UNIVERSIDAD
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DE COLOMBIA

EVALUACIÓN DEL USO DE MEZCLAS DE POLIMEROS PARA EL DESARROLLO DE MICRO/NANOFIBRAS CON INCORPORACIÓN DE UN PÉPTIDO ANTIMICROBIANO

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Universidad Nacional de Colombia
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Bogotá D.C., Colombia
2020

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Tesis presentada como requisito parcial para optar al título de:

Magister en Ciencia y Tecnología de Alimentos

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Línea de Investigación:

Calidad de los alimentos

Universidad Nacional de Colombia

Facultad de Ciencias Agrarias

Bogotá D.C., Colombia

2020

Dedicatoria

Dedico este trabajo primeramente a Dios por darme vida, salud, por permitirme obtener este logro y por la oportunidad de conocer personas excepcionales en este proceso de aprendizaje.

A mi madre y mi familia por su paciencia y apoyo incondicional, por ser el motor que me impulsa a seguir adelante todos los días en el cumplimiento de mis sueños y metas.

Agradecimientos

A mi mamá y hermana por ser mi más grande apoyo. Por enseñarme a ser responsable, honesta y comprometida con las metas que me propongo y por brindarme una voz de aliento en los momentos difíciles. A mis tíos y abuela por su confianza, su respaldo y por creer en mí. A mi novio por enseñarme que cada día hay que hacer aquello que te llena de felicidad y satisfacción.

A mis directores de tesis el profesor Carlos Mario Zuluaga, la profesora Dianney Clavijo, y el profesor Carlos Fuenmayor por su constante orientación, colaboración y comprensión durante todo el desarrollo del proyecto, por sus enseñanzas, por todo su tiempo y su inmenso apoyo para que este proyecto pudiera llevarse acabo, y por su exigencia, que me impulsa a demostrarme que siempre se puede ser mejor. Gracias porque en dos años hicieron de mi una mejor persona y profesional. Un agradecimiento especial a Caroll Cortes, por los consejos académicos y personales que recibí de su parte durante el desarrollo de mi investigación.

A la Universidad Nacional, docentes del ICTA, estudiantes, administrativos y al grupo de investigación de carotenoides por su colaboración en poder llevar acabo este proyecto, en los aprendizajes académicos y de formación. A la Dirección de Investigación (DIB), al Instituto de Ciencia y Tecnología de Alimentos, y a las Facultades de Ciencias Agrarias, Medicina y Ciencias de la Universidad Nacional de Colombia por su constructivo conocimiento científico, soporte técnico y financiación para el desarrollo de este proyecto.

A mis amigos del Laboratorio de Poscosecha: Yaneth, Diego, Jaslady, Rolando, Sandra y a mis amigas de la maestría Carmen y Magreth por su apoyo incondicional, sus alientos y su alegría que hicieron de mi maestría una experiencia gratificante. Gracias por brindarme su valiosa amistad.

Resumen

Actualmente se generan pérdidas en los alimentos debido a la presencia de microorganismos alteradores, por lo tanto, se hace necesario el uso de empaques activos que funcionen como barrera de protección. Recientemente, se ha propuesto el uso de membranas ultrafinas incorporadas con compuestos antimicrobianos que potencialmente sirvan como adjuntos en empaques activos y favorezcan la liberación sostenida del compuesto. Dentro de los antimicrobianos incorporados se encuentran los péptidos, los cuales han demostrado su actividad antimicrobiana frente a un amplio espectro de microorganismos. Entre tanto, las membranas pueden ser elaboradas a partir de polímeros biodegradables mediante el aprovechamiento de nanotecnologías, siendo de particular interés la técnica de electrohilado (o *electrospinning*). No obstante las ventajas del uso de biopolímeros para la fabricación de membranas ultrafinas como dispositivos para empaques activos, estos presentan con frecuencia características indeseables, en especial una baja resistencia al agua, lo cual compromete su estabilidad estructural para este tipo de aplicaciones. Una alternativa de mejora consiste en la combinación de polímeros de distinta naturaleza que mejoren las propiedades de interacción con el agua de membranas ultrafinas, sin comprometer su biodegradabilidad y biocompatibilidad.

En este estudio, se desarrollaron membranas ultrafinas mediante la técnica de electrohilado a partir de una mezcla de polímeros para la incorporación del péptido palindrómico LfcinB $(21\text{-}25)_{Pal}$, sintetizado a partir de la lactoferrina bovina, que ha demostrado tener actividad antimicrobiana frente a virus, bacterias y hongos. En primer lugar, se evaluó la factibilidad de producir membranas electrohiladas de pululano (PUL) –un polisacárido altamente hidrofílico-, de policaprolactona (PCL) –un poliéster biodegradable hidrofóbico, y de mezclas de PCL con polisacáridos poco solubles en agua (almidón modificado de papa y β-glucano). Estas membranas se caracterizaron morfológicamente por la técnica de Microscopía Electrónica de Barrido (SEM), para observar la orientación de las fibras, su diámetro y la presencia de imperfecciones. Las

características químicas se evaluaron por espectroscopia de infrarrojo (FTIR-ATR) para evaluar la presencia de los grupos funcionales característicos para cada fibra. Y por último, la humectabilidad se evaluó por medio de la técnica de ángulo de contacto. Una vez conocidas las propiedades de los materiales obtenidos, se realizó una membrana multicapa, en donde la capa externa estuvo compuesta de policaprolactona (PCL) y la capa interna de pululano (PUL), las cuales fueron caracterizadas adicionalmente por Calorimetría Diferencial de Barrido (DSC) para determinar la cristalinidad de las fibras.

Las membranas multicapa (PCL-PUL-PCL) poseen características estructurales de fibras cilíndricas y lisas, con un diámetro aproximado de 100 nm y una estabilidad térmica a temperaturas entre 200ºC y 300ºC. Los espectros FTIR de las membranas confirmaron que el electrohilado no generó modificaciones en la estructura de los polímeros. Basado en lo anterior, estas membranas fueron elegidas para la encapsulación del péptido, el PUL se usó como agente encapsulante del péptido y la PCL se empleó para recubrir al PUL debido a que el primero posee un carácter altamente hidrofóbico, lo que mantuvo la integridad de la membrana en presencia de agua.

Posteriormente, se logró encapsular, con una eficiencia de 65%, el péptido LfcinB (21-25)$_{Pal}$ dentro de las fibras de PUL a una carga máxima de 50 mg péptido/g de PUL para luego recubrirlas con PCL. Estas membranas fueron caracterizadas estructural, física y morfológicamente. El análisis FTIR evidenció que no hubo modificaciones químicas en los polímeros ni el péptido después del electrohilado. La evaluación de la actividad antioxidante por DPPH mostró que las membranas a la mayor carga del péptido poseen una actividad antiradicalaria de 5.88x10^{-4} mg de ácido gálico/mg membrana. En estudios previos realizados por el grupo de investigación se encontró que la concentración mínima inhibitoria del péptido, cuando se encapsula en una membrana polimérica (PUL), fue de 17 µM frente a una cepa de *Escherichia coli*. Lo anterior sugiere el potencial aplicativo de estas membranas para ser incorporadas en empaques activos que prolonguen la vida útil de los alimentos a través de la liberación sostenida del péptido antimicrobiano.

Palabras clave: Pululano, policaprolactona, electrohilado, membranas multicapa, péptido LfcinB (21-25)$_{Pal}$.

Abstract

Currently, food losses are generated due to the presence of spoilage microorganisms, therefore the use of active packaging that functions as a protective barrier is necessary. To produce this type of packaging, it has been recently proposed the use of ultrafine membranes incorporated with antimicrobial compounds that potentially serve as attachments in active packaging and favor the controlled release of the compound. Peptides are one of the most commonly incorporated antimicrobials, having a demonstrated antimicrobial activity against a wide spectrum of microorganisms. Meanwhile, the membranes can be made from biodegradable polymers by taking advantages of nanotechnologies, the electrospinning technique being of particular interest. Despite the advantages of the use of biopolymers for the manufacture of ultra-thin membranes as a devices for active packaging, they often have undesirable characteristics, especially low resistance to water, which compromises their structural stability for this type of application. An improvement alternative consists in the combination of polymers of different nature that improve the properties of interaction with water of ultra-thin membranes, without compromising their biodegradability and biocompatibility.

In this study, ultrafine membranes were developed using electrospinning from a polymer mixture for the incorporation of the palindromic peptide LfcinB(21-25)$_{Pal,}$ synthesized from bovine lactoferrin, which has been shown to have antimicrobial activity against viruses, bacteria, and fungi. Firstly, the feasibility of producing membranes of pullulan (PUL) a highly hydrophilic polysaccharide, polycaprolactone (PCL) a hydrophobic biodegradable polyester, and PCL mixtures with poorly water-soluble polysaccharides (modified starch of potato and β-glucan).The membranes were morphologically characterized by Scanning Electron Microscopy (SEM) to observe the orientation of the fiber, its diameter, and the presence of imperfections. The structural characteristics were evaluated by Differential Scanning Calorimetry (DSC) to determine fiber crystallinity. Chemical characteristics were evaluated by infrared spectroscopy (FTIR-ATR) to evaluate the presence of characteristic functional groups for each fiber. As a final point, the wettability was evaluated by measuring the contact angle.

Multilayer membranes (PCL-PUL-PCL) have structural characteristics of cylindrical and smooth fibers, with an approximate diameter of 100 nm and thermal stability at

temperatures between 200ºC and 300ºC. The FTIR spectra of the membranes confirmed electrospinning did not generate modifications in the structure of polymers. Based on the above, these membranes were chosen for peptide encapsulation, PUL was used as an encapsulation agent for the peptide and PCL was used to coat PUL since the highly hydrophobic character of PCL which maintained the integrity of the membrane in the presence of water.

Subsequently, it was possible to encapsulate up to 65% of the LfcinB (21-25)$_{Pal}$ within the PUL fibers at a maximum load of 50 mg peptide/g of PUL, to then coat them with PCL. The membranes were characterized by structural, physical, and morphological properties. FTIR analysis showed that there were no chemical changes in polymers or peptide after electrospinning. The evaluation of antioxidant activity by DPPH showed that membranes at the highest load of the peptide possess an antiradical activity of 5.21 x 10^{-4}mg ± 1.12 x 10^{-5} of gallic acid/mg membrane. Finally, in previous studies carried out by the research group, it was found that the Minimum Inhibitory Concentration of the peptide, when encapsulated in a polymer membrane, was 17 µM against a strain of *Escherichia coli.* The above suggests the potential application of these membranes be incorporated in active packaging that prolongs the shelf life of foodstuff through the controlled release of the antimicrobial peptide.

Keywords: Pullulan, polycaprolactone, electrospinning, multilayer membranes, LfcinB (21-25)$_{Pal}$ peptide.

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Símbolo	Término	Escala
µm	Micrómetro	10^{-6}m
nm	Nanómetro	10^{-9}m

Abreviaturas

Abreviatura	Término
%p/p	*Porcentaje en peso/peso*
% p/v	*Porcentaje en peso/volumen*
AMP	*Péptidos antimicrobianos*
ATR-FTIR	*Espectroscopia infrarroja por transformada de Fourier por la técnica de reflectancia total atenuada*
CF	*Cloroformo*
DPPH	*2,2-difenil-1-picrylhydrazyl*
DSC	*Calorimetría Diferencial de Barrido*
ETA	*Enfermedad transmitida por Alimentos*
HPLC	*Cromatografía de Capa Líquida*
LfcinB	*Lactoferricina Bovina*
LOD	*Límite de Detección*
LOQ	*Límite de Cuantificación*
MPS	*Almidón modificado de papa*
PEO	*Oxido de polietileno*
PCL	*Policaprolactona*
PUL	*Pululano*
SEM	*Microscopia electrónica de barrido*
TGA	*Análisis Termogravimétrico*

Introducción

Las pérdidas de alimentos constituyen una preocupación económica en la industria alimentaria. Para el año 2018, tales desperdicios se estimaban en alrededor de 1.300 millones de toneladas al año, que junto a brotes de enfermedades transmitidas por alimentos (ETA) representan una gran amenaza a la salud pública y la seguridad alimentaria (1),(2). La presencia de microorganismos de deterioro en las materias primas y en los alimentos frescos y procesados es una de las principales causas de pérdida, que típicamente surge como resultado de malas prácticas de cosecha, manipulación, almacenamiento y empaque. Esto genera alteraciones en las características sensoriales y nutricionales de los productos. Es por lo anterior que la industria busca que los empaques actuales no solo desempeñen una barrera física entre el producto y el ambiente (3), sino que tengan propiedades tales como la eliminación de sustancias indeseables o la liberación de agentes conservantes, como es el caso de los empaques activos (4), y de esta manera obtener alimentos frescos y con una mayor vida útil.

El principio de los empaques activos es el uso de materiales poliméricos que contienen agentes que imparten características antioxidantes, antimicrobianas y propiedades de eliminación de compuestos indeseables en los alimentos. Estas matrices funcionan como empaques secundarios que tienen el potencial de liberar diferentes compuestos para aplicaciones diversas en la industria, siento la liberación de sustancias con capacidad antimicrobiana una de las más usadas (4). Una de las estrategias para elaborar este tipo de empaques consiste en desarrollar un proceso de encapsulación por medio del cual se atrape un agente activo en una matriz polimérica produciendo membranas ultrafinas por la unión de fibras de tamaño micro o nanométrico que posteriormente son incorporadas en un empaque con el fin de brindarle atributos que permitan el control de crecimiento de microorganismos (4),(5). Las fibras pueden obtenerse por medio de la técnica de electrohilado o *electrospinning*, un proceso electrohidrodinámico mediante el cual un polímero en solución puede ser hilado en fibras continuas con diámetros que varían desde un micrómetro hasta unos pocos nanómetros utilizando una diferencia de voltaje (6), (7), (8).

Sin embargo, en la actualidad existen limitantes en el uso y aplicación de las membranas micro/nanofibrosas, entre estos la solubilidad de las membranas en medios acuosos especialmente si éstas son elaboradas de polímeros con un carácter hidrofóbico, puesto

que se quiere emplear en alimentos que naturalmente cuentan con una alto contenido de humedad y actividad de agua. El contacto de una membrana elaborada a partir de un polímero hidrófilico que a su vez contiene un agente activo, va a provocar una liberación inmediata de dicho agente y por ende la eficacia del tratamiento se perderá al cabo de un corto tiempo de almacenamiento. Es por esto, que el reto tecnológico es desarrollar una membrana con menor grado de solubilidad en agua, pero con la capacidad de liberar de manera progresiva el agente activo durante el máximo tiempo posible de almacenamiento del producto.

Uno de los polímeros que ha sido utilizado para la elaboración de estas estructuras es el pululano (PUL), un polisacárido de origen fúngico altamente hidrofílico, que en soluciones acuosas solubiliza rápidamente. A partir de éste es posible obtener materiales con fibras de tamaños muy finos sin necesidad de usar disolventes tóxicos, debido a que al ser muy soluble en agua las soluciones son preparadas en este solvente (9). En ese sentido, se requiere profundizar en el empleo de mezclas de polímeros con características similares al PUL pero con solubilidad en agua más baja. Por lo tanto, en este trabajo se desarrollaron membranas ultrafinas con una potencial aplicación como empaque secundario mediante la técnica de electrohilado, a partir de mezclas de un polímero natural como β-glucano, almidón modificado de papa (AMP) y PUL con un polímero sintético como la policaprolactona (PCL), debido a que esta última posee una alta hidrofobicidad y una buena capacidad de adsorción de los compuestos encapsulados para la fabricación de fibras ultrafinas en medio acuoso (10), mejorando así la encapsulación y liberación prolongada de un péptido que actúa como agente antimicrobiano (11). Se realizó una caracterización de la morfología, estructura química y estabilidad térmica de las membranas obtenidas con el fin de obtener la mejor formulación.

Posteriormente, se determinó si el PUL junto con la PCL eran capaces de soportar la inclusión de un péptido antimicrobiano (LfnB) sintetizado a partir de lactoferricina bovina , una glicoproteína de unión al hierro excretada a partir se secreciones de mamíferos como leche materna, saliva, moco bronquial y cervical. Esta proteína tiene una alta actividad biológica puesto que se ha encontrado que tiene actividades antimicrobianas, antitumorales y antiinflamatorias (12). Luego de realizar el ensayo de incorporación de este tipo de compuestos se volvió a realizar una evaluación de las características de estas membranas ultrafinas, pero con el péptido LfcinB (21-25)$_{Pal}$ incorporado. Lo anterior es

importante debido a que estas membranas pueden ser potencialmente empleadas como dispositivos secundarios en los empaques, en alimentos con alto contenido de agua como por ejemplo frutas y hortalizas, bridándole a la industria de alimentos y del embalaje una alternativa en la prolongación de la vida útil de este tipo de alimentos sin afectar sus características iniciales.

Este trabajo se divide en cuatro capítulos, los cuales están compuestos de la siguiente manera:el primer capitulo es un artículo de revisión corto sobre los estudios más relevantes en el uso de membranas nanofibrosas para encapsular agentes antimicrobianos y su evaluación *in vitro* e *in vivo* con el objetivo de extender la vida útil de los productos alimenticios, el cual fue publicado en el *Italian Journal of Food Science*. El segundo capítulo es un artículo de revisión sobre el estado del arte hasta la fecha en la incorporación de agentes antimicrobianos en fibras obtenidas a partir de polímeros sintéticos, naturales y mezclas de ellos para su uso en empaques activos, el cual fue publicado en el *International Journal of Polymeric Materials and Polymeric Biomaterials*. El tercer capítulo corresponde a un artículo científico, en donde se presentan los resultados de los ensayos de electrohilado elaborados a partir de mezclas de polímeros (naturales y sintéticos) y su caracterización estructural, química y térmica, publicado en el *Journal of Bioactive and Compatible Polymers*. Finalmente, el cuarto capítulo es un artículo científico en el que se presentan los resultados referentes a la incorporación del péptido antimicrobiano a diferentes concentraciones en las matrices nanofibrosas y la actividad antimicrobiana que tienen estas membranas frente a cepas ATCC de *Escherichia coli,* a someter en el *Journal of Food Processing and Preservation.*

Adicionalmente, a partir de los resultados obtenidos se realizó una presentación en modalidad oral titulada: "*The Use of Electrospun Nanofibers for Encapsulation of Antimicrobial Agents: Opportunities and Challenges in Food Shelf-Life Extension*" en el congreso *Shelf Life International Meeting* (SLIM 2019) (Soportes presentados en el Anexo A). De igual manera, se hizo una presentación en modalidad oral en modalidad oral titulada "*Development of nanofibers from polymer mixtures as controlled release systems of bioactive compounds in foodstuff*" en el congreso *Food Science 2019-Magnus Corpus* (Soportes presentados en el Anexo B).

Esta tesis de maestría hace parte del proyecto “Desarrollo de membranas nanofibrosas antimicrobianas con potencial aplicación en el campo de empaques activos alimentarios y productos farmacéuticos (código Hermes: 41882)” elegido en la Convocatoria para el Fortalecimiento de Alianzas Interdisciplinares de Investigación y Creación Artística de la Sede Bogotá de la Universidad Nacional, en la modalidad de apoyo a la conformación de alianzas entre dos o más investigadores pertenecientes a diferentes facultades o institutos interfacultades de la Universidad Nacional de Colombia del año 2018. Para este proyecto se cuenta con la participación de profesores de las facultades de Ciencias, Medicina, Ciencias Agrarias y el Instituto de Ciencia y Tecnología de Alimentos. Adicionalmente esta tesis recibió un apoyo de la Convocatoria para el Apoyo a la Financiación de Proyectos de Tesis para fortalecer y consolidar los programas de Doctorado y Maestría de la Facultad de Ciencias Agrarias, Sede Bogotá (Código Hermes: 46384).

Objetivos

Objetivo general

Desarrollar micro/nanofibras mediante la técnica de electrohilado a partir de la mezcla de polímeros para la incorporación de un péptido con capacidad antimicrobiana.

Objetivos específicos

- Elaborar y caracterizar micro/nanofibras a partir de la formulación de distintas mezclas de polímeros.

- Evaluar el nivel de inclusión de un péptido sintetizado a partir de Lactoferricina bovina (LfinB) en las micro/nanofibras elaboradas, en función de características fisicoquímicas y estructurales.

- Determinar la actividad antioxidante y antimicrobiana de las membranas micro/nanofibrosas con inclusión del péptido.

Capítulo 1: Incorporation of antimicrobial agents in electrospun nanofibers for use in food packaging: A review (Italian Journal of Food Science 2019, 31, 122-127)

ANTIMICROBIAL NANOFIBERS IN FOOD ACTIVE PACKAGING

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ABSTRACT

Among encapsulation techniques of antimicrobial substances, electrospinning outstands for enabling the synthesis of polymeric nanofibers that act as micro- or nanocapsules, and release systems. A myriad of polymers has been successfully electrospun to obtain ultra- thin fibers, including synthetic polymers, biomacromolecules, and their composites. In the context of food packaging, these materials have advantages such as mechanical resistance, large surface areas/ porosities, and responsiveness to external stimuli. Ultra-thin fibers have been used to incorporate antimicrobial substances. The resulting functionalized nanofibers have been proved as promising devices in a shelf-life extension of various food models. The target microorganisms against which these materials have been tested comprise *S. aureus*, *E. coli*, *Salmonella* spp., *Pseudomonas* sp., *Rhizoctonia*. In this review, a brief literature examination of the experimental evidence of electrospun nanofibers containing antimicrobials as food shelf life extenders is presented

Keywords: nanofibers, electrospinning, food active packaging, antimicrobial compounds

1. INTRODUCTION

Active packaging materials and devices are designed in order to have a relevant role in the conservation of food, beyond being a barrier between the product and the external environment (MAJID *et al*., 2016). Essential oils, polyphenol-rich plant extracts, silver nanoparticles, and antimicrobial polymers and peptides are among antimicrobial agents that have been used for active packaging purposes. These agents inhibit microorganism cellular processes, thus contributing to the improvement of food shelf life, and might represent an alternative to the use of preservatives or even thermal processing; however, many of these substances are themselves prompt to deterioration (ZHANG *et al*., 2018).
The use of nanomaterials to encapsulate antimicrobial substances represents a step forward in the design of packaging systems with controlled release of food protective agents. In this context, electrospinning is an effective and versatile electrohydrodynamic technique, used to manufacture fibers on a sub-micron to nano-scale from various polymeric materials (FUENMAYOR and COSIO, 2016). For the elaboration of these structures, a solution containing the dissolved polymer is pumped at a controlled flow rate towards a needle (spinneret) where a high voltage is applied (Sullivan *et al*., 2014). In this polymer solution, antimicrobial agents can be incorporated, in order to obtain functionalized polymer nanofibers. Thus, structures with hydrophilic or hydrophobic character will be obtained, depending on the features of the polymer and bioactive compounds incorporated in the electrospinning solution/ dispersion, offering an advantage in terms of controlled release of active agents (SOARES *et al*., 2018). The principle and mechanism of the technique have been widely studied (ZHANG *et al*., 2018; WEN *et al*., 2017), and are beyond the scope of this review. Therefore, this article presents current developments in the field of antimicrobial compounds encapsulation in nanofibers intended for food shelf life extension.

2. ENCAPSULATION AND RELEASE OF NATURAL ANTIMICROBIALS

Antimicrobial substances, especially those of natural origin, such as natural essential oils, absolutes, essences, extracts, resins, infusions, etc. are of great interest for the active packaging industry. Nevertheless, their efficient encapsulation and release represent a major challenge, considering the fact that they are very sensitive to heat, oxygen, and light. Because of their submicron to nano-scale diameter and very large surface area, electrospun fibers may offer additional advantages compared to film and sheet carriers, as they are more responsive to changes in the surrounding atmosphere, which enhances a tunable release of the entrapped compounds (VEGA-LUGO and LIM, 2009). Moreover, since the electrospinning process takes place at ambient conditions, the produced fibers are more suitable to encapsulate thermally-labile substances than fibers prepared by conventional processes, or other encapsulation methods, such as spray drying and fluid bed coating (LESMES and MCCLEMENTS 2009; XU *et al*., 2006). In this framework, electrospun nanofibers of PVA have been used to encapsulate allyl isothiocyanate (AITC) (AYTAC *et al*., 2014). Pullulan and β-cyclodextrin emulsions in water have also been electrospun for the encapsulation of volatile antimicrobials limonene (FUENMAYOR *et al*., 2013) and perillaldehyde (MASCHERONI *et al*., 2013), allowing for a controlled release of the antimicrobial, triggered by humidity. Other examples include electrospun zein, which was used to encapsulate rosehip seed oil (REO). Electrospun REO-loaded zein fibers improved the shelf-life of peeled and segmented bananas, demonstrating its potential as active packaging material (YAO *et al*., 2016).

