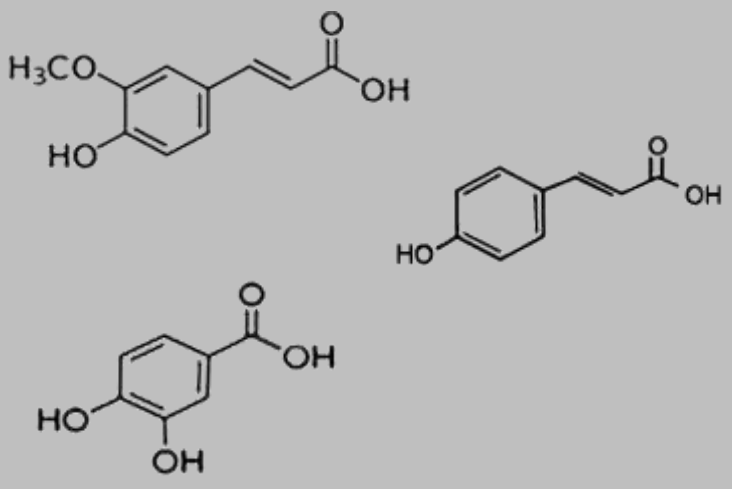
DOCTORAL THESIS

UNIVERSITAT POLITÈCNICA DE VALENCIA

BIODEGRADABLE MULTILAYER FILMS FOR ACTIVE FOOD PACKAGING, BASED ON STARCH AND POLYESTERS WITH PHENOLIC ACIDS



Presented by:

Eva Hernández García

Supervisors:

Amparo Chiralt Boix

María Vargas Colás

Valencia, February 2022

DOCTORAL THESIS

BIODEGRADABLE MULTILAYER FILMS FOR ACTIVE FOOD PACKAGING, BASED ON STARCH AND POLYESTERS WITH PHENOLIC ACIDS

UNIVERSITAT POLITÈCNICA DE VALENCIA

Instituto Universitario de Ingeniería de Alimentos para el Desarrollo

Presented by:

Eva Hernández García

Supervisors:

Amparo Chiralt Boix

María Vargas Colás

Valencia, February 2022

Dra. Amparo Chiralt Boix, Catedrática de Universidad, perteneciente al Departamento de Tecnología de Alimentos de la Universitat Politècnica de València (Valencia, España).

Dra. Maria Vargas Colás, Profesora Titular de Universidad, perteneciente al Departamento de Tecnología de Alimentos de la Universitat Politècnica de València (Valencia, España).

Hacen constar que:

La memoria titulada "**BIODEGRADABLE MULTILAYER FILMS FOR ACTIVE FOOD PACKAGING, BASED ON STARCH AND POLYESTERS WITH PHENOLIC ACIDS"** que presenta Dª **Eva Hernández García** para optar al grado de Doctor por la Universitat Politècnica de València, ha sido realizada en el Instituto Universitario de Ingeniería de Alimentos para el Desarrollo (IuIAD – UPV) bajo su dirección y que reúne las condiciones para ser defendida por su autora.

Valencia, febrero de 2022

Fdo. Amparo Chiralt Boix

Fdo. María Vargas Colás

A mi familia, lo mejor de mi vida

Y en especial a mis padres, mi hermano, mi sobrina y mi abuela Pilar

AGRADECIMIENTOS

Antes de comenzar este trabajo de Tesis Doctoral no puedo dejar pasar la oportunidad de manifestar mi gratitud a las personas que, de muy diversa manera, han contribuido a hacerlo realidad.

En primer lugar, me gustaría dar las gracias a mis directoras, **Amparo Chiralt** por darme la oportunidad de formar parte de este grupo de investigación tan prestigioso, por toda su dedicación y trabajo durante estos tres años y a **María Vargas** por su gran ayuda, paciencia y por confiar en mí. Siempre cercana y disponible. También quiero dar las gracias a **Chelo** por toda la dedicación y ayuda recibida y por haberme dejado impartir docencia en una de sus asignaturas.

Gracias a todas las personas que han formado parte del "Laboratorio de films", en especial a **Johana, Ramón, Pedro, Carla, Eva, Emma, Alina, Mayra** y **Sofía.** A **Johana**, **Ramón** y **Pedro** muchas gracias por estar siempre dispuestos a ayudar y por nuestra gran amistad. A **Carol** por su apoyo recibido.

***

Gracias al Ministerio de Ciencia, Innovación y Universidades del Gobierno de España por el apoyo económico a través del contrato predoctoral FPI: BES-2017-082040.

***

La distancia me enseñó que el corazón es más fuerte que los kilómetros. Muchas gracias a mi mayor tesoro, mi familia. Gracias a mis tías **Tere, Mavy, Lola** y **Pili**, a mis abuelos y abuelas, a mis primos/as y a mi cuñada **Irene**.

Gracias a mi abuela **Pilar** "Pitu". Esta Tesis va dedicada a ti allá donde estés. Siempre serás mi referente como madre y mujer trabajadora del mundo rural. Gracias por haber podido disfrutar de ti hasta los 102 años y por todos los grandes momentos vividos a tu lado.

Muchas gracias a mi gran referente, mi hermano **Víctor**. Siempre he estado y estaré muy orgullosa de todo lo que has conseguido a base de esfuerzo, pasión, trabajo y dedicación Doctor Víctor. Eres mi fuente de admiración y te doy las gracias por haberme dado a una sobrina tan maravillosa. **Ainhoa**, te quiero mucho.

Pero en especial a mis mayores tesoros, **María Benita** y **Nicolás**, mis padres. Muchas gracias por haberme forjado como la persona que soy en la actualidad; todos mis logros os los debo a vosotros entre los que se incluye este. Me habéis educado en un entorno de valores, en el que la educación, el cariño, la humildad, la solidaridad, el trabajo, la constancia y el esfuerzo son clave. Siempre me habéis apoyado de forma incondicional en todos los aspectos de mi vida y entre ellos está mi constante lucha con la Dermatitis Atópica y las alergias. Sois lo mejor de mi vida y mis referentes como grandes personas, siempre humanos, solidarios, trabajadores incansables y luchadores en el medio rural. Al igual que mi hermano y mi sobrina, sois lo mejor de mi vida. ¡Este trabajo es gracias a vosotros!

¡A todos, muchísimas gracias!

***

Con esta tesis culmino una etapa formativa de mi vida. El doctorado ha sido una experiencia muy enriquecedora, que me ha permitido crecer tanto a nivel académico como personal, desarrollando habilidades muy valiosas para mi futura vida profesional.

ABSTRACT

Active biodegradable multilayer films have been developed by thermoprocessing for food packaging purposes, combining improved starch films and a blend of polyesters (PLA-PHBV), with different phenolic acids (ferulic, *p*-coumaric and protocatechuic).

Into the cassava or maize starch films, gums of microbial origin (xanthan and gellan) were incorporated (10%) to improve their functional properties. The gums improved the mechanical and barrier properties to water vapor and oxygen of the starch films. These films were combined with PLA:PHBV blend films in starch-polyester bilayers by thermocompression. The bilayers exhibited high barrier capacity to oxygen and water vapor compared to their respective monolayers. The polyester layer contributes to the mechanical reinforcement of the bilayer, providing high water vapor barrier capacity, while the starch layer provided high oxygen barrier capacity to the bilayer. The bilayer with cassava starch and gellan gum showed the best interlayer adhesion, with adequate functional properties for food packaging applications.

Ferulic, *p*-coumaric and protocatechuic acids, with antimicrobial and antioxidant properties, were incorporated (2%) in the PLA: PHBV blend films to obtain active films. Phenolic acids positively modified the properties of the polyester blend, increasing its elastic modulus and resistance to break and its barrier capacity to water vapor and oxygen, while slightly increasing the Tg of the material. Protocatechuic acid caused the greatest effects, affecting the crystallization of PHBV. The release of these compounds in different food simulants (with high and intermediate polarity) was very limited in terms of release rate and released amount, which reduced the ability of the films to significantly inhibit the growth of *Listeria innocua* inoculated in culture medium. These films, with and without active compounds, disintegrated under composting conditions, without significant effect of phenolic acids. Films without active compounds and with ferulic acid biodegraded completely after 20 days of composting, whereas films containing *p*-coumaric and protocatechuic acids did so in 21 and 26 days, respectively. Therefore, none of the incorporated phenolic acids inhibited the biodegradation process, but the process was delayed, depending on the degree of retention of the compound in the polymeric matrix.

The biodegradable bilayer films with a layer of starch-gellan and another of PLA: PHBV, with and without phenolic acids, were characterized as to their mechanical properties and barrier capacity to water vapor and oxygen and were used for packaging of pork meat whose quality development was analysed throughout storage time at 5 °C. The presence of phenolic acids decreased the elastic modulus and resistance to break of the bilayers and improved their barrier capacity to water vapor and oxygen. The latter, together with the active effect of the acids, contributed to improving the preservation of the meat during storage, reducing the levels of lipid oxidation, changes in pH and weight losses of the packed samples, as well as microbial growth, especially total coliforms and lactic acid bacteria.

Biodegradable bilayer films with phenolic acids, based on starch and polyesters, appeared as a suitable strategy to obtain active packaging materials, with functional properties close to those of some synthetic plastics commonly used in food packaging. These materials can extend the shelf-life of foods, mitigating the environmental impact of plastic packaging since they can be composted.

RESUMEN

Se han desarrollado mediante termoprocesado películas multicapa biodegradables activas para el envasado de alimentos, combinando películas de almidón mejoradas y de una mezcla de poliésteres (PLA-PHBV), con diferentes ácidos fenólicos (ferúlico, *p*-cumárico y protocatecuico).

En las películas almidón de yuca o de maíz se incorporaron gomas de origen microbiano (xantana y gelano) (10%) para mejorar sus propiedades funcionales. Las gomas mejoraron las propiedades mecánicas y de barrera al vapor de agua y al oxígeno de los films de almidón. Estos films se combinaron con films mezcla de PLA:PHBV en bicapas almidón-poliésteres por termocompresión. Las bicapas presentaron una alta capacidad barrera al oxígeno y al vapor de agua comparado con sus respectivas monocapas. La capa de poliéster contribuyó al refuerzo mecánico de la bicapa, aportando alta capacidad de barrera al vapor de agua, mientras que la capa de almidón aportó alta capacidad de barrera al oxígeno a la bicapa. La bicapa con almidón de yuca y goma gelano presentó la mejor adhesión entre capas, con propiedades funcionales adecuadas para el envasado de alimentos.

Los ácidos ferúlico, *p*-cumárico y protocatecuico, con propiedades antimicrobianas y antioxidantes, se incorporaron (2%) en los films mezcla de PLA:PHBV para obtener films activos. Los ácidos fenólicos modificaron positivamente las propiedades de la mezcla de poliésteres, incrementando su módulo de elasticidad y resistencia a la fractura y su capacidad de barrera al vapor de agua y al oxígeno, al tiempo que aumentaron levemente la Tg del material. El ácido protocatecuico provocó los mayores efectos, afectando a la cristalización del PHBV. La liberación de estos compuestos en diferentes simulantes alimentarios (con polaridad alta e intermedia) fue muy limitada en cuanto a velocidad y cantidad liberada, lo que disminuyó la capacidad de las películas para inhibir de forma significativa el crecimiento de *Listeria innocua* inoculada en medio de cultivo. Estos films, con y sin compuestos activos, se desintegraron en condiciones de compostaje, sin efecto significativo de los ácidos fenólicos. Los films sin activos y con ácido ferúlico se biodegradaron completamente después de 20 días de compostaje, mientras que los films que contenían ácido *p*-cumárico y protocatecuico lo hicieron en

21 y 26 días, respectivamente. Por lo tanto, ninguno de los ácidos fenólicos incorporados inhibió el proceso de biodegradación, pero se retardó el proceso, dependiendo del grado de retención del compuesto en la matriz polimérica.

Los films bicapa biodegradables constituidos por una capa de almidón-gelano y otra de PLA:PHBV, con y sin ácidos fenólicos, se caracterizaron en sus propiedades mecánicas y de barrera al vapor de agua y al oxígeno y se utilizaron para el envasado de carne de cerdo, evaluando su calidad durante el almacenamiento a 5 °C. La presencia de ácidos fenólicos disminuyó el módulo elástico y la tensión de fractura de las bicapas y mejoró su capacidad de barrera al vapor de agua y al oxígeno. Esto último, junto al efecto activo de los ácidos, contribuyó a mejorar la conservación de la carne durante el almacenamiento, reduciendo los niveles de oxidación lipídica, cambios de pH y pérdidas de peso de las muestras envasadas, así como el crecimiento microbiano, especialmente coliformes totales y bacterias ácido-lácticas.

Los films bicapa biodegradables con ácidos fenólicos, a base de almidón y poliésteres, se muestran como una estrategia adecuada para obtener materiales de envasado activo, con propiedades funcionales próximas a las de algunos plásticos sintéticos comúnmente utilizados en el envasado de alimentos. Estos materiales pueden alargar la vida útil de los alimentos, mitigando el impacto ambiental de los envases plásticos ya que pueden ser compostados.

RESUM

S'han desenvolupat, mitjançant termoprocesat, pel·lícules multicapa biodegradables actives per a l'envasament d'aliments, combinant pel·lícules de midó millorades i d'una mescla de polièsters (PLA-PHBV), amb diferents àcids fenòlics (ferúlic, *p*-cumàric i protocatecuic).

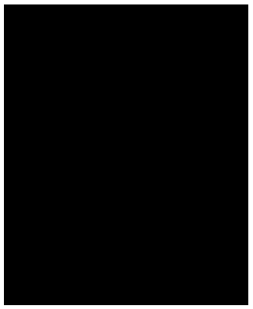
En les pel·lícules midó de iuca o de dacsa es van incorporar gomes d'origen microbià (xantana i gellan) (10%) per a millorar les seues propietats funcionals. Les gomes van millorar les propietats mecàniques i de barrera al vapor d'aigua i a l'oxigen dels films de midó. Aquests films es van combinar amb films mescla de PLA:PHBV en bicapes midó-polièsters per termocompresió. Les bicapes van presentar una alta capacitat barrera a l'oxigen i al vapor d'aigua comparat amb les respectives monocapes. La capa de polièster va contribuir al reforç mecànic de la bicapa, aportant alta capacitat de barrera al vapor d'aigua, mentre que la capa de midó va aportar alta capacitat de barrera a l'oxigen a la bicapa. La bicapa amb midó de iuca i goma gellan va presentar la millor adhesió entre capes, amb propietats funcionals adequades per a l'envasament d'aliments.

Els àcids ferúlic, *p*-cumàric i protocatecuic, amb propietats antimicrobianes i antioxidants, es van incorporar (2%) en els films mescla de PLA:PHBV per a obtindre films actius. Els àcids fenòlics van modificar positivament les propietats de la mescla de polièsters, incrementant el seu mòdul d'elasticitat i resistència a la fractura i la seua capacitat de barrera al vapor d'aigua i a l'oxigen, al mateix temps que van augmentar lleument la Tg del material. L'àcid protocatecuic va provocar els majors efectes, afectant la cristal·lització del PHBV. L'alliberament d'aquests compostos en diferents simulants alimentaris (amb polaritat alta i intermèdia) va ser molt limitada en quant a velocitat i quantitat alliberada, la qual cosa va disminuir la capacitat de les pel·lícules per a inhibir de manera significativa el creixement de *Listeria innocua* inoculada en medi de cultiu. Aquests films, amb i sense compostos actius, es van desintegrar en condicions de compostatge, sense efecte significatiu dels àcids fenòlics. Els films sense actius i amb àcid ferúlic es biodegradaren completament després de 20 dies de compostatge, mentre que els films que contenien àcid *p*-cumàric i protocatecuic ho van fer en 21 i 26 dies, respectivament. Per tant, cap dels àcids fenòlics incorporats va inhibir el procés de

biodegradació, però es va retardar el procés, depenent del grau de retenció del compost en la matriu polimèrica.

Els films bicapa biodegradables constituïts per una capa de midó-gellan i una altra de PLA:PHBV, amb i sense àcids fenòlics es van caracteritzar en les seues propietats mecàniques i de barrera al vapor d'aigua i a l'oxigen i es van utilitzar per a l'envasament de carn de porc, avaluant la qualitat a llarg del emmagatzematge a 5 °C. La presència d'àcids fenòlics va disminuir el mòdul elàstic i la tensió de fractura de les bicapes i va millorar la seua capacitat de barrera al vapor d'aigua i a l'oxigen. Això últim, junt a l'efecte actiu dels àcids, va contribuir a millorar la conservació de la carn durant l'emmagatzematge, reduint els nivells d'oxidació lipídica, canvis de pH i pèrdues de pes de les mostres envasades, així com el creixement microbià, especialment coliformes totals i bacteris àcid-làctics.

Els films bicapa biodegradables amb àcids fenòlics, a base de midó i polièsters, es mostren com una estratègia adequada per a obtindre materials d'envasament actiu, amb propietats funcionals pròximes a les d'alguns plàstics sintètics comunament utilitzats en l'envasament d'aliments. Aquests materials poden allargar la vida útil dels aliments, mitigant l'impacte ambiental dels envasos plàstics ja que poden ser compostats.



PREFACE

DISSERTATION OUTLINE

The Doctoral Thesis has been structured in five sections, as described in Figure 1: **Introduction, Objectives, Chapters, General Discussion** and **Conclusions.**

The **INTRODUCTION** section discusses the state of the art concerning antimicrobial materials based on biodegradable polymers, as well as the influence of the incorporation of these compounds on the biodegradation behaviour of the active materials. All these aspects have been examined in a review entitled "Biodegradable Antimicrobial Films for Food Packaging: Effect of Antimicrobials on Degradation".

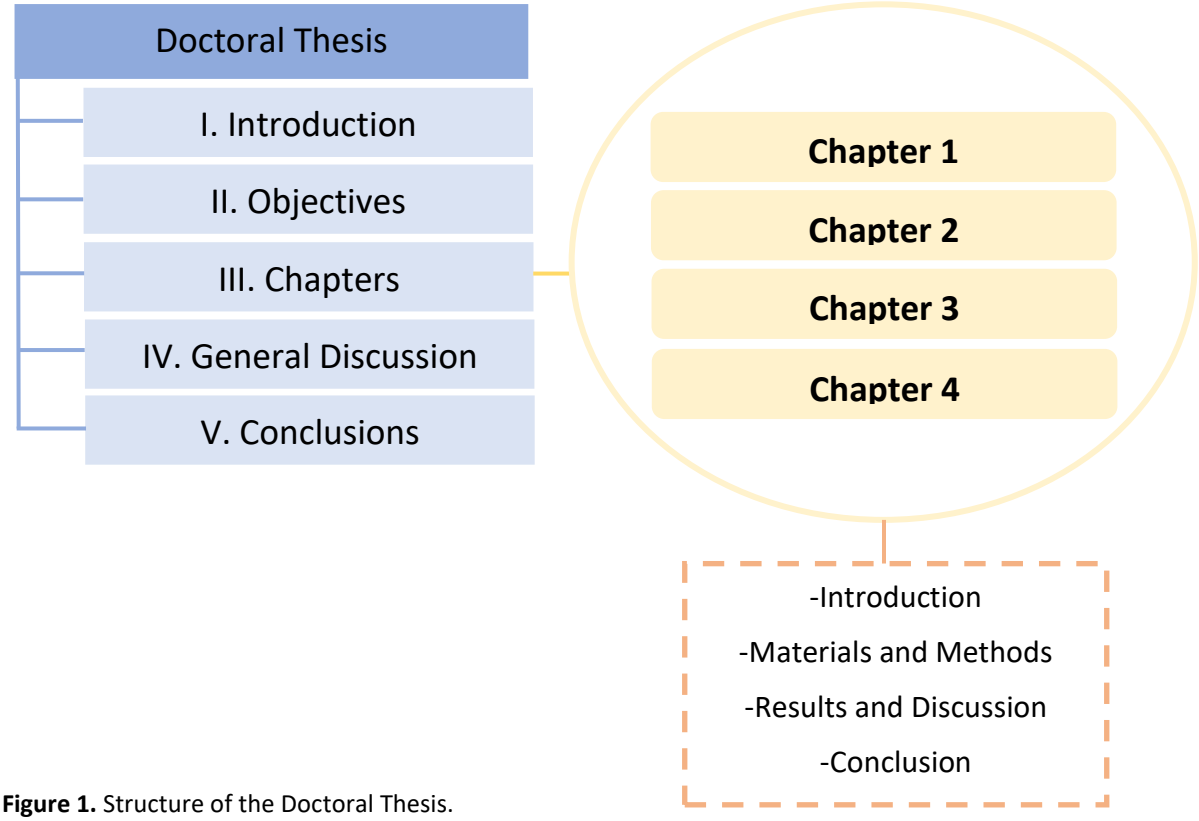


Figure 1. Structure of the Doctoral Thesis.

The **OBJECTIVE** section presents the general and specific objectives of the Thesis, which is focused on the development of biodegradable and active multilayer films based on starch and polyesters (PLA and PHBV) with phenolic acids for food packaging applications.

The obtained results are organized in four **CHAPTERS** as a collection of scientific publications with the usual sections: Introduction, Materials and Methods, Results and Discussion and Conclusion (Figure 1).

Chapter 1, entitled "**Thermoprocessed starch-polyester bilayer films as affected by the addition of gellan or xanthan gum**", aimed to analyse the physical and microstructural properties of the starch films, from maize and cassava, as affected by the xanthan or gellan gum incorporation. Thermo-compression-sealed bilayers of the different starch-based films and PLA-PHBV blend sheets were obtained and analysed in order to select the best combination for food packaging purposes. The results showed that the incorporation of gellan gum and xanthan gum improved the mechanical properties of starch-based films and decreased the water vapour (WVP) and oxygen (OP) permeabilities. Starch based-polyester bilayers presented a high oxygen and water vapour barrier capacity, as compared to their individual monolayers. Bilayer films with cassava starch including the gums showed the lowest OP and WVP values and the highest elastic modulus and tensile strength, with extensibility values in the range of the corresponding monolayers and slight changes in their physical properties throughout time. The bilayer formed with cassava starch with gellan gum and a PLA-PHBV appeared as the best option for food packaging purposes, taking into account its functional properties and the good layer adhesion in the bilayer.

Chapter 2, entitled **"Use of phenolic acids to obtain active films based on PLA- PHBV**", analysed the microstructure, thermal behaviour and functional properties of PLA-PHBV blend films incorporating phenolic acids (ferulic, *p*-coumaric and protocatechuic) as antibacterial compounds, as well as the antibacterial effectiveness of the films against *Listeria innocua*. The release kinetics of the phenolic acids in different food simulants was also analysed. The results showed that phenolic acids provoked an increase in the glass transition temperature of PLA and Polymer matrices with phenolic acids were stiffer and more resistant to break than the polyester blend, but with similar extensibility. Oxygen and water vapour barrier capacity were also improved by phenolic acids, especially for protocatechuic acid. The release rate and ratio of phenolic acids increased when polarity of the food simulant decreases, although very slow delivery was observed in all cases. The incorporation of phenolic acids into the polyester blend films

did not significantly inhibit the growth of *Listeria innocua* due to the limited release of active compounds.

Chapter 3, entitled "**Biodegradation of PLA-PHBV blend films as affected by the incorporation of different phenolic acids"**, evaluated the effect of incorporating ferulic, *p*-coumaric and protocatechuic acids, with antimicrobial activity, into PEG plasticized PLA/PHBV (75:25) blend films, on their disintegration and biodegradation behaviour under laboratory composting conditions. The sample mass loss, thermogradation behaviour and visual appearance were analysed at different times of the composting period. No effect of phenolic acids was observed on the film disintegration pattern, the films being completely disintegrated at the end of composting period. The PLA:PHBV sample without active compounds and with ferulic acid showed complete biodegradation after 20 composting days while the samples with *p*-coumaric and protocatechuic acids biodegraded 21 and 26 days, respectively.

Chapter 4, entitled "**Starch-polyester bilayer films with phenolic acids for pork meat preservation"**, analysed the physical properties of starch-polyester bilayer films incorporating ferulic, *p*-coumaric and protocatechuic acids, as well as the effect of this active bilayer films at extending the shelf life of pork meat during cold storage. The incorporation of phenolic acids significantly improved the water vapour and oxygen permeabilities and the antioxidant capacity of the active bilayer films, reducing lipid oxidation and decreasing the microbial counts in packaged pork meat during storage.

The most relevant results obtained in the different chapters are analysed from a global perspective, in the **GENERAL DISCUSSION** section. Finally, in the last section the final **CONCLUSIONS** of the Thesis are presented.

DISSEMINATION OF RESULTS

INTERNATIONAL JOURNALS JRC

Published

Thermoprocessed starch-polyester bilayer films as affected by the addition of gellan or xanthan gum. Eva Hernández-García; María Vargas; Amparo Chiralt. *Food Hydrocolloids* (2021), 113, 106509.

D.O.I: https://doi.org/10.1016/j.foodhyd.2020.106509

Biodegradable Antimicrobial Films for Food Packaging: Effect of Antimicrobials on Degradation. Eva Hernández-García; María Vargas; Chelo González-Martínez; Amparo Chiralt. *Foods* (2021), 10 (6), 1256.

D.O.I: https://doi.org/10.3390/foods10061256

Biodegradation of PLA-PHBV blend films as affected by the incorporation of different phenolic acids. Eva Hernández-García; María Vargas; Amparo Chiralt; Chelo González-Martínez. *Foods* (2022), 11 (2), 243.

D.O.I: https://doi.org/10.3390/foods11020243

Submitted

Use of phenolic acids to obtain active films based on PLA- PHBV. Eva Hernández-García; María Vargas; Amparo Chiralt. *Food Packaging and Shelf Life.*

Starch-polyester bilayer films with phenolic acids for pork meat preservation. Eva Hernández-García; María Vargas; Amparo Chiralt. *Food Chemistry.*

BOOK CHAPTERS

Use of by-products in edible coatings and biodegradable packaging materials. Amparo Chiralt; Carolin Menzel; Eva Hernández-García; Sofía Collazo-Bligiardi; Chelo González-Martínez. *Sustainability of the Food System: Sovereignty, Waste, and Nutrients Bioavailability* (2020), 101-127. Ed Elsevier.

D.O.I: https://doi.org/10.1016/B978-0-12-818293-2.00006-9

COMMUNICATIONS IN INTERNACIONAL CONGRESSES

Poster: **Obtaining cassava and maize starch films with gellan and xanthan gum by melt-blending-compression moulding**. Eva Hernández-García; María Vargas; Amparo Chiralt. "7th International Conference on Biobased and Biodegradable Polymers. BIOPOL-2019". Stockholm, Sweden (2019).

Poster: **Release kinetics and antibacterial activity of phenolic acids from PLA-PHBV films**. Eva Hernández-García; María Vargas; Amparo Chiralt. "6th International ISEKI_Food Conference. ISEKI_FOOD 2021". Online (2021).

COMMUNICATION IN SCIENTIFIC EVENTS

Oral communication: **Films multicapa biodegradables y activos a base de almidón y poliésteres (PLA y PHBV) para el envasado de alimentos.** Eva Hernández García, Maria Vargas, Amparo Chiralt. "Jornada de Investigación en Ciencia y Tecnología de Alimentos 2021". Valencia, Spain (Julio de 2021).

CO- ADVISOR in:

- Bachelor's Thesis:

Biodegradación de films en medios acuáticos. Egea Doménech, Ana María (2020). Grado en Ciencia y Tecnología de los Alimentos (UPV).

Efecto de la incorporación de antimicrobianos en la biodegradación y compostaje de films activos para el envasado de alimentos. Alventosa Carbó, Javier (2020). Grado en Ciencia y Tecnología de los Alimentos (UPV).

Cinética de liberación y actividad antibacteriana de ácidos fenólicos incorporados en films de PLA-PHBV. Peñalver Sánchez, María (2021). Grado en Ciencia y Tecnología de los Alimentos (UPV).

- Master's Thesis**:**

Efecto de la adición de gomas de origen microbiano en films bicapa biodegradables de almidón-poliésteres. Vilar Rambla, Jordi (2019). Máster Universitarito en Ciencia e Ingeniería de los Alimentos (UPV).

Materiales para el envasado activo de alimentos basados en PLA o PHBV. Cárdenas Acosta, Lissett Consuelo (2020). Máster Universitarito en Ciencia e Ingeniería de los Alimentos (UPV).

COLLABORATION IN TEACHING ACTIVITIES:

Subject: "**Internships in Research Institutes**". (Bachelor's Degree in Biotechnology, UPV, 3rd Year): Topic: Development of active PLA:PHBV based films with phenolic acids. Student: Schulze-Steinen Sebastián, Sofía (Academic Year: 2020-2021).

Subject: "**Physical Properties of Food I**". (Bachelor's Degree in Food Science and Technology, UPV, 2nd Year). Topic: Laboratory practical and computer lab sessions. (Academic year: 2019-2020 and 2020-2021).

TABLE OF CONTENTS

NOMENCLATURE

% E	Percentage of elongation at break
ASTM	American Society for Testing and Materials
C_{ab}*	Chroma
CECT	Spanish Type Culture Collection
CFU	Colony Forming Unit
Ch	Chitosan
CIN	Cinnamaldehyde
CMC	Cellulose Microcrystalline
CNC	Cellulose Nanocrystal
CS	Cassava Starch
D	Diffusion coefficient
Da	Dalton
DMSO	Dimethyl Sulfoxide
DS	Dry solids
DSC	Differential Scanning Calorimetry
DTG	Thermal Weight Loss Derivative
EM	Elastic Modulus
ENR	Epoxidized Natural Rubber
FESEM	Field Emission Scanning Electron Microscopy
G	Gellan
GU	Gloss Units
GSE	Grapefruit Seed Extract
h_{ab}*	Hue
HV	Hydroxyvalerate
ISO	International Organization for Standardization
k	Rate constant of Korsmeyer-Peppas model
k_1	Kinetic constant of Peleg model
k_2	Constant of the Peleg model
L*	Luminosity
LAB	Lactic Acid Bacteria
LAE	Ethyl Lauroyl Arginate
LSD	Least Significant Difference
M_∞	Amount of active compound release at equilibrium
MC	Methylcellulose
MIC	Minimum Inhibitory Concentration
MS	Maize Starch
MTT	Thiazolyl Blue Tetrazolium Bromide Assay
n	Difussional exponent of Korsmeyer-Peppas model
NO	Neem Oil
OEO	Oregano Essential Oil
OP	Oxygen Permeability
PBAT	Polybutyrate Adipate Terephthalate
PBS	Polybutylene Succinate

PBS	Phosphate-Buffered Saline
PBSe	Polybutylene Sebacate
PBSet	Polybutylene Sebacate-co-terephthalate
PCL	Polycaprolactone
PEG600	Polyethylene Glycol 600 Da
PEG1000	Polyethylene Glycol 1000 Da
PET	Polyethylenetetraphthalate
PHAs	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxivalerate)
PHMG	Poly-(hexamethylene-guanidine)
PHBHHx	Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)
PHPG	Polyhexamethylene guanidine hidrochloridre
PHV	Polyhydroxyvalerate
PI	Peroxide Index
PLA	Polylactic Acid
PLGA	Poly(lactic-co-glycolic acid)
POPs	Persistent Organic Pollutants
PP	Polypropylene
PSPH	Phosphoserine Phosphatase
PVA	Poly (vinyl alcohol)
R	Volatile-solid Content
REDV	Short chain peptide
RH	Relative Humidity
S	Starch
SSR	Synthetic Solid Residue
t	time
T	Temperature
$\mathbf{T_g}$	Glass transition temperature
TGA	Thermogravimetric Analysis
$\mathbf{T_i}$	Internal transmittance
$\mathbf{T_m}$	Melting Temperature
$\mathbf{T_{max}}$	Temperature of maximum degradation rate
$\mathbf{T_{peak}}$	Maximum degradation rate
TPS	Thermoplastic starch
$\mathbf{T_{onset}}$	Onset Temperature
TVC	Total Viable Count
TSB	Trypto-Casein Soy Broth agar
TS	Tensile Strength
UV	Ultraviolet
VS	Volatile Solids
WTR	Water Vapour Transmission Rate
WVP	Water Vapour Permeability
X	Xanthan
ZnONPs	Zin Oxide Nanoparticles
$\mathbf{\Delta E}$	Color Difference
$\mathbf{\Delta H_m}$	Melting enthalpy

I. INTRODUCTION

Biodegradable Antimicrobial Films for Food Packaging: Effect of Antimicrobials on Degradation

Eva Hernández-García[1], María Vargas[1*], Chelo González-Martínez[1], Amparo Chiralt[1]

Foods (2021), 10 (6), 1256

[1]Institute of Food Engineering for Development, Universitat Politècnica de València, Valencia, Spain

evherga1@upvnet.upv.es

ABSTRACT

The environmental problem generated by the massive consumption of plastics makes necessary the developing of biodegradable antimicrobial materials that can extend food shelf-life without having a negative impact on the environment. The current situation regarding the availability of biodegradable food packaging materials has been analysed, as well as different studies where antimicrobial compounds have been incorporated into the polymer matrix to control the growth of pathogenic or spoilage bacteria. Thus, the antimicrobial activity of active films based on different biodegradable polymers and antimicrobial compounds has been discussed. Likewise, relevant information on biodegradation studies carried out with different biopolymers in different environments (compost, soil, aquatic), and the effect of some antimicrobials on this behavior, are reviewed. In most of the studies, no relevant effect of the incorporated antimicrobials on the degradation of the polymer were observed, but some antimicrobials can delay the process. The changes in biodegradation pattern due to the presence of the antimicrobial are attributed to its influence on the microorganism population responsible for the process. More studies are required to know the specific influence of the antimicrobial compounds on the biodegradation behavior of polymers in different environments. No studies have been carried out or marine media to this end.

Keywords: biopolymer; active packaging; biodegradation; composting; antimicrobial.

1. INTRODUCTION

The food industry is responsible for a high consumption of plastics for food packaging, which is essential to maintain food safety. Traditionally, food companies have packaged their products in metal and glass containers, but nowadays the use of plastic containers to pack food has become global [1] due to the great advantages of plastic. It is light, with versatile mechanical and optical properties, moldable, impermeable to water and gases, resistant to corrosive chemicals, with low density and low cost, and allows for the printing of relevant information for the consumer. Moreover, plastic-based packaging materials favor the preservation of food through the application of different strategies such as the incorporation of antimicrobials and antioxidants or the development of modified atmospheres. However, a great part of synthetic plastics generates a large amount of waste that decomposes very slowly, accumulating in terrestrial and marine ecosystems, causing the known great environmental problem. In addition to the physical impacts on terrestrial and marine ecosystems, there is growing concern about the impact on human health because of the toxic substances (flame retardants, pigments, plasticizers, compatibilizers, etc) used in plastic fabrication, which can migrate to water or other contact media including food. Likewise, one of the main problems is microplastics that, unlike larger plastics, are not easily seen at naked eye and once these particles end up in the ocean, their recovery is no longer possible. Microplastics enhance the transport and bioavailability of toxic, bio-accumulative and persistent organic pollutants (POPs) that could enter the food chain through consumption of marine products [2].

In recent years, the greater environmental awareness of citizens and the new European regulations on this issue, has given rise to the new concept of sustainable packaging. Thus, research in renewable raw materials, biotransformation process, structural design and biodegradability has been extended. In this sense, the interest in the so called bioplastics has rose with new developments that provide an alternative to traditional polymers [3]. Bioplastics are polymers that come from renewable natural sources or are biodegradable, or both, such as starch or cellulose [4]. The global production of bioplastic was 2.1 million tons in 2018, with prevision of 2.4 million tons for 2024. From that, only about 55% are biodegradable, the main polymers being poly(butylene

adipate-co-terephthalate): PBAT (13.4 %), Polybutylene succinate: PBS (4.3%), polylactic acid: PLA (13.9%), polyhydroxyalkanoates: PHAs (1.2%) and starch blends (21.3%) [5]. An increase in the bioplastic production as well as the adequacy of the properties of for determined target applications are necessary to promote the sustainable use of plastics.

The development of biodegradable and sustainable packaging also requires adding other substances to the polymer matrix to adapt its properties to the specific needs, which could affect biodegradation behavior. Plasticizers are used to improve the mechanical performance of the polymer matrix since they weaken the cohesion forces between polymer chains, increasing their mobility and improving the flexibility of the polymer matrix. Polyols, such as glycerol, polyethylene glycol, propylene glycol, sorbitol, sucrose or glucose are commonly used plasticizers in the formulations based on hydrophilic polymers. Other plasticizers of hydrophobic nature can also be used, such as fatty acids and their derivatives and oils [6].

Biodegradable food packaging materials can include antimicrobial and/or antioxidant components that improve their performance, extending the shelf life of the pack-aged food. The incorporated antimicrobial agents prevent the undesirable growth of microorganisms on the surface of the food more efficiently than their direct incorporation into the food [7], due to their controlled release from the packaging to the product. The controlled release can extend the antimicrobial action over time in a more constant way, thus requiring lower doses of active compounds [8]. However, the presence of antimicrobial compounds in the material could seriously affect the biodegradation pattern of the carrying bioplastic by interfering the action of the natural microbial population responsible for the biodegradation process.

The aim of this work was to analyze different studies developing antimicrobial materials based on biodegradable polymers, as well as the influence of the incorporation of these compounds on the biodegradation behavior of the active materials.

2. ANTIMICROBIAL PACKAGING MATERIALS BASED ON BIODEGRADABLE POLYMERS

Biodegradable polymers for developing active packaging are classified in three main groups, as described in **Figure 1** [9]. The first group corresponds to those obtained from

biomass, such as the biopolymers extracted from agrofood resources or waste such as polysaccharides (starch, cellulose or chitosan) and proteins (dairy and soy proteins or gelatin). The second group corresponds to synthetic polymers obtained from monomers from renewable sources (such as PLA), or from oil (such as polycaprolactone: PCL or poly (vinyl alcohol): PVA). The third group consists of polymers produced by microorganisms, obtained from biotechnological processes through the extraction of cultures, such as PHAs.

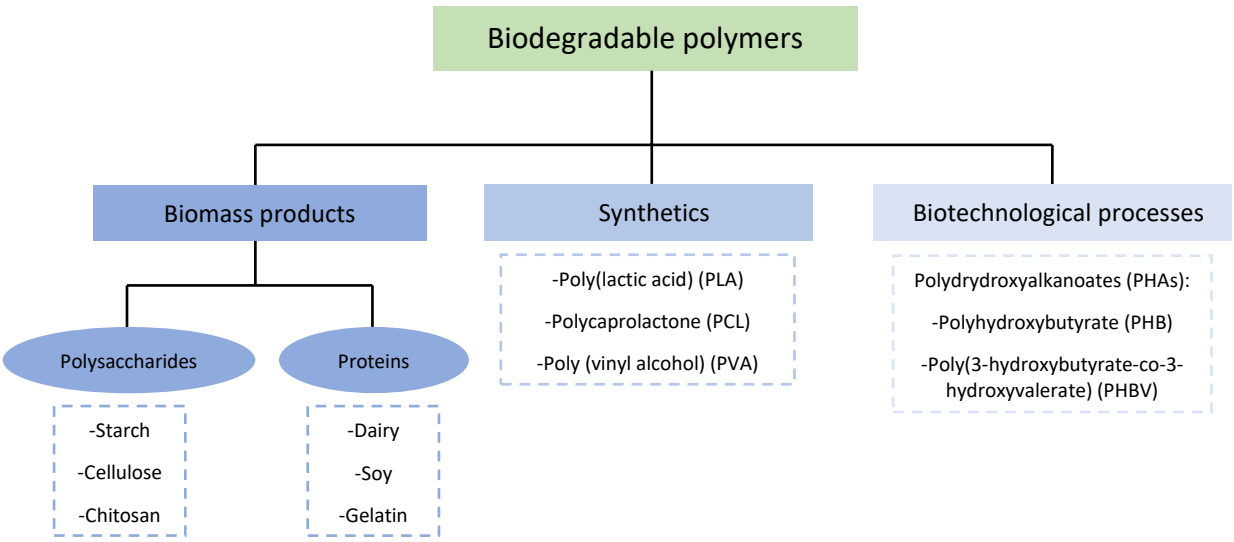


Figure 1. Classification of biodegradable polymers according to their origin.

Different studies have been carried out to obtain antimicrobial packaging materials by incorporating active components into biodegradable polymer matrices, as commented below.

2.1. Polymers from biomass

Starch is the biopolymer obtained from biomass that shows the highest production and number of studies due to its high availability, low cost and suitable properties for food contact. As shown in **Table 1**, numerous studies have been carried out on the development of active films with starch from different sources incorporating antimicrobials. In most cases, these films were obtained by casting, although some authors have also reported interesting results for thermo-processed films with broader industrial applications [10–12]. Different authors incorporated essential oils as antimicrobial agents in starch matrices to obtain active films, as can be seen in **Table 1**.

Table 1. Different studies on antimicrobial starch-based films.

Biodegradable Polymer	Antimicrobial	Microbiological Tests	Microorganisms	Results	Reference
Cassava starch/cellulose nanofibers	Tea tree essential oil	*In vitro*	*E. coli* *S. aureus* *C. albicans*	*S. aureus*: inhibition of 73% *C. albicans*: inhibition of 65% *E. coli*: no effect	[13]
Cassava starch	Cinnamon essential oil	*In vitro*	*P. commune* *E. amstelodami*	Mayor inhibition of *E. amstelodami*	[14]
Pea starch/PVA	Silver nanoparticles	*In vitro*	*L. innocua* *E. coli* *A. niger* *P. expansum*	Microbial growth inhibition	[15]
Brown rice starch/ chitosan	Chitosan	*In vitro*	*E. coli* *S. aureus*	Microbial growth inhibition	[16]
Cassava starch/chitosan	Oregano and cinnamon leaf essential oils	Pork meat	Total aerobic and coliform	No growth inhibition	[10]
Cassava starch/chitosan	Chitosan	Pork meat	Total aerobic and coliform	Microbial growth inhibition	[11]
Sugar palm starch/ nanocrystalline celulose	Cinnamon essential oil	*In vitro*	*B. susbtilis* *S. aureus* *E. coli*	Microbial growth inhibition	[17]
Tapioca starch	Chitosan	*In vitro*/Cherry *tomato*	*B. cereus* *S. aureus* *E. coli* *S.typhimurium*	Microbial growth inhibition	[18]
Corn starch/bovine gelatin	N-α-lauroyl-l-arginine ethyl ester monohydro-chloride	Chicken breast	Psychotrophic bacteria, lactic acid bacteria, anaerobic, total coliforms, *E.coli*	Microbial growth inhibition	[12]
Pea starch/PVA	Neem and oregano essential oils	*In vitro*	*L. innocua* and *E. coli*	Microbial growth inhibition	[19]
Cassava starch/chitosan	Lemongrass essential oil	*In vitro*	Mesophillic bacteria	Microbial growth inhibition	[20]
Sago starch/guar gum	Carvacrol and citral	*In vitro*	*B. cereus* *E. coli*	Microbial growth inhibition	[21]
Oxidized and Acetylated Corn Starch/ Sodium Alginate	Sodium dehydroacetate and rosemary extract	*In vitro*	*E. coli* *A. niger*	Microbial growth inhibition	[22]

Biodegradable Polymer	Antimicrobial	Microbiological Tests	Microorganisms	Results	Reference
Hydroxypropyl-high-amylose starch	Pomegranate peel powder	*In vitro*	*S. aureus* *Salmonella*	Greater action against *S. aureus* than against *Salmonella*	[23]
Antimicrobial starch	Sodium benzoate and citric acid	Cheddar cheese	*L. innocua*	Microbial growth inhibition	[24]
Corn starch/bovine gelatine	Ethyl lauroyl arginate (LAE)	Marinated salmon	*L. innocua*	Microbial growth inhibition	[25]

Silveira et al. [13] incorporated tea tree essential oil (0.08, 0.8, and 1.5% v/v) with cellulose nanofibers by casting technique, obtaining notable growth inhibition for *S. aureus* (73%) and *C. albicans* (63%), but without significant inhibition of the growth of *E. coli* in *in vitro* tests. Dhumal et al. [21] incorporated carvacrol (0.75% w/w) and citral (1.0% w/w) and both (0.75% carvacrol and 1.0% citral) in sago starch films with guar gum, prepared by casting, which exhibited good antimicrobial activity against *B. cereus* and *E. coli* in *in vitro* tests. The Petri plates were placed at 40 °C for sago starch/guar gum films and 26 °C for sago starch/guar gum/essential oil films. Valencia-Sullca et al. [10] obtained starch-chitosan bilayer films by thermo-compression, either containing essential oils (oregano or cinnamon leaf at 0.25% w/w) of not, incorporated into the chitosan monolayer that was obtained by casting. These bilayers were effective at controlling the bacterial growth in coated pork meat samples, but the thermal treatment used to form the bilayers reduced the antimicrobial effect of chitosan as compared to the chitosan monolayers. Moreover, the incorporation of essential oils into the chitosan layer did not improve the antimicrobial action of the films. Syafiq et al. [17] obtained films with palm sugar starch and palm sugar nanocrystalline cellulose, incorporating cinnamon essential oil (0.8, 1.2, 1.6 and 2.0 wt%) by casting, which showed inhibition of *B. subtilis*, *S. aureus* and *E. coli* growth in *in vitro* tests. Cano et al. [19] obtained starch/PVA films containing neem (NO) or oregano essential (OEO) oil. These were incorporated into the films, obtained by casting, at two different ratios with respect to the starch, 1:0.125 (S-PVA-1OEO and S-PVA-1NO) and 1:0.5 (S-PVA-2OEO, S-PVA-2NO). The films exhibited antibacterial effect against *L. innocua* and *E. coli*, and antifungal

properties against *A. niger* and *P. expansum*. Perdana et al. [20] developed starch-based composite films and coatings with lemongrass, obtained by casting. Lemongrass was added separately to obtain 1%, 1.5% and 2% of concentration to the film-forming solution of cassava starch (3% w/v). The coatings were effective at reducing mesophilic bacteria count and fungi count in cold-stored chilies. Other active compounds, such as sodium dehydroacetate and rosemary extract [22], silver nanoparticles [15], pomegranate peel [26], chitosan [10,16,18], N-α-lauroyl-l-arginine ethyl ester monohydrochloride [12] or sodium benzoate and citric acid [24] have also been incorporated into starch-based matrices for the purposes of obtaining antimicrobial packaging materials. Yan et al. [22] incorporated sodium dehydroacetate and rosemary extract to oxidized and acetylated corn starch films with sodium alginate that were prepared by casting. Sodium dehydroacetate was added to reach a final content of 0, 0.1, 0.3, 0.5 and 0.7% (w/w), and rosemary extract was added at a concentration of 0, 0.3, 0.6, 0.9 and 1.2% (w/w). All films showed antimicrobial effect against *E. coli*, whereas an effective inhibition of *Aspergillus niger* was only observed when sodium dehydroacetate was incorporated in the films. Ali et al. [26] incorporated pomegranate peel powder at different concentration based on the dry starch content (0, 2, 4, 6, 8, 10, 12, and 14 wt%) in high-amylose hydroxypropylated starch films plasticized with glycerol and observed the growth inhibition of both gram-positive (*S. aureus*) and gram-negative (*Salmonella*) bacteria in *in vitro* tests; the greatest effect being observed against *S. aureus*. Valencia-Sullca et al. [11] obtained cassava starch-chitosan films by melt-bending and compression moulding, using glycerol and polyethylene glycol as plasticizers. The incorporation of the highest amount of chitosan (70:30, starch/chitosan ratio) in the films led to the reduction in coliforms and total aerobic counts of cold-stored pork meat slices, thus extending their shelf-life. Hasan et al. [16] developed films based on brown rice starch and chitosan (starch/chitosan ratio: 70:30, 50:50 and 30:70), plasticized with palm oil and prepared by casting. Films showed a remarkable antimicrobial *in vitro* effect against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*). Shapii' et al. [18] obtained tapioca starch/chitosan films with different concentration of chitosan nanoparticles (0, 5, 10, 15, 20% w/w), which showed bacterial growth inhibition both in *in vitro* tests (*B. cereus, S. aureus, E. coli* and *S. typhimurium*) and when the films were applied to wrapped cherry tomatoes. Moreno et al. [12]

developed films based on corn starch and bovine gelatin with N-α-lauroyl-l-arginine ethyl ester monohydrochloride (10 wt%) that were capable of extending the shelf-life of chicken breast fillets, without affecting meat oxidation. These films were also effective at reducing the total viable counts, which remained below the legal limit, in marinated salmon samples stored for 45 at 5 °C [25]. De Moraes et al. [24] developed starch films with sodium benzoate (0.001 g/100 g of tapioca flour), citric acid (30 g/100 g tapioca flour) and the mixture of both, which reduced the growth of *L. innocua* in inoculated Cheddar cheese samples.

2.2. Synthetic Polymers

In this group PVA and polyesters, such as PLA and PCL are included. PLA is a thermoplastic biopolymer obtained from lactic acid by the starch fermentation. Due to its biodegradability in a compost medium (compostability) and its biocompatibility, PLA has found numerous applications since it also shows good barrier properties against water vapor, O_2 and CO_2, high mechanical resistance and photostability [27]. PVA is a highly hydrophilic polymer obtained through the hydrolysis of poly (vinyl acetate) [28,29]. PCL was first synthesized in the 1930s by the ring-opening polymerization of ε-caprolactone and it is highly hydrophobic with longer degradation times than PLA [30], making it suitable for applications where long degradation times are required. Due to its low melting temperature, PCL is easily processed using conventional melting techniques. Its mechanical properties can be improved by different fillers (particles or fibers) [31]. Although it is a biodegradable material, PCL comes from non-renewable petrochemical sources and is frequently used as a copolymer with PLA to prepare degradable blends with specific properties [31].

Different studies have been carried out aimed to obtain antimicrobial packaging materials based on PLA, PVA or PCL (**Table 2**).

Table 2. Different studies on antimicrobial films based on synthetic biodegradable polymers (PLA, PVA and PCL).

