UCAM

UNIVERSIDAD CATÓLICA
DE MURCIA

ESCUELA INTERNACIONAL DE DOCTORADO
Programa de Doctorado en Ciencias de la Salud

Envases activos con β-ciclodextrinas para liberación de agentes antimicrobianos naturales en alimentos de IV gama.

Autora:
Dña. Friné Velázquez Contreras

Director:
Dr. D. José Antonio Gabaldón Hernández

Murcia, septiembre de 2022

UCAM
UNIVERSIDAD CATÓLICA DE MURCIA

ESCUELA INTERNACIONAL DE DOCTORADO
Programa de Doctorado en Ciencias de la Salud

Envases activos con β-ciclodextrinas para liberación de agentes antimicrobianos naturales en alimentos de IV gama.

Autora:
Dña. Friné Velázquez Contreras

Director:
Dr. D. José Antonio Gabaldón Hernández

Murcia, septiembre de 2022

La presente Tesis Doctoral titulada "Envases activos con β-ciclodextrinas para liberación de agentes antimicrobianos naturales en alimentos de IV gama" adopta el formato de **COMPENDIO DE PUBLICACIONES**. En ella se recoge una síntesis de conceptos derivados de trabajos de revisión bibliográfica, estudios *in vitro* e *in vivo*. El compendio consta de tres artículos ya publicados. A continuación, se muestran los tres artículos que forman parte de la Tesis:

ARTÍCULO 1:

Velázquez-Contreras, F.; Acevedo Parra, H.; Nuño Don Lucas, S.; Núñez-Delicado, E.; Gabaldón J.A. (2019). Development and Characterization of a Biodegradable PLA Food Packaging Hold Monoterpene–Cyclodextrin Complexes against Alternaria alternata. Polymers, 11, 1720. https://doi.org/10.3390/polym11101720

Factor de impacto 2019 (JCR): 3.426. Categoría (JCR): Polymer Science. Posición de la revista: 16/89 (Q1).

ARTÍCULO 2:

Velázquez-Contreras, F.; García-Caldera, N.; Padilla de la Rosa, J.D.; Martínez-Romero, D.; Núñez-Delicado, E.; Gabaldón, J.A. (2021). Effect of PLA Active Packaging Containing Monoterpene-Cyclodextrin Complexes on Berries Preservation. Polymers, 13, 1399. https://doi.org/10.3390/polym13091399

Factor de impacto 2021 (JCR): 4.329. Categoría (JCR): Polymer Science. Posición de la revista: 18/90 (Q1).

ARTÍCULO 3:

Velázquez-Contreras; F.; Zamora-Ledezma, C.; López-González, Iván; Meseguer-Olmo, L.; Núñez-Delicado; E.; Gabaldón J.A. (2022). Cyclodextrins in Polymer-Based Active Food Packaging: A Fresh Look at Nontoxic, Biodegradable, and Sustainable Technology Trends. Polymers, 14 (1), 104. https://doi.org/10.3390/polym14010104

Factor de impacto 2021 (JCR): 4.329. Categoría (JCR): Polymer Science. Posición de la revista: 18/90 (Q1).

Los resultados de esta investigación también han sido presentados en varias reuniones científicas internacionales:

- Friné Velázquez Contreras; José Antonio Gabaldón Hernández. *Desarrollo de envases activos con β-ciclodextrina para liberación de agentes antimicrobianos naturales en alimentos de IV gama, ensaladas y berries.* IV Jornadas de Investigación y Doctorado: Women in Science, 2018, Murcia, España.

- Friné Velázquez Contreras; José Antonio Gabaldón Hernández. *Biodegradable food packaging development and characterization against pathogen Botrytis cinerea.* Institute of Food Technologists: IFT, 2020, Chicago, USA.

- Friné Velázquez Contreras; José Antonio Gabaldón Hernández. *Effect of PLA Active packaging containing monoterpene-cyclodextrin complexes on berries preservation.* VII Jornadas de Investigación y Doctorado: ODS con Ciencia (Modalidad Virtual), 2021, Murcia, España.

Además, parte de los resultados de esta investigación se han utilizado para la solicitud de dos patentes al Instituto Mexicano de la Propiedad Intelectual (IMPI):

- Encapsulado timol y carvacrol en **β**-ciclodextrinas para empaques activos de matriz polimérica de ácido poli-láctico.

- Proceso de inyección de matriz polimérica de ácido poli-láctico con encapsulado de timol y carvacrol en **β**-ciclodextrinas.

UCAM

UNIVERSIDAD CATÓLICA
DE MURCIA

AUTORIZACIÓN DE LO/S DIRECTOR/ES DE LA TESIS
PARA SU PRESENTACIÓN

El Dr. D. José Antonio Gabaldón Hernández como Director de la Tesis Doctoral titulada "Envases activos con β-ciclodextrinas para liberación de agentes antimicrobianos naturales en alimentos de IV gama" realizada por Dña. Friné Velázquez Contreras en el Departamento de Ciencias de la Salud, **autoriza su presentación a trámite** dado que reúne las condiciones necesarias para su defensa. Lo que firmo, para dar cumplimiento al Real Decreto 99/2011, en Murcia a 26 de septiembre de 2022.

Dr. D. José Antonio Gabaldón Hernández

RESUMEN

Título: Envases activos con β-ciclodextrinas para liberación de agentes antimicrobianos naturales en alimentos de IV gama.

Introducción: El aumento en el consumo de alimentos mínimamente procesados, la demanda de productos libres de conservantes de síntesis química, así como los cambios en las prácticas de distribución de alimentos derivadas de la globalización, son algunas de las razones que han motivado el desarrollo e innovación de nuevas tecnologías de envasado de alimentos, focalizándose mayoritariamente en la extensión de la vida útil, sin que se produzcan mermas en la calidad y seguridad de los alimentos. El envasado de alimentos es uno de los retos más importantes de la industria alimentaria, particularmente en el área de alimentos mínimamente procesados y de IV gama, ya que el envasado contribuye a la conservación de los mismos, evitando ataques por microorganismos y deterioro del producto. Así, desde fechas recientes se están desarrollando envases que, además de cumplir con las funciones básicas de contener y proteger al alimento, interaccionan con el alimento para disminuir la degradación. La tecnología de envasado activo se refiere a la adición de ciertas sustancias capaces de mantener o mejorar la calidad y seguridad del alimento, las cuales son incorporadas en el material de envasado o dentro del propio envase. Éstas interaccionan directamente con el alimento o con su entorno, actuando más allá del uso convencional de barrera con el medio externo, aumentando la vida útil del alimento; así, el envasado activo puede ser considerado como una tecnología emergente de conservación de alimentos. El envasado activo "antimicrobiano", es una alternativa para proteger alimentos de IV gama. Uno de los enfoques más comunes de este tipo de envases, se basa en la liberación de compuestos antimicrobianos del empaque. Entre las alternativas, el empleo de extractos de aceites esenciales de plantas que contienen compuestos naturales como timol y carvacrol, los cuales presentan una amplia actividad frente a microorganismos patógenos, incluidas especies gram negativas y gram positivas; así como las levaduras y los mohos, son muy atractivos para su aplicación en la industria alimentaria, además de ser considerados como aditivos GRAS (*Generally Recognized as Safe*), generalmente reconocidos como seguros por la FDA (*U.S. Food and Drug Administration*).

Objetivos: El Objetivo principal de esta Tesis Doctoral ha sido el desarrollo y caracterización un envase activo biodegradable de ácido poli láctico (PLA), que incorpora complejos de timol o carvacrol en β-ciclodextrinas (*β*-CD), evaluando su capacidad para controlar el crecimiento de microorganismos en ensayos *in vitro*, y posteriormente, evidenciar la aplicabilidad *in vivo* del envase en alimentos de IV gama, utilizando como modelo zarzamoras y frambuesas.

Metodología: Se desarrollaron y caracterizaron envases activos de PLA, aditivados con complejos de inclusión de timol y carvacrol en *β*-CD, para evaluar su aplicabilidad potencial como materiales antibacterianos en empaques de alimentos. En primer lugar, se prepararon complejos de inclusión de timol y carvacrol en β-ciclodextrinas (*β*-CD), obteniendo posteriormente complejos en estado sólido tras pasar las disoluciones por Spray Dryer, como vía para facilitar su almacenamiento y manejo. Caracterizados los complejos obtenidos, atendiendo a los valores de constante de complejación, eficiencia y rendimiento, éstos se mezclaron con PLA a diferentes proporciones (0,0 %, 1,5 %, 2,5 % y 5,0 % en peso) para conformar un envase biodegradable mediante inyección, evaluando posteriormente si la aditivación con los complejos en estado sólido, mejora la propiedades físicas y químicas del envase, y si ejerce algún efecto antimicrobiano, a ser posible de amplio espectro, sobre bacterias y hongos identificados habitualmente en alimentos, mediante ensayos *in vitro*. Por último, se evaluaron las prestaciones del envase activo en condiciones reales (refrigeración a 4 °C durante 21 días), comparando con un envase comercial y un control (PLA sin aditivar), utilizando moras y frambuesas por tener una vida comercial corta, con respecto a los parámetros de calidad (peso, color, sólidos solubles, contenido fenólico total), seguridad (carga microbiana) y aceptación del consumidor, objetivo para este tipo de alimentos.

Resultados: La presencia de estos complejos confiere características plastificantes a la matriz polimérica, mejorando así el punto de rotura. Los resultados obtenidos por Espectroscopia de infrarrojo transformada de Fourier (FTIR) confirman la inclusión de carvacrol y timol en la cavidad apolar de las *β*-CDs, obteniendo una eficiencia de complejación significativamente mayor para carvacrol (105,6) que para timol (69,3). Con respecto al comportamiento térmico del envase biodegradable, el análisis termogravimétrico (TGA), demuestra que la presencia de

complejos sólidos de timol o carvacrol en β-CD, disminuye ligeramente la temperatura de degradación térmica del polímero, en comparación con PLA puro. Los empaques con encapsulados de ambos compuestos al 5,0 %, mostraron mayor actividad antimicrobiana y antifúngica por el método de difusión en fase vapor, inhibiendo el desarrollo de *Alternaria alternata* y *Botritis cinerea* después de 10 días de incubación.
Respecto a las prestaciones de los empaques aditivados sobre la vida útil de moras y frambuesas, el que contiene 5,0 % de β-CD-timol mostró una inhibición del 51,6 % de levaduras y mohos, en comparación con el empaque comercial y el PLA control. Los empaques activos obtuvieron las mejores puntuaciones en la evaluación sensorial de los parámetros color, olor, sabor y textura. El empaque de PLA/β-CD-timol 5,0 % mostró una cinética de liberación controlada de monoterpeno (30 % del valor inicial a los 7 días), disminuyendo hasta el final del ensayo; mientras que, para el resto de los empaques activos de PLA, los niveles de monoterpeno comenzaron a disminuir mucho más rápidamente, alcanzando la línea de base, sin que se detecte concentración de ninguno de ellos, a partir de la primera semana de estudio. Este mecanismo de liberación observado para carvacrol y timol en el paquete de PLA, modificó la composición de la atmósfera inicial dentro del envase, mejorando la calidad y la vida útil postcosecha de las moras y frambuesas, almacenadas en refrigeración a temperatura controlada.

Conclusiones: Los empaques de PLA que contienen encapsulados de β-CD-timol y β-CD-carvacrol al 5,0 %, mostraron su efectividad para mejorar la calidad y seguridad alimentaria de las berries durante un período de almacenamiento de 21 días a 4 °C, extendiendo en consecuencia su vida útil 7 días más, en comparación con los empaques que se utilizan actualmente en el mercado. Sin embargo, se recomiendan estudios adicionales relacionados con la durabilidad y versatilidad de los empaques, que demuestren si estos biopolímeros pueden reutilizarse varias veces y para diferentes aplicaciones prácticas.

Palabras Clave: Empaques activos, ácido poliláctico, timol, carvacrol, β-ciclodextrina, actividad antimicrobiana, berries, vida de anaquel.

ABSTRACT

Title: Active packaging with β-cyclodextrin for release of natural antimicrobial agents in ready to eat products.

Introduction: The increase of consumption of minimally processed foods, the demand for products free of artificial preservatives, as well as the changes in food distribution practices derived from globalization, are some of the reasons that have motivated the development and innovation of new technologies for food packaging, focusing mainly on the extension of the useful life without reducing the quality and safety of the food. Food packaging is one of the most important challenges in the food industry, particularly in the area of minimally processed and ready to eat products, since packaging contributes to their preservation, avoiding attacks by microorganisms and deterioration of the product. Thus, since recently, packaging is being developed that, in addition to fulfilling the basic functions of containing and protecting food, interact with food to reduce degradation. Active packaging technology refers to the addition of certain substances capable of maintaining or improving the quality and safety of food, which are incorporated into the packaging material or within the packaging itself. These interact directly with the food or with its environment, acting beyond the conventional use of barrier with the external environment, increasing the useful life of the food, therefore active packaging can be considered as an emerging technology for food preservation. Active "antimicrobial" packaging is an alternative to protect ready to eat products. One of the most common approaches to this type of packaging is based on the release of antimicrobial compounds from the packaging. Among the alternatives, the use of herbs and essential oil extracts that contain natural compounds such as thymol and carvacrol, which have broad activity against pathogenic microorganisms, including gram negative and gram positive species; as well as yeasts and molds, they are very attractive for application in the food industry, in addition to being recognized as GRAS additives (Generally Recognized as Safe), generally recognized as safe by the FDA (U.S. Food and Drug Administration).

Objectives: The main goal of this Doctoral Thesis has been the development and characterisation of a biodegradable active packaging of poly lactic acid (PLA), which incorporates thymol or carvacrol complexes in β-cyclodextrins (*β*-CD), evaluating its capacity to control the growth of microorganisms in in vitro tests, and subsequently, to demonstrate the in vivo applicability of the packaging in fresh-cut foods, using blackberries and raspberries as a model.

Methodology: Active PLA containers, riched with thymol and carvacrol inclusion complexes in *β*-CD, were developed and characterised to evaluate their potential applicability as antibacterial materials in food packaging. Firstly, thymol and carvacrol inclusion complexes in β-cyclodextrins (*β*-CD) were prepared, subsequently obtaining solid state complexes after passing the solutions through Spray Dryer, as a way to facilitate their storage and handling. Once the complexes obtained were characterised, according to the values of complexation constant, efficiency and yield, they were mixed with PLA at different proportions (0.0 %, 1.5 %, 2.5 % and 5.0 % by weight) to form a biodegradable packaging by injection, and subsequently evaluating whether the enrichment with the complexes in solid state improves the physical and chemical properties of the packaging, and whether it exerts any antimicrobial effect, if possible with a broad spectrum, on bacteria and fungi commonly identified in food, by means of in vitro tests. Finally, the performance of the active packaging was evaluated under real conditions (refrigerated at 4 °C for 21 days), compared with a commercial packaging and a control (PLA without additives), using blackberries and raspberries as they have a short shelf life, with respect to quality parameters (weight, colour, soluble solids, total phenolic content), safety (microbial load) and consumer acceptance, which is the objective for this type of foods.

Results: The presence of these complexes confers plasticizing characteristics to the polymeric matrix, thus improving the breaking point. The results obtained by Fourier Transform Infrared Spectroscopy (FTIR) confirm the inclusion of carvacrol and thymol in the apolar cavity of the *β*-CDs, obtaining a significantly higher complexation efficiency for carvacrol (105.6) than for thymol (69.3). Regarding the thermal behavior of the biodegradable packaging, thermogravimetric analysis (TGA) shows that the presence of solid complexes of thymol or carvacrol in β-CD slightly decreases the thermal degradation temperature of the polymer compared

to pure PLA. Packages encapsulated with both compounds at 5.0 % showed higher antimicrobial and antifungal activity by the vapour phase diffusion method, inhibiting the development of *Alternaria alternata* and *Botrytis cinerea* after 10 days of incubation.

Regarding the performance of the additive packages on the shelf life of blackberries and raspberries, the one containing 5.0 % β-CD-thymol showed 51.6 % inhibition of yeasts and moulds, compared to the commercial package and the PLA control. The active packages obtained the best scores in the sensory evaluation of colour, odour, taste and texture parameters. The PLA/β-CD-thymol 5.0 % packaging showed a controlled release kinetics of monoterpene (30 % of the initial value at 7 days), decreasing until the end of the trial; whereas, for the rest of the PLA active packaging, monoterpene levels started to decrease much faster, reaching the baseline, with no detectable concentration of any of them, from the first week of the study. This release mechanism observed for carvacrol and thymol in the PLA package modified the composition of the initial atmosphere inside the package, improving the quality and post-harvest shelf life of the blackberries and raspberries, stored under temperature-controlled refrigeration.

Conclusions: PLA packaging containing encapsulated β-CD-thymol and β-CD-carvacrol at 5.0% was shown to be effective in improving the quality and food safety of berries during a storage period of 21 days at 4 °C, thus extending their shelf life by 7 days compared to packaging currently used on the market. However, further studies related to the durability and versatility of the packaging are recommended to show whether these biopolymers can be reused several times and for different practical applications.

Keywords: Active packaging, polylactic acid, thymol, carvacrol, β-cyclodextrin; antimicrobial activity, berries, shelf life.

AGRADECIMIENTOS

Durante la elaboración de este trabajo de investigación, el cual ha requerido de gran esfuerzo y dedicación, he recibido el apoyo y ayuda de muchas personas, por ello quiero aprovechar estas líneas para expresarles mi más sincero agradecimiento.

A Dios por su presencia en mi vida, por su guía, por disponer los medios y sobre todo las personas en este proceso, quienes han sido pilares fundamentales para poder culminar esta etapa tan importante en mi vida profesional.

A mi director José Antonio, gracias por la confianza otorgada, por la oportunidad de trabajar en equipo a distancia, gracias por la ayuda, la paciencia y el aprendizaje obtenidos; sobre todo por la enseñanza del mundo fascinante de la investigación.

A la Universidad Católica de Murcia por recibirme en este programa doctoral y a la Universidad Panamericana por todo su apoyo incondicional durante el desarrollo de lo que sin duda significa uno de los proyectos más importantes de mi carrera profesional.

Al grupo de investigadores que me apoyaron durante la etapa de investigación, muy especialmente a Daniel Padilla por impulsarme a constatar que con mucho esfuerzo si se pueden obtener resultados favorables, a la Dra. Elisa Cabrera por acogerme en su equipo de investigación y permitirme desarrollar los estudios microbiológicos de patógenos en sus laboratorios, a Gaby por tus consejos y ayuda en el desarrollo de esta tesis doctoral.

A ti Gerardo, compañero incansable e incondicional de quien he recibido el amor, la paciencia y la capacidad para entender la dificultad de este reto en todas sus dimensiones, a mis mayores fuentes de inspiración, Álvaro y Rodrigo, mis entrañables hijos, chispas de energía en los momentos complicados.

¡Gracias, gracias, gracias han sido todos piezas fundamentales en este invaluable proceso de crecimiento personal y profesional!

"La ciencia y la vida no pueden ni deben ser separadas" Rosalind Franklin (1920-1958).

ÍNDICE GENERAL

AUTORIZACIÓN DEL DIRECTOR
RESUMEN/ABSTRACT
AGRADECIMIENTOS
ÍNDICE GENERAL
ÍNDICE DE TABLAS Y FIGURAS **23**
SIGLAS Y ABREVIATURAS **25**
I - INTRODUCCIÓN **27**
1.1 ENVASADO ACTIVO DE ALIMENTOS **29**
1.1.1 Empaques Antimicrobianos 31
1.1.2 Utilización de biopolímeros en envases para alimentos 35
1.1.2.1 Ácido Poliláctico 37
1.1.2.2 Estructura química y propiedades del ácido poliláctico 37
1.1.2.3 Producción y procesado de ácido poliláctico 39
1.1.2.4 Aplicación en envasado de alimentos 42
1.2 ADITIVOS NATURALES **42**
1.2.1. Aceites Esenciales 43
1.2.2 Extracto de semilla de uva 45
1.2.3 Bacteriocinas 45
1.2.4 Lisozimas 46
1.2.5 Ácidos orgánicos 47
1.2.6 Quitosano 48
1.3 CARVACROL Y TIMOL **48**
1.3.1 Carvacrol 49
1.3.2 Timol 50
1.3.3 Utilización de carvacrol y timol en empaques para alimentos 52
1.4 SISTEMAS DE ENCAPSULACIÓN PARA ENVASADO DE ALIMENTOS **53**
1.4.1 Ciclodextrinas 54
1.5 EMULSIONES **58**
1.6 OTROS SISTEMAS **60**
II - OBJETIVOS **63**
III – COMPENDIO DE ARTÍCULOS **67**
ARTÍCULO 1. Development and Characterization of a Biodegradable PLA Food Packaging Hold Monoterpene–Cyclodextrin Complexes against Alternaria alternata. **69**
ARTÍCULO 2. Effect of PLA Active Packaging Containing Monoterpene-Cyclodextrin Complexes on Berries Preservation. **87**

ARTÍCULO 3. Cyclodextrins in polymer-based active food packaging: a fresh look about non-toxic, biodegradable, and sustainable technologies trends. ... 107
IV.- METODOLOGÍA ... 139
V.- RESULTADOS Y DISCUSIÓN ... 145
5.1 FORMACIÓN DE COMPLEJOS DE INCLUSIÓN ... 147
5.2 OBTENCIÓN DE LOS ENVASES ACTIVOS ... 149
5.2.1 Obtención de envases por extrusión ... 149
5.2.2 Obtención de envases por inyección ... 149
5.3 EVALUACIÓN *IN VIVO*, DE LOS EMPAQUES DE PLA CON ENCAPSULADOS DE TIMOL Y CARVACROL EN β-CICLODEXTRINAS .. 155
5.3.1 Pérdida de peso de las zarzamoras y frambuesas ... 155
5.3.2 Color ... 156
5.3.3 Sólidos solubles y Contenido fenólico total ... 157
5.3.4 Análisis de espacio de cabeza del empaque activo ... 158
5.3.5 Análisis microbiológico de las bayas ... 159
5.3.6 Atributos sensoriales ... 161
5.4 USO DE CICLODEXTRINAS (CD) EN TECNOLOGÍAS DE ENVASADO ... 161
VI – CONCLUSIONES ... 165
VII – LIMITACIONES Y FUTURAS LÍNEAS DE INVESTIGACIÓN ... 169
VIII - REFERENCIAS ... 173

ÍNDICE DE TABLAS Y FIGURAS

TABLAS

Tabla 1.1 Microencapsulación de AE con efecto antimicrobiano en CD..... 57

Tabla 1.2 Materiales de empaque antimicrobianos cargados con nanoemulsiones o microemulsiones .....¡Error! Marcador no definido.**59**

Tabla 5.1 Carvacrol y Timol. Solubilidad acuosa (S0), constante de complejación (Kc) con β-CDs, eficiencia de complejación (CE) y relación molar (MR) a pH de 7,0 .....¡Error! Marcador no definido.**147**

Tabla 5.2 Eficiencia de encapsulación (EE) y desempeño del proceso (PP) de los complejos β-CD-carvacrol y *β*-CD-timol en estado sólido¡Error! Marcador no definido.**148**

Tabla 5.3 Propiedades mecánicas de los empaques aditivados β-CD-thymol (1,5%, 2,5%, 50%) y PLA (Control 0%) .....¡Error! Marcador no definido.**150**

Tabla 5.4 Propiedades mecánicas de los empaques aditivados β-CD-carvacrol (1,5%, 2,5%, 5,0%) y PLA (Control 0%) .....¡Error! Marcador no definido.**150**

Tabla 5.5 Valores paramétricos de DSC obtenidos a partir de PLA puro y β -CD-carvacrol o β -CD-timol al 1,5 %, 2,5 % y 5,0 %, (% en peso).¡Error! Marcador no definido.**152**

FIGURAS

Figura 1.1 Mecanismos de liberación de compuestos antimicrobianos incorporados a envases activos por distintas vías (a)(b)(c):, (d): compuestos activos en contacto con la superficie del alimento..... **33**

Figura 1.2 Formas estereoquímicas de lactida.....¡Error! Marcador no definido.**38**

Figura 1.3 Diferentes rutas para producir PLA. ....**¡Error! Marcador no definido.**40

Figura 1.4 Estructura química de carvacrol y timol**¡Error! Marcador no definido.**49

Figura 1.5 (a) Estructura química de las ciclodextrinas (b) Forma geométrica de las ciclodextrinas .....¡Error! Marcador no definido.**55**

Figura 4.1 Metodología desarrollo y análisis de empaque activo con complejos de inclusión de carvacrol y timol .....¡Error! Marcador no definido.**141**

Figura 4.2 Caracterización fisico-química del envase, actividad antimicrobiana in vitro y evalaución de las prestaciones del envase activo en condiciones reales atendiendo a parámetros objetivo de calidad, seguridad y aceptación del consumidor.. **.....141**

Figura 5.1: Resultados del ensayo de difusión en fase vapor (diez días incubación) de PLA-timol-β-CDs (5%), frente a *Botrytis cinérea*..….. **..... …….154**

SIGLAS Y ABREVIATURAS

AE: Aceites esenciales

β-CD: β ciclodextrina

CD: Ciclodextrinas

CE: Eficiencia de complejación

CFT: Contenido fenólico total

ClO_2: Dióxido de cloro

DSC: Calorimetría diferencial de barrido

E: Elongación

EA: Envasado activo

EE: Eficiencia de encapsulación

FDA: U.S. Food and Drug Administration

FTIR: Espectroscopia infrarroja por transformada de Fourier

GC-FID: Cromatografía de gases con detector FID

GRAS: Generally Recognized as Safe

HS-GC-MS: Cromatografía de gases de espacio de cabeza con espectrometría de masas

Kc: Constante de complejación

MIC: Concentración mínimamente inhibitoria

MR: Relación molar radio

PCL: Poli ε-caprolactona

PE: Polietileno

PET: Polietilentereftalato

pH: Potencial de Hidrógeno

PHA: Polihidroxialcanoato

PLA: Ácido poliláctico

PP: Desempeño del proceso

ROP: Método de apertura de anillo

SEM: Microscopio electrónico

S_0: Solubilidad acuosa

SSC: Contenido de sólidos solubles

TC: Coliformes totales

Tg: Temperatura de transición vítrea

TGA: Análisis termogravimétrico

Tm: Temperatura de fusión

TPC: Contenido fenólico total

TS: Fuerza de tensión

WVTR: Tasa de trasmisión de vapor de agua

YM: Hongos y levaduras

I - INTRODUCCIÓN

I - INTRODUCCIÓN

1.1ENVASADO ACTIVO DE ALIMENTOS

El aumento en el consumo de alimentos mínimamente procesados, así como la demanda de productos libres de conservantes de síntesis química y los cambios en las prácticas de distribución de alimentos asociadas a la globalización, son algunas de las razones que han motivado el auge en la investigación e innovación de nuevas tecnologías de envasado de alimentos, las cuales se dirigen a alargar la vida útil del producto, sin alterar los parámetros objetivos de calidad y seguridad. Así, el envasado de alimentos es uno de los retos más importantes a los que se enfrenta la industria alimentaria, particularmente en el área de alimentos mínimamente procesados y de cuarta gamma, ya que el envase en sí, contribuye a la conservación de los mismos, al actuar como contenedor y barrera frente a factores ambientales externos y determinados microorganismos (Han, 2014)..

Hasta hace pocos años, un empaque cumplía la función de proteger al alimento de la contaminación por microorganismos, suciedad e insectos, así como del efecto de algunos factores físicos que intervienen en las reacciones de degradación. Sin embargo, en la actualidad, la funcionalidad ha evolucionado de acuerdo a las necesidades y exigencias de la industria alimentaria. El empaque tiene que cumplir con las normativas relacionadas con los alimentos y el medio ambiente, además de captar la atención del consumidor. Así, bajo estos condicionantes surgen nuevas tendencias en el envasado de alimentos, como los envases ecológicos, activos e inteligentes, entre otros (Catalá y Gavara, 2001).

La tecnología de envasado activo (EA) hace referencia a la adición de moléculas capaces de mantener o mejorar la calidad y seguridad del alimento, las cuales son incorporadas al material de envasado, o bien dentro del propio envase. Éstas moléculas interaccionan directamente con el alimento o con su entorno, incorporando en este caso el envase nuevas propiedades, que trascienden al uso convencional de barrera con el medio externo, al alargar la vida útil del alimento. Por ello, el EA puede ser considerado como una tecnología emergente de conservación de alimentos (Ozdemir y Floros, 2004).

En este tipo de envasado tanto el ambiente interior y el propio empaque interactúan el alimento, ya que pueden incorporar sustancias que bien absorben compuestos que afectan a la vida útil del alimento, como oxígeno, etileno, humedad, dióxido de carbono; o bien liberar determinadas sustancias con carácter antimicrobiano, antioxidante o aromas, entre otras, que mejoran tanto la vida útil como las características organolépticas (Suppakul et al., 2003).

Algunas vías para desarrollar envases activos, se centran habitualmente en la incorporación al envase, de algún tipo de sustancia que reaccione o libere un componente determinado, cuya presencia ayude a mantener la calidad del alimento que se envasa. Por tanto, se requiere que esta sustancia presente una cinética de liberación adecuada entre el envase y el alimento.

También es habitual la utilización de una pequeña bolsa o "pad", con el contenido del componente activo, que se deposita junto al producto en el interior del envase. Algunas bolsas se fabrican con materiales poliméricos, que muestran baja capacidad de barrera frente al componente que se requiere liberar o absorber del medio, para facilitar así que desarrolle su actividad sobre el alimento. Indudablemente, el principio activo utilizado debe ser inocuo, es decir, no debe suponer un riesgo para la seguridad del producto y, por ende, para la del consumidor final. Algunos ejemplos de bolsas que actualmente se utilizan en el empaque activo de alimentos contienen: bicarbonato sódico para liberar dióxido de carbono; zeolitas, las cuales absorben el etileno de algunos empaques de frutas. También se utilizan las de partículas de hierro para retirar oxígeno del medio; sin embargo, el hecho de colocar esta bolsa en el empaque ha generado cierta desconfianza en el consumidor, no habituados a encontrar en el interior del envase objetos extraños en contacto con el alimento, repercutiendo negativamente en la motivación de compra de los mismos y por tanto, en su éxito comercial (López-Rubio et al., 2004).

En este sentido, las investigaciones respecto a este tipo de envases se han dirigido a la introducción del componente activo en el material con el que se elabora el envase, o bien incorporándolo en la superficie del producto a modo de recubrimiento. Recapitulando, se persigue la liberación del componente activo a la atmosfera del empaque o bien al producto envasado, por lo que el material con el que se fabrica el envase debe exhibir unas propiedades determinadas, para no

alterar la funcionalidad y características del empaque que contiene el compuesto activo (Kerry et al., 2006).

Los EA más utilizados en la industria de alimentos son aquellos capaces de eliminar oxígeno del espacio de cabeza, reduciendo así los procesos de deterioro derivados del crecimiento bacteriano, o malos olores y sabores debidos a procesos oxidativos debidos a la presencia de este gas. Los compuestos más utilizados para este fin son aquellos capaces de reaccionar con el oxígeno y reducir, en consecuencia, su concentración en el espacio de cabeza del empaque. Entre ellos, el óxido de hierro es el más empleado, aunque también se pueden utilizar otros como el ácido ascórbico, catecoles o bien la enzima glucosa oxidasa; estos compuestos se incorporan en las etiquetas del empaque, o bien directamente a la propia estructura del envase, como el desarrollado por Farkas (1998), que incorporó hierro en polvo a polietileno de baja densidad, evitando así que el activo, en este caso hierro, fuese visible al consumidor.

Se han descrito algunas investigaciones con diferentes compuestos activos incorporados al material de envasado o al propio alimento, a modo de recubrimiento, que reducen el crecimiento de microorganismos. Así, Han et al., (2008), obtuvieron resultados muy prometedores al incorporar agentes antioxidantes en películas de ácido poliláctico (PLA), tales como: α-tocoferol y resveratrol, ácido ascórbico, catequinas, extractos de té verde, hidroxianisol butilado, hidroxitolueno butilado, galato de propilo y tertbutilhidroquinona (Jamshidian et al., 2013).

1.1.1 Empaques Antimicrobianos

Este tipo de envases tienen un interés particular, ya que responden a las necesidades de la industria para la producción de alimentos más seguros e inocuos, al minimizar la aparición de brotes infecciosos y problemas de salud pública de origen alimentario. En este caso, los compuestos con efecto antimicrobiano, interactúan con el alimento o bien con el espacio de cabeza del envase, consiguiendo reducir, retrasar e incluso inhibir el crecimiento de microorganismos patógenos (Silva-Weiss et al., 2013).

La aplicación de películas o envases antimicrobianos, es por tanto, de suma importancia para la industria de alimentos, ya que el ataque de microorganismos

puede ocurrir en diferentes etapas: desde su elaboración, procesado, distribución y almacenamiento, dando como resultado alteraciones en las características organolépticas de los alimentos, como por ejemplo la generación de olores y sabores desagradables, que pueden representar pérdidas millonarias para la industria, además de originar enfermedades causadas por el consumo de alimentos contaminados, lo que conlleva a un aumento de los gastos en salud pública y a la disminución de la productividad (Mousavi Khaneghah et al., 2018).

Para el desarrollo de este tipo de empaques, los compuestos antimicrobianos que se utilizan habitualmente pueden ser de origen natural o sintético, y se incorporan al empaque de diferentes maneras: directamente a la matriz polimérica, recubriendo la superficie del empaque, o bien se aplican directamente en el alimento. También los podemos encontrar separados físicamente del envase, en su interior en forma de sobres, almohadillas o cápsulas (Limbo y Khaneghah, 2015). Sin embargo, determinados recubrimientos antimicrobianos presentan algunas desventajas, ya que algunos compuestos no muestran actividad al incorporarlos al alimento, ya sea por inmersión en una disolución del compuesto activo o bien, rociando la superficie del alimento con una disolución del mismo, debido a la rápida difusión o volatilización de los compuestos con actividad antimicrobiana y a las reacciones que ocurren al interaccionar con el alimento. Otra desventaja de su aplicación directa estriba en la generación de sabores extraños que alteran en mayor o menor grado, la percepción sensorial del producto, por lo que existen investigaciones encaminadas a la protección de los agentes antimicrobianos con materiales poliméricos, que permiten controlar la velocidad de difusión de estos compuestos, asegurando así una concentración constante sobre la superficie de los alimentos, que permite controlar el crecimiento microbiano, sin provocar alteraciones en las características organolépticas del alimento (Huang et al., 2019).

La efectividad de estos EA depende de diferentes factores: el tipo de compuesto antimicrobiano y sus interacciones con la matriz polimérica, el microorganismo cuyo crecimiento se desea controlar o inhibir, y la correspondiente concentración mínima inhibitoria (MIC) requerida, además de la cinética de liberación del activo (Figueroa-Lopez et al., 2020).

Las posibles interacciones del compuesto activo con los componentes de los alimentos también afectarán, en gran medida, a la efectividad antimicrobiana. En este sentido, los siguientes aspectos den ser tenidos en cuenta:

a) La velocidad de liberación del agente antimicrobiano desde la película plástica o bien del polímero, debe ser constante y hacerse en el tiempo requerido para maximizar su eficacia. Habitualmente, la liberación rápida del agente antimicrobiano del material de empaque hacia el alimento es muy frecuente, y es una etapa limitante porque disminuye el tiempo de contacto entre ambos sistemas.

b) Se deben utilizar agentes antimicrobianos seguros e inocuos, que estén aceptados por las regulaciones internacionales, para ser utilizados en alimentos.

En la Figura 1.1 se representan diferentes mecanismos de interacción entre agentes activos, el material de envasado y el alimento (Bastarrachea et al., 2011):

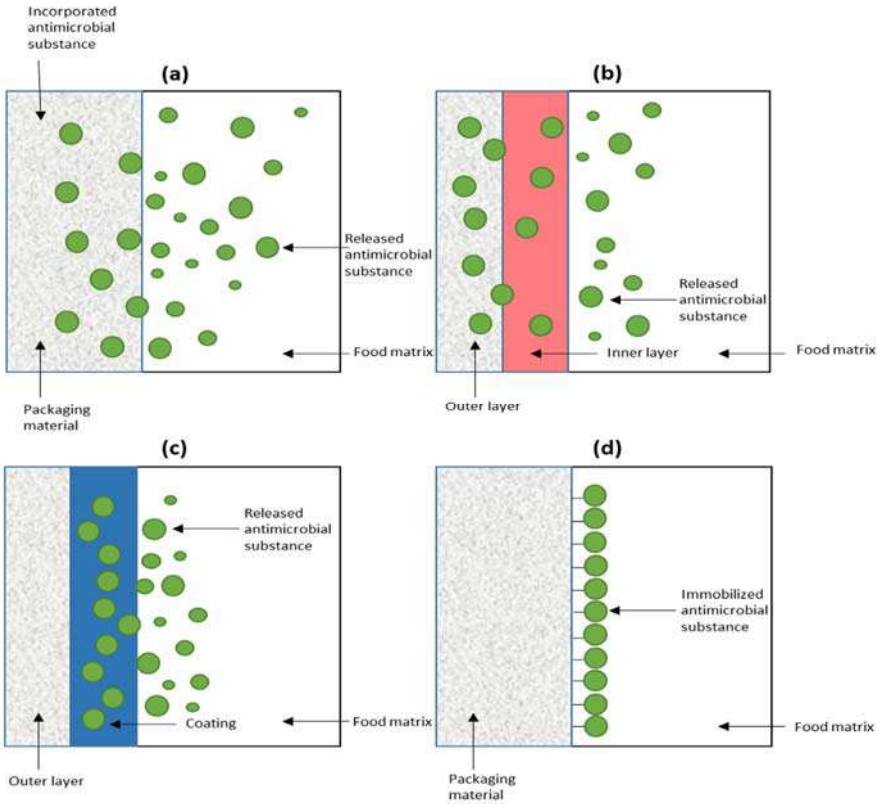


Figura 1.1. Mecanismos de liberación de compuestos antimicrobianos incorporados a envases activos por distintas vías (a)(b)(c):, (d): compuestos activos en contacto con la superficie del alimento (Bastarrachea et al., 2011).

Como se muestra en la Figura 1.1, el propio envase o bien las películas situadas en el interior, permiten la liberación del compuesto activo conforme a su cinética. Éste se puede incorporar al material de empaque, dentro de la matriz, o bien en la superficie, interactuando por una de estas vías con el alimento:

a) Sistemas de envasado que incorporan el agente activo en una capa única, lo que permite una liberación gradual hacia el alimento.
b) Sistemas con una capa interna, a fin de controlar la velocidad de liberación de agentes activos hacia la capa externa.
c) Sistemas de envasado con una capa de recubrimiento que contiene el agente activo.
d) Empaques o películas que no liberan el agente activo, pero muestran efectividad al estar en contacto directo con el alimento, por lo que se inhibe el crecimiento microbiano en la superficie del alimento.

Si bien se han utilizado diferentes metodologías para la incorporación de compuestos activos al material de embalaje, que han demostrado su eficacia como empaques antimicrobianos; se han evidenciado ciertos inconvenientes como la degradación rápida de los compuestos activos o una cinética de liberación no adecuada, que han condicionado la búsqueda de metodologías alternativas. Así, investigaciones recientes describen el empleo de técnicas de encapsulación como una alternativa viable. Se trata de un proceso mediante el cual, pequeñas partículas de compuestos activos, se empaquetan dentro de diferentes materiales para formar cápsulas, que protejan a los compuestos activos de determinados factores o procesos que puedan degradarlos, permitiendo además una liberación controlada de los mismos (Wen et al., 2016).

En concreto y, en relación a los empaques antimicrobianos, la capacidad para ralentizar o inhibir el crecimiento de microorganismos, depende en gran medida de la estructura química del agente empleado, pudiendo clasificarlos en naturales y sintéticos. Si bien, las tendencias de mercado a nivel internacional se centran en la utilización de compuestos antimicrobianos de origen natural, no tóxicos, como por ejemplo los aceites esenciales (AE) o determinados extractos de plantas (Periago et al., 2001), péptidos y bacteriocinas (Thallinger et al., 2013).

De entre los citados anteriormente, numerosas investigaciones se han centrado en la aplicación de AE directamente en el alimento o bien, incluyéndolos en matrices poliméricas, como vía para la evaluación de su actividad antimicrobiana. De hecho, tanto los AE como sus componentes individuales, representan una alternativa natural a los aditivos de síntesis química utilizados en alimentos, debido a su capacidad para inhibir el crecimiento de una amplia variedad de microorganismos patógenos o no, presentes en alimentos (Solórzano-Santos y Miranda-Novales, 2012). Los AE presentan una composición compleja, y sus componentes mayoritarios pertenecen a la familia de los terpenoides y compuestos fenólicos, que presentan propiedades antimicrobianas y antioxidantes (Atarés y Chiralt, 2016). Estas propiedades han sido evidenciadas en la literatura por González y Alvarez Igarzabal (2013), logrando inhibir el crecimiento de *Staphylococcus aureus, Escherichia coli,* y *Aspergillus sp.,* utilizando películas de ácido poliláctico (PLA) conteniendo diferentes concentraciones del monoterpeno timol.

Sin embargo, se requieren habitualmente altas concentraciones de AE o de alguno de sus componentes mayoritarios, para alcanzar la efectividad deseada, incidiendo desfavorablemente en las características organolépticas del alimento. Además, la naturaleza altamente volátil de estos compuestos, junto con su gran sensibilidad a la oxidación, los hace muy vulnerables al deterioro cuando se encuentran desprotegidos, limitando por tanto su aplicación en la superficie de los envases alimentarios. Este inconveniente puede solventarse incluyendo o encapsulando estos compuestos. Así, diferentes aproximaciones descritas en la literatura revelan una mejora de la actividad antimicrobiana de los AE tras ser complejados y aditivados posteriormente a diferentes biopolímeros, evidenciando su eficacia *in vitro* mediante ensayos de difusión en agar (Hernández-Figeroa et al., 2013).

1.1.2 Utilización de biopolímeros en envases para alimentos

En la actualidad, una vía para disminuir los problemas de contaminación debida a los desechos de empaques plásticos, consiste en el desarrollo de materiales alternativos como los biopolímeros, los cuales son elaborados a partir de fuentes renovables, y por tanto son biodegradables o compostables. Los biopolímeros se pueden usar para sustituir plásticos no biodegradables por otros materiales más

naturales y ecológicos, llamados bioplásticos, reduciendo así el impacto ambiental y la dependencia del petróleo (Geueke, 2015).

Habitualmente, los biopolímeros se producen a partir de recursos renovables y presentan ciertas ventajas, como la reducción de emisiones de dióxido de carbono y su marcada capacidad de degradación, siendo tanto el almidón como el PLA los más utilizados en la industria (Barnett, 2011). De acuerdo a Byun y Kim (2013), se pueden clasificar en cuatro categorías, atendiendo a su composición química, origen y método de síntesis:

1. Biopolímeros que se obtienen directamente de la biomasa (almidón, proteína, celulosa, etc.).
2. Biopolímeros producidos por síntesis química a partir de sus monómeros (PLA, PE de base biológica, etc.).
3. Biopolímeros producidos por fermentación microbiana, como los polihidroxialcanoatos.
4. Biopolímeros producidos por síntesis química a partir de sus monómeros bioderivados y monómeros obtenidos del petróleo, como polisuccinato de butileno o politereftalato de trimetileno, entre otros (Mittal, 2012; Robertson, 2008).

Atendiendo a su ciclo de vida, se pueden dividir en tres categorías:

1. Biopolímeros compostables (PLA).
2. Biopolímeros biodegradables (almidón, PHA, Polisuccinato de butileno, etc.).
3. Bioplásticos reciclables (PET de base biológica, PP de base biológica, PE de base biológica, etc.).

Las propiedades funcionales (mecánicas y de barrera) de los biopolímeros, deben adaptarse indudablemente a los requerimientos del alimento a empacar, empleando para ello diferentes técnicas de naturaleza química, mezclas con otros componentes, sustancias plastificantes o protectoras como las ciclodextrinas.

Para la formulación de empaques biodegradables, se necesita utilizar al menos un material capaz de formar una matriz con suficiente continuidad y cohesión para poder empacar un determinado producto, es decir, debe formar una película. Además, es necesario que la matriz presente permeabilidad al oxígeno y

sea capaz de regular la difusión del vapor de agua (WVTR). Además, debe exhibir determinadas propiedades mecánicas, como la fuerza de tensión (TS), la elongación (E) o el alargamiento antes de la ruptura. Generalmente, los biopolímeros forman estructuras continuas, que pueden ser cristalinas o amorfas, y éstas actúan como una barrera frente a determinadas sustancias, que se desea evitar que interactúen con el alimento.

1.1.2.1 Ácido Poliláctico

El ácido poliláctico (PLA) es un biopolímero derivado del ácido láctico, termoplástico y biocompatible, que se obtiene de recursos renovables como el azúcar de caña, maíz, betabel, almidón de papa y arroz. Si bien el PLA es el biopolímero más utilizado para el envasado de alimentos, también ha sido ampliamente utilizado en el sector biomédico, ya que sus propiedades son adecuadas para la elaboración de prótesis óseas y el cierre de heridas (Mano et al., 2005).

La aplicación de este biopolímero en alimentación presenta numerosas ventajas: Se obtiene de fuentes renovables como el maíz, la producción de PLA consume grandes cantidades de dióxido de carbono y proporciona importantes ahorros de energía; además es reciclable y compostable. Presenta unas propiedades térmicas, mecánicas y de barrera comparable al de los polímeros sintéticos más utilizados para el empaque de alimentos. Varios estudios han demostrado que la migración de ácido láctico del empaque a los alimentos es mínima, por lo que los polímeros derivados del ácido láctico son una buena alternativa para el desarrollo de empaques de alimentos, en especial para alimentos de vida de anaquel corta, como por ejemplo ensaladas, frutas y verduras frescas. (Auras et al., 2004).

1.1.2.2 Estructura química y propiedades del ácido poliláctico

El PLA puede obtenerse mediante la conversión química de diferentes carbohidratos en dextrosa; ésta es fermentada en ácido láctico seguido de una policondensación de monómeros de ácido láctico o lactida. El PLA de alto peso molecular se produce a partir del éster de dilactato (lactida), la cual es una molécula quiral que existe en las dos formas ópticas *L*-lactida y *D*-lactida (Wei et al., 2011). Si bien existen diferentes rutas de síntesis del PLA, la polimerización de la lactida con

apertura de anillo es la más habitual, que da lugar a la formación de polímeros PLA semicristalinos (Auras et al., 2004).

El PLA presenta tres estructuras estereoquímicas de lactida (Figura 1.2); *L*, *D*- o ambas, *D*-Lactida (Meso-lactida). Cada una tiene su propia temperatura de fusión. En aplicaciones de envasado, se ha usado ampliamente la poli (*D*, *L*-lactida) conteniendo un 90% de *L*-lactida. A pesar de que el aumento de la concentración de *D*-lactida da lugar a la formación de polímeros de PLA con una estructura más cristalina, y películas de PLA con mejor estabilidad térmica, resistencia mecánica y propiedades de barrera; sin embargo, el PLA con una alta concentración de *D*-lactida no es comercialmente viable, debido a su elevado coste (Auras et al., 2004).

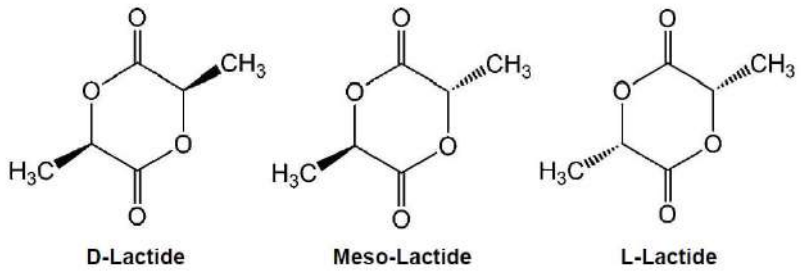


Figura 1.2 Formas estereoquímicas del anillo lactida (Auras et al., 2004).

El PLA es insoluble en agua, etanol, metanol e hidrocarburos alifáticos y soluble en cloroformo, benceno, acetonitrilo, acetona, acetato de etilo y diclorometano. Dependiendo de su peso molecular y estereoquímica, su vida media de degradación oscila entre seis meses y dos años, tal y como se ha evidenciado en ensayos *in vitro* e *in vivo* (Ho et al., 1999). Puede procesarse mediante extrusión, moldeo por inyección o soplado, termoformado, hilado de fibras y formación de películas (Rasal et al., 2010). Presenta un comportamiento en procesos térmicos que mejora el observado para otros bioplásticos, como el polihidroxialcanoato (PHA), o la poli (*ε*-caprolactona) (PCL). Esta propiedad está relacionada con el cociente entre sus formas D y L, en especial con la forma *L*-PLA, que presenta mayor cristalinidad y permite alcanzar temperaturas de fusión más altas.

El PLA presenta ciertas propiedades similares a otros polímeros. Tiene una temperatura de transición vítrea (Tg) relativamente alta, y una temperatura de

fusión (Tm) baja, en comparación con otros materiales termoplásticos. El PLA tiene propiedades similares a las del celofán, el polipropileno orientado y el polietileno orientado. La resistencia a la tracción y el módulo elástico del PLA son comparables a las del polietilentereftalato (PET). Si bien las propiedades de una botella de PLA son muy similares a las de una botella de PET convencional; en su fabricación se genera un 44% menos de dióxido de carbono, y consume un 36% menos de energía que en la producción de una botella de PET (Auras et al., 2004).

El PLA con un contenido de *L*-lactida superior al 90% tiende a ser cristalino, presenta un temperatura de fusión entre 173-178 °C y un módulo de tracción en el intervalo 16-27 GPa (Södergård & Stolt, 2010). Estas propiedades son adecuadas para la producción de un polímero transparente, con resistencia mecánica similar al PET y poliestireno (PS). También presenta baja toxicidad, lo cual permite ampliar las aplicaciones de este biopolímero al envasado de alimentos, ya que está clasificado como GRAS (*Generally Recognized as Safe*) por la Administración de Drogas y Alimentos de los Estados Unidos (U.S FDA).

1.1.2.3 Producción y procesado de ácido poliláctico

La unidad básica del PLA es el ácido láctico, puede ser obtenido por fermentación de carbohidratos o síntesis química. El ácido láctico (ácido 2-hidroxipropiónico), es un ácido hidroxílico simple con un átomo de carbono asimétrico; presenta dos configuraciones ópticamente activas, la L (+) y la D (-), las cuales son isómeros y se producen en sistemas bacterianos.

La mayor parte del ácido láctico se produce mediante fermentación bacteriana de carbohidratos, clasificando habitualmente el proceso de fermentación en función del tipo de bacterias utilizadas, siendo los heterofermentativos y homofermentativos los más comunes (Auras et al., 2004).

A nivel industrial, el método más utilizado es el homofermentativo ya que produce 1,8 moles de ácido láctico por mol de hexosa, y genera menos subproductos. Actualmente, este proceso utiliza un género de lactobacilos que produce una alta tasa de ácido láctico.

El PLA tiene un peso molecular variable que oscila entre 1000 y 100.000 Da. Como se muestra en la Figura 1.3, el PLA de alto peso molecular se puede obtener por diferentes rutas sintéticas:

1. Reacciones de policondensación:

a) Condensación directa.
b) Condensación directa en soluciones azeotrópicas.
c) Condensación en estado sólido.

2. Polimerización mediante la formación de lactida, que implica la apertura posterior del anillo.

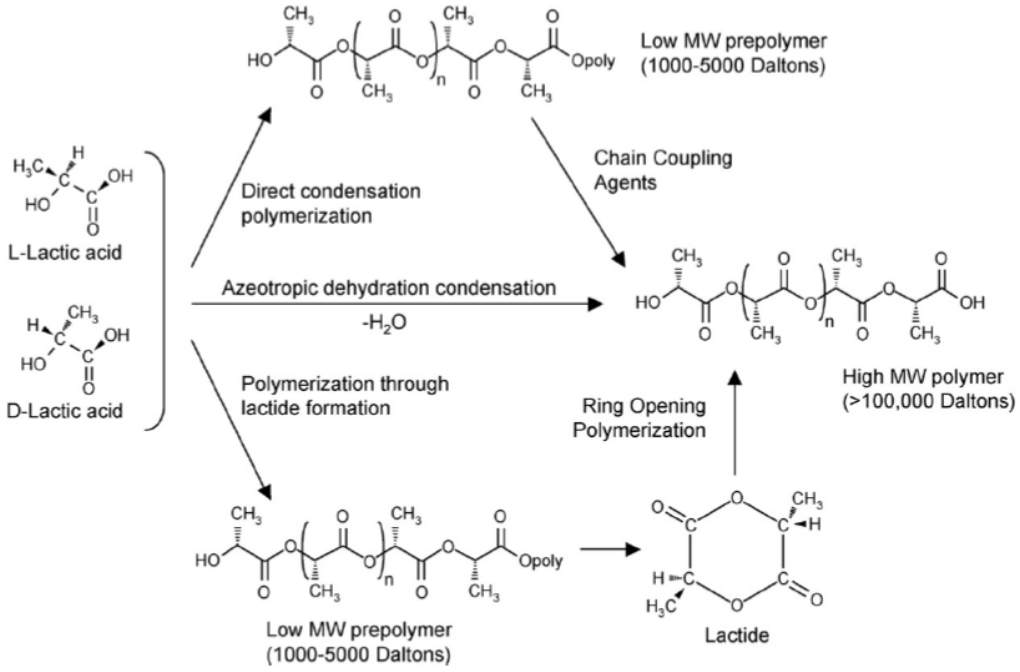


Figura 1.3 Diferentes rutas para producir PLA. (Adaptado de Auras et al. 2004).

La condensación directa se basa en la polimerización del ácido láctico (LA), en presencia de catalizadores, a bajas presiones y altas temperaturas. Los catalizadores más utilizados en este proceso son los óxidos de antimonio y compuestos organometálicos. Sin embargo, el PLA que se obtiene es de bajo peso molecular, y no es apto para aplicaciones de envasado de alimentos.

Como se ha indicado, el PLA también se puede obtener por condensación directa en soluciones azeotrópicas. Este método permite la obtención de polímeros

de alto peso molecular, ya que disminuye el punto de fusión del polímero, evitando reacciones de despolimerización (Gupta & Kumar, 2007). Las soluciones azeotrópicas utilizadas habitualmente son mezclas de dos o más líquidos, de modo que su composición no varía por destilación simple, y ayudan a eliminar el agua como subproducto, dando como resultado la obtención de PLA con alto peso molecular, llegando hasta 300 kDa.

El método de apertura de anillo (ROP) es el más utilizado para producir PLA, ya que permite un control riguroso del proceso, para obtener polímeros con elevado peso molecular y baja dispersión. Se basa en la síntesis de la lactida, un dímero cíclico de ácido láctico con tres formas diferentes: *L*-lactida, *D*-lactida, meso-lactida, que pueden generar mezclas poliméricas, dando como resultado la obtención de PLLA, PDLA y poli (ácido láctico) (PDLLA), que se sintetizan a partir de *L*-lactida, *D*-lactida y *DL*-lactida respectivamente. Los pesos moleculares, así como las propiedades mecánicas y de degradación se pueden controlar mediante el proceso ROP (Auras et al., 2004).