3. ELECTROSPUN NANOFIBERS WITH *IN VITRO* EVIDENCE AS ANTIMICROBIAL FOOD PACKAGING MATERIALS

Most of the existing evidence on the potential of electrospun nanofibers as encapsulation systems for food preservation against microbial spoilage, relies on *in vitro* antimicrobial testing, as presented in Table 1.

Interestingly, most of the electrospun polymer materials within this group of reports are edible or food-grade biopolymers. *S. aureus* and *E. coli* are the most common target microorganisms for testing the antimicrobial activity of electrospun nanofibers, being representative of Gram-positive and Gram-negative bacteria, respectively (Jenab *et al*., 2017). The *in vitro* analytical methods for evaluating the antimicrobial activity of electrospun nanofibers are diverse semi-quantitative tests aimed at measuring the microbial growth after contact with the antimicrobial packaging material.

The disk diffusion method is a technique often selected. However, according to Espitia and Andrade (2015), this method might present slight variations from one experiment to another due to target microorganisms, and experimental variables, such as incubation time and temperature. Other microbiological techniques include the optical density (OD) measurement, the colony counting method and the broth microdilution method for determining inhibitory minimum concentration (MIC). In other studies, viability and bacterial adherence have been determined qualitatively in terms of biofilm formation capacity (CLAVIJO-GRIMALDO *et al*., 2019).

Table 1. Electrospun nanofibers intended as antimicrobial food packaging tested *in vitro*.

Polymeric nature of nanofibers	Incorporated compounds	Target microorganisms	Antimicrobial test*	Reference
Chitosan nanofibers	Poly(ethylene oxide) and silver nitrate, as a co-electrospinning polymer and silver nanoparticle precursor	*E. coli*	Disk diffusion method	(Annur *et al*., 2015)
Blend of Poly(lactide-co-glycolide) (PLGA) and Chitosan	The nanofiber was functionalized with graphene oxide decorated with silver nanoparticles	*E. coli* *Pseudomonas aeruginosa* *S. aureus*	Colony counting method	(Faria *et al*., 2015)
Chitosan–polyethylene oxide	zeolitic imidazolate framework nanoparticles	*S. aureus* *E. coli*	Colony counting method - AATCC test method 100–2004	(Kohsari *et al*., 2016)
PLA and glycidyl methacrylate	Cellulose nanocrystals Lignin nanoparticles	*Pseudomonas syringae pv. tomato*	Colony counting method	(Yang *et al*., 2016)
Cellulose	Lysozyme	*Listeria monocytogenes* *Yersinia enterocolitica*	OD measurement	(Bayazid *et al*., 2018)
Pullulan	Palindromic peptide Lfcin B	*E. coli* *S. aureus*	Disk diffusion method to determinate the MIC	(Román *et al*., 2019)
Tragacanth	Peppermint oil	*E. coli* *S. aureus*	Disk diffusion method	(Ghayempour and Montazer, 2019)
Zein	Curcumin	*E. coli* *S. aureus*	OD measurement	(Wang *et al*., 2019)

*OD: Optical density; TSB broth: Tryptic soy broth; Minimum Inhibitory Concentration (MIC).

4. ELECTROSPUN NANOFIBERS FOR FOOD SHELF LIFE EXTENSION

The application of these materials in food matrixes is more important, but still limited, as presented in Table 2. Target microorganisms include Gram-positive such as *L. monocytogenes* and *S. aureus*, as well as Gram-negative such as *E. coli* and *Salmonella* (Erbay *et al*., 2017). Antimicrobial studies in food matrixes also include the electrospun nanofibers effect against fungi, such as *Aspergillus niger* and *Penicillium* (Table 2).

Table 2. Electrospun nanofibers potential applications in food preservation.

Polymeric nature of the electrospun nanofiber	Incorporated substances	Target microorganisms	Food matrix	Reference
Polyvinyl alcohol	cinnamon essential oil /β-cyclodextrin inclusion complex	*E. coli* *S. aureus*	Strawberries	(Wen *et al*., 2016b)
Poly(ε-caprolactone)	*Urtica dioica* L. extract incorporated into Whey Protein Isolated complex at different concentrations	Total aerobic mesophilic bacteria lactic acid bacteria	Rainbow trout fillets	(Erbay *et al*. 2017)
Blend of polyvinyl alcohol and β-cyclodextrin	Cinnamon essential oil and lysozyme	*Penicillium* *Salmonella enteritidis* *Aspergillus niger*	Strawberries	(Feng *et al*. 2017)
Gelatin	Rosemary essential oil	*Campylobacter jejuni*	Chicken	(Lin *et al*., 2018)
Chitosan	Chrysanthemum essential oil	*L. monocytogenes*	Meat	(Lin *et al*. 2019)
Gelatin	Moringa oil	*L. monocytogenes* *S. aureus*	Cheese	(Lin *et al*. 2019)
Silk fibroin	Thyme essential oil	*Salmonella typhimurium*	Poultry meat	(Lin *et al*. 2019)

Results from the application of antimicrobial nanofibers in packaging with food matrixes ensure the potential application of these materials in food preservation. However, more studies are needed in order to elucidate the release mechanism of antimicrobial agents from the packaging material to the food matrix, as well as the molecular interaction between the antimicrobial agent, the target microorganisms and the macro and microstructural components of the food matrix.

CONCLUSIONS AND FUTURE PERSPECTIVES

There is substantial evidence on the potential of antimicrobial nanofibers for food preservation. However, further experimental evidence on wider variety of food models is needed. Moreover, studies on the use of nanofibers for bioactive peptides and beneficial microorganism encapsulation is limited, which highlights a future trend. Finally, the lack of actual evidence on the toxicity of nanofibers as food contact materials, as well as the availability of electrospinning setups for industrial applications, are important challenges of this technology.

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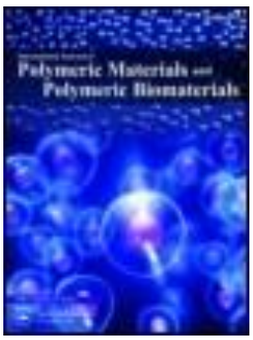
Capítulo 2: Electrospinning of ultra-thin membranes with incorporation of antimicrobial agents for applications in active packaging: A review (International Journal of Polymeric Materials and Polymeric Biomaterials)



International Journal of Polymeric Materials and Polymeric Biomaterials

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gpom20

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To cite this article: Ingrid Juliet Rodríguez-Sánchez , Carlos Alberto Fuenmayor , Dianney Clavijo-Grimaldo & Carlos Mario Zuluaga-Domínguez (2020): Electrospinning of ultra-thin membranes with incorporation of antimicrobial agents for applications in active packaging: a review, International Journal of Polymeric Materials and Polymeric Biomaterials, DOI: 10.1080/00914037.2020.1785450

To link to this article: https://doi.org/10.1080/00914037.2020.1785450

Published online: 03 Aug 2020.

INTERNATIONAL JOURNAL OF POLYMERIC MATERIALS AND POLYMERIC BIOMATERIALS
https://doi.org/10.1080/00914037.2020.1785450

Electrospinning of ultra-thin membranes with incorporation of antimicrobial agents for applications in active packaging: a review

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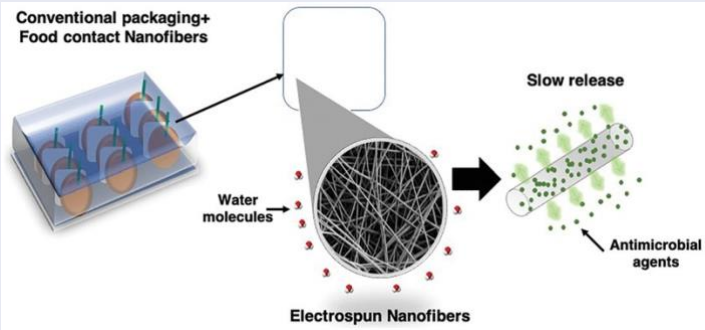
ABSTRACT

Electrospinning refers to a technique for producing nano- and micrometric-scale fibers using high voltage. It is a novel and useful technique applied over the last few years in the areas of food processing and medicine due to its versatility and low cost in the development of ultra-thin struc- tures from natural and synthetic biopolymers, facilitating the incorporation, transport, and release of preservation agents, in particular antimicrobial compounds. These membranes find application in food and medical areas where antimicrobials inhibit cellular processes of harmful biological agents. In that sense, this study provides a comprehensive review of some recent developments and technological aspects of antimicrobial ultra-thin electrospun membranes for potential use in the food and pharmaceutical industry, with a particular approach based on biopolymers.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 14 January 2020
Accepted 17 June 2020

KEYWORDS
Active packaging; antimicrobial agents; electrospinning; nanofib- ers; polymers

1. Introduction

Ultra-thin electrospun materials have been frequently studied given their applications in medical and food fields due to their high surface area, diversity of polymers, process simplicity and ability to encapsulate active agents. This has allowed these structures to be used in the development of food packaging, wound dressings, and in the administration of drugs to respond to the growing challenges reported in the last decade for each one of those areas[1]. The advance- ment in the use of ultra-thin membranes has led to intro- ducing nanotechnology for the manufacture of packaging systems and wound dressings with controlled release of active agents, using natural components with no effect on human health. In particular, electrospinning is an effective and versatile electrohydrodynamic technique, used to elabor- ate fibers on a micro-nanoscale from various polymeric materials. For the elaboration of these structures, a solution containing the dissolved polymer is pumped at a controlled flow rate toward a needle (spinneret) where a high voltage is applied. At certain conditions, this produces a phenomenon known as Taylor cone, which causes the ejection of fine solution jets, with the consequent formation of ultra-thin solid polymer fibers as a consequence of the rapid solvent evaporation. In the end, the fibers are deposited on grounded collectors as nanofibrous materials[2]. The process is carried out at room temperature and the incorporation of bioactive substances is done by adding the compound in the polymeric solution, which is later electrospun and encapsu- lated in the polymer matrix. The principle and mechanism of the technique have been extensively studied, being these aspects beyond the scope of this review and available in spe- cialized bibliography[3–7]. According to the physicochemical properties of the polymer and other substances included in

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Table 1. Comparison of methods for the elaboration of nanofibers[17].

Technique	Advantages	Disadvantages
Electrospinning	• Easy to install • Economic • Wide selection of materials • High level of versatility (diameter, microstructure)	• Toxic solvents often used • Pore with two-dimensional microstructure
Drawing	• Wide selection of materials • Simple procedure	• Low productivity (one fiber at a time) • Difficult to form fibers with a consistent diameter
Self-assembly	• Three-dimensional pore layout • Useful for *in-vivo* mounting • Easy incorporation of cells during fibers formation	• Complex procedure • Limited fiber diameter (2-30 nm)

the electrospinning solution, fiber structures with hydrophilic or hydrophobic character would be obtained. This offers an advantage in terms of a more controlled release of active agents, which in turn ensures greater action on food preservation and wound recovery[8].

According to the data presented by the United Nations Food and Agriculture Organization (FAO), in 2018 the world wasted 1.300 billion tons of food, of which 54% corresponded to losses generally produced in postharvest, storage, and industrial processing stages, and 46% corresponded to waste generated in the stages of distribution, sale, and consumption. These factors are determinant in food safety since they are associated with agricultural practices, food composition, manipulators, consumer practices, chemical products, environment, and etiologic agents such as viruses, bacteria, and fungi[9]. In the particular case of the spoiled food caused by the presence of microorganisms, products tend to have a greater deterioration when there are scarce controls in the conditions of temperature, oxygen or humidity, which represents a threat to public health since this could lead to the increase in foodborne diseases[10]. The presence of altering microorganisms in food entails changes in nutritional and sensory characteristics, such as oxidation, the production of unpleasant flavors and aromas, as well as undesirable modifications in texture and color that compromise shelf life and the perception of food from consumers[11]. Based on the above, the recent technological advances in material science have led to the development of active packages: polymeric materials containing additives with the potential to release active agents (antioxidants, antimicrobials) and/or to retain spoiling compounds (ethylene, oxygen, and water). The active package is designed to allow a controlled release of the agent, having a relevant role in the conservation of food beyond being a passive barrier between the product and the external environment[12].

On the other hand, chronic wounds generate a notable burden on the global health system representing expenses of up to 20.4 billion USD because skin wounds are susceptible to be invaded by bacteria causing infections that impedes tissue regeneration. Nanofibrous dressings have been developed to decrease the likelihood of the appearance of infections, by accelerating the healing process, in part thanks to their biocompatibility. Nanofibrous dressings are mainly made from biodegradable compounds and are intended to be biomimetic in the structure of the cell matrix[13]. Additionally, with the constant evolution of resistant bacteria, the administration systems of antibiotics have been gradually replaced by nanofibrous antimicrobial dressings. This emerging class of wound dressing comprises biopolymeric nanofibers containing antibacterial nanoparticles, nature-derived compounds, and biofunctional agents[14].

The substances with greater potential to act as encapsulated active agents include enzymes, peptides, essential oils, volatiles, metal nanoparticles, amongst others. These substances inhibit cellular processes of microorganisms, being a great alternative to food preservation[8] and associated infectious risks. In particular, peptides are the most common group of compounds used as antimicrobial agents, whose main function is to inhibit the proliferation of pathogenic microorganisms. Those antimicrobials are capable of disturbing microbial metabolism, synthesis of nucleic acids and proteins and thus achieve the inhibition of enzymatic activity[15].

Based on the above, and considering the advances of electrospinning for encapsulation of antimicrobial compounds, this review introduces the following objectives: (1) To present a global overview of recent developments on ultra-thin membranes with the inclusion of antimicrobial compounds, and (2) To recognize the potential use in the food and medical areas as an alternative to food preservation and disease treatment, respectively. Therefore, a review of the concept of ultra-thin fibers, polymeric materials with a particular focus on biopolymers, and antimicrobial compounds commonly used in the food industry in active packaging systems and the medical area through the use of wounds dressings and delivery drugs. This will provide an updated perspective of the advantages these structures offer for encapsulating active agents.

2. Functionalization of nanofibers

Nanofibers are nano-scale, elongated, thin strands obtained by techniques, such as (i) self-assembly: a process by which molecules are organized into patterns or structures through non-covalent forces, such as hydrogen bonds, hydrophobic forces and electrostatic interactions; (ii) drawing: an automated technique using a polymer solution dispensed in a pipette while contacting a substrate and moving in an x-y direction in order to form suspended nanofibers; and (iii) electrospinning: the most widely used technique, because it can control more variables in the production of nanofibers. These structures are elaborated from the use of an electric field in polymer solutions[16]. In Table 1, the comparison of techniques for the production of nanofibers is shown[17].

In particular, the electrospinning equipment consists in a syringe which serves as the reservoir for the polymer

solution, metallic needle to dispense the solution and also works as a positive electrode, a high voltage power supply unit and a grounded metallic collector to collect the nanofibers[18]. The principle work of the nanofibers elaborated by electrospinning are formed from a liquid polymer solution, and fed through a capillary tube in a region of high electric field, which is generated by connecting a high voltage power source in the range of kilovolts to the tip of the capillary, producing electrostatic forces that exceed the surface tension of the liquid and creating a Taylor cone and a thin jet stream. Some strong movements are produced that lengthen and thin the jet, and then nanofibers are obtained in a collector[17].

Moreover, the electrospun nanofibers offer advantages in structural terms because they have characteristics such as submicrometer and nanometer size, high porosity, high surface-to-volume-ratio, and intertwined fibrous structure which favors not only the contact of the fibers with the product of interest but a greater diffusion of the encapsulated products on the matrix in which the fibers are tested. Similarly, the nanofibers offer functional advantages such as sustained and controlled release, reduced denaturation, efficient encapsulation, enhanced stability of bioactives and this structure can be elaborated from food-grade polymers or biopolymers this contributes to greater protection and guarantee the stability of active compounds that are encapsulated[6]. The morphologies and characteristics of electrospun nanofibers can be modulated by the optimization of technological parameters (flow rate, voltage, spinning distance) and solution parameters (viscosity, surface tension, electric inductivity)[19].

3. Electrospinning of nanofibers from polymers in active packaging

The nanofibers can be composed of synthetic or natural polymers, having these latter structures biodegradable characteristics[20]. Therefore, electrospinning offers advantages in terms of ease in scaling, reproducibility, repeatability, and application in different areas, also allowing the production of long and continuous fibers with diameters between 10 and 1,000 nm, with high strength, elasticity and surface area, facilitating the loading of bioactive molecules and the transport of nutrients[21].

In spite of this, it is a process affected by some processing parameters, since varying the conditions of the technique may conduce to obtaining different morphologies, such as fibers with pores, flattened, branched, elliptical and hollow[18]. Similarly, the properties of the polymer solution, mainly nature, and solubility, affect the process because it might be obtained fibers with different morphology and size, in addition generating changes in the conditions of viscosity, electrical conductivity, load and surface tension[3].

Some materials used in electrospinning include natural or synthetic ingredients, or mixtures of them. Specifically, within natural polymers or biopolymers are found polysaccharides, proteins, and lipids widely used in the food industry because they exhibit low toxicity, high biocompatibility and biodegradability, compared to synthetic polymers[4].

There are currently technological challenges that limit the use and application of nanofibers in food packaging, wound dressing and drug delivery, including the solubility of the same fiber because most of the polymers used for manufacture have a high hydrophilic character, which hinders the prolonged release of the compounds and limits the protective action of this compounds[22]. Despite this, nanofibers present an attractive potential, thus, within the latest trends in this technique is the use of dietary fiber because these compounds are highly hydrophobic, offering an alternative in reducing solubility. Additionally, due to the high adsorption capacity, dietary fibers could be useful in the stability of the nanofiber since they would improve the encapsulation and release of bioactive compounds[23].

3.1. Natural polymers

The natural polymers are compounds from nature whose fundamental components are the macromolecules formed by the repetition of small molecules called monomers. Among these large molecules are polysaccharides and proteins.

Polysaccharides are the most important biopolymers in food, formed by monosaccharides linked together by glycoside bonds. Polysaccharides can be applied in the food industry given their advantages of nontoxicity, edibility, and biodegradability[24]. Similarly, films made from polysaccharides are transparent, odorless, tasteless, semi-permeable to carbon dioxide and resistant to the transmission of oxygen, functioning as a barrier[25].

Polysaccharides have a wide range of molecular properties in weight, structure and solubility, important factors when applied in biopolymer-based management systems. Delivery systems can be used not only to prevent degradation of the encapsulated components during processing and storage but also to promote a continuously and slowly release[26]. Among the most used polysaccharides for the elaboration of nanofibers are pullulan, *b*-glucan, modified starch, chitosan, or dextran, among others.

For other hand the production of nanofibers from proteins by electrospinning still remains a challenge, because their chemical composition is extremely complex, thus, their three-dimensional structure and polyelectric characteristics is often a disadvantage[27]. Currently, proteins are used in electrospinning by making mixtures with other polymers and several solvents[28]. Following, some proteins used in the elaboration of nanofibers for food and medical purposes are presented.

3.1.1. Pullulan

It is an extracellular polysaccharide produced by the fungus *Aureobasidium pullulans*. It is a nontoxic, non-mutagenic, tasteless and edible compound, mainly composed of maltotriose units linked by *a*-(1.4) and *a*-(1.6) bonds in a 2:1 ratio. This linkage provides oxygen with adhesive properties

to form fibers and films strong and impervious. In addition, pullulan increases the viscosity, decreases surface tension and electrical conductivity favoring also the formation of fibers[29].

Pullulan has been widely used in the food industry in the form of edible food bags that dissolves in water or in hot liquids, that is, pullulan functioned as an edible coating applied directly to the surface of the food that could be eaten with the product. Similarly, it has been used as an encapsulant for drugs due to its biocompatibility and low cytotoxicity. That is why, since then, research has been car- ried out on the functional properties of pullulan in films, coatings, and elaboration of fibers for application in food and medical packaging (see Table 2)[37].

According to Cordenonsi et al., electrospun nanofibers made from pullulan can be used in wound healing by encapsulating a lysate of human platelets for skin repair. Because this polymer is biocompatible and biodegradable, offering adequate mechanical properties in terms of elasti- city, good cellular adhesion, and controlled release of plate- lets due to the hydrophilic character in mediums with high water content[38].

3.1.2 b-Glucan

b-Glucan is a natural polysaccharide formed by more than 250,000 units of D-glucose joined by a *b*-glucosidic bond. This compound is used in the elaboration of fibers given its antioxidant properties, high viscosity and water solubility[39]. According to Jayachandran et al., it has been seen that *b*-glucans are nontoxic compounds and thus are widely used in the food industry as present in various vegetable sources such as oats, algae, barley, fungi and yeasts[40]. Moreover, *b*-glucan is used in functional foods since it helps to regu- late intestinal microbial flora, and also has a notable func- tion as emulsifiers, stabilizers, and gelling agent. *b*-Glucans from oats and barley are the most used for these purposes[41].

b-Glucans have been used in biomedical fields due to their antitumor, immunomodulating and metabolic proper- ties as they reduce cholesterol and glucose levels, demon- strating the usefulness as a dietary supplement and as a polymer in the manufacture of bandages and films for drug administration[42]. In the foodstuff, it has been used to improve the stability, texture and shelf life of foods, replac- ing certain additives or artificial texturing agents. However, in the food packaging industry, there are not many studies exploring in depth the properties for the elaboration of nanofibers[40].

According to Grip et al., electrospun nanofibers from *b*-glucan can be used as dressings to improve wound heal- ing, because this polymer induces the response of the innate immune system, improving immunostimulant activity in human and animal cell lines by promoting tissue regener- ation. In turn, the nanofibers of this polymer, due to their high surface-to-volume ratio, allows nanocarriers or active agents to be encapsulated and released in a controlled man- ner on wounds thus reducing the risk of infection and accel- erating the healing process[39].

3.1.3. Modified starch

It is a polysaccharide produced from natural starch by phys- ical, enzymatic or chemical treatment, improving properties such as heat resistance and reducing the sensitivity to pH and salt concentrations. The enzymic modification of starch implies the use of debranching enzymes to hydrolyze amylo- pectin and produce short linear starch molecules that result in high amounts of resistant starch. This polysaccharide is widely used in the food industry as a gelling agent, thickener and stabilizer[43].

According to Thillaipandian et al., starch is an emergent polymer in biomedical research because of its easy availabil- ity, low cost, and biological values. It has been used in appli- cations such as biomedical engineering in drug administration and tissue engineering. Gutiérrez-Sánchez et al. used starch scaffolds obtained by electrospinning for potential bone regeneration, offering sufficient mechanical, elastic stability, as well as biocompatibility, biodegradability. Membranes did not generate toxic effects, being usable in biomedical applications mixed with biosynthetic polymers for cell proliferation, differentiation, and genetic expression[44].

However, it has currently been difficult to achieve fibrous forms from starch because of starch low resistance, water resistance, thermal instability and low processing cap- acity[45]. Similarly, for nanofiber formation, pure native starch has low stability to water and weak mechanical prop- erties, thus, it is difficult to develop nanofibers which limit applications[46]. According to Guodong et al., there are few reports of nanofibers made from modified starch, since when amylose and amylopectin are modified, a limited molecular movement occurs with insufficient freedom or ability to align or associate. These factors negatively influ- ence the process of electrospinning and the resistance of the nanofibers[47].

On the contrary, according to Liu et al., modified starch compared to synthetic polymers is more hydrophilic and can be absorbed by the human body without causing toxic effects[48]. The amylose present in this structure has enough freedom of molecular movement which allows molecules to form hydrogen bonds and therefore facilitates electrospin- ning since it has been reported that when using pure modi- fied starch, continuous and smooth fibers are obtained on several nanoscales[47]. In consequence, the development of modified starches may be an adequate approach to create nanofibers with better properties in terms of solubility and resistance[41].

3.1.4. Chitosan

It is a cationic alkaline polysaccharide, obtained from the deacetylation of chitin. Chitosan has good film-forming abil- ity, biodegradability, and antibacterial activity. Therefore, it is an ideal polymer for applications in the food industry, being non- soluble in water or in alkaline solution. However, by having a polycation nature, chitosan promotes an increase in the surface tension making difficult the spinning process, thus it is usually mixed with other types of poly- mers in order to improve the capacity for fiber

Table 2. Previous studies on the use of pullulan in the elaboration of nanofibers by the electrospinning technique.

