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DESENVOLVIMENTO DE FILMES BIODEGRADÁVEIS E NANOFIBRAS A
PARTIR DE RESÍDUOS DE CÁPSULAS GELATINOSAS

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PARTIR DE RESÍDUOS DE CÁPSULAS GELATINOSAS**

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos do Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, como um dos requisitos para a obtenção do grau de Doutor em Ciência e Tecnologia de Alimentos.

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RESUMO

A aplicação de polímeros biodegradáveis em substituição aos plásticos comuns derivados do petróleo representa uma oportunidade promissora de desenvolver materiais e tecnologias mais sustentáveis. Nesse contexto, resíduos de cápsulas nutracêuticas de gelatina foram utilizados para produzir filmes biodegradáveis pelo processo de *casting* e desenvolver nanofibras por *electrospinning*. O efeito de tratamento reticulante químico, da adição de nanoargila como agente reforçador e de aquecimento ôhmico à matriz polimérica dos filmes foi investigado, bem como o potencial de produzir nanofibras com o uso de solventes menos tóxicos. Os filmes desenvolvidos foram caracterizados quanto às suas propriedades mecânicas, dinâmico-mecânicas, físico-químicas, estruturais, morfológicas, ópticas, e térmicas, enquanto as nanofibras foram morfolologicamente analisadas. De modo geral, as estratégias empregadas contribuíram para o aprimoramento das propriedades dos filmes, especialmente solubilidade em água e permeabilidade ao vapor de água para o caso dos filmes reticulados, e as propriedades mecânicas dos filmes incorporados de nanoargila e produzidos por aquecimento ôhmico. Além disso, os filmes reticulados com genipina demonstraram seu potencial em manter a qualidade de azeite de oliva durante armazenamento em condições aceleradas. Os bionanocompósitos apresentaram alta taxa de biodegradabilidade e tiveram o efeito na qualidade do solo avaliado que comprovou a ação de microrganismos no processo de biodegradação. Em relação às nanofibras, foi possível desenvolver os materiais utilizando uma combinação de ácido acético e ácido cítrico como solvente. Tais resultados cooperam para melhorar a aplicabilidade e o desempenho de filmes à base de gelatina no campo de embalagens de alimentos, bem como expandir as possíveis aplicações do resíduo de cápsulas gelatinosas para o ramo da nanotecnologia, agricultura e biorremediação.

Palavras-chave: gelatina; resíduos; *crosslinking*; nanocompósito; nanoargila; nanofibra.

ABSTRACT

The application of biodegradable polymers to substitute commonly used plastics derived from petroleum represents a promising alternative to develop sustainable materials and technologies. In this context, residues of nutraceutical gelatin capsules were used to produce biodegradable films by casting and nanofibers by electrospinning. The effect of crosslinking treatment, incorporation of nanoclay as a reinforcing agent and ohmic heating in the properties of films was investigated, along with the potential of producing nanofibers by using less toxic solvents. The developed films were characterized regarding their mechanical, dynamic-mechanical, physicochemical, structural, morphological, optical, and thermal properties, while nanofibers were morphologically analyzed. In general, the employed strategies contributed to the improvement of the properties of the films, especially concerning the total soluble matter and water vapor permeability for the crosslinked films, and the mechanical properties of nanocomposites and ohmic treated films. The genipin-gelatin films demonstrated the potential to maintain the quality attributes of extra-virgin olive oil during storage under accelerated conditions. Bionanocomposites presented a high rate of biodegradability and had their effect on the soil quality evaluated, which proved the action of microorganisms in the biodegradation process. In regards to the nanofibers, it was possible to produce them by using a combination of acetic and citric acid as solvent. Such results cooperate to improve the applicability and performance of gelatin-based films in the field of food packaging and contribute to expanding the application of gelatin capsule residues in the field of nanotechnology, agriculture, and bioremediation.

Keywords: gelatin; residues; reinforcing; crosslinking; nanocomposite; nanoclay.

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AA – ácido acético

AC – ácido cítrico

BaCl₂ – cloreto de bário

BMS-C – carbono da biomassa microbiana do solo

BS – base seca

CO₂ – dióxido de carbono

GLU – glutaraldeído/glutaraldehyde

GEN – genipina/genipin

HCl – ácido clorídrico

HPLC – cromatógrafo líquido de alta eficiência

H₂SO₄ – ácido sulfúrico

H₃PO₄ – ácido fosfórico

K₂Cr₂O₇ – dicromato de potássio

K₂SO₄ – sulfato de potássio

MMT – montmorilonita/montmorillonite

NaBr – brometo de sódio

NaOH – hidróxido de sódio

PBS – poli (succinato de butileno)

PCL – poli (ϵ -caprolactona)

PGA – poliglicólido

PLA – poli (ácido láctico)

PVA – permeabilidade ao vapor de água

qCO₂ – quociente metabólico do solo

RBS – respiração basal do solo

RGL – resíduo de cápsula de gelatina

SF – solução filmogênica

SFN – solução filmogênica de nancompósito

SG – solução baseada em resíduo de cápsula de gelatina

SN – solução baseada em nanoargila

TPA – tereftalaldeído

UR – umidade relativa

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1 INTRODUÇÃO

Os plásticos são empregados em diferentes áreas, sendo que o setor que mais demanda sua produção é o de embalagens. Entretanto, devido aos impactos negativos causados pelo acúmulo e permanência desses materiais no meio ambiente, a pesquisa e produção de polímeros biodegradáveis de fontes renováveis têm despertado o interesse do meio acadêmico e industrial. Esses materiais representam uma alternativa para substituir e/ou reduzir o emprego de plásticos derivados do petróleo convencionalmente utilizados.

Os polímeros de origem agrícola são um importante grupo de polímeros biodegradáveis que incluem materiais oriundos de biomassa como proteínas, polissacarídeos e lipídios, cuja capacidade em formar filmes vem sendo descrita na literatura. Dentre eles, a gelatina é uma proteína que tem recebido destaque devido a sua habilidade de formar filmes, além de outras características vantajosas como biodegradabilidade, biocompatibilidade e plasticidade. A gelatina é obtida pela desnaturação de colágeno presente em ossos, couro e tendões animais, principalmente de origem bovina, suína e peixes, o que a configura como uma matéria prima abundante e de baixo custo.

Nesse contexto, a utilização de resíduos da indústria nutracêutica representa uma ótima oportunidade para o desenvolvimento de filmes e nanofibras biodegradáveis, pois esse material é constituído basicamente por gelatina, água e plastificante. Esses resíduos são gerados pela produção de cápsulas gelatinosas utilizadas para encapsular compostos nutracêuticos (promotores de saúde), cuja demanda por parte dos consumidores aumentou muito nos últimos anos. Com isso, o reaproveitamento desse material colabora com a redução de impactos causados pelo amplo uso de plásticos sintéticos e com a reutilização de um material que é descartado pela indústria.

Devido a sua interessante composição, esse resíduo pode ser empregado para produzir filmes e embalagens biodegradáveis de alimentos com o objetivo de aumentar a vida útil, melhorar a qualidade dos produtos e também reduzir o acúmulo de resíduos sólidos não biodegradáveis. Apesar das características vantajosas apresentadas pelos biopolímeros de gelatina, em geral eles apresentam propriedades mecânicas, físico-químicas e de barreira inferiores quando comparados aos sintéticos. Tal fato acaba por restringir sua aplicação e resulta na busca por estratégias que possam contribuir com o aprimoramento das propriedades desses materiais, tais como crosslinking, blendas com outros compósitos, incorporação de fibras e adição de nanoargilas.

Nesse sentido, a reticulação química com o componente natural genipina representa a possibilidade de substituir agentes reticulantes tóxicos como o glutaraldeído e aprimorar propriedades mecânicas e de barreira dos filmes gelatinosos. A incorporação de nanoargilas tais como a montmorilonita como agente reforçador em filmes gelatinosos também tem demonstrado potencial de aumentar a resistência mecânica desses materiais. Alguns estudos relataram o uso de aquecimento ôhmico em substituição ao aquecimento convencional para promover alterações conformacionais na estrutura de filmes biodegradáveis que podem ocasionar melhoria nas suas propriedades finais.

Além disso, outras aplicações tecnológicas para essa proteína apresentam grande potencial, como o desenvolvimento de nanofibras para utilização em aplicações que incluem engenharia de tecidos, encapsulação de compostos bioativos e embalagens ativas. Diversos estudos relatam a obtenção de nanofibras de gelatina com o emprego de solventes tóxicos. Porém, o reaproveitamento do resíduo de cápsulas de gelatina em combinação com o uso de solventes de grau alimentício como ácido acético e ácido cítrico ainda não foi explorada.

Deste modo, a obtenção de filmes e nanofibras biodegradáveis a base de resíduos gelatinosos da indústria nutracêutica representam uma oportunidade de desenvolver tecnologias sustentáveis a partir de resíduos industriais, assim como a aplicação de reticulação química, agentes reforçadores e aquecimento ôhmico aos filmes podem contribuir para aprimorar as propriedades dos filmes e alcançar competitividade com plásticos convencionais.

Este trabalho está organizado na forma de capítulos. O Capítulo 1 compreende uma revisão bibliográfica e fundamentação teórica a respeito de polímeros biodegradáveis, gelatina, filmes e nanofibras de gelatina, processamento desses materiais, além de tratamentos como estratégia para o aprimoramento de suas propriedades. No Capítulo 2 os materiais e métodos empregados para a realização deste trabalho são explicados detalhadamente. Os quatro artigos produzidos a partir dos resultados obtidos com esta pesquisa são apresentados no Capítulo 3. A discussão e conclusão gerais são apresentadas nos Capítulos 4 e 5, respectivamente, enquanto as perspectivas futuras são apresentadas no Capítulo 6.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito de reticulação química, adição de montmorilonita como agente reforçador e aquecimento ôhmico em filmes biodegradáveis e desenvolver nanofibras elaborados a partir de resíduos de cápsulas gelatinosas.

2.2 Objetivos específicos

- Obtenção os filmes biodegradáveis a partir de resíduos de cápsulas gelatinosas através do processo de *casting*.
- Obtenção de nanofibras biodegradáveis a partir de resíduos de cápsulas gelatinosas por *electrospinning*.
- Investigar a obtenção de filmes biodegradáveis reticulados a partir de tratamento químico com os agentes genipina e glutaraldeído.
- Avaliar a obtenção de bionanocompósitos pela incorporação de montmorilonita à matriz polimérica.
- Analisar o efeito de aquecimento ôhmico nas propriedades dos filmes biodegradáveis.
- Caracterizar os filmes quanto às propriedades: mecânicas, dinâmico mecânicas, físico-químicas, estruturais, morfológicas, ópticas e térmicas.
- Caracterizar as nanofibras quanto às propriedades morfológicas.

CAPÍTULO 1: REVISÃO BIBLIOGRÁFICA E FUNDAMENTAÇÃO TEÓRICA

3 REVISÃO BIBLIOGRÁFICA E FUNDAMENTAÇÃO TEÓRICA

3.1 Polímeros biodegradáveis

O plástico é amplamente utilizado em uma gama de setores, que incluem embalagens alimentícias, eletrônicos, agricultura, roupas, construção, automobilística, saúde, lazer, dentre outros, e o tornam um importante pilar econômico dentro da sociedade. Em 2017, 348 milhões de toneladas de plástico foram produzidas no mundo, e a principal origem é o petróleo, matéria-prima de fonte fóssil (PLASTICSEUROPE, 2018). Em vista dos problemas ambientais ocasionados pelo uso crescente de plásticos, a aplicação de polímeros biodegradáveis de fontes renováveis tem despertado o interesse de pesquisadores e da indústria, pois apresentam a possibilidade de substituir os plásticos derivados do petróleo (CLARK, 2018).

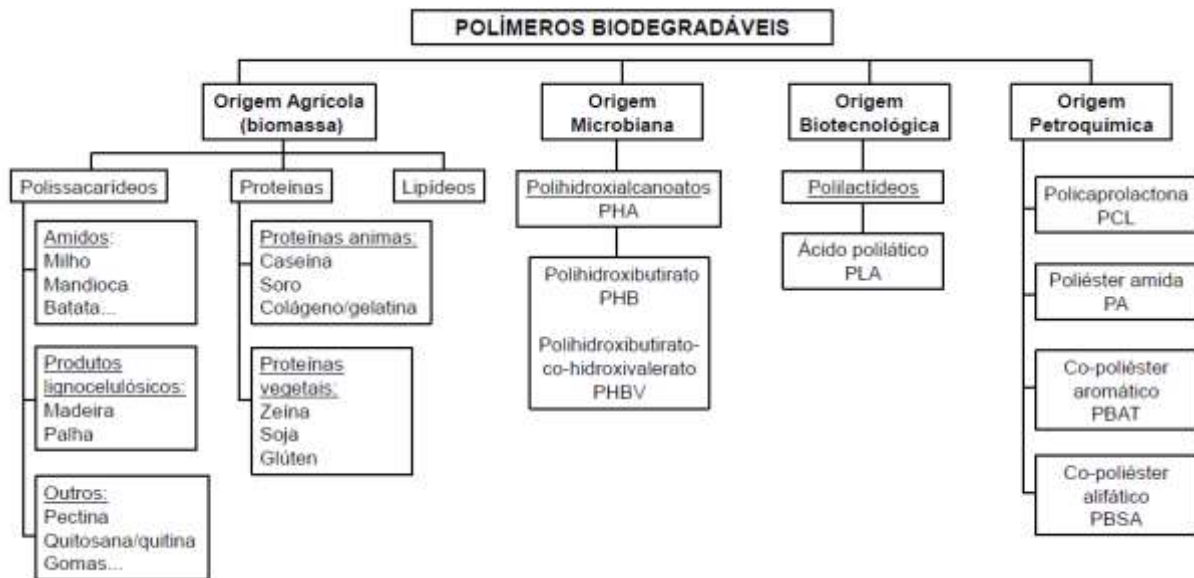
As principais vantagens da substituição dos plásticos comuns pelos biodegradáveis estão relacionadas com a redução de impactos ambientais, e o seu desenvolvimento é impulsionado pela crescente tendência de conceitos relativos à sustentabilidade, responsabilidade ambiental e tecnologias “eco-friendly”. Dependendo da fonte sustentável de carbono orgânico, biodegradabilidade e biocompatibilidade, estes polímeros têm demonstrado aplicações promissoras na indústria, agricultura, biomedicina e vida diária do consumidor (WANG & WENG, 2017).

A biodegradação é um processo biológico em que substâncias orgânicas são fragmentadas por organismos vivos e convertidas em substâncias naturais como dióxido de carbono, metano, água e biomassa (GROSS & KALRA, 2002; SHAH et al., 2008). Os polímeros biodegradáveis exibem essa característica e, conseqüentemente, podem servir de matéria orgânica para o processo de compostagem. Além disso, o adequado manejo e gerenciamento desses materiais também são capazes de contribuir com a redução do exagerado acúmulo de resíduos sólidos, constituído em grande parte pelo descarte de embalagens. Outra vantagem pode ser atribuída ao seu tempo de permanência no meio ambiente, que é imensamente menor quando comparado a outros materiais como vidro, metal e plástico não biodegradável (GROSS & KALRA, 2002; SHAH et al., 2008).

De acordo com o processo de síntese (Figura 1), os polímeros biodegradáveis podem ser classificados em quatro categorias: (I) polímeros a partir de biomassa, de origem agrícola; (II) polímeros obtidos por produção microbiana; (III) polímeros convencionalmente e quimicamente sintetizados a partir de monômeros obtidos a partir de recursos de origem

agrícola; e (IV) os polímeros obtidos a partir de fontes fósseis (AVEROUS & BOQUILLON, 2004).

Figura 1 - Classificação dos polímeros biodegradáveis de acordo com sua síntese.



Fonte: Adaptado de Averous e Boquillon, 2004.

Os setores onde a aplicação de polímeros biodegradáveis é promissora incluem: embalagens, médico, agricultura, e indústria automotiva (KUMAR, KARTHICK & ARUMUGAM, 2011). Dentre os polímeros de origem agrícola proteicos, a gelatina tem sido amplamente utilizada no desenvolvimento de filmes biodegradáveis devido ao seu alto potencial para aplicação comercial como filmes para embalagens alimentícias (NUR HANANI, ROOS & KERRY, 2014). Além disso, devido a sua alta biocompatibilidade, hidrofobicidade e bioatividade, associadas a sequências peptídicas específicas, nanofibras de gelatina também têm sido investigadas quanto à sua aplicação como material de suporte no setor biomédico de engenharia de tecidos, na encapsulação de compostos bioativos e em embalagens ativas (DENG et al., 2018).

3.2 Gelatina

A gelatina é obtida a partir da desnaturação do colágeno presente em ossos, pele e tendões animais. A sua extração é principalmente efetuada através de pré-tratamento em banho ácido, que dá origem à gelatina tipo A, ou alcalino, que gera a gelatina tipo B, seguida de processamento térmico. Dependendo do processo de fabricação, a proteína extraída é em

seguida deionizada, esterilizada e seca, mas o processo pode ser adicionado de outras etapas. O material obtido após a secagem é então chamado de gelatina (DUCONSEILLE et al., 2015).

As fontes mais abundantes de produção de gelatina são pele de porco, couro bovino, e ossos suínos e bovinos, embora outras fontes de gelatina, como a de peixe, venham crescendo na última década (GÓMEZ-GUILLÉN et al., 2009). Diferentes fatores podem influenciar as propriedades funcionais e térmicas, e a capacidade da gelatina de formar filme, dentre eles: origem da espécie (mamífero, peixe de água fria, peixe de água quente); parte do animal que forneceu o colágeno (ossos, pele, tendões); idade do animal (afetam as ligações cruzadas do colágeno); e severidade do processo de extração (pH, temperatura) (GÓMEZ-ESTACA et al., 2009a). A força de gel, também conhecida como valor *Bloom*, é outra propriedade que diferencia os diferentes tipos de gelatina; é uma medida da resistência e dureza da gelatina que reflete o peso molecular médio de seus constituintes e é em geral entre 30 e 300 *Bloom*, sendo que altos valores de *Bloom* indicam maior força (NUR HANANI, ROOS & KERRY, 2014).

A estrutura de colágeno consiste de três distintas cadeias alfa (α) de polipeptídios estabilizadas na forma de uma tripla hélice principalmente por ligações de hidrogênio intra- e intermoleculares e também por interações de van der Waals e ligações cruzadas covalentes. As ligações de hidrogênio podem ser tanto entre os grupos de CO e NH ou entre dois grupos de dois CO da cadeia polipeptídica. Cada uma das cadeias polipeptídicas consiste da repetição sequencial de três aminoácidos, Gly-Xaa-Yaa, onde Gly é glicina, e Xaa e Yaa podem ser qualquer resíduo de aminoácido, mas em geral são prolina e hidroxiprolina. Nessa estrutura, os resíduos de glicina estão orientados para o centro, enquanto os resíduos Xaa e Yaa ficam expostos ao solvente (GHOSHAL et al., 2010; TONGDEESOONTORN & RAWDKUEN, 2019).

A desnaturação do colágeno para obtenção da gelatina envolve a perda de sua estrutura nativa, em que a gelatina mantém a estrutura primária da proteína, mas envolve o rompimento total ou parcial da estrutura de tripla hélice e da estrutura secundária, provocados pelo rompimento das ligações de hidrogênio. As fibras de colágeno que formam hélices perdem a sua conformação durante o aquecimento, podendo recuperar parcialmente a sua estrutura durante o resfriamento. Consequentemente, a composição de ambos é muito similar, porém com diferentes estruturas. Durante a gelificação, a estrutura de gelatina muda, e de acordo com o estado de gel, cadeias têm diferentes arranjos espaciais e diferentes interações. Estas duas características dependem da concentração de gelatina, da temperatura e da energia

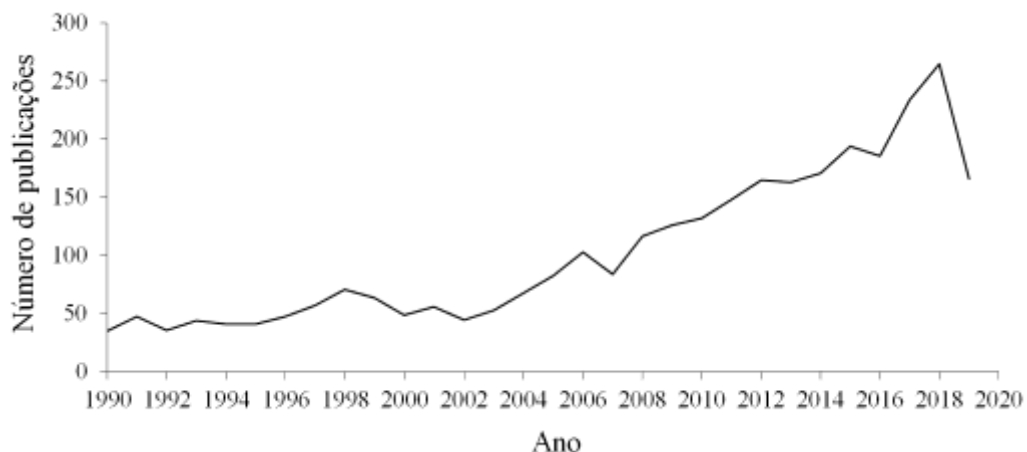
necessária para a formação da estrutura secundária (DUCONSEILLE et al., 2015; GHOSHAL et al., 2010).

Um estudo realizado por Coppola, Djabourov e Ferrand (2012) constatou três diferentes estados estruturais para a gelatina de filmes de gelatina de couro bovino tipo B: o estado amorfo, correspondente a uma estrutura em espiral com estrutura primária; o estado semicristalino, composto de triplas hélices e uma estrutura em espiral; e o cristalino, correspondente ao empacotamento das triplas hélices e uma estrutura em espiral. De acordo com os autores, a velocidade de secagem dos filmes tem grande influência na estrutura obtida, onde o estado amorfo é obtido quando os filmes de gelatina são secos rapidamente, enquanto que o estado cristalino corresponde a uma taxa de secagem lenta.

3.3 Filmes biodegradáveis de gelatina

Um extenso relato a respeito de filmes de gelatina pode ser encontrado na literatura e o desenvolvimento de tais materiais tem testemunhado crescente expansão em publicações (artigos, artigos de revisão, livros e etc.) desde a década de noventa (Figura 2).

Figura 2 - Número de publicações relacionadas a filmes à base de gelatina, desde 1990 até agosto de 2019.



Fonte: Web of Science, 2019.

Devido ao elevado número de dados da literatura relacionados a filmes à base de gelatina, a Tabela 1 ilustra uma extensa lista de estudos realizados até então sobre o tema, bem como identifica a fonte da gelatina, e o método e plastificantes empregados. A utilização de gelatina para a produção de filmes apresenta características vantajosas como

biodegradabilidade, biocompatibilidade, propriedades gelificantes e plasticidade Além disso, os filmes de gelatina podem oferecer proteção aos alimentos da ação da luz, oxigênio e umidade, bem como funcionar como barreira a gases e aromas (GÓMEZ-ESTACA et al., 2009b; GÓMEZ-GUILLÉN et al, 2009; TONGDEESOONTORN & RAWDKUEN, 2019).

Entretanto, devido ao caráter hidrofílico da gelatina, a propriedade de barreira ao vapor de água dos filmes compostos por essa proteína é desfavorável, uma vez que se deve considerar que, para a maioria dos alimentos, é necessário que essa propriedade apresente valores baixos a fim de manter as características do produto embalado. Além disso, em geral as propriedades mecânicas oferecidas por filmes gelatinosos são inferiores a filmes sintéticos, tornando-se interessante a investigação de alternativas que possam superar essas limitações. Com o objetivo de reverter essa situação e também para aprimorar suas diversas propriedades, diferentes estratégias têm sido desenvolvidas, dentre elas: *crosslinking* (reticulação), filmes laminados, blendas, e compósitos com nanomateriais e fibras naturais (MARTUCCI & RUSECKAITE, 2010a).

Quanto aos agentes plastificantes, os mais comumente empregados são o glicerol e sorbitol, como pode ser observado na Tabela 1. Em geral, a adição de plastificantes afeta as propriedades da matriz polimérica, levando ao aumento da flexibilidade, extensibilidade e redução da fragilidade do filme (SUDERMAN, ISA & SARBON, 2018). Entretanto, outros plastificantes, como ácido oleico, ácido cítrico, ácido tartárico, ácido málico, etileno glicol, dietilenoglicol, trietileno glicol, etanolamina, dietanolamina, e trietanolamina foram aplicados com sucesso em filmes de gelatina de osso bovino (CAO, YANG & FU 2009). Além disso, o potencial uso de hidrolisados de gelatina como agente plastificante foi constatado ao ser incorporado em filmes de proteína miofibrilar de peixe (NUANMANO, PRODPRAN & BENJAKUL, 2015).

Tabela 1- Listagem compreensiva de artigos sobre filmes à base de gelatina publicados até agosto de 2019.

Fonte	Método	Plastificante	Referência
Pele suína	<i>Casting</i>	Glicerol, sorbitol, sacarose	Arvanitoyannis et al., 1998
Pele suína	<i>Casting</i>	-	Bigi et al., 2001 Bigi et al., 2002 Bigi et al., 2004 Prasertsung et al., 2010 Peña et al., 2010 Soradech et al., 2012 Coimbra et al., 2014
Pele suína e osso bovino	<i>Casting</i>	-	Pavlov et al., 2001
Pele suína	<i>Casting</i>	Glicerol, propileno glicol, di-etileno glicol e etilenoglicol	Vanin et al., 2005
Pele suína	<i>Casting</i>	Glicerol, sorbitol	Thomazine et al., 2005
Pele suína	<i>Casting</i>	Glicerol	Bergo & Sobral, 2007 Wang et al., 2009 Alves et al., 2011 Molinaro et al., 2015 Flaker et al., 2015 Valencia et al., 2016 Shi et al., 2017 Lupina et al., 2019
Couro suíno	<i>Casting</i>	Lecitina e extrato de Yucca	Andreuccetti et al., 2011

Fonte	Método	Plastificante	Referência
Suína	<i> Casting </i>	Sorbitol	Kowalczyk et al., 2015
Osso bovino	<i> Casting </i>	-	Matsuda et al., 1999
Couro bovino	<i> Casting </i>	Sorbitol	Sobral et al., 2001
Pele bovina	<i> Casting </i>	Glicerol	Zheng et al., 2002 Acosta et al., 2015 Liu et al., 2016 Wu et al., 2017
Couro bovino	<i> Casting </i>	Glicerol	Carvalho & Grosso, 2004 Martucci & Roseckaite, 2010 Mu et al., 2012 Ma et al., 2013 Guo et al., 2014 Li et al., 2015
Couro bovino	<i> Casting </i>	Triacetina	Bertan et al., 2005
Couro bovino	<i> Casting </i>	-	Martucci et al., 2006
Osso bovino	<i> Casting </i>	Glicerol	Cao et al., 2007b
Couro bovino e pele de atum	<i> Casting </i>	Glicerol/sorbitol	Gómez-Estaca et al., 2009c
Bovina	<i> Casting </i>	Glicerol	Rivero et al., 2010 Díaz et al., 2011 Kavoosi et al., 2014 Kadam et al., 2015 Musso et al., 2016; 2019 Rasid et al., 2018 Chentir et al., 2019 Wang et al., 2019

Fonte	Método	Plastificante	Referência
Pele bovina	<i>Casting</i>	-	Wang et al., 2012
Bovina	<i>Casting</i>	Glicerol e/ou sorbitol	Nafchi et al., 2014 Moreno et al., 2017
Couro bovino	<i>Casting</i>	Propileno glicol	Martucci et al., 2015
Pele de atum	<i>Casting</i>	Glicerol	Gómez-Guillén et al., 2007
Pele de tilápia	<i>Casting</i>	-	Pranoto et al., 2007 Liu et al., 2012
Pele de merluza do Alasca e salmão rosa do Alasca	<i>Casting</i>	-	Chiou et al., 2008
Pele de alabote do Atlântico (<i>Hippoglossus hippoglossus</i>)	<i>Casting</i>	Sorbitol	Carvalho et al., 2008
Pele de lula (<i>Sepia pharaonis</i>)	<i>Casting</i>	Glicerol	Hoque et al., 2010
Pele de atum e couro bovino	<i>Casting</i>	Glicerol/sorbitol	Gómez-Estaca et al., 2009b
Pele de bacalhau	<i>Casting</i>	-	Staroszczyk et al., 2012
Peixe de água morna	<i>Casting</i>	Glicerol e sorbitol	Núñez-Flores et al., 2013
Pele de lula (<i>Loligo formosana</i>)	<i>Casting</i>	Glicerol	Nagarajan et al., 2013
Pele de tilápia	<i>Casting</i>	Glicerol	Tongnuanchan et al., 2013 Nagarajan et al., 2014 Ahmad et al., 2015 Dou et al., 2018 Menezes et al., 2019 Nilsuwan et al., 2019
Bacalhau	<i>Casting</i>	Glicerol	Etxabide et al., 2015

Fonte	Método	Plastificante	Referência
Pele de tubarão	<i>Casting</i>	Glicerol	He et al., 2016
Pele de peixe de água fria	<i>Casting</i>	Glicerol	Hosseini et al., 2016 Wang et al., 2017
Pele de salmão	<i>Casting</i>	Glicerol	Staroszczyk et al., 2017 Quero et al., 2018
Pele suína	Extrusão/moldagem por compressão	Glicerol, sorbitol	Park et al., 2008
Pele de bacalhau, arinca e merluza do Alasca	Extrusão/moldagem por compressão	Glicerol	Krishna et al., 2012
Pele bovina, suína e de peixe	Extrusão	Glicerol	Nur Hanani et al., 2012
Pele bovina	Extrusão	Glicerol	Nur Hanani et al., 2013, 2014
Pele suína	Extrusão	Glicerol	Andreuccetti et al., 2017

A exploração de diferentes espécies de animais como fonte de gelatina e a otimização da extração de colágeno e gelatina tem sido alvo de inúmeras publicações nos últimos anos, e uma das principais razões é o crescente interesse pela valorização de subprodutos industriais (GÓMEZ-GUILLÉN et al., 2011). O desenvolvimento de filmes biodegradáveis de gelatina a partir de resíduos industriais representa uma possibilidade de transformar subprodutos e resíduos em novos produtos viáveis com diferentes aplicações e são uma oportunidade futura a partir de uma perspectiva global, conforme relatado por Etxabide et al. (2017). De acordo com Flôres et al. (2017), que relataram a aplicação de diferentes resíduos industriais como matriz para a produção de filmes biodegradáveis, filmes de gelatina podem ser obtidos principalmente a partir de resíduos e subprodutos originados do abate de animais, do processamento de peixes e de fontes farmacêuticas.

Em relação ao campo farmacêutico, a gelatina tem sido amplamente utilizada para a fabricação de cápsulas macias e duras, expansores de plasma e em materiais para cuidados com feridas (KARIM & BHAT, 2009). O processamento de cápsulas gelatinosas macias implica na formação de partes remanescentes de material, também referida como uma rede, que é então coletada para recuperação e reciclagem de gelatina ou descarte (GULLAPALLI, 2010). A eliminação e gestão deste resíduo implicam problemas ambientais e econômicos e, portanto, é importante buscar alternativas para reduzir a quantidade de resíduos e/ou reutilizar este material para o desenvolvimento de novos produtos.

Nesse sentido, tal material remanescente foi empregado como matriz na produção de filmes por Kenawy et al. (1999), que desenvolveram misturas e compósitos baseados em resíduos de gelatina e outros materiais de resíduos naturais ou sintéticos. Seus resultados demonstraram possibilidade de produzir filmes biodegradáveis flexíveis baseados nesse resíduo. Com o mesmo propósito da utilização de resíduos e matérias-primas de subprodutos agrícolas e industriais, Chiellini et al. (2001) desenvolveram filmes baseados nesse mesmo tipo de resíduo oriundo da fabricação de cápsulas farmacêuticas e avaliaram os efeitos da adição de poli (álcool vinílico) (PVA), uma matéria-prima lignocelulósica natural (LIG) e glutaraldeído (GLU) como agente de reticulação na taxa de biodegradação, sensibilidade à água, propriedades térmicas e termomecânicas dos filmes. Os filmes à base de resíduos de gelatina apresentaram alta biodegradabilidade, e os tratamentos efetuados melhoraram as propriedades dos filmes desenvolvidos.

Recentemente, alguns estudos também relataram a obtenção de filmes biodegradáveis de gelatina a partir de resíduos oriundos da fabricação de cápsulas nutracêuticas. Em geral,

esse material é composto por gelatina, glicerol e água, o que oportunizou seu uso como matriz polimérica. Iahnke et al. (2015) combinaram resíduos derivados da fabricação de cápsulas nutracêuticas de óleo de linhaça e do processamento mínimo de cenoura para produzir filmes à base de gelatina. De acordo com análises de DPPH e teste de conservação de óleo de girassol, os filmes desenvolvidos apresentaram propriedades antioxidantes pela ação de compostos bioativos que estavam presentes no pó de cenoura adicionado à matriz de gelatina. O filme controle formulado apenas com o resíduo gelatinoso exibiu estrutura homogênea e compacta, semelhante à de outros filmes à base de gelatina de diferentes fontes relatados na literatura.

O uso de diferentes resíduos industriais para desenvolver misturas ou compósitos com propriedades melhoradas ou embalagens ativas parece ter atraído cada vez mais atenção nos últimos anos. Crizel et al. (2016) empregaram resíduos da produção de cápsulas nutracêuticas de óleo de chia como biopolímero para produzir filmes biodegradáveis, transparentes e flexíveis. Também foi avaliada a incorporação de extratos de fibra e etanólicos de resíduos de processamento de mirtilo, e a adição desses ingredientes resultou em filmes ativos. Iahnke et al. (2016) prepararam um pó a partir de cascas, talos e aparas obtidos a partir do processamento mínimo de beterraba e estudaram o efeito de sua incorporação em filmes à base do resíduo derivado do processamento das cápsulas de gelatina. O filme controle sem a adição do pó apresentou propriedades comparáveis a outros filmes de gelatina relatados na literatura, enquanto o filme composto apresentou atividade antioxidante provavelmente devido ao conteúdo de betalaína no pó adicionado. O potencial antioxidante dos filmes foi demonstrado pelo efeito de proteção contra o processo de oxidação primária do óleo de girassol.

