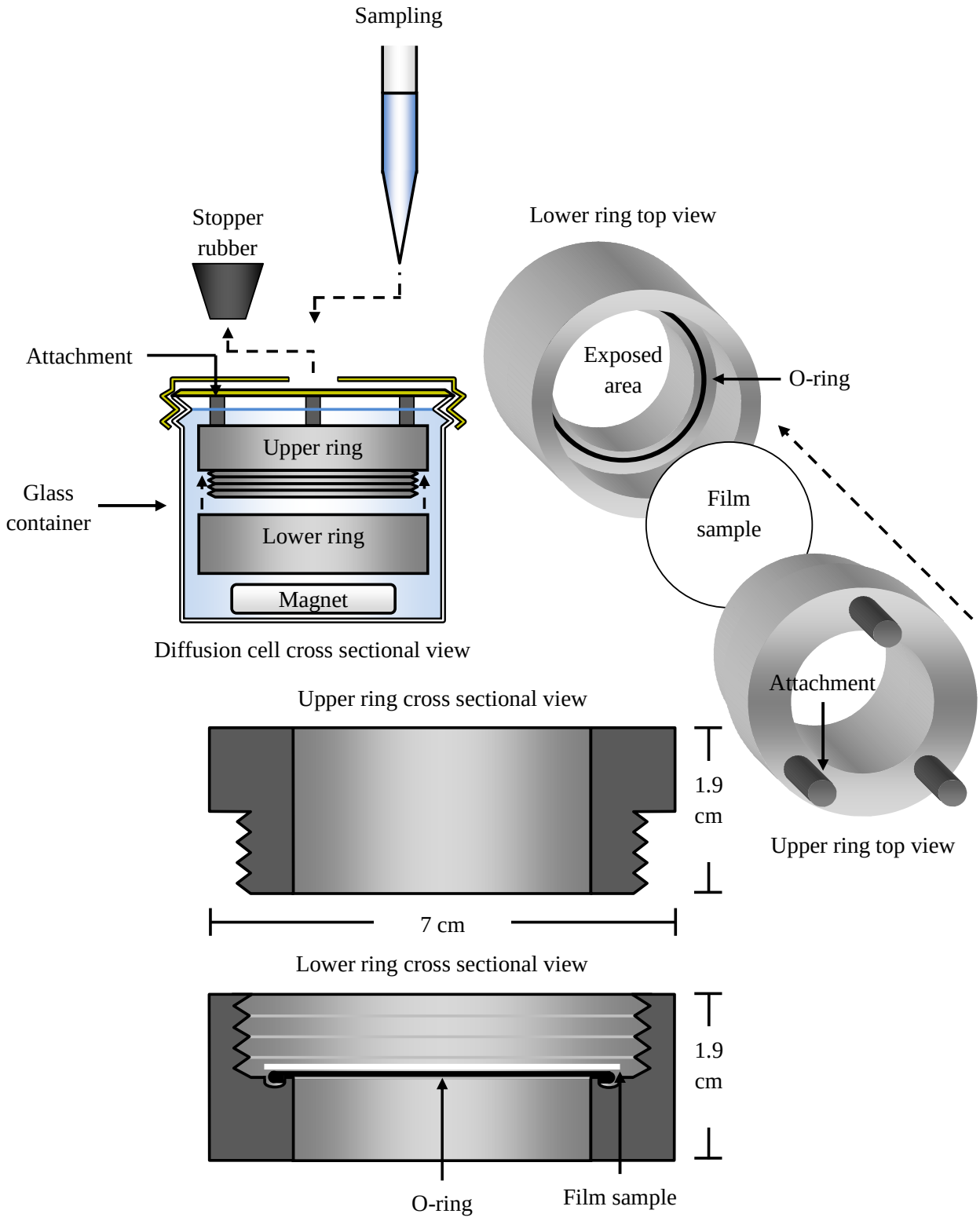

Figure 3 – Components of the diffusion cell.