La polimerización a través de la formación de lactida es el método utilizado en la actualidad por Cargill Dow LLC (Auras et al., 2004), para obtener polímeros de alto peso molecular con fines comerciales. A partir de la fermentación con dextrosa, el ácido *D*-láctico, el ácido *L*-láctico, o una mezcla de los dos, se pre-polimerizan para obtener un poli (ácido láctico) intermedio de bajo peso molecular, para posteriormente, en condiciones de presión baja, transformarlos con la ayuda de un catalizador, en una mezcla de estereoisómeros de lactida. La lactida, el dímero cíclico del ácido láctico, se forma por la condensación de dos moléculas de ácido láctico como se describe a continuación: L-lactida (dos moléculas de ácido L-láctico), *D*-lactida (dos moléculas de ácido *D*-láctico) y meso- lactida (un ácido *L*-láctico y una molécula de ácido *D*-láctico). Posteriormente, se realiza una destilación al vacío de la lactida, para obtener un PLA de alto peso molecular, con pureza óptica específica.

El PLA exhibe buenas prestaciones para ser procesado térmicamente por diferentes vías, como por ejemplo el moldeo por inyección, extrusión de láminas, moldeo por soplado, termo formado, formación de película o hilado de fibras. Dependiendo del tipo de proceso que se vaya a utilizar, se deben controlar algunos parámetros como el contenido de isómero D y distribución de peso molecular. Los estereoisómeros L, D o meso-lactida, pueden incorporarse al esqueleto del

polímero, obteniendo así diferentes tipos de PLA para aplicaciones específicas (Auras et al., 2004).

Aunque el PLA es hidrofóbico, los gránulos generalmente deben secarse a temperaturas entre 60 y 100 °C durante varias horas antes del procesamiento, para evitar una hidrólisis excesiva y modificaciones en las propiedades físicas del polímero (Lim et al., 2008).

La técnica más habitual para procesar PLA es la extrusión. De hecho, una extrusora permite que los gránulos se mezclen de manera homogénea a alta temperatura. El PLA también se puede disolver en cloroformo y otros disolventes orgánicos como el diclorometano, cloruro de metileno o acetonitrilo y, posteriormente, puede ser moldeado para obtener unas películas con elevada transparencia y brillo (Hughes et al., 2012).

1.1.2.4 Aplicación en envasado de alimentos

La utilización de PLA presenta numerosas ventajas, ya que es un material biodegradable, renovable y biocompatible, y ha sido aprobado por la FDA para el contacto directo con alimentos y fluidos biológicos. Presenta buenas propiedades de barrera y es bastante transparente. Tiene características muy parecidas a las de los plásticos sintéticos obtenidos del petróleo, como el tereftalato de polietileno (PET) o el poliestireno (PS), pudiendo ser una alternativa natural para el envasado de alimentos.

Debido al desarrollo de nuevas tecnologías de producción industrial, el PLA se obtiene a un precio muy competitivo. No obstante, el PLA tiene una capacidad de barrera a los gases limitada, debido a su naturaleza hidrofóbica. A pesar de su fuerte resistencia a la tracción (de 17 a 74 MPa), es muy frágil, y presenta una capacidad de alargamiento a la rotura inferior al 10% (Hughes et al., 2012). Se ha empleado en numerosas aplicaciones biomédicas, como ingeniería de tejidos y biomateriales, debido a que presenta una adecuada capacidad de absorción de determinadas sustancias (Armentano et al., 2013).

1.2 ADITIVOS NATURALES

Los agentes antimicrobianos son compuestos químicos, presentes o añadidos a los alimentos, que retardan el crecimiento microbiano o bien causan la muerte de

los microorganismos (Alzamora et al., 2000). Entre los antimicrobianos naturales más estudiados se encuentran los compuestos de origen vegetal, presentes en las plantas, hierbas y especias, que se utilizan habitualmente en alimentos sustituyendo a los de origen sintético, debido a la tendencia de los consumidores por el consumo de productos naturales, dependiendo la capacidad antimicrobiana del EA, de la naturaleza del compuesto empleado.

Así, la elección del tipo de compuesto dependerá en gran medida del microorganismo diana, de la compatibilidad del antimicrobiano con los alimentos envasados, de las posibles interacciones con el oxígeno, así como otros factores como: la liberación del compuesto activo durante su almacenamiento, su distribución, las propiedades físicas y mecánicas de los materiales de embalaje, así como su posible repercusión sobre las características organolépticas del alimento.

Los conservadores de naturaleza sintética y actividad antimicrobiana se utilizan en la actualidad con relativa frecuencia en el envasado de alimentos. Por lo general, son compuestos químicos que presentan en su formulación ácido etilendiaminotetraacético (EDTA), fungicidas y parabenos, entre otros. Sin embargo, hoy en día existe un particular interés por el consumo de productos libres de aditivos sintéticos. Por esta razón, se ha generado un interés particular en la investigación de sustancias de origen natural que ayuden a extender la vida útil de los alimentos y aseguren la inocuidad de los mismos. Por lo general, este tipo de compuestos proceden de diferentes fuentes de origen animal y vegetal, que incluyen plantas, bacterias, algas, hongos, levaduras, etc.; por lo general son relativamente seguros y fáciles de obtener. A continuación, se describen algunos de ellos.

1.2.1. Aceites Esenciales

Los aceites esenciales (AE) son lipídicos complejos producidos de forma natural a través del metabolismo secundario de fuentes vegetales, y los podemos encontrar en flores, brotes, semillas, hojas, madera, frutas, raíces, ramas y corteza. Las AE contienen una mezcla compleja de compuestos volátiles de origen natural, obtenidos habitualmente mediante técnicas de extracción más o menos complejas, a partir de material vegetal (Huang et al., 2019), incrementando la pureza mediante procesos de destilación. Los extractos presentan numerosos compuestos de baja

masa molecular y concentración variable, entre ellos monoterpenos, diterpenos, triterpenos y compuestos fenólicos, los cuales confieren propiedades antimicrobianas, antifúngicas, antioxidantes, antivirales, antimicóticas, antitoxigénicas, antiparasitarias, antibióticas y antisépticas, además de ser reconocidos como sustancias seguras (GRAS), por la U.S. FDA.

Los componentes de los aceites esenciales afectan por lo general, a funciones metabólicas importantes en la célula microbiana, como por ejemplo la respiración o la capacidad para producir determinadas toxinas; sin embargo, su efectividad estará condicionada por la estructura de la molécula y capacidad de unión a los sitios de reconocimiento específico presentes en la célula microbiana (López-Malo et al., 2020). Numerosos estudios han evidenciado que los AE pueden provocar un deterioro importante en la pared celular y la integridad de la membrana citoplasmática de los microorganismos, afectando tanto a la funcionalidad de las proteínas de membrana, como a los sustratos involucrados en la síntesis de ATP, desencadenando una serie cambios que conducen a la muerte de las células, y por ende, de los microorganismos (Gutierrez et al., 2008; Hernández-Figeroa et al., 2013).

Los AE han demostrado su potencial, situándose como una alternativa eficaz a los antimicrobianos de síntesis; sin embargo, la aplicación directa de la mayoría de los AE se ve limitada por las elevadas concentraciones requeridas para inhibir el crecimiento de microorganismos, dosis que modifican el sabor de los alimentos, superando el umbral de aceptación organoléptica de los consumidores.

La utilización de AE en el envasado de alimentos tiene como principal objetivo alargar la vida de anaquel y se emplean habitualmente tres procedimientos para el desarrollo de EA que los contengan: (i) Se introducen en bolsitas dentro del envase; (ii) Se aplican directamente al alimento; (iii) O bien al envase que lo contiene; evidenciando actualmente una marcada tendencia al empleo de polímeros biodegradables como el PLA, gelatinas y quitosano, todos ellos respetuosos con el medioambiente. Así, Llana-Ruiz-Cabello et al. (2016), introdujeron diferentes concentraciones del aceite esencial de *Origanum vulgare L.* en películas de PLA, evaluando la actividad antimicrobiana de la película resultante para su empleo en ensaladas listas para el consumo, obteniendo como resultado un nuevo envase activo que reveló buenas propiedades antimicrobianas frente a levaduras y hongos, a concentraciones de aceite esencial de orégano del 5%

y 10%, respectivamente. En otro estudio, Abdollahi, Rezaei, y Farzi (2012) incorporaron el aceite esencial de romero a una película de quitosano, biopolímero obtenido de la quitina y utilizado habitualmente en el envasado de alimentos debido a sus propiedades absorbentes, evidenciando mejores propiedades antimicrobianas que la película de quitosano sin aditivar con el AE.

1.2.2 Extracto de semilla de uva

El extracto de semilla de uva contiene abundantes compuestos fenólicos como catequinas, epicatequinas, ácido gálico y procianidinas, que exhibe un amplio espectro de acción antimicrobiana, llegando a inhibir a determinadas concentraciones el crecimiento de bacterias Gram positivas y Gram negativas (Cvetnic & Vladimir-Knezevic, 2004). Este efecto ha sido evidenciado por Tan et al. (2015), que incorporaron el extracto de semilla de uva en envases alimentarios obtenidos con polímeros sintéticos (PET) y naturales (policaprolactona y quitosano), observando una mejora en la cristalinidad en el envase de policaprolactona aditivado con el extracto de uva, obteniendo películas más suaves y homogéneas y con una marcada actividad antimicrobiana frente a *Pseudomonas aeruginosa*. También mejoro las propiedades del quitosano a concentraciones del 0,5%, 1,0% y 1,5% v/v, sin alterar la transparencia y mejorando el alargamiento a la rotura de la película obtenida, que mostraron efectividad in vitro frente al crecimiento fúngico.

1.2.3 Bacteriocinas

Las bacteriocinas son sustancias bactericidas producidas por determinadas bacterias, codificadas por genes y sintetizadas por ribosomas. Presentan varias estructuras, y entre las más comunes destacan la nisina y la pediocina.

La nisina es un péptido antibacteriano producido por la bacteria ácido láctica *Lactococcus lactis*, de amplio uso en alimentos, ya que su empleo está aprobado por la FDA, y se puede combinar fácilmente con diferentes materiales poliméricos para el desarrollo de EA con propiedades antimicrobianas.

Se ha descrito que la nisina puede inhibir el crecimiento de un amplio espectro de microorganismos gram positivos, como *Listeria monocytogenes* (Soto et

al., 2016), así como la germinación de esporas (Vessoni Penna et al., 2002). Presenta en su estructura cargas positivas y grupos hidrofóbicos, que le confieren una actividad específica. Así, las cargas positivas provocan la interacción electrostática entre el péptido y las membranas celulares cargadas negativamente de las bacterias, lo que permite que el péptido interaccione con a la membrana celular bacteriana al tratarse de cargas de distinto signo (Jenssen et al., 2006). Dado que la membrana celular bacteriana tiene carácter hidrofóbico, el aminoácido hidrofóbico presente en la nisina puede insertarse en la célula bacteriana, cambiando así la permeabilidad de la membrana celular bacteriana haciéndola inestable. Ello promueve la salida al exterior de diferentes estructuras celulares, como el ADN, provocando la muerte bacteriana. Su efectividad antimicrobiana, derivada de su presencia en EA, ha sido evidenciada en diferentes estudios (Cao-Hoang et al., 2010; Gharsallaoui et al., 2015).

Recientemente, Divsalar et al., (2018) desarrollaron una película biodegradable a partir de quitosano y celulosa, incorporando nisina, para alargar la vida útil de queso, comparando con un control sin aditivar. Si bien la película plástica de quitosano-celulosa no mostró actividad antimicrobiana frente a *Listeria monocytogenes*, la que incorporaba nisina mostró un incremento significativo en la actividad antimicrobiana frente a este microorganismo, llegando a inhibir su crecimiento.

La pediocina es otra bacteriocina de uso frecuente, producida mayoritariamente por *Pediococcus acidilactici.* Se trata de una proteína de pequeño tamaño, no modificada, que muestra una buena estabilidad térmica y un amplio espectro antimicrobiano. Su funcionalidad se mantiene en un amplio rango de pH, y es relativamente estable a las variaciones de temperatura. Tras ser ingerida, se puede degradar en el cuerpo humano debido a la naturaleza del polipéptido y puede inhibir diversas bacterias patógenas trasmitidas por los alimentos, como a *Listeria monocytogenes*, por lo que muestra un alto potencial para el desarrollo y aplicación de EA biodegradables.

1.2.4 Lisozimas

La lisozima, es una enzima abundante en numerosas secreciones como la saliva, lágrimas y la mucosidad, que encontramos también a concentraciones

importantes (40 mg/100 mL) en la leche materna. Está formada por una cadena mono-peptídica hidrófila (Muriel-Galet et al., 2013). Puede inhibir infecciones bacterianas, especialmente las causadas por bacterias Gram positivas. La actividad antimicrobiana de la lisozima se debe a su capacidad para hidrolizar los enlaces glucosídicos beta-1-4, entre el ácido N-acetilmurámico y la N-acetil-glucosamina en los peptidoglucanos (Irkin & Esmer, 2015). Esta enzima destruyen la pared celular ya que hidroliza el peptidoglucano, provocando así la muerte de la célula bacteriana (Cha & Chinnan, 2004).

Estas enzimas se han utilizado para el desarrollo de diferentes EA con efecto antimicrobiano (Gemili et al., 2009; Mousavi Khaneghah et al., 2018; Zhang et al., 2018), incorporándolas al material polimérico a través de una mezcla física o mediante enlace químico. Así, Gemili et al. (2009) consiguieron regular la velocidad de liberación de la lisozima incorporada a una película de acetato de celulosa, inicialmente asimétrica, convirtiéndola en más porosa y compacta. La estructura y morfología de la superficie de la película influye notablemente en la pauta y mecanismo de liberación de la enzima por difusión.

1.2.5 Ácidos orgánicos

Los ácidos de naturaleza orgánica como el ácido propiónico, el ácido láctico, el ácido málico, el ácido sórbico y el ácido tartárico, se usan habitualmente como aditivos alimentarios, ya que están reconocidos como GRAS. Se ha descrito la capacidad antimicrobiana de numerosos compuestos pertenecientes a esta familia, como el benzoato de sodio, ácido sórbico y el sorbato de potasio, presentan actividad antimicrobiana de amplio espectro, inhibiendo el crecimiento de bacterias y mohos (Jideani y Vogt, 2016). Otros como el citrato de sodio han resultado ser eficaces frente a *Listeria monocytogenes* y *Escherichia coli* O157: H7 (Morey et al., 2014).

Apoyándose en la marcada actividad antimicrobiana descrita para estos compuestos, Hu et al. (2017) desarrollaron películas poliméricas de Etilen-Vinil-Alcohol (EVOH), aditivandolas con ácido sórbico libre, o bien con micro cápsulas de ácido sórbico en quitosano, y las utilizaron para mejorar la vida útil de filetes de pescado; observando una acusada inhibición del crecimiento de *Escherichia coli, Salmonella enteritidis* y *Listeria monocytogenes*, en los filetes de pescado recubiertos

con las películas que incorporaban las microcápsulas, superior a las muestras que incorporaban el sórbico libre, probablemente debido a una liberación sostenida en el tiempo (menor velocidad), cuando el ácido sórbico está encapsulado.

1.2.6 Quitosano

El quitosano es un biopolímero de aminopolisacáridos, y está compuesto por unidades de *β*-(1-4), *D*-glucosamina (unidades desacetiladas) y N-acetil-*D*-glucosamina (unidad acetilada), que se distribuyen aleatoriamente. Exhibe propiedades antimicrobianas y se usa habitualmente en medicina, el envasado de alimentos y en compartimentos medioambientales, dada su capacidad para retener compuestos de síntesis química como los contaminantes emergentes (Lago et al., 2014).

El quitosano es un buen material de partida para el desarrollo de empaques antibacterianos, ya que permite desarrollar películas muy delgadas y no presenta toxicidad, sin embargo, se disuelve rápidamente en soluciones ácidas y sus propiedades mecánicas no son idóneas para el envasado de alimentos en contacto con ambientes húmedos, es decir, refrigerados.

Así, estas desventajas pueden solventarse combinándolo con otros polímeros o aditivándolo con determinados compuestos, encapsulados o no. Esta aporximación fue llevada a la práctica por Ojagh et al. (2010), obteniendo películas biodegradables de quitosano y AE de canela, que mostraron buena actividad antimicrobiana y baja afinidad al agua.

1.3 CARVACROL Y TIMOL

Carvacrol (2-metil-5-(1-metiletil)fenol) y timol (2-isopropil-5-metilfenol), son dos monoterpenos de naturaleza fenólica, ampliamente utilizados como aditivos naturales. Ambos compuestos son isómeros y se obtienen de los aceites esenciales de plantas aromáticas de la familia Lamiáceas (*Lamiaceae*), incluyendo los géneros de *Origanum, Satureja, Thymbra, Thymus* y *Corydothymus* (Nostro y Papalia, 2012).

Ambos compuestos han demostrado tener una marcada actividad antifúngica, antibacteriana, antimutagénica y antitumoral, siendo también utilizados como insecticidas. Ambos monoterpenos han sido ampliamente

utilizados, evidenciando su actividad antimicrobiana de amplio espectro frente a bacterias gran positivas y gram negativas, hongos y levaduras (Babili et al., 2011; Chizzola et al., 2008; Kordali et al., 2008; Xu et al., 2008).

Tanto carvacrol como timol, pueden ser utilizados como aditivos alimentarios en el envasado de alimentos, ya que ambos han sido reconocidos como seguros (GRAS) por la administración de alimentos y bebidas (FDA) de los Estados Unidos; además, la Comisión Europea los han clasificado como sustancias aromatizantes de aplicación alimentaria, ya que su empleo no supone un riesgo para la salud del consumidor, tal y como establece el Reglamento de la Comisión/(UE)/(No-10/2011).

La presencia de un hidroxilo en las estructuras de ambos isómeros, potencia sus propiedades antimicrobianas y antioxidantes, ya que, en ambos casos, el grupo hidroxilo actúa como un intercambiador de protones, promoviendo la deslocalización de electrones y reduciendo el gradiente de pH en la membrana citoplasmática. Esta interacción provoca el colapso de la fuerza motriz de protones, dificultando la permeabilidad de la membrana, y la consiguiente pérdida del contenido interno celular, conduciendo en última instancia, a la muerte de las células microbianas (Figura 1.4).

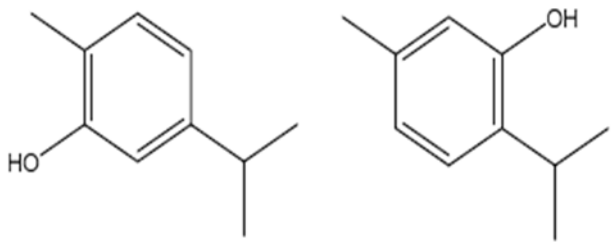


Figura 1.4 Estructura química de carvacrol y timol.

1.3.1 Carvacrol

Carvacrol es un monoterpeno mayoritario en los aceites esenciales de orégano (entre un 60 y 70%), y tomillo (aproximadamente un 45%). Se encuentra presente en otras plantas aromáticas y especias como el comino negro (*Nigella sativa*

L.) y la mejorana (*Origanum majorana* L.) (López-Mata et al., 2013), a menor concentración.

Su estructura química es de tipo fenólico (Fig.1.3). Es un líquido denso de color amarillo, poco soluble en agua (1,25 g/L a 25 °C), pero muy soluble en diversos disolventes orgánicos (Pow: log P 3,49). Presenta una marcada actividad antimicrobiana, ejerciendo un efecto disruptor sobre la membrana externa de las bacterias Gram negativas, provocando así la salida de lipopolisacáridos al exterior; además, incrementando la permeabilidad de la membrana citoplasmática, condicionando la salida del ATP; inhibe la actividad de las ATPasas y reduce la fuerza motriz de protones (Burt, 2004).

Lambert et al. (2001) evidenciaron que carvacrol causa un daño acusado en la membrana celular de *Staphylococcus aureus* y *Pseudomonas aeruginosa*, provocando la pérdida de: los componentes responsables de la fuerza motriz de protones, el gradiente de pH y el potencial eléctrico; pudiendo magnificarse el daño a la membrana en presencia de nutrientes, síntesis de ácidos nucleicos y actividad de las enzimas ATPasas. Este hecho fue corroborado años más tarde por Burt (2004), demostrando que las células de *Escherichia coli*, en presencia de concentraciones de carvacrol próximas a 5 mM, suprimen la movilidad y mueren.

Carvacrol presenta una toxicidad aguda (DL50) por vía oral de 810 mg kg^{-1}, y administrado a ratas por vía intravenosa o intraperitoneal, su dosis letal media es de 80 y 73,3 mg kg^{-1} de peso, respectivamente (Anderson, 2006). Sin embargo, en humanos es muy tóxico, con una dosis letal por vía oral de 500 mg kg^{-1}, ya que provoca la irritación de las mucosas de la boca, nariz y ojos, e incluso puede provocar náuseas, diarrea y vómitos (Rodríguez-López, 2017). La utilización de carvacrol como conservador en alimentos está limitada por su sabor intenso, cuando se añade en grandes cantidades, lo cual afecta negativamente a las propiedades organolépticas de los alimentos y por ende, al umbral sensorial del consumidor (Zhou et al., 2007).

1.3.2 Timol

El timol (2-isopropil-5-metilfenol) es el monoterpeno más abundante en los aceites esenciales de las plantas pertenecientes a la familia *Lamiaceae*, entre las que

se encuentran: *Thymus*, *Ocimum*, *Origanum*, *Satureja*, *Thymbra* y *Monarda* (Licata et al., 2015; Mancini et al., 2015; Sarwar & Latif, 2015).

También está presente en otras especies como *Lippia gracilis, Schauer*, (*Verbenaceae*), *Euphrasia officinalis, Hayne* (*Scrophulariaceae*), *Nigella sativa L. (Ranunculaceae),* y *Trachyspermum Ammi L., Sprague (Apiaceae*) (Moein et al., 2015; Novy et al., 2015; Raj et al., 2015).

Se encuentra en el aceite esencial de tomillo en porcentajes superiores al 50%, así como en el aceite esencial de orégano y en otras fuentes naturales como el aceite esencial de mandarina y tangerina. Su estructura química es similar a la del carvacrol, cambiando únicamente la posición del grupo hidroxilo (Figura 1.3). Es un sólido cristalino de color blanco, ligeramente soluble en agua (0,98 g/L a 25 °C), y bastante soluble en la mayoría de disolventes orgánicos (Pow: logP 3,30) (Nieddu et al., 2014). Sus disoluciones acuosas son estables en medios neutros y débilmente ácidos. Presenta una presión de vapor de 2,5 hPa a 50 °C.

Si bien es considerado como GRAS, por lo que por lo que puede usarse como aditivo alimentario; presenta una toxicidad aguda (DL50) por vía oral de 980 mg kg^{-1} y por absorción cutánea >2000 mg/kg en ratas. En humanos, puede provocar irritaciones en la piel tras absorción cutánea, o en los ojos por contacto ocular. Por ingestión, puede provocar irritaciones en mucosas de la boca, garganta, esófago y el tracto intestinal. A dosis elevadas, puede provocar efectos sistémicos en el sistema nervioso central, problemas hepáticos o renales y trastornos cardiovasculares (Anderson, 2006).

El mecanismo de acción de timol es semejante al de carvacrol, ya que su estructura química es similar. Provoca una alteración de la membrana externa de las bacterias Gram negativas, incrementa permeabilidad de la membrana citoplasmática permitiendo la salida de los lipopolisacáridos (Helander et al., 1998).

La capacidad antimicrobiana de timol depende de ciertos factores como el tipo de microorganismo, el pH del medio y temperatura de incubación. Investigaciones realizadas por Falcone, et al. (2005) demostraron que en presencia de timol, algunos microorganismos alterantes como *Lactobacillus curvatus*, *Lactobacillus plantarum, Bacillus cereus, Candida lusitaniae y Sacharomices cerevisiae* cambian la permeabilidad de su membrana celular en presencia de timol, permitiendo la salida de constituyentes químicos esenciales para su metabolismo,

provocando un incremento de la fase lag del microorganismo y del crecimiento microbiano.

1.3.3 Utilización de carvacrol y timol en empaques para alimentos

La utilización de carvacrol y timol en el envasado activo de alimentos ha sido investigado en diversos productos alimenticios como queso, carne, frutas y vegetales. Estos estudios han adicionado diferentes concentraciones de estos monoterpenos a varios tipos de materiales poliméricos, evaluando su efecto en las propiedades térmicas, mecánicas y ópticas del material polimérico. También se han determinado los mecanismos de liberación de estos compuestos desde el polímero al alimento (Khaneghah et al., 2018).

La bibliografía referida argumenta que la adición de carvacrol y timol a la matriz polimérica, puede modificar sus propiedades físicas y químicas del envase. Por ejemplo, las películas comestibles basadas en gelatina bovina enriquecidas con carvacrol, mostraron una disminución en la fuerza de tensión, capacidad de hinchamiento y absorción de agua, en comparación con las películas sin aditivar (Kavoosi et al., 2013). Estos cambios en las propiedades del material se relacionan directamente con el carácter hidrofílico de la gelatina y las interacciones de tipo químico con el carvacrol, interacción que satura la red interfacial de gelatina con moléculas de carvacrol, impidiendo que las moléculas de agua difundan a la gelatina, minimizando así la capacidad de hinchamiento y la absorción de agua.

Además, carvacrol ha mostrado cierto efecto plastificante cuando se adiciona a matrices comestibles, observando cierta ductilidad cuando se mezcla con determinados polímeros (Velázquez-Contreras et al., 2021).

Otra característica relacionada con la incorporación de timol y carvacrol a los sistemas de envasado para alimentos, es la reducción de la velocidad de difusión hacia el producto, manteniendo su actividad antimicrobiana en el tiempo (Ramos et al., 2013), aunque al requerir concentraciones relativamente altas, pueden provocar efectos organolépticos adversos, que superan el umbral de aceptabilidad del consumidor.

Su escasa solubilidad acuosa, unido a la relativa volatilidad e inestabilidad frente a factores ambientales como la luz, oxígeno y temperatura, ha potenciado el desarrollo de estrategias encaminadas a resolver los inconvenientes intrínsecos

inherentes a sus propiedades fisicoquímicas, que hasta la fecha han supuesto un obstáculo a uso como antimicrobianos naturales.

Así, entre las posibles soluciones se plantea el empleo de unos azúcares cíclicos que forman complejos de inclusión con diferentes compuestos, como los monoterpenos descritos anteriormente, pudiendo incorporar los complejos obtenidos a materiales poliméricos, estrategia que podría mejorar las propiedades fisicoquímicas de estos compuestos, modular las propiedades del material polimérico utilizado en la elaboración del EA, conseguir una liberación controlada y constante en el tiempo de ambos compuestos, reduciendo la concentración a emplear.

1.4 SISTEMAS DE ENCAPSULACIÓN PARA ENVASADO DE ALIMENTOS

Los alimentos inician un proceso de degradación desde el momento de su envasado hasta el final de su vida útil, generando diversos compuestos como aldehídos o cetonas, que son sintetizados por oxidación lipídica o bien mediante glicolisis anaeróbica; también aparecen con frecuencia sulfuros y aminas, a consecuencia de la degradación de proteínas, que confieren al alimento olores no agradables, llevando a su rechazo por parte del consumidor.

La presencia de estos compuestos no deseados, al igual que el crecimiento bacteriano, pueden controlarse en muchos casos mediante la incorporación al envase de alguna sustancia absorbente que atrape en su interior al compuesto responsable del olor no deseado (Del Nobile et al., 2009; López-Rubio et al., 2004; Rooney, 1995; Suloff et al., 2003); y en el caso de empaques antimicrobianos se pueden liberar sustancias que ralenticen, o bien inhiban, el crecimiento de microorganismos.

Para que estos sistemas de envasado activo puedan lograr su objetivo, es necesario estabilizar, solubilizar y liberar las sustancias que secuestran los compuestos indeseables, o que se liberan al espacio de cabeza del empaque. Una vía adecuada para lograrlo es mediante la tecnología de encapsulación, que permite la inclusión de una sustancia (agente activo) dentro de otra (agente encapsulante), obteniendo pequeñas partículas, que liberan su contenido a velocidades controladas, durante periodos de tiempo prolongados bajo condiciones específicas (Nedovic et al., 2011).

Respecto al empaque antimicrobiano para alimentos, la encapsulación de los compuestos antimicrobianos es muy importante, ya que mejora su funcionalidad:

a) Protege a los compuestos antimicrobianos de la degradación, oxidación, volatilización o interacciones indeseables con el material de empaque.
b) Mejora la compatibilidad entre el polímero y la sustancia antimicrobiana.
c) Aumenta la disponibilidad del antimicrobiano y proporciona una liberación controlada.

Por tanto, encapsular las sustancias con efecto antimicrobiano a emplear, es esencial para solucionar determinados problemas que limitan su aplicación en el empacado. Por ejemplo, los aceites esenciales necesitan encapsularse antes de su aplicación, para reducir pérdidas por volatilización o degradación durante la fabricación o almacenamiento del empaque. También contribuye a matizar el aroma y sabor, mejorando la percepción del consumidor (Simionato et al., 2019).

El encapsulado también mejora la compatibilidad del antimicrobiano con el biopolímero a emplear, al aumentar su solubilidad. En consecuencia, se han desarrollado diferentes sistemas de encapsulación de compuestos bioactivos, que utilizan liposomas, emulsiones, nanopartículas, microcápsulas y ciclodextrinas, cuyos desarrollos se han aplicado en el sector alimentario y farmacéutico (Pisoschi et al., 2018).

A pesar de ello, no todos los sistemas descritos anteriormente pueden aplicarse en el desarrollo de empaques para alimentos, ya que el material del empaque no debe modificar las propiedades mecánicas y físicas, para preservar su función principal, que es la de proteger al alimento.

1.4.1 Ciclodextrinas

Las ciclodextrinas (CD) son una familia de oligosacáridos cíclicos de α-D-glucopiranosa unida por enlaces glucosídicos α-1,4 (Figura 1.5 a) que pueden producirse debido a la biotransformación del almidón por ciertas bacterias como *Bacillus macerans* (Astray et al., 2009).

Las ciclodextrinas naturales más comunes son las α-ciclodextrinas (6 subunidades de glucosa), β- ciclodextrinas (7 subunidades de glucosa) y ɣ -

ciclodextrinas (8 subunidades de glucosa), siendo la β-CD la más económica y, por lo tanto, la más utilizada.

Las CDs presentan una conformación circular con fondo en forma de tazón o cono truncado con una cavidad no polar interna, y una superficie externa polar, por lo que son capaces de encapsular sustancias hidrofóbicas (Figura 1.5).

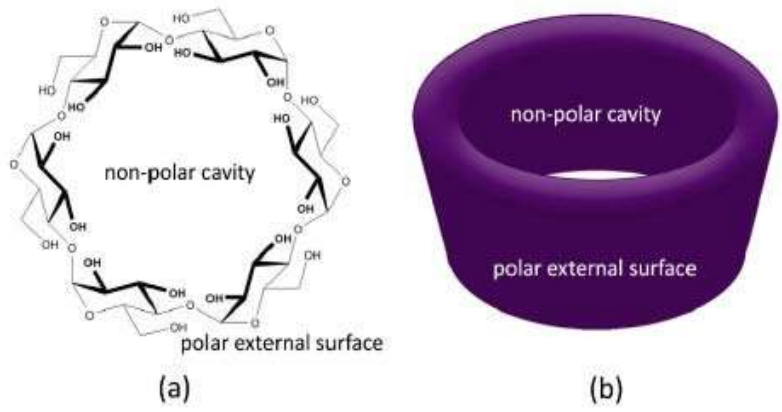


Figura 1.5. (a) Estructura química de las ciclodextrinas (b) Forma geométrica de las ciclodextrinas (Becerril et al., 2020).

La cavidad hidrófoba de las ciclodextrinas y la presencia de anillos hidrofílicos le otorgan a las ciclodextrinas la capacidad para formar complejos de inclusión con una amplia variedad de moléculas orgánicas en medio acuoso (Marques, 2010). Así, las ciclodextrinas (CD) actúan como moléculas anfitrión y encapsulan en su cavidad apolar interna huéspedes hidrofóbicos, mediante la formación de complejos de inclusión CD-huésped (Bilensoy & Hincal, 2009; Laza-Knoerr et al., 2010; Pinho et al., 2014; Valente & Söderman, 2014). Hay que tener en cuenta que la formación de los complejos de inclusión no es rígida, sino que muestran una relativa flexibilidad, la cual les permite asociarse con una gran variedad de moléculas.

Las CDs se complejan con determinadas moléculas huésped de forma selectiva, por lo que se dice que poseen la capacidad para reconocer moléculas (Davis y Higson, 2011). Si bien la formación del complejo se ve favorecida por la polaridad de la molécula huésped, se ha postulado que la formación del complejo responde mayoritariamente a factores geométricos, más que a interacciones de tipo

químico. En este sentido, puede afirmarse que el proceso de inclusión entre la molécula huésped y la CD, transcurre porque es energéticamente favorable; el huésped es relativamente apolar, y la cavidad hidrofóbica de la CD está imperfectamente solvatada. Para la formación de los complejos de inclusión, se tiene que generar una interacción entre la molécula huésped, generalmente hidrofóbica, y la CD.

La capacidad de las ciclodextrinas para formar complejos de inclusión, está condicionada por los siguientes factores:

a) El impedimento estérico, el cual es función directa del tamaño de la molécula huésped y al tamaño de la cavidad interna de la ciclodextrina.
b) Las interacciones termodinámicas entre los diferentes componentes del sistema (ciclodextrinas, huésped y solvente).

Cramer fue el primero en describir la secuencia que conduce a la formación de complejos de inclusión, dividiéndola en 5 etapas (Kfoury et al., 2015):

a) En primer lugar, la molécula huésped se acerca a la CD provocando la salida de moléculas de agua de la cavidad interna, alcanzando un nivel de energía de estado gaseoso, ya que adquieren libertad de traslación y rotación y, por tanto, una disminución de las fuerzas de Van der Waals y los puentes de hidrógeno.
b) El huésped alcanza también un nivel de energía de estado gaseoso, al liberarse las moléculas de agua.
c) La parte apolar de la molécula huésped entra en la cavidad, donde se producen interacciones de Van der Waals y/o enlaces de hidrógeno, etapa que conduce a la estabilización del complejo de inclusión.
d) Las moléculas de agua liberadas por el huésped y la cavidad de las CDs se reorganizan, formando puentes de hidrogeno.
e) La zona de la molécula huésped que queda fuera de la cavidad de las CDs se hidrata al interaccionar con las moléculas de agua, quedando inmersa en la capa de hidratación de las CDs (Crini, 2014).

La estabilidad del complejo es proporcional al carácter hidrofóbico de los sustituyentes sobre la CD. Así, los sustituyentes metilo o etilo aumentan la estabilidad de los complejos, mientras que los hidroxilos la disminuyen, al igual que ocurre con los grupos ionizables (Del Valle, 2004).

Las β-Ciclodextrinas presentan un tamaño de cavidad adecuado y buena eficacia para encapsular y proteger moléculas aromáticas y/o heterocíclicas con actividad antimicrobiana, además de un precio razonable, en comparación con las demás ciclodextrinas nativas (Helena y Marques, 2019). Algunas aplicaciones recientes se muestran en la Tabla 1.1.

Tabla 1.4.1 Microencapsulación de AE con efecto antimicrobiano en CD.

Material	Complejo de Inclusión	Actividad antimicrobiana
Gelatina	AE Tomillo/β-CD	Reducción de los recuentos bacterianos en la carne de pollo recubierta con el film. Sin cambio en color, textura y sabor (Lin et al., 2018).
Alcohol polivinílico	AE Canela/β-CD	Actividad antimicrobiana frente a *Escherichia coli* y *Staphylococcus aureus in vitro* y aumento de la vida de anaquel de las fresas envasadas (Wen, Zhu, Wu, et al., 2016).
Ácido Poliláctico	AE Canela/β-CD	Actividad antimicrobiana frente a *Escherichia coli* y *Staphylococcus aureus in vitro*, reducción de recuentos bacterianos en carne de cerdo envasada con la película (Wen, Zhu, Feng, et al., 2016)(Wen, Zhu, Wu, et al., 2016).
Zein	Timol/β-CD	Mayor actividad antimicrobiana frente a *Escherichia coli y Staphylococcus auereus in vitro* que las nanofibras con timol no encapsulado Reducción del recuento bacteriano en la carne almacenada hasta 5 días a 4 °C (Aytac et al., 2017).
Zein	AE Eucalipto/β-CD	Actividad antimicrobiana *in vitro* frente a *Staphylococcus aureus* y *Listeria monocytogenes* (Dias Antunes et al., 2017).

Otras investigaciones demuestran la capacidad que tienen estos complejos para la conservación de vegetales mínimamente procesados. La utilización de ciclodextrinas nativas y sus respectivas modificadas, es en la actualidad una de las

estrategias más utilizadas en el área de envasado de alimentos para proteger compuestos con actividad específica.

1.5 EMULSIONES

Las emulsiones se suelen utilizar para encapsular compuestos activos en cantidades significativas, ya que los líquidos inmiscibles se dispersan en otro líquido en forma de pequeñas gotas. Los compuestos lipofílicos pueden encapsularse en emulsiones de aceite en agua (O/W), mientras que los compuestos hidrofílicos pueden encapsularse en emulsiones de agua en aceite (W/O) o viceversa (Espitia et al., 2019).

Respecto al uso de emulsiones en empaques antimicrobiano, se utilizan para incorporar aceites esenciales o componentes químicos en polímeros solubles en agua, generalmente son de origen natural, obteniendo así una emulsión de aceite en agua O/W. La incorporación de aceites esenciales en emulsiones mejora su compatibilidad con los polímeros a base de agua, proporciona películas más transparentes y protege a los AE de la volatilización, permitiendo una liberación más controlada (Robledo, Vera, et al., 2018).

Las microemulsiones se definen como dispersiones coloidales de aceite y agua estabilizadas por una capa interfacial de moléculas tensoactivas con tamaños de partículas que oscilan entre 1 y 100 nm. Este tipo de emulsiones presentan algunas ventajas, como la estabilidad termodinámica y la transparencia, que las convierten en buenos vehículos para incorporar compuestos hidrófobos antimicrobianos en diferentes matrices poliméricas; sin embargo, necesitan una gran cantidad de surfactante para que sean estables (Fu et al., 2016).

Por su parte, las nanoemulsiones se definen como emulsiones que contienen partículas muy pequeñas, inferiores a 200 nm. Al igual que las emulsiones convencionales, son termodinámicamente inestables, pero su tamaño es más bajo lo cual le confiere estabilidad a largo plazo, una mejor biodisponibilidad y transparencia. Estas nanoemulsiones también requieren el empleo de tensioactivos, pero en una proporción más baja en comparación con las microemulsiones.

Una de las desventajas que presentan es que tienen una baja estabilidad en condiciones ácidas, y generalmente se preparan por homogeneización aplicando altas presiones, ultrasonidos o técnicas microfluídicas de alta presión. Las

nanoemulsiones son las más utilizadas para encapsular antimicrobianos en empaques activos (Tabla 1.2).

Tabla 1.2. Materiales de empaque con antimicrobianos cargados en nanoemulsiones o microemulsiones.

Material de empaque	**Antimicrobiano Encapsulado**	**Tensoactivo**	**Actividad antimicrobiana**
Película de carboximetil quitosano	Carvacrol	Alcohol graso poli-oxi etilen éter, ácido carboxílico	Pan de trigo expuesto a la película, mostró reducción de bacterias mesófilas aerobias, crecimiento de mohos y levaduras (Lei et al., 2019).
Película de quitosano en recubrimiento comestible	Isotiocianato de alilo o éster de arginato láurico	Bio-fibra de maíz	Las fresas que se recubrieron mostraron reducción de los inóculos de *Escherichia coli* O157: H7 y *Salmonella spp*., especialmente con películas de isotiocianato (Guo et al., 2017).
Películas de Quitosano reforzado con nanocristales de celulosa	Tomillo-orégano (AE)	Lecitina y Tween 80	El arroz envasado con esta película mostro inhibición del crecimiento fúngico. El efecto inhibitorio aumentó cuando se aplicó radiación gamma (Hossain et al., 2019)
Recubrimiento comestible de pectina	Canela, aceite esencial de ajo, curcumina	Tween 80	Filete de pechuga de pollo con recubierta mostró reducción de bacterias, levaduras y mohos (Abdou et al., 2018)
Quínoa / quitosano recubrimiento comestible	Timol	Tween 80 / Miglyol 812	Fresas recubiertas mostraron reducción del crecimiento de levaduras y hongos (Robledo, López, et al., 2018)

Las emulsiones con bajo tamaño de partículas, a escala nanométrica o micrométrica, presentan ventajas sobre los sistemas que contienen partículas más grandes (Chen et al., 2016), como una mejor estabilidad, disminución de la agregación de partículas, mayor transparencia, propiedades reológicas mejoradas y mayor biodisponibilidad de las sustancias encapsuladas. Por lo tanto, las películas o empaques antimicrobianos que contienen emulsiones de menor tamaño

de partícula, serán más homogéneas, transparentes y eficaces que las preparadas con emulsiones convencionales.

Así, Guo, Yadav, y Jin (2017), observaron que las películas que contenían microemulsiones de isocianato de alilo mostraban mayor más homogeneidad y actividad antimicrobiana que las que contenían emulsiones convencionales. Del mismo modo, Oh et al. (2017) encontraron que las películas comestibles de quitosano que contenían nanoemulsiones de aceite esencial de hierba de limón, mostraron mejor actividad antimicrobiana sobre bayas de uva y menor variación de los parámetros sensoriales con respecto a las bayas de uva recubiertas con una película de mayor tamaño sin aditivar.

Los materiales de embalaje que contienen emulsiones como estrategia de encapsulación de compuestos antimicrobianos, utilizan polímeros de origen natural y emulsionantes de origen sintético, en particular, polisorbatos como Tween 20 (Sugumar et al., 2015) o Tween 80 (Jantrawut et al., 2018).

Los emulsionantes naturales como la lecitina (Lei et al., 2019), la proteína de soja (Ghani et al., 2018), o el arabinoxilano (Guo et al., 2015) apenas se han utilizado hasta la fecha en combinación con polisorbatos, por lo cual, se requieren más investigaciones sobre el empleo de emulsionantes naturales en materiales de envasado de origen biológico, para satisfacer la creciente demanda de ingredientes naturales en la industria alimentaria.

1.6 OTROS SISTEMAS

Además de las técnicas de encapsulación mencionadas, podemos encontrar otras micro o nano partículas como microcápsulas, nanocápsulas, portadoras de lípidos, las cuales se han utilizado para encapsular sabores, vitaminas, antioxidantes, colorantes alimentarios o antimicrobianos para su empleo en la industria alimentaria (Rezaei et al., 2019).

Se han descrito ciertos desarrollos en los que incorporan microcápsulas y nanocápsulas que contienen agentes antimicrobianos a determinados polímeros, para controlar la liberación y mejorar su eficacia, ya que estas estructuras están sujetas a cierta tensión mecánica, provocando la ruptura de la pared y la consiguiente liberación del compuesto activo. Algunas microcápsulas se han incorporado a envases de papel a modo de recubrimiento, demostrando actividad

antimicrobiana frente a *Escherichia coli y Sacharomyces cerevisiae* en fase vapor (Šumiga et al., 2019). También se han diseñado microcápsulas de poliamida ligeramente reticulada fotosensibles, con aceite esencial de tomillo; la cubierta responde a los estímulos luminosos debido a la isomerización cis-trans de los residuos azo fotocrómicos, lo que provoca una contracción de la cadena polimérica que conduce a la liberación del compuesto encapsulado (Marturano et al., 2019), que evidencian su eficacia antimicrobiana. Recientemente Huang et al. (2018) desarrollaron una microcápsula capaz de incorporar al medio ClO_2, gracias a la reacción del $NaClO_2$ con agua y ácido tartárico. Las cápsulas de poli (lactida) se cargaron con un núcleo de gelatina y $NaClO_2$, y posteriormente se incorporaron a una película de PLA que contenía ácido tartárico. En presencia de agua, se produce ClO_2 (gas) y se libera de la película, reduciendo la población de *Escherichia coli* y *Staphylococcus aureus*.

II - OBJETIVOS

II. OBJETIVOS

El aumento en el consumo de alimentos mínimamente procesados, la demanda de productos libre de conservantes artificiales, así como los cambios en las prácticas de distribución de alimentos derivadas de la globalización, son algunas de las razones que han motivado el desarrollo e innovación de nuevas tecnologías de envasado de alimentos, focalizándose especialmente en la extensión de la vida útil sin que se produzcan mermas por la calidad y seguridad de los alimentos. La tecnología de envasado activo contribuye a mantener y mejorar la calidad y seguridad de los alimentos, por lo que puede ser considerado como tecnología emergente de conservación de alimentos.

Por tanto, el objetivo principal del presente estudio es el desarrollo, caracterización y aplicación de un envase activo biodegradable para controlar el proceso de degradación y contaminación microbiana en alimentos de cuarta gamma, utilizando como modelo moras y frambuesas, para posteriormente evaluar el efecto protector *in vivo* de timol y carvacrol complejados en β-ciclodextrinas, incorporados a polímeros biodegradables.

Los objetivos secundarios que se desprenden de la investigación son los siguientes:

a) Obtener películas de biopolímeros de PLA, así como de mezclas de PLA, a las cuales se incorporen complejos de ciclodextrinas con componentes mayoritarios de aceites esenciales.
b) Caracterizar las películas de PLA y sus mezclas con y sin la incorporación ciclodextrinas complejadas por atomización en Spray-dryer.
c) Caracterizar las propiedades físico-químicas de interés de los materiales desarrollados y evaluar el efecto de la incorporación de los complejos (ciclodextrina/monoterpeno) en la matriz polimérica, así como optimizar el proceso de obtención del empaque.
d) Evaluar la liberación de aceites esenciales incluidos en la ciclodextrina desde los materiales desarrollados, mediante análisis del espacio de

cabeza del empaque y comprobando la actividad antimicrobiana del mismo.

e) Estudiar la aplicación práctica de los materiales para el envasado de alimentos sensibles a degradación y contaminación, determinando su vida útil.

III – COMPENDIO DE ARTÍCULOS

ARTÍCULO 1. Development and Characterization of a Biodegradable PLA Food Packaging Hold Monoterpene–Cyclodextrin Complexes against Alternaria alternata.

Velázquez Contreras, F.; Acevedo Parra, H.; Nuño Don Lucas, M.; Núñez Delicado, E.; Gabaldón, J. A. (2019). Development and Characterization of a Biodegradable PLA Food Packaging Hold Monoterpene–Cyclodextrin Complexes against *Alternaria alternata*. Polymers, 11, 1720. https://doi.org/10.3390/polym11101720

polymers

MDPI

Article

Development and Characterization of a Biodegradable PLA Food Packaging Hold Monoterpene–Cyclodextrin Complexes against *Alternaria alternata*

Velázquez-Contreras Friné [1,2], Acevedo-Parra Hector [2], Nuño-Donlucas Sergio Manuel [3], Núñez-Delicado Estrella [1] and Gabaldón José Antonio [1,*]

1 Campus de los Jerónimos, Universidad Católica San Antonio de Murcia, 135, 30107 Guadalupe, Murcia, Spain; fvelazqu@up.edu.mx (V.-C.F.); enunez@ucam.edu (N.-D.E.)
2 Universidad Panamericana ESDAI, Álvaro del Portillo 49, Zapopan 45010, Jalisco, Mexico; hector.acevedo@up.edu.mx
3 Departamento de Ingeniería Química, Universidad de Guadalajara, Blvd, Marcelino García Barragán 1421, Guadalajara 44430, Jalisco, Mexico; gigio@cencar.udg.mx
* Correspondence: jagabaldon@ucam.edu; Tel.: +34-968-278-622

Received: 15 September 2019; Accepted: 15 October 2019; Published: 21 October 2019

Abstract: The fungi of the genus *Alternaria* are among the main pathogens causing post-harvest diseases and significant economic losses. The consumption of *Alternaria* contaminated foods may be a major risk to human health, as many *Alternaria* species produce several toxic mycotoxins and secondary metabolites. To protect consumer health and extend the shelf life of food products, the development of new ways of packaging is of outmost importance. The aim of this work was to investigate the antifungal capacity of a biodegradable poly(lactic acid) (PLA) package filled with thymol or carvacrol complexed in β-cyclodextrins (β-CDs) by the solubility method. Once solid complexes were obtained by spray drying, varying proportions (0.0%, 1.5%, 2.5%, and 5.0 wt%) of β-CD–thymol or β-CD–carvacrol were mixed with PLA for packaging development by injection process. The formation of stable complexes between β-CDs and carvacrol or thymol molecules was assessed by Fourier-transform infrared spectroscopy (FTIR). Mechanical, structural, and thermal characterization of the developed packaging was also carried out. The polymer surface showed a decrease in the number of cuts and folds as the amount of encapsulation increased, thereby reducing the stiffness of the packaging. In addition, thermogravimetric analysis (TGA) revealed a slight decrease in the temperature of degradation of PLA package as the concentration of the complexes increased, with β-CD–carvacrol or β-CDs–thymol complexes acting as plasticisers that lowered the intermolecular forces of the polymer chains, thereby improving the breaking point. Packages containing 2.5% and 5% β-CD–carvacrol, or 5% β-CD–thymol showed *Alternaria alternata* inhibition after 10 days of incubation revealing their potential uses in agrofood industry.

Keywords: food packaging; poly(lactic acid); thymol; carvacrol; β-cyclodextrin; antifungal activity

1. Introduction

Nowadays, the increasing consumer demand for healthy, freshly prepared, and convenient fruits and vegetables has driven the rapid growth of the fresh-cut produce industry worldwide, with benefits in multi-billion dollars [1]. In addition, new lifestyle drivers such as health and aging of population has stimulated the agrofood industry to enhance the offer and delivery of value-added products, such as minimally processed fruits and vegetables packaged in sealed polymeric films or on trays, ready for immediate consumption or direct cooking.

However, this trend has disturbed the scenario of foodborne diseases worldwide caused by pathogenic microorganisms, with important economic and social impacts [2], since fresh and minimally processed foods may undergo negative qualitative changes related to high respiratory rate, moisture loss, rapid enzymatic browning, and microbial contamination which lead to the rapid deterioration of the products [3]. In addition, fungal contamination of crops through latent infections usually occurs in the fields; nevertheless, the rotting arises later, during the storage and transport before marketing. The fungi of the genus *Alternaria* are among the main pathogens causing post-harvest diseases and significant economic losses. These fungi also represent a serious toxicological risk as they produce a broad spectrum of mycotoxins and secondary metabolites, which can cause problems in humans and animals [4].

This issue has raised considerable challenges for food packaging companies and researchers that specifically use biodegradable materials or bio-based packaging for food preservation. Despite the good properties of petroleum-based plastics, their widespread use and accumulation cause serious environmental problems and dependence on fossil resources. In fact, packaging applications contribute to 63% of the current plastic waste, and it is estimated that less than 14% are recyclable [5].

To overcome the described drawbacks, different approaches have been carried out to obtain bioplastics with analogous functionalities to petrochemical polymers. Poly(lactic acid) (PLA), a biodegradable aliphatic polyester which can be obtained by fermentation of renewable resources such as corn, tapioca, and sugarcane [6], meets several requirements such as high mechanical strength, biodegradability, biocompatibility, bio-absorbability, transparency, low toxicity, and easy process ability [7] to be thoroughly employed in agricultural films, biomedical devices, and food packaging [8,9] and used as a suitable carrier of active compounds to yield antioxidant or antimicrobial effects [10,11].

Currently, consumer concerns about the potential toxicity to humans of synthetic antimicrobials such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) have resulted in the increased use of natural antimicrobials, which receive a good deal of attention for a number of microorganism control issues [12]. As a result, different antimicrobials have been added to different packaging materials. In particular, essential oils and their bioactive molecules such as carvacrol and thymol have been thoroughly tested in vitro [13,14] or in different food systems such as meat, dairy or vegetable samples [15] due to their insecticidal, antiviral, antimicrobial, and antifungal activities [16]; however, their high volatility and reactivity limits their application as food preservatives. In fact, long storage time and temperature could magnify volatilization and drastically lessen their activity, requiring as consequence high concentrations to ensure antimicrobial activity, which is a detrimental praxis for organoleptic attributes (flavor, taste, and aroma) and acceptability of foods, so this strategy is not considered in practice.

In order to increase the applicability of natural antimicrobial formulations, these drawbacks could be overcome by microencapsulation or complexation techniques using cyclodextrins (CDS), which are cyclic oligosaccharides derived from starch made up of 6, 7, or 8 units of D-glucose monomers linked by α(1,4) bonds, shaped as a truncated hollow cone [17] that presents an internal hydrophobic cavity to interact with non-polar active constituents of essential oils or their bioactive molecules such as carvacrol and thymol, whereas the external face is hydrophilic, improving their water solubility and gradually increasing their effectiveness using lower concentrations of these compounds.

As a preliminary stage to subsequently evaluate the antifungal capacity of a biodegradable poly(lactic acid) (PLA) package carrying as preservatives carvacrol or thymol complexed in CDs (as described here), their complexation was carried out with native and modified CDs [18] and the antimicrobial and antifungal effects of their respective complexes was verified by comparison with hydroxypropyl-β-cyclodextrins (selected due to their highest Kc values) against *Escherichia coli*, *Staphylococcus aureus*, and *Galactomyces citri-aurantii* [19,20]. However, only native CDs (α, β, and γ) are considered as GRAS (generally recognized as safe) and are the only ones authorized to come into contact with foods.

Therefore, the present study focuses on the design and optimization of a controlled release system of antifungal carvacrol or thymol volatiles encapsulated in β-CD to be incorporated into a biodegradable polymeric matrix of PLA by industrial injection. The optimization of stable complexes between β-CDs and carvacrol or thymol molecules and characterization by Fourier-transform infrared spectroscopy (FTIR) were carried out. Mechanical, structural, and thermal characterization of developed packaging was carried out and materials behavior against *Alternaria alternata* growth was also investigated.

2. Materials and Methods

2.1. Materials

Carvacrol (CAS: 499-75-2, 99.5% purity), thymol (CAS: 89-83-8, 98.7% purity), and β-cyclodextrin (β-CD >95%, food grade) were purchased from Sigma-Aldrich Corp (Saint Louis, MO, USA). The chemical structures of the two monoterpenes are shown in Figure 1. Poly(lactic acid) (PLA, Ingeo™ Biopolymer ref. code: 3251D) with a weight-average molecular weight ($\overline{M}w$) of 5.5×10^4 g/mol, polydispersity index (PI) of 1.62, and low D-isomer content (99% L-lactide/1% D-lactide), provided by PromaPlast Co (Guadalajara, Jalisco, Mexico) and manufactured by Nature Works LLC (Blair, NE, USA), was selected for injection moulding applications since it has a higher flow capability (relative viscosity 2.5, glass transition temperature Tg = 55–60 °C, melting temperature Tm = 155–170 °C, and processing temperature 188–210 °C) than other resins currently available in the marketplace. *Alternaria alternata* strain ATCC 42761 (isolated from blackberries in Georgia, USA) was purchased from SENNA laboratories, Mexico City. Potato dextrose agar (PDA) was provided by Bioxon, Mexico. The rest of the chemical products were of analytical grade.