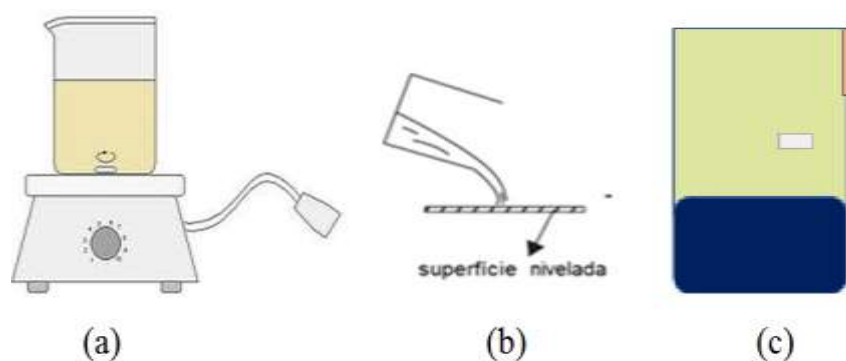
Em outro estudo, Campo et al., (2016) investigaram as propriedades mecânicas de filmes produzidos a partir de resíduos de cápsulas de óleo de cártamo e amido de milho em diferentes proporções. Observou-se que o maior teor de resíduos de gelatina na mistura de biopolímeros resultou em filmes mais flexíveis, com menor resistência à tração. Estes resultados foram atribuídos ao aumento do teor de glicerol na formulação, que foi intrínseca ao resíduo bruto e, como consequência, aumentou a mobilidade das cadeias de polímero devido ao efeito plastificante. De acordo com os estudos publicados na área de reaproveitamento de resíduos farmacêuticos para obtenção de filmes biodegradáveis à base de gelatina, o emprego desse tipo de material é uma alternativa viável para recuperação de

resíduos e representa uma estratégia promissora para a produção de embalagens sustentáveis e ativas.

3.3.1 Obtenção de filmes de gelatina por casting

Os filmes biodegradáveis são estruturas membranosas finas pré-formadas que são utilizados após serem formadas separadamente, enquanto que para o caso de revestimentos e coberturas, este filme fino é formado diretamente sobre o alimento. Em geral, os filmes são produzidos pela técnica de *casting*, que consiste em despejar a solução filmogênica em um recipiente adequado, seguido por um processo de secagem em condições ambientes ou sob uma umidade relativa controlada (Figura 3). Diversos tipos de materiais são empregados como placa-base, tais como acrílico, silício e teflon, e a sua escolha é importante para a obtenção de filmes que devem ser facilmente removidos sem serem danificados. Em geral, os filmes são secos por ar quente, energia infravermelha, energia de micro-ondas ou sob condições ambientais (DANGARAN, TOMASULA & QI, 2009; THARANATHAN, 2003). Tal técnica é amplamente empregada para obtenção de filmes gelatina, como pode ser observado na Tabela 1.

Figura 3 - Esquema ilustrativo do processo de formação de filmes pela técnica de casting. (a) Preparo da solução filmogênica; (b) acondicionamento da solução em placa acrílica; (c) secagem em estufa.



O processamento pela técnica de *casting* não requer equipamento específico e tende a consumir menor quantidade de matérias-primas durante a fabricação. No entanto, apresenta algumas limitações, pois requer espaço físico maior e também demanda considerável consumo de energia e tempo. Portanto, é justo dizer que esse método se torna menos prático para a produção normal em escala comercial (NUR HANANI et al., 2012). Em contrapartida,

técnicas empregadas para produção de polímeros sintéticos, como extrusão e sopro, podem oferecer dificuldades para o desenvolvimento de filmes biodegradáveis a partir de biopolímeros naturais, pois eles não têm pontos de fusão definidos e podem sofrer decomposição por aquecimento (THARANATHAN, 2003).

3.3.2 Estratégias para melhorar as propriedades dos filmes de gelatina

De maneira geral, filmes biopoliméricos apresentam propriedades mecânicas e de barreira inferiores quando comparados a filmes de materiais sintéticos convencionais. Tais atributos são importantes para sua aplicação como embalagens, pois garantem a integridade do material e minimizam interação com o ambiente, favorecendo, assim, a proteção e conservação do alimento embalado. Desse modo, algumas estratégias podem ser consideradas com o intuito de contribuir com aperfeiçoamento de tais características e possibilidade de aplicações, tais como: *crosslinking* (reticulação química), reforço com nanoargila e aquecimento ôhmico.

3.3.2.1 Reticulação química

A reticulação é o processo de formar redes tridimensionais ligando cadeias de polímero por ligações covalentes ou não covalentes e pode envolver ligações intra- e intermoleculares; é considerada uma das principais estratégias para modificação e melhoria de propriedades de filmes e revestimento, e é realizada através de processos químicos, físicos ou enzimáticos (AZEREDO & WALDRON, 2016). A reticulação restringe o deslizamento das cadeias umas sobre as outras e gera elasticidade em polímeros amorfos. Em geral, torna o polímero mais resistente ao calor, luz e outros fatores físicos, conferindo-lhe um elevado grau de estabilidade dimensional, resistência mecânica, e resistência química a solventes. Os efeitos da reticulação nas propriedades físicas dos polímeros são principalmente influenciados pelo grau de reticulação, a regularidade da rede formada, e a presença e a ausência de cristalinidade no polímero (BHATTACHARYA & RAY, 2009).

Os agentes de reticulação são adequados para materiais biopoliméricos, particularmente aqueles originados de carboidratos ou proteínas, proporcionando uma redução na permeabilidade ao vapor de água e gases em materiais destinados para embalagem de alimentos. O emprego dessa técnica pode auxiliar a superar as deficiências inerentes nas

propriedades mecânicas e de barreira dos biopolímeros, tornando-os mais aplicáveis em comparação aos materiais originados do petróleo (GARAVAND et al., 2017).

O glutaraldeído (GLU) é um dos agentes químicos mais utilizados para reticulação de filmes gelatinosos devido a sua eficiência em estabilizar materiais colagenosos. Sua ação envolve principalmente a formação de base de Schiff pela condensação de grupos formila do aldeído e grupos ϵ -amina presentes nos resíduos de aminoácidos lisina e hidroxilisina da gelatina. A reticulação da gelatina ocorre através da formação de ligações covalentes e envolve os grupos $-\text{NH}_2$ (principalmente) e $-\text{COOH}$ da cadeia principal da gelatina, e os grupos $-\text{OH}$ do agente reticulante químico (CHAIBI et al., 2015).

Em 2001, Bigi e colaboradores publicaram um dos primeiros estudos que relatou o efeito da concentração de GLU (0,125 a 2,5%) nas propriedades mecânicas, térmicas e de inchamento de filmes de gelatina. Os pesquisadores reportaram que a utilização de baixas concentrações de GLU é desejável a fim de prevenir efeitos tóxicos e que também é capaz de obter materiais estáveis e com diferentes possibilidades de aplicação. Com isso, constataram que com o emprego de até 1% de GLU foi possível aumentar a resistência à tração, Módulo de Young e estabilidade térmica e reduzir os valores de alongação e inchamento dos filmes gelatinosos (BIGI et al., 2001). Chiou et al. (2015) prepararam filmes de gelatina de peixe e GLU (0,25% a 0,75%) e constataram que o agente reticulante nas concentrações estudadas não afetou as propriedades mecânicas e temperaturas de fusão.

As propriedades térmicas e mecânicas, bem como a resistência à umidade e de permeabilidade ao vapor de água de filmes de gelatina bovina plastificados com sorbitol e reticulado com baixas quantidades de GLU (0-2 g/100 g gelatina) foram investigadas por Martucci, Accareddu e Ruseckaite (2012). Os resultados revelaram que grupos de cadeias laterais de aminoácidos livres de gelatina diminuíram com o GLU, confirmando a ocorrência de reticulação entre a gelatina e GLU. O efeito de GLU como agente reticulante em filmes de gelatina de pele de salmão e zeína foi investigado por Fan et al (2019), que obtiveram filmes com propriedades mecânicas e físico-químicas otimizadas com a adição de 0,02% GLU e 3% de zeína.

Os relatos de toxicidade relacionados a alguns agentes químicos reticulantes comumente empregados levaram a busca por compostos alternativos. Cao, Fu e He (2007a) utilizaram dois tipos compostos naturais, ácido ferúlico e ácido tânico, como agentes reticulantes em filmes de gelatina bovina. Os agentes reticulantes adicionados diminuíram a capacidade de absorção de água dos filmes, enquanto a permeabilidade ao vapor de água não

foi afetada. A resistência à tração foi afetada pelo pH das soluções filmogênicas e resultou em valores máximos para o valor de pH 7 da solução com ácido ferúlico, e de pH 9 para a solução com ácido tânico. Em outro trabalho, a utilização de ácido tânico e ácido cafeico foi investigada. Sua ação como agente reticulante em filmes de gelatina foi evidenciada pela técnica de ressonância nuclear magnética, que confirmou a ocorrência de reações químicas entre os sítios reativos dos compostos fenólicos e grupos amina da gelatina, formando ligações covalentes (ZHANG et al., 2010). Ácido tânico também foi empregado como agente reticulante em filmes compósitos de gelatina/nanopartículas de prata, em que foi relatado melhoramento nas propriedades mecânicas e de barreira à água com a adição de 5% do agente (MENEZES et al., 2019).

Outro agente biocompatível e de caráter atóxico que vem despertando o interesse por sua ação reticulante é a genipina. *Genipa americana* L. é uma árvore, também conhecida como jenipapo, cujas frutas contêm genipina, um composto de reticulação iridoide capaz de reagir espontaneamente com grupos de aminoácidos primários, peptídeos ou proteínas (CHIONO et al., 2008). Na reação de reticulação, uma substituição bimolecular ocorre e inclui uma substituição de um grupo éster na molécula genipina por uma amida secundária. A segunda reação é uma substituição nucleofílica do agrupamento éster. As reações de reticulação levam à formação de pigmentos azuis que incluem outras reações com um nível mais elevado de complexidade (RENHE et al., 2009).

Bigi et al. (2002) avaliaram o efeito de diferentes concentrações de solução de genipina nas propriedades de filmes de gelatina de pele de porco. Com o aumento da concentração, ocorreu diminuição da elongação e da capacidade de absorção de água, aumento do Módulo de Young e melhoria da estabilidade térmica. Com isso, esse agente natural foi considerado como uma alternativa de reticulador para aplicação em materiais à base de gelatina. Yao e colaboradores (2004) também avaliaram o efeito da concentração de solução de genipina nas propriedades de filmes de gelatina, e verificaram que a mínima concentração de genipina deve ser 0,5 g/100 g gelatina se o objetivo for alcançar uma completa reação de reticulação entre as moléculas de gelatina e genipina. Filmes de gelatina submetidos à reticulação por genipina resultaram em aumento dos valores de Módulo de Young e diminuição da elongação dos filmes, enquanto a resistência à tração permaneceu inalterada (GIOFFRÈ et al., 2012).

Um estudo desenvolvido por Ma e colaboradores (2013) teve como o objetivo produzir filmes de gelatina reticulados com genipina para atuarem como carregadores de

lisozima com liberação controlada do agente antimicrobiano. A reticulação ocasionada pela genipina retardou a liberação de lisozima e foi benéfica para modular as propriedades de liberação de lisozima a partir dos filmes de gelatina. Outro trabalho produziu compósitos de gelatina/montmorilonita e investigou o efeito de genipina na estabilização dos filmes. Foi constatada uma ação sinérgica entre montmorilonita e genipina, que impediu a dissolução dos nanocompósitos em solução aquosa e melhorou as suas propriedades mecânicas (PANZAVOLTA et al., 2013).

3.3.2.2 Nanoargila como agente reforçador

Os nanocompósitos são formados por uma matriz (polímeros, cerâmicas, metais) reforçada por um ou mais materiais que apresentem uma dimensão característica menor que 100 nm (HU, ONYEBUEKEA & ABATAN, 2010). A montmorilonita é um argilomaterial hidrofílico do grupo filossilicato comumente empregado na produção de bionanocompósitos, cuja estrutura em camadas de sua partícula é fundamentalmente constituída de uma folha de metal óxido/hidróxido octaédrica (Al-O) situada entre duas folhas de silicato tetraédrico (Si-O). A montmorilonita apresenta propriedades interessantes, tais como: grande área de superfície devido a sua estrutura em camadas; capacidade de intumescimento e potencial para a delaminação; partículas naturalmente nanométricas, que possibilitam seu uso em diferentes processos com foco em nanotecnologia; e partículas naturalmente carregadas, que levam a interações eletrostáticas relativamente fortes (ZHOU & KEELING, 2013).

Até o início da década de 2000, a produção de nanocompósitos do tipo polímero/montmorilonita foi amplamente investigada, e diversos estudos relataram a capacidade do uso de cargas inorgânicas para melhorar propriedades mecânicas e físico-químicas de polímeros sintéticos como náilon, poliestireno, resinas epóxi, poliamidas e policarbonato (ZHENG, LI & YAO, 2002). Entretanto, os impactos ambientais causados pelo consumo de plásticos sintéticos de origem não renovável geraram o interesse pelo desenvolvimento de compósitos silicatos biodegradáveis, e com isso, polímeros como PLA, PBS, PCL e PHAs passaram a ser estudados como matriz para incorporação de montmorilonita (RAY & OKAMOTO, 2003). A gelatina foi o primeiro polímero biodegradável de origem agrícola a ser estudado como matriz para incorporação de montmorilonita, a fim de obter nanocompósitos gelatinosos com melhores propriedades mecânicas e ampliar seu campo de aplicação. Pesquisadores estudaram as propriedades de

filmes de gelatina de pele bovina e MMT obtidos por *casting* e constataram que a adição de 5% de carga resultou em compósitos reforçados com maior resistência à tração e Módulo de Young, assim como propriedades térmicas aprimoradas (ZHENG et al., 2002).

Desde então, diversos estudos relataram a obtenção de nanocompósitos de gelatina/montmorilonita e investigaram o efeito de diferentes fatores nas propriedades dos filmes, como tipo e concentração da carga, método de dispersão, pH e velocidade de homogeneização da solução. Em geral, pequenas quantidades de MMT (menos de 10%) são suficientes para fortalecer as propriedades funcionais dos filmes. Em relação à aparência, a adição de MMT afeta a cor e propriedades ópticas dos filmes de transparente para levemente opaco (SOTHORNVIT, 2019).

Nagarajan e colaboradores (2014) concluíram que os diferentes tipos de montmorilonita (Cloisite® Na+, Cloisite® 15A, Cloisite® 20A e Cloisite® 30B) e concentração (0; 0,5; 1; 2,5; 5; e 10% p/p, em base seca de proteína) incorporados a filmes de gelatina de pele de tilápia afetam diretamente as propriedades do nanocompósito, e que em geral a adição de nanocarga hidrofílica resulta em melhores propriedades mecânicas que a hidrofóbica. Outro estudo avaliou o efeito da adição de diferentes concentrações de montmorilonita em filmes de gelatina de pele de porco, e constatou que a concentração crítica para a nanocarga atuar como agente de reforço está limitada a 5 g de MMT / 100 g de gelatina (FLAKER et al., 2015).

Bae et al. (2009) verificaram o efeito das nanocargas em propriedades de barreira e observaram que a maior concentração de carga estudada (9 g de MMT/100 g de gelatina de peixe) resultou em significativo decréscimo em valores de permeabilidade ao vapor de água e ao oxigênio. Quanto às propriedades térmicas, formulações contendo 3-10 g de MMT/100 g de gelatina resultaram em maior estabilidade térmica dos compósitos, atribuída a interações estabilizadoras entre os componentes, como ligações de hidrogênio (MARTUCCI, VÁZQUEZ & RUSECKAITE, 2007). Martucci e Ruseckaite (2009) desenvolveram filmes laminados de três camadas pelo processo de moldagem por compressão, em que as camadas externas eram compostas por filmes de gelatina reticulados com amido dialdeído e a camada interna por filme de gelatina reforçado com MMT.

Outro estudo utilizou MMT em diferentes concentrações (0-6,5%) para investigar o efeito na estabilização dos pigmentos antociânicos em filmes de gelatina contendo suco de acerola (RIBEIRO et al., 2018). A adição de 3,9% MMT resultou na estabilização da cor

durante o armazenamento e promoveu melhorias na resistência à tração e módulo de Young dos filmes, enquanto a elongação foi reduzida.

3.3.2.3 Aquecimento ôhmico

O aquecimento ôhmico oferece diversas vantagens, que incluem processamento contínuo sem superfície de transferência de calor, aquecimento uniforme do produto, melhor qualidade produto, menor tempo de cozimento, menor custo de capital, melhor eficiência energética, entre outros. Com isso, a aplicação dessa tecnologia no setor de alimentos vem sendo cada vez mais estudada e inclui: pasteurização, branqueamento, descongelamento, detecção online de gelatinização de amido, fermentação, descamação, evaporação, desidratação, fermentação e extração (VARGHESE et al., 2014).

O sistema para aquecimento ôhmico é representado por inúmeras possibilidades, porém alguns elementos e características são indispensáveis, tais como a presença de uma fonte de alimentação (gerador) para produzir a eletricidade e de eletrodos conectados à fonte de alimentação que devem estar em contato físico com a substância para passar a corrente elétrica. A distância entre os eletrodos no sistema pode variar de acordo com o tamanho do sistema, mas ao alterar esta distância, a intensidade do campo elétrico, expressa em volts por centímetro [Vcm^{-1}], pode ser variada (VARGHESE et al., 2014).

O aquecimento ôhmico é o fenômeno de dissipação de energia elétrica em calor que ocasiona o aumento de temperatura de materiais com resistência elétrica pela passagem de campos elétricos sem a necessidade de transferência de calor através de interfaces sólido-líquido (SASTRY, 2008). A principal diferença em relação a outros métodos de aquecimento elétrico é a presença de eletrodos que entram em contato com o material, pela frequência e pela forma onda do campo elétrico empregadas (SASTRY & BARACH, 2000).

Até o momento, poucas publicações puderam ser encontradas relatando o uso de campos elétricos na produção de filmes biodegradáveis. Um estudo de Lei et al. (2007) investigou os diferentes efeitos de aquecimento ôhmico e aquecimento por banho-maria na produção de filme proteico-lipídico a base de extrato de soja. Foi constatado que o rendimento, taxas de formação de filmes, eficiência de incorporação de proteínas, e capacidade de reidratação dos filmes foram aprimorados nos filmes tratados por aquecimento ôhmico em comparação ao convencional. Tais resultados foram atribuídos ao aquecimento uniforme do leite de soja promovido pelo aquecimento ôhmico, o que evitou o

superaquecimento parcial e assegurou a boa qualidade do filme proteico-lipídico. O extrato de soja foi passado por corrente elétrica alternada, de modo que a penetração de calor foi mais rápida, o que resultou em menos danos térmicos e maior capacidade de reidratação.

O efeito de campo elétrico (50, 100, 150, 200 Vcm^{-1}) nas propriedades de transporte e estrutura de filme de quitosana foi avaliado por Souza et al. (2009). Os resultados mostraram que o aquecimento ôhmico teve efeitos significativos nas propriedades físicas e estruturais do filme. Em geral, o efeito mais pronunciado foi observado para os tratamentos feitos com força de campo igual ou superior a 100 Vcm^{-1} , encontrando-se uma correlação positiva entre a solubilidade em água, e permeabilidade ao vapor de água, oxigênio e dióxido de carbono. Os resultados de microscopia de força atômica mostraram que a superfície dos filmes de quitosana foi mais uniforme quando um campo elétrico foi aplicado, o que pode estar relacionado com a estrutura de gel mais uniforme que levou às diferenças observadas em termos de propriedades de transporte.

Pereira et al. (2010) investigaram o efeito da aplicação de campos elétricos em soluções filmogênicas de proteína de soro de leite e compararam as propriedades físicas e estruturais dos filmes obtidos pelo método de aquecimento ôhmico e convencional. Um campo elétrico nominal de 10 Vcm^{-1} foi aplicado por 30 min a 85 °C. Os resultados demonstraram que o aquecimento ôhmico pode ter determinado menor taxa de desnaturação da proteína do soro de leite, levando à agregação menos pronunciada e menor ativação de grupos sulfidril livres quando em comparação com o aquecimento convencional sob perfil térmico semelhante nas soluções filmogênicas. O aquecimento ôhmico induziu alterações conformacionais de proteína ao aumentar o conteúdo de estruturas de folha- β na rede de filme, o que resultou em filmes proteicos com propriedades distintas, onde os filmes por aquecimento ôhmico apresentaram menor espessura e permeabilidade ao vapor de água.

Mais recentemente, a aplicação de campo elétrico moderado em filmes de quitosana e amidos incorporados de microcelulose cristalina foi avaliada por Coelho e colaboradores (2017). Foi observado que incorporação da microcelulose em combinação com a aplicação do campo elétrico (7,6 Vcm^{-1}) na solução filmogênica mantida a 70 °C provocou mudanças nas propriedades físicas e estruturais dos filmes, sendo esse efeito dependente do polissacarídeo usado. Baseado nos resultados do trabalho, os autores constataram que o aquecimento ôhmico altera a forma como a estrutura dos polissacarídeos é distribuída nos filmes, podendo impor a reorientação de moléculas. Para os filmes de quitosana, o tratamento parece ter promovido a reorientação de moléculas e redução da exposição de grupos hidrofílicos na superfície,

enquanto para os filmes de amido parece ter ocorrido um aumento da exposição de grupos hidrofílico em direção à superfície.

3.4 Nanofibras de gelatina

Em geral, o termo nanofibra refere-se a uma fibra com um diâmetro inferior a 1 μm . Nanofibras produzidas através da técnica de *electrospinning* apresentam características singulares que permitem seu emprego em diversas áreas, como aplicações médicas, biotecnologia, sensores, compósitos, têxteis, meios filtrantes, dentre outros (AGARWAL, GREINER & WENDORFF, 2013; AHMED, LALIA & HASHAIKEH, 2015b). No campo biomédico, é um fato estabelecido que quase todos os tecidos e órgãos como a pele, colágeno, dentes, cartilagem e osso, de uma forma ou de outra, têm algum tipo de semelhança com estruturas fibrosas altamente organizadas, hierárquicas e nanoestruturadas. Por conseguinte, a investigação sobre aplicações biomédicas centrou-se em: desenvolvimento de suportes fibrosos para engenharia de tecidos, curativo de feridas, mecanismos de administração de fármaco, e imobilização enzimática para obter taxas de reação mais rápidas em reações biológicas (AGARVAL et al., 2013).

O campo da medicina regenerativa tem demonstrado com sucesso seu uso como suporte para células para regenerar nova matriz extracelular que foi destruída por doenças, lesões ou defeitos congênitos sem estimular qualquer resposta imune. Nesses casos, os requisitos para um material a ser usado para fins de engenharia de tecidos são biocompatibilidade e biodegradabilidade, pois o suporte deve se degradar com o tempo e ser substituído por tecidos recém-regenerados (AGARWAL, WENDORFF & GREINE, 2008).

Além das aplicações biomédicas, as nanofibras vêm sendo amplamente estudadas como um material de filtro potencial no campo de proteção ambiental. Com base na concepção e construção da membrana e no tamanho dos contaminantes, os filtros são de dois tipos principais: nanofiltros e microfiltros. Para conseguir a remoção fácil de um contaminante específico, a membrana do filtro deve ter poros ou canais de passagem. Esses canais permitem que líquidos e partículas com a dimensão apropriada passem, enquanto prende as partículas ou contaminantes com um tamanho de partícula maior (HAIDER, A; HAIDER, S; & KANG, 2015).

A aplicação de nanofibras no setor alimentício também vem sendo investigada, principalmente nas áreas de encapsulamento de compostos, embalagens e coberturas

comestíveis. Nestes casos, as nanofibras podem ser usadas como sistema de entrega de nutrientes em alimentos, a fim de protegê-los durante o processamento e armazenamento, ou em sistemas para transferir os componentes para o alvo de interesse do corpo (BHUSHANI & ANANDHARAMAKRISHNAN, 2014; GHORANI & TUCKER, 2014).

De modo geral, os polímeros sintéticos oferecem maior facilidade de processamento por *electrospinning* e a morfologia das nanofibras obtidas é mais facilmente controlada do que quando comparada aos polímeros naturais (YOO, KIM & PARK, 2009). Entretanto, a aplicação no campo biomédico requer biocompatibilidade, onde os biopolímeros naturais apresentam melhor essa característica em relação aos homólogos sintéticos, e, portanto, são mais adequados para o corpo humano (ELSABEE, NAGUIB & MORSI, 2012). Com isso, diversos polímeros biodegradáveis vêm sendo estudados para obtenção de nanofibras por *electrospinning*, incluindo: polímeros naturais como colágeno, gelatina, seda, quitosana e alginato; polímeros sintéticos tais como poliglicólido (PGA), poli (ϵ -caprolactona) (PCL), poli (ácido láctico) (PLA) e seus copolímeros P (LLA-CL) e PLGA (KAI, LIOU & LOH, 2014).

A gelatina é um biopolímero que apresenta composição e propriedades biológicas bastante semelhantes às do colágeno, do qual é derivado. Esta proteína é biocompatível, biodegradável e facilmente disponível, sendo um dos biopolímeros mais utilizados pela FDA. Nanofibras de gelatina obtidas por *electrospinning* têm sido empregadas em aplicações que incluem engenharia de tecidos, encapsulamento de bioativos e embalagem ativas (DENG et al., 2018).

Apesar de a gelatina ser facilmente dissolvida em água, o processamento por *electrospinning* de soluções aquosas é complicado devido à viscosidade e propriedades de gel que apresenta a solução polimérica (SCHIFFMAN & SCHAUER, 2008). Com isso, solventes que dissolvem a gelatina à temperatura ambiente e apresentam alta volatilidade relativa são geralmente escolhidos, e incluem principalmente solventes tóxicos e agressivos, tais como 2,2,2-trifluoroetanol, dimetilsulfóxido, 1,1,1,3,3,3-hexafluoro-2-propanol e ácido fórmico (BHARDWAJ & KUNDU, 2010; LEIDY & XIMENA, 2019).

O estudo de Huang e colaboradores (2003) foi possivelmente o primeiro a empregar gelatina como biopolímero para a produção de nanofibras por *electrospinning*. Para isso, foi investigado o uso de solvente orgânico 2,2,2-trifluoroetanol na produção da solução polimérica de diferentes concentrações de gelatina de pele bovina, visto que a água como

solvente impossibilita o processamento. As soluções contendo 5 e 12% de gelatina (p/v) foram processadas com sucesso, e resultaram em nanofibras de diâmetro entre 100 e 340 nm.

Nanofibras foram produzidas a partir de soluções de gelatina (7-12%) em ácido fórmico por KI e colaboradores (2005). A viscosidade da solução obtida foi avaliada e constatou-se degradação da gelatina após 5 h de preparo, porém isso não afetou o processamento ou a morfologia das fibras obtidas. Parâmetros como campo elétrico, distância de rotação e concentração de solução, foram examinados para estudar os efeitos sobre o processamento por *electrospinning* e morfologia da estrutura nanofibrosa. Nanofibras de gelatina com morfologia uniforme e muito finas, com diâmetro entre 70 a 170 nm, foram produzidas pelo controle da concentração da solução e aplicação de um campo elétrico de 1,0 kV cm⁻¹ a uma distância de 10 cm. Além disso, as nanofibras apresentaram uma mistura de conformação helicoidal e aleatória, que resultou em estrutura amorfa com baixa cristalinidade.

Okutan, Terzi e Altay (2014) investigaram a influências de diferentes parâmetros nas propriedades de nanofibras de gelatina de pele bovina por *electrospinning*. Eles empregaram concentrações de polímero de 7 e 20% (p/v) em ácido acético e tensão aplicada de 28 ou 35 kV. A taxa de alimentação foi de 1 ou 0,1 mL/h. A partir da solução de gelatina a 7% (p/v) não foi possível produzir nanofibras, enquanto a concentração 20% (p/v) de gelatina resultou em nanofibras de acordo com a análise morfológica. A crescente tensão levou a diâmetros de fibra maiores com menor potencial zeta e menor coeficiente de difusão. A taxa crescente de alimentação levou a fibras com maior diâmetro e formação de grânulos. Nanofibras uniformes sem a formação de grânulos foram obtidas com tensão aplicada de 35 kV, taxa de alimentação de 0,1 mL/h, distância da placa de 10 cm, e concentração de gelatina na solução de 20% (p/v).

Soluções de gelatina comercial de pele de porco e mistura de ácido acético e ácido fórmico foram empregadas num estudo que avaliou o potencial de utilizar *electrospinning* para produzir nanofibras de gelatina (STEYAERT et al., 2016). Baseado em testes preliminares, os parâmetros do processo utilizaram solução de gelatina 13% (p/v) na mistura de solventes ácidos. As estruturas nanofibrosas foram produzidas em uma instalação multi-agulha, distância do coletor de 15 cm, taxa de alimentação de 1 mL/h, e tensão de 15 e 20 kV. Os ensaios foram realizados em ambiente com umidade relativa controlada de 35 ± 5% e uma temperatura de 21 ± 3 °C. De acordo com as medições de viscosidade, análise térmica e reologia, os resultados indicaram que não houve degradação das cadeias polipeptídicas das soluções. Quanto à morfologia, foi caracterizada por uma sua área superficial específica de

cerca 1250 vezes maior do que a gelatina original em pó. Além disso, a análise térmica mostrou que a solução a frio das nanofibras de gelatina não é completamente amorfa.

3.4.1 *Electrospinning*

As nanofibras podem ser produzidas por diferentes técnicas, como desenho, síntese de modelo, separação de fases, montagem automática e *electrospinning*. Essa última técnica tem sido crescentemente estudada e empregada, pois é um método simples e confiável para a preparação de nanofibras suaves com morfologia controlável e a partir de uma variedade de polímeros. As nanofibras obtidas por *electrospinning* apresentam diâmetros de tamanho muito pequeno, que lhes confere características específicas e únicas, como uma grande superfície específica, poros de tamanho pequeno, alta porosidade, flexibilidade nas funcionalidades de superfície e desempenho mecânico superior (AHMED, LALIA & HASHAIKEH, 2015a; HUANG et al., 2003).

Em geral, o sistema de *electrospinning* consiste de configuração de quatro componentes principais: uma fonte de alta tensão (1-30 kV) geralmente operada em modo de corrente contínua, embora o modo atual alternativo também seja possível; uma agulha ou capilar de aço inoxidável sem corte; uma bomba de seringa; e um coletor aterrado ou placa plana ou tambor rotativo (GHORANI & THUCKER, 2015). Os sistemas de *electrospinning* de agulha simples que utilizam coletor plano em geral são utilizados em escala laboratorial, a fim de inicialmente avaliar as condições que reproduzam um processo estável. Os sistemas multi-agulhas já são empregados com sucesso para a produção de grandes volumes de nanofibras, e essa técnica tornou-se a mais promissora em termos de produção de membranas nanofibrosas em larga escala (DARKO et al., 2016).

O processo envolve o uso de forças eletrostáticas que geram uma gota pendente de solução de polímero e a transformam em uma fibra fina seguida por deposição em um coletor aterrado. O polímero em fase líquida é extrudido através da agulha a uma taxa constante por uma bomba de seringa ou a uma pressão constante de um tanque de cabeçalho, formando uma gotícula na ponta. Quando um pequeno volume de líquido polimérico é exposto a um campo elétrico, a gotícula se alonga em direção ao ponto de menor potencial mais próximo, formando uma estrutura conhecida como cone de Taylor. Quando o campo elétrico atinge um valor crítico no qual as forças elétricas superam a tensão superficial da ponta da gota do cone

de Taylor, uma corrente de fluido carregada ou jato do polímero líquido é ejetado (GHORANI & THUCKER, 2015; HUANG et al., 2003).

Diversos fatores afetam o tamanho do material obtido por *electrospinning*, os quais em geral podem ser controlados. Dentre eles: o peso molar e a estrutura do polímero utilizado; o tipo e concentração do polímero e solvente empregado, que determinarão as propriedades da solução polimérica obtida, como pH, condutividade, a viscosidade e tensão superficial; as condições do processamento, como o potencial elétrico aplicado, a taxa de fluxo da solução, a distância entre a ponta da agulha e o coletor e ocasionalmente a natureza do material coletor; as condições ambientais como temperatura, umidade e velocidade do ar na câmara de processo (BHUSHANI & ANANDHARAMAKRISHNAN, 2014; DOSHI & RENEKER, 1995).

CAPÍTULO 2: MATERIAIS E MÉTODOS

4 MATERIAIS E MÉTODOS

4.1 Material

Os resíduos gerados pelo processamento de cápsulas nutracêuticas de óleo de linhaça (RGL), cuja composição é apresentada na Tabela 2 em base seca (BS), foram cedidos pelo Laboratório Químico Farmacêutico Tiaraju (Rio Grande do Sul, Brasil). A aparência desse resíduo pode ser verificada na Figura 4.

Tabela 2 - Composição química (g/100 g BS) do resíduo de cápsulas de gelatina (RGL).

RGL	
Proteína (Gelatina)	$60,79 \pm 3,15$
Lipídios	$1,40 \pm 0,07$
Cinzas	$0,290 \pm 0,004$
Glicerol*	$37,52$

Valores médios \pm desvio padrão.

Umidade = 35,00%

* Calculado por diferença.