Biodegradable Polymer	Antimicrobial	Microbiological Tests	Microorganisms	Results	Reference
PLA	Essential oils (clove, cinnamon and garlic)	*In vitro*	*C. jejuni* *S. aureus*	Garlic oil: limited antimicrobial activity Clove and cinnamon oils: more effective against *C. jejuni* than against *S. aureus*	[32]
PLA/PBAT	Cellulose-silver nanocrystals	*In vitro*	*E. coli* *S. Aureus*	Limited antimicrobial activity	[33]
PLA/PBS	Chitin nanofrils	*In vitro*	*S. aureus* *Enterobacter spp.*	No microbial inhibition	[34]
PLA	Propolis ethanolic extract	*In vitro*	Gram positive Gram negative	No efective	[35]
PLA	Propolis ethanolic extract Essential oil of Tanacetum balsamita	*In vitro*	*B. cereus* Gram positive Gram negative	Limited antimicrobial activity	[35]
PLA	Propolis ethanolic extract Tanacetum balsamita essential oil	Sausages	Lactic acid, aerobic mesophilic and psychotrophic bacterias	Extended shelf-life of sausages	[35]
PLA/Starch	Cinnamaldehyde	*In vitro*	*E. coli* *L. innocua*	Inhibition of microbial growth	[36]
PLA	Chitosan	Pork meat	Total aerobes and coliform	Inhibition of microbial growth	[37]
PVA	poly (hexamethylene guanidine)	*In vitro*	*S. aureus* *E. coli*	Inhibition of microbial growth	[38]
PVA-Chitosan	Ethyl Lauroyl Arginate (LAE)	*In vitro*	*C. jejuni, S.typhimurium E.coli, L.monocytogenes*	Inhibition of microbial growth with 5–10% LAE	[39]

Biodegradable Polymer	Antimicrobial	Microbiological Tests	Microorganisms	Results	Reference
PVA	Lactic, tartaric and malic acids	*In vitro*	*S. aureus* *E. coli*	Greater inhibition with lactic acid, followed by malic and tartaric acids	[40]
PVA-Chitosan	Chitosan	Minimally processed tomato	*S. aureus* *E. coli* *B. subtilis*	Greater inhibitory effect in *E. coli* and *B. subtilis* than in *S. aureus*	[41]
PCL/short chain peptide (REDV)	Eugenol	*In vitro*	*E. coli* *S. aureus*	Limited antimicrobial activity	[42]
PCL	Solid extract of sage	*In vitro*	*E. coli* *S. aureus*	Limited antimicrobial activity. More effective against *S. aureus*	[43]
PCL	Organic acids, rosmarinic acid extract and Asian essential oil blend.	Broccoli	*E. coli* *S. typhimurium*	Inhibition of microbial growth	[44]
PCL	Grapefruit seed extract	*In vitro*	*L. monocytogenes*	Inhibition of microbial growth	[45]
PCL/starch	Pomegranate rind powder	*In vitro*	*S. aureus*	Inhibition of microbial growth at high concentrations	[46]
PCL	Cinnamaldehyde	*In vitro*	*E. coli* *S. aureus*	Inhibition of microbial growth	[47]
PCL	Methanolic extract of pomegranate	*In vitro*	*E. coli* *S. aureus*	6–7 days growth delay	[47]
PCL	Freeze dried pomegranate arils	*In vitro*	*E. coli* *S. aureus*	2-day growth delay	[47]
PCL	Pomegranate seed flour	*In vitro*	*E. coli* *S. aureus*	2-day growth delay	[47]
PCL/starch	Carvacrol	*In vitro*	*E. coli* *L. innoua*	Inhibition of *E.coli*	[48]

Ahmed et al. [32] developed films based on PLA with polyethylene glycol (PEG) and essential oils of cinnamon (0.4, 0.8, 1.2 and 1.6 mL were poured to the PLA/PEG solution), garlic (1.6 mL in the PLA/PEG solution) or clove (1.6 mL in the PLA/PEG solution) that were obtained by casting. These films showed antimicrobial activity against different bacteria. *C. jejuni* showed greater sensitivity (7 log reduction) than *S. aureus* (2 log reduction) to cinnamon and clove oils incorporated into PLA films. Garlic oil incorporated into the PLA films exhibited limited antimicrobial activity against both bacteria. Khodayari et al. [35] developed PLA films with an ethanolic extract of propolis (0, 1 and 2% *v/v*) and essential oil of *Tanacetum balsamita* (0, 1 and 2% *v/v*), which were able to control the growth of gram-positive and gram-negative bacteria. The *in vitro* results showed that the ethanolic extract of propolis had no significant antimicrobial activity, but when the extract was added in combination with the essential oil of *Tanacetum balsamita* an effective antimicrobial effect was achieved. The gram-positive bacteria were more sensitive than the gram-negative, especially, *B. cereus*, which was the most sensitive to *Tanacetum balsamita* essential oil. Furthermore, the same authors studied the antimicrobial effect of these films on precooked sausages. During cooking, the counts of heat-sensitive bacteria, such as *Enterobacteriaceae* or *S. aureus*, dropped below the limit of detection. However, psychrotrophic bacteria went from being below the detection limit on day 0 to a detectable level, below the permissible limit after 50 days of storage, evidencing the protective effect of the films. Jiang et al. [33] developed films of PLA, PBAT (poly(butylene adipate-co-terephthalate)) and cellulose silver nanocrystals (0 to 8 wt% based on the total weight of the PLA/ PBAT component), which were prepared by casting and were effective at inhibiting the growth of *E. coli* and *S. aureus*. The film without the cellulose-silver nanoparticles did not show any inhibitory effect. Coltelli et al. [34] developed PLA and PBS (poly(butylene succinate)) films with chitin nanofibrils (2 wt%) used as filler material. The antimicrobial tests did not revealed effectiveness against *S. aureus* and *Enterobacter* spp. Muller et al. [36] incorporated cinnamaldehyde (CIN) in PLA films (PLA:CIN ratio of 10:2.5) to obtain starch-PLA active bilayers. The PLA monolayers exhibited antibacterial effect against *E. coli* and *L. innocua*, whereas the bilayers were more effective when the starch side was in contact with the culture medium. Extruded PLA films with different amounts of chitosan powder were

effective at controlling the growth of total aerobes and coliforms in meat, especially when the particle size of chitosan was more reduced [37].

Olewnik-Kruszkowska et al. [38] developed films composed of PVA and chitosan (Ch) with the addition of poly- (hexamethylene-guanidine) (PHMG) (0.5 or 1 wt% of the PVA or PVA:Ch polymer mass) that were obtained by casting. This study confirmed the biocidal potential of PVA films with PHMG, showing their antimicrobial potential against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. Haghighi et al. [39] developed films of chitosan-PVA mixtures with different concentrations of ethyl lauroyl arginate (LAE) (1, 2.5, 5 and 10 % *w/w* of biopolymer). Films were obtained by casting and drying at 25 ± 2 °C overnight. These films inhibited the growth of four foodborne bacterial pathogens, *Campylobacter jejuni*, *Salmonella typhimurium*, *Escherichia coli*, and *Listeria monocytogenes*, being the films with a content of 5 to 10% of the antimicrobial the most effective. Suganthi et al. [40] used organic acids (tartaric, lactic and malic) as crosslinking agents in PVA films (10 wt% of acid in proportion to PVA) that were obtained by casting. The films containing lactic acid exhibited the highest bacterial inhibition, which was largely attributed to its ability to modify the local pH and alter the permeability of the microbial membrane, interrupting the bacterial-substrate interaction. Tripathi et al. [41] developed films based on PVA and chitosan, which was previously dissolved into 2 wt% acetic acid to prepare a 1 wt% chitosan solution and evaluated their antimicrobial effect on minimally processed tomato. The antimicrobial films were obtained by mixing chitosan and PVA with glutaraldehyde as a crosslinking agent. A greater growth inhibitory effect was observed in *E. coli* and *B. subtilis* as compared to *S. aureus*.

As regards active films based on PCL, Li et al. [42] developed an active film using electrospun membranes composed of PCL and a short-chain peptide called REDV, with eugenol as antimicrobial agent (5, 10, 20 and 30 wt%). Eugenol films were effective against gram-positive bacteria, such as *S. aureus* and gram-negative bacteria, such as *E. coli*. Salević et al. [43] produced PCL films with sage extract (5%, 10%, and 20% *w/w* with respect to the polymer content) incorporated as an antimicrobial agent and using the electrospinning technique followed by annealing treatments. The films were effective against gram-negative bacteria (*E. coli*) and gram-positive bacteria (*S. aureus*), being

more effective against the gram-positive. Takala et al. [44] prepared trilayer bioactive films with methylcellulose (MC) and PCL. Two antimicrobial formulations named A (60 g/L organic acids mixture, rosmarinic acid extract and 6 g/L Asian essential oil blend) and B (60 g/L organic acids mixture, rosmarinic acid extract and 6 g/L Italian essential oil blend) were added to the MC films during melt blending, and the three-layer films (PCL/MC/PCL) were obtained by compression molding. These films were applied to broccoli samples that were stored at 4 °C for 12 days. The films significantly reduced the growth of *Escherichia coli* in the broccoli samples from day 4, and there was a total inhibition on day 12. Similarly, a significant reduction in *Salmonella typhimurium* counts was observed from 2 days and a total inhibition on day 7. Lyu et al. [45] also conducted a study with PCL composite films with different concentrations of grapefruit seed extract (GSE) (0, 1, 3 and 5 %) added as antimicrobial agent. The antimicrobial activity of the films increased as the concentration of GSE increased; with a greater inhibitory activity against *Listeria monocytogenes* reported for the films that incorporate 5% GSE. When these films were applied to commercial cheddar cheese packages, a delay in microbial growth of the samples was observed. Mixed PCL/starch/pomegranate rind powder films (5, 10, 15, 20, 30 and 40 % of pomegranate rind powder) were also developed for antimicrobial packaging, observing antimicrobial effectiveness for high concentrations of pomegranate rind (40%) [46]. Tampau et al. [48] developed three-layer starch films with carvacrol (15 wt% with respect to the polymer), which were loaded in electrospun PCL fibers placed between the two starch sheets. *In vitro* microbiological tests did not show growth inhibition of *Listeria innocua*, but the films were effective against *E. coli*. The total carvacrol load in the three-layer determined the film effectiveness depending on the minimal inhibitory concentration of the bacteria.

2.3. Polymers from Microorganisms

Among the polymers obtained by fermentation, PHAs are linear polyesters produced by bacteria by the fermentation of sugars or lipids. They are produced by a wide variety of bacteria throughout a carbon and energy storage mechanism. Thus, PHAs can be synthesized from renewable carbon sources, are biodegradable (they can be assimilated by many microorganisms, either from soils, seas, lakes or sewage) and are biocompatible (without toxic effects). These properties make PHAs very interesting as

substitutes for conventional plastics such as PP and PET with similar physical characteristics [49].

The two most common PHAs are polyhydroxybutyrate (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). PHB is a crystalline, biodegradable polyester with a melting temperature between 173 °C and 180 °C, close to that of PLA, which facilitates their blending in order to modulate the properties. PHB is a relatively rigid and fragile bioplastic and has a low resistance to thermal degradation, which limits its thermoprocessing [50]. PHBV contains units of 3-hydroxyvalerate (HV) inserted in the PHB polymer. PHBV is an aliphatic polyester, non-toxic, 100% biodegradable and biocompatible with many types of cells. PHBV is characterized by its high degree of crystallinity and resistance to ultraviolet radiation and acceptable amounts of alcohols, fats and oils. However, it is a rigid and quite fragile polymer, with a melting temperature of 153 °C, which is lower than that of PHB. PHBV has high viscosity in liquid state (which favors the extrusion processes) and its films show good barrier capacity to oxygen, and better mechanical properties and greater flexibility than PHB films. Despite some of the improvements that PHBV offers over PHB, this polymer continues to exhibit high brittleness, low impact resistance, and poor thermal stability as compared to petroleum-based polymers [51].

Different studies have incorporated antimicrobials into PHAs to obtain active food packaging materials (**Table 3**).

Table 3. Different studies on antimicrobial films based on biopolymers obtained from microorganisms (PHA, PHB and PHBV).

Biodegradable Polymer	Antimicrobial	Microbiological Tests	Microorganisms	Results	Reference
PHA	Lime oil	*In vitro*	*E. coli* *S. aureus*	Antimicrobial effectiveness against *S. aureus*	[52]
PHBV	Silver nanoparticles	*In vitro*	*S. enterica* *L. monocytogenes*	Effective against *S. enterica* and not effective against *L. monocytogenes*	[53]

Biodegradable Polymer	Antimicrobial	Microbiological Tests	Microorganisms	Results	Reference
PHA	Alkyl quaternary ammonium salts	*In vitro*	*E. coli* *S. aureus*	Inhibition of microbial growth	[54]
PHB	Vanillin	*In vitro*	*E. coli* *S. typhimurium* *S. flexneri* *S. aureus*	Minimum concentration to reduce microbial activity: 80µg/g PHB	[55]
PHB/PCL/Organic clays	Nisin	*In vitro/* ham slices	*L. plantarum*	Inhibition of microbial growth	[56]
PHB	Eugenol and pediocin	*In vitro*	*S. aureus* *E. coli* *S. typhimurium* *B. cereus*	Inhibition of microbial growth	[57]
PHB/PSPH	Chlorine	*In vitro*	*S. aureus* *E. coli*	Inhibition of microbial growth	[58]
PHBV	ZnO nanoparticles and oregano essential oil	*In vitro*	*E. coli* *S. aureus*	Significant microbial growth inhibition	[59]
PHBV	ZnO nanoparticles and oregano essential oil acting synergistically	*In vitro*	*E. coli* *S. aureus*	Greater microbial inhibition than that of pure antimicrobials	[59]
PHBV	Oregano essential oil	*In vitro*	*E. coli* *L. innocua*	Significant microbial inhibition	[60]
PHBV	Carvacrol	*In vitro*	*E. coli* *L. innocua*	Significant microbial inhibition	[60]
PHBV/Silica mesoporous support	Eugenol essential oil	*In vitro*	*E. coli* *S. aureus*	Microbial inhibition	[61]
PHBV	Triclosan	*In vitro*	*E. coli* *S. aureus*	Effective microbial inhibition	[62]

Biodegradable Polymer	Antimicrobial	Microbiological Tests	Microorganisms	Results	Reference
PHBV	Carvacrol/Eugenol	*In vitro*	*E. coli* *L.innocua*	Effective microbial inhibition	[63]

Basnett et al. [52] obtained PHA films with lime oil (5 wt% in the polymer solution) that were effective against *S. aureus*, even after one year of preparation. In contrast, these films did not inhibit the growth of gram-negative bacteria such as *E. coli*. Castro-Mayorga et al. [53] developed active antimicrobial suspensions in situ and by physical mixing (mix) of stabilized silver nanoparticles (0.5 mM, 1 mM and 2 mM). The antimicrobial effect of the film against *Listeria monocytogenes* was not effective after 24 h of exposure, but it markedly reduced the growth of *Salmonella enterica*, consistently with the described greater susceptibility of gram-negative bacteria. Xu et al. [54] developed PHA films with graphene oxide nanocomposites and long alkyl chain quaternary salt functionalized graphene oxide at 1, 3, 5 and 7 wt%. which showed 99.9% effectiveness against gram-negative and gram-positive bacteria. Xavier et al. [55] produced PHB from *Bacillus mycoides*, isolated from garden soil, and prepared antimicrobial films with the resulting PHB and vanillin (20, 40, 50, 80, 100, or 200 µg per gram of PHB) by solvent casting. Films were tested against *E. coli*, *S. typhimurium*, *S. flexneri* and *S. aureus* and results showed that the minimum vanillin concentration to reduce the microbial activity was 80 µg/g PHB. Correa et al. [56] developed blend nanocomposite films of PHB/PCL, organo-clays (Cloisite® 30 B and 10A) and nisin. The organic clays exerted antimicrobial activity against *Lactobacillus plantarum* CRL691 although their inclusion in the polymer blend did not lead to antimicrobial films. The presence of clays did not affect the adsorption kinetics of nisin on PHB/PCL films. The PHB/PCL nisin-activated film was effective against *L. plantarum* (used as a model of processed meat spoilage bacteria) inoculated into sliced ham samples, thus extending its shelf-life. Narayanan et al. [57] prepared PHB-based antimicrobial films incorporating eugenol (10, 20, 40, 80, 100 and 200 µg/g polymer), and its antimicrobial activity against foodborne pathogens, spoilage bacteria and fungi was evaluated. The synergistic antimicrobial activity of the films was also investigated in the presence of crude pediocin. The culture broth containing pediocin, as well as the

PHB antimicrobial film, showed a prolonged lag phase and a significant reduction in bacterial growth at 24 h. The culture broth with pediocin and eugenol incorporated into the PHB film worked synergistically. Fan et al. [58] developed active films of PHB with phosphoserine phosphatase (PSPH) (2 wt% of PSPH based on weight of PHB) by electrospinning and a bleached chlorine treatment, these films showed biocidal efficacy against *Staphylococcus aureus* (92.10% inhibition) and *Escherichia coli* (85.04%) in *in vitro* tests.

Figueroa-Lopez et al. [59] developed PHBV films using electrospinning, incorporating oregano oil (at 10 wt% in relation to the polymer) and zinc oxide nanoparticles (1, 3, 6 and 10 wt% of ZnONPs in PHBV). Both antimicrobials demonstrated their efficacy against *E. coli* and *S. aureus*, which was reduced after 15 days. The films containing a mixture of both antimicrobials (2.5 wt% OEO + 2.25 wt% ZnONPs) showed the best results, since the activity was maintained for longer time periods, being the gram-positive bacteria the most susceptible to the antimicrobial effect. Requena et al. [63] developed antimicrobial PHBV films with carvacrol, eugenol, oregano or clove essential oil (13% *w/w*, in the film) that were sprayed between two layers of PHBV. For *L. innocua*, the most effective antimicrobial agent was carvacrol followed by oregano essential oil whereas clove oil or eugenol were less effective. The antibacterial effect of PHBV films with oregano or clove essential oil, or their main compounds, carvacrol and eugenol, respectively, was analyzed in different food matrices (cheese, chicken breast, pumpkin and melon) and *in vitro* tests with *Escherichia coli* and *Listeria innocua* were also performed. The reported antimicrobial activity in foods was less remarkable than that detected in the *in vitro* tests. No antilisterial effect was observed in the evaluated food matrices. The most significant antibacterial action against *E. coli* was observed in cheese and pumpkin samples, whereas the highest migration of both compounds took place in melon slices. A lack of correlation between the antibacterial effect and the active compound migration to the food matrix was observed, which suggested that many compositional factors affect the availability of the active compound to exert its antibacterial action in a specific food [63]. The same authors also obtained active bilayer films with PLA:PHBV (75:25) blend with carvacrol (25 g carvacrol/100 g polymer matrix) and starch sheets in a previous study [64]. These active bilayers inhibited the growth of

L. innocua and *E. coli* from both contact sides of bilayers, depending on carvacrol internal diffusion through the film and subsequent release into the culture medium. Likewise, *E. coli* showed greater sensitivity than *Listeria* to all of antimicrobials tested.

Meléndez-Rodríguez et al. [61] developed antimicrobial PHBV films with a mesoporous support of silica and essential oil with eugenol that were evaluated in *in vitro* tests with *E. coli* and *S. aureus*. Microbial activity was reduced while the best efficiency was obtained for *S. aureus*. The eugenol-containing nanoparticles were loaded in the 2.5–20 wt% range into PHBV by electrospinning. Sabharwal et al. [62] incorporated triclosan (0.2, 0.4, 0.6, 0.8 and 1 g *w/w*) to PHBV films obtained by casting that were very effective against *Escherichia coli* and *Staphylococcus aureus*.

3. POLYMER BIODEGRADATION STUDIES IN DIFFERENT MEDIA

Polymer biodegradation involves biological activity and comprises of three main stages as seen in **Figure 2**: (1) biodeterioration or modification of the chemical, physical and mechanical properties of the polymer due to the growth of microorganisms on the surface of the material, (2) biofragmentation or conversion of polymers into oligomers or monomers by the action of the enzymes of microorganisms and (3) assimilation of the resulting compounds by microorganisms, as a source of carbon, energy and nutrients, and its conversion into CO_2, water and biomass [65]. Some studies have been conducted to investigate the biodegradability of bioplastics under different environmental conditions, such as soil, compost, marine, and other aquatic environments [66]. Although most of the plastic waste is present in landfills, the biodegradation of plastics in landfills has not been significantly studied.

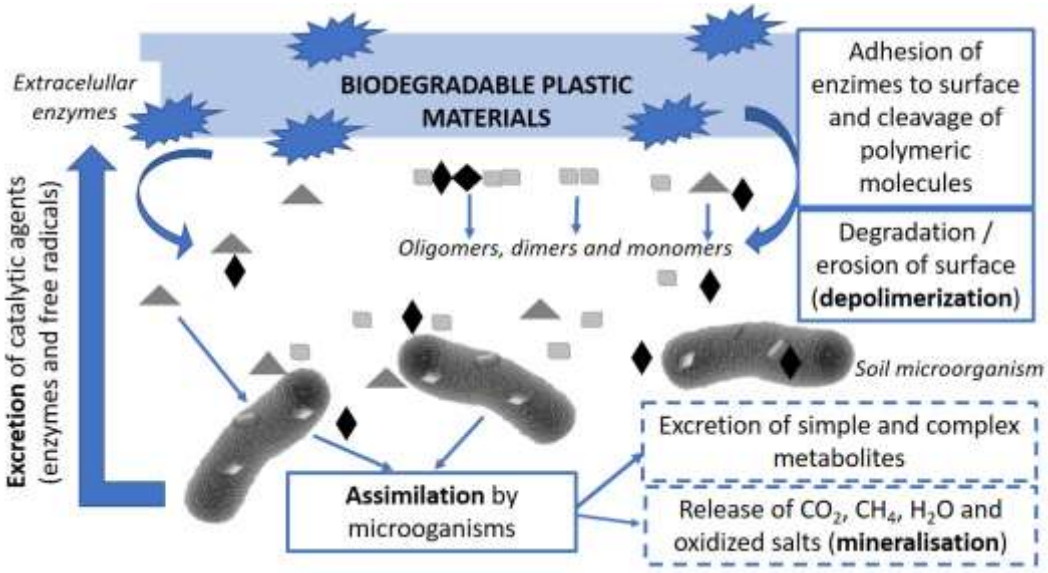


Figure 2. Polymer biodegradation pathway (Adapted from Youssef & El Sayed [67] and Lucas et al. [65]).

Composting and recycling are the two most widely considered procedures for managing plastic waste. Composting is a process in which organic matter is converted into CO_2 and a soil-like material (humus) by the activity of a mixed group of microorganisms under controlled conditions [68]. This process allows to transform organic waste and by-products into quality materials used as soil improvers and/or fertilizers. In this way, the environmental impact that this waste generates is eliminated and the use of the abundant resources that they often contain is made possible. Then, biodegradation studies in compost media have been more extensively studied than in other media.

As defined by the American Society for Testing and Materials (ASTM), a compostable plastic undergoes degradation by biological processes during composting to produce CO_2, water, inorganic compounds, and biomass at a rate consistent with other known compostable materials and leaves no visually-distinguishable or toxic residue. Therefore, a compostable plastic is biodegradable, while a biodegradable plastic is not always compostable [68].

Since plastic waste is also present in soil or aquatic environments, it is important to know the degradation behavior in these different media. Soil habitat contains a great biodiversity of microorganisms, which allows plastic biodegradation to be more feasible as compared to other environments, such as water or air [69]. On the other hand, plastic debris accumulates to a great extent uniformly in the marine environment. Due to its

semi-permanent stability in marine ecosystems, plastic debris causes marine pollution, which has an impact on marine animals [70]. However, fewer studies have been conducted on the biodegradable potential of bioplastics in soil or aquatic environments. Furthermore, given the global accumulation of plastic waste in rivers, lakes, coastal waters and sediments, polar waters and deep waters, there is a need for more experimental data on polymer biodegradation in most aquatic ecosystems [71].

Factors that affect biodegradation processes include the chemical structure of the polymer, the type of chain and molecular complexity, the degree of crystallinity, and the enzymes secreted by microorganisms that act specifically on certain types of bonds. In general, polymers with a shorter chain, more amorphous and with less molecular complexity are more sensitive to the action of microbial enzymes. Likewise, the characteristics of the environment where the process takes place notably affect to biodegradation processes; humidity, temperature, pH, and oxygenation conditions are the most relevant factors for the microbial action responsible for the process. The optimal values or intervals of each parameter are influenced by the environmental conditions, the type of waste to be treated and the composting system. Biodegradation studies of polymeric materials have been carried out mainly in compost medium, basically, through the control of the amount of CO_2 generated in the system because of the biodegradative action and mass loss of the samples due to the disintegration of the material, as a function of time. For the proper comparison of the behavior of different materials, standardized methods have been defined for carrying out the experiments under controlled conditions [72].

The moisture of the materials is considered the most important variable in composting [73] since the presence of water is essential for the physiological needs of the microorganisms. To ensure a good circulation of oxygen and other gases produced in the reaction, the humidity of the composting mass must be the required so that the water does not fully occupy the pores of the composting mass [74]. Optimal humidity for microbial growth is between 50–70% while temperature, pH, and oxygenation also play an important role. The increase in temperature of the composting mass is the clearest indication of the existence of microbial activity [74]. In the aerobic decomposition process an initial mesophilic phase ($T < 45$ °C) and a final thermophilic

phase (T > 45 °C) can be distinguished, considering the process finished when the initial temperature is reached again. pH monitoring throughout the process allows for obtaining an indirect measurement of the aeration control of the mixture, and the existing relationships between pH-aeration-microorganisms, since the organic degradation process is inhibited at low pH. A pH that remains above 7.5 during the process, indicates a good decomposition [75]. Several authors divide the evolution of composting into three phases: (1) the initial mesophilic phase, where there is a decrease in pH due to the action of microorganisms on the most labile organic matter, releasing organic acids; (2) a second phase where there is a gradual alkalinization of the medium, and (3) the third phase where the pH tends to neutrality due to the formation of humic compounds [76]. During the composting process it is necessary to ensure the presence of oxygen and avoid insufficient aeration since it would cause the substitution of aerobic microorganisms by anaerobes, retarding decomposition and giving rise to the appearance of bad odors and compounds such as hydrogen sulfide [77].

Table 4 summarizes the values of the percentage and time of biodegradation of different bioplastics in different media and conditions reported by several authors, where the effect of the environmental conditions, such as average pH, moisture and oxygen contents, and temperature on the polymer biodegradation behavior, can be observed.

Table 4. Biodegradation studies of some bioplastics in compost, soil or aquatic environments.

Bioplastics	Type of Environment	Conditions	Control Method	Biodegradation Period (days)	Biodegradation (%) Others Data	Reference
			PLA			
PLA	Compost	58 °C	Produced CO_2	80	78.9	[78]
PLA/TiO_2 nanocomposites	Compost	58 °C	Produced CO_2	80	Between 85 and 97.8	[78]
PLA	Compost	58 °C, 50% humidity	Weight loss	14	>90	[79]
PLA/CNC nanocomposites	Compost	58 °C, 50% humidity	Weight loss	14	>90	[79]
PLA	Soil	-	Weight loss	70	0.15	[80]
PLA/Starch	Soil	-	Weight loss	70	16	[80]
PLA	Sea water and freshwater	25 °C and fluorescence light (16 h light and 8 h dark)	Weight loss	365	Non-significant degradation	[81]
PLA	Compost	58 °C	Produced CO_2	130	90	[82]
PLA	Sea water	Without sediment, in euphotic and aphotic conditions	Weight loss	365	PLA > PET	[83]

Bioplastics	Type of Environment	Conditions	Control Method	Biodegradation Period (days)	Biodegradation (%) Others Data	Reference
PLGA	Sea water and fresh water	25 °C and fluorescence light (16 h light and 8 h dark)	Weight loss	270	100	[81]
			PHAs			
3-PHB	Sea water	28.75 ± 1.65 °C 53% salinity pH 7.0–7.5	Weight loss	160 (films) 80–160 (pellets)	58 (films) 38 (pellets)	[70]
PHB/PHBV	River water	Eutrophic recreation. (1 m depth)	Weight loss Degradation rate (DR)	31–42	34.6–43.5% DR: 0.011–0.014 d^{-1}	[84]
PHB	River water	Eutrophic recreation (1 m depth)	Weight loss Degradation rate (DR)	22–45	93% DR: 0.008–0.174 d^{-1}	[84]
PHB	Sea water and fresh water	25 °C and fluorescence light (16 h light and 8 h dark)	Weight loss	365	8.5	[81]
3-PHB/3-PHV	Sea water	28.75 ± 1.65 °C 53% salinity pH 7.0–7.5	Weight loss	160 (films) 80–160 (pellets)	54 (films) (13 pellets)	[70]
PHBV	Sea water	Laboratory (static), 30 °C. (With sediment, 75 mL) Aquarium (dynamic): 12–22 °C. With and without sediment.	Produced CO_2 and Weight loss (WL)	38–90	% CO_2: 70% WL static: 75–85% WL dynamic: 33–50%	[85]

Bioplastics	Type of Environment	Conditions	Control Method	Biodegradation Period (days)	Biodegradation (%) Others Data	Reference
PHBV	Sea water	Laboratory (static), 30 °C. (With sediment, 75 mL) Aquarium (dynamic): 12–22 °C. With and without sediment.	Produced CO_2 and Weight loss (WL)	38–90	% CO_2: 70% WL static: 75–85% WL dynamic: 33–50%	[85]
PHB	Sea water	Laboratory (static): 30 °C. (With sediment, 75 mL) Aquarium (dynamic): 12–22 °C. With and without sediment.	Produced CO_2 and weight loss (WL)	18–100	% CO_2: 80–90% WL static: 90% WL dynamic: <90%	[85]
PHB	Sea water	Intertidal zone, pelagic (10 m depth), benthic (20 m depth).	Degradation rate (DR)	-	DR: Benthic > Intertidal > Pelagic	[86]
PHBV	Soil	-	Weight loss	112	0.5	[87]
PHBV flax	Soil	-	Weight loss	112	6	[87]
PHBV/PBAT/ flax	Soil	-	Weight loss	112	9	[87]
PHBV/ENR flax	Soil	-	Weight loss	112	17	[87]
PHBV	Compost	58 °C	Produced CO_2	100	63.2	[88]
PHBV/flaxseed fibers	Compost	58 °C	Produced CO_2	100	85.6	[88]

Bioplastics	Type of Environment	Conditions	Control Method	Biodegradation Period (days)	Biodegradation (%) Others Data	Reference
PHBV/flax/ alginic	Compost	58 °C	Produced CO_2	100	88.0	[88]
PHBHHx/PBAT	Sea water	-	Weight loss	28	31 (ratio 100/0) 19 (ratio 80/20) 10 (ratio 60/40) 3 (ratio 40/60) 1 (ratio 0/100)	[89]
PHBHHx/PBS	Sea water	-	Weight loss	28	51 (ratio 100/0) 41(ratio 80/20) 18 (ratio 60/40) 5 (ratio 40/60) 1 (ratio 0/100)	[89]
PHBHHx/PLA	Sea water	-	Weight loss	28	34 (ratio 100/0) 33 (ratio 80/20) 32 (ratio 60/40) 26 (ratio 40/60) 1 (ratio 0/100)	[89]
			PCL			
PCL	Compost	58 °C	Produced CO_2	72	~100	[90]
PCL/TPS	Compost	58 °C	Produced CO_2	72	~ 90 (ratio 50/50) ~ 95 (ratio 30/70)	[90]
PCL	Soil	30 °C	Weight loss	90	2.5	[45]
PCL	Sea water	Depth: 321 m, 350 m, 612 m. Low temperatures and high hydrostatic pressure.	Resistance to break, (RB) and surface morphology (SM)	270–360	RB decrease: 0–20% SM: abundant pores and heterogeneous cracks	[91]

Bioplastics	Type of Environment	Conditions	Control Method	Biodegradation Period (days)	Biodegradation (%) Others Data	Reference
PCL	Sea water and fresh water	25 °C and fluorescence light (16 h light and 8 h dark)	Weight loss	365	Non-significant degradability	[81]
Others						
PBS/Starch	Soil	25 °C, 60% humidity	Weight loss	28	7 (films) 24 (powdered)	[92]
PBS	Soil	25 °C, 60% humidity	Weight loss	28	1 (films) 16.8 (powdered)	[92]
PVA	Compost	-	Iodometric analysis	8	51–79	[23]
PBS	Sea water	Depth: 321 m, 350 m, 612 m. Low temperatures and high hydrostatic pressure.	Resistance to break, RB) and surface morphology (SM)	360	RB decrease≈ 100% SM: rough surface with many stains	[91]
PBSe	Sea water	Intertidal zone, pelagic (10 m depth), benthic (20 m depth).	Weight loss and Degradation rate (DR)	-	DT: Benthic > Intertidal > Pelagic	[86]
PBSet	Sea water	Intertidal zone, pelagic (10 m depth), benthic (20 m depth).	Weight loss Degradation rate (DR)	-	DT: Benthic > Intertidal > Pelagic	[86]

CNC: cellulose nanocrystal, ENR: epoxidized natural rubber, PBSe: Polybutylene sebacate, PBSet: Polybutylene sebacate-co-terephthalate, PHBHHx: poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate), PHV: polyhydroxyvalerate, PLGA: poly(lactic-co-glycolic acid), TPS: thermoplastic starch.

The structure and composition of the biomaterials extremely affect the biodegradation process in its different stages [93]. In this sense, the range of values observed in **Table 4** for PLA is attributed to the effect that different fillers used have on biodegradation behavior [69]. In general, it has been observed that those fillers enhancing the hydrophilicity of the composite material promote the polymer hydration capacity and the effectiveness of the degradative action of microorganisms. In contrast, the increase in the material hydrophobicity decreases the rate of biodegradation, Likewise, those additives or mechanisms that inhibit the crystallization of the polymer promote degradation rate. PHBV, where the copolymerization with hydroxyvalerate decreases the crystallinity with respect to the PHB, exhibited a more effective biodegradation than PHB [94]. However, most biodegradation studies have been carried out on pure biopolymers or mixtures, without taking into account the different additives that are added to enhance the functionality of the material. The addition of plasticizers, many of them non-biodegradable, increases the longevity of the bioplastic in the environment. In general, degradation in a compost medium is more effective than in soil or aquatic environments due to the richness of the active microbial population and the ability to adjust environmental conditions. In marine environments, different marine habitats with very different biodegradation conditions must be considered [95]. Thus, in deep waters, the lack of UV radiation and low temperatures and O_2 concentration make the biodegradation process slower, being the extensive degradation less likely than in other environments [96]. For this reason, some authors have suggested the need of biodegradation studies in six different aquatic habitats: supralittoral (splash zone), eulittoral (or intertidal), sub-coastal (subtidal), benthic region (marine), pelagic and buried in sediments. These habitats mainly differ in temperature, UV light, pressure, density and oxygen and nutrient content. Thus, different authors have found that biodegradation in pelagic habitat (near surface seawater) is more efficient than in eutrophic habitat (lakes and reservoirs with excess phytoplankton) and that the maximum rate of biodegradation occur at the water-sediment interface for being richer in microorganisms [95].

The flow conditions of the water mass (static or dynamic) also affected the biodegradation pattern. Some studies showed that the biodegradation rate is higher

under static than dynamic conditions surely because of the broader temperature changes and the limited supply of nutrients under dynamic conditions. Likewise, when plastics are buried, in close contact with sediments, the biodegradation is usually positively influenced [85].

Although in aquatic systems temperature presents great variations depending on the depth, the season of the year and the geographical area, most of the studies shown in **Table 4** were carried out between 12–22 °C (pH between 7.9 and 8.1). Among the microorganisms capable of degrading plastics in aquatic systems (marine and freshwater), *Pseudomonas*, *Bacillus, Alvanivorax*, *Tenacibaculum*, *Lepthotrix*, *Enterobacter*, *Variovorax* and *Actinomyces*, including *Streptomyces,* have been found. Volova et al. [70] evaluated the effect of the shape/3D dimension of the polymeric material, concluding that PHA films degraded faster than PHA pellets due to their greater surface area, which facilitates the adhesion of microorganisms to their surface, this being beneficial for the polymer degradation.

As can be observed in **Table 4**, most of the studies found in aquatic systems do not evaluate the conversion of polymer carbon into CO_2 by microorganisms, but rather use other techniques that indirectly allows for estimating the total degradation of the plastic (carried out in a biotic and abiotic processes). In this way, these studies were carried out by measuring the changes in the material physicochemical properties, such as mechanical, molecular weight or mass loss, which became altered because of the degradative process. Thus, these can be not strictly considered as biodegradation but degradation studies.

4. EFFECT OF ANTIMICROBIALS ON THE BIODEGRADATION OF POLYMER BASED ACTIVE PACKAGING MATERIALS

Different studies have analyzed the influence of antimicrobials, on the biodegradation behavior of active packaging materials, as shown in **Table 5**. The viability and growth of relevant microorganisms that are involved in the biodegradation process can be affected by the released antimicrobial compounds after swelling or disintegration of the polymeric matrix. The action mechanism that takes place in the composting medium will depend on the nature of the antimicrobial (volatility, level of persistence in the medium,

etc.), and coincides with the mechanisms that confers the antimicrobial action to the films. Usual action mechanisms include the alteration of the cell permeability leading to disrupted bacterial cell membranes and releasing the cellular content.

Table 5. Some effects of the incorporated antimicrobial compounds on the biodegradation of polymeric films.

Polymer	Antimicrobial	Type of Environment	Main Feature	Reference
Starch/PVA	Sodium propionate	Soil	The antimicrobial did not interfere with biodegradation. 90% degradation in 28 days	[97]
PLA	Propolis (crude propolis and its ethanolic extract)	Soil	Propolis promoted biodegradation	[98]
PHBV	Silver nanoparticles	Soil	Biochar accelerated biodegradation. Silver nanoparticles significantly reduced biodegradability	[99]
Maize starch/chitosan	Chitosan	Compost	In 15 days, the chitosan did not negatively affect the biodegradation	[100]
Brown rice starch/chitosan	Chitosan	Compost	Biodegradation was faster with higher proportion of starch	[16]
Starch/PVA	Neem oil, oregano essential oil and silver nanoparticles	Compost	The oils improved the biodegradation of films Silver nanoparticles inhibited biodegradation	[101]
PBAT/ thermoplastic starch	Polyhexamethylene Guanidine Hydrochloride (PHPG)	Soil	Antimicrobial delayed the biodegradation	[102]
Pectin	Copaiba oil	Soil	Delay biodegradation of polymer	[103]

Polymer	Antimicrobial	Type of Environment	Main Feature	Reference
Starch/PCL	Carvacrol	Compost	Carvacrol delayed biodegradation	[104]
PHBV/PLA-PHB	Catechin	Compost	Catechin delayed disintegration process Lactic acid accelerated it	[105]
Starch/PVA	Silicon oxide nanoparticles	Soil	Silicon oxide nanoparticles did not affected biodegradation	[106]
Ecoflex®	Zinc oxide nanoparticles and microcapsules with ionic zinc	Soil	Zinc compounds did not affect biodegradation process	[107]
PCL	Grapefruit seed extract (GSE)	Soil	Biodegradation was faster as the incorporated amount of GSE increased	[45]

Sen & Das [97] observed 90% biodegradation in soil of PVA films formulated with starch and sodium propionate (1.791 g/100 g of polymer) throughout 28 days. In this study, the antimicrobial compound did not interfere with the action of the soil microflora and did not hinder the biodegradation of the film, which simultaneously contributed to the increase of nutrients in the soil. Furthermore, the pH was kept within the accepted limit for plant growth. Therefore, the presence of sodium propionate in the film did not prevent their biodegradation, and the films exerted a positive effect on plant growth. Ulloa et al. [98] carried out a biodegradability test in soil, with PLA films containing propolis at different concentrations: powdered raw propolis and ethanolic extract of propolis at concentrations of 5, 8.5, and 13% *w/w* of PLA. The authors observed a higher weight loss in the films incorporating active compounds, with values ranging between 2.5 and 5% for films with crude propolis powder and 9–24% after 314 days for films with ethanolic extract of propolis. The presence of propolis supposed a contribution of nutrients for the microorganisms, thus enhancing film degradation.

Costa et al. [99] studied the biodegradation of PHBV with silver nanoparticles (Ag/PHBV was 0.024 wt%) in a tropical soil under laboratory conditions incorporating Biochar® (charcoal obtained from plant debris and waste biomass of sugarcane bagasse). Biochar® was used as a tool to accelerate the compound degradation. The addition of 5–10% Biochar® in the soil increased the degradation of these polymeric materials 2 to 3 times after 30 days of incubation ground. However, the presence of silver nanoparticles significantly reduced the potential for degradability of the nanocomposite by the microbial community of the soil.

Pavoni et al. [100] studied the biodegradation of starch/chitosan blend films. The mixture of starch:chitosan solutions were prepared using different proportions of 1:1, 2:1 and 3:1 (*v/v*). Incorporating chitosan did not interfere with the behavior of biodegradation under the conditions considered in compost medium. However, Hasan et al. [16] concluded that the biodegradation rate of the starch/chitosan films (at 30:70, 50:50 and 70:30 starch/chitosan ratios) was strongly influenced by the content of chitosan; the greater the chitosan content the lower the biodegradation rate of the films in the composting medium, thus reflecting the antimicrobial effect of chitosan on the compost population.

Cano et al. [101] obtained films of starch, PVA and mixtures of both polymers with different concentrations of neem oil, oregano essential oil (1:0.5 and 1:0.125 starch:oil ratio) and silver nanoparticles at different weight ratios with respect to starch (1:0.006, 1:0.06, 1:0.16 and 1:0.32). The analysis of their decay behavior and biodegradation in compost medium during 73 and 45 days showed that the presence of neem and oregano oils improved disintegration levels and biodegradation. However, the biodegradability of films incorporating silver nanoparticles was seriously diminished, reflecting the influence of silver nanoparticles on the activity of the microbial population of the compost as silver lasts longer in the environment in comparison with the essential oils. Wang et al. [102] investigated the biodegradation behavior of PBAT (poly(butylene adipate-co-terephthalate) films and thermoplastic starch. The decay data (loss weight) showed that both PBAT and starch could be degraded, even with the presence of the antimicrobial substances (polyhexamethylene guanidine hydrochloride) at 1.0 and 0.99 wt%, but antimicrobials reduced the biodegradation rate.

Norcino et al. [103] incorporated copaiba oil nano-emulsion as an antimicrobial in pectin films that were prepared from aqueous solutions (6 wt%.) of pectin combined at 1:1 (weight ratio) with the previously prepared nanoemulsions (1%–6% *w/w*). Reported results showed a gradual decrease in the production of CO_2 as the concentration of the antimicrobial increased, thus indicating that the active compounds of the copaiba oil nano-emulsion interfered with the biodegradation pattern of the pectin films in soil. Tampau et al. [104] evaluated the biodegradation behavior of multilayer films of thermoplastic starch and PCL, with carvacrol (15 g carvacrol/100 g PCL) incorporated into the PCL layer, through weight loss and CO_2 measurements. All carvacrol-free films completely biodegraded after 25 days of composting. However, the presence of carvacrol notably affected the activity of the inoculum, thus limiting the biodegradability of the carvacrol loaded multilayers to a maximum value of about 85% after 45 days. Arrieta et al. [105] obtained various bilayer formulations of PHBV/PLA with catechin (1 and 3 wt%) and lactic acid oligomers. After 23 days of disintegration in compost, the bilayer systems began to separate. The incorporation of catechin slightly delayed the disintegration process, while lactic acid accelerated it. Tang et al. [106] prepared several starch/PVA/nano-SiO_2 (0.5, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.5 g SiO_2/10 g starch/PVA) blend films by casting. The soil burial test showed that the addition of nano-SiO_2 did not have a significant influence on biodegradability of the films. Capelezzo et al. [107] evaluated the biodegradation in soil of Ecoflex® polymers with zinc oxide nanoparticles (1 and 2% *w/w*), as an antimicrobial. The addition of zinc compounds to the biodegradable polymer did not affect its behavior biodegradation. Lyu et al. [45] developed PCL films incorporating different concentrations of grapefruit seed extract (GSE: 0, 1, 3 and 5 %) as antimicrobial agent and evaluated the biodegradation in soil. Biodegradation was faster as the amount of antimicrobial incorporated increased, in line with the lower cohesiveness of the PCL matrix when active compound was present and the lack of effective interferences of GSE with the microbial population activity.

In most of studies, no great effect of the incorporated antimicrobials on film degradation was observed, although in some cases a delay of the process was caused by the influence of active compounds on the microbial population and activity of the medium. The potential changes in the biodegradation behavior of the polymer in the antimicrobial

materials will depend on the doses of the active compounds in the films, their release kinetics to the medium, and the sensitivity of the different microorganism responsible for the degradative process. Moreover, the incorporation of antimicrobials modifies the physical structure of the matrix, their permeability properties and wetting capacity. This can favor, in some cases, its disintegration and biodegradation process. When the active compounds promote the hydrophilic nature of the polymeric matrix and so, its wetting capacity, its sensitivity to the microbial action is enhanced. No biodegradation studies of antimicrobial films have been found in aquatic environments. Therefore, specific studies are necessary in each case to know the influence of a determined antimicrobial on the degradation behavior of a specific polymeric matrix in different media.

5. FINAL REMARKS

Biodegradation of different bioplastics (from biomass, synthetic or produced by microorganisms) depends on the polymer molecular structure, crystallinity and fillers or incorporated compounds, as well as on the environmental conditions, such as microorganism population, pH, humidity and temperature of the compost, soil or aquatic media. Antimicrobial materials have been obtained from these biopolymers, by incorporating active compounds, such as plant extracts, essential oils or their compounds and inorganic compounds, such as silver or zinc nanoparticles. These materials have high potential for food packaging since they allow to extend the product shelf-life but can negatively affect the material biodegradation in determined environmental conditions. More biodegradation studies in different media (compost, soil, and marine environments) are needed to ensure the safety of these materials exposed to different ecosystems. Composting is a sustainable strategy for the management of these biopolymer-based active packaging materials, but it is necessary to ensure that the effect of the incorporated antimicrobials does not affect the process. On the other hand, the biodegradation of bioplastics in aquatic and marine ecosystems requires in-depth studies since the accumulation of bioplastics can inevitably occur in these environments.

Funding: This research was funded by *Ministerio de Ciencia e Innovación* of Spain through the Project AGL2016-76699-R, PID2019-105207RB-I00, and the predoctoral research grant #BES-2017-082040.

6. REFERENCES

1. Alvarado Mendoza, D.L. Envasado en la Industria de Alimentos y sus Nuevas Tendencias. Bachelor's Thesis, Universidad Autónoma Agraria, Saltillo, México, 2009.
2. Asociación Española de Basuras Marinas (AEBAM). ¿Qué Son Las Basuras Marinas? 2020**.** Available online: https://aebam.org/basuras-marinas/ (accessed on 14 November 2020).
3. Arrieta, M.P.; Peltzer, M.A.; Garrigós Selva, M.C.; Jímenez Migallón, A. Envases alimentarios sostenibles. Biopelículas activas obtenidas a partir de proteínas lácteas. *Segur. Medio Ambient.* **2011**, *121*, 46–56.
4. Labeaga, A. Polímeros Biodegradables. Importancia y potenciales Aplicaciones. Master's Thesis, Universidad Nacional de Educación a Distancia, Madrid, Spain, 2018**.**
5. European Bioplastics, Nova Institute. 2019**.** Available online: www.european-biplastics.org/market and www.bio-based.eu./markets (accessed on 8 September 2020).
6. Tubón, I. Formulación, Elaboración y Evaluación de Bioenvase para Caramelos a Base de Almidón de Yuca, Sacarosa y Gelatina. Bachelor's Thesis, Universidad Nacional de Chimborazo, Riobamba, Ecuador, 2013.
7. Vermeiren, F.L.; Devlieghere, J.D. Effectiveness of some recent antimicrobial packaging concepts. *Food Aditives Contam.* **2002**, *19*, 163–171, doi:10.1080/0265203011010485 2.
8. Rivas, C.F. Envases activos e inteligentes: Control de la calidad y seguridad del producto. *Instituto Tecnólogico Embalaje Logística*. **2012**, *34*.
9. Ross, G.; Ross, S.; Tighe, B.J. *Bioplastics: New Routes, New Products*; Elsevier Ltd.: Amsterdam, The Netherlands, 2017; ISBN 9780323358248. doi: 10.1016/B978-0-323-35824-8.00023-2.
10. Valencia-Sullca, C.; Vargas, M.; Atarés, L.; Chiralt, A. Thermoplastic cassava starch-chitosan bilayer films containing essential oils. *Food Hydrocoll.* **2018**, *75*, 107–115, doi:10.1016/j.foodhyd.2017.09.008.

11. Valencia-Sullca, C.; Atarés, L.; Vargas, M.; Chiralt, A. Physical and Antimicrobial Properties of Compression-Molded Cassava Starch-Chitosan Films for Meat Preservation. *Food Bioprocess Technol.* **2018**, *11*, 1339–1349, doi:10.1007/s11947-018-2094-5.
12. Moreno, O.; Atarés, L.; Chiralt, A.; Cruz-Romero, M.C.; Kerry, J. Starch-gelatin antimicrobial packaging materials to extend the shelf life of chicken breast fillets. *Lwt* **2018**, *97*, 483–490, doi:10.1016/j.lwt.2018.07.005.
13. Silveira, M.P.; Silva, H.C.; Pimentel, I.C.; Poitevin, C.G.; da Costa Stuart, A.K.; Carpiné, D.; de Matos Jorge, L.M.; Jorge, R.M.M. Development of active cassava starch cellulose nanofiber-based films incorporated with natural antimicrobial tea tree essential oil. *J. Appl. Polym. Sci.* **2020**, *137*, 1–11, doi:10.1002/app.48726.
14. Souza, A.C.; Goto, G.E.O.; Mainardi, J.A.; Coelho, A.C.V.; Tadini, C.C. Cassava starch composite films incorporated with cinnamon essential oil: Antimicrobial activity, microstructure, mechanical and barrier properties. *LWT-Food Sci. Technol.* **2013**, *54*, 346–352, doi:10.1016/j.lwt.2013.06.017.
15. Cano, A.; Cháfer, M.; Chiralt, A.; González-Martínez, C. Development and characterization of active films based on starch-PVA, containing silver nanoparticles. *Food Packag. Shelf Life* **2016**, *10*, 16–24, doi:10.1016/j.fpsl.2016.07.002.
16. Hasan, M.; Gopakumar, D.A.; Olaiya, N.G.; Zarlaida, F.; Alfian, A.; Aprinasari, C.; Alfatah, T.; Rizal, S.; Khalil, H.P.S.A. Evaluation of the thermomechanical properties and biodegradation of brown rice starch-based chitosan biodegradable composite films. *Int. J. Biol. Macromol.* **2020**, *156*, 896–905, doi:10.1016/j.ijbiomac.2020.04.039.
17. Syafiq, R.; Sapuan, S.M.; Zuhri, M.R.M. Antimicrobial activity, physical, mechanical and barrier properties of sugar palm based nanocellulose/starch biocomposite films incorporated with cinnamon essential oil. *J. Mater. Res. Technol.* **2021**, *11*, 144–157, doi:10.1016/j.jmrt.2020.12.091.
18. Shapi'i, R.A.; Othman, S.H.; Nordin, N.; Kadir Basha, R.; Nazli Naim, M. Antimicrobial properties of starch films incorporated with chitosan nanoparticles: *In vitro* and *in vivo* evaluation. *Carbohydr. Polym.* **2020**, *230*, 115602, doi:10.1016/j.carbpol.2019.115602.

19. Cano, A.; Cháfer, M.; Chiralt, A.; González-Martínez, C. Physical and Antimicrobial Properties of Starch-PVA Blend Films as Affected by the Incorporation of Natural Antimicrobial Agents. *Foods* **2015**, *5*, 3, doi:10.3390/foods5010003.
20. Perdana, M.I.; Ruamcharoen, J.; Panphon, S.; Leelakriangsak, M. Antimicrobial activity and physical properties of starch/chitosan film incorporated with lemongrass essential oil and its application. *Lwt* **2021**, *141*, 110934, doi:10.1016/j.lwt.2021.110934.
21. Dhumal, C.V.; Ahmed, J.; Bandara, N.; Sarkar, P. Improvement of antimicrobial activity of sago starch/guar gum bi-phasic edible films by incorporating carvacrol and citral. *Food Packag. Shelf Life* **2019**, *21*, 100380, doi:10.1016/j.fpsl.2019.100380.
22. Yan, Q.; Zhang, J.; Dong, H.; Hou, H.; Guo, P. Properties and antimicrobial activities of starch-sodium alginate composite films incorporated with sodium dehydroacetate or rosemary extract. *J. Appl. Polym. Sci.* **2013**, *127*, 1951–1958, doi:10.1002/app.37570.
23. Liu, Y.; Deng, Y.; Chen, P.; Duan, M.; Lin, X.; Zhang, Y. Biodegradation analysis of polyvinyl alcohol during the compost burial course. *J. Basic Microbiol.* **2019**, *59*, 368–374, doi:10.1002/jobm.201800468.
24. de Moraes, J.O.; Hilton, S.T.; Moraru, C.I. The effect of Pulsed Light and starch films with antimicrobials on Listeria innocua and the quality of sliced cheddar cheese during refrigerated storage. *Food Control* **2020**, *112*, 107134, doi:10.1016/j.foodcont.2020.107134.
25. Moreno, O.; Gil, À.; Atarés, L.; Chiralt, A. Active starch-gelatin films for shelf-life extension of marinated salmon. *LWT-Food Sci. Technol.* **2017**, *84*, 189–195, doi:10.1016/j.lwt.2017.05.005.
26. Ali, A.; Chen, Y.; Liu, H.; Yu, L.; Baloch, Z.; Khalid, S.; Zhu, J.; Chen, L. Starch-based antimicrobial films functionalized by pomegranate peel. *Int. J. Biol. Macromol.* **2019**, *129*, 1120–1126, doi:10.1016/j.ijbiomac.2018.09.068.
27. Serna, C.L.; Rodríguez de S.A.; Albán, A.F. Ácido Poliláctico (PLA): Propiedades y Aplicaciones. *Ing. Compet.* **2011**, *5*, 16, doi:10.25100/iyc.v5i1.2301.
28. Chen, J.; Wei, D.; Gong, W.; Zheng, A.; Guan, Y. Hydrogen-Bond Assembly of Poly(vinyl alcohol) and Polyhexamethylene Guanidine for Nonleaching and

Transparent Antimicrobial Films. *ACS Appl. Mater. Interfaces* **2018**, *10*, 37535–37543, doi:10.1021/acsami.8b14238.

