UNIVERSIDADE
ESTADUAL DE LONDRINA

Jéssica Fernanda Pereira

Biocompósitos obtidos a partir de polímeros biodegradáveis e resíduos lignocelulósicos

Londrina
2023

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biotecnologia, do Departamento de Bioquímica e Biotecnologia, da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutora em Biotecnologia.

Orientadora: Profª. Dra. Suzana Mali de Oliveira

Londrina
2023

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Pereira, Jéssica Fernanda.

Biocompósitos obtidos a partir de polímeros biodegradáveis e resíduos lignocelulósicos / Jéssica Fernanda Pereira. - Londrina, 2023.
124 f. : il.

Orientador: Suzana Mali de Oliveira.
Tese (Doutorado em Biotecnologia) - Universidade Estadual de Londrina, Centro de Ciências Exatas, Programa de Pós-Graduação em Biotecnologia, 2023.
Inclui bibliografia.

1. Biopolímeros - Tese. 2. Biocompósitos - Tese. 3. Resíduos Lignocelulósicos - Tese. I. Mali de Oliveira, Suzana. II. Universidade Estadual de Londrina. Centro de Ciências Exatas. Programa de Pós-Graduação em Biotecnologia. III. Título.

CDU 66

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BANCA EXAMINADORA

Profª. Drª. Suzana Mali de Oliveira
Universidade Estadual de Londrina – UEL

Profª. Drª. Franciely Grose Colodi
Universidade Estadual de Londrina – UEL

Profª. Drª. Janaina Montovan
Universidade Estadual de Londrina – UE

Profª. Drª. Gizilene Maria de Carvalho
Universidade Estadual de Londrina – UEL

Profª. Drª. Marianne Ayumi Shirai
Universidade Tecnológica Federal do Paraná – UTFPR

Londrina, 01 de Setembro de 2023.

Dedico este trabalho à meus pais, meu irmão, minha irmã, e toda a minha família e amigos que sempre me apoiaram em todos os momentos dessa caminhada, sempre me incentivando e acreditando em minhas escolhas.

AGRADECIMENTOS

Agradeço primeiramente a Deus por sempre ter me abençoado, protegido e dado força, principalmente nos momentos de dificuldades, mostrando que com sua ajuda tudo é possível.

À minha querida e amada Universidade Estadual de Londrina que me ofereceu a oportunidade de realizar meu sonho de ser doutora, e que foi minha casa por mais de dez anos.

Agradeço muito a minha orientadora a Prof. Dr. Suzana Mali por sempre ter acreditado em mim, e em todos os meus sonhos durante todos esses anos que convivemos. Sempre vou ter o prazer de dizer que muito do que sei vem dos seus ensinamentos.

Aos meus pais Meire e Ademir, que são minha base e exemplo de minha vida. Sempre estiveram presentes em todos os momentos me guiando e estimulando para eu desenvolver o meu melhor. Sempre me colocando em primeiro lugar para eu conseguir realizar esse grande sonho. Sem eles, posso dizer com toda certeza que não teria chego até aqui. Agradeço a Deus pelo o privilegio de ter os melhores pais deste mundo.

Aos meus irmãos Jaqueline e Wellington que sempre acreditaram em mim e em meu potêncial. Em especial ao meu irmão por todo o investimento e confiança depositados em mim.

A todos os meus amigos que foram de extrema importância nessa fase da minha vida, amigos os quais que dividi muitas noites de choros e incertezas, mas também muitas noites de alegrias e felicidades. Em especial, gostaria de agradecer a Beatriz, Luciane e diego que foram de extrema importancia em todo esse tempo percorrido.

Também gostaria de agradecer a CAPES-PDSE pelo financimaneto de meu doutorado sanduíche na Espanha. Também gostaria de agradecer ao Instituto de Agroquímica y Tecnologia de Alimentos (IATA), aos professores e alunos que me receberam tão bem, posso dizer que essa etapa foi de extrema importancia para meu desenvolvimento pessoal e intelectual.

Por fim, gostaria de agradecer a todos que de alguma forma foram presentes e contribuiram nesses anos de estudos e aprendizado, a vocês o meu muito obrigada!

PEREIRA, Jéssica Fernanda. **Biocompósitos obtidos a partir de polímeros biodegradáveis e resíduos lignocelulósicos.** 2023, 124 p. Tese (Doutorado em Biotecnologia) – Universidade Estadual de Londrina, Londrina, 2023.

Resumo

A pesquisa e desenvolvimento de novos produtos biodegradáveis e provindos de fonte renovável tem aumentado nos últimos anos atendendo à uma tendência de mercado, tanto em função da maior compreensão dos consumidores em relação à procedência das matérias-primas, como dos processos empregados na sua manufatura. Com base nisso, novas estratégias tecnológicas têm sido utilizadas no desenvolvimento de novos produtos e processos que resultem em menores impactos ambientais. No presente trabalho, o objetivo geral foi produzir e caracterizar biocompósitos na forma de hidrogéis (filmes) a partir de misturas de gelatina, celulose extraída da casca de aveia, e goma xantana, assim como, filmes à base de poli (3-hidroxibutirato-co-3-hidroxivalerato) (PHBV), poli (adipato-co-tereftalato de butileno) (PBAT), e fibras de cânhamo, com vistas à sua aplicação na área cosmética e de alimentos, respectivamente. Com base nisto, a primeira etapa do presente estudo consistiu na produção e caracterização dos hidrogéis. Inicialmente, a celulose da casca de aveia foi extraída utilizando o ácido peracético como agente de deslignificação, e os ácidos cítrico e succínico foram empregados para modificação da celulose como agentes esterificantes em diferentes concentrações (0, 5, 12,5 e 20%) empregando-se o processo de extrusão reativa. Os resultados mostraram que a esterificação ocorreu em todas as amostras para ambos os ácidos, e que as amostras modificadas apresentaram maior hidrofobicidade, porém não sofreram alteração quanto à sua estrutura morfológica ou padrão de cristalinidade. Este estudo mostrou ser possível modificar a celulose através da reação com ácidos orgânicos utilizando um processo simples e ecologicamente correto baseado na extrusão reativa, que possui baixa geração de efluentes, tempos de reação curtos, e é escalonável para larga escala. Em seguida, amostras de celulose nativa e modificada foram empregadas como reforço em matrizes de gelatina e goma xantana para obtenção de hidrogéis na forma de filmes pelos processos de extrusão reativa e termoprensagem. A estratégia empregada neste estudo para obter os hidrogéis foi eficaz, resultando em materiais com propriedades promissoras para serem usados como formas farmacêuticas em potencial para liberação de compostos ativos em produtos cosméticos. A goma xantana atuou como um agente de reticulação alternativo na formação da matriz polimérica, resultando em hidrogéis que apresentaram intumescimento dependente do pH, e os maiores valores foram obtidos em pH 4, variando de 5,3 a 7,9 g/g. A celulose nativa ou modificada atuou como agente de reforço para os hidrogéis, aumentando a estabilidade térmica e a resistência à tração dos filmes quando empregada no nível mais alto (7%). Maior teor de celulose também resultou em maior capacidade de adsorção de água e permeabilidade ao vapor de água dos hidrogéis. Na segunda etapa, fibras de cânhamo (*Cannabis sativa*) foram caracterizadas quanto ao seu teor de proteínas, lipídios, umidade e lignina, e foram utilizadas na produção de filmes biodegradáveis a partir de misturas contendo diferentes concentrações de PHBV e PBAT, com a adição de cera de abelha ou de carnaúba. Os filmes foram produzidos por extrusão e termoprensagem, e foram caracterizados por suas propriedades mecânicas, ângulo de contato, MEV e permeabilidade ao vapor de água. A incorporação das fibras de cânhamo resultou em filmes com maior permeabilidade ao vapor de água e menor resistência mecânica em comparação aos materiais sem adição das fibras. Os filmes com a adição das ceras apresentaram propriedades mecânicas semelhantes às dos filmes preparados sem a cera, mas exibiram ângulo de contato significativamente maior (89º), e ainda, menor permeabilidade ao vapor de água, indicativo de sua maior hidrofobicidade. De forma geral, nas duas etapas deste estudo, tanto a celulose nativa ou modificada extraída da casca de aveia, como as fibras de cânhamo, resultaram em reforço das matrizes poliméricas obtidas, assim como, os processos empregados se mostraram adequados para a produção dos biocompósitos obtidos, que podem ser considerados como alternativas em potencial para a aplicação nos setores cosméticos e de alimentos.

Palavras Chaves: Celulose, casca de aveia, cânhamo, extrusão reativa, termoprensagem, biocompostos.

PEREIRA, Jessica Fernanda. **Biocomposites obtained from biodegradable polymers and lignocellulosic residues,** 2023, 124 p. Thesis (Doctorate in Biotechnology) – State University of Londrina, Londrina, 2023.

