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IMPROVEMENT OF ACTIVE PACKAGING MATERIALS
BASED ON POLY(LACTIC ACID) CARRYING
ENCAPSULATED ANTIMICROBIAL VOLATILES

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IMPROVEMENT OF ACTIVE PACKAGING MATERIALS BASED ON POLY(LACTIC ACID) CARRYING ENCAPSULATED ANTIMICROBIAL VOLATILES

By

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ABSTRACT

IMPROVEMENT OF ACTIVE PACKAGING MATERIALS BASED ON POLY(LACTIC ACID) CARRYING ENCAPSULATED ANTIMICROBIAL VOLATILES

By

MinJung Joo

Bio-based blends made of poly(lactic acid) (PLA), an aliphatic thermoplastic polyester, and β-cyclodextrins (β-CDs), an enzymatically modified starch, are stiff and brittle due to the incompatibility which limits their applications. The same limitations are expected for bio-based antimicrobial materials created by blending PLA with inclusion complexes (ICs) which serve as a carrier for natural antimicrobial volatile, trans-2-hexenal based on β-CDs. The objective of this study was to overcome limitations by enhancing the compatibility of the carrier with PLS by using a masterbatch. The study was divided into two phases. In phase one, the interfacial adhesion of PLA and β-CDs at various ratios was investigated and the effectiveness of using a masterbatch to improve the adhesion was studied. In phase two, the materbatch was used to develop an antimicrobial material based on a PLA/ICs blend carrying trans-2-hexenal. The use of the masterbatch significantly enhanced the compatibility between PLA and β-CDs, and improved the thermal, mechanical, optical, and barrier properties of the blends. The antimicrobial PLA/ICs-trans-2-hexenal blend has been shown to be effective against *Alternaria Solani*. The exposure of the ICs to high heat and relative humidity during processing caused a premature loss of the antimicrobial compound encapsulated in the β-CD molecules for later release, and resulted in a reduced antimicrobial activity.

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ABBREVIATIONS

ASTM	American Society of Testing and Materials
ANOVA	univariate analysis of variance
β-CDs	β-cyclodextrins
BOPP	biaxially oriented poly(propylene)
CD	cyclodextrin
CDs	cyclodextrins
DMA	dynamic mechanical analysis
DSC	differential scanning calorimeter
EVA	ethylene vinyl acetate
GFSE	grapefruit seed extract
HDPE	high density poly(ethylene)
HSD	honestly significant difference
ICs	inclusion complexes
LDPE	low density poly(ethylene)
LLDPE	linear low density poly(ethylene)
MB	masterbatch
OTR	oxygen transmission rate
PE	poly(ethylene)
PET	poly(ethylene terephthalate)
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolide)

PP	poly(propylene)
PS	poly(styrene)
PVC	poly(vinyl chloride)
PVOH	poly(vinyl alcohol)
RH	relative humidity
SEM	scanning electron microscopy
TGA	thermogravimetric analysis
TPS	thermoplastic starch
WVPC	water vapor permeability coefficient
WVTR	water vapor transmission rate

CHAPTER 1. INTRODUCTION

1.1 Introduction and motivation

Active packaging is a technology that promotes product quality and safety by adding specific compounds to the package. Many different types of active packaging have been proposed and these include the control of gases (oxygen, carbon dioxide, ethylene, etc.), moisture, and microbial contamination [1].

Antimicrobial packaging is a type of active packaging that retards/inhibits mold or bacteria growth, generally by contacting or releasing active compounds with antimicrobial properties [2]. Since the use of antimicrobial packaging offers some advantages compared with the direct addition of antimicrobial agents to food products as the preservative agents are applied to the packaging material in a way than only low levels of preservative come into contact with the food [3], the food and packaging industry has shown a growing interest in antimicrobial packaging in the last years [4]. The number of recently published articles and patents suggest that research on the incorporation of antimicrobials into packaging for food applications has more than doubled in the past 5 years [2]. There are many ways to make packages antimicrobial, but just two basic mechanisms: (1) placing the antimicrobial compound inside the package along with the product to be packed (e.g.: sachets and labels), and (2) making the antimicrobial compound part of the materials that form the package itself. The latter can be achieved by entrapping antimicrobial agents into the polymeric matrix, or by polymer surface modification, or by coating [5].

Thermal processing methods such as extrusion can be used to incorporate antimicrobials into polymers [2]. The choice of the antimicrobial is often limited by the incompatibility of the component with the packaging material or by the heat liability of the component during extrusion [6-7]. For example, thermally unstable antimicrobials such as volatiles cannot be directly extruded. They need to be processed in a protected state.

Microencapsulation is a relatively new technology that is used for protection, stabilization and slow release of food ingredients. The encapsulating material generally consist of starch derivatives, proteins, gums, lipids or any combination thereof [8]. Cyclodextrins (CDs) are cyclic oligosaccharides produced by enzymatic fermentation of starch that have successfully been used for pharmaceutical formulation and delivery [9-10]. The most notable feature of cyclodextrins is their ability to form inclusion complexes (host-guest complexes) (ICs) in solution with a very wide range of solid, liquid and gaseous compounds by a phenomenon of molecular complexation. Among the different types of CDs, β-cyclodextrins (β-CDs) are the most accessible, the lowest priced and generally useful. [9]. β-CDs have been used for encapsulation and controlled release of naturally occurring volatile compounds such as hexanal, and acetaldehyde in order to reduce fungal growth [11-12]. In this study, β-CDs have been chosen to develop antimicrobial packaging materials due to their capability to: (1) carry antimicrobial volatiles by forming ICs, (2) release these depending on the surrounding conditions (temperature and relative humidity) [13], and (3) protect the antimicrobial volatile from the high temperatures achieved during polymer processing.

Antimicrobial compounds have been incorporated into paper, thermoplastics and thermosets, and have been tested against a wide variety of bacteria and molds [2]. Most

of these studies have been done using commercial petroleum-based polymeric matrixes such as linear low density poly(ethylene) (LLDPE), ethylene vinyl acetate (EVA), nylon, etc. Bio-based polymers have the potential to become viable alternatives to the petroleum-based ones for active packaging applications. Bio-based polymers contain raw materials originating from agricultural and marine sources and they can be directly extracted from biomass like starch, cellulose, or synthesized from bio-derived monomers, or produced directly by natural or genetically modified microorganisms [14]. Bio-based polymers with antimicrobial activity have been developed by using chitosan, nisin, starch, etc [15-17]. Poly(lactic acid), PLA, is a biocompatible and biodegradable semi-crystalline polyester with promising further developments because its mechanical and barrier properties are comparable to those of conventional polymers [18-19]. In this study, ICs based on β-CDs carrying trans-2-hexenal, a naturally occurring antimicrobial volatile found in plant tissue and products, have been blended with PLA to develop a bio-based antimicrobial polymeric material. The antimicrobial capacity is based on the release of the antimicrobial volatile in function of temperature and relative humidity during storage. In the development of polymer blends, polymer compatibility is an important factor since this governs the morphology of the blends and therefore, affects the mechanical and physical properties of these [20]. Blending starch with PLA reduces the cost and increases the biodegradability of the blend, however, the mechanical strength of the blend is reduced due to the poor interfacial adhesion between materials [18]. The resulting blend is brittle and rigid, both major drawbacks in many fields of application. Similar results were expected for blend of CDs and PLA. In this study, the interfacial adhesion of PLA and β-CDs at different weight ratios was investigated and the approach of using a

masterbatch (PLA with high β-CD content) to improve the adhesion was studied. In addition, it was studied whether the exposure to relative humidity and to high heat during processing causes a premature loss of the antimicrobial compounds encapsulated in the β-CD molecules for later release, as this would reduce the antimicrobial properties of the active material.

1.2 Objectives

The main objective of this study was to improve active packaging materials based on poly(lactic acid) carrying encapsulated antimicrobial volatiles by enhancing the dispersion of the active compound and the compatibility between phases.

Specific objectives were to:

- characterize the effect of the β-CDs content on the mechanical, thermal, barrier, morphological, and optical properties of the PLA/β-CDs blends,
- study the effect of the masterbatch on the mechanical, thermal, barrier, morphological, and optical properties of blends made from PLA and β-CDs,
- reduce the stiffness and brittleness of the polymer material,
- improve the dispersion of the ICs,
- develop an antimicrobial polymer material based on bio-based materials, and to verify its antimicrobial capacity,
- study the effects of processing on the antimicrobial activity of the films, and to quantify this,
- enhance the commercial applicability of this new polymeric material.

CHAPTER 2. LITERATURE REVIEW

2.1 Active packaging

There is a need of new food conservation technologies to ensure quality and safety of packaged food. Modified atmosphere packaging (MAP), which is the most commonly used packaging technology to prolong food shelf life by surrounding the food product with and optimal atmosphere, can be adversely affected by changes in temperature and relative humidity throughout the cold chain. These changes can negatively affect the quality and safety of the packaged food. Active packaging transforms the concept of packaging from being a mere container or 'passive package' to an 'active package' that plays an active role in maintaining or even improving the quality of the packed product [1, 21]. Active packaging can be described as a new food conservation technology based on the interactions within a food/package/environment system to improve the quality and safety of the packaged food and to increase its shelf life [1, 5].

2.1.1 Active packaging market

Active packaging technologies have received a great deal of attention during the last decade. Changes in retail and distribution practices such as centralization of activities, new trends (e.g. internet-shopping), and globalization of markets, all resulting in increased distribution distances and longer storage times of different products with different temperature requirements, are putting huge demands on the packaging industry.

In addition, consumers are increasingly demanding mildly preserved convenience foods that have better fresh-like qualities [22-23]. Thus, active packaging is gaining interest from researchers and industry due to its potential to satisfy these needs. The number of packaging products that are developed and used in commercial applications is growing. Active packaging comprised approximately 27% of the global market in 2008 [24]. In 2010, the US market for "active, controlled and smart" packaging for food and beverages is an estimated $38 billion and is expected to surpass $54 billion by 2015 [25].

2.1.2 Types of active packaging

A variety of active packaging technologies have been developed to provide better quality, wholesome and safe foods, and also to limit package related environmental pollution and disposal problems [26]. Different types of active packaging include oxygen scavengers, carbon dioxide emitters/absorbers, moisture regulators, ethylene absorbers, ethanol emitters, flavor releasing/absorbing systems, as well as antioxidant release, antimicrobial containing, and gas permeable/breathable films [21-22, 24, 26].

2.2 Antimicrobial packaging

Antimicrobial packaging is a system that can kill or inhibit the growth of micro-organisms to extend the shelf life and to enhance the safety of packed products [3, 27].. Research on antimicrobial packaging started with the development of packaging materials containing chemicals with antimicrobial capacity in their macromolecular

structures using common packaging materials [27]. In recent years, a lot of research is being devoted to the development of different types of antimicrobial delivery systems and packaging material-product combinations to maximize the efficacy of the system [28].

2.2.1 Antimicrobials

There is a wide variety of substances with antimicrobial activity like organic acids, spice extracts, enzymes, proteins, antibiotics and metals [29]. They can be classified into three groups: chemical agents, natural agents, and probiotics [30]. Chemical agents are the most common substances used in the industry and some examples of these include organic aids, and alcohols [27]. They offer additional benefits in preventing post-process contamination and in strengthening inhibitory effects against microorganisms. Natural agents are mostly extracted from plant and animal sources and they have been proposed and/or tested for antimicrobial activity in packaging [31]. Probiotics are naturally produced antimicrobials that can inhibit the growth of other bacteria. They can effectively control competitive undesirable microorganisms [30]. The effectiveness of antimicrobial agents can differ as follows [30]:

Microbiocidal effect

The antimicrobial agent is expected to kill the target spoilage and pathogenic microorganism. This type of antimicrobial agent can eliminate micro-organisms from a food/packaging system. The specific nomenclature is bactericidal and fungicidal for those that kill bacteria and fungi, respectively.

Microbiostatic effect

The antimicrobial agent, applied above a certain critical concentration, can inhibit the growth of microorganisms. However, at concentrations below the critical one, or if the agent is removed from the packaging systems through a seal defect, leakage, opening or any other means, the suppressed microorganisms can grow or their spores germinate. The specific nomenclature is bacteriostatic and fungistatic for those that kill bacteria and fungi, respectively.

2.2.1.1 Naturally occurring antimicrobials

Natural antimicrobial compounds have been used for centuries in food preservation. Research concerning plant-origin compounds has increased since the 1990s [32]. The mechanism of natural antimicrobials is to interrupt metabolic pathways in the cell wall membrane/structure. For example, nisin interacts with the sulfur containing compounds in the bacterial membrane, disrupting their function as a semipermeable membrane and causing lysing of the cells. Antimicrobials also can reduce the water activity within the cell. Since water is a key compound in the metabolism of a cell, any disruption of the fluid flow causes growth suppression [31].

2.2.1.1.1 Trans-2-hexenal

Trans-2-hexenal (Figure 1) is a naturally occurring compound of plant origin that plays an important role in the aroma of many fruits and vegetables including pome fruit, stone fruit, strawberries, grapes and vegetables [33-34].

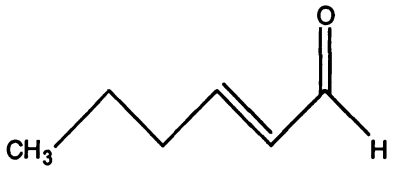

Figure 1. Chemical structure of trans-2-hexenal [35]

The antimicrobial effectiveness of trans-2-hexenal has been widely studied *in vitro* [36-39]. The antifungal activity of trans-2-hexenal vapor *in vivo* has been confirmed against *Pichia subpelliculosa* in sliced apples [40], *Penicillium expansum* in pears and apples [37, 41-42] *Botrytis cinerea* in strawberries [38], and *Escherichia coli, Salmonella enteritidis* and *Listeria monocytogenes* in sliced apples [43].

Table 1 summarizes the main characteristics of the compound trans-2-hexenal. The vapor pressure of trans-2-hexenal is 10mmHg at 20°C (www.sigmaaldrich.com) and the antimicrobial activity of Trans-2-hexenal may be due to its high reactivity and volatility [37]. The precise mechanism of the protective action of trans-2-hexenal is not clear yet, but likely they permeate by passive diffusion across the plasma membrane, and once inside the cell, the α, β-unsaturated aldehyde moiety reacts with biologically important nucleophilic groups which play a key role in living cells [44]. The combination of its antimicrobial capacity and the fact that it is approved as food additive permitted for direct addition to food for human consumption by the US Food and Drug Administration [45] makes trans-2-hexenal particularly interesting as an antimicrobial compound for food

applications. According to US Food and Drug Administration, toxic LD_{50} of trans-2-hexenal is 780 mg/kg [46].

Table 1. Main characteristics of trans-2-hexenal [35]

Linear structure	$C_6H_{10}O$
Boiling point	146-149°C
Flash point	35°C
Density	0.846g/mL at 25°C
Molecular weight	98.14 g/mol

2.2.2 Antimicrobial polymers

Antimicrobial polymers are one of the most promising concepts for active packaging. Antimicrobial polymers are mainly classified into the following types: (1) those where the antimicrobial agent is directly incorporated into the packaging material and (2) coatings which act as a carrier for the antimicrobial agent. The antimicrobial agent is released from the polymer into the product to inhibit microbial growth [47]. Some antimicrobial agents can migrate from the surface of the package material to the product and others are effective without migration of the active agents to the product [2, 26, 31]. Sachets and pads are the main trend to deliver antimicrobials due to their high effectiveness in suppressing surface microbial growth [26]. However, they are not broadly accepted by consumers due to fears of ingestion by children and accidental consumption with package contents. [2]. The development and use of antimicrobial polymers in the form of thin films is expected to increase in the next decade [26].There are promising alternatives such as immobilization of antimicrobials by ion or covalent

linkages to polymers and polymers that are inherently antimicrobial polymers are more effective than the direct addition of the antimicrobial agent since they slow the migration of the agents away from the surface, thus helping to maintain high concentrations where these are needed [48].

2.2.2.1 Antimicrobial polymer market

Research in the use of antimicrobial packaging materials as an alternative method for controlling microbial contamination has significantly increased during the last few years [3, 49]. The global market demand for specialty antimicrobials in plastics was $130 million at manufacturing level and $231 million at end-user level in 2001. The overall growth of the industry was around 3.5-4% per year. The expected growth of the global plastic antimicrobial industry was 4% per year up to 2005 [50]. In 2006, Japan was the leader in the commercialization of antimicrobial films. The commercialization of these films was limited outside of Asia because of their strong odor due to the allylisothiocyanate content [51].

2.2.2.2 Antimicrobial polymers and processing

Polymer processing can be defined as a group of operations carried out on polymeric materials to shape them into useful products [52]. Classic polymer technological processes (such as extrusion) are generally used to process antimicrobial polymers [53]. Thermally stable antimicrobials such as silver substituted zeolites are used with thermal

processing methods such as extrusion and injection molding [2]. However, other antimicrobial compounds are thermally instable and therefore, these active agents may partially or completely be lost, or may partially or completely lose its antimicrobial activity if incorporated into the polymeric matrix due to the high pressure and temperature conditions in the extruder [7, 53]. In addition, the antimicrobial activity of an incorporated active substance in a polymeric matrix may deteriorate during converting, storage, and/or distribution of the active packaging material. The residual antimicrobial activity is defined as the effective activity of the antimicrobial agent after the casting (extrusion or coating) and the converting processes [31]. Besides chemical degradation, the loss of the antimicrobial substances by volatilization is another reason for antimicrobial activity reduction during casting and storage [7]. For example, the volatility of antimicrobial compounds like naturally occurring plant volatiles cases them to be lost when exposed to high temperatures. The treatment of the polymer or the derivatization of active agents prior to their addition to the polymer may be necessary to increase the compatibility between the active agent and the polymer [26].