29. Hajji, S.; Chaker, A.; Jridi, M.; Maalej, H.; Jellouli, K.; Boufi, S.; Nasri, M. Structural analysis, and antioxidant and antibacterial properties of chitosan-poly (vinyl alcohol) biodegradable films. *Environ. Sci. Pollut. Res.* **2016**, *23*, 15310–15320, doi:10.1007/s11356-016-6699-9.
30. Cameron, R.E.; Kamvari-Moghaddam, A. *Degradation Rate of Bioresorbable Materials*; Woodhead Publishing Limited: Sawston, UK, 2008; Synthetic Bioresorbable Polymers, pp. 43-66. ISBN 9781845693299.
31. Perveen, R.; Inamuddin; Nasar, A. *Multiwalled Carbon Nanotube-Based Nanocomposites for Artificial Bone Grafting*; Elsevier Inc.: Amsterdam, The Netherlands, 2018; ISBN 9780128137574.
32. Ahmed, J.; Hiremath, N.; Jacob, H. Antimicrobial, Rheological, and Thermal Properties of Plasticized Polylactide Films Incorporated with Essential Oils to Inhibit Staphylococcus aureus and Campylobacter jejuni. *J. Food Sci.* **2016**, *81*, E419–E429, doi:10.1111/1750-3841.13193.
33. Ma, P.; Jiang, L.; Yu, M.; Dong, W.; Chen, M. Green Antibacterial Nanocomposites from Poly(lactide)/Poly(butylene adipate-co-terephthalate)/Nanocrystal Cellulose-Silver Nanohybrids. *ACS Sustain. Chem. Eng.* **2016**, *4*, 6417–6426, doi:10.1021/acssuschemeng.6b01106.
34. Coltelli, M.B.; Aliotta, L.; Vannozzi, A.; Morganti, P.; Panariello, L.; Danti, S.; Neri, S.; Fernandez-Avila, C.; Fusco, A.; Donnarumma, G.; et al. Properties and skin compatibility of films based on poly(lactic acid) (PLA) bionanocomposites incorporating chitin nanofibrils (CN). *J. Funct. Biomater.* **2020**, *11*, 21, doi:10.3390/jfb11020021.
35. Khodayari, M.; Basti, A.A.; Khanjari, A.; Misaghi, A.; Kamkar, A.; Shotorbani, P.M.; Hamedi, H. Effect of poly(lactic acid) films incorporated with different concentrations of Tanacetum balsamita essential oil, propolis ethanolic extract and cellulose nanocrystals on shelf life extension of vacuum-packed cooked sausages. *Food Packag. Shelf Life* **2019**, *19*, 200–209, doi:10.1016/j.fpsl.2018.11.009.
36. Muller, J.; Casado Quesada, A.; González-Martínez, C.; Chiralt, A. Antimicrobial properties and release of cinnamaldehyde in bilayer films based on polylactic acid

(PLA) and starch. *Eur. Polym. J.* **2017**, *96*, 316–325, doi:10.1016/j.eurpolymj.2017.09.009.

37. Bonilla, J.; Fortunati, E.; Vargas, M.; Chiralt, A.; Kenny, J.M. Effects of chitosan on the physicochemical and antimicrobial properties of PLA films. *J. Food Eng.* **2013**, *119*, 236–243, doi:10.1016/j.jfoodeng.2013.05.026.
38. Olewnik-Kruszkowska, E.; Gierszewska, M.; Jakubowska, E.; Tarach, I.; Sedlarik, V.; Pummerova, M. Antibacterial films based on PVA and PVA-chitosan modified with poly(hexamethylene guanidine). *Polymers* **2019**, *11*, 93, doi:10.3390/polym11122093.
39. Haghighi, H.; Leugoue, S.K.; Pfeifer, F.; Siesler, H.W.; Licciardello, F.; Fava, P.; Pulvirenti, A. Development of antimicrobial films based on chitosan-polyvinyl alcohol blend enriched with ethyl lauroyl arginate (LAE) for food packaging applications. *Food Hydrocoll.* **2020**, *100*, 105419, doi:10.1016/j.foodhyd.2019.105419.
40. Suganthi, S.; Vignesh, S.; Kalyana Sundar, J.; Raj, V. Fabrication of PVA polymer films with improved antibacterial activity by fine-tuning via organic acids for food packaging applications. *Appl. Water Sci.* **2020**, *10*, 1–11, doi:10.1007/s13201-020-1162-y.
41. Tripathi, S.; Mehrotra, G.K.; Dutta, P.K. Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications. *Int. J. Biol. Macromol.* **2009**, *45*, 372–376, doi:10.1016/j.ijbiomac.2009.07.006.
42. Li, Z.; Zhou, P.; Zhou, F.; Zhao, Y.; Ren, L.; Yuan, X. Antimicrobial eugenol-loaded electrospun membranes of poly(ε-caprolactone)/gelatin incorporated with REDV for vascular graft applications. *Colloids Surfaces B Biointerfaces* **2018**, *162*, 335–344, doi:10.1016/j.colsurfb.2017.12.004.
43. Salević, A.; Prieto, C.; Cabedo, L.; Nedović, V.; Lagaron, J.M. Physicochemical, antioxidant and antimicrobial properties of electrospun poly(ε-caprolactone) films containing a solid dispersion of sage (Salvia officinalis L.) extract. *Nanomaterials* **2019**, *9*, 270, doi:10.3390/nano9020270.
44. Takala, P.N.; Vu, K.D.; Salmieri, S.; Khan, R.A.; Lacroix, M. Antibacterial effect of biodegradable active packaging on the growth of Escherichia coli, Salmonella

typhimurium and Listeria monocytogenes in fresh broccoli stored at 4 °C. *LWT Food Sci. Technol.* **2013**, *53*, 499–506, doi:10.1016/j.lwt.2013.02.024.

45. Lyu, J.S.; Lee, J.S.; Han, J. Development of a biodegradable polycaprolactone film incorporated with an antimicrobial agent via an extrusion process. *Sci. Rep.* **2019**, *9*, 1–11, doi:10.1038/s41598-019-56757-5.
46. Khalid, S.; Yu, L.; Feng, M.; Meng, L.; Bai, Y.; Ali, A.; Liu, H.; Chen, L. Development and characterization of biodegradable antimicrobial packaging films based on polycaprolactone, starch and pomegranate rind hybrids. *Food Packag. Shelf Life* **2018**, *18*, 71–79, doi:10.1016/j.fpsl.2018.08.008.
47. Uzunlu, S.; Niranjan, K. Laboratory antimicrobial activity of cinnamaldehyde and pomegranate-based polycaprolactone films. *J. Appl. Polym. Sci.* **2017**, *134*, 1–9, doi:10.1002/app.45347.
48. Tampau, A.; González-Martínez, C.; Chiralt, A. Release kinetics and antimicrobial properties of carvacrol encapsulated in electrospun poly-(ε-caprolactone) nanofibres. Application in starch multilayer films. *Food Hydrocoll.* **2018**, *79*, 158–169, doi:10.1016/j.foodhyd.2017.12.021.
49. Steinbüchel, A.; Hustede, E.; Liebergesell, M.; Pieper, U.; Timm, A.; Valentin, H. Molecular basis for biosynthesis and accumulation of polyhydroxyalkanoic acids in bacteria. *FEMS Microbiol. Lett.* **1992**, *103*, 217–230, doi:10.1016/0378-1097(92)90313-D.
50. Erceg, M.; Kovačić, T.; Klarić, I. Thermal degradation of poly(3-hydroxybutyrate) plasticized with acetyl tributyl citrate. *Polym. Degrad. Stab.* **2005**, *90*, 313–318, doi:10.1016/j.polymdegradstab.2005.04.048.
51. Rivera-Briso, A.L.; Serrano-Aroca, Á. Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate): Enhancement strategies for advanced applications. *Polymers* **2018**, *10*, 732, doi:10.3390/polym10070732.
52. Basnett, P.; Marcello, E.; Lukasiewicz, B.; Nigmatullin, R.; Paxinou, A.; Ahmad, M.H.; Gurumayum, B.; Roy, I. Antimicrobial materials with lime oil and a poly(3-hydroxyalkanoate) produced via valorisation of sugar cane molasses. *J. Funct. Biomater.* **2020**, *11*, 24, doi:10.3390/jfb11020024.
53. Castro-Mayorga, J.L.; Martínez-Abad, A.; Fabra, M.J.; Olivera, C.; Reis, M.; Lagarón, J.M. Stabilization of antimicrobial silver nanoparticles by a polyhydroxyalkanoate

obtained from mixed bacterial culture. *Int. J. Biol. Macromol*. **2014**, *71*, 103–110, doi:10.1016/j.ijbiomac.2014.06.059.

54. Xu, P.; Yang, W.; Niu, D.; Yu, M.; Du, M.; Dong, W.; Chen, M.; Jan Lemstra, P.; Ma, P. Multifunctional and robust polyhydroxyalkanoate nanocomposites with superior gas barrier, heat resistant and inherent antibacterial performances. *Chem. Eng. J.* **2020**, *382*, 122864, doi:10.1016/j.cej.2019.122864.
55. Xavier, J.R.; Babusha, S.T.; George, J.; Ramana, K.V. Material Properties and Antimicrobial Activity of Polyhydroxybutyrate (PHB) Films Incorporated with Vanillin. *Appl. Biochem. Biotechnol.* **2015**, *176*, 1498–1510, doi:10.1007/s12010-015-1660-9.
56. Correa, J.P.; Molina, V.; Sanchez, M.; Kainz, C.; Eisenberg, P.; Massani, M.B. Improving ham shelf life with a polyhydroxybutyrate/polycaprolactone biodegradable film activated with nisin. *Food Packag. Shelf Life* **2017**, *11*, 31–39, doi:10.1016/j.fpsl.2016.11.004.
57. Narayanan, A.; Neera; Mallesha; Ramana, K.V. Synergized antimicrobial activity of eugenol incorporated polyhydroxybutyrate films against food spoilage microorganisms in conjunction with pediocin. *Appl. Biochem. Biotechnol.* **2013**, *170*, 1379–1388, doi:10.1007/s12010-013-0267-2.
58. Fan, X.; Jiang, Q.; Sun, Z.; Li, G.; Ren, X.; Liang, J.; Huang, T.S. Preparation and characterization of electrospun antimicrobial fibrous membranes based on polyhydroxybutyrate (PHB). *Fibers Polym.* **2015**, *16*, 1751–1758, doi:10.1007/s12221-015-5108-1.
59. Figueroa-Lopez, K.J.; Torres-Giner, S.; Enescu, D.; Cabedo, L.; Cerqueira, M.A.; Pastrana, L.M.; Lagaron, J.M. Electrospun active biopapers of food waste derived poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with short-term and long-term antimicrobial performance. *Nanomaterials* **2020**, *10*, 506, doi:10.3390/nano10030506.
60. Requena, R.; Jiménez, A.; Vargas, M.; Chiralt, A. Poly[(3-hydroxybutyrate)-co-(3-hydroxyvalerate)] active bilayer films obtained by compression moulding and applying essential oils at the interface. *Polym. Int.* **2016**, *65*, 883–891, doi:10.1002/pi.5091.

61. Melendez-Rodriguez, B.; Figueroa-Lopez, K.J.; Bernardos, A.; Martínez-Máñez, R.; Cabedo, L.; Torres-Giner, S.; Lagaron, J.M. Electrospun antimicrobial films of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) containing eugenol essential oil encapsulated in mesoporous silica nanoparticles. *Nanomaterials* **2019**, *9*, 227, doi:10.3390/nano9020227.
62. Sabharwal, P.K.; Chattopadhyay, S.; Singh, H. Preparation and characterization of antimicrobial, biodegradable, triclosan-incorporated polyhydroxybutyrate-co-valerate films for packaging applications. *J. Appl. Polym. Sci.* **2018**, *135*, 1–10, doi:10.1002/app.46862.
63. Requena, R.; Vargas, M.; Chiralt, A. Eugenol and carvacrol migration from PHBV films and antibacterial action in different food matrices. *Food Chem.* **2019**, *277*, 38–45, doi:10.1016/j.foodchem.2018.10.093.
64. Requena, R.; Vargas, M.; Chiralt, A. Obtaining antimicrobial bilayer starch and polyester-blend films with carvacrol. *Food Hydrocoll.* **2018**, *83*, 118–133, doi:10.1016/j.foodhyd.2018.04.045.
65. Lucas, N.; Bienaime, C.; Belloy, C.; Queneudec, M.; Silvestre, F.; Nava-Saucedo, J.E. Polymer biodegradation: Mechanisms and estimation techniques—A review. *Chemosphere* **2008**, *73*, 429–442, doi:10.1016/j.chemosphere.2008.06.064.
66. Muniyasamy, S.; Anstey, A.; Reddy, M.M.; Misra, M.; Mohanty, A. Biodegradability and compostability of lignocellulosic based composite materials. *J. Renew. Mater.* **2013**, *1*, 253–272, doi:10.7569/JRM.2013.634117.
67. Youssef, A.M.; El-Sayed, S.M. Bionanocomposites materials for food packaging applications: Concepts and future outlook. *Carbohydr. Polym.* **2018**, *193*, 19–27, doi:10.1016/j.carbpol.2018.03.088.
68. Kale, G.; Kijchavengkul, T.; Auras, R.; Rubino, M.; Selke, S.E.; Singh, S.P. Compostability of bioplastic packaging materials: An overview. *Macromol. Biosci.* **2007**, *7*, 255–277, doi:10.1002/mabi.200600168.
69. Emadian, S.M.; Onay, T.T.; Demirel, B. Biodegradation of bioplastics in natural environments. *Waste Manag.* **2017**, *59*, 526–536, doi:10.1016/j.wasman.2016.10.006.
70. Volova, T.G.; Boyandin, A.N.; Vasiliev, A.D.; Karpov, V.A.; Prudnikova, S.V.; Mishukova, O.V.; Boyarskikh, U.A.; Filipenko, M.L.; Rudnev, V.P.; Bá Xuân, B.; et al.

Biodegradation of polyhydroxyalkanoates (PHAs) in tropical coastal waters and identification of PHA-degrading bacteria. *Polym. Degrad. Stab.* **2010**, *95*, 2350–2359, doi:10.1016/j.polymdegradstab.2010.08.023.

71. Harrison, J.P.; Boardman, C.; O'Callaghan, K.; Delort, A.M.; Song, J. Biodegradability standards for carrier bags and plastic films in aquatic environments: A critical review. *R. Soc. Open Sci.* **2018**, *5*, doi:10.1098/rsos.171792.
72. UNE-EN 13432. Requirements for Packaging Recoverable through Composting and Biodegradation Test Scheme and evaluation Criteria for the Final Acceptance of Packaging. 2001. Available online: https://www.une.org/encuentra-tu-norma/busca-tu-norma/norma?c=N0024465 (accessed on 10 January 2021).
73. Madejón, E.; Jesús Díaz, M.; López, R.; Cabrera, F. New approaches to establish optimum moisture content for compostable materials. *Bioresour. Technol.* **2002**, *85*, 73–78, doi:10.1016/S0960-8524(02)00030-5.
74. Miyatake, F.; Iwabuchi, K. Effect of compost temperature on oxygen uptake rate, specific growth rate and enzymatic activity of microorganisms in dairy cattle manure. *Bioresour. Technol.* **2006**, *97*, 961–965, doi:10.1016/j.biortech.2005.04.035.
75. Suler, D.J.; Finstein, M.S. Effect of temperature, aeration, and moisture on CO_2 formation in bench scale, continuously thermophilic composting of solid waste. *Appl. Environ. Microbiol.* **1977**, *33*, 345–350, doi:10.1128/aem.33.2.345-350.1977.
76. Sánchez-Monedero, M.A.; Roig, A.; Paredes, C.; Bernal, M.P. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. *Bioresour. Technol.* **2001**, *78*, 301–308, doi:10.1016/S0960-8524(01)00031-1.
77. Bidlingmaier, W.; Müsken, J. *Compost Science and Technology,* Volume 8; Elsevier, Amsterdam, The Netherlands, 2007; Odor Emissions from Composting Plants, pp. 215-328. ISBN 9780080439600 .
78. Luo, Y.; Lin, Z.; Guo, G. Biodegradation Assessment of Poly (Lactic Acid) Filled with Functionalized Titania Nanoparticles (PLA/TiO_2) under Compost Conditions. *Nanoscale Res. Lett.* **2019**, *14*, 56.
79. Luzi, F.; Fortunati, E.; Puglia, D.; Petrucci, R.; Kenny, J.M.; Torre, L. Study of disintegrability in compost and enzymatic degradation of PLA and PLA

nanocomposites reinforced with cellulose nanocrystals extracted from Posidonia Oceanica. *Polym. Degrad. Stab.* **2015**, *121*, 105–115, doi:10.1016/j.polymdegradstab.2015.08.016.

80. Lv, S.; Zhang, Y.; Gu, J.; Tan, H. Physicochemical evolutions of starch/poly (lactic acid) composite biodegraded in real soil. *J. Environ. Manage.* **2018**, *228*, 223–231, doi:10.1016/j.jenvman.2018.09.033.
81. Bagheri, A.R.; Laforsch, C.; Greiner, A.; Agarwal, S. Fate of So-Called Biodegradable Polymers in Seawater and Freshwater. *Glob. Challenges* **2017**, *1*, 1700048, doi:10.1002/gch2.201700048.
82. Balaguer, M.P.; Aliaga, C.; Fito, C.; Hortal, M. Compostability assessment of nano-reinforced poly(lactic acid) films. *Waste Manag.* **2016**, *48*, 143–155, doi:10.1016/j.wasman.2015.10.030.
83. Beltrán-Sanahuja, A.; Casado-Coy, N.; Simó-Cabrera, L.; Sanz-Lázaro, C. Monitoring polymer degradation under different conditions in the marine environment. *Environ. Pollut.* **2020**, *259*, doi:10.1016/j.envpol.2019.113836.
84. Volova, T.G.; Gladyshev, M.I.; Trusova, M.Y.; Zhila, N.O. Degradation of polyhydroxyalkanoates in eutrophic reservoir. *Polym. Degrad. Stab.* **2007**, *92*, 580–586, doi:10.1016/j.polymdegradstab.2007.01.011.
85. Thellen, C.; Coyne, M.; Froio, D.; Auerbach, M.; Wirsen, C.; Ratto, J.A. A processing, characterization and marine biodegradation study of melt-extruded polyhydroxyalkanoate (PHA) films. *J. Polym. Environ.* **2008**, *16*, 1–11, doi:10.1007/s10924-008-0079-6.
86. Briassoulis, D.; Pikasi, A.; Briassoulis, C.; Mistriotis, A. Disintegration behaviour of bio-based plastics in coastal zone marine environments: A field experiment under natural conditions. *Sci. Total Environ.* **2019**, *688*, 208–223, doi:10.1016/j.scitotenv.2019.06.129.
87. Zaidi, Z.; Mawad, D.; Crosky, A. Soil Biodegradation of Unidirectional Polyhydroxybutyrate-Co-Valerate (PHBV) Biocomposites Toughened With Polybutylene-Adipate-Co-Terephthalate (PBAT) and Epoxidized Natural Rubber (ENR). *Front. Mater.* **2019**, *6*, 1–12, doi:10.3389/fmats.2019.00275.
88. Muniyasamy, S.; Ofosu, O.; Thulasinathan, B.; Thondi Rajan, A.S.; Ramu, S.M.; Soorangkattan, S.; Muthuramalingam, J.B.; Alagarsamy, A. Thermal-chemical and

biodegradation behaviour of alginic acid treated flax fibres/ poly(hydroxybutyrate-co-valerate) PHBV green composites in compost medium. *Biocatal. Agric. Biotechnol.* **2019**, *22*, 101394, doi:10.1016/j.bcab.2019.101394.

89. Sashiwa, H.; Fukuda, R.; Okura, T.; Sato, S.; Nakayama, A. Microbial degradation behavior in seawater of polyester blends containing poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx). *Mar. Drugs* **2018**, *16*, 34, doi:10.3390/md16010034.
90. Nevoralová, M.; Koutný, M.; Ujčić, A.; Starý, Z.; Šerá, J.; Vlková, H.; Šlouf, M.; Fortelný, I.; Kruliš, Z. Structure Characterization and Biodegradation Rate of Poly(ε-caprolactone)/Starch Blends. *Front. Mater.* **2020**, *7*, 1–14, doi:10.3389/fmats.2020.00141.
91. Sekiguchi, T.; Saika, A.; Nomura, K.; Watanabe, T.; Watanabe, T.; Fujimoto, Y.; Enoki, M.; Sato, T.; Kato, C.; Kanehiro, H. Biodegradation of aliphatic polyesters soaked in deep seawaters and isolation of poly(ε-caprolactone)-degrading bacteria. *Polym. Degrad. Stab.* **2011**, *96*, 1397–1403, doi:10.1016/j.polymdegradstab.2011.03.004.
92. Adhikari, D.; Mukai, M.; Kubota, K.; Kai, T.; Kaneko, N.; Araki, K.S.; Kubo, M. Degradation of Bioplastics in Soil and Their Degradation Effects on Environmental Microorganisms. *J. Agric. Chem. Environ.* **2016**, *5*, 23–34, doi:10.4236/jacen.2016.51003.
93. Lambert, S.; Wagner, M. Environmental performance of bio-based and biodegradable plastics: The road ahead. *Chem. Soc. Rev.* **2017**, *46*, 6855–6871, doi:10.1039/c7cs00149e.
94. Weng, Y.X.; Wang, X.L.; Wang, Y.Z. Biodegradation behavior of PHAs with different chemical structures under controlled composting conditions. *Polym. Test.* **2011**, *30*, 372–380, doi:10.1016/j.polymertesting.2011.02.001.
95. Tosin, M.; Weber, M.; Siotto, M.; Lott, C.; Innocenti, F.D. Laboratory test methods to determine the degradation of plastics in marine environmental conditions. *Front. Microbiol.* **2012**, *3*, 225, doi:10.3389/fmicb.2012.00225.
96. Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605, doi:10.1016/j.marpolbul.2011.05.030.
97. Sen, C.; Das, M. Biodegradability of Starch Based Self-Supporting Antimicrobial Film and Its Effect on Soil Quality. *J. Polym. Environ.* **2018**, *26*, 4331–4337, doi:10.1007/s10924-018-1304-6.

98. Ulloa, P.A.; Vidal, J.; Lopéz de Dicastillo, C.; Rodriguez, F.; Guarda, A.; Cruz, R.M.S.; Galotto, M.J. Development of poly(lactic acid) films with propolis as a source of active compounds: Biodegradability, physical, and functional properties. *J. Appl. Polym. Sci.* **2019**, *136*, 1–11, doi:10.1002/app.47090.

99. Gonçalves, S.P.C.; Strauss, M.; Martinez, D.S.T. The Positive Fate of Biochar Addition to Soil in the Degradation of PHBV-Silver Nanoparticle Composites. *Environ. Sci. Technol.* **2018**, *52*, 13845–13853, doi:10.1021/acs.est.8b01524.

100. Pavoni, J.M.F.; Luchese, C.L.; Tessaro, I.C. Impact of acid type for chitosan dissolution on the characteristics and biodegradability of cornstarch/chitosan based films. *Int. J. Biol. Macromol.* **2019**, *138*, 693–703, doi:10.1016/j.ijbiomac.2019.07.089.

101. Cano, A.I.; Cháfer, M.; Chiralt, A.; González-Martínez, C. Biodegradation behavior of starch-PVA films as affected by the incorporation of different antimicrobials. *Polym. Degrad. Stab.* **2016**, *132*, 11–20, doi:10.1016/j.polymdegradstab.2016.04.014.

102. Wang, H.; Wei, D.; Zheng, A.; Xiao, H. Soil burial biodegradation of antimicrobial biodegradable PBAT films. *Polym. Degrad. Stab.* **2015**, *116*, 14–22, doi:10.1016/j.polymdegradstab.2015.03.007.

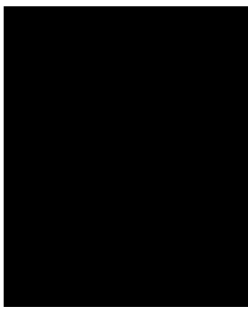
103. Norcino, L.B.; Mendes, J.F.; Natarelli, C.V.L.; Manrich, A.; Oliveira, J.E.; Mattoso, L.H.C. Pectin films loaded with copaiba oil nanoemulsions for potential use as bio-based active packaging. *Food Hydrocoll.* **2020**, *106*, 105862, doi:10.1016/j.foodhyd.2020.105862.

104. Tampau, A.; González-Martínez, C.; Chiralt, A. Biodegradability and disintegration of multilayer starch films with electrospun PCL fibres encapsulating carvacrol. *Polym. Degrad. Stab.* **2020**, *173*, doi:10.1016/j.polymdegradstab.2020.109100.

105. Arrieta, M.P.; García, A.D.; López, D.; Fiori, S.; Peponi, L. Antioxidant bilayers based on PHBV and plasticized electrospun PLA-PHB fibers encapsulating catechin. *Nanomaterials* **2019**, *9*, 346, doi:10.3390/nano9030346.

106. Tang, S.; Zou, P.; Xiong, H.; Tang, H. Effect of nano-SiO_2 on the performance of starch/polyvinyl alcohol blend films. *Carbohydr. Polym.* **2008**, *72*, 521–526, doi:10.1016/j.carbpol.2007.09.019.

107. Capelezzo, A.P.; Mohr, L.C.; Dalcanton, F.; Barreta, C.R.D.M.; Martins, M.A.P.M.; Fiori, M.A.; De Mello, J.M.M. Polimero biodegradavel antimicrobiano através da aditivacao com compostos a base de zinco. *Quim. Nova* **2018**, *41*, 367–374, doi:10.21577/0100-4042.20170187.



II. OBJECTIVES

General objectives

The **general objective** of this Doctoral Thesis was to develop biodegradable active multilayer materials (laminates) based on improved starch layers (with high oxygen barrier capacity) and PLA-PHBV polyester blends (with high water vapour barrier) in order to obtain packaging systems useful to meet food packaging requirements. Laminates were provided with antimicrobial/antioxidant capacity by incorporating phenolic acids (ferulic, *p*-coumaric and protocatechuic acids) as active compounds. The active multilayer systems were evaluated as to their capacity to extend the shelf- life of pork meat.

Specific objectives

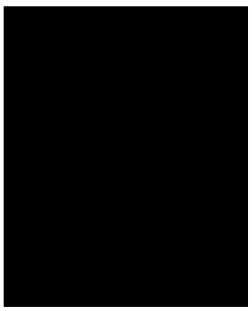
1. To improve the functional properties of starch films (maize and cassava) obtained by melt-blending and compression moulding by incorporating two different microbial gums (xanthan and gellan).

2. To obtain and characterize multilayer biodegradable films combining polar (starch) and less-polar (polyesters blend) sheets, to optimize the material functionality: barrier and mechanical properties.

3. To incorporate phenolic acids (ferulic, *p*-coumaric and protocatechuic acids) into polyester blend films based on PLA and PHBV, and analyse: a) the effect of the incorporation of phenolic acids on the microstructure, thermal behaviour and functional properties of the films, b) the release kinetics of active compounds from the films in different foods simulants and c) the *in vitro* antimicrobial activity of the films.

4. To study the biodegradation behaviour of the polyesters blend films containing, or not, phenolic acids, analysing the potential effect of antimicrobial compounds on the film disintegration and biodegradation pattern in standard composting conditions.

5. To study the functional properties of laminates based on starch layers plus PLA/PHBV blend sheets, incorporating ferulic, *p*-coumaric and protocatechuic acids into the polyester blend, and to analyse the effectiveness of active laminates at preserving fresh pork meat quality and at extending its self-life during cold storage.



III. CHAPTERS

CHAPTER 1

Thermoprocessed starch-polyester bilayer films as affected by the addition of gellan or xanthan gum.

CHAPTER 2

Use of phenolic acids to obtain active films based on PLA-PHBV polyesters.

CHAPTER 3

Biodegradation of PLA-PHBV blend films as affected by the incorporation of different phenolic acids.

CHAPTER 4

Starch-polyester bilayer films with phenolic acids for meat preservation.

Chapter 1

Thermoprocessed starch-polyester bilayer films as affected by the addition of gellan or xanthan gum

Eva Hernández-García[1*], María Vargas[1], Amparo Chiralt[1]

Food Hydrocolloids (2021), 113, 106509

[1]Institute of Food Engineering for Development, Universitat Politècnica de València, Valencia, Spain

evherga1@upvnet.upv.es

ABSTRACT

Monolayer films based on cassava starch (CS) or maize starch (MS), with and without 10 % of gellan or xanthan gum, and PLA-PHBV (75:25) blend films, were obtained by melt-blending and compression moulding, using glycerol (for starch blends) and PEG1000 (for polyester blends) as plasticisers. Bilayer films were obtained by thermo-compression of the different starch based sheets with the polyester sheet. Both mono and bilayers were characterised as to their mechanical and barrier properties, equilibrium moisture, water solubility and microstructure. The incorporation of gellan gum and xanthan gum improved the mechanical properties of starch-based films, especially in the case of MS, although the highest EM and TS values were obtained for CS-gum films. The incorporation of either gellan or xanthan gum decreased the water vapour and oxygen permeability of starch-based films; the CS films with gums being the least permeable to oxygen. The lowest changes in mechanical properties throughout storage were obtained in cassava starch-based films, especially those containing xanthan gum. Starch based-polyester bilayers presented a high oxygen and water vapour barrier capacity, as compared to their individual monolayers. Bilayer films with cassava starch including the gums showed the lowest OP and WVP values and the highest elastic modulus and tensile strength, with extensibility values in the range of the corresponding monolayers and slight changes in their physical properties throughout time. The bilayer formed with cassava starch with gellan gum and a PLA-PHBV appeared as the best option for food packaging purposes taking into account its functional properties and the good layer adhesion of the bilayer.

Keywords: biodegradable bilayer films; PLA; PHBV; starch; gellan; xanthan.

1. INTRODUCTION

Over the last few decades, there has been a growing need to find alternatives to petroleum-based non-biodegradable products due to environmental concerns (Martín et al. 2001). This has increased interest in developing biodegradable food packaging materials based on biopolymers, such as starch. The use of thermoplastic starch (TPS) to develop biodegradable packaging materials has several advantages, such as low cost, renewability, sustainable production, good processability by means of conventional techniques, good oxygen barrier capacity and stretchability, as well as suitable transparency, odour and taste (Muller et al., 2017). However, starch materials are water sensitive and exhibit poor water vapour barrier properties, which are greatly affected by their moisture content (Vieira et al., 2011). Additionally, the phenomenon of retrogradation modifies the mechanical behaviour of starch-based materials throughout time, depending on the amylose/amylopectin ratio and moisture content (Cano et al., 2014; López et al., 2013; Ortega-Toro et al. 2014). Glycerol is usually employed as a plasticiser in starch films in order to facilitate thermo-processing, by reducing the intermolecular forces and increasing the flexibility of starch-based films (Savadekar & Mhaske, 2012). Different strategies have been applied to improve the functional properties of starch based materials, such as blending it with different additives or other biopolymers (Cano et al., 2017; Ortega-Toro et al., 2017; Samsudin & Hani, 2017). Sapper et al. (2019) observed that the cassava starch mixture with 10 or 20% of different gums of microbial origin, such as gellan, xanthan or pullulan, permitted the improvement of the properties of starch-based films obtained by casting, while maintaining the competitive cost of the material. In general, the addition of such gums improved the mechanical properties of starch films and their storage stability (Kim et al., 2015; Sapper et al, 2019). Xanthan gum enhanced the tensile properties of the films but led to a less extensible matrix (Arismendi et al., 2013).

Developing bilayer films based on biodegradable monolayers formulated with different biopolymers with complementary properties is an innovative approach to improving the performance of the material (laminate), as compared to the use of monolayers, while also meeting the food packaging requirements better (Slavutsky et al., 2018). In this sense, the combination of starch films with sheets of hydrophobic polyesters represents

a good alternative means of accomplishing this purpose. Poly(lactic acid) (PLA) and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are biodegradable polyesters obtained from natural resources which can be used for food packaging purposes, due to their ability to form food contact plastic materials at a relatively competitive cost. PLA is a biodegradable thermoplastic linear aliphatic polyester, of great potential in the packaging industry because of its optical properties, good thermal behaviour and water vapour barrier properties (Bonilla et al., 2013; Chaiwutthinan et al., 2015; Muller et al., 2017). However, PLA shows limited gas barrier capacity and is very brittle, with less than 10% elongation at break (Rasal et al., 2010). Therefore, PLA has been combined with different plasticisers and other polyesters, such as PHBV. Although PHBV has physical properties that are comparable to some synthetic polymers, such as polypropylene and polyethylene, the PHBV materials are more brittle with lower elongation at break (Laycock et al., 2013). PHBV and PLA blends have been studied in order to improve the functional properties of the materials. The polymers exhibited low miscibility, so PHBV-PLA blend films had low transparency, but their mechanical resistance was significantly improved as compared with pure PLA or PHBV films (Liu et al., 2015). Different plasticisers, such as acetyl tributyl citrate, limonene, and PEG have been used to improve the extensibility of mixtures of PLA and PHB (Armentano et al., 2015). The addition of PEG1000 and PEG600 at concentrations lower than 10% improved the thermal properties of PLA-PHBV based materials (Thongpina et al., 2017).

Obtaining bilayer structures consisting of a PLA-PHBV blend film layer and a starch-based film layer could represent a good alternative to obtain the target materials with improved mechanical and barrier properties suitable for food packaging applications. The polyester layer would contribute to the strengthening of the bilayer while reducing water vapour permeability, whereas the starch layer would help to control the oxygen and gas barrier capacity of the bilayer assembly. In previous studies, Requena et al., (2018) combined a PLA-PHBV (75:25) monolayer with glycerol-plasticised starch sheets to develop bilayer food packaging materials. In this study, a good layer adhesion was obtained for starch/PLA-PHBV sheets, but the elastic modulus and mechanical resistance of polyester-cassava starch bilayer films were lower than that of the corresponding polyester monolayers due to the weaker strength of starch monolayers.

In this sense, the incorporation of xanthan or gellan gum to a thermo-processed starch layer could improve both the monolayer and bilayer functional properties for food packaging purposes. The origin of the starch may also affect both the film and bilayer properties, since different starches provide the films with more or less mechanical resistance and stability, mainly depending on their amylose/amylopectin ratio (Cano et al., 2014).

The aim of this study was to analyse the physical and microstructural properties of the melt blended and compression moulded starch films, from maize and cassava, as affected by the xanthan or gellan gum incorporation. Thermo-compression-sealed bilayers of the different starch-based films and PLA-PHBV blend sheets were obtained and analysed in order to select the best combination for food packaging purposes.

2. MATERIALS AND METHODS

2.1. Materials

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) ENMAT Y1000P with 3% hydroxyvalerate was supplied by Helian Polymers B.V. (Belfeld, Holland). Amorphous PLA 4060D, density of 1.24g/cm^3 and average molecular weight of 106,226 D with 40% of low molecular weight fraction (275 D) as reported by Muller et al. (2017), was supplied by Natureworks (U.S.A). Maize starch (MS, 27% amylose) and cassava starch (CS, 9% amylose) were supplied by Roquette (Roquette Laisa, Benifaió, Spain) and Quimidroga S.A. (Barcelona, Spain), respectively. Xanthan gum (X) (high molecular weight;~ 10^6 Da), was supplied by EPSA (Valencia, Spain). Negatively charged, low acyl gellan gum (G) KELGOGEL F (MW 3-5x10^5 Da), was purchased from Premium Ingredients (Murcia, Spain). The plasticiser, poly(ethylene glycol) with a molecular weight of 1000 Da (PEG1000), was purchased from Sigma-Aldrich (Steinheim, Germany), and the glycerol was obtained from Panreac Química S.L.U. (Castellar del Vallés, Barcelona, Spain). For sample conditioning purposes, phosphorus pentoxide (P_2O_5) and magnesium nitrate-6-hydrate ($Mg(NO_3)_2$) were supplied by Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain).

2.2. Preparation of films

2.2.1. Starch monolayer films

For the preparation of maize (MS) and cassava (CS) starch monolayer films, the starch solutions were mixed in the adequate proportion with which to obtain a starch:gum ratio of 90:10, using glycerol (0.30 g/g of starch) as a plasticiser, by melt blending and compression moulding. The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 130 °C, rotor speed 50 rpm, for 10 min and 50 g of blend were processed in each batch. After processing, blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and the powder conditioned at 25 °C and 53% relative humidity (RH) for one week. Four g of the conditioned powder was required to obtain each film (160 mm in diameter) that were put onto Teflon sheets and preheated at 160 °C or 150 °C (for CS or MS) for 1 (CS) or 5 (MS) min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). Films were obtained by compressing at 160 °C (CS) or 150 °C (MS) for 2 min at 50 (CS) or 30 (MS) bars, followed by 6 min at 100 (CS) or 130 (MS) bars and a final cooling cycle for 3 min until the temperature reached about 70 °C, according to that described by other authors for CS (Requena et al., 2018) and MS (Silva-Guzmán et al., 2018). The obtained films were conditioned at 25 °C and 53% RH until used to obtain bilayer films.

2.2.2. Polyester monolayer films

PLA-PHBV blend monolayers were obtained by melt blending and compression moulding in a ratio of 75:25, using PEG1000 (15 g/100 g polymer) as a plasticiser. The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 170 °C, rotor speed 50 rpm, for 12 min. After processing, blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and conditioned at 25 °C. Only 3 g of the conditioned powder were required to obtain each film (160 mm in diameter) due to the higher flowability of the polyester blends. The powder was put onto Teflon sheets and preheated at 200 °C for 5 min in a hot plate press (Model LP20, Labtech Engineering, Thailand). Films were obtained by compressing at 200 °C for 4 min at 100 bars, and a final cooling cycle for 3 min until the

temperature reached about 70 °C (Requena et al., 2018). The obtained films were conditioned at 25 °C and 53% RH until used to obtain bilayer films.

2.2.3. Starch-polyester bilayer films

Starch monolayers and polyester monolayers were submitted to compression moulding in a hydraulic press (Model LP20, Labtech Engineering, Thailand) at 180 °C and 100 bars for 2 min and cooled down until 80 °C in 2 min, thus obtaining starch-polyester bilayer films. All bilayer films were stored at 25 °C and 53% RH till their analyses.

2.3. Film characterisation

2.3.1. Tensile properties and thickness

The mechanical behaviour of the films was tested by using a universal testing machine (TA-XT plus, Stable Micro Systems, Surrey, United Kingdom) according to the ASTM D882 standard method (ASTM, 2001). The mechanical parameters, tensile strength (TS), elastic modulus (EM) and elongation at break (E), were obtained from the stress–strain curves of the various samples. Equilibrated samples (1 or 5 weeks at 25 °C and 53% RH) of 2.5 cm wide and 10 cm long were mounted in the film extension grips of the testing machine 5 cm apart and the samples were stretched at 50 mm/min until fracture. Eleven replicates were performed for each film formulation. The film thickness was measured to the nearest 0.0025 mm with a Palmer digital micrometer (Electronic Digital Micrometer, Comecta S.A., Barcelona, Spain) at six random positions around the film.

2.3.2. Water vapour permeability (WVP)

The water vapour permeability (WVP) of the films was determined following the gravimetric method ASTM E96-95 (ASTM, 1995), considering the modification proposed by McHugh et al. (1993). Three round film samples (35 mm in diameter) of each formulation were placed on Payne permeability cups (3.5 cm in diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium). The temperature was 25 °C and the relative humidity gradient was 53-100%, which was obtained using magnesium nitrate-6-hydrate and distilled water, respectively. The cup's weight loss was controlled every 1 h and 30 min using an analytical balance (±0.00001 g), until the steady state was reached. WVP was calculated from the slope of the curves of weight loss versus time as described

by Ortega-Toro et al. (2016), taking into account the film thickness. An apparent value of WVP was also determined for bilayer films, considering their total thickness value.

2.3.3. Oxygen permeability (OP)

The oxygen permeability (OP) of the conditioned films was evaluated by measuring the oxygen permeation rate by means of an OX-TRAN 1/50 system (Mocon, Minneapolis, USA) at 53% RH and 25 °C (ASTM Standard Method D3985-05, 2002). The transmission values were determined every 20 min until equilibrium was reached. The exposure area during the tests was 50 cm^2 for each formulation. In order to obtain the oxygen permeability (OP), the film thickness was considered in every case. At least two replicates per formulation were made. An apparent value of OP was also determined for bilayer films, considering their total thickness value.

2.3.4. Moisture content

The moisture content of film samples previously conditioned at 53% RH and 25 °C was determined. Four samples of each formulation were dried in a vacuum oven (VaciotermT, JP Selecta S.A., Barcelona, Spain) at 60 °C for 24h, and afterwards, the samples were placed into a desiccator with P_2O_5 at 25 °C for 2 weeks, until constant weight was reached.

2.3.5. Film water solubility

The solubility was evaluated by a modification of the method described by Balaguer et al. (2011). Film samples (3 cm x 3 cm), previously conditioned in P_2O_5, were weighed and then the dry films were immersed in glass containers in 10 mL of distilled water and kept at 25 °C for 24 h. Then, the solvent was poured into a filter, retaining the film sample, the remaining surface water was removed and the final wet weight was measured. These wet samples were dried till constant weight to evaluate the mass of residual solids in the film after soaking. Each film formulation was analysed in triplicate.

2.3.6. Microstructural analyses

Microstructural analyses of the films were carried out by using a Field Emission Scanning Electron Microscope (FESEM Ultra 55, Zeiss, Oxford Instruments, U.K). Film samples were kept in desiccators with P_2O_5 for two weeks at 25 °C in order to eliminate film

moisture. Then, film samples were cryofractured with nitrogen liquid in order to observe the cross-sections and adequately placed on support stubs and coated with platinum. The samples were observed using an accelerating voltage of 2 kV.

2.3.7. Statistical analysis

The statistical analysis of the results was performed through an analysis of variance (ANOVA) using Statgraphics Centurion XVII-X64. Both a One-way and multifactor ANOVA were used to analyse the influence of composition variables and storage time on the properties of the films. Fisher's least significant difference (LSD) procedure was used at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Properties and microstructure of monolayer films

3.1.1. Thickness, equilibrium moisture content and water solubility

Table 1 shows the thickness, moisture content and water solubility of monolayer films. Significant differences were observed as regards thickness, maize starch (MS) films being slightly thicker (about 220 μm) than those made from cassava starch (CS) (about 190 μm), and both are thicker than the polyester film (130 μm). Differences in the film thickness can be attributed to the different flowability of the material during the hot compression, which is dependent on both the viscosity under the temperature and pressure conditions and on the mass of pellets used in each case (4 or 3 g for starch and polyesters, respectively). The equilibrium moisture content of CS films was slightly higher than that of MS films and the incorporation of the gums slightly decreased the water adsorption capacity. This could be attributed to the formation of hydrogen bonds between the chains of starch and the gums, which could reduce the number of active points for water sorption. A similar trend was observed by Sapper et al. (2019) in cassava starch-gellan films obtained by casting. The polyester films showed lower moisture content values, as expected from their hydrophobic nature.

About 90% of the total solids of every starch film were solubilised in water without there being any significant differences between the samples. Thus, the water solubility of maize and cassava starch films was not affected by the incorporation of gum. However,

Sapper et al. (2019) observed lower solubility values after the addition of gellan or xanthan gums in cassava starch films obtained by casting, probably due to the establishment of more gum-starch hydrogen bond interactions in the polymer aqueous solution than in the blend melt. Thus, the method of obtaining the films affected polymer chain interactions with water molecules and, therefore, their solubility. The polyester films exhibited very low solubility in water, coherently with their hydrophobic nature. The solubilised solids would probably be made up of 15% PEG1000 (plasticiser) and some small oligomers of PLA or PHBV.

Table 1. Thickness, equilibrium moisture content and water solubility of cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P). Mean values ± standard deviation.

Formulation	Thickness (µm)	Equilibrium Moisture (g water/100 g dried film)	Water Solubility (g/ 100 g dried film)
CS	189 ± 14$^{(d)}$	9.1 ± 0.3$^{(a)}$	89.5 ± 0.1$^{(a)}$
CS-G	191 ± 18$^{(d)}$	8.0 ± 0.4$^{(b)}$	89.2 ± 0.2$^{(a)}$
CS-X	200 ± 16$^{(c)}$	7.7 ± 0.8$^{(b)}$	89.6 ± 0.1$^{(ab)}$
MS	221 ± 17$^{(a)}$	8.3 ± 0.3$^{(b)}$	89.0 ± 0.3$^{(a)}$
MS-G	219 ± 20$^{(a)}$	8.1 ± 0.3$^{(b)}$	89.6 ± 0.1$^{(ab)}$
MS-X	210 ± 20$^{(b)}$	8.1 ± 0.9$^{(b)}$	89.7 ± 0.1$^{(ab)}$
P	130 ± 0$^{(e)}$	0.3 ± 0.1$^{(c)}$	27.0 ± 3.0$^{(c)}$

Different superscript letters (a - e) within the same column indicate significant differences among formulations ($p < 0.05$).

3.1.2. Tensile properties and barrier properties

Table 2 shows tensile parameters (Elastic modulus: EM, tensile strength: TS and percentage deformation E% at break) and barrier properties (water vapour: WVP and oxygen permeability: OP) of monolayer films after 1 week and 5 weeks of storage at 25 °C and 53% RH.

Table 2. Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E) and barrier properties (water vapour (WVP) and oxygen (OP) permeability) of cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P). Mean values after 1 and 5 storage weeks ± standard deviation.

	EM (MPa)		TS (MPa)		E (%)		WVP ($g \cdot mm \cdot kPa^{-1} \cdot h^{-1} \cdot m^{-2}$)		OPx10^{14} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	
	Week 1	**Week 5**	**Week 1**	**Week 5**	**Week 1**	**Week 5**	**Week 1**	**Week 5**	**Week 1**	**Week 5**
CS	500	600	12.0	13.0	4.0	3.0	13.4	13.5	3.7	3.9
	$\pm 70^{(c,2)}$	$\pm 70^{(c,1)}$	$\pm 1^{(bc,2)}$	$\pm 2^{(d,1)}$	$\pm 0.4^{(c,1)}$	$\pm 0.4^{(b,2)}$	$\pm 1.2^{(a,2)}$	$\pm 0.7^{(b,1)}$	$\pm 0.1^{(c,1)}$	$\pm 0.1^{(d,1)}$
CS-G	600	900	18.0	23.0	5.0	3.0	11.0	11.0	2.6	2.7
	$\pm 70^{(b,2)}$	$\pm 30^{(a,1)}$	$\pm 2^{(a,2)}$	$\pm 2^{(a,1)}$	$\pm 0.2^{(c,1)}$	$\pm 0.4^{(b,2)}$	$\pm 1.6^{(b,2)}$	$\pm 0.7^{(c,1)}$	$\pm 0.3^{(d,1)}$	$\pm 0.1^{(e,1)}$
CS-X	700	700	14.0	17.0	3.0	3.0	11.4	13.4	2.4	2.6
	$\pm 40^{(a,2)}$	$\pm 80^{(b,1)}$	$\pm 4^{(b,2)}$	$\pm 1^{(b,1)}$	$\pm 1^{(c,1)}$	$\pm 0.3^{(b,2)}$	$\pm 0.9^{(b,2)}$	$\pm 1.1^{(b,1)}$	$\pm 0.1^{(d,1)}$	$\pm 0.3^{(e,1)}$
MS	140	374	7.0	13.0	30.0	13.0	15.0	19.8	10.1	11.2
	$\pm 60^{(e,2)}$	$\pm 20^{(d,1)}$	$\pm 1^{(e,2)}$	$\pm 1^{(cd,1)}$	$\pm 8^{(a,1)}$	$\pm 5^{(a,2)}$	$\pm 0.8^{(a,2)}$	$\pm 1^{(a,1)}$	$\pm 0.7^{(a,1)}$	$\pm 0.4^{(a,1)}$
MS-G	360	600	9.0	14.0	11.0	4.0	13.4	12.9	5.3	5.8
	$\pm 70^{(d,1)}$	$\pm 70^{(c,1)}$	$\pm 1^{(d,2)}$	$\pm 1^{(c,1)}$	$\pm 6^{(b,1)}$	$\pm 0.4^{(b,2)}$	$\pm 0.8^{(ab,2)}$	$\pm 2^{(b,1)}$	$\pm 0.3^{(b,1)}$	$\pm 0.2^{(b,1)}$
MS-X	500	660	11.0	15.0	40.0	3.0	11.8	12.0	4.6	4.9
	$\pm 100^{(c,2)}$	$\pm 30^{(bc,1)}$	$\pm 2^{(c,2)}$	$\pm 1^{(c,1)}$	$\pm 0.6^{(c,1)}$	$\pm 0.3^{(b,2)}$	$\pm 0.6^{(b,2)}$	$\pm 1.2^{(b,1)}$	$\pm 0.1^{(b,1)}$	$\pm 0.1^{(c,1)}$
P	780	860	15.0	16.0	2.4	3.0	0.20	0.3	410	432
	$\pm 190^{(a,1)}$	$\pm 80^{(a,2)}$	$\pm 2^{(b,1)}$	$\pm 1^{(bc,1)}$	$\pm 0.2^{(d,1)}$	$\pm 0.2^{(b,1)}$	$\pm 0.03^{(e,1)}$	$\pm 0.1^{(e,2)}$	$\pm 7^{(e,1)}$	$\pm 3^{(e,2)}$

Different superscript letters (a-e) within the same column indicate significant differences among formulations ($p < 0.05$). Different superscript numbers (1-2) within the same row for each parameter and sample indicate significant differences between the two storage times ($p < 0.05$).

EM values were significantly ($p<0.05$) affected by the type of starch, the type of gum and the storage time, as well as the interactions between these factors. EM values were higher for cassava starch films than maize starch films. In both cases the EM increased over time, this increase being more noticeable in maize starch. Although the incorporation of gums increased EM in both of the films formulated with starches, the incorporation of gellan gum was more effective in cassava starch films while xanthan gum was more effective in films prepared with maize starch. Tensile strength (TS) showed a similar trend to EM, but there was no statistically significant interaction

between storage time and the type of gum ($p > 0.05$), which means that the effect of storage time was similar for films with both kinds of gums. TS was higher for cassava starch films than for maize starch and increased to a greater extent during storage in MS films. This was coherent with the higher amylose content (27%) of maize starch than cassava starch (9%), which is more affected by the retrogradation phenomena during storage (Cano et al., 2014). The incorporation of both gums led to higher TS values as compared to those obtained in pure starch films, this increase being more significant in the films containing gellan gum. The films formulated with gums exhibited more stable TS values over time than pure starch films, regardless of the type of gum used. Gellan gum was more effective than xanthan gum at increasing the resistance to break in cassava starch films, while both gums had a similar effect on maize starch-based films.

The percentage of deformation at break (E%) was significantly ($p<0.05$) affected by the three parameters (type of starch, gum and storage time) and their interactions. Maize starch- based films were more stretchable than cassava starch films, but the extensibility was reduced by more than half in maize starch-based films during storage whereas the ability to stretch slightly decreased over time in cassava starch-based films. The incorporation of gums decreased the extensibility of all the starch-based films, but more significantly in films prepared with xanthan gum. Throughout the 5 weeks of storage, the stretchability of the films formulated with gellan gum decreased to almost half, while in the films formulated with xanthan gum it remained constant.