Abstract

Research and development of new biodegradable products from renewable sources has increased in recent years, due to the better understanding of consumers of the origin of the materials used to manufacture the products. Based on this, new technological strategies have been used in the development of new products and processes that result in lower environmental impacts. In the present work, the general objective was to produce and characterize biocomposites in the form of hydrogels (films) from mixtures of gelatin, cellulose extracted from oat hulls, and xanthan gum, as well as films based on poly (3-hydroxybutyrate-co -3-hydroxyvalerate) (PHBV), poly (adipate-co-butylene terephthalate) (PBAT), and hemp fibers, with a view to their application in the cosmetic and food areas, respectively. Based on this, the first stage of the present study consisted of the production and characterization of the hydrogels. Initially, the cellulose was extracted from the oat hulls using peracetic acid as a delignification agent, and citric and succinic acids were used to modify the cellulose as esterifying agents at different concentrations (0, 5, 12.5 and 20%) using the reactive extrusion process. The results showed that esterification occurred in all samples for both acids, and that the modified samples showed greater hydrophobicity, but did not change their morphological structure or crystallinity pattern. This study showed that it is possible to modify cellulose through reaction with organic acids using a simple and ecologically correct process based on reactive extrusion, which has low effluent generation, short reaction times, and is scalable for large scale. Then, native and modified cellulose samples were used as reinforcement in gelatin, and xanthan gum matrices to obtain hydrogels in the form of films by reactive extrusion and thermopressing processes. The strategy employed in this study to obtain the hydrogels was effective, resulting in materials with promising properties to be used as potential pharmaceutical forms for the release of active compounds in cosmetic products. Xanthan gum acted as an alternative crosslinking agent in the formation of the polymeric matrix, resulting in hydrogels that showed pH-dependent swelling, and the highest values were obtained at pH 4, ranging from

5.3 to 7.9 g/g. Native or modified cellulose acted as a reinforcing agent for the hydrogels, increasing the thermal stability and tensile strength of the films when used at the highest level (7%). Higher cellulose content also resulted in higher water adsorption capacity and water vapor permeability of the hydrogels. In the second step, hemp (*Cannabis sativa)* fibers were characterized for their protein, lipid, moisture and lignin content, and were used in the production of biodegradable films from mixtures containing different concentrations of PHBV and PBAT, with the addition of beeswax or carnauba wax. The films were produced by extrusion and thermopressing, and were characterized by their mechanical properties, contact angle, SEM and water vapor permeability. The incorporation of hemp fibers resulted in films with higher permeability to water vapor, and smaller mechanical strength compared to materials without the addition of fibers. The films with the addition of waxes presented similar mechanical properties to the films prepared without the wax, but exhibited a significantly higher contact angle (89º), and even lower permeability to water vapor, indicative of its greater hydrophobicity. In general, in both stages of this study, both native or modified cellulose extracted from oat hulls, as well as hemp fibers, resulted in reinforcement of the polymeric matrices obtained, as well as the processes employed proved to be adequate for the production of biocomposites obtained, which can be considered a potential alternatives for application in the cosmetic and food sectors.

Key words: Cellulose, oat hulls, hemp fiber, reactive extrusion, thermopressing, biocomposites.

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LISTA DE ABREVIATURAS E SIGLAS

AC	Ácido cítrico
AS	Ácido succínico
DRX	Difração de Raios-X
FDA	Food and Drug Administration
FT-IR	Espectroscopia no infravermelho com transformada de Fourier
MEV	Microscopia eletrônica de varredura
PBAT	Poli (adipato-co-tereftalato de butileno)
PET	Polietileno tereftalato
PI	Ponto isoelétrico
PHAs	Poliésteres alifáticos
PHBV	Poli (3-hidroxibutirato-co-3-hidroxivalerato)
PLA	Ácido poliláctico
PVA	Permeabilidade ao vapor de água
TGA	Análise termogravimétrica
UEL	Universidade Estadual de Londrina

SUMÁRIO

1. INTRODUÇÃO

O avanço na tecnologia de polímeros e biopolímeros tem levado ao crescente desenvolvimento de produtos obtidos a partir matérias-primas de fonte renovável (Cascone; Lamberti, 2019). Nesse sentido, hidrogéis e filmes obtidos a partir de materiais poliméricos têm se destacado (Cascone; Lamberti, 2019; Garcia *et al*., 2018; Gutiérrez; Valencia, 2021; Kalendova *et al*., 2021; Mitura; Sionkowska; Jaiswal, 2020; Podshivalov *et al*., 2017; Rodríguez-Rodríguez *et al*., 2020; Vercelheze *et al*., 2019).

Os hidrogéis podem ser obtidos a partir de polímeros naturais ou sintéticos, são uma classe única de materiais que são formados por redes poliméricas viscoelásticas tridimensionais hidrofílicas, que permitem a difusão e fixação de moléculas e células. Essas redes são mantidas devido à presença de ligações cruzadas (reticulação) entre as cadeias dos polímeros, apresentando capacidade inerente de intumescer e reter água na sua estrutura de gel. O processo de reticulação pode ser de natureza física ou química. Nos géis físicos, as cadeias poliméricas são mantidas por ligações de hidrogênio, hidrofóbicas ou van forças de Van der Waals. Os hidrogéis químicos apresentam ligações covalentes entre cadeias poliméricas, e são mais estáveis às alterações de temperatura, pressão ou estresse mecânico (Deng *et al*., 2019; Frachini; Petri, 2019; Gutiérrez; Valencia, 2021; Varaprasad *et al*., 2017).

Os filmes biodegradáveis podem ser produzidos a partir de um único polímero, natural ou sintético, dentre os quais amido, gelatina, celulose, e gomas em geral, como também a partir de suas misturas. Os filmes biodegradáveis vêm sendo estudados ao longo dos anos com diversas aplicações, em especial como embalagens de alimentos (Alves *et al*., 2009, Fakhouri *et al*., 2003, Lucena *et al*.,2017), revestimentos comestíveis (Krochta *et al*., 1994; Fakhouri *et al*., 2007; Romio *et al*., 2017), ou ainda, em sistemas de liberação de fármacos (Dixil; Puthili, 2009; Ketul *et al*., 2013; Patil; Shrivastava, 2014).

Os biopolímeros são polímeros de fonte renovável, dentre os quais podemos citar os polissacarídeos e proteínas, macromoléculas que podem ser utilizadas na obtenção de matrizes poliméricas para a liberação de princípios ativos, incluindo na obtenção de hidrogéis e filmes. Em geral, os polissacarídeos e proteínas apresentam elevada capacidade de absorção de água, o que está relacionado à presença de grupamentos hidrofílicos presentes na sua estrutura química, tais como grupamentos amina (NH_2), hidroxila (-OH), amida (-CONH-, $-CONH_2$), carboxila (-COOH), dentre outros (Mitura *et al*., 2020).

A gelatina é uma proteína de baixo custo, obtida através da hidrólise ácida ou alcalina do colágeno isolado a partir de pele, ossos ou tecido conjuntivo de animais, amplamente empregada pela indústria farmacêutica. É facilmente solúvel em água a temperatura média de 40 °C, formando uma solução viscosa e com capacidade de formação de filmes e géis termoreversíveis, e esta característica vem sendo amplamente explorada em estudos envolvendo a produção de hidrogéis e filmes biodegradáveis e/ou comestíveis com as mais diversas aplicações (Borges *et al*., 2013; Laffleur; Strasdat, 2019; Rodríguez-Rodríguez *et al*., 2020).

A celulose é um homopolissacarídeo linear formado pela repetição de unidades de β-D-glicose, que são unidas por ligações β-(1→4), com três grupos hidroxila por unidade de monossacarídeo (Gan *et al*., 2017), o que faz da celulose uma ótima plataforma para modificações químicas. A celulose pode ser facilmente modificada para a utilização em matrizes poliméricas (Capanema *et al*., 2018; Maaloul *et al*., 2021; Tao; Nonaka, 2021).

A goma xantana é um biopolímero de origem microbiana, produzido pela bactéria *Xanthomonas campestris* que vem sendo muito utilizado na indústria farmacêutica e cosmética. Possui boa viscosidade e capacidade de formar filmes, podendo ser utilizado como cadeia hidrofílica para a liberação de princípios ativos em hidrogéis (Kola; Kumar, 2013; Silva; Cristiano, 2017). A goma xantana é um heteropolissacarídeo ramificado que apresenta uma cadeia principal semelhante à da celulose, constituída de moléculas de glicose ligadas através de ligações *β*-(1–4). A sua cadeia lateral é formada por um trissacarídeo ligado à segunda unidade glicose da cadeia principal, sendo constituída de uma molécula de ácido glicurônico entre duas unidades de D-manose. A D-manose ligada à cadeia principal contém um grupo acetila na posição 6 e, aproximadamente, metade das D-manoses terminais contém um resíduo de ácido pirúvico ligado através de um grupo cetônico aos carbonos 4 e 6 (Cristiano, 2017; Garcia-Ochoa, 2000).

O poli (3-hidroxibutirato-co-3-hidroxivalerato) (PHBV) é um polímero pertencente à família dos polihidroxialcanoatos (PHAs), que são polímeros biodegradáveis e não tóxicos produzidos por diversos microrganismos. PHBV tem atraído atenção devido às suas características de desempenho favoráveis em relação aos outros membros da família dos PHAs, e preço mais baixo em relação a outros polímeros da família dos PHAs. O PHBV é formado pela adição de poli (3-hidroxivalerato) (PHV) à molécula de PHB (Gupta *et al*., 2022; Meereboer *et al*., 2020; *Pal et al*., 2020). No entanto, o PHBV tem algumas desvantagens inerentes, incluindo fragilidade e custos mais elevados em comparação com outros polímeros biodegradáveis e tradicionais.