Application	Test conditions	Result	Reference
Ultrafine fibers obtained by electrospinning from different mixtures of amaranth protein isolate (API) and pullulan with or without surfactant Tween 80	Solvent: formic acid at 95% Voltage: 15 kV Feed Flow: 0.4 mL/h Collector distance: 10 cm	Pullulan improved physicochemical properties such as viscosity, surface tension and conductivity, in the fibers obtained. Fiber diameters around 300 nm.	[30]
Ultrafine electrospun fibers from two different mixes of amaranth protein isolate (API) and pullulan, loaded with two different concentrations of curcumin (0.05% and 0075%)	Solvent: formic acid at 95% Voltage: 22 kV Feed flow: 0.4 mL/h Collector distance: 10 cm	Pullulan showed high efficiency in terms of viscosity and surface tension. A rapid release of the active compound was generated in the first 50 min. Fiber diameters between 200 and 500 nm.	[31]
Different concentrations (0.5%, 1%, 1.5% w/v) of tea polyphenols were incorporated in pullulan-carboxilmetil sodium cellulose solutions for the conservation of strawberries	Solvent: Distilled water Voltage: 21 kV Feed flow: 0.6 mL/h Collector distance: 15 cm	There was a significant effect on life prolongation with a concentration of 1.5% w/v polyphenols, of up to 10 days. Fiber diameters between 80 and 200 nm.	[32]
Electrospun fibers of pea protein mixtures (*Pisum sativum*) and pullulan	Solvent: HCl 1N o NaOH 1N Voltage: 15 and 22 kV Feed flow: 0.4 and 1 mL/h Collector distance: 10 and 15 cm	Pullulan improved viscosity, and decreased surface tension and electrical conductivity, which favored the formation of more resistant fibers. Fiber diameters of 149 and 170 nm.	[29]
Pullulan aqueous solution fibers (10%) and alginate obtained by electrospinning	Solvent: Distilled water Voltage: 30 kV Feed flow: 0.3 mL/h Collector distance: 15cm	Alginate increased crosslinking in nanofibers, giving more stable, smooth and ultrafine structures. Fiber diameters of 247 nm.	[22]
Fast dissolving oral films for drug delivery prepared from pullulan/chitosan electrospinning nanofibers	Solvent: Distilled water Voltage: 15 kV Feed flow: 0.5 mL/h Collector distance: 10cm	The combination of the polymers generated a good stability of the films which allowed aspirin to be encapsulated and successfully released into the oral mucosal. Fibers diameters from 70 to 134 nm.	[33]
Development of pullulan nanofibers such as alternative for ocular drug delivery	Solvent: Distilled water Voltage: 12 kV Feed flow: 0.75 mL/h Collector distance: 10cm	The high porosity and interconnectivity of the fibers allowed the penetration of water and subsequently release of the encapsulated drug. Fibers diameters of 225 to 450 nm.	[34]
Application of tanic acid/ pullulan nanofibers in wound dressing	Solvent: Ethanol:methanol (7:3) Voltage: 15 kV Feed flow: 2 mL/h Collector distance: 12 cm	Resistant, flexible fibers were obtained with adhesive properties. Cytotoxicity in fibroblast cells was not evident. Fibers diameters of 650 to 750 nm.	[35]
Carboxymethyl pullulan membranes in vascular endothelial cell proliferation	–	Scaffolds composed of pullulan were able to adhere to vascular cells and promoted cell proliferation while being biocompatible with this tissue.	[36]

formation[49]. Geng et al. noted that the increase in the concentration of acetic acid reduced the surface tension of the chitosan solution, making it an important additive for electrospinning[49].

Moreover, this polymer also has such medical applica- tions as in the treatment of wound infections. Abid et al. used nanofibers made with chitosan and polyethylene oxide to manage burn infections. Chitosan had a notorious antimicrobial activity, which enhanced when the fibers were loaded with drugs. These structures generated a sustained release of the encapsulated antibacterial agent and addition- ally provided a moist environment helping to facilitate heal- ing, a hemostatic effect by the presence of chitosan, and good absorption of the exudate from the wound. It was con- cluded that 1 g/mL of the drug allows a similar response as when high doses of antibiotics were used[50].

Based on this, the development of pure and stable chitosan nanofibers has been considered a challenge due to the aforementioned parameters. Table 3 shows some of the conditions for electrospinning and the types of solvents that were used to achieve the spinning of this polymer, in add- ition to the applications of these fibers for food and med- ical purposes.

3.1.5. Dextran

Dextran is a natural branched bacterial polysaccharide produced by *Leuconostoc mesenteroides*, which consists of α-1,6-D-glucopyranose bonds with side chains attached to other α-1,2, α-1,3 or α-1,4 chains. It is a soluble in water and organic solvents polymer, it is biodegradable and biocompat- ible. In the presence of water, it is able to form a high vis- cosity solution and thus it has been widely used as a thickening and stabilizing agent[58].

For the elaboration of nanofibers by electrospinning, it is necessary to prepare a solution with glutaraldehyde in order to generate a cross-linking, or it can be used a mixture of ethanol, deionized water and, phosphate buffer saline when nanofibers of sizes between 200 and 250 nm are required, all this to generate a better ability of the solution to produce nanofibers with a controlled release of encapsulated com- pounds in time ranges between 1 week and 1 month[4].

On the other hand, nanofibers elaborated from dextran have been used in the encapsulation of compounds such as vitamin E, where it has been seen that they offer greater stability to the mechanical properties and a good visual acceptability thanks to the ability to retain water. In addition, ultrafine fibers of dextran can be used to trap hydrophobic compounds, thus, they have a higher potential in the design of new functional products for the food industry and delivery drugs[59].

Furthermore, dextran has been used in the development of wound dressings. Rajan et al. developed dressings made from dextran nanofiber mats loaded with estradiol for the treatment of postmenopausal wounds because this polymer was biodegradable, non-immunogenic and non-antigenic, therefore, useful in the administration of drugs and scaffolding for tissue engineering. It was evident that the nanofibers composed of dextran promoted the process of wound cicatrization because it contributed to cell proliferation and the production of epithelial growth factors[60].

Innocenti et al. found that dextran presents a limitation related to a high solubility in water which can be overcome by the use of crosslinkers to form three-dimensional structures. In this study, boric acid worked as an effective, ecological and nontoxic crosslinker in high concentrations, therefore it was found that the fibers of pure dextran produce soft fibers without pearls, with good thermal stability and capacity for encapsulating and releasing active compounds, under controlled conditions[61].

3.1.6. Gelatin

Gelatin has been widely studied alone and in combination with synthetic polymers, since it is a promising material for tissue engineering, however, due to the gelling processes that occur in this polymer, gelatin is able to form a triple helix that generates difficulties during electrospinning, therefore it becomes necessary to use a combination of solvents and parameters such as temperature and pH to destabilize the formation of the triple helix. In Table 4, some examples of solvents commonly used in the elaboration of gelatin-based nanofibers and the conditions in the electrospinning process are presented.

3.1.7. Zein

The main maize storage protein is zein, which contains a large α-type helix. This α-helix is formed by hydrogen bonds between the hydroxyl groups and the amino groups in the main peptide chains, therefore, zein has a great hydrophobi- city[62,63]. It has been seen that nanofibers made from this protein exhibit high stability against heat and have a low absorbance of water during storage stages, thus, they are suitable for packing dried food[58].

Additionally, Jiang et al. reported zein has been used as a release system for different drugs because it has good elasticity, biodegradability, and cellular biocompatibility. Likewise, it is also resistant to digestion processes, which favors the delivery of medicines in a specific site providing desired profiles for drug release[68]. Aslam et al. encapsulated resver- atrol in zein due to its chemical instability, finding an improvement in the photostability, release, and bioaccessibil- ity of the bioactive compound for the treatment of chronic diseases[28].

Neo et al. have reported it is necessary to make solutions of 25% (p/p) of zein in an 80% ethanol aqueous solution and adding gallic acid in the mixture to develop nanofibers, varying their size between 327 and 387 nm. In addition, the electrospun structures of zein can have a potential applica- tion in dried-food packaging, exhibiting good stability against heat and chemicals, as well as having a low absorb- ance of water during storage at 21 °C for 60 days with a rela- tive humidity of approximately 58%[69]. Similarly, Fuenmayor and Cosio reported that it was necessary to make a solution of (25% w/w) in a hydroalcoholic solution (ethanol: water 4:1) to encapsulate phenolic compounds as gallic acid (GA) and naringenin (NAR), the nanofibers

Table 3. Studies on the application of chitosan in the food or medical areas.

Application	Test conditions	Result	Reference
Optimization of the electrospinning process for the elaboration of nanofibers of chitosan solutions of acetic acid in aqueous solution of 70–90%	Solvent: Acetic acid Voltage: 17 kV Collector distance: 16 cm	Complex process due to the polycationic character of the chitosan and its high molecular weight. Nanofibers were strongly affected by the conditions of electrospinning, as well as the concentration of solvent. Fibers diameters of 140 nm.	[47]
Preparation of *e*-poly-lysine/chitosan nanofibers for inhibition of *Salmonella typhimurium* and *Salmonella enteritidis* in chicken	Solvent: Acetic acid Voltage: 15 kV Feed flow: 0.5 mL/L Collector distance: 15 cm	Fibers with uniform characteristics, smooth and without granules or fractures were obtained. Values of significant inhibition of approximately 24 mm corresponding to 3 log/ CFU with pullulan and *e*-poly-lysine fibers in a ratio 5:1. Fibers diameters of 346 nm.	[51]
Effect of mango leaf extract (MLE) (1–5%) In morphology, optical nature, water exposure and mechanical characteristics of films composed with chitosan	Solvent: Acetic acid	The conservation of walnuts for 28 days of storage showed 56% higher oxidation resistance for the 5% film compared to a polyamide/commercial polyethylene film. Films with a thickness from 0 to 25 mm	[52]
Preparation of stable pure chitosan nanofibers by electrospinning in presence of ethylene oxide	Solvent: Acetic acid 90% and ethylene oxide Voltage: 20 kV Feed flow: 0.5 mL/L Collector distance: 15 cm	Ethylene oxide improved spinning capacity because it produced nanofibers without pearls or defects, and with tubular characteristics of diameter between 85 and 147 nm when a pullulan concentration of 25 mg/ mL was selected.	[53]
Applications of chitosan/PVA nanofibers in cell attachment for skin tissue regeneration	Solvent: Acetic acid 5% Voltage: 15 kV Feed flow: 0.2 mL/L Collector distance: 6 cm	Developed nanofibers presented biocompability with cells without affecting cytotoxicity. Fibers diameters of 197–227 nm.	[54]
Tranexamic acid-loaded chitosan electrospun nanofibers as drug delivery system for hemorrhage control applications	Solvent: Acetic acid 2% Voltage: 27 kV Feed flow: 0.5 mL/L Collector distance: 12 cm	Uniform fibers were evidenced without beads and with a resistance of 3 to 5 MPa. Also were hemocompatible contributing to the time of blood clotting. Fibers diameters of 160–220 nm.	[55]
Chitosan nanofibers containing carboxymethyl-hexanoyl chitosan nanoparticles for skin cancer treatment	Solvent: Acetic acid 2% Voltage: 8 kV Feed flow: 0.1 mL/L Collector distance: 13 cm	Chitosan allowed the formation of continuous fibers, without beads and with release of nanoparticles in a controlled manner for up to 120 h. Fibers diameters of 600–1,200 nm.	[56]
Colorimetric point-of-care detection of cholesterol using chitosan nanofibers	Solvent: Methanol 10% Voltage: 30 kV Feed flow: 0.5 mL/L Collector distance: 15 cm	Membranes without the presence of beads, uniform and malleable were obtained, which allowed the immobilization of enzymes by adsorption to determine cholesterol levels in biological fluids Fibers diameters of 60–90 nm.	[57]

Table 4. Conditions and solvents used for the elaboration of gelatin-based nanofibers and their applications.

Application	Test conditions	Result	Reference
Cytocompatibility of collagen gelatin and keratin nanofibers	Solvent: PHBV (Polihidroxibutirate-co-hydroxyvalerate) Voltage: 7 kV Collector distance: 15 cm	Gelatin provided good mechanical properties and had cellular compatibility, useful for pharmaceutical and food purposes Fibers diameters of 815 nm.	[64]
Electrospun nanofibers for food and food packaging technology	–	Electrospinning of gelatin can only be done with polar solvents such as acetic acid, formic acid or water and ethanol, given its low resistance to water, requiring a crosslinking process, which allows to retain the three-dimensional structure of the fiber without dissolution. Fibers average diameter of 60–120 nm.	[19]
Elaboration and characterization of electrospun gelatin nanofibers reticulated with oxidized phenolic compounds	Solvent: acetic acid 40% Voltage: 25 kV Collector distance: 13 cm Feed flow: 0.3 mL/h	A nanofiber was manufactured incorporating functional groups such as tannins, gallic, caffeic, hydroxyl and ferulic with an improvement in its mechanical properties. Fibers diameters of 280 nm.	[65]
Effect of glucose content as a ligand in the production of nanofibers	–	Gelatin nanofibers could be thermally reticulated by Maillard reaction to improve mechanical properties, because the addition of a reducing sugar alters the conformation and interaction of proteins, which results in nanofibers with adjustable properties. Fiber diameters between 280 and 575 nm.	[66]
Development of an electrospun-gelatin-based antibacterial material	Solvent: acetic acid Voltage: 15 kV Collector distance: 13 cm Feed flow: 0.02 mL/h	Gelatin nanofibers were developed combined with glycerol and glucose. The fibers showed a strong antibacterial capacity of 20% against *E. coli* and *S. aureus* Fibers diameters of 700 nm.	[67]
Gelatin nanofibers encapsulated with Amphotericin-B	Solvent: water-acetic acid (7:3 v/v) Voltage: 14 kV Collector distance: 8 cm Feed flow: 50 mL/min	The fibers obtained a good stability in acid medium allowing the incorporation of the drug and avoiding their degradation. Furthermore, the fibers maintained a prolonged release after 12 h. Fibers diameters of 123 and 119 nm.	[68]
Production of bactericidal wound dressing material based on gelatin nanofiber Dual nanofibers scaffolds composed by polyurethane-gelatin for bone tissue engineering	Nanofibers of PCL/ gelatin loaded with ciprofloxacin and quercetin: A potential antibacterial dressing material	Solvent: formic acid-acetic acid (3:1 v/v) Voltage: 25 kV Collector distance: 10 cm Feed flow: 30 mL/min Solvent: Formic acid 80% with 20% acidic acid Voltage: 20 kV Collector distance: 14 cm Feed flow: 0.8 mL/h Solvent: Acetic acid Voltage: 14 kV Collector distance: 12 cm Feed flow: 0.5 mL/h	Membranes with homogeneous distribution, smooth and without defects were obtained. Also, it was determined that are

biodegradable and do not present cytotoxicity. Fibers diameters of 201 and 662 nm.	[69]
The scaffolds presented good mechanical and biocompatible properties because gelatin plays a fundamental role in cellular metabolism promoting cell proliferation, growth and binding that favors nanofibers application in tissue engineering Fibers diameters of 70 and 109 nm.	[70]
Smooth nanofibers without beads were obtained and with good mechanical resistance, gelatin contributed to the construction of the extracellular matrix due to the fact that contains a cell recognition site which promotes cell binding Fibers diameters of 234 nm.	[71]

present a ribbon-like structure of variable dimensions, characteristically ranging between 230 and 396 nm, the incorporation on the antioxidant did not visibly affect this morphology and also the nanofibers had a burst release tendency of the active agents and for this reason the zein can be a promising polymer for the phenolic compounds encapsulation[74]. Additionally, according to previous studies (see Table 5), there are other solvents capable of producing nanofibers.

3.2. Mixtures of biopolymers

In order to improve mechanical resistance, biodegradability, biocompatibility, absorption capacity, hydrophilicity, and hydrophobicity, it has been proposed to elaborate combined nanofibers, in which, two or more polymeric substances are mixed for electrospinning. The mixtures of biocompatible polymers (see Table 6) can improve the mechanical properties of nanofibers because they share similar functionalities.

At present, there are technological challenges that limit the use and application of nanofibers in food and medical packaging. Some of them are related to the high solubility and low mechanical resistance when active agents are incorporated, which makes it difficult to release these compounds. Therefore, in recent years some alternatives such as the use of mixtures of polymers, mainly polysaccharides, and proteins have been studied, in order to reduce solubility and obtain a greater capacity of encapsulation of active agents in nanofibers.

4. Active agents incorporated in nanofibers

Currently, the use of natural active agents has been a great alternative in food processing since consumers demand foodstuff without synthetic additives[88]. Nanofibers obtained by electrospinning offer an advantage, as they allow food producers to encapsulate a range of natural ingredients including hydrophilic and hydrophobic compounds such as vitamins, carotenoids, polyphenols, enzymes, oils, flavors, probiotics, peptides, therapeutic agents (see Table 7).

Due to a high porosity characteristics and large surface area, the incorporation of active compounds in nanofibers is possible, offering protection and avoiding their deterioration by external factors such as temperature, oxygen, and moisture[95]. In turn, these structures incorporated with active agents represent a great interest in the packaging industry because they function as an external component of food, thus playing an important role in the food industry, avoiding the deterioration of perishable products during distribution to final consumers, through the suppression of the growth of microorganisms, resistance to oxidation, stability against environmental conditions, masking unpleasant aromas, and conservation of flavors[19].

In the case of the medical area, active agents, especially antimicrobials, offer protection against pathogenic microorganisms that produce severe infections compromising the health of patients and are therefore used in the development of wound dressings (see Figure 1). These therapeutic agents result in a very useful alternative for the treatment of diseases due to their antimicrobial, antioxidant, and anti-inflammatory properties, and encapsulation resulted in a proper technique to create controlled release systems[99].

Among the most commonly incorporated active agents are antimicrobials such as nisin, lactoferrin, essential oils and chitosan; antioxidant compounds such as essential oils, plant extracts, lignin and phenolic compounds; oxygen scavengers such as ascorbic acid, laccase and glucose oxidase and carbon dioxide emitters such as citric acid, sodium bicarbonate and iron bicarbonate[12]. Moreover, antimicrobial agents are widely used because of their action on etiologic agents that constitute risks to the health of consumers and, at the same time, are the main causatives of food losses; some examples are antimicrobial peptides, plant extracts, essential oils, and chitosan.

4.1. Antimicrobial peptides

Antimicrobial peptides (AMP) are compounds known primarily as natural antibiotics, due to their rapid and efficient antimicrobial effects against a wide range of microorganisms, including Gram-negative and Gram-positive bacteria, yeasts, filamentous fungi and to a lesser extent protozoa and viruses[76]. These compounds have been widely studied because they cause inhibition of enzymatic activities and synthesis of proteins, as well as affect the cell wall of microorganisms, being the rupture of the membrane the main mechanism of the AMP since, depending on the structure of the peptide and the binding of the peptide to the membrane, they can generate a destabilization by the formation of pores that will later generate lysis[102].

In order to carry out those alterations, as a first stage, the interaction between a peptide and a target cell bacterial type should be generated, by the attraction between the peptide and cell membrane. This is caused by electrostatic interactions among the remains of positively charged peptides, due to the presence of basic amino acids and negative charge constituents on the surface of the bacterial membrane as well as phosphate groups of the lipopolysaccharide and the lipoteichoic acid present in Gram-negative and Gram-positive bacteria[15].

Similarly, the mechanism of antifungal action of peptides is described as a fungal cell lysis generated by interference in the synthesis of the wall, including the permeabilization of the membrane, the union to ergosterol/cholesterol present in the fungal membrane, the attack to mitochondria or other intracellular organelles, and the deformation of the cell membrane[75]. These aspects support the fact they are widely used in the food industry (see Table 8), having a fundamental role in the inhibition of etiologic agents.

4.2. Plant extracts

Plant extracts show antiallergic, antioxidant, antibacterial, anti-inflammatory and antiviral properties. Most of these properties can be attributed to their content of biological compounds such as polyphenols, isothiocyanates, etc.

Table 5. Test conditions and applications of nanofibers made from zein.

Application	Test conditions	Result	Reference
Electrospun nanofibers of zein incorporated with cyclodextrin for the encapsulation of active agents	Solvent: acetic acid Voltage: 12 kV Collector distance: 12 cm	The films, nanofibers and nanocapsules elaborated with zein are able to encapsulate essential oils, aromas and flavors, allowing a controlled release of active agents. Fibers diameters of 170 nm.	[75]
Incorporation of gallic acid in ultrafine fibers of zein (5, 10% and 20%)	Solvent: acetic acid Voltage: 20 kV Collector distance: 12 cm	Nanofibers were obtained with a diameter of less than 100 nm, tubular form and little formation of pearls, which favors the encapsulation and release of phenolic compound in all concentrations studied. Fibers diameters of 100 nm.	[76]
System of administration of active compounds based on electrospun nanofibers of zein	Solvent: ethanol Voltage: 25 kV Collector distance: 18 cm	Nanofibers obtained showed good mechanical resistance at a pH lower than 7. Fiber diameters between 720 and 912 nm.	[77]
Zein nanofibers obtained by electrospinning for application in active agent administration systems	Solvent: ethanol Voltage: 20 kV Collector distance: 12 cm	Zein was able to encapsulate tannins to be used as an antimicrobial in the treatment of diseases from the digestive tract. Fibers diameters of 100 nm.	[78]
Sesamol incorporated zein nanofiber membrane: An efficient strategy to accelerate diabetic wound healing	Solvent: Acetic acid: water (70:30 v/v) Voltage: 17 kV Collector distance: 10 cm	The nanofibers showed a smooth tubular morphology that allows the encapsulation of sesamol, promoting wound healing in diabetic mice, reducing inflammation factors (IL-10 and IL-6). Fibers diameters of 98 nm.	[79]
Electrospun zein nanofibers dual drug delivery system for simultaneous delivery of aceclofenac and pantoprazole	Solvent: Ethanol and methanol Voltage: 25 kV Collector distance: 18 cm	Ultra-thin fibers, with a smooth surface, semi-transparent and uniform with a high efficacy of drug administration were obtained because zein was able to resist proteolytic degradation and gastric pH Fibers diameters of 50 to 200 nm.	[80]
Electrospun zein nanofibers for therapeutic delivery of essential oil	Solvent: Ethanol 80%v/v Voltage: 25 kV Collector distance: 150 mm	Smooth and uniform nanofibers with an efficient delivery due its low digestibility were evidenced, making useful in the administration of oil for intestinal diseases Fibers diameters of 410 to 788 nm	[81]

Table 6. Nanofibers obtained from mixtures of polymers for use in food or medical areas.

Application	Test conditions	Polymer Mix	Result	Reference
Creation of electrospun fibers from aqueous solutions of calcium or sodium caseinate and pullulan	Voltage: 11 to 23 kV Power flow: 3mL/h Collector distance: 10cm Solvent: deionized water	Pullulan and casein with a mass ratio of 2:1−1:4	A 1:4 casein in pullulan ratio produced fibers with good mechanical resistance, unlike pullulan that presented deficiencies in terms of viscosity, consistency in diameters and physical appearance of nanofibers. Fibers diameters of 301 nm.	[82]
Manufacture of food grade nanofibers of whey protein isolate and guar gum	Voltage: 20 kV Power flow: 0.6 mL/min Collector distance: 8cm Solvent: mixture of water and acetic acid in proportion 90:10	Food grade whey protein and guar gum	Pearl-free, ultrafine nanofibers were obtained. Guar gum at a concentration of 0.9% by weight, contributed in the reduction of viscosity of the solution. The protein helped in obtaining nanofibers with good mechanical resistance. Fiber diameters between 466 and 510 nm.	[83]
Electrospinning of nanofibers made from gelatin/zein cross-linked with glucose by the Maillard reaction	Voltage: 15 kV Feeding flow: 1 mL/h Collector distance: 10cm Solvent: mixture of acetic acid and water in proportion 8:2	Gelatin and zein	Nanofibers obtained from 30% w/v of gelatin in various proportions of zein showed good water resistance and acceptable wettability, as well as good biocompatibility and non-cytotoxicity. Fiber diameters between 451 and 780 nm.	[84]
Fabrication of electrospun poly (vinyl alcohol)/dextran nanofibers as drug delivery system	Voltage: 15 kV Feeding flow: 0.5 mL/h Collector distance: 15cm Solvent: Distilled water	98%w/w PVA with 2 %w/w of dextran	Smooth and cylindrical fibers were evident. Dextran polymer generated a desirable slower release mechanism compared when only PVA fibers were used. Fiber diameters between 200 and 600 nm.	[85]
Fabrication of doxycycline-loaded electrospun PCL/PEO (poly ethylene oxide) membranes for a potential drug delivery system	Voltage: 10 kV Feeding flow: 100mL/h Collector distance: 10 cm Solvent: Dichloromethane	75% w/w PCL, 25% w/w of PEO	Thin fibers of different diameters and stable for drug incorporation were evident. A prolonged release was obtained which is desirable for this type of application. Fiber diameters between 801 and 878 nm.	[86]
Electrospun pullulan/ poly (vinyl alcohol)/silver hybrid nanofibers: preparation and property characterization for antibacterial activity	Voltage: 15 kV Feeding flow: 0.5 mL/h Collector distance: 15 cm Solvent: Distilled water	Total polymer concentration 14% w/w with 60/40 mass ratio of pullulan to PVA	The thermal stability and tensile strength were enhanced. The pullulan nanofibers possess good antibacterial performance, making them a potential practical use as a new preservative Fiber diameters of 100 nm.	[87]

Table 7. Compounds commonly incorporated in nanofibers by electrospinning.