The obtained tensile behaviour of the different thermoprocessed films revealed a greater structural toughness in films based on CS, with lower amylose ratio, than in MS based films. This could be related with the higher molecular weight of the highly branched amylopectin that could offer the possibility of a greater chain entanglement in the melt, thus forming a more cohesive, less extensible polymer matrix, with lower retrogradation degree during storage. The incorporation of gums with high molecular weight (10^5-10^6 Da) will contribute to reinforce the starch polymer matrix, creating association domains in the matrix where gums and starch polymers could participate through the aggregation of the helical conformations of the different chains. Gellan gum is an anionic polysaccharide hydrogel-forming polymer that comprises a tetrasaccharide repeat unit of two β-D-glucoses, one β-D-glucuronate, and one a-L-rhamnose. It forms

a physical gel by undergoing a random coil to double helix transition upon cooling. This molecular characteristic could provide a greater reinforcing effect in the CS matrix, with lower amylose (with potential helical associations) content, whereas its effect was less appreciable in the MS matrix with higher amylose ratio. In contrast, xanthan gum consists of a main chain of D-glucopyranosyl with a β 1-4 bond, as in cellulose, with trisaccharide side chains composed of D-mannopyranosyl and D-glucopyranosyluronic acid residues. Different interactions of xanthan gum and starch have been described, depending on the starch source and the amylose/amylopectin ratio (Sikora et al., 2008). Differences in the molecular structure of the gums and the amylose/amylopectin ratio in starch may explain the observed tensile behaviour of the blend films, depending on their composition. The linear structure of gellan chains could better reinforce the CS matrix with a lower ratio of amylose, providing it with more regions with glucose helical associations, while this contribution could be less noticeable in MS matrices, with higher amylose content. Despite the structural differences, both gums enhanced the toughness of the starch matrix structure, giving rise to a better mechanical performance of the starch-based films.

As regards water vapour permeability (WVP), significant effects as a result of the type of starch, the type of gum and storage time were observed, as were interactions between the type of starch and gum. Maize starch films were more permeable to water vapour than cassava starch films. Over time there was a slight increase in WVP, which was more significant in maize starch films. The incorporation of gums significantly reduced WVP, xanthan gum being more effective in maize starch-based films. Both gums minimised the impact of storage time on the WVP values of the films and, in general, there were no significant changes as far as WVP is concerned throughout storage.

The OP values were significantly affected by the type of starch and gum together with the storage time and the interactions between the type of starch and the type of gum. The incorporation of both gums reduced the OP of starch films, which coincides with the results obtained by Sapper et al. (2019) for cassava starch films prepared by casting. Maize starch films were more permeable to oxygen and were more affected by storage time, with a more noticeable increase in the OP at the end of the 5-week storage.

The observed effects of gums on the starch film barrier properties were coherent with that commented on above as regards the reinforcing effect of gums in the starch matrices. This reinforce implied the formation of a tougher, more cohesive network that limited the mass transfer phenomena to greater extent than pure starch matrix. Different effects of each gum depending on the starch source was also observed, as previously described for its influence in the film tensile behaviour.

The changes in the properties of starch films over time are attributable to the recrystallisation of amylose, or starch retrogradation, (Cano et al. 2017) whose proportion is higher in maize starch as mentioned above. Crystallisation led to more rigid (with greater EM) and less extensible (lower %E) films, while hydration promoted the plasticisation of the amorphous fraction of the films and led to an increased molecular mobility, thus favouring the diffusion-dependent phenomena, such as the mass transport associated with the permeation of water or gas molecules through the amorphous regions of the films. In general, storage time had a more significant effect on tensile properties than on barrier properties. The WVP showed an increase throughout storage in maize starch films without gums, which also presented a slightly higher equilibrium moisture content. As concerns the tensile properties, changes over time led to an increase in the EM and TS and a decrease in the film extensibility, which was more marked in maize starch films, with a higher amylose content and, thus, greater sensitivity to crystallisation. This was mitigated to a greater extent by xanthan gum. The most resistant films were those formulated with cassava starch with gellan gum, which also showed a more limited increase in strength and toughness during storage time, while exhibiting reduced extensibility of 3-5%.

PLA: PHBV blend films (P) showed high EM and TS values and low extensibility E(%), all of which were in the range of those of cassava starch films with gums, the most rigid, resistant and least extensible starch-based films. The EM values increased slightly over time, which could be attributed to the progressive crystallisation of PHBV (Arismendi et al., 2013).

3.1.3. Microstructural analysis of the monolayer films

Figure 1 shows FSEM micrographs of the cross-section of the monolayer films. Gums were only partially miscible with starch and gum-rich domains appeared dispersed in the starch-rich continuous phase. The different cryofracture behaviour of the starch continuous phase of the films reveals the partial miscibility of the gums in the starch phase, which reinforced the starch matrix, as revealed by the higher structural toughness deduced from the tensile parameters of blend films. The lack of polyester miscibility can also be observed in **Figure 1** where different domains of PLA and PHBV can be observed, as previously reported (Gasmi et al., 2019).

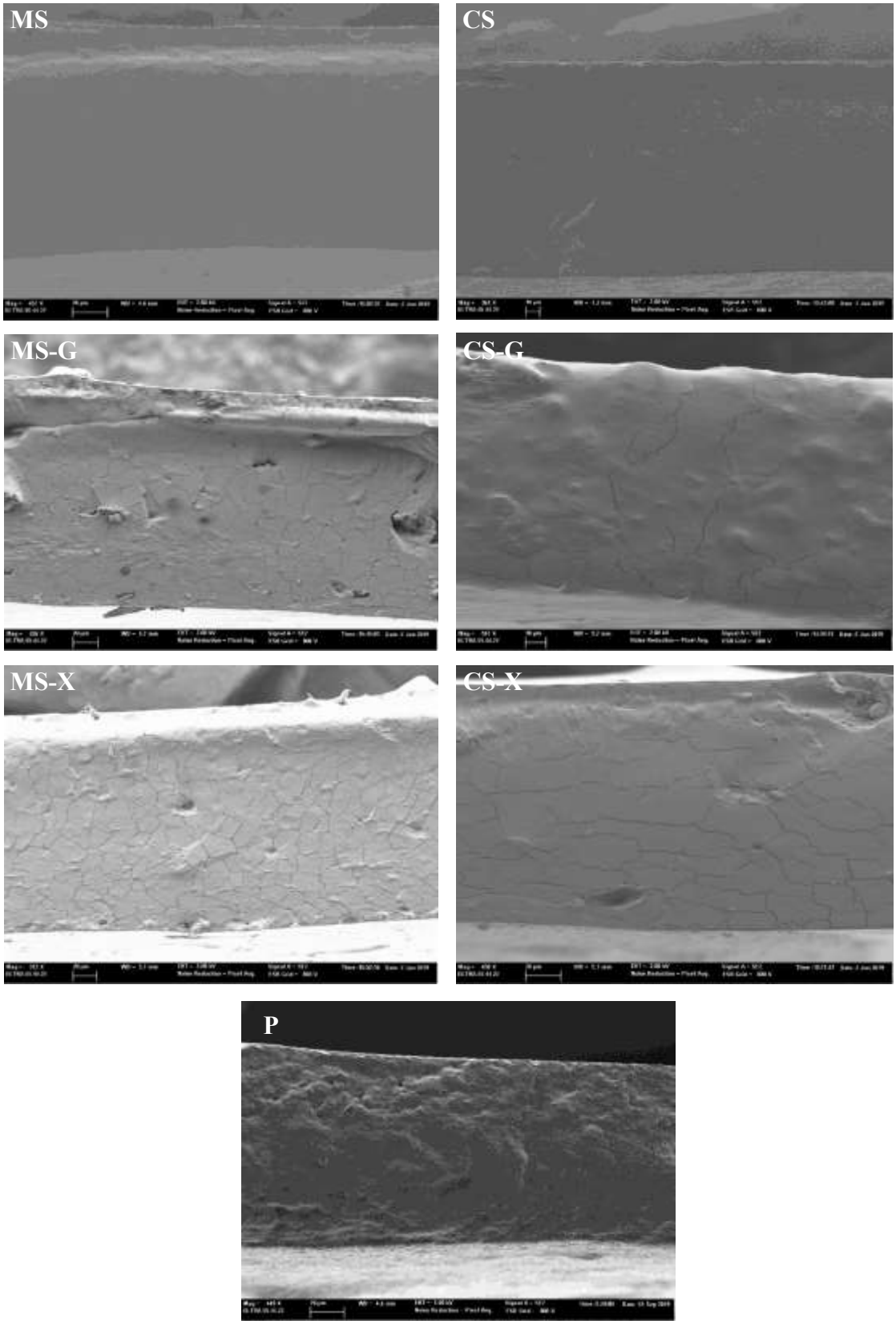


Figure 1. FSEM micrographs of monolayer films (cross section) from maize starch (MS) and Cassava starch (CS) and films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P).

3.2. Properties and microstructure of bilayer films

Table 3 shows the thickness values, moisture content and solubility of starch-polyester bilayer films. The bilayer films were not as thick as expected from the values of the monolayers, which indicates creep phenomena (flow of material) during the thermo-compression carried out to adhere the sheets. This creep was higher in the bilayers prepared with maize starch monolayers than in those made with cassava starch monolayers, probably due to the smaller amount of flow provoked in the maize starch monolayers obtained at milder compression and lower temperatures. In fact, a second thermo-compression of the different monolayers under the conditions used for the laminate thermo-sealing revealed a 20-30% reduction in thickness for MS-based films and only a 15-25% reduction for CS-based films. A mere 5% reduction in thickness was observed for the polyester films during the second thermo-compression. However, the thickness values observed for the bilayers were still lower than that predicted from the sum of the corresponding thicknesses of monolayers submitted to an equivalent second thermo-compression. This indicates that the creep phenomenon occurred to a greater extent when both layers were in contact and could imply different interactions between the polymers in contact associated with the thermal compression of the bilayers. In all likelihood, the proximity to the softening point of the polymers and the migration of some components of the respective monolayer, such as plasticisers, promoted the mobility of the polymers in the different sheets, thus encouraging flowability during compression.

Table 3. Thickness (experimental value and sum of the corresponding monolayer thicknesses submitted to the same thermocompression process used to obtain bilayers), equilibrium moisture content and water solubility of bilayer films obtained from cassava starch (CS) and maize starch (MS) sheets, containing or not gellan (G) and xanthan (X) gums, thermo-compressed with PLA-PHBV blend films (P).

Formulation	Thickness (μm)	Sum of Monolayer Thickness (μm)	Equilibrium Moisture (g water/100 g dried film)	Water Solubility (g/ 100 g dried film)
CS-P	205 ±15$^{(ab)}$	280	8.6 ± 0.6$^{(a)}$	74 ± 10$^{(a)}$
CS-G-P	230 ± 20$^{(a)}$	264	7.7 ± 0.2$^{(b)}$	75 ± 3$^{(a)}$
CS-X-P	222 ± 16$^{(a)}$	296	7.4 ± 0.7$^{(b)}$	71 ± 13$^{(a)}$
MS-P	190 ± 15$^{(b)}$	301	7.4 ± 0.7$^{(b)}$	72 ± 3$^{(a)}$
MS-G-P	230 ± 20$^{(a)}$	275	9.0 ± 0.5$^{(a)}$	77 ± 11$^{(a)}$
MS-X-P	220 ± 20$^{(ab)}$	286	8.2 ± 0.7$^{(b)}$	77 ± 8$^{(a)}$

Different superscript letters (a-b) within the same column indicate significant differences among formulations ($p < 0.05$).

In **Figure 2**, the FSEM images of the bilayer cross-section can be observed, where the polyester sheet can be seen to be much less thick, which indicates that the flow of the polyester layer during the thermo-sealing of the layers was much more intense and variable than that undergone by the starch-based layers. The thickness of the polyester film ranged between 20-30 μm in most of the cases, whereas the starch-based layers ranged between 160-180 μm for MS sheets and 110-140 μm for CS sheets. Although the polyester-starch mass ratio in the laminate was 3:4, the thickness ratio of the sheets fell sharply, thus reflecting the greater flowability of polyester as compared to starch, when both are in contact during thermo-compression. In fact, the variable, low thickness values of the polyester layers observed in **Figure 2** are remarkable. Likewise, cryo-fracture provoked a partial detachment of the layers, as shown in **Figure 2**, except for the laminate with cassava starch-gellan and polyesters, which exhibited a clear well-adhered interface. The laminates with cassava starch-xanthan exhibited an irregular interface where a partial detachment could also be observed. Therefore, from the structural point of view, the bilayers with cassava starch and gellan with the polyester layer were the best option.

The total thickness values of bilayers estimated from the FSEM micrographs are, in general, lower than those directly measured with the calliper (**Table 3**), which can be explained in terms of the film swelling with water adsorption when conditioned at 53% RH (values from **Table 3**); in FSEM analyses, however, the films were completely dried (P_2O_5 conditioned).

The water solubility of the bilayers was reduced from about 90% in the starch monolayers to about 75%, without there being any significant differences between bilayers. Although this implied a notable reduction in water solubility with respect to that of starch monolayers, this was slightly lower than that expected from the mass ratio and the respective solubility of starch-based and polyester sheets, which would suppose a solubility of only 60-65%. Therefore, using thermo-compression to obtain the laminate could promote the water solubility of the hydrophobic polyester monolayer due to the interactions between layer components at high temperatures and pressures. Particularly, the water diffusion from the starch layer to the polyester layer could promote the chain hydrolysis, giving rise to water soluble oligomers.

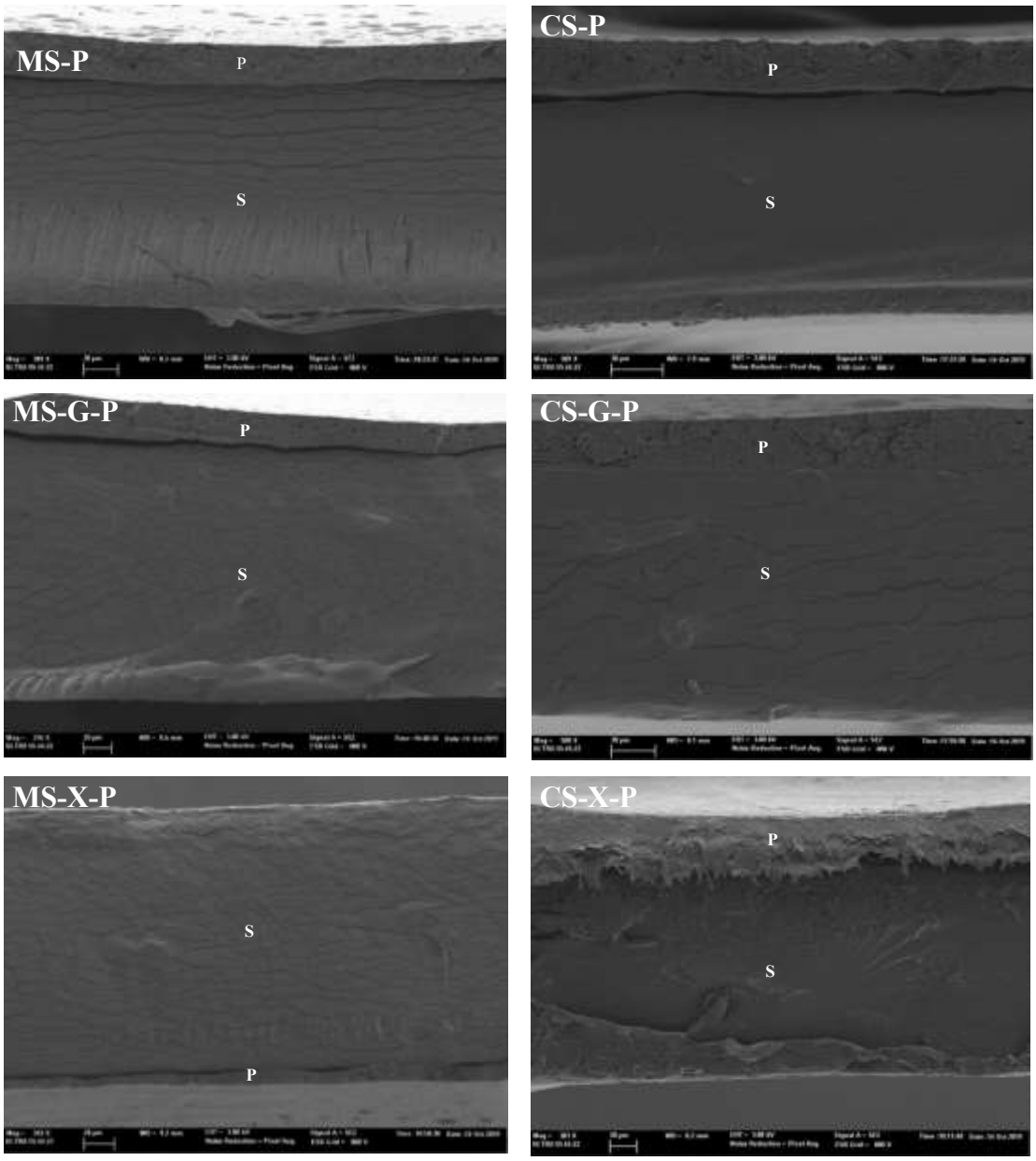


Figure 2. FSEM micrographs of bilayer films (cross section) obtained by the thermocompression of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P). Polyester (P) and starch (S) sheets are marked.

Figure 3 shows the tensile properties of the starch-polyester bilayer films as compared to the corresponding values of the starch monolayers. The cassava starch-polyester bilayer films with gums, with EM values near those of the polyester sheet, presented values of EM slightly greater than those of their corresponding starch monolayers. However, in the rest of the cases, EM was slightly lower, or in the range of, the corresponding hydrophilic monolayer. This indicates that the thicker starch monolayer

mostly determined the stiffness of the bilayer. The cassava starch-polyester bilayer films with gums presented the highest elastic modulus.

The cassava starch-polyester bilayers with gums presented similar TS values to those of corresponding starch monolayers, whereas lower values were observed for the rest of bilayers. In general, the extensibility of the bilayers was similar to or lower than that of the corresponding starch monolayers and in the range of the extensibility of the polyester sheet which limited the stretchability of the bilayers in every case.

The EM of bilayers decreased over time in practically every case, which is contrary to that observed in monolayers (**Table 2**). This could be associated with the diffusion of compounds, such as water, glycerol, PEG1000 or oligomers, present or formed during the polymer processing, which could affect the tensile behaviour of each sheet, and so of the assembly. In particular, water migration from the starch based sheet to the polyester sheet could provoke a partial hydrolysis, reducing the toughness of the matrix. The maize starch bilayer films without gums showed EM values that were stable over time. In the same way, the TS of bilayers decreased over time in every case, whereas the extensibility was more stable, but with some fluctuations. The cassava starch-polyester films with gums exhibited the highest values of EM and TS, regardless of the storage time.

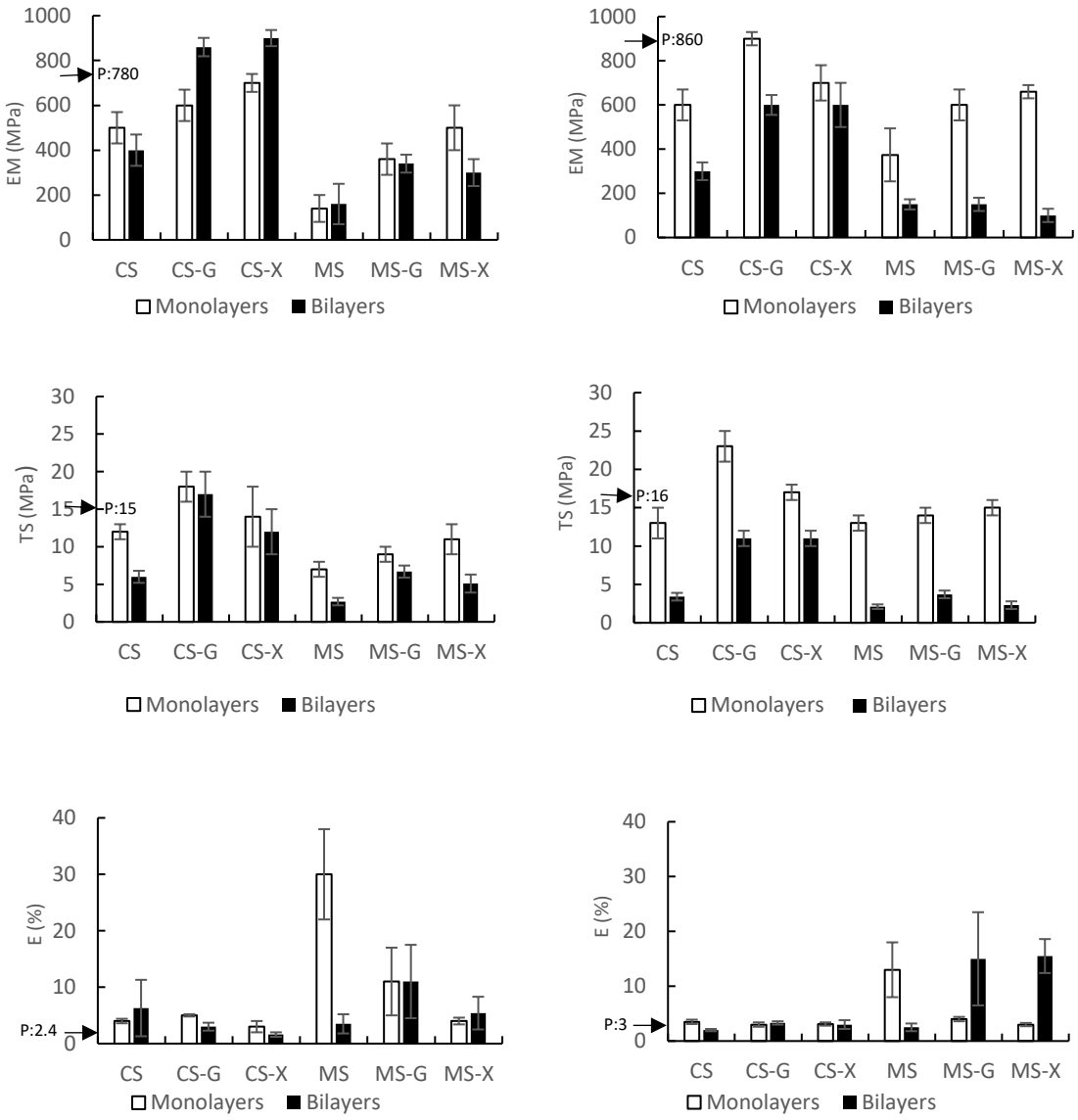


Figure 3. Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E) bilayer films (Black bars) of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P), in comparison with the corresponding values of the respective starch monolayers (white bars). Values after 1 (left) and 5 (right) storage weeks. Arrow indicates the values for the polyester monolayer: EM (week 1: 780, week 5: 860), TS (week 1: 15, week 5: 16) and E (week 1: 2.4, week 5: 3).

Figure 4 shows the barrier properties (WVP and OP) of the different bilayer films, compared to the corresponding starch monolayer. In every case, a significant decrease in both apparent permeability values (to water vapour and oxygen) was observed for the bilayer assemblies, with respect to the values of the corresponding starch monolayers, as previously observed in other starch-polyester laminates (Ortega-Toro et

al., 2015; Muller et al. 2017; Tampau et al. 2018). The values did not significantly change during the 5-week storage of the films, which reflects the fact that the barrier capacity of the assemblies remained stable during the storage time.

The parallel assembly of the hydrophilic and hydrophobic layers explains the reduction in WVP with respect to the starch films, since the polyester sheet controlled the water transfer through the laminate. In fact, the apparent WVP values of the bilayers were in the range of those of the polyester films for most cases, except for the MS films where an increase (promoted by storage time) was observed. This could be explained by the migration phenomena, previously commented on, that can reduce the water barrier capacity of the hydrophobic layer by plasticisation, coherently with that observed in the case of tensile parameters.

As concerns oxygen permeability, the apparent values of the bilayers were also reduced with respect to those of the corresponding values of starch monolayers which, in turn, are the controlling sheets for the oxygen transfer according to their lower OP values. The migration of compounds from the polyester sheet to the starch matrices, or the greater compactness of the starch matrix provoked by the second compression, implied a still greater reduction in the OP values of the bilayers, reaching values below those of the initial starch layer. This effect was particularly remarkable for the bilayers of cassava starch with gums that exhibited the lowest apparent oxygen permeability.

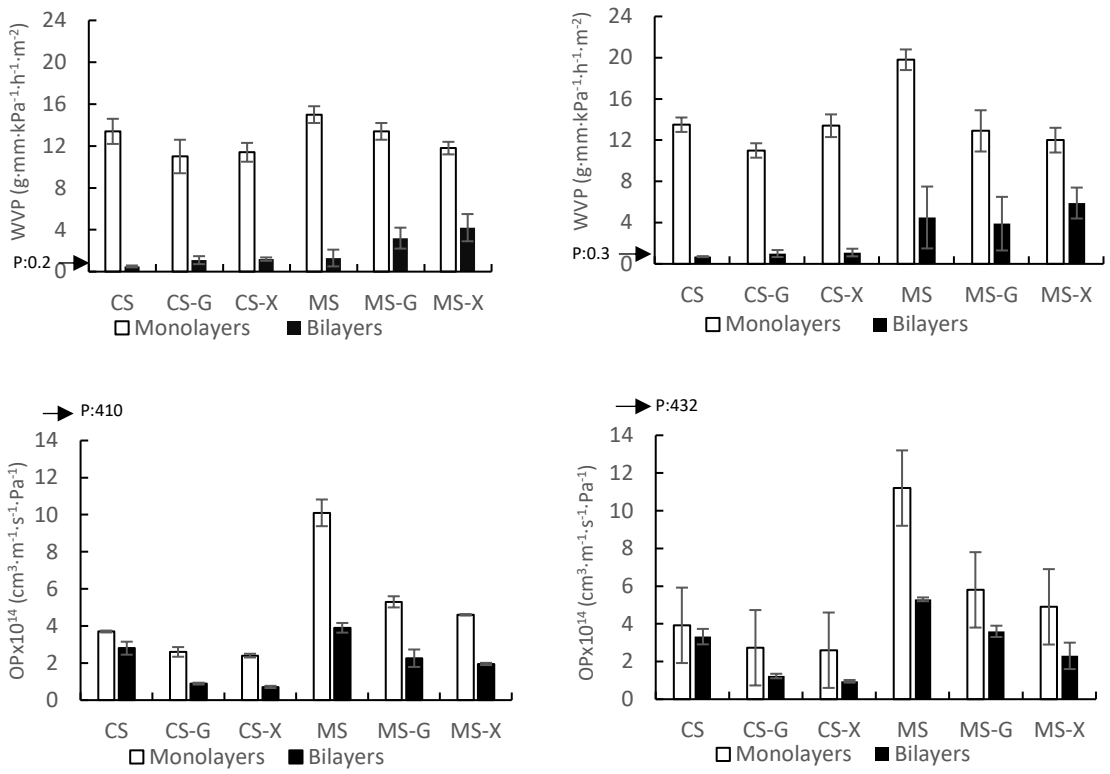


Figure 4. Barrier properties (water vapour: WVP and oxygen (OP) permeability) of bilayer films (Black bars) of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P), in comparison with the corresponding values of the respective starch monolayers (white bars). Values after 1(left) and 5 (right) storage weeks. Arrow indicates the values of the polyester film: WVP (week 1: 0.2, week 5: 0.3), OP (week 1: 410, week 5: 432).

4. CONCLUSIONS

The incorporation of gellan and xanthan gums into thermo-processed cassava and maize starch slightly reduced the water adsorption capacity of starch-based films and improved their mechanical properties. This improvement was more noticeable in maize starch films, although the highest EM and TS values were obtained for cassava starch-gum films. The incorporation of either gellan or xanthan gum decreased the water vapour and oxygen permeabilities of starch films, the cassava starch films with gums being the least permeable to oxygen. The cassava starch films were more stable in their mechanical properties over time, especially those incorporating xanthan gum.

The starch based-polyester laminates exhibited improved oxygen and water vapour barrier capacity with respect to both starch and polyester monolayers. The laminates

with cassava starch with gums showed the lowest OP and WVP values and the highest elastic modulus and tensile strength, with extensibility values in the range of the corresponding monolayers and a reasonable degree of stability throughout time. When also taking the layer adhesion into account, the bilayer formed with the cassava starch with gellan gum and the PLA-PHBV sheet appeared as the best option for food packaging purposes.

Acknowledgements

The authors would like to thank the Ministerio de Ciencia e Innovación of Spain, for funding this study through the Project AGL2016-76699-R and PID2019-105207RB-I00, and the predoctoral research grant # BES-2017-082040.

5. REFERENCES

Arismendi, C., Chillo, S., Conte, A., Del Nobile, M. A., Flores, S., & Gerschenson, L. N. (2013). Optimization of physical properties of xanthan gum/tapioca starch edible matrices containing potassium sorbate and evaluation of its antimicrobial effectiveness. *LWT - Food Science and Technology*, *53*(1), 290–296. https://doi.org/10.1016/j.lwt.2013.01.022

Armentano, I., Fortunati, E., Burgos, N., Dominici, F., Luzi, F., Fiori, S., Jiménez, A., Yoon, K., Ahn, J., Kang, S., & Kenny, J. M. (2015). Bio-based PLA_PHB plasticized blend films: Processing and structural characterization. *LWT - Food Science and Technology*, *64*(2), 980–988. https://doi.org/10.1016/j.lwt.2015.06.032

ASTM. (1995). Standard test methods for water vapor transmission of materials. Standard designations: E96-95. In ASTM, *Annual book of* ASTM *standards* (pp. 406-413). Philadelphia, PA: American Society fot Testing and Materials.

ASTM. (2001). Standard test method for tensile properties of thin plastic sheeting. Standard designations: D882. *In Annual book* of ASTM *standards* (pp. 162-170). Philadelphia, PA: American Society for Testing and Materials.

ASTM. (2002). Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor. Standard designations: 3985-95. *In Annual book* of ASTM *standards* (pp. 472-477). Philadelphia, PA: American Society

for Testing and Materials.

Balaguer, M. P., Gómez-Estaca, J., Gavara, R., & Hernandez-Munoz, P. (2011). Biochemical properties of bioplastics made from wheat gliadins cross-linked with cinnamaldehyde. *Journal of Agricultural and Food Chemistry*, *59*(24), 13212–13220. https://doi.org/10.1021/jf203055s

Bonilla, J., Fortunati, E., Vargas, M., Chiralt, A., & Kenny, J. M. (2013). Effects of chitosan on the physicochemical and antimicrobial properties of PLA films. *Journal of Food Engineering*, *119*(2), 236–243. https://doi.org/10.1016/j.jfoodeng.2013.05.026

Cano, A., Chafer, M., Chiralt, A., & Gonzalez-Martinez, C. (2017). Strategies to improve the functionality of starch-based films. *Handbook of Composites from Renewable Materials*, *1–8*, 311–337. https://doi.org/10.1002/9781119441632.ch74

Cano, Amalia, Jiménez, A., Cháfer, M., Gónzalez, C., & Chiralt, A. (2014). Effect of amylose:amylopectin ratio and rice bran addition on starch films properties. *Carbohydrate Polymers*, *111*, 543–555. https://doi.org/10.1016/j.carbpol.2014.04.075

Chaiwutthinan, P., Pimpan, V., Chuayjuljit, S., & Leejarkpai, T. (2015). Biodegradable Plastics Prepared from Poly(lactic acid), Poly(butylene succinate) and Microcrystalline Cellulose Extracted from Waste-Cotton Fabric with a Chain Extender. *Journal of Polymers and the Environment*, *23*(1), 114–125. https://doi.org/10.1007/s10924-014-0689-0

Gasmi, S., Hassan, M. K., & Luyt, A. S. (2019). Crystallization and dielectric behavior of PLA and PHBV in PLA/PHBV blends and PLA/PHBV/TIO 2 nanocomposites. *Express Polymer Letters*, *13*(2), 199–212. https://doi.org/10.3144/expresspolymlett.2019.16

Kim, S. R. B., Choi, Y. G., Kim, J. Y., & Lim, S. T. (2015). Improvement of water solubility and humidity stability of tapioca starch film by incorporating various gums. *LWT - Food Science and Technology*, *64*(1), 475–482. https://doi.org/10.1016/j.lwt.2015.05.009

Laycock, B., Halley, P., Pratt, S., Werker, A., & Lant, P. (2013). The chemomechanical properties of microbial polyhydroxyalkanoates. *Progress in Polymer Science*, *38*(3–

4), 536–583. https://doi.org/10.1016/j.progpolymsci.2012.06.003

Liu, Q., Wu, C., Zhang, H., & Deng, B. (2015). Blends of polylactide and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with low content of hydroxyvalerate unit: Morphology, structure, and property. *Journal of Applied Polymer Science*, *132*(42), 1–9. https://doi.org/10.1002/app.42689

López, O. V., Zaritzky, N. E., Grossmann, M. V. E., & García, M. A. (2013). Acetylated and native corn starch blend films produced by blown extrusion. *Journal of Food Engineering*, *116*(2), 286–297. https://doi.org/10.1016/j.jfoodeng.2012.12.032

Hugh, T. H., Avena-Bustillos, R., & Krochta, J. M. (1993). Hydrophilic Edible Films: Modified Procedure for Water Vapor Permeability and Explanation of Thickness Effects. *Journal of Food Science*, *58*(4), 899–903. https://doi.org/10.1111/j.1365-2621.1993.tb09387.x

Martin, O., Schwach, E., Avérous, L., & Couturier, Y. (2001). Properties of biodegradable multilayer films based on plasticized wheat starch. *Starch/Staerke*, *53*(8), 372–380. https://doi.org/10.1002/1521-379X(200108)53:8<372::AID-STAR372>3.0.CO;2-F

Muller, J., González-Martínez, C., & Chiralt, A. (2017). Poly(lactic) acid (PLA) and starch bilayer films, containing cinnamaldehyde, obtained by compression moulding. *European Polymer Journal*, *95*(July), 56–70. https://doi.org/10.1016/j.eurpolymj.2017.07.019

Ortega-Toro, R., Bonilla, J., Talens, P., & Chiralt, A. (2017). Future of Starch-Based Materials in Food Packaging. In *Starch-Based Materials in Food Packaging: Processing, Characterization and Applications*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-809439-6.00009-1

Ortega-Toro, R., Jiménez, A., Talens, P., & Chiralt, A. (2014). Properties of starch-hydroxypropyl methylcellulose based films obtained by compression molding. *Carbohydrate Polymers*, *109*, 155–165. https://doi.org/10.1016/j.carbpol.2014.03.059

Ortega-Toro, R., Morey, I., Talens, P., & Chiralt, A. (2015). Active bilayer films of thermoplastic starch and polycaprolactone obtained by compression molding. *Carbohydrate Polymers*, *127*, 282–290.

https://doi.org/10.1016/j.carbpol.2015.03.080

Rasal, R. M., Janorkar, A. V., & Hirt, D. E. (2010). Poly(lactic acid) modifications. *Progress in Polymer Science (Oxford)*, *35*(3), 338–356. https://doi.org/10.1016/j.progpolymsci.2009.12.003

Requena, R., Vargas, M., & Chiralt, A. (2018). Obtaining antimicrobial bilayer starch and polyester-blend films with carvacrol. *Food Hydrocolloids*, *83*, 118–133. https://doi.org/10.1016/j.foodhyd.2018.04.045

Samsudin, H., & Hani, N. M. (2017). Use of Starch in Food Packaging. In *Starch-Based Materials in Food Packaging: Processing, Characterization and Applications* (Vol. 2019). Elsevier Inc. https://doi.org/10.1016/B978-0-12-809439-6.00008-X

Sapper, M., Talens, P., & Chiralt, A. (2019). Improving functional properties of cassava starch-based films by incorporating xanthan, gellan, or pullulan gums. *International Journal of Polymer Science*, *2019*(6), 1–9. https://doi.org/10.1155/2019/5367164

Savadekar, N. R., & Mhaske, S. T. (2012). Synthesis of nano cellulose fibers and effect on thermoplastics starch based films. *Carbohydrate Polymers*, *89*(1), 146–151. https://doi.org/10.1016/j.carbpol.2012.02.063

Sikora, M., Kowalski, S., Piotr Tomasik, P. (2008). Binary hydrocolloids from starches and xanthan gum. Food Hydrocolloids, 22(5), 943-952. ttps://doi.org/10.1016/j.foodhyd.2007.05.007.

Silva-Guzmán, J. A., Anda, R. R., Fuentes-Talavera, F. J., Manríquez-González, R., & Lomelí-Ramírez, M. G. (2018). Properties of Thermoplastic Corn Starch Based Green Composites Reinforced with Barley (Hordeum vulgare L.) Straw Particles Obtained by Thermal Compression. *Fibers and Polymers*, *19*(9), 1970–1979. https://doi.org/10.1007/s12221-018-8023-4

Slavutsky, A. M., Gamboni, J. E., & Bertuzzi, M. A. (2018). Formulation and characterization of bilayer films based on Brea gum and Pectin. *Brazilian Journal of Food Technology*, *21*(December). https://doi.org/10.1590/1981-6723.21317

Tampau, A., González-Martínez, C., & Chiralt, A. (2018). Release kinetics and antimicrobial properties of carvacrol encapsulated in electrospun poly-(ε-

caprolactone) nanofibres. Application in starch multilayer films. *Food Hydrocolloids*, *79*, 158–169. https://doi.org/10.1016/j.foodhyd.2017.12.021

Thongpina, C., Tippuwanan, C., Buaksuntear, K., & Chuawittayawuta, T. (2017). Mechanical and thermal properties of PLA melt blended with high molecular weight PEG modified with peroxide and organo-clay. *Key Engineering Materials, 751, 337-343.* https://doi.10.4028/www.scientific.net/KEM751.337

Vieira, M. G. A., Da Silva, M. A., Dos Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European Polymer Journal*, *47*(3), 254–263. https://doi.org/10.1016/j.eurpolymj.2010.12.011

Chapter 2

Use of phenolic acids to obtain active films based on PLA- PHBV polyesters

Eva Hernández-García[1], María Vargas[1*], Amparo Chiralt[1]

Submitted to Food Packaging and Shelf Life

[1]Institute of Food Engineering for Development, Universitat Politècnica de València, Valencia, Spain

evherga1@upvnet.upv.es

ABSTRACT

Phenolic acids (ferulic, *p*-coumaric and protocatechuic) have been incorporated into polylactic (PLA): Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (75:25) blend films that were obtained by melt-blending and compression moulding, using PEG1000 as plasticizer. Film microstructure, thermal behaviour, functional properties, and the release kinetics of phenolic acids in different food simulants were analysed, as well as the film antimicrobial activity against *Listeria innocua*. Phenolic acids led to an increase in the glass transition temperature of PLA while PHBV supercooling occurred in the films containing protocatechuic acid, which affected their thermal degradation behaviour. Polymer matrices with phenolic acids were stiffer and more resistant to break than the polyester blend, but with similar extensibility, while oxygen and water vapour barrier capacity were also improved, especially in films containing protocatechuic acid. The release rate and ratio of phenolic acids increased when polarity of the food simulant decreases, although very slow delivery was observed in all cases. The limited release of active compounds in aqueous media provoked that films did no inhibit significantly the growth of *Listeria innocua* in inoculated culture medium.

Keywords: biodegradable active films; PLA; PHBV; ferulic acid; *p*-coumaric acid; protocatechuic acid

1. INTRODUCTION

In the last few years, there is a trend to reduce as much as possible the addition of synthetic preservatives in food products while consumers demand healthy and easy-to prepare foods with high nutritional value (Diblan & Kaya, 2018). To face this challenge and avoid deteriorative processes in food, active packaging materials are being developed, incorporating compounds that absorb or release substances from or on the packaged foods or in the head-space of the container (Malhotra et al., 2015). In this sense, the use of active packaging films has been proved to be an alternative to maintain food quality and extend its shelf-life (Moreno et al., 2018; Ngo et al., 2020; Szabo et al., 2020; Esmaeili et al., 2020) at the same time that the drawbacks associated with the direct application of active compounds in the food product surface are reduced (Castillo et al., 2017). To increase the shelf-life and maintain the storage-keeping quality of the packaged foods, active compounds such as essential oils (Krepker et al., 2017; Jiang et al., 2020; Zhang et al., 2020; Esmaeili et al., 2020; Figueroa-López et al., 2020), organic acids (Wang et al., 2020; Sharma et al., 2020; Narasagoudr et al., 2020), and plant extracts (Choulitoudi et al., 2020; Szabo et al., 2020), among others, have been incorporated into active films. Within organic acids, phenolic acids have been shown to have significant antimicrobial and antioxidant properties (Moreno et al., 2006; Porras-Loaiza & López- Malo, 2009).

Phenolic acids consist of an aromatic ring and at least one hydroxyl substituent. They are found in plant tissues, plant foods and metabolites (Badui Dergal, 2006). Phenolic acids, such as ferulic acid, *p*-coumaric acid and protocatechuic acid have been shown to have noticeable antimicrobial activity. Ferulic acid showed an inhibitory effect against *Listeria monocytogenes* (Takahashi et al., 2013; Takahashi et al., 2015) and its incorporation by solvent casting methods into blend poly(lactide)-poly(butylene adipate-co-terephthalate) (PLA-PBAT) was effective against *Listeria monocytogenes* and *Escherichia* coli (Sharma et al., 2020). *P*-coumaric acid has been shown to be effective against different bacteria (Lou et al., 2012), such as enterobacteria (Mitani et al., 2018) and *E. coli* and *Staphylococcus aureus* (Contardi et al.,2019). Protocatechuic acid inhibited the growth of *Listeria monocytogenes* in cream cheese (Stojković et al., 2013),

and of *E. coli*, *S. typhimurium, L. monocytogenes, S. aureus,* and *B. cereus* in ground beef and apple juice (Chao & Yin, 2009).

As concerns the development of new food packaging materials, one of the techniques that has been recently used to improve their functional properties is the combination of two or more polyesters in the production phase (Kanda et al., 2018). This strategy, which is cheaper and more rapid than the use of new monomers and/or new polymerization routes, allows for obtaining films with improved properties as compared to the pure polymer films. However, the improvement of one property can result in the weakening of another (Zhang et al., 2012). For example, PLA based films show low ductility and high permeability to water vapor and O_2 (Auras et al., 2004), and to overcome these drawbacks PLA has been blended with a highly crystalline biopolymer with high melting point such as PHB (Noda et al., 2004). Previous studies have reported an improvement in the crystallization of PLA due to the positive effect of PHB/PHBV crystals (Zembouai et al., 2013; Liu et al., 2015; Arrieta et al., 2019), thus improving the water vapor and oxygen barrier properties (Zembouai et al., 2013), the thermal stability and mechanical resistance (Zhang & Thomas, 2011; Liu et al., 2015) as compared to pure PLA films. In the same sense, Requena et al. (2018) obtained compression-molded PLA:PHBV (75:25) blend films, with 15% PEG1000, with improved extensibility and water barrier capacity. However, to the best of our knowledge, phenolic acids have not been incorporated to thermoplastic PLA-PHBV blend films to develop active packaging materials.

The aim of this study was to analyze the functional properties of PLA-PHBV blend films obtained by melt-blending and compression molding incorporating phenolic acids (ferulic, *p*-coumaric and protocatechuic) as antibacterial compounds. The antibacterial effectiveness of the films against *L. innocua* and the release kinetics of the phenolic acids in different food simulants have been also analyzed.

2. MATERIALS AND METHODS

2.1. Materials

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) ENMAT Y1000P with 3% hydroxyvalerate was supplied by Helian Polymers B.V. (Belfeld, Holland). Amorphous PLA 4060D, density of 1.24g/cm^3 and average molecular weight of 106,226 D with 40%

of low molecular weight fraction (275 D) as reported by Muller et al. (2017) was purchased from Natureworks (U.S.A). The plasticizer poly(ethylene glycol) with molecular weight of 1000 Da (PEG1000) was purchased from Sigma-Aldrich (Steinheim, Germany). Ferulic, *p*-coumaric and protocatechuic acids were purchased from Sigma-Aldrich (Madrid, Spain). UV methanol, UV ethanol, phosphorus pentoxide (P_2O_5) and magnesium nitrate-6-hydrate ($Mg(NO_3)_2$) were supplied by Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain).

2.2. Preparation of blend films

PLA-PHBV blend films were obtained by melt blending and compression moulding in a polymer ratio of 75:25, using PEG1000 (15 g/100 g polymer) as a plasticizer, and without or with a constant amount of antibacterial compound (ferulic, *p*-coumaric and protocatechuic acids) of 2% (w/w) with respect to the polymer matrix (polymer plus plasticizer). The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 170 °C and 50 rpm, for 12 min. The obtained pellets were conditioned at 25 °C and 53% RH. Four grams of the conditioned pellets were put onto Teflon sheets and preheated at 200 °C for 5 min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). Films were obtained by compressing at 200 °C for 4 min at 100 bars, and a final cooling cycle for 3 min until the temperature reached about 70 °C (Requena et al., 2018). The obtained films were conditioned at 25 °C and 53% RH.

The thickness of the films was measured in triplicate in six random points of each sample by a hand-held digital micrometer (Electronic Digital Micrometer, Comecta S.A., Barcelona, Spain).

2.3. Microstructural and thermal analyses

The microstructure of films was observed by Field Emission Scanning Electron Microscope (FESEM Ultra 55, Zeiss, Oxford Instruments, UK). The samples were maintained with P_2O_5 for two weeks to ensure the total dehydration of the films. Film samples were cryofractured with liquid nitrogen to obtain cross-sections, which were mounted on support stubs and coated with platinum prior performing the microstructural observations at 2 kV.

Differential scanning calorimetry analyses were carried out using a Differential Scanning Calorimeter (DSC823^e Star^e Mettler-Toledo Inc., Switzerland). Films samples (10 mg) were placed into aluminium pans and tightly sealed. Samples were heated from room temperature to 180 °C at 10 °C /min. Then, samples were cooled until -30 °C at -50 °C /min and heated again to 180 °C at 10 °C /min as reported by Requena et al. (2018). As reference, an empty aluminum pan was used. Each sample was analysed in duplicate under a nitrogen stream of 20 mL/ min.

The thermal stability was analyzed using a thermogravimetric analyser (TGA 1 Star^e System analyser, Mettler-Toledo, Switzerland), by sample heating from 25 to 600 °C at 10 °C/min under nitrogen flow (10 mL/min). Samples (3 mg maximum) were conditioned in desiccators with P_2O_5, using at least two replicates per formulation. In each test, the corresponding mass curve was obtained as a function of temperature. From theses curves, the derived curves (DTG) were obtained as a function of temperature, and the corresponding values of T_{onset} (initial degradation temperature) and T_{max} (temperature of maximum degradation rate) using the STAR^e Evaluation Software (Mettler-Toledo, Switzerland).

2.4. Tensile properties and thickness

Mechanical properties (elastic modulus (EM), tensile strength (TS), and elongation at break (E) were determined using a Universal Testing Machine (Stable Micro System TA. XT plus, Haslemere, England), according to ASTM standard method D882 (ASTM, 2001). EM, TS, and E were determined from the stress strain curves, estimated from force-distance data obtained for eleven pre-conditioned (53% RH and 25 °C) film samples of each formulation (2.5 cm wide and 10 cm long), which were mounted in the extension grip of the equipment and stretched at 50 mm·min^{-1} until breaking.

2.5. Moisture content and barrier properties

In order to determine the moisture content of the films, three samples of each formulation (previously conditioned at 53% RH and 25 °C) were dried at 60 °C for 24h in a vacuum oven (VaciotermT, JP Selecta S.A., Barcelona, Spain). Afterwards, the samples were equilibrated in a desiccator containing P_2O_5 at room temperature, until constant weight was reached.

The oxygen permeability (OP) was determined using an OX-TRAN equipment (Model 1/50, Mocon, Minneapolis, USA). Two film samples per formulation were previously conditioned at 53% RH and 25 °C (ASTM Standard Method D3985-05, 2002). The tests were performed at 53% RH and the film exposure area was 50 cm^2. OP was calculated by dividing the oxygen transmission rate by the difference in the oxygen partial pressure between the two sides of the film and multiplying by the film thickness.

The water vapor permeability (WVP) of films was measured according to a modification of the ASTM E96-95 (ASTM, 1995) gravimetric method, at 25 °C and a RH gradient of 53%-100% using Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium) of 3.5 cm in diameter. This gradient was generated by using an oversaturated $Mg(NO_3)_2$ solution and pure water, respectively. Three circular samples of each formulation were prepared, and the thickness of each sample was measured in six random points with an electronic digital micrometer (Comecta S.A., Barcelona, Spain). To determine WVP, the cups were weighed periodically using an analytical balance (ME36S, Sartorius, Germany; ±0.00001 g) at intervals of 1.5 h for 24 h after the steady state had been reached. The slope of the weight loss versus time was plotted and the water vapor transmission rate (WVTR) and water vapor permeability were calculated according to Vargas et al. (2009).

2.6. Optical properties

The optical properties (transparency and CIE-L*a*b* color coordinates) were determined in triplicate by measuring the reflection spectrum of the samples using a Konica Minolta spectrocolorimeter, model CM-5 (Minolta CO., Tokyo, Japan). The transparency of the films was determined by means of the internal transmittance (Ti), applying the Kubelka-Munk theory (Hutchings, 1999) for multiple scattering to the reflection spectra (400 to 700 nm), given the reflection spectra of both black and white backgrounds. The CIE-L*a*b* color coordinates were obtained from the reflectance of an infinitely thick layer of the material by considering illuminant D65 and observer 10°. Psychometric coordinates Chroma (C_{ab}*) and hue (h_{ab}*) were also determined using Eq. (1) and (2) (Hutchings, 1999).

$$h_{ab}^{*}=\arctan(b^{*}/a^{*}) \qquad \text{Eq. (1)}$$

$$c_{ab}^{*}=\sqrt{(a^{*})^{2}+(b^{*})^{2}} \qquad \text{Eq. (2)}$$

Moreover, to evaluate the color differences between ferulic, *p*-coumaric and protocatechuic films and the control film, the following equation was used Eq. (3):

$$\Delta E=\sqrt{(\Delta L^{*})^{2}+(\Delta a^{*})^{2}+(\Delta b^{*})^{2}} \qquad \text{Eq. (3)}$$

The film gloss was determined at an incidence angle of 60º, according to the ASTM standard method D523 (ASTM, 1999), using a flat surface gloss meter (Multi Gloss 268, Minolta, Germany). Measurements of each sample were taken in triplicate and three films of each formulation were considered. All results are expressed as gloss units (GU), relative to a highly polished surface of black glass standard with a value near to 100 GU.

2.7. Retention of active compounds and release kinetics in different food simulants

The final phenolic acids content in the films was determined by methanol extraction, followed by spectrophotometric quantification using a UV-visible spectrophotometer (Thermo Scientific Evolution 201, EEUU). Standard calibration curves for ferulic, *p*-coumaric and protocatechuic acids were obtained to determine their concentration from the absorbance values by using an initial solution with 500 $\mu g \cdot mL^{-1}$ and subsequent dilutions. Ferulic, *p*-coumaric and protocatechuic acids were quantified through absorbance measurements at 320, 309 and 208 nm, respectively, using the methanol extract of active-free PLA-PHBV films as blank solution. Films samples of 100 mg were cut in strips and placed in flasks with 10 ml of UV methanol, under stirring for 48 h at 20 °C. After that, samples were filtered and appropriately diluted to obtain absorbance values between 0.2 and 0.8. All analyses carried out in samples from four different positions of four different films to analyze the degree of homogeneity of the active distribution throughout the film.