O poli (adipato-co-tereftalato de butileno) (PBAT) é um copoliéster amorfo, de cadeia alifático-aromática, formado pela policondensação do 1,4-butanodiol com os ácidos adípico e tereftálico (Jian *et al*., 2020; Pal *et al*., 2020). Embora o PBAT não seja um polímero totalmente de origem natural, sua capacidade de sofrer biodegradação o torna uma boa alternativa para ser utilizado na produção de materiais que substituam os plásticos convencionais (Pal *et al.,* 2020).

As fibras naturais são materiais de fonte renovável e de baixo custo que estão disponíveis em todo o mundo a partir de diferentes fontes, e são matérias-primas em potencial para a aplicação como reforços de matrizes poliméricas biodegradáveis para a obtenção de biocompósitos. Os biocompósitos podem ser definidos como uma classe de materiais provindos de fonte renovável que são heterogêneos, com no mínimo duas fases distintas, uma delas sendo descontínua chamada de reforço, responsável por fornecer a principal resistência ao esforço. A outra fase é contínua, chamada de matriz, correspondendo ao meio de transferência deste esforço (Khalili *et al*., 2023; Lamsaf *et al*., 2023).

Os resíduos lignocelulósicos são fontes de fibra em potencial para a aplicação na obtenção de biocompósitos, alguns exemplos são madeira, juta, miscanthus, cânhamo, linho e sisal. O uso de resíduos lignocelulósicos está cada vez mais frequente, pois além de ser uma alternativa às fibras sintéticas de base petroquímica, oferecem características que ajudam no melhoramento de várias propriedades, tais como, resistência mecânica, rigidez, juntamente com uma redução no custo final de produção (Pal *et al*., 2020; Wu *et al.,* 2018).

A fibra de cânhamo (*Cannabis sativa*) é uma fibra natural longa, com fibrilas de celulose cristalina, o que as torna uma fonte em potencial de fibras lignocelulósicas naturais (Wu *et al*., 2018). Entre todas as fibras naturais, o cânhamo tem os maiores valores de módulo de Young e possui uma das fibras de celulose de maior resistência. Além disso, as fibras de cânhamo possuem elevada razão de aspecto (comprimento/largura), que é outra característica que as torna adequadas para uso como reforço em biocompósitos (Lamsaf *et al*., 2023; Promhuad, *et al*., 2020). O cânhamo é altamente utilizado como plantação industrial com diversas aplicações, principalmente na Europa e na China, o que o torna uma fonte de material lignocelulósico barato originado de resíduos industriais (Wu *et al.,* 2018).

A produção de hidrogéis e filmes podem acontecer a partir do uso de um polímero, ou do uso de uma mistura polimérica. Ao se optar pela mistura polimérica algumas características podem ser melhoradas, tais como a hidrofilicidade, resistência mecânica e capacidade de intumescimento (Antoniolia *et al*., 2018; Garcia *et al*., 2018; Grabska; Sionkowska, 2019). A extrusão reativa é uma tecnologia que apresenta diversas vantagens, se classificando como um processo eco-amigável, contínuo, e sem a geração de efluentes. A extrusão reativa pode ser utilizada na modificação de polissacarídeos através da combinação de calor, pressão e atrito mecânico, assim como, para a mistura e obtenção de matrizes de biocompósitos.

A modificação e obtenção de matrizes poliméricas empregando o processo de extrusão trazem vários benefícios, tais como a utilização de matérias-primas com baixa umidade, possibilidade de adicionar reagentes e aditivos como auxiliares de processamento e estabilizadores, resultando em baixo custo, simplicidade, e possibilidade de produção de materiais em larga escala (Cai *et al*., 2019; Liu *et al*., 2016; Ye *et al*., 2019).

Dentro deste contexto, a proposta deste trabalho foi o desenvolvimento de biocompósitos a partir de matérias-primas biodegradáveis, indo ao encontro a uma tendência de mercado que prioriza matérias-primas e processos de baixo impacto ambiental. Os biocompósitos foram obtidos a partir da utilização de gelatina, goma xantana, PHBV, PBAT como matrizes poliméricas, e celulose da casca de aveia e fibras de cânhamo como materiais de reforço, tendo em vistas suas aplicações em indústrias cosméticas e de embalagens alimentícias.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Produzir e caracterizar biocompósitos na forma de hidrogéis (filmes) a partir de misturas de gelatina, celulose extraída da casca de aveia goma xantana, assim como, filmes à base de PHBV, PBAT e fibras de cânhamo, com vistas à sua aplicação na área cosmética e de alimentos, respectivamente.

2.2 OBJETIVO ESPECÍFICOS

- Extrair celulose da casca de aveia utilizando ácido peracético;
- Modificar a celulose através da introdução de ligações cruzadas via extrusão reativa com ácidos succínico e cítrico;
- Desenvolver processo para a produção dos hidrogéis biopoliméricos a partir das misturas poliméricas de celulose nativa e modificada, gelatina, e goma xantana;
- Caracterizar os hidrogéis produzidos em relação à sua microestrutura, densidade, estabilidade térmica, capacidade de absorção de água e capacidade de adsorção de água;

- Fazer a caracterização das fibras de cânhamo em relação à quantidade de proteínas, gorduras, teor de umidade, lignina, cinza e carboidratos totais.

- Desenvolver processo de produção de filmes a partir das misturas de PHBV, PBAT e das fibras de cânhamo, e caracterizar os filmes produzidos quanto às suas propriedades mecânicas, ângulo de contato, permeabilidade ao vapor de água e microscopia eletrônica de varredura.

3. REVISÃO DA LITERATURA

3.1. MATERIAIS BIODEGRADÁVEIS

3.1.1.Biopolímeros

Os biopolímeros são polímeros ou copolímeros produzidos a partir de matérias-primas de fontes renováveis (ABNT-NBR 15448-1, 2008). As matérias-primas obtidas de fontes renováveis apresentam um ciclo de vida muito mais curto quando são comparadas com as de fontes fósseis como, por exemplo, os polímeros sintéticos plásticos derivados do petróleo (Brito *et al*., 2011). Além de serem de fonte renovável, na maioria das vezes apresentam outras vantagens, como ser biodegradáveis e biocompatíveis, ou seja, são matérias-primas que não oferecem riscos, tais como reações adversas, tóxicas e carcinogênicas ao paciente (ISO 10993-1, 2009). São empregados como espessantes, excipientes, em revestimentos, filmes biodegradáveis e microencapsulação de fármacos, e no preparo de hidrogéis (Afonso *et al*., 2019; Pereira *et al*., 2022).

Apesar de a maioria dos biopolímeros ser biodegradável, nos últimos anos houve um aumento na produção de biopolímeros não-biodegradáveis. Esses plásticos não-biodegradáveis que são produzidos empregando carbono orgânico de fontes renováveis (via processo fermentativo) não são biodegradáveis, uma vez que a biodegradabilidade está relacionada estrutura do polímero e não à fonte de carbono, esses polímeros apresentam propriedades idênticas às suas versões petroquímicas convencionais, ajudando a reduzir pegada de carbono do produto, mas eles permanecem não biodegradáveis (Andreeßen; Steinbüchel, 2019; Taiatele *et al*., 2019).

Os biopolímeros biodegradáveis podem ser separados em três grupos de acordo com a sua origem. Esses grupos são: origem agrícola (por exemplo, amido, celulose, gelatina); origem microbiana (por exemplo, goma xantana e celulose bacteriana), e polímeros de origem biotecnológica, tais como o ácido polilático e os polihidroxialcanoatos (Kalhid, Arif, 2022; Shamsuri *et al*., 2021; Taiatele *et al*., 2019).

De acordo com Arif *et al.* (2022), os biopolímeros têm apresentado um enorme potencial de aplicação devido à diversidade de compostos, o que resulta em materiais com uma grande variedade de propriedades físicas e mecânicas. Os biopolímeros têm sido efetivamente utilizados em muitas aplicações biomédicas e de engenharia, incluindo liberação controlada de medicamentos, embalagens de alimentos, na construção civil, medicina regenerativa, eletrônicos, implantes ortopédicos, dentre outras aplicações. Particularmente, na última década, os biopolímeros experimentaram um renascimento devido à limitação dos recursos fósseis em combinação com as demandas públicas por processos ambientalmente amigáveis e sustentáveis, o que levou à formação de um mercado para materiais poliméricos de base biológica.

Os materiais à base de biopolímeros também são responsáveis pela redução da pegada de carbono durante sua fabricação e sua degradação microbiana, resultando em menores impactos ambientais quando comparados aos materiais sintéticos de origem fóssil empregados com a mesma finalidade (Andreeßen, Steinbüchel, 2019).

O uso de biopolímeros biodegradáveis tem sido relatado para o desenvolvimento de novos materiais direcionados para o uso em cosméticos, pois geralmente são materiais atóxicos e não irritantes. Além disso, existem várias características que podem ser controladas com a escolha do polímero, como tempo de desintegração, resistência mecânica e flexibilidade. Portanto, a escolha do polímero ou polímeros para ser usado é uma etapa crítica da pesquisa (Afonso *et al*., 2019; Karki *et al.,* 2016; Pereira *et al*., 2022). Os biopolímeros podem ser empregados em vários produtos cosméticos, com as mais diferentes aplicações, como espessantes ou matrizes poliméricas para liberação de compostos ativos (Arif *et al*., 2022).