Figura 4 - Aparência da rede de resíduo de cápsulas gelatinosas derivadas do processamento de cápsulas nutracêuticas de óleo de linhaça.



O composto glutaraldeído (GLU) 25% p/v em água foi adquirido da Nuclear Company (Brazil), enquanto a genipina (GEN) foi gentilmente extraída e cedida pelo Laboratório de Enzimologia do ICTA/UFRGS. A nanoargila montmorilonita organicamente modificada

(MMT Cloisite® 30B) foi fornecida pela Southern Clay Products, Inc (Texas, USA). Os solventes e reagentes utilizados para o processo de *electrospinning* foram adquiridos da Sigma-Aldrich e utilizados como recebidos (ácido acético e ácido fórmico), enquanto uma solução 1% em peso de ácido cítrico foi preparada. Todos os outros reagentes utilizados foram de grau analítico.

4.2 Processos de obtenção, reticulação e reforço dos filmes

4.2.1 *Casting*

As soluções filmogênicas (SF) baseadas em RGL, que já contêm glicerol como plastificante, foram obtidas pela mistura do resíduo com água destilada, de acordo com a proporção desejada para cada tratamento. A mistura foi colocada em um becker e mantida em temperatura de 70-75 °C com agitação magnética por cerca de 30-60 minutos, até que o completo derretimento da rede de RGL e homogeneização da SF fossem obtidos. No caso dos filmes reticulados, a temperatura foi mantida em um sistema de banho-maria (Fisatom, 752A, Brazil), enquanto para os filmes com MMT a solução foi mantida diretamente sobre chapa aquecedora (IKA-Works Inc, Ret Basic C, USA) a 75 °C até derretimento da rede, e após a temperatura foi mantida a 45 °C durante o tempo de homogeneização de 30 minutos.

Os agentes específicos (reticulante químico e reforçador) foram incorporados à matriz polimérica como descrito nos itens 4.2.2 e 4.2.3. Em geral, após a obtenção da SF, o processo de *casting* envolveu a distribuição da SF em Placas de Petri de poliestireno na concentração de 0,086 g/cm² para os filmes reticulados e produzidos por aquecimento ôhmico, e 0,1 g/cm² para os filmes reforçados com MMT. A secagem em estufa com circulação de ar ocorreu a 45 °C por 4 h para os filmes reticulados e produzidos por aquecimento ôhmico, e 35 °C por 20 h para os filmes reforçados com MMT. Antes da caracterização, os filmes foram armazenados em dessecadores contendo solução saturada de NaBr, mantidos a 58% UR/25 °C por dois dias.

4.2.2 Filmes reticulados com genipina

4.2.2.1 Obtenção da genipina

A genipina (GEN) foi extraída do fruto jenipapo (*Genipa americana* L.), utilizando enzimas celulolíticas em um sistema bifásico aquoso, consistindo em tampão e acetato de etila (1:1), como descrito por Winotapun et al. (2013). Depois da extração, a secagem de GEN ocorreu com a utilização de gás nitrogênio para evaporar o solvente orgânico (ZHOU et al., 2010). A pureza alcançada de 97% foi determinada por cromatografia líquida de alta eficiência (HPLC) (BELLÉ et al., 2018).

4.2.2.2 Obtenção do filme reticulado

O processo de reticulação química foi realizado em duas etapas, através da incorporação de soluções aquosas contendo os agentes químicos GLU ou GEN diretamente sobre a SF com concentração de 70 g RGL/100 g água. Primeiramente, a SF foi distribuída em Placas de Petri de poliestireno (0,086 g/cm²) e permaneceu em condições ambientais (25 ± 2 °C) por 30 min para ocorrer gelificação do sistema. Em paralelo, foram preparadas duas soluções aquosas de cada agente reticulante na concentração de 0,5% (w/v). Os filmes reticulados foram produzidos através da distribuição das soluções de GEN ou GLU sobre a superfície dos filmes gelificados (0,3 mL/g SF), baseado no método descrito por Klein et al. (2016). Depois de 15 h reagindo em temperatura ambiente (25 ± 2 °C), o sistema obtido foi lavado com água destilada (KLEIN et al., 2016), e submetido à secagem a 45 °C durante 4 h em forno com circulação de ar (DeLeo, B5AFD, Brasil). Os filmes controles seguiram o mesmo processo de preparação, sem a etapa de reticulação.

4.2.2.3 Grau de reticulação dos filmes

O grau de reticulação dos filmes reticulados com GLU e GEN foi determinado pelo ensaio da ninidrina, seguindo o método descrito por Sun e colaboradores (2016). A ninidrina pode reagir com grupos NH₂ (primários e secundários) de proteínas, produzindo uma cor púrpura, que é detectável por espectrofotômetro a 570 nm. Calculando a quantidade de grupos NH₂ nas amostras antes e depois da reticulação, pode-se calcular a quantidade de

grupos NH_2 que participam no processo de reticulação. Para isso, as amostras de filme controle e reticulados foram congeladas a $-80\text{ }^\circ\text{C}$ e, após, foram liofilizadas por 3 dias. Posteriormente, as amostras liofilizadas foram aquecidas a $90\text{ }^\circ\text{C}$ por 30 minutos sob agitação em uma solução de ninidrina em etanol. Solução de ninidrina foi utilizada como branco. Após resfriamento, a absorvância óptica foi medida por um espectrofotômetro (Shimadzu, UV-1800, Japão) a 570 nm . Uma curva padrão de glicina foi usada para determinar a concentração de grupos amino livres (NH_2).

4.2.2.4 Aplicação dos filmes reticulados com genipina

Após o desenvolvimento e caracterização dos filmes reticulados com genipina, foi avaliado o seu potencial de aplicação como embalagem alimentícia na manutenção dos atributos de qualidade de azeite de oliva extra virgem mantido sob condições de armazenamento acelerado de temperatura e iluminação. Para isso, foi empregada a metodologia descrita por Stoll et al. (2017), com algumas modificações. Os filmes foram produzidos para desenvolver sachês de azeite designados para uso único, similar aos disponibilizados em redes de lanchonetes e restaurantes. Os filmes foram cortados em retângulos e selados para formar sachês de $7\text{ cm} \times 5,5\text{ cm}$, que consistiram em uma área total de 77 cm^2 . Em seguida, 7 g de azeite (Capellini, Itália) foram adicionados ao sachê, cuja borda aberta foi então selada. Para meios de comparação, o azeite foi mantido em vidro aberto e três sachês fechados com a mesma área superficial: filme de polipropileno transparente, filme laminado de alumínio e o filme reticulado com genipina.

As amostras de azeite contidas nos diferentes tipos de embalagem foram armazenadas aleatoriamente em uma câmara (Tecnal, TE-402, Brasil) por 14 dias, a $40 \pm 2\text{ }^\circ\text{C}$, 54% UR e exposição à luz fluorescente de 900-1000 lux (Luxometer, V & A Instrument, MS6610, China). As amostras de óleo foram analisadas nos dias 0, 3, 7 e 14 quanto às seguintes propriedades:

- Índice de peróxidos, expresso em miliequivalentes de oxigênio ativo por quilograma de óleo (mEq/Kg), determinado de acordo com a AOCS (1993).
- Coeficientes de absorção específicos (K_{232} e K_{268}), determinados de acordo com AOCS (2009).
- Cor do azeite, avaliada através de colorímetro (modelo Miniscan XE, Hunterlab, Estados Unidos) e determinação dos parâmetros de cor CIE $L^*a^*b^*$ dos filmes, com operação

à luz do dia com D65. O padrão foi um disco branco com L_0^* : 97,5; a_0^* : 0,13 e b_0^* : 1,7. Para esse propósito, o azeite foi colocado em uma placa de Petri de vidro e coberto por um vidro fino.

4.2.3 Filmes reforçados com montmorilonita

4.2.3.1 Preparação dos filmes reforçados

A preparação da solução filmogênica de nanocompósito (SFN) foi realizada em três etapas, baseado em método adaptado de Rhim (2011) e Alexandre et al. (2016). Para isso, duas soluções foram preparadas separadamente, uma solução baseada em RGL (SG) e uma solução baseada na nanoargila (SN), que após foram combinadas nas proporções de acordo com a Tabela 3. Primeiramente, a nanoargila foi precisamente pesada, dispersa em água destilada (0,125 g/g de água) e hidratada utilizando um agitador magnético (IKA-Works Inc, Ret Basic C, EUA) durante 30 minutos a temperatura ambiente. A SN foi homogeneizada usando um misturador de alto cisalhamento (Heidolph Instruments, RZR 2021, Alemanha) a 1000 rpm por 4 h. A solução foi tratada num banho de ultrassom (Bandelin Eletronic, 400, Alemanha) a 55 °C durante 15 min para eliminar as bolhas de ar.

Tabela 3 - Proporção das soluções baseadas no resíduo de gelatina e de nanoargila empregadas para obtenção dos filmes nanocompósitos contendo diferentes concentrações de montmorilonita.

Filme	SG		SN (0,125g/g água)		SFN
Concentração de nanoargila	RGL (g)	Água (g)	SN (g)	Gelatina (g/100 g SFN)	Nanoargila (g/100g proteína)
0%	30,8	50	0	25	0
0,1%	30,8	49,9	0,1	25	0,1
0,5%	30,8	49,5	0,5	25	0,5
2 %	30,8	48	2	25	2
6%	30,8	44	6	25	6
10%	30,8	40	10	25	10

SG: solução baseada no resíduo de gelatina; SN: solução baseada na nanoargila; RGL: resíduo de gelatina; SFN: solução filmogênica de nanocompósito.

A SG foi preparada separadamente. Não foi adicionado qualquer agente plastificante, visto que o próprio RGL já contém uma quantidade considerável de glicerol (Item 4.1). O RGL foi dissolvido em água destilada (0,616 g RGL/g SFN) a 70 °C durante 5 min usando uma placa de aquecimento com agitador magnético (IKA-Works Inc, Ret Basic C, EUA) e depois a mistura foi homogeneizada a 45 °C por 1 h. Depois disso, as soluções SN e SG foram misturadas de acordo com a Tabela 3, e homogeneizadas por um misturador de alto cisalhamento a 1000 rpm durante 2 h. O filme controle foi preparado da mesma maneira, sem incorporação de nanoargila. As concentrações finais de nanoargila, considerando a composição de RGL da Tabela 2, foram: 0, 0,1 g, 0,5 g, 2,0 g, 6,0 g e 10,0 g /100 g de proteína. Os filmes foram colocados em placas de Petri (0,1 g/cm²) e secos em estufa a 35 °C por 20 h.

4.2.3.2 Avaliação da biodegradabilidade dos bionanocompósitos

A avaliação da biodegradabilidade foi realizada para os filmes contendo 0% e 2% de MMT, os quais foram selecionados após a caracterização dos filmes. O método empregado foi baseado na avaliação da biodegradação dos filmes quando os mesmos são enterrados e expostos à microflora natural encontrada no solo, de acordo com metodologia empregada por Martucci e Ruseckaite (2009). Para isso, caixas de plástico compartimentadas (5,5 cm × 6 cm × 6,5 cm) e com área superficial de 33 cm² foram preenchidas com solo orgânico natural (Vida Ecological Development Ltda, Brasil), que foi utilizado como meio de degradação de substrato para filmes.

Os filmes foram recortados em retângulos (2 cm x 3 cm) e secos em estufa (modelo TLK48, DeLeo, Brasil) a 60 °C até peso constante (m_0). Em seguida, eles foram acondicionados separadamente em recipientes (3 cm × 4 cm) feitos de malha de nylon de 2 mm, que foram previamente secos durante 48 h a 60 °C e pesados antes de serem adicionados da amostra de filme. Os recipientes contendo as amostras foram adicionados aos compartimentos de plástico contendo solo (Figura 5) e enterrados a uma profundidade de 4 cm da superfície do solo. A cada 5 dias, o conjunto malha/filme foi retirado do solo, lavado com água destilada para remover as partículas do solo e seco a 60 °C até peso constante (m_t). O solo foi mantido em 40% de teor de água. A perda de peso dos filmes (%) após a biodegradação do solo foi determinada pela equação:

$$WL(\%) = [(m_t - m_0)/m_0] \times 100$$

onde m_0 é a massa inicial da amostra seca de filme e m_t é a massa seca remanescente no tempo 5 ou 10 dias.

Figura 5 - Recipiente plástico compartimentado com solo orgânico e adicionado das amostras de filme acondicionadas em malha de nylon.



4.2.3.3 Efeito dos bionanocompósitos selecionados no solo

O efeito dos filmes previamente selecionados (contendo 0% e 2% de MMT) na qualidade do solo foi avaliado por respiração basal do solo (RBS), carbono da biomassa microbiana do solo (BMS-C) e quociente metabólico do solo (qCO_2) durante a biodegradabilidade dos filmes. O substrato foi o mesmo utilizado no ensaio de biodegradação. Ele foi peneirado através de uma malha de 2 mm para eliminar detritos orgânicos maiores e ajustado para 60% de capacidade de retenção de água. A RBS foi determinada para cada tempo de amostragem a partir de biodegradação como a evolução de dióxido de carbono (CO_2) aprisionada em 10 mL de solução de NaOH 1,0 M, pela adição de $BaCl_2$ e após a titulação com HCl 0,5 M, de acordo com Silva, Azevedo e De-Polli (2007a) com algumas adaptações. Para isso, recipientes ($D = 4,8$ cm) foram preenchidos com 25 g de substrato. Amostras de filme ($1,8$ cm \times $1,8$ cm) foram enterradas a uma profundidade de 1 cm da superfície do solo. A área de relação filme/superfície foi mantida a mesma que no teste de biodegradação.

A determinação do BMS-C foi realizada no solo orgânico natural (dia 0) e na mesma amostra utilizada para análise de biodegradação (dia 10), seguindo o método de extração de fumigação descrito por Vance et al. (1987) e modificado por Silva, Azevedo e De-Polli (2007b), com algumas adaptações. Para isto, 10 g do substrato incubado foi adicionado

diretamente de 1 mL de clorofórmio isento de etanol e fumigado durante 24 h a 25 ± 3 °C. Após esse período, 25 mL de 0,5M K_2SO_4 foram adicionados às amostras, as quais foram mantidas sob agitação orbital a 220 rpm por 30 min a 25 °C. As amostras foram deixadas em repouso durante 30 min. Os extratos foram obtidos por filtração do sobrenadante (papel 80 g/m²; porosidade 3 µm). A extração de amostras não fumigadas foi usada como controle. O extrato do solo (8 mL), $K_2Cr_2O_7$ 0,066 M (2 mL), H_2SO_4 (10 mL) e H_3PO_4 (5 mL) foram misturados.

Após resfriamento, a mistura foi adicionada de água deionizada (70 mL), resfriada novamente e titulada com sulfato de amônio ferroso (II) 0,033 M, utilizando difenilamina como indicador. O teor de carbono orgânico foi determinado após a oxidação do dicromato. O BMS-C (mg de substrato microbiano C kg⁻¹) foi calculado segundo a equação

$$BMS - C = [C(\text{fum}) - C(\text{n.fum})]/k_c$$

onde C (fum) é o conteúdo de carbono orgânico da amostra fumigada, C (n.fum) é o conteúdo de carbono orgânico da amostra não fumigada e fator $k_c = 0,33$, conforme descrito por Sparling e West (1988). O qCO_2 (mg C-CO₂.g⁻¹ BMS-C.h⁻¹) foi calculado de acordo com a seguinte equação (ANDERSON et al., 1993):

$$qCO_2 = RBS/BMS - C$$

4.2.4 Filmes produzidos por aquecimento ôhmico

Este processo é diferenciado do descrito anteriormente no Capítulo 4, item 4.2.1 devido ao método de aquecimento da solução filmogênica, que emprega campo elétrico ao invés de banho de aquecimento ou placa aquecedora. Para isso, foi utilizado um aparato experimental desenvolvido pelo Laboratório de Química de Alimentos/ICTA (Figura 6). O aparato é constituído de uma célula ôhmica, um estabilizador (Forceline, EV 1000 T/2-2, Brasil), um transformador variável (Sociedade Técnica Paulista LTDA, Varivolt, Brasil), fonte de alimentação com uma frequência alternada de 60 kHz, um computador, sistema de aquisição de dados (Novus, modelo Field logger, Brasil) e agitador magnético com aquecimento, conforme ilustrado na Figura 7.

A célula ôhmica (Figura 8) foi produzida a partir de uma vasilha de vidro Pyrex de 400 ml (8,0 cm de diâmetro e 13,5 cm de altura) e foi equipada com uma camisa de água. A tampa do recipiente continha quatro aberturas para sensores de temperatura e duas para os

eletrodos. Os eletrodos de titânio possuíam 5 cm de altura. A distância mínima e máxima entre os eletrodos foi de 5,7 cm e 7,0 cm, respectivamente.

As soluções filmogênicas (SF) foram obtidas pela mistura do resíduo gelatinoso e água destilada, na proporção de 80 g RGL/100 g água. As soluções obtidas foram avaliadas quanto à condutividade elétrica, a fim de verificar se a solução possuía condutividade necessária para a realização da técnica. O conjunto foi colocado dentro da célula ôhmica e mantido sobre um agitador magnético (IKA-Works Inc, C-MAG HS 10, Brasil) a fim de promover a homogeneização da solução durante o processo.

Figura 6 - Aparato experimental para a produção de filmes através de aquecimento ôhmico.

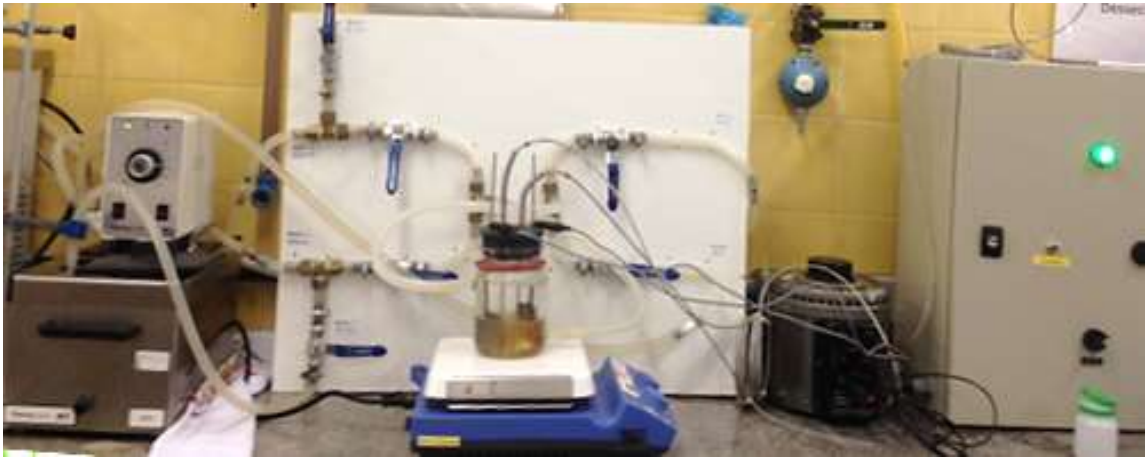
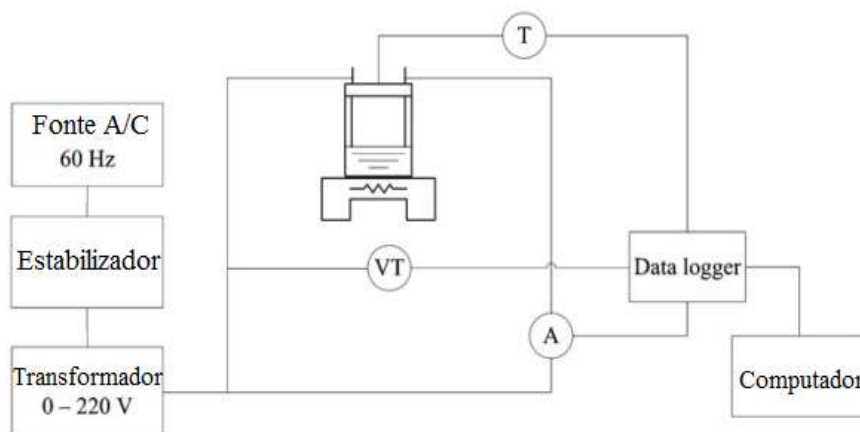


Figura 7 - Esquema ilustrativo do sistema de aquecimento ôhmico (T= sensor de temperatura; VT= medidor de tensão; A=medidor de corrente elétrica).



Fonte: Adaptado de Mercali et al., 2012.

Figura 8 - Célula ôhmica contendo o resíduo de gelatina e água para formação da solução filmogênica.



Um sistema de bypass foi usado para permitir o uso de dois banhos termostáticos para alimentar a camisa da célula: um (Lauda, modelo T, Alemanha) para promover o aquecimento inicial da mistura através da passagem de água quente ($90\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$) pela célula. Quando a solução atingiu $70\text{ }^{\circ}\text{C}$, o banho quente foi desligado e a temperatura de $75\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ foi mantida por 30 min pela aplicação de aquecimento ôhmico e pela circulação de água fria ($3\text{ }^{\circ}\text{C}$ - $15\text{ }^{\circ}\text{C}$) advinda do outro banho de água com temperatura controlada (Lauda, RM 12, Brasil). Os filmes foram produzidos com a aplicação de duas tensões diferentes: 60 V (intensidade média do campo elétrico de $9,5\text{ Vcm}^{-1}$) e 120 V (intensidade média do campo elétrico de $19,0\text{ Vcm}^{-1}$).

Após a obtenção da SF, o processo de *casting* envolveu a distribuição da SF em Placas de Petri de poliestireno ($0,086\text{ g/cm}^2$) e secagem em estufa com circulação de ar a $45\text{ }^{\circ}\text{C}$ por 4 h. Antes da caracterização, os filmes foram armazenados em dessecadores contendo solução saturada de NaBr, mantidos a 58% UR/ $25\text{ }^{\circ}\text{C}$ por dois dias. O filme de controle foi produzido por método convencional, sem aplicação de campo elétrico. Para isso, foi empregado o mesmo sistema, sem a conexão dos eletrodos, e a temperatura de $75\text{ }^{\circ}\text{C}$ foi mantida pela circulação de água quente através da camisa da célula ôhmica.

4.2.5 Obtenção das nanofibras

Diferentes sistemas de solventes foram investigados, baseados em ácido acético, ácido fórmico, ácido cítrico (1%) e água. Baseado em testes, o sistema escolhido foi o de ácido acético (AA)/ácido cítrico (AC) contendo 50% em peso de cada (AA/AC), visto que esse foi o

que propiciou melhor dissolução de gelatina à temperatura ambiente, garantindo assim um processo de *electrospinning* estável sem obstrução agulha ou a necessidade de uma instalação aquecida complexa.

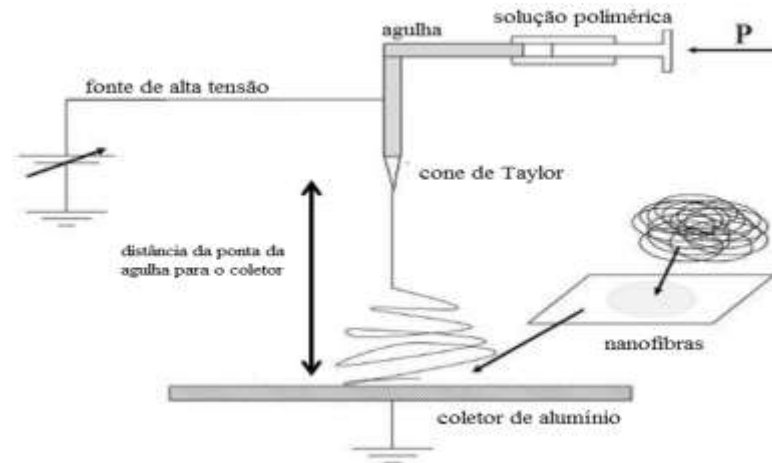
A concentração de gelatina na solução foi testada em valores que variaram de 13% a 30%, de onde se obteve que a concentração que facilitou o processo foi a de 25% em peso em relação ao solvente AA/AC (50/50). Para produzir essa concentração, considerou-se que: cada 100 g do resíduo das cápsulas de gelatina contêm 40,6 g de gelatina, como avaliado anteriormente; e que a densidade em água dos solventes ácido acético e ácido cítrico 1% são 1,05 g/cm³ e 0,9996 g/cm³, respectivamente.

As soluções de *electrospinning* foram preparadas pela mistura do resíduos de gelatina com o solvente AA/AC em um becker, e mantidas sob agitação magnética a 75 °C (IKA-Works Inc, Ret Basic C, EUA) até o derretimento da rede e prévia homogeneização dos componentes (5 min). Após, o sistema foi mantido sob agitação em temperatura ambiente para completa homogeneização por 30 min. As soluções obtidas foram guardadas em recipientes de vidro e mantidas em condições ambientes. O processamento por *electrospinning* foi realizado no dia posterior ao da obtenção das soluções.

As nanofibras foram produzidas em uma instalação desenvolvida pelo laboratório de Gante, conforme ilustrado na Figura 9. Para isso, foi utilizado um sistema de escala laboratorial que consiste de uma agulha, uma bomba de infusão (KD Scientific Syringe Pump Series 100) e uma fonte de alta tensão (Glassman High Voltage Series EH). Uma folha de alumínio foi utilizada como coletor (DE VRIEZE et al., 2009). A agulha de 15,24 cm de comprimento e diâmetro interno de 1,024 mm foi conectada a uma seringa de 12 mL que continha a solução de gelatina, de onde a solução foi bombeada e processada, conforme Figura 10. Todos os parâmetros, como a concentração de gelatina, proporção de solvente, distância da ponta da agulha para o coletor, vazão e tensão aplicada, foram adaptados e investigados para permitir um processo estável e, assim, a produção de nanofibras uniformes. Todos os ensaios de *electrospinning* foram realizados em condições ambiente, com umidade relativa de $65 \pm 5\%$ e uma temperatura de 21 ± 3 °C.

Em geral, foram empregadas as seguintes condições: concentração de gelatina de 25%; distância de coleta entre 10 cm e 20 cm; vazão de solução entre 1 mL.h⁻¹ a 2 mL.h⁻¹; tensão entre 12 e 20 kV. Os resultados foram avaliados para determinar os parâmetros que resultam nas nanofibras com melhores propriedades.

Figura 9 - Esquema ilustrativo do sistema de *electrospinning*.



Fonte: Adaptado de Darko et al., 2016.

Figura 10 - Agulha conectada à seringa e coletor de alumínio durante *electrospinning* de solução de gelatina.



4.3 Caracterização dos filmes

4.3.1 Propriedades mecânicas

Um texturômetro (modelo TA-XT2, Stable Micro Systems, United Kingdom) foi utilizado para medir a resistência à tração (MPa), a porcentagem de alongação na ruptura (%) e o Módulo de Young (MPa) de acordo com a ASTM D882-09 (2009). A separação inicial das garras foi de 50 mm e a velocidade do probe de 0,8 mm/s. Dez tiras de cada amostra (100 mm × 10 mm) foram analisadas.

O ensaio dinâmico mecânico foi realizado no analisador DMA-Q800 (TA Instruments, Japão). As amostras foram cortadas em retângulos de 10,7 mm × 6,4 mm. O experimento foi conduzido na faixa de temperatura de – 120 °C a 100 °C (isoterma -30 °C/5 min) a uma taxa de aquecimento de 2 °C/min e frequência de 1 Hz. Ar atmosférico foi usado como gás de purga. As propriedades determinadas a partir desse ensaio em função da variação de temperatura foram o módulo de armazenamento, módulo de perda, tan Delta e temperatura de transição vítrea (T_g), em função da variação de temperatura.

4.3.2 Propriedades físico-químicas

4.3.2.1 Espessura

Um micrometro digital (modelo IP40, Digimess, Brasil) (Figura 11) foi utilizado para determinar a espessura dos filmes, cuja escala é de 0-25 mm e precisão de 0,001 mm. Cinco medidas aleatórias foram feitas de cada amostra (em triplicata), e então foi calculada a média dos valores.

Figura 11 - Ilustração da medição de espessura efetuada em micrômetro digital (modelo IP40, Digimess, Brasil).



4.3.2.2 Umidade e solubilidade em água

O conteúdo de umidade das amostras (2 cm diâmetro) foi determinado por gravimetria, de acordo com a A.O.A.C. (2005). Uma estufa com circulação forçada de ar (modelo TE-394/2, Tecnal, Brasil) foi utilizada para a secagem dos filmes até peso constante, a 105 °C. Após pesagem das amostras, foi dada continuidade de análise para determinação da solubilidade. Para isso, os recipientes que continham filme seco foram adicionados de 30 mL de água destilada e mantidos sob agitação (modelo NT145, Novatecnica, Brasil). Após 24 h, a

água foi retirada e os recipientes com a amostra foram submetidos à secagem a 105 °C por 24 h. A determinação da matéria seca que não se dissolveu em água foi determinada por gravimetria (peso antes e depois da desidratação do filme). Essa propriedade também foi determinada para as amostras úmidas, e para o cálculo da solubilidade foi descontada a umidade anteriormente obtida.

4.3.2.3 Permeabilidade ao vapor de água

A permeabilidade ao vapor de água dos filmes (PVA) foi avaliada com base na ASTM E96 (2001) e em método descrito por Talja et al. (2008). Para isso, células de permeação de alumínio com CaCl₂ anidro granular (0% UR, 25 °C) foram utilizadas para fixar os filmes em sua superfície. O conjunto (Figura 12) foi armazenado por 24 h dentro de uma cuba de vidro com solução de cloreto de sódio (75% UR, 25 °C). A PVA foi determinada pela equação:

$$PVA = (w \times FT) / (A \times t \times \Delta p)$$

onde *w* é a massa de água permeada através do filme (g), *FT* é a espessura do filme (mm), *A* é a área de permeação, *t* é o tempo de permeação (h), e Δp é a diferença de pressão de vapor entre os dois lados do filme (Pa).

Figura 12 - Células de permeação armazenadas em cuba de vidro para análise de PVA.



4.3.3 Propriedades estruturais e morfológicas

A estrutura dos filmes foi analisada em microscópio eletrônico de varredura (MEV) (modelo JSM 5800, JEOL, Japão), operado a 5 KV e com aumento de imagem entre 500 a 2000 vezes. As amostras de filme foram fixadas com fita adesiva dupla-face em stubs de bronze e cobertas por fina camada de ouro. As análises realizadas nos laboratórios da Bélgica utilizaram um microscópio eletrônico de varredura Phenom-World, Phenom Pro X, Holanda.

As amostras foram montadas em stubs de alumínio com adesivo dupla face, e a tensão de aceleração de 15 kV. A partir dessa análise foi possível verificar a presença de bolhas, rachaduras, uniformidade, homogeneidade e outras características do filme que podem ser afetadas pelos processos realizados.

O comportamento estrutural e morfológico dos filmes nanocompósitos foi averiguado por difratômetro de raios-X (modelo D500, Siemens, Estados Unidos), utilizando radiação Cu K α ($\lambda = 0,15418$ nm) e com operação angular de 2° a 40° e taxa de 0,05 °/min. Os materiais poliméricos também foram avaliados por espectroscopia na região do infravermelho com Transformada de Fourier (FTIR), em espectrofotômetro (Thermo Scientific, Nicolet 6700 FTIR, USA), com uma resolução de 4 cm⁻¹ e 32 varreduras/espectro na região entre 4000 e 600 cm⁻¹.

4.3.4 Propriedades ópticas

Um colorímetro (modelo Miniscan XE, Hunterlab, Estados Unidos) foi utilizado para determinar os parâmetros de cor CIE L*a*b* dos filmes, com operação à luz do dia com D65. O padrão foi um disco branco com L₀*: 97,5; a₀*: 0,13 e b₀*: 1,7. A equação (ROTTA *et al.*, 2009) seguinte auxiliou na determinação da diferença de cor entre o disco padrão/filme controle e os filmes:

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

A transmitância de luz (%) foi obtida por espectrofotometria (modelo UV-1800, Shimadzu, Japão). Retângulos de 4 cm × 1 cm dos filmes foram colocados em cubetas de quartzo, posicionados no equipamento e analisadas em comprimentos de luz entre 200 nm e 800 nm, o que compreende as regiões ultravioleta e visível. A partir dos dados de transmitância a 600 nm foi calculada (HAN & FLOROS, 1997): a opacidade dos filmes:

$$\text{Opacidade (A/mm)} = (-\log T_{600}) / x$$

onde T₆₀₀ é a transmitância fracional a 600 nm, e x é a espessura do filme (mm).

4.3.5 Propriedades térmicas

As amostras (± 6 mg) foram avaliadas quanto à estabilidade térmica em um analisador termogravimétrico (modelo TGA-Q500, TA Instruments, Japão), e submetidas a uma faixa de

temperatura crescente (10 °C/min) desde 40 °C (isoterma de 5 min) até 600 °C (isoterma de 5 min). Ar atmosférico foi utilizado como gás de purga. O TGA permite determinar dados como a perda de massa da amostra na faixa de temperatura estudada, bem como os diferentes estágios associados e as temperaturas máximas de degradação dos componentes, e massa residual, referente ao conteúdo de carbono residual ao final do experimento.

As transições térmicas dos filmes foram investigadas por um calorímetro de varredura diferencial modulado (TA Instruments, MDSC Q1271, Estados Unidos) calibrado com índio. Aproximadamente 10 mg de cada amostra foram selados em placas de alumínio e escaneados na faixa de temperatura de -120 °C a 100 °C. A placa de alumínio vazia foi utilizada como referência. A taxa de aquecimento foi de 5 °C min⁻¹, com uma modulação de ± 0,5 °C de amplitude e 60 s de período. Dados como a temperatura de fusão (T_f), temperatura de cristalização (T_c), e a entalpia de fusão (ΔH_f) e de cristalização (ΔH_c) foram determinados.