Active agent	Compound	Incorporation matrix	Reference
Vitamins	Vitamin A	Poly Vinyl Alcohol	[89]
	Vitamin D3	Polyacrylonitrile	[90]
Carotenoids	*b*-Carotene	Polyvinylpyrrolidone	[91]
	Carotenoids extracted from tomato peel	Gelatin	[92]
Enzymes	Ficin	Poly Vinyl Alcohol	[16]
	Lipases	Poly (Acrylonitrile-co-maleic acid)	[93]
Essential Oils	Oil of *Perilla frutescens*	Poly Vinyl Alcohol	[9]
	Carvacrol	Starch	[94]
Flavors and Colors	Gallic acid	Poly Vinyl Alcohol	[95]
	Curcumin	Xanthan gum– Chitosan	[96]
Probiotics	*Lactobacillus acidophilus* y *Bifidobacterium animalis*	Poly Alcohol Vinyl	[97]
Peptides	Peptide with stem cell affinity	Polycaprolactone	[98]
Therapeutic agents	Levofloxacin	Poly Vinyl Alcohol	[99]
	Ciprofloxacin	Gelatin	[100]
	Moxifloxacin	Chitosan	[101]
	Cefazolin	Pullulan	
	Imipenem		

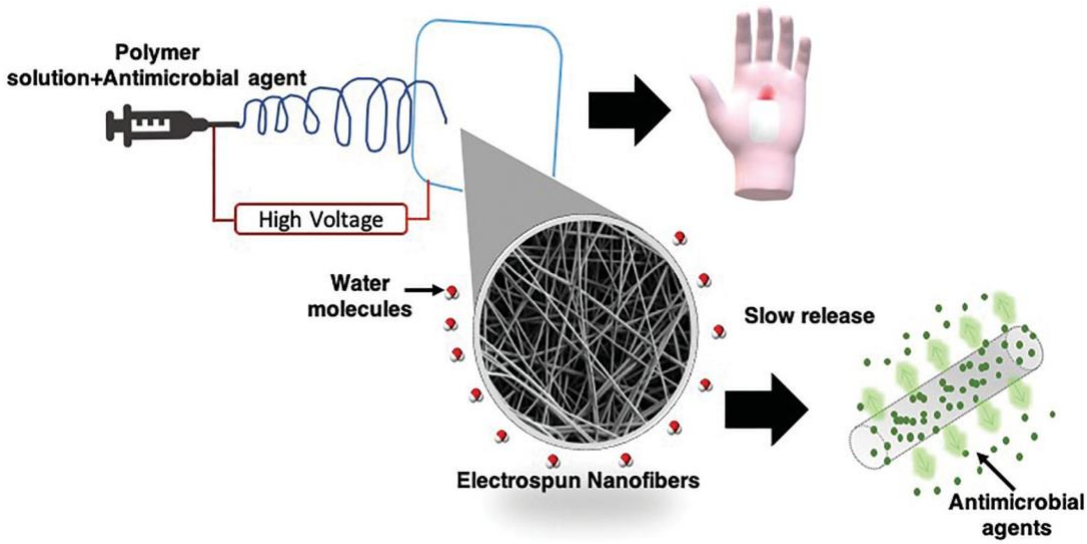

Figure 1. Graphical representation of electrospun nanofibers to be employed in wound dressings.

Similarly, plant extracts contain nutrients such as vitamins, proteins, minerals, and antivirals of potential application in food and pharmaceutical areas[107].

It has been possible to extract essential oils from plants, which are complex mixtures of volatile compounds with strong antimicrobial and antioxidant properties. However, their use is limited due to rapid oxidation processes and degradation, as well as low solubility in water and high volatility[74]. Therefore, several studies are found in the literature (see Table 9).

4.3. Silver nanoparticles

Nanoparticles have been used in electrospinning to be applied to in several fields such as medicine, analytical sci- ences, and materials engineering. These structures have shown antimicrobial properties against several bacterial spe- cies since silver has a large biocide effect, by generating an interruption of the enzyme activity and membrane synthesis in microorganisms, acting as antiseptics[108]. It is also known silver ions can destabilize and increase the permeability of bacterial membranes, inhibiting the functions of essential proteins and respiratory enzymes, which in turn generates a rupture of the replication of nucleic acids (RNA and DNA) of bacteria and obstructs the process of ion transport (see Table 10)[110].

5. Use of antimicrobial agents encapsulated in nanofibers for food packaging

Due to industrialization and globalization, foodstuff requires a longer shelf life and maintain quality and safety parameters during transport, storage and consumption. Therefore, nanofibers are widely used since they can preserve several properties of food and beverages, such as flavor, color, appearance, texture, consistency, as well as mechanical and antimicrobial barrier properties during stages of transport and storage through its application in food containers[12]. The advances in food processing and packaging materials have mainly focused on protecting flavors and maintaining

Table 8. Applications of antimicrobial peptides.

Application	Result	Load	Array	Reference
Application of nisin and other peptides in active food packaging	Nisin is a peptide with antimicrobial and antifungal properties extending the life of food.	Concentrations between 25 and 50 μmol/mL provide bacteriostatic and bactericidal effects against *L. monocytogenes* and *S. aureus*	Cellulose nanofibers, starch or chitosan	[15]
Mechanism of action and control of pediocin against *Listeria monocytogenes*	*In vivo* and in *vitro* tests showed that pediocin inhibited the growth of *Listeria monocytogenes* up to 50%.	Incorporation in the polymer matrix in a 20, 40 and 50% w/w against *Listeria monocytogenes*	Cellulose acetate nanofibers	[103]
Immobilization of an antimicrobial cecropin peptide in cellulose nanofibers	The antimicrobial activity against *Bacillus subtilis* was demonstrated	The peptide was immobilized in amounts between 6.5 mg and 13 mg in 1 mL of polymer solution	Nanofibers and cellulose films	[104]
Development of antimicrobial nanofibers of a peptide (Ple) derived from the mucosal skin of winter fluke, electrospun with poly vinyl alcohol	Nanofibers showed a temperature-dependent peptide release. A decrease by almost 50% in the antimicrobial activity of the pathogens *Escherichia coli* and *Staphylococcus aureus* was found, assessed for 14 days.	Incorporation in the polymer matrix in 15% w/w	Electrospun Polyvinyl alcohol nanofibers	[105]
Pullulan nanofibers containing antimicrobial palindromic peptide LfinB obtained via electrospinning	The peptide incorporated pullulan nanofibers completely inhibited the bacterial grown of *S.aureus* and *P.aeruginosa* at concentration of the peptide to 50 mg/mL and 100 mg/mL, respectively.	The pullulan-peptide solution contained pullulan 20% w/w and peptide (13.2 mg/mL)	Nanofibers of pullulan	[106]

Table 9. Applications of plant extracts incorporated in nanofibers.

Compound	Result	Load	Array	Reference
Encapsulation of a saffron extract in nanofibers of zein and tragacanth	Encapsulation reduced the evaporation rate, protected against moisture and avoided the loss of volatile components.	3 and 9% p/p	Zein and tragacanth nanofibers	[109]
Essential oil incorporated in chitosan nanofibers	Inhibition against *Listeria monocytogenes*.	150 mg of the compound to 0.45 g of Chitosan	Chitosan nanofibers	[110]
Cellulose bagasse extracted from *Agave tequilana* Weber	Fibers showed a fibrillar form, with a high surface area and diameters between 30 and 100 nm.	Polymer concentrations between 4 and 14% w/v	Cellulose 42%, hemicellulose 18% and lignin 14%.	[111]

Table 10. Applications of silver nanoparticles.

Compound	Result	Load	Array	Reference
Nanofibers of polyvinyl alcohol with nanoparticles coated with starch	The fibers showed an inhibition in the growth of *Staphylococcus aureus*	Concentration of 8% w/w	Polyvinyl alcohol	[112]
Synthesis of polyvinyl alcohol nanofibers incorporated with silver nanoparticles	An inhibition was observed in both *E. coli* and *B. circulans* compared to control	Concentration of 1% w/w	Polyvinyl alcohol	[113]
Incorporation of silver nanoparticles in nanofibers from chitosan	A decrease in 5 log/CFU of *Staphylococcus aureus* was observed through *in vitro* tests.	Concentration of 1% w/w	Chitosan	[114]

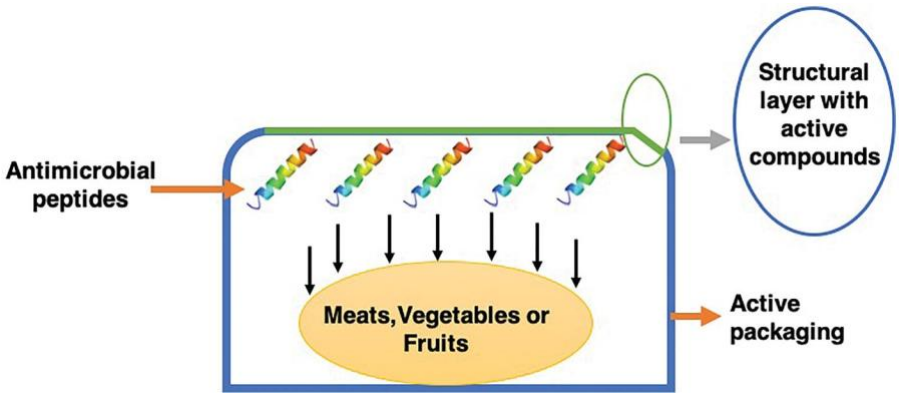

Figure 2. Electrospun fibers used in active packaging for food preservation.

the quality of packaged food, through special packaging that prevents food-borne diseases by a release of active biocides to increase shelf life[111].

Most recent studies on packaging technology have focused their attention on the elimination of oxygen, incorp- oration of antimicrobial substances and low moisture absorption, which today are characteristics of what is known as active packaging. The basic principle of this type of pack- aging is the addition of polymeric materials containing some additives that provide antimicrobial characteristics. These matrices have the potential to release active agents (anti- microbial, antioxidants), to retain compounds (ethylene, oxygen, and water) or to eliminate undesirable components in food[12] (see Figure 2). Therefore, an encapsulation pro- cess is required to protect those compounds when incorpo- rated into packages, where a substance (active agent) is trapped in a matrix, producing micro-or nanometric- sized fibers[112].

In this sense, the basic principle of this type of packaging depends on the type of active component incorporated, which can vary according to the functional properties they can fulfill, such as absorption, elimination, antioxidant, anti- microbial, preservative, among others[113]. Traditionally, these substances are placed inside a polymer, which is used as a vehicle for diffusion inside the packaging. The con- trolled supplies of this type of active agents into foodstuff through packaging films generate a prolonged release and in turn restrict the development of undesirable flavors. In con- sequence, there are ample applications of this type of packaging as shown in Table 11, where the nanofibers have proven to be appropriate technologies in the food industry[7].

Currently, active packages are being more studied and applied in the industry since they constitute a promising alternative in the extension of shelf life without having any effect in the sensory quality and safety of food. Similarly, nanofibers are structures becoming increasingly more useful because of their ability to encapsulate active agents charac- terized by potential antimicrobial, antioxidant and conserva- tion applications for later being used inactive packaging. These aspects offer an advantage with respect to the trad- itional packaging, meanwhile, the active packaging are made mostly made with biodegradable, nontoxic materials and contribute to functions such as the inhibition of microbial growth, inhibition of oxidation processes, etc., which ensures a safer food in benefit to consumers.

6. Parameters used for the evaluation of the stability of nanofibers with incorporation of antimicrobial agents

In order to evaluate the stability of the nanofibers with and without incorporation of active agents, several techniques are used in order to determine the chemical, structural and thermal characteristics of these structures. The systems com- monly used in the determination of these properties are pre- sented below.

Table 11. Applications of nanofibers of real food matrices.

Packed food	Encapsulating agent and matrix	Test conditions	Objective	Reference
Cheese	Nanofibers of chitosan against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* y *Salmonella*	Solvent: Distilled water Voltage: 26 kV Feed flow: 0.6 mL/h Collector distance: 15 cm Nisin concentration: 40% w/v	A satisfactory antimicrobial effect and an insignificant impact on the sensory quality of the cheese was found.	[119]
Foodstuff	Protein nanofibers (gelatin and zein) to encapsulate curcumin.	Solvent: Acetic acid 20% Voltage: 12 kV Feed flow: 0.15 and 1mL/h Collector distance: 10 cm Curcumin concentration: 40% w/v	90% of efficiency of encapsulation of curcumin in nanofibers. A release of agent in periods above 24 h in hydrophobic foods was found.	[120]
Chicken	Gelatin nanofibers/rosemary essential oil for active packaging against *Campylobacter jejuni*	Solvent: Acetic acid 20% Voltage: 20 kV Feed flow: 0.4 mL/h Collector distance: 15 cm Oil concentration: 1.5% v/v	Nanofibers reduced *Campylobacter* growth by approximately 50%. No adverse impact on color, texture or sensory evaluation was found.	[121]
Strawberries	Nanofibers of cellulose modified with resin as an reinforcement agent in a film composed of polylactic acid (PLA) and chitosan	Solvent: Distilled water Voltage: 19 and 21 kV Feed flow: 0.6 mL/h Collector distance: 15 cm	A significant enhance in shelf life of up to 10 days was found, without affecting the firmness and color of the fruit.	[32]
Meat	Nanofibers containing chrysanthemum oil and their application for meat packaging	Solvent: Glacial acetic acid Voltage: 19 and 25 kV Feed flow: 0.2 mL/h Collector distance: 15 cm Oil concentration: 5 mg/mL.	Inhibition of *Listeria monocytogenes*. No impact on the quality of meat as a function of parameters such as color and texture.	[110]
Rainbow trout fillets	Electrospun poly (*e*-caprolactone) nanofibers *with Urtica diotica* extract	Solvent: Glacial acetic acid Voltage: 15 kV Feed flow: 1 mL/h Collector distance: 12 cm Oil concentration: 2% p/p	Inhibition of lactic acid bacteria that could extend the shelf life and quality of the fish fillets up to 15 days	[122]
Poultry meat	Nanofibers of silk fibroin and thyme essential oil against *Salmonella typhimurium* in poultry meat	Solvent: Water Voltage: 18 kV Feed flow: 1 mL/h Collector distance: 15 cm Oil concentration: 0.25% p/p	Nanofibers reduced *Salmonella typhimurium* growth approximately in 2.24 Log CFU/g	[123]

6.1. Thermal characteristics

6.1.1. Thermogravimetric analysis (TGA)

Thermogravimetry is a technique that controls the mass of a sample against time or temperature, performed with a thermogravimetric analyzer or thermobalance. Through this method is possible to determine degradation temperatures, the level of organic and inorganic components in materials, the temperature of decomposition peaks and residues[88]. In nanofibers, TGA is employed in order to measure changes in the mass of the sample, the weight loss of material due to breakdown, oxidation or loss of volatile substances, also, it is used to obtain information on the thermal and oxidative stability, the content of moisture and the content of volatile compounds of the fibers[88].

Aman et al. used a TGA analysis in the manufacture of nanofibers of whey protein isolated from gum-guar. The thermal stability of the gum corresponded to 0.7% by weight, where the decomposition peak was found at 250 °C due to the excision of the galactose and mannose units present in the structure of this gum, therefore, it was concluded that the stability of these fibers is given at temperatures below 250 °C[83]. Similarly, Hadas et al. determined that the weight loss of nanofibers made from flax mucilage occurred between 46 and 123 °C, and the greatest degradation and weight loss occurred at temperatures between 275 and 329 °C, this latter found when polyvinyl alcohol was used in the polymer mixture, improving the thermal stability of the fiber[124]. On the other hand, Lin et al. elaborated nanofibers of glycerin and *e*-poly-lysine in gelatin to control *Listeria monocytogenes* in meat. It was possible to observe the presence of carbonyl, carboxyl and amine groups, corresponding to NH_3, C_2H_4, HCN and CH_4 groups from the chemical structures of the three polymers used[125].

6.1.2. Differential scanning calorimetry (DSC)

This is a technique used to determine a series of tempera- ture transitions in materials, such as glass transition

temperature (T_g) and melting temperature (T_m). The calorimeter measures the difference in heat flow between the sample and a reference, either during a temperature scan or through the course of non-isothermal phenomena such as polymerization[126]. The determination of the glass transition temperature is one of the most important applications, where the material goes from a vitreous to gummy state. In the glass transition the polymer experiences changes in vol- ume and expansion, heat flux and thermal capacity, where the DSC measures the change in heat capacity[122].

In nanofibers, this technique is used to measure and ana- lyze the reaction of polymers to heat. In turn, it is used to determine the transition enthalpy, the degree of crystallinity and the thermal conductivity of polymeric materials, all this in order to obtain the thermal characteristics of the nanofib- ers and the degree of crystallization of materials[128]. Lin et al. obtained *Tg* values for nanofibers made of glycerin and ε-poly-lysine in gelatin between 331.2 °C and 338.8 °C. The increase of temperature could be due to the crystalline structure of nanofibers joined together and formed a con- tinuous crystal-like form in all the material[125]. Similarly, Dehcheshmeh et al., in the production of zein nanofibers with the addition of saffron extract, obtained a *Tg* of 172 °C, due to the main thermal degradation of this protein. However, the loss temperature of nanofibers was 250 °C, the point of evaporation of saffron[109]. Likewise, Hadad et al. found a *Tg* for nanofibers manufactured using flax mucilage at 191 °C, being a related to the melting point of flaxseed (70 °C), thus, these structures have high thermal stability in

comparison with polyvinyl alcohol[129].

6.2. Structural characteristics

6.2.1. Scanning electron microscopy (SEM)

Scanning electron microscopy is used to capture and interpret some signals emitted during the interaction of the electron beam with the sample. Within these signals, there are secondary and retro dispersed electrons, X-rays, light (ultraviolet, visible and infrared), heat, electrons driven through the sample and electrons absorbed by the sample. With some of these signals, it is possible to observe and characterize the sample in terms of surface morphology, structural organization, and chemical composition. To carry out this technique, a fine and uniform coating must be made with a conductive metal such as gold or by the use of evaporation techniques with carbon[130]. Thanks to this method, it is possible to evaluate the main characteristics that should have a nanofiber and terms of uniformity in the surface, consistency, quality, absence of pearls and size of the diameters, which are parameters that condition the functionality of the fiber, in turn, this technique allows to evaluate if the parameters used in electrospinning (voltage, flow of power, distance of the collector) were suitable in obtaining the nanofibers[131].

Lin et al. in the preparation of nanofibers of chitosan with ε-poly-lysine, could establish nanofibers had an average diameter range between 100 and 500 nm with a mean of 273 nm. Fibers were tubular without fractures and with protuberances by the encapsulation of the compound[51]. Lin et al., when prepared nanofibers of gelatin, glycerin, and ε-poly-lysine, found uniform and smooth fibers, without the presence of beads, with the diameter of 45% of the fibers of approximately 200 nm[125]. Bayat et al. observed that fibers made with chitosan had a smooth surface with a diameter of 200 nm, however some beads were observed in a concen- tration of 4% w/v[132]. Finally, Agheb et al., in the elabor- ation on gelatin nanofibers observed an average nanofiber diameter range from 230 to 250 nm and pore size to 75 μm which corresponded to a porosity of 85%[133].

6.2.2. Transmission electron microscopy (TEM)

Transmission electron microscopy works with a highly focused high-voltage electron beam (80–200 keV), which passes through a thin, solid sample of thickness approximately between 100 and 200 nm. However, sample factors such as density and composition have an impact on the transmission of the electron beam. The principle of this technique is related to the collision of electrons with the sample, depending on its thickness and the type of atoms that form it, some of them are scattered, that is, some electrons directly traverse the sample and other are diverted. All of them are driven and modulated by a few lenses to form an image on a digital ocular camera. In order to carry out this technique, a previous thickness reduction in the sample is required, thus, the electron beam can cross it. Among the methods used for this reduction are the mechanical cut and frosted[134].

TEM is a technique used in nanofibers to examine characteristics of samples that have sizes lower than 100 nm, in turn, it is useful to study morphology, crystalline structure and elementary information of fibers[135]. It is appropriate also to observe the distribution of active agents when they are incorporated into these structures as a visual monitoring tool[135]. Dehcheshmeh et al., in the production of core-shell nanofibers from zein and tragacanth for the encapsulation of saffron extract, found through TEM the core-covered structure of the nanofibers, where there was a clear distinc- tion between the clearest and darkest areas corresponding to the zein shell and the central region where the compound was previously encapsulated[109].

Wang et al. found irregular fibrillary fibers in the elaboration of polyaniline nanofibers, with diameters between 30 and 120 nm, then they could obtain nanofibers of more uniform diameters as changing the polymer concentration of 0.1–0.6 mol/L[136]. Zhuang et al., in the elaboration of electrospun nanofibers of chitosan/gelatin containing silver nanoparticles, obtained nanofibers with diameters between 220 and 400 nm without defects and, in addition, it was determined that most nanoparticles have sizes that are in the range of 1 and 5 nm[137].

6.3. Physical-chemical characteristics

6.3.1 X-Ray diffraction (XRD)

X-Ray diffraction is an analytical technique used in nanofib- ers to characterize the crystalline structure of nanofibers.

XRD can provide information on the dimensions and properties of the fibers. Bragg's law ($n\lambda = 2d\sin(\theta)$), describes the principle of XRD analysis, where scattered rays from the parallel planes interact with each other to create constructive interference for structural analysis, where is determined to correspond to the wavelength (λ) of X-rays, and θ refers to the angle of the X-ray diffracted, that allows to deduce the distances of the crystals. The phenomenon of X-ray diffraction is conditioned by the number and distribution of the electrons involved in the structure in order to determinate the crystalline nature of the samples[138].

Ghaderi et al. found this method useful in determining the apparent crystallinity in bagasse nanofibers, where a peak intensity at 2θ 18° was obtained, which increases according to the duration of the fiber in the solution, because the effect of dissolution time induced a loss of crys- tallinity and reduced crystallite size by the solvent penetrat- ing the spaces between the crystallites and amorphous section[107]. Hadad et al. produced nanofibers from flax mucilage, and observed acute crystalline peaks of approxi- mately 2θ 19.42 and 21.92° indicating a semi-crystalline structure of the nanofibers by internal and external hydro- gen limits[129]. González et al. evaluated cellulose nanofibers made by using soy by-products, finding an increase in the proportion of crystalline cellulose. The indices of crystallin- ity were 81.70 and 90.70, which indicates a high degree of crystallinity[139].

6.3.2. Nuclear magnetic resonance (NMR)

The NMR technique provides information on atomic environments based on different resonance frequencies exhibited by nuclei in a strong magnetic field. Many nuclei are observable by NMR, however, those of hydrogen and carbon atoms are the most studied. NMR measurements in solid- state are useful for characterizing crystalline forms of solid specimens without requiring any sample treatment[54]. The nuclei typically analyzed in this technique include ^{13}C, ^{31}P, ^{15}N, ^{25}Mg and ^{23}Na[140]. The different crystalline structures of a compound can cause disturbance of the chemical envir- onment of each nucleus, resulting in a unique spectrum for each shape. Once the resonances have been assigned to mol- ecule-specific atoms, information about the nature of the sample can be obtained[54].

This technique is used in nanofibers to obtain detailed information on the structure of the molecules that make up the fibers. Additionally, it allows determining the physical and chemical properties of the atoms and, in turn, of mole- cules[141]. Therefore, the properties of the nanofibers in chemical terms and the distribution of the molecules in the structures could be described[55]. According to Sun et al., NMR was useful to rectify the chemical structure of starch and polyacrylonitrile nanofibers where were observed ^{13}C, CN, CH, and CH_2 groups belonging to starch[142]. Agheb et al. determined the structure of fibers made from modified gelatin, observing the presence of peaks of methyl groups of lysine and arginine as well as triazole, characteristic of the modification of the polymer[133]. On the other hand, Mateon et al. developed nanofibers composed of a mixture of tannic acid/chitosan/pullulan in aqueous solution. NMR was used to observe the chemical structure of nanofibers; there were found glucosamine peaks characteristic of tannic acid and CH, CH_2 groups that corresponded of the pullulan structure[35].

6.3.3. Fourier transform infrared spectroscopy (FT-IR)

FT-IR is used in the evaluation of nanofibers since it allows analyzing the composition of organic and inorganic materials (solids, liquids or gases) depending on the chemical bonds having different energy levels, and the type of linkage conformation. This technique is also useful to obtain information on the chemical composition of the fibers since it generates a spectrum coming from the vibrations of the functional groups and the consecutive interactions of these groups with other atoms[36]. FT-IR is a technique where the IR radiation passes through the sample, where some of this radiation is absorbed by the sample and part of it is transmitted[143]. The resulting spectrum represents the absorption and molecular transmission, creating a molecular fingerprint of the sample, with absorption peaks corresponding to the vibration frequencies between the bonds of the atoms that compose the sample. Since each sample has a unique composition and, consequently a different arrangement of atoms, there are no two compounds that produce exactly the same IR spectrum. Similarly, the size of a peak in the spectrum is a direct indication of the amount of material present[144].