2.2.2.2.1 Extrusion

Extrusion is the process of shaping a molten polymeric material by forcing it though a die [54] using a piece of equipment called an extruder. The extruder takes the raw material and compresses, heats, melts, and conveys it [55]. The parts of an extruder are hopper, screw, barrel, and die. The screw performs a number of functions such as conveying, melting and mixing. L/D ratio indicates the relative length of the screw and 24:1 is the

most common value. This could go up to 32:1 for better mixing or more output. Depending on the number of screws, extruders are divided into single, and twin screw extruders [54]. The barrel is the reservoir that hosts the extruder. The barrel of any extruder has three sections: feed, compression, and metering. The feed section is the first part of the barrel that conveys the pellets and where the melting process starts; the compression section is where the material gets fully melted and mixed, and the metering section is the area where the molten material is conveyed. The die is the part of the extruder where the molten material is shaped and this varies in size, shape and format [55].

Most plastic resins are processed through the extrusion process. This process is accountable for significant quantities of plastic products, such as films, sheets and profiles, and it is also used to produce the plastic pellets that are later used by other plastics manufacturing processes [56].

There are different types of extrusion. Blow and cast extrusion are the most commonly used to process sheets and films. The major difference between blown and cast films is the method by which the film is formed, which is dictated by the shape of the die. [56]. Blow film extrusion involves extruding a polymer through a ring-shaped die, through either the bottom or the side. The melt is forced through the die opening and forms a comparatively thick-walled tube [56]. Cast extrusion is widely used to produce polymer films. In cast extrusion, a molten polymer is extruded through a flat die, then stretched in air and cooled on a chill roll [57]. Cast extrusion allows a polymer to be manufactured into a film with better tolerance control than can be achieved in blow film extrusion.

2.2.2.2.1.1 Factors affecting extrusion

The control of extrusion factors such as temperature, time, and pressure results in a higher-quality, and a more efficient extrusion of the plastic material. Temperature is a difficult factor to control because of the various heating elements throughout the extruder. Time is also critical since this parameter determines the effect of processing on the resulting extrudate [58]. Extrusion conditions can be tailored to produce extrudates with the desired properties [59].

2.2.2.3 Antimicrobial polymers and materials

2.2.2.3.1 Petroleum-based antimicrobial polymers

Most antimicrobial films have been reported in the literature as made from synthetic polymers such as poly(ethylene) (PE), low density poly(ethylene) (LDPE), ethylene vinyl acetate/linear low density poly(ethylene) (EVA/LLDPE), poly(vinyl chloride) (PVC), poly(vinyl alcohol) (PVOH), nylon, or biaxially oriented poly(propylene) (BOPP) [60]. Most research has been carried out with LDPE films with incorporated antimicrobial components such as silver-substituted zeolite [61], imazalil [62], sorbate [7], nisin [63], chitosan [47], grapefruit seed extract (GFSE) [64-65], poly(propylene) (PP) with essential oils [66], poly(vinyl alcohol) (PVOH) with iysozyme [67], and poly(styrene) (PS), and poly(ethylene terephthalate) (PET) with sodium benzoate [29].

2.2.2.3.2 Bio-based antimicrobial polymers

Bio-based polymers are defined as polymers that contains natural raw materials which could be extracted directly from natural raw materials such as starch, and protein, or produced by chemical synthesis from bioderived monomers, or produced by micro-organisms [68]. The desire for natural ingredients and the realization that plants harbor compounds of an antimicrobial nature has led to the production of a number of films with extracts from plants [60]. Currently, incorporating food preservatives [69] and natural antimicrobial agents into bio-based polymers is becoming a popular area of packaging research [31]. Bio-based polymers are being widely studied as edible coatings or film materials. Antimicrobial polymers have been developed by blending polylactide-co-polycaprolactone with encapsulated allyl isothiocyanate [70], and by blending l-polylactide and l-polylactide-polycaprolactone with nanoclays [71]. Other bio-based materials with a potential for developing antimicrobial polymers are chitosan [15] and starch [72]

2.2.2.3.2.1 Poly(lactic acid), PLA

Poly(lactic acid), PLA (Figure 2), is a linear aliphatic thermoplastic polyester obtained from the fermentation of sugar feed stocks [73] and produced by controlled depolymerization of the lactic acid monomer [74]. PLA is biodegradable and compostable. It breaks down to biomass and CO_2 and water in a given time period and environment under the action of biological enzymes [75]. The use of PLA may be a

promising way to reduce the solid waste disposal problem [76-77] so it is attractive as plastic substitute.

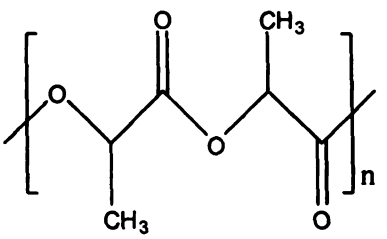

Figure 2. Chemical structure of PLA [76]

The stereochemistry of PLA is complex because of the chiral nature of the lactic acid monomer. The L/D ratio of the lactic acid unit varies PLA properties such as melting temperature and crystallinity [18, 74].

PLA has comparable mechanical and barrier performance to that of some petroleum-based polyester. It has also a good processability with good shaping and molding capability which are desirable for a number of applications [18, 78]. The use of PLA has been limited because of its high cost, and low availability [78], however, the increase of the oil price and the implementation of environmental policies such as green tax might expand the use of this polymer [78]. Main drawbacks of PLA are rigidity, brittleness, and a low glass transition temperature [18, 79].

2.2.2.3.2.1.1. PLA blends and masterbatches

Blending PLA with other polymers (biodegradable and non-biodegradable) or with additives is probably the most extensively used methodology to improve PLA mechanical

properties such as brittleness [80]. Polymers blends are produced when the physical, chemical, or rheological properties required for processing or for application cannot be achieved using a single polymer. Blends are also produced to reduce cost. The blending of polymers is a complicated process due to possible incompatibilities between them.

Starch has been used as filler for environmentally friendly plastics because it is inexpensive and renewable [81]. PLA has been blended with starch mostly because PLA is still more expensive than conventional plastics and the degradation rate of PLA is not completely satisfactory in some instances [82]. In previous studies, as a low-cost alternative, native starch has been added to PLA with poly(ethylene glycol) [83] and thermoplastic starch to PLA with glycerol [81] to create a low-cost alternative material without sacrificing mechanical properties. Starches with different amylase levels [82], moisture contents [83] and ratios [79] have been blended with PLA to investigate the water sensitivity of PLA-starch blends. Numerous studies have been done on the influence of plasticizers [84] and compatabilizers [85] on the thermal, mechanical and morphological properties of PLA.

A masterbatch can be used to improve the blending process. A thermoplastic masterbatch is a complex blend based on a thermoplastic resin containing a high amount of additive which is then blended with a polymer to improve its properties [86]. The addition of a masterbatch to the PLA has been shown to improve the mechanical properties of the blend by reducing the brittleness, and increasing the impact strength [87]. Investigations of the effect of a masterbatch on the PLA properties are relatively scarce, and further studies on the influence of the masterbatch on the PLA processing conditions and others are necessary.

2.2.2.3.2.2 Cyclodrextrins

Starch is a renewable, degradable, carbohydrate biopolymer derived from different sources like rice, wheat, corn, cassava and potatoes. All these properties allow it to be considered as a possible substitute for petroleum-based plastics [79]. Starch has been blended with petroleum-/bio-based polymers to increase their biodegradability, and to reduce the usage of the petroleum-based or more expensive bio-based polymer. Blends made from PLA-starch [88], and LDPE-starch [89] have been developed and characterized.

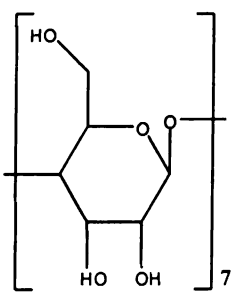

Figure 3. Chemical structure of β-cyclodextrin [90]

Table 2. Physicochemical characteristics of cyclodextrins [91]

Attribute	α -cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Glucopyranose (units)	6	7	8
Molecular weight (g/mol)	972	1135	1297
Solubility H_2O (g/100mL)	14.5	1.85	23.2
Cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
Cavity volume $(Å)^3$	174	262	427

Cyclodextrins, CDs (Figure 3), produced by fermentation of starch, are cyclic, non-reducing oligosaccharides built up from six, seven, or eight glucopyranose units. β-cyclodextrin, β-CDs (Table 2), the most important and most readily accessible member of this group, is comprised of seven glucopyranose units [92].

CDs are typical "host" molecules that can trap a great variety of molecules that have the size of one or two benzene rings, or a side chain of comparable size in the case of even larger compounds, to form crystalline inclusion complexes [92]. Water molecules filling the CD cavity are replaced by the "guest" when this is less polar than water and its size and shape are compatible with that of the host. As long as the complexes do not dissociate in a highly diluted aqueous solution, the enclosed molecules are protected [92].

2.2.2.3.2.2.1 Micro-encapsulation

Microencapsulation is a process by which a substance or a mixture is coated or entrapped in another material [93]. There is a lot of research done in a wide range of cores (encapsulatants), wall-forming materials (encapsulating agents), and technologies for controlling the interaction of ingredients for manufacturing microcapsules and microparticles of different size and shape [94]. The retention of flavor compounds is governed by factors related to the chemical nature of the core, including its molecular weight, chemical functionality, polarity and relative volatility, to the wall material properties, and to the nature and the parameters of the encapsulation technology [95]. Inclusion encapsulation generally refers to the supra-molecular association of a ligand (the 'encapsulated' ingredient) into a cavity-bearing substrate ('shell' material) by an

hydrophobic effect [96]. Different aroma compounds have been encapsulated into CDs [93, 97]. Some encapsulated aroma compounds have been shown to prevent or inhibit fungal growth [13].

2.2.2.3.2.2.2 Controlled release

The European Directive 3AQ19a defines controlled release as a "modification of the rate or place at which an active substance is released [94]. The aim of controlled release systems is to transfer the antimicrobial agent to the packed food at a specific rate in order to maintain a predetermined concentration of the active compound in the release medium for a predetermined period of time [98]. For matrix systems encapsulating volatile compounds, the release depends on several mutually dependent processes, such as diffusion of the volatile compound through the matrix, type and geometry of the particle, transfer from the matrix to the environment and degradation/dissolution of the matrix material [99]. CDs are used to provide a controlled release of volatiles over the last years. The release can be affected by the surrounding relative humidity and temperature.

PLA has been investigated as a biodegradable polymeric device for controlled drug release /delivery. Drug release from PLA and poly(lactic-co-glycolide) (PLGA) microspheres has been examined at various temperatures in order to clarify the effect of temperature on mechanisms drug release [100-101]. PLA blends such as PLA-poly(ethylene glycol) has been also investigated as a biodegradable drug delivery system [102].

CHAPTER 3. PREPARATION AND CHARACTERIZATION OF BLENDS MADE OF POLY(LACTIC ACID) AND AN ENZYMATICALLY MODIFIED STARCH WITH A CAPACITY TO CARRY HYDROPHOBIC MOLECULES: IMPROVEMENT OF THE BLEND PROPERTIES BY USING A MASTERBATCH*

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ABSTRACT: Cyclodextrins (CDs) are enzymatically modified starch with a hydrophilic exterior and a hydrophobic cavity, which enables them to form inclusion complexes with a variety of hydrophobic molecules. The incorporation of CDs in a polymer matrix enables new applications for polymers in the food and pharmaceutical packaging area. In this study, β-CDs, one of the most common CDs, were mixed with poly(lactic acid) (PLA) with the goal of creating a biodegradable and bio-based polymer with the capacity to carry hydrophobic molecules. The materials were extruded, pelletized, and cast into sheets. The effect of different β-CD contents (0, 15, or 30%), and of the use of a masterbatch (MB) on the morphology and the physical properties of the PLA/CDs blends was investigated. The MB consisted of pellets containing 30% β-CD content and was used in an attempt to improve the dispersion of the β-CDs into the PLA matrix. Testing of mechanical, thermal, and barrier properties revealed that β-CDs and PLA are incompatible. Thermal stability, tensile strength, modulus of elasticity, elongation at break, and oxygen and water-barrier capacity of the PLA decreased with the β-CD content. In all cases, the decrease was notably reduced when the β-CDs were added as a

masterbatch and this showed that the use of the masterbatch increased the compatibility of the PLA and the β-CDs. Also the crystalline content and color change increased with the β-CD content but not in the blend obtained using the masterbatch. In SEM images the blends showed an uneven distribution of the β-CDs in the polymeric matrix.

KEYWORDS: Poly(lactic acid), β-cyclodextrins, masterbatch, preparation, characterization

INTRODUCTION

Public concern about the environment have stimulated interests in biodegradable polymers as alternatives to conventional polymers [79]. Poly(lactic acid) (PLA) is one of the more widely studied biodegradable polymers, because many of its properties are equivalent or superior to those of some petroleum-based plastics, which makes this polymer suitable for a variety of applications [80].

PLA is a linear aliphatic thermoplastic polyester derived from lactic acid and it is mainly synthesized from the commercial fermentation of sugar feedstock [73] which means it is a compostable polymer at specific conditions [103]. PLA has a transparent and glossy finish [104] and heat seal capacity [104]. PLA in its semicrystalline form is used in applications where high mechanical strength and toughness are required [87]. The CO_2, O_2, and N_2, permeability coefficients of PLA are reasonable: they are lower than those for poly(styrene) (PS), but higher than those for poly(ethylene terephthalate) (PET) [76]. PLA is suitable for a wide range of processing technologies including injection molding, blow molding, thermoforming, cast film and sheet, extrusion blow film, foaming, and

others [78]. The toxicity of PLA is low, which makes it an adequate material for food packaging applications [105]. All these properties make PLA more attractive than other bio-based materials as a biodegradable plastic substitute. However, PLA is more expensive than commonly used plastics [78]. PLA has a relatively low glass transition temperature and any further reduction thereof limits the application of the polymer further [106] and its impact strength and elongation at break are smaller than those of widely used polymers such as high density poly(ethylene), poly(propylene), and poly(styrene) [104]. Based on some of these properties, PLA offers vast potential for the development of biodegradable packaging systems that carry active compounds for food and pharmaceutical applications. While some aspects of the potential for active packaging have been explored [107], further research and development is needed.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of β-(1,4) linked glucopyranose subunits. They are produced by enzymatic degradation of starch by bacteria. CDs have a cage-like supramolecular structure that allows them to interact with molecules, ions, and radicals [9]. CDs also enable the controlled release of these molecules as shown by [11-13]. Therefore, the blending of this modified starch with other polymers might be a new approach to increase the number of food and pharmaceutical applications for these polymers.

Generally, CDs are used to synthesize copolymers with PLA for drug delivery or for improving the miscibility of PLA with others [108-109], however, information on blending this modified starch with PLA is scarce. In contrast, PLA/starch blends have been widely studied since blending starch with PLA is a promising approach [110]: starch is abundant and cheap, and PLA has good mechanical properties and can reduce

the sensitivity of starch to moisture [82, 111]. In addition, the resulting blend is biodegradable and is derived from renewable sources. However, hydrophobic PLA and hydrophilic starch are thermodynamically immiscible because of the hydroxyl and carboxyl end groups in the PLA and the numerous hydroxyl groups in the starch [18]. Starch remains in a separate conglomerate form in a PLA matrix [112]. Due to this weak interfacial adhesion between phases, the mechanical strength of PLA/starch blends is low and the material is weak and brittle [18, 79]. Numerous studies have been undertaken to improve the interfacial interactions of these blends through the use of compatibilizers and other additives [113-114].

Masterbatch is another way to improve properties in a polymer blend and it has been broadly used with polymers like polypropylene [115], poly(ethylene terephthalate) [116], and others. Four commercial masterbatches have been blended with PLA and some properties of PLA such as clarity and impact resistance have been improved by the addition of the masterbatch. However, the four masterbatches had a slight negative effect on the tensile strength of PLA [87]. PLA and poly(butylenes adipate-co-butylene terephthalate) has been blended by using masterbatches as muticompatibilizer to enhance compatibility [117].

In this study, β-CDs were mixed with PLA in order to create a bio-based polymer with a capacity of carrying hydrophobic molecules. Different blends were created with the objective to achieve specific properties that make these blends fit for a number of packaging applications. Due to the expected weak interfacial adhesion between phases (PLA and modified starch), a masterbatch was tested in an attempt to improve the dispersion of the β-CDs into the PLA matrix and thus to enhance the compatibility and

mechanical properties of the blend. The effect of the β-CD content (0, 15, or 30%), and the use of a masterbatch (MB) on the morphology and on the physical properties of the blends PLA-β-CDs has been investigated.