4.4 Caracterização das nanofibras

As soluções para *electrospinning* foram previamente caracterizadas para determinação de viscosidade nos tempos 0, 4 h, 24 h e 48 h após seu preparo, usando um reômetro (Bohlin, CS50). A condutividade elétrica das soluções foi avaliada por um medidor de condutividade (WTW, inoLAB Cond 730, Alemanha). Os materiais obtidos por *electrospinning* foram caracterizados quanto às propriedades morfológicas (MEV), conforme descrito no item 4.3.

4.5 Análise dos resultados

Os experimentos foram realizados em triplicata. Os resultados foram estatisticamente analisados por ANOVA e teste de comparação de médias de Tukey ao nível de 5% de significância, através do programa Statistica 12.0.

CAPÍTULO 3: ARTIGOS CIENTÍFICOS

ARTIGO 1: Gelatin residue-based films crosslinked with the natural agent genipin**Artigo publicado em:** Packaging Technology and Science (A2)

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Gelatin capsule residue-based films crosslinked with the natural agent genipin

ABSTRACT

This study focused on the development and characterization of biodegradable films based on gelatin capsule residue crosslinked with the natural agent genipin extracted from genipap (*Genipa americana* L.). Their potential on maintaining the quality attributes (level of peroxides, conjugated dienes and trienes, color) of extra-virgin olive oil under accelerated storage conditions was investigated. Results showed that the genipin-crosslinked films had a dark blue appearance. Their total soluble matter, water vapor permeability, tensile strength, UV and visible light barriers, and thermal properties were improved in relation to the uncrosslinked film, whereas their moisture content and microstructure were not affected. In general, films presented comparable properties to those of glutaraldehyde-crosslinked films. The developed films successfully protected the packed oil from primary and secondary oxidation, and maintained the color over the storage period. Therefore, this study demonstrated the effectiveness of genipin on improving the properties of gelatin-based films and the promising application of films as food packaging.

KEYWORDS

BIODEGRADABLE, FILM PROPERTIES, CROSSLINKING, WASTE, GELATIN, PACKAGING.

1 Introduction

The development and application of bio-based materials in the food packaging field has increasingly gained attention as they are an alternative to non-biodegradable petroleum-based plastics, which in turn have been proven to cause serious environmental impacts.^{1,2} As reported by Averous and Boquillon,³ biodegradable polymers may be divided according to their origin into four families, as from biomass products, from micro-organisms, from biotechnology and from petrochemical products. The agro-polymers are obtained from biomass, such as proteins, polysaccharides, and lipids.

As recently reported in the literature,^{4,5,6} one interesting source of protein to develop biodegradable films is the residue generated by the production of gelatin soft capsules, which are used to deliver bioactive compounds in the nutraceutical sector. The resulting scraps are not reused by the industries, which imply waste treatment and disposal. This gelatin capsule residue is mainly composed of gelatin, a biopolymer that has been reported to present adequate properties to produce edible films and coatings.⁷ Therefore, the reuse of this material to produce biodegradable films may contribute to scale down the amount of solid waste disposal and to reduce the use of non-biodegradable synthetic packaging.

Despite its advantageous characteristics, gelatin and other biopolymer-based films usually present poor mechanical and barrier properties, and are sensitive to water, which limit their application when compared to petroleum-derived packaging.^{8,9} One promising strategy to overcome these drawbacks is the chemical crosslinking.¹⁰ This technique involves the formation of tridimensional networks by linking polymer chains by covalent or noncovalent bonds and has been commonly applied to gelatin-based films due to their large amount of functional side groups that can be crosslinked.⁹

Gelatin-based matrices have been frequently crosslinked with glutaraldehyde, predominantly by the formation of a strong Schiff's base through a reaction between ϵ -amino groups present in lysine and hydroxylysine gelatin residues, and formyl groups of the aldehyde.^{11,12} Despite its efficiency in stabilizing collagenous materials, glutaraldehyde has been considered cytotoxic due to its potential to leave un-reacted toxic molecules that will release upon in vivo biodegradation.^{13,14} Consequently, the use of natural agents that are effective in crosslinking gelatin and present non-toxic characteristic is an important factor when considering the application in food packaging materials.

Genipin, an iridoid that can be obtained from *Genipa americana* and *Gardenia jasminoides*, has been proposed as a crosslinking agent for diverse applications in substitution of glutaraldehyde, being 5.000 to 10.000 times less cytotoxic.¹⁵ This compound is soluble in different solvents, such as water, ethyl-acetate, propylene-glycol and alcohol, and is able to spontaneously react with primary amine groups, which results in dark blue pigments.^{16,17} Despite its potential applicability, the extraction process usually results in low recovery ratio and high commercial cost. According to the literature, the highest efficiency of genipin extraction from genipap was 34 mg.g⁻¹, obtained by high pressure processing.¹⁷ In this sense, Bellé et al.¹⁸ have recently used an efficient and inexpensive method based on enzyme-assisted extraction in two-phase aqueous system to extract genipin from genipap fruits (*Genipa americana* L.). This method resulted in the highest genipin concentration (196 mg.g⁻¹) reported until now, and may represent a possibility to broaden the application of genipin in the packaging field.

As far as we are concerned, the production of gelatin-based films crosslinked with genipin has been investigated by only two research groups,^{13,19,20,21} who used commercial gelatin from pigskin and bovine sources, and commercial genipin. Additionally, the application of gelatin-genipin films as food packaging has not been explored. The use of extracted genipin was considered in the present work due to the abundance of genipap in Brazil, whose pulp presents higher concentrations of geniposide than gardenia fruit.²² An attempt to present a comprehensive characterization of the resulting films was performed to offer further knowledge in the field of gelatin-genipin films and to understand their possible applications as food packaging. In view of the above mentioned, the aims of this research were to investigate the effect of genipin extracted from genipap (*Genipa americana* L.) on the final properties (physicochemical, mechanical, optical, thermal and morphological) of a gelatin capsule residue-based film and its potential as packaging to extra-virgin olive oil.

2 Materials and methods

2.1 Materials

The gelatin capsule residue (GCR) (Figure 1) was provided by the Chemical Pharmaceutical Tiaraju Laboratory (Rio Grande do Sul, Brazil), generated from the processing of linseed oil nutraceutical capsules. Its approximate composition is gelatin from bovine source (40.6%), water (35.0%) and glycerol (24.4%). Glutaraldehyde (GLU) 25 wt. %

in water was obtained from Nuclear Company (Brazil). Genipap fruits (*Genipa americana* L.) were purchased from a local market (São Paulo, Brazil). Commercial enzyme Celluclast was kindly donated by LFN Latinoamericana® (Brazil). Ethyl acetate was purchased from Dinâmica® (Brazil). The extra-virgin olive oil (EVOO) (Capellini, Italy) was purchased at a local market. All other used reagents were of analytical grade.



FIGURE 1 Visual appearance of the gelatin capsule residue (GCR).

2.2 Genipin extraction

Genipin (GEN) was extracted from genipap (*Genipa americana* L.) according to the method described by Bellé et al.,¹⁸ by using the commercial enzyme Celluclast 10% (36 °C, pH 3.7) in a liquid-liquid two-phase aqueous system (TPAS) consisting of distilled water and ethyl acetate. This method was reported to be simple, efficient and inexpensive. Dried GEN was obtained by using nitrogen gas to evaporate the organic solvent.²³ The attained purity of the extracted GEN was 97%, determined by high performance liquid chromatography (HPLC) analysis.

2.3 Preparation of the crosslinked films

Crosslinked and control films were prepared by the casting method, based on the methodology and conditions described by Iahnke et al.²⁴ The GCR was first dissolved in distilled water (70 g GCR/100 g water) in a water bath (70 °C). The film-forming solutions (FS) were obtained after homogenization of the mixture (55 °C/30 min) by a hotplate with magnetic stirrer (350 rpm) (Fisatom, 752A, Brazil). An ultrasonic treatment (Unique, USC-

1400, Brazil) was used to remove remaining air bubbles. The final FS had 16.7 g gelatin/100 g FS and 60 g glycerol/100 g gelatin. Glycerol was naturally present in the raw material.

The FS was spread onto polystyrene Petri dishes (0.086 g/cm^2) and gelation occurred at environmental conditions ($25 \pm 2 \text{ }^\circ\text{C}$) for 30 minutes. The genipin crosslinked film (GCR-GEN) were achieved by spreading a 0.5% (w/v) GEN solution over the top surface area of the films, based on the method described by Bigi et al.¹³ The GEN concentration of 0.15% (w/w) was chosen based on studies that produced gelatin-genipin films, which demonstrated that 0.15%¹³ and 0.1%²⁵ GEN were sufficient to crosslink 61% and 55% of the ϵ -amino groups, respectively, and to significantly improve the properties of the materials. The films were left to react for 15 h at room temperature and then dried at $45 \text{ }^\circ\text{C}$ for 4 h in a ventilated oven (DeLeo, B5AFD, Brazil). For a comparison purpose, glutaraldehyde crosslinked (GCR-GLU) film was prepared by the same methodology. The same concentration as GEN and time-temperature conditions were used. Control films were produced without the crosslinking step. Samples were stored under controlled relative humidity ($50 \pm 5\%$) and room temperature ($25 \pm 2 \text{ }^\circ\text{C}$) for at least 48 h before characterization.

2.4 Evaluation of crosslinking degree

The crosslinking degree of GCR-GLU and GCR-GEN films was determined by the ninhydrin assay.²⁶ Lyophilized film samples were heated ($90 \text{ }^\circ\text{C}/30 \text{ min}$) with a ninhydrin solution and the optical absorbance was measured by a spectrophotometer (Shimadzu, UV-1800, Japan) at 570 nm. A standard curve of glycine was used to determine the concentration of free amino groups.

2.5 Characterization of the films

2.5.1 Thickness and mechanical properties

Film thickness (d) was measured to the nearest 0.001 mm by a digital micrometer (Digimess, IP40, Brazil) and calculated from the average of five measurements from each specimen.²⁷ Tensile strength (σ_{max} ; MPa) and percent elongation at break (ϵ_f ; %) were evaluated by a texture analyzer (TA.XT2i e Stable Micro Systems, UK), according to ASTM D882-09²⁸ standard procedures. The initial grid separation was 50 mm and the crosshead speed $0.8 \text{ mm}\cdot\text{s}^{-1}$. Ten strips of each film ($100 \text{ mm} \times 25 \text{ mm}$) were evaluated.

2.5.2 Water-related properties

Samples of each film (2 cm diameter) were dried at 105 °C for 24 h in an oven (DeLeo, TLK48, Brazil) to determine the moisture content (MC) gravimetrically.²⁹ The total soluble matter (TSM) was determined based on the method described by Rhim et al.,²⁷ by directly immersing wet film samples (2 cm diameter) in recipients containing 30 mL of distilled water. Occasional soft stirring was applied by a shaker (Novatecnica, NT145, Brazil). After 24 h, the water was carefully removed from the recipients and the films were dried at 105 °C/24 h to obtain the TSM gravimetrically. The MC previously determined was used for calculations.

The water vapor permeability (WVP) was evaluated as described by Iahnke et al.³⁰ and based on the ASTM E 96-00.³¹ To that purpose, aluminum permeation cells (height: 25 mm; inner diameter: 63 mm) were filled with granular anhydrous CaCl₂ and weighed. The films were mounted into the cells and stored inside a chamber (25 °C, 75% RH). The setup was weighed at intervals 0, 1, 2 and 24 h in order to evaluate the gained mass and determine WVP gravimetrically.

2.5.3 Color measurements

The analysis of color was performed in a colorimeter (Minolta Co. Ltd., CR-300, Japan) with illuminant D₆₅. Results were expressed as the CIELab parameters L* (0: black; 100: white), a* (-60:green; +60: red), and b* (-60: blue; +60: yellow). Film samples were placed on the top of a white standard plate (L₀* = 97.83, a₀* = 0.13 and b₀* = 1.66) and the color parameters were measured. The difference in color (ΔE*) between films and the white standard plate was calculated by the equation³²:

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

2.5.4 Light transmittance and transparency

A UV-vis spectrophotometer (Shimadzu, UV-1800, Japan) was used to evaluate the ultraviolet (UV) and visible light barrier properties of the developed films at the wavelength range between 200 nm and 800 nm.³³ Transparency of the films was obtained from the following equation³⁴:

$$\text{Transparency value} = (-\log T_{600}) / d$$

where T_{600} is the fractional transmittance measured at 600 nm and d is the correspondent film thickness (mm).

2.5.5 Thermal stability

The thermal stability of films was evaluated from 40 °C to 600 °C by a thermogravimetric analyzer (TA Instruments, TGA Q500, USA), at a rate of 10 °C min⁻¹ using air as the purge gas (25 mL min⁻¹).

2.5.6 Modulated differential scanning calorimetry (MDSC)

Modulated differential scanning calorimetry (TA Instruments, MDSC Q1271, USA) calibrated with indium was used to determine the thermal transitions of the films. The film samples (10 ± 1 mg) were placed in aluminum pans, sealed and scanned from -120 °C to 100 °C at a heating rate of 5 °C min⁻¹ with a modulation of ± 0.50 °C amplitude and 60 s period.

2.5.7 Film surface characteristics

The surface morphology of films was observed under a scanning electron microscope (SEM) (JEOL, JSM 6060, Japan), following the same procedures as Pagno et al.³⁵ The micrographs were observed at an acceleration voltage of 15 kV and magnification of 1000×.

2.5.8 Fourier transform infrared (FTIR) spectroscopy

A smart attenuated total reflection (ATR) with a ZnSe crystal was used in conjunction with the FTIR spectroscopy (Thermo Scientific, Thermo Nicolet 6700 FTIR Spectrophotometer, USA). The film samples were analyzed in the range of 600-4000 cm⁻¹, with 4 cm⁻¹ spectral resolution and collection of 32 scans/spectrum. The spectra of the samples were rationed against a background spectrum previously obtained from the clean empty cell.

2.6 Application of the GCR-GEN films

The effect of GCR-GEN films on maintaining the quality attributes of EVOO under accelerated storage conditions was investigated following the methodology described by Stoll et al.³⁶ with some modifications. Films were produced to develop EVOO sachets designed for single use. They were cut into rectangles and heat sealed to form 7 cm x 5.5 cm film pouches,

which consisted of a total surface area of 77 cm². Next, 7 g of oil were filled into the pouch, whose open edge was heat-sealed. For a comparison purpose, EVOO was kept in open dish glass (OG), and three closed pouches with the same surface area: transparent polypropylene film (PP), Al-laminated film (ML), and the genipin crosslinked film (GCR-GEN), as illustrated in Supplementary Figure 1.

The packed EVOO samples were randomly stored in a chamber (Tecnal, TE-402, Brazil) for 14 days, at 40 ± 2 °C, 54% RH and fluorescent light exposure of 900-1000 lux (Luxometer, V&A Instrument, MS6610, China). Oil samples were analyzed at days 0, 3, 7 and 14. Peroxide value (PV), expressed as milliequivalents of active oxygen per kilogram of oil (mEq/kg), and specific absorption coefficients (K_{232} and K_{268}) were determined according to AOCS.^{37,38} The oil color was measured as described in section 2.5.3. To that purpose, EVOO was placed in a glass petri dish and covered by a thin glass.



SUPPLEMENTARY FIGURE 1 Visual aspect of extra-virgin olive oil packed in open dish glass (OG), polypropylene film (PP), Al-laminated (ML) and genipin crosslinked film (GCR-GEN).

2.7 Statistical analysis

The difference between the average values of the properties of the films was compared by analysis of variance (ANOVA) and Tukey's test of multiple comparisons. Software Statistica 12.0 (Statsoft Inc., Tulsa, USA) was used for data analysis at significance level 5%. When not cited, analyses were performed in triplicate.

3 Results and Discussion

3.1 Crosslinking degree

As evaluated by the ninhydrin assay, the crosslinking degree of the GCR-GLU and GCR-GEN films crosslinked with 0.15% (w/w) of GLU or GEN were 50% and 41%,

respectively. As observed by Bigi et al.,^{13,39} GLU is more effective in reducing the amount of the ϵ -amino groups, which can be associated to the different mechanism of crosslinked between both chemical agents. Consequently, higher crosslinking degree is achieved in comparison to GEN at the same concentration. Whereas GLU was reported to reach 100% degree of crosslinking, GEN is not able to link all the ϵ -amino groups even at high concentrations. This is possibly due to the shielding effect caused by the formation of the tertiary structure, which prevents some lysine groups to react with genipin.⁴⁰

The crosslinking of genipin and gelatin, which contains primary amino groups, involves two reactions. In the first one there is the substitution of an ester group of the crosslinker molecule by a secondary amide. The second occurs lately and regards to the formation of an intermediate amine group due to a nucleophilic attack of the primary amino group to the C3 carbon of the genipin. Next, the dihydropyran ring is opened and the resulting aldehyde group is attacked by the secondary amino group. The exact mechanism that leads to the formation of blue pigments is of high complexity and not completely understood, but it is believed that it occurs through the dehydrogenation of intermediary pigments and oxygen radical-induced polymerization.^{17,25}

Yao et al.²⁵ developed gelatin networks containing different concentration of GEN from 0.01 to 1.75 wt% and found that below 0.075 wt% the degree of crosslinking was very low and not calculated. For 0.1 wt% and 0.25 wt% GEN, the degree of crosslinking was 55% and 64%, respectively. Above 0.5 wt%, no significant difference was observed among values, which presented about 73% of crosslinking content and indicated that the reaction reached a plateau. A similar behavior was found for gelatin-genipin films added of 0.07-2% GEN.¹³ The maximum crosslinking degree was about 85%, without significant variations at GEN greater than 1%. It was reported that 0.15% GEN was able to achieve 61% of crosslinking degree, which resulted in significant effects on the film properties.

According to another work, the crosslinking degree presented slightly higher values for GLU than GEN, which were 42% and 34%, respectively, after reaction with 0.15% of the agent.¹⁹ In general, the mechanical, chemical and thermal properties of the films at this concentration of agents resulted in films with comparable characteristics, similarly to results obtained at the present study as discussed along the paper.

Differences among the crosslinking degree from different studies is related to different factors, such as the source of gelatin and amino acid content, concentration of gelatin in the

film-forming solution, concentration of GLU or GEN, purity of the chemical agent, time of reaction, among others.

3.2 Characterization of the films

3.2.1 Film thickness and mechanical properties

The developed films showed no statistical difference among their thickness values (~ 0.168 mm). Tensile strength (σ_{\max}) and elongation at break (ϵ_f) of the control and crosslinked films are presented in Figure 2. As shown in Figure 2.A, GCR-GLU and GCR-GEN films had their σ_{\max} value significantly increased ($p < 0.05$) when compared to the control film (2.72 MPa). The GCR-GEN films presented ϵ_f values that reached approximately 244%, whereas both control and GCR-GLU films displayed values around 168% that did not differ statistically (Figure 2.B).

In general, crosslinking reactions are accompanied by increasing mechanical resistance. This effect was observed for other gelatin-based films crosslinked with GLU, likewise observed by Martucci et al.⁴¹ and Bigi et al.³⁹ Pigskin gelatin films crosslinked with GLU and GEN at different concentrations (0.15, 0.30 and 0.67 g /100 g FS) had their σ_{\max} enhanced by both agents, but 0.67% GLU expressed a cytotoxic effect.¹⁹ Another study obtained gelatin-genipin films with different concentrations of GEN (0.07, 0.15, 0.33, 0.67, 1.00, 1.50, 2.00 g GEN/ g FS) and concluded that the concentration of 0.15% was sufficient to induce improvements on the mechanical, chemical and thermal properties of films.¹³

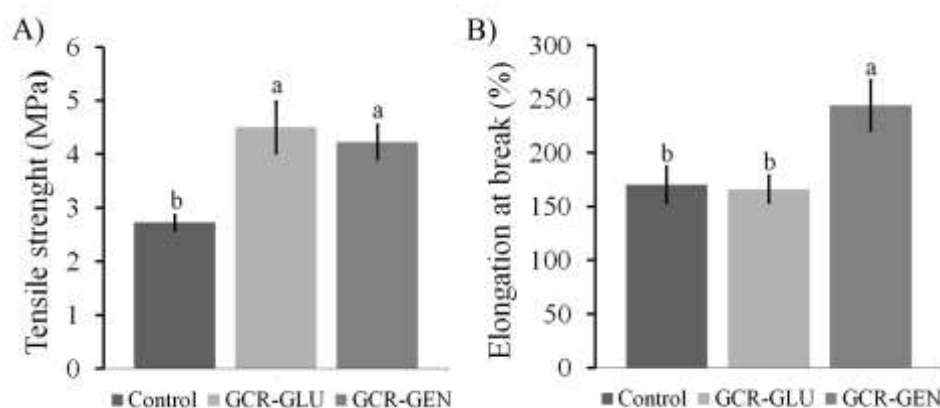


FIGURE 2 Tensile strength (A) and elongation at break (B) of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

Different superscript letters indicate statistically significant differences ($p < 0.05$).

Usually, it is expected that the increasing σ_{\max} values would be accompanied by decreasing ε_f values, but that was not observed in the present study. Soy protein films crosslinked with GEN (0.1, 1.0, 2.5, 5.0, 7.5 and 10.0%) presented a similar behavior, where films added of up to 1% GEN had their σ_{\max} and ε_f increased, but greater amounts decreased ε_f and maintained σ_{\max} constant.⁴² The crosslinking degree increased linearly with GEN concentration up to about 90%, but the film properties did not follow this linear behavior. It was suggested that the mechanical properties of films were enhanced by the addition of up to 1% GEN, associated to increased intermolecular crosslinking, whereas greater concentrations increased the intramolecular crosslinking. The effect of formaldehyde and glyoxal on the properties of gelatin films was investigated by Carvalho et al.,¹⁰ who verified that the mechanical properties were only affected by formaldehyde. Tensile strength was significantly increased from 15 MPa to 23 MPa, whereas ε_f remained around 38%.

The response of ε_f could be associated to the amount of glycerol naturally present in the raw material. Plasticizers such as glycerol and sorbitol are commonly added to gelatin films to promote mobility of the chains and flexibility and are usually added up to 30 g plasticizer/100 g gelatin.⁴³ In the present study, the concentration of glycerol is 60 g/100 g gelatin. Therefore, it is believed that the tensile strength was enhanced by the chemical crosslinking, but the great amount of plasticizer still allowed the plasticizing effect of glycerol and water to expand the network and produce more flexible films. In the case of GCR-GEN, the ε_f suffered the influence from plasticizer and the mechanism of reaction, which yielded films with increased ε_f values.

Overall, results indicated that despite GCR-GLU presented a higher degree of crosslinking, both GCR-GLU and GCR-GEN films showed a stronger network in comparison to the control film, which demonstrated the reinforcent action of the chemical agents and the potential use of GEN as an alternative to GLU to crosslink GCR-based films.

3.2.2 Water-related properties

In general, gelatin-based films show poor resistance towards water due to their intrinsic hydrophilic characteristic related to the presence of polar protein groups in the gelatin structure.⁴⁴ Results for moisture content (MC), total soluble matter (TSM) and water vapor permeability (WVP) of the films are shown in Table 1. As can be seen, MC values

ranged from 22.4% to 23.9%, which is in accordance to other gelatin-based films reported in the literature by Iahnke et al.⁶ (MC: 24.6%) and Tongnuanchan et al.⁴⁵ (MC: 20.2%).

The TSM is an important parameter that may provide insight into how films would behave under high moisture environment, including their resistance and degradability under this condition.⁴⁶ Results showed that the control film presented the highest solubility in water and lost its integrity after 24 h, which would probably limit their application to dry food products. Crosslinked films had their TSM significantly reduced to 35.8% (GCR-GLU) and 36.3% (GCR-GEN) when compared to control film (62.4%), which suggests their soluble fraction was mostly corresponded to water and glycerol. The WVP values of GCR-GLU and GCR-GEN were significantly reduced. Crosslinking with GLU decreased WVP to a greater extent in comparison to GEN, which might be associated to the greater crosslinking degree achieved by GCR-GLU.

TABLE 1 Moisture content (MC), total soluble matter (TSM) and water vapor permeability (WVP) of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

Sample	MC (%)	TSM (%)	WVP (g mm/ h ⁻¹ m ² kPa)
Control	22.4 ± 0.7 ^a	62.4 ± 3.5 ^a	1.02 ± 0.05 ^a
GCR-GLU	22.8 ± 0.6 ^a	35.8 ± 2.2 ^b	0.76 ± 0.06 ^c
GCR-GEN	23.9 ± 0.9 ^a	36.3 ± 4.5 ^b	0.88 ± 0.00 ^b

Mean values ± standard deviation (n = 3).

Different superscript letters in the same column indicate statistically significant differences (p < 0.05).

Results indicated that both agents had a beneficial effect in reducing the TSM and WVP of the films. In this study, the use of low concentration of GEN probably led to the formation of mainly intermolecular cross-linking. This resulted in a more complex three dimensional structure, which may explain the reductions in TSM and WVP.⁴² Regarding the effect of GLU, the reaction involves the formation of new covalent bonds between gelatin molecules, and, consequently, the improvement of the water resistance of films.⁴⁷

3.2.3 Color measurements

Crosslinking with GEN and GLU significantly influenced the CIELab color parameters (L^* , a^* and b^*) of the films (Table 2). Control film presented the highest L^* value, which was decreased by the crosslinking action with GEN and indicated loss of lightness. The higher b^* values revealed a yellowish color tendency for GCR-GLU films due to the formation of Schiff's base during crosslinking, similar to the appearance of bovine gelatin film crosslinked with GLU.⁴¹ The GCR-GEN films presented negative b^* values and higher ΔE^* , indicating the formation of blue pigments and evidencing the occurrence of the crosslinking reaction triggered by GEN.¹⁷ These results are in accordance with the visual appearance of the films, as shown in Figure 3. The formation of a colored film with dark blue pigmentation could be considered as an interesting achievement where the application of transparent packaging is not required in the food industry.

TABLE 2 CIELab color parameters (L^* , a^* and b^*) and difference in color (ΔE^*) of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

Film	L^*	a^*	b^*	ΔE^*
Control	85.50 ± 0.97^a	0.70 ± 0.03^b	1.75 ± 0.15^b	11.26 ± 0.97^c
GCR-GLU	81.83 ± 0.67^b	-0.59 ± 0.04^c	12.95 ± 0.77^a	18.57 ± 0.88^b
GCR-GEN	21.6 ± 1.63^c	1.81 ± 0.09^a	-4.86 ± 0.50^c	75.40 ± 1.65^a

Mean values \pm standard deviation (n = 3).

Different superscript letters in the same column indicate statistically significant differences ($p < 0.05$).



FIGURE 3 Visual appearance of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

3.2.4 Light transmittance and transparency

Table 3 shows the light transmittance at different wavelengths as well as the transparency values for the control and crosslinked films. The light transmittance in the range of 200-300 nm was low for all the samples, which means that the studied GCR-based films presented protection against middle ultraviolet radiations that may lead to oxidative deterioration. This protective ability of gelatin-based films has been reported elsewhere, and might be associated to the high content of aromatic amino acids that can absorb UV light.^{2,30} In the visible range, GCR-GEN film showed the lowest light transmittance, probably as a consequence of the blue pigments that caused the light scattering or hindrance of light passage. A study monitored the absorption evolution of the crosslinking reaction between genipin and chitosan amino groups by spectroscopy.⁴⁸ It was reported the appearance of a peak at 605 nm correspondent to the formation of a dark blue coloration. This fact can be related to the low transmittance value found for GCR-GEN around 600 nm, and to the crosslinking reaction that took place.

TABLE 3 Light transmittance and transparency of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

Film	Light transmittance (%) at different wavelengths (nm)*								Transparency**
	200	280	350	400	500	600	700	800	
Control	0.0	0.0	33.1	55.1	70.2	73.0	74.5	75.5	0.9 ± 0.1 ^b
GCR-GLU	0.0	0.0	31.5	60.4	77.9	85.3	87.1	87.9	0.5 ± 0.0 ^c
GCR-GEN	0.0	0.0	5.6	13.1	21.9	3.2	51.1	72.7	8.4 ± 0.3 ^a

* Each value represents the mean value of three determinations.

** Mean values ± standard deviation (n = 3).

Different superscript letters in the same column indicate statistically significant differences (p < 0.05).

Regarding the transparency, lower values indicate a more transparent appearance of the films. The greatest value was obtained for the GCR-GEN film, which is in agreement with the lower L* value found for the same film formulation. These results were attributed to the formation of a bluish color that led to a more opaque appearance. The GCR-GLU and control films showed the lowest transparency values, indicating their transparent aspect. In general, GCR-GEN film presented the lowest light transmittance values at the studied wavelength range, which suggested its high potential to protect food products against UV and visible

light. Additionally, the transparency value indicated their loss of transparency, which may be a desirable characteristic for food applications that require protection from light.

3.2.5 Thermal stability

Table 4 exhibits the thermal degradation temperature (T_d), the peak temperature in the derivative of the mass loss curve (DTG) where the maximum rate of mass loss occurs (T_{dmax}), weight loss (Δw) and residual mass (%) of the control and crosslinked films. All the prepared films showed a similar behavior, with four mass loss stages. The TG and DTG thermograms are shown in Supplementary Figure 2.

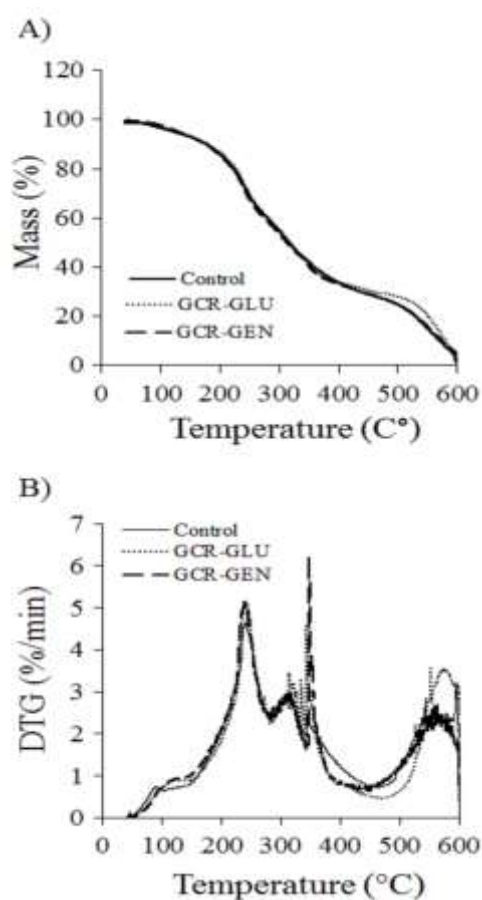
TABLE 4 Thermal degradation temperatures (T_d , °C), peak temperature (T_{dmax} , °C), mass loss (Δw , %) and residual mass (%) of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

		Sample		
		Control	GCR-GLU	GCR-GEN
Δ_1	$T_{d1,onset}$ (°C)	68	78	83
	Δw_1 (%)	6.8	6.6	6.0
	T_{d1max} (°C)	87	110	105
Δ_2	$T_{d2,onset}$ (°C)	147	149	142
	Δw_2 (%)	33.5	34.1	35.9
	T_{d2max} (°C)	239	238	239
Δ_3	$T_{d3,onset}$ (°C)	281	284	283
	Δw_3 (%)	31.6	29.6	28.7
	T_{d3max} (°C)	313	342	347
Δ_4	$T_{d4,onset}$ (°C)	463	468	450
	Δw_4 (%)	26.2	28.6	28.4
	T_{d4max} (°C)	542	550	558
Residue (%)		1.9	1.1	1.0

Δ_x : signifies the x^{th} mass loss step.

In general, the crosslinked films displayed higher T_d and T_{dmax} for the four stages when compared to the control film. This result suggested that GEN and GLU had a beneficial effect

on the thermal stability of the films provided by the crosslinking reactions. Mu et al.⁴⁹ observed a similar behavior for gelatin films crosslinked with dialdehyde carboxymethyl cellulose, where the crosslinked films were stiffer and more compact with improved thermal stability. Regarding the residual mass, all of the films presented a residual content below 2% at 600 °C. These results were expected, considering that a complete oxidation was obtained under air atmosphere. When nitrogen is used, a more stable behavior under the provided conditions is allowed and higher amounts of residual mass (18 – 23%) are usually observed for gelatin films.^{50,51,52}



SUPPLEMENTARY FIGURE 2 TG (A) and DTG (B) thermograms of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

3.2.6 Modulated differential scanning calorimetry (MDSC)

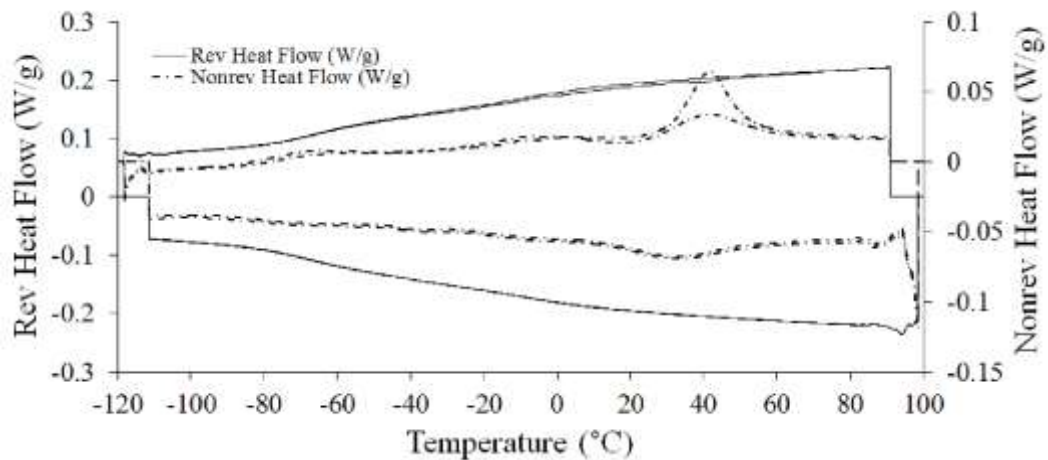
Crystallization temperature (T_c), crystallization enthalpy (ΔH_c), melting temperature (T_m), melting enthalpy (ΔH_m) and glass transition temperature (T_g) of the film samples are summarized in Table 5. All the developed films showed similar MDSC thermograms, and a typical thermogram of the control film is illustrated in Supplementary Figure 3. The total heat

flow signal is rather complex. It is split in a reversible heat flow signal, showing mainly ‘slow’ phenomena in this case probably only the heat capacity, thus T_g , and a non reversible heat flow signal showing the ‘kinetic’ phenomena such as crystallization and melting.⁵³ T_m was taken during 1st heating and T_c during subsequent cooling.