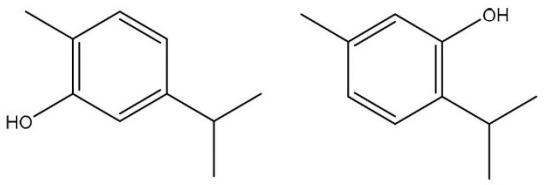


Figure 1. Chemical structures of carvacrol and thymol monoterpenes.

2.2. Preparation of β-CD Inclusion Complexes

Both β-CD–carvacrol and β-CD–thymol inclusion complexes were prepared using the solubility method [18]. For that, aqueous solutions of increasing concentrations of β-cyclodextrin (0–15 mM) were prepared in sodium phosphate buffer (100 mM, pH of 7.0) in a total volume of 100 mL. A saturating amount of carvacrol or thymol was added to each one of the solutions and kept in an ultrasound bath (Ultrasons HP, Selecta, Spain) for 60 min in the dark at 25 °C, until equilibrium was reached. After that, to remove excess monoterpene, the respective solutions were filtered through a nylon filter of 0.45 μm. Liquid complexes were used for phase solubility diagrams, determining the concentration of entrapped monoterpene by GC/MS, and posterior spray drying process to obtain powdered dehydrated complexes.

From the phase diagrams of carvacrol or thymol, complexed with β-CDs, the parameters efficiency of complexation (CE) and the molar ratio (MR) were determined. CE is the ratio between the dissolved complex and free cyclodextrin (CD) concentration. It is independent of S_0 (aqueous solubility), and was calculated from the slope of the phase solubility profiles by using Equation (1).

$$CE\ (\%) = \frac{[dissolved - complex]}{[CD]_f} \quad (1)$$

The MR of β-CD–carvacrol and β-CD–thymol inclusion complexes was calculated using CE values with Equation (2).

$$MR = \frac{1}{\left(1 + \frac{1}{CE}\right)} \quad (2)$$

2.3. Atomization Process to Obtain Complexes in Solid State

To obtain complexes in solid state, the β-CD–carvacrol and β-CD–thymol solutions were subjected to an atomization process using a laboratory-scale atomizer and Büchi B290 Mini Spray Dryer (Flawil, Switzerland) working with air as the carrier gas at a flow rate 5 mL/min, pressure of 3.2 bar, and an inlet and outlet temperature of 170 ± 2 °C and 68 ± 2 °C, respectively, using a 1.5 mm nozzle diameter. In each case, the entrapment efficiency (EE) was determined with respect to the theoretical number of monoterpenes present in the inclusion complex after atomization, using Equation (3).

$$\text{EE} = \frac{\text{Amount of active compound entrapped}}{(\text{Initial active compound amount})} \times 100 \quad (3)$$

Furthermore, the process performance (PP) was determined as follows:

$$\text{PP} = \frac{\text{Total weight obtained from solids after spray drying process (g)}}{(\text{Initial Initial weight} - \text{CD in solution (g)})} \times 100 \quad (4)$$

Carvacrol and thymol concentrations in dehydrated complexes were quantified after spray drying. For that, β-CD–carvacrol and β-CD–thymol were diluted in ethanol (complex: ethanol, 20:80, *v*/*v*), to break the complexes formed. After that, β-CDs was removed from the solution, leaving only the active compound for further quantification in triplicate, by GC/MS analysis at Agilent Technologies 7890B (Palo Alto, CA, USA) coupled to a 5977A mass spectrometer, as previously described by Rodríguez-López et al. (2019) [18].

2.4. Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra used to study changes of chemical structures of free carvacrol and thymol, and their respective complexes were acquired using a Varian FTIR 670 (Agilent Tech, Amstelveen, The Netherlands) spectrophotometer coupled with an accessory to analyze the attenuated total reflectance (ATR) with a wave number resolution of 0.10 cm^{-1} in the range of 250–4000 cm^{-1}. A minimum of 32 scans were signal-averaged with a resolution of 4 cm^{-1} in the above ranges.

2.5. Boxes Production

The PLA samples were dried in an oven at 60 °C for 4 h to avoid bubbles in the molding process. After that, physical mixtures were performed using as ingredients PLA (100%, 98.5%, 97.5%, and 95% weight percentages, wt%), and dehydrated complexes of β-CD–carvacrol or β-CD–thymol at (0%, 1.5%, 2.5%, and 5% wt%), that were introduced in a pilot extruder to produce pellets. The extruder had a screw diameter (D) of 25.4 mm, screw length (L) of 406.4 mm (L/D ratio of 16), four heating zones, and a slot 1.75 mm matrix outlet. The barrel temperature profile was set at 150/170/180/180 °C with a screw speed of 30 rpm.

The pellets produced in the previous step were thermo-pressed in the Belken BLD-68 injector from AG Plastic (Querétaro, México), optimizing the parameters of heated mold (180 °C/100 bar), to ensure the adequate fluidity of the material to produce (12 × 10 × 3.0 cm) boxes (Figure 2). Once the material reached the cooling temperature, the boxes were then released from the molds.

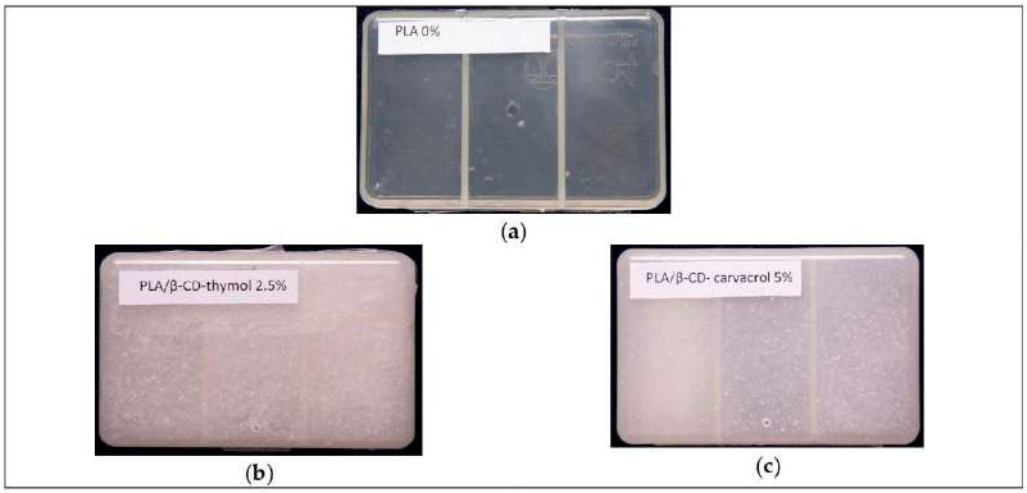


Figure 2. Boxes obtained by injection of the pellets. (**a**) (PLA); (**b**) (PLA/β-CD–thymol, 2.5%, wt%); (**c**) (PLA/β-CD–carvacrol, 5.0%, wt%).

2.6. *Packaging Characterization*

2.6.1. Mechanical Properties

Tensile proofs were carried out in the universal traction machine SFM 100 from United Testing Systems (Ontario, Canada). Previously the packages were manually cut to obtain assay pieces (ten of each formulation), according to dimensions established by the ASTM method D-638. The tests were conducted at room temperature, at 5 mm/min speed using an initial grip length of 25 mm. The parameters, namely, average of maximum stress (MPa), breaking point (%), and Young's modulus (MPa) were determined for the pieces of PLA and β-CD–carvacrol or β-CD–thymol, according to the aforementioned procedure [21].

2.6.2. Scanning Electron Microscopy (SEM)

The structure of the packaging material was determined by scanning electron microscopy MIRA3 model form TESCAN (Brno, Czech Republic). Packages were previously frozen at −80 °C, manually fractured, and later placed on the slide and gold coated during 90 s using a sputter coater. All the samples were evaluated using a voltage of 7.0 kV.

2.6.3. Thermal Characterization of the Developed Packaging

The thermal evaluation of the packaging material was done by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC assays were performed in DSC-Q100 (TA Instruments, New Castle, USA). Firstly, pieces of 5 mg were dried for 48 h in an oven at room temperature; after that, samples were placed in an aluminium capsule that was subjected to a temperature scan from 20–230 °C at a heating rate of 10 °C/min under inert nitrogen atmosphere. In addition, thermal stability of the materials was carried out by TGA using the gravimetric thermal analyzer TGA-550 (TA Instruments, New Castle, PA, USA). For that, samples of 10 mg were weighed and placed in platinum trays, which were subjected to a temperature scan of 20–600 °C at a heating rate of 20 °C/min under a nitrogen atmosphere.

2.7. *Antimicrobial Activity*

The antifungal activity of the packages with β-CD–thymol and β-CD–carvacrol was evaluated by vapor phase diffusion, in triplicates, according to Du et al. [22] using a strain of *A. alternata* (ATCC

42761). Pure fungal cultures in potato-dextrose agar medium plates with 14 days of incubation (23 °C) were suspended in 10 mL sterile distilled water containing 0.05% of Tween 20, and collected by gently scraping the surface of the agar with a sterile L-shaped glass rod. Next, the arthrospores concentration was adjusted to 10^6 spores/mL using the McFarland scale (Shumadzu, UVmini-1240), and the inoculum was used for in vitro bioassays.

For the bioassay, 3.0 µL of spore suspensions were placed in the centre of Petri dishes previously filled with inoculated potato dextrose agar (PDA). Subsequently, these boxes were incubated at 25 °C for 5 and 10 days, inverted and covered with parafilm, and were used as controls.

Packages containing different concentration of active compounds were aseptically cut into 50 mm rectangles and placed on top of the Petri dishes. Parafilm M (Bemis) was used to hermetically seal the Petri dishes, which were incubated at 25 ± 1 °C in an incubator (Binder ED), for 120–240 h. After the incubation period, the inhibition zone diameter created by the vapor and active compound (thymol or carvacrol complexed with CDs) released from the packaging into the culture medium was measured and related to the package antimicrobial activity.

The growth of fungal cultures as well as controls were daily evaluated by measuring the diameter of the colony or surface area (diameter at right angles to each other) of the plates occupied by the colony during incubation time. The measurements were carried out with a gauge on the agar surface reporting growth at 5 and 10 days. Due to the transparency of the materials used, these measurements were conducted without opening the box. Every assay was tested in triplicate and the results were statistically analyzed.

2.8. Statistical Analysis

The data corresponding to mechanical properties and the diameter of the colony in the antifungal activity were subjected to statistical analysis. Analysis of variance (ANOVA) and Tukey's multiple comparison test were performed using MINITAB 18 statistical software (Paris, France), at a 5% significance level.

3. Results and Discussion

3.1. Assessment of the Obtained Complexes

As described previously by Rodríguez-López et al., 2019 [18], phase solubility diagrams of carvacrol and thymol with β-CDs were carried out at pH 7.0 (25 °C), since the pH of the medium could condition its dissociation degree and consequently its solubility, thus determining the stability of the complexes. By using linear regression analysis of the phase solubility diagrams and considering the formation of β-CD–carvacrol and β-CD–thymol 1:1 complexes when the concentration of β-cyclodextrin was 11 mM, it was possible to determine the complexation constant (Kc), the complexation efficiency (CE), and molar ratio (MR) by applying Equations (1) and (2).

As can be seen in Table 1, β-CD–thymol and β-CD–carvacrol complexes show the same molar ratio (1:2), indicating that almost one of every two β-CDs molecules in solution is forming soluble complexes with carvacrol or thymol [18]. However, the efficiency of complexation obtained for carvacrol (105.6) is significantly higher than that obtained for thymol (69.3).

Table 1. Carvacrol and thymol aqueous solubility (S_0), complexation constant (Kc) with β-CDs, complexation efficiency (CE), and molar ratio (MR) at pH of 7.0.

Complexes	S_0 (mmol L^{-1})	K_C (L mol^{-1})	CE (%)	Molar Ratio
Carvacrol/β-CDs	5.64 ± 0.12	1871 ± 143	105.6 ± 10.3	1:2
Thymol/β-CDs	5.77 ± 0.15 *	1198 ± 115	69.3 ± 9.2	1:2

* SD, standard deviation of triplicate determinations.

For further packaging formulations with PLA, soluble complexes of β-CD–carvacrol and β-CDs–thymol were subjected to a spray drying process to obtain complexes in a solid state to improve their management.

After the dehydration process, entrapment efficiency (Equation (3)) and process performance (Equation (4)) parameters were determined (see Table 2), showing similar values for both the parameters, but slightly higher for thymol.

Table 2. Entrapment efficiency (EE) and process performance (PP) of β-CD–carvacrol and β-CD–thymol complexes in solid state.

Monoterpene	β-CD	EE (%)	PP (%)
Carvacrol	11 mM	45 ± 2.5 *	84 ± 3.2
Thymol	11 mM	47 ± 1.8	86 ± 3.7

* SD, standard deviation of triplicate determinations.

FTIR is a suitable technique for evidencing the formation of the β-CD–carvacrol and β-CD–thymol inclusion complexes (Figure 3), due the shift or vanishing of the stretching and bending vibrations of the functional groups of guest molecule once complexed.

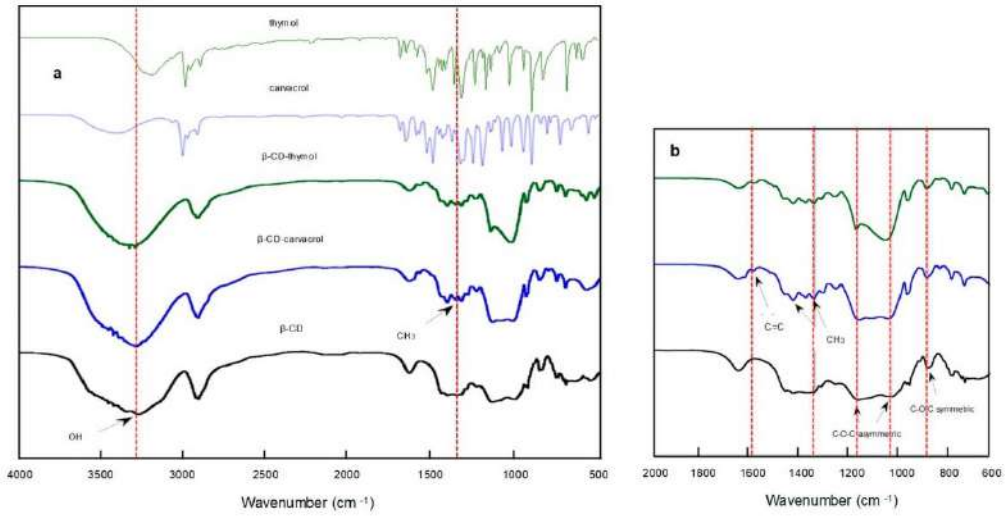


Figure 3. FTIR spectra of carvacrol (blue) and thymol (green); β-CD–thymol (green) and β-CD–carvacrol (blue) complexes, and β-CD (black) in normal (**a**) and broad view (**b**).

As can be seen in Figure 3a, the IR spectrum of thymol (structural isomer of carvacrol), shows several characteristics peaks: 3164 cm^{-1}, O-H stretching and bending vibrations (3164 cm^{-1} and 1453 cm^{-1}, respectively); C-H symmetric and asymmetric stretching bands at 2858 cm^{-1} and 2897 cm^{-1}, respectively; and three C=C stretching vibrations of weak intensity at 1624 cm^{-1}, 1592 cm^{-1}, and 1506 cm^{-1}, revealing the tri-substitution of the aromatic ring. With respect to the substituents of the aromatic ring, methyl (-CH_3) appears at 1344 cm^{-1} and a typical doubled signal (like a tooth) at 1410 cm^{-1} characteristic of isopropyl group was observed. The IR spectrum of β-CDs (Figure 3a) showed characteristic bands corresponding to stretching vibrations of O-H and C-H links, around 3268 cm^{-1} and 2875 cm^{-1}, respectively and O-H bending vibrations at 1623 cm^{-1}.

In addition, Figure 3b shows an approach of the IR spectrum of β-CDs revealing C–O–C symmetric and asymmetric vibrations at 890 cm^{-1}, 1170 cm^{-1}, and 1021 cm^{-1}; respectively. With respect to free β-cyclodextrin, the spectra of β-CD–carvacrol and β-CD–thymol inclusion complexes (Figure 3b) highlighted the presence of characteristics C=C peaks corresponding to carvacrol and thymol aromatic

ring close to 1590 cm^{-1} and vibrations of their respective methyl (-CH_3) groups appear at 1430 cm^{-1} (asymmetric) and 1360 cm^{-1} (symmetric). These shifts relative to those of respective free compounds provide a clear evidence of host-guest interactions.

3.2. Mechanical Properties of PLA Packaging Loaded with β-CD–Carvacrol or β-CD–Thymol Inclusion Complexes

In order to prevent breakages during the packaging process, polymeric materials to be used in food packaging require sufficient flexibility [23]. In this sense, the mechanical properties of the PLA boxes with different concentrations (1.5%; 2.5%, and 5%, wt%) of β-CD–carvacrol (Table 3) and β-CDs–thymol (Table 4) inclusion complexes were evaluated, using PLA boxes without solid complexes as control.

Table 3. Mechanical properties of the enriched or not PLA trays with β-CD–carvacrol complexes.

PLA Boxes with Different % of β-CD–Carvacrol				
Parameter	**0%**	**1.50%**	**2.50%**	**5%**
Young's modulus (Mpa)	2873 ± 176	2327 ± 170 *	2259 ± 53 *	1960 ± 110 *
Maximum stress (MPa)	63.6 ± 4.5	49.9 ± 6.5 *	51.3 ± 4.9 *	47.5 ± 5.1 *
Breaking point (%)	2.4 ± 0.4	2.7 ± 0.3 *	2.9 ± 0.2 *	3.2 ± 0.4 *

Results expressed in mean ± standard deviation of ten determinations; symbol (*) in the same file indicates significant differences ($p < 0.05$) according to Tukey's test.

Table 4. Mechanical properties of the enriched or not PLA trays with β-CD–thymol complexes.

PLA Boxes with Different % of β-CD–Thymol				
Parameter	**0%**	**1.50%**	**2.50%**	**5%**
Young's modulus (Mpa)	2873 ± 176	2667 ± 161 *	2382 ± 69 *	2394 ± 118 *
Maximum stress (MPa)	63.6 ± 4.5	57.9 ± 6.8 *	53.2 ± 2.3 *	55.1 ± 5.2 *
Breaking point (%)	2.4 ± 0.4	2.8 ± 0.3 *	2.9 ± 0.2 *	3.1 ± 0.3 *

Results expressed in (mean ± standard deviation) of ten determinations; Symbol (*) in the same file indicate significant differences ($p < 0.05$) according to Tukey's test.

As can be seen in Table 3, Young's modulus ranged from 2873 to 1960 MPa for β-CD–carvacrol complexes and from 2873 to 2394 MPa for β-CD–thymol complexes (see Table 4), showing lower values than the control trays (only PLA). In fact, Young's modulus gradually decreases as the concentration (weight percentage, w%) of the dehydrated complexes increases, obtaining the lowest value of Young's modulus in the sample fortified with 5% of carvacrol (see Table 3), with a significant difference respect to the average value ($p < 0.05$). The same trend was observed when evaluating the maximum stress, with the lowest value being observed in the PLA package enriched with dehydrated complexes of β-CD–carvacrol (5%), 14% lower than the value obtained for PLA fortified with thymol complexes at the same concentration (w%). The different mechanical values observed when both the complexes were added to the PLA polymer could be due to the higher CE value obtained for carvacrol–β-CDs (105.6%), 65% higher than the value obtained for thymol–β-CDs (69.3%), revealing that CE values above 100% indicate that at pH 7.0, there are more β-CDs complexing carvacrol than free in solution. In the case of thymol, the number of β-CDs complexing thymol is lower, since CE is less than 100%, and in consequence, the decrease in mechanical properties is less pronounced.

Regarding the breaking point, a significant increment of this parameter was observed as the concentration (wt%) of the dehydrated complexes increased, improving 25% and 23% of the elongation capacity of the polymeric material (control), when 5% of β-CD–carvacrol or 5% of β-CD–thymol, respectively, were added to PLA. This behavior may be attributable to a plasticizing effect triggered by the addition of β-CD complexes to the polymer matrix disrupting the crystalline structure of PLA and increasing its ductile properties [24].

As a result, the relative high elongations achieved were beneficial since the boxes presented better flexibility. These results are consistent with those obtained by Ramos and López-Rubio, wherein an increase in elongation and breaking point in plastic films composed of polypropylene/carvacrol/thymol were evidenced [24,25].

3.3. *Scanning Electron Microscopy*

The fracture micrographs of the samples are shown in Figure 4.

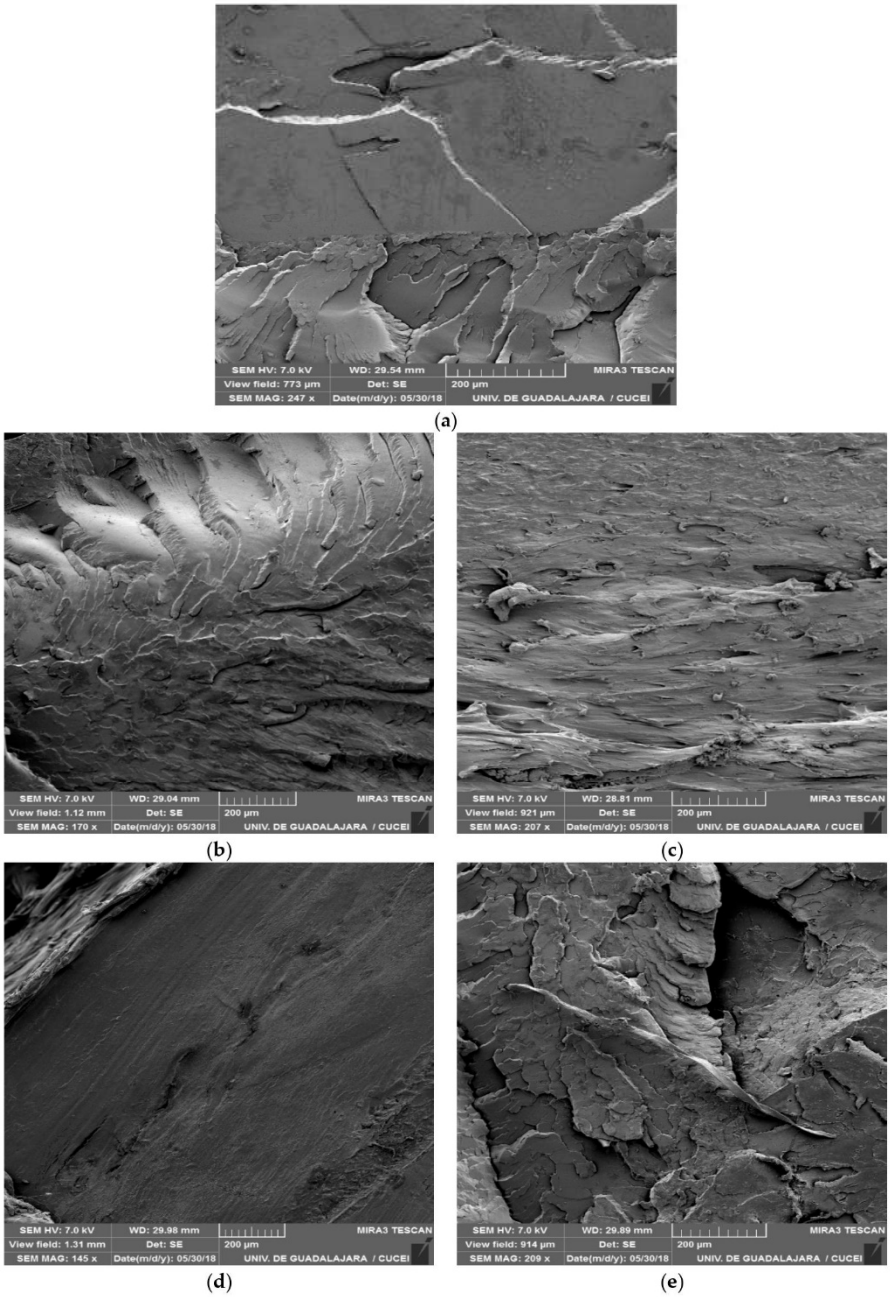
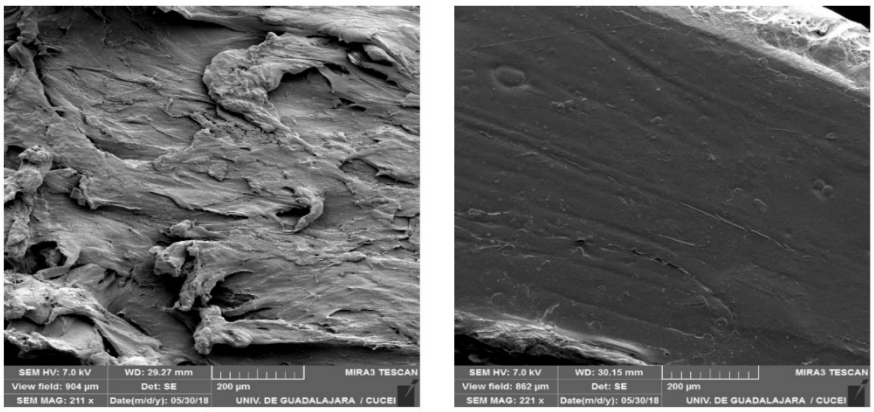


Figure 4. *Cont.*



(**f**) (**g**)

Figure 4. SEM micrographs of fracture samples: (**a**) 100% PLA; (**b**) 98.5% PLA with 1.5% β-CD–thymol; (**c**) 97.5% PLA with 2.5% β-CD–thymol; (**d**) 95.0% PLA with 5.0% β-CD–thymol; (**e**) 98.5% PLA with 1.5% β-CD–carvacrol; (**f**) 97.5% PLA with 2.5% β-CD–carvacrol; (**g**) 95.0% PLA with 5.0% β-CD–carvacrol.

As can be seen (Figure 4a), while control sample (only PLA) had an irregular surface, the PLA samples enriched with β-CD–carvacrol (Figure 4e–g) or β-CD–thymol (Figure 4b–d) complexes exhibited a more uniform surface as the concentration (wt%) increased. In this sense, the decrease in the number of cuts and folds of polymeric material was directly proportional to the concentration of added complex.

These results are in agreement with the values reported in mechanical tests (see Tables 3 and 4), evidencing that the increase of the concentration of complexes in the formulation of the plastic material favors obtaining more flexible packaging (decrease in Young's modulus), providing the formation of a smoother and continuous surface.

This fact could be due to encapsulation which helps incorporate the active compound (carvacrol or thymol) into the polymeric matrix, since different results have been described in the literature when raw essential oils (without encapsulation) were added to polymeric materials to produce heterogeneous structures with oil droplets trapped into the polymer [26,27].

3.4. Differential Scanning Calorimetry

To investigate the thermal transitions of the films studied, DSC measurements were accomplished. As can be seen in Table 5, the packages containing β-CD–carvacrol or β-CD–thymol complexes showed similar thermal properties, regardless of their concentration. The glass transition temperature (Tg) of the PLA-enriched materials was analogous to that obtained for PLA control, and similar to Tg values described in the literature [28], indicating that the amorphous phase of the PLA does not undergo any change.

On the other hand, the packaging with additives shows a significant variation in the cold crystallization temperature with respect to the control packaging (Tc = 102.7 °C; PLA 0%), increasing up to 3 °C and 5 °C for concentrations of 2.5% (wt%) of β-CD–thymol and β-CD–carvacrol, respectively, modifying the cold crystallization behavior of the PLA, and in consequence, the formation of the ordered structure of polymer matrix [29].

As can be seen in Figure 5, an endothermic peak was observed for all samples at a melting temperature, Tm, close to 168.5 °C, with slight temperature variations (lower than 1 °C), for PLA containing β-CD–carvacrol complexes. The little variations of cold crystallization and melting temperatures observed, when increasing β-CD–carvacrol and β-CD–thymol added to the PLA matrix, could be due to the increase in the chain mobility of the polymer matrix.

Table 5. Parametric values of DSC obtained from pure PLA and added β-CD–carvacrol or β-CD–thymol at 1.5%, 2.5%, and 5%, wt%.

Parameter	Control * 0%	PLA–Thymol–β-CDs (wt%)			PLA–Carvacrol–β-CDs (wt%)		
		1.5%	2.5%	5.0%	1.5%	2.5%	5.0%
Tg (°C)	59	61	59	61	60	60	60
Tcc (°C)	102.7	103.8	105.4	105.0	106.5	107.7	105.9
Tm (°C)	168.5	168.5	168.5	168.8	167.9	168.8	169.1
ΔHc Energy (J/g)	36.09	33.08	30.61	29.03	36.42	32.09	36.83
ΔHm Energy (J/g)	45.63	44.09	37.44	35.65	44.72	37.54	42.95

* Control, pure PLA without β-CD–carvacrol or β-CD–thymol; Tg, glass transition temperature; Tcc, cold crystallization transition temperature; Tm, melting transition temperature.

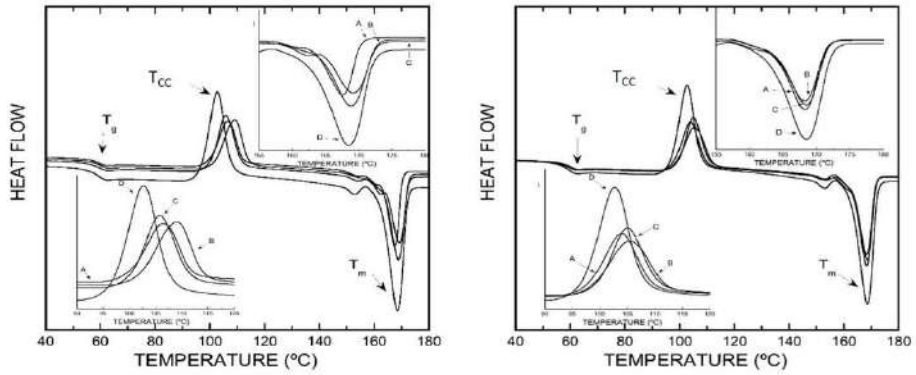


Figure 5. (**a**) DSC curves for: (**A**) PLA–(β-CD–carvacrol 1.5%, wt%); (**B**) PLA–(β-CD–carvacrol 2.5%, wt%); (**C**) PLA–(β-CD–carvacrol 5%, wt%); (**D**) PLA. (**b**) DSC curves for (**A**) PLA–(β-CD–thymol 1.5%, wt%); (**B**) PLA–(β-CD–thymol 2.5%, wt%); (**C**) PLA–(β-CD–thymol 5%, wt%); (**D**) PLA.

3.5. *Thermogravimetry (TGA)*

The thermal stability of PLA trays fortified with β-CD–carvacrol and β-CD–thymol complexes and non-fortified trays was measured by TGA, and all the samples had two weight loss steps (Figure 6).

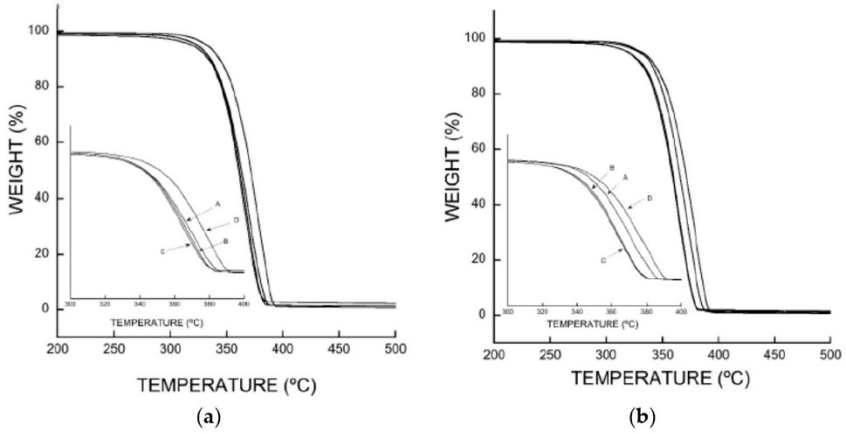


Figure 6. (**a**): Thermogravimetric analysis curves for (**A**) PLA–(β-CD–carvacrol 1.5%, wt%); (**B**) PLA–(β-CD–carvacrol 2.5%, wt%); (**C**) PLA–(β-CD–carvacrol 5, wt%); (**D**) PLA. (**b**): Thermogravimetric analysis curves for (**A**) PLA–(β-CD–thymol 1.5%, wt%); (**B**) PLA–(β-CD–thymol 2.5%, wt%); (**C**) PLA–(β-CD–thymol 5%, wt%); (**D**) PLA.

A significant mass loss between 320 °C and 390 °C could be observed, which is in agreement with PLA decomposition, following which the thermal analysis curves slow down from 390 °C up to 500 °C till the complexes achieve a constant mass. In addition, pure PLA has a slightly higher stability (Figure 6) than PLA–β-CD–carvacrol and PLA–β-CD–thymol and the thermal stability of the polymeric matrices diminishes with increasing concentrations of β-CD–carvacrol or β-CD–thymol. These results indicate that although all polymer samples are, in essence, thermally stable below 300 °C, the mixtures containing β-CD–carvacrol and β-CD–thymol have a faster weight loss rate than pure PLA at the same temperature.

In practice, the PLA-based packages used in the food industry will be at room temperature or lower; therefore, the thermal stability of the PLA–β-CD–carvacrol and PLA–β-CD–thymol materials developed herein, will not be compromised.

3.6. Antifungal Assays

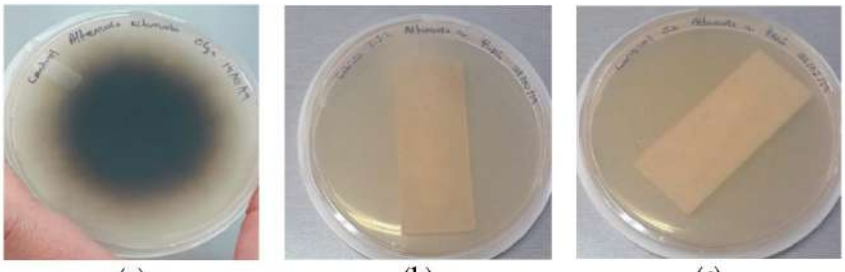
The antifungal properties of PLA packages containing monoterpene–cyclodextrin complexes was monitored for 10 days of incubation to determine their prospective potential uses in the agrofood industry, and the results are shown in Table 6.

Table 6. Antifungal activity over incubation time of developed packaging materials against *Alternaria alternate*.

Type of Packaging	Encapsulation Concentration (% *w*/*w*)	Incubation Time	
		5 Days	10 Days
PLA-control	0.0%	29.7 [a]	69.0
PLA–β-CD–thymol	1.5%	28.3	71.6
	2.5%	30.0	60.0
	5.0%	3.3 *	0.0 *
PLA–β-CD–carvacrol	1.5%	29.7	65.3
	2.5%	0.0 *	0.0 *
	5.0%	0.0 *	0.0 *

[a] Diameter of the colony or surface area in mm. For each test, * values are statistically significant ($p < 0.05$).

As can be seen in Table 6, the results showed that PLA packages containing 2.5% and 5% β-CD–carvacrol or 5% β-CD–thymol (wt%) completely inhibited *Alternaria alternata* after 10 days of incubation (see Figure 7).



(**a**) (**b**) (**c**)

Figure 7. Photographs of the antifungal test: (**a**) Control *Alternaria alternata;* (**b**) PLA–β-CD–carvacrol packaging at 2.5%, wt%; (**c**) PLA–β-CD–carvacrol packaging at 5%, wt% with inoculation of *A. alternata* after 10 days of incubation.

These results are in agreement with those obtained by Llana-Ruiz-Cabello et al. (2016), revealing the antimicrobial properties, against yeasts and moulds, of PLA films containing 5% and 10% of oregano essential oil in ready-to-eat salads [30].

The addition of carvacrol and thymol encapsulates to the polymeric matrix of PLA, as well as the optimized injection temperature (180–190 °C) to produce the packaging material, allow to play an active role against the growth of moulds. The inhibitory effect of these vapor phase assets can be attributed to the accumulation of volatile substances in the medium, followed by interaction with the hydrophobic portion of the cell membrane [31].

Other investigations associate the inhibitory effect of the active compounds of essential oils such as carvacrol and thymol with changes in the morphology of hyphae due to penetration of active compounds into the plasma membrane [32]. The antifungal activity of both the compounds was preserved following the inclusion and injection process, due to encapsulation with β-CD. Previous investigations with similar encapsulation processes such as spray drying [33], freeze drying [34], and lyophilization [35] have shown that encapsulation helps to preserve the antimicrobial and antioxidant properties, mainly of essential oils, and that they are advantageous because they improve water solubility by forming inclusion complexes.

Carvacrol and thymol are volatile compounds; therefore, they could be highly effective in removing bacteria from packaging [36]. The antimicrobial action of carvacrol and thymol released by the PLA matrix against a wide range of phytopathogens constitutes an interesting topic for further studies. Indeed, studies are being conducted regarding the measurement of the effectiveness of packaging against other microorganisms in different food products at various storage temperatures by this research group.

4. Conclusions

In this work, PLA packages filled with thymol or carvacrol complexed in β-cyclodextrins (β-CDs) were prepared and characterized to evaluate their potential use as antibacterial materials. The results obtained by FTIR confirm that the inclusion of carvacrol and thymol in the apolar cavity of β -CDs yielded a significantly higher efficiency of complexation for carvacrol (105.6) than for thymol (69.3). Different proportions of β-CD–thymol or β-CD–carvacrol (0.0%, 1.5%, 2.5%, and 5.0%, wt%) complexes were mixed with PLA for packaging development by injection process, selecting 180–190 °C as the optimal temperature. The presence of β-CD–carvacrol or β-CDs–thymol complexes confer to polymer material plasticizers features that diminish intermolecular forces of the polymer chains, thereby reducing packaging stiffness. In TGA experiments for thermal behavior analysis, the presence of thymol– or carvacrol–β-cyclodextrins solid complexes in PLA formulations slightly decreased the thermal degradation temperature of the polymer, when compared with pure PLA. The performance of the developed polymer materials against *Alternaria alternata* inhibition after 10 days of incubation provided evidence for their potential use in agrofood industry, since packages containing 2.5% and 5% β-CD–carvacrol, or 5% β-CD–thymol, completely inhibited fungal growth. Additional studies are required to evaluate the diffusion and release kinetics of carvacrol and thymol complexes in the PLA polymer matrix during food storage.

Author Contributions: V.-C.F., experimental development and writing of the article; A.-P.H., FTIR and SEM analysis interpretation, and writing and revision of the article; N.-D.S.M., mechanical, structural, and thermal evaluation of polymer materials; N.-D.E., design of antifungal tests and revision of the article; G.J.A., experimental design and revision of the article.

Funding: This work was financially supported by the Ministry of Economy and Competitiveness (MINECO), within the State Program of Research, Development and Innovation Oriented to the Challenges of the Society (Reference: RTC-2015-3321-2).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Francis, G.A.; Gallone, A.; Nychas, G.J.; Sofos, J.N.; Colelli, G.; Amodio, M.L.; Spagno, G. Factors affecting quality and safety of fresh-cut produce. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 595–610. [CrossRef] [PubMed]

2. De Oliveira, M.A.; De Souza, V.M.; Bergamini, A.M.; De Martinis, E.C. Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control* **2011**, *22*, 1400–1403. [CrossRef]
3. Abadias, M.; Usall, J.; Anguera, M.; Solsona, C.; Viñas, I. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int. J. Food Microbiol.* **2008**, *123*, 121–129. [CrossRef] [PubMed]
4. França, K.R.S.; Silva, T.L.; Cardoso, T.A.L.; Ugulino, A.L.N.; Rodrigues, A.P.M.; De Mendonça Júnior, A.F. In vitro Effect of Essential Oil of Peppermint (Mentha x piperita L.) on the Mycelial Growth of Alternaria alternata. *J. Exp. Agric.* **2018**, *26*, 1–7. [CrossRef]
5. Muller, J.; González-Martínez, C.; Chiralt, A. Combination of poly (lactic) acid and starch for biodegradable food packaging. *Materials* **2017**, *10*, 952. [CrossRef] [PubMed]
6. Murariu, M.; Dubois, P. PLA composites: From production to properties. *Adv. Drug Deliv. Rev.* **2016**, *107*, 17–46. [CrossRef] [PubMed]
7. Zhou, L.; Zhao, G.; Jiang, W. Mechanical properties of biodegradable polylactide/poly (ether-block-amide)/thermoplastic starch blends: Effect of the crosslinking of starch. *J. Appl. Polym. Sci.* **2016**, *133*, 7. [CrossRef]
8. Qi, X.; Ren, Y.; Wang, X. New advances in the biodegradation of Poly(lactic) acid. *Int. Biodeterior. Biodegrad.* **2017**, *117*, 215–223. [CrossRef]
9. Huang, T.; Qian, Y.; Wei, J.; Zhou, C. Polymeric antimicrobial food packaging and its applications. *Polymers* **2019**, *11*, 560. [CrossRef]
10. 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*, 419–429. [CrossRef]
11. Suriyatem, R.; Auras, R.; Rachtanapun, C.; Rachtanapun, P. Biodegradable rice Strach/carboxymethyl chitosan films with added propolis extract for potential use as active food packaging. *Polymers* **2018**, *10*, 954. [CrossRef] [PubMed]
12. Ribeiro-Santos, R.; Andrade, M.; de Melo, N.R.; Sanches-Silva, A. Use of essential oils in active food packaging: Recent advances and future trends. *Trends Food Sci. Technol.* **2017**, *61*, 132–140. [CrossRef]
13. Wang, L.H.; Zhang, Z.H.; Zeng, X.A.; Gong, D.M.; Wang, M.S. Combination of microbiological, spectroscopic and molecular docking techniques to study the antibacterial mechanism of thymol against Staphylococcus aureus: Membrane damage and genomic DNA binding. *Anal. Bioanal. Chem.* **2017**, *409*, 1615–1625. [CrossRef] [PubMed]
14. Ramos, M.; Jiménez, A.; Garrigós, M.C. Carvacrol-Based Films: Usage and Potential in Antimicrobial Packaging. In *Antimicrobial Food Packaging*; Academic Press: Cambridge, MA, USA, 2016; pp. 329–338. [CrossRef]
15. Raybaudi-Massilia, R.M.; Mosqueda-Melgar, J.; Soliva-Fortuny, R.; Martín-Belloso, O. Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 157–180. [CrossRef]
16. Saad, N.Y.; Muller, C.D.; Lobstein, A. Major bioactivities and mechanism of action of essential oils and their components. *Flavour Fragr. J.* **2013**, *28*, 269–279. [CrossRef]
17. Ayala-Zavala, J.F.; Soto-Valdez, H.; Gonzalez-Leon, A.; Alvarez-Parrilla, E.; Martin-Belloso, O.; Gonzalez-Aguilar, G.A. Microencapsulation of cinnamon leaf (Cinnamomum zeylanicum) and garlic (Allium sativum) oils in beta-cyclodextrin. *J. Incl. Phenom. Macrocycl. Chem.* **2008**, *60*, 359–368. [CrossRef]
18. Rodríguez-López, M.I.; Mercader-Ros, M.T.; López-Miranda, S.; Pellicer, J.A.; Pérez-Garrido, A.; Pérez-Sánchez, H.; Gabaldón, J.A. Thorough characterization and stability of HP-β-cyclodextrin thymol inclusion complexes prepared by–microwave technology: A required approach to a successful application in food industry. *J. Sci. Food Agric.* **2019**, *99*, 1322–1333. [CrossRef]
19. Rodríguez-López, M.I.; Mercader-Ros, M.T.; Pellicer, J.A.; Gómez-López, V.M.; Martínez-Romero, D.; Núñez-Delicado, E.; Gabaldón, J.A. Evaluation of monoterpene-cyclodextrin complexes as bacterial growth effective hurdles. *Food Control* **2020**, *108*, 106814. [CrossRef]
20. Serna-Escolano, V.; Serrano, M.; Valero, D.; Rodríguez-López, M.I.; Gabaldón, J.A.; Castillo, S.; Guillén, F.; Zapata, P.J.; Martínez-Romero, D. Effect of Thymol and Carvacrol Encapsulated in Hp-B-Cyclodextrin by Two Inclusion Methods against Geotrichum citri-aurantii. *J. Food Sci.* **2019**, *84*, 1513–1521. [CrossRef]

21. American Society for Testing and Materials (ASTM). *Standard Test Method for Tensile Properties of Plastics*; ASTM: West Conshohocken, PA, USA, 2014.
22. Du, W.X.; Olsen, C.W.; Avena-Bustillos, R.J.; McHugh, T.H.; Levin, C.E.; Mandrell, R.; Friedman, M. Antibacterial effects of allspice, garlic, and oregano essential oils in tomato films determined by overlay and vapour-phase methods. *J. Food Sci.* **2009**, *74*, M390–M397. [CrossRef]
23. Arrieta, M.P.; López, J.; Ferrándiz, S.; Peltzer, M.A. Characterization of PLA-limonene blends for food packaging applications. *Polym. Test.* **2013**, *32*, 760–768. [CrossRef]
24. Ramos, M.; Jiménez, A.; Peltzer, M.; Garrigós, M.C. Characterization and antimicrobial activity studies of polypropylene films with carvacrol and thymol for active packaging. *J. Food Eng.* **2012**, *109*, 513–519. [CrossRef]
25. López-Rubio, A.; Lagaron, J.M. Improvement of UV stability and mechanical properties of biopolyesters through the addition of β-carotene. *Polym. Degrad. Stab.* **2010**, *95*, 2162–2168. [CrossRef]
26. Liu, D.; Li, H.; Jiang, L.; Chuan, Y.; Yuan, M.; Chen, H. Characterization of active packaging films made from poly (lactic acid)/poly (trimethylene carbonate) incorporated with oregano essential oil. *Molecules* **2016**, *21*, 695. [CrossRef]
27. Kumari, A.; Kumar, V.; Yadav, S.K. Plant extract synthesized PLA nanoparticles for controlled and sustained release of quercetin: A green approach. *PLoS ONE* **2012**, *7*, e41230. [CrossRef]
28. Carrasco, F.; Pagés, P.; Gámez-Pérez, J.; Santana, O.O.; Maspoch, M.L. Processing of poly(lactic acid): Characterization of chemical structure, thermal stability and mechanical properties. *Polym. Degrad. Stab.* **2010**, *95*, 116–125. [CrossRef]
29. Hwang, S.W.; Shim, J.K.; Selke, S.E.; Soto-Valdez, H.; Matuana, L.; Rubino, M.; Auras, R. Poly (L-lactic acid) with added α-tocopherol and resveratrol: Optical, physical, thermal and mechanical properties. *Polym. Int.* **2012**, *61*, 418–425. [CrossRef]
30. Llana-Ruiz-Cabello, M.; Pichardo, S.; Bermudez, J.M.; Banos, A.; Nunez, C.; Guillamon, E.; Aucejo, S.; Camean, A.M. Development of PLA films containing oregano essential oil (Origanum vulgare L. virens) intended for use in food packaging. *Food Addit. Contam. Part A* **2016**, *33*, 1374–1386.
31. Laird, K.; Phillips, C. Vapour phase: A potential future use for essential oils as antimicrobials. *Lett. Appl. Microbiol.* **2012**, *54*, 169–174. [CrossRef]
32. Soylu, E.M.; Kurt, S.; Soylu, S. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. *Int. J. Food Microbiol.* **2010**, *143*, 183–189. [CrossRef]
33. Arana-Sánchez, A.; Estarrón-Espinosa, M.; Obledo-Vázquez, E.N.; Padilla-Camberos, E.; Silva-Vázquez, R.; Lugo-Cervantes, E. Antimicrobial and antioxidant activities of Mexican oregano essential oils (Lippia graveolens H. B. K.) with different composition when microencapsulated in beta-ciclodextrin. *Lett. Appl. Microbiol.* **2010**, *50*, 585–590. [CrossRef] [PubMed]
34. Santos, E.H.; Kamimura, J.A.; Hill, L.E.; Gomes, C.L. Characterization of carvacrol beta-cyclodextrin inclusion complexes as delivery systems of antibacterial and antioxidant applications. *LWT Food Sci. Technol.* **2015**, *60*, 583–592. [CrossRef]
35. Wang, T.; Li, B.; Si, H.; Lin, I.; Chen, L. Release characteristics and antibacterial activity of solid state eugenol/β-cyclodextrin inclusion complex. *J. Incl. Phenom. Macrocycl. Chem.* **2011**, *71*, 207–213. [CrossRef]
36. Goñi, P.; López, P.; Sánchez, C.; Gómez-Lus, R.; Becerril, R.; Nerín, C. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chem.* **2009**, *116*, 982–989. [CrossRef]

ARTÍCULO 2. Effect of PLA Active Packaging Containing Monoterpene-Cyclodextrin Complexes on Berries Preservation.

Velázquez-Contreras, F.; García-Caldera, N.; Padilla de la Rosa, J.D.; Martínez-Romero, D.; Núñez-Delicado, E.; Gabaldón, J.A. (2021) Effect of PLA Active Packaging Containing Monoterpene-Cyclodextrin Complexes on Berries Preservation. Polymers, 13, 1399. https://doi.org/10.3390/polym13091399

polymers

MDPI

Article

Effect of PLA Active Packaging Containing Monoterpene-Cyclodextrin Complexes on Berries Preservation

Friné Velázquez-Contreras [1,2], Nelsy García-Caldera [2], José Daniel Padilla de la Rosa [3], Domingo Martínez-Romero [4], Estrella Núñez-Delicado [1] and José Antonio Gabaldón [1,*]

1 Department of Health Sciences, Campus de los Jerónimos, Universidad Católica San Antonio de Murcia, No.135 Guadalupe, 30107 Murcia, Spain; fvelazqu@up.edu.mx (F.V.-C.); enunez@ucam.edu (E.N.-D.)
2 Escuela de Administración de Instituciones (ESDAI). Universidad Panamericana. Álvaro del Portillo 49. Ciudad Granja, Zapopan 45010, Mexico; ngarciac@up.edu.mx
3 Departamento de Tecnología Alimentaria, Centro de Investigación y Asistencia Tecnológica y Diseño del Estado de Jalisco (CIATEJ), Marcelino García Barragán 800, Guadalajara 44270, Mexico; jdpadilla@ciatej.mx
4 Department of Food Technology, University Miguel Hernández, Orihuela, 03312 Alicante, Spain; dmromero@umh.es
* Correspondence: jagabaldon@ucam.edu; Tel.: +34-968-278-622

Abstract: Blackberries and raspberries are highly perishable and fragile products, which limits their shelf life. The effect of biodegradable active packaging of blackberries and raspberries containing 2.5% and 5.0% weight (wt%) of thymol or carvacrol complexed in β-cyclodextrins (β-CDs), successively added to poly (lactic acid) (PLA), and melt-processed by injection molding was evaluated under stored conditions at 4 °C for 21 days, using as reference commercial clamshell and PLA package control samples. Thus, physicochemical, headspace, microbiological, and sensory quality studies were carried out in order to compare the efficacy of the different packages. Concerning weight loss, color, and total phenolic and soluble solids content, significant differences were detected when compared with commercial clamshell packaging. The results show that the PLA packages containing thymol and carvacrol complexes maintained the color, weight, and phenolic content of berries until day 21, with a score up to 45% better compared to commercial clamshell. The headspace analysis detected 101 mg L^{-1} (ppm) of thymol and 35 ppm of carvacrol on the first day of refrigeration; these concentrations decreased with time. This release mechanism of carvacrol and thymol into the PLA package modified the initial atmosphere composition. After 21 days of storage, the berries had 4.25 degrees of acceptance, without adverse perception of aroma or flavor for both carvacrol and thymol compounds. A general microbial inhibition was observed for yeast and molds, which increased with the concentration of monoterpene in PLA packages, and showed an inhibition of 3.5 log units for PLA packages containing thymol, and of 3 log units for those containing carvacrol. Overall results show that PLA/β-CD-thymol 5.0% packages prolonged raspberries' and blackberries' shelf life by one more week at 4 °C, compared with commercial clamshell packaging.

Keywords: active packaging; poly (lactic acid); thymol; carvacrol; β-cyclodextrin; berries; shelf life

Citation: Velázquez-Contreras, F.; García-Caldera, N.; Padilla de la Rosa, J.D.; Martínez-Romero, D.; Núñez-Delicado, E.; Gabaldón, J.A. Effect of PLA Active Packaging Containing Monoterpene-Cyclodextrin Complexes on Berries Preservation. *Polymers* **2021**, *13*, 1399. https://doi.org/10.3390/polym13091399

Academic Editor: Cédric Delattre

Received: 8 April 2021
Accepted: 21 April 2021
Published: 26 April 2021

1. Introduction

Since ancient times and before the start of agriculture, berries have been valuable as a primary food source for humans and other primates, and their consumption has increased greatly in recent years. This marked trend could be related to health as a recent motivational purchase vector of consumers since—despite berries having a number of highly appreciated nutritional (they represent an important source of macro- and micronutrients) and organoleptic attributes such as their sharp color, exclusive flavor, and delicate texture—their health-promoting properties have been largely associated with their high levels of bioactive compounds (including anthocyanins, ascorbic and phenolic acids, and flavonoids) with known benefits in the prevention of certain types of cancer, as well as heart and age-related diseases, such as neurodegeneration [1].

However, berries have a short storage life (about 3 weeks at low temperature) as a result of their high respiration and water loss, which results in the fruit shriveling and softening. In addition, they show high susceptibility to mechanical damages and decay, which is generally increased by postharvest fungal infections (mainly caused by *Botrytis cinerea* and *Colletotrichum gloeosporioides*). Hence, berries should be marketed soon after picking, since they are very perishable fruits [2], and must be rapidly cooled and kept at temperatures close to 0 °C and 90–95% of relative humidity (RH) [3] for maximum postharvest preservation, since temperature management is the most critical postharvest factor [4].

Blackberry and raspberry (*Rubus* spp.) are considered non-climacteric fruits; they must be harvested at or near full maturity, since habitually they will not continue to ripen once isolated from the plant. Therefore, after harvest, attention to detail is required throughout the handling system between the sites of production and consumption, since fruits are living organs subject to continuous changes. Some of these changes are desirable and may require specific actuation protocols (including treatments and/or techniques) to promote them, while others, such as ripening and senescence, the last stages in the development of fresh berry fruits, which are characterized by some irreversible processes that lead to breakdown and death of the fruit, are undesirable and require the application of corrective actions to delay and minimize their occurrence and severity [5]. Therefore, understanding the biological and environmental factors affecting the postharvest deterioration rate (respiration, ethylene evolution, compositional changes, temperature, CO_2, and pathogens incidence) is crucial to the implantation of suitable strategic actions for maintaining quality and extending berries' postharvest shelf life.