In the release studies two different food simulants were used: 10% (v/v) (simulant A) and 50% (v/v) (simulant D1) ethanol-water solutions (Commission regulation (EU) 10/2011). 500 mg of each sample was placed into a flask containing 100 mL of each simulant. Thereby, each film formulation-food simulant system was kept under stirring at 25 °C throughout the assay time, using a spectrophotometric method, at 311, 287 and

291 nm of wavelength (where the ferulic, *p*-coumaric and protocatechuic acid absorbance is maximum, respectively, for simulant A), and 313, 290 and 293 nm of wavelength (where the ferulic acid, *p*-coumaric and protocatechuic acid absorbance is maximum, respectively, for simulant D1). Phenolic acids quantification in the liquid phase was established using the previously obtained calibration curves in each simulant. The assay was performed in triplicate.

Two empirical models were considered to describe the behavior of the active compounds in the food simulants. To predict the release kinetics, the Peleg model was applied (Eq.4) (Peleg, 1988).

$$\frac{t}{Mt} = k_1 + k_2 t \qquad \text{Eq. (4)}$$

where: M_t is total active compound released into the simulants after contact time t; k_1 and k_2 are the model constants, where $1/k_1$ is the release rate at the beginning of the process and $1/k_2$ is the mass of active compound released at equilibrium (M_∞). In addition, the experimental results were fitted to the Korsmeyer-Peppas model (Eq. 5) (Siepmann & Peppas, 2011) to investigate the possible coupling of the relaxation of the polymer in contact with the solvent with the diffusion of the active compound through the polymer matrix.

$$\frac{Mt}{M\infty} = kt^n \qquad \text{Eq. (5)}$$

where: M_t/M_∞ is the fraction of active compound at time t (h); k is the rate constant of the film (h^{-n}); n is the diffusional exponent that provides information about the mechanisms involved in the release process which takes values in the 0-1 interval (dimensionless). Thus, a 0.5 value for the diffusional exponent means that the release takes place through Fickian diffusion, whereas if the n value is higher than 0.5 (anomalous transport) the diffusion and the polymer relaxation rates are coupled. If the n value is lower than 0.5, a quasi-Fickian diffusion for the active release can be considered (Siepmann & Peppas, 2012).

2.8. Antibacterial activity

The minimum inhibitory concentration of ferulic, *p*-coumaric and protocatechuic acids was determined as described by Requena et al. (2019). *Listeria innocua* (CECT 910,

Spanish Type Culture Collection, Burjassot, València) and *E. coli* (CECT 101, Spanish Type Culture Collection Burjassot, València) stored at -25 °C with 30% glycerol, has been used. Bacterial cultures in exponential phase of growth were prepared by inoculating the microbial stock suspensions into TSB, followed by incubation at 37 °C for 24h. The inoculums were diluted in TSB tubes to get a target inoculum of 10^5 colony-forming units mL^{-1}. Aliquots of 100 µl were taken from this bacterial suspension and deposited in each of the wells of the 96-well-plate. In parallel, stock solutions of the different active compounds were prepared in DMSO with a concentration of 3 mg active/ mL. Thus, 100 µl of each of the solutions with the active ingredients were transferred to different positions of the multiwell plate previously identified and were incubated for 24h at 37 °C. Certain positions of the plate were used to control the test. After 24 hours of incubation, a solution of the MTT reagent was prepared in PBS (5mg/ mL) and 10 µl was added to each well of the plate. The plate was incubated again for 4h at 37 °C and after this time the results were visually read. The MIC of each of the active compounds was the lowest concentration at which no color change (microbial growth) occurred.

The antibacterial effect of PLA-PHBV blend films containing the polyphenol acids was evaluated *in vitro* (liquid medium test) according to the methodology described Valencia-Sullca et al. (2016), with some modifications. *Listeria innocua* (CECT 910, Spanish Type Culture Collection, Burjassot, València) stored at -25 °C with 30% glycerol, has been used. Bacterial cultures of *Listeria innocua* in exponential phase of growth were prepared as described below. The inoculums were diluted in TSB tubes to get a target inoculum of 10^4 colony-forming units mL^{-1}. Film samples of 5.86 x 9 cm (ferulic and protocatechuic acid) and 7.54 x 9 cm (*p*-coumaric acid) obtained from the different types of film formulation were placed in TSB tubes. Inoculated tubes without film were used as control samples. Immediately after the inoculation and after 6 days at 10 °C the microbial counts on plates were determined. Serial dilutions were made and poured onto Base Palcam agar medium dishes (which contained the selective supplement for *Listeria*), which were incubated for 48 h at 37 °C. All tests were run in triplicate.

2.9. Statistical analysis

Statgraphics Centurion XVII-64 software (Manugistics Corp., Rockville, Md.) was used for carrying out the statistical analyses of data through analysis of variance (ANOVA).

Homogeneous sample groups were obtained by using LSD test (95% significance level).

3. RESULTS AND DISCUSSION

3.1. Microstructure and thermal behavior

Microstructural images show the degree of homogeneity in the distribution of film matrix components, a factor related to the functional properties of the material. The cross-section morphologies of PLA-PHBV blend films and PLA-PHBV blend films containing ferulic acid, *p*-coumaric acid and protocatechuic acid are shown in **Figure 1**.

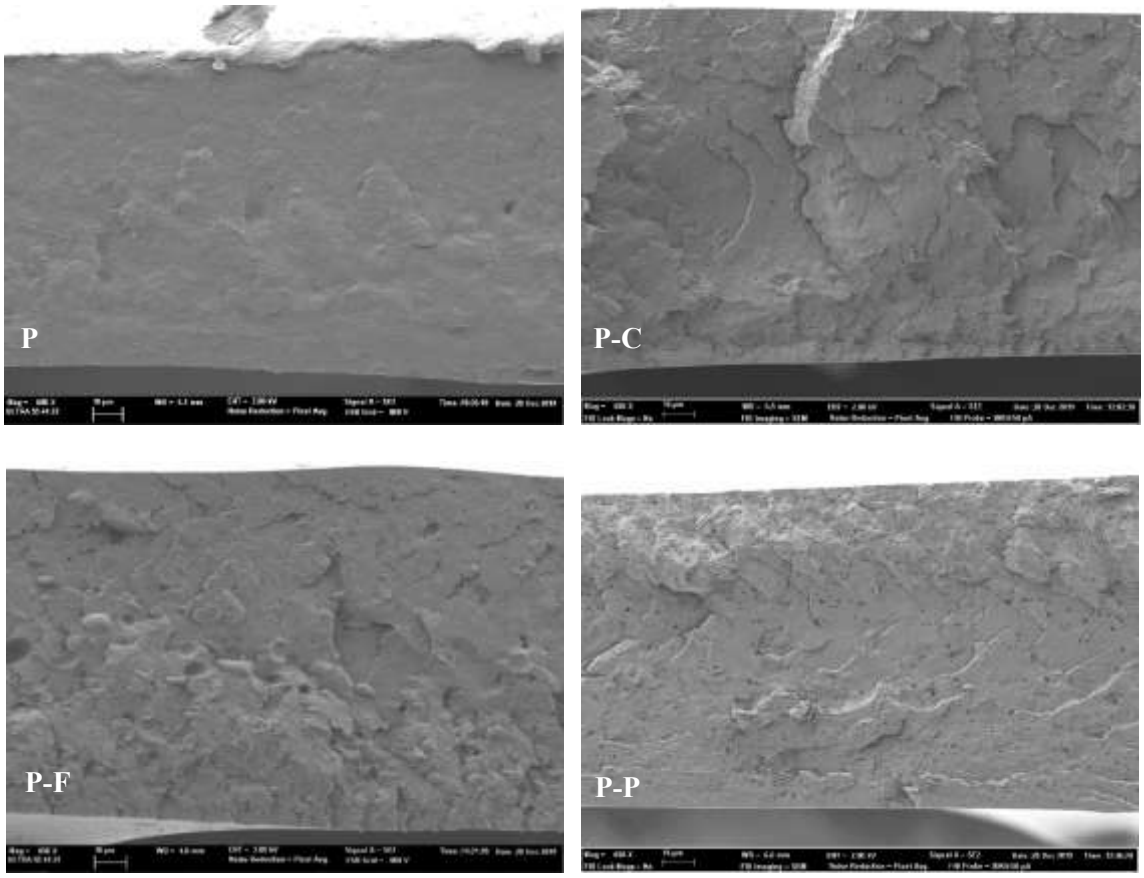


Figure 1. Field Emission Scanning Microscope (FESEM) micrographs of the cross-section of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P) (magnification: 650X; bar: 10 µm).

In the cross section of PLA-PHBV films (P), a lack of miscibility between the polyesters and the plasticizer can be observed, with different domains of PLA and PHBV, as previously reported (Hernández-García et al., 2021). The incorporation of phenolic acids led to a more heterogeneous cross section, where a more brittle fracture was observed

in different domains, in contrast with that observed by Mathew and Abraham. (2008) for starch-chitosan blend films containing ferulic acid. In all cases small particles dispersed in the more continuous phase were observed, which can be attributed to dispersed aggregates of phenolic acids, thus evidencing the lack a complete miscibility of these compounds with the polymers. This was particularly evident in films containing protocatechuic acid that showed aggregates of higher size.

Table 1 shows the results obtained from DSC analysis: glass transition temperature (T_g), melting temperature (T_m), and melting enthalpy (ΔH_m) of PLA-PHBV active films as deduced from 1st heating scan and from the second heating scan when the thermal history of the polymers was erased (**Table 1**). In **Figure 2**, the DSC curves from the 1st and the 2nd heating scan are shown.

Table 1. Thermal properties of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P) from the first and the second heating scan: Glass transition (T_g), melting temperature (T_m) and melting enthalpy (ΔH_m) of the polymers in the blend. Mean values± standard deviation.

First Heating Scan				
Formulation	**$T_{g\ PLA}$ (°C)**	**$T_{m\ 1}$ (°C)**	**$T_{m\ 2}$ (°C)**	**ΔH_m (J/g polymer)**
P	36.1 ± 1.3^{d}	162.0 ± 3.0^{a}	169.1 ± 1.7^{a}	20.0 ± 4.0^{ab}
P-F	39.4 ± 0.3b^{c}	154.2 ± 0.6^{b}	163.5 ± 0.4^{b}	18.9 ± 1.0^{b}
P-C	46.0 ± 0.0^{a}	158.8 ± 1.4^{a}	167.4 ± 1.7^{a}	20.3 ± 1.6^{b}
P-P	41.0 ± 0.3^{b}	-	163.8 ± 0.3^{b}	24.0 ± 2.0^{a}
Second Heating Scan				
	$T_{g\ PLA}$ (°C)	**$T_{m\ 1}$ (°C)**	**$T_{m\ 2}$ (°C)**	**ΔH_m (J/g polymer)**
P	29.1 ± 0.1^{d}	163.0 ± 2.0^{a}	170.0 ± 2.0^{a}	18.6 ± 3.0^{ab}
P-F	33.9 ± 0.7^{c}	154.0 ± 0.3^{c}	162.0 ± 0.1^{c}	19.4 ± 0.7^{b}
P-C	37.0 ± 0.0^{a}	159.3 ± 1.9^{b}	165.0 ± 2.0^{b}	21.4 ± 1.0^{a}
P-P	36.4 ± 0.2^{b}	-	162.9 ± 0.3^{b}	22.4 ± 1.6^{a}

T_m ferulic (°C): 172.54; T_m *p*-coumaric (°C): 214.9; T_m protocatechuic (°C): 199.15

Different superscript letters (a-c) within the same column indicate significant differences among formulations ($p < 0.05$).

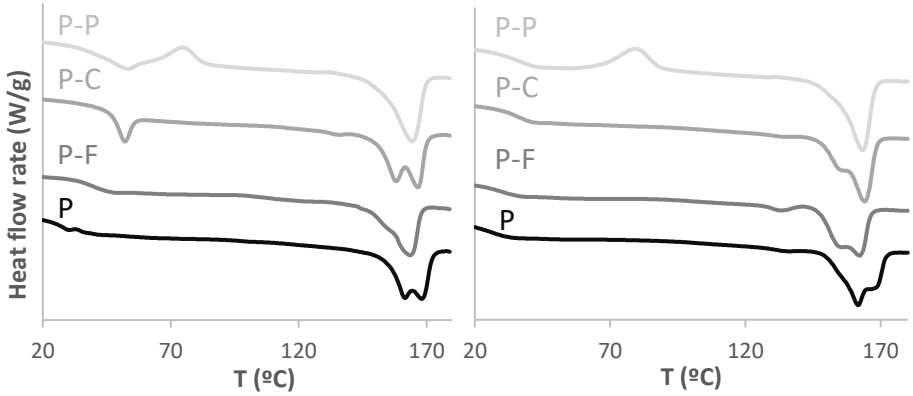


Figure 2. DSC thermograms of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P) obtained from the first heating scan (left) and the second heating scan (right).

In both first and second heating scans, a two-peak endothermic event was observed for the polymers at about 160 and 170 °C, as observed by Requena et al. (2018) for similar blend films with PLA and PHBV. Only the films containing protocatechuic acid showed a single endothermic peak at 163 °C which has been also observed in some films with similar PLA/PHBV ratio. Moreover, these films containing protocatechuic acid, exhibited a exothermic peak around 75-80 °C during both scans, which suggests that supercooling of PHBV was promoted in the presence of protocatechuic acid and, as consequence, PHBV crystalized after glass transition in the PLA matrix. Considering the slower crystallization of PLA, mainly in the amorphous form used, there are two possibilities for the origin of the two melting peaks: the melting and subsequent crystallization of the PHBV or the formation of two types of lamellae structures in the crystals, as speculated by Furukava et al. (2005) by analyzing the crystallization behavior of PHB in different blends with PLA. In fact, the total melting enthalpy values are very close in all film formulations (19-22 J/g), which considering the mass fraction of PHBV in the blend and the melting enthalpy of pure PHBV (146 J/g PHBV, González-Ausejo et al., 2017) represent a crystallinity degree of PHBV of about 55 %. However, in the films containing protocatechuic acid this crystallinity was only reached after heating of the films acid, and much less crystalline forms would be present in the supercooled obtained film. The incorporation of ferulic and *p*-coumaric acids slightly decreased the melting points of PHBV, with no significant changes in the total melting enthalpy, which showed values in

the range reported by Requena et al. (2018) for similar polymer blends. This reduction reflects the specific interactions between phenolic acid and PHBV, such as hydrogen bonds between phenolic hydroxyls and polyester carbonyls, which slightly reduces the size of crystals and so, melting point. In the case on protocatechuic acid, these interactions promoted the polymer supercooling without total crystallization in the film, but only when temperature increased above the Tg of the PLA matrix. This behavior suggests that protocatechuic acid could established stronger interactions with the polymers that affected the crystallization behavior in the matrix. The particular molecular structure of protocatechuic acid (**Table 6**) could be more able to promote interchain hydrogen bonds, enhancing the crosslinking effect thus inhibiting crystallization because of the more limited molecular mobility. This was also reflected in the Tg values of PLA commented below. The reduction of melting points due to the addition of *p*-coumaric acid was also observed by Contardi et al. (2019) in polyvinylpyrrolidone films.

The glass transition of PHBV was not recorded in the DSC analyses due to the low expected value (-1 °C, Ferreira et al., 2002; Martinelli et al., 2012) that could not be evaluated with the used equipment. For the PLA phase the T_g values were 36 °C for the first scan and 29 °C for the second scan, which agreed with those previously reported by Requena et al. (2018) for similar polymer blends. The relaxation enthalpy associated with the endothermic peak in the region of the glass transition was observed in the first heating scan for P-P and P-C films, due to the aging effects. The incorporation of phenolic acids led to an increase in the Tg values, especially for *p*-coumaric and protocatechuic acids, which exert an anti-plasticizing effect in the PLA:PHBV matrix. As previously pointed out, this could be due to the promotion of interchain interactions through hydrogen bonds between phenolic hydroxyls and carbonyl of polyesters, thus reducing the molecular mobility in the matrix and increasing the Tg. The ability of each acid to promote interchain associations will be affected by the molecular structure of phenolic acid (shown **Table 6**). In contrast, Kasmi et al. (2019) use synthesized derivatives of ferulic acid (without hydroxyl groups) as plasticizers in PLA.

The effect of phenolic acids on the thermal degradation pattern of PLA: PHBV blends is shown in **Figure 3**. All films exhibited two different degradation steps that can be attributed to the separated degradation of each polyester, PLA and PHBV, according to

the lack of polymer miscibility previously reported (Armentano et al., 2015; Requena et al., 2018), being PLA more thermostable than PHBV as reported by Ferreira et al. (2002) and Zhao et al. (2013). The initial degradation temperatures (T_{onset}) and the temperature at the maximum degradation rate (T_{peak}) for the different degradation steps of the polyester blend films containing or not phenolic acids are shown in **Table 2**.

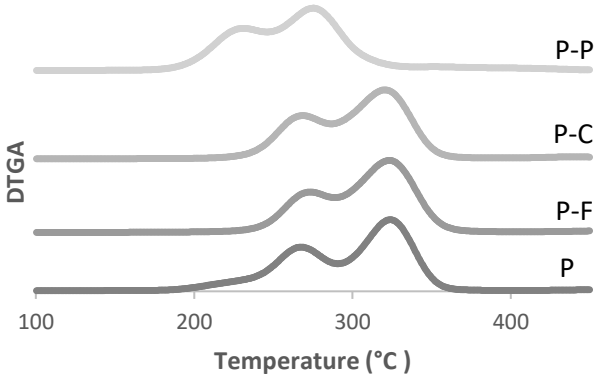


Figure 3. DGTA curves of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P).

Table 2. Initial degradation temperature (T_{onset}), temperature at the maximum degradation rate (T_{peak}) for the main degradation steps of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). Mean values ± standard deviation.

Formulation	T_{onset}	$T_{peak\ PHBV}$	$T_{peak\ PLA}$
P	190 ± 3^{c}	267 ± 3^{b}	321 ± 4^{a}
P-F	222 ± 3^{a}	273 ± 1^{a}	323 ± 1^{a}
P-C	209 ± 1^{b}	269 ± 1^{b}	321 ± 2^{a}
P-P	185 ± 7^{c}	232 ± 8^{c}	275 ± 1^{b}

Different superscript letters (a-c) within the same column indicate significant differences among formulations ($p < 0.05$).

The peak temperatures are at around 267 °C and 321 °C and can be attributed to the PHBV and PLA maximum degradation rates, respectively. The incorporation of phenolic acids into the polymeric matrix, provoked different effects on thermal behavior. Ferulic and coumaric acids led to a significant increase ($p > 0.05$) in the initial degradation temperatures of the polymer blends. However, this effect was not observed with the addition of protocatechuic acid, which caused a decrease in the thermal stability of the blends, decreasing both the onset temperature and those of the maximum degradation

rates of PHBV and PLA. These changes in thermal degradation behavior are coherent with the described patterns for glass transition and crystallization deduced from DSC analyses. Degradation temperature increases when the cohesive forces in the matrix arise which are affected by the crosslinking and crystallization degree in the matrix. The described crosslinking effect promoted by phenolic acids could explain the slight delay in onset of thermal degradation of polyesters when ferulic and coumaric acid were added. In contrast, protocatechuic acid inhibited PHBV crystallization, and therefore thermo-degradation occurred at lower temperatures.

Microstructural and thermal analyses revealed that PLA, PHBV and phenolic acids are not completely miscible but interchain interactions occurred probably due to the interchain hydrogen bonds and phenolic molecules. This modifies the molecular mobility in the matrix, giving rise to an increase in the Tg and changes in the polymer crystallization pattern, both affecting the thermal stability of the polymers. Protocatechuic acid seems to promote the strongest interactions, enhancing more the Tg value and limiting the crystallization of PHBV. In contrast, the lack of crystallization in protocatechuic acid containing films turn the PHBV matrix less thermostable.

3.2. Tensile properties and film thickness

Thickness values of the films are shown in **Table 3**. The incorporation of *p*-coumaric acid did not have a significant effect ($p > 0.05$) on film thickness, but ferulic acid led to an increase in film thickness whereas protocatechuic acid led to a significant decrease. This could be associated with the different interchain forces and subsequent film compactness established in each case, depending on the molecular structure of phenol. These interactions forces also affected the film flowability and extension during the compression-molding step.

The mechanical properties give essential information for analyzing the strength, stiffness, resistance to break and extensibility of materials (Liu et al., 2020). The mechanical behavior will be affected by the interactions between the different components of the matrix and the incorporated active compounds (Talón et al., 2017). **Table 3** shows the elastic modulus (EM) and tensile strength (TS) and deformation at break (%E) of the different films. The obtained values of tensile parameters for the

polyester blend without acids (P) were in the range of those reported by Requena et al. (2018) for similar polyester blends obtained by melt-blending and compression molding.

Table 3. Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E), thickness, barrier properties (water vapour (WVP) and oxygen (OP) permeability) and equilibrium moisture content of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). Mean values ± standard deviation.

Sample	EM (MPa)	TS (MPa)	E (%)	Thickness (μm)	WVP ($g.mm.kPa^{-1}.h^{-1}.m^{-2}$)	OPx10^{14} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	Equilibrium moisture (g water/100g dried film)
P	1050 ± 90^{c}	19.0 ± 1.5^{b}	2.3 ± 0.2^{a}	145 ± 0.015^{b}	0.21 ± 0.05^{a}	168 ± 0.3^{a}	0.160 ± 0.006^{a}
P-F	1112 ± 100^{bc}	23.0 ± 1.9^{a}	2.4 ± 0.4^{a}	155 ± 0.018^{a}	0.16 ± 0.01^{ab}	149 ± 10^{b}	0.150 ± 0.001^{a}
P-C	1176 ± 50^{ab}	23.0 ± 2.3^{a}	2.2 ± 0.3^{a}	143 ± 0.016^{b}	0.18 ± 0.02^{ab}	122 ± 1.6^{c}	0.030 ± 0.002^{b}
P-P	1231 ± 90^{a}	24.5 ± 3.6^{a}	2.3 ± 0.4^{a}	138 ± 0.018^{c}	0.14 ± 0.01^{b}	110 ± 7.7^{c}	0.100 ± 0.008^{ab}

Different superscript letters (a-c) within the same column indicate significant differences among formulations ($p < 0.05$).

Incorporation of phenolic acids significantly ($p < 0.05$) affected tensile parameters of the films. Elastic modulus (EM) and tensile strength (TS) increased with the addition of the three acids, this increase being higher for protocatechuic acid. This agrees with the above-mentioned more marked interactions of protocatechuic acid with the polymeric chains that limited the crystallization of PHBV. This differs from that obtained in other studies carried out with PLA-based films (without PHBV), in which the incorporation of ferulic acid or *p*-coumaric acid led to a decrease in the TS values (Dintcheva et al., 2018; Rigoussen et al., 2018; Sharma et al., 2020). On the other hand, no significant differences in the elongation at break (E) were observed with the incorporation of the phenolic acids. However, previous studies have shown an increase in extensibility when incorporating ferulic acid into semi crystalline PLA films (Dintcheva et al., 2018). Therefore, the addition of phenolic acids in the PLA-PHBV matrix resulted in more resistant and stiffer films, which could be attributed to the promoted interactions between phenolic acids and PHBV, but with the same extensibility as the pure polyester blend.

3.3. Moisture content and barrier properties

Water vapor permeability (WVP), oxygen permeability (OP) and moisture content of films stored for 1 week at 25 °C and 53% RH, are shown in **Table 3**. P films showed WVP

values slightly lower than that observed in previous studies (Requena et al., 2018). The incorporation of phenolic compounds in PLA:PHBV polymer matrix reduced the water vapor permeability of the films, probably due to the reaction of the end chain OH groups with acid molecules during thermal process, which further limits the water solubility in the films and then permeation capability of water molecules. The hydrogen bonding between the phenol groups and oxygen of polyester group would also affect the water permeation though the polymer matrix. This is also reflected in the values of the equilibrium moisture content of the films, which were reduced with the addition of phenolic acid. The reported slight improvement in the water barrier properties was significant ($p < 0.05$) in films containing protocatechuic acid, which is coherent with the more marked above mentioned interactions with PHBV chains.

Likewise, addition of phenolic acids to the polyester blend led to a significant decrease in the oxygen permeability. This improvement was more accentuated for films with protocatechuic acid, coherently with the above mentioned higher interactions with the polymer chains. Therefore, the decrease in oxygen permeability promoted by phenolic acids can be explained by the interactions between the phenols and polyester chains, tightening the polymer matrix and limiting mass transport phenomena through the films (Benbettaïeb et al., 2019; Contardi et al., 2019).

3.4. Optical properties

Table 4 shows color parameters, internal transmittance and gloss values (at 60°) for PLA-PHBV blend films with and without phenolic acids. The incorporation of phenolic acids promoted slight changes in the color parameters, with total color change (ΔE) inside the non-perceptible range. Likewise, the gloss and internal transmittance (T_i) values of the films slightly increased in films with phenolic acids, especially with the addition of *p*-coumaric and protocatechuic acids.

Table 4. Colour parameters, total colour difference as regards P film (**ΔE**), gloss and internal transmittance (Ti) at 550 nm. Mean values ± standard deviation.

Formulation	L*	hab*	Cab*	ΔE	Gloss 60°	T_i (λ = 550 nm)
P	77.0 ± 0.9^{b}	82.5 ± 0.8^{b}	14.8 ± 0.5^{a}	-	12.4 ± 1.3^{d}	80 ± 0.01^{c}
P-F	78.7 ± 0.7^{a}	82.5 ± 0.6^{b}	14.1 ± 0.6^{b}	1.74	18.9 ± 2.9^{c}	82 ± 0.01^{b}
P-C	78.0 ± 0.5^{a}	83.8 ± 0.5^{a}	14.6 ± 0.3^{ab}	1.03	23.6 ± 2.3^{a}	83 ± 0.01^{a}
P-P	76.7 ± 0.5^{b}	81.6 ± 0.4^{c}	13.5 ± 0.4^{c}	1.37	21.6 ± 3.2^{b}	83 ± 0.01^{a}

Different superscript letters (a-d) within the same column indicate significant differences among formulations ($p < 0.05$).

3.5. Retention of active compounds after film processing and release kinetics

The methanol extraction of phenolic acids from films with ferulic, *p*-coumaric and protocatechuic acids and the subsequent quantification by a spectrophotometric method yielded similar values of 83.0±0.03, 80.0±0.1 and 79.0±0.04 g compound retained/100 g compound incorporated, respectively. This decrease in the initially incorporated amount of acids implies a final concentration of 1.7-1.6 g of actives /100 g film, which could be considered enough to inhibit bacterial growth if it is completely released to the food substrate, taking into account the minimal inhibitory concentration of the acids against some bacteria (**Table 6**). However, the effective release of these compounds into the food substrate are necessary to exert this action. In this sense, analyses of release kinetics of these active compounds into different food simulants (with high and intermediate polarity) were carried out.

Figures 4 and 5 show the data obtained for the released amount of actives as a function of contact time, for simulants A and D1, respectively, in terms of the M_t/M_0 values (ratio of the amount of phenolic acid released at each time with respect to the initial content in the film). The fitted models (Peleg and Korsemeyer-Peppas) were also plotted in Figures 4 and 5. Likewise, **Table 5** shows the obtained kinetic parameters from Peleg and Korsmeyer-Peppas models. As expected, different release behavior was observed for the phenolic acids in both simulants according to the different chemical affinity of compounds with the polymer matrix and solubility in the both simulants, both factors defining the partition coefficient at equilibrium (Requena et al., 2017). The released ratio of phenolic acids into the simulant D1 (richer in ethanol) was higher than that obtained

in simulant A, for *p*-coumaric and protocatechuic acids whereas this was slightly lower for ferulic acid. One important fact was the film disintegration into the simulants from a determined contact time, depending on the simulant. The kinetic data were only considered up to the disintegration point, since from this time, release behavior was accelerated stochastically due to the loss of film integrity. This disintegration occurs faster for films containing acids, whereas the acid free polyester film remains whole for more than 20 days in contact with both simulants. This suggest that the acid release and the subsequent pH decrease in the aqueous media highly contribute to the film disintegration. The pKa values of the acids are 4.58, 4.64 and 4.26, respectively for ferulic, *p*-coumaric and protocatechuic acid and considering the amount released at equilibrium, pH values will be lower than 5 in the aqueous media. However, other authors also observed degradation of PLA films in contact with ethanolic simulants; the higher the ethanol concentration and temperature, the greater the PLA degradation (Jamshidian et al., 2012). Likewise, the release rate of different phenolic antioxidant increased when the ethanol concentration rose, which has been mainly attributed to the penetration of ethanol into the PLA matrix, opening the matrix and favoring the compound diffusion (Mascheroni et al., 2010). Considering the faster release of phenolic acids in D1 simulant (50% aqueous ethanol) and its lower water ratio, a faster pH decrease was expected in this case, thus implying a faster film disintegration. In fact, this disintegration occurred in all acid loaded films after about 190 h of contact with simulant A (10 % aqueous ethanol) and 12 h in contact with simulant D1 (50% aqueous ethanol). Therefore, the asymptotic release value M_∞ (considered as the equilibrium value) was determined from the fitting of Peleg's model to the M_t vs. t data inside the mentioned ranges and considered to calculate the M_t/M_∞ values used to fit the model of Korsmeyer-Peppas.

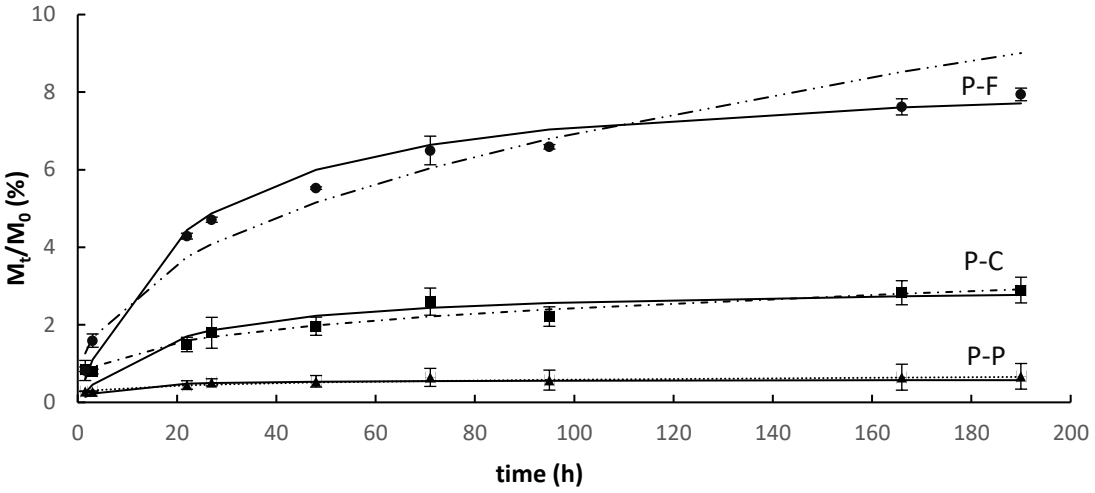


Figure 4. Values of M_t/M_0 (ratio of the amount of phenolic acid released at each time with respect to the initial mass in the film) as a function of time for the simulant A: experimental data (points) and Peleg and Korsmeyer-Peppas fitted models (continuous and discontinuous lines, respectively). Values were considered until the film started to disintegrate. Films with ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). For Peleg's model R^2 ranged between 0.996-0.974 and for Korsmeyer-Peppas R^2 ranged between 0.987-0.929.

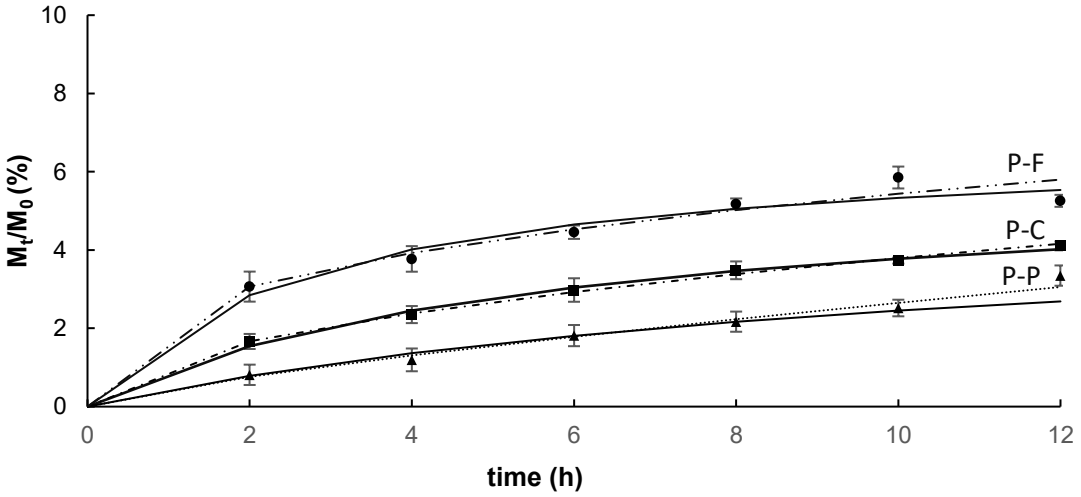


Figure 5. Value of M_t/M_0 (ratio of the amount of phenolic acid released at each time with respect to the initial mass in the film) as a function of time for the simulant D1: experimental data (points) and Peleg and Korsmeyer-Peppas fitted models (continuous and discontinuous lines, respectively). Values were considered until the film started to disintegrate. Films with ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). For Peleg's model R^2 ranged between 0.986-0.834 and for Korsmeyer-Peppas R^2 ranged between 0.983-0.904.

Table 5. Parameters of Peleg Model: $1/k_1$ (% released/h); $M_\infty/M_o=1/k_2$ (% released at equilibrium) and Korsmeyer-Peppas Model: n (dimensionless) and k (h^{-n}) in simulants A (ethanol aqueous solution, 10% v/v) and D1: (ethanol aqueous solution, 50% v/v). Mean values and standard deviation.

Formulation	Simulant A				Simulant D1			
	Parameters of Peleg Model		Parameters of Korsmeyer-Peppas Model		Parameters of Peleg Model		Parameters of Korsmeyer-Peppas Model	
	$1/k_1$	M_∞/M_o	n	K	$1/k_1$	M_∞/M_o	n	K
P-F	0.4 ± 0.04	8.5 ± 0.4	0.4 ± 0.01	0.13 ± 0.05	2.5 ± 0.6	6.8 ± 0.4	0.4 ± 0.07	0.35 ± 0.06
P-C	0.2 ± 0.04	3.0 ± 0.3	0.3 ± 0.02	0.22 ± 0.07	1.05 ± 0.05	6.0 ± 0.6	0.51 ± 0.05	0.19 ± 0.01
P-P	0.2 ± 0.1	0.7 ± 0.4	0.2 ± 0.1	0.37 ± 0.03	0.46 ± 0.03	5.4 ± 1.5	0.77 ± 0.01	0.08 ± 0.01

The highest initial release rate ($1/k_1$) and release ratio at equilibrium was obtained for ferulic acid in the two simulants, whereas protocatechuic acid was the compound most slowly released from the films in both simulants, also reaching the lowest release ratio at equilibrium (0.7 and 5.4 % of the film load, in simulants A and D1, respectively). As concerns the effect of simulant, very close values of final release (M_∞/M_o) were obtained for the three acids (6.8-5.4 %) in simulant D1, whereas these values differ more in the most polar simulant A. The maximum released amount of the acids was much lower than the compound solubility in water that, in turn, increases with the ethanol content in the simulants. Therefore, the different chemical interactions of the compounds with the polyester matrix and its different degree of relaxation in contact with the solvent can explain the differences found in the kinetic parameters. In fact, release rate and the released amount at equilibrium for all phenolic acids were higher in simulant D1, which contain higher proportion of ethanol, as observed by other authors (Jamshidian et al., 2012) for PLA films. A greater ethanol content promotes PLA relaxation and release of the incorporated compounds.

As concerns the parameters of Korsmeyer-Peppas Model, values of n coefficient were lower than 0.5 in almost all the cases, thus indicating that quasi-Fickian diffusion mechanisms are involved in the release of the three phenolic acids from the PLA:PHBV matrix. Only for protocatechuic acid in simulant D1, n value was higher than 0.5, which is associated to an anomalous transport where diffusion mechanism was coupled with polymer relaxation and swelling caused by the ethanol penetration in the matrix.

From the release study, a very slow release of the active compounds was deduced, highly affected by the polarity of simulant, being non-polar systems, such as fatty foods,

those more suitable to favor the delivery of phenolic acids. These systems are more sensitive to oxidation reactions than bacterial growth. Nevertheless, studies in real foods would be necessary to prove the active compound effectiveness. Next section analyses the antibacterial properties of the phenolic acids and the films containing these, using a gram-positive (*Listeria innocua*) and gram-negative (*Escherichia coli*) bacteria.

3.6. Minimum inhibitory concentration (MIC) of phenolic acids and antibacterial performance of the films.

As shown in **Table 6**, all the used phenolic acids exhibited antibacterial activity against *Escherichia coli* and *Listeria innocua*, with values of MIC ranging from 700 mg/L to 900 mg/L. Protocatechuic acid was the most effective at inhibiting the growth of *Escherichia coli* (lowest MIC) and showed the same MIC as ferulic acid against *Listeria innocua*. The reported MICs values were slightly lower than those found by other authors (Cueva et al., 2010; Alves et al., 2013; Borges et al., 2013; Miyague et al., 2015), which can be explained by changes in the test conditions such as inoculum volume, incubation time, temperature, culture medium and pH (Pei et al., 2009).

Table 6. Minimum inhibitory concentration (MIC) of the ferulic, *p*-coumaric and protocatechuic acids studied against *Escherichia coli* and *Listeria innocua*

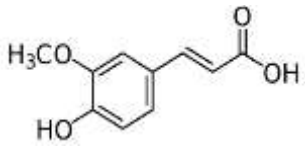
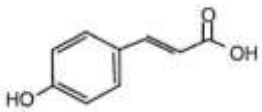
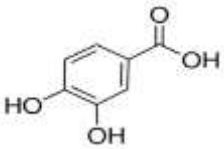
Phenolic acids	MIC (mg/L)		Other studies (mg/L)	
	Escherichia coli	*Listeria innocua*	*Escherichia coli*	*Listeria innocua*
Ferulic acid 	800	700	>1000 (Alves et al., 2013) 1250 (Borges et al., 2013)	1942 (Miyague et al., 2015) >1000 (Alves et al., 2013)
p-coumaric acid 	800	900	1000 (Alves et al., 2013)	2461 (Miyague et al., 2015) >1000 (Alves et al., 2013)
Protocatechuic acid 	750	700	>1000 (Cueva et al., 2010) 1000 (Alves et al., 2013)	3082 (Miyague et al., 2015) 1000 (Alves et al., 2013)

Figure 6 shows the results of the *in vitro* tests performed with the films in TSB culture medium inoculated with *L. innocua* after 6 days of cold storage. Results showed that the incorporated phenolic acids led to a slight significant decrease ($p<0.05$) in the growth of *Listeria innocua*, with no significant differences due to the type of phenolic acid despite the observed differences in the respective MIC values. However, the microbial growth inhibition with respect to the polyester films without phenolic acids (P, control) was lower than 2 logarithms, which is what is usually considered as being significant in microbial growth studies. This scarce efficacy of the potentially active films can be explained by the small amount of acid released from the polymeric matrix into the highly polar inoculated culture media, as can be deduced from the release study.

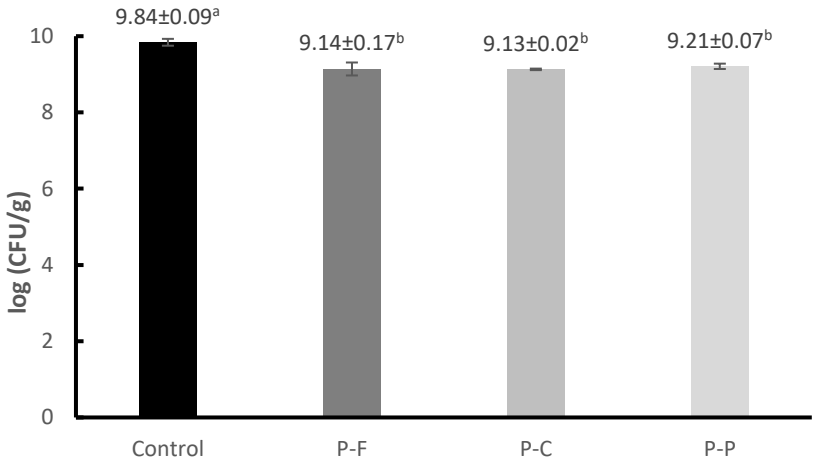


Figure 6. Microbial counts for *Listeria innocua* obtained after 6 days of incubation to 10 °C in TSB culture media (in vitro test). Control (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). Mean values ± standard deviation. Different superscript letters (a-b) indicate significant differences among formulations ($p < 0.05$).

4. CONCLUSIONS

The incorporation of 2% (w/w) of ferulic, *p*-coumaric or protocatechuic acids by melt-blending and compression molding in a polymer matrix of PLA:PHBV (75:25) led to an increase in the glass transition temperature of PLA while PHBV supercooling occurred in the films containing protocatechuic acid. These changes affected the thermal degradation behavior. The addition of ferulic acid increased the thermal stability of the polyester blend films whereas the incorporation of protocatechuic decreased it. Likewise, matrices with phenolic acids were stiffer and more resistant to break than the polyester blend, but with similar extensibility. Oxygen and water vapor barrier capacity were also improved by phenolic acids, in agreement with the higher cohesion forces of the films. Protocatechuic acid promoted the highest effects. The release rate and ratio of phenolic acids increased when polarity of the food simulant decreases, although very slow delivery was observed in all cases. The three phenolic acids exhibited antibacterial activity against gram-positive (*Listeria innocua)* and gram-negative (*Escherichia coli)* bacteria. However, their incorporation into the polyester blend films did no inhibit significantly the growth of *Listeria innocua* in inoculated culture medium, due to very

limited release of active compounds in the polar culture medium. Therefore, the target application of these potentially active packaging materials should consider the scarce release of active compounds in aqueous systems, which will affect the antibacterial performance of the films.

Acknowledgements

The authors would like to thank the Ministerio de Ciencia e Innovación of Spain, for funding this study through the Project AGL2016-76699-R and PID2019-105207RB-I00, and the predoctoral research grant # BES-2017-082040.

5. REFERENCES

Alves, M. J., Ferreira, I. C. F. R., Froufe, H. J. C., Abreu, R. M. V., Martins, A., & Pintado, M. (2013). Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. Journal of Applied Microbiology, 115(2), 346–357. https://doi.org/10.1111/jam.12196

Armentano, I., Fortunati, E., Burgos, N., Dominici, F., Luzi, F., Fiori, S., Jiménez, A., Yoon, K., Ahn, J., Kang, S., & Kenny, J. M. (2015). Bio-based PLA_PHB plasticized blend films: Processing and structural characterization. LWT - Food Science and Technology, 64(2), 980–988. https://doi.org/10.1016/j.lwt.2015.06.032

Arrieta, Marina P., García, A. D., López, D., Fiori, S., & Peponi, L. (2019). Antioxidant bilayers based on PHBV and plasticized electrospun PLA-PHB fibers encapsulating catechin. Nanomaterials, 9(3), 1–14. https://doi.org/10.3390/nano9030346

Arrieta, Marina P., López, J., Hernández, A., & Rayón, E. (2014a). Ternary PLA-PHB-Limonene blends intended for biodegradable food packaging applications. European Polymer Journal, 50(1), 255–270. https://doi.org/10.1016/j.eurpolymj.2013.11.009

Arrieta, Marina Patricia, Castro-López, M., Rayón, E., Barral-Losada, L. F., López-Vilariño., J.M. & González-Rodríguez., M.V. (2014b). Plasticized Poly(lactic acid) − Poly(hydroxybutyrate) (PLA − PHB) Blends Incorporated with Catechin Intended for Active Food- Packaging Applications. Journal of Food Agriculture and Food Chemistry, 62, 10170-10180. dx.doi.org/10.1021/jf5029812 |

Armentano, I., Fortunati, E., Burgos, N., Dominici, F., Luzi, F., Fiori, S., Jiménez, A., Yoon, K., Ahn, J., Kang, S., & Kenny, J. M. (2015). Bio-based PLA_PHB plasticized blend films: Processing and structural characterization. LWT - Food Science and Technology, 64(2), 980–988. https://doi.org/10.1016/j.lwt.2015.06.032

ASTM. (1995). Standard test methods for water vapor transmission of materials. Standard designations: E96-95. In ASTM, Annual book of ASTM standards (pp. 406-413). Philadelphia, PA: American Society fot Testing and Materials.

ASTM. (1999). Standard test method for specular gloss. Standard designations: D523. In Annual book of ASTM standards (vol.06.02). Philadelphia, PA: American Society for Testing and Materials.

ASTM. (2001). Standard test method for tensile properties of thin plastic sheeting. Standard designations: D882. In Annual book of ASTM standards (pp. 162-170). Philadelphia, PA: American Society for Testing and Materials.

ASTM. (2002). Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor. Standard designations: 3985-95. In Annual book of ASTM standards (pp. 472-477). Philadelphia, PA: American Society for Testing and Materials.