TABLE 5 Glass transition temperature (T_g), melting temperature (T_m), melting enthalpy

Sample	Glycerol rich phase					Protein rich phase				
	T_{c1} (°C)	ΔH_{c1} (J/g)	T_{m1} (°C)	ΔH_{m1} (J/g)	T_{g1} (°C)	T_{c2} (°C)	ΔH_{c2} (J/g)	T_{m2} (°C)	ΔH_{m2} (J/g)	T_{g2} (°C)
Control	-37	-0.15	-9	0.57	-68	33	-3.5	42	8.1	-13
GCR-GLU	-38	-0.48	-6	0.89	-78	30	-2.9	47	11.1	-7
GCR-GEN	-39	-0.65	-10	0.42	-78	32	-3.2	47	14.8	-5

(ΔH_m), crystallization temperature (T_c) and crystallization enthalpy (ΔH_c) of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).



SUPPLEMENTARY FIGURE 3 Typical MDSC thermogram of the control film (heat-cool-heat). Thermograms for the glutaraldehyde and genipin-crosslinked films look similar.

According to Biliaderis et al.,⁵⁴ a miscible polymer matrix would present a single T_g , whereas incompatible systems would show separate T_g 's corresponding to each component of the mixture. All the evaluated films were characterized by two different T_m , T_c and T_g , which indicates phase separation. The same phenomenon was observed by Sobral et al.,⁵⁵ who studied the properties of bovine hide and pigskin gelatin films as a function of sorbitol content

(15-65 g sorbitol/100 g gelatin). It was found that the increasing concentration of plasticizer (over 35 g/100 g gelatin) promoted phase separation between gelatin and sorbitol, which is in agreement with the present study.

T_{g1} was found at low temperatures (under $-68\text{ }^{\circ}\text{C}$), and could be related to glycerol/water rich phase, with a T_g still higher than that reported for the pure plasticizer ($-85.6\text{ }^{\circ}\text{C}$).⁵⁶ This means it still contained a protein fraction. The lower T_g for the crosslinked protein films reflected a higher degree of phase separation, thus a lower protein fraction in the glycerin rich phase. T_{g2} was observed at temperatures that ranged from $-13\text{ }^{\circ}\text{C}$ (Control) to $-5\text{ }^{\circ}\text{C}$ (GCR-GEN). These values were lower than those reported in literature for gelatin based films from tilapia skin ($41\text{ }^{\circ}\text{C}$)⁴⁵ and bovine hide ($61\text{ }^{\circ}\text{C}$),⁵⁷ but similar to that found for skin of cuttlefish gelatin films ($-6.09\text{ }^{\circ}\text{C}$ to $3.21\text{ }^{\circ}\text{C}$).⁵⁸ Although T_{g2} was mostly associated to the protein rich phase, the glycerol is able to localize between the protein chains, bind water and disrupt intermolecular polymer interactions of the film matrix, which contributed to the reduced T_g values.⁵⁹ Additionally, it is important to note that T_g values were higher for GCR-GEN and GCR-GLU films when compared to the control films, due to the crosslinking reactions that were able to reduce the chain mobility of the polymer.

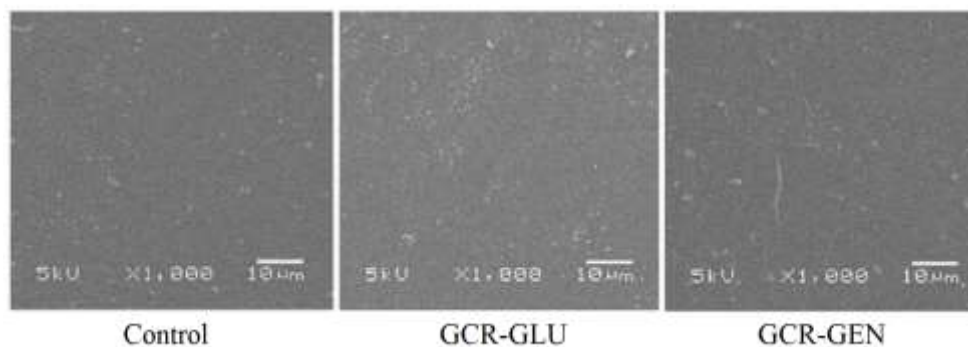
The endothermic transition peaks (T_{m1} and T_{m2}) of the samples indicated the temperature that caused the disruption of ordered molecular structures. T_{m1} , as well as T_{c1} , were linked to the crystallization/melting of water in an environment with glycerin and proteins. In Table 5, they are put together with the glycerin rich phase, although with the current knowledge it cannot be proven that only the water from that phase is able to crystallize. In the case of T_{m2} , it was associated to the disruption of the protein interaction formed during the film preparation and the rearrangement of the triple helix into a random configuration.^{56,60} Among all films tested, GCR-GEN and GCR-GLU films had the highest T_{m2} and highest corresponding enthalpies, which indicated that a greater portion of ordered structure was formed. Besides, GCR-GEN film showed the highest ΔH_{m2} (14.8 J/g), thus most triple helix formation. At first sight this might be in contradiction with the crosslinking that normally impedes crystallization. The larger extent of crystallization can be related to i) the lower amount of solvent (glycerin and water) due to more pronounced phase separation (lower T_g , comparable for GCR-GLU and GCR-GEN), ii) a low degree of crosslinking.

Regarding the exothermic transition peaks (T_{c1} and T_{c2}), all the samples presented similar T_c and ΔH . The T_{c2} was due to triple helix formation. The lower crystallization

temperatures for the crosslinked films were due to the crosslinking, hindering the movement of the chains. The enthalpies (ΔH_{c2}) were comparable, but in any case much lower than the melting enthalpies of the first heating. One reason is that it was hard to measure these values as the peaks are broad and the curves seem to bend in this region. Another reason is that during cooling much less time is given to the samples to crystallize than upon first formation. This is also reflected in a much smaller melting enthalpy during the second heating. The higher fraction of triple helices in the crosslinked films can also in part explain the increased tensile strength, as observed in Figure 1.

3.2.7 Film surface characteristics

The surface structure of GCR-GEN, GCR-GLU and control films was characterized by SEM and the obtained micrographs are shown in Supplementary Figure 4. Results pointed out that all the developed films presented a similar structure, with a smooth, compact and homogeneous surface morphology, similar to that reported for films based on tilapia scale gelatin⁶¹ and pig hide gelatin.⁶² Therefore, it was possible to assume that at the studied concentrations of GEN and GLU, the crosslinking reactions that took place in the film matrix did not affect its surface morphology in comparison to the pure GCR-based film.



SUPPLEMENTARY FIGURE 4 SEM micrographs of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

3.2.8 Fourier transform infrared (FTIR) spectroscopy

Figure 4 shows the FTIR spectra of the control, GCR-GLU and GCR-GEN films, which presented similar spectral behavior as assayed by the available FTIR Spectrophotometer. All the samples yielded three major peaks at the range $1629\text{-}1630\text{ cm}^{-1}$, $1538\text{-}1539\text{ cm}^{-1}$ and 1235 cm^{-1} , which corresponded to the absorption regions of Amide-I, II

and III respectively. These bands are characteristic of raw gelatin from different sources, such as bovine, fish and porcine. The amide-I absorption is mainly associated to the C=O stretching vibration, while Amide-II band is related to the N-H bending vibration and C-N stretching vibration, and Amide-III generally indicates the C-N stretching vibrations coupled to N-H in-bending vibrations.⁶³

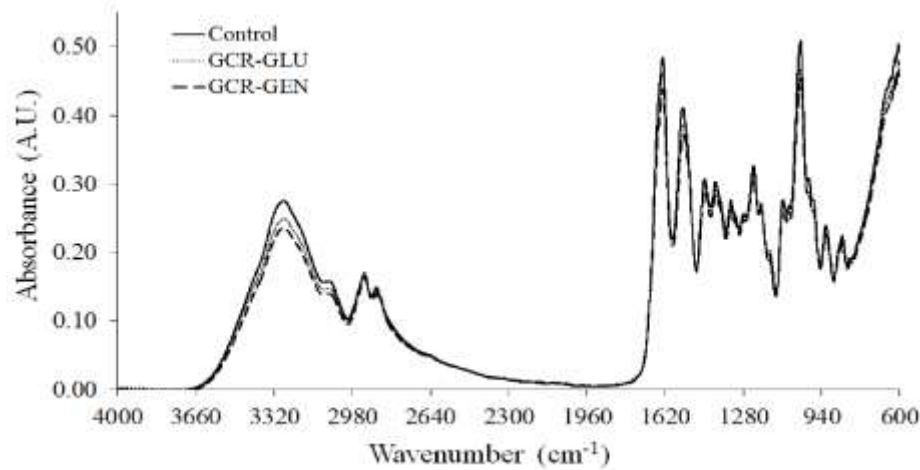


FIGURE 4 FTIR spectra of the control film and films crosslinked with glutaraldehyde (GCR-GLU) and genipin (GCR-GEN).

Other characteristic peaks of gelatin can be seen at $3277\text{--}3281\text{ cm}^{-1}$, correspondent to NH stretching coupled with hydrogen bonding (Amide-A); $2926\text{--}2930\text{ cm}^{-1}$, which regards to CH_2 asymmetrical stretching vibration; and $2873\text{--}2875\text{ cm}^{-1}$, which is related to CH_2 symmetrical stretching vibration.⁶⁴ Additionally, the FTIR spectra of all films exhibited a band at 1032 cm^{-1} due to the presence of glycerol (-OH group).⁴³ The peaks above mentioned have also been observed for other gelatin-based films from cold water fish skin,² warm-water fish,⁶⁵ pigskin⁴³ and bovine hide.⁶⁶

The FTIR spectra of crosslinked films did not show major differences from the control film. This might be attributed to the fact that characteristic peaks involved in crosslinking reactions are masked by stronger peaks from gelatin and glycerol. For the GCR-GEN films, a peak around 1080 cm^{-1} could be expected, assigned to ring C-H in plane bending and CO stretching mode of the primary alcohol on the genipin molecule, respectively.⁶⁷ However, this peak might have been masked by the strong peak at 1032 cm^{-1} due to the presence of glycerol. Regarding the GCR-GLU film, the aldehyde groups of the crosslinking agent can react with amino groups of the gelatin to form C=N groups (Schiff's base). The absorption peak of this

group is usually around $1620\text{-}1660\text{ cm}^{-1}$,⁶⁸ which might have been masked by stronger Amide-I bands.

3.3 Application of the GCR-GEN films

Taking into account the above mentioned results, the GCR-GEN films were investigated regarding their potential on maintaining the quality attributes of EVOO for 14 days under accelerated conditions. For comparison purposes, EVOO was packed in open dish glass (OG), polypropylene film (PP) and Al-laminated film (ML), as shown in Supplementary Figure 1. Results for PV are observed in Figure 5. Figure 6 shows results for K_{232} (Figure 6A) and K_{268} (Figure 6B), whereas color of EVOO is shown in Table 6. The initial values of PV, K_{232} and K_{268} were 4.3 mEq/kg, 2.3 and 0.17, respectively. These values are below the established levels according to the Codex Alimentarius ($PV \leq 20$ mEq/kg, $K_{232} \leq 2.5$ and $K_{268} \leq 0.22$).⁶⁹

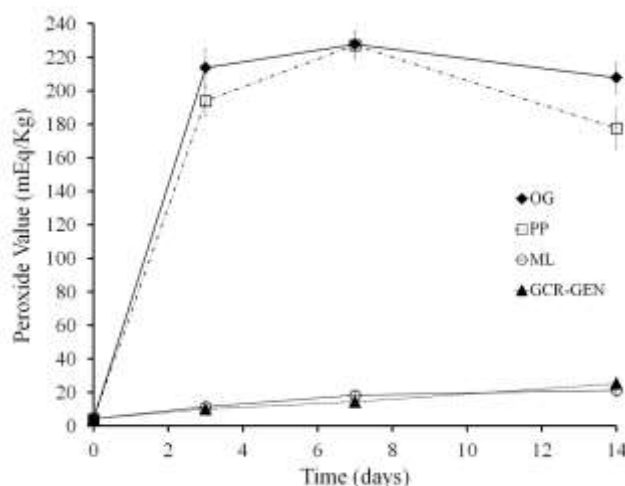


FIGURE 5 Peroxide value of extra-virgin olive oil packed in open dish glass (OG), polypropylene film (PP), Al-laminated film (ML) and genipin crosslinked film (GCR-GEN) after 14 days under accelerated conditions.

Among treatments, ML and GCR-GEN yielded the best results on protecting EVOO from deterioration and presented a similar behavior as observed in Figure 5 and Table 6. The PV is related to the primary oxidation of lipids and formation of hydroperoxides.⁷⁰ The ML and GCR-GEN maintained PV values under the limit up to the 7th day of storage and reached an average value of 23.3 mEq/kg after 14 days. The PV values for OG and PP exceeded 177 mEq/kg during the whole period of experiment and reached a maximum value of

approximately 227 mEq/kg on day 7. After this day, the level decreased, which indicated the formation of other products as observed by the increasing values of K_{232} and K_{268} .

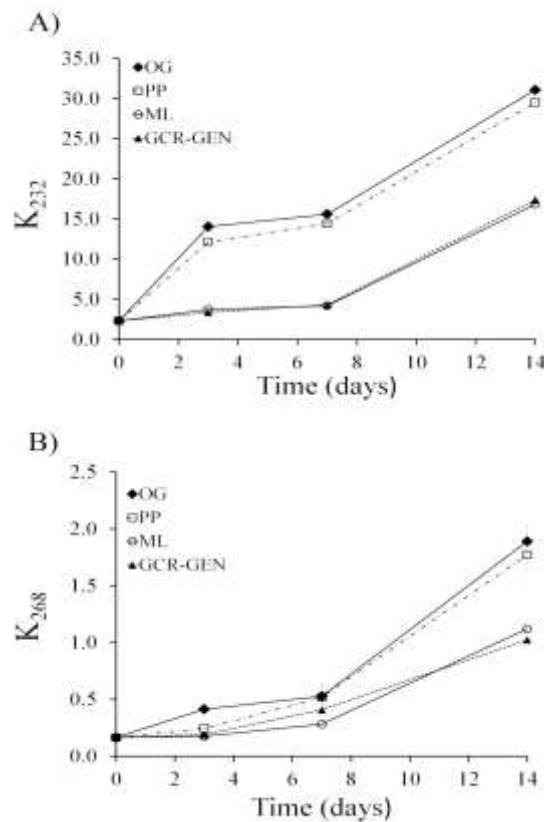


FIGURE 6 K_{232} (A) and K_{268} (B) of extra-virgin olive oil packed in open dish glass (OG), polypropylene film (PP), Al-laminated film (ML) and genipin crosslinked film (GCR-GEN) after 14 days under accelerated conditions.

Conjugated dienes and their oxidation products absorb at $\lambda = 232$ nm and are expressed by K_{232} , while conjugated trienes and their secondary products of oxidation absorb at $\lambda = 268$ nm and are expressed by K_{268} .⁷⁰ The increase of absorbance at the specific wavelength results in higher specific absorption coefficients, and consequently, higher quantity of certain oxidized compounds. The limits established for K_{232} and K_{268} by the Codex Alimentarius⁶⁹ were exceeded, regardless the kind of packaging. However, it can be observed that the initial values of K_{232} (Figure 6.A) and K_{268} (Figure 6.B) were close to the limit and that results for ML and GCR-GEN were significantly lower in comparison to OG and PP during the 14 days.

Other studies have demonstrated the potential application of biodegradable films to pack vegetable oils. The primary oxidation of sunflower oil was retarded by GCR-based films in comparison to oil packed in transparent polyethylene terephthalate (PET) bottles and unpacked, as assayed by peroxide value during 28-35 days under accelerated conditions.^{6,30} Starch-based film incorporated with encapsulated anthocyanins were effective on protecting EVOO from oxidation under accelerated conditions.³⁶ It was found that the biodegradable films maintained PV values under the limit up to day 8 (13.6 mEq/kg), whereas EVOO packed in PP was deteriorated before day 4 (326.5 mEq/kg). Another study demonstrated that films based on cassava starch and nanoencapsulated β -carotene had a positive influence on the quality maintenance (PV, K_{232} and K_{268}) of sunflower oil in comparison to polyethylene films, plastic bottles and unpacked oil under accelerated conditions.

As observed in Table 6, the color parameters of EVOO were maintained by ML and GCR-GEN, whereas oil packed in OG and PP had a^* and b^* parameters significantly affected during the period of storage. The loss of green color was evidenced by the increased a^* values, which is associated to chlorophylls degradation. The decreased values of b^* values corresponded to the shift from yellow to blue and are related to the degradation of carotenoids.³⁶ Therefore, ML and GCR-GEN films were able to maintain the organoleptic aspect of EVOO and protect it from the degradation of chlorophylls and carotenoids.

TABLE 6 Color parameters of extra-virgin olive oil packed in open dish glass (OG), polypropylene film (PP), Al-laminated film (ML) and genipin crosslinked film (GCR-GEN) after 14 days under accelerated conditions.

Parameter	Day 0	Day 14			
		OG	PP	ML	GCR-GEN
L^*	74.8 ± 1.6^b	75.5 ± 2.1^b	83.64 ± 0.33^a	73.4 ± 0.8^b	73.3 ± 0.7^b
a^*	-4.4 ± 0.8^d	-1.3 ± 0.1^b	-0.90 ± 0.0^a	-4.9 ± 0.2^d	-3.1 ± 0.5^c
b^*	13.0 ± 2.8^a	-2.1 ± 0.1^c	1.04 ± 0.08^b	13.3 ± 0.6^a	12.7 ± 1.7^a

Mean values \pm standard deviation (n = 3).

Different superscript letters in the same line indicate statistically significant differences ($p < 0.05$).

The deterioration process rate of olive oil depends on many factors that include temperature, the incidence of light and the presence of oxygen. Therefore, packaging plays an

important role on protecting the product from the effect of light and oxygen.⁷⁰ Olive oil sachets are commonly made of a combination of layers of different materials such as polyethylene, aluminum, and poly(ethylene terephthalate), which are very difficult to recycle and have become a great environmental concern.⁷¹ Results indicate that GCR-GEN films were able to maintain the quality attributes of EVOO in the same magnitude as ML, whereas for OG and PP the effect of oxygen and/or light had a great effect on the deterioration process.

4 Conclusions

Blue pigmented gelatin capsule residue-based films crosslinked with genipin were successfully developed in the present work. In comparison to uncrosslinked films, tensile strength was increased from 2.72 MPa to 4.23 MPa, whereas total soluble matter and water vapor permeability were significantly reduced from 62% to 36% and 1.02 to 0.88 g mm/h⁻¹ m² kPa, respectively. Results indicated that the employed concentration of genipin was sufficient to yield considerable improvements and had comparable properties to that of glutaraldehyde crosslinked film. Thermal properties and UV and light barriers were also improved, whereas the film thickness, moisture content and microstructure were not affected. In addition, the crosslinked films were able to maintain the quality attributes of extra-virgin olive oil under accelerated conditions, which yielded comparable results to that of Al-laminated synthetic films. Therefore, this study demonstrated the potential application of the genipin crosslinked films as biodegradable sachets to prevent quality deterioration of extra-virgin olive oil.

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ARTIGO 2: Gelatin capsule residue/modified montmorillonite-based films: characterization, biodegradation and effect on soil quality

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Gelatin capsule residue/modified montmorillonite-based films: characterization, biodegradation and effect on soil quality

Abstract

Bionanocomposites were successfully produced by casting by the combination of gelatin capsule residue and modified montmorillonite (Cloisite® 30B). The effect of different nanoclay loadings (0, 0.1, 0.5, 2, 6 and 10 g/100 g protein) on the final properties of the films was investigated. The 0% and 2% nanoclay films were further investigated regarding their biodegradation and effect on soil quality. The increasing concentration of clay resulted in thicker films, decreased total soluble matter and lower transmittance, whereas transition temperatures were not affected. Dynamic mechanical analysis revealed a great enhancement in the storage modulus caused by the clay. The incorporation of 2% clay was able to increase the storage modulus by a factor of 15 at environmental temperature. The results for SEM and X-ray diffraction analyses depended on the clay content, but in general, an exfoliated or intercalated structure was achieved. Regardless of its composition, the films were biodegraded after a 10-day soil burial experiment. Microbial biomass carbon and basal respiration of the substrate were positively related, whereas the metabolic quotient of the substrate showed no significant changes. The present work indicates that 2% nanoclay was able to improve the properties of films based on gelatin capsule residue and enhance their applicability beyond the food packaging field due to their biodegradability and effect on soil quality.

Keywords: biodegradable; films, packaging, Biopolymers and Renewable Polymers, Nanostructured Polymers

1 Introduction

Plastic materials are manufactured for a wide range of societal use, and in 2017 its global production exceeded 348 millions of tons (PlasticsEurope, 2018). Despite their many advantages, they have become a serious environmental concern due to their persistence in global environments (Barnes et al., 2009). As a consequence, there has been an increasing interest in the development of biodegradable materials to substitute and/or reduce the use of conventional petroleum-based plastics (Wihodo & Moraru, 2013). In 2016, the global production capacity of bio-based polymers was 4.160.000 tons. Important biodegradable polymer applications include industry sectors such as packaging, food service, medical, agriculture, horticulture, automotive, building, and consumer goods (Sikorska et al., 2017).

Biopolymers have demonstrated to be promising alternatives as biodegradable films and edible coatings in the food packaging field (Wihodo & Moraru, 2013), and as agricultural mulch films, where they present the potential to overcome problems associated with the contamination and accumulation of plastics in soil (Sander, 2019). Among biopolymers from agro-resources, gelatin shows unique properties such as film-forming ability, transparency, and biocompatibility, and has been extensively studied as a polymer film matrix (Suderman et al., 2018). In this sense, scraps generated by the processing of nutraceutical soft gel capsules have demonstrated to be an attractive source of gelatin to successfully produce biodegradable films (Iahnke, Costa, Rios, & Flôres, 2016; Campo, Costa, Rios, & Flôres, 2016).

Despite the many benefits of gelatin-based films, they usually present inferior characteristics when compared to synthetic plastics, especially concerning their mechanical, thermal and water-related properties (Wu; Liu, Y.; Wang; Han; & Liu, A., 2017), which might limit their application. A useful strategy to help overcome these drawbacks is the formation of nanocomposites, which are materials comprised of two or more phases, in which a continuous phase is usually the polymer, and the dispersed one is represented by at least one filler or reinforcing agent that has a dimension lower than 100 nm (Mihindukulasuriya & Lim, 2014) in at least one dimension.

Among clay minerals, montmorillonite is one of the most widely applied nanoparticles in bionanocomposites (Jorge, Alexandre, Flaker, Bittante, & Sobral, 2015). It is an alumina-silicate-layered nanoclay that belongs to the 2:1 phyllosilicates

structural family, and consists of a central octahedral sheet of aluminum hydroxide and magnesium oxide linked with two silica tetrahedral sheets (Weiss, Takhistov, & McClements, 2006).

The enhanced properties of gelatin/montmorillonite films have attracted the research focus, and these improvements have been reported in the literature (Bae et al., 2009; Flaker, Lourenço, Bittante, & Sobral, 2015; Jorge et al., 2014; Martucci, Vázquez, & Ruseckaite, 2007; Nagaranjan, Benjakul, Prodpran, & Songtipya, 2015; Ribeiro et al., 2018; Zheng, Li, Ma, & Yao, 2002). However, to the best of our knowledge, the biodegradability and the impact of these materials on the soil quality has been poorly addressed, and this is a crucial aspect when considering the application of biodegradable materials in the packaging and agricultural industry. Therefore, this study aimed to develop and characterize bionanocomposite films based on gelatin capsule residue in combination with montmorillonite organically modified, and investigate their biodegradability and impact on the soil quality.

2 Materials and methods

2.1 Materials

The gelatin capsule residue (GCR) originated from the processing of nutraceutical oil capsules was provided by the Chemical Pharmaceutical Tiaraju Laboratory (Rio Grande do Sul, Brazil). This material is mainly composed of gelatin from bovine source (40.6%), water (35.0%) and glycerol (24.4%). The plasticizer was naturally present in the raw material. The organically modified montmorillonite Cloisite® 30B (MMT) was acquired from Southern Clay Products, Inc (Texas, USA). The substituted cations of this nanoclay are known to be bis-(2-hydroxyethyl) methyl (hydrogenated tallow alkyl) quaternary ammonium. All other used reagents were of analytical grade.

2.2. Preparation of the nanocomposite film forming solutions

The bionanocomposite film solutions (NFS) were prepared based on the method described by Jorge et al. (2014) with modifications, by mixing a nanoclay based solution (NS) and a GCR-based solution (GS), as illustrated in Table 1. First, precisely weighed MMT was dispersed in distilled water (0.15 g/g NS) and hydrated using a

magnetic stirrer (IKA-Works Inc, Ret Basic C, USA) for 30 min at room temperature. The NS was homogenized using a high shear mixer (Heidolph Instruments, RZR 2021, Germany) at 1000 rpm for 4h. The solution was treated in an ultrasound bath (Bandelin Eletronic, 400, Germany) at 55 °C for 15 min to eliminate the air bubbles.

Table 1. Preparation of 100 g of the bionanocomposite film solutions (NFS) containing different nanoclay levels.

Film sample	GS		NS (g)
	GCR (g)	Water (g)	
GCR-MMT0	37	63.0	0
GCR-MMT0.1	37	62.9	0.1
GCR-MMT0.5	37	62.5	0.5
GCR-MMT2	37	61.0	2.0
GCR-MMT6	37	57.0	6.0
GCR-MMT10	37	53.0	10.0

GS: gelatin capsule residue-based solution; GCR: gelatin capsule residue; NS: nanoclay-based solution.

The GS was prepared separately by melting the GCR in distilled water at 70 °C for 5 min, using a heating plate with a magnetic stirrer. The mixture was homogenized at 45 °C for 1 h. The appropriate amount of the prepared NS was added to the GS (Table 1), and the mixture was homogenized using a high shear mixer at 1000 rpm for 2h. The final solution NFS was used to produce the bionanocomposite films (GCR-MMT). The final NFS had: 15 g of gelatin/100 g of NFS, 60 g of glycerol/100 g of gelatin (naturally present in the raw material) and 0, 0.1, 0.5, 2, 6 and 10 g of MMT/100g of gelatin.

2.3 Film casting and conditioning

The films were produced by casting the NFS onto polystyrene Petri dishes (0.1 g/cm²) and drying at 35 °C for 20 h in an oven (ThermoFisher Scientif, Heraeus, USA). After this period, they were peeled off from the dishes and maintained under a controlled atmosphere of 58 % RU/ 25 °C (kept by saturated NaBr solution) for at least 48 h before characterization.

2.4 Characterization of the films

2.4.1 Physicochemical properties

A digital micrometer (Digimess, IP40, Brazil) was used to determine the film thickness (d) to the nearest 0.001 mm. Five different measurements per sample were taken to calculate the average “ d ” (Rhim, Gennadios, Weller, Carole, & Hanna, 1998). The moisture content (MC) was evaluated gravimetrically (AOAC, 2005) by drying the film samples (2 cm in diameter) at 105 °C for 24 h in an oven (DeLeo, TLK48, Brazil).

The total soluble matter (TSM) was determined gravimetrically, based on the method described by Rhim et al. (1998). Weighed wet film samples (w_1) were immersed in 30 mL of distilled water and kept under soft stirring by a shaker at 25 °C (Novatecnica, NT145, São Paulo, Brazil). After 24h, the water was carefully removed from the recipients containing the films, which were dried (105 °C; 24 h) and weighed (w_2). The MC previously settled was used to calculate w_1 on dry basis (w_{1d}). The TSM was calculated using Equation 1:

$$\text{TSM} = [(w_{1d} - w_2)/w_{1d}] * 100 \quad (1)$$

2.4.2 Dynamic mechanical analysis

A DMA-Q800 analyzer (TA Instruments, Japan) was used to measure the storage modulus of the films. The samples were cut into rectangles of 10.7 mm × 6.4 mm. The experiment was conducted in the temperature range of -120 °C to 100 °C (isothermal - 30 °C/5 min) at a heating rate of 2 °C/min and a frequency of 1 Hz. Atmospheric air was used as the purge gas. The glass transition temperatures (T_g) were obtained from the peak of the tan delta curves.

2.4.3 X-ray diffraction

X-ray diffraction (XRD) was carried out on a Siemens D500 diffractometer (United States) using CuK α radiation ($\lambda = 0.15418$ nm). Measurements were taken from 2° to 40° at a rate of 0.05°/min.

2.4.4 Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the film samples were recorded in the range of 600–4000 cm^{-1} by a FT-IR Spectrometer (Thermo Scientific, Nicolet 6700 FTIR, USA) with

a collection of 32 scans/spectrum and a resolution of 4 cm^{-1} . A smart-ITR with a ZnSe crystal was used.

2.4.5 Film surface characteristics

A scanning electron microscope (SEM) (Phenom-World, Phenom Pro X, Netherlands) was used to observe the microstructure of films, which were mounted onto stubs with double-sided adhesive. An accelerating voltage of 15.0 kV and magnification of $500 \times$ were employed.

2.4.6 Transmittance and transparency

The light transmittance between 200 nm and 800 nm was measured by a UV-vis spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan). The transparency of the films was calculated by taking into account the fractional transmittance measured at 600 nm (T_{600}) and the correspondent thickness in mm (x), according to Equation 2 (Han & Floros, 1997):

$$\text{Transparency value} = (-\log T_{600}) / x \quad (2)$$

2.4.7 Indoor soil burial biodegradation

The GCR-MMT films containing 0 and 2% nanoclay were selected to be further evaluated regarding their biodegradation. The methodology was performed according to Martucci and Ruseckaite (2009), with some modifications. To that purpose, plastic boxes ($5.5 \text{ cm} \times 6 \text{ cm} \times 6.5 \text{ cm}$) with a superficial area of 33 cm^2 were filled with a natural organic soil (Vida Ecological Development Ltda, Brazil), which was used as the substrate degradation medium for films. Films were cut into rectangles ($2 \text{ cm} \times 3 \text{ cm}$) and dried at $60 \text{ }^\circ\text{C}$ (DeLeo, TLK48, Brazil) until constant weight. Films were separately placed into litterbags ($3 \text{ cm} \times 4 \text{ cm}$) made from 2 mm nylon mesh, which were previously dried for 48 h at $60 \text{ }^\circ\text{C}$ and weighed before filled with film sample. The litterbags containing the samples were buried at the depth of 4 cm from the surface of the soil. Every 5 days, the litterbags were removed from the soil, washed with distilled water to remove the soil particles and dried at 60°C until constant weight. The soil was maintained in 40% water content. The weight loss of films (WL; %) after soil biodegradation was determined by Equation 3:

$$WL(\%) = [(m_t - m_0) / m_0] \times 100 \quad (3)$$

where m_0 is the initial dried mass and m_t is the remaining dried mass after 10 days.

2.5 Effect of selected films on the soil quality

The effect of GCR-MMT and GCR-MMT2 on the soil quality was assayed by basal respiration (R_B), soil microbial biomass carbon (MBC) and metabolic quotient (qCO_2) during biodegradability of the films. The substrate was the same used in the biodegradation trial. It was sieved through a 2 mm mesh to eliminate larger organic debris and adjusted to 60% water-holding capacity. The R_B was determined for each sampling time from biodegradation as CO_2 evolution trapped in 10 mL of 1.0 M NaOH solution, by adding $BaCl_2$ and following titration with 0.5 M HCl, according to Silva et al. (2007a) with some adaptations. To this, vessels ($D = 4.8$ cm) were filled with 25 g of substrate. Film samples (1.8 cm \times 1.8 cm) were buried at the depth of 1 cm from the surface of the soil. The ratio area of film/surface was maintained the same as in the biodegradation test.

The determination of MBC was performed in the natural organic soil (day 0) and in the same sample used for biodegradation analysis (day 10), following the fumigation extraction method as described by Vance, Brookes and Jenkinson (1987) and modified by Silva et al. (2007b), with some adaptations. To this, 10 g of the incubated substrate was directly added of 1 mL of ethanol-free chloroform and fumigated for 24 h at 25 ± 3 °C. After this period, 25 mL of 0.5 M K_2SO_4 were added to the samples, which were kept under orbital shaking at 220 rpm for 30 min at 25 °C. The samples were left to stand for 30 min. Extracts were obtained by filtering the supernatant (paper 80 g/m²; 3 μ m porosity). Extraction of non-fumigated samples was used as control. The soil extract (8.0 mL), 0.066 M $K_2Cr_2O_7$ (2 mL), H_2SO_4 (10.0 mL) and H_3PO_4 (5 mL) were mixed. After cooling, the mixture was added of deionized water (70 mL), left to cool again, and titrated with 0.033 M ferrous (II) ammonium sulfate, using diphenylamine as an indicator. The organic carbon content was determined after dichromate oxidation. The MBC (mg microbial C kg⁻¹ substrate) was calculated following Equation 4:

$$MBC = [C(fum) - C(non.fum)] / k_c \quad (4)$$

where $C(fum)$ is the organic carbon content of fumigated sample, $C(non.fum)$ is the organic carbon content of non-fumigated sample, and k_c -factor of 0.33 as described by

Sparling and West (1988). The $q\text{CO}_2$ ($\text{mg C-CO}_2 \text{ mg}^{-1} \text{MBC h}^{-1}$) was calculated following Equation 5 (Anderson & Domsch, 1993):

$$q\text{CO}_2 = R_B / \text{MBC} \quad (5)$$

2.6 Statistical analyses

The data were analyzed by the Software Statistica 12.0 (Statsoft Inc., Tulsa, USA) at a 5% significance level. Analysis of variance (ANOVA) and Tukey's test were used to compare the means of the films and determine significant differences. When not mentioned, analyses were performed in triplicate.