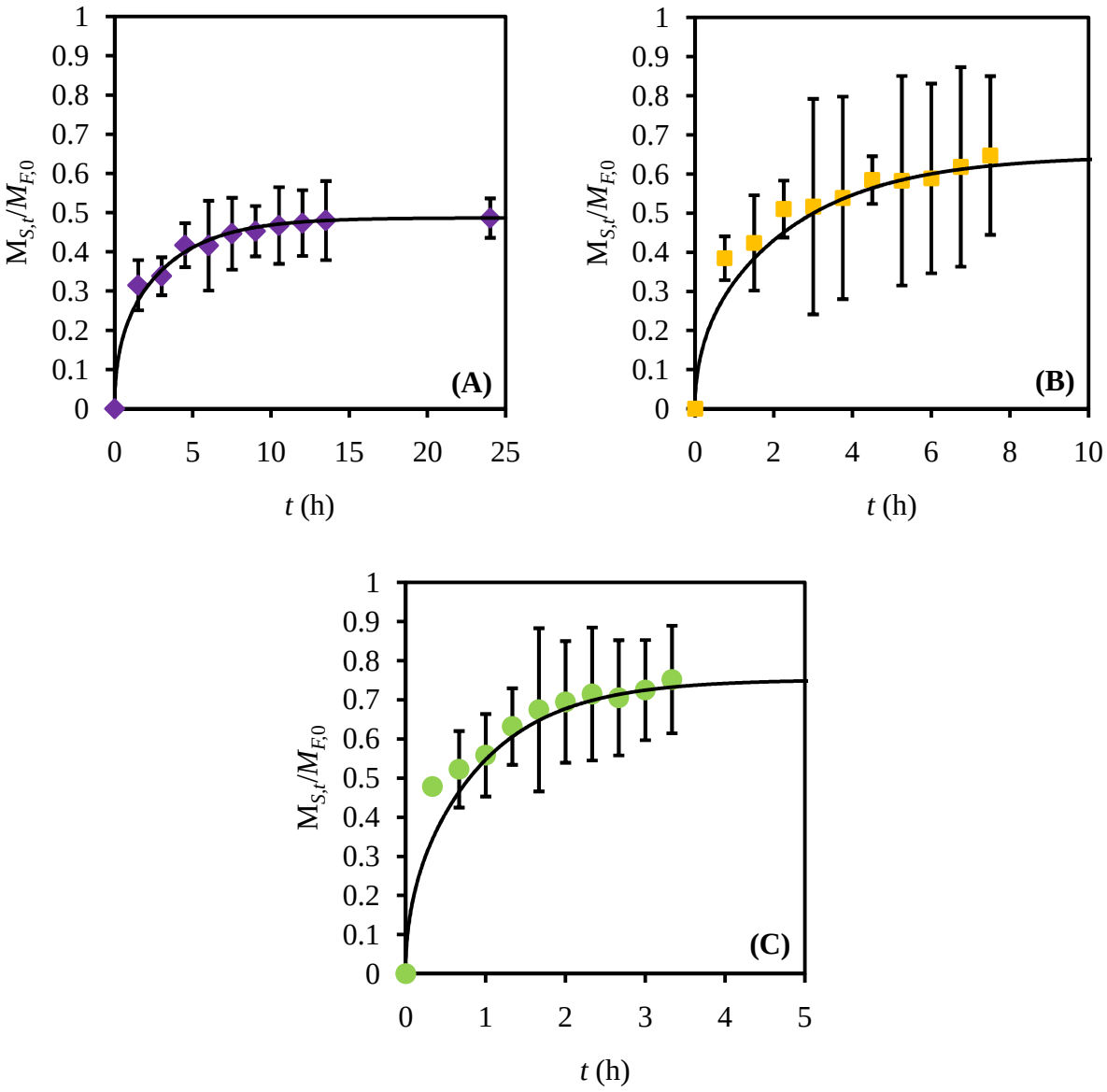

Figure 4 – Plots of $M_{S,t}/M_{F,0}$ against t at 5.6 (A), 22 (B), and 40 ºC (C), showing the observed data at 5.6 (♦), 22 (■), and 40 ºC (●) and the trend lines (—) from Equation 7 (R^2 values at 5.6, 22, and 40 ºC were, respectively: 0.981, 0.948 and 0.946). The observed values are means of at least 4 replicates.