Lin et al. used FT-IR in a wavenumber of 400−4,000 cm^{-1} to evaluate nanofibers of chitosan integrated with nanoparticles of moringa oil for a development of a packaging against *Listeria monocytogenes* and *Staphylococcus aureus* in cheese, where they could chemically characterize nanofibers obtaining vibration in wavenumber of 1,172 cm^{-1} and 1,713 cm^{-1}, mainly of carbonyl and carboxyl groups corresponding to palmitic acid and phytol from oil, and amine groups corresponding to the chitosan[145]. Similarly, Aman et al. used a spectral range of 500−4,000 cm^{-1} in the evaluation of food-grade nanofibers of whey protein isolated from gum-guar, and observed vibrations mainly of −OH and −NH groups from the gum-guar in wavenumber of 2,920 and 1,538 cm^{-1} respectively, by the presence of amides and hydrogen bonds from the helical structure of the protein[83]. Lin et al. designed nanofibers from glycerin and ε-poly-lysine, noticing peaks corresponding to the carbonyl group from gelatin. In relation to the glycerin, there were observed peaks in the groups −OH, −CH and $-CH_2$; and finally, for ε-poly-lysine, peaks were obtained in the −NH groups, $-NH_2$ and carbonyls[125].

In another study made by Hadad and Goli, in the fabrication and characterization of electrospun nanofibers using flaxseed mucilage, FT-IR was employed to determinate of interactions through infrared spectra between in flaxseed mucilage and polyvinyl alcohol in the nanofibers. Infrared spectra were recorded in the frequency range of 600−4,000 cm^{-1}, where it was observed that vibrations of carbonyl, amide and hydroxyl groups indicated the presence of the two compounds; two bands of 1,612 cm^{-1} and 3,321 cm^{-1} were found in the mucilage spectrum, and for

the case of polyvinyl alcohol bands were observed at 1,710, 1,380, and 1,100 cm^{-1}. Based on these differences, it was concluded there was no chemical interaction between basil seed mucilage and polyvinyl alcohol in the electrospun nanofibers[129].

6.3.4. Antioxidant activity

Oxidation is one of the most important processes in food deterioration, as it can affect the safety, color, taste, and tex- ture of food. Antioxidants prevent the harmful effects of free radicals by avoiding the deterioration of fats and other food components. This characteristic is studied in nanofibers used as structures in food packaging, in order to maintain the organoleptic characteristics of the product[146].

6.3.4.1. Radical scavenging test by DPPH. DPPH (2.2-diphenyl-1-picrylhydrazyl) is a stable and colorless radical widely used in the determination of antioxidant capacity. DPPH is soluble in organic solvents and has an absorption in a range from 515 to 520 nm. Generally, the decrease in the intensity of absorption in the presence of samples con- taining antioxidants is recorded at an incubation time of 30 min[147]. The current procedure of determination of free radicals by DPPH is based on the measurement of certain properties of light depending on the wavelength using a spectrophotometer. The result is expressed in terms of the antioxidant capacity of the sample[67].

Some studies of this technique have been reported, Wang et al. evaluated the antioxidant activity of polyaniline nano-fibers, where it was found that the number of colored DPPH radicals absorbed at 516 nm decreased linearly as the concentration of polyaniline increased, thus, higher antioxi-dant activity was found[136]. Li et al. studied nanofibers of cellulose with the incorporation of quercetin, which showed antioxidant effect due to the phenolic hydroxyl group from the flavonoid, which can provide hydrogen to reduce free radicals and prevent the oxidation of lipids and proteins[148].

6.3.4.2. Trolox-equivalent antioxidant capacity (TEAC). It is a test based on the quantification of the discoloration of the radical $ABTS^{\bullet+}$ due to its interaction with hydrogen donor species of electrons. The cationic radical $ABTS^{\bullet+}$ is a chromophore that absorbs at a wavelength of 734 nm, and it is generated by a radical oxidation reaction (2, 4′-azino-bis- (3-ethylbenzothiazoline-6-sulfonate of ammonium)) with potassium persulfate. The measurements are performed at a wavelength of 734 nm after 30 min of reaction at room tem-perature and in darkness[4].

Muhammad et al. observed that cellulose nanofibers had an antioxidant effect because there was an increase of almost three times in the elimination of free radicals, in comparison to control[149]. Soni et al. elaborated nanofibers of chitosan and observed a gradual increase in antioxidant activity pro-portional to the concentration of chitosan, where there was a removal of radicals in a value of 8.2 in relation to 7.9, therefore it was concluded that antioxidant activities of the films toward the radical ABTS are slightly higher than those of the radical DPPH[150].

6.4. Antimicrobial activity

This test is used in order to evaluate the antimicrobial activ- ity of nanofibers, which can come mainly from two sources, the first from the polymers themselves since they contain antimicrobial properties such as Pullulan, or the second source may be due to the incorporation of active agents in these structures such as antimicrobial peptides, essential oils, and plant extracts, which have a broad spectrum against Gram-positive and Gram-negative microorganisms. In this technique, it is performed an analysis of the death curve of the microorganisms pathogens evaluated, where it is observed the inhibitory effect of polymers and active agents on microbial growth due to the loss of cell structure, block- ing of membrane protein synthesis and nucleic acids of bio- logical agents[80].

6.4.1. Sensitivity evaluation in plate

The antimicrobial susceptibility test is a semiquantitative method, based on a disk diffusion test on an agar plate, where it is intended to expose microorganisms of interest to different concentrations of antibiotics or active agents. This test is evaluated by visual inspection, if the microorganism is sensitive to the agent, a halo of growth inhibition of the microorganism will be formed, and if it is not sensitive the halo will be nonexistent[151].

Mocanu et al. found inhibition halos against two bacteria *Escherichia coli* and *Bacillus Subtilis* of 3 mm and 1 mm, respectively, for carbon nanofibers with silver nanoparticles having a greater antimicrobial effect compared to strains such as *E. coli*[124]. Amariei et al. elaborated nanofibers with ε-Poly-lysine and found halos of inhibition of 7.5 mm against *Staphylococcus aureus,* possibly due to damage of microorganism cell membranes[152].

6.4.2. Dilution in broth

This technique allows to quantitatively measure the *in vitro* activity of an antimicrobial agent against a bacterial culture through the measurement of the inhibitory minimum con-centration (MIC), which corresponds to the lower concen-tration that is required of an agent to inhibit the visible growth of the microorganism. Moreover, it is useful to determine the minimum bactericidal concentration (MBC), which corresponds to the lower concentration of the agent that decreases the viability of the initial inoculum of the microorganism. In that sense, a death curve of the micro-organism is done at different concentrations of the anti-microbial agent in order to determine the inhibitory effect of the antimicrobial on the microorganism, which will be expressed in logarithmic units of colony-forming units per mL of culture.

Lin et al. elaborated nanofibers of chitosan with ε-poly-lysine for the packaging of food against *Salmonella* in chicken, and observed inhibition in 5.03 Log CFU/g of *Salmonella*[51]. In another study conducted by Lin et al. related to the obtaining of gelatin nanofibers, glycerin and ε-poly-lysine to control *Listeria monocytogenes* in beef, it

was observed that nanofibers had an inhibitory effect since colonies decreased from 3.12 Log CFU/g to 1.91 Log CFU/g during a storage period of 10 days[125]. Also, in another study conducted by Lin et al. in gelatin nanofibers incorpo- rated with rosemary essential oil for active packaging against *Campylobacter jejuni* in chicken, a decrease was observed on the part of nanofibers in 2.74 Log CFU/g, thus it was shown that nanofibers had an inhibitory effect against the micro-organism[121]. Bayazidi et al. prepared cellulose nanofibers by immobilizing lysozyme against to Gram-negative and Gram-positive bacteria, and observed an 1.5 Log CFU/g inhibition of *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Saccharomyces cerevisiae*[126].

7. Conclusions

Electrospinning is a simple technology that allows ultrafine membranes (nano/micro scale) be produced with a high sur-face-volume ratio, high porosity and generating great stabil- ity to encapsulated compounds. This review summarized the use of electrospun nanofibers, the formation of these fibers through carbohydrates and proteins aimed at applications in food and drug packaging systems. Although electrospun fibers are used in a variety of applications, one of the most important areas where (1) electrospun ultra-thin membranes are used as encapsulating matrices of mainly antimicrobial and antioxidant compound that can be useful in preserving and prolonging the shelf life of the food stuff mainly foods with high water content, because these are the most suscep- tible to microbial contamination and oxidative reactions and
(2) electrospun ultra-thin membranes are used as drug deliv-ery systems and development of wound dressing for treat- ment of multiple diseases.

In last decades, nanofibers have been widely studied, and in consequence, the food and medical packaging industry has been one sector that has focused its attention on these structures due to the structural and functional advantages they offer such as the encapsulation and stability of the active agents. Among the mostly encapsulate active agents are plant extracts that offer enhanced antimicrobial action due to the bactericidal activity of the chemical compounds that are present in them. In the same way, the antimicrobial peptides and silver nanoparticles although synthesized, pro- vide a specific antimicrobial mechanism because they dir- ectly affect the biochemical structure and metabolism of the microorganisms.

Therefore, in this review, the most important studies car-ried out in the application of nanofibers were shown, in order to explore the features of electrospinning and in the same way provide information about food matrices and medical treatment where electro-spun membranes have been used to date and information on the techniques that are commonly used in order to evaluate the physical-chemical characteristics of these structures. Finally, it is clear that is necessary to transform the success of electrospinning in laboratory scale to an industrial scale where its application may be more feasible.

Acknowledgements

The authors wish to thank the Research Direction (DIB), the Institute of Food Science and Technology and the Faculties of Agricultural Sciences and Medicine from the Universidad Nacional de Colombia. This review was supported by the project entitled "Development of antimicrobial nanofiber membranes with potential application in active packaging and pharmaceutical products" (Project code: 41882).

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Capítulo 3: Ultra-thin single and multilayer electrospun fibrous membranes of polycaprolactone and polysaccharides (Journal of Bioactive and Compatible Polymers)

JOURNAL OF
Bioactive and Compatible Polymers

Original Article

Journal of Bioactive and Compatible Polymers
1–12

DOI: 10.1177/0883911520944422
journals.sagepub.com/home/jbc
SAGE

Ultrathin single and multiple layer electrospun fibrous membranes of polycaprolactone and polysaccharides

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Abstract

Electrospinning was used to produce fibrous membranes, in single and multiple layers, from poly(ε-caprolactone), pullulan, and from mixtures of poly(ε-caprolactone) with potato modified starch and β-glucan. It was possible to obtain single-layer membranes from solutions of pullulan in water, poly(ε-caprolactone) in chloroform, and from mixtures of poly(ε-caprolactone)/β-glucan and poly(ε-caprolactone)/potato modified starch in chloroform. Scanning electron microscopy images showed the formation of ultrathin homogeneous fibers from electrospun poly(ε-caprolactone) and pullulan, whereas the fibers obtained from mixtures of poly(ε-caprolactone)/ β -glucan and poly(ε-caprolactone)/potato modified starch had different sizes and morphologies, as well as irregular microstructures, characterized by the presence of *beads.* Contact angle analyses showed that pullulan membranes were extremely hydrophilic, while poly(ε-caprolactone) membranes were predominantly hydrophobic. Subsequently, poly(ε-caprolactone)-pullulan-poly(ε-caprolactone) multilayer membranes, with intermediate wettability, were prepared by successive electrospinning

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steps. Infrared spectroscopy and calorimetric analyses showed the presence of both polymers and the absence of changes in their structure and stability due to electrospinning, indicating adequate compatibility between the two polymers. We foresee that the polyester-polysaccharide multilayer membrane might be used as a biodegradable vehicle for active agents with different hydrophobicity, with applications as food packaging and biocompatible scaffold materials.

Keywords
Electrospinning, polymers, membranes, layers, pullulan, poly(ε-caprolactone)

Introduction

Electrospinning is a technique for producing nano- and micrometric-scale polymeric fibers that relies on the application of a high voltage between the needle tip of a polymer solution injector, and a metal collector. It has been used to produce materials with a wide variety of applications, including active food packaging, tissue engineering, wound dressings, among others.[1,2] Some of the advantageous features of these materials are increased ability to dissipate forces along their length, greater elasticity, high porosity with small pore size, high gas permeability, and high surface-to-mass ratio.[3]

Electrospun fibrous membranes have been developed using synthetic and natural polymers, with biopolymers, such as proteins and polysaccharides, being particularly interesting for biotechnology and food-related applications.[4,5] These biopolymers can be biodegradable or nonbiodegradable, extracted from biomass, synthesized from biologically derived monomers or produced from microorganisms.[6] Polysaccharides are promising for the fabrication of electrospun materials aimed at bioengineering and food applications, due to their biocompatibility, edibility, and the feasibility of their industrial production from renewable sources.[7] Among the biopolymers used in the development of nanofibers is pullulan, a polysaccharide of microbial origin produced by *Aureobasidium pullulans* that, in aqueous solution, has presented excellent characteristics for electrospinning, producing membranes characterized by a high permeability to oxygen.[8] Although pullulan has been successfully used for producing nanofibers with enhanced functionalities, such as their inclusion with antimicrobial compounds, this polymer has a high tendency to solubilize in water.[9,10] This extremely hydrophilic behavior can be a limiting factor for electrospun pullulan fibers in food packaging or wound dressings due to high instability toward high moisture environments.[11]

On the other hand, some natural non-water-soluble biopolymers, such as potato modified starch (PMS), β-glucan, and other synthetic biopolymers, such as poly(ε-caprolactone) (PCL), could be used to obtain biodegradable and biocompatible nanofibrous materials with improved water resistance, which are desirable characteristic in the development of packaging systems for food preservation, as well as in the field of bioactive scaffolds. Starch is one of the most abundant polysaccharides; its solubility in water, that is, its hydrophilic character, can be tailored via chemical, physical, and enzymatic modifications, for instance, inducing the amylose to have greater freedom of molecular movement to form hydrogen bonds, which ultimately facilitate electrospinning.[12] Also, β-glucan is a set of high molecular weight polysaccharides present in plants, mainly barley and oats, and on the cell wall of microorganisms such as bacteria, yeasts, and fungi. The macromolecular structure and the type of links in the main chain depend on the source of the glucan and allow differentiation between them. The yeast-derived glucans, insoluble in water, are composed of glucose chains linked by bonds β-(1 → 3) and side chains β-(1 → 6). Those types of links, as well as chain length and ramifications length, influence its solubility properties.[13] Within the synthetic polymers is PCL, a linear, semi-crystalline, aliphatic polyester with repeating units of O-$(CH_2)_5$-CO. This polymer is hydrophobic, and it has been widely used, alone or in combination with other

polymers, to produce water-resistant, biocompatible, and biodegradable electrospun fibers with good mechanical properties.[14–16]

Various studies have been carried out on the preparation of nanofibers with PCL, and it has been indicated that they exhibit outstanding functionalities for the fabrication of scaffolds and in food packaging applications.[16–19] Although there are fewer reports on the electrospinning of β-glucan[20,21] and modified starches, the latter have been highlighted for biomedical and pharmaceutical applications.[22,23]

The objective of this study was to evaluate the feasibility of obtaining ultrathin membranes via electrospinning, from biodegradable polymers: PCL, pullulan, mixtures of PCL with non-water-soluble polysaccharides (PCL/β-glucan and PCL/PMS), and, subsequently, to evaluate their physical-chemical characteristics to determine which membranes would have potential use for future application as systems for encapsulation and release of active compounds.

Materials and methods

Materials

Pullulan cosmetic grade was supplied by Hayashibara Co., Ltd. (Okayama, Japan). Food grade β-glucan of *Saccharomyces cerevisiae* ~70% 1.3/1.6 was purchased from Bulk Supplements (Henderson, United States). Modified potato starch ELIANE BC 160 was acquired from Joli (Veendam, Netherlands). PCL pellets (Mn-80,000) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical-grade chloroform was acquired from EMSURE ACS (USA).

Preparation of electrospinning solutions

For this study, two types of membranes were developed. First, single-layer membranes from pullulan, PCL, and miscible solutions of PCL/β-glucan and PCL/modified starch (PCL:PMS), were prepared. Second, multilayer membranes were prepared from miscible solutions of pullulan in water and PCL in chloroform, being the external layers PCL and the internal layers pullulan. Single-layer membranes were prepared from the following solutions: (1) pullulan in water (20% w/w); (2) PCL in chloroform (9% w/w); (3) PCL/β-glucan in chloroform (15% w/w), with a mass ratio of 70/30; and (4) PCL/PMS in chloroform (15% w/w) with a mass ratio of 70/30. The PCL-pullulan-PCL multilayer membranes were made using on the inner layer a solution composed of pullulan in water (20% w/w) and for the outer layers a solution of PCL in chloroform (9% w/w). The polymers were weighed and homogenized in an ultrasound bath (Model ATM40-2LCD, Mark ATU) at 50W for 15 min. The solutions were kept in a beaker sealed with parafilm and stored at a temperature of 20ºC for a time not exceeding 8 days.

Electrospinning

The prepared solutions were pumped into the electrospinning equipment composed by syringe, dosing pump (KDS 100 to Geneq, Canada), needle (Upchurch Brand, USA), copper collector, and a high voltage source (Gamma High Voltage, USA). The electrospinning conditions for obtaining uniform pullulan membranes were: solution flow 0.3 mL/h, distance from needle to collector 11 cm, and voltage 12–14 kV. On the other hand, based on the methodology described by Van der Schueren *et al.*, the following conditions were followed for electrospinning of PCL solutions: 0.7–0.8 mL/h flow rate, 0–15 cm distance from needle to collector, and 7–20 kV voltage.[18,24] These conditions with some modifications were used in electrospinning tests until a stable Taylor cone was obtained. Single-layer membranes were prepared by deposition of the collected fibers onto a grounded copper collector covered with an aluminum foil, until approximately 0.15 mm thickness. Multilayer membranes were prepared similarly, in three electrospinning steps (PCL-pullulan-PCL) to reach a total thickness of approximately 0.20 mm. The thickness of the membranes was measured using a Vernier digital caliper (Mitutoyo, Japan).

Characterization

Scanning electron microscopy. Fiber samples were collected during 1–3 min in 1 cm × 1 cm glass slides, after an adequate Taylor cone stability was ensured (usually after 5 min since the start of an electrospinning run), and observed on an Olympus optical microscope model CX31RB-SFA (Japan), to delimit the area of analysis. Subsequently, the glass slide was coated with gold using the Quorum Q150R ES (Singapore) metallizer under the following operating conditions: spray current 60 mA, spray time 30 s, and pressure in the chamber 1000 mbar.

The coated samples were analyzed in an FEI Quanta 200 scanning electron microscope (Thermo Scientific™, USA) for 30 min at a high vacuum, with an operating voltage of 30 kV for the electron beam and magnifications from 200× to 50,000×. Microphotographs were analyzed using the ImageJ software (NIH, USA) to determine the diameter of the fibers.

Contact angle. The samples were placed on a glass support, then drops with a defined volume (10 µL) of distilled water were deposited on the membrane surface with a micropipette. The contact angle was evaluated at 1, 60, and 300 s after the drop was deposited on the surface to determine the wettability of the membranes. Photos were taken using a Canon PowerShot SX130IS digital camera (Canon Inc., Japan). The captured images were stored in a personal computer in a JPG format, and contact angles were calculated using anown-developed algorithm in MATLAB (ver. R2016a, MathWorks Inc., USA), to determine hydrophilic (angles less than 90º) or hydrophobic (angles greater than 90º) behaviors. Measurements were made from the left and right sides of the angle formed between the drop and the polymer matrix to obtain a more accurate value on wettability.[25]

Fourier transform infrared spectroscopy analy- sis. An FT/IR-4700 Type A spectrometer with an ATR PRO ONE accessory (Jasco, Japan) was used for spectral analysis of polymers. The samples were placed directly in the device window with a metal spatula and pressed with a sapphire tip until forming a thin layer. During the experimental analysis, air accumulation was eliminated to prevent interference generation. The conditions used during the analysis were: wavenumber range of 400– 4000 cm^{-1}, 24 scans, 7.1 cm aperture, meas- urement resolution of 4 cm^{-1}, measurement temperature of 22ºC and absorbance reading mode. Subsequently, smoothing treatments were applied using the means-movement method, removal of false peaks due to known source noise, baseline correction and removal of CO_2 and H_2O peaks corresponding to the absorption of water vapored and CO_2 in the infrared spectrum. Spectra Manager II soft- ware (Jasco, Japan) was used for data control and analysis.

Differential scanning calorimetry (DSC). Melting and crystallization points were determined in the multilayer membranes using a DSC1-Star System Calorimeter (Mettler Toledo, USA) with standard aluminum pan/lid sets. Thermal stability was determined in the multilayer membranes since they were the only fibers with appropriate physical-chemical character- istics. The calorimeter was pre-calibrated with nitrogen (+99% purity) at 50 mL/min and subsequently loaded with 10 mg of the solid sample. For the PCL-pullulan-PCL multilayer membrane, initial heating was performed from 25ºC to 300ºC, with a heating ramp of 10ºC/min and then cooling from 300ºC to 25ºC with a ramp of 10ºC/min.

Statistical analysis

The results of fiber thickness obtained via scanning electron microscopy (SEM) were expressed as mean ± standard deviation and the statistical comparison between means was performed by a Student's *t*-test, with a 95% confidence level. The contact angle results were analyzed by means of a one-way analysis of variance (ANOVA), and the differences between means were evaluated by Tukey test, with a 95% confidence level. A p-value less than 0.05 was considered statistically significant.

Results and discussion

Preparation and morphology of nanofibrous membranes

Electrospinning at different conditions enabled the preparation of single-layer membranes and multilayer membranes from the solutions. The pullulan solution in water presented a stable formation of Taylor cone, with a high deposition area; the membranes reached the desired thickness (0.15 mm) after 20 min of deposition, at the following conditions: voltage of 14 kV, flow rate of 0.5 mL/h and tip-to-collector distance of 12 cm. Figure 1(a) shows SEM microphotographs of the pullulan fibers obtained by electrospinning, featuring random orientation, diameters of 0.887 ± 0.087 µm. The occurrence of some beads was observable only at the lower magnifications (200×). These results are consistent with a study conducted by Qian *et al*. reporting that pullulan (20% w/w) in aqueous solution had excellent characteristics in the formation of membranes with regular micro/nanometric fibers (100– 210 nm) and without the formation of beads.[26]

The electrospinning of the PCL 9% w/w solution in chloroform was characterized by a highly stable Taylor cone, a high deposition area, and a membrane of the desired thickness (0.15 mm) was obtained after approximately 30 min at the following conditions: voltage of 7 kV, flow rate of 0.8 mL/h and tip-to-collector distance of 15 cm. Figure 1(b) shows the SEM microphotographs of the fibers obtained from PCL, showing randomly oriented, cylindrical, beads-free fibers of mean diameters of 0.279 ± 0.018 µm. As for pullulan, some bead formation was observable at the lower magnification (200×). This was consistent with the report of Autac and Uyar, in which thin and cylindrical PCL fibers were obtained, without the presence of beads and with diameters in a range from 0.1 to 0.5 µm.[27] At these conditions, PCL presented a lower fiber diameter in comparison to pullulan ($p < 0.05$). Variables, such as applied voltage, chemical nature, and interaction between polymer and solvent, define the fiber dimensions and morphology, with higher voltages commonly leading to larger fiber diameters, due to a greater transport of charges through the polymer.[28,29]

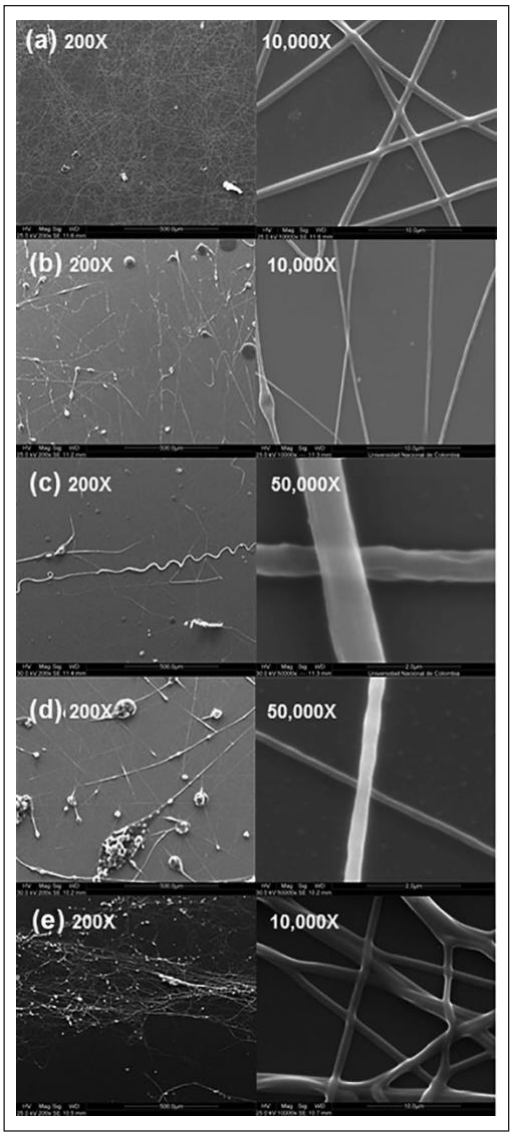

Figure 1. SEM photomicrographs of the membranes obtained in the electrospinning of solution of: (a) pullulan 20% w/w in water, (b) PCL 9% w/w in chloroform, (c) PCL/PMS 15% w/w (70:30) in chloroform, (d) PCL/β-glucan 15% w/w (70:30) in chloroform, and (e) multilayer (PCL (9% w/w in chloroform)-pullulan (20% w/w in water)-PCL (9% w/w in chloroform)).