In this context, different approaches have been investigated to extend the shelf life of these fruits, with the application of modified atmosphere packaging (MAP) being widely used. In this preservation technique, when the air surrounding the berries in the package is changed to another composition, close to 10% O_2 and 15% CO_2, the decay is significantly retarded, and the fruits show a more attractive color compared with those stored under standard conditions [6]. In addition, MAP promotes microbial load reduction, since berries keep respiring the trapped air until the concentration of CO_2 swiftly reaches the critical level (10–15%) required to inhibit microorganism growth, such as *Botrytis cinerea*. However, some negative effects include the slow-down of the cooling of the packaged products and an increased potential for water condensation within the package, as well as lower respiration and softening rates that have been described in this type of packaging, which consequently reduces the berries' shelf life. Therefore, appropriate postharvest techniques are needed, as well as a low storage temperature to maintain the quality of the fruits, to prevent physical injury, and to keep diseased or wounded berries out of packages, as rot can rapidly spread from diseased berries to the nearby healthy ones. In addition, temperature fluctuations that can occur during storage, transport, and retail display can generate an unfavorable atmosphere inside the package [7] and affect the desirable flavor and aroma organoleptic parameters [8,9].

Due to the increasing consumer awareness about residues of agrochemicals, a set of limitations on their use was imposed by many countries. However, the consumption and often uncontrolled disposal of single-use plastic packaging is regarded as one of the main environmental hazards [10]. Indeed, the forecast by 2050 is not optimistic, since oceans could contain more plastic than fish by weight, and the impacts of degraded or intentionally produced microscopic-sized plastics on human health remain unknown [11]. In fact, plastic pollution is considered to be among the greatest challenges the world faces.

Thus, to respond to growing consumer demand, some of the strategic approaches recently outlined by the European Commission under the Food 2030 umbrella are focused on the development of impactful solutions to the urgent, complex, and interconnected challenges inherent to food systems, applying the bioeconomy principles. Challenges in this area include, among others, achieving zero food waste, promoting the employment of

safer alternatives to replace synthetic pesticides, and rethinking food packaging for better biodegradable options that limit micro plastics and greenhouse gases release [12,13].

Currently, most bioplastics are produced from agricultural crop-based feedstocks, [14] like cellulose, proteins, starch, renewable polyethylene and polyvinyl chloride (PVC), polyhydroxyalkanoates (PHAs), or poly (lactic acid) (PLA). Among them, PLA exhibit good functional properties in terms of bio-absorbability, biodegradability, biocompatibility, mechanical strength, and transparency, and meet several packaging requirements to extend the shelf life and protection of these types of fruits [15]. Moreover, PLA could be enhanced with antimicrobial activity via the incorporation of natural essential oils, displaying a promising active packaging in view of reducing postharvest losses, and retaining high standards in terms of environmental sustainability [16].

The main disadvantage of using essential oils are their highly volatile nature, as they are quite sensitive to light, insoluble in water, and have an unpleasant aroma, which limits their application in active food packaging [17]. For this reason, the search for strategies that protect the active compounds from volatilization during processing, and prevent a fast release of these kinds of compounds, is required. Thus, the introduction of nanoparticles that include essential oils or their main components as a filler into biopolymer-based materials could be a successful approach to fulfil these drawbacks.

Based on this concept, recent studies have reported the antimicrobial effectiveness by in vitro tests of PLA and starch films with cinnamaldehyde against *Escherichia coli* and *Listeria Innocua* through [18]; or against *Escherichia coli* and *Staphylococcus aureus* by PLA nanofilms incorporating inclusion complex of cinnamon essential oil in β-cyclodextrins [19]. In addition, fungal growth hurdles of *Alternaria alternata* were shown to be effective, using as additives up to 10 wt% of thymol or carvacrol complexed in β-cyclodextrins, which were successively added to PLA and melt-processed by injection molding [20].

Thus, the development of PLA active packaging containing the above described materials is an innovative antimicrobial approach in the agri-food sector [16], and in particular to protect blackberries and raspberries during their storage and distribution to improve their shelf life by reducing bacterial and fungal growth [21]. In this type of packaging, one of the most common approaches is based on the release of antimicrobial compounds from the package. Although these compounds could be of synthetic or natural in origin, due to the consumer's growing refusal of synthetic additives in food, the use of natural antimicrobial substances could be promoted.

Carvacrol and thymol are two monoterpenoid compounds naturally present in some plant species such as oregano and thyme, which have shown a wide range of activity against pathogenic microorganisms, including Gram-negative and Gram-positive species [22]. Due to their hydrophobic nature, the presence of a free hydroxyl group, and a delocalized electron system, which are responsible for the disruption of the cytoplasmic membrane of bacterial cells, disturbing their functional properties, causing leakage of integrity and intracellular material [23], they are also effective against various fungi such as *Aspergillus* spp., *Botrytis cinerea*, *Fussarium* spp. [22], or *Penicillium* spp., [24], by inducing envelope damage and blocking ergosterol biosynthesis.

Recently, Zhang et al. [25] demonstrated that concentrations of thymol between 65 mg L^{-1} and 100 mg L^{-1}, and of carvacrol between 120 mg L^{-1} and 140 mg L^{-1}, showed high postharvest efficacy against *Botrytis cinerea*, changing the morphology of the cell wall, breaking the membrane by collapse, thus causing deformation and deterioration of hyphae and conidia. In addition, thymol and carvacrol were complexed in native and modified β-cyclodextrins in a previous study, and successfully applied by in vitro tests against filamentous fungi [26] and bacteria [17].

The two cyclodextrins and two monoterpenes to be added to PLA have been classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA), and included in the list of additives permitted for direct addition to food for human consumption.

In this context, the antifungal effect of a controlled release system of thymol and carvacrol encapsulated in β-cyclodextrin, incorporated into a biodegradable polymeric

matrix of PLA by industrial injection, was previously assessed in vitro [20]. At present, there are very few research results on their efficacy in extending the shelf life of fresh produce in vivo under storage conditions. The aim of this study was to evaluate the in vivo effect of the biodegradable packaging in order to enhance the quality attributes of berries during refrigerated storage, to increase their shelf life in commercial distribution, trying to demonstrated the functionality of this active packaging.

2. Materials and Methods

2.1. Materials

Thymol (CAS: 89-83-8, 98.7% purity), carvacrol (CAS: 499-75-2, 99.5% purity), and β-cyclodextrin (β-CDs > 95%, food grade) were provided by Sigma-Aldrich Corp. (Saint Louis, MO, USA).

For injection molding applications, a poly (lactic acid) (PLA, Ingeo™ ref. code: 3251D) biopolymer with required physicochemical parameters ($\overline{M}w = 5.5 \times 10^4$ g/mol; PI = 1.62 and 99% L-lactide/1% D-lactide isomers content) and flow capability performances [13] was purchased from PromaPlast Co. (Guadalajara, Jalisco, Mexico). Potato dextrose agar (PDA), plate count agar (PCA), and violet red bile agar (VRBA) were provided by Bioxon, Mexico. All other chemical products used were of analytical grade.

2.2. Packaging Preparation and Characteristics

In preliminary experiments, spray drying was used to prepare microcapsules of carvacrol and thymol complexed in β-cyclodextrins in solid state, which were directly mixed with PLA (up to 5 wt%) and extruded to produce pellets [20]. The microcapsules proved to act as plasticizers, being particularly able to reduce intermolecular forces of PLA chains, thus improving the breaking properties and stretchability, which were subsequently required for adequate melt-processing of the pellets by injection-molding to obtain (12 cm long × 10 cm width × 3.0 cm height and 0.1 cm wall thickness) boxes for the in vivo test. Briefly, from pellets containing as ingredients PLA (97.5% and 95% weight percentages, wt%) and dehydrated complexes of β-CD-carvacrol or β-CD-thymol (at 2.5% and 5% wt%), a representative number of boxes were obtained by injection of the pellets, which were identified as PLA/β-CD-carvacrol, 2.5% wt%; PLA/β-CD-carvacrol, 5.0% wt%; PLA/β-CD-thymol, 2.5% wt%; PLA/β-CD-thymol, 5.0% wt%. In addition, boxes containing 100% PLA were also formed and used as control.

2.3. Design and Application of Storage Tests

Blackberries (*Rubus ulmifolius* var. Dasha) and raspberries (*Rubus idaeus* var. Maravilla) were selected to assess the antimicrobial activity of the PLA/β-CD-carvacrol and PLA/β-CD-thymol packaging, as well as their effect on quality attributes of berries during refrigerated storage. Thus, commercial Driscoll's packed samples from the western zone of Mexico, Jalisco state, were purchased in October 2020, in a local supermarket of Zapopan (Jalisco, Mexico), and transported in coolers to the Universidad Panamericana and CIATEJ (Center of Research and Assistance of Technology and Design of Jalisco State). After that, berries were stored at 4 °C ± 2 °C for six hours until use. Samples of 47 g (6 blackberries) and 25 g (6 raspberries) were weighed using a common scale in Adventurer Pro balance (OHAUS, Parsippany, NJ, USA) and placed inside the developed boxes (Figure 1), which were divided into six groups as follow:

- PLA/β-CD—thymol, 2.5% containing blackberries and raspberries
- PLA/β-CD—thymol, 5.0% containing blackberries and raspberries
- PLA/β-CD—carvacrol 2.5% containing blackberries and raspberries
- PLA/β-CD—carvacrol 5.0% containing blackberries and raspberries
- PLA/C (packaging control) containing blackberries and raspberries
- Commercial clamshell (control) containing blackberries and raspberries

Figure 1. Boxed samples containing blackberries and raspberries. (**A**) PLA/C, closed; (**B**) PLA/β-CD-carvacrol, 2.5% wt%, open; (**C**) PLA/β-CD-thymol, 5.0% wt%, closed.

For each group, thirty replicate packages were prepared to accomplish all physicochemical, microbiological, and chromatographic determinations at initial (T0), 7 days (T1), 14 days (T2), and 21 days (T3). The packages were stored at 4 °C ± 2 °C and 85–90% RH in a conventional refrigerator (Whirlpool Mod. WS5501Q).

2.4. Physicochemical Analyses

2.4.1. Weight Loss

Weight loss of stored blackberries and raspberries was measured on day 0, and subsequently on the corresponding sampling days, in triplicate, using an Adventurer precision balance (OHAUS, Parsippany, NJ, USA), and expressed as percent of weight loss per initial fruit weight by means of the following equation:

$$WL = \left(\frac{(m1 - m2)}{m1}\right) \times 100$$

where (m_1) was the initial weight of the berries, and (m_2) the weight after the corresponding storage time.

2.4.2. Color

The surface color of blackberries and raspberries was determined by measuring at least six berries from each package, in triplicate, at the beginning and at the end of storage, using a colorimeter Konica Minolta (Chroma Meter, Tokyo, Japan). The colorimeter used the CIE standard illuminant D65 that represents daylight more completely and accurately than B and C illuminants [27]. Measurements were obtained using the CIE L*a*b*: L * indicates lightness, a * indicates chromaticity on a green (-) to red (+) axis, and b * indicates chromaticity on a blue (-) to yellow (+) axis. Results were expressed as the mean ± standard deviation of three determinations.

2.4.3. Soluble Solids Content

The determination of soluble solids (SSC) was carried as described by Almenar et al. [28]. For this, approximately 20 g of blackberries and raspberries were blended in a mortar for 60 s. SSC in the obtained juice were measured using a digital handheld refractometer (Atago, Tokyo, Japan) at 20 °C. Three measurements were taken on each sample, and the results were expressed as °Brix.

2.4.4. Total Phenolic Content

The total phenolic content (TPC) of the berries' extract was determined colorimetrically using the Folin–Ciocalteu reagent, according to a modification of the López Miranda et al. [29] method. For that, the gallic acid curve was prepared by serial solutions from 1 mg mL^{-1} standard. The berries' extracts were dissolved in ethanol 1 mg/mL (*v*/*v*). For each extract, an aliquot of 20 µL was added to 100 µL of Folin–Ciocalteu in spectrophotometer cuvettes and allowed to react for 5 min, then 80 µL of 7.5% sodium carbonate solution was added and homogenized. Then, it was incubated at room temperature in the dark for 2 h, and the optical density was measured at 750 nm using an Infinite 200 PRO Microplate Reader

from TECAN. Data were expressed as gallic acid equivalents. Results were expressed as the mean ± standard deviation of three determinations.

2.5. Study on the Sustained Release of Carvacrol and Thymol

The release rate of carvacrol and thymol from the PLA were evaluated in the headspace by gas chromatography coupled to mass spectrometry (HS-GC-MS) using a GC/MSD System Agilent Technologies 7890B/5977A (Palo Alto, CA, USA). To perform these determinations, a representative number of berry containers were properly adapted to do correct sampling (Figure 2). Briefly, the PLA packages were drilled to make a hole through which to sample the terpene phenols released during storage. The hole was sealed with a PTFE/silicone septum (20 mm, Supelco) and fixed using reinforced aluminum adhesive tape. A gas-tight syringe A-2 Series (VICI Precision, Los Ángeles, CA, USA) was used to sample 1 mL of headspace through the septum in each package, and further introduced into the injector operated in split mode (3:1) at 240 °C. The separation was carried out on an INNOWAX capillary column (60 m × 250 µm × 0.25 µm, Folsom, CA, USA) from Agilent Technologies, and helium was used as carrier gas, at a flow rate of 0.8 mL min^{-1}. The oven temperature was constant at 200 °C, held for 16 min. The MSD transfer line and ion source temperatures were operated at 260 °C and 250 °C, respectively, in electron-impact ionization mode (70 eV).

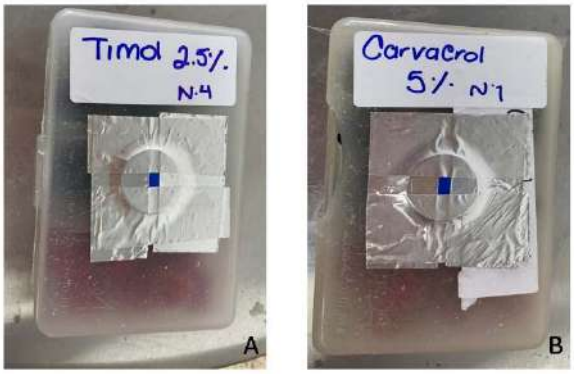


Figure 2. Boxes containing blackberries and raspberries adapted for headspace sampling. (**A**) PLA/β-CD-thymol, 2.5% wt%; (**B**) PLA/β-CD-carvacrol, 5.0%.

Carvacrol and thymol identification were performed by injection of pure standard (Sigma-Aldrich, Saint Louis, MO, USA) and spectral data from the NIST14 MS library database in a previous acquisition in full scan mode (*m/z* 29–300). Quantification was performed by using selected ion monitoring (SIM) focused on *m/z* 91, 135, and 150 characteristic ions of thymol and carvacrol. Chromatographic responses (peak area abundance) were monitored to obtain the comparative concentration of each compound in the studied samples. Three replicates for each group and sampling day were evaluated.

2.6. Microbial Analysis

Ten grams of the sample of each package (group) were ground and suspended in 90 mL peptone water (0.1% *v/v*), and the suspension was mixed in a blender (Seward Stomacher 400) for 5 min. Serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) of the homogenated berries were plated on the surface of selective media: aerobic mesophilic bacteria (AMB) on plate count agar (PCA), incubated at 35 °C for 48 h; total coliforms (TC) using violet red agar (VRBA), incubated at 35 °C for 48 h; and yeast and molds (YM) on potato dextrose agar (PDA), incubated at 25 °C for 5 days.

All culture mediums were from BD Bioxon, México, and microbial analysis was performed in triplicate, according to the Official Standard Method guidelines [30]. The colony forming units (CFU) were expressed as log CFU (colony-forming units) per gram of blackberry and raspberry. Results were expressed at the mean ± standard deviation of three determinations.

2.7. Sensory Atributes Analysis

The organoleptic parameters—color, flavor, odor, texture, and overall impression of the blackberries and raspberries packaged in the control clamshell and control PLA, as well as in PLA packages containing β-CD-thymol (2.5% and 5.0% wt%) or β-CD-carvacrol (2.5% and 5.0% wt%)—were scored using the 5-point scale (where extremely like = 5, moderately like = 4, neither like nor dislike = 3, moderately dislike = 2, and extremely dislike = 1) by a panel of 30 judges (15 men and 15 women), with experience in sensory analysis (all members of the academy of sciences at Universidad Panamericana) by means a hedonic analysis [31]. The samples were delivered coded with 3-digit numbers, in sufficient quantity at an adequate temperature (21 °C), in order to minimize the variability associated with this type of non-descriptive hedonic test. In addition, all evaluations were carried out at 0, 7, 14, and 21 days of storage, in individual cabinets that fit the design standard specifications. As consensus of opinion among judges, the end of shelf life was set for a score of 3 or less for any of the organoleptic characteristics evaluated. Results were expressed as the mean ± standard deviation of three determinations.

2.8. Statistical Data Treatment

Experiments were carried out in a randomized design, in triplicate, and the data were reported as means ± 1 standard deviation. When ANOVA was significant ($p < 0.05$), means were separated by Turkey's range test. The statistical analyses were performed using 01Statgraphics Centurion XV.

3. Results and Discussion

To investigate whether the developed active packaging releasing carvacrol or thymol would prevent deterioration of non-climacteric berries, storage stability was studied.

3.1. Weight Loss

Usually, postharvest moisture loss alters fruit appearance, flavor, and texture, and reduces its marketable weight [32]. Since raspberries and blackberries are prone to dehydration due to the lack of epicuticular wax, a maximum moisture loss of 6% is considered as commercially acceptable [32]. Figure 3 shows the weight loss of control and active packaged blackberries (A) and raspberries (B) samples with storage time.

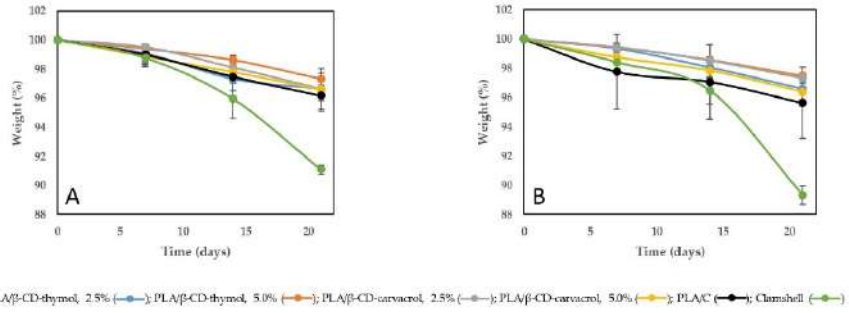


Figure 3. Effect of type of packaging and storage time (0, 7, 14, 21 days) at 4 °C on the blackberries' (**A**) and raspberries' (**B**) weight losses (expressed as percentage).

As can be seen, the weight loss in raspberries (Figure 3B) was significantly greater ($p < 0.05$) than for blackberries (Figure 3A). This fact could be due to the mass transport and diffusion of water vapor that happens during the physiological processes; raspberries have higher respiration rates (metabolic activity 49 mg CO_2 kg^{-1} h^{-1} at 10 °C) than blackberries (metabolic activity 31 mg CO_2 kg^{-1} h^{-1} at 10 °C) [33].

In addition, in the clamshell control samples (see Figure 3), a higher moisture loss of berries stored at 4 °C was observed at the end of the study ($p < 0.05$): 9% for blackberries and 11% for raspberries, both of which are higher than the commercially acceptable value of 6%.

This behavior was attributed to excessive water loss, from dry matter consuming water, and we should not be amazed, since in the fresh market, berries are commonly packed in clamshells with opening ratios between 3% and 10%, preventing ethylene, heat, and moisture accumulation [8]. However, the vents could make berries with a wide surface area ratio (like proposed here) more susceptible to freezing, chilling, and drying damage [34]. Similar moisture losses, or still higher values that are directly proportional with the diameter and number of opening ratios, have been described in the literature [35]. To overcome commercial clamshells' limitations, the active packaging here proposed (with lid closed), which has low water vapor permeability between fruits and the surrounding air, retains a relative humidity inside the package [36]. Therefore, the observed weight losses are even 7% lower in the berries inside the control packaging (PLA/C).

Taking into account the above described commercially acceptable moisture loss value for berries, all PLA active packaging containing monoterpene-cyclodextrin complexes showed a slower weight loss trend, reaching, in the worst case, a decrease of 3% at the end of the study. The higher reduction of weight loss by carvacrol or thymol would indicate a role of these compounds in lowering the dehydration process [35]. PLA β-CD-thymol 5.0 wt% showed the lowest weight loss for blackberries and raspberries, with a significant difference ($p < 0.05$) when compared to PLA/C and commercial clamshells control samples, stored under refrigeration conditions at 4 °C after 21 days of assay. Moreover, berries in commercial clamshells were unsaleable by day 16, with a weight loss of 6%.

3.2. Color

The development of the preferred red/purple-bluish color in berries is mainly due to anthocyanins, and during postharvest ripening of the fruit, numerous changes occur in pigments affecting color [37]. As can be seen in Table 1, color changes were significantly affected in blackberries as well as in raspberries (see Table 2) stored under refrigeration at 4 °C, varying by type of packaging and storage time.

The ANOVA test showed significant differences ($p < 0.05$) with respect to storage time. In the case of blackberries, the packaging treatments' decreasing lightness (L*) and modified chromatic coordinates a* and b*, increasing the red shift (a*) as well as a yellow color trend (b*), were observed in the clamshell with respect to the PLA β-CD-thymol 2.5 wt% and PLA β-CD-thymol 5.0 wt% packaging, respectively.

With respect to the raspberries, color changes were significantly affected by packaging treatments, decreasing lightness (L*), and chromatic coordinates (a* and b*). However, the raspberries packed in PLA β-CD-thymol (2.5 or 5.0 wt%) showed less loss of red color (a*). This could be due to the protective and antioxidant effect of a sustained and controlled release of encapsulated thymol inside the PLA package, as has been previously described for table grapes stored for 56 days under modified atmosphere packaging with thymol [38].

In summary, a decrease in L* value during storage could be attributed to the oxidation of phenolic compounds and other physicochemical phenomena [34], like the formation of adducts or complexes between anthocyanins and quinones, generated throughout the oxidation of polyphenols and the weight loss that occurs during storage [39].

Table 1. Changes in color parameters (L*, a*, b*) of blackberries under different packaging at 0, 7, 14, and 21 days of storage at 4 °C.

Day	Packaging	L*	a*	b*
0	-	17.91 ± 0.48^{a}	0.82 ± 0.13^{a}	-0.93 ± 0.11^{a}
7	Clamshell	15.65 ± 0.42^{bc}	1.35 ± 0.59^{b}	-0.91 ± 0.08^{a}
	PLA/C	15.98 ± 0.27^{bc}	3.35 ± 0.59^{b}	-0.48 ± 0.23^{b}
	PLA/β-CD-thymol, 2.5%	15.28 ± 0.99^{bc}	1.67 ± 0.29^{a}	-1.01 ± 0.03^{a}
	PLA/β-CD-thymol, 5.0%	18.23 ± 0.16^{a}	1.13 ± 0.11^{ab}	-0.95 ± 0.07^{a}
	PLA/β-CD-carvacrol, 2.5%	18.23 ± 0.27^{a}	1.32 ± 0.36^{ab}	-0.91 ± 0.08^{a}
	PLA/β-CD-carvacrol, 5.0%	17.01 ± 1.07^{ab}	0.92 ± 0.22^{a}	-1.08 ± 0.30^{a}
14	Clamshell	13.02 ± 0.76^{c}	1.05 ± 0.10^{ab}	-0.55 ± 024^{b}
	PLA/C	14.02 ± 0.76^{c}	2.05 ± 0.3^{b}	-0.57 ± 0.20^{b}
	PLA/β-CD-thymol, 2.5%	17.26 ± 0.87^{ab}	0.68 ± 0.16^{a}	-1.11 ± 0.04^{a}
	PLA/β-CD-thymol, 5.0%	16.12 ± 0.30^{bc}	1.09 ± 0.10^{ab}	-1.02 ± 0.03^{a}
	PLA/β-CD-carvacrol, 2.5%	15.20 ± 0.37^{c}	1.75 ± 0.74^{ab}	-0.55 ± 0.04^{b}
	PLA/β-CD-carvacrol, 5.0%	15.99 ± 0.21^{bc}	0.74 ± 0.03^{a}	-1.06 ± 0.08^{a}
21	Clamshell	12.92 ± 0.36^{c}	0.66 ± 0.18^{a}	-0.55 ± 024^{b}
	PLA/C	13.65 ± 0.42^{bc}	2.05 ± 0.63^{b}	-0.57 ± 0.20^{b}
	PLA/β-CD-thymol, 2.5%	17.10 ± 0.77^{ab}	0.63 ± 0.21^{a}	-1.15 ± 0.10^{a}
	PLA/β-CD-thymol, 5.0%	16.10 ± 0.29^{bc}	1.12 ± 0.15^{ab}	-1.11 ± 0.15^{a}
	PLA/β-CD-carvacrol, 2.5%	14.90 ± 0.80^{c}	1.72 ± 0.74^{ab}	-0.73 ± 0.34^{ab}
	PLA/β-CD-carvacrol, 5.0%	15.95 ± 0.27^{bc}	0.68 ± 0.08^{a}	-1.10 ± 0.08^{a}

* Values are reported as average ± standard deviation of triplicate determinations. Values in the same column with different letters are significantly different statistically ($p < 0.05$).

Table 2. Changes in color parameters (L*, a*, b*) of raspberries under different packaging at 0, 7, 14, and 21 days of storage at 4 °C.

Day	Packaging	L*	a*	b*
0	-	28.26 ± 0.68^{a}	26.49 ± 1.17^{a}	10.85 ± 1.09^{a}
7	Clamshell	25.29 ± 0.90^{c}	24.65 ± 0.34^{a}	8.01 ± 0.24^{b}
	PLA/C	27.46 ± 1.34^{ab}	28.10 ± 1.23^{ab}	8.05 ± 0.09^{b}
	PLA/β-CD-thymol, 2.5%	27.80 ± 1.26^{ab}	31.57 ± 2.18^{b}	8.52 ± 0.37^{b}
	PLA/β-CD-thymol, 5.0%	27.39 ± 0.90^{c}	25.78 ± 0.33^{ab}	8.93 ± 0.59^{b}
	PLA/β-CD-carvacrol, 2.5%	28.48 ± 0.09^{a}	25.44 ± 0.52^{ab}	8.77 ± 0.24^{b}
	PLA/β-CD-carvacrol, 5.0%	28.35 ± 0.65^{ab}	25.26 ± 1.99^{ab}	8.76 ± 0.56^{b}
14	Clamshell	24.08 ± 0.17^{b}	21.20 ± 1.32^{b}	6.77 ± 0.39^{b}
	PLA/C	25.73 ± 0.52^{c}	23.01 ± 0.23^{ab}	6.87 ± 0.45^{b}
	PLA/β-CD-thymol, 2.5%	27.17 ± 0.16^{ab}	24.29 ± 0.34^{a}	7.21 ± 0.87^{b}
	PLA/β-CD-thymol, 5.0%	26.92 ± 0.23^{b}	24.51 ± 0.74^{a}	8.13 ± 0.51^{b}
	PLA/β-CD-carvacrol, 2.5%	26.33 ± 0.59^{ab}	22.45 ± 1.49^{a}	7.90 ± 0.80^{b}
	PLA/β-CD-carvacrol, 5.0%	26.53 ± 0.21^{bc}	21.83 ± 2.28^{b}	7.54 ± 0.15^{b}
21	Clamshell	23.96 ± 0.54^{b}	16.57 ± 0.50^{c}	6.11 ± 0.28^{b}
	PLA/C	25.57 ± 0.59^{b}	22.08 ± 2.80^{a}	6.35 ± 0.45^{b}
	PLA/β-CD-thymol, 2.5%	26.29 ± 0.34^{b}	22.16 ± 1.71^{b}	7.09 ± 0.47^{b}
	PLA/β-CD-thymol, 5.0%	26.78 ± 0.33^{a}	22.98 ± 1.08^{ab}	8.03 ± 0.39^{b}
	PLA/β-CD-carvacrol, 2.5%	25.08 ± 0.17^{b}	21.91 ± 0.61^{b}	7.81 ± 0.59^{b}
	PLA/β-CD-carvacrol, 5.0%	25.23 ± 1.26^{b}	21.71 ± 0.60^{c}	7.50 ± 1.25^{b}

* Values are reported as average ± standard deviation of triplicate determinations. Values in the same column with different letters are significantly different statistically ($p < 0.05$).

3.3. Soluble Solids and Total Phenolic Content

The soluble solid (SSC) as well as total phenolic content (TPC) of control and treated samples is shown in Table 3. As can be seen in Table 3, a significant difference was found in blackberries' soluble solid content, regarding the value at the beginning of the study (8.93 ± 0.12 °Brix), under storage time with the different packaging treatments.

Table 3. Evolution of soluble solid content (SSC) expressed as Brix grade (°Brix) and total phenolic content (TPC) expressed as milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g) of blackberries and raspberries under different packaging at 0, 7, 14, and 21 days of storage at 4 °C.

		Blackberries		Raspberries	
Day	**Packaging**	**°Brix**	**mg GAE/100 g**	**°Brix**	**mg GAE/100 g**
0	-	8.93 ± 0.12 ^{a}	101.01 ± 6.66 ^{a}	11.80 ± 0.26 ^{a}	119.10 ± 3.09 ^{a}
7	Clamshell	8.67 ± 0.06 ^{ac}	62.58 ± 0.51 ^{d}	9.70 ± 0.20 ^{cd}	80.41 ± 0.43 ^{cd}
	PLA/C	8.33 ± 0.23 ^{cd}	61.80 ± 1.37 ^{b}	9.17 ± 0.06 ^{d}	76.55 ± 7.26 ^{d}
	PLA/β-CD-thymol, 2.5%	9.67 ± 0.21 ^{b}	71.98 ± 13.49 ^{c}	9.23 ± 0.35 ^{d}	86.08 ± 19.78 ^{bcd}
	PLA/β-CD-thymol, 5.0%	9.20 ± 0.35 ^{ab}	81.91 ± 22.41 ^{ab}	9.13 ± 0.15 ^{d}	79.10 ± 10.77 ^{abc}
	PLA/β-CD-carvacrol, 2.5%	9.00 ± 0.17 ^{a}	80.03 ± 7.79 ^{c}	10.33 ± 0.45 ^{bc}	110.5 ± 2.51 ^{ab}
	PLA/β-CD-carvacrol, 5.0%	8.07 ± 0.15 ^{d}	88.35 ± 1.25 ^{bc}	10.50 ± 0.10 ^{b}	100.88 ± 24.88 ^{a}
14	Clamshell	10.97 ± 0.21 ^{d}	60.45 ± 0.56 ^{d}	9.80 ± 0.10 ^{cd}	80.43 ± 1.26 ^{b}
	PLA/C	8.10 ± 0.10 ^{b}	80.55 ± 5.48 ^{bc}	8.53 ± 0.21 ^{c}	83.40 ± 4.32 ^{b}
	PLA/β-CD-thymol, 2.5%	11.01 ± 0.17 ^{d}	92.40 ± 15.71 ^{a}	10.27 ± 0.47 ^{bc}	69.82 ± 10.54 ^{ab}
	PLA/β-CD-thymol, 5.0%	10.50 ± 0.10 ^{ab}	92.28 ± 6.34 ^{ab}	10.90 ± 0.26 ^{ab}	82.52 ± 16.46 ^{b}
	PLA/β-CD-carvacrol, 2.5%	10.93 ± 0.25 ^{d}	72.83 ± 4.66 ^{cd}	9.10 ± 0.69 ^{de}	106.09 ± 4.79 ^{ab}
	PLA/β-CD-carvacrol, 5.0%	9.73 ± 0.38 ^{c}	81.70 ± 10.64 ^{bc}	10.63 ± 0.31 ^{bc}	103.38 ± 8.54 ^{ab}
21	Clamshell	9.57 ± 0.45 ^{a}	58.07 ± 0.76 ^{c}	9.17 ± 0.57 ^{c}	79.30 ± 0.53 ^{b}
	PLA/C	8.83 ± 0.15 ^{a}	68.59 ± 1.00 ^{bc}	9.80 ± 0.44 ^{bc}	83.90 ± 13.34 ^{b}
	PLA/β-CD-thymol, 2.5%	8.43 ± 1.08 ^{a}	57.57 ± 7.07 ^{c}	9.97 ± 0.15 ^{bc}	100.92 ± 11.86 ^{ab}
	PLA/β-CD-thymol, 5.0%	8.83 ± 0.06 ^{a}	68.29 ± 2.98 ^{bc}	10.07 ± 0.06 ^{bc}	102.84 ± 15.37 ^{ab}
	PLA/β-CD-carvacrol, 2.5%	8.23 ± 0.21 ^{a}	70.27 ± 7.91 ^{bc}	10.03 ± 0.12 ^{bc}	84.86 ± 11.46 ^{ab}
	PLA/β-CD-carvacrol, 5.0%	8.30 ± 0.40 ^{a}	83.09 ± 3.40 ^{b}	10.53 ± 0.57 ^{b}	116.47 ± 2.58 ^{a}

* Values are reported as average ± standard deviation of triplicate determinations. Values in the same column with different letters are significantly different statistically ($p < 0.05$).

Overall, SSC content in all treatment groups increased during storage, showing higher values that ranged from 9.73 ± 0.38 °Brix for PLA/β-CD-carvacrol 5.0% to 11.01 ± 0.17 °Brix for PLA/β-CD-thymol 2.5% on day 14, with the exception of packaging control PLA/C, which decreased SSC (8.10 ± 0.10 °Brix).

Similar compositional changes have been described by Forney et al. [40], with an increase in sugar concentration from about 2.2–3.2% as berry ripening progresses. However, a trend change was observed on day 21 of storage, and a slight decrease in SSC was observed, showing that at the end of the study, all PLA packaging treatments maintained the SSC content in blackberries compared to commercial clamshell.

Regarding SSC of raspberries at the beginning of the study (11.80 ± 0.26 °Brix), at day 21, the SCC in PLA/β-CD-thymol 2.5%, PLA/β-CD-thymol 5.0%, PLA/β-CD-carvacrol 2.5%, and PLA/β-CD-carvacrol 5.0% active packaging decreased by 15.5%, 14.7%, 15.0%, and 10.8%, respectively, which was slightly lower than that obtained for packaging control PLA/C (17%), and considerably lower than that obtained for commercial clamshell (22.3%).

This shows that all PLA active packaging, and specifically the PLA/β-CD-carvacrol 5.0%, maintained a sustained SSC loss through 21 storage days. As has been described in the literature using coating films containing microcapsules of oregano essential oil to preserve fresh purple yam [41], our results indicated that PLA active packaging containing microcapsules of carvacrol or thymol effectively slowed down the metabolism of berries.

Regarding total phenolic content (TPC), although it decreased gradually in all groups with storage time (see Table 3), the PLA groups containing carvacrol and thymol microcapsules showed a less pronounced decline. The control groups, both the commercial clamshell and PLA/C, had the largest TPC decrease in berry samples. For blackberries, with a TPC initial value of 101.01 ± 6.66 mg gallic acid/100 g weight, it decreased up to 58.07 ± 0.76 mg gallic acid/100 g weight on day 21 in commercial clamshell, which represents a 43% TPC loss with respect to its initial value; while the PLA/β-CD-carvacrol 5.0% packaging revealed 83.09 ± 3.40 mg gallic acid/100 g weight on day 21 of storage, with only a 17.74% loss of TPC, suggesting a slower decline process than that observed in the control group. Concerning the TPC initial value for raspberries (119.10 ± 3.09 mg Gallic acid/100 g weight), the best results at the end of the study were obtained again for the PLA/β-CD-carvacrol 5.0% packaging (116.47 ± 2.58 mg gallic acid/100 g weight), which saw a loss of 2.21%, much lower than that determined for commercial clamshell (34% TPC loss), which showed the largest decrease. This slow decline was attributed to the presence, inside the PLA packages, of free carvacrol or thymol compounds, which exert a marked antioxidant effect, protecting phenolic compounds present in berries from oxidation due to their delocalized electron system and aromatic structure—results that agree with those previously described by Ramos et al. [42].

3.4. Headspace Analysis

Determination of volatile compounds from carvacrol and thymol was monitored by head space with gas chromatography–mass spectrometry (HS-GC-MS). At each sampling time, the syringe was introduced through the corresponding package septum, withdrawing 1 mL of fluid gas, and immediately released in a splitless injector at 250 °C, obtaining the results showed in Figure 4, corresponding to three replicates for each group and sampling day.

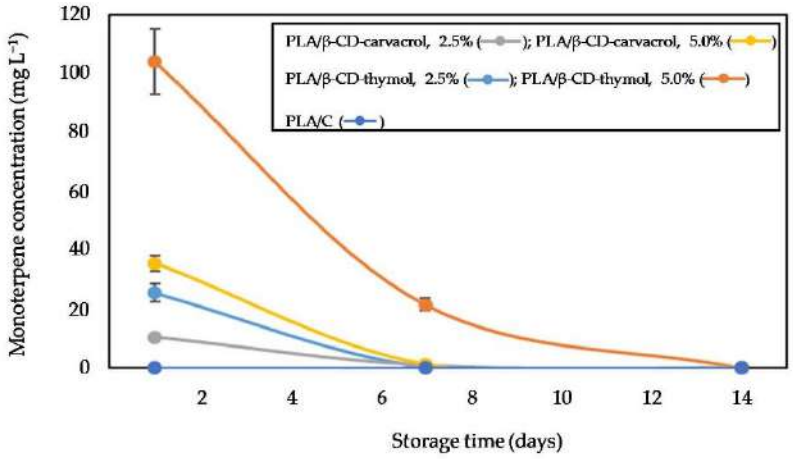


Figure 4. Carvacrol and thymol concentrations in mg L^{-1} (ppm) in the headspace of PLA packages (see inset legend in the figure), by GC-MS at 0, 7, 14, 21 days of storage at 4 °C. Data points represent averages and standard deviations of three replicate samples.

At the initial stage, PLA/β-CD-thymol 5.0% packaging showed the higher volatile concentration, 75% higher than that determined for PLA/β-CD-carvacrol 5.0% and PLA/β-CD-thymol 2.5% packaging. As the storage time was prolonged, only PLA/β-CD-thymol 5.0% packaging maintained a controlled release of monoterpene (30% of initial level at day 7), decreasing asymptotically until the end of the trial, whereas for the rest of the PLA active packaging, the monoterpene levels started to decrease much more quickly until reaching the baseline, with no concentration of any of them detected from the first week of study. A similar behavior was reported by Higueras et al. [43], using films of chitosan containing carvacrol cyclodextrin complexes, evidencing a fast release of carvacrol after the first three days of storage, the concentration of which was maintained over time since packages were hermetically sealed, providing an infinite barrier to carvacrol.

However, in our case, the packages were not hermetically sealed, and the gases could be released outside or a proportion could be absorbed by the fruits without altering their organoleptic properties, as was described by Higueras et al. [43], who argued that the large amount of antimicrobial absorbed or that reacted with the sample caused an unacceptable sensory deterioration.

This release mechanism, observed for carvacrol and thymol in the PLA package, modified the initial atmosphere composition inside the packages, improving the quality and postharvest shelf life of blackberries and raspberries stored under controlled temperature.

3.5. Microbiological Quality

Microorganisms counts allow for studying the spoiling degree of a particular food during a determined postharvest period. Figure 5 shows the count for aerobic mesophilic bacteria (AMB), total coliforms (TC), and yeasts and molds (YM) for blackberry (Figure 5A) and raspberry (Figure 5B), respectively.

As can be seen in Figure 5A for blackberry samples, a sustained increase is observed in the AMB count during the first two weeks under refrigerated storage conditions, from 1 to approximately 2.5 log *cfu*, independently of the packaging type and concentration of monoterpene, with a marked increase noticeable for both control samples (PLA/C and clamshell), showing at the end of the study a 1 log higher value than that obtained for PLA containing microencapsulates of carvacrol and thymol. Regarding the AMB count for raspberries (Figure 5B), a marked increase for clamshell control samples was observed between the first and the second sampling week (a 2 log *cfu* increase), which was 1.5 units higher than the value obtained for PLA/C, and no appreciable AMB was detected growing in PLA/β-CD-carvacrol 5.0%, PLA/β-CD-thymol 5.0%, or PLA/β-CD-thymol 2.5% packaging. For these same types of packaging, the AMC value increased 2.0 log *cfu* at the end of the study (being 1.5 log *cfu* lower that the values obtained for both control samples).

Coliform growth for blackberries showed a slow increase (close to 0.8 log units) for all PLA packaging materials after seven trial days, whereas this increase was lower by half in clamshell control samples (Figure 5A). However, at day 14, it reached a value of 3.5 log *cfu*, which was higher by 1 log unit compared to the level achieved in all PLA samples, with a consensus value for all groups after three weeks of assay, which was 3 log units higher that the initial value. Regarding coliform growth for raspberries (Figure 5B), in general, a delay was observed through the first week of the test, with better inhibition results for coliforms obtained with PLA/β-CD-thymol 5.0% and PLA/β-CD-thymol 2.5% packaging, both at 14 and 21 days of trial, and it was approximately 0.5 log units lower than the values achieved with the PLA packaging containing microencapsulates of carvacrol at the same percentages.

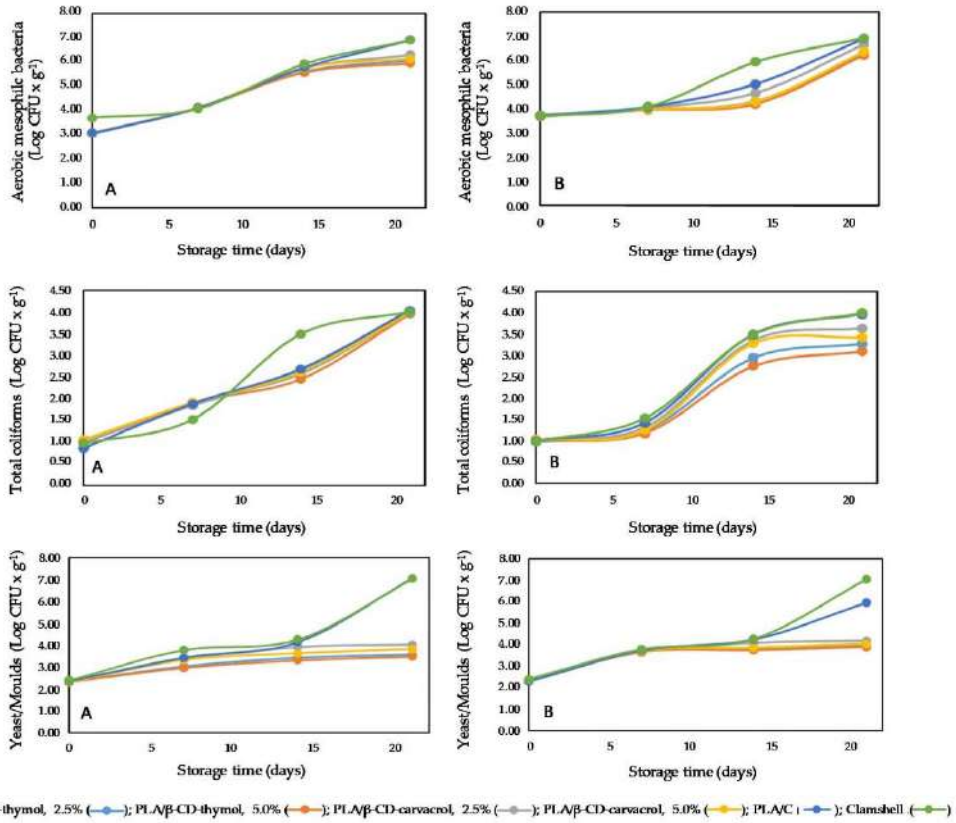


Figure 5. Effect of the type of packaging and storage time (0, 7, 14, 21 days) at 4 °C on the grow of aerobic mesophilic bacteria, totals coliforms, and yeast and molds in blackberries (**A**) and raspberries (**B**).

On the other hand, yeast and molds growth was stopped in raspberries after the first week with all PLA packages containing microencapsulates of carvacrol or thymol (Figure 5B), showing a reduction of 2.2 and 3.2 log units for PLA/C and clamshell control samples, respectively. For blackberries, the growth curve was first low, then gradually increased up to day 14, and subsequently remained constant up to the end of the study for PLA/β-CD-thymol/carvacrol packages (Figure 5A), achieving a slightly better antifungal activity for PLA/β-CD-thymol 5.0% and PLA/β-CD-thymol 2.5% than for PLA packaging containing microencapsulates of carvacrol at the same percentages, and 4.0 log units lower than PLA/C and clamshell control samples, with the berries still fit for consumption. These results are in agreement with those previously described for *Alternaria alternata*, achieving a complete inhibition with PLA packages containing 2.5% and 5% β-CD-carvacrol or 5% β-CD-thymol (wt%) after 10 days of incubation [20], and those obtained against yeasts and molds with PLA films containing 5% and 10% of oregano essential oil in ready-to-eat salads, allowing for the extension of its commercial life [44].

It has been described in the literature that thymol displays a bacteriostatic effect on a wide range of different bacteria and fungi, for which this compound interacts with the lipids on the cell membrane, causing permeabilization and ion leaking, and thus, death [17,26], slightly improving its antimicrobial activity (see Figure 5) as thymol concen-

tration increases [45]. Carvacrol has been reported to present a lower minimum inhibitor concentration (MIC) for different bacterial strains than other phytochemicals, and its synergistic activity with antibiotics has been previously described [46]. This compound exhibits a bacteriostatic effect on microorganisms, with a similar mechanism exerted by thymol. Iron (II) chelating properties of both compounds have been previously described [45,47]; this activity has importance in lipid oxidation reactions, which could explain the inhibition depicted in this study. In addition, the activity displayed for both berries could be related with the amounts of phenolic compounds determined (see Table 3.)

3.6. Sensory Atributes

The flavor is one of the most valuable sensory attributes in berries for consumers. As can be seen in Figure 6, after 21 days of storage at 4 °C, both products reveal a texture decrease as storage days increased. The highest flavor scores obtained at the end of the study were for samples packed in active PLA packaging containing carvacrol and thymol, and adverse aroma or flavor notes of thymol and carvacrol were not noticed by trained referees. The highest acceptance value (4.25 degrees of acceptance at 21 days) for both berries was assigned to PLA packages containing 5% β-CD-carvacrol, which was the same as that obtained for the control sample at the beginning of the study.

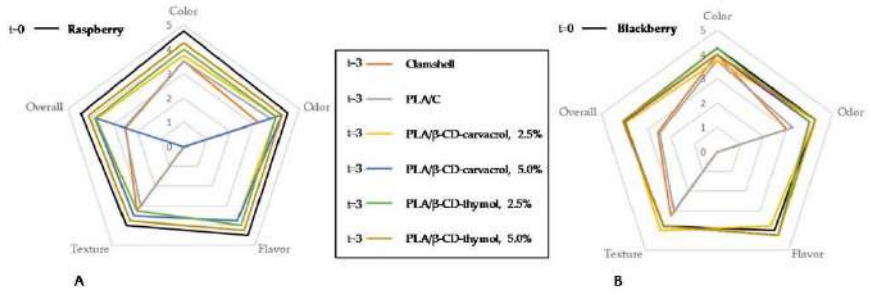


Figure 6. Effect of the type of packaging and storage time (0, 7, 14, 21 days) at 4 °C on sensory attributes of raspberries (**A**) and blackberries (**B**).

The blackberries and raspberries contained in the clamshell and PLA control packages could not be sensory evaluated at T3, that is, after 21 days of storage, as they showed decomposition and severe fungal attack. Raspberries' and blackberries' aroma scores increased during storage due to ripening processes, while a reduction of the texture parameter value was observed. Fruit control color showed 1.5 less degrees of acceptance compared to the fruits in packages containing thymol and carvacrol cyclodextrin complexes. In addition, fruit packaged with both compounds retained the highest degree of color acceptance, with slightly better values for the packages containing thymol. Therefore, the packages that showed the highest overall scores after 21 days of storage at 4 °C for the flavor, odor, color, and texture parameters were, in this order: PLA/β-CD-thymol 5.0%, PLA/β-CD-thymol 2.5%, and PLA/β-CD-carvacrol. These results are in accordance with those previously described in the literature by Viacava et al. [48], since thymol and carvacrol delayed the physiological processes of the fruit and reduced the loss of quality.

4. Conclusions

In this work, active PLA packages filled with thymol or carvacrol complexed in β-cyclodextrins (β-CDs) were prepared to evaluate their potential uses to improve berries' preservation. The results obtained showed the effectiveness of these monoterpenes in improving the shelf life of these fruits. When comparing the results obtained using the developed active PLA packaging materials with control samples along 21 days of storage at

4 °C, a visible growth of microbiological load (mainly yeast and molds) and deterioration in berries' sensory parameters were observed in control samples, which were higher than those obtained for PLA packaging containing both carvacrol and thymol. The PLA packages containing 5% β-CD-thymol showed a higher inhibition of yeasts and molds (51.57%), with a very good sensory score, with the berries still fit for consumption. Consequently, these results indicated the advantages and potential uses of these biodegradable active packages, to replace the conventional plastics and also the direct addition of preservatives in food formulations, in consonance with bioeconomy principles. The PLA packages containing 5% β-CD-thymol or 5% β-CD-carvacrol showed a promising potential to improve the food quality and safety of berries during a storage period of 21 days at 4 °C, extending their shelf life by one more week, compared to the packages currently used in market. However, more studies are also needed regarding the the durability of the packaging, and to evaluate if these polymers can be reusable several times and for different practical applications.

Author Contributions: F.V.-C.: formal analysis and writing original draft preparation; N.G.-C.: validation and supervision; J.D.P.d.l.R.: investigation; D.M.-R.: writing, review, and editing, E.N.-D.: methodology, review, and editing, J.A.G.: conceptualization, funding acquisition, review, and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Ministry of Economy and Competitiveness (MINECO), within the State Program of Research, Development and Innovation Oriented to the Challenges of the Society (Reference: RTC-2015-3321-2).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nile, S.H.; Park, S.W. Edible berries: Bioactive components and their effect on human health. *Nutrition* **2014**, *30*, 134–144. [CrossRef] [PubMed]
2. Kim, M.J.; Lee, M.Y.; Shon, J.C.; Kwon, Y.S.; Liu, K.H.; Lee, C.H.; Ku, K.M. Untargeted and targeted metabolomics analyses of blackberries. Understanding postharvest red drupelet disorder. *Food Chem.* **2019**, *300*, 125169. [CrossRef] [PubMed]
3. Krüger, E.; Dietrich, H.; Schöpplein, E.; Rasim, S.; Kürbel, P. Cultivar, storage conditions and ripening effects on physical and chemical qualities of red raspberry fruit. *Postharvest Biol. Technol.* **2011**, *60*, 31–37. [CrossRef]
4. Perkins-Veazie, P.; Pattison, J.; Fernandez, G.; Ma, G. Fruit quality and composition of two advanced North Carolina strawberry selections. *Int. J. Fruit Sci.* **2016**, *16*, 220–227. [CrossRef]
5. Kumar, S.; Baghel, M.; Yadav, A.; Dhakar, M.K. Postharvest biology and technology of berries. In *Postharvest Biology and Technology of Temperate Fruits*; Mir, S.A., Shah, M.A., Mir, M.M., Eds.; Springer International Publishing: Berlin/Heidelberg, Germany, 2018; pp. 349–370.
6. Giuggioli, N.R.; Briano, R.; Baudino, C.; Peano, C. Effects of packaging and storage conditions on quality and volatile compounds of raspberry fruits. *CyTA J. Food.* **2015**, *13*, 512–521. [CrossRef]
7. Matar, C.; Guillard, V.; Gauche, K.; Costa, S.; Gontard, N.; Guilbert, S.; Gaucel, S. Consumer behaviour in the prediction of postharvest losses reduction for fresh strawberries packed in modified atmosphere packaging. *Postharvest Biol. Technol.* **2020**, *163*, 111119. [CrossRef]
8. Almenar, E.; Samsudin, H.; Auras, R.; Harte, B.; Rubino, M. Postharvest shelf life extension of blueberries using a biodegradable package. *Food Chem.* **2008**, *110*, 120–127. [CrossRef]
9. Fortunati, E.; Luzi, F.; Puglia, D.; Petrucci, R.; Kenny, J.M.; Torre, L. Processing of PLA nanocomposites with cellulose nanocrystals extracted from Posidonia oceanica waste: Innovative reuse of coastal plant. *Ind. Crop. Prod.* **2015**, *67*, 439–447. [CrossRef]
10. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef] [PubMed]
11. Karan, H.; Funk, C.; Grabert, M.; Oey, M.; Hankamer, B. Green bioplastics as part of a circular bioeconomy. *Trend Plant Sci.* **2019**, *24*, 237–249. [CrossRef]
12. European Commission. *Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. A New Circular Economy Action Plan For a Cleaner and More Competitive Europe*; COM(2020) 98 Final; European Commission: Brussels, Belgium, 2020. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM:2020:98:FIN&WT.mc_id=Twitter (accessed on 1 April 2021).