Gupta *et al.* (2022) relatam que biopolímeros como goma xantana, goma carragena, pectina, ágar, ácido hialurônico, amidos, goma de guar, alginato, gelatina, colágeno e queratina têm sido utilizados na pesquisa e desenvolvimento de produtos cosméticos direcionados para uso dermatológico, dentre outros usos, pois são seguros e biocompatíveis. Adicionalmente, Rao *et al.* (2022) relatam que a biocompatibilidade, biodegradabilidade, não carcinogenicidade e não imunogenicidade torna os biopolímeros materiais em potencial para uso em cosméticos, podendo ser aplicados tanto em formulações de hidratantes, cremes, emulsões, filmes e adesivos dérmicos.

De acordo com a sua origem, o biopolímero pode apresentar baixo custo e alta disponibilidade, como exemplo tem-se os amidos de diferentes fontes e a gelatina. Entretanto, apesar de todas as vantagens que apresentam, os biopolímeros que possuem origem biológica apresentam algumas limitações técnicas que tornam mais difícil sua processabilidade e seu uso como produto final. Entre essas limitações encontramos a processabilidade, resistência térmica, propriedades mecânicas, propriedades reológicas, permeabilidade a gases e a elevada taxa de degradação (Brito *et al.*, 2011).

Em função destas limitações, o emprego de misturas poliméricas se torna uma alternativa viável e bastante buscada nos últimos anos por diferentes pesquisadores, para diferentes aplicações. De acordo com Shamsuri*,* Abdan e Kaneko (2021), o emprego de misturas biopoliméricas pode ser interessante, sendo uma estratégia para melhorar as propriedades destes materiais. Rogovina e Vikhoreva (2006) relatam que as propriedades físico-químicas e mecânicas de misturas poliméricas são determinadas pelo tipo de ligações estabelecidas entre os componentes, sua compatibilidade e as características de uma estrutura supramolecular em formação.

Mitura, Sionkowska e Jaiswal (2022) relatam que o emprego de misturas de biopolímeros pode ser importantes alternativas para liberação de compostos bioativos em formulações cosméticas. Sionkowska *et al.* (2016) observaram misturas ternárias à base de colágeno, quitosana e ácido hialurônico apresentam boas propriedades de intumescimento quando empregadas na formulação de matrizes poliméricas para uso cosmético. Estes autores relatam que a mistura de polissacarídeos e proteínas pode ser benéfica devido à sua compatibilidade química.

Singh *et al.* (2022) relataram a produção de uma máscara facial a partir da mistura polimérica composta de alginato de sódio, hidroxipropilmetil-celulose ou hidroxietilcelulose, com a adição de nanopartículas de prata. Pereira *et al.* (2021) relataram que a mistura binária de amido e gelatina pode ser promissora para a produção de filmes biodegradáveis de uso cosmético a serem aplicados como matriz para liberação de compostos antioxidantes. De acordo com Seth *et al.* (2022), a mistura binária de amido e gelatina resulta em hidrogéis com maior hidrofilicidade e resistência mecânica em comparação aos hidrogéis obtidos a partir dos materiais puros.

3.1.1.1 Gelatina

A gelatina é uma proteína de origem animal que resulta de hidrólise ácida ou alcalina a partir do colágeno que é proveniente da pele, ossos e tecidos conectivos de bovinos e suínos. Possui alta solubilidade em água a temperatura média de 50 °C. De acordo com a matéria prima usada em sua produção, a gelatina poderá apresentar um tipo de mistura de polipeptídios (Garcia *et al.,* 2018; Rodriguez *et al.,* 2020). Grande parte da gelatina produzida no mercado não tem massa molecular homogênea por ser obtida a partir da degradação de cadeias longas (Garcia *et al.,* 2018; Wang *et al.,* 2016).

A representação (GLY-XPRO)^n é a responsável pela estrutura de tripla hélice da gelatina, onde o X representa os aminoácidos lisina, arginina, metionina e valina. Um terço da cadeia é composta por glicina (aproximadamente 33%), o outro um terço é composta por prolina ou hidroxiprolina (aproximadamente 33%), e o restante da cadeia é composta por outros resíduos de aminoácidos. A gelatina exibe um comportamento anfotérico devido a presença tanto de grupos básicos como ácidos (Laffleur, Strasdat, 2019; Rodriguez *et al.,* 2020; Su; Wang, 2015).

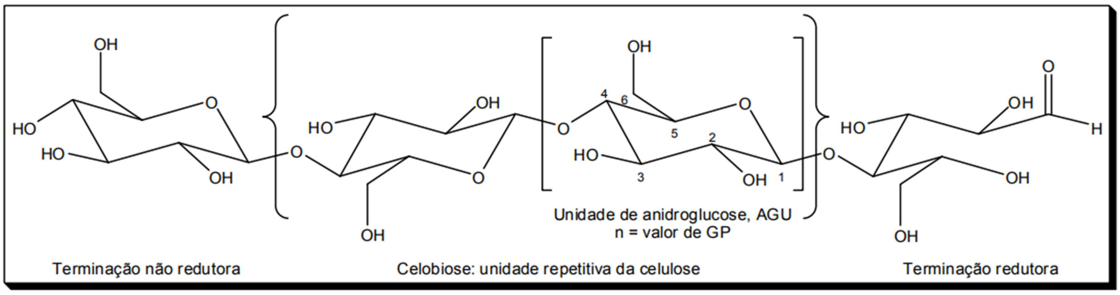
Os géis de gelatina são capazes de formar uma rede tridimensional, sendo formados géis em baixas concentrações de sólidos, a partir de 1%. São géis termorreversíveis, que fundem em temperaturas entre 30 – 40 °C. O mecanismo mais aceito para a gelatinização é a reversão das hélices enroladas aleatoriamente, onde as regiões ricas em determinados tipos de aminoácidos provenientes das diferentes cadeias polipeptídicas atuam como potenciais zonas de junção, na medida em que, ao resfriar a solução, elas assumam uma conformação helicoidal, o que resulta em um gel tridimensional (Mariod, Adam, 2013).

A gelatina é amplamente utilizada na indústria alimentícia e farmacêutica como estabilizante, espessante, formador de filmes, protetor coloidal, emulsificante, agente espumante e clarificante de bebidas (Laffleur; Strasdat, 2019; Rodríguez-Rodríguez *et al*., 2020). Devido a excelente capacidade de se dissolver na água e formar solução viscosa com capacidade de formação de filmes e géis, a gelatina vem sendo amplamente explorada em estudos envolvendo a produção de filmes biodegradáveis e/ou comestíveis com as mais diversas aplicações (Borges, 2013; Wang *et al.,* 2016), incluindo a produção de matrizes poliméricas para liberação de princípios ativos.

3.1.1.2 Celulose

A celulose é um homopolímero natural, não ramificado e fibroso de origem vegetal ou bacteriana. É composta por unidades de β- D glicopiranose unidas por ligações glicosídicas β- D (1- 4), que ao se unirem liberam uma molécula de água a partir das hidroxilas presentes nos carbono 1 e 4. A celobiose é a unidade de repetição da molécula de celulose (Klemm *et al*., 2005, Gan *et al*.,2017). Na figura 1 tem-se a ilustrada a estrutura química da celulose.

Figura 1: Estrutura química da celulose.



Fonte: MORGADO, 2009.

Na estrutura da celulose, a molécula de glicose possui 3 hidroxilas livres que estão ligadas aos carbonos 2,3 e 6 (Morgado, 2009). As moléculas de celulose são capazes de formar ligações de hidrogênio inter e intramoleculares, dessa forma podem se agregar e levar a formação de microfibrilas. Tais microfibrilas possuem zonas ordenadas que são chamadas de regiões cristalinas, e zonas não ordenadas que são chamadas de regiões amorfas (Claro, 2017). Nas regiões cristalinas, as fibras possuem maior resistência à tração, ao alongamento e a solvatação (Morgado, 2009).

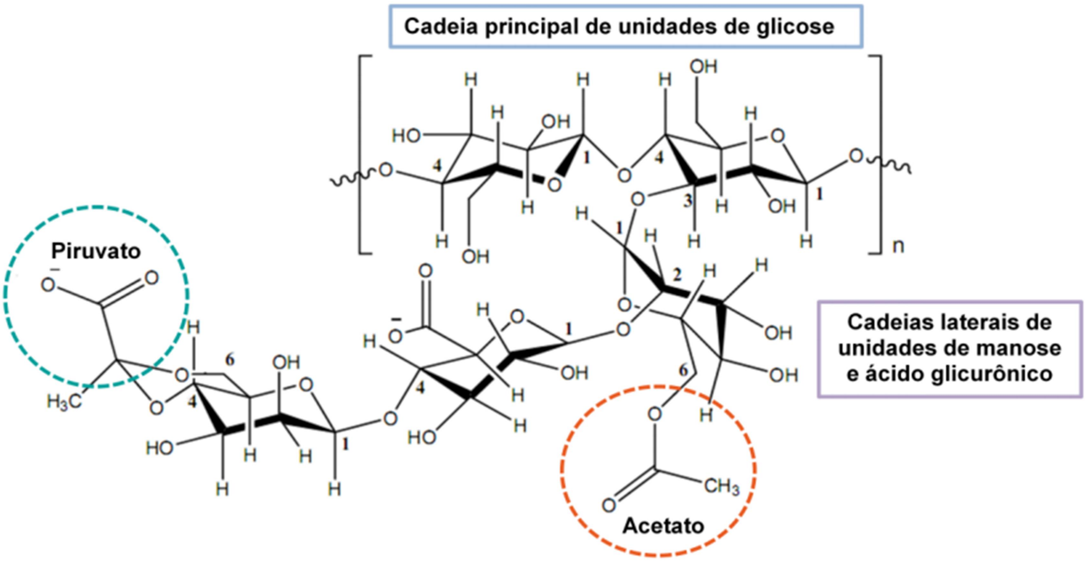
Entre a vasta quantidade de polímeros naturais no mercado, a celulose e seus derivados apresentam vários benefícios, como a biocompatibilidade e biodegradabilidade, e têm sido extensivamente utilizados como matéria-prima para a produção de hidrogéis para a aplicação em diversos setores, tais como, tratamentos de feridas, liberação de medicamentos e aplicações cosméticas (Capanema *et al*., 2018; Gan *et al*., 2017).