During the electrospinning process of the mixture PCL/PMS (70/30) in chloroform, a pseudo-stable and very thin Taylor cone was observed, with a membrane of adequate thickness being

obtained after 30 min, at the following conditions: voltage of 20 kV, flow rate of 0.8 mL/h and tip-to-collector distance of 10 cm. Figure 1(c) shows the SEM microphotographs of the membrane obtained from PCL/PMS in chloroform. In this membrane, two types of fibers were observed: smooth fibers with diameters of 0.968 ± 0.052 µm, and striated fibers that were significantly thinner (0.646 ± 0.031 µm) ($p < 0.05$), as well as the presence of homogeneous beads. Jukola *et al.* used mixtures of PCL with starch at the same concentration (15% w/w), and described highly porous fibers with lower diameters of 0.180 µm for PCL, and large particles along the fibers, which were assumed to be starch. The differences with these findings might be partially attributed to the electrospinning parameters, which in the work of Jukola *et al.* were characterized by a lower electric field (voltage of 15 kV and a distance of 25 cm), arguably leading to thinner fibers.[30]

In the case of PCL/β-glucan (70/30) solutions in chloroform, the electrospinning presented a thin, unstable jet, with a low deposition area at the following conditions: voltage of 7 kV, flow rate of 0.7 mL/h and tip-to-collector distance of 10 cm. Figure 1(d) shows the SEM microphotographs of the fibers obtained. As well was for PCL/PMS, two fiber morphologies were observed, with significant differences in diameter: smoother fibers (0.176 ± 0.018 µm) and a grooved, larger fibers with diameters of 1.847 ± 0.188 µm, as well as the presence of large clusters of beads. There are no previous reports of the electrospinning of this mixture of polymers; however, similar diameters have been reported in a range of 80–120 nm for fibers made from glucans.[31]

In sum, pure solutions of PCL (in chloroform) and the polysaccharide pullulan (in water) presented excellent electrospinnability, and homogeneous fibers with submicron diameters were obtained, with low bead formation, in agreement with previous evidence. In contrast, chloroform solutions of mixtures of PCL with the polysaccharides PMS and β-glucan allowed for obtaining electrospun fibers; however, the electrospinning process was unstable, and the materials obtained presented a more irregular morphology and exhibited the presence of beads.

Finally, a multilayer membrane composed of electrospun pullulan (for the inner layer) and PCL (for the outer layers). A total of 60 min of electrospinning were required to achieve an adequate thickness (approximately 0.20 mm), by means of step-by-step electrospinning of PCL (9% w/w in chloroform) for the external layers, and pullulan (20% w/w in water) for the internal layer. SEM microphotographs are shown in Figure 1(e), in which different diameters corresponding to PCL (0.279 ± 0.018 µm) and pullulan (0.887 ± 0.087 µm) were observed. Moreover, for both types of fibers, cylindrical, elongated, and unsupported beads morphologies were evidenced, similar to those obtained with the pure polymers.

Contact angle

The results of contact angle analyses of the membranes are shown in Table 1. For single-layer membranes of pullulan, a contact angle of less than 25º was observed, indicating a hydrophilic behavior. This was expected due to the high water-solubility of pullulan, which is enhanced by the typical increase of its surface-to-mass ratio when the polymer is structured in the form of ultrathin fibers. Similar results have been described previously, demonstrating that electrospun pullulan has a great affinity with water, therefore, a highly hydrophilic character.[26]

The PCL membrane had an average contact angle of 132.20° on the left side, and 130.84° on the right side at 0 s, both slightly decreasing with time. These results indicate PCL fiber had a highly hydrophobic behavior. Gutiérrez-Sánchez *et al.* found a similar initial contact angle (135.64º ± 1.26º) for PCL, which after 5 min decreased by less than 8° due to its low wettability or hydrophobic character of the polymer.[32]

For the PCL/PMS membrane, a hydrophobic behavior was observed, with average contact angles of 140.53° on the left side and 137.68° on the right side at 0 s. A significant reduction in angles was found at 60 s ($p < 0.05$) on both sides. Nonetheless, no significant differences were found between 60 s and 300 s ($p = 0.28$). In this membrane, a lower tendency to wetting over

Table 1. Contact angles between the membranes and the water drop at different times.

Membrane	Time (s)	Left side	Right side
PCL	1	132.20° ± 0.95^{c,B}	130.84° ± 0.75°^{c,B}
	60	129.54° ± 0.69^{b,B}	129.15° ± 1.55°^{b,B}
	300	126.05° ± 0.46^{a,B}	127.42° ± 1.23°^{a,B}
PCL/PMS	1	140.53° ± 1.86^{b,C}	137.68° ± 0.35°^{b,C}
	60	134.37° ± 1.09^{a,C}	133.46° ± 0.43°^{a,C}
	300	132.45° ± 2.03^{a,C}	132.74° ± 1.06^{a,C}
PCL/β-glucan	1	131.25° ± 1.01^{c,B}	131.28° ± 1.31^{c,B}
	60	123.51° ± 1.84^{b,B}	124.72° ± 2.70^{b,B}
	300	117.57° ± 0.87^{a,B}	118.18° ± 0.86^{a,B}
Multilayer PCL/pullulan/PCL	1	120.35° ± 0.90^{b,A}	118.27° ± 1.16^{b,A}
	60	117.52° ± 1.29^{a,A}	117.21° ± 0.15^{a,A}
	300	113.41° ± 1.22^{a,A}	114.26° ± 0.10^{a,A}

*^{a,b,c}Tukey test between times ($\alpha = 0.05$).
**$^{A, B,C}$Tukey test between treatments ($\alpha = 0.05$).

time may be due to the presence of the electrospun PMS, which is only slightly soluble in water.[32] In PCL/PMS membranes, the presence of PMS led to a more hydrophobic character compared to the pure PCL membranes, suggesting that hydrophobic interactions between the two polymers induces a greater hydrophobic behavior.[33] In the case of the PCL/β-glucan solution, the membrane had a contact angle statistically similar to PCL ($p = 0.46$). However, this membrane tended to moisturize over time because the contact angle presented the largest decreased (14º after 5 min), probably due to water-polysaccharide interactions.

The multilayer membrane had a mean contact angle of 120.35º on the left side, and 118.27° on the right side, at 0 s. This angle was similar after 60 s ($p = 0.11$). However, differences were found after 300 s, when the contact angle dropped to a mean of 113.41° on the left side, and 114.26° on the right side ($p < 0.05$). A total decrease of 7º was observed after 300 s, which was related to the migration of water through the pores of the external layer of PCL, and the subsequent wetting of pullulan. There are no previous studies on PCL and pullulan in a multilayer structure; however, Takala *et al.* evaluated the diffusion of antimicrobials through a membrane composed of two external layers of PCL and an internal layer of methylcellulose (MC), which exhibited high resistance to wetting in addition to a controlled release of antimicrobials.[34] In the same way, the PCL/pullulan/PCL electrospun membranes have the potential to be used as encapsulating agents for hydrophobic and hydrophilic antimicrobial compounds. This, in addition to the improved barrier properties provided by PCL (resistance to moisture), make this membrane promising for application as food active packaging materials and active wound dressings.

A clear correlation between the morphology and the hydrophilic/hydrophobic character of the fibers was not observed because the wettability depends more on the nature of the polymer and its solubility in water. However, according to Yu *et al.*, the porosity of electrospun nanofibers should affect the dissolution rate,[35] with higher porosities allowing for greater water diffusion within the membrane, and therefore, lower contact angles.

Fourier transform infrared spectroscopy analysis (ATR-FTIR)

The identification of functional groups was based on Fourier transform infrared spectroscopy analysis (FTIR) peaks attributed to stretch and bend vibrations. According to Madi *et al.*, Shigel, Singh y Saini, Trovatti *et al.*, Sugumaran *et al.*, and Saber-Samandari *et al.*, the following key bands are characteristic of FTIR pullulan spectra: 3305–3431–3435 cm^{-1}; 2923–2928–2929 cm^{-1};

1636–1645 cm^{-1}; 1354–1366–1368 cm^{-1}; 1148–1154–1155 cm^{-1}; 851–848 cm^{-1}; 738–755cm^{-1}; 918–929 cm^{-1}; and 1080–1081 cm^{-1}. All these bands were observed for both pullulan and pullulan electrospun membranes (Figure S1), and indicate vibrations of the type: OH; C-H sp3; O-C-O; C-O-H; C-O-C; α-D-glucopyranoside; α- (1,4)-D-glucosidase; α-(1,6)-D-glucoside; bonds CO and C_6-OH, respectively.[34,36–40] Similarly, according to Elzein *et al.*, and Correa *et al.*, the following key bands are present in the FTIR spectrum of PCL: 2949 cm^{-1}; 2865 cm^{-1}; 1727 cm^{-1}; 1240 cm^{-1}; and 1170 cm^{-1}, associated to symmetric CH_2, asymmetric CH_2, carbonyl tension, symmetric C-O-C, and asymmetric C-O-C;[15,41] these characteristic bands were observed in both PCL and electrospun PCL (details in supporting information: Table S1 and Figure S1). The similarity of the FTIR spectra of the electrospun membranes and the corresponding non-electrospun polymers indicates that no chemical changes are induced in the polymers as a consequence of the electrospinning process.

The spectrum of the membrane obtained from the PCL/PMS mixture in chloroform, and the spectra of pure PCL and PMS are shown in Figure 2(a). The spectrum of PCL/ PMS was very similar to the characteristic absorption spectrum of pure PCL, which con- firms the predominance of this polymer in the membrane. An absorption peak at 1022 cm^{-1} in the PCL/PMS spectrum, which is associated to ordered amorphous structures present in starch,[42] confirmed the presence of PMS in this membrane. Similarly, in the membrane obtained from the mixture of PCL/β-glucan in chloroform Figure 2(b), the representative bands of PCL were observed, and the band at 1044.02 cm^{-1}, which corresponds to the vibration of the C-O bond of polysaccharides,[43] confirmed the presence of β-glucan.

In the FTIR spectrum of the PCL-pullulan-PCL composite multilayer membrane Figure 2(c), the position of the bands in the membrane is similar to that observed in PCL. This is expected because the external layers were made of electrospun PLC. However, two characteristic bands of pullulan (1368.13 and 1636.96 cm^{-1}) were observed in the multilayer membrane spectrum, which confirms the presence of this polymer, and suggests that there was a good entanglement between the pullulan and PCL fibers. It was evidenced that the electrospinning did not generate changes in the structure of the polymers for any of the solutions.

Furthermore, the functional groups observed in the spectra of pullulan and PMS corroborated the result obtained by the contact angle analysis. In pullulan, links associated with hydrophilicity and water-solubility are α-(1→4) and α-(1→6) glycosidic bonds, which are associated with the observed 755 cm^{-1} and 932 cm^{-1} bands, respectively.[44,45] In PCL, PMS and β-glucan, the bands associated with hydrophobicity are: stretching of the carbonyl group of the ester C = O at 1730 cm^{-1}; stretching of the bond hydroxyl group OH at 3000 cm^{-1} and OH flexion at 1645 cm^{-1} present in the anhydroglucose units that conform the amyl- ose and amylopectin chains available for the formation of (1→ 3) (1→6)-β-D-glucans at 1044 cm^{-1}, respectively.[46–48]

Differential scanning calorimetry

In the case of the multilayer membrane, temperatures of crystallization (Xc) and melting (T_m) were evaluated from 25ºC to 300ºC (Figure S2) (details in supporting information). In the heating phase, a single endothermic peak was observed at 57.92ºC corresponding to the melting point (T_m) with a correlated enthalpy per unit mass of 2.59 J/g. Similarly, Correa *et al.* reported a range of melting temperatures for PCL from 58ºC to 60ºC.[41] These results suggest that neither electrospinning, nor its interaction with pullulan affect significantly the PCL melting point. A crystallization was observed near 270ºC, arguably caused by the decrease in the mobility of the polymer chains due to a molecular rearrangement.[41]

In the cooling ramp, a phase transition near 33.14 ºC was observed with an enthalpy per unit mass of 1.71 J/g; given the peak thinness, this transition might be due to a high-speed cold crystallization.[49] Mascheroni et al.[10] reported that this pullulan presents a good stability up to temperatures near 200ºC, however, small peaks were evident in a temperature range from 265ºC to 300ºC. According to Qin et al.[50] pullulan exhibits weight

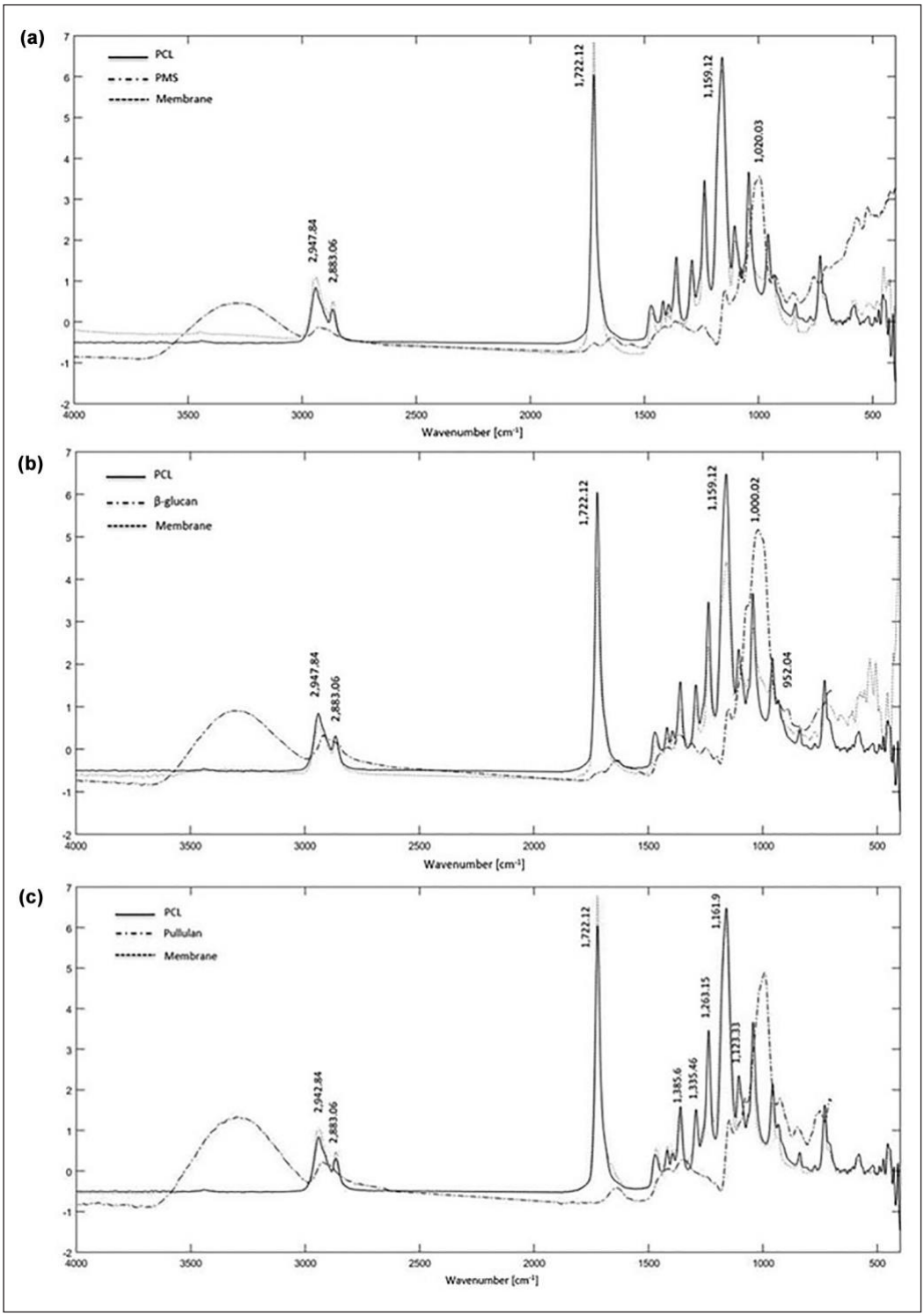

Figure 2. FTIR spectra of (a) pure PCL, pure PMS and PCL/PMS membrane; (b) pure PCL, pure β-glucan and PCL/β-glucan membrane; and (c) pure PCL, pure pullulan and PCL-pullulan-PCL multilayer membrane.

loss, attributed to the degradation of internal covalent bonds of biopolymer monomers, in temperatures ranging from 250°C to 350ºC. These results show that ultrathin fibers of PCL and pullulan exhibit a good thermal stability.

Conclusion

It was possible to obtain ultrathin fibers with diameters in the submicron scale via electrospinning of natural and synthetic biodegradable polymers, pure (pullulan, PCL) or mixed (PCL/β-glucan, PCL/PMS), forming single-layer or multilayer (PCL-pullulan-PCL) membranes of 0.15–0.20 mm thickness. In agreement with pre- vious reports, pullulan and PCL exhibited excel- lent electrospinnability in solutions of water and chloroform, respectively. The fibers of PCL and pullulan, in single-layer or multilayer membranes, were homogeneous, cylindrical and smooth, with diameters ranging between >100 nm and 0.1 µm. On the contrary, PCL/PMS (70:30) and PCL/β-glucan (70:30) in chloroform solutions, presented an unstable Taylor cone during electrospinning, and the fibers obtained were characterized by heterogeneous shapes and the presence of beads.

Pullulan membranes were extremely hydrophilic, while PCL, PCL/PMS, and PCL/β-glucan membranes were predominantly hydrophobic, as expected from each polymer or polymer combination. On the other hand, PCL-pullulan-PCL multilayer had an intermediate hydrophobicity. Calorimetric analyses indicated an adequate thermal stability of PCL-pullulan-PCL membranes in high-temperature ranges (200–300ºC). The FTIR spectra confirmed that electrospinning did not cause changes in the structure of the polymers, and showed the presence of functional groups related to both polymers in the multilayer membrane (PCL-pullulan-PCL).

We foresee that the polyester-polysaccharide multilayer membranes could be used as encapsulating agents of either hydrophilic or hydrophobic bioactive substances, while PCL will help maintain the structural integrity of the membranes in aqueous media, or in high-water activity environments, compared to the pure pullulan membranes. This enables their potential applications in active food packaging and antimicrobial scaffolds.

Acknowledgements

The authors thank the Institute of Food Science and Technology (ICTA), and the Faculties of Agricultural Sciences, Medicine and Sciences of Universidad Nacional de Colombia.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by Universidad Nacional de Colombia, through the projects “Development of antimicrobial nanofiber membranes with possible application in active packaging and pharmaceuticals” (Code: 41882) and “Evaluation of the use of polymer mixtures for the development of micro / nanofibers with the incorporation of an antimicrobial peptide” (Code: 46384).

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Supplemental material

Supplemental material for this article is available online.

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Capítulo 4. Multilayer polycaprolactone - pullulan membranes incorporated with the antimicrobial palindromic peptide LfcinB (21-25)$_{Pal}$ as a potential application in active packaging

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Abstract

Food losses are currently generated by microbial contamination due to the lack of packaging that not only functions as a barrier but releases compounds against such organisms. Electrospinning was used to develop multilayer membranes using poly-(ε-caprolactone) (PCL) in the outer layer and encapsulating the peptide LfcinB (21-25)$_{Pal}$ in an inner pullulan (PUL) layer. The membranes were characterized by Fourier Transformed Infrared Spectroscopy (FTIR). FTIR showed the presence of the peptide in PUL membranes, the presence of both polymers in multilayer membranes, and the absence of chemical changes in the peptide structure. In addition, a High Performance Liquid Chromatography (HPLC) was performed for peptide quantification, where an encapsulation efficiency of 65% in the membrane was observed, and a maximum load of 50 mg peptide / g PUL, meanwhile at higher concentrations there was no stability during the electrospinning process. The antioxidant activity of the peptide, evaluated through the inhibition of radical DPPH, showed an antiradical activity of 5.21 x 10^{-4}mg ± 1.12 x 10^{-5} gallic acid/mg membrane. Finally, the antimicrobial activity showed a minimum inhibitory concentration of 15 ± 0.01 µM against a strain of *Escherichia coli.* In conclusion, the multilayer membrane can potentially be used as an active packaging to prolong the shelf life of foodstuff susceptible to microbial contamination.

Keywords: Electrospinning, Nanofibers, Encapsulation, Antimicrobial Peptide, Antioxidant Capacity.

1. Introduction

Foodborne illnesses, caused by biological agents such as bacteria, viruses, and fungi, are of great concern in food safety. According to the World Health Organization (2019), annually 600 million people get sick due to the consumption of food contaminated by biological agents, and approximately 420 000 die globally each year (1). Raw products such as fruits and vegetables are more susceptible to microbial deterioration due to their high water content and lack of processes that improve their safety (2),(3). For this reason, it is

necessary to reduce the risks of contamination through packaging systems that guarantee appropriate food quality standards.

Packaging has a fundamental role in the food industry because it improves the shelf life of products. Packages function as protective barriers against physicochemical damages caused by changes in pH, water content, oxygen, and carbon dioxide concentrations during the storage and transport stages (4). Additionally, oxidation is one of the most important processes in food spoilage, since it can affect the quality, color, and texture (5). Based on the above, and in response to expectations from consumers to buy healthy and safe foods, researchers have focused their attention on developing active packaging to maintain the quality and sensory properties of foodstuff over time (6).

Active packaging, made from polymeric materials containing active agents (e.g. antioxidants or antimicrobials) incorporated by encapsulation techniques, is designed to allow a controlled release of the agent, and therefore plays a relevant role in food preservation beyond being a barrier between the product and the external environment (7),(8). These polymeric materials added to the packaging are made from nanofibers using an electrospinning technique, having the advantage of a high surface-to-mass ratio, excellent mechanical properties, and high porosity with small pore sizes (9).

Electrospinning is known to be an economical, fast and simple technique, which can be performed at room temperature, useful for producing nano and micrometric scale fibers with a large surface area and good mechanical properties such as greater elasticity and malleability, high porosity with small pore sizes and high gas permeability (10),(11),(12). This technique has been widely used to encapsulate compounds such as peptides, antibiotics, vitamins, essential oils, and proteins in polymer matrices (13),(14),(15). Typically, bioactive compounds and antimicrobials have low chemical stability and are susceptible to degradation, where encapsulation protects these compounds from enzymes and digestive pH until reaching areas of intestinal absorption. In the food industry, such compounds are encapsulated to guarantee a controlled release (16),(17). Electrospinning offers advantages in terms of protecting active agents against external factors that can decrease or eliminate the bioactivity and in turn, allows active compounds to be administered at the right time and the desired site. This technique has been used in the encapsulation of antimicrobial

peptides, very sensitive compounds prone to degradation by environmental factors, allowing a controlled release for a prolonged period of time (18).

Antimicrobial peptides (AMP) are compounds known primarily as natural antibiotics, due to their fast and efficient antimicrobial effects against a wide range of microorganisms (19). These compounds generate inhibition in the enzymatic activities and protein synthesis of the cell wall of microorganisms, being the rupture of the membrane the main mechanism of AMP (20). Bovine lactoferrin (LfcinB) is an AMP with antimicrobial, antifungal, antiparasitic activity. LfcinB interacts electrostatically with the bacterial membrane, i.e. between the positive charges of the side chains of the LfcinB and the negative loads of the bacterial surface. Subsequently, the hydrophobic lateral chains of the peptide interact with the lipid of the membrane causing its rupture, and therefore cell lysis (21), (22).