3 Results and Discussion

3.1 Physicochemical properties

The film thickness (d), moisture content (MC) and total soluble matter (TSM) of the GCR-MMT bionanocomposites are shown in Table 2. The lowest d values were found for GCR-MMT01 and GCR-MMT01 films, whereas GCR-MMT10 presented the greatest d value. Jorge et al. (2015) also observed that the d values of pigskin gelatin films (0.071 mm) were significantly increased by the incorporation of 5 g (0.077 mm) and 10 g (0.080 mm) of MMT/100 g of gelatin. The moisture content, MC, was not affected by the incorporation of MMT, regardless the concentration. The average MC was 21%, similar to that of other films prepared from a bovine hide (MC: 19.3%; Ma et al., 2012) and tilapia skin (MC: 20.2%; Tongnuanchan, Benjakul, Prodpran, & Nilsuwan, 2015) gelatin.

The pure GCR-based film presented the highest solubility in aqueous environment. This result is associated to the hydrophilic nature of its compounds, which usually limits the application for food products with high moisture content (Hosseini, Rezaei, Zandi, & Farahmandghavi, 2015). Results indicated that increasing MMT concentration decreased the TSM, which is a positive effect when considering the application in the food packaging field. Similar behavior was observed for gelatin-nanoclay films produced with the incorporation of Cloisite®20A (0, 2, 6, 10, 14, 18 g clay/100 g gelatin), which is natural montmorillonite modified with a quaternary ammonium salt (Farahnaky, Dadfar, & Shahbazi, 2014). The authors reported that this

effect might be due to the MMT particles that act as a barrier and reduce the interactions between gelatin and water.

Table 2. Film thickness (d), moisture content (MC), and total soluble matter (TSM) of the GCR-MMT bionanocomposites with different clay content.

Sample	d (mm)	MC (%)	TSM (%)
GCR-MMT0	0.189 ± 0.011 ^c	21.5 ± 0.8 ^a	62.3 ± 2.0 ^a
GCR-MMT0.1	0.191 ± 0.007 ^c	20.9 ± 1.1 ^a	59.4 ± 0.7 ^b
GCR-MMT0.5	0.206 ± 0.010 ^b	20.8 ± 0.4 ^a	56.9 ± 1.1 ^b
GCR-MMT2	0.215 ± 0.007 ^b	21.7 ± 0.8 ^a	56.1 ± 3.2 ^{bc}
GCR-MMT6	0.220 ± 0.009 ^b	20.5 ± 1.6 ^a	56.5 ± 1.3 ^{bc}
GCR-MMT10	0.255 ± 0.011 ^a	21.6 ± 0.4 ^a	48.6 ± 1.1 ^c

Mean values ± standard deviation (n = 3).

Different superscript letters in the same column indicate statistically significant differences ($p < 0.05$).

3.2 X-ray diffraction

Different aspects may affect the formation and properties of nanocomposites, such as the presence of additives, type of clay modification, processing temperature, mixing speed, shear rates, polymer viscosity, among others. Therefore, the achievement of exfoliation/intercalation of the nanoclay has been considered a key parameter to reach improvements in the final nanocomposite (Albdiry, Yousif, Ku, & Lau, 2012). In this sense, XRD was used to examine the morphology of the materials and more specific to check if the clay has been exfoliated. The XRD patterns of the organically modified MMT (Cloisite® 30B) powder and GCR-MMT films are shown in Figure 1. When an exfoliated structure is formed in the nanocomposite, no diffraction peak regarding the clay is visible in the XRD, due to the enlargement of spacing between the layers and/or disordering of the polymer. In the case of intercalation, usually, a shift of the diffraction peak is observed, which leads to lower angles values. An ordered multilayered structure is achieved, where layers of the polymer and the clay are alternated (Alexandre & Dubois, 2000; Sinha Ray & Bousmina, 2005).

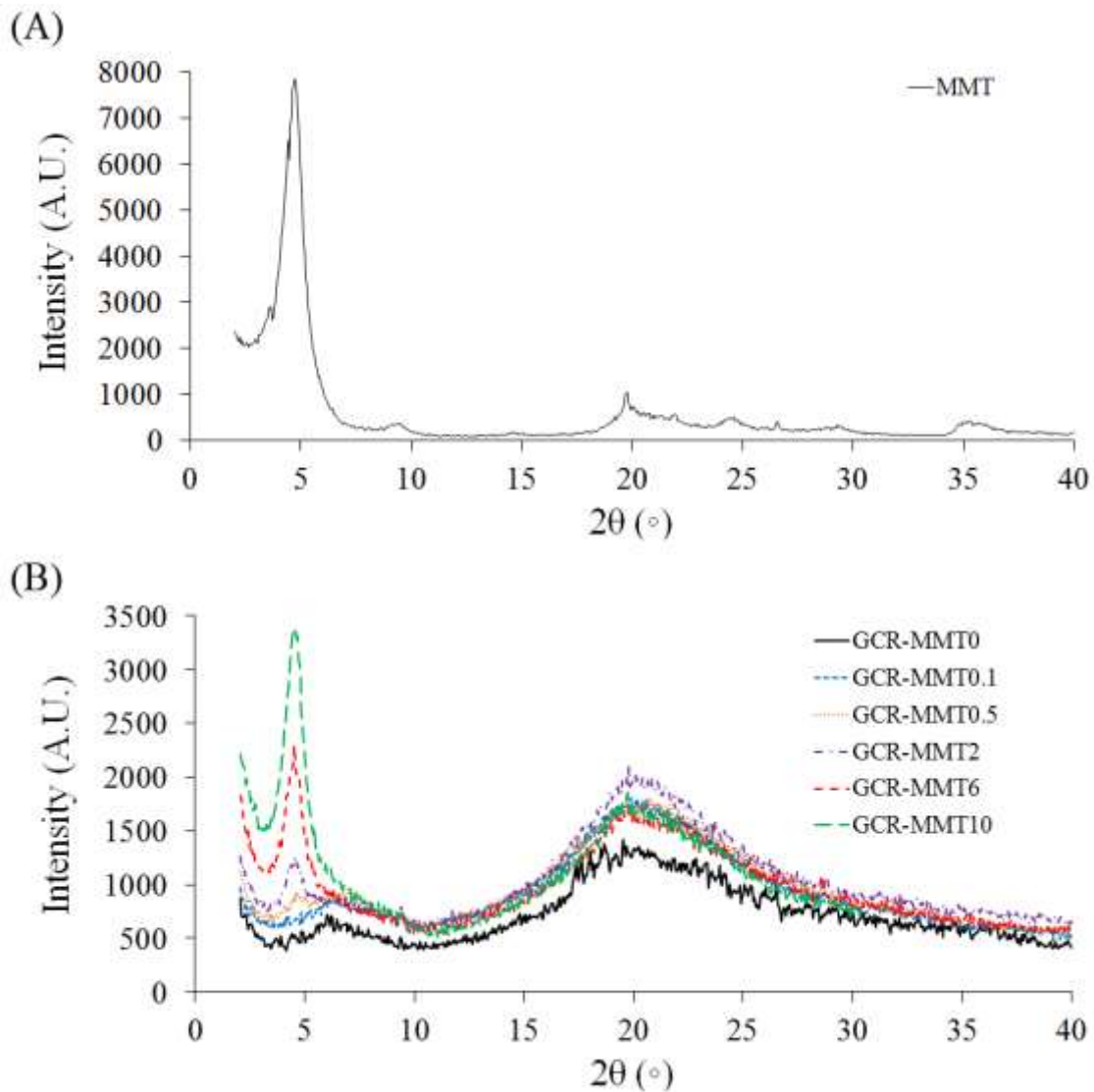


Figure 1 X-ray diffractograms of the modified nanoclay (A) and the GCR-MMT bionanocomposites with different clay content (B).

The pure GCR-based film presented a diffraction peak at $2\theta = 6^\circ$ and one at $2\theta = 19.7^\circ$, which are characteristic of the crystalline triple-helix structures of gelatin and amorphous phase, respectively, as similarly reported by Pereda, Ponce, Marcovich, Ruseckaite, and Martucci (2011). Montmorillonite Cloisite® 30B showed a sharp peak at $2\theta = 4.75^\circ$. For the bionanocomposite films with 0.1 and 0.5% MMT, there was no evidence of this reflection peak, which proved the formation of an exfoliated structure. The GCR-MMT film with 2% of clay showed a small peak at $2\theta = 4.55^\circ$, suggesting an intercalated/exfoliated structure. The increasing addition of clay (GCR-MMT6 and

GCR-MMT10) resulted in intercalated structures, as illustrated in Figure 1 by the sharper peak at $2\theta = 4.55^\circ$, which resembled the Cloisite® 30B peak. These results are in agreement with those found for fish gelatin films added of Na^+ MMT (1, 3, and 5 g/100 g gelatin), where the higher concentrations of clay resulted in intercalated structures, whereas the lower ones resulted in exfoliated structures (Rostamzad, Paighambari, Shabanpour, Ojagh, & Mousavi, 2016).

Another research investigated chitosan-based nanocomposites with Cloisite® 30B, and a sharp peak at $2\theta = 4.9^\circ$ was found for the clay powder, which is in agreement with this study. The pure chitosan film had characteristic peaks around $2\theta = 8^\circ$, 11° , and 18° . After the formation of nanocomposites with 5 % MMT, the appearance of a peak at 4.56° (a peak of the clay shifted from $2\theta = 4.9^\circ$) was noticed, which indicated significant intercalation in the hybrid structure (Rhim, Hong, Park, & Ng, 2006).

3.3 Dynamic-mechanical analysis

Dynamic-mechanical analysis (DMA) was used to determine the storage modulus (E') and tan delta, which was useful to determine the glass transition temperatures of the films. The DMA curves are shown in Figure 2. From Figure 2.A, it is possible to note that the E' of all the nanocomposite films was higher than that of GCR-MMT0 over the entire temperature range. GCR-MMT2 exhibited the highest E' values, followed by GCR-MMT6 and GCR-MMT10, GCR-MMT05, GCR-MMT01, and finally GCR-MMT0. From 60°C the films presented a significant decrease in their E' , associated with the increase of the molecular chain mobility.

The storage modulus of the pure gelatin film at environmental condition temperature (20°C) was approximately 4.5 MPa, whereas for the bionanocomposite film with 2% Cloisite 30B was 74 MPa. This increase with a factor of 15 can be considered a large improvement of the modulus, with an addition of only 2 wt% nanoclay to the polymer matrix. Increased E' values were found for the other clay loadings, but only to a lower extent. To the best of the authors' knowledge, DMA is not frequently used to investigate the properties of gelatin-MMT films, and comparison between the acquired data with literature is rather difficult. Kumar et al. (2010) reported a similar behavior for soy protein isolate-based films added of 0, 5 and 15% Cloisite

30B, where the addition of 5% nanoclay yielded the greater E' enhancement for the films (from 337 MPa to 870 MPa).

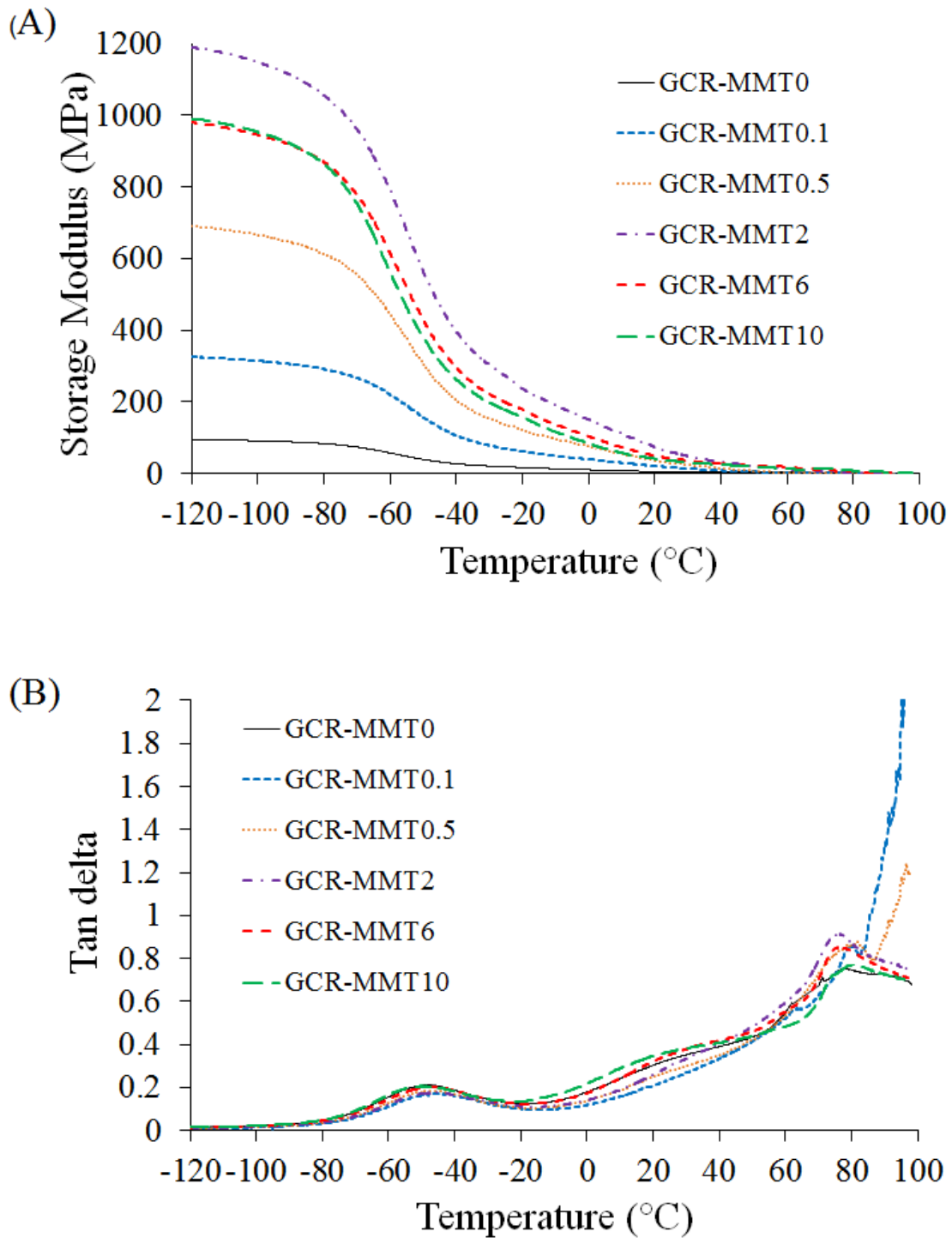


Figure 2 Storage modulus (A) and Tan delta (B) curves of the of the GCR-MMT bionanocomposites with different clay content.

The improvements in the storage modulus are probably associated to the aspect ratio of MMT and its rigidity, as well as with the good interfacial interactions between the clay and the polymer matrix. In general, even low concentrations of nanoclay (<5 wt%) have been proving to cause noticeable enhancements in the mechanical properties of various bionanocomposites (Rhim, Park, & Ha, 2013). Additionally, a previous study reported that higher E' values and tensile strength were found for soy protein isolate-based films incorporated with modified MMT in comparison to natural MMT (Cloisite Na⁺) at the same concentration of 5% (Kumar et al., 2010). This fact was associated to the higher volume fraction of Cloisite 30B in the bionanocomposite at the same clay content and to its longer d-spacing values as compared to that of natural MMT.

From Figure 2.B, it can be observed that all the film samples presented two peaks for each tan delta curve. This fact is related to the occurrence of two different T_g 's for each film sample, which could be attributed to a phase separation between the components resulting in a glycerol-rich fraction and a protein-rich fraction. The same phenomenon was observed for gelatin films (Sobral et al., 2001; Sobral & Habitante, 2001), as well as gelatin-based bionanocomposites (Jorge et al., 2015). The negative temperature transitions were found around -48 °C for the pure GCR-base film and the nanocomposites with different MMT concentrations, and could be associated to the glycerol-rich phase. The T_g of the gelatin-rich phase was not affected by the addition of nanoclay, regardless the concentration, and was observed at approximately 77 °C for all the films. Alexandre, Lourenço, Bittante, Moraes, and Sobral (2016) investigated the effect of incorporating 5% MMT into pigskin gelatin films and also reported that the T_g of the naocomposites were very similar to that of the pure pigskin gelatin films.

3.4 Fourier transform infrared (FTIR) spectroscopy

Figure 3 displays the FTIR spectrum of GCR-MMT0, which illustrates the typical spectral behavior of all the produced. This result implied that the incorporation of MMT did not cause any obvious changes in the intensity or position of the peaks, and consequently the chemical composition of gelatin was not altered at a molecular perception. All the samples yielded three major peaks corresponding to the absorption regions of amide-I (1629-1630 cm⁻¹), amide-II (1538-1539 cm⁻¹) and amide-III (1235 cm⁻¹). These bands are commonly found for gelatin-based films from different

sources, and are associated to the C=O stretching vibration (amide-I), N-H bending vibration and C-N stretching vibration (amide-II), and C-N stretching vibrations coupled to N-H in-bending vibrations (amide-III) (Cebi, Durak, Toker, Sagdic, & Arici, 2016).

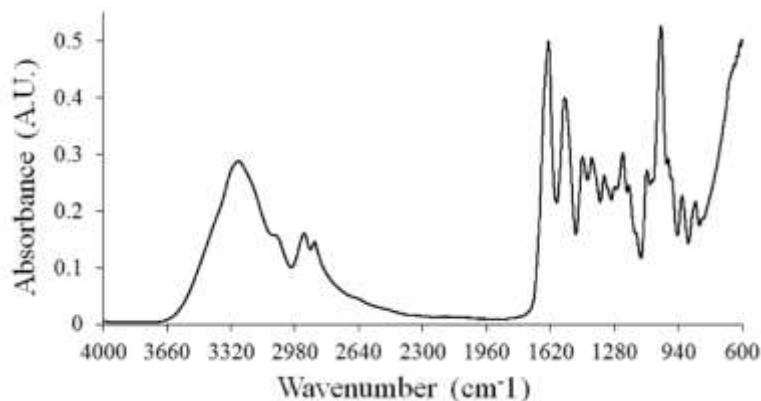


Figure 3 FTIR spectrum of the GCR-MMT bionanocomposites with different clay content.

Other peaks were observed in the range $3277\text{--}3281\text{ cm}^{-1}$, $2926\text{--}2930\text{ cm}^{-1}$, and $2873\text{--}2875\text{ cm}^{-1}$. They corresponded to NH stretching coupled with hydrogen bonding (Amide-A), CH_2 asymmetrical stretching vibration, and symmetrical stretching vibration, respectively (Muyonga, Cole, & Duodu, 2004). Some authors found similar FTIR spectra with the above mentioned absorptions for gelatin films from cold water fish skin (Hosseini, Rezaei, Zandi, & Farahmandghavi, 2015), shark skin (He, Zhang, Cai, & Wang, 2016), pigskin (Bergo & Sobral, 2007) and bovine skin (Nur Hanani, McNamara, Roos, & Kerry, 2013). The intense absorption observed at 1032 cm^{-1} is associated to the C-OH stretching of glycerol (Bergo & Sobral, 2007), which is a plastificant and was intrinsic to the raw material used to produce the films.

3.5 Film surface characteristics

The micrographs obtained by SEM analysis are presented in Figure 4. They revealed that the surface of the films were homogeneous, without the presence of any cracks or holes. The GCR-MMT0 (Figure 4.A) presented a smoother surface in comparison to the nanocomposite films. The addition of MMT resulted in the appearance of “dots” (as indicated by the bright areas), which become more numerous at higher loadings. The pure gelatin film also shows some white spots due to air bubbles. These extra dots at higher loadings are due to the aggregation of MMT upon

increasing concentration. The MMT particles were uniformly dispersed and, as the concentration of clay increased, the content of dispersion increased gradually as well. A similar result was observed by Wu et al. (2017), who produced bovine skin gelatin films incorporated with different concentrations of nanoscale hydroxyapatite (0%, 5%, 10%, 15%, 20%) as an inorganic filler.

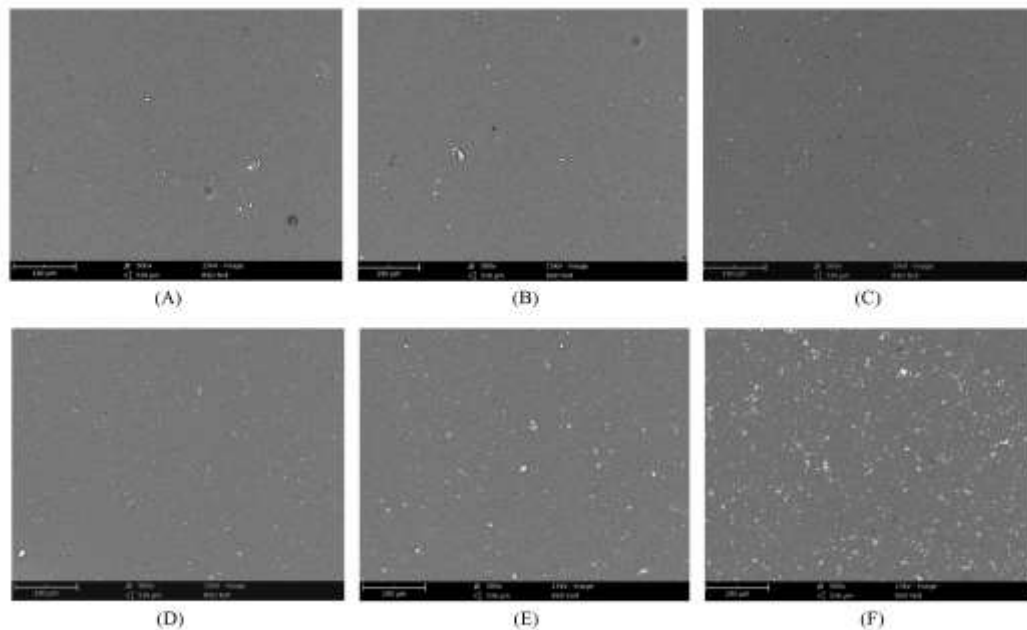


Figure 4 Surface micrographs of the GCR-MMT bionanocomposites with different clay content: 0 g (A), 0.1 g (B), 0.5 g (C), 2.0 g (D), 6.0 g (E) and 10.0 g (F) per 100g of gelatin.

3.6 Transmittance and transparency

Light transmittance and transparency of the films prepared from GCR containing MMT at different levels are illustrated in Figure 5. All the films presented negligible transmittance at 200 nm and 280 nm, which demonstrated they acted as excellent UV barrier. This characteristic is associated with the high content of aromatic amino acids in protein films, which are capable of absorbing UV light (Hamaguchi, WuYin, & Tanaka, 2007). This is an interesting feature that could help to prevent discoloration and lipid oxidation caused by UV light incidence in packed food stuff (Ramos et al., 2013).

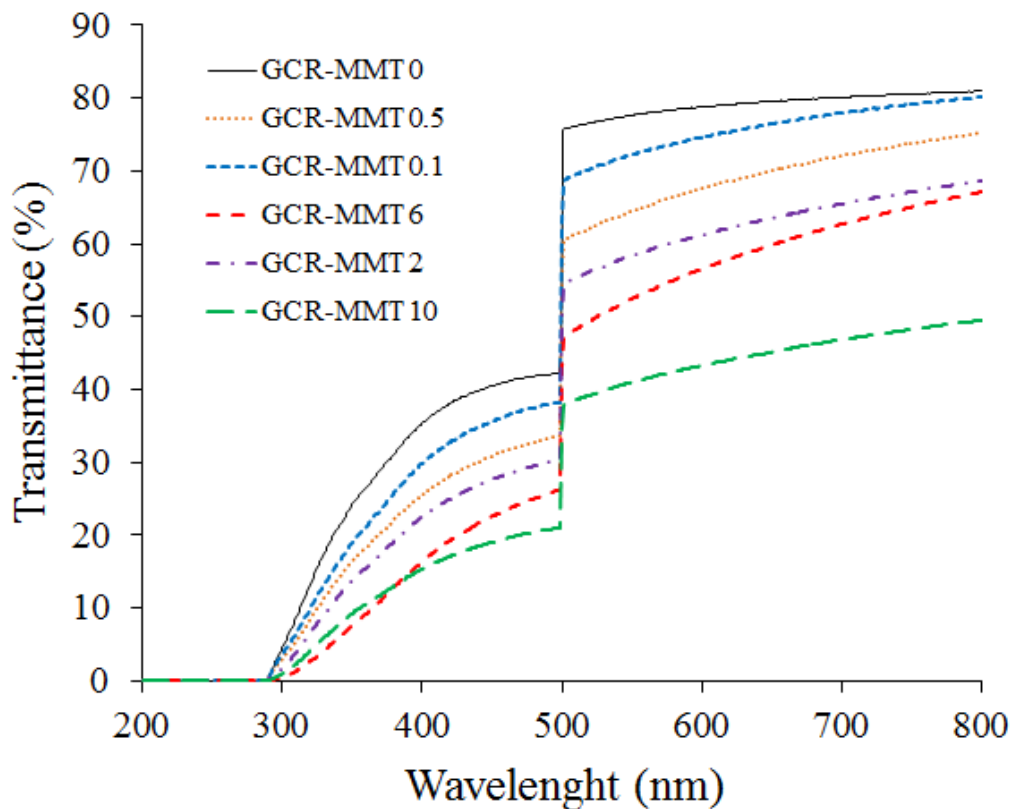


Figure 5 Light transmittance of the GCR-MMT bionanocomposites with different clay content.

From 300 nm to 800 nm, the higher the concentration of clay, the lower transmittance value (%) was observed, probably due to the more significant amount of dispersed nanoparticles in the polymer matrix that hindered the light passage (Kanmani & Rhim, 2014). Sothornvit, Hong, An, and Rhim (2010) studied the properties of whey protein isolate (WPI) films added of montmorillonite (Cloisite[®] 30B) and reported the same effect of the increasing concentration of clay (0, 5, 10, and 20 g/100 g WPI) in the transparency values. This behavior, as well as the transmittance, is associated with the aggregation of nanoparticles and obstruction of light transmission, resulting in reduced film transparency. Despite the significant differences among the transparency values of the films, they all presented low values and were apparently transparent, which may play an important role regarding their visual aspect when considering their application in the food packaging field.

3.7 Indoor soil burial biodegradation

The indoor soil burial experiment was carried out for 10 days because beyond this point the recovery and evaluation of samples became unpractical. This method was employed to simulate the naturally occurring conditions of biodegradation, which is comprised of two phases: disintegration and mineralization. The first one is associated to mechanical, chemical, or thermic degradation. The second involves the action of microorganisms and production of carbon dioxide and/or methane, water, and biomass (Kyrikou & Briassoulis, 2007).

Weight loss of films was used herein only to qualitatively analyze the biodegradability of the GCR-MMT films. At the end of the experiment, GCR-MMT0 and GCR-MMT2 lost over than 98% of their initial weight. This result was in agreement with the visual aspect of films observed after 10 days, when their structural integrity was wholly lost, and samples disappeared from the soil mass. These results indicate that GCR-MMT0 and GCR-MMT2 could be considered as entirely biodegraded after 10 days, and that the formation of bionanocomposite with 2% MMT did not influence the biodegradation process when compared to the pure GCR-based film. In the present study, the fast rate of biodegradability of GCR-MMT0 and GCR-MMT2 can be associated to the hydrophilic character of the films, which turn them into water-wettable materials susceptible to microbial attack. Additionally, their organic nature favors the process under aerobic conditions (Kyrikou & Briassoulis, 2007).

Dalev et al. (2000) investigated the soil burial biodegradation of pure and chemically crosslinked gelatin films and obtained similar results to the present work. It was observed great changes in the samples even on the first day, and on the 10th day, all the samples were considered biodegraded. Another study evaluated the biodegradation of pure gelatin films and the effect of crosslinking with dialdehyde starch, compounding with 5% MMT and formation of multilayer film (Martucci & Ruseckaite, 2009). After 10 days, gelatin film was the most sensitive to biodegrade (40% weight loss), followed by the bionanocomposite, multilayer, and crosslinked film, respectively. Results indicated that the addition of MMT had a smaller effect on the biodegradation of gelatin films in comparison to the formation of networks by the crosslinking. Despite the differences, all the films lost their structural integrity after 10 days and were considered as quite rapidly degradable materials.

3.8 Effect of biodegradation of selected films on soil quality

Soil health, or quality, was broadly defined by Doran (2002) as: “the capacity of a living soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health”. It can be accessed by a combination of different approaches regarding the microbiological, chemical and physical properties of the soil, which reflect management-induced changes in soil conditions due to land use changes or tillage (Raiesi & Kabiri, 2016). From a biological perspective, the most used indexes include respiration, microbial biomass, enzyme activities, metabolic quotient, nematodes, etc., although the integration of physical and chemical parameters may help to provide more sufficient information (Bastida et al., 2008).

The present work dealt with the biodegradation of the GCR-MMT films and the effect of this process on the soil quality. The biodegradation of such materials involves the action of microorganisms that use hydrolysis products as substrates to synthesize biomolecules and respire (Sander, 2019). Therefore, the basal respiration (R_B), soil microbial biomass carbon (MBC), and metabolic quotient (qCO_2) were determined to establish a relation between the biodegradation of films and its effect on soil quality.

Results showed that the biodegradation of GCR-MMT0 and GCR-MMT2 in soil significantly increased the R_B when compared to the control soil ($5.1 \text{ mg kg}^{-1} \text{ h}^{-1}$). The highest CO_2 -C efflux rates were observed on day 5, which reached 10.3 and $12.6 \text{ mg kg}^{-1} \text{ h}^{-1}$ for soils added of GCR-MMT0 and GCR-MMT2, respectively. The efflux rates tended to decrease with increasing incubation time. According to Ibrahim et al. (2015), higher R_B rates at the early stages may be due to the rapidly decomposable carbon and other substrates induced by the material biodegradation, stimulating the action of microbial activities and communities.

Initially, the MBC was 577 mg kg^{-1} substrate and it was significantly increased by the biodegradation process of the films after the 10-day experiment up to 976 mg kg^{-1} (GCR-MMT0) and 992 mg kg^{-1} (GCR-MMT2). The increased values of MBC in soil with the addition of feedstock may be associated with increased values of the potentially labile carbon (oxidized using $K_2Cr_2O_7$) (Al-Wabel et al., 2017), which is in accordance with the obtained results for soil containing GCR-based films.

Regarding the $q\text{CO}_2$, results demonstrated that $q\text{CO}_2$ for the soil used as reference was $8.9 \text{ mg C-CO}_2 \text{ mg}^{-1} \text{ MBC h}^{-1}$ and 8.2 and $8.0 \text{ mg C-CO}_2 \text{ mg}^{-1} \text{ MBC h}^{-1}$ for soils containing GCR-MMT0 and GCR-MMT2, respectively. Moreira et al. (2018) developed biodegradable polymers (polyvinyl-alcohol and cassava starch) added fillers and nutrients to improve the soil quality. In their study, the reference control soil presented $q\text{CO}_2$ of $10.3 \text{ mg C-CO}_2 \text{ mg}^{-1} \text{ MBC h}^{-1}$. During the 35-day experiment, the values from the substrate in which the biodegradation occurred were increased, decreased, or maintained depending on the sample.

In general, enhanced $q\text{CO}_2$ and reduced microbial efficiency are related to soil disturbance and stress because more energy is demanded to convert C-sources into biomass (Leita et al., 1999). In the present study, no statistical differences were observed among $q\text{CO}_2$ results. This fact might be associated to the higher R_B results for the soil containing GCR-MMT films, which yielded comparable values of $q\text{CO}_2$ among all the samples. As reported by Chiellini et al. (2007), the carbon content of an organic matter which was metabolized into microbial biomass or humus can be also mineralized to carbon dioxide.

4 Conclusions

In this research, bionanocomposite films were successfully produced by combining modified montmorillonite, and gelatinous residues originated from the processing of nutraceutical capsules. According to XRD results, an exfoliated and/or intercalated structure was achieved for the bionanocomposites, which contributed to the large improvements regarding the dynamic mechanical properties. The total soluble matter of the pure gelatin film (62%) was significantly reduced by the increasing concentration of MMT and reached 49% solubility when 10% MMT was added. This result is an important achievement to hydrophilic films, which cooperates to broaden their applicability as food packaging to dry and low moisture food products. Light barrier properties of films were also improved by the addition of MMT, which enhanced their protective effect against UV and visible light. Moreover, the visual aspect of all bionanocomposites was transparent, regardless of the MMT content.

Results demonstrated that the bionanocomposites had improved properties and were rapidly biodegraded in a 10-day soil burial experiment. Additionally, the R_B and

MBC values of soil in which the biodegradation occurred were increased, confirming the action of microorganisms in the biodegradation process. Further studies are necessary to improve our knowledge about the effects of biodegradation of protein-based polymers on the quality of different soils and the possible applications in areas such as agriculture and bioremediation.