Auras, R., Harte, B., & Selke, S. (2004). An overview of polylactides as packaging materials. Macromolecular Bioscience, 4(9), 835–864. https://doi.org/10.1002/mabi.200400043

Badui Dergal, S. (2006). Salvador Badui Dergal. In Química de los alimentos. Fourth edition. Pearson Education. México, 2006. ISBN: 970-26-0670-5

Benbettaïeb, N., Debeaufort, F., & Karbowiak, T. (2019). Bioactive edible films for food applications: mechanisms of antimicrobial and antioxidant activity. Critical Reviews in Food Science and Nutrition, 59(21), 3431–3455. https://doi.org/10.1080/10408398.2018.1494132

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microbial Drug Resistance, 19(4), 256–265. https://doi.org/10.1089/mdr.2012.0244

Castillo, L. A., Farenzena, S., Pintos, E., Rodríguez, M. S., Villar, M. A., García, M. A., & López, O. V. (2017). Active films based on thermoplastic corn starch and chitosan oligomer for food packaging applications. Food Packaging and Shelf Life, 14(August), 128–136. https://doi.org/10.1016/j.fpsl.2017.10.004

Chao, C. Y., & Yin, M. C. (2009). Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. Foodborne Pathogens and Disease, 6(2), 201–206. https://doi.org/10.1089/fpd.2008.0187

Choi, I., Lee, S. E., Chang, Y., Lacroix, M., & Han, J. (2018). Effect of oxidized phenolic compounds on cross-linking and properties of biodegradable active packaging film composed of turmeric and gelatin. Lwt, 93(March), 427–433. https://doi.org/10.1016/j.lwt.2018.03.065

Choulitoudi, E., Velliopoulou, A., Tsimogiannis, D., & Oreopoulou, V. (2020). Effect of active packaging with Satureja thymbra extracts on the oxidative stability of fried potato chips. Food Packaging and Shelf Life, 23(March 2019), 100455. https://doi.org/10.1016/j.fpsl.2019.100455

Commission, E. (2011). EU Guidance to the Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food (Vol. 450, pp. 1e26). Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta

Contardi, M., Heredia-Guerrero, J. A., Guzman-Puyol, S., Summa, M., Benítez, J. J., Goldoni, L., Caputo, G., Cusimano, G., Picone, P., Di Carlo, M., Bertorelli, R., Athanassiou, A., & Bayer, I. S. (2019). Combining dietary phenolic antioxidants with polyvinylpyrrolidone: Transparent biopolymer films based on: *P*-coumaric acid for controlled release. Journal of Materials Chemistry B, 7(9), 1384–1396. https://doi.org/10.1039/c8tb03017k

Cueva, C., Moreno-Arribas, M. V., Martín-Álvarez, P. J., Bills, G., Vicente, M. F., Basilio, A., Rivas, C. L., Requena, T., Rodríguez, J. M., & Bartolomé, B. (2010). Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. Research in Microbiology, 161(5), 372–382. https://doi.org/10.1016/j.resmic.2010.04.006

Dintcheva, N. T., Baiamonte, M., & Spera, M. (2018). Assessment of pro-oxidant activity of natural phenolic compounds in bio-polyesters. Polymer Degradation and Stability, 152, 280–288. https://doi.org/10.1016/j.polymdegradstab.2018.05.003

Dıblan, S., & Kaya, S. (2018). Antimicrobials Used in Active Packaging Films. Food and Health, 4(1), 63–79. https://doi.org/10.3153/jfhs18007

Esmaeili, H., Cheraghi, N., Khanjari, A., Rezaeigolestani, M., Basti, A. A., Kamkar, A., & Aghaee, E. M. (2020). Incorporation of nanoencapsulated garlic essential oil into edible films: A novel approach for extending shelf life of vacuum-packed sausages. Meat Science, 166(October 2019), 108135. https://doi.org/10.1016/j.meatsci.2020.108135

Figueroa-Lopez, K. J., Cabedo, L., Lagaron, J. M., & Torres-Giner, S. (2020). Development of Electrospun Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Monolayers Containing Eugenol and Their Application in Multilayer Antimicrobial Food Packaging. Frontiers in Nutrition, 7(September), 1–16. https://doi.org/10.3389/fnut.2020.00140

Furukawa, T., Sato, H., Murakami, R., Zhang, J., Duan, Y. X., Noda, I., Ochiai, S., & Ozaki, Y. (2005). Structure, dispersibility and crystallinity of poly (hydroxybutyrate)/ poly(l-lactic acid) blends studied by FT-IR microspectroscopy and differential scanning calorimetry. Macromolecules, 38 (15), 6445-6454. 10.1021/ma0504668

González-Ausejo, J., Sánchez-Safont, E., Lagarón, J. M., Balart, R., Cabedo, L., & Gámez-Pérez, J. (2017). Compatibilization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)–poly(lactic acid) blends with diisocyanates. Journal of Applied Polymer Science, 134(20), 1–11. https://doi.org/10.1002/app.44806

Hernández-García, E., Vargas, M., & Chiralt, A. (2021). Thermoprocessed starch-polyester bilayer films as affected by the addition of gellan or xanthan gum. Food Hydrocolloids, 113 (December 2020). https://doi.org/10.1016/j.foodhyd.2020.106509

Hutchings, J.B. (1999). Food and colour appearance, second edition. Gaithersburg, Maryland: Chapman and Hall Food Science Book, Aspen Publication

Jamshidian, M., Arab Tehrany, E., & Desobry, S. (2012). Release of synthetic phenolic antioxidants from extruded poly lactic acid (PLA) film. Food Control, 28, 445–455. https://doi.org/10.1016/j.foodcont.2012.05.005

Jiang, J., Gong, L., Dong, Q., Kang, Y., Osako, K., & Li, L. (2020). Characterization of PLA-P3,4HB active film incorporated with essential oil: Application in peach preservation. Food Chemistry, 313(November 2019). https://doi.org/10.1016/j.foodchem.2019.126134

Kanda, G. S., Al-Qaradawi, I., & Luyt, A. S. (2018). Morphology and property changes in PLA/PHBV blends as function of blend composition. Journal of Polymer Research, 25(9). https://doi.org/10.1007/s10965-018-1586-3

Kasmi, S., Gallos, A., Beaugrand, J., Paës, G., & Allais, F. (2019). Ferulic acid derivatives used as biobased powders for a convenient plasticization of polylactic acid in continuous hot-melt process. European Polymer Journal, 110(March 2018), 293–300. https://doi.org/10.1016/j.eurpolymj.2018.11.036

Krepker, M., Shemesh, R., Danin Poleg, Y., Kashi, Y., Vaxman, A., & Segal, E. (2017). Active food packaging films with synergistic antimicrobial activity. Food Control, 76, 117–126. https://doi.org/10.1016/j.foodcont.2017.01.014

Liu, Q., Wu, C., Zhang, H., & Deng, B. (2015). Blends of polylactide and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with low content of hydroxyvalerate unit: Morphology, structure, and property. Journal of Applied Polymer Science, 132(42), 1–9. https://doi.org/10.1002/app.42689

Liu, W., Wang, Z., Liu, J., Dai, B., Hu, S., Hong, R., Xie, H., Li, Z., Chen, Y., & Zeng, G. (2020). Preparation, reinforcement and properties of thermoplastic starch film by film blowing. Food Hydrocolloids, 108(February), 106006. https://doi.org/10.1016/j.foodhyd.2020.106006

Lou, Z., Wang, H., Rao, S., Sun, J., Ma, C., & Li, J. (2012). *P*-Coumaric acid kills bacteria through dual damage mechanisms. Food Control, 25(2), 550–554. https://doi.org/10.1016/j.foodcont.2011.11.022

Malhotra, B., Keshwani, A., & Kharkwal, H. (2015). Antimicrobial food packaging: Potential and pitfalls. Frontiers in Microbiology, 6(JUN), 1–9.

https://doi.org/10.3389/fmicb.2015.00611

Mascheroni, E., Guillard, V., Nalin, F., Mora, L., & Piergiovanni, L. (2010). Diffusivity of propolis compounds in polylactic acid polymer for the development of antimicrobial packaging films. Journal of Food Engineering, 98, 294-301. 10.1016/j.jfoodeng.2009.12.028

Mathew, S., & Abraham, T. E. (2008). Characterisation of ferulic acid incorporated starch-chitosan blend films. Food Hydrocolloids, 22(5), 826–835. https://doi.org/10.1016/j.foodhyd.2007.03.012

Mitani, T., Ota, K., Inaba, N., Kishida, K., & Koyama, H. A. (2018). Antimicrobial activity of the phenolic compounds of prunus mume against enterobacteria. Biological and Pharmaceutical Bulletin, 41(2), 208–212. https://doi.org/10.1248/bpb.b17-00711

Miyague, L., Macedo, R. E. F., Meca, G., Holley, R. A., & Luciano, F. B. (2015). Combination of phenolic acids and essential oils against Listeria monocytogenes. LWT - Food Science and Technology, 64(1), 333–336. https://doi.org/10.1016/j.lwt.2015.05.055

Moreno, O., Atarés, L., Chiralt, A., Cruz-Romero, M. C., & Kerry, J. (2018). Starch-gelatin antimicrobial packaging materials to extend the shelf life of chicken breast fillets. Lwt, 97(February), 483–490. https://doi.org/10.1016/j.lwt.2018.07.005

Moreno, S., Scheyer, T., Romano, C. S., & Vojnov, A. A. (2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research, 40(2), 223–231. https://doi.org/10.1080/10715760500473834

Muller, J., González-Martínez, C., & Chiralt, A. (2017). Poly(lactic) acid (PLA) and starch bilayer films, containing cinnamaldehyde, obtained by compression moulding. European Polymer Journal, 95(July), 56–70. https://doi.org/10.1016/j.eurpolymj.2017.07.019

Nanda, M. R., Misra, M., & Mohanty, A. K. (2011). The effects of process engineering on the performance of PLA and PHBV blends. Macromolecular Materials and Engineering, 296(8), 719–728. https://doi.org/10.1002/mame.201000417

Narasagoudr, S. S., Hegde, V. G., Chougale, R. B., Masti, S. P., & Dixit, S. (2020). Influence of boswellic acid on multifunctional properties of chitosan/poly (vinyl alcohol) films for active food packaging. International Journal of Biological Macromolecules, 154, 48–61. https://doi.org/10.1016/j.ijbiomac.2020.03.073

Ngo, T. M. P., Nguyen, T. H., Dang, T. M. Q., Tran, T. X., & Rachtanapun, P. (2020). Characteristics and antimicrobial properties of active edible films based on pectin and nanochitosan. International Journal of Molecular Sciences, 21(6). https://doi.org/10.3390/ijms21062224

Noda, I., Satkowski, M. M., Dowrey, A. E., & Marcott, C. (2004). Polymer alloys of nodax copolymers and poly(lactic acid). Macromolecular Bioscience, 4(3), 269–275. https://doi.org/10.1002/mabi.200300093

Pei, R. S., Zhou, F., Ji, B. P., & Xu, J. (2009). Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against E. coli with an improved method. Journal of Food Science, 74(7), 379–383. https://doi.org/10.1111/j.1750-3841.2009.01287.x

Peleg, M. (1988). An Empirical Model for the Description of Moisture Sorption Curves. Journal of Food Science, 53(4), 1216–1217. https://doi.org/10.1111/j.1365-2621.1988.tb13565.x

Porras-Loaiza, L.-M. A. (2009). Importancia de los grupos fenólicos en los alimentos. In Temas Selectos de Ingeniería de Alimentos (Vol. 3, Issue 1, pp. 121–134). https://www.udlap.mx/WP/tsia/files/No3-Vol-1/TSIA-3(1)-Porras-Loaiza-et-al-2009.pdf

Requena, R., Vargas, M., & Chiralt, A. (2017). Release kinetics of carvacrol and eugenol from poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) films for food packaging applications. European Polymer Journal, 92 (May), 185–193. https://doi.org/10.1016/j.eurpolymj.2017.05.008

Requena, R., Vargas, M., & Chiralt, A. (2018). Obtaining antimicrobial bilayer starch and polyester-blend films with carvacrol. Food Hydrocolloids, 83, 118–133. https://doi.org/10.1016/j.foodhyd.2018.04.045

Requena, R., Vargas, M., & Chiralt, A. (2019). Study of the potential synergistic

antibacterial activity of essential oil components using the thiazolyl blue tetrazolium bromide (MTT) assay. Lwt, 101(June 2018), 183–190. https://doi.org/10.1016/j.lwt.2018.10.093

Rigoussen, A., Verge, P., Raquez, J. M., & Dubois, P. (2018). Natural Phenolic Antioxidants As a Source of Biocompatibilizers for Immiscible Polymer Blends. ACS Sustainable Chemistry and Engineering, 6(10), 13349–13357. https://doi.org/10.1021/acssuschemeng.8b02999

Sharma, S., Jaiswal, A. K., Duffy, B., & Jaiswal, S. (2020). Ferulic acid incorporated active films based on poly(lactide) /poly(butylene adipate-co-terephthalate) blend for food packaging. Food Packaging and Shelf Life, 24(October 2019). https://doi.org/10.1016/j.fpsl.2020.100491

Siepmann, J., & Peppas, N. A. (2012). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Advanced Drug Delivery Reviews, 64(SUPPL.), 163–174. https://doi.org/10.1016/j.addr.2012.09.028

Siepmann, Juergen, & Peppas, N. A. (2011). Higuchi equation: Derivation, applications, use and misuse. International Journal of Pharmaceutics, 418(1), 6–12. https://doi.org/10.1016/j.ijpharm.2011.03.051

Stojković, D. S., Živković, J., Soković, M., Glamočlija, J., Ferreira, I. C. F. R., Janković, T., & Maksimović, Z. (2013). Antibacterial activity of Veronica montana L. extract and of protocatechuic acid incorporated in a food system. Food and Chemical Toxicology, 55, 209–213. https://doi.org/10.1016/j.fct.2013.01.005

Szabo, K., Teleky, B. E., Mitrea, L., Călinoiu, L. F., Martău, G. A., Simon, E., Varvara, R. A., & Vodnar, D. C. (2020). Active packaging-poly (vinyl alcohol) films enriched with tomato by-products extract. Coatings, 10(2), 1–18. https://doi.org/10.3390/coatings10020141

Takahashi, H., Kashimura, M., Koiso, H., Kuda, T., & Kimura, B. (2013). Use of ferulic acid as a novel candidate of growth inhibiting agent against Listeria monocytogenes in ready-to-eat food. Food Control, 33(1), 244–248. https://doi.org/10.1016/j.foodcont.2013.03.013

Takahashi, H., Takahashi, T., Miya, S., Yokoyama, H., Kuda, T., & Kimura, B. (2015).

Growth inhibition effects of ferulic acid and glycine/sodium acetate onListeria monocytogenes in coleslaw and egg salad. Food Control, 57, 105–109. https://doi.org/10.1016/j.foodcont.2015.03.037

Talón, E., Trifkovic, K. T., Nedovic, V. A., Bugarski, B. M., Vargas, M., Chiralt, A., & González-Martínez, C. (2017). Antioxidant edible films based on chitosan and starch containing polyphenols from thyme extracts. Carbohydrate Polymers, 157, 1153–1161. https://doi.org/10.1016/j.carbpol.2016.10.080

Valencia-Sullca, C., Jiménez, M., Jiménez, A., Atarés, L., Vargas, M., & Chiralt, A. (2016). Influence of liposome encapsulated essential oils on properties of chitosan films. Polymer International, 65(8), 979–987. https://doi.org/10.1002/pi.5143

Vargas, M., Chiralt, A., Albors, A., & González-Martínez, C. (2009). Effect of chitosan-based edible coatings applied by vacuum impregnation on quality preservation of fresh-cut carrot. Postharvest Biology and Technology, 51(2), 263–271. https://doi.org/10.1016/j.postharvbio.2008.07.019

Wang, L., Dekker, M., Heising, J., Fogliano, V., & Berton-Carabin, C. C. (2020). Carvacrol release from PLA to a model food emulsion: Impact of oil droplet size. Food Control, 114(January), 107247. https://doi.org/10.1016/j.foodcont.2020.107247

Woranuch, S., Yoksana, R., & Akashi, M. (2015). Ferulic acid-coupled chitosan: Thermal stability and utilization as an antioxidant for biodegradable active packaging film. Carbohydrate Polymers, 115, 744–751. https://doi.org/10.1016/j.carbpol.2014.06.074

Zembouai, I., Bruzaud, S., Kaci, M., Benhamida, A., Corre, Y. M., Grohens, Y., Taguet, A., & Lopez-Cuesta, J. M. (2014). Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate)/Polylactide Blends: Thermal Stability, Flammability and Thermo-Mechanical Behavior. Journal of Polymers and the Environment, 22(1), 131–139. https://doi.org/10.1007/s10924-013-0626-7

Zembouai, I., Kaci, M., Bruzaud, S., Benhamida, A., Corre, Y. M., & Grohens, Y. (2013). A study of morphological, thermal, rheological and barrier properties of Poly(3-hydroxybutyrate-Co-3-Hydroxyvalerate)/polylactide blends prepared by melt mixing. Polymer Testing, 32(5), 842–851.

https://doi.org/10.1016/j.polymertesting.2013.04.004

Zhang, H., Liang, Y., Li, X., & Kang, H. (2020). Effect of chitosan-gelatin coating containing nano-encapsulated tarragon essential oil on the preservation of pork slices. Meat Science, 166(February), 108137. https://doi.org/10.1016/j.meatsci.2020.108137

Zhang, H. Y., Arab Tehrany, E., Kahn, C. J. F., Ponot, M., Linder, M., & Cleymand, F. (2012). Effects of nanoliposomes based on soya, rapeseed and fish lecithins on chitosan thin films designed for tissue engineering. Carbohydrate Polymers, 88(2), 618–627. https://doi.org/10.1016/j.carbpol.2012.01.007

Zhang, M., & L., T. N. (2011). Blending Polylactic Acid with Polyhydroxybutyrate: The Effect on Thermal, Mechanical, and Biodegradation Properties. Advances in Polymer Technology, 32(2013), 474–485. https://doi.org/10.1002/adv.20235

Chapter 3

Biodegradation of PLA-PHBV blend films as affected by the incorporation of different phenolic acids

Eva Hernández-García[1], María Vargas[1*], Amparo Chiralt[1], Chelo González-Martínez[1]

Foods (2022), 11 (2), 243

[1]Institute of Food Engineering for Development, Universitat Politècnica de València, Valencia, Spain

evherga1@upvnet.upv.es

ABSTRACT

Films based on of polylactic acid 75:25 (PLA) and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) blend, containing 2% (w/w) of different phenolic acids (ferulic, *p*-coumaric or protocatechuic acid), and plasticized with 15 wt. % polyethylene glycol (PEG 1000), were obtained by melt blending and compression moulding. The disintegration and biodegradation of the film under thermophilic composting conditions was studied throughout 35 and 45 days, respectively, in order to analyse the effect of the incorporation of the antimicrobial phenolic acids into the films. Sample mass loss, thermodegradation behavior and visual appearance were analyzed at different times of the composting period. No effect of phenolic acids was observed on the film disintegration pattern, and the films were completely disintegrated at the end of the composting period. The biodegradation analysis through the CO_2 measurements revealed that PLA-PHBV blend films without phenolic acids, and with ferulic acid, completely biodegraded after 20 composting days, while *p*-coumaric and protocatechuic slightly retarded full biodegradation (21 and 26 days, respectively). Phenolic acids mainly extended the induction period, especially protocatechuic acid. PLA-PHBV blend films with potential antimicrobial activity could be used to preserve fresh foodstuff susceptible to microbial spoilage, being their biodegradation under composting conditions ensured.

Keywords: ferulic; *p*-coumaric; protocatechuic ; disintegration; TGA, biodegradation

1. INTRODUCTION

Biodegradable polymers and their compounds have a wide range of applications and are in high demand since they can be good alternative means of reducing the global environmental impact caused by non-biodegradable polymers and the concerns derived from their use [1]. It has been shown that this environmental impact has led to undesirable consequences on marine fauna, the climate, human health and even the economy. Also, the benefits of these biodegradable materials in terms of their renewable origin, safety, low cost and potential role for extending the shelf life and maintaining quality of many food products have to be taken into account [2].

The global production of bioplastics was 2.11 million tonnes in 2020, and the forecast is for 2.87 million tonnes by 2025. Biodegradable plastics (PLA, PHA, starch blends and others) represent 60% of the capacity (more than 1.2 million tonnes) of global bioplastics production. This production of biodegradable plastics is expected to increase to 1.8 million tonnes in 2025. Of the 60% of biodegradable plastics, 18.7% correspond to PLAs and 1.7% to PHAs [3].

PLA is a biodegradable polyester that is obtained from lactic acid during the fermentation of renewable crops; it is readily available and low cost. It is characterized by its rigidity, transparency, processability and biocompatibility [4], and can be degraded by abiotic and biotic processes and even by a great variety of microorganisms [5]. Due to its low resistance to oxygen permeation and its brittleness [6], recent studies have focused on improving its functional properties, i.e, by combining PLA with other plasticisers and polyesters, such as PHBV [7-10]. Moreover, PHBV is one of the most widely used polyhydroxyalkanoates (PHAs) due to its lower degree of rigidity, compatibility and biodegradability [11].

In order to obtain active biodegradable films, active compounds are usually incorporated so as to confer antimicrobial and antioxidant properties on the final blend films [2,7,8,12]. Of the active compounds, phenolic acids, such as ferulic acid, *p*-coumaric and protocatechuic acids [13-18], have demonstrated significant antimicrobial and antioxidant properties while exhibiting a milder sensory impact than other natural compounds extracted from plants, such as essential oils.

Composting and recycling are the most common processes for the recovery of plastics. Composting is a subset of biodegradation [19] in which organic matter is transformed into CO_2 and soil-like material (humus) by the activity of different microorganisms under aerobic conditions [20]. Changes in the formulation of bioplastics, including the incorporation of filler compounds, blends, active compounds as antimicrobials, among others, could promote changes in their biodegradation pattern in different environments; however, these potential changes are a largely unexplored frontier [19]. Few studies can be found about the influence of the incorporation of antimicrobial compounds on the biodegradation behaviour of films under composting conditions. In some of these studies [21,22], no changes in the film´s biodegradation behaviour were found, such as was observed when sodium propionate [21] or silicon oxide nanoparticles [22] were incorporated as antimicrobials into starch/PVA blends. Other authors showed that the effect of the antimicrobials on the biodegradation profiles of the films depended on the type and concentration of antimicrobial compound [23]. Thus, these changes were greater when using non-volatile substances with strong antimicrobial power, such as silver species, which slowed down the degradation rate and reduced the degradation extent, suggesting a partial alteration of the compost inoculum. Other authors [9,24] also showed that the presence of catechin [9] or silver nanoparticles [24] in PHBV-based films promoted a remarkable delay in the biodegradation process. The possible changes in the biodegradation pattern of the films when incorporating antimicrobials depended on their release kinetics into the medium, the sensitivity of the different microorganisms responsible for the degradative process and the dose of active compounds in the films [23].

The aim of the present study was to analyse the effect that the incorporation of ferulic, *p*-coumaric and protocatechuic acids with antimicrobial activity into PEG plasticised PLA/PHBV (75:25) blend films had on their disintegration and biodegradation behaviour under laboratory composting conditions.

2. MATERIALS AND METHODS

2.1. Materials

Amorphous polylactide (PLA, 4060D) with a density of 1.24 g/cm^3, average molecular weight of 106,226 D (40% low molecular weight fraction (275 D) as reported by [25]), was purchased from Natureworks (Minnetonka, MN, USA). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV, ENMAT Y1000P) with 3% hydroxyvalerate was supplied by Helian Polymers B.V. (Belfeld, Holland). Poly(ethylene glycol) with a molecular weight of 1000 Da (PEG1000) and ferulic, *p*-coumaric and protocatechuic acids were purchased from Sigma-Aldrich (Madrid, Spain). Phosphorus pentoxide (P_2O_5) and magnesium nitrate-6-hydrate ($Mg(NO_3)_2$) were supplied by Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain).

For the biodegradation and disintegration studies, ripe compost (no more than 3 months old) from a local solid waste treatment plant (Valencia, Spain) was used. Vermiculite (Leroy Merlin, Spain) and cellulose microcrystalline (CMC) (Sigma Aldrich, Madrid, Spain) were used for the biodegradation test. Other components used in the disintegration test were refined corn germ oil (Koipe, Córdoba, Spain), corn starch (Roquette Laisa, Beniafio, Valencia, Spain), sucrose (Azucarera Ebro, Madrid, Spain), urea (Urea 46% Prill, Antonio Tarazona S.L., Silla, Valencia, Spain), rabbit food (Super Feed S.L, Madrid, Spain) and sawdust (Productos de limpieza Adrián, Valencia, Spain).

2.2. Obtaining active blend films

PLA-PHBV blend monolayers were obtained by melt blending and compression moulding in a polymer ratio of 75:25, using PEG1000 (15 g/100 g polymers) as plasticiser, according to a previous study [8], incorporated with 2% (w/w with respect to the film mass) antibacterial compound (ferulic, *p*-coumaric or protocatechuic acids). These phenolic acids were chosen on the basis of their previously proved antimicrobial activity [13,26]. The melt blending process was carried out in an internal mixer (HAAKE^{TM} PolyLab^{TM} QC, Thermo Fisher Scientific, Germany) at 170 °C and 50 rpm, for 12 min. Four grams of dried pellets were placed onto Teflon sheets and preheated at 200 °C for 5 min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). The films were obtained by compressing at 200 °C for 4 min at 100 bars, followed by a final cooling cycle for 3 min until the temperature reached about 70 ºC [8]. The films were then conditioned at 25 °C and 53% RH.

2.3. Film characterisation

2.3.1. Moisture content, thickness, elementary composition and visual appearance.

The moisture content was determined in previously conditioned films in triplicate. To this end, four samples per formulation were dried in a vacuum oven (J.P. Selecta, S.A. Barcelona, Spain) at 60 °C for 24 hours and, subsequently, the samples were placed into a desiccator with P_2O_5 at 25 °C for 2 weeks, until they reached a constant weight [10]. The film thickness was measured at 5 different points using a Palmer digital micrometer (Comecta, Barcelona, Spain).

In order to determine the C composition of the samples, an elemental analysis was performed by means of a Euro EA3000 analyser (EUROVECTOR, Milan, Italy). The analyses were carried out in triplicate.

The morphological changes that samples underwent throughout the disintegration experiments were recorded by means of photographs taken at different times of the process. For this purpose, specific samples extracted from the reactors were dried in a vacuum oven at 40 °C for a week and the pictures were taken using a digital camera (EOS 5D Mark II, Canon, Japan).

2.4. Thermogravimetric analysis

The thermal analysis of film samples was carried out in triplicate using the thermogrametric analyser (TGA 1 Star^e System analyser, Mettler-Toledo, Switzerland) at 0, 14, 21, and 35 disintegration days. Previously, the samples were conditioned in desiccators with P_2O_5. For the analysis, the samples were heating from 25 to 600 °C at 10 °C /min under a nitrogen atmosphere (10 mL/min). From the curves obtained in each test, the derived curves (DTGA) as a function of temperature and the corresponding values of T_{max} (temperature of maximum degradation rate) were obtained using the STAR^e Evaluation Software (Mettler-Toledo, Switzerland).

2.5. Compost and synthetic solid residue (SSR)

For the disintegration and biodegradation tests, ripe compost supplied from a local solid waste treatment plant was used, which was previously prepared by removing any inert pieces and then sieved. Its pH was assessed by mixing one part of compost to 5 parts de-ionised water and measured immediately. A synthetic solid residue (SSR) was prepared for the disintegration test by manually mixing ripe compost, corn germ oil, corn starch, sucrose, urea, rabbit food and sawdust, according to ISO 20200 [27].

For both tests, the water content of the ripe compost and of the SSR was adjusted to 55% (w/w) throughout the experimental period by incorporating de-ionised water and gently stirring. The characterisation of dry (DS) and volatile (VS) solids was carried out in triplicate at the beginning and at the end of the composting process, following the ISO 20200:2004 International Standard [27]. DS was obtained by drying the sample in a convection oven at 105 °C until constant weight (Eq. 1) and VS was determined by calcining the samples previously dried in a muffle (Selecta, Barcelona, Spain) at 550 °C until constant weight (Eq. 2). The ripe compost used for the biodegradation test was also characterised in terms of DS and VS at initial time.

$$DS(\%) = \frac{w_d^{105}}{w_w^i} \times 100 \qquad \text{Eq. (1)}$$

$$VS(\%) = \frac{w_d^{105} - w_d^{550}}{w_d^{105}} \times 100 \qquad \text{Eq. (2)}$$

where W^i_w is the initial weight of the sample, W^{105}_d is its weight after drying at 105 °C and W^{550}_d is the weight of the ashes after the treatment at 550 °C.

2.6. Disintegration test

The film disintegration test was performed on a laboratory scale, following an adapted method based on the current ISO 20200:2004 International Standard [27].

Film samples (cut into 25 x 25 mm squares), included in mesh bags (1x1 mm mesh size) were weighed using an analytical balance (± 0.00001 g) and placed into reactors (less than 10 g per reactor) with 1 kg of SSR. Three reactors were prepared per film formulation, which were stored in an oven (Selecta, J.P. Selecta S.A., Barcelona, Spain) at 58 ± 2 °C, to ensure controlled thermophilic conditions, for 35 days. A 5 mm diameter

hole was made on each narrow side of the reactor to allow gas exchange between the atmospheres on the inside and the outside. At the initial time and throughout the 35 days of testing, the reactors were weighed, and if necessary, de-ionised water was added to restore the initial mass as specified by the ISO 20200:2004 International Standard [27]. One of the samples with around 5 g (cut into 25x25 mm squares) was used to control the final time sample weight loss, according to the standard guidelines, and the other mesh bags, each with one sample square, were extracted from the reactor at different control times in order to carry out both the TGA and the visual morphological analysis. Prior to these analyses, the mesh bags containing samples were gently cleaned with a soft brush to eliminate the adhered compost residues.

The disintegration percentage after 35 days composting period was determined according to Eq. (3), on the basis of the mass loss of the film.

$$D_{35}(\%) = \frac{m_0 - m_{35}}{m_0} \times 100 \qquad \text{Eq. (3)}$$

where m_0 is the sample dry mass at the start of the test and m_{35} is the dry mass of the final disintegrated samples after 35 composting days.

2.7. Biodegradation test

The adapted ISO 14855:1-2012 International Standard [28] method was used for the analysis of the aerobic biodegradability of films under controlled composting conditions, by measuring the amount of generated CO_2. Prior to the test, the C, N and H contents of the different samples were determined. In this test, ripe compost was mixed with vermiculite (compost/vermiculite ratio: 3:1) to prevent the compaction of the compost and to ensure good oxygen access. For this analysis, reactors (glass jars of 2L) containing two polypropylene flasks (60 mL) were used: one of them contained 3 g of dry compost mixed with 1 g of vermiculite and a sample quantity (previously cut into 2 mm^2 squares) equivalent to 50 mg of carbon; the other flask contained de-ionised water to ensure 100% relative humidity. The bioreactors were closed and incubated for 45 days at 58 ± 2 °C. A blank reactor containing only compost and another containing cellulose microcrystalline (CMC) as a reference sample [29], mixed with the compost, were also prepared.

The percentage of CO_2 generated inside the reactors was measured in triplicate using a CO_2 analyser throughout the biodegradation process. The theoretical amount of CO_2 that could be generated from the sample ($CO_2^{Th}S$) was estimated from its carbon content by applying the following equation (Eq. 4).

$$CO_2^{Th}S = DWs \text{ x } C_S \text{x} \frac{Mwco_2}{Mw_C} \qquad \text{Eq. (4)}$$

where DWs is the dry weight of the sample (g); C_S is the proportion of carbon in the dry sample, as determined by elementary analysis (%); $Mwco_2$ and Mw_C are the molecular weights of CO_2 and of C, respectively.

The percentage of biodegradation was calculated using the following equation (Eq. 5) [29], assuming that all sample carbon was converted into CO_2.

$$B(\%) = \frac{\sum CO_{2S} - \sum CO_{2B}}{CO_2^{Th}S} \text{x}100 \qquad \text{Eq. (5)}$$

where ΣCO_{2S} is the accumulative amounts of CO_2 produced in the sample bioreactor and $\sum CO_{2B}$ is the accumulative amounts of CO_2 produced in the blank bioreactor.

To describe the kinetics of the process, the experimental data obtained from the biodegradation test were modeled using the Hill equation (Eq. 6).

$$\%B = \%B_{max} \times \frac{t^n}{k^n + t^n} \qquad \text{Eq. (6)}$$

where $\%B_{max}$ is the percentage of biodegradation at infinite time (%), t is the time, k is the time at which 50% of the maximum biodegradation occurred (0.5% B_{max}) and n is the curve radius of the sigmoid function.

2.8. Statistical analysis

Statgraphics Centurion XVII-X64 software (Manugistics Corp., Rockville, Md.) was used to perform the statistical analyses of the results by means of an analysis of variance (ANOVA). Fisher's least significant difference (LSD) procedure was used at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Moisture content, thickness and elementary composition

Table 1 shows the initial moisture content, thickness and carbon content of PLA-PHBV control films with and without phenolic acids. The moisture content of all the films was very low, coinciding with the hydrophobic nature of the polymeric matrix. The incorporation of the phenolic acids slightly reduced ($p < 0.05$) the moisture content of the films, the sample containing protocatechuic acid exhibiting the lowest moisture content.

Film thickness ranged between 140-150 μm and the incorporation of phenolic acids had a significant effect on the thickness of the films. Ferulic acid led to an increase in the film thickness, whereas protocatechuic acid led to a significant decrease. This could be associated with the different interchain forces established between polymers and the antimicrobial agents, depending on the phenol molecular structure, which could affect the flowability of the material during the thermo-compression process and thus, the final film thickness. Ferulic and *p*-coumaric acids are hydroxycinnamic acids with a para OH group, but ferulic acid also contain a meta methoxy group in the molecule. This could promote higher interchain distance when phenol-polymer hydrogen bonds are formed. In contrast, protocatechuic is a hydroxybenzoic acid with two hydroxyls in meta and para positions, which could promote lower interchain distances by the hydrogen bonding.

The carbon content of the samples (**Table 1**) was about 52% for all the samples, none of which differed significantly, as expected from their very similar composition (only 2 % wt. of the different phenolic acids in the films).

Table 1. Moisture content (MC), thickness and elemental carbon (C%) of the films prior to the composting test. PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). Mean values and standard deviations.

Sample/reactor	MC (%)	Thickness (μm)	C (%)
P	0.1600 ± 0.0006^{a}	146 ± 0.015^{b}	52.0 ± 0.2^{a}
P-F	0.1500 ± 0.0010^{b}	153 ± 0.018^{a}	52.0 ± 0.6^{a}
P-C	0.0300 ± 0.0002^{d}	144 ± 0.016^{c}	52.1 ± 0.3^{a}
P-P	0.1000 ± 0.0008^{c}	137 ± 0.018^{d}	52.0 ± 0.4^{a}

Different superscript letters (a-d) within the same column indicate significant differences between formulations ($p < 0.05$).

3.2. Compost characteristics

The ripe compost used as inoculum in both tests showed an initial organic matter content (VS) of 93.4 ±1.2 %, expressed as volatile solids with respect to dried solids. The content of total dry solids (DS) was 85±1% and pH =7.8, measured according to ISO 14855 [28]. At the end of the disintegration test, the content of organic matter in the compost changed and the values are shown in **Table 2** for the different reactors without films and with the different film samples. All the values decreased with respect to the initial by more than 40%, in line with the conversion of the organic matter into CO_2 brought about by the microflora of the compost throughout time. The percentage of organic matter reduction was higher in reactors containing films, which suggests that inoculum could be stimulated by the film substrates. The standard method establishes a reduction in the volatile content in the sample after the composting period (R values in Table 2) of over 30% as well as a standard deviation of the disintegration values of the samples (D_{35}(%), in **Table 2**) of less than 10 units. Likewise, the appearance of the composting material changed over time, from an initial yellow to a brownish colour after 10 days of composting, according to what is described in the ISO 20200 standard [27]. Therefore, the test could be validated.

Table 2. Volatile solids (VS) after the composting period of the disintegration test, the reduction in the volatile content (R) and disintegration percentage for the samples after 35 composting days of the SSR, control (P) and P films incorporating ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). Mean values and standard deviations.

Reactor	VS (g volatiles/100 g DS) Post-composting	R (%)	Disintegration D_{35} (%)
SSR (blank)	90.5 ± 0.9^{a}	40 ± 1.8^{d}	-
P	86 ± 1.3^{b}	58 ± 1.5^{b}	89 ± 2^{ab}
P-F	82 ± 1.0^{c}	64 ± 1.6^{a}	92 ± 5^{a}
P-C	85 ± 1.2^{b}	53 ± 0.6^{c}	85 ± 3^{b}
P-P	89 ± 1.6^{a}	54 ± 2.0^{c}	88 ± 4^{b}

Different superscript letters (a-d) within the same column indicate significant differences between formulations ($p < 0.05$). (-) Reactor without sample.

3.3. Disintegration test

Figure 1 shows the development of the disintegration (mass loss) of the different films as a function of time. As can be observed, all the films showed similar disintegration patterns. During the first 10 days, the mass losses occurred very fast in every case, reaching %D values of around 63-67%. This disintegration rate was greater than those found by other authors for PLA-PHBV blends [30,31], probably due to the presence of plasticiser (PEG1000) and to the amorphous nature of PLA, which both lead to an acceleration of the process. The disintegration rate for PLA-PHB blend and PLA-PHB blend with limonene in the first 10 days was less than 10% [30] while for PLA-PHBV blend was 13.2 % after 30 days [31].

From 10 days on, the disintegration rate slowed down, reaching a plateau after around 30 days, and the sample weight losses reached values of close to 90% (**Table 2**). In general, the incorporation of antimicrobial agents did not induce significant changes ($p>0.05$) in the disintegration behaviour of P films.

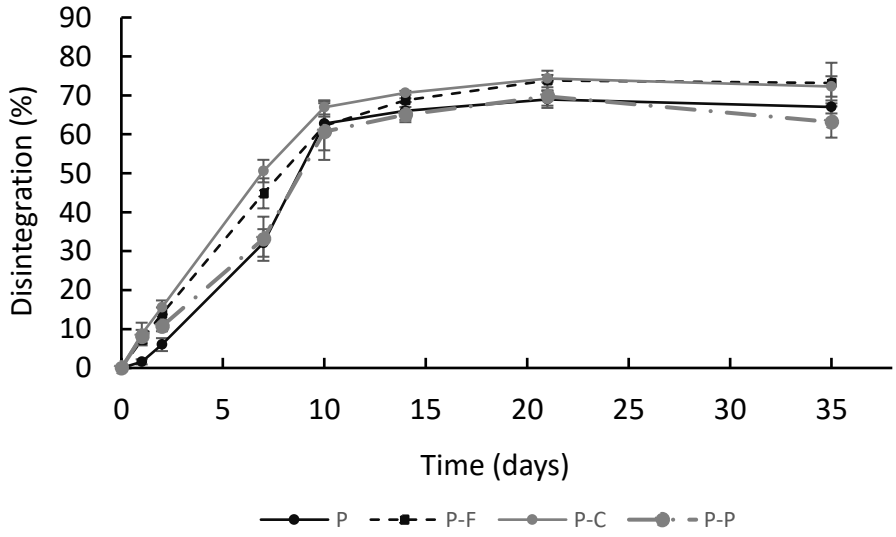


Figure 1. Development of sample disintegration as a function of time for the different films. PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). Mean values and standard deviations.

Photographs after different composting times (**Figure 2**) show the morphological changes in film samples associated with their disintegration. As can be observed,

considerable erosion and fractures were found in the films after 4 composting days, and after 12 days, the samples appeared broken into small pieces, especially the P-P films. After 20 composting days, the film's appearance in the mesh was no longer distinguishable from compost due to the fact that the aggregates were brown, making the weighing process more difficult. Afterwards, the mass particles disaggregated becoming smaller than the mesh size, leading to a very small residue, coinciding with the complete disintegration of the films. From these observations, it can be concluded that the film disintegrated completely before 30 days, although small fragments were adhered to the mesh contributing to the values of residual mass shown in **Figure 1**.

The colour of the formulations incorporating ferulic and protocatechuic acids became more yellowish and brownish throughout the composting period, respectively, due to the promotion of the oxidation of these phenolic compounds at the beginning of the disintegration process.

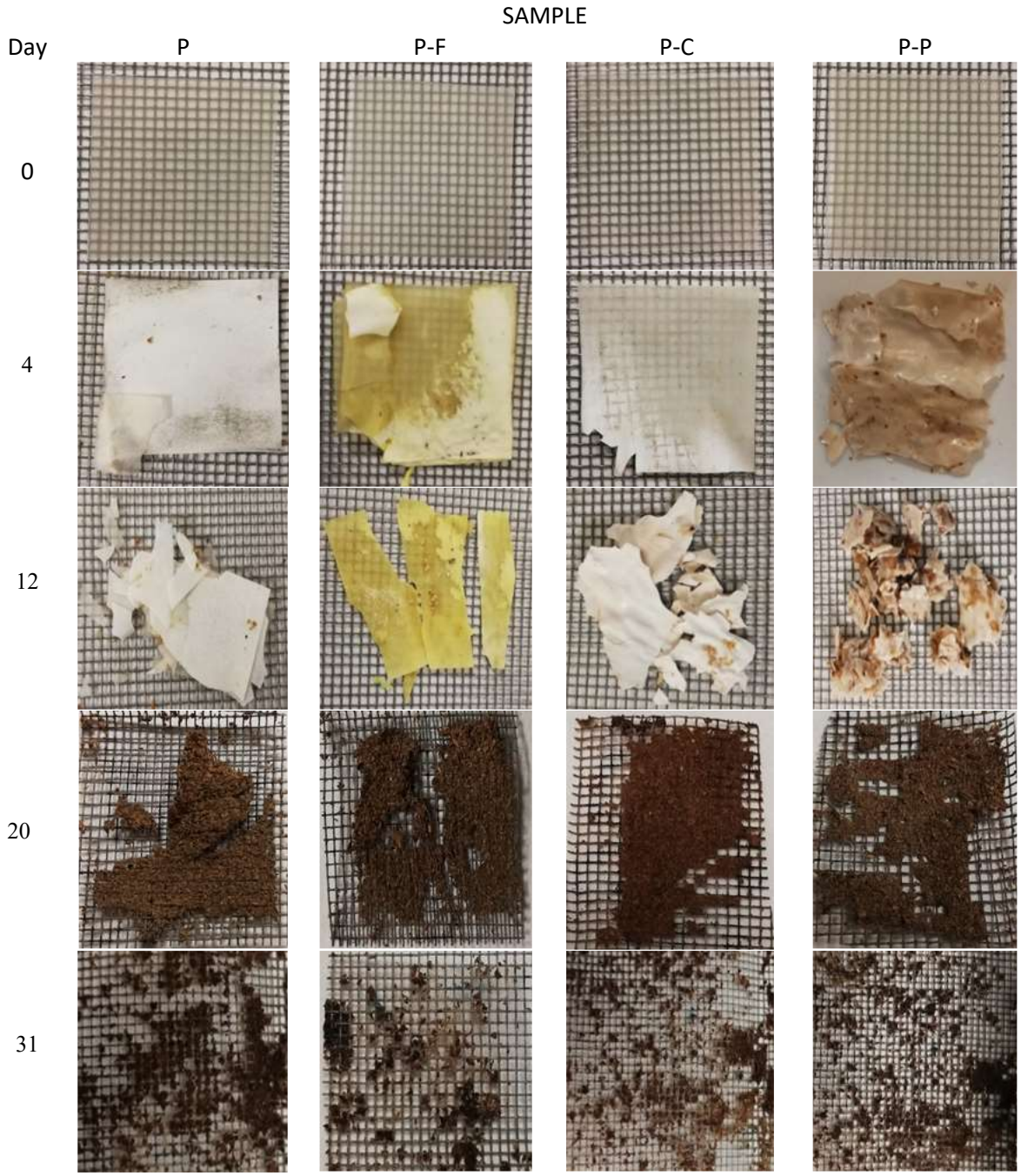


Figure 2. Photographs of the film samples after different composting times. PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P).

3.4. Thermogravimetric analysis

The thermal degradation behaviour of PLA-PHBV blend films and of those containing phenolic acids was studied by TGA at different times of the disintegration period to observe the changes in the polymer structure. The TGA curves and their first derivatives are depicted in **Figure 3**. The onset (T_0) temperature of degradation and the

temperature at which the maximum degradation rate (T_{peak}) occurred for the different degradation steps of the films at different times of the disintegration test, are also summarised in **Table 3**.

As can be observed, all the films exhibited a two-step mass loss before the composting process (t=0). These steps can be attributed to the separate thermodegradation of PLA and PHBV, in line with what has previously been reported [8], which is a typical pattern when blending immiscible polymers with different thermal stabilities. Thus, PLA, which was more thermostable than PHBV [31], showed a maximum degradation rate at 320 °C, at which temperature the cleavage of bonds on the backbone to form cyclic oligomers, lactide and carbon monoxide as products occurs. As concerns the PHBV, the maximum degradation rate occurred at 268 °C, corresponding to a random intramolecular chain scission by cis-elimination, which leads to a remarkable molecular weight reduction and, to a lesser extent, to an intermolecular chain trans-esterification [32]. Similar thermodegradation patterns were obtained by other authors for plasticised PHBV [33]. In general, the incorporation of ferulic acid and *p*-coumaric acid increased the onset temperature ($p<0.05$) of the thermal degradation process, whereas the incorporation of protocatechuic acid caused a significant decrease in the thermal stability of the film, in agreement with that previously observed [34]. Phenolic acids usually promote a crosslinking effect between polyester chains, which could explain the observed changes in the onset of the thermal degradation of polymers [35]. Also, as suggested by other authors, the protective effect of the antioxidant agents against polymer thermal degradation at the first heating stages has to be considered [36]. In contrast, protocatechuic acid is found to inhibit PHBV crystallisation, thus favouring its thermodegradation at lower temperatures [35]. Likewise, the phenolic acids affected the peak temperature of the polymer thermal degradation, in the same sense as that observed for the onset temperature.

As shown in **Figure 3**, the effect of the composting time was noticeable for the different degradation steps. Degradation peaks shift towards lower degradation temperatures throughout the first 21 composting days, and completely disappeared in samples composted for longer times. This was consistent with the remarkable reduction in the molecular weight of both polymers throughout this period. Thus, both the amorphous-

PLA fraction and the semicrystalline PHBV fraction were partially hydrolyzed, thus reducing their thermal stability. After 21 composting days, only one thermo-degradation peak was observed, which could indicate that amorphous PLA was completely disintegrated, and only the partially degraded crystalline PHBV remained, although both partially degraded polymers could be present with similar thermo-degradation pattern. After 35 days, the characteristics peaks of both PLA and PHBV completely disappeared as a consequence of the full degradation of the material. The residual components were more thermostable, exhibiting higher degradation temperatures (450 and 550 °C), as was observed in other studies [30,37].

Table 3 also shows the residual mass at 600 °C for each sample, which exhibited very low pyrolysis residual mass. This mass increased ($p<0.05$) throughout the composting time, regardless of the incorporation of the phenolic acids; this was coherent with the increase in the mass ratio of the mineral content in the partially degraded polymeric film, as previously observed by other authors [37].

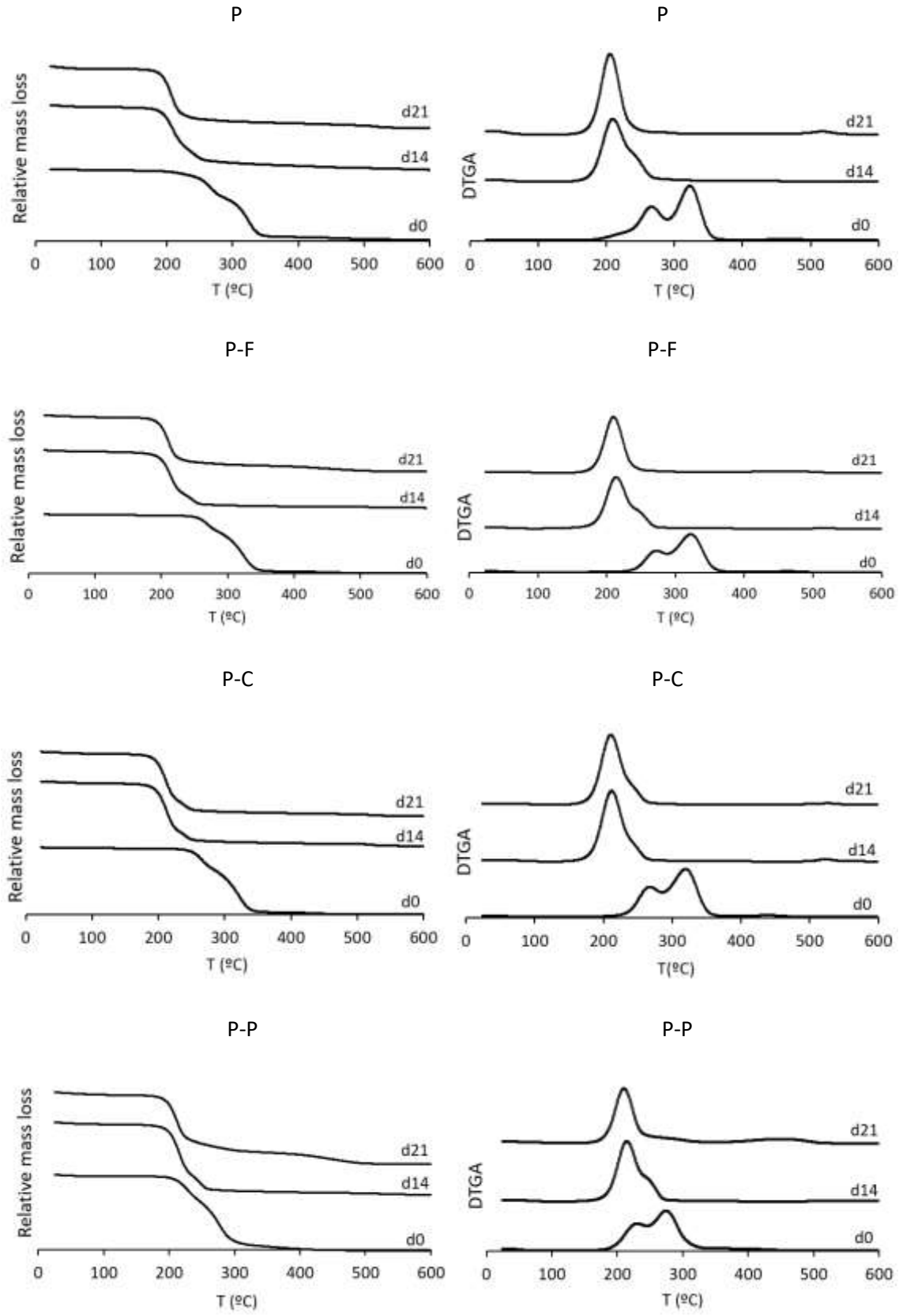


Figure 3. TGA and DGTA curves of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P), after different composting times (0, 14 and 21 days).

Table 3. TGA parameters obtained from the TGA analysis throughout the composting process: onset (To) and peak (Tp) temperatures, and pyrolysis residual mass at 600 °C of PLA-PHBV blend films (P) and PLA-PHBV blend films containing ferulic acid (P-F), *p*-coumaric acid (P-C) and protocatechuic acid (P-P). Mean values and standard deviation.

Sample	Day	T_o	T_p		Residual mass (%)
			PHBV	PLA	
P	0	197.0 ± 3.0^{c,1}	268.0 ± 3.0^{b,1}	320.0 ± 3.0^{a,1}	0.4 ± 0.1^{b,2}
	14	181.0 ± 4.0^{a,2}	210.0 ± 4.0^{bc,2}	240.0 ± 4.0^{c,2}	2.5 ± 0.5^{b,1}
	21	180.8 ± 0.3^{b,2}	207.4 ± 1.1^{c,2}	-	4.0 ± 1.5^{abc,1}
P-F	0	242.8 ± 2.0^{a,1}	273.0 ± 1.0^{a,1}	324.0 ± 3.0^{a,1}	0.5 ± 0.1^{ab,3}
	14	186.5 ± 0.1^{b,2}	214.7 ± 0.2^{b,2}	248.0 ± 0.7^{ab,2}	1.1 ± 0.1^{c,2}
	21	184.5 ± 1.0^{a,3}	210.8 ± 2.0^{b,3}	-	3.6 ± 0.2^{b,1}
P-C	0	207.0 ± 3.0^{b,1}	269.0 ± 1.0^{b,1}	321.0 ± 1.0^{a,1}	0.7 ± 0.1^{a,2}
	14	184.9 ± 0.1^{b,2}	212.9 ± 0.4^{c,2}	247.3 ± 1.0^{b,2}	1.9 ± 0.1^{b,1}
	21	184.2 ± 0.5^{a,3}	211.8 ± 0.5^{a,3}	-	2.3 ± 0.5^{c,1}
P-P	0	176.0 ± 2.0^{d,3}	232.0 ± 3.0^{c,1}	275.0 ± 1.0^{b,1}	0.6 ± 0.1^{ab,3}
	14	186.9 ± 0.2^{b,1}	215.2 ± 0.1^{a,2}	249.1 ± 0.5^{a,2}	3.8 ± 0.6^{a,2}
	21	183.6 ± 1.0^{a,2}	210.6 ± 0.1^{b,3}	-	5.0 ± 0.5^{a,1}

Different superscript letters (a, b, c, d) within the same column indicate significant differences between formulations after the same analysis time ($p < 0.05$).

Different superscript numbers (1–3) within the same column indicate significant differences between the composting times for a given formulation ($p < 0.05$).

3.5. Biodegradation kinetics under laboratory-scale composting conditions

Biodegradation is promoted by abiotic (surface erosion, temperature, humidity, pH…) and biotic factors, where the microorganisms present in the environment surrounding the material consume oxygen under aerobic conditions and release enzymes, which allow the complete or partial decomposition of the material into carbon dioxide, water, inorganic compounds and biomass [38].

The biodegradation behaviour of the films in a composting environment was analysed by submitting the samples to laboratory-scale composting conditions for more than 40 days, using a constant temperature of 58 °C, following the ISO 14855 standard [28]. The theoretical maximum quantity of carbon dioxide that can be produced by the total biodegradation of the samples was calculated from their carbon content. The biodegradation profile of cellulose microcrystalline (CMC) was also analysed to be used as reference.

Figure 4 shows the biodegradation kinetics in terms of percentage biodegradation (Eq. 5) as a function of time for the PLA/PHBV blend films, containing or not phenolic acids, in comparison with the CMC sample. In order to validate the quality of the inoculum used in the biodegradation test, ISO 14855 establishes that the degree of biodegradation of the reference material must be more than 70% after 45 days. As can be observed in **Figure 4**, the degree of biodegradation of the positive reference material (CMC) was 91% after 45 days of composting, which validates the experimental data.

All the PLA/PHBV blend films, containing or not phenolic acids, showed the characteristic sigmoid profiles of the respirometry test with the three different phases that were also found by other authors [37,39,40]: an initial delay/induction period (extended up to 10-22 d, depending on the type of film), followed by a biodegradation phase (extended up to 22-28 d), and a plateau. It is known that the degradation of PLA in compost takes place in two main, consecutive stages. The process starts at surface level with the hydrolysis of the polymer chains induced by the diffusion of water from the materials, causing a random polymer decomposition to form oligomers and lactic acid. Subsequently, once the molecular weight reaches approximately 10,000-20,000 D, the enzymatic degradation takes place, leading to the formation of carbon dioxide, water, and humus [41]. On the other hand, the degradation of PHBV is firstly caused by microorganisms through a mechanism of surface erosion, gradually spreading to the bulk [42]. So, both water-induced hydrolysis in the polymer films and enzymatic degradation caused by microorganism contributed to biodegradation, [43] with abiotic hydrolysis being the main depolymerisation mechanism according to the higher ratio of PLA in the films. Therefore, any factor affecting the rate of hydrolysis could either accelerate or retard the whole degradation process [44].

Amorphous-PLA/PHBV films presented an initial delay period of 10 days and completely biodegraded (B=100%) after 20 composting days, in contrast to what was found by other authors for full crystalline-PLA/PHBV films [31], which reached 90% biodegradation within 180 days. This difference can be explained by the amorphous nature of the PLA used in the film, as the crystalline regions of PLA are hydrolysis-resistant compared to the amorphous regions due to the highly restricted access of water molecules to chains inside the rigid crystalline regions [45]. So, the use of amorphous PLA favours the hydrolytic cleavage of PLA chains, thus promoting a faster degradation pattern.

The addition of phenolic acids modified the biodegradation profile of PLA/PHBV films, especially when using protocatechuic acid. Thus, longer initial induction periods (lag phase) and a more active composting stage were detected for P-P films. The initial delay periods were of around 10-12 days for all the films except for those containing protocatechuic acid, when this period was extended to 22 days. This could be linked to the different bonding capacity of the phenolic acids to the polymer chains that affects their release from the matrix and dilution in the compost medium. The debonding of the phenolic acids will also contribute to the hydrolysis process, given their low pK_a values (4.5-4.6) and capacity to acidify the aqueous environment. The release kinetics of these acids from the PLA/PHBV matrix into aqueous food simulants of differing polarities (controlled by the ethanol content) revealed that a greater amount of ferulic acid than *p*-coumaric was released and at a faster rate; the difference was even more marked if compared with protocatechuic acid, whose release was very limited [35]. The faster release of ferulic acid could have contributed to the acceleration of the hydrolyses/hydrolysis of the PLA amorphous matrix, shortening the induction time. In contrast, the antimicrobial capacity of these phenolic compounds and their bonding capacity to the polyester blend through hydrogen bonds with carboxylic oxygens [35] could also limit the accessibility of polymer matrices for an attack by microorganisms. This bonding capacity was greater in the case of protocatechuic acid, the only acid exhibiting two hydroxyl groups in the phenolic ring able to establish interchain hydrogen bonds with the polyester carbonyl groups.

PLA-PHBV blend films containing phenolic acids completely biodegraded after 20-27 days (B=100%). Moreover, after 30 days, these films reached biodegradation

percentages of over 100%. This effect is attributed to the priming effect brought about by the overstimulation of the organic matter mineralization when using easily-decomposable polymers, as was also found by other studies [46]. How the mechanism by priming effect occurs is unknown [47] but it is believed to be the result of the interaction among the microorganisms and the small molecules released into the medium due to polymer degradation [29].