According to previous studies conducted by Vargas *et al.* (2017), RRWQWR is the minimum sequence of the LfcinB peptide with antimicrobial activity. This sequence contains aromatic amino acids such as tryptophan and arginine residues that give it amphipathic properties and interact electrostatically with the bacterial cell wall allowing the peptide to approach the membrane and induce cell lysis. Short palindromes such as LfcinB (21-25)$_{Pal,}$ with a sequence RWQWRWQWR, have shown both antifungal and antibacterial activity because it repeatedly contains the same amino acids that are present in the LfcinB peptide (23).

Based on the above, the objective of this study was to evaluate the level of inclusion of the peptide LfcinB (21-25)$_{Pal}$ in PUL-PCL-PUL multilayer membranes, which were selected based on their physical-chemical and structural characteristics. PUL is a biopolymer commonly used in the development of nanofibers but it has a high tendency to solubilize in water (14), therefore PCL was used as a hydrophobic polymer to decrease the solubility of PUL to have a longer release of the peptide over time. The multilayer membrane was developed using a PCL solution for the outer layers and a PUL-peptide solution at different concentrations of peptide for the inner layer. In addition, based on the antimicrobial properties of the peptide, its activity was tested against a Gram Negative strain having into consideration there are already previous studies showing activity against Gram Positive bacteria (15),(23).

Similarly, it was assessed the antioxidant activity of the peptide to evaluate the capacity to counteract such reactive oxygen species as free radicals, peroxides, and/or oxygen ions, as a result of the food cellular metabolism. This gives an additional value to ultrafine membranes for a potential application in the food industry, not only as an antimicrobial agent but also as an anti-radical, since typically products such as fruits and vegetables are susceptible to microbial contamination and oxidation due to their high water content, being multilayer membranes a complementary tool in food storage and preservation.

2. Materials and methods

2.1 Materials

Pullulan cosmetic grade was supplied by Hayashibara Co., Ltd. (Okayama, Japan). The PCL pellets (Mn~80,000) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The chloroform for analysis was acquired from EMSURE ACS (ISO Reag. Ph Eur of the United States). The DPPH reagent (2,2-Diphenyl-1-picryl-hydrazyl) was purchased from Sigma Aldrich (Germany). The methanol and ethanol used were J.T.Baker (Xalostoc, Mexico). The gallic acid used was 98% purity, AK Scientific (California, USA). Acetonitrile (ACN), trifluoroacetic acid (TFA) and Ninhydrin were acquired from Merck (Darmstadt, Germany). The SPE SupelcleanTM LC-18 column was acquired from Sigma-Aldrich (St. Louis, MO, USA). Mueller Hinton medium was acquired from Merck (Darmstadt, Germany).

2.2 Synthesis of peptide

The Palindromic peptide LfcinB (21-25)$_{Pal}$, was synthesized manually by solid phase, purified and evaluated by RP-HPLC following the methodology described by Román et *al.* (2017) (15).

2.3 Preparation of electrospinning solutions

For the elaboration of the multilayer membrane, a PCL solution was used for the outer layers and a pullulan-peptide solution at different concentrations of peptide for the inner layer. Three pullulan-peptide solutions were prepared: (a) PUL (1g) and LfcinB (21-25)$_{Pal}$ (10mg/g); (b) PUL (1g) and LfcinB (21-25)$_{Pal}$ (50mg/g); (c) PUL (1g) and LfcinB (21-25)$_{Pal}$ (100mg/g). The solutions were diluted with deionized water to a PUL concentration of 20% w/w. In addition, the PCL solution was prepared in chloroform at a concentration of (9%

w/w). The solutions were stored in beakers sealed with parafilm and stored at a temperature of 20ºC for a time not exceeding two days.

The polymers were weighed on an analytical scale (Model AX1246, OHASUS, China), mixed and homogenized at room temperature (21 ºC) in an ultrasound bath (Model ATM40- 2LCD, USA) at a power of 50 W, for 15 and 30 minutes for PCL and PUL-peptide solution, respectively.

2.4 Electrospinning

Prepared solutions were pumped into the electrospinning consisted of a syringe, dosing pump (KDS 100 to Geneq, Canada), needle (Upchurch, USA), copper collector, and high voltage source (Gamma High Voltage, USA). The electrospinning conditions determined to obtain uniform PUL-peptide nanofibrous membranes were carried out according to established by Rodríguez *et al.* (2020): solution flow 0.5 mL/h, needle distance to collector 12 cm, and voltage 12-15 kV. The conditions for the electrospinning of PCL solutions were a flow of 0.8 mL/h, needle distance to 15 cm collector, and 7 kV voltage. The multilayer membranes were deposited in a grounded copper collector covered with an aluminum sheet. The membranes were covered with aluminum and stored in a glass desiccator at room temperature until characterization.

2.5 Qualitative analysis of fibers

Membranes were analyzed using the Kaiser test to determine the existence of free amino groups, and thus, the presence of the peptide. To perform the Kaiser test, three solutions were prepared: (i) phenol in absolute ethanol (4 g/mL); (ii) 1 mL of an aqueous solution of KCN (0.65 mg/mL) with 49 mL of pyridine; (iii) 1.25 g of ninhydrin dissolved in 25 mL of absolute ethanol. Solutions (i) and (ii) were mixed (1:1 v/v), stored and labeled as solution A, the solution (iii) was labeled as solution B and stored at 4ºC in darkness. To carry out the test, a fraction of the dried resin-peptide or fibers (~ 1 mg) and 150 µL of the mixture of solutions A and B (2:1 v/v) were added. The mixture was heated for 5 min at 105 ºC in a thermostated bath. Blue color indicates a positive test for the presence of primary amines and yellow coloration indicates a negative test.

2.6 Quantification of LfcinB (21-25)Pal by reverse phase liquid chromatography (RP-HPLC)

The quantification of the peptide in the fibers was performed by RP-HPLC (Agilent 1200 series). The chromatographic system consisted of a monolithic column Chromolith® C18 (50 x 4.6 mm), injection volume 10 µL with a linear gradient elution applying 5% to 50% solvent B (0.05% TFA in ACN) in solvent A (0.05% TFA in water), at a flow of 2 mL/min at room temperature for 8 min. Three repetitions were performed per analysis. The reading was made at 210 nm on an HPLC-UV detector (Agilent, Nebraska, USA).

Three stock solutions were prepared, a solution (1B) of the pure LfcinB (21-25)$_{Pal}$ peptide to prepare the calibration curve, in which 1.5 mg of LfcinB (21-25)$_{Pal}$ was dissolved in 1.5 mL of water. The second (2B) and third (3B) solutions were prepared for quantification. The solution 2B was made from 5 mg of the fiber and dissolved in 1 mL of water, to obtain a concentration of 10 mg of the LfcinB(21-25)$_{Pal}$ peptide per g of PUL. Finally, the solution 3B was prepared from 5.2 mg of the fiber and dissolved in 1 mL of water, to obtain a concentration of 50 mg of the peptide LfcinB(21-25)$_{Pal}$ per g of PUL.

The calibration curve was performed using concentrations of 50.1, 163.07, 262.52, 386.58, 492.99, and 988.03 ppm of LfcinB peptide (21-25)$_{Pal}$ in water. The limit of detection (LOD) was 35 ppm and the limit of quantification (LOQ) was 106 ppm.

2.7 Characterization of the multilayer membranes incorporated with the LfcinB(21-25)$_{Pal}$ peptide

2.7.1 Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Using the ATR-FTIR technique, both PUL and PCL polymers, as well as LfcinB(21-25)$_{Pal}$ peptide and the membranes produced by electrospinning were analyzed to confirm the presence of polymers and peptide in the prepared membranes. This technique was also employed to observe if electrospinning generated modifications in the chemical structure of any of the polymers. An FT/IT-4700 Type A spectrometer (Jasco Inc., USA) provided with an ATR PRO ONE accessory and Spectra Manager II software for data control and analysis were used.

The samples were placed directly in the equipment window with a metal spatula and pressed with a sapphire tip to form a thin layer. During the experimental analysis, air accumulation

was eliminated to avoid interference generation. The conditions used during the analysis were: 400 – 4 000 nm wavenumber range, 24 scans, 7.1 cm aperture, 4 cm^{-1} measurement resolution, 22 ºC measurement temperature, and absorbance reading mode. Subsequently, smoothing treatments were applied using the Means-Movement method, the removal of false peaks due to known source noise, baseline correction, and removal of CO_2 and H_2O peaks corresponding to the absorption of water steam and CO_2 in the infrared spectrum.

2.8 Antioxidant activity

Antioxidant activity was determined as the percentage of inhibition of DPPH radicals expressed in gallic acid equivalents. To measure the antioxidant power of the membranes incorporated into the peptide, a calibration curve was performed related to the percentage of inhibition (24). Initially, a stock solution of 0.3 mg/mL of gallic acid in methanol was prepared and, from it, diluted points at a range from 0.002 to 0.008 mg/mL were obtained. The correlation coefficient between the two variables of the curve was 0.9916.

Subsequently, a stock solution of DPPH 0.01 M in methanol was prepared in darkness at room temperature and left in agitation for 30 min, then the DPPH solution was adjusted to an absorbance range from 0.70 to 0.75 (25). The reactions were carried out by adding 200 µL of the sample with 2 mL of adjusted DPPH solution. The blank was prepared by replacing the sample with methanol. The absorbance was read after 30 minutes in an UV-VIS spectrophotometer (Jasco model V-530, Japan) at a wavelength of 517 nm after the standardization of the reading time. The percentage of antioxidant activities were calculated according to Equation 1.

$$Antioxidant\ activity\ (\%) = \frac{(Acontrol - Asample)}{A\ control} x\ 100 \quad (1)$$

Where Acontrol and Asample represent the absorbance values of the DPPH solution with methanol and the DPPH solution with the sample, respectively.

For the quantification of the antioxidant activity of the samples, initially, for the pure peptide, a solution was prepared by taking 1 mg of the peptide and 500 µL of methanol. After 200 µL of the solution was taken and reacted for 5 minutes with the DPPH solution until achieving a colored formation of the compound, then the absorption value was recorded. In the case

of the membranes incorporated with the peptide, 10 mg were taken from each membrane and 500 µL of methanol was added to extract the peptide and precipitate the PUL due to its low solubility in this solvent. The incorporated membranes were homogenized by vortex and left for 24 h at room temperature, after that period 200 µL of the extract was reacted with the DPPH solution. The recorded absorbance of the samples was replaced in the equation and interpolated into the inhibition curve to determine the antioxidant activity equivalent to the concentration in mg/mL of gallic acid.

2.9 Antimicrobial activity

The minimum inhibitory concentration (MIC) was determined based on the methodology described by Román *et al*. (2017). To determine the MIC a microdilution test was used (26),(27). For the antimicrobial activity assay, a bacterial strain of *Escherichia coli* (Migula) Castellani and Chalmers ATCC 25922 (Manassas, VA, USA) was employed. The strain was attached in a KWIK-STIK$_{TM}$ unit, which contained a lyophilized pellet of the microorganism. Subsequently, the strain was grown in Tryptone Soy Agar (primary culture) and kept at 35°C in an aerobic atmosphere for up to 48 h. To assess viability, three serial peals of the primary culture were carried out. A colony count was done and purity was evaluated with a Gram staining. The first pass was kept as a reserve strain and passes two and three were working strains, these latter were kept in refrigeration at a temperature of 8°C.

To obtain the inoculum, the bacterial strain of *Escherichia coli* was incubated for up to 24 h at 37°C in Müller-Hinton broth to an optic density of 0.15 to 0.30, read at an absorbance of 620 nm, using an Elisa Asys Expert Plus reader (Biochrom, UK), equivalent to an approximate concentration of 5 x 10_6 UFC/mL. Then, 90 µL of PUL-LfcinB(21-25)$_{Pal}$ (33mg/250 µL) mixture was added to a microtiter plate, and this mixture was serially diluted to final concentrations of the peptide in each well (200, 100, 50, 25, 12.5 and 6.2 g/mL) where 10 µL of the previous inoculum was added.

The minimum bactericidal concentration (MBC) was determined by taking a small sample of each well where there was no visible growth, and spread on Müller-Hinton Agar plates (MHA) and incubated overnight at 37°C. The minimum bactericidal concentration (MBC) was considered as the plaque that did not exhibit bacterial growth (15).

3. Results and discussion

3.1 Fourier Transform Infrared Spectroscopy Analysis (ATR-FTIR)

Figure 4.1 presents the FTIR spectra of PUL-10 mg LfcinB $(21\text{-}25)_{Pal}$, and PUL-50mg LfcinB $(21\text{-}25)_{Pal}$. The identification of functional groups was based on peaks attributed to stretching and bending vibrations.

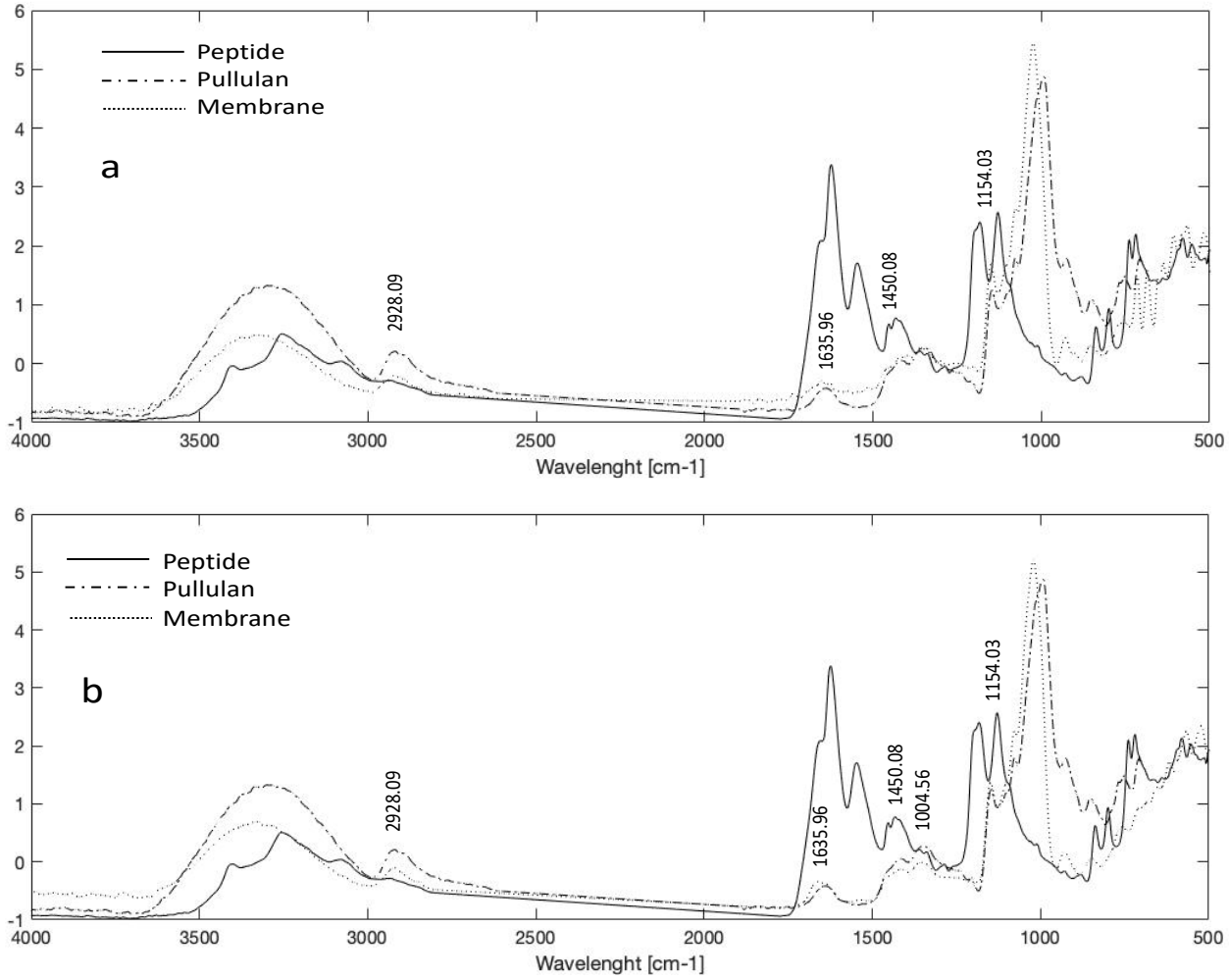

Figure 1. FTIR spectra of (a) LfcinB(21-25)$_{Pal}$ peptide, pure PUL, and PUL-peptide (10mg) membrane and (b) LfcinB(21-25)$_{Pal}$ peptide, pure PUL, and PUL-peptide (50mg) membrane, made by electrospinning.

According to Shai (2013), peptides absorb infrared radiation in specific frequency ranges depending on the type of chemical bond (single or double) and the quality of the link vibration (stretching or bending) (28). The absorbance in the amide I region was found at 1600-1700 cm^{-1} and corresponded to the stretching of the C=O bonds of the individual amino acids (28). In addition, the amide II region, typical in these types of compounds, was found between 1400-1545 cm^{-1} and corresponds to the stretches of the carbonyl peptide groups (29), while NH bending vibrations were obtained around wavelengths of 3000 - 3600 cm^{-1} (30). Among the amino acids that make up the peptide LfcinB $(21\text{-}25)_{Pal}$, some aromatic amino acids such as tryptophan were found, whose representative bands were located at 1334 and 1455 cm^{-1} and corresponded to a C-H tension vibration and a C-N tension vibration, respectively. Additionally, this peptide is also made up of glutamine, an amino acid with an amide group in its lateral chain, with representative bands at 1586 cm^{-1} by the bending vibration of the NH_2 group and at 1659 and 1696 cm^{-1} for stress vibration C=O. Finally, this peptide contains arginine, an amino acid with positive lateral chains found in the range between 1688 and 1695 cm^{-1} for asymmetrical stretching vibrations of the CN_3H_{5+} group and symmetrical stretching vibrations of the CN_3H_{5+} group in bands between 1576 and 1577 cm^{-1}(31),(32).

In Figure 4.1 (a) corresponding to the spectrum of PUL-10mg LfcinB $(21\text{-}25)_{Pal}$ membrane, the characteristic bands of the PUL polymer were observed at wavelengths of 2928.09, 1635.96 and 1154.03 cm^{-1}, corresponding to vibrations in links C-H sp_3,O-C-O, and C-O- C, respectively (33),(34). Based on this, the presence of PUL in the membrane was corroborated. Regarding the peptide LfcinB$(21\text{-}25)_{Pal,}$ only one band was found at 1450.08 cm^{-1} which corresponds to the amide II region, related to the stretching of carbonyl in the peptide bond (29). It is possible that due to the low concentration of the peptide compared to PUL, no more bands characteristic of this active agent were observed. Therefore, the presence of PCL and PUL polymers, as well as the peptide in the nanofibrous membrane were evidenced, and no modifications in polymer structure were observed after electrospinning.

On the other hand, according to Madi *et al*. (1997); Shigel (2002); Singh and Saini. (2008); Trovatti *et al*. (2012); Sugumaran *et al*. (2013) and Saber-Samandari *et al*. (2014), the following key bands are present in PUL spectrum: 3305-3435; 2928-2929; 1636-1645; 1366-1368; 1148-1154-1155; 851-848; 738-755; 918-929; 1080-1081 $cm^{-1.}$ These bands

indicate vibrations of the type: OH; C-H sp_3; O-C-O; C-O-H; C-O-C; α-D-glucopyranoside; α-(1,4)-D-glucosidic; α-(1,6)-D-glucosidic; as well as CO and C_6-OH bonds (33), (34), (35), (36), (37), (38). Likewise, according to Elzein *et al*. (2004) and Correa *et al.* (2019), the following key bands are present in the FTIR spectrum of PCL: 2949; 2865; 1727; 1240; 1170 cm_1. These bands indicate stretches of type: asymmetry CH_2, symmetric CH, carbonyl, asymmetry C-O-C, symmetrical C-O-C (39),(40).

Moreover, for the PUL-50mg LfcinB $(21\text{-}25)_{Pal}$ membrane, the characteristic bands of PUL were observed in the spectrum in Figure 4.1 (b). The presence of PUL in the membrane was thus corroborated, however, for this case, two bands corresponding to the peptide LfcinB $(21\text{-}25)_{Pal,}$ at the wavelengths of 1004.56 and 1450.08 were observed and related to the amide II region for the stretching of carbonyl in the peptide bond (29). It was evident the presence of the peptide within PUL, suggesting the peptide was effectively encapsulated in the polymer matrix at the two load concentrations evaluated for the antimicrobial agent.

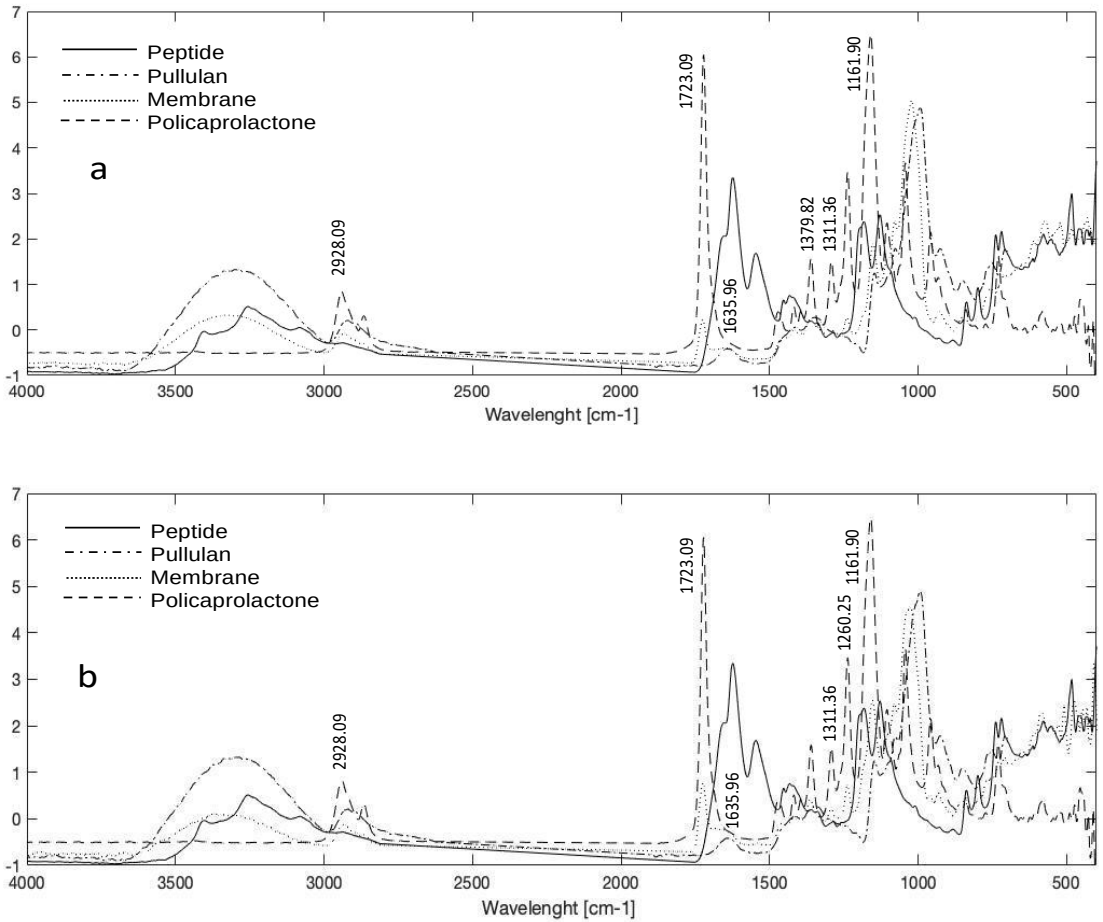

Figure 2 FTIR spectra of (a) LfcinB(21-25)$_{Pal}$ peptide, pure PUL, pure PCL and PCL- PUL/10mg LfcinB (21-25)Pal multilayer membrane, and (b) LfcinB(21-25)$_{Pal}$ peptide, pure PUL, pure PCL and PCL- PUL/50mg LfcinB (21-25)Pal multilayer membrane, made by electrospinning.

In Figure 4.2, in the case of the multilayer membranes, the representative peaks of the PCL were observed at wavelengths of 1729.09, 1311.35, and 1161.90 cm^{-1}, which corresponded to the symmetrical stretches CH, carbonyl, asymmetric C-O-C, and symmetric C-O-C, respectively (39). Also, the characteristic bands of PUL at 2928.09 and 1635.96 cm^{-1} were found, which confirms the presence of the two polymers in the membrane; it was evidenced a lack of chemical changes in the structure. Based on the above, the FTIR spectra confirmed electrospinning as an effective method for peptide encapsulation in polymeric membranes.