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ARTIGO 3: Effect of moderate electric field on the properties of gelatin capsule residue-based films

Artigo publicado em: Food Hydrocolloids (A1)

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Effect of moderate electric field on the properties of gelatin residue-based films

Abstract

This work focused on the development of edible films based on gelatin capsule residue by ohmic heating. The effect of the electric field strength (9.5 Vcm^{-1} and 19 Vcm^{-1}) on the mechanical, thermal, physicochemical and morphological properties of films was investigated and compared to films produced by the conventional heating method. Results demonstrated that the application of 9.5 Vcm^{-1} during ohmic heating was able to improve tensile strength from 2.66 MPa to 6.62 MPa in comparison to the conventional method, whereas the film developed using 19 Vcm^{-1} reached a value of 3.26 MPa. Young's Modulus was also significantly increased, while an opposite effect was observed for elongation at break. Modulated differential scanning calorimetry analysis revealed that all samples had two transition temperatures. Electrically treated film (9.5 Vcm^{-1}) presented increased crystallinity and thermal stability. Thickness, morphology and water-related properties were not affected by the application of electric field. Films produced by ohmic heating, especially when 9.5 Vcm^{-1} was applied, presented improved mechanical and thermal properties, with increased crystallinity.

Keywords: ohmic heating; gelatin film; waste; packaging; moderate electric field.

1. Introduction

Biodegradable materials have been reported to be an interesting alternative to conventional petroleum-based polymers, especially concerning environmental aspects. In the food packaging sector, edible films and coatings have also drawn attention due to the increasing consumer demand for more natural methods of food protection and conservation (Enujiugha & Oyinloye, 2018). These materials are produced from edible biopolymers: proteins, polysaccharides, and lipids. Other ingredients and additives incorporated into the formulation should be categorized as food-grade components (López et al., 2017). Although the functional properties of edible and synthetic films are still not equivalent, the intrinsic characteristics of proteins turn them into an excellent polymer matrix to develop films and coatings. Additionally, due to their amino acid composition, they present multiple sites for chemical interactions that may induce improvements in the final properties of the films (Dangaran, Tomasula, & Qi, 2009).

Among proteins, gelatin has been one of the most studied biopolymers because of some attributes, such as film-forming ability, biocompatibility, non-toxicity, abundance, transparency and potential use as carriers to different compounds and additives (Nur Hanani, Roos, & Kerry, 2014). The obtainment of gelatin consists of collagen denaturation by acidic or alkaline treatment, being mainly extracted from pig skin, bovine hide, and pork and cattle bones (Gómez-Guillén et al., 2009). In this context, the gelatinous residue originated from the processing of nutraceutical soft gel capsules is another interesting source of gelatin that has been successfully used to produce edible films (Campo, Costa, Rios, & Flôres, 2016; Crizel, Costa, Rios, & Flôres, 2016; Iahnke, Costa, Rios, & Flôres, 2015).

In addition to the intrinsic characteristics of the film matrix and its components, the extrinsic processing factors also play an important role in the final properties of the films. Protein films may be formed by two main methods: wet (casting) and dry (extrusion and thermopressing) processing, which differently influence the film properties mainly depending on the processing temperature and drying conditions (Gómez-Estaca, Gavara, Catalá, & Hernández-Muñoz, 2016). The casting process is the most widely used technique to develop gelatin-based films and usually involves the formation of a film-forming solution, where gelatin is dissolved in warm water, followed by spreading over Petri dishes and solvent evaporation (Flôres et al., 2017). In

general, the first step involves heat denaturation of protein by breaking the triple helix structure into random coils, whereas dehydration promotes a partial renaturation of the structure that exists in the native collagen (Bigi, Panzavolta, & Rubini, 2004).

The production of gelatin-based film-forming solutions is carried out by conventional heating systems that use indirect water baths or hotplates (Flôres et al., 2017). In this context, ohmic heating (OH) has recently gained attention as an alternative to conventional heating for films or coatings production made from whey protein (Pereira, Souza, Cerqueira, Teixeira, & Vicente, 2010; Pereira, Teixeira, & Vicente, 2011), chitosan (Souza et al., 2009; 2010) and starch/chitosan (Coelho et al., 2017). It is relevant to highlight that these studies were conducted matching the temperature profiles of the ohmic and conventional treatments, which made possible the evaluation of the non-thermal effects of the electricity. Overall, the properties of the films were influenced and improved by the application of this treatment, which depended on parameters such as the strength of the applied electric field. The ohmic heating consists of internally heating food liquids and solids that have electrical resistance by passing an alternating current through them, offering advantages that include uniform heating of liquid and solid samples and high heating rates (Sastry, 2008).

To the best of our knowledge, very few works have dealt with the potential use of ohmic heating to produce edible films. It is important to point out that the aforementioned studies were all performed by the same research group, and none has reported the application of a moderate electric field to gelatin-based films. Therefore, the aims of the present study were to investigate the potential use of ohmic heating for the development of gelatin residue-based films for the first time, and understand its effect on their mechanical, thermal, morphological and physicochemical properties. For this, the electrically treated films were compared to films obtained by conventional heating, both conducted under the same temperature profile.

2. Materials and methods

2.1. Materials

The gelatin capsule residue (GCR), mainly composed of bovine gelatin (40.6%), water (35.0%) and glycerol (24.4%), was supplied by the Chemical Pharmaceutical

Tiaraju Laboratory (Rio Grande do Sul, Brazil). This material came from the processing of linseed oil nutraceutical capsules.

2.2. *Film production*

The GCR-based film-forming solutions (GS) were prepared by mixing GCR and distilled water (80 g of GCR/100 g of water). Two heating treatments were applied to produce the films: ohmic and conventional heating processes. The film-forming solutions were kept at 75 °C for 30 min, based on the methodology described by Wang, Liu, Holmes, F. Kerry, & P. Kerry (2007). Films were obtained by casting the heated GS onto polystyrene Petri dishes (0.086 g/cm²) and drying at 45 °C for 4 h in an oven (DeLeo, B5AFD, Brazil). These conditions were chosen based on preliminary tests, in which the drying curves from different drying temperatures (from 30 °C to 60 °C) were obtained. The drying time was established when the weight loss became constant. The temperature of 45 °C was chosen in order to optimize the processing time and because the film properties were considered adequate.

The produced films were conditioned in desiccators (25 ± 2 °C; 50 ± 5% RH) for at least 48 h before characterization. The final GS had 18 g of gelatin/100 g of GS and 60 g of glycerol/100 g of gelatin. Glycerol was naturally present in the GCR. The chosen parameters ensured an optimal starting electrical conductivity of the GS (~2200 μS · cm⁻¹; Digimed, MD-3P, Brazil), which allowed the ohmic heating effect to take place.

2.3. *Heating treatments*

2.3.1. Ohmic heating

The experimental ohmic heating apparatus was described in detail by Mercali, Jaeschke, Tessaro, and Marczak (2012). It consisted of a stabilizer (Forceline, EV 1000 T/2-2, Brazil), a power supply with an alternating frequency of 60 kHz, a variable transformer (Sociedade Técnica Paulista LTDA, Varivolt, Brazil), a computer, a data acquisition system and an ohmic cell (a 400 ml water jacket glass vessel). The lid of the cell was designed to operate with four temperature sensors and two curved titanium electrodes with 5 cm height. The minimum and maximum inter-electrode gaps were 5.7 cm and 7.0 cm, respectively.

A bypass system was used to enable the use of two thermostatic baths to feed the jacket vessel: one for the come-up phase and another for the isothermal phase. One (Lauda, model T Germany) was used to promote initial heating of the mixture, by passing hot water ($90\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$) through the jacket. When the solution reached $70\text{ }^{\circ}\text{C}$, the hot bath was switched off, and the temperature of $75\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ was held for 30 min by the application of ohmic heating and by circulating cool water ($3\text{ }^{\circ}\text{C}$ to $15\text{ }^{\circ}\text{C}$) from another temperature-controlled water bath (Lauda, RM 12, Brazil). From $70\text{ }^{\circ}\text{C}$ to $75\text{ }^{\circ}\text{C}$, the solution was heated by the circulating hot water through the jacket of the cell. The water baths were switched at $70\text{ }^{\circ}\text{C}$ because the time required for the sample to raise from $70\text{ }^{\circ}\text{C}$ to $75\text{ }^{\circ}\text{C}$ was exactly the time necessary to switch the water in the hoses and in the jacket of the cell from hot to cool water. The GCR-based films were produced with the application of two different voltages: 60 V (average electric field strength of 9.5 Vcm^{-1}) and 120 V (average electric field strength of 19.0 Vcm^{-1}), named OH9.5 and OH19, respectively. The GS was kept under stirring by a magnetic stirrer plate (IKA-Works Inc, C-MAG HS 10, Brazil) to promote homogenization of the solution during heating.

2.3.2. Conventional heating

An analogous procedure to the ohmic heating was followed to produce films by conventional heating (CON), without the application of electric field. The conventional heating process was performed in the same ohmic cell but without the presence of electrodes. To that purpose, a thermostatic water bath ($90\text{ }^{\circ}\text{C}$) connected to the cell was used to heat the samples up to $75\text{ }^{\circ}\text{C}$. The samples were kept at this temperature by the circulating water from another water bath (set at $75\text{ }^{\circ}\text{C}$) for 30 minutes, under magnetic stirring. This procedure was necessary to regulate the heating rate of the come-up phase and to match the temperature profiles of the conventional and ohmic heating, allowing the assessment of the non-thermal effects of electricity. This strategy was important to guarantee that any differences between the treatments would be exclusively because of the electric field application. To that purpose, a close match of the temperature profiles was desired and acquired, as can be observed in Fig. 1.

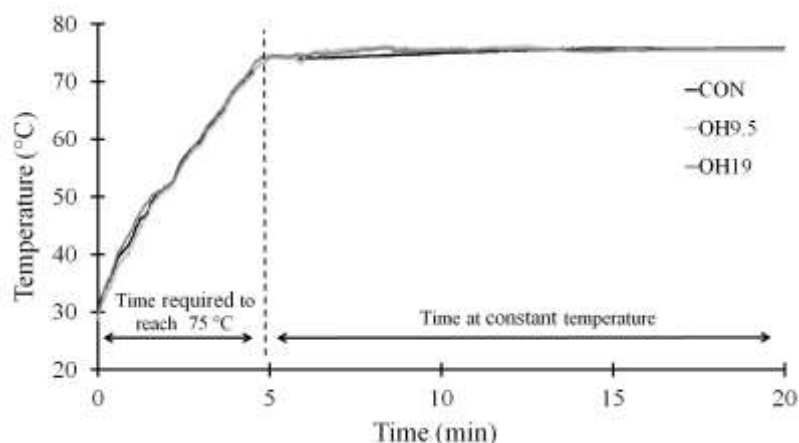


Figure 1. Temperature profiles of the gelatin capsule residue-based film-forming solutions during conventional (CON) and ohmic heating (OH9.5: 9.5 Vcm⁻¹; OH19: 19 Vcm⁻¹).

2.4. Characterization of the films

2.4.1. Modulated differential scanning calorimetry (MDSC)

Thermal transitions of the film samples (10 ± 1 mg) were examined by a modulated differential scanning calorimeter (MDSC Q1271, TA Instruments, USA), calibrated with indium. The samples were weighed into aluminum pans, sealed and scanned from -120 °C to 100 °C, at a heating rate of 5 °C min⁻¹, with modulation of ± 0.50 °C amplitude and 60 s period. An empty aluminum pan was used as a reference.

2.4.2. Thermogravimetric analysis (TGA)

Films samples (10 ± 1 mg) were scanned from 40 °C to 600 °C by a thermogravimetric analyzer (TGA Q500, TA Instruments, USA), at a heating rate of 10 °C min⁻¹ and using air as the purge gas (25 mL min⁻¹).

2.4.3. Thickness and mechanical properties

Thickness was evaluated by a micrometer (Digimes, IP40, Brazil) to the nearest 0.001 mm, and expressed as the average of five random measurements taken from each film sample (Rhim, Gennadios, Weller, Carole, & Hanna, 1998). A texture analyzer (TA.XT2i e Stable Micro Systems, Godalming, UK) was used to determine the tensile strength (TS; MPa), percent elongation at break (EAB; %) and Young's Modulus (YM; MPa), according to the ASTM D882-09 (ASTM, 2009). To that purpose, ten strips

(100 mm × 25 mm) of each sample were analyzed, and an initial grid separation of 50 mm and a crosshead speed of 0.8 mm s⁻¹ were used.

2.4.4. Film surface characteristics

Micrographs were observed with a scanning electron microscope (JEOL, JSM 6060, Japan) at an accelerating voltage of 5.0 kV and magnification of 1000 ×. A double-sided adhesive was used to fix the film samples onto cylindrical bronze stubs.

2.4.5. Moisture content and water solubility

The moisture content (MC) was determined gravimetrically by drying the films (2 cm diameter) in an oven (DeLeo, TLK48, Brazil) at 105 °C for 24 h (AOAC, 2005). Water solubility (WS) was evaluated gravimetrically, following the method described by Rhim, Gennadios, Weller, Carole, & Hanna (1998). Films samples (2 cm diameter) were immersed in 30 mL of distilled water and softly stirred by a shaker (Novatecnica, NT145, Brazil) for 24 h. After this period, the water was discarded, and the samples were dried at 105 °C for 24 h. The MC previously obtained was used to calculate the WS.

2.4.6. Water vapor permeability

The water vapor permeability (WVP) analysis was performed based on the methodologies described by ASTM E 96-00 (ASTM, 2001) and Iahnke, Costa, Rios and Flôres (2016). The films were mounted into aluminum permeation cells (height: 25 mm; inner diameter: 63 mm) containing granular anhydrous CaCl₂. The system was stored in a chamber (25 °C; 75% RH) and weighed at intervals 0, 1, 2 and 24 h for the gravimetric determination of WVP.

2.5. *Statistical analyses*

Analysis of variance (ANOVA) and Tukey's test of multiple comparisons were applied to evaluate the difference among the average values of the film properties. Data analysis was performed using Software Statistica 12.0 (Statsoft Inc., Tulsa, USA) at significance level 5%. When not mentioned, analyses were done in triplicate.

3. Results and Discussion

3.1. Modulated differential scanning calorimetry

Data obtained from the MDSC analysis are displayed in Table 1. The total heat flow was split into: reversible heat flow signal, which facilitates the perception of the “slow” phenomena, such as glass transition temperature (T_g); and non-reversible heat flow signal that shows the ‘kinetic’ phenomena, such as crystallization temperature (T_c), crystallization enthalpy (ΔH_c), melting temperature (T_m) and melting enthalpy (ΔH_m) (De Meuter, Rahier, & Van Mele, 1999). The degree of crystallinity (X_c) was obtained by relating ΔH_m from the protein rich phase with the denaturation enthalpy of native collagen (62.05 Jg^{-1}), according to Arvanitoyannis, Psomiadou, Nakayama, Aiba, & Yamamoto (1997). Despite the differences among results from CON, OH9.5, and OH19, each of them presented two T_c , T_m and T_g . Therefore, an MDSC thermogram of CON was used to illustrate this typical behavior observed for samples produced either by conventional and ohmic heating, as shown in Fig. 2.

Table 1. Glass transition temperature (T_g), melting temperature (T_m), melting enthalpy (ΔH_m), crystallization temperature (T_c), crystallization enthalpy (ΔH_c) and degree of crystallinity (X_c) of gelatin-residue based films produced by conventional (CON) and ohmic heating at 9.5 Vcm^{-1} (OH9.5) and 19 Vcm^{-1} (OH19).

Sample	Glycerol rich phase					Protein rich phase					
	T_{c1} (°C)	ΔH_{c1} (J/g)	T_{m1} (°C)	ΔH_{m1} (J/g)	T_{g1} (°C)	T_{c2} (°C)	ΔH_{c2} (J/g)	T_{m2} (°C)	ΔH_{m2} (J/g)	T_{g2} (°C)	X_c (%)
Control	-37 ^a	-0.55 ^a	-8 ^a	0.49 ^a	-65 ^{ab}	29 ^a	-4.6 ^b	45 ^b	19.8 ^b	-12 ^b	32 ^b
OH9.5	-36 ^a	-0.63 ^a	-7 ^a	0.45 ^a	-61 ^a	29 ^a	-6.6 ^c	54 ^a	23.9 ^a	-8 ^a	39 ^a
OH19	-37 ^a	-0.66 ^a	-10 ^a	0.47 ^a	-73 ^b	30 ^a	-2.3 ^a	47 ^b	19.4 ^b	-9 ^{ab}	31 ^b

Mean values \pm standard deviation (n = 3).

Different superscript letters in the same column indicate statistically significant differences (p < 0.05).

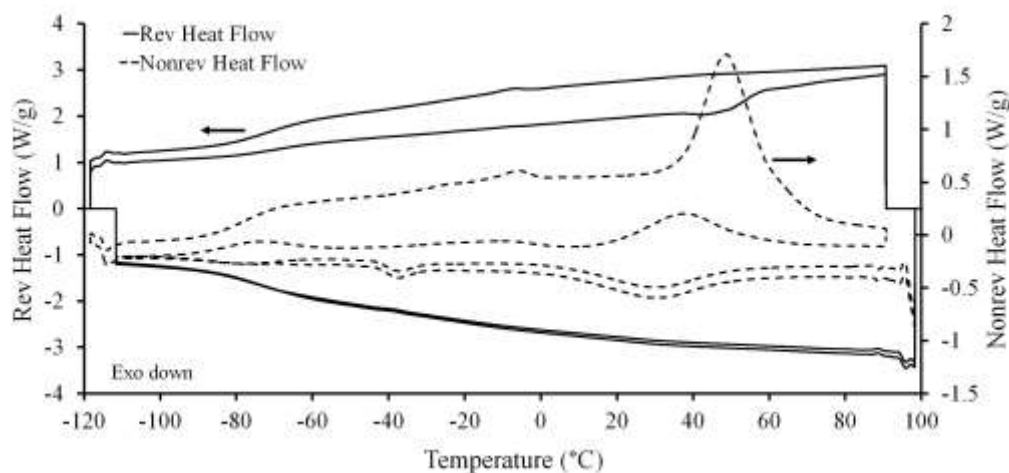


Figure 2. Typical MDSC thermogram of the control film (CON). Thermograms of films produced by ohmic heating have a similar pattern.

The T_{g1} was found at temperatures below -61 °C, associated to a glycerol/water rich phase, whereas T_{g2} was observed at temperatures from -12 °C (CON) to -8 °C (OH9.5), mostly related to the protein rich phase. This result suggested phase separation between the polymer matrix components, as also reported for pigskin gelatin-based films that presented two T_g 's at approximately -75 °C and 45 °C (Valencia, Lourenço, Bittante, & Sobral, 2016). Values found for T_{g2} are comparable to those found for cuttlefish gelatin films (-6.09 °C to 3.21 °C) (Hoque, Benjakul, & Prodpran, 2010) but lower than that of bovine hide (61 °C) and tilapia skin (52 °C) gelatin films, as reported by Ma et al. (2012) and Ahmad et al. (2015), respectively. The reduced T_{g2} values can be attributed to the great amount of glycerol present in GCR, which was able to localize between the protein chains of the protein rich phase, hindering their interaction and increasing the mobility of the molecules (Dangaran et al., 2009).

The disruption of ordered molecular structures was related to the endothermic (T_{m1} and T_{m2}) and exothermic (T_{c1} and T_{c2}) transition. All samples had similar T_{m1} and T_{c1} regarding the glycerol rich phase, which were associated to water melting/crystallization in the phase. T_{m2} was associated to the disruption of the protein chains and rearrangement of the triple helix from an ordered to a random configuration (Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006), whereas T_{c2} was related to a process that involves the reorganization of protein chains from the melt. Symmetry, intermolecular bonding, molecular weight, tacticity and branching are

factors that influence crystallinity and T_m . Higher melting point and greater rigidity are thus a reflex of increased crystallinity, which are accompanied by greater enthalpy values (Chandas, 2013).

The gradient voltage of 19 Vcm^{-1} resulted in films with comparable characteristics to CON regarding MDSC analysis. On the other hand, the higher T_{m2} , ΔH_{m2} , and X_c of OH9.5 indicated that a greater portion of the ordered structure was formed due to its enhanced crystallinity behavior, which also resulted in reduced mobility of the molecules. Owing to this fact, more thermal energy was required to initiate the movements of large segments of the gelatin chain, resulting in higher T_{g2} (Chandas, 2013). The transition from glassy to rubberlike state affects properties such as stiffness, hardness, elasticity and brittleness of materials (Chandas, 2013), which agrees with the mechanical results from OH9.5 that yielded improved tensile strength and Young's Modulus, whereas flexibility was reduced.

The effect of electric field on unfolding and aggregation of whey protein solution films was investigated by Pereira et al. (2010). An electric field of 10 Vcm^{-1} was applied to the film-forming solution, which was held at $85 \text{ }^\circ\text{C}$ for 30 min. In comparison to the conventional method, lower concentration and less aggregation of free sulphhydryls in the solutions was induced by ohmic heating. Additionally, protein conformational changes were promoted, which resulted in a film with increased content of β -sheet structures. Higher contents of β -sheet are associated with a more crystalline structure (Lefèvre, Subirade, & Pérolet, 2005), and this behavior may explain results from the present work. Souza et al. (2010) investigated the effect of ohmic heating on the properties of chitosan films and found analogous results, where electric treated films presented higher crystallinity and greater ordered structure in comparison to films prepared by conventional heating.

Another research compared the effects of conventional and ohmic heating on the denaturation of whey proteins in solution (Pereira et al., 2011). It was demonstrated that electric field had the potential to reduce the whey protein denaturation, which suggested that this treatment is less aggressive for protein's structure and may generate products with improved final quality. The authors associated the lower rates of protein denaturation to the unique characteristics of ohmic heating, such as the absence of hot surfaces and less overheating, which enables uniform heating with rapid rates without

inducing excessive denaturation or coagulation (Sastry, 2008). These statements are in accordance with results obtained in this work, which culminated with an OH9.5 film with higher T_{g2} , T_{m2} , ΔH_{m2} , and X_c values. It was believed that the non-thermal effects of the electricity were more intense at higher electric field strength (19 Vcm^{-1}), which promoted a similar response to that of conventional heating. This might be associated to higher aggregation and denaturation levels that led to higher irreversible denaturation rates (Rodrigues et al., 2015).

3.2. Thermogravimetric analysis (TGA)

Table 2 shows data obtained from the mass loss curve and its derivative. The maximum rate of mass loss (T_{dmax}) was taken from the peak temperature in the derivative of the mass loss curve. All films presented four mass loss stages, as observed in the thermograms of Fig. 3. The first stage of weight loss (Δ_1) was observed at the onset temperature ($T_{d1, onset}$) of 64-74 °C and T_{d1max} of 80-88 °C, mostly associated to the vaporization of free water. The $T_{d2, onset}$ ranged from 197 °C (CON) to 211 °C (OH9.5). Similar results were found by Hoque, Benjakul, & Prodpran (2011), who developed cuttlefish skin gelatin films ($T_{d2, onset}$: 196-216 °C) and reported that the second mass loss step (Δ_2) corresponded to the degradation of structurally bound water, glycerol and protein fractions of smaller size.

The third stage of weight loss (Δ_3) was mainly related to the loss of protein fractions of high molecular weight. For CON and OH19, T_{d3max} was around 310 °C, while $T_{d3, onset}$ was observed at approximately 281 °C, similarly to that found for gelatin films from cuttlefish skin ($T_{d3, onset}$: 271-290 °C) and tilapia skin (290 °C), as reported by Hoque et al. (2011) and Ahmad et al. (2015). The highest T_{d3max} (351 °C) and $T_{d3, onset}$ (343 °C) were found for OH9.5, which was accompanied by the lower weight loss (Δw_3 : 27.6%). A fourth mass loss stage was observed above 495 °C, and all samples presented less than 2% of residual mass (char content) at the end of the experiment. This fact was attributed to the complete decomposition and oxidation under air atmosphere of the molecules of the matrix components.

Overall, OH9.5 presented superior heat resistance due to its higher $T_{d, onset}$ and T_{dmax} , and lower Δw , especially regarding the degradation of higher interacted protein fractions. This suggested that more energy was required to degrade the film, which

might be associated to the higher degree of crystallinity, being in agreement with results from MDSC analysis and mechanical improvements. A few works have dealt with this research topic, and further research would help with the understanding of this effect.

Table 2. Thermal degradation temperatures ($T_{d,onset}$, °C), peak temperature in the derivative of the mass loss curve ($T_{d,max}$, °C), mass loss (Δw , %) and residual mass (%) of gelatin-residue based films produced by conventional (CON) and ohmic heating at 9.5 Vcm^{-1} (OH9.5) and 19 Vcm^{-1} (OH19).

		Sample		
		CON	OH9.5	OH19
Δ_1	$T_{d1,onset}$	64 ^b	74 ^a	66 ^b
	Δw_1	7.14 ^a	7.47 ^a	4.93 ^b
	$T_{d1,max}$	80 ^a	86 ^a	88 ^a
Δ_2	$T_{d2,onset}$	197 ^b	211 ^a	206 ^a
	Δw_2	36.7 ^a	36.6 ^a	36.9 ^a
	$T_{d2,max}$	238 ^a	230 ^a	232 ^a
Δ_3	$T_{d3,onset}$	280 ^b	343 ^a	281 ^b
	Δw_3	28.9 ^b	27.6 ^c	29.9 ^a
	$T_{d3,max}$	309 ^b	351 ^a	310 ^b
Δ_4	$T_{d4,onset}$	501 ^a	507 ^a	499 ^a
	Δw_4	26.3 ^b	27.4 ^a	26.3 ^b
	$T_{d4,max}$	531 ^b	584 ^a	510 ^c
Residue	2.6	0.96 ^b	0.93 ^b	1.97 ^a

Δ_x means the x^{th} mass loss step. Mean values \pm standard deviation ($n = 3$).

Different superscript letters in the same line indicate statistically significant differences ($p < 0.05$).

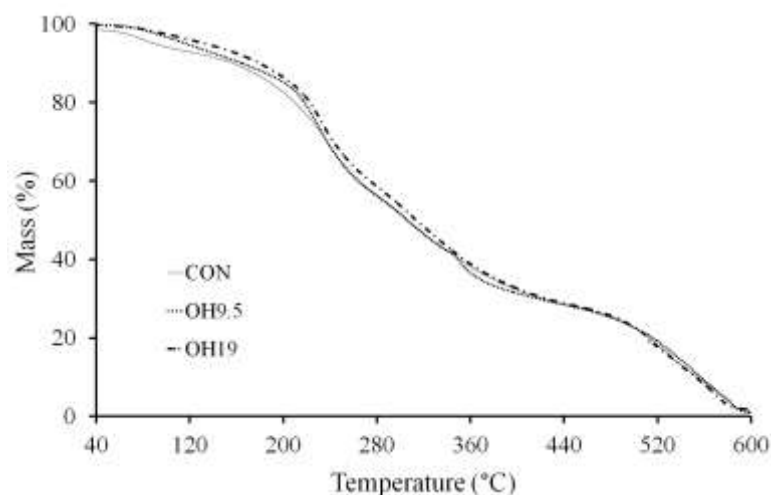


Figure 3. TGA thermograms of the gelatin capsule residue-based films produced by conventional (CON) and ohmic heating (OH9.5: 9.5 Vcm^{-1} ; OH19: 19 Vcm^{-1}).

3.3. Thickness and mechanical properties

The data from thickness (FT), tensile strength (TS), elongation at break (EB) and Young's Modulus (YM) of films produced by conventional and ohmic heating are shown in Table 3. Among CON, OH9.5, and OH19, no statistical difference in FT ($\sim 0.195 \text{ mm}$) was observed. The values of TS and EB for CON were similar to those reported by Iahnke et al. (2015), who also developed biodegradable films based on GCR produced by casting (TS: 2.57 MPa ; EB: 283%). Slight differences between both studies might be related to distinct parameters used, such as the proportion of GCR and the drying temperature.

Table 3. Film thickness (FT), tensile strength (TS), elongation at break (EB) and Young's Modulus (YM) of gelatin-residue based films produced by conventional (CON) and ohmic heating using 9.5 Vcm^{-1} (OH9.5) and 19 Vcm^{-1} (OH19).

Film	FT (mm)	TS (MPa)	EB (%)	YM (MPa)
CON	0.194 ± 0.004^a	2.66 ± 0.36^c	235 ± 12^a	4.07 ± 0.21^c
OH9.5	0.194 ± 0.003^a	6.62 ± 0.26^a	197 ± 9^b	10.29 ± 1.10^a
OH19	0.198 ± 0.008^a	3.26 ± 0.08^b	220 ± 7^a	7.89 ± 0.90^b

Different superscript letters in the same column indicate statistically significant differences ($p < 0.05$).

Ohmic heated films had their TS and YM significantly increased when compared to CON, which suggested a benefic influence triggered by the electric field. The most pronounced effect was observed for OH9.5, which presented the higher TS (6.62 MPa) and

YM (10.29 MPa) values. Amongst all films, CON exhibited the greatest EB, followed by OH19 and OH9.5. Higher TS values were accompanied by higher YM and lower EB values, which is an expected phenomenon as related by Mu, Guo, Li, Lin, and Li (2012).

The mechanical improvements observed for OH9.5 and OH19 might be due to potential disturbances on tertiary protein structures probably caused by the alternating movement of electrical charges, which interfere in interactions between molecules of protein that include hydrogen bonds, ionic bonds, hydrophobic interactions and electrostatic interactions (Rodrigues et al., 2015). As reported by Havea, Carr, and Creamer (2004), the heat-induced gelation of whey protein concentrate solutions was dominated by these non-covalent interactions, which appeared to maintain the rigid characteristic of protein structure. Furthermore, the application of 9.5 Vcm^{-1} influenced the crystallinity degree and produced films with a more ordered structure, as discussed along with MDSC results. Bigi et al. (2004) studied the influence of triple helix content on the mechanical properties of gelatin-based films and reported that a higher degree of crystallinity was usually related to the improvement of mechanical properties.

When studying the effect of the electric field (100 Vcm^{-1} and 200 Vcm^{-1}) on thermal and mechanical properties of chitosan films, Souza et al. (2010) have not found significant differences for thickness values between control and treated samples, whereas TS and EB values were increased by 9% and 18%, respectively. This influence was associated to the higher crystallinity degree of films produced by the application of electric field. Pereira et al. (2010) reported that whey protein isolated films produced by the application of electric field ($\sim 10 \text{ Vcm}^{-1}$) presented lower FT and EB values in comparison to conventional heating, whereas TS was not affected. A few works have investigated the effect of ohmic heating on the mechanical properties of films, and the exact pattern of influence is still uncertain.

3.4. Film surface characteristics

The SEM micrographs of CON, OH9.5 and OH19 are illustrated in Fig. 4. As can be observed, the surface morphology of all produced films was similar, which indicates that the application of electric field during heating did not cause any apparent changes as accessed by this analysis. In general, films had a homogeneous and smooth surface, without presence of cracks or holes. All samples presented “dots” homogeneously distributed. This can be associated to the high concentration of glycerol in GCR, which resulted in a network structure

where the excess of glycerol molecules was distributed as droplets, or to the remaining presence of air bubbles.

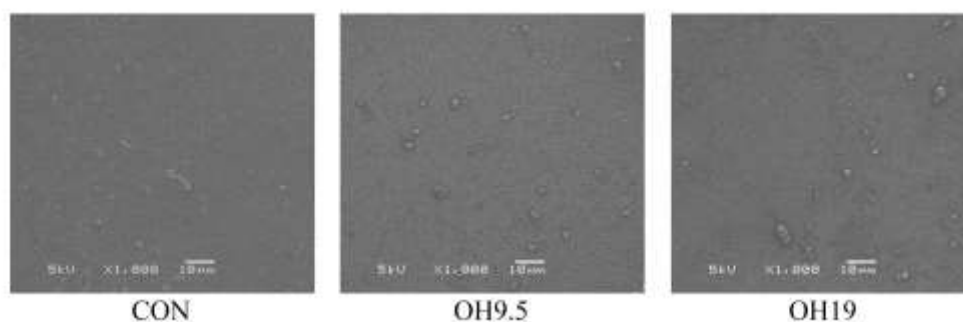


Figure 4. SEM micrographs of gelatin capsule residue-based films produced by conventional (CON) and ohmic heating (OH9.5: 9.5 Vcm^{-1} ; OH19: 19 Vcm^{-1}).

3.5. Moisture content, total soluble matter and water vapor permeability

Results for MC, WS, and WVP are shown in Table 4. The MC of films remained in the range of 20.9%, which is similar to that found by Andreuccetti, Carvalho, Galicia-García, Martínez-Bustos, & Grosso (2011) for pig hide gelatin film (23%). The WS was over 95% for CON, OH9.5, and OH19, with no significant differences between values. Regardless of the heating treatment, all the film samples disintegrated almost completely after 24 h, and this result is comparable to those found for bovine (Martucci, Accareddu, & Ruseckaite, 2012), giant squid (Giménez, Gómez-Estaca, Alemán, Gómez-Guillén, & Montero, 2009) and cod (Carvalho et al., 2008). Values for WVP for GCR-based films prepared by conventional and ohmic heating at both studied voltages showed no statistical difference and were around $1.04 \text{ g mm h}^{-1} \text{ m}^{-2} \text{ kPa}^{-1}$. Avena-Bustillos et al. (2006) found similar results for cold ($0.932 \text{ g mm h}^{-1} \text{ m}^{-2} \text{ kPa}^{-1}$) and warm ($1.309 \text{ g mm h}^{-1} \text{ m}^{-2} \text{ kPa}^{-1}$) water fish gelatin films.

The application of the electric field did not affect the water related properties of the GCR-based films, despite the observed influence on the crystalline content. In general, it would be expected that increasing crystallinity should reflect in a decrease of the barrier permeability due to the potential of crystallites to act as impermeable inert fillers (Duan & Thomas, 2014). However, the lack of correlation among crystallinity, water diffusivity and water solubility in biodegradable polymers might take place when the water–cluster model is followed, rather than the solution–diffusion model, during the permeation of water molecules in the polymer matrix (Siparsky, Voorhees, Dorgan, & Schilling 1997; Yoon, Jung, Jim, & Park, 2000).