3.1.1.3 Goma xantana

A goma xantana é um polissacarídeo de origem microbiana, sua síntese ocorre a partir da fermentação de açucares pela bactéria fitopatogênica *Xanthomonas campestris*. Possui elevada viscosidade, com comportamento pseudoplástico, sendo estável em uma grande faixa de temperatura (10 – 60 °C) e pH (3-12), com estabilidade máxima de pH entre 4-10, essas características são altamente exploradas nas indústrias farmacêuticas, cosméticas e alimentícias (Fitzpatrick *et al.,* 2013; Nunes, 2016; Raschip *et al*., 2015).

A goma xantana é um heteropolissacarídeo aniônico (Figura 2), com a cadeia principal constituída de resíduos de D-glicose que são unidos por ligações β(1→4) e uma cadeia lateral trissacarídea que contém uma unidade de ácido D-glucurônico entre duas unidades de D-manose ligadas a resíduos alternativos de D-glicose. Metade da D-manose terminal está ligada a um ácido pirúvico, e a unidade D-manose que está ligada a cadeia principal pode conter um grupo acetil ligado (Alizadeh-Sani *et al*., 2019).

Figura 2: Estrutura química da goma xantana.



Fonte: BUENO *et al*., 2013 e SILVA; CRISTIANO, 2017.

A natureza aniônica da goma xantana se deve à presença de grupamentos carboxílicos no ácido glicurônico e pirúvico, que estão presentes na sua cadeia lateral (Horn *et al*., 2015). Aspectos de segurança e tecnológicos referentes a esse polímero foram extensivamente estudados para a sua aplicação nos setores farmacêuticos e alimentícios, sendo considerado um produto atóxico, que não causa sensibilização ou irritação (Garcia-Ochoa, 2000; Silva; Cristiano, 2017).

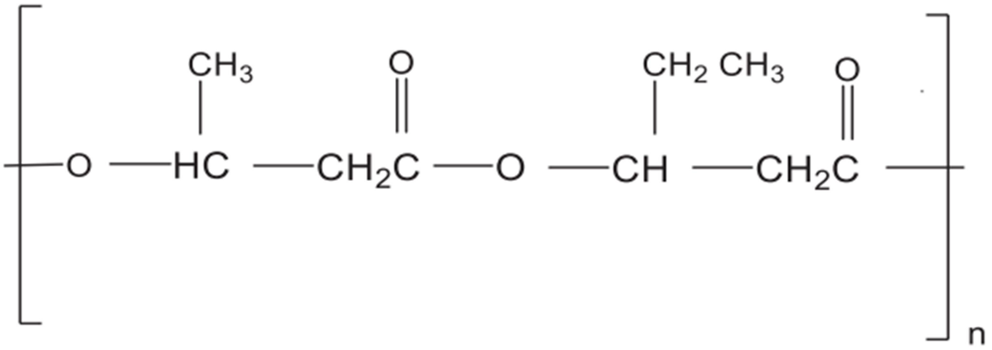
Joshy *et al*. (2020) usaram a goma xantana para a produção de filmes reforçados com óxido de zinco para revestimentos comestíveis em frutas, e observaram que os tomates e maçãs revestidos apresentaram menor perda de massa quando comparados com frutas e vegetais não revestidos. Raschip *et al*. (2015) produziram hidrogéis de goma xantana e lignina para a liberação controlada de bisoprolol fumarato e os resultados demostraram que essa é uma excelente matriz para liberação controlada de compostos ativos.

3.1.2 Poliésteres biodegradáveis

3.1.2.1 Poli (3-hidroxibutirato-co-3-hidroxivalerato) PHBV

Os PHAs são poliésteres alifáticos conhecidos por suas propriedades biodegradáveis e sua origem de fonte microbiana. O poli(3-hidroxibutirato-co-3-hidroxivalerato) (PHBV) é um membro da família dos PHAs que é formado pela adição dos monômeros de poli(3-hidroxivalerato) (PHV) à monômeros da molécula de PHB. O PHBV vem sendo muito estudado devido ao seu melhor desempenho e custo mais baixo quando comparado com outros polímeros da família dos PHAs (Gupta *et al*., 2022; Meereboer *et al*., 2020).

Figura 3: Estrutura química do PHBV.



$$\left[-\mathrm{O}-\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{HC}}}-\mathrm{CH_2}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}-\mathrm{O}-\overset{\mathrm{CH_2\,CH_3}}{\overset{|}{\mathrm{CH}}}-\mathrm{CH_2}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}- \right]_n$$

Fonte: Briso & Aroca 2018.

O PHBV é um excelente candidato para substituir plásticos convencionais não biodegradáveis. No entanto, junto com todas as suas vantagens, o PHBV apresenta alta fragilidade, apresenta custo elevado em comparação com outros polímeros biodegradáveis e convencionais (Pal *et al*., 2020). Assim, a incorporação de outros agentes de reforço pode ser uma abordagem adequada para sobrepor essas desvantagens apresentadas pelo PHBV (Gupta *et al*., 2022).

Paul *et al*. (2020) utilizaram uma mistura de PHBV com PBAT, com a adição de nanoargila modificada organicamente para produzir embalagens a partir de duas técnicas, por compressão e extrusão de filme, e por *casting*. Como resultado, observaram que os filmes produzidos por extrusão de filme apresentaram melhores propriedades de barreira. Nakayama *et al.* (2018) trabalharam com uma mistura de PHBV com PLA (ácido poliláctico) e fibras de seda, para a produção de biocompósitos. Observaram que as fibras de seda não tiveram uma boa interação com os polímeros, apresentando zonas de aglomeração, entretanto observaram que estes materiais poderiam ser aplicados em aplicações que exigiam alto impacto e força.

3.1.2 Poli (butileno adipato-co-tereftalato) PBAT

O PBAT é um copoliéster alifático-aromático que exibe biodegradabilidade completa. Ele sofre degradação em um curto espaço de semanas, o qual é facilitado por processos enzimáticos (Jian *et al*., 2020). O PBAT pode ser incorporado em misturas poliméricas para promover melhoria na elongação de ruptura (Larsson *et al*., 2016).

Jiang *et al.* (2006) utilizaram uma mistura de PBAT com PLA para a produção de materiais biodegradáveis pelo método de extrusão. Nos resultados, analisaram que a adição de PBAT na mistura melhorou as propriedades dos filmes em relação aos filmes de PLA, obtendo filmes menos rígidos e quebradiços, sem perder a biodegradabilidade dos mesmos. Moustafa *et al.* (2017) trabalharam com uma mistura de PLA com PBAT, com a adição de nanoargilas com propriedades antibacterianas para a produção de embalagens.

De acordo com Pal *et al*., (2022), filmes obtidos pela mistura entre PHBV e PBAT por extrusão são candidatos em potencial para serem utilizados como embalagens flexíveis. Pal *et al*., (2023) desenvolveram filmes à base de PBAT e PHBV, com a incorporação de 5% de amido, que foi tratado com silano como agente acoplador empregando-se o processo de extrusão. Zytner *et al*., (2023) relataram a produção de materiais biodegradáveis de PBAT e PHBV via injeção termoplástica, e observaram que estes poliésteres são naturalmente imiscíveis, porém dependendo das proporções empregadas de cada polímero, assim como das condições de processamento, suas propriedades e aplicações podem ser largamente melhoradas.

3.2 FIBRAS DE CÂNHAMO

Recentemente muitas fibras lignocelulósicas, tais como, cânhamo, juta, e fibra de sisal vêm sendo estudadas como reforço em matrizes poliméricas (Dixit *et al*., 2022). Muito cultivada na América Latina, Europa e China, a fibra de cânhamo apresenta alta concentração em celulose cristalina (55-72%), lignina (2-5%) e hemicelulose (8-19%), o que torna essa fibra muito atrativa para a utilização como reforço polimérico (Promhuad *et al*., 2022; Yingji *et al*., 2018). Se submetidas a tratamentos físicos, químicos, ou a uma combinação de ambos, as fibras de cânhamo podem apresentar uma melhora significativa em suas propriedades mecânicas e de barreira, o que torna este material ainda mais interessante para a sua aplicação como agente de reforço em matrizes poliméricas (Dixit *et al*., 2022).

Gupta *et al.* (2022) produziram biocompósitos a partir da mistura de fibras de cânhamo em matrizes de PBAT, PBAT modificado com anidrido maleico e PHBV pelo processo de extrusão. Como resultado, obtiveram filmes com melhor resistência mecânica em comparação com o PHBV original, o que possivelmente ocorreu porque o processo de extrusão reativa melhorou a interação interfacial entre a matriz fibrosa, o PHBV e o PBAT.