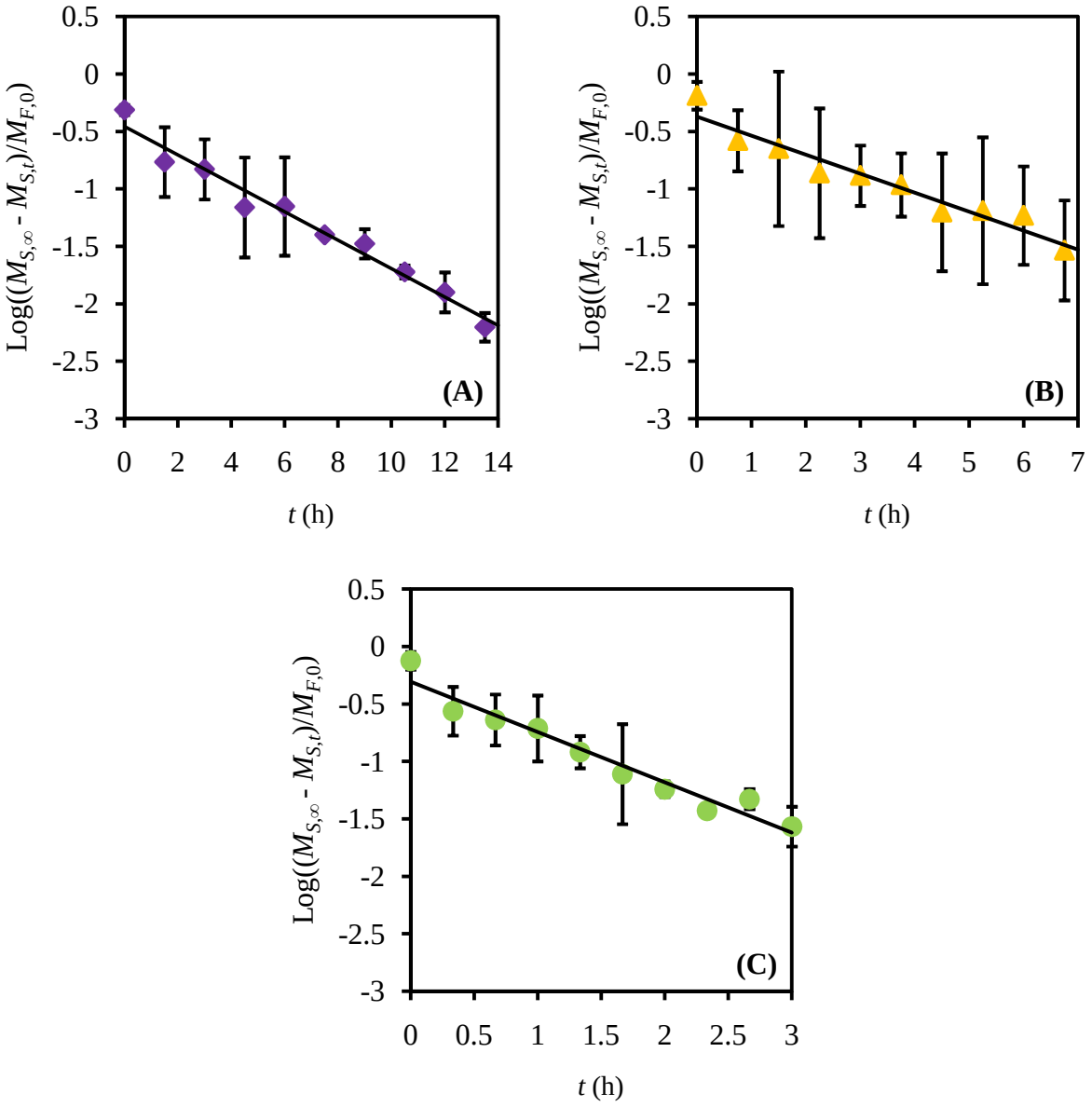

Figure 5 – Plots of the logarithm of $(M_{S,\infty} - M_{S,t})/M_{F,0}$ against t at 5.6 (A), 22 (B), and 40 °C (C), showing the observed data at 5.6 (♦), 22 (■), and 40 °C (●), with the trend lines (—) from Equation 9 (R^2 values at 5.6, 22, and 40 °C were, respectively: 0.974, 0.936 and 0.950). The observed data are means of at least 4 replicates.