Phenolic acids exert antimicrobial activity by diffusion of the undissociated acid across the membrane, resulting in the acidification of the cytoplasm and, eventually, cell death [48-51]. There are several factors affecting this activity, such as the pK_a and the lipophilicity of the phenolic acid (related with their molecular structure), since these determine the solubility of phenolic acids in bacterial membranes. Ferulic and *p*-coumaric acids (hydroxycinnamic acids) are more lipophilic than protocatechuic (which is a hydroxybenzoic acid) because of their side unsaturated chain and, thus, they could exhibit a greater antimicrobial activity. On the other hand, the methoxy group in ferulic acid makes this acid more lipophilic than *p*-coumaric [52], which could promote its antimicrobial activity in the inoculum. Nevertheless, apart from the different length of the induction period, the potential antimicrobial effect did not notably interfere in the biodegradation of the PLA/PHBV films, which reach total biodegradation during a relatively short composting time (20-27 days). Protocatechuic acid was the compound that promoted the longest delay, probably due to its greater persistency in the polymer matrix, as deduced in previous studies [35].

The biodegradation curves were fitted to the Hill equation, and the fitting parameters are reported in **Table 4**. The time required to reach 50% of the maximum biodegradation level (k values) in the blend films ranged from 17 to 26 days, the longest time being for films with protocatechuic acid. The maximum biodegradation percentage (B_{max}) ranged between 158 and 230, the highest value corresponding to the PLA/PHBV blend films, while the values decreased in proportion to how difficult it is for the compound to gain release from the matrix deduced in previous studies [35]. The faster the release kinetics of the compound in aqueous media, the lower the B_{max} values, and the shorter the induction period of the biodegradation process. Therefore, although the presence of any of the studied phenolic acids does not inhibit the biodegradation process of the

PLA/PHBV films, they tended to prolong the induction period, slightly reducing the percentage of final biodegradation when the compound was more tightly retained in the polymer matrix; this was probably due to there being greater difficulties for it to dilute in the media, making the antimicrobial action more effective. Similar results were obtained by other authors [37] analysing films loaded with non-volatile antimicrobial compounds.

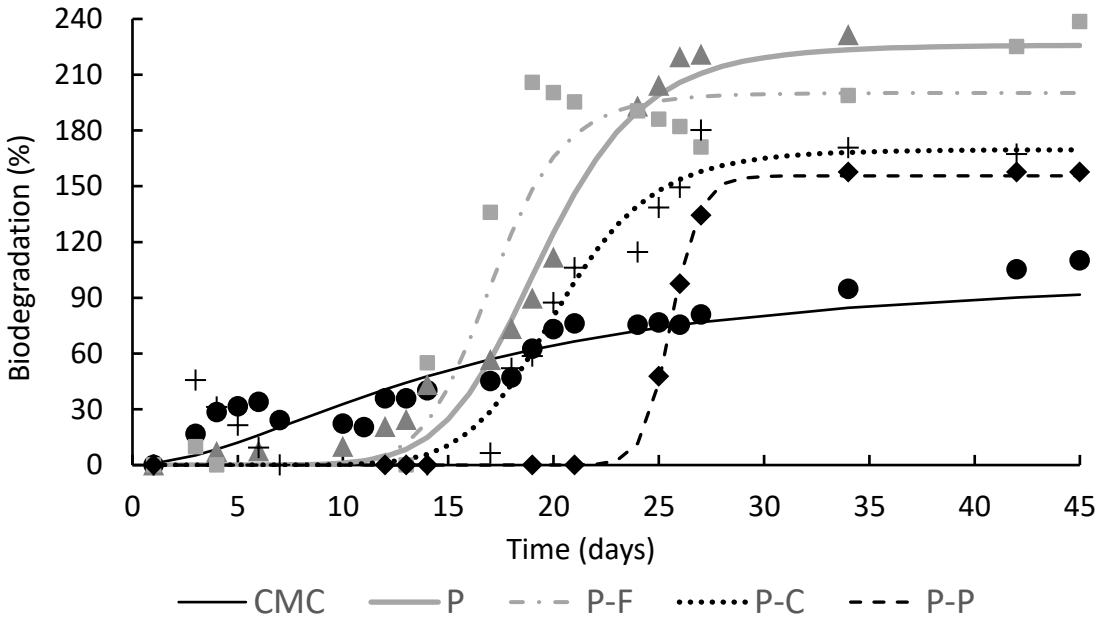


Figure 4. Biodegradation kinetics of CMC (●) and PLA-PHBV films P (▲), PLA-PHBV films containing ferulic acid P-F (■), *p*-coumaric acid P-C (+) and protocatechuic acid P-P (◆) throughout the composting time. Experimental data (symbols) and Hill´s fitted model (lines).

Table 4. Hill's parameters (%B_{max}, k and n) for the different films and cellulose microcrystalline (CMC, reference). k: time needed to reach 50% of Bmax and Bmax: percentage of biodegradation at infinite time.

Sample	n	k (days)	B_{max} (%)	R^2
CMC	1.8	15.5	105	0.92
P	8.0	19.5	226	0.89
P-F	10.1	17.1	200	0.80
P-C	9.0	20.3	170	0.83
P-P	39.1	25.6	156	0.78

n: curve radius of the sigmoid function; k: time required to reach 50% of the maximum biodegradation; B_{max}(%): Percentage of biodegradation at infinite time; R^2: correlation coefficient.

4. CONCLUSIONS

This study provided relevant information regarding the effect that the incorporation of phenolic acids into PLA/PHBV blend films had on their disintegration and biodegradation processes. A complete disintegration of the PLA/PHBV blend films was observed during the 35 days of the composting test, regardless of the presence of phenolic acids. From TGA analyses, the faster disintegration of the amorphous PLA fraction could be deduced, while the process was barely affected by the presence of phenolic acids. As concerns the biodegradation process, the incorporation of the phenolic acids delayed the biodegradation period, extending the induction period, especially when using protocatechuic acid. This could be related with the greater persistency of the compound in the polymeric matrix. Phenolic-free PLA/PHBV blend films fully biodegraded after 20 composting days, while *p*-coumaric and protocatechuic slightly retarded full biodegradation (21 and 26 days, respectively). These results indicated that none of the antimicrobial phenolic acids inhibit the biodegradation process of the blend films, but they can retard biodegradation when these are tightly retained in the polymer matrix. Thus, these new films with potential antimicrobial activity could be used to preserve fresh foodstuff susceptible to microbial spoilage, being their biodegradation under composting conditions ensured. Further studies should focus on biodegradation studies of these films exposed to different natural environments such as soil or sea water.

Acknowledgements

The authors would like to thank the Spanish Agencia Estatal de Investigación, for funding this study through Projects AGL2016-76699-R and PID2019-105207RB-I00 and the predoctoral research grant # BES-2017-082040

5. REFERENCES

[1] O. Wilfred, H. Tai, R. Marriott, Q. Liu, V. Tverezovskiy, S. Curling, H. Tai, Z. Fan, W. Wang, Biodegradation of Polylactic Acid and Starch Composites in Compost and Soil, International Journal of Nano Research. 1 (2018) 01–11. www.innovationinfo.org.

[2] Abdin, M.; El-Beltagy, A.E.; El-sayed, M.E.; Naeem, M.A. Production and Characterization of Sodium Alginate/Gum Arabic Based Films Enriched with

Syzygium cumini Seeds Extracts for Food Application. Journal of Polymer and The Environment. (2021), , doi: 10.1007/s10924-021-02306-z.

[3] European Bioplastics, Market update 2020: Bioplastics continue to become mainstream as the global bioplastics market is set to grow by 36 percent over the next 5 years, (2020) 1–2.

[4] Y. Zhong, P. Godwin, Y. Jin, H. Xiao, Biodegradable polymers and green-based antimicrobial packaging materials: A mini-review, Advanced Industrial and Engineering Polymer Research. 3 (2020) 27–35. https://doi.org/10.1016/j.aiepr.2019.11.002.

[5] A.A. Shah, F. Hasan, A. Hameed, S. Ahmed, Biological degradation of plastics: A comprehensive review, Biotechnology Advances. 26 (2008) 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005.

[6] R.M. Rasal, A. V. Janorkar, D.E. Hirt, Poly(lactic acid) modifications, Progress in Polymer Science (Oxford). 35 (2010) 338–356. https://doi.org/10.1016/j.progpolymsci.2009.12.003.

[7] M. Boufarguine, A. Guinault, G. Miquelard-Garnier, C. Sollogoub, PLA/PHBV films with improved mechanical and gas barrier properties, Macromolecular Materials and Engineering. 298 (2013) 1065–1073. https://doi.org/10.1002/mame.201200285.

[8] R. Requena, M. Vargas, A. Chiralt, Obtaining antimicrobial bilayer starch and polyester-blend films with carvacrol, Food Hydrocolloids. 83 (2018) 118–133. https://doi.org/10.1016/j.foodhyd.2018.04.045.

[9] M.P. Arrieta, A.D. García, D. López, S. Fiori, L. Peponi, Antioxidant bilayers based on PHBV and plasticized electrospun PLA-PHB fibers encapsulating catechin, Nanomaterials. 9 (2019) 1–14. https://doi.org/10.3390/nano9030346.

[10] E. Hernández-García, M. Vargas, A. Chiralt, Thermoprocessed starch-polyester bilayer films as affected by the addition of gellan or xanthan gum, Food Hydrocolloids. 113 (2021). https://doi.org/10.1016/j.foodhyd.2020.106509.

[11] A.L. Rivera-Briso, Á. Serrano-Aroca, Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate): Enhancement strategies for advanced applications, Polymers. 10 (2018) 1–28. https://doi.org/10.3390/polym10070732.

[12] Eltabakh, M.; Kassab, H.; Badawy, W.; Abdin, M.; Abdelhady, S. Active Bio-composite Sodium Alginate/Maltodextrin Packaging Films for Food Containing Azolla pinnata Leaves Extract as Natural Antioxidant. Journal of Polymers and the Environment (2021) doi:10.1007/s10924-021-02287-z

[13] M.J. Alves, I.C.F.R. Ferreira, H.J.C. Froufe, R.M.V. Abreu, A. Martins, M. Pintado, Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies, Journal of Applied Microbiology. 115 (2013) 346–357. https://doi.org/10.1111/jam.12196.

[14] H. Takahashi, T. Takahashi, S. Miya, H. Yokoyama, T. Kuda, B. Kimura, Growth inhibition effects of ferulic acid and glycine/sodium acetate on Listeria monocytogenes in coleslaw and egg salad, Food Control. 57 (2015) 105–109. https://doi.org/10.1016/j.foodcont.2015.03.037.

[15] N.V.B. Meira, R.A. Holley, K. Bordin, R.E.F. d. Macedo, F.B. Luciano, Combination of essential oil compounds and phenolic acids against Escherichia coli O157:H7 in vitro and in dry-fermented sausage production, International Journal of Food Microbiology. 260 (2017) 59–64. https://doi.org/10.1016/j.ijfoodmicro.2017.08.010.

[16] R. Ordoñez, L. Atarés, A. Chiralt, Physicochemical and antimicrobial properties of cassava starch films with ferulic or cinnamic acid, Lwt. 144 (2021). https://doi.org/10.1016/j.lwt.2021.111242.

[17] Z. Lou, H. Wang, S. Rao, J. Sun, C. Ma, J. Li, P-Coumaric acid kills bacteria through dual damage mechanisms, Food Control. 25 (2012) 550–554. https://doi.org/10.1016/j.foodcont.2011.11.022.

[18] D.S. Stojković, J. Živković, M. Soković, J. Glamočlija, I.C.F.R. Ferreira, T. Janković, Z. Maksimović, Antibacterial activity of Veronica montana L. extract and of protocatechuic acid incorporated in a food system, Food and Chemical Toxicology. 55 (2013) 209–213. https://doi.org/10.1016/j.fct.2013.01.005.

[19] K.W. Meereboer, M. Misra, A.K. Mohanty, Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites, Green Chemistry. 22 (2020) 5519–5558. https://doi.org/10.1039/d0gc01647k.

[20] G. Kale, T. Kijchavengkul, R. Auras, M. Rubino, S.E. Selke, S.P. Singh, Compostability of bioplastic packaging materials: An overview, Macromolecular Bioscience. 7 (2007) 255–277. https://doi.org/10.1002/mabi.200600168.

[21] C. Sen, M. Das, Biodegradability of Starch Based Self-Supporting Antimicrobial Film and Its Effect on Soil Quality, Journal of Polymers and the Environment. 26 (2018) 4331–4337. https://doi.org/10.1007/s10924-018-1304-6.

[22] S. Tang, P. Zou, H. Xiong, H. Tang, Effect of nano-SiO2 on the performance of starch/polyvinyl alcohol blend films, Carbohydrate Polymers. 72 (2008) 521–526. https://doi.org/10.1016/j.carbpol.2007.09.019.

[23] E. Hernández-García, M. Vargas, C. González-Martínez, A. Chiralt, Biodegradable antimicrobial films for food packaging: Effect of antimicrobials on degradation, Foods. 10 (2021). https://doi.org/10.3390/foods10061256.

[24] S.P.C. Gonçalves, M. Strauss, D.S.T. Martinez, The Positive Fate of Biochar Addition to Soil in the Degradation of PHBV-Silver Nanoparticle Composites, Environmental Science and Technology. 52 (2018) 13845–13853. https://doi.org/10.1021/acs.est.8b01524.

[25] J. Muller, C. González-Martínez, A. Chiralt, Poly(lactic) acid (PLA) and starch bilayer films, containing cinnamaldehyde, obtained by compression moulding, European Polymer Journal. 95 (2017) 56–70. https://doi.org/10.1016/j.eurpolymj.2017.07.019.

[26] Miyague, L.; Macedo, R.E.F.; Meca, G.; Holley, R.A.; Luciano, F.B. Combination of phenolic acids and essential oils against Listeria monocytogenes. LWT - Food Science and Technology. 64 (2015) 333–336, doi:10.1016/j.lwt.2015.05.055.

[27] ISO 20200, ISO 20200, Plastics-Determination of the Degree of Disintegration pf Plastic Materials under Simulated Composting in a Laboratory -Scale Test, 2004.

[28] UNE-EN ISO 14855-1, Determinación de la biodegradabilidad aeróbica final de materiales plásticos en condiciones de compostaje controladas, in: Método según el análisis de dióxido de carbono generado, 2012. Parte 1: Método general.

[29] M.P. Balaguer, J. Villanova, G. Cesar, R. Gavara, P. Hernandez-Munoz, Compostable properties of antimicrobial bioplastics based on cinnamaldehyde cross-linked gliadins, Chemical Engineering Journal. 262 (2015) 447–455. https://doi.org/10.1016/j.cej.2014.09.099.

[30] M.P. Arrieta, J. López, A. Hernández, E. Rayón, Ternary PLA-PHB-Limonene blends intended for biodegradable food packaging applications, European Polymer Journal. 50 (2014) 255–270. https://doi.org/10.1016/j.eurpolymj.2013.11.009.

[31] S. Muniyasamy, O. Ofosu, M.J. John, R.D. Anandjiwala, Mineralization of poly(lactic acid) (PLA), Poly(3-hydroxybutyrate-co-valerate) (PHBV) and PLA/PHBV blend in compost and soil environments, Journal of Renewable Materials. 4 (2016) 133–145. https://doi.org/10.7569/JRM.2016.634104.

[32] M.A. Berthet, H. Angellier-Coussy, V. Chea, V. Guillard, E. Gastaldi, N. Gontard, Sustainable food packaging: Valorising wheat straw fibres for tuning PHBV-based composites properties, Composites Part A: Applied Science and Manufacturing. 72 (2015) 139–147. https://doi.org/10.1016/j.compositesa.2015.02.006.

[33] R. Requena, A. Jiménez, M. Vargas, A. Chiralt, Effect of plasticizers on thermal and physical properties of compression-moulded poly[(3-hydroxybutyrate)-co-(3-hydroxyvalerate)] films, Polymer Testing. 56 (2016) 45–53. https://doi.org/10.1016/j.polymertesting.2016.09.022.

[34] S. Sharma, A.K. Jaiswal, B. Duffy, S. Jaiswal, Ferulic acid incorporated active films based on poly(lactide) /poly(butylene adipate-co-terephthalate) blend for food packaging, Food Packaging and Shelf Life. 24 (2020) 100491. https://doi.org/10.1016/j.fpsl.2020.100491.

[35] E. Hernández-García, M. Vargas, A. Chiralt , Use of phenolic acids to obtain active films based on PLA-PHBV. Submitted: Food Packaging and Shelf Life.

[36] M. Arrieta, Films de PLA y PLA-PHB plastificados para su aplicación en envases de alimentos. Caracterización y análisis de los procesos de degradación, Universidad Politécnica de Valencia. (2014) 202. http://hdl.handle.net/10251/39338.

[37] A.I. Cano, M. Cháfer, A. Chiralt, C. González-Martínez, Biodegradation behavior of starch-PVA films as affected by the incorporation of different antimicrobials, Polymer Degradation and Stability. 132 (2016) 11–20. https://doi.org/10.1016/j.polymdegradstab.2016.04.014.

[38] A. El-Hadi, R. Schnabel, E. Straube, G. Müller, S. Henning, Correlation between degree of crystallinity, morphology, glass temperature, mechanical properties and biodegradation of poly (3-hydroxyalkanoate) PHAs and their blends, Polymer Testing. 21 (2002) 665–674. https://doi.org/10.1016/S0142-9418(01)00142-8.

[39] M.P. Balaguer, C. Aliaga, C. Fito, M. Hortal, Compostability assessment of nano-reinforced poly(lactic acid) films, Waste Management. 48 (2016) 143–155. https://doi.org/10.1016/j.wasman.2015.10.030.

[40] A. Tampau, C. González-Martínez, A. Chiralt, Biodegradability and disintegration of multilayer starch films with electrospun PCL fibres encapsulating carvacrol, Polymer Degradation and Stability. 173 (2020). https://doi.org/10.1016/j.polymdegradstab.2020.109100.

[41] E. Fortunati, F. Luzi, D. Puglia, F. Dominici, C. Santulli, J.M. Kenny, L. Torre, Investigation of thermo-mechanical, chemical and degradative properties of PLA-limonene films reinforced with cellulose nanocrystals extracted from Phormium tenax leaves, European Polymer Journal. 56 (2014) 77–91. https://doi.org/10.1016/j.eurpolymj.2014.03.030.

[42] Y.X. Weng, Y. Wang, X.L. Wang, Y.Z. Wang, Biodegradation behavior of PHBV films in a pilot-scale composting condition, Polymer Testing. 29 (2010) 579–587. https://doi.org/10.1016/j.polymertesting.2010.04.002.

[43] L. Husárová, S. Pekařová, P. Stloukal, P. Kucharzcyk, V. Verney, S. Commereuc, A. Ramone, M. Koutny, Identification of important abiotic and biotic factors in the biodegradation of poly(l-lactic acid), International Journal of Biological

Macromolecules. 71 (2014) 155–162. https://doi.org/10.1016/j.ijbiomac.2014.04.050.

[44] S.K. Guralp Ozkoc, Morphology, Biodegradability, Mechanical, and Thermal Properties of Nanocomposite Films Based on PLA and Plasticized PLA, Journal of Applied Polymer Science. 114 (2009) 2481–2487. https://doi.org/10.1002/app.

[45] G. Gorrasi, R. Pantani, Hydrolysis and Biodegradation of Poly (lactic acid), Advances in Polymer Science. 279 (2018) 119–151. https://doi.org/10.1007/12.

[46] Y. Kuzyakov, J.K. Friedel, K. Stahr, Review of mechanisms and quantification of priming effects, Soil Biology and Biochemistry. 32 (2000) 1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5.

[47] S. Fontaine, A. Mariotti, L. Abbadie, The priming effect of organic matter: A question of microbial competition, Soil Biology and Biochemistry. 35 (2003) 837–843. https://doi.org/10.1016/S0038-0717(03)00123-8.

[48] P.J. Herald, P.M. Davidson, Antibacterial Activity of Selected Hydroxycinnamic Acids, Journal of Food Science. 48 (1983) 1378–1379. https://doi.org/10.1111/j.1365-2621.1983.tb09243.x.

[49] M.E. Ramos-Nino, M.N. Clifford, M.R. Adams, Quantitative structure activity relationship fot the effect of benzoic acids, cinnamic acids and banzaldehydes on Listeria monocytogenes, Journal Applied Bacteriology. 80 (1996) 303-310. https://doi.org/10.1111/j.1365-2672.1996.tb03224.x

[50] T.N. Phan, P.T.M. Nguyen, J. Abranches, R.E. Marquis, Fluoride and organic weak acids as respiration inhibitors for oral streptococci in acidified environments, Oral Microbiology and Immunology. 17 (2002) 119–124. https://doi.org/10.1046/j.0902-0055.2001.00103.x.

[51] F.M. Campos, J.A. Couto, A.R. Figueiredo, I. V. Tóth, A.O.S.S. Rangel, T.A. Hogg, Cell membrane damage induced by phenolic acids on wine lactic acid bacteria, International Journal of Food Microbiology. 135 (2009) 144–151. https://doi.org/10.1016/j.ijfoodmicro.2009.07.031.

[52] A.F. Sánchez-Maldonado, A. Schieber, M.G. Gänzle, Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria, Journal of Applied Microbiology. 111 (2011) 1176–1184. https://doi.org/10.1111/j.1365-2672.2011.05141.x.

Chapter 4

Starch-polyester bilayer films with phenolic acids for meat preservation

Eva Hernández-García[1], María Vargas[1], Amparo Chiralt[1]

Submitted to Food Chemistry

[1]Institute of Food Engineering for Development, Universitat Politècnica de València, Valencia, Spain

evherga1@upvnet.upv.es

ABSTRACT

Starch (S) films containing gellan gum (90:10) and polyester (P) blend films (PLA: PHBV, 75:25) with and without ferulic, *p*-coumaric or protocatechuic acid at 2% (w/w) were obtained by melt-blending and compression moulding for the purposes of obtaining S-P bilayers by thermo-compressing both monolayers together. These were characterised as to their mechanical and barrier properties and as to their performance as packaging materials for pork meat slices. The incorporation of phenolic acids promoted the water vapour and oxygen barrier capacity of bilayers while reducing their stiffness and resistance to break, mainly in the case of protocatechuic acid. Phenolic acids significantly improved the antioxidant capacity of the bilayer films, reducing the lipid oxidation of packaged meat during storage. Phenolic acid loaded bilayers also reduced the microbial counts of meat, mainly for lactic acid bacteria. These effects positively affected the development of the sample pH and colour parameters throughout storage. Active starch-polyester bilayer films exhibited great potential as a means of extending the shelf-life and improving the quality preservation of pork meat.

Keywords: biodegradable bilayer films; active packaging; starch; PLA-PHBV blend; phenolic acids; pork meat preservation

1. INTRODUCTION

Active food packaging is an emerging technology as compared to traditional "inert packaging", since it incorporates active compounds, such as oxygen scavengers and antioxidant or antimicrobial compounds that promote the extension of the food's shelf-life (Yildirim et al., 2018). Active food packaging is a field in dynamic development that presents broad market prospects and takes advantage of the possible interactions between the packaging and the food for the benefit of improving its quality and acceptability (Kuai et al., 2021).

The use of biodegradable materials for the purposes of producing active packaging is necessary in order to reduce the environmental impact of plastics, given that landfilling of postconsumer plastic waste continuously threatens the environment and the ecological system because of the presence of plastic waste and microplastics in rivers and marine and coastal environments (Muthuraj et al., 2018). Likewise, the development of biodegradable laminates to better adapt the materials to the food packaging requirements is a useful strategy as it occurs in synthetic plastics. The combination of biodegradable polymers with complementary barrier properties, such as hydrophilic (e.g starch) and hydrophobic (e.g. polyesters) polymers, permits the production of materials with suitable barrier and mechanical properties and improved functionality for food packaging (Muller et al., 2017). Starch-based films are materials of poor resistance, materials that are highly water sensitive with a great oxygen barrier capacity (Olivato et al., 2013), whereas PLA and PHBV are hydrophobic polymers that yield stiff packaging materials with good mechanical performance and water vapour barrier capacity. However, the low chemical affinity of hydrophilic and hydrophobic polymers makes it necessary to add compatibilisers to promote their interfacial affinity (Encalada et al., 2018). In a previous study, Hernández-García et al. (2021a) obtained bilayer films that consisted of PEG-plasticised PLA-PHBV (75:25) blend film and glycerol-plasticised cassava starch films with 10% gellan gum with adequate functional properties to meet food packaging requirements. These bilayers presented improved oxygen and water vapour barrier properties, as compared to the starch-based or polyester-based monolayers, with a good mechanical performance.

Packaging represents a critical tool for providing meat with physical protection, while controlling microbiological growth and oxidative processes during distribution and retailing. During storage, meat deteriorates due to pigment oxidation, oxidative rancidity, microbial growth and surface dehydration (Bou et al., 2009; Mazzola et al., 2019). Colour, which is one of the principal attributes evaluated by consumers during the purchase, can be affected by other inherent variables, such as pH, oxidation processes or microbial contamination (Mazzola et al., 2019). The presence of microorganisms can cause enzymatic deterioration and oxidation, but bacterial growth is the most important contributing factor for meat spoilage. After microbial deterioration, oxidative processes are the most significant, since they affect lipids, pigments, proteins, and vitamins (Domínguez et al., 2019). The packaging of meat allows the exposure to oxygen to be reduced or limited and it is a good strategy to prevent and delay lipid oxidation (Xiao et al., 2011). Therefore, the development of biodegradable, active materials for meat packaging is a challenge that requires the use of materials with low oxygen transmission rates and with antioxidant/antimicrobial compounds to extend meat shelf-life, ensuring quality and safety (Jalal et al., 2018; Cenci-Goga et al., 2020). Pork is one of the most commonly-consumed meat products in the world (McGlone, 2013). Due to its high lipid content, lipid oxidation can occur during storage, reducing the quality and shortening the shelf life (Hong et al., 2009). Therefore, one of the challenges as regards pork meat packaging is the incorporation of antioxidants into the materials in order to inhibit lipid oxidation and microbial growth.

Phenolic compounds are considered effective antioxidants due to their ability to deactivate and stabilise free radicals, acting as hydrogen donors from the phenolic hydroxyls (Christaki et al., 2012; Kalogianni et al., 2020). Ferulic, *p*-coumaric and protocatechuic acids exhibited quite similar antioxidant activity, quantified as 1.9, 2.2 and 1.2 mM relative to TROLOX, respectively (Rice-Evans, et al., 1996) while also exhibiting antimicrobial activity (Alves et al., 2013; Miyague et al., 2015). In this sense, these have been used to enhance meat product preservation. Ferulic (Ijabadeniyi et al., 2021), *p*-coumaric (Chen et al., 2020) and protocatechuic (Yin et al., 2008) acids have been incorporated into meat products in order to extend their shelf-life during cold storage. Ferulic acid was incorporated into chitosan-based coatings that were applied to

extend the shelf-life of beef (El-Refai et al., 2017) and pork (Wang et al., 2021) meat. Ferulic, *p*-coumaric or protocatechuic acids were incorporated into PEG-plasticised PLA:PHBV films, obtaining antibacterial films with improved tensile and barrier properties of potential use as active food packaging materials (Hernández-García et al., 2021b). Nevertheless, to the best of our knowledge, the promotion of their oxygen barrier capacity by means of lamination with starch films was not previously studied so as to better meet the meat packaging requirements.

The aim of this study was to evaluate the physical properties of starch-polyester (PLA:PHBV blend) bilayer films incorporating phenolic acids (ferulic, *p*-coumaric and protocatechuic acid) into the polyester layer, and to evaluate the performance of such films at extending the shelf-life of pork meat during cold storage.

2. MATERIALS AND METHODS

2.1. Materials

Cassava starch (9% amylose) was purchased by Quimidroga S.A. (Barcelona, Spain). Negatively charged, low acyl gellan gum KELKOGEL F (MW 3-5x10^5) was supplied from premium ingredients (Murcia, Spain). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) ENMAT Y1000P with 3% hydroxyvalerate was supplied by Helian Polymers B.V. (Belfeld, Holland) and amorphous PLA 4060D, density of 1.24 g/cm^3 and average molecular weight of 106,226 D with 40% of low molecular weight fraction (275 D) as reported by Muller et al. (2017), was supplied by Natureworks (U.S.A). The plasticiser, poly(ethylene glycol) with a molecular weight of 1000 Da (PEG1000), was purchased from Sigma-Aldrich (Steinheim, Germany), and the glycerol was obtained from Panreac Química S.L.U. (Castellar del Vallés, Barcelona, Spain).

For sample conditioning purposes, magnesium nitrate-6-hydrate ($Mg(NO_3)_2$) was supplied by Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain). Glacial acetic acid and petroleum ether, used to carry out the different analyses, were provided by Panreac Química SLU (Barcelona, Spain).

Pork meat was purchased from a local supermarket (Consum, Valencia, Spain) and processed in the laboratory. Microbiological media: buffered peptone water, Violet Bile Red agar (VRB) and Plate Count agar (PCA) were provided by Scharlab (Barcelona, Spain)

and Man, Rogosa and Sharpe agar (MRS) was provided by Lankem-Labbox (Barcelona, Spain).

2.2. Preparation of bilayer films

For the preparation of cassava starch films, starch and gellan gum were mixed in the correct proportion to obtain a starch:gum ratio of 90:10, using glycerol (0.30 g/g of starch) as a plasticiser, and obtained by melt blending and compression moulding. The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 130 °C, rotor speed 50 rpm, for 10 min and 50 g of blend were processed in each batch. After processing, the obtained blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and the powder was conditioned at 25 °C and 53% relative humidity (RH) for one week. Four grams of the conditioned powder were required to obtain each film (160 mm in diameter); this powder was put onto Teflon sheets and preheated at 160 °C for 1 min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). The films were obtained by compressing at 160 °C for 2 min at 50 bars, followed by 6 min at 100 bars and a final cooling cycle for 3 min until the temperature reached about 70 °C, according to that described by Hernández-García et al. (2021a). The obtained films were conditioned at 25 °C and 53% RH until used to obtain bilayer films.

PLA: PHBV films were prepared by melt-blending and compression moulding in a ratio of 75:25, using PEG1000 (15 g/100 g polymer) as a plasticiser, and with or without a constant amount of phenolic acids (ferulic, *p*-coumaric or protocatechuic acid) of 2% (w/w) with respect to the polymer matrix (polymer plus plasticiser). The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 170 °C, rotor speed 50 rpm, for 12 min and 50 g of blend were processed in each batch. After processing, blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and conditioned at 25 °C. Three grams of the conditioned powder were required to obtain each film (160 mm in diameter); this powder was put onto Teflon sheets and preheated at 200 °C for 5 min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). The films were obtained by compressing at 200 °C for 4 min at 100 bars and a final cooling cycle for 3 min until the

temperature reached about 70 °C, according to that described by Hernández-García et al. (2021a).

Starch/polyester bilayers films, with or without phenolic acids, were obtained by thermocompressing the polyester film together with the cassava starch-gellan gum film in the hydraulic press (Model LP20, Labtech Engineering, Thailand) at 180 °C and 100 bars for 2 min and then cooling it down until 80 °C over 2 min. The bilayer films were stored at 25 °C and 53% RH until their analyses.

2.3. Characterisation of tensile and barrier properties of the bilayer films

The mechanical properties of the bilayer films were evaluated using a universal testing machine (Stable Micro System TA-XT plus, Surrey, United Kingdom), following standard method ASTM D882 (ASTM, 2001). The thickness of twelve preconditioned film samples at 25 °C and 53% RH of 25 mm x 100 mm was measured at six random points using an electronic digital micrometer (Comecta S.A., Barcelona, Spain). The samples were positioned in tension test clips (model A/TG, Stable Micro Systems Haslemere, United Kingdom) and subjected to a tensile test at a speed of 50 mm min^{-1} until break. The force distance curves obtained in the test were transformed into Henky stress-strain curves that made it possible to obtain the mechanical parameters: elastic modulus (EM), tensile strength (TS) and elongation at break (E).

The water vapour permeability (WVP) of the films was determined according to standard method ASTM E96-95 (ASTM, 1995), considering the modification proposed by McHugh et al. (1993). Prior to the test, the thickness of every sample was measured at six points, as described above. Three round film samples of each formulation were placed on Payne permeability cups of 3.5 cm diameter (Elcometer SPRL, Hermelle/s Argenteau, Belgium). The temperature was 25 °C and the relative humidity gradient was 53-100%. 5 mL distilled water was placed (100% RH) inside each cup, and then the cups were placed inside desiccators containing an oversaturated magnesium-nitrate solution in order to generate 53% RH. The cups were weighed periodically every 1.5h for 24h using an analytical balance (ME36S, Sartorius, Germany, ±0.00001 g), until the steady state was reached. The WVP was calculated from the steady-state permeation slopes

obtained from the regression analysis of weight loss data as a function of time, as described by Cano et al., (2014).

The oxygen permeability (OP) of the different bilayers was determined according to standard method ASTM F1927 (ASTM, 2010) by using an OX-TRAN 1/50 system (Mocon, Minneapolis, USA). Prior to the test, three samples of each formulation were conditioned to 53% RH and 25 °C. The film thickness was measured with a digital electronic micrometer (Palmer, COMECTA, Barcelona, Spain) to the nearest 0.001 mm at six random positions. The area of exposure of each sample to pure nitrogen flow on one side and pure oxygen flow on the other side was 50 cm^2. The equipment took oxygen transmission rate measurements at intervals of 20 minutes until equilibrium was reached. The oxygen transmission rate was transformed into oxygen permeability using average film thickness and oxygen pressure in the equipment.

2.4. Application of the bilayer films for pork meat packaging purposes

Pork meat was purchased from a local supermarket (Consum, Valencia, Spain). To avoid cross contamination during sample preparation, devices and work surfaces were disinfected with 96% ethanol (Panreac S.A, Barcelona, Spain) and every film was sterilized by exposure to ultraviolet (UV) light for 1h in a laminar flow cabinet (Bio II Advance, Telstar, Terrasa, Spain).

Packaging bags (8 cm x 11 cm) were prepared with the different bilayer films by thermo-sealing (SAECO Vacio Press Elite, Barcelona, Spain). The pork meat was cut into 10 g slices using a professional slicer (Smarty 250 IX, Manconi, Italy) and immediately placed inside the bags that were heat sealed and vacuum packed (SAECO Vacio Press Elite, Barcelona, Spain). All of these procedures were carried out in a laminar flow cabinet (Bio II Advance, Telstar, Terrasa, Spain). All of the samples were stored at 5 °C and 48% RH for 3, 7, 11 and 15 days, after which times the bags were weighed to determine the sample weight loss with respect to the initial weight, by using an analytical balance (Sartorius, Goettingen, Germany). Likewise, throughout cold storage, the pH, lipid oxidation, colour changes and microbial counts were monitored in the packaged pork meat slices.

The pH was determined using a digital pH meter by means of the direct insertion of the electrode probe (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) into the pork meat slice. Six measurements were taken, in duplicate for each packaging treatment and time.

The oxidative stability of pork meat was measured by means of the peroxide index (PI) and thiobarbituric acid reactive substances (TBARs) on day 0 and after 15 storage days. Prior to taking the PI measurements, fat was extracted from the pork meat samples. For this purpose, 5 g of minced pork sample were weighed and mixed with 10 g of sea sand and dried for 24 h at 100 °C. Then, Soxhlet equipment was used to extract the meat fat with petroleum ether using for 3 h and the solvent was evaporated to obtain solvent free fat. All of the extractions were performed in triplicate and the peroxide index was also evaluated in triplicate using the method described by Talón et al. (2019). The results were expressed as meq O_2/kg extracted fat. The TBARs in the meat samples were measured in triplicate as described by Siu and Draper (1978). The results were expressed as mg of malondialdehyde (MDA)/kg of meat sample.

The CIE L* a* b* colour coordinates of the packaged pork meat were obtained using the illuminant D65/ 10° observer from the reflection spectra of the sample surface measured at six random points using the MINOLTA colorimeter spectrum (model CM-5, Minolta Co., Tokyo, Japan) and using a black and a white standard background to calculate the L*, a* and b* coordinates from the reflectance of an infinite thickness sample (Hutchings, 1999). The colour parameters, chroma (Cab*) and hue (hab*) were also determined by using Equations (1) and (2), respectively. The total colour difference after packaged meat samples were cold stored for different times with respect to day 0 was calculated using Equation (3). Three packaged pork meat samples with each bilayer film were analysed in duplicate after each storage time.

$$C^*_{\mathrm{ab}} = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h^*_{ab} = arctg\frac{b^*}{a^*} \quad (2)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

Microbiological analyses of the packaged pork meat slices were carried out after different storage times (0, 3, 7, 11 and 15 days). A total of 10 g of meat sample were aseptically taken from the film bags using sterile forceps in the laminar flow cabinet (Bio II Advance, Telstar, Terrassa, Spain) and subsequently placed in sterile bags (Stomacher 440 Classic Strainer Bags, Worthing, UK) with 90 mL of peptone water (Scharlab, Barcelona, Spain). The Stomacher bags were homogenised for 3 min using a homogeniser (IUL Instruments, Barcelona, Spain) and the homogenate was then 10-fold serially diluted using TSB and used to enumerate total viable counts, total coliforms and lactic acid bacteria. The total viable counts and total coliforms were enumerated in PCA and VRB plates, respectively, after incubation at 37 °C for 48h. The lactic acid bacteria were enumerated using MRS plates after incubation at 30 °C for 72h. The results of bacterial counts were converted to $\log_{10}$ colony-forming units per gram of sample (log CFU/g) prior to statistical analyses.

2.5. Statistical analysis

The statistical analyses of the data were performed through an analysis of variance (ANOVA) using Statgraphics Centurion XVII-X64 software (Manugistics Corp., Rockville, Md.). Both a one-way and multifactor ANOVA were used to analyse the influence of the kind of packaging and storage time on the properties of packaged meat. Fisher's least significant difference (LSD) procedure was used at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Tensile and barrier properties of the films

Films with good mechanical strength and extensibility are generally required for food packaging applications to withstand external stress during the processing, transportation, handling and storage of packaged food products (Aloui et al., 2021). **Figure 1** shows the typical stress-strain curves of the obtained bilayers and the mechanical parameters (elastic modulus, tensile strength, deformation at break) are shown in **Table 1**.

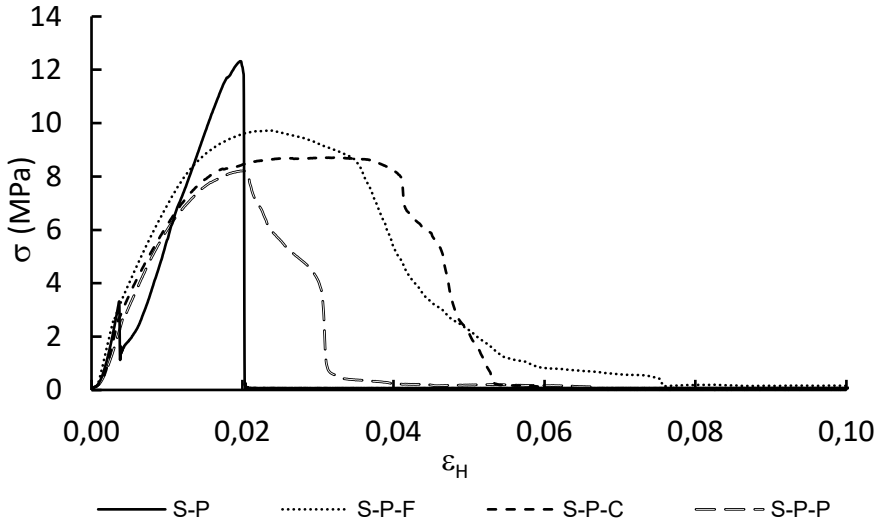


Figure 1. Stress-strain curves of starch-gellan/PLA-PHBV bilayer films (S-P) and those containing ferulic acid (S-P-F), *p*-coumaric acid (S-P-C) and protocatechuic acid (S-P-P).

S-P films did not exhibit plastic deformation, since after elastic deformation these films broke without any signs of deformation exceeding the elastic limit. These bilayers exhibited a small peak at low deformation that can be attributed to a partial layer detaching during the film's extension until the simultaneous fracture of both layers. In contrast, bilayer films containing phenolic acids presented plastic deformation when exceeding the elastic limit, especially with the incorporation of ferulic and *p*-coumaric acids. The fracture of the layers in the laminate occurred separately, the starch layer being more extensible after the first fracture of the P layer. Therefore, the incorporation of phenolic acids yielded more plasticised polyester-starch bilayer films, while mainly promoting the extensibility of the starch layer. Although the tensile behaviour of laminates is determined by the particular properties of each bilayer and the interlayer adhesion forces, it would also be affected by the partial compound migration between layers during thermocompresion that could modify the initial properties of each layer. Specifically, water and glycerol could migrate from the starch sheet to the polyester sheet and PEG and phenolic acids could migrate into the starch layer, all of which would affect the expected tensile behaviour. The less extensible, more resistant and stiffer polyester sheet will mainly determine the bilayer's tensile properties; however, these can also be affected by the starch layer's contribution to the tensile strength, interlayer adhesion forces and compound migration.

Table 1. Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E), thickness, barrier properties (WTR: water transmission rate, WVP: water vapour permeability, OTR: oxygen transmission rate and OP: oxygen permeability) of starch-gellan/PLA-PHBV bilayer films (S-P) and bilayer films containing ferulic acid (S-P-F), *p*-coumaric acid (S-P-C) and protocatechuic acid (S-P-P). Mean values ± standard deviation.

Sample	EM (MPa)	TS (MPa)	E (%)	Thickness (µm)
S-P	834 ± 75^{a}	12.0 ± 2.0^{a}	2.0 ± 0.2^{a}	244 ± 3^{a}
S-P-F	822 ± 157^{ab}	9.0 ± 1.0^{ab}	2.0 ± 1.0^{a}	217 ± 2^{b}
S-P-C	718 ± 90^{ab}	8.0 ± 2.0^{ab}	3.0 ± 1.0^{a}	204 ± 2^{c}
S-P-P	633 ± 60^{b}	8.0 ± 1.0^{b}	2.0 ± 1.0^{a}	215 ± 1^{b}
Sample	**WTR ($g/d.m^2$)**	**WVP ($g \cdot mm \cdot kPa^{-1} \cdot h^{-1} \cdot m^{-2}$)**	**OTR ($cm^3/d.m^2$)**	**OPx10^{14} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)**
S-P	190 ± 30^{a}	1.14 ± 0.20^{a}	0.354 ± 0.02^{ab}	0.93 ± 0.09^{a}
S-P-F	70 ± 14^{b}	0.40 ± 0.04^{b}	0.347 ± 0.01^{a}	0.76 ± 0.01^{b}
S-P-C	58 ± 7^{b}	0.41 ± 0.05^{b}	0.325 ± 0.01^{bc}	0.73 ± 0.04^{bc}
S-P-P	86 ± 30^{b}	0.54 ± 0.20^{b}	0.280 ± 0.05^{c}	0.62 ± 0.11^{c}

Different superscript letters (a-c) within the same column indicate significant differences between formulations ($p < 0.05$).

Even though the incorporation of phenolic acids slightly promoted the extensibility of bilayers (measured at the first fracture point), it led to a decrease in the elastic modulus and tensile stress at break. In contrast, these compounds promoted the stiffness and resistance to break (by about 17 and 30 %, respectively) in the polyester blend monolayers (Hernández-García et al., 2021b). This suggests that the acid migration into the starch sheets notably affected the mechanical contribution of the starch layers to the laminate. Previous studies showed a marked decrease in the elastic modulus and resistance to break, with highly enhanced elongation, when phenolic acids were incorporated into the film matrix (Ordoñez et al., 2021). Therefore, the partial migration of phenolic acids into the starch layer could explain the tensile behaviour of the active bilayers.

As shown in **Table 1**, the incorporation of the three acids caused a significant decrease ($p < 0.05$) in the thickness of the films, with the films containing *p*-coumaric acid being the thinnest. This could be associated with the changes promoted by phenolic acids in the interchain forces of the polymeric matrix, which affect the flowability and extension of the films during the first and the second compression moulding steps.

As concerns the barrier properties, the water vapour and oxygen permeability of packaging materials are critical parameters for the shelf-life of the packaged foods since

they can affect the microbial growth, fat oxidation and texture and water loss (Keery & Tyuftin, 2017). As shown in **Table 1**, the incorporation of the phenolic acids into the polyester layer resulted in a significant improvement ($p < 0.05$) in the barrier properties of the bilayers. The polyester layer provides the laminate with a greater water vapour barrier capacity, whereas the starch layer contributes to an enhancement of the oxygen barrier capacity. Despite the fact that the phenolic acids reduced the WVP and OP values of polyester monolayers by about 30 % (Hernández-García et al., 2021b), they promoted the OP in starch monolayers, without there being any notable effect on WVP; this was mainly due to matrix plasticisation (Ordoñez et al., 2021). The significant reduction in WVP caused by phenolic acids in the bilayers reflects the positive effect of the phenolic acids in the polyester sheet. This has been explained both by the possible reaction of the end chain OH groups with phenolic acid molecules during thermal processing, as well as by the hydrogen bonding between the phenol groups and the oxygen of the polyester group, which further limits the water solubility in the films and so the permeation capability of water molecules. The decreased oxygen permeability promoted by the addition of phenolic acids to the polyester monolayer could also explain the observed reduction in the laminates. The interactions between the phenols and the polyester chains, tightening the polymer matrix, limit mass transport phenomena through the films (Benbettaïeb et al., 2019; Contardi et al., 2019).

The obtained bilayer films exhibited mechanical and barrier properties that were adequate for the purposes of meeting the meat packaging requirements (Nguyen et al., 2021) while the barrier properties were notably improved by the incorporation of phenolic acids. The reduction in WVP and OP could impact positively on how well these materials preserve meat during storage. Additionally, the potential antimicrobial and antioxidant action of these compounds would also contribute to enhancing meat quality and safety during storage.

3.2. Changes in the quality parameters of packaged pork meat throughout cold storage

The weight losses of the pork meat samples packaged in the bilayer films are shown in **Figure 2**. The greatest weight losses throughout the cold storage period were obtained

in the samples packaged in S-P films, which exhibited significantly higher WTR, as compared to bilayer films with phenolic acids. Coherently, the samples packaged in films with *p*-coumaric or ferulic acid, which exhibited the lowest WTR, had the smallest weight loss.

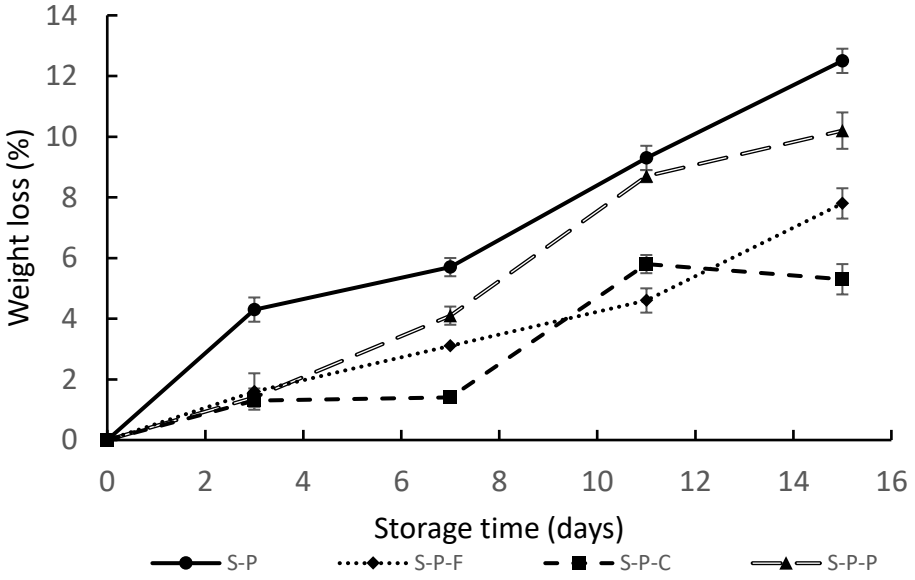


Figure 2. Development of sample weight loss (%) during storage. Mean values and standard deviation.

Figure 3 shows the changes in the pH of the different packaged pork meat samples during cold storage. The pH values were significantly ($p < 0.05$) affected by the bilayer composition and the storage time, as can be observed in **Figure 3**. The pH of fresh pork meat typically ranges between 5.10 and 6.36 and consumers are more likely to prefer a pH of 5.7 to 6.1 (Xiong et al., 2020; Kim et al., 2016). As shown in **Figure 3**, the initial pH value of pork meat was 5.6, which was consistent with the values reported by other authors (Zhang et al., 2018, Xiong et al., 2020 and Wang et al., 2021). The pH value of all of the samples increased over storage time, which is coherent with the production of alkaline compounds due to proteolytic reactions during storage and microbial activity (Arancibia et al., 2015; Daniloski et al., 2019). As compared with samples packaged in phenolic-free S-P bilayer films, the samples packaged in bilayers with phenolic acids (S-P-F, S-P-C and S-P-P) showed lower pH values throughout storage, which suggests a better meat preservation and is coherent with the lower oxidation level and total microbial counts of these samples, commented on below.

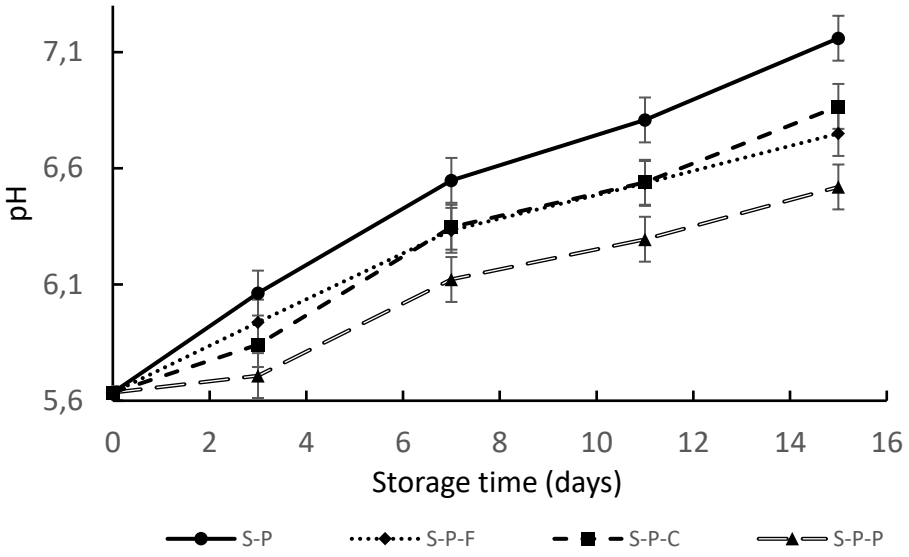


Figure 3. Development of the pH of the pork meat samples packaged in starch-gellan/PLA-PHBV bilayer films without phenolic acids and with ferulic acid (S-P-F), *p*-coumaric acid (S-P-C) and protocatechuic acid (S-P-P), throughout 15 days at 5 °C. Average values and 95% LSD intervals.

When applied to pork meat samples, the antioxidant properties of the starch-polyester bilayer films, containing or not phenolic acids, were assessed by monitoring the PI and TBARs values at the beginning of cold storage and after 15 days. Unsaturated fatty acids react with molecular oxygen through a radical mechanism, giving rise to odourless, highly unstable, first oxidation products known as hydroperoxides; these, in turn, give rise to secondary compounds, such as hydrocarbons, aldehydes and esters, among others (Ross & Smith., 2006), which cause off-flavours and off-odours in meat (Dominguez et al., 2019). As shown in **Table 2**, the PI of pork meat samples increased after 15 days of storage, the PI of the control samples (S-P) being the highest at the end of storage. The PI values of the samples packaged in films with phenolic acids were lower, especially those packaged in the films containing protocatechuic acid, which is coherent with the fact that these films exhibited the lowest OTR values (**Table 1**).