EXPERIMENTAL

Materials

Poly(lactic acid) 4050D (PLA 4050D) resin (95% L-lactide, Density=1.24g/cm^3) was kindly provided by Wilkinson Inc. (Calhoun, NE, US). β-cyclodextrin (β-CD) (purity >99%, Density=0.5g/cm^3, and Food grade) was purchased from Wacker Chemical Corporation (Adrian, MI, US).

Formulation and preparation of pellets

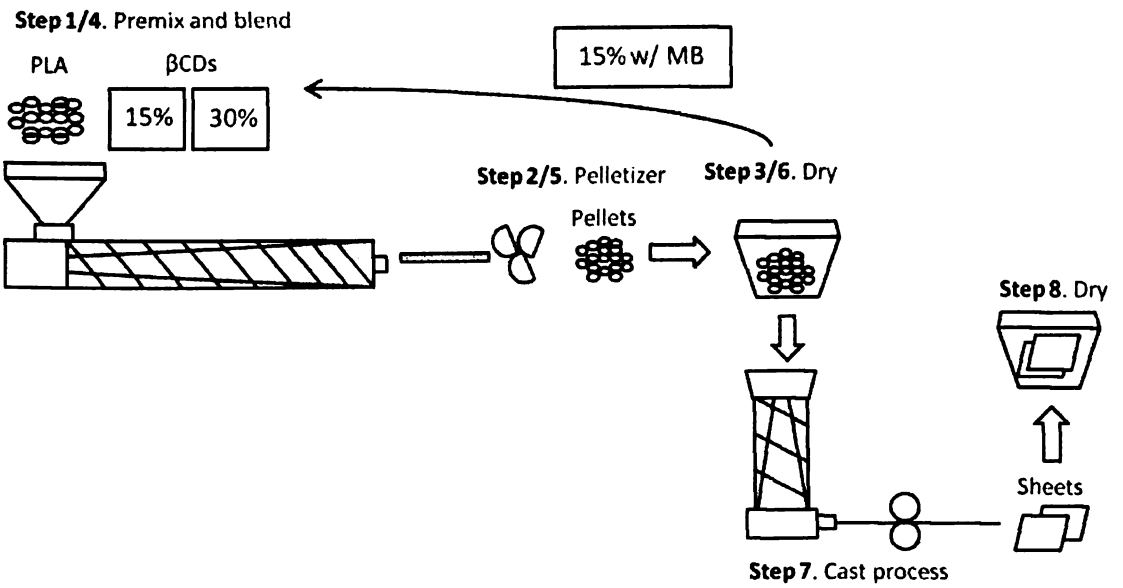

Figure 4. Preparation steps of the four different types of sheets: plain PLA (steps 1, 2, 3, 7, 8), PLA-β-CDs (steps 1, 2, 3, 7, 8), PLA-β-CDs-MB (steps 1, 2, 3, 4, 5, 6, 7, 8)

PLA 4050D resin and β-CDs were dried in a vacuum oven (1430 VMR International, Cornelius, OR, US) at 65°C for 4 and 24 hrs, respectively . Quantities of PLA resin and β-CDs according to the calculated compositions (Table 3) were premixed and subsequently blended (Figure 4. Step 1). The premix was extruded using a pilot scale extruder (Century extruders CX-30, Traverse city, MI, US) with 30 mm diameter twin screws with a L/D ratio of 42:1, and ten separate barrel zones temperature controlled with a water system and set from feeder to die to temperatures of 27 °C, 81 °C, 129 °C, 144 °C, 150 °C, 161 °C, 161 °C, 160 °C, 161 °C, and 161 °C. The screw speed was 100 rpm and the level of torque was controlled at 70~75%. The blend was collected from the die and allowed to cool down prior to being granulated into pellets using a pelletizer (Scheer bay Co. N. Euclid, Bay city, MI, US) ((Figure 4. Step 2). The pellets containing 0 (PLA), 15 (PLA-β-CD15%), and 30% (PLA-β-CD30%) CDs were stored until use in a desiccator at 23 °C (Figure 4. Step 3).

The masterbatch (MB) consisted of pellets containing 30% β-CD content. Quantities of PLA and MB pellets according to the calculated compositions (Table 3) were premixed and subsequently blended (Figure 4. Step 4) using the same equipment and processing as described above. The blend was collected from the die and allowed to cool down prior to being granulated into pellets using the same equipment as previously described (Figure 4. Step 5). The pellets containing 15% β-CDs (PLA-β-CD15%-MB) were stored until use in a desiccator at 23 °C (Figure 4. Step 6).

Table 3. Sample codes for neat PLA and PLA/β-CDs blends (produced with or without masterbatch (MB))

Sample Code	PLA (wt%)	β-CDs (wt%)	PLA-β-CD30% (wt%)	β-CD content (wt%)*
PLA	100	0	0	0
PLA-β-CD15%	85	15	0	15
PLA-β-CD30%	70	30	0	30
PLA-β-CD15%-MB	50	0	50	15

* nominal values

Preparation of sheets

All four different pellets (Table 3) were first dried in a vacuum oven prior to use to remove remaining moisture (Figure 4. Step 3/6). The pellets were converted into sheets by cold cast process (Figure 4. Step 7). A single screw microextruder (Randcastle RCP-0625 Microextruder, Randcastle extrusion system Inc., Cedar Grove, NJ, US) with a screw diameter of 15mm and a length to diameter ratio (L/D ratio) of 24:1 was used. The screw speed of the extruder was 35 rpm. The temperature profile of the extruder was controlled by a water cooling system and set from the feeder zone to the die zone at 149° C, 160° C, 177° C, 177° C and 177° C. Chill rolls temperature was 52° C. Ambient air was used to cool down the sheets on top of the roll. All samples were stored in a desiccator at 23° C to prevent moisture absorption (Figure 4. Step 8).

Analyses

Mechanical characterization

Neat PLA and PLA/β-CDs sheets were shaped into specimens with a width of 1 in and a length of 8 in prior to use. The thickness of the specimens (0.17 mm) was determined by averaging of three readings taken at different points. The tensile properties: tensile strength, elongation at break, and modulus of elasticity were measured according to ASTM D882-02 [118], using a Instron Universal Testing Machine (Model 5565, Instron, Norwood, MA, US) with a crosshead speed of 0.5 in/min, and a gage length of 5 in. Tensile properties of neat PLA and PLA/β-CDs blends were determined from an average of 10 samples.

Thermal characterization

Thermal transitions of neat PLA and PLA/β-CDs sheets were determined using a TA Instruments Q100 V 9.8 Differential Scanning Calorimeter (TA instruments, New Castle, DE, US). The temperature calibration of the equipment was performed in accordance with ASTM E967-03 [119] and the heat flow calibration was performed in accordance with ASTM E968-02 [120] Transition glass temperature (T_g), and melting temperature (T_m) were measured and calculated in accordance with ASTM D3418-03 [121]. The degree of crystallinity ($\%X_c$) for neat PLA and PLA/β-CDs sheets was calculated as follows [82]:

$$\%\chi_c = \left(\frac{\Delta H_m + \Delta H_c}{93 \times X_{PLA}} \right) \times 100$$

where ΔH_m, ΔH_c, and X_{PLA} are the melting enthalpy, crystallization enthalpy, and PLA content, respectively. A value of 93 J/g for the melting enthalpy for 100% crystalline PLA was obtained from the literature [122].

An amount between 7-9 g of PLA and PLA/β-CDs sheets was used for each experience. Samples were heated from 40 to 190 °C at a rate of 10 °C/min. The β-CD content was taken into consideration when determining $\%X_c$. Three replications of each type of sheet were tested.

Mass loss or gain due to the decomposition of the neat PLA and PLA/β-CDs sheets was measured using a thermogravimetric analyzer (Hi Res TGA 2950, TA instruments, New Castle, DE, US) under a nitrogen flow of 70 in/min. The samples were scanned with a constant heating rate of 10°C/min from 40°C to 600°C.

Dynamic mechanical analysis was used to characterize and to compare the viscoelastic nature of neat PLA and PLA/β-CDs sheets. Storage modulus (E'), loss modulus (E'') and damping coefficient (tanδ) of all materials were measured as a function of temperature in accordance to ASTM D4065-06 [123] by using a TA Instruments Model Q800 dynamic mechanical analyzer equipped with tension clamps and operating at a heating rate of 3°C/min (from -20°C to 120°C), and a frequency of 1.0 Hz. All specimens were 40 mm long, 5 mm wide, and 0.17 mm thick. Three samples for each type of material were tested.

Optical characterization

The color of the neat PLA and of the PLA/β-CDs sheets was measured using a colorimeter (LabScan XE, HunterLab, Reston, VA, US) with a 17-mm-diameter measuring area. The CIELAB color system was used to characterize the color. The different sheets were square-shaped (60 x 60 mm^2) and then placed on the measuring area where CIE L*, a*, and b* values were measured. The three color coordinates ranged from L*=0 (black) to L*=100 (white), -a* (greenness) to +a* (redness), and –b* (blueness) to +b* (yellowness). Total color difference (ΔE) was calculated by using the equation below with respect to neat PLA as reference [76].

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Morphological characterization

A scanning electron microscope (JSM-model 6400 SEM, JEOL, Peabody, MA, US) with an accelerating voltage of 12 kV and a working distance of 25 mm at X500 magnification was used to assess the morphology of plain PLA and PLA/β-CDs sheets. Prior to analysis, the samples were fractured, stored in a desiccator, and finally coated with 3 nm of gold.

Barrier characterization

Water vapor transmission rates (WVTR) of neat PLA and PLA/β-CDs sheets were measured in accordance to ASTM F1249-05 [124] using a Permatran W Model 3/33 Water Permeability Analyzer (MOCON, Minneapolis, MN, US). Three to five sheets of each type were tested at 23 and 37.8°C, and 100% RH (using wet sponges). Oxygen transmission rates (OTR) of the sheets were measured in accordance to ASTM D3985-05

[125] using a 8001 Oxygen Permeation Analyzer (Mocon, Minneapolis, MN, US). Three to five sheets of each type were tested at 23 °C and 0% RH. All samples were masked with adhesive type aluminum foil (McMaster-carr, Aurora, Ohio, US), leaving an uncovered test area of 3.14 cm^2.

Statistical methods

Statistical analysis of the results was performed using a univariate analysis of variance (ANOVA). Means were separated using the Tukey honestly significant difference (HSD) test ($p < 0.05$) in the analytical software SPSS version 15 (SPSS Inc., Chicago, IL, USA). The data were analyzed and graphically plotted using Sigma-plot software version 10 (Systat Software Inc., Richmond, CA).

RESULTS AND DISCUSSION

Mechanical characterization

The mechanical properties tensile strength (machine direction (MD) and cross-machine direction (CD)), modulus of elasticity, and elongation at break of neat PLA and PLA/β-CDs blends have been measured and they are listed in Table 4. In general, all mechanical properties of the PLA changed significantly with the addition of the β-CDs. When the β-CDs were directly blended with the PLA (PLA-β-CD15% and PLA-β-CD30%), the tensile strength, elongation at break, and modulus of elasticity of the PLA decreased by about a factor of three, two, and two, respectively. This happened because β-CDs acted as fillers in the PLA continuous matrix and due to the poor interfacial interaction between

the hydrophilic β-CD and hydrophobic PLA the continuous matrix became discontinuous. These perturbations of the otherwise continuous matrix resulted in changes of the mechanical properties. A similar incompatibility has been reported for blends of PLA and starch [81]. A reduction of the strength and of the elongation at break in the PLA/starch blends has been observed regardless of starch type and composition [79, 126]. It has also been reported that the reduction of the tensile strength and of the elongation of the PLA/starch blends is related to the nucleating effect of the starch on the PLA matrix [127]. This could also be applicable to β-CDs since those plays a role as a nucleating agent for PLA [128].

With increasing starch content in the PLA/starch blends, the PLA matrix become more discontinuous resulting in decreases of strength and elongation [79, 126]. In contrast, strength and elongation at break in the PLA/β-CDs blends did not depend on the β-CD content (15% vs. 30%). The tensile strength of the PLA/β-CDs blends PLA-β-CD15% and PLA-β-CD30% was 11.55±1.26 MPa vs. 11.31±1.34 MPa, and 6.60±0.78 MPa vs. 3.82±1.16 MPA, for MD and CD, respectively. No effect of the CDs content on the elongation at break of the PLA/β-CD blends observed. All samples ranged around a value of 2% and 1% for MD and CD, respectively. The tensile strength and elongation at break of other bio-based blends, like blends of PLA and TPS have been reported to depend on its composition. In contrast, the modulus of elasticity of the blends was slightly affected by the amount of β-CDs. This was more evident in the CD results.

The mechanical properties of the blends improved with the use of the masterbatch to incorporate the β-CDs. This indicated better mixing between PLA and β-CDs. The tensile strength of the blends was increased by the use of the masterbatch. PLA-β-CD15%-MB

showed approximately twice the tensile strength of the blends PLA-β-CD15% and PLA-β-CD30%. The modulus of elasticity of the blends was also improved with the use of the masterbatch. In this case, PLA-β-CD15%-MB showed 2-3 times the modulus of elasticity of the blends PLA-β-CD15% and PLA-β-CD30%. However, no effect of the masterbatch on the elongation at break of the blends was observed. These results indicate that the blends were less breakable and more flexible in the presence of the masterbatch.

Table 4. Tensile strength, modulus of elasticity and elongation at break of the neat PLA and the PLA/β-CDs blends

	Sample code	Tensile strength (MPa)	Elongation at break (%)	Modulus of elasticity (GPa)
MD	PLA	37.9±0.9 a	3.5±0.4 a	2.4±0.0 a
	PLA-β-CD15%	11.6±1.3 b	1.6±0.1 b	0.9±0.1 b
	PLA-β-CD15%-MB	19.7±2.3 c	1.9±0.1 b	1.4±0.1 c
	PLA-β-CD30%	11.3±1.3 b	2.1±0.5 b	0.5±0.2 b
CD	PLA	29.2±1.8 a	1.7±0.3 a	2.3±0.0 a
	PLA-β-CD15%	6.6±0.8 bc	1.1±0.1 b	0.8±0.1 b
	PLA- β-CD15%-MB	10.5±1.5 b	1.3±0.2 b	1.3±0.1 c
	PLA-β-CD30%	3.8±1.2 c	1.1±0.0 b	0.7±0.2 d

a,b,c, and d indicate significant differences between samples

Thermal characterization

Table 5 summarizes some of the DSC results for the neat PLA and for the PLA/β-CDs blends. The neat PLA exhibited a glass transition temperature (T_g) of 57 °C and a melting temperature (T_m) of 148 °C. The addition of β-CDs slightly decreased both of these

thermal transitions in the PLA. The T_g of all the PLA/β-CDs blends were about a value of 54°C and all T_m ranged about a value of 147°C. Similarly, Ke and Sun (2000) did not observe a significant difference in T_g among polymer blends of PLA and corn or wheat starches at various ratios [79]. Figure 5 shows in detail how crystallinity and melting peaks were modified with the addition of β-CDs. As the amounts of β-CDs increased, the melting peak become broader and deeper and the crystallization peak become larger than those of the neat PLA. The broader melting peak was possibly caused by a wide range of sizes of the crystals formed by the interaction between the PLA and the β-CDs. The latter had a T_m of 155°C (data not shown). These broader melting peaks in the PLA/β-CDs blends may lead to higher hot tacks under a broader range of T_m, especially at lower ones. The larger crystallinization peak was caused by the nucleating capacity of the β-CDs in the PLA matrix [128]. The fraction of crystallinity of the neat PLA and of the blends PLA/β-CDs is presented in figure 6. In the calculation of the crystallinity of the blends only PLA was considered since PLA was the main load-bearing phase. The crystallinity of the blends was relatively low but higher than that of the neat PLA. The slight increase was correlated to the amount of β-CDs. The higher amount of β-CDs, the higher the crystallinity. This agrees with Almenar, Auras, Harte, Rubino (2009) who reported that the crystallinity of PLA increased with the increase of the β-CD content. Starch has been identified as a nucleating agent for PLA, too [88, 128-129]. However, it has been reported that the crystallinity of blends with starch contents of <20% is the same as that of extruded pure PLA, and that the crystallinity decreases slightly as the starch content increases to 40% [79]. The crystalline contents of PLA-β-CD15%-MB and neat PLA did not differ. The reduction of the crystalline content of the PLA in the PLA-β-CD15%-MB

blend compared with that of the PLA/β-CD15% blend was due to the use of the MB. Lower crystalline contents have been related to higher impact resistance and ductivility [130]. A reduction in crystallinity of the PLA with the addition of commercial masterbatches has been reported [87].

A hysteresis peak (63°C) associated with the T_g of neat PLA and its blends is also shown in Figure 5. This peak was an aging peak [79] and resulted from an increase in the excess enthalpy of relaxation during processing [131]. The use of the masterbatch did not affect the thermal properties of the PLA in the blends. The same T_g and T_m were observed for PLA-β-CD15%-MB and for PLA-β-CD15% (Figure 5 and Table 5). Similarly, Byrne, Ward, Kennedy, Imaz, Hughes, Dowling (2000) who evaluated four commertial masterbatches in order to improve the impact strenght, flexibility, and clarity of PLA reported no effect of the masterbatch on the T_g and on the T_m of the PLA [87].