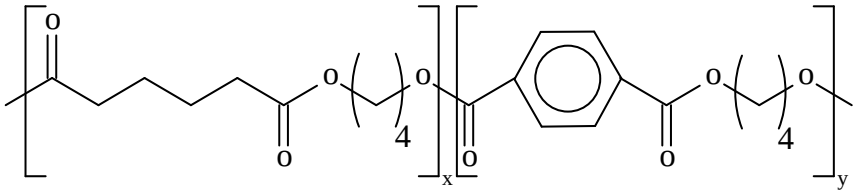

Figure 6 – PBAT molecule (Chivrac and others 2006).

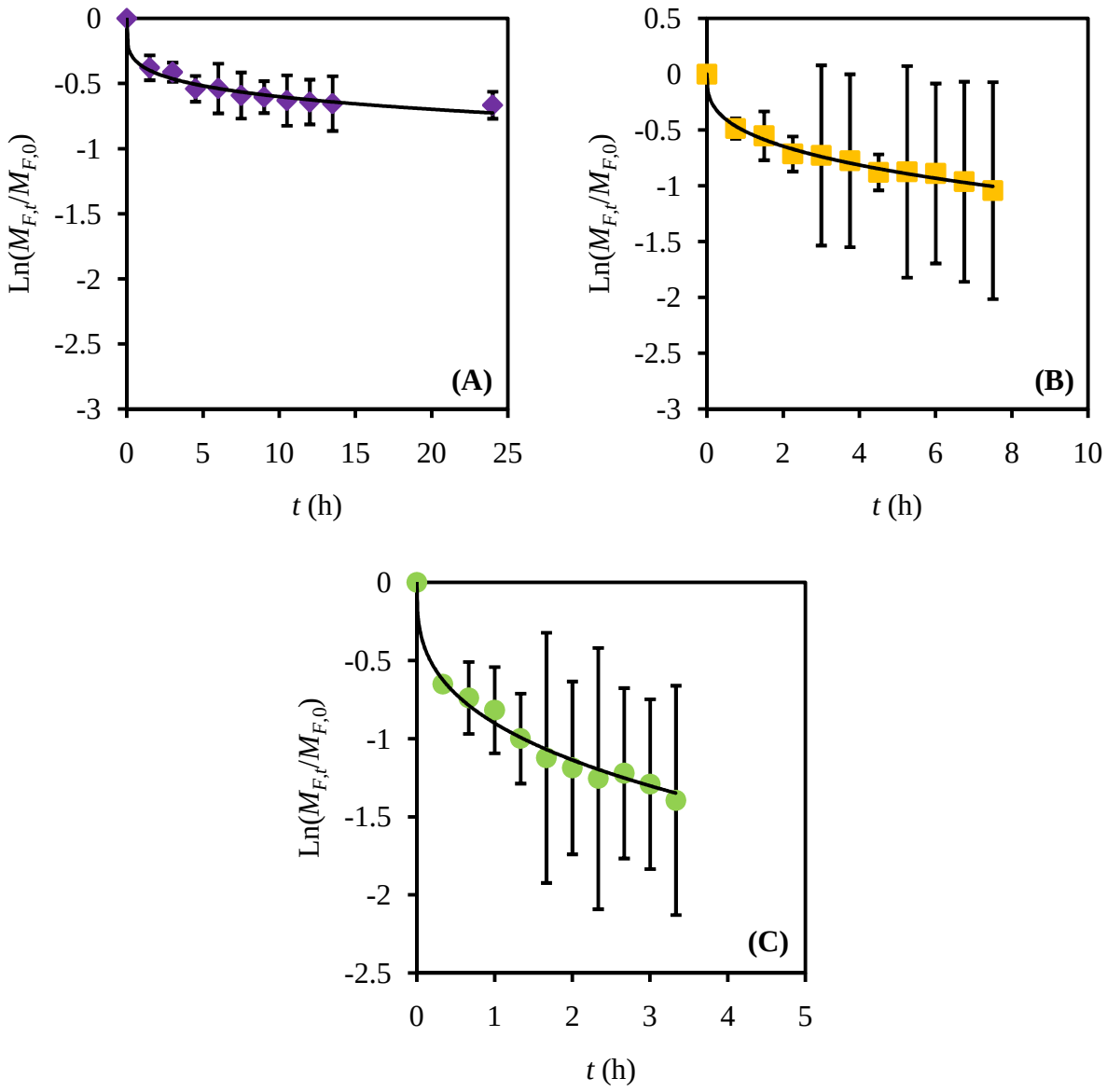

Figure 7 – Release kinetics of nisin at 5.6 (A), 22 (B), and 40 ºC (C), showing the observed data at 5.6 (♦), 22 (■), and 40 ºC (●), fitted with the Weibull's model (—). Observed data are means of at least 4 replicates (R^2 and RMSE values at 5.6, 22, and 40 ºC were, respectively: 0.976 and 0.030, 0.990 and 0.028, and 0.989 and 0.042).