Table 2. TBARs and Peroxide Index of fresh samples (t = 0) and of those stored for 15 days at 5 °C, packaged in bilayer films without phenolic acids (S-P), with ferulic acid (S-P-F), *p*-coumaric acid (S-P-C) and protocatechuic acid (S-P-P). Mean values ± standard deviation.

	t=0 d	t =15 d			
	Fresh sample	**S-P**	**S-P-F**	**S-P-C**	**S-P-P**
TBARs	0.31	1.01	0.80	0.75	0.68
(mg MDA/kg meat sample)	± 0.05	± 0.04^{a}	± 0.07^{b}	± 0.05^{b}	± 0.06^{b}
Peroxide index	0.6	4.4	4.1	4.0	3.1
(meq O_2/ kg extracted fat)	± 0.1	± 0.5^{a}	± 0.5^{ab}	± 0.6^{ab}	± 0.6^{b}

Different superscript letters (a-b) within the same row indicate significant differences between formulations ($p < 0.05$).

The TBARs assay measures the amount of malondialdehyde (MDA) produced by the secondary products of polyunsaturated fatty acid peroxidation (Song et al., 2014). The off-flavours in pork meat can generally be detected by consumers when the TBARs value is above the threshold of 0.5 mg MDA/kg (Sheard et al., 2000). As shown in **Table 2**, the TBARs value of packaged pork meat increased after 15 days of storage and surpassed the abovementioned limit. Nevertheless, although the samples packaged with S-P films exhibited the highest TBARs value, the addition of phenolic acids to the bilayers led to a significant reduction ($p < 0.05$) in the TBARs value of pork meat samples. The samples packaged in films with protocatechuic acid (S-P-P) exhibited the lowest TBARs value, which also agrees with the fact that this bilayer had the lowest OTR values (**Table 2**).

In terms of lipid oxidation, the results are coherent with the oxygen permeability values of the films and also suggest that ferulic, *p*-coumaric and protocatechuic acids exerted their antioxidant activity from the laminate, thus effectively retarding lipid oxidation. A similar effect was reported in previous studies into the incorporation of ferulic acid into chitosan-based films (Wang et al., 2021) when applied to refrigerated pork meat.

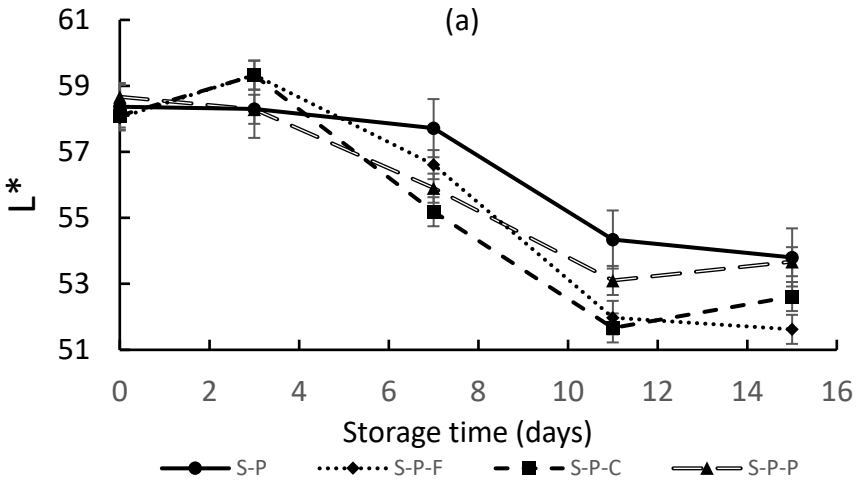
Surface colour is an important visual quality indicator of fresh meat (Pathare et al., 2013). **Figure 4** shows the changes in the colour parameters, lightness (L*), chroma (Cab*) and hue (hab*), as a function of storage time. All of the colour parameters were significantly ($p < 0.05$) affected by the type of film and storage time, as shown in **Figure 4**. After three days of storage, L* significantly decreased in every sample, indicating that the packaged meat became darker. This could be, in part, explained by the changes in

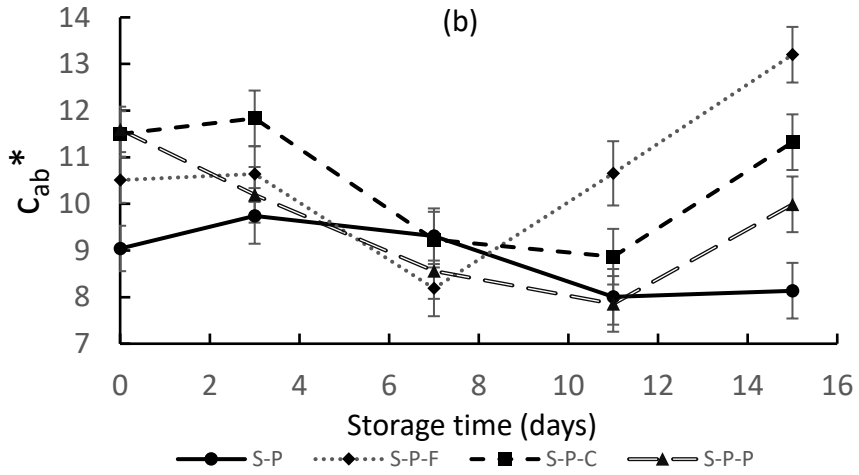
pH throughout the storage time (**Figure 2**), which affect the meat water holding capacity (Barbut et al., 2005) and the sample weight loss, which mainly occur at surface level (**Figure 4**). This led to changes in the selective light absorption on the sample surface, due to the changes in the refractive index of the material and the surface concentration of the pigments (Hutchings, 1999). These changes also affect the values of the chromatic parameters and so, the chroma and hue values. The oxidation of lipids and myoglobin in meat also leads to discoloration, and these processes often appear to be linked (the oxidation of one of these compounds produces chemical species that promote the oxidation of the other) (Faustman et al., 2010). In fact, several studies have reported that antioxidants act to preserve the fresh meat colour.

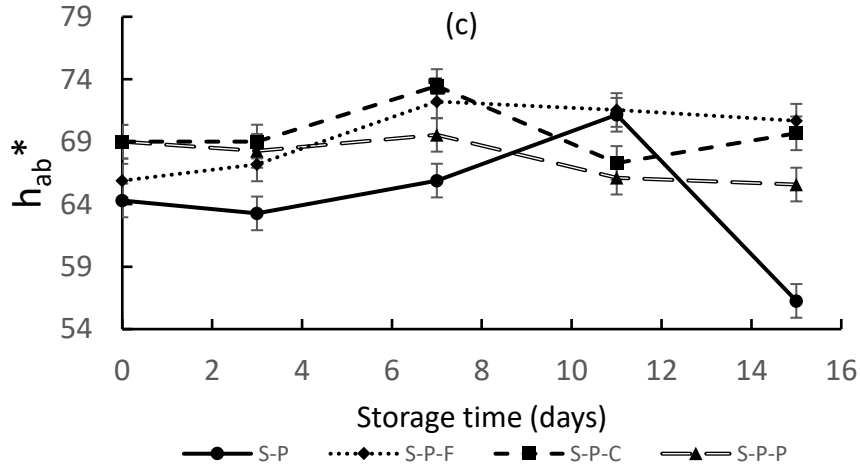
The pH influence on meat colour has also been described. A lower pH in pork meat has been associated with greater reflectance, which leads to an increase in lightness (L*) and a decrease in the relative amount of the reduced form of myoglobin (Mb). At the same time, a lower pH is accompanied by a greater susceptibility of muscle pigments to oxygenation and oxidation (Karamucki et al., 2013). Meat yellowness increases due to an increase in the relative amounts of the oxygenated and oxidised forms of myoglobin (MbO_2 and MetMb) at the expense of the reduced form.

The type of film packaging slightly affected the colour development of meat during storage. Significant differences could be observed in the case of the samples packaged in films incorporating phenolic acids, mainly at the end of the storage period. The pork meat samples packaged in the active bilayers exhibited hue and chrome values that were significantly higher than the samples packaged in the S-P film, with lower hue and a less saturated colour.

The total colour difference after each storage time with respect to the initial values is shown in **Figure 4d**. The total colour differences in the pork meat samples packaged in films containing phenolic acids did not exceed the usual tolerance limit for food products ($\Delta E<5$) (Hutchings, 1999), thus indicating a better colour preservation in the samples packaged in antioxidant films with lower OTR values.


(a)
L*
61
59
57
55
53
51
0
2
4
6
8
10
12
14
16
Storage time (days)
S-P
S-P-F
S-P-C
S-P-P


(b)
C*ab
14
13
12
11
10
9
8
7
0
2
4
6
8
10
12
14
16
Storage time (days)
S-P
S-P-F
S-P-C
S-P-P


(c)
h*ab
79
74
69
64
59
54
0
2
4
6
8
10
12
14
16
Storage time (days)
S-P
S-P-F
S-P-C
S-P-P

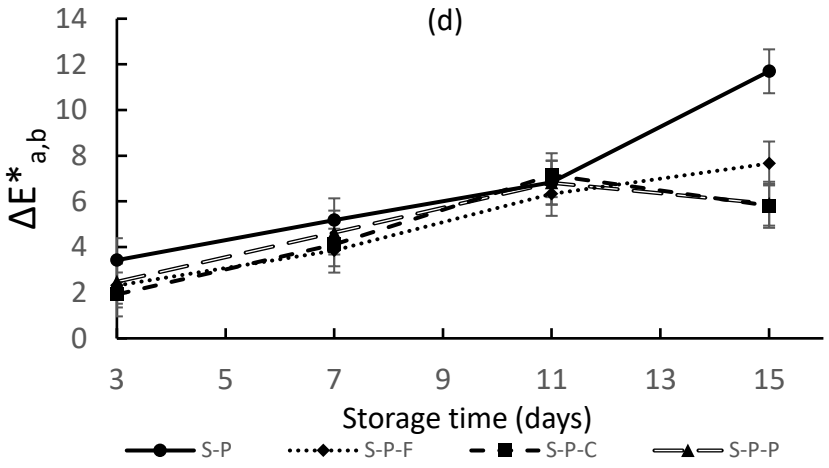


Figure 4. Development of the colour parameters of pork meat samples packaged in starch-gellan/PLA-PHBV bilayer films without (S-P) and with ferulic acid (S-P-F), *p*-coumaric acid (S-P-C) and protocatechuic acid (S-P-P), throughout 15 days at 5 °C. a) L*, lightness; b) Cab*, chroma c) hab*, hue d) total colour difference, ΔE*. Average values and 95% LSD intervals.

Figure 5 shows the progress throughout the storage (up to 15 days) of the total aerobic counts, coliforms, and lactic acid bacteria of pork meat packaged in starch-polyester bilayer films containing or not phenolic acids. The total viable counts are an important microbiological indicator for the purposes of evaluating the sanitary quality and safety of meat. It is the quantitative sanitary standard used to identify the process conditions and contamination degree of meat (European Commission, 2005). Total viable counts (**Figure 4a**) were significantly ($p < 0.05$) affected by the type of food packaging and the storage time. In all of the meat samples, the total viable counts increased during the storage time, which is mainly related to the proliferation of psychotrophic bacteria (Ercolini et al., 2009; Xiong et al., 2020). The initial total viable count of the fresh samples was 2.8 log CFU/g. Similarly, Xiong et al. (2020) and Wang et al. (2018) reported counts of fresh pork of around 2.51 and 2.2-2.7 log CFU/g respectively. On days 3, 7 and 15, the incorporation of phenolic acids had a significant effect ($p > 0.05$) on the total viable counts. S-P-P was the formulation that showed the greatest reduction in total viable counts. The incorporation of protocatechuic acid led to a log reduction of 0.7, 0.5, 0.8 and 0.6 log on days 3, 7, 11 and 15, respectively. These results suggested that active bilayers incorporated with ferulic acid, *p*-coumaric acid and protocatechuic acids were capable of reducing the total viable counts of pork during cold storage, with the

protocatechuic acid being slightly more effective. However, the decrease in microbial growth as compared with that of the bilayer films without phenolic acids (control) was smaller than 2 log, which is what is usually considered as being significant in microbial growth studies. This coincides with the results obtained in a previous study in which the antimicrobial performance of polyester films with the same phenolic acids was evaluated in vitro (Hernández-García et al., 2021b). The limited antimicrobial action of phenolic acids was attributed to the slow and scarce release of the active compounds into the culture medium. According to Huang et al. (2013), a total viable count of 7 log can be used as the threshold for the quality of pork meat. All of the samples, except S-P, were below this threshold after the 15-day storage period, indicating that the incorporation of phenolic acids was effective at preventing the pork from microbial spoilage, despite their slow release.

The microbial counts of total coliforms throughout the storage time are shown in **Figure 5b**. Total coliform counts were significantly ($p < 0.05$) affected by the type of packaging and the storage time. While the incorporation of phenolic acids did not have a significant effect ($p > 0.05$) on total coliform counts on day 3, it did have a significant effect on days 7 and 15, which is coherent with the slow compound release. Protocatechuic acid was the most effective, showing log reductions of 1.1 and 0.8 log on days 7 and 15 of storage, respectively.

Lactic acid bacteria are the dominant group of microorganisms isolated from meat and vacuum-packed meat products (Xu et al., 2018). The initial count of lactic acid bacteria in the fresh pork samples was 1.79 log CFU/g (**Figure 5c**), in the range of that reported in other studies (Xu et al., 2018). Lactic acid bacteria counts were also significantly ($p < 0.05$) affected by the type of food packaging and the storage time. At the end of the storage, the greatest log reduction (1.2 log) also occurred in samples packaged in the bilayers with *p*-coumaric acid.

Protocatechuic acid was the phenolic acid that led to the greatest microbial inhibition, especially against total coliforms and lactic acid bacteria. The inhibition did not occur at the beginning, coherently with the slow release of these kinds of compounds from the polyester matrix, as previously reported (Hernández-García et al., 2021b). In fact, the antibacterial action was mainly detected from the 7th storage day onwards.

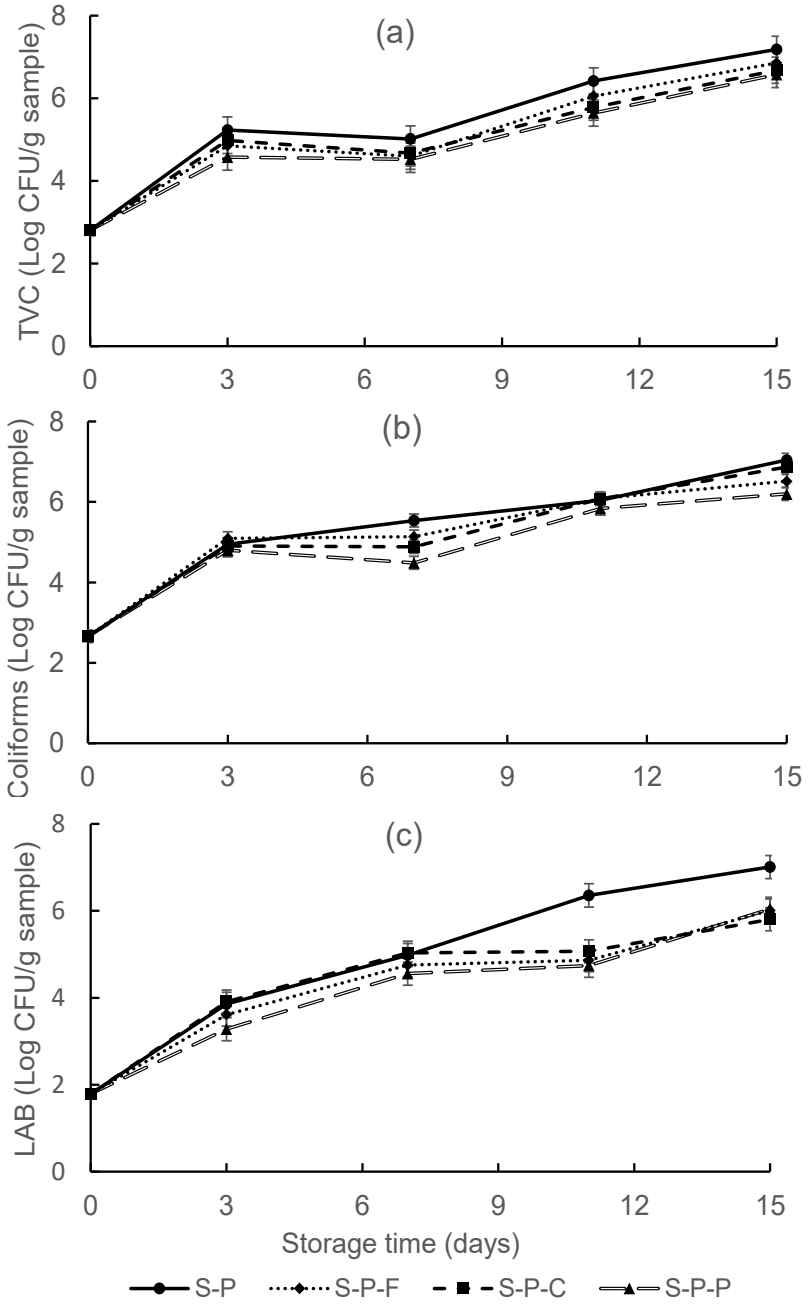


Figure 5. Microbial counts obtained for the pork meat samples packaged in starch-gellan-/PLA-PHBV bilayer films (S-P) and those containing ferulic acid (S-P-F), *p*-coumaric acid (S-P-C) and protocatechuic acid (S-P-P) throughout 15 days at 5 °C. a) total aerobic counts, b) total coliforms, c) lactic acid bacteria. Average values and 95% LSD intervals.

4. CONCLUSION

The incorporation of 2% (w/w) ferulic, *p*-coumaric or protocatechuic acid into starch-polyester bilayer films, obtained by melt blending and compression moulding, reduced

their stiffness and resistance to break but significantly improved their barrier capacity to water vapour and oxygen. The films containing protocatechuic acid experienced the greatest improvement. This implied that the functional properties of bilayers with phenolic acids were better for meat packaging purposes. Additionally, the antioxidant and antimicrobial properties of phenolic acids may also contribute to an improvement in meat preservation. In fact, ferulic, *p*-coumaric and protocatechuic acids in the films led to a reduction in the meat weight loss and less lipid oxidation during storage, while the increase in sample pH was smaller than that in the samples packaged without phenolic acids. Microbial growth was also inhibited in meat samples packaged in films with phenolic acids, with the active films with protocatechuic acid being the most effective, especially against total coliforms and lactic acid bacteria. It would be necessary to carry out further studies in order to achieve a faster release of phenolic acids from the films for the purposes of reaching greater microbial growth inhibition, thus better ensuring the shelf-life extension of pork meat.

Acknowledgements

The authors would like to thank the Spanish Agencia Estatal de Investigación, for funding this study through Project PID2019-105207RB-I00 and the predoctoral research grant # BES-2017-082040.

5. REFERENCES

Aloui, H., Deshmukh, A. R., Khomlaem, C., & Kim, B. S. (2021). Food Hydrocolloids Novel composite films based on sodium alginate and gallnut extract with enhanced antioxidant , antimicrobial , barrier and mechanical properties. *Food Hydrocolloids*, *113*, 106508. https://doi.org/10.1016/j.foodhyd.2020.106508

Alves, M. J., Ferreira, I. C. F. R., Froufe, H. J. C., Abreu, R. M. V., Martins, A., & Pintado, M. (2013). Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *Journal of Applied Microbiology*, 115(2), 346–357. https://doi.org/10.1111/jam.12196

Arancibia, M. Y., López-Caballero, M. E., Gómez-Guillén, M. C., & Montero, P. (2015). Chitosan coatings enriched with active shrimp waste for shrimp preservation. *Food*

Control, *54*, 259–266. https://doi.org/10.1016/j.foodcont.2015.02.004

ASTM. (1995). Standard test methods for water vapor transmission of materials. Standard designations: E96-95. In ASTM, Annual book of ASTM standards (pp. 406-413). Philadelphia, PA: American Society fot Testing and Materials.

ASTM. (2001). Standard test method for tensile properties of thin plastic sheeting. Standard designations: D882. In Annual book of ASTM standards (pp. 162-170). Philadelphia, PA: American Society for Testing and Materials.

ASTM. (2010). Standard Test Method for Determination of Oxygen Gas Transmission Rate, Permeability and Permeance at Controlled Relative Humidity Through Barrier Materials Using a Coulometric Detector, ASTM F1927; ASTM international: West Conshohocken, PA, USA, 2010; Volume 07, pp. 1–6.

Barbut, S., Zhang, L., & Marcone, M. (2005). Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. *Poultry Science*, *84*(5), 797–802. https://doi.org/10.1093/ps/84.5.797

Benbettaïeb, N., Debeaufort, F., & Karbowiak, T. (2019). Bioactive edible films for food applications: mechanisms of antimicrobial and antioxidant activity. *Critical Reviews in Food Science and Nutrition*, *59*(21), 3431–3455. https://doi.org/10.1080/10408398.2018.1494132

Bou, R., Codony, R., Tres, A., Decker, E. A., & Guardiola, F. (2009). Dietary strategies to improve nutritional value, oxidative stability, and sensory properties of poultry products. *Critical Reviews in Food Science and Nutrition*, *49*(9), 800–822. https://doi.org/10.1080/10408390902911108

Cano, A., Jiménez, A., Cháfer, M., Gónzalez, C., & Chiralt, A. (2014). Effect of amylose:amylopectin ratio and rice bran addition on starch films properties. *Carbohydrate Polymers*, *111*, 543–555. https://doi.org/10.1016/j.carbpol.2014.04.075

Cenci-goga, B. T., Lulietto, M. F., Sechi, P., Borgogni, E., Karama, M., & Grispoldi, L. (2020). New Trends in Meat Packaging. *Microbiology Research*, *11*, 56–67. https://doi.org/doi:10.3390/microbiolres11020010

Chen, X., Yu, F., Li, Y., Lou, Z., Toure, S. L., & Wang, H. (2020). The inhibitory activity of p-coumaric acid on quorum sensing and its enhancement effect on meat preservation. *CYTA - Journal of Food*, *18*(1), 61–67. https://doi.org/10.1080/19476337.2019.1701558

Christaki, E., Bonos, E., Giannenas, I., & Florou-Paneri, P. (2012). Aromatic plants as a source of bioactive compounds. *Agriculture*, *2*, 228–243. https://doi.org/10.3390/agriculture2030228

Contardi, M., Alfaro-Pulido, A., Picone, P., Guzman-Puyol, S., Goldoni, L., Benítez, J. J., Heredia, A., Barthel, M. J., Ceseracciu, L., Cusimano, G., Brancato, O. R., Di Carlo, M., Athanassiou, A., & Heredia-Guerrero, J. A. (2019). Low molecular weight ϵ-caprolactone-pcoumaric acid copolymers as potential biomaterials for skin regeneration applications. *PLoS ONE*, *14*(4), 1–18. https://doi.org/10.1371/journal.pone.0214956

Commission Regulation, 2005 (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. In Official Journal of the European Union pp 338/1–338/26

Daniloski, D., Trajkovska, A., Galić, K., Ščetar, M., Kurek, M., Vaskoska, R., Kalevska, T., & Nikolovska, D. (2019). The effect of barrier properties of polymeric films on the shelf-life of vacuum packaged fresh pork meat. *Meat Science*, *158*, 107880. https://doi.org/10.1016/j.meatsci.2019.107880

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants*, *8*(429). doi:10.3390/antiox8100429

El-Refai, A., Hassan, A., Nagy, K., & Rabie, M. (2017). Antimicrobial Effect for Both of Carboxy Methyl Cellulose and Chitosan Treated with Ferulic Acid or Nanosilver Particles as Edible Coatings used for Some Refrigerated Beef Samples. *Journal of Food and Dairy Sciences*, *8*(2), 87–91. https://doi.org/10.21608/jfds.2017.37128

Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science*, *86*(1), 86–94.

https://doi.org/10.1016/j.meatsci.2010.04.025

Encalada, K. Aldás, M.B., Proaño, E., Valle, V. (2018). An overview of starch-based biopolymers and their biodegradability. *Ciencia e Ingeniería*, *39*(3), 245–258.

Ercolini, D., Russo, F., Nasi, A., Ferranti, P., Villani, F., & Universita, V. (2009). Mesophilic and Psychrotrophic Bacteria from Meat and Their Spoilage Potential In Vitro and in Beef. *Applied and Environmental Microbiology*, *75*(7), 1990–2001. https://doi.org/10.1128/AEM.02762-08

Hernández-García, E., Vargas, M., & Chiralt, A. (2021a). Thermoprocessed starch-polyester bilayer films as affected by the addition of gellan or xanthan gum. *Food Hydrocolloids*, *113*. https://doi.org/10.1016/j.foodhyd.2020.106509

Hernández-García, E., Vargas, M., & Chiralt, A. (2021b).Use of phenolic acids to obtain active films based on PLA-PHBV. *Food Packaging and Shelf Life* (Sutmitted).

Hong, Y.H., Lim, G.O., Song, K. . (2009). Physical Properties of Gelidium corneum – Gelatin Blend Films Containing Grapefruit Seed Extract or Green Tea Extract and Its Application in the. *Journal of Food of Science*, *74*(1). https://doi.org/10.1111/j.1750-3841.2008.00987.x

Huang, L., Zhao, J., Chen, Q., & Zhang, Y. (2013). Rapid detection of total viable count (TVC) in pork meat by hyperspectral imaging. *Food Research International*, *54*(1), 821–828. https://doi.org/10.1016/j.foodres.2013.08.011

Hugh, T. H., Avena-Bustillos, R., & Krochta, J. M. (1993). Hydrophilic edible films: Modified procedure for water vapor permeability and explanation of thickness effects. Journal of Food Science, 58(4), 899–903. https://doi.org/10.1111/j.1365-2621.1993.tb09387.x

Hutchings, J. B. (1999). Instrumental specification. In Food colour and appearance (pp. 199–237). https://doi.org/10.1007/978-1-4615-2373-4_7

Ijabadeniyi, O.A. Govender, A., Olagunju, O.F., Oyedeji, A. B. (2021). The antimicrobial activity of two phenolic acids against foodborne. *Italian Journal of Food Science*, *33*(1), 39–45. https://doi.org/10.15586/ijfs.v33i1.1933

Jalal, H., Salahuddin, M., Para, P. A., Pal, M., & Wani, S. (2018). Bio-based Packaging Materials for Preservation of Processed Meat Products. *Recent Research Trends in Veterinary Sciences and Animal Husbandry*, *January*, 31–48. https://doi.org/10.22271/ed.book01.a03

Kalogianni, A. I., Lazou, T., Bossis, I., & Gelasakis, A. I. (2020). Natural phenolic compounds for the control of oxidation, bacterial spoilage, and foodborne pathogens in meat. In *Foods* (Vol. 9, Issue 6). https://doi.org/10.3390/foods9060794

Karamucki, T., Jakubowska, M., Rybarczyk, A., & Gardzielewska, J. (2013). The influence of myoglobin on the colour of minced pork loin. *Meat Science*, *94*(2), 234–238. https://doi.org/10.1016/j.meatsci.2013.01.014

Kerry, J. P., & Tyuftin, A. A. (2017). Storage and Preservation of Raw Meat and Muscle-Based Food Products: IV Storage and Packaging. In *Lawrie's Meat Science: Eighth Edition*. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-100694-8.00010-8

Kim, T. W., Kim, C. W., Kwon, S. G., Hwang, J. H., Park, D. H., Kang, D. G., Ha, J., Yang, M. R., Kim, S. W., & Kim, I. S. (2016). PH as analytical indicator for managing pork meat quality. *Sains Malaysiana*, *45*(7), 1097–1103

Kuai, L., Liu, F., Chiou, B. Sen, Avena-Bustillos, R. J., McHugh, T. H., & Zhong, F. (2021). Controlled release of antioxidants from active food packaging: A review. *Food Hydrocolloids*, *120*(February), 106992. https://doi.org/10.1016/j.foodhyd.2021.106992

Mazzola, N., & Sarantópoulos, C.I.G.L. (2019). Packaging Design Alternatives for Meat Products. In book: Insights on Novel Meat Processing and Preservation Methods. 10.5772/intechopen.88586

Mcglone, J. J. (2013). The Future of Pork Production in the World: Towards Sustainable, Welfare-Positive Systems. *Animals*, *3*, 401–415. https://doi.org/10.3390/ani3020401

Miyague, L., Macedo, R. E. F., Meca, G., Holley, R. A., & Luciano, F. B. (2015). Combination of phenolic acids and essential oils against Listeria monocytogenes.

LWT - Food Science and Technology, 64(1), 333–336. https://doi.org/10.1016/j.lwt.2015.05.055

Muller, J., González-Martínez, C., & Chiralt, A. (2017). Poly(lactic) acid (PLA) and starch bilayer films, containing cinnamaldehyde, obtained by compression moulding. *European Polymer Journal*, *95*(July), 56–70. https://doi.org/10.1016/j.eurpolymj.2017.07.019

Muthuraj, R., Misra, M., Mohanty, A. K. (2018). Biodegradable compatibilized polymer blends for packaging applications: A literature review. *Journal of Applied Polymer Science*, *135*(24). https://doi.org/10.1002/app.45726

Nguyen, H. L., Tran, T. H., Hao, L. T., Jeon, H., Koo, J. M., Shin, G., Hwang, D. S., Hwang, S. Y., Park, J., & Oh, D. X. (2021). Biorenewable, transparent, and oxygen/moisture barrier nanocellulose/nanochitin-based coating on polypropylene for food packaging applications. *Carbohydrate Polymers*, *271*, 118421. https://doi.org/10.1016/j.carbpol.2021.118421

Olivato, J. B., Nobrega, M. M., Müller, C. M. O., Shirai, M. A., Yamashita, F., & Grossmann, M. V. E. (2013). Mixture design applied for the study of the tartaric acid effect on starch / polyester films. *Carbohydrate Polymers*, *92*(2), 1705–1710. https://doi.org/10.1016/j.carbpol.2012.11.024

Ordoñez, R., Atarés, L., & Chiralt, A. (2021). Physicochemical and antimicrobial properties of cassava starch films with ferulic or cinnamic acid. *Lwt*, *144*. https://doi.org/10.1016/j.lwt.2021.111242

Pathare, P. B., Opara, U. L., & Al-said, F. A. (2013). Colour Measurement and Analysis in Fresh and Processed Foods : A Review. *Food Bioprocess Technol*, *6*, 36–60. https://doi.org/10.1007/s11947-012-0867-9

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, *20*(7), 933–956. https://doi.org/10.1016/0891-5849(95)02227-9

Ross, C. F., & Smith, D. M. (2006). Use of volatiles as indicators of lipid oxidation in muscle foods. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 5,

Issue 1, pp. 18–25). https://doi.org/10.1111/j.1541-4337.2006.tb00077.x

Sheard, P. R., Enser, M., Wood, J. D., Nute, G. R., Gill, B. P., & Richardson, R. I. (2000). Shelf life and quality of pork and pork products with raised n-3 PUFA. *Meat Science*, *55*(2), 213–221. https://doi.org/10.1016/S0309-1740(99)00145-X

Siu, G., & Draper, H.H. (1978). A survey of malonaldehyde content of retail meats and fish. *Journal of Food Science, 43*,1147-1149.

Song, N., Lee, J., Mijan, M. Al, & Song, K. Bin. (2014). LWT - Food Science and Technology Development of a chicken feather protein fi lm containing clove oil and its application in smoked salmon packaging. *LWT - Food Science and Technology*, *57*(2), 453–460. https://doi.org/10.1016/j.lwt.2014.02.009

Talón, E., Vargas, M., Chiralt, A., & González-Martínez, C. (2019). Antioxidant starch-based films with encapsulated eugenol. Application to sunflower oil preservation. *Lwt*, *113*, 108290. https://doi.org/10.1016/j.lwt.2019.108290

Wang, W., & Lihua, S. (2018). Effect of high-oxygen modified atmosphere packaging on the tenderness , lipid oxidation and microbial growth of cooked pork. *Food Measure*, *12*, 395–402. https://doi.org/10.1007/s11694-017-9652-9

Wang, G., Liu, Y., Yong, H., Zong, S., Jin, C., & Liu, J. (2021). Effect of ferulic acid-grafted-chitosan coating on the quality of pork during refrigerated storage. *Foods*, *10*(6). https://doi.org/10.3390/foods10061374

Xiao, S., Zhang, W. G., Lee, E. J., Ma, C. W., & Ahn, D. U. (2011). Effects of diet , packaging , and irradiation on protein oxidation , lipid oxidation , and color of raw broiler thigh meat during refrigerated storage. *Diet, Packaging, and Irradiation on Meat Quality*, 1348–1357. https://doi.org/10.3382/ps.2010-01244

Xiong, Y., Chen, M., Warner, R. D., & Fang, Z. (2020). Incorporating nisin and grape seed extract in chitosan-gelatine edible coating and its effect on cold storage of fresh pork. *Food Control*, *110*, 107018. https://doi.org/10.1016/j.foodcont.2019.107018

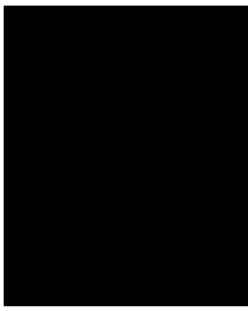
Xu, F., Wang, C., Wang, H., Xiong, Q., Wei, Y., & Shao, X. (2018). Antimicrobial action of flavonoids from Sedum aizoon L . against lactic acid bacteria in vitro and in

refrigerated fresh pork meat. *Journal of Functional Foods*, *40*, 744–750. https://doi.org/10.1016/j.jff.2017.09.030

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., Radusin, T., Suminska, P., Marcos, B., & Coma, V. (2018). Active Packaging Applications for Food. *Comprehensive Reviews in Food Science and Food Safety*, *17*(1), 165–199. https://doi.org/10.1111/1541-4337.12322

Yin, M., & Chao, C. (2008). Anti- Campylobacter , anti-aerobic , and anti-oxidative effects of roselle calyx extract and protocatechuic acid in ground beef. *International Journal of Food Microbiology Journal*, *127*(1), 73–77. https://doi.org/10.1016/j.ijfoodmicro.2008.06.002

Zhang, H., He, P., Kang, H., & Li, X. (2018). LWT - Food Science and Technology Antioxidant and antimicrobial e ff ects of edible coating based on chitosan and bamboo vinegar in ready to cook pork chops. *LWT - Food Science and Technology*, *93*, 470–476. https://doi.org/10.1016/j.lwt.2018.04.005



IV. GENERAL DISCUSSION

The environmental problem generated by the massive consumption of plastics, and the need for improving food preservation, makes developing of active biodegradable or compostable materials necessary. Research on biodegradable materials is focused on synthetic and natural polymers. Of the natural biodegradable bioplastics, the most studied is starch because of its high availability and low cost, non-toxicity, biocompatibility and the ability of yielding thermoplastic materials with affordable plasticizers. However, the high water sensitivity and water vapour permeability and the limited mechanical yield of starch-based materials constitute serious drawbacks for their food packaging applications.

To meet food packaging requirements, the development of multilayer films based on biodegradable monolayers with complementary properties, is an innovative approach that allows for obtaining materials (laminates), with improved performance as compared to the use of monolayers, as also occurs in the conventional plastic laminates. In this sense, the combination of starch films with sheets of biodegradable polyesters represents an alternative for accomplishing this purpose. Polylactic acid (PLA) and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) are biodegradable polyesters obtained from natural resources that can be used for food packaging purposes due to their ability to develop food contact plastic materials at a relatively competitive cost. PLA is a biodegradable thermoplastic aliphatic polyester that is compostable, non-toxic, easy to process, transparent, with relatively low cost and good thermal behaviour and good water vapour barrier capacity as compared to starch, although with limited gas barrier properties. PHBV is an interesting polyester since it is obtained from biosynthesis, using mixed bacteria cultures, from different agri-food residues, which represent a sustainable solution for obtaining bioplastics. Nevertheless, previous studies demonstrated poor adhesion properties of PHBV and starch for developing bilayers, whereas amorphous PLA exhibited good adhesion capacity with starch sheets by thermocompression. Likewise, blend films of amorphous PLA and PHBV (75:25) exhibited better mechanical and barrier performance than the pure polyester films.

In this context, the present Doctoral Thesis focuses on the development of biodegradable multilayer active films consisting of a starch-based layer and a PLA-PHBV blend film layer, incorporating phenolic acids as active compounds, with antimicrobial

and antioxidant capacity. This general objective was addressed through different strategies developed in the different chapters. First, in **Chapter 1**, the starch film properties were improved to better meet the mechanical and barrier requirements of the bilayer assembly. To this end, maize and cassava starch films were obtained by incorporating xanthan and gellan gum, and analysed in their properties as both monolayers and bilayer combination with a polyester sheet. From this study, cassava starch films with gellan gum was selected as the starch film with better properties. In **Chapter 2**, the ability of polyester films as food contact layer carrying the active compounds was studied. Termoprocessed PLA-PHBV blend films with different phenolic compounds (ferulic, *p*-coumaric and protocatechuic acids) were developed and characterized as to their physical properties, active compound release kinetics and antibacterial activity. Likewise, the influence of antimicrobial compounds on the biodegradation behaviour of PLA-PHBV blend films was studied in **Chapter 3**. Finally in **Chapter 4**, the active starch-polyester bilayer films with phenolic acids were characterized and tested in pork meat preservation.

As concerns the results shown in **Chapter 1**, the addition of the gums slightly decreased the water adsorption capacity of the starch films, which represent an improvement in the starch functionality. This was attributed due to the formation of hydrogen bonds between the starch chains and gellan or xanthan that could reduce the number of active points for the water adsorption. Nevertheless, the incorporation of gums did not affected the water solubility of maize and cassava starch films. As concerns barrier properties, maize starch films were more permeable to water vapour and oxygen than cassava starch films and the incorporation of gums significantly reduced both water vapour permeability (WVP) and oxygen permeability (OP) of the starch films. The tensile behaviour of the films revealed a greater structural toughness in films based on cassava starch, with lower amylose ratio, as compared to maize starch-based films. This could be related with the higher molecular weight of the highly branched amylopectin that could offer the possibility of a greater chain entanglement in the melt, thus forming a more cohesive, less extensible polymer matrix, with lower retrogradation degree during storage. The incorporation of gums with high molecular weight (10^5–10^6 Da) contributed to reinforce the starch polymer matrix, creating association domains in the matrix where

gums and starch polymers could participate through the aggregation of the helical conformations of the different chains. Differences in the molecular structure of the gums and the amylose/amylopectin ratio in starch may explain the observed tensile behaviour of the blend films, depending on their composition. The linear structure of gellan chains could better reinforce the cassava starch matrix, with a lower ratio of amylose, providing it with more regions with glucose helical associations, while this contribution could be less noticeable in maize starch matrices, with higher amylose content. Despite the structural differences, both gums enhanced the toughness of the starch matrix structure, giving rise to a better mechanical performance of the starch-based films.

Over storage time there was a slight increase in WVP, which was more significant in maize starch films. Both gums minimised the impact of storage time on the WVP values of the films and, in general, there were no significant changes as far as WVP is concerned throughout storage. The incorporation of both gums also reduced the oxygen permeability of starch films. The observed effects of gums on the starch film barrier properties were coherent with that commented on above as regards the reinforcing effect of gums in the starch matrices. The changes in the properties of starch films over time were attributed to the starch retrogradation, through the recrystallisation of amylose, whose proportion is higher in maize starch. In general, storage time had a more significant effect on tensile properties than on barrier properties.

Except for cassava starch films with gums, the values of elastic modulus and resistance to break of the bilayers were notably reduced with respect to those of the stiffest and hardest polyester film. This suggested the partial interlayer migration of water and plasticisers, mainly during the thermocompression step, that highly affected the mechanical resistance of the polyester sheet, probably by a hydrolytic effect. This reduction progressed throughout storage time and bilayer with cassava starch with gums exhibited the highest EM and TS values, regardless of the storage time. As concerns barrier properties of the different bilayer films, a significant decrease in water the vapour and oxygen permeability values was observed, with respect to respective values of the starch and polyester monolayers, thus revealing the effective assembly of both layers to meet food packaging barrier requirements. The values did not significantly

change during the 5-week storage of the films, which reflects the fact that the barrier capacity of the assemblies remained stable throughout storage.

Results shown in the **Chapter 2**, revealed that PLA, PHBV and phenolic acids are not completely miscible, as deduced from the microstructural and thermal analyses. PHBV-rich domains were observed in a continuous PLA-rich matrix. Nevertheless, interchain and compound interactions occurred due to hydrogen bonding between the polymers and the phenolic molecules. Protocatechuic acid appears to promote the strongest interactions, further increasing the PLA Tg value, and limiting the PHBV crystallization, which reduced the thermal degradation temperature of the PHBV fraction. The incorporation of phenolic acids into the PLA-PHBV matrix resulted in more resistant and stiffer films, which could be attributed to the promoted interactions between phenolic acids and polymers, but with the same film extensibility as the pure polyester blend. Likewise, phenolic acids reduced the water vapour and oxygen permeability of the bilayers. This improvement was more accentuated in films containing protocatechuic acid, coherently with the abovementioned higher interactions with the polymer chains. Phenolic acids also promoted slight changes in the colour parameters and slightly increased the gloss and internal transmittance of the films.

The release of the phenolic acids from the polyester matrix into the food simulants (high and intermediate polarity) was very slow while release rate and ratio was favoured in less polar simulants. In fact, the release rate and the amount released at equilibrium for all phenolic acids were higher in simulant D1 (50% v/v ethanol) than in simulant A (10% v/v ethanol). The higher ethanol content promotes the relaxation of PLA matrix and the release of the incorporated compounds. The highest release rate and the equilibrium release ratio was obtained for ferulic acid in both simulants, while protocatechuic acid was the compound that was released more slowly in both simulants, with the lowest equilibrium release ratio.

The incorporation of phenolic acids led to a significant decrease in the growth of *Listeria innocua* without significant differences between acids. However, the reduction was less than 2 logarithms, which is what is usually considered as being significant in microbial growth studies. The poor efficacy of the potentially active films could be explained by the small amount of acid released from the polymeric matrix into the aqueous

inoculated culture media, as inferred from the release study. However, studies in real food are necessary to demonstrate the true efficacy of the active films. To this end, the active films were tested as to their preservation capacity in cold-stored pork meat (**Chapter 4**).

As concerns the incorporation of antimicrobial agents into biodegradable food packaging materials, it is important to evaluate the impact of such components in the film biodegradation behaviour (**Chapter 3**). The study of disintegration and biodegradation of the PLA-PHBV blend films containing phenolic acids was carried out under thermophilic composting. The disintegration trend was similar in all films reaching values of 85-92% at the end of the assay. During the first 10 days, the mass losses occurred very fast in every case, reaching values of disintegration of around 63-67%. From 10 days on, the disintegration rate slowed down, reaching a plateau after around 30 days, and the sample weight losses reached values if close to 90%. In general, the incorporation of the active agents did not induce significant changes in the disintegration behaviour of PLA-PHBV films.

The biodegradation test revealed that amorphous-PLA/PHBV films presented an initial delay period of 10 days and completely biodegraded (B=100%) after 20 composting days. The addition of phenolic acids modified the biodegradation profile of PLA/PHBV films, especially when using protocatechuic acid. The initial delay periods were around 10-12 days for all the films except for those containing protocatechuic acid, when this period extended to 22 days. This could be linked to the different bonding capacity of the phenolic acids to the polymer chains that affect their release from the matrix and dilution in the compost medium. PLA/PHBV blend films containing phenolics acids completely biodegraded after 20-27 days, and after 30 days, these films reached biodegradation percentages of over 100%. Nevertheless, apart from the different length of the induction period, the potential antimicrobial effect of active compounds did not notably interfered in the biodegradation of the PLA/PHBV films, which reach total biodegradation during a relatively short composting time (20-27 days).

In **Chapter 4**, laminates that consist of a starch-based layer and a PLA/PHBV blend sheet, with ferulic, *p*-coumaric and protocatechuic acids were studied and the effectiveness of

these active laminates at preserving fresh pork meat quality and at extending its shelf-life during cold storage was analysed.

The incorporation of phenolic acids yielded more plasticized starch-polyester bilayers. The incorporation of ferulic and *p*-coumaric acids did not significantly affect the elastic modulus and tensile at break of the films, whereas the addition of protocatechuic acid caused a significant decrease in EM and TS as compared to starch-polyester multilayer films. In contrast, the opposite trend was observed for PLA:PHBV monolayer when phenolic acids were incorporated. The differences can be explained by the interlayer migration of low molecular compounds, such water and glycerol from starch films and phenolic compounds from the polyester layer, mainly during the thermocompression step. Water could cause hydrolysis of polyester and phenolic acids could negatively affect mechanical properties of starch.

The incorporation of phenolic acids into the polyester layer resulted in a significant improvement in the barrier capacity of the bilayers. Both, water vapour and oxygen permeability were markedly reduced in the bilayer, compared with the active-free laminate. This can be explained by the interactions between the phenols and the polyester chains, tightening the polymer matrix and limiting mass transport phenomena through the films. This improvement, and the active nature of the phenolic acids, were effective at enhancing the preservation capacity of these bilayers, extending the meat shelf-life.

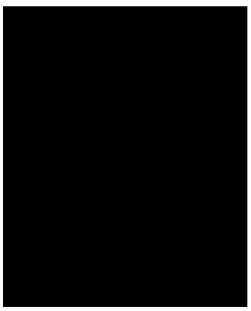
The pork meat samples packaged with the starch-polyester bilayer films without active compounds showed the highest weight losses throughout cold storage, which was coherent with the higher values of water vapour permeability of these films. In contrast, bilayers with phenolic acids, reduced the meat sample weight loss during storage. Likewise, these meat samples exhibited lower pH increase and microbial counts than samples packaged with active-free bilayers. The addition of phenolic acids also significantly reduced the TBARs and peroxide values of the meat samples, protocatechuic acid being the most effective. This was coherent with the lower oxygen permeability of these films and the antioxidant capacity of the compounds; all this effectively retarding lipid oxidation.

The type of film packaging slightly affected the colour of meat during storage, the differences being significant at the end of the storage period where meat samples packaged in the active bilayers showed hue and chrome values significantly higher than samples packaged in the active-free films, which showed darker and less saturated colour. The total colour difference, with respect to the initial colour, of packaged meat samples in active bilayers with phenolic acids, did not exceed the usual tolerance limit for food products at the end of storage period.

As concerns the antimicrobial effect of the bilayers, those containing ferulic, *p*-coumaric and protocatechuic acids were capable of reducing the total viable counts of pork meat during cold storage, the films with protocatechuic acid being the most effective. However, the growth inhibition with respect the sample packaged in active-free films was lower than 2 log, which is what is usually considered as being significant in microbial growth studies. This coincides with that found in **Chapter 2** for the antimicrobial performance of polyester films with phenolic acids, evaluated through *in vitro* tests, and it was attributed to the limited release of active compounds into the food surface. In fact, the inhibition did not occur at the beginning of storage but delayed in time, coherently with that it takes a few days for the active compound to be released into the food matrix. Protocatechuic acid was the active compound that led to the highest microbial inhibition, especially against total coliforms and lactic acid bacteria.

The development of laminates of starch layers and PLA/PHBV blend sheets, incorporating ferulic, *p*-coumaric and protocatechuic acids into the polyester blend represents a good strategy to obtain active bilayer films based on biodegradable polymers with complementary barrier properties, so as to improve the performance of the material (laminate), as compared to the monolayers. The polyester layer contributes to the strengthening of the multilayer while reducing water permeability, whereas the starch layer help to control the oxygen and gas barrier capacity of the multilayer assembly. Thus, bilayer films exhibited functional properties close to those of some synthetic plastics commonly used in food packaging. These materials can extend the shelf-life of foods sensitive to oxidation and microbial contamination, such as meat products, mitigating the environmental impact of the single-use plastic packaging, since they are compostable.

However, further studies are necessary to evaluate other techniques for incorporating the active compounds into the polymeric matrix, or to test other polymer blends to incorporate the actives in order to achieve a more effective release of the active compounds and better antimicrobial performance.



V. CONCLUSIONS

1

The incorporation of gellan or xanthan gums into thermo-processed cassava and maize starch slightly reduced the water adsorption capacity of starch-based films and improved their mechanical properties, especially in maize starch films. The incorporation of either gellan or xanthan gum decreased the water vapour and oxygen permeabilities of starch-based films, being cassava starch films with gums the least permeable to oxygen and the more stable in their mechanical properties throughout storage time, especially those incorporating xanthan gum. The starch based-polyester laminates exhibited improved oxygen and water vapour barrier capacity with respect to the respective monolayers. The laminates based on cassava starch with gums showed the lowest OP and WVP values and the highest elastic modulus and tensile strength, with extensibility values in the range of the corresponding monolayers and a reasonable degree of stability throughout time. When also taking the layer adhesion into account, the bilayer formed with the cassava starch with gellan gum and the PLA-PHBV sheet was the best option for food packaging purposes.

2

The incorporation of ferulic, *p*-coumaric or protocatechuic acids by melt- blending and compression moulding in a polymer matrix of PLA:PHBV led to an increase in the glass transition temperature of PLA while PHBV supercooling occurred in the films containing protocatechuic acid. These changes affected the thermal degradation behaviour. The addition of ferulic acid increased the thermal stability of the polyester blend films whereas the incorporation of protocatechuic decreased it. Likewise, PLA:PHBV films with phenolic acids were stiffer and more resistant to break and shows better oxygen and water vapor barrier capacity, as compared to phenolic acid-free films, in agreement with the higher cohesion forces of the active films. Protocatechuic acid promoted the highest effects. The release rate and ratio of phenolic acids increased when polarity of the food simulant decreases, although very slow release was observed in all cases. The incorporation of phenolic acids into the polyester blend films did no significantly inhibit the growth of *Listeria innocua* in inoculated culture medium, due to very limited release of active compounds in the polar culture medium. Therefore, the target application of

these potentially active packaging materials should consider the scarce release of active compounds in aqueous systems, which in turn will affect the antibacterial performance of the films.

3

A complete disintegration of the PLA:PHBV blend films was observed during the composting test, regardless of the presence of phenolic acids. A faster disintegration of the amorphous PLA fraction was inferred from thermo gravimetric analysis, while the process was barely affected by the presence of phenolic acids. As concerns the biodegradation process, the incorporation of the phenolic acids delayed the biodegradation period, extending the induction period, especially when using protocatechuic acid. This could be related with the greater persistency of the compound in the polymeric matrix. Phenolic-free PLA:PHBV blend films fully biodegraded after 20 composting days, while *p*-coumaric and protocatechuic slightly retarded full biodegradation (21 and 26 days, respectively). These results indicated that none of the antimicrobial phenolic acids inhibit the biodegradation process of the blend films, but they can retard biodegradation when these are tightly retained in the polymer matrix.

4

The incorporation of phenolic acids into the starch-polyester laminates reduced the stiffness and resistance to break but significantly improved their barrier capacity to water vapour and oxygen. This implied that the functional properties of bilayers with phenolic acids were better for meat packaging purposes. Additionally, the antioxidant and antimicrobial properties of phenolic acids may also contribute to an improvement in meat preservation. In fact, ferulic, *p*-coumaric and protocatechuic acids in the films led to a reduction in the meat weight loss and lipid oxidation during storage, while the increase in sample pH was smaller than that in the samples packaged without phenolic acids. Microbial growth was also inhibited in meat samples packaged in films with phenolic acids, the active films with protocatechuic acid being the most effective, especially against total coliforms and lactic acid bacteria. It would be necessary to carry out further studies in order to achieve a faster release of phenolic acids from the films for the purposes of reaching greater microbial growth inhibition, thus better ensuring the shelf-life extension of pork meat.

As a general conclusion, biodegradable bilayer films with phenolic acids, based on cassava starch and PLA-PHBV blend films, appears to be a suitable strategy to obtain active packaging materials, with functional properties close to those of some synthetic plastics commonly used in food packaging. These materials can extend the shelf-life of foods sensitive to oxidation and microbial spoilage, mitigating the environmental impact of the single-use plastic packaging, since they are compostable. Nevertheless, further studies are necessary to improve the release of active compounds and to ensure the stability of the materials, analysing the potential effects of the interlayer compound migration throughout time that can contribute to the loss of the material performance.

DOCTORAL THESIS