Table 5. Glass transition temperatures and melting temperatures of the neat PLA and the PLA/β-CDs blends

Sample code	T_g (°C)	T_m (°C)
PLA	57.0±0.9 a	148.2±0.4 a
PLA-β-CD15%	53.9±0.4 b	147.5±0.3 ab
PLA-β-CD15%-MB	54.3±0.5 b	147.9±0.2 ab
PLA-β-CD30%	54.2±0.5 b	147.1±0.6 c

a, b, and c indicate significant differences between samples

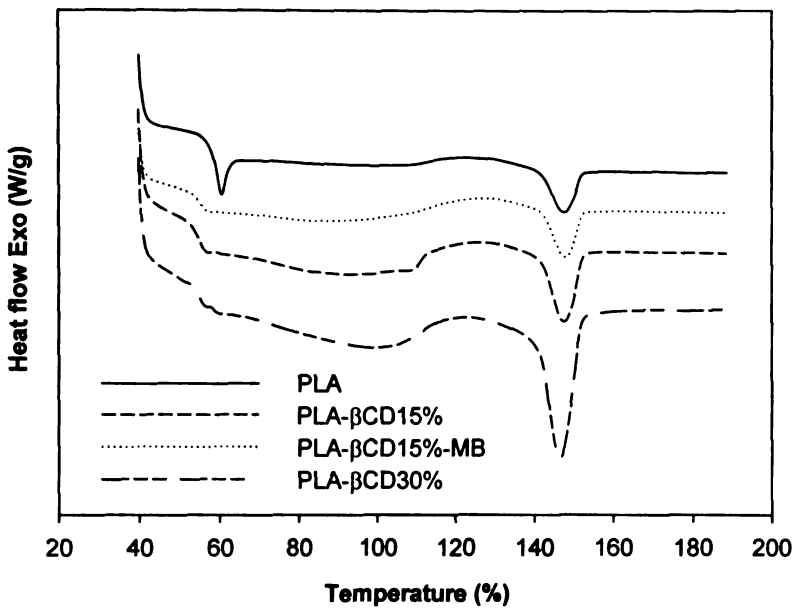

Figure 5. DSC thermograms of the neat PLA and the PLA/β-CDs blends (1st cycle)

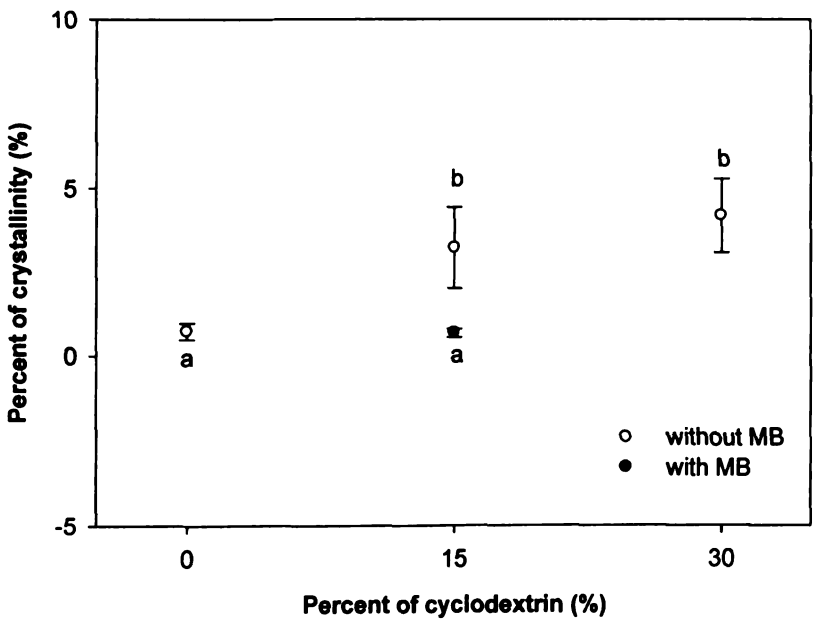

Figure 6. Percent of crystallinity (%) of the neat PLA and the PLA/β-CDs blends (a, and b indicate significant differences between samples)

TGA thermograms of the neat PLA and of the PLA/β-CDs blends are presented in Figures 7 and 8. The decomposition of the neat PLA started at around 366°C and ended at 411°C (Figure 7).The PLA/β-CDs blends showed a lower thermal stability than that of the neat PLA and this was β-CDs content dependent. The higher the β-CDs content the lower the thermal stability. No effect of the masterbatch on the thermal stability of the blends was observed. Figure 8 shows the rates of decomposition for the neat PLA and for the PLA/βCDs blends. The rates of decomposition for PLA/βCDs blends were faster than for the neat PLA. PLA-β-CD 30% showed the fastest rate of decomposition. The derivative thermogravimetric curve shows the temperature at the maximum rate of weight loss, and this corresponds to the decomposition temperature [81]. PLA-β-CD15% and PLA-β-CD15%-MB showed similar rates of decomposition as observed in figure 8. The remaining water in the β-CDs was possibly the reason for a faster decomposition rate for the blends than for the plain PLA. It has been reported that the initial moisture content of the starch had a significant effect on the decomposition of PLA during processing of PLA/starch blends. The PLA gets slightly degraded in the presence of water under the high levels of heat used during processing [82]. The derivative thermogravimetric curves of the PLA/β-CDs blends showed a shoulder which results from the degradation of the β-CDs. This has been confirmed by performing the same study on β-CDs (data not shown) which resulted in the same bimodal peak. Further confirmation comes from a study of the derivative weight of poly(β-CD/2 benzoxazine IC) under nitrogen which showed a bimodal peak at about 300~350°C [132].

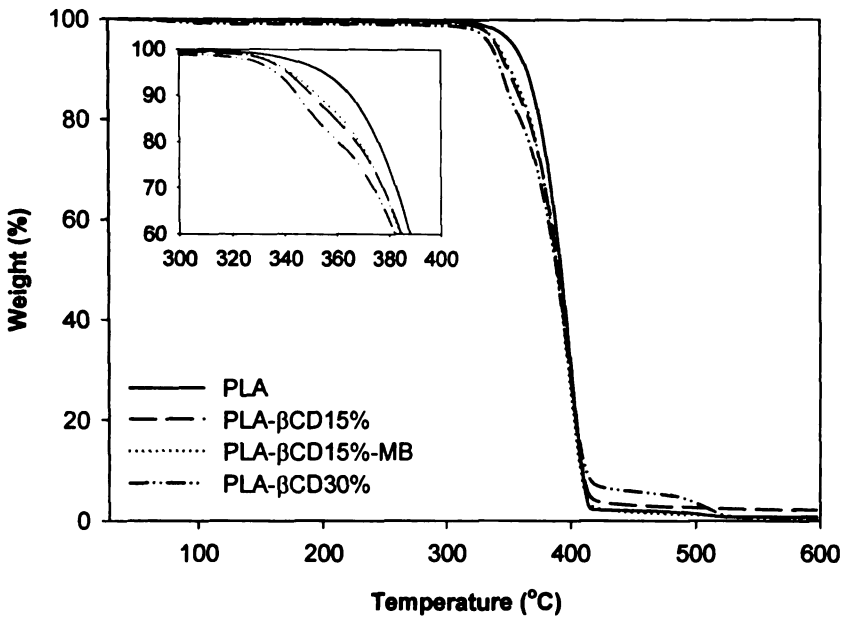

Figure 7. Weight change (%) as a function of the temperature (°C) of the neat PLA and the PLA/β-CDs blends

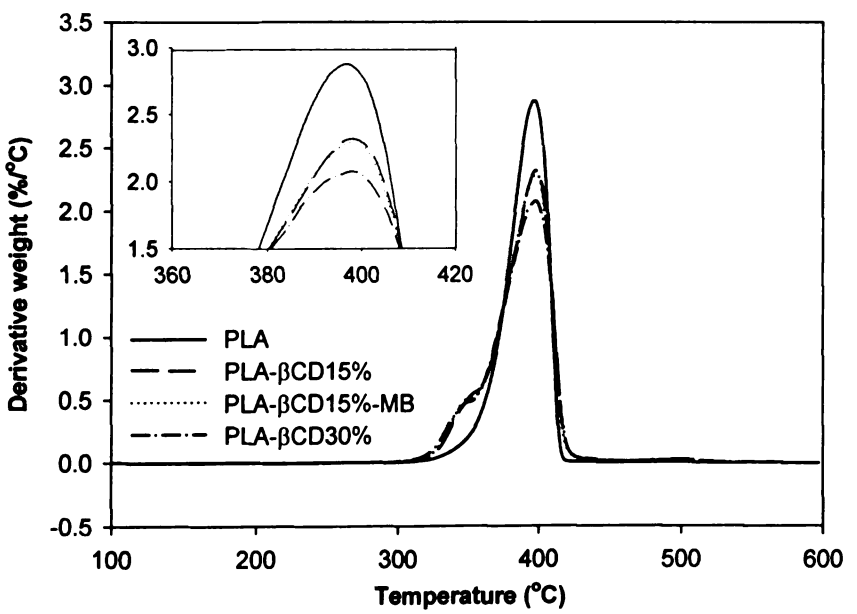

Figure 8. Derivative weight (%/°C) as a function of the change of temperature (°C) of the neat PLA and the PLA/β-CDs blends

Dynamic mechanical characterization was performed on the neat PLA and on its blends. Dynamic mechanical analysis mainly measures viscoelastic behaviors and compares the stress and strain signals as the in-phase (storage) and out-of-phase (loss) components, from which storage or elastic (E'), loss (E'') moduli as well as the tan $\delta = E''/E'$ are obtained as functions of temperature [81]. E' and tan δ of all samples are presented as a function of temperature in figures 9 and 10.

As shown in figure 9, the PLA blends showed higher E' than that of the plain PLA over the entire temperature range. This confirms that the PLA matrix is restrained by the loading of β-CDs because of the incompatibility between components. Therefore, E' increased proportional to the concentration of β-CDs. These results are in agreement with those reported by Almenar et al (2009). No effect of the masterbatch on E' was observed. Therefore, the use of the masterbatch didn't improve the applicability of the blends in applications that require rigorous processing conditions. E' dropped at 60~65°C because of the increase in the segment mobility of the polymer chain at around the glass transition temperature.

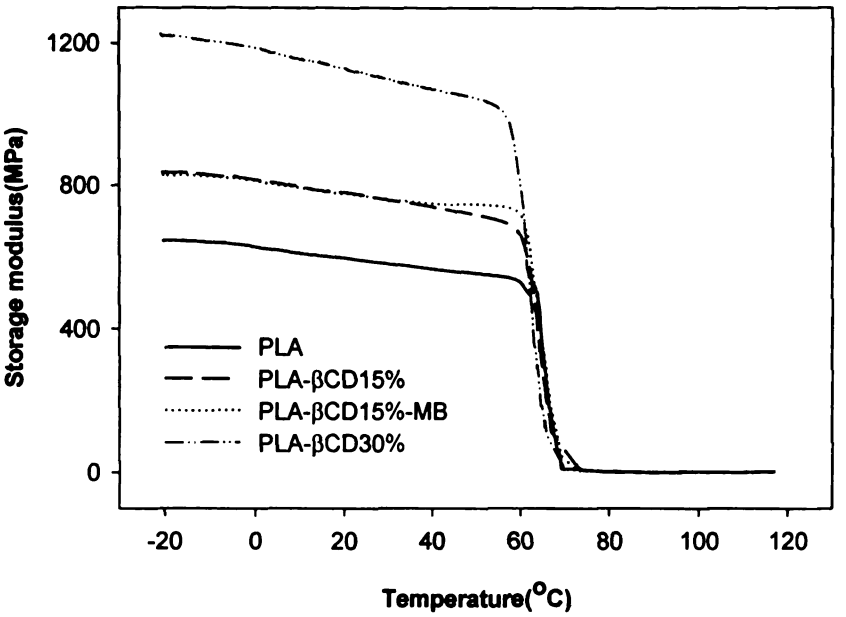

Figure 9. Storage modulus changes of neat PLA and PLA/β-CDs blends

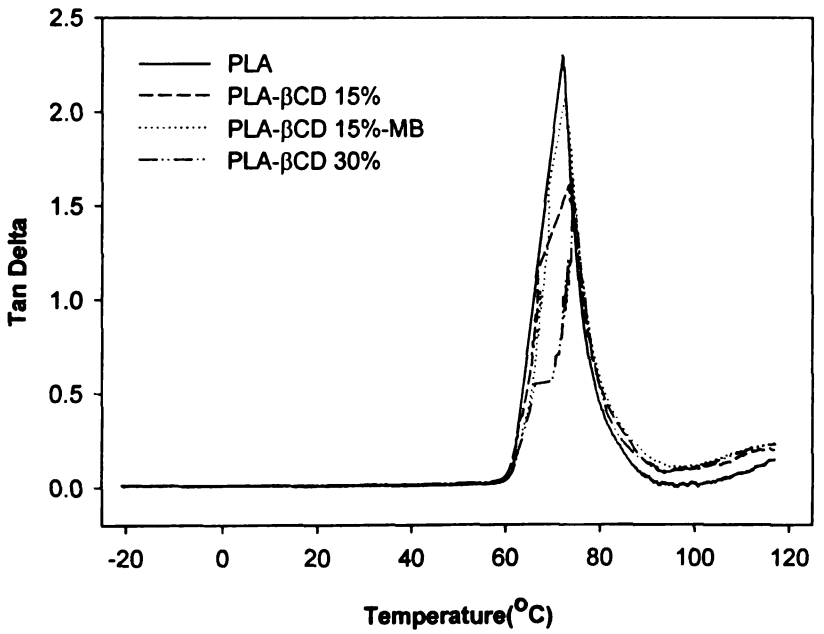

Figure 10. Tan delta changes of neat PLA and PLA/β-CDs blends

The neat PLA showed the highest value of tan delta and this decreased in the following order: PLA-β-CD15%-MB>-β-CD15%>-β-CD30%. It has been reported that the neat PLA showed a very sharp and intense peak because there was no restriction to the chain motion [133]. The reduction of height of tan delta confirmed the hindrance of the chain mobility by the presence of β-CDs. Less reduction in height was observed in the blend containing the masterbatch. The masterbatch seems to improve the chain motion and therefore, the processability of the PLA/β-CDs blends. The glass transition temperature of the blends obtained from the DMA curve was a little higher than that obtained from the DSC curve. DMA monitors the temperature dependence of mechanical stress/strain and loss behavior (tan delta) as a function of the frequency of the oscillating deformation force applied. For a perfectly elastic material, the applied stress and measured strain is perfectly in phase and thus no losses occur. As Tg is approached, mobility of the polymer chains increases until, at a given temperature, the relaxation time for chain mobility coincides with the frequency of the applied stress. At this particular point, the polymer absorbs mechanical energy and the damping factor (loss tan delta) reaches a maximum value. Thus the glass transition temperature can be identified as the point of maximum damping on a plot of tan delta versus temperature at the particular frequency under consideration [134].

Optical characterization

CIELAB describes the optical properties using the parameter L*, a*, and b*. In the CIELAB space, the parameter L* is a measure of the lightness of a sample, and ranges from 0 (black) to 100 (white) [135]. Figure 11 shows the lightness of the neat PLA and of

the PLA/β-CDs blends. As observed, the blends presented a higher lightness than the neat PLA (92.53±0.16 vs. 93.24±0.15 and 93.34±0.21, for PLA, PLA-β-CD15%, and PLA-β-CD30%). There are two reasons for this lightness increase. The first one is the natural white color of the β-CDs which became part of the polymer matrix during processing and, the second reason is the higher crystallinity content in the blends due to the nucleating agent capacity of the β-CDs. In general, the higher the crystalline content in a polymer, the higher its lightness is. However, there were no significant differences between PLA-β-CD15%, and PLA-β-CD30% because the reduced mobility of the PLA molecules caused a similar crystallinity of the material in spite of the different amounts of nucleating agent. Similarly, extrudates of PLA/starch are opaque and of white color [82]. Ke et al (2000) reported PLA/starch blends with 20% of starch as light gray and it turned into off-white as the starch contents increased to ≥40% [79]. PLA-β-CD15%-MB showed less lightness compared with PLA-β-CD15%. This is in agreement with the reduction of the crystallinity resulting from the use of the masterbatch (figure 6). Therefore, the use of the masterbatch improved to see through the new material. .

In the CIELAB space, variables a* and b* define the degree of greenness (- a*) or redness (+ a*), and blueness (- b*) or yellowness (+ b*) [76]. Figure 12 shows the color variables a* and b* of the neat PLA and of the PLA/β-CDs blends. All samples exhibited a* high b* value due to the natural yellow color of the PLA [76]. Both b* and a* increased as β-CD content increased. Therefore, the blends became redder and more yellow and with a total color more saturated. Of the blends, PLA-β-CD15%-MB showed the lowest redness, close to that of the neat PLA, while at the same time PLA-β-CD15%-MB showed the same yellowness as PLA-β-CD30%. This was due to the presence of β-

CDs in the polymer matrix. Byrne et al (2009) also reported an increase of the yellowness index of PLA with the use of the commercial masterbatches Biomax strong, and PLA dcS515-N [87].