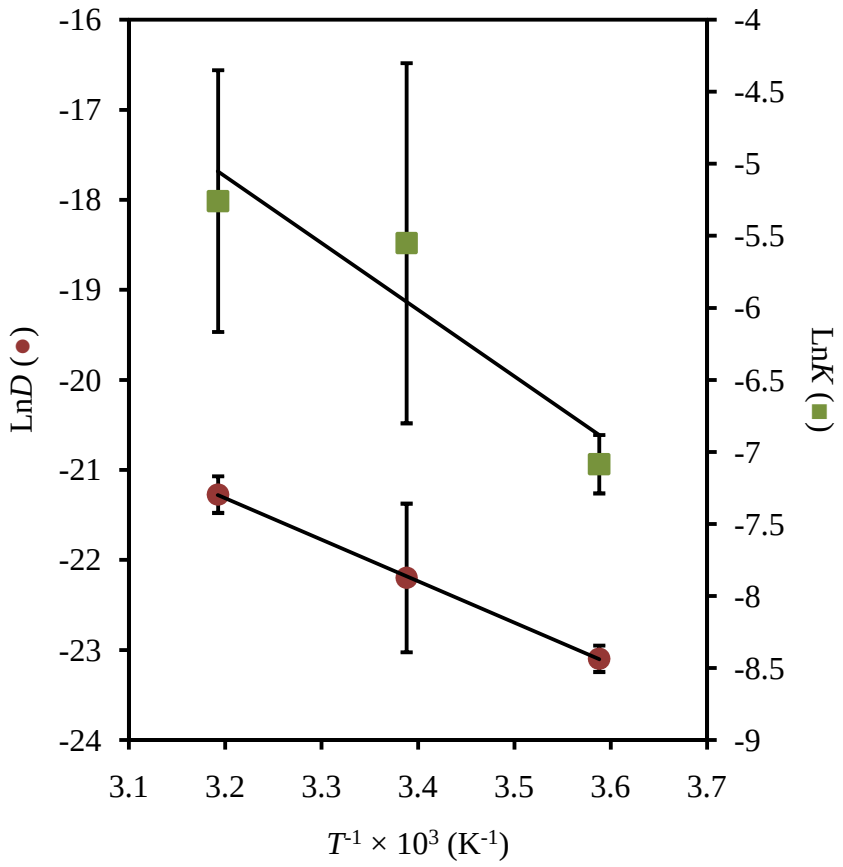

Figure 8 – Arrhenius plot for *D* (●) and *K* (■), fitted with their corresponding Arrhenius equations (—) (R^2 values for *D* and *K* were, respectively: 1 and 0.871).