13. Ioannidou, S.M.; Pateraki, C.; Ladakis, D.; Papapostolou, H.; Tsakona, M.; Vlysidis, A.; Kookos, I.K.; Koutinas, A. Sustainable production of bio-based chemicals and polymers via integrated biomass refining and bioprocessing in a circular bioeconomy context. *Bioresour. Technol.* **2020**, *307*, 123093. [CrossRef]
14. Jancikova, S.; Dordevic, D.; Jamroz, E.; Behalova, H.; Tremlova, B. Chemical and physical characteristics of edible films, based on κ-and ι-carrageenans with the addition of lapacho tea extract. *Foods* **2020**, *9*, 357. [CrossRef]
15. Valdés, A.; Ramos, M.; Beltrán, A.; Jiménez, A.; Garrigós, M. State of the art of antimicrobial edible coatings for food packaging applications. *Coatings* **2017**, *7*, 56. [CrossRef]
16. Scaffaro, R.; Lopresti, F.; Marino, A.; Nostro, A. Antimicrobial additives for poly (lactic acid) materials and their applications: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 7739–7756. [CrossRef] [PubMed]
17. Rodríguez-López, M.I.; Mercader-Ros, M.T.; Pellicer, J.A.; Gomez-Lopez, V.M.; Martínez-Romero, D.; Núñez-Delicado, E.; Gabaldon, J.A. Evaluation of monoterpene-cyclodextrin complexes as bacterial growth effective hurdles. *Food Control* **2020**, *108*, 106814. [CrossRef]
18. Muller, J.; González-Martínez, C.; Chiralt, A. Poly(lactic) acid (PLA) and starch bilayer films, containing cinnamaldehyde, obtained by compression moulding. *Eur. Polym. J.* **2017**, *95*, 56–70. [CrossRef]
19. Wen, P.; Zhu, D.H.; Feng, K.; Liu, F.J.; Lou, W.Y.; Li, N.; Zong, M.H.; Wu, H. Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/β-cyclodextrin inclusion complex for antimicrobial packaging. *Food Chem.* **2016**, *196*, 996–1004. [CrossRef]
20. Velázquez-Contreras, F.; Acevedo-Parra, H.; Nuño-Donlucas, S.M.; Núñez-Delicado, E.; Gabaldón, J.A. Development and characterization of a biodegradable PLA food packaging hold monoterpene-cyclodextrin complexes against *Alternaria altern*. *Polymers* **2019**, *11*, 1720. [CrossRef]
21. López, P.; Sánchez, C.; Batlle, R.; Nerín, C. Development of flexible antimicrobial films using essential oils as active agents. *J. Agric. Food Chem.* **2007**, *55*, 8814–8824. [CrossRef]
22. Taghavi, T.; Kim, C.; Rahemi, A. Role of natural volatiles and essential oils in extending shelf life and controlling postharvest microorganisms of small fruits. *Microorganisms* **2018**, *6*, 104. [CrossRef]
23. Bajpai, V.K.; Baek, K.H.; Kang, S.C. Control of Salmonella in foods by using essential oils: A review. *Food Res. Int.* **2012**, *45*, 722–734. [CrossRef]
24. Pérez-Alfonso, C.O.; Martínez-Romero, D.; Zapata, P.J.; Serrano, M.; Valero, D.; Castillo, S. The effects of essential oils carvacrol and thymol on growth of *Penicillium digitatum* and *Penicillium italicum* involved in lemon decay. *Int. J. Food Microbiol.* **2012**, *158*, 101–106. [CrossRef] [PubMed]
25. Zhang, J.; Ma, S.; Du, S.; Chen, S.; Sun, H. Antifungal activity of thymol and carvacrol against postharvest pathogens *Botrytis cinerea*. *J. Food Sci. Technol.* **2019**, *56*, 2611–2620. [CrossRef]
26. Serna-Escolano, V.; Serrano, M.; Valero, D.; Rodríguez-López, M.I.; Gabaldón, J.A.; Castillo, S.; Guillén, F.; Zapata, P.J.; Martínez-Romero, D. Effect of thymol and carvacrol encapsulated in Hp-β-cyclodextrin by two inclusion methods against *Geotrichum citri-aurantii*. *J. Food Sci.* **2019**, *84*, 1513–1521. [CrossRef]
27. Pathare, P.B.; Opara, U.L.; Al-Said, F.A.J. Colour measurement and analysis in fresh and processed foods: A review. *Food Bioproc. Technol.* **2013**, *6*, 36–60. [CrossRef]
28. Almenar, E.; Hernández-Muñoz, P.; Lagarón, J.M.; Catalá, R.; Gavara, R. Controlled atmosphere storage of wild strawberry fruit (Fragaria vesca L.). *J. Agric. Food Chem.* **2006**, *54*, 86–91. [CrossRef]
29. López-Miranda, S.; Serrano-Martínez, A.; Hernández-Sánchez, P.; Guardiola, L.; Pérez-Sánchez, H.; Fortea, I.; Gabaldón, J.A.; Núñez-Delicado, E. Use of cyclodextrins to recover catechin and epicatechin from red grape pomace. *Food Chem.* **2016**, *203*, 379–385. [CrossRef]
30. ISO–ISO 7954:1987–Microbiology. General Guidance for Enumeration of Yeasts and Moulds-Colony Count Technique at 25 Degrees C. Available online: https://www.iso.org/standard/14931.html (accessed on 4 April 2021).
31. Stone, H.; Bleibaum, R.N.; Thomas, H.A. *Sensory Evaluation Practices*; Elsevier Inc: London, UK, 2020; pp. 1–457.
32. Paniagua, A.C.; East, A.R.; Hindmarsh, J.P.; Heyes, J.A. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biol. Technol.* **2013**, *79*, 13–19. [CrossRef]
33. Robbins, J.; Sjulin, T.M.; Patterson, M. Postharvest storage characteristics and respiration rates in five cultivars of red raspberry. *Hort. Sci.* **1989**, *24*, 980–982. Available online: http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=6712045 (accessed on 1 April 2021).
34. Pathare, P.B.; Opara, U.L.; Vigneault, C.; Delele, M.A.; Al-Said, F.A.J. Design of packaging vents for cooling fresh horticultural produce. *Food Bioproc. Technol.* **2012**, *5*, 2031–2045. [CrossRef]
35. Van der Steen, C.; Jacxsens, L.; Devlieghere, F.; Debevere, J. Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biol. Technol.* **2002**, *26*, 49–58. [CrossRef]
36. Wu, Y.; Qin, Y.; Yuan, M.; Li, L.; Chen, H.; Cao, J.; Yang, J. Characterization of an antimicrobial poly (lactic acid) film prepared with poly (ε -caprolactone) and thymol for active packaging. *Polym. Adv. Technol.* **2014**, *25*, 948–954. [CrossRef]
37. Mannozzi, C.; Tylewicz, U.; Chinnici, F.; Siroli, L.; Rocculi, P.; Dalla-Rosa, M.; Romani, S. Effects of chitosan based coatings enriched with procyanidin by-product on quality of fresh blueberries during storage. *Food Chem.* **2018**, *251*, 18–24. [CrossRef]

38. Valero, D.; Valverde, J.M.; Martínez-Romero, D.; Guillén, F.; Castillo, S.; Serrano, M. The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety and functional properties of table grapes. *Postharvest Biol. Tech.* **2006**, *41*, 317–327. [CrossRef]
39. Cortés Rodríguez, M.; Villegas Yépez, C.; Gil González, J.H.; Ortega-Toro, R. Effect of a multifunctional edible coating based on cassava starch on the shelf life of Andean blackberry. *Heliyon* **2020**, *6*, e03974. [CrossRef] [PubMed]
40. Forney, C.F.; Kalt, W.; Jordan, M.A.; Vinqvist-Tymchuk, M.R.; Fillmore, S.A. Blueberry and cranberry fruit composition during development. *J. Berry Res.* **2012**, *2*, 169–177. [CrossRef]
41. Huang, H.; Huang, C.; Yin, C.; Khan, M.R.; Zhao, H.; Xu, Y.; Huang, L.; Zheng, D.; Qi, M. Preparation and characterization of β-cyclodextrin–oregano essential oil microcapsule and its effect on storage behavior of purple yam. *J. Sci. Food Agric.* **2020**, *100*, 4849–4857. [CrossRef] [PubMed]
42. Ramos, M.; Beltrán, A.; Valdés, A.; Peltzer, M.A.; Jiménez, A.; Garrigós, M.C.; Zaikov, G.E. Carvacrol and thymol for fresh food packaging. *J. Bioequiv. Availab.* **2013**, *5*, 154–160. [CrossRef]
43. Higueras, L.; López-Carballo, G.; Hernández-Muñoz, P.; Catalá, R.; Gavara, R. Antimicrobial packaging of chicken fillets based on the release of carvacrol from chitosan/cyclodextrin films. *Int. J. Food Microbiol.* **2014**, *188*, 53–59. [CrossRef]
44. Llana-Ruiz-Cabello, M.; Pichardo, S.; Bermudez, J.M.; Banos, A.; Nunez, C.; Guillamon, E.; Aucejo, S.; Camean, A.M. Development of PLA films containing oregano essential oil (*Origanum vulgare L. virens*) intended for use in food packaging. *Food Addit. Contam. A* **2016**, *33*, 1374–1386. [CrossRef]
45. Marchese, A.; Orhan, I.E.; Daglia, M.; Barbieri, R.; Di Lorenzo, A.; Nabavi, S.F.; Gortzi, O.; Izadi, M.; Nabavi, S.M. Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chem.* **2016**, *210*, 402–414. [CrossRef]
46. Magi, G.; Marini, E.; Facinelli, B. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. *Front. Microbiol.* **2015**, *6*, 165. [CrossRef] [PubMed]
47. Leyva-López, N.; Gutiérrez-Grijalva, E.P.; Vázquez-Olivo, G.; Heredia, J.B. Essential oils of oregano: Biological activity beyond their antimicrobial properties. *Molecules* **2017**, *22*, 989. [CrossRef] [PubMed]
48. Viacava, G.E.; Ayala-Zavala, J.F.; González-Aguilar, G.A.; Ansorena, M.R. Effect of free and microencapsulated thyme essential oil on quality attributes of minimally processed lettuce. *Postharvest Biol. Technol.* **2018**, *145*, 125–133. [CrossRef]

ARTÍCULO 3. Cyclodextrins in polymer-based active food packaging: a fresh look about non-toxic, biodegradable, and sustainable technologies trends.

Velázquez-Contreras, F.; Zamora-Ledezma Camilo; López-González Ivan; Meseguer-Olmo Luis; Núñez-Delicado, E., and Gabaldón J. A. (2022) Cyclodextrins in polymer-based active food packaging: a fresh look about non-toxic, biodegradable, and sustainable technologies trends. Polymers, 14, 104. https:// doi.org/10.3390/polym14010104.

polymers

MDPI

Review

Cyclodextrins in Polymer-Based Active Food Packaging: A Fresh Look at Nontoxic, Biodegradable, and Sustainable Technology Trends

Friné Velázquez-Contreras [1,2], Camilo Zamora-Ledezma [3], Iván López-González [3], Luis Meseguer-Olmo [3], Estrella Núñez-Delicado [1] and José Antonio Gabaldón [1,*]

1 Molecular Recognition and Encapsulation Research Group (REM), Health Sciences Department, UCAM-Universidad Católica de Murcia, Campus de los Jerónimos 135, 30107 Murcia, Spain; fvelazqu@up.edu.mx (F.V.-C.); enunez@ucam.edu (E.N.-D.)

2 Escuela de Administración de Instituciones (ESDAI), Universidad Panamericana, Álvaro del Portillo 49, Ciudad Granja, Zapopan 45010, Mexico

3 Tissue Regeneration and Repair Group Orthobiology, Biomaterials and Tissue Engineering, Health Sciences Department, UCAM-Universidad Católica de Murcia, Campus de los Jerónimos 135, 30107 Murcia, Spain; czamora9@ucam.edu (C.Z.-L.); ilopez27@ucam.edu (I.L.-G.); lmeseguer@ucam.edu (L.M.-O.)

* Correspondence: jagabaldon@ucam.edu; Tel.: +34-968-278-622

Abstract: Using cyclodextrins (CDs) in packaging technologies helps volatile or bioactive molecules improve their solubility, to guarantee the homogeneous distribution of the complexed molecules, protecting them from volatilization, oxidation, and temperature fluctuations when they are associated with polymeric matrices. This technology is also suitable for the controlled release of active substances and allows the exploration of their association with biodegradable polymer targeting to reduce the negative environmental impacts of food packaging. Here, we present a fresh look at the current status of and future prospects regarding the different strategies used to associate cyclodextrins and their derivatives with polymeric matrices to fabricate sustainable and biodegradable active food packaging (AFP). Particular attention is paid to the materials and the fabrication technologies available to date. In addition, the use of cutting-edge strategies, including the trend of nanotechnologies in active food packaging, is emphasized. Furthermore, a critical view on the risks to human health and the associated updated legislation is provided. Some of the more representative patents and commercial products that currently use AFP are also listed. Finally, the current and future research challenges which must be addressed are discussed.

Keywords: active packaging; biodegradable polymers; cyclodextrins; foods; shelf life

Citation: Velázquez-Contreras, F.; Zamora-Ledezma, C.; López-González, I.; Meseguer-Olmo, L.; Núñez-Delicado, E.; Gabaldón, J.A. Cyclodextrins in Polymer-Based Active Food Packaging: A Fresh Look at Nontoxic, Biodegradable, and Sustainable Technology Trends. *Polymers* **2022**, *14*, 104. https://doi.org/10.3390/polym14010104

Academic Editor: Sergio Torres-Giner

Received: 24 November 2021
Accepted: 21 December 2021
Published: 28 December 2021

1. Introduction

Nowadays, most food packaging is used to guarantee food quality and safety by protecting it from external factors, including but not limited to odors, temperature, light exposure, and microorganisms [1]. Research and innovation in food packaging technologies have become an important part of the industry because they seek to improve safety and preserve the food's organoleptic properties while maintaining product quality; they are also important due to the increased consumption of minimally processed foods, the demand for products without artificial preservatives, and the changes in food distribution practices associated with globalization [2–4]. As a result, food packaging is one of the most significant challenges in the food industry, particularly in the area of minimally processed foods, in which appropriate packaging helps to extend the food's shelf life, avoiding microorganism invasion and deterioration. In this sense, the container, beyond fulfilling its basic functions of containing and protecting food, is evolving towards a multifunctional preservation

receptacle that limits degradation while maintaining the food's organoleptic properties and product quality [5].

Active food packaging (AFP) plays an active role in preserving foods, extending their shelf life and improving their safety and sensory properties while maintaining food quality. They are classified into two main groups, active-scavenging packaging (ASP) and active-releasing packaging (ARP). Typically, ASP acts as a moisture, carbon dioxide, oxygen, and unpleasant-odor absorber. On the contrary, ARP acts as an antioxidant and antimicrobial and is able to release carbon dioxide and ethanol. The most common AFP technology is focused on the development of labels, sachets, sheets, trays, coating, and pads that can absorb or release gases in a package or headspace, as well as materials containing the active components themselves. [6]. A typical representation of AFP and its main features are shown in Figure 1.

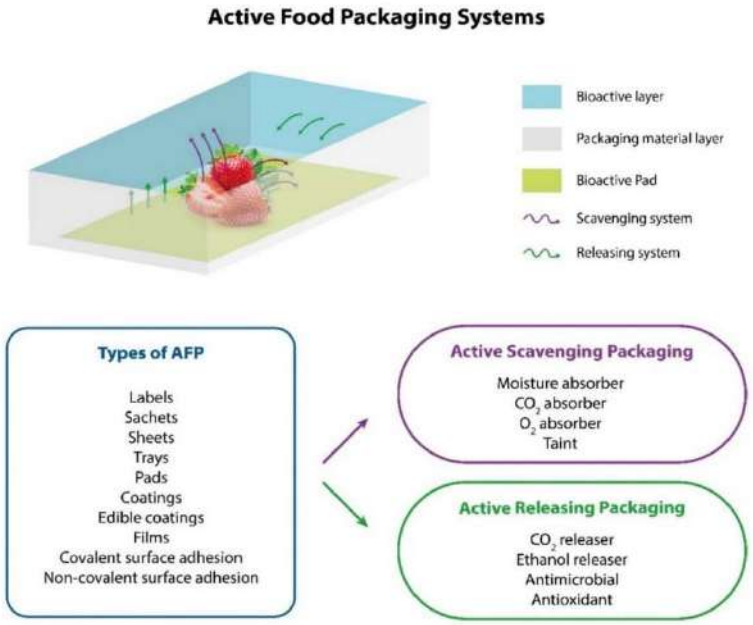


Figure 1. Schematization of the active food packaging (AFP) systems and their main features.

Currently, the active packaging most frequently implemented in the industry involves the use of bioactive molecules which can be directly incorporated into the host matrix, wrapped within an envelope, placed on the host surface as a coating, immobilized on the host surface, or layered into a permeable material and placed alongside the food, allowing the release of volatile agents without direct contact with the food product, simultaneously protecting it against contamination and degradation [7]. Likewise, emerging active packaging technologies focus on the incorporation of natural or synthetic volatile and nonvolatile antimicrobial agents into a polymeric matrix [8]. In this framework, Mallardo et al. reported the customizable fabrication of AFP that incorporated antimicrobial and/or antioxidant agents for the controlled release of substances, or for the absorption of the unwanted substances from both the food and the container's internal atmosphere, which positively impacted the packaged food [9]. Thus, controlling the active molecule evaporation during processing remains one of the most important technological challenges for the AFP industry. As has been reported in the literature, these drawbacks can be readily overcome by different routes. One of the most promising technological pathways for the fabrication of AFP is based on the association of synthetic or natural polymers with the encapsulation of active molecules (natural volatile essential oils; their main components such as thymol,

carvacrol, and triclosan; or synthetic nanoparticles, among others) [10]. As a consequence, the cyclodextrins have been incorporated into food packaging either alone (the so-called "empty" cyclodextrins) or by forming host–guest complexes, named cyclodextrin inclusion complexes (CICs) [11–14].

The most recent technology advancements from the past few decades have also promoted an increased interest in the research in this area. This can be readily appreciated after a brief bibliometric study. In fact, the number of publications per year in this area demonstrates an exponential growth trend; this can be seen in Figure 2, which shows the evolution of the scientific publications per year found by searching in the Scopus database using the separate keywords "Cyclodextrin" and "Cyclodextrin and Packaging". From this overview, it can be seen that when only the word "Cyclodextrin" was used, more than 50,000 related papers published in this area were obtained. Additionally, an exponential trend is evident if these results are compared with the previous three decades. Interestingly, when we included "Cyclodextrin and Packaging" in our keywords, we only computed 253 published papers. This result demonstrates the growing interest in developing cutting-edge research related to the use of cyclodextrins and their derivatives in the food packaging industry. It is also worth noting that in both cases, China, the United States, India, and a few countries from Europe such as Italy, France, and Spain are in the top ten countries with the most papers published per year in this research field.

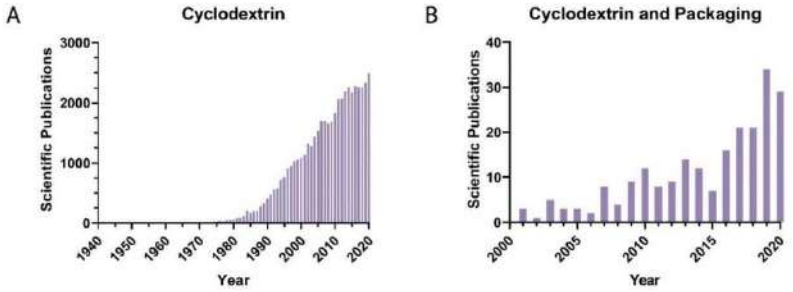


Figure 2. Evolution of scientific publications per year by searching in the Scopus database using the keywords (**A**) "Cyclodextrin" and (**B**) "Cyclodextrin and Packaging".

Given the significance of this topic for the agro-food sector, in this review article, we discuss the current status and future prospects regarding the association of cyclodextrins and their derivatives with synthetic or natural polymeric matrices to fabricate cost-efficient, sustainable, and biodegradable active food packaging (AFP). We pay particular attention to the materials and the affordable fabrication technologies available to date, including the trend of nanotechnologies in active food packaging. In addition, we provide a critical view on the possible risks to human health and the updated legislation. Moreover, some patents and commercial products that currently apply AFP technology are also listed and finally, the current and future research challenges which must be addressed are discussed.

2. Cyclodextrins (CDs) and Their Derivatives

Cyclodextrins were discovered about two centuries ago (1891) by Villiers, who named them cellulosine [15–17]. A few years later, Schardinger identified the bacteria capable of degrading starch to produce CDs [18]. Since then, these molecules have been extensively investigated due to their structure and chemical properties. However, it was not until the 1960s and 1970s that Higuchi and Connors proposed a mathematical model to represent the mechanism of the formation of the inclusion complexes used today [19]. CDs are a family of cyclic α-D-glucopyranose oligosaccharides linked by α-1,4 glycosidic bonds that are produced thanks to the biotransformation of starch by microorganisms such as *Bacillus macerans* [20]. The most common cyclodextrins are α-cyclodextrins (six glucose

subunits), β-cyclodextrins (seven glucose subunits), and γ-cyclodextrins (eight glucose subunits), recognized as native CDs, with β-CD being the most frequently applied to food [15]. In Figure 3, the schematic representation of the typical geometrical shape of native cyclodextrins and their chemical structures is shown. CDs have a natural origin because they are produced by the enzymatic degradation of starch [15–17]. Structurally, CDs have a truncated conical cylinder shape with a nonpolar inner cavity (hydrophobic) and a polar outer surface (hydrophilic) that confers on them the ability to encapsulate hydrophobic substances. The β-CD molecule possesses a complete secondary belt formed by hydrogen bonds, which confers its rather rigid structure and its concomitant lowest water solubility. This hydrogen-bond belt is totally incomplete in the α-CD molecule because one of the glucopyranose units is located at a distorted position. Therefore, only four hydrogen bonds can be fully established, and their solubility in water increases significantly if compared with β-CD. In contrast, the γ-CD possesses a noncoplanar intrinsic structure, which leads to a much more flexible configuration, and for this reason it is the more soluble of the three aforementioned CDs. The thermal and mechanical properties of CDs are also important. The melting points of CDs are relatively high (see Table 1) and their differential scanning calorimetry (DSC) is almost identical, with two heat absorption peaks: the first, at 100 °C, is usually attributed to the water evaporation from the crystals, and the second, located at 250 °C, is attributed to the crystal melting and thermal decomposition. In Table 1, we summarize the main physical and chemical properties of α-, β- and γ-CDs [21–23].

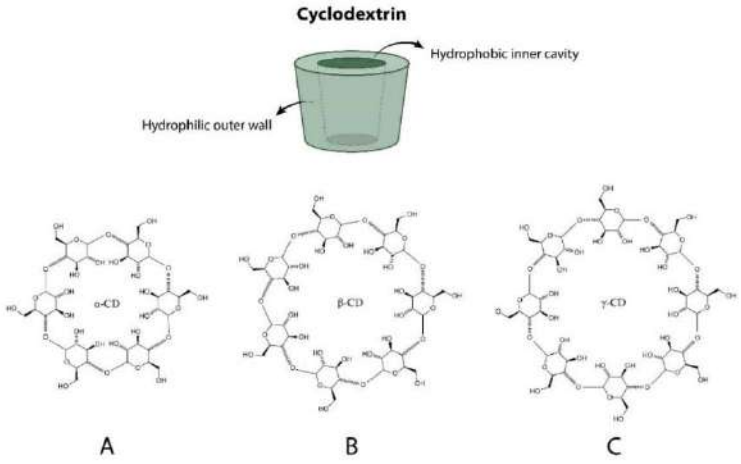


Figure 3. Schematic representation of the typical geometrical shape of native cyclodextrins and their chemical structures for (**A**) α-cyclodextrins, (**B**) β-cyclodextrins, and (**C**) γ-cyclodextrins.

Native cyclodextrins are safe to apply in foods; in the USA, they have obtained the GRAS (Generally Recognized as Safe) status as approved by the FDA (US Food and Drug Administration). In Europe, they are considered food additives and labeled as E-457 (α-CDs), E-458 (γ-CDs), and E-459 (β-CDs) [24,25]. The FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) Joint Expert Committee on Food Additives (JECFA) set the maximum advisable level of β-CD in food at 5 mg/kg of body weight per day; however, α-CD and γ-CD do not have an established Acceptable Daily Intake (ADI), due to their benign toxicological profiles [6]. Current regulations suggest that the migration of cyclodextrins from packaging to food does not have harmful effects on consumers if they are part of the packaging; this is why cyclodextrins are recognized as safe additives for use in food [6,26].

Table 1. Main physical and chemical properties of α-, β-, and γ-CDs. Data from [21–23].

Properties	α-CDs	β-CDs	γ-CDs
Number of glucose units	6	7	8
Molecular weight (g/mol)	972	1135	1297
Melting point (°C)	275	280	275
Solubility in water at 25 °C (%, *w/v*)	14.5	1.9	23.2
Enthalpy; ΔH (kJ/mol)	32.1	34.7	32.3
Entropy; ΔS (J/°K mol)	57.7	48.9	61.4
Cavity diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3
External diameter (Å)	14.6	15.4	17.5
Approximate volume of cavity ($Å^3$)	174.0	262.0	427.0
Crystal forms (from water)	Hexagonal plates	Monoclinic parallelograms	Quadratic prisms
European trade name as food additives	E-457	E-459	E-458
Solubility in:			
Tetrahydrofuran, methyl isobutyl ketone, methyl isopropyl ketone, acetone, alcohols	0.0	0.0	0.0
Solubility in propylene glycol	0.5	0.5	0.5
Solubility in pyridine	3.5	3.5	3.5
Solubility in ethyleneglycol	7.0	7.0	7.0
Solubility in N-methylpyrrolidone	14.8	14.8	14.8
Solubility in dimethylformamide	28.3	28.3	28.3
Solubility in dimethylsulfoxide	>41	>41	>41

The host–guest complexes obtained by the association of CDs with other molecules are defined as cyclodextrin inclusion complexes (CICs), chemical equilibriums governed by the law of mass action, where the CDs are the host molecules [14,27]. The use of inclusion complexes in food packaging contributes to maintaining and releasing active substances in a controlled manner, acting as preservatives and, therefore, reducing the required number of food additives. CICs can also be used on the container's outer surface to protect against environmental factors [28]; however, it would greatly increase the final price of the active packaging if the internal surface had already been functionalized. Similarly, empty CDs have been used to complex hydrophobic compounds inside packaging, to decrease off-flavors, and to reduce plasticizer migration [29]. Gonzalez-Pereira et al. recently discussed the main applications of CDs in the food industry, including their toxicity impact on human health. However, the active packaging applications involving the use of CDs or CICs were outside the scope of their study [30].

Despite the huge advantages and benefits of the use of cyclodextrins in food packaging formulation, there are, however, a few drawbacks and limitations that are worthy of being taken into account. CDs possess intrinsically limited solubility; therefore, large volumes of water have to be used during any intended formulation. Thus, the reservoir capacity, time, and energy required for heating and cooling may become non-negligible and important factors that would increase the formulation and scale-up cost factors [17]. Likewise, the introduction of novel composites based on CDs and their derivatives in the food packaging industry would be subject to strict regulatory constraints, which would often limit, delay, or hinder its industrial exploitation [15]. Another aspect that must be considered during the fabrication of food packaging composite materials is related to the undesired diffusion of harmful and contaminant substances from the packaging into the food or beverages, which is promoted unintentionally by CDs. Thus, there are necessary issues associated with the detection limit of contaminants in the food [31]. Last but not least, as far as CICs are concerned, the size of the guest molecules must fit within the cyclodextrin's internal cavity; additionally, the thermodynamic interactions between the CD components (host/guest and solvent) modulate their stability. Furthermore, the stability of the inclusion complex is governed by its formation constant (K_F) or dissociation constant (K_D) [32,33].

3. Polymers Used in Active Packaging

Food packaging (FP) materials can have different shapes and functions relative to their intrinsic physicochemical properties, and they must guarantee a synergistic balance between their shape and their intended function [1]. Food packages can be customizable depending on their intended use, weight, and shelf life. They might be rigid to produce bottles or trays; flexible to obtain films, wraps, or bags; or semiflexible to produce caps or boxes [34]. The most common food packaging materials used currently in the food industry vary between a wide range of natural and synthetic elements such as metals, paper, glass, and plastics. Nowadays, it is critical to select a suitable material for packaging, depending on the type of food and the final specific functions that the packaging is aimed to fulfill [35]. Although there is a wide range of raw materials that can be used, polymer-based composites for packaging applications remain by far the most suitable material for the food packaging industry. These polymers are classified as biodegradable and nonbiodegradable polymers. We will detail below the most commonly used polymers in the manufacture of films, containers, trays, and other types of packaging [36].

3.1. Nonbiodegradable Polymers

The use of nonbiodegradable polymers in the food packaging industry has economical and industrial advantages such as transparency, flexibility, and easy processability. In addition, they are relatively cheap and can be thermally processed and easily manipulated to obtain different forms of packaging. However, their use also exhibits several limitations. According to the 2021–2028 plastic market size, share, and trends analysis report, it is expected that the global plastic market size will increase to more than USD 600 billion by 2021, with an estimated annual growth of 3.4% for the more than 400 million tons of plastics produced per annum worldwide (*Plastic Market Size, Growth, and Trends Report, 2021–2028*, s. f.).The packaging end-use has the largest share, representing more than 36% of the market in 2020. Furthermore, the environmental impacts of plastics-based packaging are an extremely important issue. In fact, plastic-based products persist and pollute long after their intended use. The prospects for 2050 are not optimistic, since the oceans could contain more plastic than fish by weight, and the impacts of degraded or intentionally produced microscopic-sized plastics on human health remain unknown. As claimed in a recent European report, about 40% of the total pollution is directly related to packaging waste [37]. These facts are the driving force behind the current challenges to the packaging industry, which pursues efficient, innovative, and biodegradable packaging solutions with environmental advantages [38].

Among the most commonly nonbiodegradable polymers used for the manufacture of food packaging, we can list the following: (i) polypropylene (PP), which is a thermoplastic resin with a high melting point (160 °C), a low density, and a high water-vapor resistance, and thus very suitable for packaging applications that have to withstand high temperatures; (ii) polyethylene-co-vinyl acetate (EVA), which is nontoxic, transparent, flexible, and suitable for the fabrication of thin films composed of copolymerized polyethylene (PE) and amorphous poly(vinyl acetate) (PVA) [39,40], commonly used in refrigerated food packaging because they are highly adhesive, display low vapor permeability, and exhibit heat-sealing features [41–44]; (iii) high-density polyethylene (HDPE), which is a linear ethylene polymer with small branching, more rigid and less transparent than the low-density variety, and is suitable for the fabrication of film that acts as a barrier against gas and water vapor but is permeable to oxygen and carbon dioxide; (iv) low-density polyethylene (LDPE), which is widely used worldwide due to its low cost, can resist water vapor, but does not retain oxygen, carbon dioxide, and other vapors in food packaging applications [45]; and (v) polyethylene terephthalate (PET), which is a polymer with a high crystalline melting temperature, suitable for bottles and food packaging materials in the food industry, since it exhibits relatively good functional strength and toughness besides its safety and easy processability [46].

Usually, in polymer-based composite packaging fabrication, the bioactive substance is added during the transformation processes. This dictates the type of polymer to be used. The common routes for the fabrication of food packaging include solution–melt mixing, the mold-casting method, thermocasting and -pressing, extrusion blown films, electro spinning, and extrusion or coextrusion technologies. Among these, extrusion stands out as the most frequently used in the industry and involves high temperatures. Consequently, the concomitant properties of the bioactive molecules are compromised due to the high temperatures required to extrude the polymer composite. This is a clear indication that temperature plays a key role in the food industry. Indeed, the temperature at which food is stored can also affect the release rate and shelf life of bioactive components. For these reasons, the direct incorporation of bioactive molecules such as antioxidants or antimicrobials into polymers during the extrusion step has been limited [39,47]. Therefore, at present, innovative, cost-efficient, and eco-friendly alternative pathways are highly appealing to the food packaging industries.

3.2. Biodegradable Polymers

As was mentioned above, the increasing awareness of sustainability has motivated the food packaging industry to turn toward the development of innovative technologies that promote the minimization of the environmental impact. This is mainly due to the fact that the products persist and pollute long after their intended use. Thus, the progressive replacement of plastics that are harmful to the environment by biodegradable polymers has become of paramount importance in the last decade. These bioplastics mainly degrade into CO_2 by the natural enzymatic action of microbes [48,49]. In addition, biodegradable polymers would reduce the pollution problems that are due to plastic packaging waste, since these materials are made mostly from renewable sources that are biodegradable or compostable. So far, they have been classified into four categories based on their chemical composition, origin, and synthesis method, as follows [50]:

i. Those obtained directly from biomass (for example, starch, protein, and cellulose).
ii. Those produced through chemical synthesis from bioderived monomers (for example, polylactic acid (PLA) and biobased polyethylene (PE)).
iii. Those produced through microbial fermentation (for example, polyhydroxy-alkanoates).
iv. Those produced through chemical synthesis from bioderived monomers and monomers based on petroleum, butylene polysuccinate (PBS), or polytrimethylene terephthalate (PTT) [51].

Among the biodegradable polymers most commonly used for the manufacture of food packaging, we can list the following.

Polylactic acid (PLA), which is the main biopolymer used in food packaging; its advantages include the fact that it is an excellent thermoplastic and that it is biodegradable and compostable [52,53]. PLA is classified as "Generally Recognize As Safe" (GRAS) by the FDA. It can be rigid or flexible depending on the intended use, being suitable for different applications within the packaging industry; furthermore, its barrier properties are considered similar to those of synthetic polyethylene terephthalate (PET) [54]. Given its useful traits, e.g., that it is nontoxic, noncarcinogenic, biocompatible, hydrophilic, water-soluble, and chemically stable, it is generally mixed with different polymers and used in packaging technologies to produce films, containers, and molding for food and medicine packaging applications.

Natural starch also belongs to the group of the most widely used biopolymers in the food packaging industry and exhibits environmental advantages such as biodegradability and a reduction in the production of carbon dioxide [55]. Starch can act as a thickener, adhesive, or additive. Starch-based films have physical properties similar to those fabricated with synthetic polymers; they are transparent, exhibit no odor or taste, are semi-permeable to carbon dioxide, and are resistant to oxygen transmission. However, in order to fulfill the physical properties required for packaging, starch must be plasticized, that is, subjected to a process that deconstructs the granules, which often results in brittle film materials

when dehydrated [56]. The most common polymers used in food packaging applications are summarized in Table 2 [39,48,57]. Table 3 summarizes the oxygen/moisture barrier properties of the polymers most frequently used in the active food packaging industry [58].

Table 2. Polymers used in food packaging applications.

Origin	Types
Natural and biodegradable	Polysaccharides (starch, cellulose, chitin); proteins (gelatin, casein, silk); polyhydroxy alkanoates (PHA), polylactic acid (PLA)
Natural and nonbiodegradable	Polyamides; polyesteramides; unsaturated polyesters;epoxy and phenolic resins
Synthetic and biodegradable	Aliphatic polyesters (polyglycolic acid (PGA), polycaprolactone (PCL), polybutylene succinate (PBS)); polyvinyl alcohol (PVA); polyalkylene dicarboxylates (polyethylene succinate(PES), polybutynel adipate (PBA)); polyanhydrides

Table 3. Summary of the oxygen/moisture barrier properties of polymers. Data from [58].

	Polymer	Oxygen Permeation (cc.mil/m^2-day-atm)	Water Vapor Permeation (g.mil/m^2-day-kPa)
Biodegradable	PHA	8 (23 °C/85%)	106 (23 °C/50%)
		85 (23 °C/0%)	30 (25 °C/100%)
		230 (25 °C/80%)	26 (37.8 °C/100%)
	PLA	132–590 (23 °C/50% or 0%)	63~342 (23 °C/85%)
	PPC	230	162 (23 °C/90%)
	PLA/Chitosan	72 (25 °C/0%)	319 (37.8 °C/95%)
	PBS	208 (23 °C/50%)	175 (25 °C)
		340 (20 °C/90%)	-
	PCL	1990 (25 °C/0%)	137 (23 °C/48%)
	PBAT	2440 (23 °C/50%)	173 (23 °C/75%)
	PGA	1 (30 °C/80%)	10 (40 °C/90%)
Non Biodegradable	HDPE	2325 (23 °C/0%)	6 (40 °C/90%)
	PP	2500–3000 (23 °C/0%)	5–10 (40 °C/90%)
	PET	40 (23 °C/0%)	15–20 (37.8 °C/90%)
	PVDC	~1 (23 °C/75%)	2 (38 °C/90%)
	PEF	~18 (25 °C/50%)	~30 (25 °C/90%)
	Bio-PE	2140 (23 °C/0%)	~3 (38 °C/90%)
	Nylon 6	40 (23 °C/0%)	295–310 (37.8 °C/90%)
	Polystyrene	4030 (23 °C/0%)	132 (40 °C/90%)
	EVOH	0.5 (23 °C/0%)	33 (40 °C/90%)

Cellulose is another biopolymer widely used in food packaging applications and is considered the most abundant natural polymer; it can be extracted from corn cobs and is eco-friendly and biodegradable [59]. Cellulose by itself is limited to form films; however, cellophane can be produced from it, which has favorable functional properties but is sensitive to moisture [60].

Another type of biopolymer less exploited in the packaging industry is chitosan, which has a linear structure obtained from chitin and is the second most abundant natural polysaccharide. Chitin is converted into chitosan through an enzymatic or chemical deacetylation process. This biopolymer also exhibits antimicrobial properties and can be used either as an antimicrobial agent or substrate. Chitosan films have superior optical, mechanical, and oxygen-barrier properties compared to films derived from other similar polysaccharides. They have been successfully used to extend shelf life and keep food fresh in food packaging applications [61]. The chemical structures of the polymers most frequently used in the active food packaging industry are shown in Figure 4.

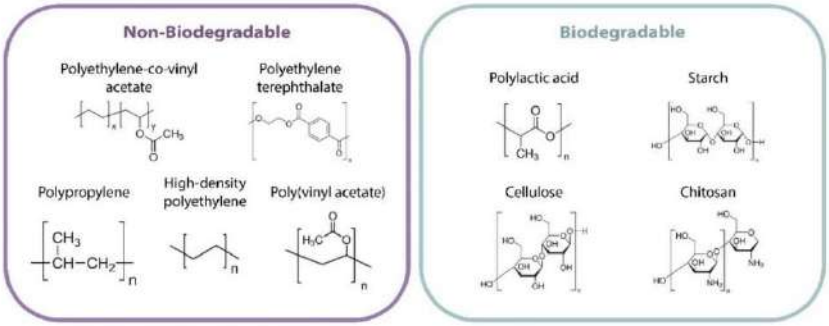


Figure 4. Chemical structures of the nonbiodegradable and biodegradable polymers most frequently used in the active food packaging industry.

Biopolymers themselves can form continuous structures that can be crystalline or amorphous, and they can act as a barrier against different substances that should be prevented from interacting with food. Additionally, they are be able to preserve food while still being environmentally friendly. Most of the biodegradable polymers described above have been used to produce flexible packaging and fabricate molds, films, and containers to store fruits and vegetables. Furthermore, these materials can also be used to fabricate modified atmospheric storage (MAP) products [48]. In Figure 4, we list the main nonbiodegradable and degradable polymers used in the active food packaging industries [62]. The aforementioned reasons, alongside the principles of a circular economy and sustainability, are the driving force behind the current and future emergent research trends regarding plastics within food packaging technologies. Indeed, related industries must increase their awareness of replacing the use of nonbiodegradable plastic raw materials with biofriendly and biodegradable ones. Thus, future efforts should be focused on the development of eco-friendly and compostable materials.

4. Incorporation of Active Substances in Polymeric Matrix Composites for Active Packaging Applications

The physicochemical properties and the functionality of the biopolymers must be adapted to the market, consumer needs, and the type of food to be packed. This has been commonly carried out through the use of different routes including but not limited to chemical modifications and associations with other substances or molecules, plasticizers, or cyclodextrins [50,55]. The formulation of modern biodegradable packaging must fulfill specific physicochemical requirements. These materials must be capable of forming a mold with sufficient continuity and cohesion in order to protect or pack a product with tailored tensile strength (TS) and elongation (E), but also an adequate oxygen permeability and water vapor transmission rate (WVTR) [63,64]. According to the most recent literature, the current and future research trends in plastics are focused on the development of eco-friendly and compostable materials [63,64].

As was mentioned above, one of the most promising technological pathways for the fabrication of active food packaging is based on the fortification of synthetic or natural polymers with nano/micro capsules containing active molecules, preferably of a natural source. However, it is still challenging to preserve the properties associated with packaging materials, such as the color, odor, antimicrobial or antioxidant activity, and permeability. Various technologies have been used so far to incorporate active substances into a polymeric matrix via chemical encapsulation. In fact, classical technologies used in the food packaging industry are also being used or considered for use in the efficient development of innovative active packaging. Common extrusion and coextrusion, solution–melt mixing, the mold-casting method, thermocasting and -pressing, extrusion-blown films, and electrospinning technologies, to mention just a few, are highly suitable approaches for active food

packaging applications [1,35,65–68]. In a typical procedure, the active molecules can be directly incorporated into the polymeric matrix, wrapped within an envelope, applied to the surface as a coating, immobilized on the polymer surface, or layered into a permeable material and placed alongside the food. Any of these routes must face several drawbacks during its processing. The temperature is one of the most important parameters that must be taken into account. As a result, most of the packaging used in the food industry is produced at high temperatures, between 100 °C and 200 °C, making it difficult to incorporate active substances. Such temperatures are responsible for a molecule's volatilization and material degradation and other considerable losses of its physical and chemical properties. For example, Suppakul et al. reported that the association of basil essential oil within low-density polyethylene (LDPE) films resulted in very little antimicrobial activity in cheese samples due to the volatilization of the active ingredient during extrusion processing [46]. Similarly, Boonruang et al. recently reported the antifungal properties of polymer composites based on the association of thymol and (R)-(-)-carvone within poly(lactic acid) (PLA). Although they reported acceptable antifungal efficiency, the authors also highlighted that thermal processing within the 80–150 °C range of temperatures resulted in the loss of antifungal properties [3,69]. In addition, Chen et al. reported the advantages of encapsulating antimicrobial agents such as citral (CI) and trans-cinnamaldehyde (TC) into packaging film composites, to minimize the losses with the temperature increment [70]. Since these molecules are easily degraded by the temperatures commonly used in extrusion processing, the encapsulation of these active molecules prevented their degradation via the temperature increase and preserved their bioactive effectiveness during and after the extrusion process. They also highlighted that this route would not only offer an improvement in the thermal sensitivity of such compounds but would also be able to control their release rate.

To solve the temperature and processing drawbacks, the fabrication of tailored, encapsulated polymer-based composites has been widely studied and seems to be one of the most promising and industrially scalable routes so far [4]. Additionally, in line with the EU's recent research and innovation policy, Food 2030, which aimed to make our food systems ready for the future, linking multiple sectors such as packaging, waste, and recycling, following a bioeconomy strategy, the food packaging industry is also making an effort to replace the use of nonbiodegradable plastic raw materials with biofriendly and biodegradable ones. Indeed, future research challenges will be focused on the production of cost-efficient, eco-friendly, and industrially scalable materials based on the association of biodegradable polymers with molecular encapsulation technologies. In this context, the fabrication of cyclodextrin inclusion complexes (CICs) has been widely used in the literature [11]. These complexes can be applied in a coating layer, in which they are suspended over the polymeric mold to avoid the agglomerations that would limit its functionality [7,71]. To date, various research groups have already demonstrated the development of biodegradable packaging with positive results in terms of its functional characteristics and activity. For example, Friné et al. recently reported the fabrication of an antimicrobial PLA biodegradable packaging material by incorporating thymol and carvacrol inclusion complexes into β-cyclodextrin through an injection technique. These customized receptacles showed an appropriate functional characteristic, as they were able to inhibit *Alternaria alternata* growth [41]. Similarly, Yang et al. demonstrated that starch-based coatings that incorporated natamycin inclusion complexes in methyl-β-cyclodextrin lengthened the shelf life of tomatoes, significantly inhibiting the *Botrytis cinerea* activity [42]. Other interesting examples can be found in literature for when the active ingredient must be released in a controlled way, as in the case of antimicrobial substances, antioxidants, or fragrances [72]. In such composite materials, a miscible polymer with adequate kinetics must be achieved, because a very slow release will not often produce the desired effect. In this circumstance, hydrophobic polymers such as polylactic acid, cellulose, or chitosan are most frequently used to stimulate the release of active substances [28]. On the other hand, masterbatch (MB) has been used to improve the compatibility of some inclusion complexes with less-hydrophilic polymers, to obtain films with enhanced mechanical properties [73].

These facts make it clear that the current and future research efforts into incorporating cyclodextrins are focused on facilitating the biodegradability of packaging materials. In particular, the inclusion complex route is very promising, because it generates some weak points in the structure that facilitates the degradation of the material. This effect would be highly enhanced if the packaging were made of biopolymers [74].

4.1. Cyclodextrins in Food Packaging Technologies

The development of active food packaging with cyclodextrins is highly appealing nowadays, mainly due to their truncated conical cylinder shape, with its nonpolar inner cavity and polar outer surface [17]. This configuration makes them especially suitable for the encapsulation of hydrophobic substances, protecting them from volatilization, oxidation, and overheating and simultaneously improving their solubility. Furthermore, they are also highly biocompatible, eco-friendly, and not harmful to human health [14]. Cyclodextrins and their host–guest-complex derivatives such as cyclodextrin inclusion complexes (CICs) are among the most commonly used encapsulation approaches for the development of active food packaging [12,13,34]. As was mentioned above, this technology allows the protection and extension of the shelf life of a variety of foods but also offers a positive environmental impact due to its biodegradable nature [6,30,75]. When CDs are associated with biodegradable-polymer-based hosts, their potential applications increase and the packaging becomes entirely eco-friendly and biodegradable [76,77].

CDs have been incorporated into active food packaging through different routes: (i) alone, the so-called "empty cyclodextrins" that are commonly used to retain the hydrophobic substances released inside the package; (ii) as cyclodextrin inclusion complexes (CICs), which can keep the active substance in the inner cavity and control its release to extend the product's shelf life without losses in its quality, safety, or organoleptic parameters, but are also used to trap molecules on the outer surface of the package, providing protection against environmental factors; and (iii) CDs can form themselves - supramolecular polymer assemblies. In fact, the intrinsic structure of CDs allows the synthesis and design of CD-based polymers with specific functions and assemblies [78]. This route is suitable to improve the properties and the activity of different active substances [27,79].

4.1.1. Empty Cyclodextrins

CDs can be introduced in empty form into food packaging. Their main function involves trapping or encapsulating undesirable volatile compounds such as hydrophobic molecules that are present either inside or outside the packaging, but they are also used to prevent taste/aroma losses from the packaging and to slow volatile substances' migration into and out of the packaging [3,6,11,15,24,30,80]. Recently, this technology has been applied for capturing flavors and eliminating unpleasant odors, such as those generated by irradiated meat. An interesting work by Shin et al. reported the versatility of empty CDs in food packaging applications for sulfur-based odor elimination. They reported the use of an electrospun fiber mat based on low-density polyethylene (LDPE) and triacetyl-β-cyclodextrin (TA-β-CD) to eliminate three sulfur odor compounds: (i) dimethyl disulfide (DMDS), up to 90%; (ii) dimethyl sulfide (DMS); and (iii) carbon disulfide (CDS) [29]. Similarly, López de Dicastillo et al. reported the fabrication of polyvinyl alcohol-free standing films doped with β-CD in order to reduce milk cholesterol. They reported an important cholesterol reduction and showed that most of the cholesterol molecules were trapped on the packaging walls used during storage [81]. The same group also demonstrated, in a separate work, the immobilization of β-cyclodextrin in an ethylene–vinyl alcohol copolymer (EVOH) by extrusion with glycerol. Such composite films were flexible and transparent, with a maximum load of β-CD of 30%, and showed increased barrier properties against water vapor, oxygen (O_2), and carbon dioxide (CO_2) when compared with those of pure EVOH. These permeability enhancements were ascribed to the presence of discontinuities and hydrophobic cavities within the matrix provided by the β-CD inclusion [82]. In this case, empty CDs applied to plastic containers during the heat-

sealing process would also help to eliminate the smell of decomposition derived from this process [11]. Figure 5 shows a typical schematic representation of an empty cyclodextrin accompanied by the main CD functionalities for active packaging applications (AFP).

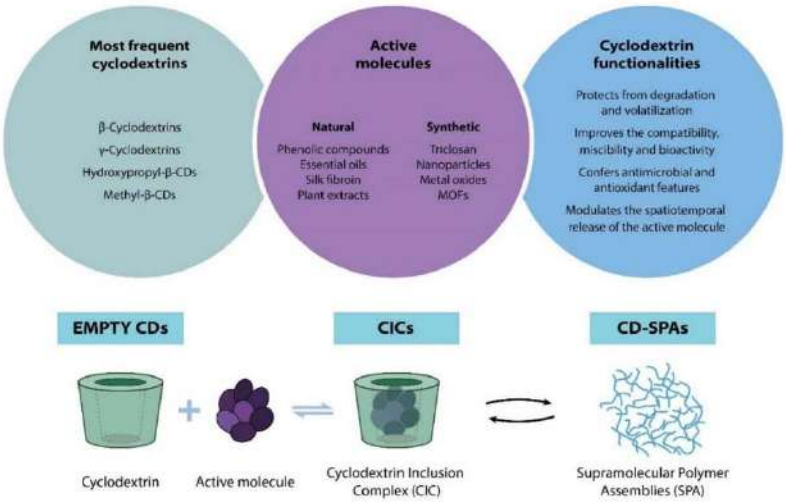


Figure 5. Schematic representation of the cyclodextrin inclusion complex (CIC) formation. The most frequent CICs, the active molecules, and the main CD functionalities for active packaging applications (AFP) are also listed.

4.1.2. Active Molecule Encapsulation and Cyclodextrin Inclusion Complex for Active Food Packaging Applications

Encapsulation in food packaging is defined as the process of trapping an active molecule within another macromolecule, producing customized micro- and macroparticles that release their contents during prolonged periods in a controlled way [83]. These substances can be active molecules or not, responsible for conferring the active food packaging characteristics of the material, such as: (i) protecting the active molecule from degradation, volatilization, and undesirable interactions with packaging materials; (ii) improving the compatibility, miscibility, and synergy between the packaging polymer and the active molecule; (iii) confering the antimicrobial/antioxidant and bioactivity enhancement; and (iv) modulating the spatiotemporal control over the release of the active molecule, in order to extend the shelf life and reduce the changes in the sensory properties of foods [20,84].

The chemical complex obtained from the association of CDs with a target molecule is commonly named a cyclodextrin inclusion complex (CIC) in the literature [31]. Due to their versatility, CD inclusion complexes are one of the most promising formulation routes in the active food packaging industry [11,17,80,85]. A schematic representation of the cyclodextrin inclusion complex (CIC) formation by a simple combination of cyclodextrin with a target active compound is shown in Figure 5.

In recent years, several investigations have focused on the use of CICs to stabilize active substances, aiming to confer on the material its antioxidant, antimicrobial, and bioactive properties for food packaging applications. These complexes have also been applied to produce tailored packages as food preservatives [76,80,86]. In such materials, the release rate is of paramount importance and depends on: (i) the final concentration of the active substance within the polymer; (ii) the affinity for the cyclodextrin; (iii) the diffusion coefficient in the polymer; (iv) the partition coefficient between the polymer and the cyclodextrin, and between the polymer and the packaged product; and (iv) the temperature and time [28,34].

Among the CDs most frequently used to obtain inclusion complexes for active food packaging applications are the native β-CD and the modified hydroxypropylated and methylated β-CDs. These are highly efficient at encapsulating and protecting aromatic and/or heterocyclic molecules compared to other classes of cyclodextrins [87–89]. In the case of essential oils (EOs) and their active ingredients, such as thymol, carvacrol, linalool, and cinnamaldehyde, the encapsulation step is essential to reducing volatilization or degradation losses during manufacturing or storage. The encapsulation also helps to improve their compatibility and miscibility with biopolymers by increasing their solubility, and it promotes the reduction of the organoleptic impact on food products caused by their strong odor or taste [90,91]. EOs have been widely used in food packaging to extend the shelf life of a variety of foods [92]. Various methods have been used to incorporate them into polymeric molds. Polymer-blend solution stands as the most commonly used so far. They have also been used, directly or via chemical encapsulation, as a coating for containers and for the fabrication of bags [8]. As revealed through an interesting work by Lin et al., gelatin nanofibers containing thyme essential oil were fabricated to form inclusion complexes of β-cyclodextrin in ε-polylysine (TCPN) [93]. These fibers were obtained via ionic gelation, and they exhibited excellent antimicrobial activity against *Campylobacter jejuni* in chicken samples with no adverse effect on the color, texture, and sensory properties. In the same way, Wen et al. reported the incorporation of a β-CD inclusion complex with cinnamon EO into polylactic acid (PLA) nanofibers fabricated by electrospinning [89]. They produced two series of samples with and without EO encapsulation and demonstrated the extended shelf life of pork when they encapsulated the cinnamon EO. Similarly, Santos et al. reported the fabrication of inclusion complexes of carvacrol with HP-β-CD and β-CD. They reported that these CICs showed improved antimicrobial activity against *Escherichia coli K12* and *Salmonella enterica* serovar Typhimurum LT2 [94]. They also demonstrate that a greater antimicrobial capacity was obtained for the encapsulated carvacrol when compared with the nonencapsulated carvacrol. In the same way, Velazquez-Contreras et al. evidenced that PLA packaging containing 5% β-CD-thymol or 5% β-CD-carvacrol extended the shelf life of berries during a storage period of 21 days at 4 °C, revealing a good sensory score and the higher inhibition of yeasts and molds [53].

It is worth mentioning that both carvacrol and thymol remain as the most frequently used natural active components in the food industry. They are naturally present in oregano and thyme EOs and exhibit a high antimicrobial activity. However, both possess a very low solubility in water, which limits their application in food science. To summarize, the most relevant studies regarding the use of cyclodextrin inclusion complexes (CICs) in active food packaging applications are listed in Table 4. It is worth noting that, according to a recent bibliometric study regarding the distribution of the biological applications of cyclodextrins, a significant share (around 20%) of these items concern the antimicrobial activity, just behind the antioxidant activity [79].

Table 4. Inclusion complexes with cyclodextrin and essential oils used in active food packaging applications.

Inclusion Complex	Material	Application	Reference
Mustard essential oil/β-cyclodextrin	Cellulose, sulfate film	Antimicrobial edible films, against *E. coli* and *S. aureus*.	[13]
Thymol/γ-cyclodextrin	Zein, nanofibrous web	Antimicrobial food packaging, inhibiting the growth of *E. coli* and *S. aureus* in meat.	[12]
Eucalyptus/β-cyclodextrin	Zein, ultrafine fibers	Antimicrobial, against *S. aureus* and *L. monocytogenes*.	[95]
Carvacrol/HP-β-cyclodextrin	Chitosan, Film	Antimicrobial packaging for chicken filet.	[96]

Table 4. *Cont.*

Inclusion Complex	Material	Application	Reference
Thyme/β-CD ε-polylysine	Gelatine, nanofiber film	Antimicrobial packaging, reduction of the activity against *C. jejuni* in coated chicken.	[93]
Tea tree oil/β-cyclodextrin	Poly(ethylene oxide), nanofiber film	Antimicrobial packaging, antibacterial activity against *E. coli* O157:H7, tested on beef.	[97]
Cinnamon–oregano EO/β-cyclodextrin	Chitosan/ poly(vinyl alcohol), nanofiber film	Antifungal activity against *Botrytis* sp.	[98]
Cinnamon EO/β-cyclodextrin	Poly(vinyl alcohol), nanofiber film	Antimicrobial packaging, antibacterial activity against *E. coli* and *S. aureus* in mushrooms.	[99]
D-Limonene/β-cyclodextrin	Poly(butylene succinate), composite film	Antimicrobial food packaging, antibacterial properties against different bacteria straws.	[9]
Thyme/β-cyclodextrin	Inclusion complex extract	Natural antioxidant and antibrowning activities.	[100]
Curcumin, carvacrol/β-cyclodextrin	Cellulose nanocrystals, film	Antimicrobial food packaging, microbial activity against *B. subtilis*.	[87]
Palmarosa EO/β-cyclodextrin	Polyethylene terephthalate (PET)	Antifungal packaging, extends apple shelf life by slowing *P. expansum* growth.	[101]
Galangal root oil/β-cyclodextrin	Gelatin, nanofibers	Inhibitory effect against *E. coli* O157:H7 in beef.	[102]
Basil and pimenta dioica/β-cyclodextrin	Sachets	Potential to be used as food preservative against *S. aureus*, *E. coli*, *L. monocytogenes*, and *P. aeruginosa*.	[103]
Cinnamon EO/CD-nanosponges	α-nanosponges and β-nanosponges	Antimicrobial activity against foodborne bacteria.	[104]
Oregano EO/(α-CD and γ-CD)	PHBV, film	Higher antimicrobial activity against *S. aureus* and *E. coli*.	[105]
Litsea cubeba EO/β-cyclodextrin	Dandelion polysaccharide, nanofiber	Sustained release and long-lasting antibacterial effect against *S. aureus*.	[106]
Clove EO/β-cyclodextrin	Chitosan/ β-cyclodextrin citrate/ oxidized nanocellulose	Higher activity over Gram-negative bacteria (*E. coli* and *P. aeruginosa*).	[107]
Carvacrol, thymol/β-cyclodextrin	Poly(lactic acid) (PLA)	Microbial inhibition of mesophiles, yeast, molds, and coliforms. Extended the shelf life of raspberries and blackberries.	[53]
Carvacrol, oregano, and cinnamon EOs/β-cyclodextrin	Cardboard box	Reduction in microbial growth of mesophiles, psychrophiles, enterobacteria, yeast, and molds. Extended the shelf life of mandarins.	[108]

4.1.3. Cyclodextrin and Their Derivatives for the Formation of Supramolecular Polymer Assemblies

Another approach to improving the properties and the activity of different active substances in food packaging research is focused on the formulation of CD–polymer supramolecular assemblies. Indeed, the intrinsic structure of CDs allows the synthesis of supramolecular polymeric assemblies based on the association of CDs with monomers or polymers to produce novel composites with tailored structures, properties, and functionalities [78]. So far, CDs and their derivatives had been used to synthesize several linear polymers, polyrotaxanes, rings, helices, and brushes, among others [16,80,109–111].