Lamsaf *et al*. (2023) adicionaram fibras de cânhamo a matrizes de PBAT, resultando em aumento da resistência mecânica dos filmes de PBAT, exercendo efeito de agete reforçador da matrz polimérica. Gheribi *et al*. (2023) relataram o emprego de fibras de cânhamo como reforço em materiais de embalagem à base de quitosana, resultando em aumento da resistência mecânica e decréscimo da permeabilidade ao vapor de água, indicativo de que os biocompósitos desenvolvidos tiveram suas propriedades melhoradas. Both *et al*. (2023), relataram o uso de fibras de cânhamo como reforço em biocompósitos por meio de uma reação de reticulação em matriz polimérica à base de pectina. As fibras de cânhamo foram reticuladas por meio de um processo de transesterificação entre a lignina presente nas fibras de cânhamo e a pectina, resultando em compósitos de fibra de cânhamo em um processo de etapa única.

3.3 HIDROGÉIS BIODEGRADÁVEIS

Os hidrogéis podem ser definidos como materiais formados por rede polimérica de estrutura tridimensional formada a partir da reticulação de polímeros hidrofílicos, com o objetivo de manter a sua integridade estrutural, permitindo a absorção e retenção de grande quantidade de água ou fluidos biológicos sem se desintegrar, podendo ser obtidos a partir de polímeros sintéticos ou biopolímeros (Cagnin *et al*., 2021; Chang *et al*., 2021; Deng *et al*., 2019; Erdagi; Ngwabebhoh, Yildiz, 2020; Heidarian *et al*., 2020; Nascimento *et al*., 2018; Tang *et al*., 2022).

Os hidrogéis podem ser preparados como membranas, filmes, hidrogéis injetáveis, microgéis, nanogéis, ou microesferas, dependendo da técnica de polimerização e preparação (Ahmed, 2015; Yazdi *et al*., 2020). A resistência à dissolução acontece em função das ligações cruzadas entre as cadeias poliméricas formadoras da rede tridimensional. Os hidrogéis, quando secos apresentam-se quebradiços, quando em contato com água, tornam-se um gel elástico, e a sua forma original é preservada (Ahmed, 2015; Biduski *et al*., 2018; Shahzamani, *et al*., 2020; Simões *et al*., 2020).

A interação entre as cadeias poliméricas e a água e/ou fluidos biológicos acontecem de três formas diferentes, por osmose, forças de hidratação ou forças capilares. Essas forças são contra equilibradas e acabam por causar uma expansão das redes poliméricas. Com isso, o equilíbrio na formação do hidrogel depende da magnitude dessas forças contrárias que também irão determinar algumas propriedades inerentes dos hidrogéis, tais como, resistência mecânica, transporte interno e difusão (Buwalda *et al*., 2014; Ye; Chang; Zhang, 2019; Varaprasad *et al*., 2017).

Os hidrogéis podem ser produzidos a partir de homopolímeros, que são formados apenas por um tipo de unidade monomérica; ou por copolímeros, que são formados por dois ou mais monômeros diferentes; ou por misturas poliméricas, que também podem ser utilizadas (Deng *et al*., 2019). Vários polímeros naturais e sintéticos têm sido relatados nas últimas duas décadas para a obtenção de hidrogéis com diferentes aplicações.

Os hidrogéis podem ser classificados com base em suas diferentes propriedades, como fonte de polímero, método de preparação, cargas iônicas, fontes, taxa de biodegradação, natureza e densidade da reticulação (Ahmed, 2015; Ullah *et al*., 2015). Independentemente do tipo ou origem do polímero ou do método de preparação do hidrogel, a introdução de ligações cruzadas (reticulação) deve ser realizada na matriz polimérica do hidrogel. De acordo com Ahmed (2015), qualquer técnica que pode ser usada para criar um polímero reticulado pode ser usada para produzir um hidrogel.

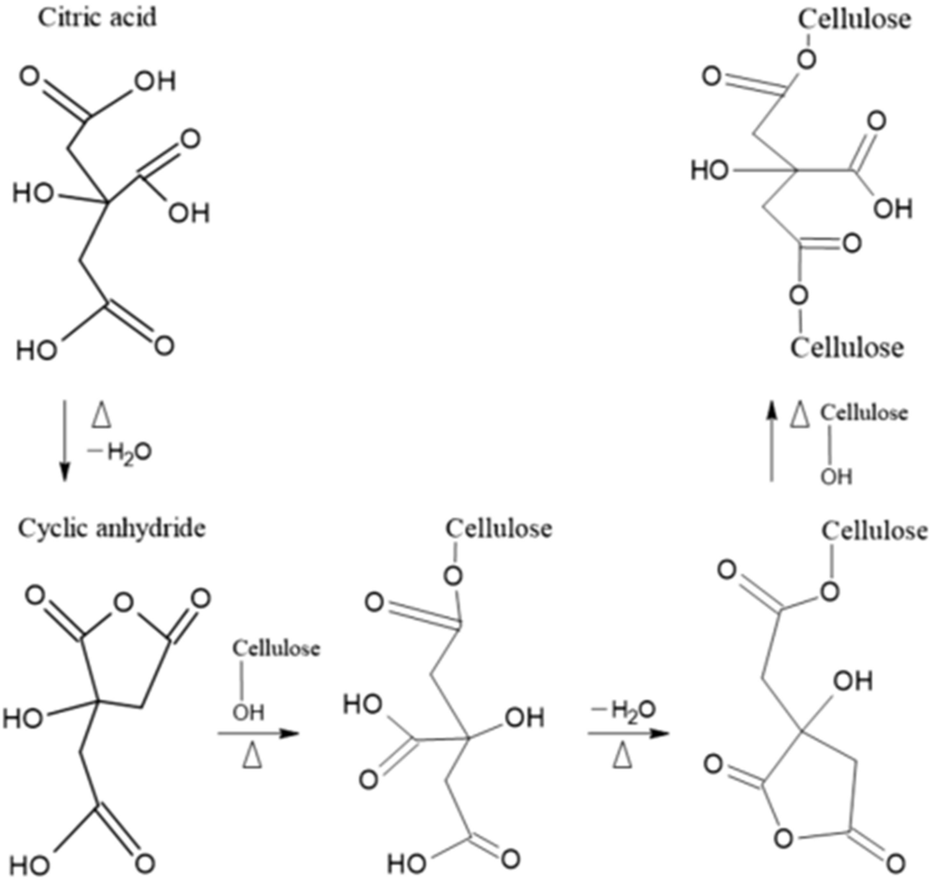
Os processos de reticulação física ou química são relatados na literatura. Os hidrogéis reticulados fisicamente são formados por interações transitórias formadas a partir do enovelamento das cadeias dos polímeros, ou a partir de interações físicas, como interações iônicas, ligações de hidrogênio ou interações hidrofóbicas. Essas interações são fracas e podem se interromper quando as condições favoráveis são alteradas (Ahmed *et al.,* 2015; Nascimento *et al*., 2018; Shahzamani, *et al*., 2020; Ullah *et al.,* 2015; Varaprasad *et al*. 2017).

Em matrizes poliméricas reticuladas quimicamente são introduzidas ligação covalentes permanentes entre as cadeias poliméricas, o que confere uma maior estabilidade em comparação aos hidrogéis obtidos por reticulação física (Heidarian *et al*., 2020). Um agente reticulante é uma molécula de massa molecular muito menor que as cadeias poliméricas envolvidas na formação do hidrogel, e que deverá apresentar pelo menos dois grupamentos reativos capazes de estabelecer ligações (pontes) entre as cadeias dos polímeros. Os grupamentos funcionais presentes nos polímeros que são responsáveis pelas ligações com os agentes reticulantes são os grupamentos amínicos (NH_2), hidroxila (-OH), amida (-CONH-, $-CONH_2$) e carboxila (-COOH), entre outros (Maitra; Shukla, 2014).

Vários agentes reticulantes têm sido empregados para a introdução de ligações covalentes em matrizes de hidrogéis, dentre os quais o formaldeído, glutaraldeído e epicloridrina. No entanto, estes reticulantes exibem um certo nível de toxicidade fisiológica tanto devido à presença de frações do reticulante, quanto dos subprodutos gerados durante a reação de síntese, havendo interesse no uso de novos agentes reticulantes de menor toxicidade (Erdagi, Ngwabebhoh, Yildiz, 2020; Maitra, Shukla, 2014).

Os ácidos orgânicos, em especial os ácidos policarboxílicos, tais como os ácidos cítrico e succínico, são considerados uma alternativa viável e ecoamigável como agentes reticulantes, sendo de baixo custo e a atóxicos (Golachowski *et al*., 2020; Miskeen *et al.,* 2021). O ácido cítrico é um ácido tri-carboxílico que vem sendo muito utilizado na funcionalização de biopolímeros, como o amido e a celulose (Garcia *et al*., 2014; Gil-Giraldo *et al*., 2021; Simões *et al*., 2020; Tao *et al*., 2021). O ácido cítrico vem sendo largamente explorado no campo da biomedicina por ser um material biocompatível e biodegradável (Salihu *et al*., 2021). A Figura 4 demonstra a reação de reticulação da celulose com ácido cítrico.