A slightly modified way to calculate ΔE was used [136]. In this study, ΔE denotes the overall difference in color between the samples (blends) and a reference (neat PLA), and the larger the value of ΔE, the larger the differences in color. According to Sharma (2003), a ΔE of approximately 2.3 corresponds to a just noticeable difference [137]. The values of ΔE were less than 2.3 for all the blends and therefore, there were no differences in overall color among them. Although the color values L*, a*, and b* of the blends have been shown to be dependent on the β-CDs content, this did not happen for the overall color of the blends.

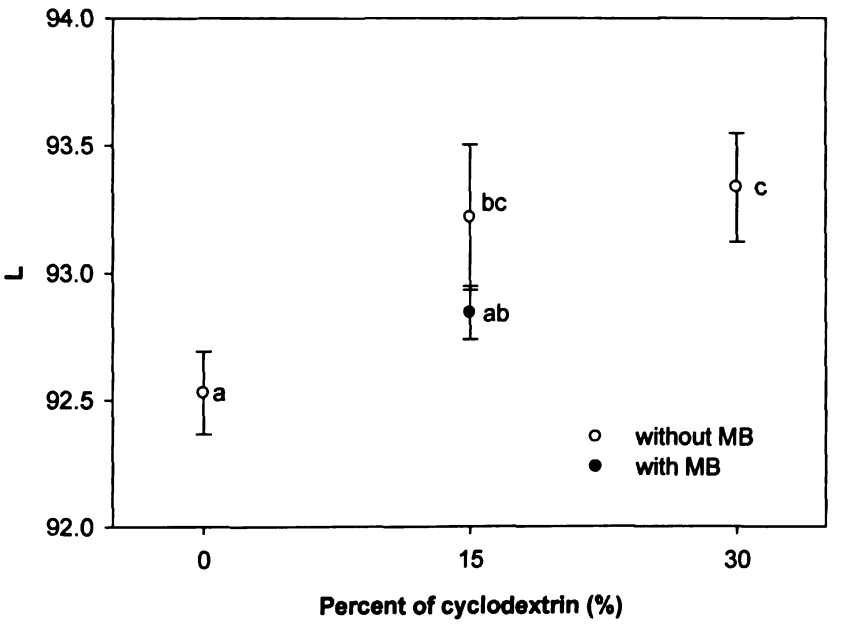

Figure 11. Lightness of neat PLA and PLA/β-CDs blends (a,b and c indicate significant differences between samples)

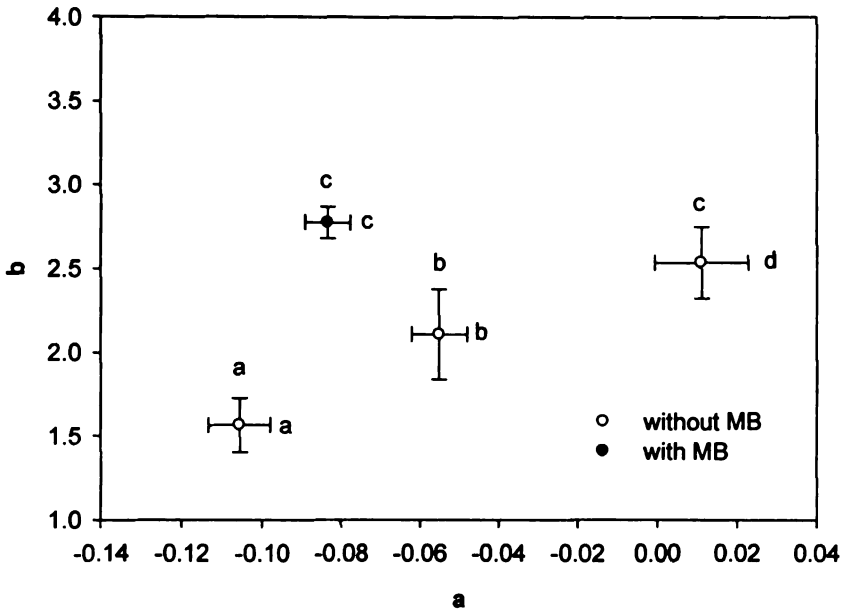

Figure 12. a* and b* values of neat PLA and PLA/β-CDs blends (a, b, c, d and A, B, and C indicates significant differences between samples)

Table 6. ΔE of the PLA-βCDs blends with respect to PLA as reference

	PLA	PLA-β-CD15%	PLA-β-CD15%-MB	PLA-β-CD30%
ΔE	-	0.91	1.25	1.27

Morphological characterization

Scanning electron micrographics of the surface of fractured neat PLA and PLA/β-CDs blends were taken to better understand the interaction between PLA and β-CDs. All samples were prepared in the same way, and all micrographics were taken under the same conditions. The results are shown in Figure 13. Panel (a) shows the continuous phase of the neat PLA, while panels (b), (c), and (d) show the micrographics for PLA with different β-CD contents and how this continuous phase became discontinuous with the addition of β-CDs. The mix of PLA and β-CDs produced a two-phase system. This claim

was based on: (1) β-CDs were not completely covered by the PLA phase. A larger amount of uncovered β-CDs is present in the blend with the highest β-CD content (30%) (d) which might be due to the increased size of the β-CDs conglomerate in this material. It seems that larger β-CDs conglomerates (approximately 15 μm width) were formed by interaction between smaller ones during processing and that higher amounts of β-CDs lead to the formation of larger conglomerates (figure 13, arrows). This would be in agreement with previous study done with CDs that reports the ability of CDs to form supramolecular complexes by linking covalently or non covalently specifically to other CDs [9]. (2) The presence of cavities between the PLA and the β-CDs. The higher the amount of β-CDs, the larger the cavities were. This illustrates the poor adhesion between the phases PLA and β-CDs. These results are supported by similar ones published for PLA/starch blends. Microscopic observations revealed non-uniformly dispersed holes in TPS/PLA blends which indicated a separation of phases between TPS and PLA [84]. Some gaps between PLA and starch in PLA/starch blends were observed which indicated likely poor adhesion between the two phases [79]. Comparing the micrographics of PLA/starch with those of PLA/ β-CDs for the same content of filler shows that the miscibility between PLA and β-CDs was higher than that of PLA and starch.

The micrographic of PLA-βCD15%-MB (c) looks like a combination of the micrographic of the PLA-βCD30% (d) and of the neat PLA (a) since some areas still showed conglomerates although these were smaller than the ones in the PLA-βCD30% blend. Also, the number of cavities was lower. Therefore, processing improved the adhesion between the two phases due to the reduction of the conglomerate size and of the number

of cavities. The micrographic of PLA-βCD15%-MB (c) does not show improved interfacial adhesion compared to PLA-βCD15% (b).

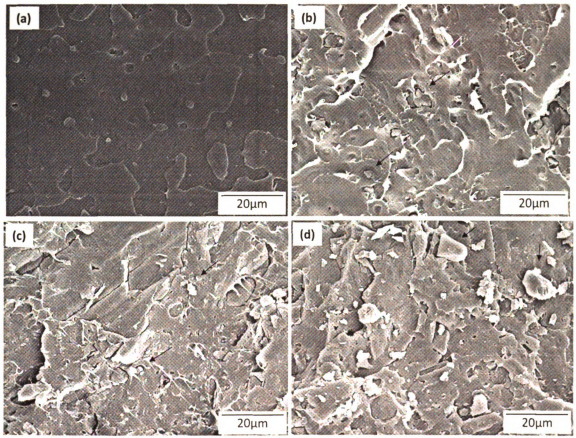

Figure 13. Scanning electron micrographics of the surface of fractured neat PLA and PLA/β-CDs pellets. Panel (a) shows neat PLA while panels (b), (c), (d) show PLA-β-CD15%, PLA-β-CD15%-MB, PLA-β-CD30 (arrows pointing to the conglomerates)

Barrier characterization

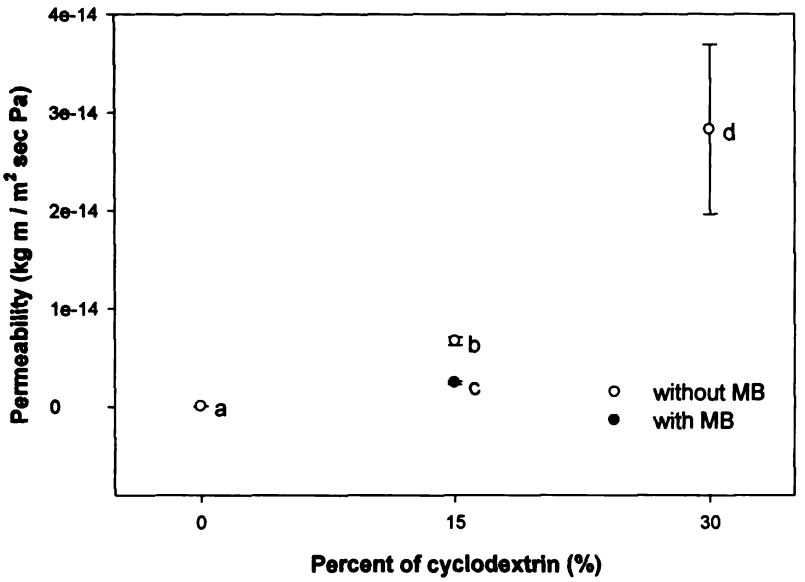

Figure 14. Oxygen permeability coefficient of the neat PLA and of the PLA/β-CDs blends at 23°C and 0%RH (a,b,c, and d indicate significant differences between samples)

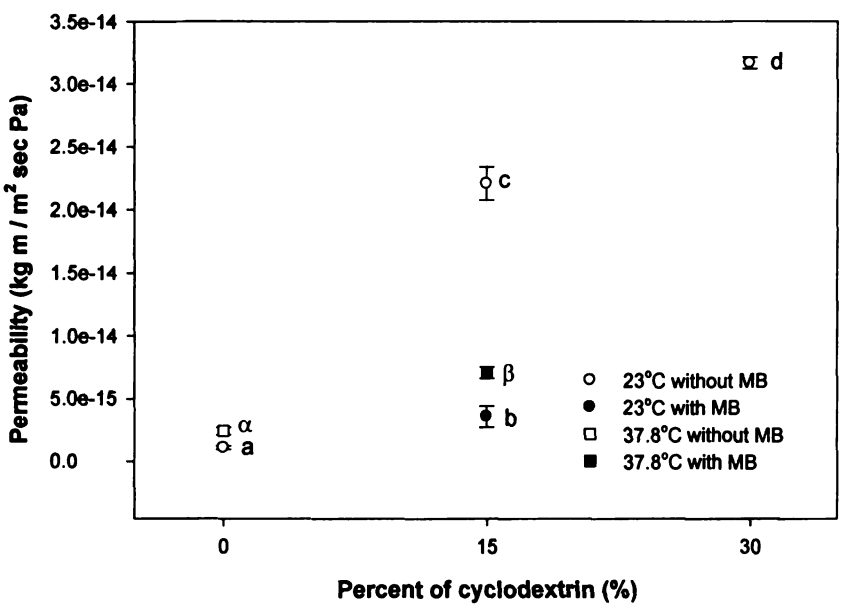

Figure 15. Water permeability coefficient of the neat PLA and of the PLA/β-CDs blends at 23°C and 37.8°C, and 100%RH (Not data for PLA-βCDs15%, PLA-βCDs30% at 37.8°C) (a, b, c, d and A B indicate statistical difference among samples)

The oxygen permeability coefficients of neat PLA and its blends are presented in figure 14. The permeability of the neat PLA was $8.71\times10^{-18}\pm1.17\times10^{-18}$ kg·m/m^2·s·Pa at 23°C and this value was in agreement with the value of 6.0×10^{-18} kg·m/m^2·s·Pa reported in the literature for PLA films exposed to the same temperature [76]. For higher temperature (25°C), oxygen permeability of PLA films has been reported as 3.3×10^{-17} kg·m/m^2·s·Pa [138]. The values for the PLA/β-CDs blends were over several orders of magnitude larger than the neat PLA, and this difference was more pronounced for the higher β-CD content ($6.69\times10^{-15}\pm4.10\times10^{-16}$ and $2.83\times10^{-14}\pm8.62\times10^{-15}$ kg·m/m^2·s, for PLA-β-CD15% and PLA-β-CD30%, respectively). The increased permeability was caused by the discontinuity between the β-CDs and the PLA. The voids between phases allowed the oxygen molecules to diffuse faster through the matrix. The use of the masterbatch improved the barrier properties of the blends due to the reduction of discontinuities in the polymer matrix. PLA-β-CD15%-MB showed a lower permeability than PLA-β-CD15% ($2.43\times10^{-15}\pm1.64\times10^{-16}$ kg·m/m^2·s·Pa vs. the data above).

Figure 15 shows the water vapor permeability coefficients of neat PLA and of the PLA/β-CDs blends. The permeability of neat PLA was $1.11\times10^{-15}\pm1.34\times10^{-16}$ kg·m/m^2·s·Pa and $2.43\times10^{-15}\pm3.00\times10^{-16}$ kg·m/m^2·s·Pa, at 23°C and at 37.8°C, respectively. Higher permeability values for water vapor have been reported in the literature (2.0×10^{-14} kg·m/m^2·s Pa at 20°C and 90%RH) [76]. This might be due to differences in processing history, and other parameters. The permeability of the blends increased over that of the neat PLA with the increase of the β-CD content. The immiscibility of phases in the blends allowed more water molecules to penetrate. Some of the water molecules were possibly absorbed by the polymer matrix since part of them was removed in the drying

process before the processing of the material. Similarly, it has been reported that water can penetrate the PLA/starch blends through the voids between phases and that some waster was absorbed by the starch (<20%) [79]. The permeability of the PLA-β-CD30% blends at 37.8°C was so high that it couldn't be measured. The use of the masterbatch increased the barrier properties of the blends. The PLA-β-CD15%-MB blend has a water vapor permeability of $3.58\times10^{-15}\pm8.34\times10^{-16}$ kg·m/m^2·s·Pa and $7.07\times10^{-15}\pm4.64\times10^{-16}$ kg·m/m^2·s·Pa at 23°C, and at 37.8°C. Both values were lower than $2.21\times10^{-14}\pm1.31\times10^{-15}$, the value obtained for PLA-β-CD15%.

In general, oxygen and water vapor permeability coefficients were a little lower than those reported in the literature, which might be caused by differences in thickness, processing and parameters.

CONCLUSIONS

In this study, a bio-based polymer with the capacity to carry hydrophobic molecules has been developed and characterized. Testing of mechanical, thermal, barrier, optical, and morphological properties revealed that β-CDs and PLA are incompatible. The higher the β-CD content, the higher the incompatibility due to the increase in size of the β-CDs conglomerates, and the poor adhesion between phases. The use of a masterbatch (PLA containing high β-CD content) is an effective way to improve the compatibility between PLA and β-CDs, and the properties of PLA/β-CDs blends.

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CHAPTER 4. DEVELOPMENT AND CHARACTERIZATION OF A BIO-BASED ANTIMICROBIAL POLYMER MADE OF POLY(LACTIC ACID) AND ENZYMATICALLY MODIFIED STARCH CONTAINING TRAPPED TRANS-2-HEXENAL*

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*to be submitted for publication to BIOMACROMOLECULES

The present study aims at developing and characterizing a bio-based antimicrobial polymer with potential use in food and pharmaceutical packaging applications, but also as an inherently sterile material by itself. Trans-2-hexenal, a naturally occurring plant volatile with antimicrobial capacity, was trapped into β-cyclodextrins (β-CDs), enzymatically modified starch, and indirectly incorporated into a poly(lactic acid) (PLA) matrix in order to develop a bio-based antimicrobial polymer. A masterbatch was used prior to casting of the polymer in order to improve the dispersion of the antimicrobial compound in the PLA matrix. Physical, mechanical, barrier, and antimicrobial properties of antimicrobial and neat PLA sheets were determined in order to study the effect of the antimicrobial on the polymer properties. The antimicrobial capacity of the sheets was tested against the fungus *Alternaria solani*. Incorporation of trans-2-hexenal trapped into β-CDs to the PLA matrix did not affect the physical properties, but did affect the mechanical, barrier and antimicrobial capacity, and the new polymer was effective in inhibiting the growth of micro-organisms. Processing operations resulted in a dramatic loss of antimicrobial capacity. The masterbatch showed 90% more antimicrobial capacity than the polymer sheet.

1. Introduction

Antimicrobial polymers are a type of active polymers that can reduce, retard or inhibit the growth of spoilage and pathogenic micro-organisms, generally by contacting or releasing active compounds with antimicrobial properties. The antimicrobials can be incorporated into the polymer, coated or immobilized onto the polymer, or be an inherent part of the polymer [2, 139-140]. There are many antimicrobials that potentially can be used to develop antimicrobial polymers. In recent years, much interest has been attracted by the ones that can be found in nature.

Some naturally occurring plant volatiles are well-know to provide protection against microbial proliferation in wounded areas of plants [33]. Their use as antimicrobial has been thoroughly explored in *in vitro* and *in vivo* systems, and among these the highest effectiveness has been reported for aldehydes [141]. The precise mechanism of the protective action of aldehydes is not clear yet, but likely they permeate by passive diffusion across the plasma membrane, and once inside the cell, the α, β-unsaturated aldehyde moiety reacts with biologically important nucleophilic groups which play a key role in living cells [44]. Trans-2-hexenal is one of the aldehydes most successfully tested to date. This naturally occurring six-carbon plant volatile produced through the lipoxygenase pathway has been successfully employed against bacteria and molds in *in vitro* and *in vivo* systems [36, 43-44, 142-143]. The combination of its antimicrobial capacity and the fact that it is approved as food additive permitted for direct addition to food for human consumption by the US Food and Drug Administration [45] makes trans-2-hexenal particularly interesting as an antimicrobial compound for food applications.