The principal synthesis methods for the formation of such supramolecular assemblies are:

(i) ring-opening polymerization, (ii) Cu-catalyzed azide–alkyne cycloaddition reaction, (iii) atom transfer radical polymerization, and (iv) reversible addition–fragmentation transfer polymerization [112].

It is worth mentioning that the synthesis of polymers with CDs dramatically improves their intrinsic properties, and they can be readily customizable for their intended application. An interesting work was recently published by Zou et al. They reported the fabrication of mechanically reinforced composite films based on the association of high-amylose corn starch/konjac glucomannan (HCS/KGM) with β-cyclodextrin (β-CD), for the development of degradable active packaging materials. They demonstrated that the β-CD was systematically segregated from the polymer chains to form crystals. This promoted a more compact HCS/ KGM film. These materials were transparent and exhibited a reduced moisture formation, with an improved water vapor permeability compared with the native films [113]. Similarly, Huang et al. reported the fabrication of a novel food packaging material based on molecularly imprinted polymers (MIP), using β-CD as a monomer. They combined allyl isothiocyanate MIP (AITC-MIP), chitosan (CS), β-CD, and toluene diisocyanate (TDI) as crosslinkers for beef preservation. These composite coating films exhibited tailored antimicrobial properties and promoted the extended shelf life of the beef, evidenced by the reduction of the muscle deterioration compared with the native films [114]. Using a similar approach, Joo et al. reported the fabrication of biodegradable free-standing films via a cold-casting process based on poly(lactic acid) (PLA) and β-CDs at different loads (up to 30 wt.%). Surprisingly, they claimed that the PLA and β-CDs were immiscible. This effect increased as the CD content increased. They reported that the cyclodextrin tended to form agglomerates at higher loads, promoting a poorer adhesion between both phases. This incompatibility was responsible for the deterioration of the mechanical and oxygen/water-barrier capacity of the PLA. They also demonstrated that a possible route to overcome these undesired effects consisted of the addition of CDs at 30 wt.% in the form of a masterbatch [73]. It should also be noted that the inclusion complex immobilized in a polymeric matrix by physical entrapment is considered a CD–polymer even though the CD units are not linked by covalent bonds [111].

5. Methods of Incorporating CDs in Polymer-Based Active Food Packaging

As was mentioned above, the most promising technological pathways to associating CDs and their derivatives with polymers for the fabrication of active food packaging include extrusion or coextrusion, coating, spraying, casting, solution–melt mixing, the mold casting method, layer-by-layer deposition, thermocasting and -pressing, adsorption, extrusion blown films, and electrospinning, to mention just a few [1,65–67] (Figure 6). These techniques exhibit technological advantages and disadvantages depending on the intended use, and they have been used to fabricate different types of active food packaging [68,86,115]. In the next section, we will describe in detail the most promising technologies.

5.1. Electrospun Micro-/Nanofibers and Mats

Electrospinning is one of the most appealing technologies for the incorporation of CDs within micro-/nanofibers and mats. This low-cost technique allows the transformation of a given viscoelastic fluid into fibrous membranes under the influence of an electric field and was observed for the first time more than a century ago [116,117]. The final performance of these fibrous materials is influenced by the physicochemical properties of the precursor solution, the experimental variables used during the electrospinning, and the environmental conditions. These factors modulate the resulting membrane structure and its concomitant physicochemical properties. The versatility of this technique lies in the fact that it not only allows the control of the dimensions and diameters of the microparticles and fibers but also permits the modulation of the morphology and texture of their surfaces, with structures ranging from smooth fibers to rough, porous, and hollow structures. The experimental setup of the classical electrospinning technique is quite simple and consists of atomizing a conductive solution by applying a strong electric field. The stabilization of the

electrospray process occurs when the electrostatic forces exceed the surface tension of the liquid. Most applications focus on the stable regimen or cone-type jet, which turns out to be the simplest and most reliable method for the production of microparticles, fibers, and mats. It is worth mentioning that the experimental electrospinning setup can be readily tailored for industrial purposes as a pilot line production tool that provides all of the capabilities needed to develop the continuous and customizable manufacture of products, making this technology ideal for the fabrication of scalable pre-production volumes of high-quality tailored materials. To date, different experimental setup configurations exist in the market. These range from typical electrospinning (solution electrospinning) to other customizable setups, such as gas jet electrospinning, magnetic-field-assisted electrospinning, conjugate electrospinning, and centrifugal electrospinning [118–120].

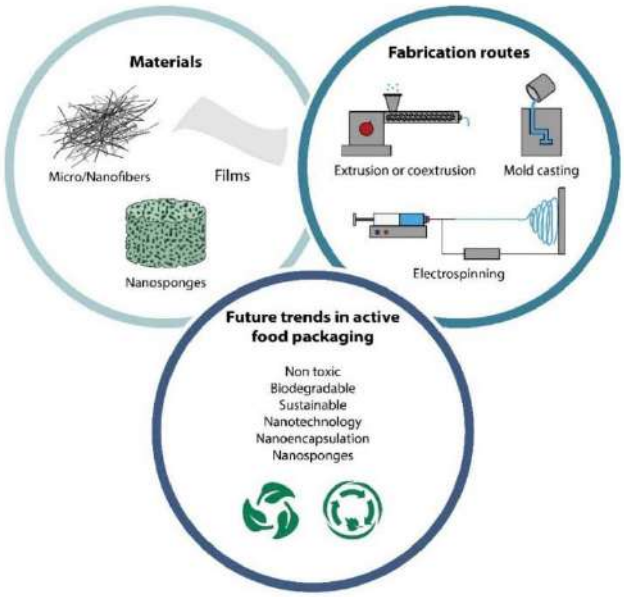


Figure 6. Cyclodextrins and polymers in active food packaging applications: materials and routes for incorporating CDs in polymers.

As far as food packaging and electrospinning are concerned, the conductive polymeric solution precursor can be made from different polymers such as PVA, PLA, PGA, PLGA PP, HDPE, chitosan, cellulose, gelatin, and zein [68,121]. Moreover, this technology has been successfully applied to melted polymers, polymer solutions, and molecule–nanoparticle mixtures [122–124]. Owing to its feasibility and versatility, the electrospinning system solution has been commonly employed in the development of food packaging materials. It provides a high loading capacity and greater stability for the bioactive molecules without the temperature drawback of other techniques, promoting the sustained and controlled release of the embedded molecules [125]. It is also worth noting that, at present, there exist controversies about electrospinning-based materials in food packaging applications. Indeed, there are critical concerns due to the loss of fibers that can penetrate the food; however, this can be avoided by fusing the fibers onto a base substrate that forms a barrier between the food and the fibers. Typically, electrospinning-based materials exhibit low barrier rates against oxygen and water vapor due to their highly porous matrix, which in turn is a critical problem. Aytac et al. used this technique to produce electrospun nanofibers containing thymol inclusion complexes in γ-cyclodextrin (TCICs), with improved antimicrobial activity as demonstrated through the decreased bacterial count in meat stored for

5 days at 4 °C. This effect was not observed in the other nanofibers studied [12]. More recently, the same group reported the development of electrospun fiber mats for potential application as packaging materials [126]. They reported different fibers made of cellulose nanocrystals (CNCs), zein (protein), and starch combined with either pure thyme oil, citric acid, and nisin or their complexed forms as cyclodextrin inclusion complexes (CICs). These composite materials were highly responsive to relative humidity (RH) and were antimicrobial against *E. coli*, *L. innocua*, and *A. fumigatus* [126]. Recently, Sharif et al. demonstrated the antibacterial potential of electrospun fiber mats based on the association of cuminaldehyde (CUM) with the hydroxypropyl-β-cyclodextrin (HP-βCD) inclusion complex. Their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* confirmed the suitability of this method for the development of films for active packaging applications. It is worth mentioning that they also studied the inclusion complex formation and stability via computational molecular docking [127]. Similarly, Li et al. reported the fabrication of a novel food packaging material based on electrospun nanofibers using a combination of soy lecithin phospholipids, poly(ethylene oxide), and the cinnamaldehyde (CA)/hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion complex. The phospholipids acted as a surfactant and substantially reduced the surface tension of the precursor solution, which led to a dramatic decrease in the polydispersity of the fibers. These nanofibers exhibited significant antibacterial activity against *Listeria monocytogenes* on fresh-cut cucumber, without altering their sensory properties [128]. Another recently published work by Narayan et al. reported the use of electrospinning to fabricate antibacterial polymeric fiber mats for active food packaging applications. Their formulation was based on the association of poly(vinyl alcohol) (PVA) with caffeic acid (CA)/β-CD and γ-CD inclusion complexes. Its antibacterial activity was demonstrated against the Gram-positive bacteria of *E-coli* and Gram-negative bacteria of *S. aureus* [129]. The versatility of the combination of electrospinning with CD encapsulation has been widely discussed in the recent literature [68].

5.2. Nanosponges

An interesting type of supramolecular polymeric material based on cyclodextrins has been recently labeled as cyclodextrin-based nanosponge (CD-NS). This supramolecular assembly is based on cross-linked cyclodextrin polymers nanostructured into a three-dimensional network, and it is considered a new type of biocompatible cross-linked polymer which possesses particular properties and advantages in terms of its biocompatibility, greater protection of encapsulated compounds, higher loading capacity, better solubility, and provision of an efficient method for controlling the release of molecules [104,130]. Depending on the precursors, the nanosponges can be completely nontoxic, and they are able to form stable formulations over a wide range of pH values and temperatures [130,131]. CD-NSs are highly porous nanoparticles characterized by a crystalline or amorphous structure, spherical shape, and swelling properties. They can be formulated indistinctly from any cyclodextrins and their derivatives and can be cross-linked with different molecules such as di-aldehydes, epoxides, epichlorohydrin, and diacyl chlorides, depending on the intended application [12,132]. These nanostructures have been widely used in the pharmaceutical, cosmetic, food, and environmental industries, targeting drug-delivery applications. To date, it has been demonstrated that they are very suitable within the food industry for the encapsulation of antimicrobial agents in packaging materials [24,76,130]. In this context, Simionato et al. recently reported the encapsulation of cinnamon essential oil with α-CD and β-CD nanosponges, demonstrating their ability to encapsulate higher amounts of EOs [104]. They also claimed an improved bacteriostatic effect against *B. thermosphacta*, *L. monocytogenes*, and *E. coli*. Similarly, Silva et al. reported the incorporation of coriander essential oil in CD nanosponges and proved their ability to provide a controlled EO release. They reported an improved bacterial growth inhibition for those materials fabricated from β-CD-derived NSs [130].

Despite the various examples that can be found in the literature, many authors agree on the fact that most of the nanosponge's potential applications within the food packaging industry have not been fully exploited due to the technological challenges that are still present during its manufacturing scale-up. These technological and industrial drawbacks are mostly related to the poor stability of the active compounds during processing and their uncontrolled molecular release from the packaging material [30,110,130]. These facts constitute the current driving forces behind active food packaging research.

5.3. Nanotechnology and Cyclodextrins in Active Food Packaging Applications

Nanotechnology is one of the most appealing and modern transdisciplinary research areas for the formulation of customized materials for a wide range of potential applications in different industrial sectors [133–135]. This discipline is more and more present in our day-to-day life and deals with the formulation of cost-efficient, innovative, and well-performing materials and devices within the specific scale-range of atoms and molecules. By definition, nanomaterials are those in which at least one of their constituents are within the range of 1 to 100 nanometers (1 nanometer is defined as one billionth of a meter). At this scale, these materials exhibit novel, enhanced, and tunable physicochemical and biological properties, which can be exploited in many industrial applications. Most nanoparticles exhibit a very high specific surface area and thus have a large potential for trapping or releasing active or bioactive substances and molecules. In this context, the food packaging industry also represents an important niche for the development of modern and competitive nanomaterials. To date, many authors have already reported interesting findings regarding the development of multifunctional materials for food packaging [66,88,136]. Many of these research studies have been focused on the development of smart and active packaging [133,136,137]. One of the most promising routes is also related to the encapsulation, or nanoencapsulation, of specific active/bioactive nanometric particles. In this context, cyclodextrins can adopt a variety of nanometric forms beside their intrinsic ability to act as capping or reducing agents for metallic nanoparticles. CDs can also form stable nanoparticles/fibers or nanomicelles, which can be subsequently utilized for plenty of applications, including active food packaging [138]. An interesting work in this regard has been reported by Lin et al., who detailed different nanoencapsulation strategies for natural compounds based on liposomes and chitosan nanogels [139]. Nanomaterials could play an important role within the food packaging materials industry when a combination of specific active molecules is needed to control the capture or release of molecules, to confer antimicrobial/antioxidant properties, or to extend a food's shelf life, among other things [133]. Concerning surface microbial food contamination, it is important to highlight that some nanomaterials, such as nanocarbon (carbon nanotubes, graphene, and other nanofullerene derivatives); metallic/magnetic nanoparticles, such as silver, gold, and zinc; and several nano-oxides, such as zinc, magnesium, and titanium (ZnO, MgO, and TiO) exhibit effective and cost-efficient antimicrobial effects against several different bacteria, as well as against various strains of fungi, algae, and certain viruses [134,140–143]. This represents a huge advantage compared with other natural/synthetic antimicrobial agents that are capable of inhibiting only specific organisms. Due to the nanoscale range of these particles, they are able to disrupt the barriers of lipopolysaccharides and proteins and pierce into the outer and inner membranes of the cells. However, many of the potential applications of nanomaterials in food science are still under debate due to the contradictory results reported regarding the potential risks and cytotoxic effects on human cells [141,144]. As far as cyclodextrins and nanotechnology are concerned, different examples can readily be found in the literature which demonstrate the effectiveness of combining both elements [24,80,145]. A recent study by Kathuria et al. demonstrated the feasibility of producing nanoporous structures with a high surface area, tunable pore size, and selective molecule sorption. They fabricated a nanocrystalline structure using γ-cyclodextrin (γ-CD) and potassium ions by a vapor diffusion process. Such materials, which are commonly known as nano metal–organic frameworks (nano-MOFs), proved to have the ability to encapsulate ethanol through host–guest interactions. This

is crucial in the development of active packaging [146]. Another interesting approach is the fabrication of functional nanosponges based on CDs and essential oils, such as those reported by the group of Silva et al. [130]. Very recently, Adeli et al. demonstrated that the application of a simple bioactive coating based on edible gelatin/hydroxypropyl-β-cyclodextrin (HP-βCD) enriched with a nanoemulsion of mustard essential oil extracted from mustard (*Brassica juncea*) seeds inhibited lipid oxidation and significantly delayed the microbial contamination of turkey meat, extending its shelf life substantially [147]. Furthermore, nanofibers based on the association of natural/synthetic polymers and natural essential oils, synthetic antimicrobial components, or nanomaterials seem to be a highly promising route for the fabrication of active packaging [67,68]. Moreover, nanocellulose and biopolymeric silk fibroin nanoparticles (SFNPs) have great potential for the development of innovative food packaging [57,133,148]. SFNPs are particularly interesting due to their unique combination of intrinsic mechanical and biological properties such as biocompatibility and biodegradability [149,150]. However, concerning their industrial processing, the main drawback of these regenerated particles lies in the fact that they are almost insoluble spontaneously in water and in most common organic solvents. This is why their association with cyclodextrin polysaccharides would enhance their potential applications. So far, silk fibroin nanoparticles have been largely used as a carrier for a wide range of bioactive molecules. Additionally, SFNP can act as a natural enzyme immobilizer. This has been crucial in debittering naringin-containing packaging juices [149]. Finally, all these aforementioned materials are the most outstanding precursors for food packaging applications (Figure 6).

6. Toxicity and Legislation of Cyclodextrins in Polymer-Based Active Food Packaging

The pioneers on the biological effects, toxicity, and risk assessment of dextrin-derivative-based materials in animals were Pringsheim and French, thanks to their works published almost a century ago [11,151]. In fact, the first trials of Pringsheim and coworkers carried out by the direct administration of cyclodextrins demonstrated their nontoxic nature and their potential for use as a source of energy for diabetics. These results were based on the fact that they were unable to measure significant increases in the sugar levels in the animals' urine. However, a few years later, French and coworkers reported that, depending on the dose and the chosen dextrin derivative used, the result could be totally different, with a negative impact on the biological response of the animals. Thus, it is important to note the extreme complexing ability of cyclodextrin when interacting with other molecules and biological systems. The hydrophilic CD groups can penetrate, with considerable difficulty, the lipophilic membranes and form complex crystallizations in some organs such as kidneys and eye corneas. Even the lipophilic derivative methylated β-cyclodextrin does not readily permeate lipophilic membranes [17]. However, several studies demonstrated their inertness, attributed to their similarity to other inert macromolecules such as starch and linear dextrin and their lack of absorption from the gastrointestinal tract. Despite the scarce adverse effects reported, CD's toxicity, as well as its immunogenicity, remains fairly low. In fact, its use has grown continuously over recent decades, not only in the food packaging industry but also in other key industrial sectors such as the pharmaceutical and chemical industries [15,16,30]. There are still a lot of limitations and challenges regarding the use of CDs in food packaging applications. In fact, it has been demonstrated that a high oral intake of CDs above 200 mg/kg/day causes adverse effects and health issues in the digestive system, such as diarrhea [34]. Today, cyclodextrins and most of their derivatives are considered totally safe and nontoxic in controlled doses. However, their toxicity and associated regulations differ from one country to another. In Asia, the United States, and Europe, the α-, β-, and γ-cyclodextrins are considered safe. The β-cyclodextrins are the only ones with well-established safe consumption doses, due to their innocuous effect. In the United States, CDs are considered a GRAS food additive. In Australia and New Zealand, CDs are considered food. In Europe, CDs have been approved as an additive (E-459).

Concerning the current legislation, an interesting work has recently been published by Petitjean et al. They reported on the legal aspect of using CDs in the food industry worldwide [76]. It is also worth noting that the Scientific Committee on Food (SCF), in a report published in 2016, concluded that, based on the available toxicological database, there is no reason to revise the current ADI of 5 mg/kg bw per day for β-cyclodextrin (E 459) [25]. They did, however, recommend that a few actions must be carried out: (i) including the microbiological specifications for β-cyclodextrin (E 459) and (ii) reducing to the lowest level (according to SCF recommendations) the presence of the carcinogenic trichloroethylene as a residual solvent in β-cyclodextrin (E-459) (SCF, 2002a,b,c,d, as referred to by EFSA Scientific Committee (2005) [37,76]).

As far as the food packaging industry is concerned, the harmful effects and risks for human health associated with the direct consumption of CDs and their derivatives are minimized due to their indirect exposure. In fact, CDs in AFP applications are usually cross-linked within the material used as a package, limiting in this way the direct contamination of the food and the final consumers.

7. Commercial Products and Patents

Due to the importance and use of CDs in several industries, a large number of patents have been registered. Table 5. presents the recent patents using CDs in active food packaging. The data were obtained from online databases, which include Scopus, WIPO, Worldwide Espacenet, and Google Patents. The results show that most of the patents are focused on the development of sustainable packaging that extends the shelf life of the product. The patents mostly include films, extrusion, and coatings. The intellectual property of the patents has mainly concentrated on the method of preparation and the application. The inventions contemplate the inclusion of CDs with compounds such as essential oils, fibers, and plastics.

Table 5. Some patents using CDs in active food packaging.

Patent Title	Application	Description	Reference
Active packaging film based on essential oil/β-cyclodextrin inclusion compound and preparation method for active packaging film	Active packaging film	Beta-cyclodextrin; essential oil with broad-spectrum antibacterial performance; by weight, essential oil/ benexate hydrochloride is 4–24% of the total amount.	[152]
Functional gelatin food packaging film and preparation method	Gelatin food packaging film	Using gelatin as the carboxylated beta-cyclodextrin of main raw material compound and natural active matter.	[153]
Cyclodextrin compositions, articles, and methods	A selectively permeable packaging material	Cyclodextrin inclusion complex and a polymer, the composition obtained with electromagnetic irradiation of a cyclodextrin composition comprising one or more radiation-polymerizable monomers and a cyclodextrin complex, the cyclodextrin complex comprising a cyclodextrin compound, and an olefinic inhibitor comprising a cyclopropane.	[154]
Antibacterial quality-guarantee food packaging bag and preparation method thereof	An antibacterial food packaging	Low-density polyethylene, zinc stearate, monoglyceride, polylactic resin, propylene glycol, dioctyl phthalate, ethoxylated alkylamine, porous hydroxyapatite, medical stone, beta-cyclodextrin, chitosan, lanthanum-loaded zinc oxide, 3–6 parts lanthanum-loaded titanium dioxide, and 0.5–1 part natamycin.	[155]

Table 5. *Cont.*

Patent Title	Application	Description	Reference
Degradable packaging film for fruit and vegetables	Packaging film	Based on a polyolefin selected from polyethylene (PE), polypropylene (PE), polystyrene (PS), and ethyl vinyl acetate (EVA) and essential oil antimicrobial agents or said essential agent, microencapsulated in an encapsulating agent selected from the group consisting of cyclodextrin (β- or γ-).	[156]
Clove essential oil contained sterilization plastic wrap and preparation method thereof	Clove essential oil contained sterilization plastic wrap	Clove essential oil, beta-cyclodextrin	[157]
Method for preparing antibacterial food packaging preservation film by doping garlic-oil-beta-cyclodextrin inclusion compound with clove oil	An antibacterial food packaging preservation film	Garlic-oil-beta-cyclodextrin inclusion compound with clove oil	[158]
Environmentally friendly food packaging plastic and preparation method thereof	Environmentally friendly food packaging plastic	25–35 parts epoxy modified hyperbranched poly (beta-cyclodextrin) containing azide and vinyl groups, 8–12 parts vinyl polylactic acid, 4–6 parts (Z)-2-(2-aminothiazole 4yl) 2-pentenoic acid, 1–3 parts coupling agent, 0.5–0.9 part fullerene nano/microfibers, and 0.4–0.6 part initiator.	[159]
Application of hydroxypropyl-beta-cyclodextrin in preparation of antibacterial material, food packaging, and preparation method of food packaging	The food packaging includes, but is not limited to, packaging boxes and bags for packaging edible substances, and packaging bottles for packaging edible substances	Hydroxypropyl-β-cyclodextrin is prepared by enzymatically hydrolyzing starch with *Bacillus*, and the *Bacillus* expresses cyclodextrin glucosyltransferase.	[160]
Food packaging films containing natural antibacterial component	Edible films and, more particularly, a method for preparing a food packaging film with antibacterial activity	Perilla oil and cyclodextrin in the mixed dispersion of high-amylose corn starch and konjac glucomannan to prepare an active film.	[161]
Packaging material	Film or sheet for use in "active" packaging systems, capable of inhibiting the growth of microorganisms on the surface of the food product packaged therein	Encapsulated ethanol and a polymeric component selected from chitosan grafted with polyethylene glycol or cyclodextrin, a mixture of chitosan and polyethylene glycol, and a polymer or mixture of polymers for printable paint applied to the other side of the base layer.	[162]

Although several patents have been published so far relating to active food packaging applications, commercially available products are still scarce. Only a few companies from the USA, Japan, Germany, Finland, Spain, France, and South Africa are actually leading the active food packaging market (AFP) [4]. They are mainly commercializing different products, such as moisture absorbers, oxygen scavengers, carbon dioxide emitters, and antimicrobial packaging in the form of films, wraps, trays, pads, sachets, and masterbatches, among others. This lack of commercial AFP products is in total agreement with the bibliometric study and the abovementioned research trends in this area (see Figure 2). This also demonstrates that the topic is still open and extremely important, with high commercial potential.

8. Conclusions and Future Trends

The use of cyclodextrins and their inclusion complex derivatives for the development of innovative, nontoxic, biodegradable, sustainable, and cost-efficient food packaging has

many advantages. First, it improves the compatibility, miscibility, and synergy between the packaging polymer and the active molecule. Second, it protects the active molecule from degradation, volatilization, and undesirable interactions with the packaging materials. Third, it acts as a vehicle to confer on the packaging the antimicrobial/antioxidant and bioactivity enhancement. For most active molecules, the miscibility is crucial, because most are hydrophobic and almost insoluble spontaneously in water or common organic solvents. This is especially important for those natural or synthetic bioactive molecules intended to confer the antimicrobial or antioxidant characteristics on the active food packaging applications. Fourth, it allows the modulation of the spatiotemporal control over the release of active molecules in order to extend the shelf life and reduce the changes in the sensory properties of foods. For their part, cyclodextrin complexes are stable at high temperatures due to the high thermal degradation of CDs (~280 °C). This is responsible for protecting against the degradation of the active ingredients due to the high temperatures of the packaging production methods. Another advantage is their concomitant biodegradability, which allows the development of new environmentally friendly packaging materials that harmonize with the current sustainable market and commercial exigencies.

The use of cyclodextrins in active food packaging applications needs further study in order to mitigate their environmental and human health impact. Their low toxicity and immunogenicity drive the continuously growing research interest not only in the food packaging industries but also in other key industrial sectors such as the pharmaceutical and chemical industries. According to the latest trends reported in the literature, everything points to a focus in the future food packaging trends on a combination of the classic methods with nanotechnology, nanoencapsulation, or the use of nanosponges. Among the processing techniques, the versatility of electrospinning offers a unique opportunity to design and industrially fabricate tailored polymer/CD-based composite materials for the food packaging industry. So far, CDs have been incorporated into active food packaging as empty cyclodextrins, complexed through cyclodextrin inclusion complex derivatives (CICs), or by the supramolecular polymer assemblies of CDs. According to the literature reviewed, the inclusion complex derivatives stand out as the most appealing and promising route for encapsulating bioactive molecules in active packaging.

As far as the legislation is concerned, despite the marked differences in the current policies applied to different regions such as America, Europe, and Asia, most agree on the safety of using CDs and their derivatives in the food sciences, and even more so in the food packaging industries, due to their indirect contact mechanisms. However, it seems highly appealing to consciously re-examine their impact on the environment and human health due to the extreme complexing ability of cyclodextrin when interacting with other molecules and biological systems.

It is worth mentioning that the patents reported so far in this area are focused on the development of sustainable packaging that extends the shelf life of the product and is produced mainly via film extrusion and coatings. Despite the existence of various patents for active food packaging applications, commercially available products are still scarce. Only a few companies from America, Europe, Africa, and Asia are actually leading the market and commercializing products such as moisture absorbers, oxygen scavengers, carbon dioxide emitters, or antimicrobial packaging in the form of films, wraps, trays, pads, sachets, and masterbatches, among others. This lack of commercial AFP products is in total agreement with the bibliometric study and research trends in this area and can be considered as another demonstration that the development of active food packaging based on biodegradable polymers combined with cyclodextrins is still an open and extremely important issue, with high commercial potential.

Finally, the inclusion of CDs in active packaging would play a crucial role in future trends regarding the implementation of the ultimate packaging. Future development shall focus on the fabrication of cost-efficient and sustainable multifunctional packaging combining the advantages of this CD active packaging (intended to maintain or improve its intrinsic properties and extend the shelf life of the packaged food), boosted by the

advantages of smart packaging (intended to monitor the condition of the packaged food and provide safety information to stakeholders such as manufacturers, retailers, and consumers), with the ultimate interest of exploiting sustainable packaging (intended to reduce the environmental impact of packaging waste).

Funding: This work was financially supported by the Ministry of Economy and Competitiveness (MINECO) within the State Program of Research, Development, and Innovation Oriented to the Challenges of Society (Reference: RTC-2015-3321-2).

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ADI	Acceptable daily intake
AFP	Active food packaging
ARP	Active-releasing packaging
ASP	Active-scavenging packaging
CDs	Cyclodextrins
CD-NSs	Cyclodextrin-based nanosponges
CI	Citral
CICs	Cyclodextrin inclusion complexes
DMDS	Dimethyl disulfide
EOs	Essential oils
EVA	Polyethylene-co-vinyl acetate
FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe
HDPE	High-density polyethylene
JECFA	Joint Expert Committee on Food Additives
LDPE	Low-density polyethylene
MAP	Modified atmospheric storage
MB	Masterbatch
PBS	Butylene polysuccinate
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Polylactic acid
PP	Polypropylene
PTT	Polytrimethylene terephthalate
PVA	Poly(vinyl acetate)
SCF	Scientific Committee on Food
TA-β-CD	Triacetyl-β-cyclodextrin
TC	Trans-cinnamaldehyde

References

1. Han, J.W.; Ruiz-Garcia, L.; Qian, J.P.; Yang, X.T. Food Packaging: A Comprehensive Review and Future Trends. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 860–877. [CrossRef] [PubMed]
2. Moeini, A.; Germann, N.; Malinconico, M.; Santagata, G. Formulation of secondary compounds as additives of biopolymer-based food packaging: A review. *Trends Food Sci. Technol.* **2021**, *114*, 342–354. [CrossRef]
3. Beltran Sanahuja, A.; Valdes Garcia, A. New Trends in the Use of Volatile Compounds in Food Packaging. *Polymers* **2021**, *13*, 1053. [CrossRef] [PubMed]
4. Soltani Firouz, M.; Mohi-Alden, K.; Omid, M. A critical review on intelligent and active packaging in the food industry: Research and development. *Food Res. Int.* **2021**, *141*, 110113. [CrossRef] [PubMed]
5. Hosseinnejad, M. Active packaging for food applications—A review. *Int. J. Adv. Biol. Biomed. Res.* **2014**, *2*, 1174–1180.
6. Matencio, A.; Navarro-Orcajada, S.; García-Carmona, F.; López-Nicolás, J.M. Applications of cyclodextrins in food science. A review. *Trends Food Sci. Technol.* **2020**, *104*, 132–143. [CrossRef]
7. Mousavi Khaneghah, A.; Hashemi, S.M.B.; Limbo, S. Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions. *Food Bioprod. Process.* **2018**, *111*, 1–19. [CrossRef]
8. Limbo, S.; Khaneghah, A.M. Active packaging of foods and its combination with electron beam processing. In *Electron Beam Pasteurization and Complementary Food Processing Technologies*; Woodhead Publishing: Cambridge, UK, 2015; pp. 195–217.

9. Mallardo, S.; De Vito, V.; Malinconico, M.; Volpe, M.G.; Santagata, G.; Di Lorenzo, M.L. Poly(butylene succinate)-based composites containing β-cyclodextrin/d-limonene inclusion complex. *Eur. Polym. J.* **2016**, *79*, 82–96. [CrossRef]
10. Troise, A.D.; Fogliano, V. Reactants encapsulation and Maillard Reaction. *Trends Food Sci. Technol.* **2013**, *33*, 63–74. [CrossRef]
11. Szente, L.; Fenyvesi, E. Cyclodextrin-Enabled Polymer Composites for Packaging (dagger). *Molecules* **2018**, *23*, 1556. [CrossRef] [PubMed]
12. Aytac, Z.; Ipek, S.; Durgun, E.; Tekinay, T.; Uyar, T. Antibacterial electrospun zein nanofibrous web encapsulating thymol/cyclodextrin-inclusion complex for food packaging. *Food Chem.* **2017**, *233*, 117–124. [CrossRef] [PubMed]
13. Chen, G.; Liu, B. Cellulose sulfate based film with slow-release antimicrobial properties prepared by incorporation of mustard essential oil and β-cyclodextrin. *Food Hydrocoll.* **2016**, *55*, 100–107. [CrossRef]
14. Marques, H.M.C. A review on cyclodextrin encapsulation of essential oils and volatiles. *Flavour Fragr. J.* **2010**, *25*, 313–326. [CrossRef]
15. Astray, G.; Gonzalez-Barreiro, C.; Mejuto, J.C.; Rial-Otero, R.; Simal-Gándara, J. A review on the use of cyclodextrins in foods. *Food Hydrocoll.* **2009**, *23*, 1631–1640. [CrossRef]
16. Harada, A.; Takashima, Y.; Yamaguchi, H. Cyclodextrin-based supramolecular polymers. *Chem. Soc. Rev.* **2009**, *38*, 875–882. [CrossRef]
17. Del Valle, E.M.M. Cyclodextrins and their uses: A review. *Process Biochem.* **2004**, *39*, 1033–1046. [CrossRef]
18. Schardinger, F. Über thermophile Bakterien aus verschiedenen Speisen und Milch. *Zeitschrift für Untersuchung der Nahrungs- und Genußmittel* **1903**, *6*, 865–880. [CrossRef]
19. Higuchi, T.; Connors, K.A. Phase Solubility Techniques. *Adv. Anal. Chem. Instrum.* **1965**, *4*, 117–212.
20. Becerril, R.; Nerin, C.; Silva, F. Encapsulation Systems for Antimicrobial Food Packaging Components: An Update. *Molecules* **2020**, *25*, 1134. [CrossRef]
21. Szejtli, J. Introduction and General Overview of Cyclodextrin Chemistry. *Chem. Rev.* **1998**, *98*, 1743–1754. [CrossRef]
22. Kfoury, M.; Geagea, C.; Ruellan, S.; Greige-Gerges, H.; Fourmentin, S. Effect of cyclodextrin and cosolvent on the solubility and antioxidant activity of caffeic acid. *Food Chem.* **2019**, *278*, 163–169. [CrossRef]
23. Hedges, A. Cyclodextrins: Properties and Applications. In *Starch: Chemistry and Technology*, 3rd ed.; Academic Press: Cambridge, MA, USA, 2009; pp. 833–851.
24. Cravotto, G.; Binello, A.; Baranelli, E.; Carraro, P.; Trotta, F. Cyclodextrins as Food Additives and in Food Processing. *Curr. Nutr. Food Sci.* **2006**, *2*, 343–350. [CrossRef]
25. Mortensen, A.; Aguilar, F.; Crebelli, R.; Di Domenico, A.; Dusemund, B.; Frutos, M.J.; Galtier, P.; Gott, D.; Gundert-Remy, U.; Leblanc, J.C.; et al. Re-evaluation of β-cyclodextrin (E 459) as a food additive. *EFSA J.* **2016**, *14*, e04628.
26. Martina, K.; Binello, A.; Lawson, D.; Jicsinszky, L.; Cravotto, G. Recent Applications of Cyclodextrins as Food Additives and in Food Processing. *Curr. Nutr. Food Sci.* **2013**, *9*, 167–179. [CrossRef]
27. Matencio, A.; Garcia-Carmona, F.; Lopez-Nicolas, J.M. The inclusion complex of oxyresveratrol in modified cyclodextrins: A thermodynamic, structural, physicochemical, fluorescent and computational study. *Food Chem.* **2017**, *232*, 177–184. [CrossRef]
28. Poverenov, E.; Granit, R.; Gabai, S. Encapsulation and controlled release of antifungal propionic acid utilizing biodegradable active films based on natural polymers. *Eur. Food Res. Technol.* **2013**, *237*, 19–26. [CrossRef]
29. Shin, J.; Lee, E.J.; Ahn, D.U. Electrospinning of tri-acetyl-β-cyclodextrin (TA-β-CD) functionalized low-density polyethylene to minimize sulfur odor volatile compounds. *Food Packag. Shelf Life* **2018**, *18*, 107–114. [CrossRef]
30. Gonzalez Pereira, A.; Carpena, M.; Garcia Oliveira, P.; Mejuto, J.C.; Prieto, M.A.; Simal Gandara, J. Main Applications of Cyclodextrins in the Food Industry as the Compounds of Choice to Form Host-Guest Complexes. *Int. J. Mol. Sci.* **2021**, *22*, 1339. [CrossRef]
31. Tian, B.; Xiao, D.; Hei, T.; Ping, R.; Hua, S.; Liu, J. The application and prospects of cyclodextrin inclusion complexes and polymers in the food industry: A review. *Polym. Int.* **2020**, *69*, 597–603. [CrossRef]
32. Mura, P. Analytical techniques for characterization of cyclodextrin complexes in aqueous solution: A review. *J. Pharm. Biomed. Anal.* **2014**, *101*, 238–250. [CrossRef]
33. Mura, P. Analytical techniques for characterization of cyclodextrin complexes in the solid state: A review. *J. Pharm. Biomed. Anal.* **2015**, *113*, 226–238. [CrossRef] [PubMed]
34. Liu, Y.; Sameen, D.E.; Ahmed, S.; Wang, Y.; Lu, R.; Dai, J.; Li, S.; Qin, W. Recent advances in cyclodextrin-based films for food packaging. *Food Chem.* **2021**, *370*, 131026. [CrossRef]
35. Yildirim, S.; Rocker, B.; Pettersen, M.K.; Nilsen-Nygaard, J.; Ayhan, Z.; Rutkaite, R.; Radusin, T.; Suminska, P.; Marcos, B.; Coma, V. Active Packaging Applications for Food. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 165–199. [CrossRef]
36. Rooney, M.L. *Active Food Packaging*; Springer: Boston, MA, USA, 1995; p. 260.
37. Da Costa, J.P.; Rocha-Santos, T.; Duarte, A.C. *The Environmental Impacts of Plastics and Micro-Plastics Use, Waste and Pollution: EU and National Measures*; European Parliament: Strasbourg, France, 2020.
38. Silvestre, C.; Duraccio, D.; Cimmino, S. Food packaging based on polymer nanomaterials. *Prog. Polym. Sci.* **2011**, *36*, 1766–1782. [CrossRef]
39. Tajeddin, B.; Arabkhedri, M. Polymers and food packaging. In *Polymer Science and Innovative Applications*; Elsevier: London, UK, 2020; pp. 525–543.

40. Wang, K.; Deng, Q. The Thermal and Mechanical Properties of Poly(ethylene-co-vinyl acetate) Random Copolymers (PEVA) and its Covalently Crosslinked Analogues (cPEVA). *Polymers* **2019**, *11*, 1055. [CrossRef]
41. Frine, V.C.; Hector, A.P.; Manuel, N.S.; Estrella, N.D.; Antonio, G.J. Development and Characterization of a Biodegradable PLA Food Packaging Hold Monoterpene-Cyclodextrin Complexes against Alternaria alternata. *Polymers* **2019**, *11*, 1720. [CrossRef]
42. Yang, Y.; Huan, C.; Liang, X.; Fang, S.; Wang, J.; Chen, J. Development of Starch-Based Antifungal Coatings by Incorporation of Natamycin/Methyl-beta-Cyclodextrin Inclusion Complex for Postharvest Treatments on Cherry Tomato against Botrytis cinerea. *Molecules* **2019**, *24*, 3962. [CrossRef]
43. Boonnattakorn, R.; Chonhenchob, V.; Siddiq, M.; Singh, S.P. Controlled Release of Mangiferin Using Ethylene Vinyl Acetate Matrix for Antioxidant Packaging. *Packag. Technol. Sci.* **2015**, *28*, 241–252. [CrossRef]
44. Sonia, A.K.; Dasan, K.P. Feasibility studies of cellulose microfiber (CMF) reinforced poly(ethylene-co-vinyl acetate) (EVA) composites for food packaging applications. *Sci. Eng. Compos. Mater.* **2016**, *23*, 489–494. [CrossRef]
45. Reesha, K.V.; Panda, S.K.; Bindu, J.; Varghese, T.O. Development and characterization of an LDPE/chitosan composite antimicrobial film for chilled fish storage. *Int. J. Biol. Macromol.* **2015**, *79*, 934–942. [CrossRef]
46. Suppakul, P.; Sonneveld, K.; Bigger, S.W.; Miltz, J. Efficacy of polyethylene-based antimicrobial films containing principal constituents of basil. *LWT-Food Sci. Technol.* **2008**, *41*, 779–788. [CrossRef]
47. Wang, L.; Mu, R.-J.; Li, Y.; Lin, L.; Lin, Z.; Pang, J. Characterization and antibacterial activity evaluation of curcumin loaded konjac glucomannan and zein nanofibril films. *Lwt* **2019**, *113*, 108293. [CrossRef]
48. Mangaraj, S.; Yadav, A.; Bal, L.M.; Dash, S.K.; Mahanti, N.K. Application of Biodegradable Polymers in Food Packaging Industry: A Comprehensive Review. *J. Packag. Technol. Res.* **2018**, *3*, 77–96. [CrossRef]
49. Siracusa, V.; Rocculi, P.; Romani, S.; Rosa, M.D. Biodegradable polymers for food packaging: A review. *Trends Food Sci. Technol.* **2008**, *19*, 634–643. [CrossRef]
50. Byun, Y.; Kim, Y.T. Bioplastics for Food Packaging. In *Innovations in Food Packaging*; Elsevier: Tokyo, Japan, 2014; pp. 353–368.
51. Mittal, V. *Characterization Techniques for Polymer Nanocomposites*; Wiley VCH: Singapore, 2012.
52. Mano, J.F.; Gómez Ribelles, J.L.; Alves, N.M.; Salmerón Sanchez, M. Glass transition dynamics and structural relaxation of PLLA studied by DSC: Influence of crystallinity. *Polymers* **2005**, *46*, 8258–8265. [CrossRef]
53. Velazquez-Contreras, F.; Garcia-Caldera, N.; Padilla de la Rosa, J.D.; Martinez-Romero, D.; Nunez-Delicado, E.; Gabaldon, J.A. Effect of PLA Active Packaging Containing Monoterpene-Cyclodextrin Complexes on Berries Preservation. *Polymers* **2021**, *13*, 1399. [CrossRef]
54. Auras, R.; Harte, B.; Selke, S. An overview of polylactides as packaging materials. *Macromol. Biosci.* **2004**, *4*, 835–864. [CrossRef] [PubMed]
55. Barnett, I. *The Global Outlook for Biodegradable Packaging*; Business Insights Ltd.: Burnaby, BC, Canada, 2011.
56. Gotro, J. Thermoplastic Starch: A Renewable, Biodegradable Bioplastic. In *Polymer Innovation Blog*; InnoCentrix, LLC: Rancho Santa Margarita, CA, USA, 2013.
57. Restrepo-Osorio, A.; Cruz Riaño, L.J.; Alvarez-Lopéz, C.; Rios Osorio, A.D. Revisión: Fibroína de seda y sus potenciales aplicaciones en empaques biodegradables para alimentos/Review: Silk fibroin and their potential applications on biodegradable food packaging. *Prospectiva* **2017**, *15*, 7–15. [CrossRef]
58. Wu, F.; Misra, M.; Mohanty, A.K. Challenges and new opportunities on barrier performance of biodegradable polymers for sustainable packaging. *Prog. Polym. Sci.* **2021**, *117*, 101395. [CrossRef]
59. Wei, X.W.; Guo, G.; Gong, C.Y.; Gou, M.L.; Yong Qian, Z. Biodegradable Polymers: Research and Applications. In *A Handbook of Applied Biopolymer Technology*; Springer: Berlin/Heidelberg, Germany, 2011; Chapter 12; pp. 365–387.
60. Tang, X.Z.; Kumar, P.; Alavi, S.; Sandeep, K.P. Recent advances in biopolymers and biopolymer-based nanocomposites for food packaging materials. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 426–442. [CrossRef]
61. Södergård, A.; Stolt, M. *Poly(lactic acid): Synthesis, Structures, Properties, Processing, and Applications*; John Wiley & Sons: Hoboken, NJ, USA, 2010.
62. Goni-Ciaurriz, L.; Senosiain-Nicolay, M.; Velaz, I. Aging Studies on Food Packaging Films Containing beta-Cyclodextrin-Grafted TiO2 Nanoparticles. *Int. J. Mol. Sci.* **2021**, *22*, 2257. [CrossRef] [PubMed]
63. Hughes, J.; Thomas, R.; Byun, Y.; Whiteside, S. Improved flexibility of thermally stable poly-lactic acid (PLA). *Carbohydr. Polym.* **2012**, *88*, 165–172. [CrossRef]
64. Rasal, R.M.; Janorkar, A.V.; Hirt, D.E. Poly(lactic acid) modifications. *Prog. Polym. Sci.* **2010**, *35*, 338–356. [CrossRef]
65. Almasi, H.; Jahanbakhsh Oskouie, M.; Saleh, A. A review on techniques utilized for design of controlled release food active packaging. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 2601–2621. [CrossRef] [PubMed]
66. Azeredo, H.M.C.d. Nanocomposites for food packaging applications. *Food Res. Int.* **2009**, *42*, 1240–1253. [CrossRef]
67. Echegoyen, Y.; Fabra, M.J.; Castro-Mayorga, J.L.; Cherpinski, A.; Lagaron, J.M. High throughput electro-hydrodynamic processing in food encapsulation and food packaging applications: Viewpoint. *Trends Food Sci. Technol.* **2017**, *60*, 71–79. [CrossRef]
68. Patino Vidal, C.; Lopez de Dicastillo, C.; Rodriguez-Mercado, F.; Guarda, A.; Galotto, M.J.; Munoz-Shuguli, C. Electrospinning and cyclodextrin inclusion complexes: An emerging technological combination for developing novel active food packaging materials. *Crit. Rev. Food Sci. Nutr.* **2021**, 1–16. [CrossRef] [PubMed]
69. Jawaid, M.; Boonruang, K.; Chinsirikul, W.; Hararak, B.; Kerddonfag, N.; Chonhenchob, V.; Kenawy, E.-R. Antifungal Poly(lactic acid) Films Containing Thymol and Carvone. *MATEC Web Conf.* **2016**, *67*, 6107.

70. Chen, H.; Li, L.; Ma, Y.; McDonald, T.P.; Wang, Y. Development of active packaging film containing bioactive components encapsulated in β-cyclodextrin and its application. *Food Hydrocoll.* **2019**, *90*, 360–366. [CrossRef]
71. Mousavi Khaneghah, A.; Hashemi, S.M.B.; Es, I.; Fracassetti, D.; Limbo, S. Efficacy of Antimicrobial Agents for Food Contact Applications: Biological Activity, Incorporation into Packaging, and Assessment Methods: A Review. *J. Food Prot.* **2018**, *81*, 1142–1156. [CrossRef]
72. Sanches-Silva, A.; Costa, D.; Albuquerque, T.G.; Buonocore, G.G.; Ramos, F.; Castilho, M.C.; Machado, A.V.; Costa, H.S. Trends in the use of natural antioxidants in active food packaging: A review. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess* **2014**, *31*, 374–395. [CrossRef] [PubMed]
73. Joo, M.; Auras, R.; Almenar, E. Preparation and characterization of blends made of poly(l-lactic acid) and β-cyclodextrin: Improvement of the blend properties by using a masterbatch. *Carbohydr. Polym.* **2011**, *86*, 1022–1030. [CrossRef]
74. Wen, P.; Wen, Y.; Zong, M.H.; Linhardt, R.J.; Wu, H. Encapsulation of Bioactive Compound in Electrospun Fibers and Its Potential Application. *J. Agric. Food Chem.* **2017**, *65*, 9161–9179. [CrossRef] [PubMed]
75. Sánchez-Guijarro, M.; García-Gómez, P.; Almela, L.; Nuñez-Delicado, E.; Gabaldón, J.A. Improvement of the shelf life of minimally processed artichoke through antimicrobial and antioxidants agents. *Acta Hortic.* **2020**, *1284*, 205–220. [CrossRef]
76. Petitjean, M.; Garcia-Zubiri, I.X.; Isasi, J.R. History of cyclodextrin-based polymers in food and pharmacy: A review. *Environ. Chem. Lett.* **2021**, *19*, 3465–3476. [CrossRef] [PubMed]
77. Arruda, T.R.; Marques, C.S.; Soares, N.F.F. Native Cyclodextrins and Their Derivatives as Potential Additives for Food Packaging: A Review. *Polysaccharides* **2021**, *2*, 825–842. [CrossRef]
78. Morin-Crini, N.; Winterton, P.; Fourmentin, S.; Wilson, L.D.; Fenyvesi, É.; Crini, G. Water-insoluble β-cyclodextrin–epichlorohydrin polymers for removal of pollutants from aqueous solutions by sorption processes using batch studies: A review of inclusion mechanisms. *Prog. Polym. Sci.* **2018**, *78*, 1–23. [CrossRef]
79. Matencio, A.; Hoti, G.; Monfared, Y.K.; Rezayat, A.; Pedrazzo, A.R.; Caldera, F.; Trotta, F. Cyclodextrin Monomers and Polymers for Drug Activity Enhancement. *Polymers* **2021**, *13*, 1684. [CrossRef]
80. Hu, Y.; Qiu, C.; Qin, Y.; Xu, X.; Fan, L.; Wang, J.; Jin, Z. Cyclodextrin–phytochemical inclusion complexes: Promising food materials with targeted nutrition and functionality. *Trends Food Sci. Technol.* **2021**, *109*, 398–412. [CrossRef]
81. Lopez-de-Dicastillo, C.; Jorda, M.; Catala, R.; Gavara, R.; Hernandez-Munoz, P. Development of active polyvinyl alcohol/beta-cyclodextrin composites to scavenge undesirable food components. *J. Agric. Food Chem.* **2011**, *59*, 11026–11033. [CrossRef]
82. López-de-Dicastillo, C.; Gallur, M.; Catalá, R.; Gavara, R.; Hernandez-Muñoz, P. Immobilization of β-cyclodextrin in ethylene-vinyl alcohol copolymer for active food packaging applications. *J. Membr. Sci.* **2010**, *353*, 184–191. [CrossRef]
83. Nedovic, V.; Kalusevic, A.; Manojlovic, V.; Levic, S.; Bugarski, B. An overview of encapsulation technologies for food applications. *Procedia Food Sci.* **2011**, *1*, 1806–1815. [CrossRef]
84. González-Louzao, R.; Lucas-Abellán, C.; Pérez-Sánchez, H.; Pedro Cerón-Carrasco, J.; Antonio Gabaldón, J.; López-Miranda, S.; Josefa Yáñez-Gascón, M.; Asín-Llorca, M.; Núñez-Delicado, E. Encapsulation of finasteride with native and modified γ-cyclodextrins. Extensive characterization of the complexes. *Int. J. Pharm.* **2020**, *587*, 119619. [CrossRef] [PubMed]
85. Folch-Cano, C.; Yazdani-Pedram, M.; Olea-Azar, C. Inclusion and functionalization of polymers with cyclodextrins: Current applications and future prospects. *Molecules* **2014**, *19*, 14066–14079. [CrossRef] [PubMed]
86. Charasphat, P.; Warinyupa, M.; Manchumas, P.; Ekasit, N.; Krisana, S. Enhancing stability and antioxidant efficacy of fisetin by encapsulating as β-cyclodextrin inclusion complex with porous polylactic acid film from breath figure. *J. Met. Mater. Miner.* **2021**, *31*, 81–87.
87. de Castro, D.O.; Tabary, N.; Martel, B.; Gandini, A.; Belgacem, N.; Bras, J. Controlled release of carvacrol and curcumin: Bio-based food packaging by synergism action of TEMPO-oxidized cellulose nanocrystals and cyclodextrin. *Cellulose* **2018**, *25*, 1249–1263. [CrossRef]
88. Lavoine, N.; Givord, C.; Tabary, N.; Desloges, I.; Martel, B.; Bras, J. Elaboration of a new antibacterial bio-nano-material for food-packaging by synergistic action of cyclodextrin and microfibrillated cellulose. *Innov. Food Sci. Emerg. Technol.* **2014**, *26*, 330–340. [CrossRef]
89. Wen, P.; Zhu, D.H.; Feng, K.; Liu, F.J.; Lou, W.Y.; Li, N.; Zong, M.H.; Wu, H. Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/beta-cyclodextrin inclusion complex for antimicrobial packaging. *Food Chem.* **2016**, *196*, 996–1004. [CrossRef]
90. Prakash, B.; Kujur, A.; Yadav, A.; Kumar, A.; Singh, P.P.; Dubey, N.K. Nanoencapsulation: An efficient technology to boost the antimicrobial potential of plant essential oils in food system. *Food Control* **2018**, *89*, 1–11. [CrossRef]
91. Zanetti, M.; Carniel, T.K.; Dalcanton, F.; dos Anjos, R.S.; Gracher Riella, H.; de Araújo, P.H.H.; de Oliveira, D.; Antônio Fiori, M. Use of encapsulated natural compounds as antimicrobial additives in food packaging: A brief review. *Trends Food Sci. Technol.* **2018**, *81*, 51–60. [CrossRef]
92. Atarés, L.; Chiralt, A. Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends Food Sci. Technol.* **2016**, *48*, 51–62. [CrossRef]
93. Lin, L.; Zhu, Y.; Cui, H. Electrospun thyme essential oil/gelatin nanofibers for active packaging against Campylobacter jejuni in chicken. *LWT* **2018**, *97*, 711–718. [CrossRef]