3.2 Qualitative analysis of the peptide LfcinB (21-25)$_{Pal}$

PUL-LfcinB(21-25)$_{Pal}$ membranes with peptide concentrations 10 and 50 mg were evaluated using the Kaiser test, to observe the presence of primary amines (41). A positive control (blue coloration) was used, which corresponded to the peptide LfcinB (21-25)$_{Pal}$, meanwhile a negative control (yellow coloration) corresponded to the PUL membrane. In relation to the membrane PUL-10mg LfcinB (21-25)$_{Pal}$, a yellow coloration was observed, which indicates there was no presence of the peptide, since it was not homogeneously incorporated into the fiber and its presence within the polymer matrix could not be evident. For the membrane PUL-50mg LfcinB (21-25)$_{Pal}$ a blue coloration was observed, indicating that the fiber contained primary amino groups of the incorporated peptide, confirming PUL effectively incorporated the peptide (26). However, the results showed that to encapsulate the peptide in the polymer matrix, relatively high concentrations of the active agent are required, since at low concentrations the encapsulation method is not effective due to the lack of peptide content in relation to the polymer and therefore its adherence to the matrix is reduced.

3.3 Reverse Phase Liquid Layer Chromatography Analysis (RP-HPLC)

An RP-HPLC was used to quantify the peptide LfcinB (21-25)$_{Pal}$ incorporated into the ultrafine membrane, after evidencing the absence of chromophoric groups that could interfere in the chromatographic process. The samples showed peptide retention times of 5.8 min, which was confirmed with a previous study carried out by Vargas *et al*. (2017) (23).

For the PUL-50mg LfcinB (21-25)$_{Pal}$ membrane, it was determined a peptide concentration of 164 ppm (0.164 mg of peptide/mL of solution) and a recovery percentage of 64-65%. On the contrary, the PUL-10mg LfcinB(21-25)$_{Pal}$ membrane had a concentration lower than LOD. From this result, it can be corroborated that although the electrospinning process is stable with up to 50 mg of peptide incorporation and an encapsulation percentage greater than 50%, the compound was not homogeneously incorporated within the polymer, which is evidenced by a low quantification of the active compound within the matrix. These results were corroborated by Roman *et al*. (2019), in which 13 mg of the peptide was incorporated into a PUL membrane, and a concentration of only 0.026 mg/mL and a recovery rate of 12-30% was obtained (15). In a similar study of encapsulation of an amphiphile peptide in polyvinyl alcohol matrices by electrospinning, percentages of encapsulation between 30 and 80% were found, for which the interest to use this technique for the encapsulation of active compounds have an increasing trend (42). To improve the distribution of the peptide in the membrane, different ultrasound times can be tested in the polymer-peptide solution, because with a longer time there would be a greater shear of the liquid particles to decrease the formation of bubbles and, in this way, the stability and the uniformity of the mixture and the peptide can be increased.

3.4 Antioxidant activity

Living organisms have a complex protection system that is activated to prevent oxidative damage. In this line of defense, some peptides can naturally exert antioxidant activity, playing a key role in the inhibition of oxidation and removing free radicals present in the medium (43),(44). Antioxidant activities of peptides are often attributed to their amino acid composition. Residues from hydrophobic amino acids such as leucine, proline, and isoleucine, as well as residues of aromatic amino acids such as tyrosine and tryptophan are frequently responsible for this activity (45).

According to previous studies, bovine lactoferrin has shown antioxidant properties, mainly because the iron present in milk is bound to lactoferrin giving the ability to capture free Fe^{3+} and the iron bound to the protein cannot participate as a catalyst for the generation of highly toxic hydroxyl radicals. These phenomena inhibit the Fenton reaction responsible for forming a hydroxyl radical and a hydroxide ion from the oxidation of organic substrates by

Fe^{2+} and H_2O_2 and, thus, reduce the availability of absorbing iron necessary for the milk of neonates (46),(47).

To evaluate the antioxidant activity, a sample of 5 mg of pullulan membrane was initially taken to observe whether the PUL itself has antioxidant activity, for which inhibition of 0% was observed, indicating the antioxidant activity registered in the subsequent tests corresponded exclusively to the peptide. Regarding the samples with peptide inclusion, 10 mg membrane sample of PUL-10mg LfcinB $(21\text{-}25)_{Pal}$ was taken, finding an inhibition percentage of 15.83%, and an anti-radical activity of $1.59 \times 10^{-4} \pm 1.51 \times 10^{-5}$ mg gallic acid/mg membrane.

Similarly, 10mg of PUL-50mg LfcinB$(21\text{-}25)_{Pal}$ membrane was taken, an inhibition percentage of 51.31%, and anti-radical activity of $5.21 \times 10^{-4} \pm 1.12 \times 10^{-5}$ mg gallic acid/mg membrane were established. The previous data suggest that antioxidant activity was related to the concentration of peptide since the membrane that had the maximum amount of peptide supported by the polymeric matrix increased its antiradical activity by almost three times.

The previous results corroborate the presence of antioxidant activity in the synthesized peptide from lactoferrin for the two cases in which it was evaluated, being dependent on the concentration of the peptide in the samples since the higher concentration of peptide incorporated, the greater the antiradical activity. This suggests that the palindromic peptide, in addition to its high reported antimicrobial activity (23), had antioxidant activity. These factors are advantages to use these membranes in the food industry since contribute to the reduction of oxidation reactions, avoiding changes to physicochemical or sensory aspects in terms of variation in taste, odor, color, and/or growth of microorganisms (48).

3.5 Antimicrobial activity

Foodborne diseases are the result of ingesting products contaminated with pathogenic microorganisms and/or their toxins. Among the various types of microorganisms, *E. coli* is the most prone to generate infections and diseases in humans (49). This bacterial strain was used in this study given its high pathogenic capacity and has been used as a research model for its sensitivity to peptides derived from bovine lactoferricin (27). According to the

results obtained (see Table 1), membranes composed of PUL and loaded with the peptide LfcinB(21-25)$_{Pal}$ showed antimicrobial activity against an *E.coli* strain.

Table 4.1 Determination of the MIC of the PUL-LfcinB(21-25)$_{Pal}$ membrane against *E.coli* ATCC 25922. Positive control (+), negative control (-), technique control (T1), growth control (CT). Inoculum of 500 000 UFC/mL; (n=2).

Matrix	**Peptide concentration (µg/mL)**					**Controls**				**CMI/CMB µM**
Pul-LfcinB (21-25)$_{Pal}$	200	100	50	25	12.5	**(+)**	**(-)**	**T1**	**CT**	***E.coli* 25922**
	0.512	0.275	0.145	**0.131**	0.542	0.105	0.598	0.889	0.108	17 (25)/68 (100)
	0.489	0.204	0.123	**0.130**	0.458	0.108	0.609	0.910	0.108	

Based on the above, a MIC of 17 µM and an MBC of 68 µM were found (see Figure 4.4). This is consistent with reported by Huertas *et al.* (2017), where the LfcinB(21-25)$_{Pal}$ palindrome peptide exhibited antimicrobial activity against an *E.coli* strain, establishing a MIC of 6.25 µM and an MCB of 12.5 µM. In another study conducted by León *et al.* (2015), the peptide showed antimicrobial activity against a strain of *Enterococcus faecalis* ATCC 29212, with a MIC of 27 µM and an MCB of 34 µM (26). Similarly, Vargas *et al.* (2017) showed that the palindrome peptide exhibited antimicrobial activity against *Salmonella enteritidis* ATCC 13076 with a MIC and MCB of 16 µM. Moreover, the Lfcin(21-25)$_{Pal}$ peptide had shown antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus* with a MIC of 135 µM (25). In summary, the peptide can inhibit the growth of Gram-negative and Gram-positive bacteria.

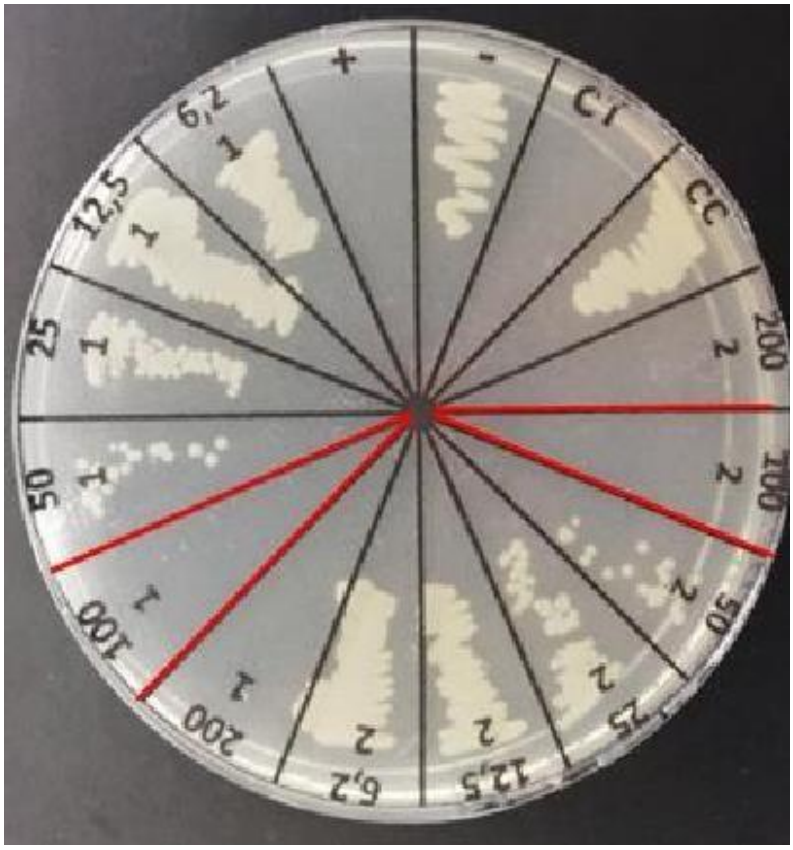

Figure 43 Determination of MBC. The effect of PUL-LfcinB $(21\text{-}25)_{pal}$ nanofiber at different concentrations on bacterial growth against *E.coli* 25922 was evidenced. Sections in red contained the lowest peptide concentration for which no bacterial growth was observed.

Antimicrobial peptides (AMP) are molecules with a variety of sizes and structures that are part of the innate immune response, i.e. they act as the first line of defense in several organisms including plants, animals, and humans, for which they have a low antigenicity and exhibit antimicrobial activities against a broad spectrum of pathogens (50),(51). The mechanism of action of these peptides on bacteria starts with an initial electrostatic interaction between the lateral chains of positively charged amino acids (arginine) and the negative chains of molecules of the surface of the bacterial wall, mainly lipopolysaccharides and teichoic acid present in the Gram-negative and Gram-positive bacteria respectively. Subsequently, a second interaction is generated between the lateral chains of hydrophobic residues of tryptophan and the lipid bilayer of the bacteria that lead to a destabilization of the membrane and, therefore, cellular lysis (52).

It was evidenced that the process of incorporation of an antimicrobial peptide LfcinB(21-25)$_{Pal}$ in PUL using electrospinning did not affect its antimicrobial activity. The results showed when the peptide is incorporated into a polymeric matrix, can inhibit the growth of pathogenic microorganisms in a similar way as when it is free. Therefore, this peptide is promising to be used as an active agent in the development of secondary devices for active packaging to prolong the shelf life of foodstuff.

4. Conclusion

Smooth ultra-thin multilayer membranes were obtained using electrospinning, with a thickness of approximately 0.20 mm, made from PCL solutions for the outer layer and PUL with LfcinB (21-25)$_{Pal}$ for the inner layer. In the multilayer membrane, it was possible to incorporate up to 50 mg of LfcinB (21-25)$_{Pal}$, in which the formation of ultrafine and cylindrical fibers was evident, with slight beads corresponding to the encapsulated peptide. FTIR spectra confirmed electrospinning did not generate modifications either the structure or in any chemical bonds of the LfcinB peptide (21-25)$_{Pal}$. Additionally, the multilayer membranes showed antimicrobial activity against *E.coli* and antiradical activity of $5.21 \times 10^{-4} \pm 1.12 \times 10^{-5}$ mg gallic acid/mg membrane at the highest load of the peptide. These types of technique and membranes can be potentially applied in the development of active packaging or controlled release systems to contribute to the preservation of foodstuff susceptible to microbial contamination mainly due to their high water content and special storage conditions.

5. Acknowlegments

The authors thank the Research Vice-Rectory, the Institute of Food Science and Technology, and the Faculties of Agricultural Sciences, Medicine and Sciences of the Universidad Nacional de Colombia for their scientific technical support. This research was funded by the Universidad Nacional de Colombia, through the projects "Development of antimicrobial nanofiber membranes with possible application in active packaging and pharmaceuticals" (Code: 41882) and “Evaluation of the use of polymer mixtures for the development of micro / nanofibers with the incorporation of an antimicrobial peptide” (Code: 46384).

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Conclusiones Generales

En el presente trabajo fue posible obtener membranas multicapa ultrafinas a partir de soluciones que contenían polímeros naturales y sintéticos, luego de que estas fueron electrohiladas. Las membranas multicapa de PCL-PUL-PCL fueron las más promisorias debido a que se evidenciaron fibras tubulares y lisas con un diámetro promedio de 0.1 µm, sin discontinuidades y se evidenció una buena estabilidad en el cono de Taylor durante el proceso de electrohilado. Los análisis DSC indicaron la estabilidad de las membranas PCL-PUL-PCL en rangos de altas temperaturas (200°C-300°C), los espectros de FTIR permitieron corroborar que las membranas multicapa contenían los polímeros de PCL y PUL, adicionalmente se evidenció que el proceso de electrohilado no produjo modificaciones químicas en la matriz polimérica. Lo anterior permitió elegir este tipo de configuración para la encapsulación del péptido antimicrobiano, debido a que el PUL pudo usarse como un agente encapsulante, mientras que la PCL ayudó a mantener la integridad estructural de la membrana.

La capacidad máxima de encapsulación del péptido LfcinB (21-25)$_{Pal}$ en la membrana multicapa elaborada fue 50 mg de péptido por g de PUL, ya que a concentraciones mayores se evidenció una baja estabilidad en el proceso de electrohilado debido a que no hubo formación de un *jet* de solución sino de un goteo constante lo que impidió la formación de fibras. Las membranas multicapa evidenciaron fibras ultrafinas y cilindricas, con unas ligeras protuberancias correspondientes al péptido encapsulado. De igual manera, los espectros de FTIR confirmaron que el electrohilado no generó modificaciones en la estructura o enlaces químicos de los polímeros, confirmando la presencia tanto de PCL como de PUL y el péptido.

Las membranas multicapa mostraron tener una actividad antiradicalaria de $5.21 \times 10^{-4} \pm 1.12 \times 10^{-5}$ mg ácido gálico/mg de membrana a la mayor carga de péptido posible la cual fue de 50 mg/g de PUL concluyendo que cuando se aumenta la concentración del péptido de 10 a 50 mg/g de PUL la actividad se incrementa casi tres veces. Adicionalmente se evidenció actividad antimicrobiana contra *E. coli* con una concentración mínima inhibitoria

de 17 µM, siendo eficiente contra bacterias Gram Negativas, así como a Gram Positivos de acuerdo a lo reportado a la fecha en literatura. La actividad antioxidante es una propiedad que hasta la fecha aún no se había evaluado para este péptido, pero que le brinda no solo un carácter antimicrobiano sino también un carácter antioxidante, el cual es útil en el área de alimentos ya que puede funcionar como defensa al estrés oxidativo. La técnica de electrohilado se presenta entonces como una alternativa útil y apropiada para la incorporación de péptidos antimicrobianos, puesto que permite un porcentaje de recuperación y encapsulación alto dentro de la matriz polimérica sin afectar su actividad biológica, principalmente en términos de la actividad antimicrobiana y antioxidante.

Finalmente, este tipo de fibras pueden ser empleados en la industria alimentaria como empaques activos y sistemas de liberación controlada de compuestos activos con características antimicrobianas y antioxidantes como el péptido LfcinB (21- 25)$_{Pal,}$ que funcionen como barrera complementaria al empaque tradicional para, de esta manera, contribuir en la conservación de alimentos susceptibles a la contaminación microbiana. Su aplicación tiene cabida principalmente en alimentos perecederos como frutas, hortalizas y productos cárnicos que poseen una alta actividad de agua y requieren adicionalmente de condiciones especiales de conservación bajo refrigeración o congelación.

Recomendaciones

Se recomienda hacer un estudio de análisis termogravimétrico (TGA) en las membranas multicapa (PCL-PUL-PCL) con el fin de complementar los ensayos de DSC y determinar de manera más concreta las propiedades químicas de los polímeros en términos de su estabilidad a diferentes temperaturas, debido a que en la elaboración de este tipo de estructuras la caracterización térmica ayuda a definir si funcionan como agentes encapsulantes de compuestos activos.

Adicionalmente, con el objetivo de evidenciar el potencial de aplicación de estas membranas ultrafinas obtenidas por la incorporación del péptido palindrómico LfcinB (21-25)$_{Pal}$ como empaques activos, se recomienda realizar un ensayo *in vivo* de liberación del péptido encapsulado en las membranas sobre diferentes matrices alimenticias como por ejemplo hortalizas, frutas o productos cárnicos, debido a que tienen un alto contenido de agua y una alta susceptibilidad a la contaminación microbiana. Esto permitiría determinar el tiempo de liberación máximo del péptido antimicrobiano y evaluar si efectivamente prolonga la vida útil del alimento, para con esto complementar la información proporcionada por esta investigación y abordar de manera más precisa cual es la matriz más adecuada para emplear este tipo de membranas como potencial dispositivo complementario en empaques activos.

De igual manera, sería interesante poder probar estas membranas ultrafinas como dispositivos secundarios en diferentes tipos de empaques elaborados a partir de polímeros como poliamida o polietileno, entre otros, con el fin de evaluar si hay una efecto favorable o desfavorable, y observar con cual empaque se presenta una mayor afinidad para tener una visión más detallada de su funcionamiento en la industria del embalaje.

Por otra parte, en la fabricación de membranas ultrafinas se podrían probar otros polímeros de origen natural y sintético que se encuentren aprobados para uso en alimentos con el fin de desarrollar fibras que funcionen como alternativa en la elaboración de dispositivos complementarios de empaques y que a su vez permitan encapsular otros péptidos antimicrobianos o mezclas de estos con el objetivo de evaluar si es posible incorporar mayor cantidad de estos agentes activos para de esta manera potenciar su acción frente

a un mayor espectro de microrganismos y por ende contribuir en la conservación de diversos alimentos.

Finalmente, se recomienda para las membranas multicapa (PCL-PUL-PCL) incorporadas con el péptido LfcinB (21-25)$_{Pal}$ realizar pruebas de caracterización térmica como DSC y TGA con el fin de evaluar la estabilidad del péptido cuando se encuentra incorporado dentro de la matriz polimérica. De igual manera, realizar ensayos de actividad antimicrobiana de las membranas frente a algún patógeno presente en alimentos como *Escherichia coli*, *Staphylococcus aureus* o *Salmonella*, con el objetivo de evaluar si la actividad antimicrobiana del péptido se ve afectada cuando se encuentra incorporado en una matriz multicapa.

Anexo A: Certificado de participación en el congreso Shelf Life International Meeting (SLIM2019)

SLIM 2019

SHELF LIFE INTERNATIONAL MEETING

9° Shelf Life International Meeting

CERTIFICATE OF *ATTENDANCE*

We hereby confirm that

Naples, Italy
June 17 - 20th 2019
Congress Center Federico II

Carlos Alberto Fuenmayor, Carlos Mario Zuluaga-Domínguez, Paula Judith Pérez Espitia, Ingrid Juliet Rodríguez-Sánchez, Dianney Clavijo Grimaldo, Maria Stella Cosio

attended the

9° SHELF LIFE INTERNATIONAL MEETING | 17-20 June 2019 | Naples, Italy

for oral presentation

"The Use of Electrospun Nanofibers for Encapsulation of Antimicrobial Agents: Opportunities and Challenges in Food Shelf-Life Extension"

organized by

GSICA

GSICA President
Prof. Luciano Piergiovanni

Federico II

Department of Agricultural Science
Prof. Elena Torrieri

iPCB
Consiglio Nazionale delle Ricerche

Institute of Polymers, Composites and Biomaterials
Dr. Giovanna G. Buonocore

THE USE OF ELECTROSPUN NANOFIBERS FOR ENCAPSULATION OF ANTIMICROBIAL AGENTS: OPPORTUNITIES AND CHALLENGES IN FOOD SHELF-LIFE EXTENSION

Abstract

Among encapsulation techniques of antimicrobial substances, electrospinning outstands for enabling the simple, room-temperature, and single-step synthesis of polymeric nanofibers that act as micro- or nanocapsules. A myriad of polymers has been successfully electrospun to obtain ultra-thin fibers, including synthetic polymers, biomacromolecules, and their composites. In the context of food packaging, these materials combine advantages of laminate materials, such as “continuity” and mechanical resistance, with the advantages of nanomaterials, such as large surface areas/porosities, and responsiveness to external stimuli. Ultra-thin fibers have been used to incorporate antimicrobial substances including: allyil isothiocyanate, diallyl sulfide, allicin, eugenol, triclosan, limonene, perillaldehyde, tea extract, curcumin, chitosan, and silver nanoparticles, among others. The resulting functionalized nanofibers have been proved as promising devices in shelf-life extension of various food models, such as rainbow trout fillets, pork meat, apple cider and strawberries. The target microorganisms against which these materials have been tested comprise *S. aureus, E. coli, Salmonella spp., Pseudomonas sp., Rhizoctonia*. Current trends include the encapsulation and release of viable microorganisms, as well as the incorporation of bioactive peptides with antimicrobial potential. Despite recent advances, the scaling up to commercial applications, by optimization of the setups for large processing volumes, remains a challenge. Moreover, the real application of these materials is still limited due to toxicity concerns, with more studies being needed to assess the risk caused by exposure to food products packaged in electrospun materials. We present, both from our experimental evidence and a literature review, the main aspects of this technology in the context of food packaging, including the main nanofiber-encapsulation approaches, relevant case studies on its use for shelf-life extension, trends, and drawbacks to be overcome.

Keywords: *nanofibers, electrospinning, active food packaging, antimicrobial compounds.*

Anexo B: Certificado de Participación en el congreso International Conference on Food Science and Nutrition 2019

Magnus Corpus Conferences and
Organizing Committee wish to thank

Prof/Dr/Mr/Ms Ingrid Juliet Rodríguez-Sánchez
Universidad Nacional de Colombia, Colombia

for her phenomenal and worthy Oral presentation on

Development of nanofibers from polymer mixtures as controlled release systems of bioactive compounds in foodstuff

at the "International Conference on Food Science and Nutrition"
held on October 23-25, 2019 in Rome, Italy

Claude Billeaud
University of Bordeaux, France

Massimo Collino
University of Turin, Italy

DEVELOPMENT OF NANOFIBERS FROM POLYMER MIXTURES AS CONTROLLED RELEASE SYSTEMS OF BIOACTIVE COMPOUNDS IN FOODSTUFF

Abstract

Food loss constitutes an economic concern in the industry. Among the factors that present a greater influence on food loss is composition, pH, water activity, redox potential, biological structure and the presence of etiologic agents. For this reason, delivery systems of bioactive compounds based on nanofibers are widely applicable in the food industry, favoring the release of substances in order to inhibit the growth of microorganisms, control oxidation processes or incorporate compounds into foods, in order to improve their sensory and nutritional characteristics. These structures could be obtained by electrospinning from a solution of a synthetic or natural-type polymer, that is subsequently subjected to an electric field. The obtained nanofibers offer an advantage due to their high porosity and surface ratio per unit area. The aim of this research was to assemble and characterize mixtures of polycaprolactone with starch and β-glucan, in order to evaluate their mechanical strength, absorption capacity, and structural characteristics. The experimental design has as factors some variables in electrospinning such as voltage, feed flow and distance to the collector, in order to obtain the adequate parameters for nanofiber formation. The best conditions obtained for polycaprolactone/β-glucan were a flow of 0,7 mL/h, a distance of 10 cm and a voltage of 7 kV, meanwhile, for polycaprolactone/starch a flow of 0,8 mL/h, a distance of 10 cm and a voltage of 20 kV. It was observed by scanning electron microscopy (SEM) that polycaprolactone/β-glucan nanofibers did not present beads, defects and have a continuous fibrillar structure in comparison with the polycaprolactone/starch mixture that showed irregular fibers, presence of beads, did not have tubular structure, and in addition, did not form a stable Taylor cone. Similarly, differential scanning calorimetry (DSC) showed coherent stability of nanofibers at different temperatures. Finally, the chemical composition of the fibers was determined through Fourier Transform Infrared Spectroscopy (FT-IR). The evaluation of these properties and stability is of great importance to design delivery systems of active agents in the food industry that contribute to improving food quality and safety.

Keywords: Electrospinning, polymers, nanofibers, release systems.

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