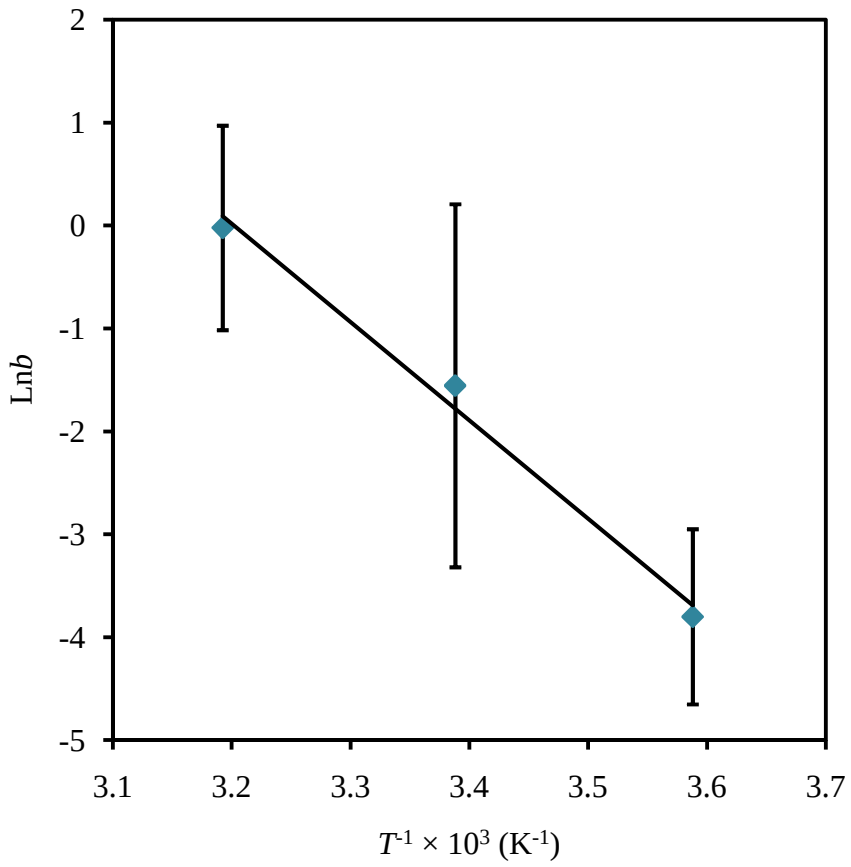

Figure 9 – Arrhenius plot for the scale parameter *b* (♦), fitted with the Arrhenius equation (—) (R^2 = 0.956).

CHAPTER 4

Summary and recommendations for future work

Nisin incorporated PBAT film was able to inhibit *Listeria innocua* in agar media. Some of the properties of PBAT films were affected after the addition of nisin. The elastic modulus and the tensile strength (E and σ_s, respectively) were significantly altered after the incorporation of nisin, as well as crystallization temperature (T_c), the enthalpy of crystallization (ΔH_c), the melting enthalpy (ΔH_m), and the crystallinity (χ). On the other hand, there was not statistical evidence of change in the gas barrier properties (oxygen permeability and water vapor permeability), the elongation at break (ε_b), the glass transition temperature (T_g), and the melting temperature (T_m). The Environmental Scanning Electron Microscpy (ESEM) exhibited formation of pinholes in the nisin containing PBAT films, and the *X*-Ray diffraction patterns confirmed the decrease in χ.

The release of the antimicrobial substance nisin from amorphous PBAT films occurred at a fast rate compared to what has been observed in previous works. The diffusion of nisin through PBAT films was described using Fick's law and Weibull equation. The temperature dependence of the diffusion coefficient, the partition coefficient and the scale parameter of the Weibull model agrees modeled using the Arrhenius equation.

Based on the results obtained, the following recommendations can be made for future work:

1. Evaluate the effectiveness of nisin incorporated PBAT films with the selected food groups in terms of potential increase in the shelf life and quality changes in the foods. Some food products might be more suitable for this kind of polymeric structure

incorporated with nisin. Animal origin products could be good candidates for evaluation of nisin incorporated PBAT films.

2. Fabricate PBAT films incorporated with other antimicrobials. Evaluate its effectiveness with selected pathogenic and spoilage microorganisms, and quantify possible changes in tensile, thermal and barrier properties. Among the antimicrobials that could be incorporated in PBAT are natamycin, other bacteriocins, sorbates, propionates, and so on. This could diversify the applications of PBAT for food packaging.
3. Develop PBAT films with antimicrobials using commercial method of manufacturing films such as extrusion. The extrusion method of fabrication may influence tensile, thermal and gas barrier properties and the effectiveness of antimicrobials in a different way in comparison to solution casting, and so on.
4. Explore ways to improve the tensile, thermal, and barrier properties of PBAT films with nisin and other antimicrobials. As it was confirmed in the present study, some properties may be negatively affected after the incorporation of antimicrobials, which makes it necessary to find ways to maintain the original characteristics, either by modifying the production techniques or by incorporating additives that could help overcome those drawbacks.
5. Explore the possibility of incorporated antimicrobial PBAT film in a multilayer structure and evaluate the resulting properties and effectiveness against selected microorganisms and foods. This study showed that the release of nisin from PBAT takes place rapidly. PBAT could be a good option to be part of a multilayer system as a reservoir layer, so that the release of nisin can take place in a slower rate. On the other hand, different food products may require certain rates of nisin release (due to their particular shelf-life), so

that different multilayer systems could be tested in different food products to identify the most suitable for each one.