94. Ribeiro-Santos, R.; Carvalho-Costa, D.; Cavaleiro, C.; Costa, H.S.; Albuquerque, T.G.; Castilho, M.C.; Ramos, F.; Melo, N.R.; Sanches-Silva, A. A novel insight on an ancient aromatic plant: The rosemary (*Rosmarinus officinalis* L.). *Trends Food Sci. Technol.* **2015**, *45*, 355–368. [CrossRef]
95. Dias Antunes, M.; da Silva Dannenberg, G.; Fiorentini, A.M.; Pinto, V.Z.; Lim, L.T.; da Rosa Zavareze, E.; Dias, A.R.G. Antimicrobial electrospun ultrafine fibers from zein containing eucalyptus essential oil/cyclodextrin inclusion complex. *Int. J. Biol. Macromol.* **2017**, *104 Pt A*, 874–882. [CrossRef]
96. Higueras, L.; Lopez-Carballo, G.; Hernandez-Munoz, P.; Catala, R.; Gavara, R. Antimicrobial packaging of chicken fillets based on the release of carvacrol from chitosan/cyclodextrin films. *Int. J. Food Microbiol.* **2014**, *188*, 53–59. [CrossRef]
97. Cui, H.; Bai, M.; Lin, L. Plasma-treated poly(ethylene oxide) nanofibers containing tea tree oil/beta-cyclodextrin inclusion complex for antibacterial packaging. *Carbohydr. Polym.* **2018**, *179*, 360–369. [CrossRef]
98. Munhuweyi, K.; Caleb, O.J.; van Reenen, A.J.; Opara, U.L. Physical and antifungal properties of β-cyclodextrin microcapsules and nanofibre films containing cinnamon and oregano essential oils. *LWT* **2018**, *87*, 413–422. [CrossRef]
99. Pan, J.; Ai, F.; Shao, P.; Chen, H.; Gao, H. Development of polyvinyl alcohol/beta-cyclodextrin antimicrobial nanofibers for fresh mushroom packaging. *Food Chem.* **2019**, *300*, 125249. [CrossRef]
100. Favre, L.C.; Dos Santos, C.; Lopez-Fernandez, M.P.; Mazzobre, M.F.; Buera, M.D.P. Optimization of beta-cyclodextrin-based extraction of antioxidant and anti-browning activities from thyme leaves by response surface methodology. *Food Chem.* **2018**, *265*, 86–95. [CrossRef]
101. da Rocha Neto, A.C.; Beaudry, R.; Maraschin, M.; Di Piero, R.M.; Almenar, E. Double-bottom antimicrobial packaging for apple shelf-life extension. *Food Chem.* **2019**, *279*, 379–388. [CrossRef]
102. Zhou, C.; Abdel-Samie, M.A.; Li, C.; Cui, H.; Lin, L. Active packaging based on swim bladder gelatin/galangal root oil nanofibers: Preparation, properties and antibacterial application. *Food Packag. Shelf Life* **2020**, *26*, 100586. [CrossRef]
103. Marques, C.S.; Carvalho, S.G.; Bertoli, L.D.; Villanova, J.C.O.; Pinheiro, P.F.; Dos Santos, D.C.M.; Yoshida, M.I.; de Freitas, J.C.C.; Cipriano, D.F.; Bernardes, P.C. beta-Cyclodextrin inclusion complexes with essential oils: Obtention, characterization, antimicrobial activity and potential application for food preservative sachets. *Food Res. Int.* **2019**, *119*, 499–509. [CrossRef] [PubMed]
104. Simionato, I.; Domingues, F.C.; Nerin, C.; Silva, F. Encapsulation of cinnamon oil in cyclodextrin nanosponges and their potential use for antimicrobial food packaging. *Food Chem. Toxicol.* **2019**, *132*, 110647. [CrossRef]
105. Figueroa-Lopez, K.J.; Enescu, D.; Torres-Giner, S.; Cabedo, L.; Cerqueira, M.A.; Pastrana, L.; Fuciños, P.; Lagaron, J.M. Development of electrospun active films of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by the incorporation of cyclodextrin inclusion complexes containing oregano essential oil. *Food Hydrocoll.* **2020**, *108*, 106013. [CrossRef]
106. Cui, H.; Zhang, C.; Li, C.; Lin, L. Preparation and antibacterial activity of Litsea cubeba essential oil/dandelion polysaccharide nanofiber. *Ind. Crops Prod.* **2019**, *140*, 111739. [CrossRef]
107. Adel, A.M.; Ibrahim, A.A.; El-Shafei, A.M.; Al-Shemy, M.T. Inclusion complex of clove oil with chitosan/β-cyclodextrin citrate/oxidized nanocellulose biocomposite for active food packaging. *Food Packag. Shelf Life* **2019**, *20*, 100307. [CrossRef]
108. Lopez-Gomez, A.; Ros-Chumillas, M.; Buendia-Moreno, L.; Navarro-Segura, L.; Martinez-Hernandez, G.B. Active Cardboard Box with Smart Internal Lining Based on Encapsulated Essential Oils for Enhancing the Shelf Life of Fresh Mandarins. *Foods* **2020**, *9*, 590. [CrossRef]
109. Muñoz-Shugulí, C.; Vidal, C.P.; Cantero-López, P.; Lopez-Polo, J. Encapsulation of plant extract compounds using cyclodextrin inclusion complexes, liposomes, electrospinning and their combinations for food purposes. *Trends Food Sci. Technol.* **2021**, *108*, 177–186. [CrossRef]
110. Reque, P.M.; Brandelli, A. Encapsulation of probiotics and nutraceuticals: Applications in functional food industry. *Trends Food Sci. Technol.* **2021**, *114*, 1–10. [CrossRef]
111. Rajkumar, T.; Kukkar, D.; Kim, K.-H.; Sohn, J.R.; Deep, A. Cyclodextrin-metal–organic framework (CD-MOF): From synthesis to applications. *J. Ind. Eng. Chem.* **2019**, *72*, 50–66. [CrossRef]
112. Yao, X.; Huang, P.; Nie, Z. Cyclodextrin-based polymer materials: From controlled synthesis to applications. *Prog. Polym. Sci.* **2019**, *93*, 1–35. [CrossRef]
113. Zou, Y.; Yuan, C.; Cui, B.; Sha, H.; Liu, P.; Lu, L.; Wu, Z. High-Amylose Corn Starch/Konjac Glucomannan Composite Film: Reinforced by Incorporating beta-Cyclodextrin. *J. Agric. Food Chem.* **2021**, *69*, 2493–2500. [CrossRef] [PubMed]
114. Huang, Y.A.; Zeng, X.; Zhu, Q.; Lu, K.; Xu, Q.; Ye, C. Development of an active packaging with molecularly imprinted polymers for beef preservation. *Packag. Technol. Sci.* **2018**, *31*, 213–220. [CrossRef]
115. Del Nobile, M.A.; Conte, A.; Buonocore, G.G.; Incoronato, A.L.; Massaro, A.; Panza, O. Active packaging by extrusion processing of recyclable and biodegradable polymers. *J. Food Eng.* **2009**, *93*, 1–6. [CrossRef]
116. El Fawal, G. Polymer nanofibers electrospinning: A review. *Egypt. J. Chem.* **2019**, *63*, 1279–1303. [CrossRef]
117. Narvaez-Muñoz, C.P.; Carrion-Matamoros, L.M.; Vizuete, K.; Debut, A.; Arroyo, C.R.; Guerrero, V.; Almeida-Naranjo, C.E.; Morales-Flórez, V.; Mowbray, D.J.; Zamora-Ledezma, C. Tailoring Organic–Organic Poly(vinylpyrrolidone) Microparticles and Fibers with Multiwalled Carbon Nanotubes for Reinforced Composites. *ACS Appl. Nano Mater.* **2019**, *2*, 4302–4312. [CrossRef]
118. Peng, H.; Liu, Y.; Ramakrishna, S. Recent development of centrifugal electrospinning. *J. Appl. Polym. Sci.* **2017**, *134*. [CrossRef]
119. Lin, Y.; Yao, Y.; Yang, X.; Wei, N.; Li, X.; Gong, P.; Li, R.; Wu, D. Preparation of poly(ether sulfone) nanofibers by gas-jet/electrospinning. *J. Appl. Polym. Sci.* **2008**, *107*, 909–917. [CrossRef]

120. Xu, J.; Liu, X.; Zhang, Z.; Wang, L.; Tan, R.; Zhang, D. Controllable generation of nanofibers through a magnetic-field-assisted electrospinning design. *Mater. Lett.* **2019**, *247*, 19–24. [CrossRef]
121. Zhang, C.; Feng, F.; Zhang, H. Emulsion electrospinning: Fundamentals, food applications and prospects. *Trends Food Sci. Technol.* **2018**, *80*, 175–186. [CrossRef]
122. Dalton, P.D.; Grafahrend, D.; Klinkhammer, K.; Klee, D.; Möller, M. Electrospinning of polymer melts: Phenomenological observations. *Polymer* **2007**, *48*, 6823–6833. [CrossRef]
123. Topuz, F.; Uyar, T. Cyclodextrin-functionalized mesostructured silica nanoparticles for removal of polycyclic aromatic hydrocarbons. *J. Colloid Interface Sci.* **2017**, *497*, 233–241. [CrossRef]
124. Topuz, F.; Uyar, T. Antioxidant, antibacterial and antifungal electrospun nanofibers for food packaging applications. *Food Res. Int.* **2020**, *130*, 108927. [CrossRef]
125. Tian, J.; Deng, H.; Huang, M.; Liu, R.; Yi, Y.; Dong, X. Electrospun Nanofibers for Food and Food Packaging Technology. In *Electrospinning: Nanofabrication and Application*; William Andrew Publishing: Norwich, NY, USA, 2019; pp. 455–516.
126. Aytac, Z.; Xu, J.; Raman Pillai, S.K.; Eitzer, B.D.; Xu, T.; Vaze, N.; Ng, K.W.; White, J.C.; Chan-Park, M.B.; Luo, Y.; et al. Enzyme- and Relative Humidity-Responsive Antimicrobial Fibers for Active Food Packaging. *ACS Appl. Mater. Interfaces* **2021**, *13*, 50298–50308. [CrossRef]
127. Sharif, N.; Golmakani, M.-T.; Hajjari, M.M.; Aghaee, E.; Ghasemi, J.B. Antibacterial cuminaldehyde/hydroxypropyl-β-cyclodextrin inclusion complex electrospun fibers mat: Fabrication and characterization. *Food Packag. Shelf Life* **2021**, *29*, 100738. [CrossRef]
128. Li, C.; Chen, W.; Siva, S.; Cui, H.; Lin, L. Electrospun phospholipid nanofibers encapsulated with cinnamaldehyde/HP-β-CD inclusion complex as a novel food packaging material. *Food Packag. Shelf Life* **2021**, *28*, 100647. [CrossRef]
129. Narayanan, V.; Alam, M.; Ahmad, N.; Balakrishnan, S.B.; Ganesan, V.; Shanmugasundaram, E.; Rajagopal, B.; Thambusamy, S. Electrospun poly (vinyl alcohol) nanofibers incorporating caffeic acid/cyclodextrins through the supramolecular assembly for antibacterial activity. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2021**, *249*, 119308. [CrossRef]
130. Silva, F.; Caldera, F.; Trotta, F.; Nerín, C.; Domingues, F.C. Encapsulation of coriander essential oil in cyclodextrin nanosponges: A new strategy to promote its use in controlled-release active packaging. *Innov. Food Sci. Emerg. Technol.* **2019**, *56*, 102177. [CrossRef]
131. Trotta, F.; Zanetti, M.; Cavalli, R. Cyclodextrin-based nanosponges as drug carriers. *Beilstein J. Org. Chem.* **2012**, *8*, 2091–2099. [CrossRef]
132. Sherje, A.P.; Dravyakar, B.R.; Kadam, D.; Jadhav, M. Cyclodextrin-based nanosponges: A critical review. *Carbohydr. Polym.* **2017**, *173*, 37–49. [CrossRef]
133. Gokularaman, S.; Stalin Cruz, A.; Pragalyaashree, M.; Nishadh, A. Nanotechnology Approach in Food Packaging Review. *J. Pharm. Sci. Res.* **2017**, *9*, 1743–1749.
134. Kolahalam, L.A.; Kasi Viswanath, I.V.; Diwakar, B.S.; Govindh, B.; Reddy, V.; Murthy, Y.L.N. Review on nanomaterials: Synthesis and applications. *Mater. Today Proc.* **2019**, *18*, 2182–2190. [CrossRef]
135. Laux, P.; Tentschert, J.; Riebeling, C.; Braeuning, A.; Creutzenberg, O.; Epp, A.; Fessard, V.; Haas, K.H.; Haase, A.; Hund-Rinke, K.; et al. Nanomaterials: Certain aspects of application, risk assessment and risk communication. *Arch. Toxicol.* **2018**, *92*, 121–141. [CrossRef] [PubMed]
136. Kumar, P.; Mahajan, P.; Kaur, R.; Gautam, S. Nanotechnology and its challenges in the food sector: A review. *Mater. Today Chem.* **2020**, *17*, 100332. [CrossRef]
137. Mlalila, N.; Kadam, D.M.; Swai, H.; Hilonga, A. Transformation of food packaging from passive to innovative via nanotechnology: Concepts and critiques. *J. Food Sci. Technol.* **2016**, *53*, 3395–3407. [CrossRef]
138. Narayanan, G.; Shen, J.; Matai, I.; Sachdev, A.; Boy, R.; Tonelli, A.E. Cyclodextrin-based nanostructures. *Prog. Mater. Sci.* **2021**, 100869. [CrossRef]
139. Lin, L.; Xue, L.; Duraiarasan, S.; Haiying, C. Preparation of ε-polylysine/chitosan nanofibers for food packaging against Salmonella on chicken. *Food Packag. Shelf Life* **2018**, *17*, 134–141. [CrossRef]
140. Galdiero, S.; Falanga, A.; Vitiello, M.; Cantisani, M.; Marra, V.; Galdiero, M. Silver nanoparticles as potential antiviral agents. *Molecules* **2011**, *16*, 8894–8918. [CrossRef]
141. Hegab, H.M.; ElMekawy, A.; Zou, L.; Mulcahy, D.; Saint, C.P.; Ginic-Markovic, M. The controversial antibacterial activity of graphene-based materials. *Carbon* **2016**, *105*, 362–376. [CrossRef]
142. Magne, T.M.; de Oliveira Vieira, T.; Costa, B.; Alencar, L.M.R.; Ricci-Junior, E.; Hu, R.; Qu, J.; Zamora-Ledezma, C.; Alexis, F.; Santos-Oliveira, R. Factors affecting the biological response of Graphene. *Colloids Surf. B Biointerfaces* **2021**, *203*, 111767. [CrossRef]
143. Zamora-Ledezma, C.; Chicaiza-Zambrano, A.; Santiago Vispo, N.; Debut, A.; Vizuete, K.; Guerrero, V.H.; Almeida, C.E.; Alexis, F. Frequency Based Control of Antifouling Properties Using Graphene Nanoplatelet/Poly(Lactic-co-Glycolic Acid) Composite Films. *Compos. Interfaces* **2021**, *28*, 1137–1153. [CrossRef]
144. Boutillier, S.; Fourmentin, S.; Laperche, B. Food additives and the future of health: An analysis of the ongoing controversy on titanium dioxide. *Futures* **2020**, *122*, 102598. [CrossRef]
145. Ju, J.; Chen, X.; Xie, Y.; Yu, H.; Guo, Y.; Cheng, Y.; Qian, H.; Yao, W. Application of essential oil as a sustained release preparation in food packaging. *Trends Food Sci. Technol.* **2019**, *92*, 22–32. [CrossRef]

146. Kathuria, A.; Pauwels, A.-K.; Buntinx, M.; Shin, J.; Harding, T. Inclusion of ethanol in a nano-porous, bio-based metal organic framework. *J. Incl. Phenom. Macrocycl. Chem.* **2019**, *95*, 91–98. [CrossRef]
147. Adeli, M.; Ghobadi, M.; Ghanbarzadeh, B.; Alizadeh, A.; Ghasemi, P. Effect of novel bioactive coating enriched with nanoemulsion of mustard essential oil on the quality of turkey meat. *J. Food Nutr. Res.* **2020**, *59*, 71–80.
148. Aliabbasi, N.; Emam-Djomeh, Z.; Amighi, F. Active food packaging with nano/microencapsulated ingredients. In *Application of Nano/Microencapsulated Ingredients in Food Products*; Academic Press: Cambridge, MA, USA, 2021; pp. 171–210.
149. Wu, M.-H.; Zhu, L.; Zhou, Z.-Z.; Zhang, Y.-Q. Coimmobilization of Naringinases on Silk Fibroin Nanoparticles and Its Application in Food Packaging. *J. Nanopart.* **2013**, *2013*, 901401. [CrossRef]
150. Lozano-Pérez, A.A.; Montalbán, M.G.; Aznar-Cervantes, S.D.; Cragnolini, F.; Cenis, J.L.; Víllora, G. Production of silk fibroin nanoparticles using ionic liquids and high-power ultrasounds. *J. Appl. Polym. Sci.* **2014**, *132*. [CrossRef]
151. Morin-Crini, N.; Fourmentin, S.; Fenyvesi, É.; Lichtfouse, E.; Torri, G.; Fourmentin, M.; Crini, G. 130 years of cyclodextrin discovery for health, food, agriculture, and the industry: A review. *Environ. Chem. Lett.* **2021**, *19*, 2581–2617. [CrossRef]
152. Jing, D.; Wen, L. Active Packaging Film Based On Essential Oil/Beta-cyclodextrin Inclusion Compound and Preparation Method for Active Packaging Film. Patent No. CN102585412A, 18 July 2012.
153. Ge, L.; Li, D.; Mu, C.; Ye, Y. Functional Gelatin Food Packaging Film and Preparation Method. Patent No. CN104693811A, 10 June 2015.
154. Keute, J.; Kuduk, W.; Wood, W. Cyclodextrin Compositions, Articles, and Methods. Patent No. AU2016206334A1, 4 August 2016.
155. Xu, J. Antibacterial Quality-Guarantee Food Packaging Bag and Preparation Method Thereof. Patent No. CN105623067A, 1 June 2016.
156. Zapata Ramirez, P.A.; Yañez Sanchez, M.E. Degradable Packaging Film for Fruit and Vegetables. U.S. Patent Application No WO 2017/106984 Al, 22 December 2017.
157. Li, J. Clove Essential Oil Contained Sterilization Plastic Wrap and Preparation Method Thereof. Patent No. CN106967226A, 21 July 2017.
158. Li, W.; Yang, Y. Method for Preparing Antibacterial Food Packaging Preservation Film by Doping Garlic Oil-Beta Cyclodextrin Inclusion Compound-Clove Oil. Patent No. CN109233161A, 18 January 2019.
159. Xie, Q. Environment-Friendly Food Packaging Plastic and Preparation Method Thereof. Patent No. CN112048102A, 8 December 2020.
160. Bian, X.; Liang, N.; Shen, J.; Sun, Y.; Wang, X. Application of Hydroxypropyl-beta-cyclodextrin in Preparation of Antibacterial Material, Food Package and Preparation Method of Food Package. Patent No. CN111820219A, 27 October 2020.
161. Cui, B.; Fang, Y.; Guo, L.; Yu, B.; Yuan, C.; Zou, Y. Food Packaging Films Containing Natural Antibacterial Component. U.S. Patent US20200337357A1, 29 October 2020.
162. Romano, I. Packaging Material. U.S. Patent US11110694, 7 September 2021.

IV.- METODOLOGÍA

IV.- METODOLOGÍA

La primera fase de esta investigación, consistió en la selección de los agentes antimicrobianos naturales para la encapsulación de los mismos en β-CD, para posteriormente aplicarlos a la matriz polimérica.

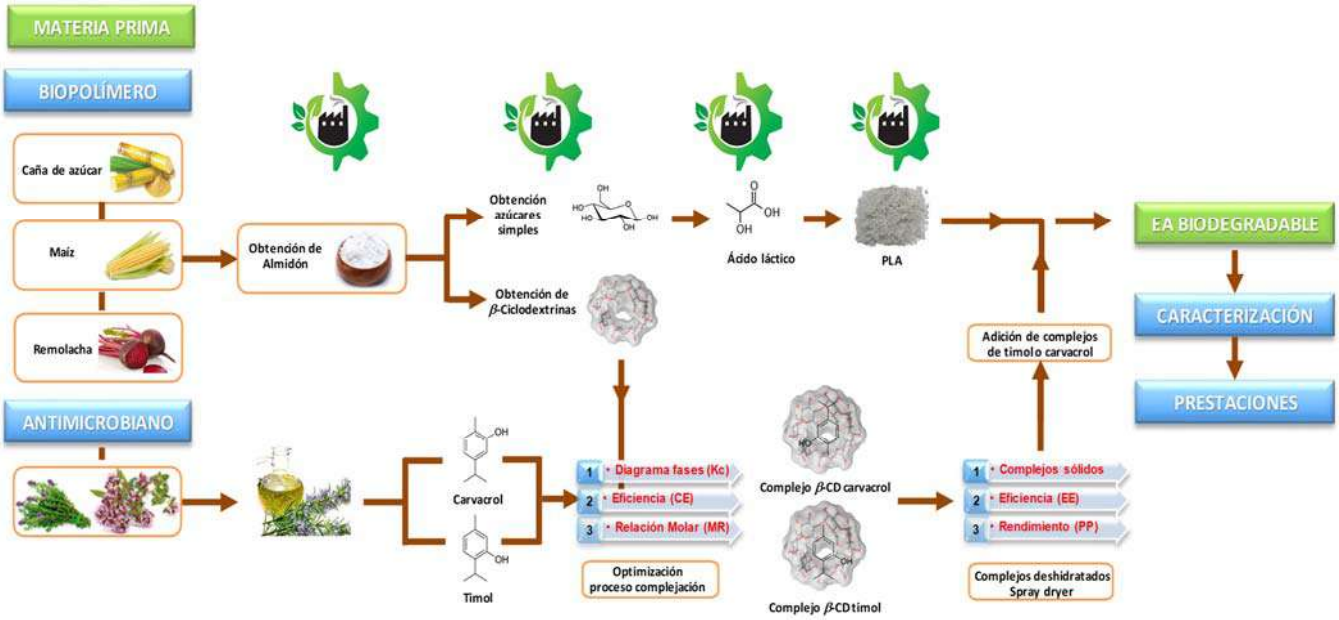


Figura 4.1 Metodología desarrollo y análisis de empaque activo con complejos de inclusión de carvacrol y timol.

Se seleccionaron timol y carvacrol para evaluar sus prestaciones, que son dos monoterpenos presentes de manera natural, en algunas especies vegetales como el orégano y el tomillo. Ambos compuestos poseen actividad antimicrobiana, antifúngica y antioxidante, bastante probada; sin embargo, son sensibles a la luz, poco solubles en agua y con un aroma poco agradable para su aplicación en la industria alimentaria, por lo cual se llevó a cabo su complejación en β-CD por el método de solubilidad, de acuerdo a lo descrito por Rodríguez-López et al., 2019. Para ambos monoterpenos, se determinó la constante de complejación (Kc) utilizando la pendiente y ordenada en el origen de los respectivos diagramas de fases, así como la eficiencia de complejación (CE) y su relación Molar (MR).

Además, para los complejos en estado sólido se determinó la Eficiencia (EE) y rendimiento del proceso (RP), como paso previo a su adición al polímero biodegradable para conformar el envase activo por inyección, utilizando diferentes proporciones de β-CD-timol y β-CD-carvacrol (0,0 %, 1,5 %, 2,5 % y 5,0 % en peso), seleccionando (180–190 °C) como temperatura óptima.

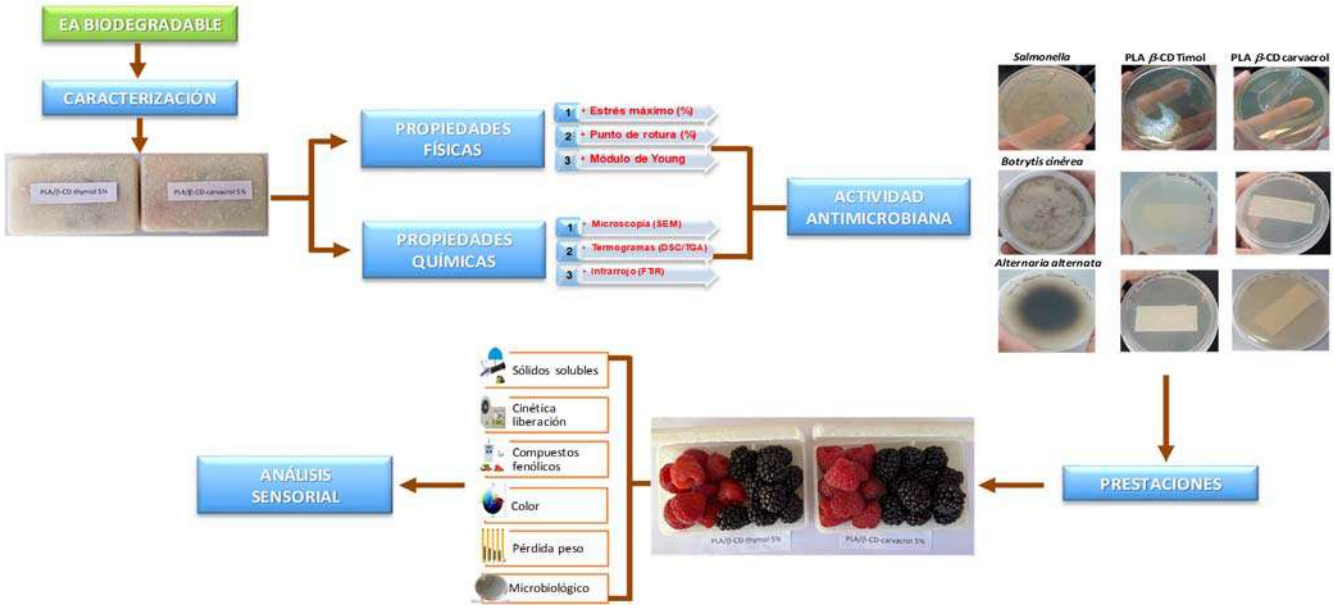


Figura 4.2 Caracterización fisico-química del envase, actividad antimicrobiana *in vitro* y evalaución de las prestaciones del envase activo en condiciones reales atendiendo a parámetros objetivo de calidad, seguridad y aceptación del consumidor.

El nuevo material biodegradable obtenido se sometió a ensayos de tipo mecánico (módulo de Young, estrés máximo y punto de ruptura) y pruebas de caracterización empleando diferentes técnicas instrumentales (espectrometría infrarroja por transformada de Fourier, microscopía electrónica, calorimetría diferencial y análisis termogravimétrico), con el fin de comprobar si la aditivación con los complejos en estado sólido, mejora la propiedades físicas y químicas del envase. Además, se evaluó la actividad antimicrobiana del nuevo material sobre bacterias (*Salmonella typhimurium*, *Salmonella agona* y *Listeria monocytogenes*) y hongos (*Alternaria alternata* y *Botrytis cinérea*), identificados habitualmente en

alimentos, mediante ensayos *in vitro*, como requisito para sustentar una futura aplicación comercial.

Por último, se evaluaron las prestaciones del envase activo en condiciones reales (refrigeración a 4 °C durante 21 días), comparando con un envase comercial (clamshell) y un control (PLA sin aditivar), utilizando moras y frambuesas por tener una vida comercial limitada, con respecto a los parámetros de calidad (peso, color, sólidos solubles, contenido fenólico total), seguridad (carga microbiana) y aceptación del consumidor, objetivo para este tipo de alimentos.

V.- RESULTADOS Y DISCUSIÓN

V.- RESULTADOS Y DISCUSIÓN

5.1 FORMACIÓN DE COMPLEJOS DE INCLUSIÓN

Los valores obtenidos para las constantes de complejación (K_C) fueron: 1592,27 M^{-1} para timol y 1423,50 M^{-1} para carvacrol, obteniendo en ambos casos complejos 1:1 y valores de constante más altos cuando la concentración de β-ciclodextrina fue 11 mM.

Los diagramas de fases para carvacrol y timol complejados con β-ciclodextrinas muestran una tendencia lineal, ya que conforme aumenta la concentración de ciclodextrina también aumenta la concentración del monoterpeno, indicando que la estequiometría de los complejos de inclusión formados es 1:1. En ambos casos se observó un descenso en la concentración de monoterpeno, cuando la concentracion de β-ciclodextrina fue 13 mM. De acuerdo a los resultados obtenidos, se decidió utilizar una concentración de β-ciclodextrina 11 Mm para el secado de los complejos por spray-dryer, ya que a esta concentración se obtuvieron los mayores valores de constante de complejación (Kc).

Como se observa en la Tabla 5.1, los complejos β-CD-timol y β-CD-carvacrol muestran la misma relación molar radio (1:2), lo que indica que casi una de cada dos moléculas de β-CD en solución está formando complejos solubles con carvacrol o timol. Sin embargo, la eficiencia de complejación obtenida para carvacrol (105,6 %), es significativamente mayor que la obtenida para timol (69,3 %).

Tabla 5.1. Carvacrol y Timol, Solubilidad acuosa (S_0), constante de complejación (Kc) con β-CDs, eficiencia de complejación (CE) y relación molar (MR) a pH de 7,0.

Complejo	S_0 (mmol L^{-1})	K_C (L mol^{-1})	CE (%)	MR
β-CD-carvacrol	5,77 ± 0,15*	1198 ± 115	69,3 ±9,2	1:2
β-CD-timol	5,64 ± 0,12	1871 ± 143	105,6 ±10,3	1:2

SD: Desviación estándar de las mediciones por triplicado

Se determinaron los parámetros de eficiencia de encapsulación (EE) y rendimiento del proceso (PP) (Tabla 5.2) para los complejos en estado sólido, obteniendo valores similares para ambos parámetros, aunque significativamente superiores para el timol.

Los valores de eficiencia de encapsulación para carvacrol (45 ± 2,5 %) y timol (47 ± 1,8 %), fueron similares a los descritos en la bibliografía por otros autores (Anaya-Castro et al., 2017).

Tabla 5.2. Eficiencia de encapsulación (EE) y desempeño del proceso (PP) de los complejos β-CD-carvacrol y *β*-CD-timol en estado sólido.

Monoterpeno	EE (%)	PP (%)
Carvacrol	45 ± 2,5*	84 ± 3,2
Timol	47 ± 1,8	86 ± 3,7

SD: Desviación estándar de las mediciones por triplicado

Para corroborar la inclusión de los respectivos monoterpenos en la cavidad interna de β-ciclodextrina, los reactantes (β-ciclodextrina, timol y carvacrol) y los respectivos productos (complejos formados), se evaluaron por espectroscopía infrarroja por transformada de fourier FT-IR.

Como se puede observar en la Figura 3A del Artículo 1, el espectro IR del timol (isómero estructural del carvacrol), muestra varios picos característicos a 3164 cm^{-1}; vibraciones de estiramiento y flexión de OH de 3164 cm^{-1} y 1453 cm^{-1}, respectivamente; bandas de estiramiento simétricas y asimétricas C-H a 2858 cm^{-1} y 2897 cm^{-1}, respectivamente; y tres vibraciones de estiramiento C=C de intensidad débil a 1624 cm^{-1}, 1592 cm^{-1} y 1506 cm^{-1}, revelando la tri-sustitución del anillo aromático. Con respecto a los sustituyentes del anillo aromático, aparece metilo al 1344 cm^{-1}. También se observó una señal doble típica a 1410 cm^{-1}, característica del grupo isopropilo. Además, el espectro IR de *β*-CD (Articulo 1, Figura 3B) revela vibraciones C–O–C simétricas y asimétricas en 890 cm^{-1} y 1170 cm^{-1} y 1021 cm^{-1}; respectivamente.

En relación a la *β*-ciclodextrina libre, en los espectros de los complejos de inclusión β-CD-carvacrol y *β*-CD-timol (Articulo 1, Figura 3B), se destacó la presencia de picos de característicos C=C correspondientes a carvacrol y timol de anillo aromático cercano a 1590 cm^{-1} y vibración de sus respectivos grupos metilo aparecen a 1430 cm^{-1} (asimétrico) y 1360 cm^{-1} (simétrico). Estos cambios en relación con los de los respectivos compuestos libres, proporcionan una clara evidencia de las interacciones huésped-huésped.

5.2 OBTENCIÓN DE LOS ENVASES ACTIVOS

La segunda fase del estudio consistió en la obtención de los empaques, para lo que se evaluaron dos tipos de procesos, extrusión e inyección, y dos biopolímeros, policaprolactona (PCL) y ácido poli láctico (PLA).

El primero se descartó debido a que los bajos pesos moleculares de la PCL, no permitieron el procesamiento a temperaturas de extrusión y de inyección, ya que con la fibra obtenida con PCL, fue imposible formar el molde para empaque, decidiendo trabajar solo con la PLA. Así, se formaron envases utilizando una matriz polimérica de PLA, aditivada con complejos sólidos de β-CD-Timol y β-CD-Carvacrol, a diferentes concentraciones (0; 1,5; 2,5 y 5,0 % en peso), respectivamente.

5.2.1 Obtención de envases por extrusión

Los filamentos que se obtienen a la salida de la extrusora son muy poco manejables, en la etapa posterior de enfriamiento y solidificación no se conseguió un diámetro homogéneo. Éstos se vuelven quebradizos y no permiten una manipulación adecuada en la impresora 3D. Así, el proceso de extrusión no es adecuado para obtener envases de PLA aditivados con los complejos de β-ciclodextrina con timol y carvacrol.

5.2.2 Obtención de envases por iinyección

En este caso, fue posible la formación de envases por inyección. Para ello, se hizo una mastermix que contenía los pellets de PLA y los respectivos complejos (β-CD-Timol o β-CD-Carvacrol), a diferentes concentraciones (0; 1,5; 2,5 y 5,0 % en peso). Inicialmente se optimizó el rango de temperatura de la inyectora (175-180 °C), para conseguir la fluidez adecuada de la mastermix (PLA/complejo), hasta llegar a formar el empaque.

El proceso de desmoldeado de los empaques obtenidos se llevó a cabo cuando el material alcanzó la temperatura de enfriamiento entre 20 – 25 ° C.

Los resultados de las pruebas mecánicas del empaque activo desarrollado se muestran en las Tablas 5.3 y 5.4:

Tabla 5.3 Propiedades mecánicas de los empaques aditivados β-CD-thymol (1,5%, 2,5%, 50%) y PLA (Control 0%)

Empaques de PLA con diferentes % β-CD-timol				
Parámetro	**0%**	**1,50%**	**2,50%**	**5,0%**
Módulo de Young (Mpa)	2873 ± 176	2667 ± 161	2382 ± 69*	2394 ± 118*
Esfuerzo máximo (MPa)	63,6 ± 4,5	57,9 ± 6,8	53,2 ± 2,3	55,1 ± 5,2
Punto de ruptura (%)	2,4 ± 0,4	2,8 ± 0,3	2,9 ± 0,2	3,1 ± 0,3*

Resultados expresados en (media ± desviación estándar) de diez determinaciones.
**: Diferencias significativas (p <0.05) según la prueba de Tukey.*

Tabla 5.4. Propiedades mecánicas de los empaques aditivados β-CD-carvacrol (1,5%, 2,5%, 5,0%) y PLA (Control 0%)

Empaques de PLA con diferentes % β-CD-carvacrol				
Parámetro	**0%**	**1,50%**	**2,50%**	**5%**
Módulo de Young (Mpa)	2873 ± 176	2327 ± 170	2259 ± 53*	1960 ± 110*
Esfuerzo máximo (MPa)	63,6 ± 4,5	49,9 ± 6,5	51,3 ± 4,9	47,5 ± 5,
Punto de ruptura (%)	2,4 ± 0,4	2,7 ± 0,3*	2,9 ± 0,2*	3,2 ± 0,4*

Resultados expresados en (media ± desviación estándar) de diez determinaciones.
**: Diferencias significativas (p <0.05) según la prueba de Tukey.*

Como se puede observar en las Tablas 5.3 y 5.4, el módulo de Young tuvo una variación de 2873 a 1960 Mpa para los complejos β-CD-carvacrol, y de 2873 a 2394 Mpa para los complejos β-CD-timol, mostrando valores más bajos en comparación con el empaque control (PLA sin aditivar).

De hecho, el módulo de Young disminuye gradualmente a medida que aumenta la concentración de los complejos deshidratados, obteniendo el valor más bajo del módulo de Young en la muestra aditivada con 5,0 % de carvacrol, con una diferencia significativa respecto al valor medio (p <0,05).

La misma tendencia se advirtió al evaluar el esfuerzo máximo, observándose el valor más bajo en el paquete de PLA con complejos de β-CD-carvacrol (5,0 %),

14 % menor que el valor obtenido para PLA adicionado con complejos de timol en la misma concentración.

Estos valores obtenidos en las pruebas mecánicas, cuando ambos complejos se agregaron al polímero de PLA, podrían deberse al incremento en el valor de eficiencia de complejación (CE) obtenido para carvacrol-β-CD (105,6 %) y 65 % superior al valor obtenido para timol-β-CD (69,3 %), revelando que valores de CE superiores al 100 % indican que a pH 7,0 hay más β-CD formando complejos de inclusión de carvacrol que libres en solución. En el caso del timol, el número de β-CD que se compleja es menor, ya que la CE es inferior al 100% y, en consecuencia, la disminución de valores en las propiedades mecánicas es menos pronunciada.

En cuanto al punto de rotura, se observó un incremento significativo de este parámetro a medida que aumenta la concentración (% en peso) de los complejos deshidratados, mejorando en un 25 % y un 23 % la capacidad de elongación de la matriz polimérica (control), cuando se adiciona el 5,0 % de β-CD-carvacrol o el 5,0 % de β-CD-timol. Este comportamiento puede atribuirse a un efecto plastificante provocado por la adición de complejos de β-CD a la matriz polimérica alterando la estructura cristalina del PLA y aumentando sus propiedades dúctiles (Ramos et al., 2012).

La caracterización estructural de los empaques por SEM, muestra una disminución en la cantidad de cortes y pliegues del material polimérico, los cuales fueron directamente proporcionales a la concentración del complejo adicionando, como se muestra en el Articulo 1, Figura 4.

La muestra de PLA control tiene una superficie irregular, mientras que las muestras de PLA enriquecidas con los complejos β -CD-carvacrol y β -CD-timol revelan una superficie más uniforme a medida que aumenta la concentración del complejo. En este sentido, la disminución en el número de cortes y pliegues del material polimérico fue directamente proporcional a la concentración de complejo agregado.

Estos resultados concuerdan con los valores reportados en los ensayos mecánicos (Articulo 1, Tablas 3 y 4), evidenciando que el aumento de la concentración de complejos en la formulación del material plástico favorece la obtención de envases más flexibles (disminución del módulo de Young), proporcionando la formación de una superficie más lisa y continua.

Este hecho podría deberse a que la encapsulación ayuda a incorporar los compuestos activos (carvacrol o timol) a la matriz polimérica, ya que en la literatura se han descrito diferentes resultados cuando se añadieron aceites esenciales sin encapsular a materiales poliméricos, obteniendo estructuras heterogéneas con gotitas de aceite atrapadas en el polímero (Liu et al., 2016; López-Rubio y Lagaron, 2010).

Al objeto de investigar las transiciones térmicas de los empaques, se realizaron mediciones de Calorimetría Diferencial de Barrido (DSC, por sus siglas en inglés). Como se puede observar en la Tabla 5.5, los empaques que contienen complejos β-CD-carvacrol o β-CD-timol mostraron propiedades térmicas similares, con independencia de la concentración. La temperatura de transición vítrea (Tg) de los materiales enriquecidos con PLA fue análoga a la obtenida para el control de PLA, y similar a los valores de Tg descritos en la literatura por Kumari et al. (2012), lo que indica que la fase amorfa del PLA no sufre ningún cambio.

Tabla 5.5. Valores paramétricos de DSC obtenidos a partir de PLA puro y β -CD-carvacrol o β -CD-timol al 1,5 %, 2,5 % y 5,0 %, (% en peso).

Parámetro	**PLA**	**PLA-timol-β-CDs**			**PLA-carvacrol-β-CDs**		
	100%	**1.5%**	**2.5%**	**5%**	**1.5%**	**2.5%**	**5%**
T_g (°C)	59	61	59	61	60	60	60
T_c (°C)	102,7	103,8	105,4	105,0	106,5	107,7	105,9
T_m (°C)	168,5	168,5	168,5	168,8	167,9	168,8	169,1
ΔHc Energía (J/g)	36,09	33,08	30,61	29,03	36,42	32,09	36,83
ΔHm Energía (J/g)	45,63	44,09	37,44	35,65	44,72	37,54	42,95

Tg: temperatura de transición vítrea; Tc: temperatura de transición de cristalización, Tm: temperatura de transición de fusión.

Por otro lado, los empaques con aditivos muestran una variación significativa en la temperatura de cristalización, con respecto al empaque de control (Tc = 102,7 °C; PLA 0%), aumentando hasta 3 °C a 5 °C para concentraciones de 2,5 % en peso de β-CD-timol y β -CD-carvacrol, respectivamente, modificando el comportamiento de cristalización del PLA y, en consecuencia, la formación de la estructura ordenada de la matriz polimérica.

Como puede observarse en la Figura 5 del Artículo 1, se evidencia un pico endotérmico para todas las muestras a una temperatura de fusión, Tm cercano a 168,5 °C, con ligeras variaciones de temperatura (inferiores a 1 °C), para el PLA que contiene complejos β -CD-carvacrol. Las pequeñas variaciones de las temperaturas

de cristalización y fusión observadas al aumentar el contenido de β-CD-carvacrol y β-CD-timol, podrían deberse al aumento en la movilidad y ocupación de la cadena de la matriz polimérica.

Al realizar el análisis termogravimétrico (TGA) se observó una leve disminución en la temperatura de degradación en los empaques aditivados con los complejos. En consecuencia, se deduce que los complejos β-CD-carvacrol o β-CD-timol actúan como plastificantes, disminuyendo las fuerzas intermoleculares de las cadenas poliméricas, mejorando así el punto de rotura.

El TGA demostró una pérdida de masa significativa entre 320 °C y 390 °C, lo cual coincide con la descomposición del PLA a 500 °C. Las curvas de análisis térmico se ralentizan, para terminar con el paso de descomposición hasta lograr una masa constante. Además, el PLA puro tiene una estabilidad ligeramente superior (Articulo 1, Figura 6), en comparación con el PLA-β-CD-carvacrol y el PLA-β-CD-timol, disminuyendo la estabilidad térmica de la matriz polimérica con el aumento de la concentración. Estos resultados indican que, todas las muestras de polímero son, en esencia, térmicamente estables por debajo de 300 °C, puesto que, a la misma temperatura, las mezclas que contienen β-CD-carvacrol y β-CD-timol, tienen una pérdida de peso más rápida que el PLA puro.

En la práctica, los envases a base de PLA utilizados en la industria alimentaria estarán a temperatura ambiente o inferior (refrigerados), por lo tanto, la estabilidad térmica de los materiales PLA β-CD-carvacrol y PLA β-CD-timol desarrollados, no se verá comprometida.

Los resultados de los análisis microbiológicos revelan que los empaques aditivados con de PLA-β-CD-carvacrol al 5,0 %, son los únicos que presentan inhibición para *Salmonella typhimurium*. Sin embargo, no mostraron efecto inhibitorio frente a *Listeria monocytogenes*.

Con respecto a los hongos, se observa inhibición de *Alternaria alternata* Articulo 1, Figura 7 en los empaques de PLA-β-CD-carvacrol al 2,5 y 5,0 %, tras 10 días de incubación. También mostraron efecto inhibitorio frente a *Botritis cinérea* durante el mismo tiempo de incubación (Figura 5.1).

La incorporación de los encapsulados de carvacrol y timol a la matriz polimérica de PLA, permitió protegerlos de la temperatura de inyección, favoreciendo la actividad frente al crecimiento de microorganismos patógenos. Como han descrito otros autores (Laird y Phillips, 2012), el efecto inhibitorio de

estos activos en fase vapor puede atribuirse a la acumulación de sustancias volátiles en el micelio, seguido de la interacción con la porción hidrófoba de la membrana celular.

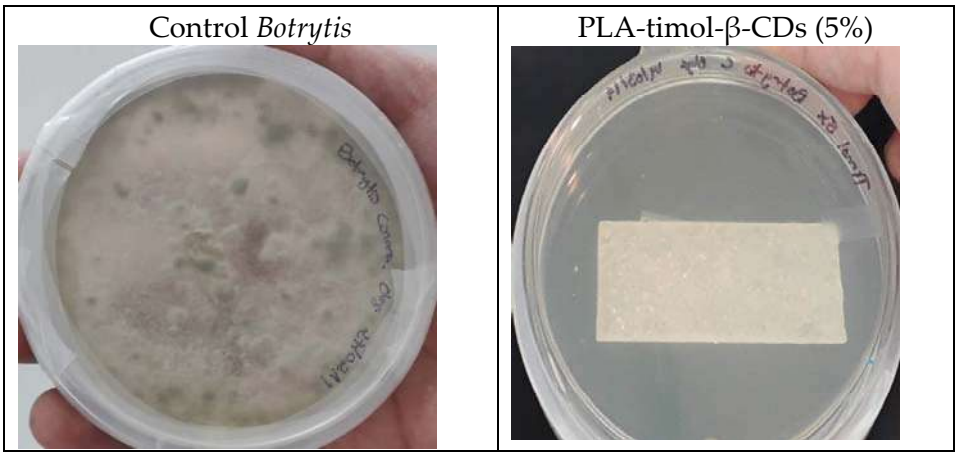


Figura 5.1: Resultados del ensayo de difusión en fase vapor (diez días de incubación) de PLA-timol-β-CDs (5%), frente a *Botrytis cinérea*.

Otras investigaciones relacionan el efecto inhibidor de los principios activos de los aceites esenciales, como son carvacrol y timol, con cambios en la morfología de las hifas, debido a la penetración de compuestos activos en la membrana plasmática (Soylu et al., 2010).

La actividad antimicrobiana de ambos compuestos se conservó después del proceso de inclusión y el de inyección, debido a la encapsulación con β-CD. Trabajos previos con procesos similares de encapsulación como el secado por aspersión (Arana-Sánchez et al., 2010) secado por congelación (Santos et al., 2015) y liofilización (Wang et al., 2011), también han demostrado que la encapsulación contribuye a conservar las propiedades antimicrobianas y antioxidantes, principalmente de los aceites esenciales, e incluso presentan ventajas ya que al formar complejos de inclusión, mejoran la solubilidad en agua y reducen la cantidad necesaria ya que se limitan las mermas por volatilización. De hecho, carvacrol y timol son compuestos volátiles y, por lo tanto, podrían ser altamente efectivos en el espacio de cabeza del empaque activo, reduciendo así la proliferación microbiana (Santos et al., 2015).

5.3 EVALUACIÓN *IN VIVO,* DE LOS EMPAQUES DE PLA CON ENCAPSULADOS DE TIMOL Y CARVACROL EN β-CICLODEXTRINAS

Para cumplir con otro de los objetivos de este trabajo de investigación se evaluaron las prestaciones del envase sobre diferentes parámetros objetivo de calidad en frutos sensibles al deterioro; haciendo especial hincapié en los valores microbiológicos y características organolépticas de frambuesas y moras, envasadas en condiciones de refrigeración, comparando con un envase comercial y un envase de PLA sin aditivar.

5.3.1 Pérdida de peso de las zarzamoras y frambuesas

Por lo general, la pérdida de humedad postcosecha altera la apariencia, el sabor y la textura de la fruta, y reduce su peso comercial. Dado que las frambuesas y las moras son propensas a la deshidratación debido a la falta de cera epicuticular, una pérdida máxima de humedad del 6% se considera comercialmente aceptable (Paniagua et al., 2013). La pérdida de peso en las frambuesas fue significativamente mayor (p <0,05) que en las moras (Artículo 2, Figuras 3A y 3B). Este hecho podría deberse al transporte masivo y difusión de vapor de agua, que ocurre durante los procesos fisiológicos; ya que las frambuesas tienen una mayor tasa de respiración (actividad metabólica 49 mg CO_2 kg^{-1} h^{-1} a 10 °C) que las moras (actividad metabólica 31 mg CO_2 kg^{-1} h^{-1} a 10 °C) (Robbins et al., 1989).

Se observó una pérdida de peso mayor en las bayas almacenadas a 4 °C en el empaque control (clamshell) al final del estudio (p <0,05), un 9 % para moras y un 11 % para frambuesas, ambas superiores al valor considerado comercialmente aceptable (6 %). Este comportamiento se atribuyó a una pérdida excesiva de agua por la materia seca, y no debemos sorprendernos, ya que, en el mercado de productos frescos, las bayas se envasan comúnmente en clamshells con ratios de apertura entre 3 % y 10 %, evitando el etileno, el calor, y acumulación de humedad. Sin embargo, las rejillas de ventilación podrían hacer que las bayas, con una relación de superficie amplia, sean más susceptibles a daños por congelación, enfriamiento y secado (Pathare et al., 2012). Pérdidas de humedad similares, o valores aún más altos que son directamente proporcionales al diámetro y el número

de relaciones de apertura de los empaques tipo clamshell, se han descrito en la literatura por Van Der Steen et al. (2002).

Sin embargo, el empaque activo desarrollado cuenta con una tapa cerrada y baja permeabilidad al vapor de agua entre las frutas y el aire circundante, por lo que retiene una humedad relativa dentro del envase. Por tanto, la pérdida de peso observado es incluso un 7 % menor en las bayas dentro del envase control (PLA/C).

Teniendo en cuenta el valor de pérdida de humedad comercialmente aceptable descrito anteriormente para las bayas, todos los envases activos de PLA que contienen complejos monoterpeno-ciclodextrina mostraron una tendencia de pérdida de peso más lenta, alcanzando, en el peor de los casos, una disminución del 3 % al final del ensayo.

El empaque de PLA β-CD-timol con un 5,0% en peso, mostró la menor pérdida de peso para moras y frambuesas, con una diferencia significativa (p <0,05) en comparación con PLA/C control y las del envase comercial clamshell, almacenadas en condiciones de refrigeración a 4 °C después de 21 días de ensayo.

5.3.2 Color

El desarrollo del color rojo en el caso de las frambuesas y púrpura azulado de las moras se debe principalmente a las antocianinas y, durante la maduración postcosecha de la fruta, producen numerosos cambios en los pigmentos que afectan al color.

Como se puede observar en la Tabla 1 del Artículo 2, los cambios de color fueron significativos tanto en moras como en frambuesas almacenadas en refrigeración a 4 °C, variando de acuerdo al tipo de envasado y tiempo de almacenamiento. El resultado más favorable se obtuvo en las frambuesas empacadas en PLA β-CD-timol (2,5 o 5,0 % en peso) mostraron menos pérdida de color rojo (a*). Esto podría deberse al efecto protector y antioxidante de una liberación sostenida y controlada de timol encapsulado dentro del paquete de PLA, como se ha descrito previamente para las uvas de mesa almacenadas durante 56 días en un empaque de atmósfera modificada conteniendo timol (Valero et al., 2006). Respecto a la disminución del parámetro L* durante el almacenamiento, podría atribuirse a la oxidación de compuestos fenólicos y otros fenómenos fisicoquímicos (Pathare et al., 2012), como la formación de complejos entre

antocianinas y quinonas, generados a través de la oxidación de polifenoles y la pérdida de peso, lo cual ocurre durante el almacenamiento.

5.3.3 Sólidos solubles y Contenido fenólico total

El contenido de sólidos solubles (SSC) en todos los grupos evaluados aumentó durante el almacenamiento, mostrando valores que oscilaron entre 9,73 ± 0,38 y 11,01 ± 0,17 °Brix para PLA/β-CD-carvacrol 5,0% mientras que para el PLA/β-CD-timol 2,5 % disminuyó el contenido a 8,10 ± 0,10 °Brix en el día 14. En este sentido, Forney et al., (2012) describieron cambios de composición similares, con un aumento en la concentración de aproximadamente 2,2 a 3,2 %, a medida que avanza la maduración de las bayas. Por otro lado, se observó un cambio en la tendencia el día 21 de almacenamiento, mostrando una ligera disminución de los SSC, lo que demuestra que al final del estudio, todos los tratamientos de empaques activos de PLA mantuvieron el contenido de SSC en las bayas, en comparación con el empaque comercial y el control.

Los resultados muestran que todos los envases activos de PLA, y específicamente PLA/β-CD-carvacrol al 5,0 %, mantuvieron una pérdida sostenida de SSC durante 21 días de almacenamiento, e igualmente se observó que los envases activos de PLA que contienen encapsulados de carvacrol o timol, ralentizan eficazmente el metabolismo de las bayas.

Respecto al contenido fenólico total (CFT), disminuyeron gradualmente en todos los grupos con el tiempo de almacenamiento (Artículo 2, Tabla 3), los empaques de PLA que contienen encapsulados de carvacrol y timol mostraron una disminución menos pronunciada, en comparación con los envases control.

De hecho, tanto el envase comercial (clamshell), como el control sin aditivar (PLA/C), mostraron la mayor disminución de CFT. Para las moras, con un valor inicial de CFT de 101,01 ± 6,66 mg de ácido gálico/100 g de peso, disminuyó hasta 58,07 ± 0,76 mg de ácido gálico/100 g de peso en el día 21 en el empaque clamshell comercial, lo que representa una pérdida de CFT del 43 % con respecto a su valor inicial; en comparación con el empaque de PLA/β-CD-carvacrol 5,0 % se obtuvo un valor 83,09 ± 3,40 mg de ácido gálico/100 g de peso el día 21 de almacenamiento, con tan solo una pérdida del 17,74 % de CFT, lo que demuestra una disminución más lenta que el observado en el grupo control.

En cuanto al valor inicial de CFT para frambuesas (119,10 ± 3,09 mg ácido gálico/100 g peso), los mejores resultados al final del estudio se obtuvieron nuevamente para el envase PLA/β-CD-carvacrol 5,0 % (116,47 ± 2,58 mg gálico ácido/100 g peso), registrando una pérdida del 2,21 %, muy inferior a la determinada para la clamshell comercial (34 % TPC pérdida), lo cual representa la mayor disminución. Este lento descenso se atribuyó a la presencia, dentro de los envases de PLA, de carvacrol o timol en estado libre, que ejercen un marcado efecto antioxidante, protegiendo los compuestos fenólicos presentes en las bayas, disminuyendo la oxidación debida a su estructura aromática y sistema de electrones deslocalizados, resultados que concuerdan con los descritos previamente por (Ramos et al., 2013).

5.3.4 Análisis de espacio de cabeza del empaque activo

Se analizó el espacio de cabeza del empaque activo para determinar la presencia de compuestos volátiles (carvacrol y timol en estado libre), mediante cromatografía de gases-espectrometría de masas (HS-GC-MS). En cada muestreo, se introdujo la jeringa por la correspondiente apertura del empaque, extrayendo 1 mL de gas, que se introdujo inmediatamente en el inyector (splitless) a 250 °C, obteniendo los resultados mostrados (Articulo 2, Figura 4), correspondientes a tres réplicas, para cada grupo de empaques objeto de estudio.

En la etapa inicial, el empaque de PLA/β-CD-timol al 5,0 % mostró una concentración de volátiles, un 75 % más elevada que la determinada para el empaque de PLA/β-CD-carvacrol al 5,0 % y PLA/β CD-timol al 2,5 %. Como el tiempo de almacenamiento se prolongó hasta 21 días, solo el empaque de PLA/β-CD-timol 5,0% mantuvo una liberación controlada de monoterpeno (30% del nivel inicial en el día 7), disminuyendo hasta el final del ensayo, mientras que, para el resto de los empaques activos de PLA, los niveles de monoterpeno comenzaron a disminuir mucho más rápidamente, alcanzando la línea de base sin que se detecte concentración, de ninguno de ellos, en la primera semana de estudio. Un comportamiento similar fue descrito por (Higueras et al., 2014), utilizando películas de quitosano que contienen complejos carvacrol ciclodextrina, evidenciando una rápida liberación de carvacrol después los primeros tres días de almacenamiento,

cuya concentración se mantuvo en el tiempo, ya que los envases se sellaron herméticamente, proporcionando así una barrera al carvacrol.

Sin embargo, en nuestro caso, los empaques no estaban sellados herméticamente, y los gases podían ser liberados al exterior, o bien, una proporción podía ser absorbida por los frutos sin alterar sus propiedades organolépticas, tal y como describen (Higueras et al., 2014), quienes argumentaron que la gran cantidad de antimicrobiano absorbido o que reaccionó con la muestra, provocó un deterioro sensorial inaceptable. Este mecanismo de liberación, observado para carvacrol y timol en el empaque de PLA, modificó la composición de la atmósfera inicial en el interior, mejorando la calidad y la vida útil postcosecha de las moras y frambuesas almacenadas a temperatura controlada de refrigeración.

5.3.5 Análisis microbiológico de las bayas

Se analizaron las moras y frambuesas al inicio del estudio, a los 7, 14, 21 días de almacenamiento en refrigeración en los diferentes empaques, realizando un recuento de microorganismos indicadores: aerobios mesófilos (AMB), coliformes totales (TC), hongos y levaduras (YM), los cuales permiten estudiar el grado de deterioro de los alimentos durante un periodo determinad.

Se observó un aumento sostenido en el recuento de AMB durante las dos primeras semanas para las muestras de moras, independientemente del empaque, mostrando un aumento significativo en los empaques de PLA/C y clamshell obteniendo un valor superior a una unidad logarítmica de unidades formadoras de colonias (1 log *ufc*), al obtenido por los empaques de PLA aditivados con los encapsulados de carvacrol y timol.