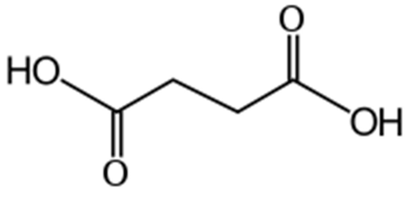
Figura 4 - Reação de reticulação entre celulose e ácido cítrico.



Fonte: SALIHU *et al.*, 2021.

O ácido succínico é um ácido di-carboxílico (Figura 5), que pode ser considerado um agente promissor para a modificação da celulose, uma vez que ele permite a introdução de ligações cruzadas entre as cadeias do polímero. Leszczyńska *et al*. (2019) relataram que a reação do ácido succínico com a celulose resultou em alterações nas suas propriedades físicas e químicas.

Figura 5- Estrutura do ácido succínico.



Fonte: Oliveira *et al*., 2013.

Os processos convencionais de reticulação têm sido realizados em reatores descontínuos ou de tanque agitado contínuo, com longos tempos de processamento, resultando em impactos ambientais dos efluentes gerados em todos os processos; portanto, o uso de métodos físicos associados aos métodos químicos pode ser uma alternativa interessante para minimizar o uso excessivo de reagentes (Cai *et al*., 2019).

3.4 Filmes Poliméricos

Os filmes biodegradáveis produzidos a partir de biopolímeros vêm sendo estudados ao longo dos anos com diversas aplicações, em especial como embalagens de alimentos (Alves *et al*., 2009; Fakhouri *et al*., 2003; Lucena *et al*.,2017,), como revestimentos comestíveis (Fakhouri *et al*., 2007; Krochta *et al*.,1994; Romio *et al*., 2017). Nos últimos anos, vários trabalhos têm relatado o uso dos filmes biodegradáveis na produção de biomateriais para aplicações médicas, como na produção de enxertos e próteses (Ulery *et al.,* 2011), ou ainda, em sistemas de liberação de fármacos (Dixil; Puthili, 2009; Ketul *et al.,* 2013; Patil; Shrivastava, 2014).

Os polissacarídeos e proteínas apresentam excelentes propriedades para a sua utilização como filmes em produtos direcionados à aplicação em alimentos, cosméticos e medicamentos. Além disso, são biocompatíveis, biodegradáveis, bioestáveis, abundantes e suscetíveis à digestão enzimática no corpo humano, resultando em materiais não citotóxicos (Rodriguez *et al.,* 2020; Vaparasad *et al*., 2017).

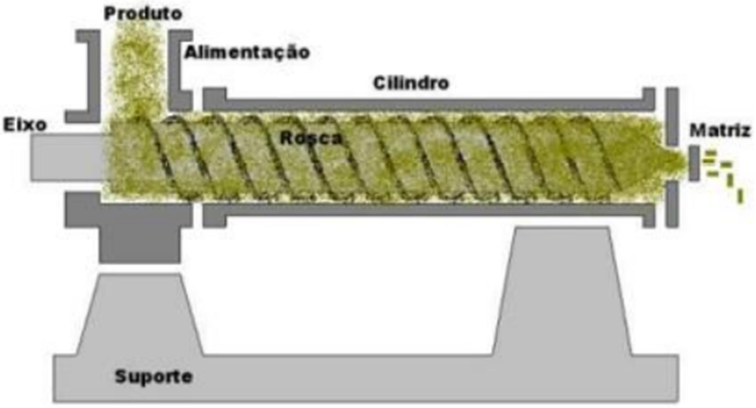
Os filmes devem possuir fácil manuseio e transporte, eles podem ser produzidos a partir de uma alta diversidade de macromoléculas, levando em consideração as propriedades fisico-quimicas do componente ativo de interesse (Dixit, Puthli, 2009; Hashide *et al.,* 2012; Nishigaki *et al*., 2012).

3.5 Extrusão Reativa Para Produção De Hidrogéis e Filmes

A extrusão é caracterizada como um processo térmico, que combina calor, umidade e trabalho mecânico a fim de gerar modificações estruturais nos materiais poliméricos, na forma física e nas suas características funcionais. Trata-se de um processo de transporte e mistura dos polímeros por uma rosca sem fim, no qual a rosca tem a capacidade de fundir, amolecer, homogeneizar e plastificar o polímero (Samaniego *et al*., 2022; Vahabi *et al*., 2022).

A extrusora monorosca (Figura 6) é constituída de uma rosca sem fim que fica dentro de um cilindro, o que possibilita o fácil manuseio. A mistura polimérica ocorre através da ação combinada de calor, pressão e atrito mecânico, as quais apresentam vantagens tais como, alta produtividade e ausência de efluentes (Merci *et al.,* 2015; Ye *et al*., 2019).

Figura 6. Esquema simplificado de uma extrusora monorosca.



Fonte: Merci *et al*., 2015.

A extrusão reativa é considerada uma técnica ecologicamente correta que pode ser usada para modificação química de polímeros, uma vez que nenhum solvente é empregado no processo, são empregados tempos de reação curtos, e o processo é escalonável para escala industrial (Gil-Giraldo *et al.,* 2021; Mantovan; Yamashita; Mali, 2022).

A utilização de extrusão reativa é um método possível de mistura de polímeros, como também de modificações químicas nos polímeros utilizados. Na literatura a utilização de extrusão reativa para a produção de hidrogéis ainda é limitada (Crippa, 2016; Todd,1988; Nascimento, 2016; Simões *et al*., 2020). Simões *et al*. (2020) utilizaram a extrusão reativa para a obtenção de hidrogéis a base de amido de mandioca e goma xantana, e a técnica se mostrou eficiente na produção dos hidrogéis. Nascimento (2016) utilizou a extrusão reativa para a produção de hidrogéis superabsorventes de amido e álcool polivinílico para aplicação agrícola na agricultura. Cagin *et al*. (2021) obtiveram hidrogéis de amido com carboxilmetil-celulose através da utilização de extrusão reativa e relataram resultados satisfatórios. Gil-Giraldo *et al.* (2021) relataram que o uso da extrusão reativa foi eficiente para introdução de ligações cruzadas na estrutura da celulose através da reação com ácido cítrico. Após a modificação, a celulose apresentou mudanças importantes na sua solubilidade, com aumento da hidrofobicidade e da capacidade de absorção de óleo.

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Artigo 1

O artigo 1 foi publicado em 2022:

Pereira, J.F. Chemical Modification of Cellulose Using a Green Route by Reactive Extrusion with Citric and Succinic Acids. Polysaccharides 2022, 3, 292-305. https://doi.org/10.3390/polysaccharides3010017

Abstract

Cellulose is a natural, unbranched, and fibrous homopolymer that is a major component in several agroindustrial residues. The aim of this study was to extract cellulose from oat hulls and then to modify it using a green route to obtain esterified cellulose through reaction with organic acids employing the reactive extrusion process. Citric (CA) and succinic (SA) acids were employed as esterifying agents in different concentrations (0, 5, 12.5 and 20%). Modified cellulose samples were characterized by their degree of substitution (DS), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (DRX), scanning electron microscopy (SEM), wettability, oil and water absorption capacities, and thermal stability. DS of modified samples ranged from 2.28 to 3.00, and FTIR results showed that the esterification occurred in all samples for both acids by observation of important bands at 1720 and 1737 cm^{-1} for samples modified with CA and SA, respectively. All modified samples presented improved hydrophobicity. The modification did not have influence on the morphological structure or crystallinity pattern of all samples. This study proved to be possible to modify cellulose using a simple and ecofriendly process based on reactive extrusion with organic acids, which has low effluent generation, short reaction times and scalable for large scale, and also expand its use in the industry.

Keywords: Reactive extrusion; Esterification; Lignocellulosic residues; Oat hull.

1. Introduction

Cellulose is one of the most abundant natural, renewable, and biodegradable polymers, it is an unbranched and fibrous homopolymer that can be obtained from plants or synthetized by bacterial. The cellulose chain consists of β-D-glucose units repetition joined by β (1-4) glycosidic bonds, with three hydroxyl groups per monosaccharide unit (Nguyen *et al*., 2020; Raucci *et al*., 2015; Zainal *et al*.,2020), which makes cellulose an excellent platform for chemical modifications (Gil-Giraldo *et al*., 2021). So, cellulose can be modified to be used in the cosmetic, pharmaceutic and food industries and in agricultural systems, among others (Capanema *et al*., 2018; Liu *et al*., 2006; Javanmard *et al*., 2020).

Agroindustrial residues can be considered interesting sources for cellulose extraction, and in the last few years there is an increased interest in the obtainment of cellulose from these materials using different approaches, which can be considered a promising alternative for the production of sustainable products at affordable prices to reduce the dependency on petroleum-based products (Das *et al*., 2021). The use of agroindustrial residues to obtain new products is inserted into the concept of biorefineries, meeting the vision of a sustainable economy using biological resources, maximizing benefits and profits through strategies to add value to the plant biomass chain (De Bhowick *et al*., 2018; Zuin *et al*., 2018).

Oat hull is a by-product from oat grain milling and represents 25 to 30% of oat grain weight, with approximately 28 - 35% of cellulose, 18 - 28% of hemicellulose and 18 - 22% of lignin (Debliagi *et al*., 2018; Debliagi *et al*., 2021; Gil-Giraldo *et al*., 2021), being considered an interesting raw material for cellulose obtainment.