However, its direct application on a substrate has limited benefits because of its rapid volatilization. Cyclodextrins (CDs) are a possible alternative to trap, and to control the release of trans-2-hexenal.

CDs are cyclic oligosaccharides composed of α-1,4-coupled D-glucose units produced enzymatically from starch. They have a hydrophilic exterior and a hydrophobic cavity, which enables them to form inclusion complexes (ICs) with a variety of hydrophobic molecules [144]. The trapping of naturally occurring volatiles into CDs allows for great application flexibility. For example, CDs have been used as carriers and releasers of aldehydes like acetaldehyde and hexanal to reduce and/or inhibit spoilage micro-organisms [11-12].

Antimicrobials can be directly incorporated into a polymeric matrix leading to antimicrobial polymers as mentioned above [139]. This direct insertion is not possible for naturally occurring plant volatiles because of their rapid volatilization. The use of CDs will allow the incorporation of naturally occurring plant volatiles into the polymeric matrix since the volatiles are handled as a powder. To date, most antimicrobial polymers are petroleum-based. Polymers made from natural resources with antimicrobial properties are practically restricted to edible films and coatings [60, 68]. Bio-based polymers with antimicrobial activity have been developed by using starch, cellulose, alginates, carrageenan, chitosan, agar, wey, zein, gluten, soy, gelatin, waxes, and glycerides [68].

Poly(lactic acid), PLA, is a biocompatible and biodegradable semi-crystalline polyester with potential for further developments because its mechanical and barrier properties are comparable to those of conventional polymers [18-19]. PLA is a plastic material made from natural resources such as corn or sugar cane and is transparent and glossy [76], is

approved for food contact by the US Food and Drug Administration [145], and can be degraded in a composting system [146]. These properties make PLA particularly interesting as a bio-based viable alternative matrix for antimicrobial polymers for food and pharmaceutical packaging applications. In this study, trans-2-hexenal was trapped into CDs and was indirectly incorporated into a poly(lactic acid) matrix, yielding a bio-based antimicrobial polymer. Since ICs are sensitive to external stimuli, such as temperature changes, shear forces, and the presence of competing CD-binding molecules [91, 144], the antimicrobial capacity of the polymer may change during processing [7] and the immiscibility between PLA and β-CDs affects the characteristics of antimicrobial polymer. Joo et al. (2010) reported that the use of a masterbatch made of PLA containing high β-CD content improved the distribution of the CDs in the polymeric matrix and the miscibility between PLA and CDs [147]. In addition, Han (2000) recommend the use of a masterbatch to process antimicrobial polymers to minimize the deterioration of the antimicrobial agent during film fabrication, distribution and storage [27]. Thus, a masterbatch was used prior to casting of the antimicrobial polymer in order to improve the dispersion of the antimicrobial compound in the PLA matrix.

The purpose of this study was: (1) to develop a bio-based antimicrobial polymer using a masterbatch, (2) to characterize the new polymer in terms of mechanical, physical, barrier, and antimicrobial properties, and (3) to study the effect of processing on the antimicrobial capacity of the new polymer.

2. Experimental Section

2.1 Materials. The volatile compound, trans-2-hexenal (purity >95%, Food grade), was purchased from Sigma-Aldrich Corp. (Saint Louis, MO, US) (figure 16 1a). Poly(lactic acid) 4042D (PLA 4042 D) (92% L-lactide, D=1.24g/cc) resin was purchased from NatureWorks, LLC (Blair, NE, US) (figure 16b). β-cyclodextrin (β-CDs) (purity >99%, Food grade) were purchased from Wacker Chemical Corporation (Adrian, MI, US) (figure 16c).

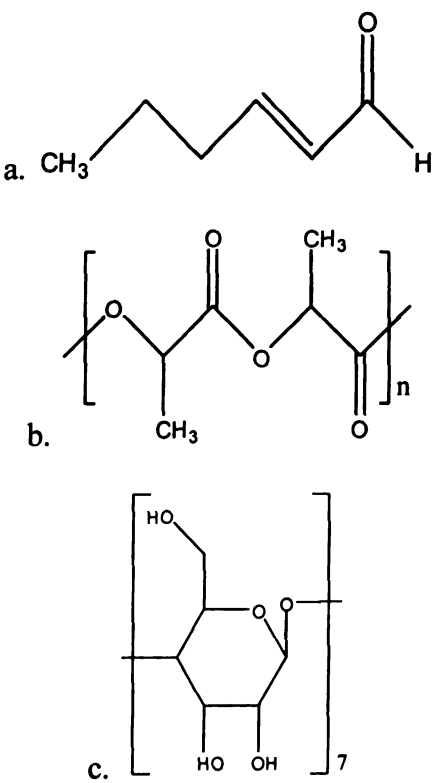

Figure 16. Chemical structures: a. trans-2-hexenal, b. PLA, and c. β-CD

2.2 Microbial strains. Single spore isolates of *Botrytis cinerea*, and *Colletotrichum acutatum* were originally isolated from diseased blueberry fruit, and of *Penicillium* sp. from spoiled bread. *Aspergillus niger* and *Alternaria solani* spore isolates were provided by the Department of Plant Pathology, MSU, East Lansing, MI, USA.

2.3 Synthesis and characterization of inclusion complexes (ICs). An amount of 5.67 g of β-CDs was weighed using an electronic balance (Adventurer™ precision balance, OHAUS, NJ, US) and then added to a beaker containing 56 ml of 100 °C distilled water (figure 17a). The mix was stirred for a few seconds on a hot plate stirrer (Thermolyne® Mirak™ hot plate stirrer; Sigma-Aldrich Corp.(Saint Louis, MO, US)) prior to the addition of 315µl of pure-trans-2-hexenal. The molar ratio of β-CDs and trans-2-hexenal was 5:3. The solution was stirred for two hours at 130 rpm, and then cooled down using a second stirrer without heating (Thermolyne Nuova II stir plate, Barnstead International, Testware, Sparks, NY, US). The mix was centrifuged at 1600 rpm for 1 hr and then dried at the 90°C in a conventional oven for 24hr (10-180 Incubator, QL, Quincy Lab, Inc, Chicago, US). The ICs were maintained in a desiccator at 23°C until use.

To determine the amount of trapped volatile (figure 17b), 2 ml glass vials were filled with ICs (0.01 g) and then positioned at the bottom of 40 ml glass vials containing 1 ml of distilled water (placed at the bottom) each. The vials immediately were tightly closed with Mininert® valves (Supelco, Bellefonte, PA, US). Approximately 24 hours later, a 65-µm PDMS/DVB SPME fiber (Supelco, Bellefonte, PA) was exposed to the vial headspace for 10 minutes, and then was inserted into the splitless injection port of a GC Hewlett-Packard 6890 series esc (Agilent technology, Palo Alto, CA, US) equipped with FID and an HP-5 column (30 m×0.32 mm×0.25 µm, Hewlett–Packard, Agilent Technology, Palo Alto, CA, US). The initial oven temperature was 40 °C for 5 min. It was then increased to 230 °C at 5 °C/min and maintained for 10 min. The injector and detector temperatures were 230 and 270 °C, respectively. The amount of trans-2-hexenal released from the ICs was evaluated in triplicate, and quantified on the basis of prepared

calibration curve. A calibration curve was prepared by measuring the chromatographic area of accurately known trans-2-hexenal solutions and by plotting the different amounts of trans-2-hexenal (concentration) on the X-axis vs. their corresponding measured responses (chromatographic area) on the Y-axis.

A scanning electron microscope (JSM-model 6400 SEM, JEOL, Peabody, MA, US) with an accelerating voltage of 12 kV and a working distance of 25 mm at x500 magnification was used to assess the morphology of the β-CDs and of the ICs during their synthesis, simulating their synthesis but without trans-2-hexenal, and after the release of the trans-2-hexenal. Prior to analysis, the samples were coated with Osmium.

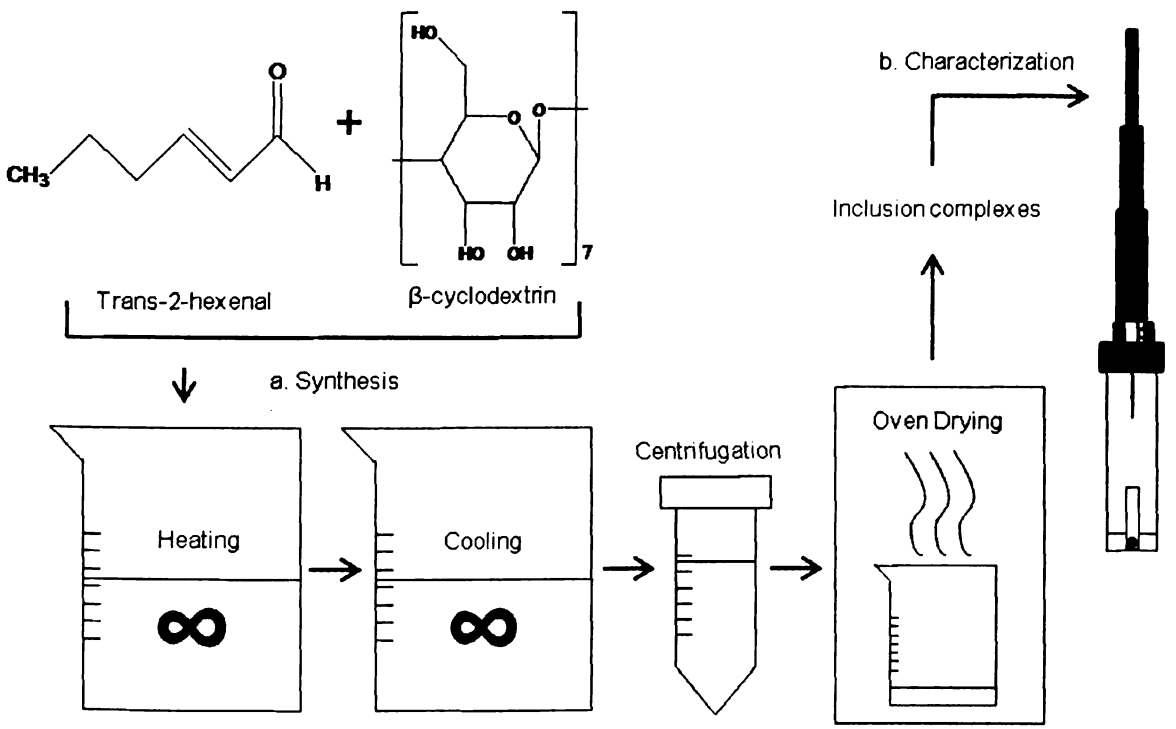

Figure 17. Synthesis and characterization of the ICs

2.4 Preparation of the antimicrobial masterbatch. A masterbatch was prepared to improve the dispersion of the antimicrobial compound and the properties of the PLA matrix. Antimicrobial masterbatch was prepared as follows (figure 18a):

PLA 4042D dried for 4 hrs at in a conventional oven (1430 VMR International, Cornelius, OR, US), was mixed with ICs (70:30 w/w) using a 32 mm conical counter-rotating twin-screw extruder (C.W. Brabender Instruments, South Hackensack, NJ, US) with a L/D ratio of 13:1 and four separated temperature controlled barrel zones set from feeder to die at 170 °C, 175 °C, 175 °C, and 170 °C. The screw speed was set at 40 rpm. The blend was collected from the die and allowed to cool down prior to being granulated into pellets (JC-5, Conair Wortex, Franklin, PA, US). The resulting pellets were used as antimicrobial masterbatch pellets for the casting process. The pellets were maintained in a desiccator at 23 °C until use.

2.5 Preparation of the antimicrobial sheet. The antimicrobial masterbach and plain PLA 4042D pellets were mixed (50:50 w/w) and pelletized using the same processing conditions as described in figure 18b. The pellets were transferred to the hopper of a casting film twin extruder (RCP-0625 Microtuder control panel, RandCastle extrusion system, INC, Cedar Grove, NJ, US) with four separated temperature controlled barrel zones set from feeder to die at 160 °C, 165 °C, 170 °C, 170 °C and 170 °C, and a screw speed of 35 rpm. The resultant melt was extruded through a slit die to form a sheet, and the extrudate was cooled on chill rolls kept at a constant temperature of 52 °C. The width and thickness of the resulting antimicrobial sheet were 19 cm and 0.019 cm, respectively. The antimicrobial sheets were maintained in a desiccator at 23 °C until use.

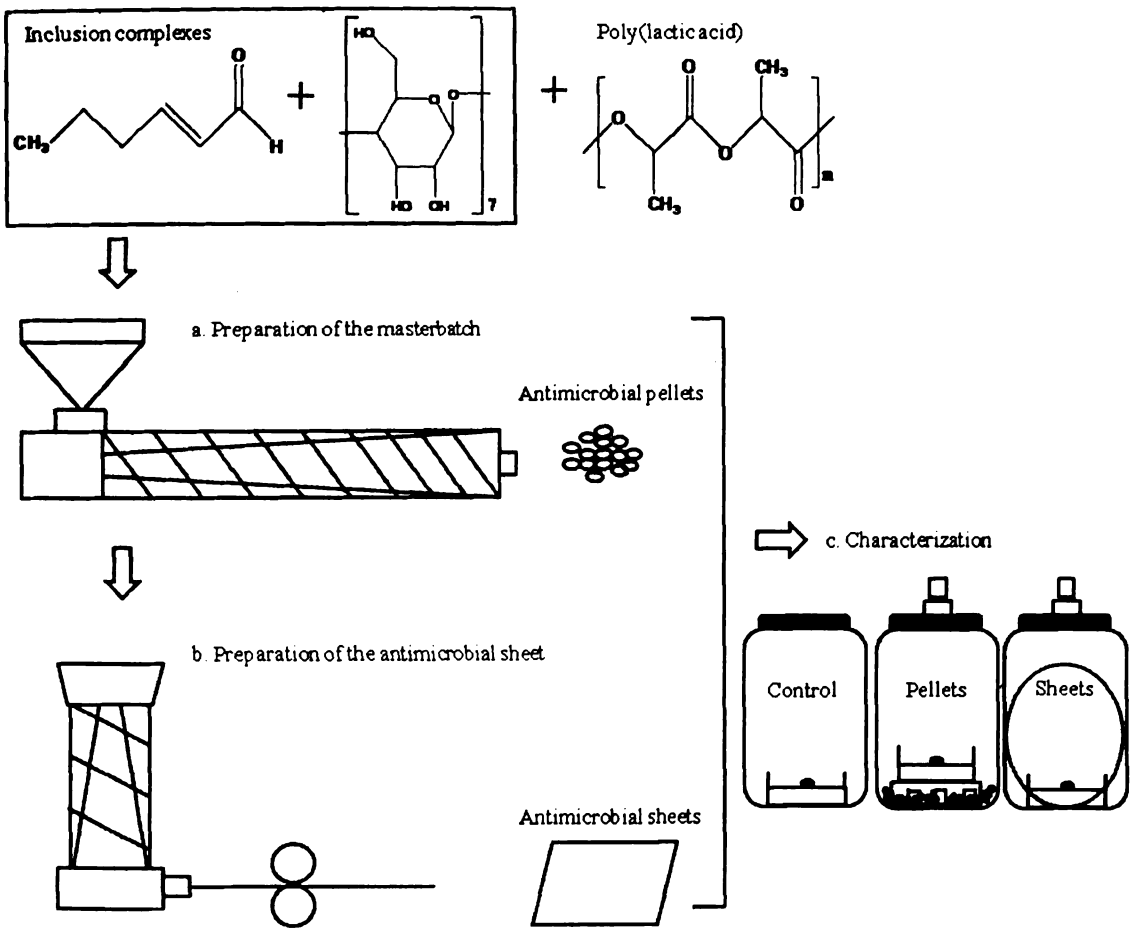

Figure 18. Processing and characterization of pellets and antimicrobial sheets

2.6 Antimicrobial effectiveness of ICs, pellets and sheets. Culture preparation and bioassay (figure 18c): Five kinds of fungi: *Alternaria Solani, Aspergillus niger, Botrytis cinerea, Colletotrichum acutatum, Penicilium spp.* were cultured on Potato Dextrose Agar (PDA) (Sigma-Aldrich Corp. Saint Louis, MO, USA) in plastic petri dishes (Ø 9 cm) for 14 days at 23 °C. An amount of 10mL of sterile distilled water and of 1 mL of glycerol were added to each petri dish in order to allow the removal of mycelia by means of a spatula. Each conidial suspension was transferred to a plastic tub and then vigorously shaken to dislodge the spores from the mycelia. The number of spores was concentrated to 1 x 10^6 spores/mL using the Neubauer improved method (Bright-Line Hemacytometer,

Hausser Scientific, Horshan, PA, USA). Each conidial suspension was divided into several microtubes and then frozen in a refrigerator.