Respecto a los resultados de AMB en frambuesa, se observó un marcado aumento para las muestras de PLA/C y clamshell entre la primera y la segunda semana de muestreo, con un aumento de hasta 2 log *ufc*. El crecimiento de AMB en los empaques activos de PLA/β-CD-carvacrol 5,0 %, PLA/β-CD-timol 5,0 %, o PLA/β-CD timol 2,5 % no fue significativo, y al final del estudio se observó que el incremento fue 1,5 log ufc menor, que los valores obtenidos para ambas muestras control.

El crecimiento de coliformes para las moras mostró un aumento lento (cerca de 0,8 unidades logarítmicas) para todos los materiales de empaque de PLA con

encapsulados a los siete días de almacenamiento; mientras que a los 14 días de almacenamiento se obtuvo 1 unidad logarítmica menor en los empaques activos, con respecto a los empaques control.

En general, se observó un descenso a lo largo de la primera semana de ensayo en el recuento de coliformes totales en frambuesas, obteniendo mejores resultados de inhibición para coliformes en los empaques de PLA/β-CD-timol 5,0 % y PLA/β-CD-timol 2,5 %, tanto a los 14 como a los 21 días de ensayo, siendo aproximadamente 0,5 unidades logarítmicas inferior, en los envases de PLA que contienen los encapsulados de carvacrol a los mismos porcentajes.

Respecto al crecimiento de levaduras y mohos, éste se detuvo en frambuesas tras la primera semana de ensayo, en todos los envases de PLA que contienen encapsulados de carvacrol o timol. Para las moras, la curva de crecimiento fue baja inicialmente, para posteriormente aumentar de forma gradual hasta el día 14 y, finalmente, mantenerse constante hasta el final del estudio. Se alcanzó una actividad antifúngica ligeramente mejor para empaques de PLA/β-CD-timol al 5,0 % y PLA/β-CD-timol al 2,5 % que para los empaques de PLA que contienen encapsulados de carvacrol, en los mismos porcentajes, y 4,0 unidades logarítmicas más bajas en comparación con las muestras de PLA/C y clamshell, siendo las bayas todavía aptas para el consumo. Estos resultados concuerdan con los descritos previamente para *Alternaria alternata*, logrando una inhibición completa con envases de PLA que contienen 2,5 % y 5,0 % de β-CD-carvacrol o 5,0 % β-CD-timol tras 10 días de incubación (Velázquez et al., 2019), y los obtenidos contra levaduras y mohos en películas de PLA que contienen 5,0 % y 10 % de aceite esencial de orégano, en ensaladas listas para consumir, permitiendo la extensión de su vida comercial (Llana-Ruiz-Cabello et al., 2016).

Se ha descrito en la literatura que el timol presenta un efecto bacteriostático sobre una amplia gama de bacterias y hongos, ya que este compuesto interactúa con los lípidos de la membrana celular, provocando la permeabilización y salida de iones, y, por tanto, la muerte del microorganismo (Rodríguez-López et al., 2020). Respecto a carvacrol, éste presenta una concentración mínima inhibitoria más baja que otros fitoquímicos, para diferentes cepas bacterianas; además, su actividad sinérgica con antibióticos ha sido descrita previamente por (Magi et al., 2015). Este compuesto presenta un efecto bacteriostático sobre los microorganismos, con un mecanismo similar al ejercido por timol.

Las propiedades quelantes del hierro (II) de ambos compuestos también ha sido descrita con anterioridad (Leyva-López et al., 2017; Marchese et al., 2016). Esta actividad quelante, tiene importancia en las reacciones de oxidación de lípidos, lo que podría explicar la inhibición microbiana observada en este estudio. Además, la actividad mostrada para ambas bayas podría estar relacionada con las cantidades de compuestos fenólicos determinadas en este estudio.

5.3.6 Atributos sensoriales

El sabor es uno de los atributos sensoriales que más aprecian los consumidores en las bayas. Como se puede observar en la Figura 6, Artículo 2, después de 21 días de almacenamiento a 4 °C, ambas frutas revelan una disminución en la textura a medida que aumentan los días de almacenamiento.

Las puntuaciones de sabor más altas obtenidas al final del estudio fueron para las muestras de bayas envasadas en los empaques activos de PLA que contenían los encapsulados de timol y carvacrol, los jueces entrenados no percibieron notas adversas de aroma o sabor asociadas a timol o carvacrol.

El valor de aceptación más alto (4,25 grados de aceptación a los 21 días) lo obtuvo el empaque de PLA con encapsulado de β-CD-carvacrol al 5 % en peso, siendo el mismo valor que obtuvo el empaque control PLA/C al inicio del estudio.

Además, las bayas envasadas con ambos encapsulados mostraron el mayor grado de aceptación del color, con valores ligeramente menores para los envases que contienen timol. Por lo tanto, los empaques que obtuvieron los valores de puntuación más alto, después de 21 días de almacenamiento a 4 °C, para los parámetros de sabor, olor, color y textura fueron: PLA/β-CD-timol 5,0 %, PLA/β-CD-timol 2,5 % y PLA/ β-CD-carvacrol 5,0 %. Estos resultados están de acuerdo con los descritos previamente en la literatura por (Viacava et al., 2018), ya que el timol y el carvacrol retrasaron los procesos fisiológicos de la fruta y redujeron la pérdida de calidad.

5.4 USO DE CICLODEXTRINAS (CD) EN TECNOLOGÍAS DE ENVASADO

El uso de ciclodextrinas (CD) en tecnologías de empaque ayuda a que las moléculas volátiles o bioactivas mejoren su solubilidad, para garantizar la

distribución homogénea de las moléculas complejadas, protegiéndolas de la volatilización, oxidación y fluctuaciones de temperatura, cuando se asocian a matrices poliméricas.

Así, en el tercer artículo, se ha hecho una revisión crítica, acerca de las ventajas de la utilización de ciclodextrinas y sus complejos de inclusión en el desarrollo de envases alimentarios innovadores, no tóxicos, biodegradables, sostenibles y económicamente viables.

En primer lugar, los complejos mejoran la compatibilidad, miscibilidad y sinergia entre el polímero del empaque y la molécula activa.

En segundo lugar, se protege a la molécula activa de la degradación, la volatilización y las interacciones no deseadas con los materiales de envasado.

En tercer lugar, actúa como vehículo para conferir al envase propiedades antimicrobiana/antioxidante y de bioactividad. Para la mayoría de las moléculas activas, la miscibilidad es crucial, ya que gran parte de estas moléculas son hidrofóbicas y casi insolubles en agua o disolventes orgánicos de amplio uso. Esta propiedad es especialmente importante para aquellas moléculas bioactivas naturales o sintéticas, destinadas a conferir las características antimicrobianas o antioxidantes a las aplicaciones de envasado de alimentos activos.

En cuarto lugar, permite modular la liberación de moléculas activas para prolongar la vida útil y reducir los cambios en las propiedades sensoriales de los alimentos. Por su parte, los complejos de ciclodextrina son estables a altas temperaturas, ya que su degradación térmica acurre a ~280 °C, por lo cual protegen a las moléculas activas de la degradación y o mermas a consecuencia de las altas temperaturas requeridas en los métodos de producción de los envases alimentarios. Otra ventaja es su biodegradabilidad, lo que permite el desarrollo de nuevos materiales de embalaje respetuosos con el medio ambiente, en consonancia con las exigencias legales y comerciales vigentes en la actualidad.

Hasta el momento, las CDs se han incorporado en envases de alimentos activos como ciclodextrinas vacías, en forma de complejos de inclusión (CIC), o mediante la formación de estructuras poliméricas supramoleculares de CD, utilizando agentes entrecruzantes de distinta naturaleza. De acuerdo con la literatura revisada, los complejos de inclusión destacan como la vía más atractiva y prometedora para incorporar moléculas con actividad biológica específica en empaques activos.

En cuanto a la legislación, a pesar de las marcadas diferencias en las políticas actuales de aplicación en distintas regiones como América, Europa y Asia, la mayoría coincide en la seguridad del uso de CD y sus derivados en las ciencias de la alimentación, y más aún, en las industrias de envasado de alimentos.

VI – CONCLUSIONES

VI- CONCLUSIONES

De acuerdo con los objetivos planteados en el presente estudio se puede concluir, en base en los resultados obtenidos, que la incorporación de los encapsulados de carvacrol y timol a la matriz polimérica de PLA, permitió protegerlos de la temperatura durante el proceso de inyección para la obtención del empaque activo, permitiendo conservar las propiedades antioxidantes y bacteriostáticas de estos monoterpenos.

Los resultados obtenidos en el ensayo en condiciones reales, muestran que las bayas que estuvieron almacenadas en los empaques biodegradables de PLA, aditivados con complejos de carvacrol y timol, mejoraron la vida de anaquel, lo cual indica el uso potencial de estos empaques como sistemas de envasado activo, siendo una opción para reemplazar la adición directa de conservantes artificiales a las formulaciones de alimentos.

De acuerdo a los objetivos secundarios podemos concluir lo siguiente:

1. Respecto a los complejos β-CD-timol y β-CD-carvacrol, ambos muestran la misma relación molar radio (1:2), lo que indica que casi una de cada dos moléculas de β-CD en solución está formando complejos solubles con carvacrol o timol. Si bien la eficiencia de complejación para carvacrol es superior a la de timol, la eficiencia de encapsulación para ambos fue muy similar, apreciando solo un incremente del 5 % para timol.

2. Los resultados obtenidos por FT-IR confirman que ambos monoterpenos interaccionan con la cavidad apolar de β-CDs, apreciando una eficiencia de complejación significativamente superior para carvacrol.

3. Para el desarrollo de empaques activos de PLA con complejos de β-CD-timol o β-CD-carvacrol (0,0 %, 1,5 %, 2,5 % y 5,0 %) se optó por el proceso de inyección, seleccionando 180-190 °C como la temperatura óptima para la obtención del empaque. La presencia de estos complejos confiere características plastificantes a la matriz polimérica, reduciendo las fuerzas intermoleculares de las cadenas poliméricas, y mejorando así el punto de rotura.

4. La presencia de complejos sólidos de β-ciclodextrinas timol o carvacrol en formulaciones de PLA disminuyó ligeramente la temperatura de degradación térmica del polímero, en comparación con PLA puro, no comprometiendo la estabilidad térmica del empaque activo.

5. Los empaques activos desarrollados, presentan actividad antimicrobiana y, mostrando mayor eficacia aquellos que contienen β-CD-carvacrol 5,0 % y β-CD-timol 5,0 % ya que estos inhibieron el desarrollo de *Alternaria alternata* y *Botritis cinerea* después de 10 días de incubación, lo cual proporciona evidencia de su uso potencial en la industria agroalimentaria.

6. El empaque de PLA/β-CD-timol al 5,0 % mostró la concentración de volátiles 75 % más alta al inicio del estudio, manteniendo una liberación controlada de monoterpeno (30 % del nivel inicial en el día 7). En el resto de envases activos los niveles de monoterpeno bajaron a line base a los 7 días de ensayo.

7. Las bayas almacenadas en los empaques activos de PLA, mejoraron la vida de anaquel por una semana más, en comparación con el empaque control, el cual mostró un crecimiento visible de hongos, y deterioro de sus características organolépticas. El empaque PLA/β-CD-timol al 5,0 % mostró una inhibición de las levaduras y mohos del 51,57 %, en comparación con el control y obtuvo una valoración sensorial muy buena por el panel evaluador.

VII – LIMITACIONES Y FUTURAS LÍNEAS DE INVESTIGACIÓN

VII – LIMITACIONES Y FUTURAS LÍNEAS DE INVESTIGACIÓN

La utilización de ciclodextrinas y sus complejos en empaques activos para alimentos, tienen un papel relevante en las tendencias de envasado. El desarrollo futuro se centrará en la fabricación de envases multifuncionales, sostenibles y a precio razonable, que combinen las ventajas aportadas por los complejos de CDs, en el mantenimiento y mejora de sus propiedades y vida comercial de los alimentos envasados, prestaciones que pueden potenciarse con los envases inteligentes, diseñados para evaluar el estado de los alimentos envasados, aportando información de seguridad alimentaria a las partes interesadas, como fabricantes, minoristas y consumidores; y en última instancia, apostando por la estrategia Food 2030, desarrollando envases biodegradables partiendo de materias primas procedentes de residuos agroalimentarios, minimizando así, el problema asociado a la presencia de microplásticos en la cadena trófica.

Sin embargo, la utilización de CDs en aplicaciones de envasado de alimentos activos requiere nuevos estudios, sobre la posible migración de éstas a los alimentos y el impacto sobre la salud. Sus prestaciones y baja toxicidad han despertado el interés de otros sectores como la industria farmacéutica y la química. De acuerdo con las últimas tendencias reportadas en la literatura, todo apunta a enfocar las tendencias futuras del envasado de alimentos en una combinación de rutas clásicas con la nanotecnología, la nanoencapsulación o el uso de polímeros a base de CDs, que ya se están aplicando en la industria farmacéutica y en remediación medioambiental.

En el caso de los envases desarrollados de PLA, que contienen complejos de inclusión de timol y carvacrol, se requieren nuevos estudios para evaluar la posible reutilización del envase activo, tanto a temperatura ambiente, como en condiciones de refrigeración, lo que constituiría, sin duda, otra futura línea de investigación.

VIII - REFERENCIAS

VIII – REFERENCIAS BIBLIOGRÁFICAS

Abdollahi, M., Rezaei, M., & Farzi, G. (2012). Improvement of active chitosan film properties with rosemary essential oil for food packaging. *International Journal of Food Science and Technology*, *47*(4), 847–853. https://doi.org/10.1111/j.1365-2621.2011.02917.x

Abdou, E. S., Galhoum, G. F., & Mohamed, E. N. (2018). Curcumin loaded nanoemulsions/pectin coatings for refrigerated chicken fillets. *Food Hydrocolloids*, *83*, 445–453. https://doi.org/10.1016/j.foodhyd.2018.05.026

Alzamora, S. M., Tapia, M. S., & López-Malo, A. (2000). *Minimally processed fruits and vegetables: fundamental aspects and applications (Libro, 2000) [WorldCat.org]*. Aspen Publishers. https://www.worldcat.org/title/minimally-processed-fruits-and-vegetables-fundamental-aspects-and-applications/oclc/43859498

Anaya-Castro, M. A., Ayala-Zavala, J. F., Muñoz-Castellanos, L., Hernández-Ochoa, L., Peydecastaing, J., & Durrieu, V. (2017). β-Cyclodextrin inclusion complexes containing clove (Eugenia caryophyllata) and Mexican oregano (Lippia berlandieri) essential oils: Preparation, physicochemical and antimicrobial characterization. *Food Packaging and Shelf Life*, *14*, 96–101. https://doi.org/10.1016/J.FPSL.2017.09.002

Anderson, A. (2006). Final Report on the Safety Assessment of Sodium p -Chloro- m -Cresol, p -Chloro- m -Cresol, Chlorothymol, Mixed Cresols, m -Cresol, o -Cresol, p -Cresol, Isopropyl Cresols, Thymol, o -Cymen-5-ol, and Carvacrol1. *International Journal of Toxicology*, *25*(1_suppl), 29–127. https://doi.org/10.1080/10915810600716653

Arana-Sánchez, A., Estarrón-Espinosa, M., Obledo-Vázquez, E. N., Padilla-Camberos, E., Silva-Vázquez, R., & Lugo-Cervantes, E. (2010). Antimicrobial and antioxidant activities of Mexican oregano essential oils (Lippia graveolens H. B. K.) with different composition when microencapsulated inβ-cyclodextrin. *Letters in Applied Microbiology*, *50*(6), 585–590. https://doi.org/10.1111/j.1472-765X.2010.02837.x

Armentano, I., Bitinis, N., Fortunati, E., Mattioli, S., Rescignano, N., Verdejo, R., Lopez-Manchado, M. A., & Kenny, J. M. (2013). Multifunctional nanostructured PLA materials for packaging and tissue engineering. In *Progress in Polymer Science* (Vol. 38, Issues 10–11, pp. 1720–1747). Elsevier Ltd. https://doi.org/10.1016/j.progpolymsci.2013.05.010

Astray, G., Gonzalez-Barreiro, C., Mejuto, J. C., Rial-Otero, R., & Simal-Gándara, J. (2009). A review on the use of cyclodextrins in foods. In *Food Hydrocolloids* (Vol. 23, Issue 7, pp. 1631–1640). Elsevier. https://doi.org/10.1016/j.foodhyd.2009.01.001

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. In *Trends in Food Science and Technology* (Vol. 48, pp. 51–62). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2015.12.001

Auras, R., Harte, B., & Selke, S. (2004). An overview of polylactides as packaging materials. In *Macromolecular Bioscience* (Vol. 4, Issue 9, pp. 835–864). John Wiley & Sons, Ltd. https://doi.org/10.1002/mabi.200400043

Aytac, Z., Ipek, S., Durgun, E., Tekinay, T., & Uyar, T. (2017). Antibacterial electrospun zein nanofibrous web encapsulating thymol/cyclodextrin-inclusion complex for food packaging. *Food Chemistry*, *233*, 117–124. https://doi.org/10.1016/j.foodchem.2017.04.095

Babili, F. El, Bouajila, J., Souchard, J. P., Bertrand, C., Bellvert, F., Fouraste, I., Moulis, C., & Valentin, A. (2011). Oregano: Chemical analysis and evaluation of its antimalarial, antioxidant, and cytotoxic activities. *Journal of Food Science*, *76*(3), C512–C518. https://doi.org/10.1111/j.1750-3841.2011.02109.x

Barnett, I. (2011). *The Global Outlook for Biodegradable Packaging Key trends and developments driving the global biodegradable packaging market*.

Bastarrachea, L., Dhawan, S., & Sablani, S. S. (2011). Engineering Properties of Polymeric-Based Antimicrobial Films for Food Packaging. In *Food Engineering Reviews* (Vol. 3, Issue 2, pp. 79–93). Springer. https://doi.org/10.1007/s12393-011-9034-8

Becerril, R., Nerín, C., & Silva, F. (2020). Encapsulation Systems for Antimicrobial Food Packaging Components: An Update. *Molecules*, *25*(5), 1134. https://doi.org/10.3390/molecules25051134

Bilensoy, E., & Hincal, A. A. (2009). Recent advances and future directions in amphiphilic cyclodextrin nanoparticles. In *Expert Opinion on Drug Delivery* (Vol. 6, Issue 11, pp. 1161–1173). Expert Opin Drug Deliv. https://doi.org/10.1517/17425240903222218

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods - A review. In *International Journal of Food Microbiology* (Vol. 94, Issue 3, pp. 223–253). Elsevier. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Byun, Y., & Kim, Y. T. (2013). Bioplastics for Food Packaging: Chemistry and Physics. In *Innovations in Food Packaging: Second Edition* (pp. 353–368). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-394601-0.00014-X

Cao-Hoang, L., Chaine, A., Grégoire, L., & Waché, Y. (2010). Potential of nisin-incorporated sodium caseinate films to control Listeria in artificially contaminated cheese. *Food Microbiology*, *27*(7), 940–944. https://doi.org/10.1016/J.FM.2010.05.025

Catalá, R., & Gavara, R. (2001). Nuevos envases. De la protección pasiva a la defensa activa de los alimentos envasados. *Arbor*, *168*(661), 109–127. https://doi.org/10.3989/arbor.2001.i661.825

Cha, D. S., & Chinnan, M. S. (2004). Biopolymer-based antimicrobial packaging: A review. *Critical Reviews in Food Science and Nutrition*, *44*(4), 223–237. https://doi.org/10.1080/10408690490464276

Chen, H., Hu, X., Chen, E., Wu, S., McClements, D. J., Liu, S., Li, B., & Li, Y. (2016). Preparation, characterization, and properties of chitosan films with cinnamaldehyde nanoemulsions. *Food Hydrocolloids*, *61*, 662–671. https://doi.org/10.1016/j.foodhyd.2016.06.034

Chizzola, R., Michitsch, H., & Franz, C. (2008). Antioxidative properties of Thymus vulgaris leaves: Comparison of different extracts and essential oil chemotypes. *Journal of Agricultural and Food Chemistry*, *56*(16), 6897–6904. https://doi.org/10.1021/jf800617g

Crini, G. (2014). Review: A history of cyclodextrins. In *Chemical Reviews* (Vol. 114, Issue 21, pp. 10940–10975). American Chemical Society. https://doi.org/10.1021/cr500081p

Cvetnic, Z., & Vladimir-Knezevic, S. (2004). Antimicrobial activity of grapefruit seed and pulp ethanolic extract. *Acta. Pharm.*, *54*, 243–250.

Davis, F., & Higson, S. (2011). *Macrocycles: Construction, Chemistry and Nanotechnology Applications | Wiley*. John Wiley & Sons. https://www.wiley.com/en-us/Macrocycles%3A+Construction%2C+Chemistry+and+Nanotechnology+Applications-p-9780470714621

Del Nobile, M. A., Conte, A., Scrocco, C., & Brescia, I. (2009). New strategies for minimally processed cactus pear packaging. *Innovative Food Science & Emerging Technologies*, *10*(3), 356–362. https://doi.org/10.1016/J.IFSET.2008.12.006

Del Valle, E. M. M. (2004). Cyclodextrins and their uses: A review. In *Process Biochemistry* (Vol. 39, Issue 9, pp. 1033–1046). Elsevier. https://doi.org/10.1016/S0032-9592(03)00258-9

Dias Antunes, M., da Silva Dannenberg, G., Fiorentini, Â. M., Pinto, V. Z., Lim, L. T., da Rosa Zavareze, E., & Dias, A. R. G. (2017). Antimicrobial electrospun ultrafine fibers from zein containing eucalyptus essential oil/cyclodextrin inclusion complex. *International Journal of Biological Macromolecules*, *104*, 874–882. https://doi.org/10.1016/j.ijbiomac.2017.06.095

Divsalar, E., Tajik, H., Moradi, M., Forough, M., Lotfi, M., & Kuswandi, B. (2018). Characterization of cellulosic paper coated with chitosan-zinc oxide nanocomposite containing nisin and its application in packaging of UF cheese. *International Journal of Biological Macromolecules*, *109*, 1311–1318. https://doi.org/10.1016/j.ijbiomac.2017.11.145

Espitia, P. J. P., Fuenmayor, C. A., & Otoni, C. G. (2019). Nanoemulsions: Synthesis, Characterization, and Application in Bio-Based Active Food Packaging. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 264–285. https://doi.org/10.1111/1541-4337.12405

Falcone, P., Speranza, B., Del Nobile, M. A., Corbo, M. R., & Sinigaglia, M. (2005). A study on the antimicrobial activity of thymol intended as a natural preservative. *Journal of Food Protection*, *68*(8), 1664–1670. https://doi.org/10.4315/0362-028X-68.8.1664

Farkas, J. K. (1998). The development of iron-based oxygen absorbing systems used in food packaging and preservation. In *Theses and Dissertations Available from ProQuest*. https://docs.lib.purdue.edu/dissertations/AAI9953735

Forney, C. F., Kalt, W., Jordan, M. A., Vinqvist-Tymchuk, M. R., & Fillmore, S. A. E. (2012). Blueberry and cranberry fruit composition during development. *Journal of Berry Research*, *2*(3), 169–177. https://doi.org/10.3233/JBR-2012-034

Fu, Y., Sarkar, P., Bhunia, A. K., & Yao, Y. (2016). Delivery systems of antimicrobial compounds to food. In *Trends in Food Science and Technology* (Vol. 57, pp. 165–177). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2016.09.013

Gemili, S., Yemenicioğlu, A., & Altinkaya, S. A. (2009). Development of cellulose acetate based antimicrobial food packaging materials for controlled release of lysozyme. *Journal of Food Engineering*, *90*(4), 453–462. https://doi.org/10.1016/j.jfoodeng.2008.07.014

Geueke, B. (2015). *FPF Dossier: Bioplastics*. https://doi.org/10.5281/ZENODO.33517

Ghani, S., Barzegar, H., Noshad, M., & Hojjati, M. (2018). The preparation, characterization and in vitro application evaluation of soluble soybean polysaccharide films incorporated with cinnamon essential oil nanoemulsions. *International Journal of Biological Macromolecules*, *112*, 197–202. https://doi.org/10.1016/j.ijbiomac.2018.01.145

Gharsallaoui, A., Oulahal, N., Joly, C., & Degraeve, P. (2015). Nisin as a Food Preservative: Part 1: Physicochemical Properties, Antimicrobial Activity, and Main Uses. *Https://Doi.Org/10.1080/10408398.2013.763765*, *56*(8), 1262–1274. https://doi.org/10.1080/10408398.2013.763765

González, A., & Alvarez Igarzabal, C. I. (2013). Soy protein - Poly (lactic acid) bilayer films as biodegradable material for active food packaging. *Food Hydrocolloids*, *33*(2), 289–296. https://doi.org/10.1016/j.foodhyd.2013.03.010

Guo, M., Jin, T. Z., Yadav, M. P., & Yang, R. (2015). Antimicrobial property and microstructure of micro-emulsion edible composite films against Listeria. *International Journal of Food Microbiology*, *208*, 58–64. https://doi.org/10.1016/j.ijfoodmicro.2015.03.018

Guo, M., Yadav, M. P., & Jin, T. Z. (2017). Antimicrobial edible coatings and films from micro-emulsions and their food applications. *International Journal of Food Microbiology*, *263*, 9–16. https://doi.org/10.1016/j.ijfoodmicro.2017.10.002

Gupta, A. P., & Kumar, V. (2007). New emerging trends in synthetic biodegradable polymers - Polylactide: A critique. In *European Polymer Journal* (Vol. 43, Issue 10, pp. 4053–4074). Pergamon. https://doi.org/10.1016/j.eurpolymj.2007.06.045

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology*, *124*(1), 91–97. https://doi.org/10.1016/j.ijfoodmicro.2008.02.028

Han, J. H. (2014). Innovations in Food Packaging. In *Innovations in Food Packaging: Second Edition*. Elsevier. https://doi.org/10.1016/C2011-0-06876-X

Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., Gorris, L. G. M., & Von Wright, A. (1998). Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. *Journal of Agricultural and Food Chemistry*, *46*(9), 3590–3595. https://doi.org/10.1021/jf980154m

Helena, M., & Marques, C. (2019). *A review on cyclodextrin encapsulation of essential oils and volatiles †. February 2010*, 313–326. https://doi.org/10.1002/ffj.2019

Hernández-Figeroa, R. H., Palou-García, E., & López-Malo, A. (2013). Utilización de películas comestibles y ciclodextrinas para la liberación controlada de aceites esenciales como agentes antimicrobianos en vegetales - TSIA. *Temas Selectos de Ingeniería de Alimentos*, *1*(11). https://tsia.udlap.mx/utilizacion-de-peliculas-comestibles-y-ciclodextrinas-para-la-liberacion-controlada-de-aceites-esenciales-como-agentes-antimicrobianos-en-vegetales/

Higuchi, T., & Connors, K. A. (1965). Phase solubility techniques. *Advanced Analytical Chemistry of Instrumentation*, *4*, 117–212.

Higueras, L., López-Carballo, G., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Antimicrobial packaging of chicken fillets based on the release of carvacrol from chitosan/cyclodextrin films. *International Journal of Food Microbiology*, *188*, 53–59. https://doi.org/10.1016/j.ijfoodmicro.2014.07.018

Ho, K.-L. G., Pometto, A. L., Gadea-Rivas, A., Briceno, J. A., & Rojas, A. (1999). Degradation of Polylactic Acid (PLA) Plastic in Costa Rican Soil and Iowa State University Compost Rows 1. In *Journal of Environmental Polymer Degradation* (Vol. 7, Issue 4).

Hossain, F., Follett, P., Salmieri, S., Vu, K. D., Fraschini, C., & Lacroix, M. (2019). Antifungal activities of combined treatments of irradiation and essential oils (EOs) encapsulated chitosan nanocomposite films in in vitro and in situ conditions. *International Journal of Food Microbiology*, *295*, 33–40. https://doi.org/10.1016/j.ijfoodmicro.2019.02.009

Hu, S., Yu, J., Wang, Z., Li, L., Du, Y., Wang, L., & Liu, Y. (2017). Effects of Sorbic Acid-Chitosan Microcapsules as Antimicrobial Agent on the Properties of Ethylene Vinyl Alcohol Copolymer Film for Food Packaging. *Journal of Food Science*, *82*(6), 1451–1460. https://doi.org/10.1111/1750-3841.13731

Huang, C., Zhang, B., Wang, S., Zhang, L., Wang, J., Huang, X., Zhao, Y., & Huang, L. (2018). Moisture-triggered release of self-produced ClO2 gas from microcapsule antibacterial film system. *Journal of Materials Science*, *53*(18), 12704–12717. https://doi.org/10.1007/s10853-018-2576-x

Huang, T., Qian, Y., Wei, J., & Zhou, C. (2019). Polymeric Antimicrobial Food Packaging and Its Applications. *Polymers*, *11*(3), 560. https://doi.org/10.3390/polym11030560

Hughes, J., Thomas, R., Byun, Y., & Whiteside, S. (2012). Improved flexibility of thermally stable poly-lactic acid (PLA). *Carbohydrate Polymers*, *88*(1), 165–172. https://doi.org/10.1016/j.carbpol.2011.11.078

Irkin, R., & Esmer, O. K. (2015). Novel food packaging systems with natural antimicrobial agents. In *Journal of Food Science and Technology* (Vol. 52, Issue 10, pp. 6095–6111). Springer India. https://doi.org/10.1007/s13197-015-1780-9

Jamshidian, M., Tehrany, E. A., & Desobry, S. (2013). Antioxidants Release from Solvent-Cast PLA Film: Investigation of PLA Antioxidant-Active Packaging. *Food and Bioprocess Technology*, *6*(6), 1450–1463. https://doi.org/10.1007/s11947-012-0830-9

Jantrawut, P., Boonsermsukcharoen, K., Thipnan, K., Chaiwarit, T., Hwang, K. M., & Park, E. S. (2018). Enhancement of antibacterial activity of orange oil in pectin thin film by microemulsion. *Nanomaterials*, *8*(7). https://doi.org/10.3390/nano8070545

Jenssen, H., Hamill, P., & Hancock, R. E. W. (2006). Peptide antimicrobial agents. In *Clinical Microbiology Reviews* (Vol. 19, Issue 3, pp. 491–511). American Society for Microbiology (ASM). https://doi.org/10.1128/CMR.00056-05

Jideani, V. A., & Vogt, K. (2016). Antimicrobial Packaging for Extending the Shelf Life of Bread—A Review. *Critical Reviews in Food Science and Nutrition*, *56*(8), 1313–1324. https://doi.org/10.1080/10408398.2013.768198

Kavoosi, G., Dadfar, S. M. M., Mohammadi Purfard, A., & Mehrabi, R. (2013). Antioxidant and antibacterial properties of gelatin films incorporated with carvacrol. *Journal of Food Safety*, *33*(4), 423–432. https://doi.org/10.1111/jfs.12071

Kerry, J. P., O'Grady, M. N., & Hogan, S. A. (2006). Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: A review. *Meat Science*, *74*(1), 113–130. https://doi.org/10.1016/j.meatsci.2006.04.024

Kfoury, M., Auezova, L., Greige-Gerges, H., & Fourmentin, S. (2015). Promising applications of cyclodextrins in food: Improvement of essential oils retention, controlled release and antiradical activity. *Carbohydrate Polymers*, *131*, 264–272. https://doi.org/10.1016/j.carbpol.2015.06.014

Khaneghah, A. M., Hashemi, S. M. B., Es, I., Fracassetti, D., & Limbo, S. (2018). Efficacy of antimicrobial agents for food contact applications: Biological activity, incorporation into packaging, and assessment methods: A review. In *Journal of Food Protection* (Vol. 81, Issue 7, pp. 1142–1156). International Association for Food Protection. https://doi.org/10.4315/0362-028X.JFP-17-509

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., & Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish Origanum acutidens and its three components, carvacrol, thymol and p-cymene. *Bioresource Technology*, *99*(18), 8788–8795. https://doi.org/10.1016/j.biortech.2008.04.048

Kumari, A., Kumar, V., & Yadav, S. K. (2012). Plant Extract Synthesized PLA Nanoparticles for Controlled and Sustained Release of Quercetin: A Green Approach. *PLoS ONE*, *7*(7), e41230. https://doi.org/10.1371/journal.pone.0041230

Lago, M. A., Sendón, R., de Quirós, A. R. B., Sanches-Silva, A., Costa, H. S., Sánchez-Machado, D. I., Valdez, H. S., Angulo, I., Aurrekoetxea, G. P., Torrieri, E., López-Cervantes, J., & Paseiro, P. (2014). Preparation and Characterization of Antimicrobial Films Based on Chitosan for Active Food Packaging Applications. *Food and Bioprocess Technology*, *7*(10), 2932–2941. https://doi.org/10.1007/s11947-014-1276-z

Laird, K., & Phillips, C. (2012). Vapour phase: a potential future use for essential oils as antimicrobials? *Letters in Applied Microbiology*, *54*(3), 169–174. https://doi.org/10.1111/j.1472-765X.2011.03190.x

Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, *91*(3), 453–462. https://doi.org/10.1046/j.1365-2672.2001.01428.x

Laza-Knoerr, A. L., Gref, R., & Couvreur, P. (2010). Cyclodextrins for drug delivery. In *Journal of Drug Targeting* (Vol. 18, Issue 9, pp. 645–656). Taylor & Francis. https://doi.org/10.3109/10611861003622552

Lei, K., Wang, X., Li, X., & Wang, L. (2019). The innovative fabrication and applications of carvacrol nanoemulsions, carboxymethyl chitosan microgels and their composite films. *Colloids and Surfaces B: Biointerfaces*, *175*, 688–696. https://doi.org/10.1016/j.colsurfb.2018.12.054

Leyva-López, N., Gutiérrez-Grijalva, E. P., Vazquez-Olivo, G., & Heredia, J. B. (2017). Essential Oils of Oregano: Biological Activity beyond Their Antimicrobial Properties. *Molecules*, *22*(6), 989. https://doi.org/10.3390/molecules22060989

Licata, M., Tuttolomondo, T., Dugo, G., Ruberto, G., Leto, C., Napoli, E. M., Rando, R., Rita Fede, M., Virga, G., Leone, R., & La Bella, S. (2015). Study of quantitative and qualitative variations in essential oils of Sicilian oregano biotypes. *Journal of Essential Oil Research*, *27*(4), 293–306. https://doi.org/10.1080/10412905.2015.1045088

Lim, L. T., Auras, R., & Rubino, M. (2008). Processing technologies for poly(lactic acid). In *Progress in Polymer Science (Oxford)* (Vol. 33, Issue 8, pp. 820–852). https://doi.org/10.1016/j.progpolymsci.2008.05.004

Limbo, S., & Khaneghah, A. M. (2015). Active packaging of foods and its combination with electron beam processing. In *Electron Beam Pasteurization and Complementary Food Processing Technologies* (pp. 195–217). Elsevier Inc. https://doi.org/10.1533/9781782421085.2.195

Lin, L., Zhu, Y., & Cui, H. (2018). Electrospun thyme essential oil/gelatin nanofibers for active packaging against Campylobacter jejuni in chicken. *LWT*, *97*, 711–718. https://doi.org/10.1016/j.lwt.2018.08.015

Liu, D., Li, H., Jiang, L., Chuan, Y., Yuan, M., & Chen, H. (2016). Characterization of Active Packaging Films Made from Poly(Lactic Acid)/Poly(Trimethylene Carbonate) Incorporated with Oregano Essential Oil. *Molecules*, *21*(6), 695. https://doi.org/10.3390/molecules21060695

Llana-Ruiz-Cabello, M., Pichardo, S., Bermúdez, J. M., Baños, A., Núñez, C., Guillamón, E., Aucejo, S., & Cameán, A. M. (2016). Development of PLA films containing oregano essential oil (Origanum vulgare L. virens) intended for use in food packaging. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *33*(8), 1374–1386. https://doi.org/10.1080/19440049.2016.1204666

López-Malo, A., Alzamora, S. M., Paris, M. J., Lastra-Vargas, L., Coronel, M. B., Gómez, P. L., & Palou, E. (2020). Naturally Occurring Compounds – Plant Sources. In *Antimicrobials in Food* (pp. 527–594). CRC Press. https://doi.org/10.1201/9780429058196-17

López-Mata, M. A., Ruiz-Cruz, S., Silva-Beltrán, N. P., Ornelas-Paz, J. D. J., Zamudio-Flores, P. B., & Burruel-Ibarra, S. E. (2013). Physicochemical, antimicrobial and antioxidant properties of chitosan films incorporated with carvacrol. *Molecules*, *18*(11), 13735–13753. https://doi.org/10.3390/molecules181113735

López-Rubio, A., Almenar, E., Hernandez-Muñoz, P., Lagarón, J. M., Catalá, R., & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. In *Food Reviews International* (Vol. 20, Issue 4, pp. 357–387). Taylor and Francis Inc. https://doi.org/10.1081/FRI-200033462

López-Rubio, A., & Lagaron, J. M. (2010). Improvement of UV stability and mechanical properties of biopolyesters through the addition of β-carotene. *Polymer Degradation and Stability*, *95*(11), 2162–2168. https://doi.org/10.1016/j.polymdegradstab.2010.03.002

Magi, G., Marini, E., & Facinelli, B. (2015). Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. *Frontiers in Microbiology*, *6*(MAR). https://doi.org/10.3389/fmicb.2015.00165

Mancini, E., Senatore, F., Del Monte, D., De Martino, L., Grulova, D., Scognamiglio, M., Snoussi, M., & De Feo, V. (2015). Studies on chemical composition, antimicrobial and antioxidant activities of five Thymus vulgaris L. essential oils. *Molecules*, *20*(7), 12016–12028. https://doi.org/10.3390/molecules200712016

Mano, J. F., Gómez Ribelles, J. L., Alves, N. M., & Salmerón Sanchez, M. (2005). Glass transition dynamics and structural relaxation of PLLA studied by DSC: Influence of crystallinity. *Polymer*, *46*(19 SPEC. ISS.), 8258–8265. https://doi.org/10.1016/j.polymer.2005.06.096

Marchese, A., Orhan, I. E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S. F., Gortzi, O., Izadi, M., & Nabavi, S. M. (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. In *Food Chemistry* (Vol. 210, pp. 402–414). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2016.04.111

Marques, H. M. C. (2010). A review on cyclodextrin encapsulation of essential oils and volatiles. In *Flavour and Fragrance Journal* (Vol. 25, Issue 5, pp. 313–326). John Wiley & Sons, Ltd. https://doi.org/10.1002/ffj.2019

Marturano, V., Marcille, H., Cerruti, P., Bandeira, N. A. G., Giamberini, M., Trojanowska, A., Tylkowski, B., Carfagna, C., Ausanio, G., & Ambrogi, V. (2019). Visible-Light Responsive Nanocapsules for Wavelength-Selective Release of Natural Active Agents. *ACS Applied Nano Materials*, *2*(7), 4499–4506. https://doi.org/10.1021/acsanm.9b00882

Mittal, V. (2012). Characterization Techniques for Polymer Nanocomposites. In V. Mittal (Ed.), *Characterization Techniques for Polymer Nanocomposites*. Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/9783527654505

Moein, M. R., Zomorodian, K., Pakshir, K., Yavari, F., Motamedi, M., & Zarshenas, M. M. (2015). Trachyspermum ammi (L.) Sprague: Chemical composition of essential oil and antimicrobial activities of respective fractions. *Journal of Evidence-Based Complementary and Alternative Medicine*, *20*(1), 50–56. https://doi.org/10.1177/2156587214553302

Morey, A., Bowers, J. W. J., Bauermeister, L. J., Singh, M., Huang, T. S., & Mckee, S. R. (2014). Effect of salts of organic acids on Listeria monocytogenes, shelf life, meat quality, and consumer acceptability of beef frankfurters. *Journal of Food Science*, *79*(1). https://doi.org/10.1111/1750-3841.12220

Mousavi Khaneghah, A., Hashemi, S. M. B., & Limbo, S. (2018). Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions. In *Food and Bioproducts Processing* (Vol. 111, pp. 1–19). Institution of Chemical Engineers. https://doi.org/10.1016/j.fbp.2018.05.001

Muriel-Galet, V., Talbert, J. N., Hernandez-Munoz, P., Gavara, R., & Goddard, J. M. (2013). Covalent immobilization of lysozyme on ethylene vinyl alcohol films for nonmigrating antimicrobial packaging applications. *Journal of Agricultural and Food Chemistry*, *61*(27), 6720–6727. https://doi.org/10.1021/jf401818u

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, *1*, 1806–1815. https://doi.org/10.1016/j.profoo.2011.09.265

Nieddu, M., Rassu, G., Boatto, G., Bosi, P., Trevisi, P., Giunchedi, P., Carta, A., & Gavini, E. (2014). Improvement of thymol properties by complexation with cyclodextrins: In vitro and in vivo studies. *Carbohydrate Polymers*, *102*(1), 393–399. https://doi.org/10.1016/j.carbpol.2013.10.084

Nostro, A., & Papalia, T. (2012). Antimicrobial Activity of Carvacrol: Current Progress and Future Prospectives. *Recent Patents on Anti-Infective Drug Discovery*, *7*(1), 28–35. https://doi.org/10.2174/157489112799829684

Novy, P., Davidova, H., Serrano-Rojero, C. S., Rondevaldova, J., Pulkrabek, J., & Kokoska, L. (2015). Composition and antimicrobial activity of Euphrasia rostkoviana Hayne essential oil. *Evidence-Based Complementary and Alternative Medicine*, *2015*. https://doi.org/10.1155/2015/734101

Oh, Y. A., Oh, Y. J., Song, A. Y., Won, J. S., Song, K. Bin, & Min, S. C. (2017). Comparison of effectiveness of edible coatings using emulsions containing lemongrass oil of different size droplets on grape berry safety and preservation. *LWT*, *75*, 742–750. https://doi.org/10.1016/j.lwt.2016.10.033

Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chemistry*, *122*(1), 161–166. https://doi.org/10.1016/j.foodchem.2010.02.033

Ozdemir, M., & Floros, J. D. (2004). Active food packaging technologies. *Critical Reviews in Food Science and Nutrition*, *44*(3), 185–193. https://doi.org/10.1080/10408690490441578

Paniagua, A. C., East, A. R., Hindmarsh, J. P., & Heyes, J. A. (2013). Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biology and Technology*, *79*, 13–19. https://doi.org/10.1016/J.POSTHARVBIO.2012.12.016

Pathare, P. B., Opara, U. L., Vigneault, C., Delele, M. A., & Al-Said, F. A. J. (2012). Design of Packaging Vents for Cooling Fresh Horticultural Produce. *Food and Bioprocess Technology 2012 5:6*, *5*(6), 2031–2045. https://doi.org/10.1007/S11947-012-0883-9

Periago, P. M., Palop, A., & Fernandez, P. S. (2001). Combined Effect of Nisin, Carvacrol and Thymol on the Viability of Bacillus Cereus Heat-Treated Vegetative Cells. *Food Science and Technology International*, *7*(6), 487–492. https://doi.org/10.1106/JE3P-NYKQ-4UDQ-TJC6

Pinho, E., Grootveld, M., Soares, G., & Henriques, M. (2014). Cyclodextrins as encapsulation agents for plant bioactive compounds. In *Carbohydrate Polymers* (Vol. 101, Issue 1, pp. 121–135). Elsevier. https://doi.org/10.1016/j.carbpol.2013.08.078

Pisoschi, A. M., Pop, A., Cimpeanu, C., Turcuş, V., Predoi, G., & Iordache, F. (2018). Nanoencapsulation techniques for compounds and products with antioxidant and antimicrobial activity - A critical view. In *European Journal of Medicinal Chemistry* (Vol. 157, pp. 1326–1345). Elsevier Masson s.r.l. https://doi.org/10.1016/j.ejmech.2018.08.076

Raj, G. A., Chandrasekaran, M., Krishnamoorthy, S., Jayaraman, M., & Venkatesalu, V. (2015). Phytochemical profile and larvicidal properties of seed essential oil from Nigella sativa L. (Ranunculaceae), against Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus (Diptera: Culicidae). *Parasitology Research*, *114*(9), 3385–3391. https://doi.org/10.1007/s00436-015-4563-3

Ramos, M., Beltran, A., Valdes, A., Peltzer, M. A., Jimenez, A., Garrigos, M. C., & Zaikov, G. E. (2013). Carvacrol and thymol for fresh food packaging. *Journal of Bioequivalence and Bioavailability*, *5*(4), 154–160. https://doi.org/10.4172/jbb.1000151

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

Rezaei, A., Fathi, M., & Jafari, S. M. (2019). Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers. In *Food Hydrocolloids* (Vol. 88, pp. 146–162). Elsevier B.V. https://doi.org/10.1016/j.foodhyd.2018.10.003

Robbins, J., Sjulin, T. M., & Patterson, M. (1989). Postharvest storage characteristics and respiration rates in five cultivars of red raspberry. *Hort. Sci.*, *24*. https://agris.fao.org/agris-search/search.do?recordID=US9020978

Robertson, G. (2008). State-of-the-art biobased food packaging materials. *Environmentally Compatible Food Packaging*, 3–28. https://doi.org/10.1533/9781845694784.1.3

Robledo, N., López, L., Bunger, A., Tapia, C., & Abugoch, L. (2018). Effects of antimicrobial edible coating of thymol nanoemulsion/quinoa protein/chitosan on the safety, sensorial properties, and quality of refrigerated strawberries (Fragaria × ananassa) under commercial storage environment. *Food and Bioprocess Technology*, *11*(8), 1566–1574. https://doi.org/10.1007/s11947-018-2124-3

Robledo, N., Vera, P., López, L., Yazdani-Pedram, M., Tapia, C., & Abugoch, L. (2018). Thymol nanoemulsions incorporated in quinoa protein/chitosan edible films; antifungal effect in cherry tomatoes. *Food Chemistry*, *246*, 211–219. https://doi.org/10.1016/j.foodchem.2017.11.032

Rodríguez-López, Ma. Isabel. (2017). *Estudio de los parámetros fisicoquímicos de los complejos de timol, carvacrol y linalol en ciclodextrinas y evaluación del efecto de la complejación en su actividad antimicrobiana*. Universidad Católica de Murcia.

Rodríguez-López, María Isabel, Mercader-Ros, M. T., López-Miranda, S., Pellicer, J. A., Pérez-Garrido, A., Pérez-Sánchez, H., Núñez-Delicado, E., & Gabaldón, J. A. (2019). Thorough characterization and stability of HP- β -cyclodextrin thymol inclusion complexes prepared by microwave technology: A required approach to a successful application in food industry. *Journal of the Science of Food and Agriculture*, *99*(3), 1322–1333. https://doi.org/10.1002/jsfa.9307

Rodríguez-López, María Isabel, Mercader-Ros, M. T., Pellicer, J. A., Gómez-López, V. M., Martínez-Romero, D., Núñez-Delicado, E., & Gabaldón, J. A. (2020). Evaluation of monoterpene-cyclodextrin complexes as bacterial growth effective hurdles. *Food Control*, *108*, 106814. https://doi.org/10.1016/j.foodcont.2019.106814

Rooney, M. L. (1995). Overview of active food packaging. *Active Food Packaging*, 1–37. https://doi.org/10.1007/978-1-4615-2175-4_1

Santos, E. H., Kamimura, J. A., Hill, L. E., & Gomes, C. L. (2015). Characterization of carvacrol beta-cyclodextrin inclusion complexes as delivery systems for antibacterial and antioxidant applications. *LWT - Food Science and Technology*, *60*(1), 583–592. https://doi.org/10.1016/j.lwt.2014.08.046

Sarwar, A., & Latif, Z. (2015). GC-MS characterisation and antibacterial activity evaluation of Nigella sativa oil against diverse strains of Salmonella. *Natural Product Research*, *29*(5), 447–451. https://doi.org/10.1080/14786419.2014.947493

Silva-Weiss, A., Ihl, M., Sobral, P. J. A., Gómez-Guillén, M. C., & Bifani, V. (2013). Natural Additives in Bioactive Edible Films and Coatings: Functionality and Applications in Foods. In *Food Engineering Reviews* (Vol. 5, Issue 4, pp. 200–216). Springer. https://doi.org/10.1007/s12393-013-9072-5

Simionato, I., Domingues, F. C., Nerín, C., & Silva, F. (2019). Encapsulation of cinnamon oil in cyclodextrin nanosponges and their potential use for antimicrobial food packaging. *Food and Chemical Toxicology*, *132*, 110647. https://doi.org/10.1016/j.fct.2019.110647

Södergård, A., & Stolt, M. (2010). Industrial Production of High Molecular Weight Poly(Lactic Acid). In *Poly(Lactic Acid): Synthesis, Structures, Properties, Processing, and Applications* (pp. 27–41). John Wiley & Sons, Inc. https://doi.org/10.1002/9780470649848.ch3

Solórzano-Santos, F., & Miranda-Novales, M. G. (2012). Essential oils from aromatic herbs as antimicrobial agents. *Current Opinion in Biotechnology*, *23*(2), 136–141. https://doi.org/10.1016/J.COPBIO.2011.08.005

Soto, K. M., Hernández-Iturriaga, M., Loarca-Piña, G., Luna-Bárcenas, G., Gómez-Aldapa, C. A., & Mendoza, S. (2016). Stable nisin food-grade electrospun fibers. *Journal of Food Science and Technology*, *53*(10), 3787–3794. https://doi.org/10.1007/s13197-016-2365-y

Soylu, E. M., Kurt, Ş., & Soylu, S. (2010). In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. *International Journal of Food Microbiology*, *143*(3), 183–189. https://doi.org/10.1016/j.ijfoodmicro.2010.08.015

Sugumar, S., Mukherjee, A., & Chandrasekaran, N. (2015). Eucalyptus oil nanoemulsion-impregnated chitosan film: Antibacterial effects against a clinical pathogen, Staphylococcus aureus, in vitro. *International Journal of Nanomedicine*, *10*(Suppl 1), 67–75. https://doi.org/10.2147/IJN.S79982

Suloff, E. C., Marcy, J. E., Blakistone, B. A., Duncan, S. E., Long, T. E., & O'Keefe, S. F. (2003). Sorption Behavior of Selected Aldehyde-scavenging Agents in Poly(ethylene terephthalate) Blends. *Journal of Food Science*, *68*(6), 2028–2033. https://doi.org/10.1111/J.1365-2621.2003.TB07013.X

Šumiga, B., Šumiga, B., Ravnjak, D., & Podgornik, B. B. (2019). Antimicrobial paper coatings containing microencapsulated cymbopogon citratus oil. *Coatings*, *9*(8), 470. https://doi.org/10.3390/coatings9080470

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. In *Journal of Food Science* (Vol. 68, Issue 2, pp. 408–420). Institute of Food Technologists. https://doi.org/10.1111/j.1365-2621.2003.tb05687.x

Tan, Y. M., Lim, S. H., Tay, B. Y., Lee, M. W., & Thian, E. S. (2015). Functional chitosan-based grapefruit seed extract composite films for applications in food packaging technology. *Materials Research Bulletin*, *69*, 142–146. https://doi.org/10.1016/J.MATERRESBULL.2014.11.041

Thallinger, B., Prasetyo, E. N., Nyanhongo, G. S., & Guebitz, G. M. (2013). Antimicrobial enzymes: An emerging strategy to fight microbes and microbial biofilms. *Biotechnology Journal*, *8*(1), 97–109. https://doi.org/10.1002/biot.201200313

Valente, A. J. M., & Söderman, O. (2014). The formation of host-guest complexes between surfactants and cyclodextrins. In *Advances in Colloid and Interface Science* (Vol. 205, pp. 156–176). Adv Colloid Interface Sci. https://doi.org/10.1016/j.cis.2013.08.001

Van Der Steen, C., Jacxsens, L., Devlieghere, F., & Debevere, J. (2002). Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biology and Technology*, *26*(1), 49–58. https://doi.org/10.1016/S0925-5214(02)00005-4

Velázquez-Contreras, F., Zamora-Ledezma, C., López-González, I., Meseguer-Olmo, L., Núñez-Delicado, E., & Gabaldón, J. A. (2021). Cyclodextrins in Polymer-Based Active Food Packaging: A Fresh Look at Nontoxic, Biodegradable, and Sustainable Technology Trends. *Polymers 2022, Vol. 14, Page 104*, *14*(1), 104. https://doi.org/10.3390/POLYM14010104

Vessoni Penna, T. C., Augusto Moraes, D., & Nogueira Fajardo, D. (2002). The effect of nisin on growth kinetics from activated Bacillus cereus spores in cooked rice and in milk. *Journal of Food Protection*, *65*(2), 419–422. https://doi.org/10.4315/0362-028x-65.2.419

Viacava, G. E., Ayala-Zavala, J. F., González-Aguilar, G. A., & Ansorena, M. R. (2018). Effect of free and microencapsulated thyme essential oil on quality attributes of minimally processed lettuce. *Postharvest Biology and Technology*, *145*, 125–133. https://doi.org/10.1016/j.postharvbio.2018.07.004

Wang, T., Li, B., Si, H., Lin, L., & Chen, L. (2011). Release characteristics and antibacterial activity of solid state eugenol/β-cyclodextrin inclusion complex. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *71*(1–2), 207–213. https://doi.org/10.1007/s10847-011-9928-3

Wei, X. W., Guo, G., Gong, C. Y., Gou, M. L., & Yong Qian, Z. (2011). Biodegradable Polymers: Research and Applications. In *A Handbook of Applied Biopolymer Technology: Synthesis, Degradation and Applications* (pp. 365–387). https://doi.org/10.1039/9781849733458-00365

Wen, P., Zhu, D. H., Feng, K., Liu, F. J., Lou, W. Y., Li, N., Zong, M. H., & Wu, H. (2016). Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/β-cyclodextrin inclusion complex for antimicrobial packaging. *Food Chemistry*, *196*, 996–1004. https://doi.org/10.1016/j.foodchem.2015.10.043

Wen, P., Zhu, D. H., Wu, H., Zong, M. H., Jing, Y. R., & Han, S. Y. (2016). Encapsulation of cinnamon essential oil in electrospun nanofibrous film for active food packaging. *Food Control*, *59*, 366–376. https://doi.org/10.1016/j.foodcont.2015.06.005

Xu, J., Zhou, F., Ji, B. P., Pei, R. S., & Xu, N. (2008). The antibacterial mechanism of carvacrol and thymol against Escherichia coli. *Letters in Applied Microbiology*, *47*(3), 174–179. https://doi.org/10.1111/j.1472-765X.2008.02407.x

Zhang, C., Feng, F., & Zhang, H. (2018). Trends in Food Science & Technology Emulsion electrospinning : Fundamentals , food applications and prospects. *Trends in Food Science & Technology*, *80*(May), 175–186. https://doi.org/10.1016/j.tifs.2018.08.005

Zhou, F., Ji, B., Zhang, H., Jiang, H., Yang, Z., Li, J., Li, J., & Yan, W. (2007). The antibacterial effect of cinnamaldehyde, thymol, carvacrol and their combinations against the foodborne pathogen Salmonella typhimurium. *Journal of Food Safety*, *27*(2), 124–133. https://doi.org/10.1111/j.1745-4565.2007.00064.x