Table 4. Moisture content (MC), water solubility (WS) and water vapor permeability (WVP) of gelatin-residue based films produced by conventional (CON) and ohmic heating at 9.5 Vcm^{-1} (OH9.5) and 19 Vcm^{-1} (OH19).

Sample	MC (%)	WS (%)	WVP ($\text{g mm h}^{-1} \text{m}^{-2} \text{kPa}^{-1}$)
CON	21.0 ± 0.5^a	95.9 ± 0.2^a	1.04 ± 0.10^a
OH9.5	21.5 ± 1.2^a	96.2 ± 1.6^a	1.03 ± 0.16^a
OH19	20.1 ± 0.8^a	96.8 ± 2.6^a	1.05 ± 0.10^a

Mean values \pm standard deviation (n = 3).

Different superscript letters in the same column indicate statistically significant differences ($p < 0.05$).

Chitosan films produced by ohmic heating had their water solubility and water vapor permeability significantly reduced when compared to films produced by the conventional method (Souza et al., 2009). This effect was observed for the application of 100, 150 and 200 Vcm^{-1} , which caused a decrease in the same magnitude, whereas the field strength of 50 Vcm^{-1} had no influence on these properties. This effect was associated with the possible enhancement in the crystallinity of the material, which caused higher insolubility and improved the WVP. The film surface roughness was another factor that seemed to have a positive correlation with the reduction of WVP values.

Another study found that the application of electric field (10 Vcm^{-1}) had no significant effect on moisture content, water solubility and water vapor permeance of whey protein films, which were around 18.3%, 57.5% and $3.35 \text{ g h}^{-1} \text{m}^{-2} \text{kPa}^{-1}$, respectively (Pereira et al., 2010). As electric treated films were significantly thinner, the water vapor permeability was recalculated as the product of permeance and thickness, which yielded higher WVP values ($0.663 \text{ g mm h}^{-1} \text{m}^{-2} \text{kPa}^{-1}$) for conventional films in comparison to those electric treated ($0.602 \text{ g mm h}^{-1} \text{m}^{-2} \text{kPa}^{-1}$). It was stated that different factors may influence the values of WVP, which made it difficult to compare the permeability data. Additionally, the measurement of the contact angle showed that films produced by ohmic heating presented a more hydrophilic surface, thus higher availability of hydrophilic groups. This was attributed to the reorientation of hydrophobic clusters in the protein structure caused by the electric field, which might help to explain results from the present study.

Results suggested that MC, WS, and WVP of the GCR-based films produced by conventional and ohmic heating were mostly governed by the hydrophilic character of the

components of the film formulation, which was used to produce all films. The proportion of hydrophilic/hydrophobic components of films has a great effect on the water vapor permeability since the permeation of water usually takes place through its hydrophilic part (Souza, Cerqueira, Teixeira, & Vicente, 2010). Therefore, most edible films and coatings tend to exhibit a hydrophilic character and high WVP due to their high degree of polar parts that facilitate water molecule sorption (Gontard, Duchez, Cuq, & Guilbert, 1994). In addition to the gelatin and water influence, glycerol, which was present in a considerable amount, also contributes to increase the WVP and the susceptibility of the matrix to environmental humidity due to its hydrophilic character (Andreuccetti, Carvalho, & Grosso, 2009).

4. Conclusions

Gelatin capsule residue was used to produce edible films. This material is treated as waste by nutraceutical industries and its reuse contributes to reduce the amount of solid waste and to the development of a sustainable technology. Films were successfully produced by ohmic heating, and results demonstrated that the application of electric field on the film-forming solutions had significant impact on the film properties. In general, the most pronounced effect was observed for treatment made at 9.5 Vcm^{-1} , while the application of 19 Vcm^{-1} and the conventional method resulted in films with comparable characteristics.

The film electrically treated with 9.5 Vcm^{-1} had its tensile strength and Young's Modulus increased up to 6.62 MPa and 10.29 MPa, respectively, whereas elongation at break was reduced to 197 % in comparison to non-treated film (TS: 2.66 MPa; YM: 4.07 MPa; EB: 235%). It was observed a higher crystallinity for OH9.5, as analyzed by modulated differential scanning calorimetry, and improved thermal stability. The electric field did not affect thickness, morphology and water-related properties, and results were analogous to film produced by the conventional heating method. The reported results showed that ohmic heating may thus represent a novel method for the development of gelatin-based films with tailored properties. A few works have dealt with this topic, and, therefore, future studies are needed towards a clearer understanding of these effects.

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ARTIGO 4: Electrospun gelatin capsule residue nanofibers: feasibility study
(Short Communication)

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Electrospun Gelatin capsule residue nanofibers: feasibility study

Abstract

Gelatin nanofibrous structures have been investigated for biomedical and food applications. The present work evaluated the potential use of gelatin capsule residues (GCR) in different concentrations (13%, 20%, 25% and 30%) as polymeric matrix to produce a gelatin-based nanofibrous material. In addition, combinations of solvents that include citric acid (CA), acetic acid (AA), formic acid (FA) and water (WA) were tested. Results indicated that the lower and greater concentration of gelatin were not capable of producing nanofibers, regardless the solvent. In general, the best results were obtained for the 25% gelatin nanofiber solution in AA/CA, with the application of 17.5 kV, distance from collector to nozzle of 17.5 cm and solution flow rate of 1 mL h⁻¹.

Keywords: electrospinning; nanofiber; waste; gelatin.

1. Introduction

The electrospinning technique is performed by the application of an electric field between a collector plate, which acts as the counter electrode, and a polymer solution that is pumped through a small capillary orifice. As the solution jet travels, it is bent by the electric forces while the solvent evaporates. This process leads to the formation of the fibers, which are deposited in the collector. The solution parameters, processing parameters, and ambient parameters play an important role in the final properties of the nanofibers (De Vrieze et al., 2007; Kriegel et al., 2008).

Electrospun polymer nanofibers present unique properties such as minimal diameters, high porosity, small pore size, and high specific surface. Due to these characteristics, they can be used in a broad range of applications in areas that include: scaffolds for tissue engineering, medical applications, filter media, energy, biotechnology, sensors, defense and security, and healthcare (Agarwal, Greiner, & Wendorff, 2013; Ahmed, Lalia, & Hashaikeh; 2015). Lately, there has been an increasing interest in the investigation of tissue engineering scaffolds or medical devices using biopolymers that include proteins and carbohydrates from animal and plant origin (Sridhar et al., 2015). In addition, the potential application of nanofibers in the food science field is an under-explored area, where the electrospinning of food-grade polymers is needed (Tavassoli-Kafrani, Goli, & Fathi, 2017).

Amongst biopolymers, gelatin presents an interesting amino acid composition that includes glycine, proline and hydroxyproline groups, which promote cell adhesion and growth, making it suitable for the human body (Yao et al., 2017). As reported by Flôres et al. (2017) and Iahnke et al. (2016, 2019), one possible source of bovine gelatin to develop biodegradable materials is the scrap generated by the processing of nutraceutical softgel capsules. This residue is interestingly composed of protein, water and glycerol, but it is not reused by the industries and implies waste treatment and disposal.

Gelatin is biocompatible, biodegradable, and readily available, being one of the most used FDA approved biopolymer. It has been electrospun for applications that include tissue engineering, bioactive encapsulation, and active packaging (Deng et al., 2018). Since gelatin cannot be electrospun from aqueous solutions, solvents that dissolve gelatin at room temperature and present relative high volatility are usually chosen, and include mainly toxic and aggressive solvents such as 2,2,2-trifluoroethanol, dimethyl sulfoxide, 1,1,1,3,3,3-hexafluoro-2-propanol and formic acid (Bhardwaj & Kundu, 2010; Leidy & Ximena, 2019). However, most of these solvents are classified as class 2 and/or 3 by the FDA and present a

limited daily exposure (0.5–38.8 mg/day), whereas organic solvents such as formic acid, acetic acid, and ethanol are considered as less toxic and of lower risk to human health (Food and drug administration, 2003; Leidy & Ximena, 2019). This communication deals with our research in evaluating the potential in creating nanofibers based on gelatin capsule residues produced by electrospinning by using a combination of less toxic and explored solvents, including acetic acid, formic acid, citric acid and water.

2. Materials and methods

2.1. Materials

The source of gelatin was a residue originated from the processing of soft gel capsules: gelatin capsule residue (GCR) (Figure 1). This material is composed of gelatin from bovine source (40.6%), water (35.0%) and glycerol (24.4%). The plasticizer was naturally present in the raw material. Acetic acid (99.8 v%), formic acid and citric acid were acquired from Sigma-Aldrich (St. Louis, USA). All other used reagents were of analytical grade.



Figure 1. Gelatin capsule residue.

2.2 Preparation of solutions

The solvent systems named acetic acid/citric acid (AA/CA), acetic acid/formic acid (AA/FA) and acetic acid/water (AA/WA) were chosen to be tested for electrospinning. The citric acid solution was prepared from powder to the concentration of 1 wt%. The electrospinning solutions were prepared by melting the GCR in 50/50 AA/CA, AA/FA or AA/WA for 5 min, using a hotplate with a magnetic stirrer (IKA-Works Inc, Ret Basic C, USA). The system was homogenized at room temperature ($21\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$) for 30 min. Gelatin concentrations of 13%, 20%, 25% and 30% were tested.

Previous tests showed that GCR is electrospinnable at 25 wt% for AA/CA solutions with 50/50 ratio. These parameters allowed the dissolution of GCR at room temperature and guaranteed a stable process. The GCR electrospinning solutions were characterized prior to use by their conductivity and viscosity. Conductivity was measured by conductivity meter (WTW, inoLAB Cond 730, Germany) and viscosity was determined after 0, 4 h, 24 h and 48 h using a rheometer (Bohlin, CS50).

2.3 Electrospinning

It was initially developed an electrospinning setup, as demonstrated in Figure 2. It consisted of an infusion pump connected to a syringe (Sigma-Aldrich) equipped with a blunt-ended stainless steel capillary; a high voltage power supply, which is connected to the metallic capillary needle where the polymer solution is pumped from; a grounded target collector that allows for an electric field to be established between the capillary tip and the target. The preliminary tests were performed by applying voltage from 10-20 kV, distance from nozzle to collector varying from 10-20 cm, and solution flow rate ranging from 1 to 2 ml h⁻¹. Experiments were performed at ambient conditions (21 °C ± 3 °C; 65 ± 3 RH).

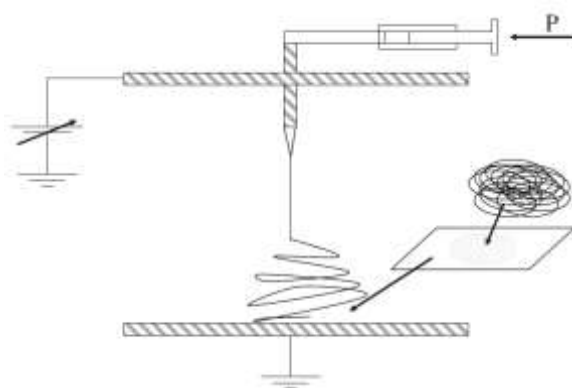


Figure 2. Schematic illustration of the electrospinning setup (De Vrieze et al., 2007).

2.4 Characterization of the nanofibers

A scanning electron microscope (SEM) (Phenom-World, Phenom Pro X, Netherlands) was used to observe the microstructure of films, which were mounted onto aluminum pin type stubs with double-sided adhesive. An accelerating voltage of 15.0 kV and magnification of 2500x were employed.

3. Results and Discussion

Results from the present study are related to a preliminary study towards electrospinnability of GCR solutions. Electrospinnable gelatin nanofibers have been obtained by using acetic acid and/or formic acid as solvents (Deng et al., 2018; Okutan, Terzi, & Altay, 2014; Steyaert et al., 2016). Therefore, a range of solutions was prepared to be used in the electrospinning equipment, as shown in Table 1. Acetic acid was chosen to be present in all solutions following the successful data found in literature, along with formic acid. Citric acid was selected as a food grade option to be evaluated for the first time as solvent to gelatin electrospinning. As reported by Leidy and Ximena (2019), the normal concentration of biopolymer used to produce nanofiber ranges from 10% to 40% w/w, and was chosen the range of 13-30% for the present study. The obtained solutions presented a conductivity in the range of 1600 $\mu\text{S cm}^{-1}$. Viscosity values remained stable up to 24 h and were in the range of 0.082 Pa.s.

Table 1. Composition of the GCR-solutions analyzed for electrospinnability.

Solution Number	Solvent (50/50)	Gelatin concentration (wt%)	Electro-spinnability
1	AA/WA	13	Negative
2	AAWA	20	Negative
3	AA/WA	25	Negative
4	AA/WA	30	Negative
5	AA/FA	13	Negative
6	AA/FA	20	Negative
7	AA/FA	25	Negative
8	AA/FA	30	Negative
9	AA/CA	13	Negative
10	AA/CA	20	Positive
11	AA/CA	25	Positive
12	AA/CA	30	Negative

AA: acetic acid; WA: water; FA: formic acid; CA: citric acid.

Visual observation during the experiments and scanning electron microscopy (SEM) of the resulting material deposited at the collector surface were used to analyze results. The success of electrospinnability was considered when the following requirements were fulfilled, according to De Vrieze et al. 2007:

- Visual observation: formation of a Taylor cone at the tip of the nozzle, formation of a stable jet, and uniform distribution of the deposited fibers at the macroscale.
- SEM: formation of fibers with diameters less than 500 nm.

The process of nanofiber formation is influenced by many parameters, such as concentration, viscosity, electrical conductivity, and surface tension of the solution; distance to the collector plate, operating voltage, solution flow rate; humidity and temperature of the ambient and so on (De Vrieze et al., 2009; Kriegel et al., 2008). As observed in Table 1, solutions 2, 3, 6, and 7 were not successful in producing the GCR-nanofiber. A Taylor cone and stable jet were initially detected for these solutions, but as time passed by these criteria were lost. These phenomena resulted in the deposition of a sort of fiber like structure surrounded by droplets or non-defined beaded fibers, which might have been caused by inhomogeneous charge distributions over the jet (De Vrieze et al., 2007), as illustrated in Figure 3.

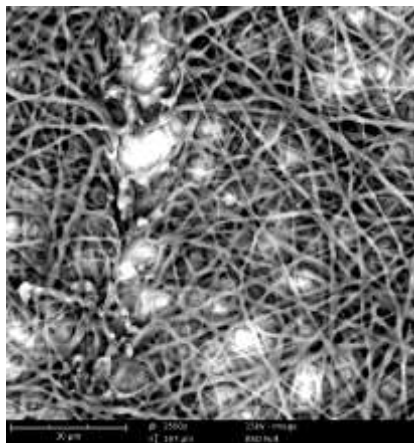


Figure 3. SEM image from a 25% gelatin in 50/50 AA/WA solution at 12.5 cm distance from nozzle to collector, solution flow rate of 1 mL h^{-1} and applied voltage of 12.5 kV.

Solutions 1, 5 and 9 containing 13% gelatin, and 4, 8 and 12 containing 30% gelatin were not electrospinnable, due to the formation of liquid droplets on the collector or the obstruction of the needle. According to Bhardwaj & Kundu (2010), the viscosity, which is dependent on the polymer concentration and solvent type, has great influence on the fiber formation. At lower values, the polymer chain entanglement is generally lower, favoring the

break up of the polymer jets and formation of droplets instead of fibers. At higher values, the viscosity may prevent the solution flow and ejection from the capillary needle (Bhardwaj & Kundu, 2010).

Successful results were obtained for solutions 10 and 11, which were produced by using the AA/CA combination with 20% or 25% gelatin. These samples yielded a well-defined Taylor cone, a stable jet and a uniform white material deposited at the top of the collector. From these results, important observations were taken into consideration to obtain the optimal parameters to produce GCR-nanofibers, which include: the absence of bead formation, uniformity of the deposited material, diameter of the obtained fibers, and distribution of the fiber thickness.

Analysis of the SEM images demonstrated that the optimal nanofibers were obtained for 25% AA/CA gelatin solutions produced by the application of the following parameters: 17.5 kV, 17 cm and 1 ml h⁻¹, as shown in Figure 4B. As can be seen from Figure 4, these parameters yielded nanofibers without the presence of beads, with lower fiber diameter and better distribution of fiber thickness. However, all the samples presented a combination of smooth, which fused at touching points, which might be associated with the incomplete evaporation of solvents (Choktaweasap et al., 2007). Since gelatin is hydrophilic, future experiments could be conducted under controlled temperature and humidity in order to prevent influences from the environment.

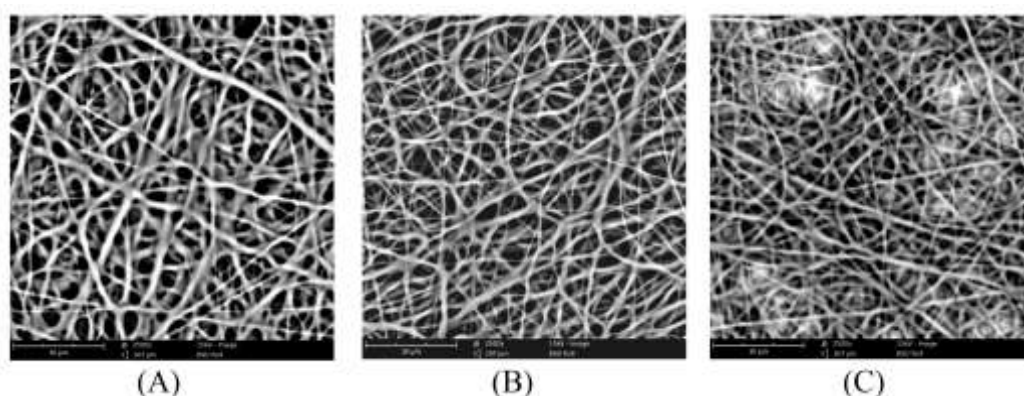


Figure 4. SEM image from a 25% gelatin in 50/50 AA/AC solution at 17.5 cm distance from nozzle to collector and solution flow rate of 1 mL h⁻¹. Applied voltage is: (A) 15 kV, (B) 17.5 kV and (C) 20 kV.

4. Conclusions

In this work, the potential of producing nanofibers from gelatin capsule residue dissolved in different solvent combinations was evaluated. The gelatin concentrations of 13%

and 30% did not produce nanofibers, regardless of the solvent. The same phenomenon was observed for the combination of acetic acid/water and acetic acid/formic acid as solvent, where no uniform and smooth fibers were obtained. The optimal conditions were found for 25% gelatin in acetic acid/citric acid solvent, by applying 17.5 kV, using a distance of 17.5 cm and flow rate of 1 mL h⁻¹. Further studies are necessary to understanding how properties are affected by the parameters on the electrospinning process of GCR-nanofibers and to tailor properties for specific applications.

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CAPÍTULO 4 - DISCUSSÃO GERAL

Devido a sua versatilidade e capacidade de inovação, materiais plásticos apresentam soluções para as necessidades de diversos produtos, aplicações e setores. Em 2017, a produção anual de plásticos alcançou a marca de 348 milhões de toneladas, tendo sua maior demanda no setor de embalagens, seguido pelo de construção, automotivo, elétrico e eletrônico, lazer e esportes, agricultura e outros (equipamentos médicos, móveis de plástico e etc.). Apesar de suas vantagens, a maior parte dos plásticos não é biodegradável, sendo de origem fóssil e produzida para utilização em um curto período de tempo, o que pode ocasionar problemas de poluição devido a sua persistência no meio-ambiente. Como consequência, pode ser observado um crescente interesse por parte de pesquisadores e da indústria no desenvolvimento de materiais biodegradáveis para substituir e/ou reduzir o uso de plásticos convencionais à base de petróleo em setores que incluem embalagens alimentícias, agricultura e aplicações médicas.

Assim, o presente trabalho desenvolveu filmes biodegradáveis e nanofibras a partir de resíduos provenientes do processamento de cápsulas nutracêuticas de gelatina (RGL). Foram investigadas aplicações de diferentes estratégias a fim de possibilitar a melhoria das propriedades dos filmes, tais como reticulação química, reforço com nanoargila e produção por aquecimento ôhmico. A partir das pesquisas efetuadas e resultados obtidos, foram produzidos quatro artigos científicos:

1. Gelatin residue-based films crosslinked with the natural agent genipin
2. Gelatin capsule residue/modified montmorillonite-based films: characterization, biodegradation and effect on soil quality
3. Effect of moderate electric field on the properties of gelatin capsule residue-based films
4. Electrospun gelatin capsule residue nanofibers: feasibility study

O primeiro artigo teve como objetivo desenvolver e caracterizar filmes biodegradáveis a base de RGL (70 g/100 g água) reticulados com o agente natural genipina RGL-GEN (0,15%), extraída do jenipapo (*Genipa americana* L.). Para fins de comparação, os filmes também foram reticulados com glutaraldeído RGL-GLU. Além disso, foi avaliado o potencial dos filmes em manter os atributos de qualidade de azeite de oliva extra virgem armazenado sob condições aceleradas de exposição à luz e

temperatura. Os resultados demonstram que filmes de coloração azul foram obtidos após a reticulação com genipina. Em comparação com os filmes controle (não reticulados), a reticulação com genipina ocasionou melhoria nas propriedades dos filmes, com aumento na resistência mecânica de 2,72 MPa pra 4,23 MPa. As propriedades relacionadas à água também foram favorecidas, tendo a permeabilidade ao vapor de água valor reduzido de 1,02 pra 0,88 g mm h⁻¹ m² kPa, e solubilidade em água de 62% para 36%. Além disso, é importante destacar que os filmes de RGL-GEN tiveram propriedades similares aos RGL-GLU, o que enfatiza seu potencial em substituir esse agente que pode apresentar toxicidade. Quanto ao estudo do filme como embalagem, foi constatado que eles mantiveram os atributos de qualidade do azeite (índice de peróxidos, dienos e trienos conjugados, e cor) durante o período de 14 dias do experimento. Os resultados obtidos foram equiparados ao azeite embalado em filme plástico multicamada não biodegradável. Tais resultados enfatizaram o potencial de aplicação dos filmes RGL-GEN como embalagem alimentícia para azeites.

O segundo artigo avaliou o efeito da incorporação de diferentes concentrações (0, 0,1, 0,5, 2, 6, e 10 g/100 g gelatina) de montmorilonita (MMT) como agente reforçador nas propriedades dos filmes a base de RGL (60 g/100 g água). Também foi analisada a biodegradação dos filmes e o efeito na qualidade do solo para os filmes selecionados (0 e 2% MMT). Foi constatado que o aumento na concentração de MMT resultou no aumento da espessura, que variou entre 0,189 mm e 0,225 mm, e da transmitância de luz, enquanto a solubilidade em água foi reduzida de 62,3% (0% MMT) para 48,6% (10% MMT). A análise dinâmico-mecânica permitiu verificar que o filme contendo 2% MMT apresentou o maior módulo de armazenamento, seguido pelos filmes contendo 6, 10, 0,5, 0,1, e 0%. A análise de raios-X sugeriu que os filmes com 0,1 e 0,5% MMT tiveram estrutura esfoliada, o com 2% MMT uma estrutura intercalada/esfoliada, e os filmes com 6 e 10% MMT demonstraram estrutura intercalada. Os filmes selecionados apresentaram biodegradabilidade em solo orgânico em 10 dias. O carbono da biomassa microbiana e a respiração basal do substrato foram positivamente relacionados, enquanto o quociente metabólico do substrato não apresentou alterações significativas.

O terceiro artigo relacionado ao emprego de estratégias para o desenvolvimento de filmes a base de RGL utilizou o aquecimento ôhmico em substituição ao aquecimento convencional por banho-maria ou placa aquecedora. Nesse processo, foi

estudado o efeito da aplicação de campo elétrico moderado ($9,5 \text{ Vcm}^{-1}$ e 19 Vcm^{-1}) nas propriedades dos filmes. Os resultados demonstraram que as propriedades mecânicas foram significativamente afetadas, onde o Módulo de Young foi aumentado, enquanto a alongação foi reduzida. A aplicação de $9,5 \text{ Vcm}^{-1}$ resultou no aumento da resistência à tração de 2,66 MPa para 6,62 MPa, enquanto para o tratamento com 19 Vcm^{-1} houve um aumento para 3,26 MPa. As propriedades térmicas também foram afetadas, tendo sua cristalinidade e estabilidade térmica aumentadas. As alterações e melhorias nas propriedades dos filmes produzidos por aquecimento ôhmico foram observadas principalmente com a aplicação do menor campo elétrico.

Para uma melhor compreensão dos efeitos das diferentes estratégias empregadas, a Tabela 4 ilustra os resultados obtidos com a caracterização dos filmes a base de resíduo de gelatina: controle RGL (sem tratamento), reticulado com genipina (RGL-GEN), reforçado com 2% MMT (RGL-MMT), e produzido com aplicação de $9,5 \text{ Vcm}^{-1}$ (RGL-AO).

De um modo geral, foi possível observar que o tratamento reticulante com genipina ocasionou as mais significativas alterações nas propriedades relacionadas à água dos filmes. Em relação ao filme controle, a solubilidade em água e permeabilidade ao vapor foram reduzidas, o que pode ser explicado pela formação de reticulação intermolecular e de uma rede tridimensional mais compacta. Tal resultado é interessante do ponto de vista em que a aplicação em alimentos com médio e alto teor de umidade requerem embalagens com boa permeabilidade ao vapor de água e que não percam sua estrutura quando em contato com a água presente no alimento. Logo, tais resultados são uma base para futuros estudos a fim de investigar estratégias que possibilitem expandir as possibilidades de aplicação dos filmes. Além disso, o tratamento reticulante ocasionou a mudança visual mais pronunciada dentre todos os tratamentos, alterando a coloração dos filmes de amarelada e transparente para azul e opaca.

Tabela 4 - Caracterização das propriedades dos filmes biodegradáveis selecionados.

Propriedade do filme	RGL	RGL-GEN	RGL-MMT	RGL-AO
Espessura (mm)	0,168-0,194	0,168	0,215	0,194
Resistência à tração (MPa)	2,69	4,23	N.A.	6,62
Elongação na ruptura (%)	177-235	244	N.A.	197
Módulo de Young (MPa)	4,07	N.A.	N.A.	10,29
Módulo de armazenamento (MPa)	4,5	N.A.	74	N.A.
Umidade (%)	21,6	23,9	21,5	21,5
Solubilidade (%)	62,3-95,9	36,3	56,1	96,2
PVA*	1,03	0,88	N.A.	1,03
Diferença de cor	11,26	75,4	12,05	N.A.
Transmitância 280 nm (%)	0	0	0	N.A.
Transmitância 600 nm (%)	73	3,2	57	70
Transparência (A/mm)	0,7	8,4	0,99	N.A.
Estrutura MEV	Homogênea	Homogênea	Homogênea	Homogênea
Biodegradabilidade	10 dias	N.A.	10 dias	N.A.
Proteção de azeite de oliva	N.A.	SIM	N.A.	N.A.

N.A. = não aplicável. *PVA: permeabilidade ao vapor de água; g mm/ h m² kPa.

Filmes: Controle RGL (sem tratamento), Reticulado com Genipina (RGL-GEN), Reforçado com 2% MMT (RGL-MMT), e produzido com aplicação de 9.5 Vcm⁻¹ (RGL-AO).

O efeito da reticulação também ocasionou aumento da resistência à tração dos filmes, porém em menor proporção quando comparado ao filme produzido por aquecimento ôhmico. A aplicação de campo elétrico originou os filmes com melhorias mais acentuadas nas propriedades mecânicas avaliadas pelo texturômetro, tendo sua resistência à tração aumentada de 2,69 para 6,62 MPa. Tal efeito pode ser associado a perturbações potenciais nas estruturas de proteínas terciárias provavelmente causadas pelo movimento alternado de cargas elétricas, que interferem nas interações entre moléculas de proteína que incluem ligações de hidrogênio, ligações iônicas, interações hidrofóbicas e interações eletrostáticas. Já as propriedades relacionadas à água dos filmes RGL-AO não sofreram alteração com o processamento.

Quanto aos filmes incorporados de MMT, foram os únicos que tiveram sua estrutura do ponto de vista da análise de raios-x modificada, visto que passaram a

apresentar estruturas esfoliadas ou intercaladas devido à presença da nanoargila. Esses filmes não foram avaliados através de texturômetro, mas sim pelo analisador dinâmico mecânico, que possibilita ter uma noção do comportamento mecânico dos filmes. Em comparação ao filme controle sem MMT, o filme com 2% MMT teve seu módulo de armazenamento aumentado de 4,5 MPa para 74 MPa, evidenciando o efeito reforçador da MMT. O filme também foi investigado quanto a sua biodegradabilidade e foi demonstrada uma rápida taxa de biodegradabilidade evidenciada pela perda de peso e completa desintegração dos filmes.

Quanto ao quarto e último artigo produzido referente aos resultados obtidos com a presente tese de doutorado, foi realizado um estudo que possibilita seguir adiante com a pesquisa relacionada à obtenção de nanofibras a partir do resíduo de gelatina (RGL). O estudo foi realizado em escala laboratorial e em condições ambientais, onde inúmeros testes foram realizados até o alcance das condições mais ideais para a obtenção de nanofibras, conforme comprovado em análises de microscopia eletrônica de varredura. É possível constatar que estudos futuros serão mais promissores caso empreguem condições controladas de processamento, a fim de que a umidade do ar ou temperatura não influenciem os resultados obtidos, além da produção em larga escala que envolva equipamentos multi-agulhas. Além disso, o estudo possibilitou o emprego de solventes de grau alimentício, utilizando a combinação de ácido acético e ácido cítrico pela primeira vez, em detrimento de solventes mais tóxicos e poluentes. As aplicações de nanofibras de gelatina incluem além do campo da biomedicina, o setor alimentício, principalmente nas áreas de encapsulamento de compostos, embalagens e coberturas comestíveis.

CAPÍTULO 5 - CONCLUSÃO FINAL

Os resultados obtidos no presente trabalho evidenciaram que resíduos gelatinosos originados da produção de cápsulas nutracêuticas podem ser empregados na produção de filmes biodegradáveis e nanofibras através das técnicas de *casting* e *electrospinning*, respectivamente. A eficácia da utilização de estratégias para a melhoria das propriedades dos filmes biodegradáveis foi comprovada através da utilização de tratamentos de reticulação com genipina, incorporação de montmorilonita e aquecimento ôhmico. Tais técnicas demonstraram capacidade em melhorar significativamente as propriedades mecânicas e físico-químicas dos filmes, principalmente no que refere à resistência à tração, solubilidade em água e permeabilidade ao vapor de água. Quanto às nanofibras, foi possível realizar a produção em escala laboratorial e empregar solventes de grau alimentício como ácido acético e ácido cítrico.

Os filmes reticulados com genipina demonstraram seu potencial de aplicação através de resultados que comprovaram sua eficácia em manter a qualidade de azeite de oliva durante armazenamento em condições aceleradas. Os filmes adicionados de montmorilonita apresentaram rápida biodegradabilidade em um experimento de 10 dias, bem como tiveram o efeito da biodegradação no solo avaliado. A utilização de aquecimento ôhmico com aplicação de campo elétrico moderado demonstrou ser uma alternativa de processo ao aquecimento convencional. O estudo das nanofibras evidenciou a possibilidade de produzir tais materiais a partir dos resíduos e utilizar solventes de grau alimentício. Deste modo, os filmes a base de resíduo de cápsula de gelatina demonstraram potencial de aplicação em áreas como embalagens alimentícias, agricultura e recuperação de solos através da utilização dos filmes como matéria orgânica, enquanto as nanofibras apresentam potencial de utilização em setores que envolvem a área médica e setor alimentício.

CAPÍTULO 6 - PERSPECTIVAS FUTURAS

Como perspectivas futuras, pode-se considerar:

- Uso conjunto de estratégias de reticulação e/ou reforço e/ou aquecimento ôhmico na produção de filmes biodegradáveis, com o intuito de combinar os benefícios oferecidos por cada técnica e obter em um mesmo filme melhorias significativas referentes às propriedades como solubilidade e permeabilidade ao vapor de água, e mecânicas.
- Desenvolvimento de filmes de resíduo de gelatina por outros métodos de processamento, como extrusão e compressão por moldagem. Devido às propriedades da gelatina e condições necessárias para obtenção de materiais por essas técnicas, poucos estudos relataram a obtenção de filmes de gelatina através de métodos além do *casting*. Entretanto, já se demonstrou ser possível. A formação de compósitos e blendas com outros polímeros também pode ser uma estratégia para viabilizar o processo.
- Avaliação de outras possíveis aplicações dos filmes como embalagens para alimentos tendo em vista suas propriedades atuais ou de acordo com futuras estratégias empregadas. As propriedades atuais permitem a aplicação em alimentos com baixa umidade e/ou oleosos, tais como oleaginosas e outros óleos vegetais. Outras aplicações dependerão das características obtidas dos materiais, em que será interessante possibilitar a aplicação dos filmes em alimentos com teor de umidade médio a alto.
- Aprofundamento da compreensão sobre o efeito de filmes biodegradáveis no solo, através de técnicas de análise do solo antes, durante e após a biodegradação dos filmes tais como: avaliação físico química, atividade enzimática, determinação de N, P e K totais e biodisponíveis, contagem e identificação de microrganismos, resposta do rendimento do manejo e colheita de determinado cultivo.
- Investigação de outras possíveis aplicações de filmes, como na recuperação de solos degradados e na agricultura.
- Otimização da técnica de produção de nanofibras de resíduo de gelatina, como utilização de ambiente com umidade e temperatura controlada.
- Avaliação de produção em larga escala das nanofibras.
- Investigação do potencial de aplicação das nanofibras de resíduo de cápsula de gelatina.

6 REFERÊNCIAS

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