For the bioassay, conidial suspensions were defrozen and then an amount of 3.5 μL of each conidial suspension was placed in the center of a small petri dish (Ø 5.5 cm) containing PDA using a 100 μL Oxford autoclavable Benchmate pipet (Nichiryo, Japan). The petri dishes were then placed inside 500 mL sterile glass jars, which were closed with screw caps and stored at 23 °C. For the control bioassay systems, regular screw caps were used. For treatment bioassay systems, modified caps were used to allow the withdrawal of the volatile [12]. Petri dishes were placed on supports made from their own lids but perforated to allow volatile flow. The supports were inserted into the jars prior to the petri dishes. Treatment bioassays systems were divided into three groups. One group was used to test the ICs, another to test the antimicrobial masterbatch, and the remaining one to test the antimicrobial sheets. The amount of ICs (4 g) added to the bioassay was determined based on previous microbial studies using pure trans-2-hexenal (data not shown) and on the amount of volatile released from the ICs during the characterization process (see above). To test the antimicrobial masterbatch pellets and sheets, same amount as that of ICs was placed inside the jars. This reduced the amount of ICs to 1.2, and 0.6 g for pellets and sheets, respectively. In all treatments, the volatile was released due to the rich humidity environment generated by the petri dish in the closed system. Controls and treatments were stored at 23 °C. Three bioassays systems were tested per fungus and per treatment.

Measurement of fungal growth and trans-2-hexenal levels: Growth of the cultures in both control and treatment bioassay systems was evaluated daily over a 7 day period at 23 °C

by measuring the diameter of the colony on the agar surface. Measurement of diameters was made using a conventional ruler and reported in centimeters. Because of the optical transparency of both the glass and Petri dish, these measurements could be made without opening the jars. Petri dishes with no fungal growth after exposure to trans-2-hexenal were moved from the bioassay systems and transferred to a new bioassay system containing a headspace atmosphere free of volatile. Prior to their insertion into the jars, the growth-free Petri dishes were modified by transferring 5 mm agar plugs from the center of the plate to new Petri dishes containing fresh agar and then inserted into the new jars. Radial growth of the cultures was evaluated over a 7 day period at 23 °C as mentioned above.

The levels of trans-2-hexenal released from ICs, materbatch pellets, and sheets were determined by using a SPEM fiber and gas chromatography as reported above.

2.7 Characterization of the antimicrobial sheet. Physical, mechanical, and barrier properties of the antimicrobial sheet and the plain PLA sheet were measured as follows (figure 18c):

Thermal transitions of the neat PLA sheet and the antimicrobial sheet were determined using a TA Instruments Q100 V 9.8 Differential Scanning Calorimeter (TA instruments, New Castle, DE, US). The temperature calibration of the equipment was performed in accordance with ASTM E967-03 [119] and the heat flow calibration was performed in accordance with ASTM E968-02 [120]. Transition glass temperature (T_g), and melting temperature (T_m) were measured and calculated in accordance with ASTM D3418-03 [148]. The degree of crystallinity (%X_c) for neat PLA and antimicrobial pellets and sheets was calculated as follows [79]:

$$\%\chi_c = \left(\frac{\Delta H_m + \Delta H_c}{93 \times X_{PLA}} \right) \times 100$$

where ΔH_m, ΔH_c, and X_{PLA} are the melting enthalpy, crystallization enthalpy, and PLA content, respectively. A value of 93 J/g for the melting enthalpy for 100% crystalline PLA was obtained from the literature [122].

An amount between 7-9 g of PLA and PLA/β-CDs sheets was used for each experience. Samples were heated from 40 to 190 °C at a rate of 10 °C/min. The β-CD content was taken into consideration when determining $\%X_c$. Three replications of each type of sheet were tested. In the calculation of the crystallinity of the blends only PLA was considered since PLA was the main load-bearing phase.

Neat and antimicrobial PLA sheets were maintained in a in a desiccator at 23 °C until use and then shaped into specimens with a width of 1 in and a length of 8 in prior to use. The thickness of the specimens (0.18 mm) was determined by averaging of three readings taken at different points. The tensile properties: tensile strength, elongation at break, and modulus of elasticity were measured according to ASTM D882-02 [118], using a Instron Universal Testing Machine (Model 5565, Instron, Norwood, MA, US) with a crosshead speed of 0.5 in/min, and a gage length of 5 in (ASTM D882-02 [118]). Tensile properties of neat and antimicrobial sheets PLA was determined from an average of 10 tests at 23°C and 50%RH. Ten replications of each type of sheet were tested.

Permeation cells made from poly(methylmethacrylate) were used to determine gravimetrically by using a modification of ASTM Standard Method E 96-80 [149] the

water vapor transmission rate (WVTR) of neat and antimicrobial PLA. Cells were filled with desiccant and placed in an environmental chamber set at 37.8°C and 85% RH. The weights of the cups were recorded daily. Linear regression-derived slopes of the steady state (linear) portion of weight loss versus time curves were used to estimate WVTR. The mean of three replications of each type of sheet were tested and the initial and final stagnant air gap height was used in the calculations. The permeability (kg-m/m^2-s-Pa) was calculated as:

$$P = \frac{WVTR \times L}{AP}$$

where L is the mean film specimen thickness (m) and AP partial water vapor pressure difference (Pa).

2.8 Statistical analysis.

Statistical analysis of the results was performed using a univariate analysis of variance (ANOVA). Means were separated using the Tukey honestly significant difference (HSD) test ($p < 0.05$) and a t-test was used to analyze statistical differences between the sheets in the analytical software SPSS version 15 (SPSS Inc., Chicago, IL, USA). The data were analyzed and graphically plotted using Sigma-plot software version 10 (Systat Software Inc., Richmond, CA).

3. Results and discussion

3.1 Antimicrobial effectiveness of ICs made of β-CD and trans-2-hexenal

Previous studies carried out by this research group have shown the effectiveness of pure trans-2-hexenal against the fungi *Alternaria solani, Botrytis cinerea, Colletotrichum acutatum, Penicillium* sp., and *Aspergillus niger* [150]. Based on these results and on the characterization of the release of trans-2-hexenal from ICs, 4 g of ICs were calculated to release enough trans-2-hexenal into a volume of 0.5 L of air (bioassay system) to prevent the growth of all the fungi listed above. The high relative humidity necessary to release the trans-2-hexenal from the ICs was provided by the culture media enclosed in the bioassay system.

Effectively, 4 g of ICs generated a concentration of approx. 1 μL trans-2-hexenal/L air figure 4 (panel on the right) which was sufficient to avoid fungal development over a seven day storage period at 23°C (figure 4, panel on the left). This occurred in all bioassay systems but the ones containing *Penicillum* sp where a lower trans-2-hexenal concentration was observed. This was possibly due to the higher affinity of this volatile for the cell membrane of the *Penicillium* sp. spores. Inouye et al (2001) showed that different essential oils have a different gaseous contact with bacteria depending on their permeability and/or interaction with the cell membrane [151]. In agreement, Almenar et al reported that toxic volatile molecules have different affinities to different fungal cell membranes [11]. Independently of the initial trans-2-hexenal concentration, the volatile decreased in all bioassays due to its absorption by the culture medium as has been reported to happen for other naturally occurring volatiles like acetaldehyde and hexanal

[11-12]. Remaining concentrations of trans-2-hexenal in the bioassay systems were similar for all fungi (figure 4, panel on the right).

The antimicrobial effect of ICs of trans-2-hexenal has been investigated earlier [36]. Trans-2-hexenal at a concentration of 30 μg/mL showed a bactericidal effect against *Staphylococcus aureus* IFO12732. The results of this study cannot be compared with our results directly since the volatile was encapsulated into a different type of CDs, and only the bactericidal capacity was investigated. ICs of other naturally occurring volatiles also have been tested against fungal growth. An amount of 1.2 g of ICs of hexanal was tested against *Colletotrichum Acutatum*. Since fungal growth was observed, it was concluded that the amount of IC necessary to prevent all growth of this fungus is higher than 1.2 g [12]. An amount of 0.7 g of ICs of acetaldehyde was tested against *Alternaria alternata*. No fungal growth was observed over a seven day storage period at 23°C [11]. In our study, the remaining amount of trans-2-hexenal in the headspace of the bioassay systems was lower than those reported for hexanal and for acetaldehyde. This is possibly explained by the higher affinity of trans-2-hexenal to the fungal membrane. A higher effectiveness has been reported for trans-2-hexenal than for hexanal or for acetaldehyde and the volatile effectiveness has been related to the affinity of the volatile to the cell membrane [11].

The fungicidal and/or fungistatic effect of trans-2-hexenal was investigated and the results are presented in Figure 19 (panel on the left). Trans-2-hexenal is considered fungistatic if growth of the fungus occurred after it was transferred from the bioassay system containing the volatile to media in a jar free of the volatile. If no growth is observed after transfer to an empty jar, then it is considered fungicidal. Figure 19 (panel

on the left) shows that trans-2-hexenal had fungicidal activity against the five fungi tested. Therefore, trans-2-hexenal can be considered fungicidal at a concentration as low as 1 μL trans-2-hexenal/L air. Higher values have been reported in the literature for other aldehydes in model systems. Hexanal, at a concentration of 3.3 μL/L air had a fungicidal effect against *C. acutatum* but not against *B. cinerea* [12]. In real systems, trans-2-hexenal has been shown more effective against fungal and bacterial growth than other naturally occurring volatiles. An amount of 20 μL/L of trans-2-hexenal proofed bactericidal for *Listeria monocytogenes* inoculated fresh-cut apples, while 150 μL/L of hexyl acetate were necessary to achieve the death of this fungus. These results show that trans-2-hexenal is more effective against major postharvest pathogens than other naturally occurring volatiles. In Figure 4 (panel on the left) is also observed that controls showed different fungal growth patterns after these were transferred to media in a jar free of the volatile. The difference resulted from the fact that the initial spores were 14 day old while the spores in the plugs transferred from one bioassay to another contained spores only 7 days old, and this affects the germination process.

It has been reported that the antifungal activity of aroma compounds is dependent on their vapor pressure rather than their concentration in the system which means that factors such as temperature which are able to increase the vapor pressure of these substances can enhance their antimicrobial activity [39]. Therefore, further research is necessary to find key factors able to increase the vapor pressure and therefore the antimicrobial activity of ICs of trans-2-hexenal.

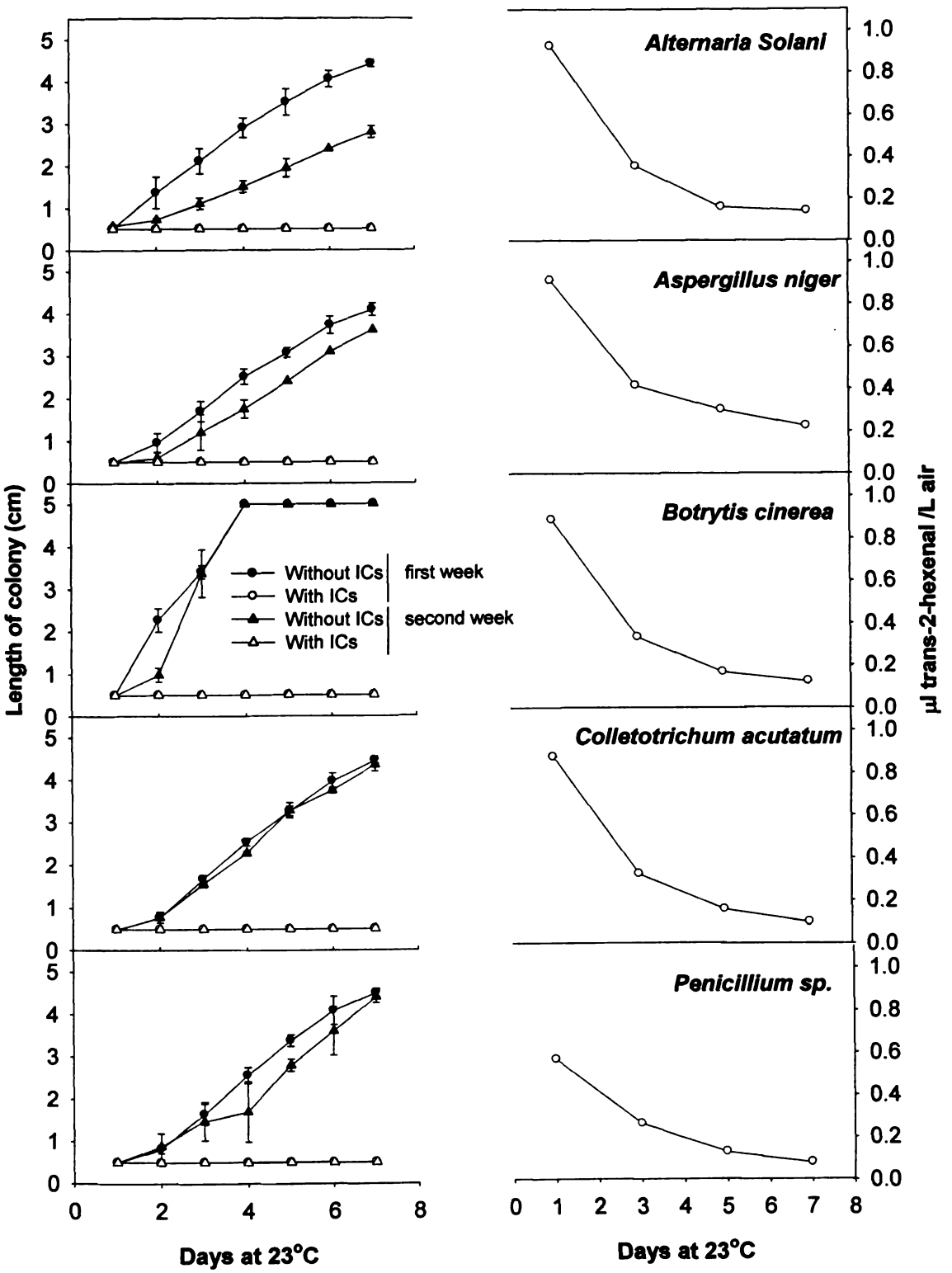

Figure 19. Left: (-o-) Inhibition of the growth of the postharvest decay fungi

Figure 19 (continued). *Alternaria solani*, *Aspergillus niger*, *Botrytis cinerea*, *Colletotrichum acutatum* and *Penicillium sp.* during exposure to trans-2-hexenal released from ICs over a seven day storage period at 23°C. (-●-) Growth rate of the same fungi without exposure to trans-2-hexenal over a seven day storage period at 23°C (controls). (-Δ-) Inhibition of the growth of the same fungi after those being transferred to trans-2-hexenal-free atmosphere bioassay systems in new media over a seven day storage period at 23°C. (-▲-) Growth rate of the controls after those being transferred to an trans-2-hexenal-free atmosphere bioassay systems in new media over a seven day storage period at 23°C. Right: (-Δ-) Concentration of trans-2-hexenal released from ICs over a seven day storage period at 23°C.

Scanning electron micrographics of the surface of fractured β-CD and ICs during their synthesis (with or without trans-2-hexenal), and after release of trans-2-hexenal were taken to better understand the interaction between β-CDs and trans-2-hexenal. All samples were prepared in the same way, and all micrographics were taken under the same conditions. The results are shown in Figure 20. Panel (a) shows β-CDs, while panels (b), (c), and (d) show the micrographics for ICs during their synthesis (with or without trans-2-hexenal) and after the release of trans-2-hexenal. β-CDs are naturally of the size of Å, which is below the resolution of SEM with a magnification of x500. Since the molecules shown in panel (a) are much larger than the individual β-CDs, it can be concluded that β-CDs occur in nature as conglomerates and not as a single molecule. This might cause due to the surrounding relative humidity which interacts with the single molecules and helps to form a more complex structure. Comparing panel (a) with panels (b) and (c) reveals

that in presence of water the β-CDs form a much larger conglomerate which seems to have a more crystal-like structure. If panels (b) and (c) are compared, panel (b) shows more similarity to the β-CDs conglomerates observed in panel (a) than can be found in panel (c). Therefore, the complexation of the trans-2-hexenal into the β-CDs results in a more pronounced change in the structure of the conglomerate, towards a more crystal-like structure. This agrees with Szejtli (1998) who reported that formed inclusion complexes can be isolated as stable crystalline substances [152]. Panel (d), which shows the ICs after the release of the entrapped volatile, illustrates the reduction of the size of the conglomerate. This reduction may be due to the breakdown of the crystalline structure when exposed to relative humidity and two possible reasons could be given: (1) the water interacts with the crystal and breaks it down, or (2) the release of the volatile creates a less stable structure which breaks down. Further studies such as X-ray diffraction are necessary to verify the crystal sizes.

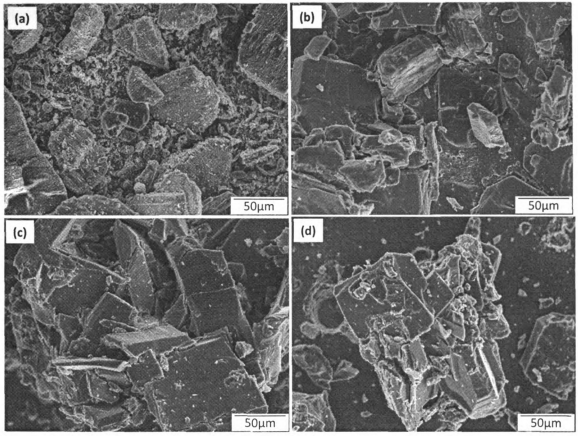

Figure 20. Scanning electron micrographics of the surface of fractured β-CDs and ICs. Panel (a) shows β-CDs, (b) shows β-CDs after simulating the IC synthesis but without using trans-2-hexenal, (c) shows ICs after complexation with trans-2-hexenal, (d) shows ICs after release of trans-2-hexenal (24 hrs exposed to 100% RH).

3.2 Antimicrobial effectiveness of pellets and sheets made of PLA and β-CD-trans-2-hexenal

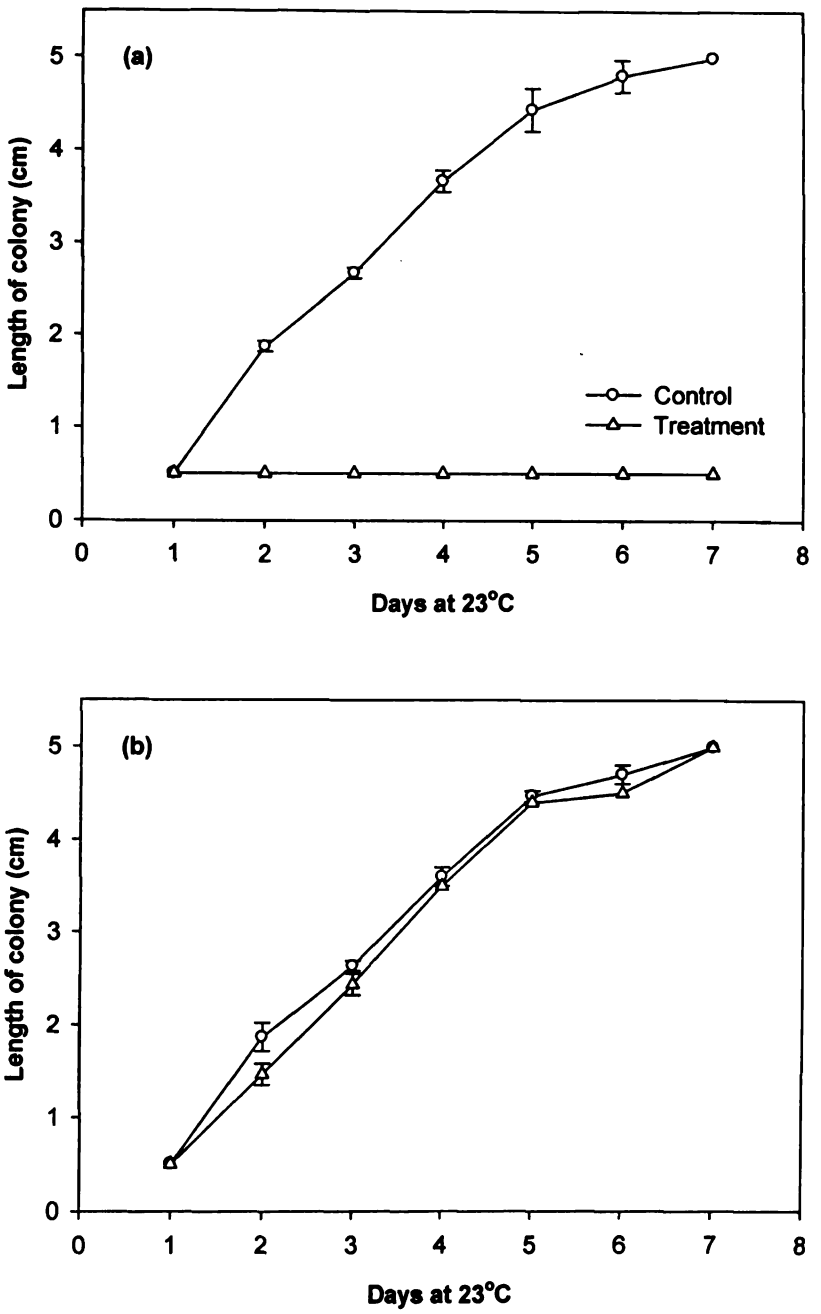

Figure 21. Colony diameter of *Alternaria Solani* cultures in the presence (-Δ-) and absence (-o-) of trans-2-hexenal released from antimicrobial pellets (a) and sheets (b) during a 7 days storage period at 23°C .

Since *Alternaria solani* was the most sensitive to trans-2-hexenal of the five fungi listed above (data not shown), the antimicrobial effectiveness of PLA/β-CD-trans-2-hexenal pellets and sheets was determined for this fungus only. Figure 21 shows the colony diameter of *Alternaria Solani* cultures in the presence and absence of trans-2-hexenal released from antimicrobial pellets (a) and sheets (b) during a 7 days storage period at 23°C. As is evident from panel (a), PLA/β-CD-trans-2-hexenal pellets inhibited completely the growth of *Alternaria Solani* during 1 week at 23°C while panel (b) shows that the PLA/β-CD-trans-2-hexenal sheet only delayed the *Alternaria* growth. The complete inhibition of growth due to the release of trans-2-hexenal from pellets is visualized in figure 22. The concentration achieved in the sealed jars containing antimicrobial pellets after 24 hrs was 0.08μL/L air (figure 23). Therefore, a concentration as low as 0.08 μL/L air can suppress *Alternaria* growth during at least 1 week at room temperature. This concentration is much lower than that reported above as effective for trans-2-hexenal but this is because the above reported IC amount was chosen high enough to inhibit all five fungi. *Alternaria* growth in the presence or absence of the antimicrobial sheet showed statistical differences until day 3. The fungal growth was reduced by 21% at day 2 and by 7% at day 3. The different antimicrobial effect between pellets and sheets was due to the different amounts of trans-2-hexenal released from the polymeric matrixes (figure 23). This difference was expected since the pellets contained twice the amount of ICs than the sheets (the pellets were used as a masterbatch to form the antimicrobial sheet). However, the concentration of trans-2-hexenal achieved in the bioassay system containing the antimicrobial sheet was four orders of magnitude lower than the expected level of 50% of the value achieved using the antimicrobial pellets. The

different concentrations of trans-2-hexenal released from pellets and from sheets were also caused by the fact that pellets were obtained using less processing (no casting process was involved since no sheet was formed). It has been reported that ICs are sensitive to external stimuli, such as temperature changes, shear forces, and the presence of competing CD-binding molecules [91, 144]. Therefore, the exposure of the ICs to high heat and relative humidity during processing caused a premature loss of the antimicrobial compound encapsulated in the β-CD molecules for later release, and resulted in a reduced antimicrobial activity. In agreement, Han et al (2003) reported that the antimicrobial capacity of the polymer may change during processing [7].

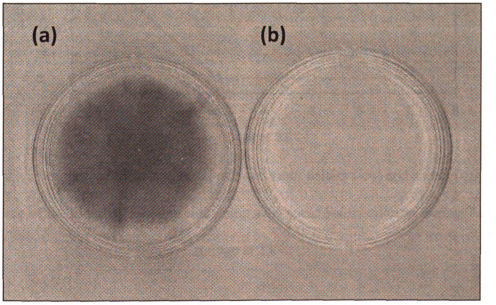

Figure 22. *Alternaria Solani* in the absence (a) and presence (b) of antimicrobial pellets after 7 days at 23°C.

The initial concentrations reached in the bioassay systems containing sheets or pellets decreased during storage due to the absorption of the antimicrobial volatile by the culture medium as has been reported above to happen for the trans-2-hexenal released from ICs.

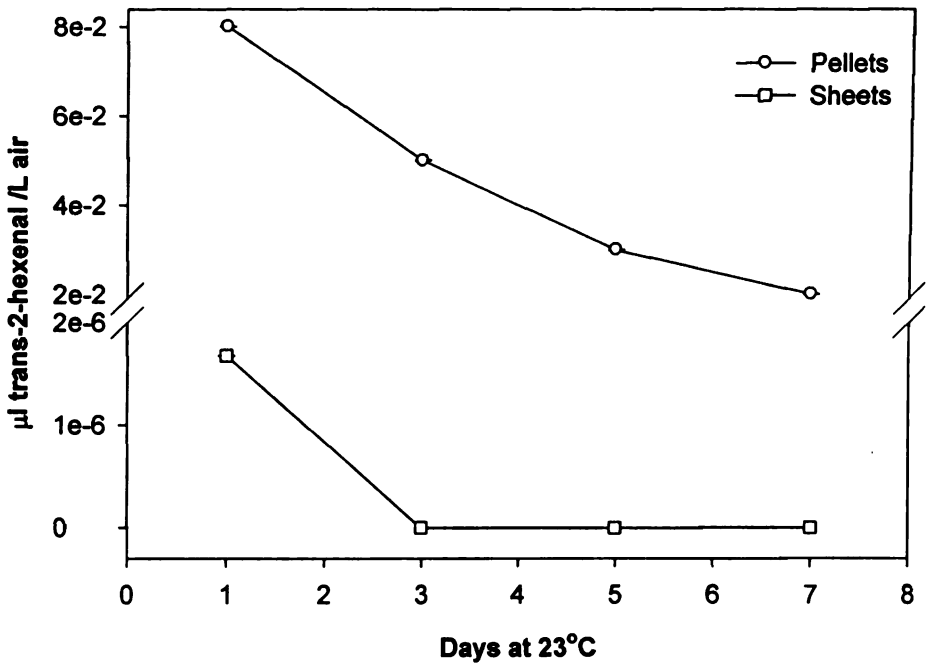

Figure 23. Evolution of trans-2-hexenal released from pellets (-○-) and sheets (-□-) in the headspace of the bioassay systems containing *Alternaria Solani* cultures during 7 days of storage at 23°C.

The release of antimicrobial compounds from a polymer can be regulated by controlling the rate of wall solubility, the swelling of the wall material, pH effects, or changes in the ionic strength of the surrounding medium [153]. More research is required to better understand the effect of processing and/or the better incorporation of trans-2-hexenal in the PLA matrix by using CDs.

3.3 Physical, mechanical, and barrier characterization of the antimicrobial sheet

The antimicrobial sheet was characterized by measuring its physical, mechanical, and barrier properties. The neat PLA sheet was used as a control. The results are presented in table 7. The DSC data shows slightly lower glass transition and melting temperatures for the antimicrobial sheet than for the neat PLA sheet (approx. 1.5 and 2 °C for T_g and T_m, respectively) but there were no statistical differences between both sheets. Byrne et al. (2009) observed no effect of the addition of several commercial masterbatches on the T_g and T_m of the PLA either [87]. The antimicrobial sheet and the neat PLA sheet showed the same crystalline content in spite of β-CDs having been reported to play a role as nucleating agents in PLA [128]. In contrast, Joo et al. (2010) observed an effect on all the thermal properties mentioned above when a masterbach formed from PLA containing high β-CD content was blended with PLA [147]. These results may differ due to the change in morphology of the β-CDs after the encapsulation of the antimicrobial as can be observed in Figure 20, panels (a) and (c), or that the presence of the antimicrobial compound affected the crystal growth during processing.PLA crystallinity has been also reported to increase when the PLA has been blended with other molecules similar to CDs like starch [88, 128-129].

The mechanical properties tensile strength (machine direction (MD) and cross-machine direction (CD)), modulus of elasticity, and elongation at break of the neat PLA sheet and the antimicrobial sheet are also presented in Table 7. There were significant differences in the tensile strength and the modulus of elasticity but not in the elongation at break between the neat PLA sheet and the antimicrobial sheet. The antimicrobial sheet showed about 50% smaller values in these properties which were caused by the filler effect of the

β-CDs in the PLA matrix as reported by Joo et al. (2010). The presence of the β-CDs produced the discontinuity between the PLA chains caused by the immiscibility between PLA and β-CDs. Similar results have been reported also for blends of PLA and starch [154]. The decrease in the tensile strength of PLA by adding a masterbatch has been previously reported iny the literature. Byrne et al. [87] reported a reduction in 3-5% of the tensile strength of the PLA by the addition of 4 different commercial masterbatches. The water vapor permeability coefficient (WVPC) of the antimicrobial sheet and of the neat PLA sheet was measured using permeation cells. The permeability coefficients were calculated as $5.0\pm0.4\times10^{-15}$ kg m/m^2 s Pa for the neat PLA sheet and $4.8\pm0.7\times10^{-14}$ kg m/m^2 s Pa for the antimicrobial sheet. These WVPC are close to the values 2.4×10^{-15} kg m/m^2 s Pa and 7.1×10^{-15} kg m/m^2 s Pa reported by Joo et al. (2010) for neat PLA and for PLA blended with β-CDs using a masterbatch. The WVPC of the neat PLA was lower than the value $1.5\sim2.2\times10^{-14}$ kg m/m^2 s Pa reported in the literature for neat PLA at 10 to 37.8°C and 40 to 90% RH [76]. This might be due to differences in thickness, processing history, and other parameters between these neat PLA samples. The permeability of the antimicrobial sheet was higher than that of the neat PLA sheet due to the immiscibility between phases and the resulting gaps. Similarly, it has been reported that water can penetrate faster in PLA/starch blends than in neat PLA through the voids between phases [79]. The antimicrobial sheet had a higher WVPC than the sheet resulting of blending PLA with β-CDs using a masterbatch reported by Joo et al. (2010), and this was caused by less adhesion between ICs and PLA than between β-CDs and PLA. This is due to differences in structure between ICs and β-CDs as shown in figure 20. Further research

needs to be done for a better understanding of the effect of the volatile on the characteristics of the polymeric matrix.

Table 7. Characterization of the neat PLA sheet and the antimicrobial sheet

Property	Type	Neat PLA sheets	Antimicrobial sheets
Thermal	T_g	55.2±2.0 a	53.4±1.0 a
	T_m	143.4±0.3 a	141.6±0.8 a
	%χ	1.2±0.9 a	1.7±1.5 a
Mechanical	Tensile strength (MPa)-MD	55.6±4.5 a	17.6±2.2 b
	Tensile strength h(MPa)-CD	54.1±4.6 a	16.8±1.4 b
	Elongation at break (%)-MD	1.0±0.2 a	0.6±0.1 b
	Elongation at break (%)-CD	0.8±0.2 a	0.5±0.4 b
	Modulus of elasticity (GPa)-MD	10.0±0.7 a	5.4±0.7 b
	Modulus of elasticity (GPa)-CD	10.1±0.5 a	5.7±0.4 b
Barrier	Water vapor permeability (kg m/m^2 s Pa)	5.0±0.4^x a	4.8±0.7^y b

a and b indicate statistical differences($P<0.05$) between neat PLA and antimicrobial sheets.

$^x \times 10^{-15}$

$^y \times 10^{-14}$

4. Conclusions

Enzymatically modified starch containing trapped trans-2-hexenal is an effective tool to control major postharvest pathogens. Its incorporation in the PLA polymeric matrix yields bio-based antimicrobial polymers. The addition of the antimicrobial didn't affect the physical properties of the polymer matrix but did affect the mechanical, and barrier

ones. The exposure of the enzymatically modified starch containing trapped trans-2-hexenal to high heat and relative humidity during processing caused a premature loss of the antimicrobial compound, and resulted in a reduced antimicrobial activity of the antimicrobial polymer.

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CHAPTER 5. CONCLUSIONS

A bio-based polymer made of PLA and β-CDs with the capacity to carry hydrophobic molecules has been developed and characterized. Testing of mechanical, thermal, barrier, optical, and morphological properties revealed that β-CDs and PLA are incompatible. The higher the β-CD content, the higher the incompatibility due to the increase in size of the β-CDs conglomerates, and the poor adhesion between phases. The use of a masterbatch (PLA containing high β-CD content) is an effective way to improve the compatibility between PLA and β-CDs, and the properties of PLA/β-CDs blends. The use of the masterbatch reduced the stiffness and brittleness of the PLA/β-CDs blend and increased their barrier properties.

Enzymatically modified starch (β-CDs) containing trapped trans-2-hexenal (ICs of trans-2-hexenal) is an effective tool to control major postharvest pathogens. Its effectiveness has been confirmed against 5 main postharvest fungi. Its incorporation in the PLA polymeric matrix yields bio-based antimicrobial polymers (pellets and sheets). Antimicrobial pellets (30% of ICs) showed fungicidal activity against *Alternaria Solani* and antimicrobial sheets delayed *Alternaria Solani* growth during day 3. The addition of the antimicrobial didn't affect the physical properties of the polymer matrix but did affect the mechanical, and barrier ones. The exposure of the enzymatically modified starch containing trapped trans-2-hexenal to high heat and relative humidity during processing caused a premature loss of the antimicrobial compound, and resulted in a reduced antimicrobial activity of the antimicrobial polymer (90%).

Future work

- To improve the understanding of the encapsulation mechanism and of the structure of the ICs (β-CD-trans-2-hexenal-water) and to correlate these data with the effect of humidity and temperature on the releasing process.
- Release kinetics of the ICs of trans-2-hexenal, pellets, and sheets as a function of temperature and relative humidity
- Effect of the presence of the antimicrobial compound on the compostability of the PLA/ β-CDs blends.
- Shelf life studies of the antimicrobial pellets and sheets (the antimicrobial agent remains above the critical inhibiting concentration).
- To explore potential commercial applications of the new bio-based antimicrobial materials (pellets and sheets).

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