Modifications in the cellulose structure have been largely studied to enhance its properties. Among all the possible modification types, esterification is one of the most reported approaches (De Cuadro *et al*., 2015; He *et al*., 2018). The esterified cellulose transfers from hydrophilic to hydrophobic nature due to the introduction of hydrophobic aliphatic branches (Ji *et al*., 2019).

In the last few years there has been great interest in green routes to obtain new materials from renewable sources, including modified cellulose. According to Liyanage *et al*. (2021), the use green technologies for the production of versatile materials can reduce carbon footprint. The use of organic acids such as citric or succinic acids has gained attention as esterifying and crosslinking agents for carbohydrate polymers in many aspects, they are safe, inexpensive, UV resistant, and biocompatible multifunctional monomers that are listed in the generally regarded as safe (GRAS) category by the US Food and Drug Administration (FDA) (Cui *et al*., 2020; De Cuadro *et al*., 2015; He *et al*., 2018; Ji *et al*., 2019; Liyanage *et al*., 2021; Quin *et al*., 2016; Salihu et al., 2021; Zainal *et al*., 2020).

Citric acid and other polycarboxylic acids had been described as efficient esterification and crosslinking agents for polysaccharides, such as cellulose. The reaction of cellulose with polycarboxylic acids occurs due to the attachment of the carboxylic group from acid via esterification with a cellulosic hydroxyl group, and also a further reaction via esterification with another cellulosic hydroxyl group can produce a crosslink between cellulose chains (Demitri *et al*., 2008; Gil-Giraldo *et al*., 2021; He *et al*., 2018; Salihu *et al*., 2021; Zainal *et al*., 2020). Although the catalytic mechanism of cellulose esterification with organic acids has not been completely elucidated, esterification catalyzed by α-hydroxy acids have been proposed to proceed via a nucleophilic attack of the acylant by the hydroxyl groups of the α-hydroxy acid, followed by second nucleophilic attack of the intermediate formed by the hydroxyl groups of celulose (Nguyen *et al*., 2020; Romeo *et al*., 2020).

According to Gil-Giraldo *et al*. (2021), reactive extrusion process is an efficient method for modifying cellulose and it is considered an ecofriendly technological solution since the extruder is used as a reactor, where chemical reactions such as esterification and crosslinking can be performed. Reactive extrusion combines the thermomechanical energy to promote the reaction between cellulose and organic acids in a single process without using other reagents. Additionally, reactive extrusion is a continuous process that has commercial viability, and it is easy to adapt to industrial large scales, offering short reaction times (2–3 min) (Formele *et al*., 2018; Moad *et al*., 2011).

The use of reactive extrusion to obtain esterified cellulose through reaction with organic acids has not been fully exploited in the literature.

The aim of this study was to extract cellulose from oat hulls and then to modify it using a green route to obtain esterified cellulose through reaction with organic acids (citric and succinic acids) employing the reactive extrusion process.

2. Materials and Methods

2.1. Materials

The oat hull was acquired from a local oat processing industry (SL Alimentos - Mauá da Serra-PR, Brazil). The citric acid (CA) and succinic acid (SA) of analytical grades were purchased from Synthlab (Synthlab, Diadema, Brazil), like all other reagents and solvents.

2.2.1. Extraction of the cellulose from oat hulls

The extraction of the cellulose was performed following the process described by Marim *et al.* (2021). Oat hulls (10 g) were treated with 250 mL of peracetic acid (50% acetic acid, 38% hydrogen peroxide, and 12% distilled water), the suspensions were maintained on a mechanical stirrer with a controlled temperature of 60 °C for 24 h. After this procedure, the samples were filtered, and washed with distilled water to reach pH 5-6 and dried at 35 °C for 24 h in an air circulation oven.

Cellulose and hemicellulose contents were determined by the Van Soest method (1965). Lignin contents were determined by the TAPPIT222 om-88 method (1999).

2.2.2. Modification of cellulose by reactive extrusion

The citric acid (CA) and succinic acid (SA) were used in different concentrations (0, 5.0, 12.5 and 20.0% – g acid/100 g cellulose) as esterifying agents. CA or SA in different concentrations were dissolved in distilled water and mixed with de cellulose (100 g), resulting in a final moisture content of 32% (g water/ 100 g cellulose). Each mixture was slowly added to sealed plastic bags and equilibrated for 1 h before extrusion. A control sample was extruded without any reagent other than water, resulting in the same final moisture content of 32%. The reactive extrusion parameters were based on a previous study of Gil-Giraldo *et al*. (2021). All samples were extruded in a single screw extruder (AX Plastics, Diadema, SP, Brazil) with a screw diameter of 1.6

cm and a screw length/diameter ratio (L/D) of 40, with four heating zones and a matrix of 0.8 cm in diameter. The temperature in all zones was 100 °C and the screw speed was 60 rpm. The modified cellulose was collected, placed in an oven, dried to constant weight at 45 °C, grounded and sieved through an 80-mesh sieve. The samples were washed three times with absolute ethanol to remove the unreacted CA or SA, and finally the washed samples were air-dried at 45 °C before being characterized. Cellulose samples prepared by reaction with CA were labeled CA5%, CA12.5% and CA20% throughout the study, while samples prepared with SA were labeled SA5%, SA12.5% and SA20% throughout the study.

2.2.3. Determination of the degree of substitution (DS)

The DS of the modified cellulose was determined using the method of Karnitiz *et al*. (2007). The carboxylic acid concentration (C_{COOH}) per gram of the modified cellulose was determined by retro titration using hydrochloride acid and sodium hydroxide. The DS was calculated according to the equation as follows: $DS = [(C_{NaOH} \cdot V_{NaOH}) - (4 \cdot C_{HCl} \cdot V_{HCl})] / Mm$”where: C_{NaOH} is the sodium hydroxide solution concentration (mmol/L), C_{HCl} is the hydrochloric acid solution concentration (mmol/L), V_{NaOH} is the sodium hydroxide volume (L), V_{HCL} is the hydrochloric acid volume spent on the titration of sodium hydroxide unreacted (L) and Mm is the cellulose mass used (g).

2.2.4. Fourier transform-infrared spectroscopy (FTIR)

The pulverized and dried samples were mixed with potassium bromide and compressed into tablets. The FTIR analyses were carried out with a Shimadzu FTIR - 8300 (Kyoto, Japan), which has a spectral resolution of 4 cm^{-1} and a spectral range of 4000–500 cm^{-1}.

2.2.5. X-ray Diffraction (XRD)

The crystallinity of each sample was investigated using XRD. The samples were finely powdered (particles 0.149 mm) using an analytical mill (IKA basic 23), and the analysis was performed using a PANalytical X'Pert PRO MPD diffractometer (Almelo, The Netherlands) with copper Kα radiation (λ = 1.5418 Å) under the operational conditions of 40 kV and 30 mA. All the assays were performed with a ramp rate of 1°/min. The relative crystallinity index (CI) was calculated as follows: CI (%) = [$(I_{200} - I_{am})$ .100/(I_{200})], where I_{200} is the intensity of the 200 peak (at approximately $2\theta = 20° - 22°$) and I_{am} is the intensity corresponding to the peak at $2\theta = 18°$.

2.2.6. Scanning electron microscopy (SEM)

SEM analyses were performed on the FEI Quanta 200 equipment (Hillsboro, USA). Samples were incubated in an air circulation oven (Marconi MA 035, Piracicaba, Brazil) at 60 °C for 3 h and then kept in desiccators containing anhydrous calcium chloride for 7 d. The dried samples were assembled for viewing on bronze stumps using double-sided tape. Afterward, the samples were covered with a thin layer of gold (40-50 nm) and an accelerated voltage of 20 kV was used for all samples.

2.2.7. Differential scanning calorimetry (DSC)

DSC analyses were performed on a Shimadzu DSC 60 (Kyoto, Japan) calorimeter. Approximately 3.0 mg of each sample were placed in platinum containers and heated from 30 to 450 °C at a heating rate of 5 °C / min in a helium atmosphere

2.2.8. Thermogravimetric analysis (TGA)

TGA of the samples were performed using the Shimadzu TGA-50 (Kyoto, Japan) equipment. The scans were performed from room temperature up to 600 °C with a heating rate of 20 °C/min under a nitrogen flow of 20 mL/min.

2.2.9. Wettability

Samples were mixed into two different systems with two immiscible solvents with different density values: 1) water (d: 1.000 g/cm^{-3}) and dichloromethane (d: 1.335 g/cm^{-3}) and water (d: 1.000 g/cm^{-3}) and chloroform (d: 1.490 g/cm^{-3}), allowing to observe the affinity of each sample for each solvent (Namazi *et al*., 2010).

2.2.10. Water absorption capacity (WAC) and oil absorption capacity (OAC)

WAC and OAC were determined following the methodology described by Gil-Giraldo *et al*. (2021). Approximately 0.5 g of each sample (M0) and 15 mL of water (or soybean oil) (M1) were added to a centrifuge tube. Then, the samples were kept in a water bath for 30 min and centrifuged for 30 min at 9000 rpm (Hettich Centrifuge, Universal model 320R, Darmstadt, Germany). The non-adsorbed water (or soybean oil) (M2) was removed, and the water (or soybean oil) absorbed by the samples was estimated as the difference between M1 and M2. WAC was calculated as: WAC (g/g) = (M1 − M2)/M0 and OAC was calculated as: OAC (g/g) = (M1 − M2)/M0.

2.2.11. Statistical Analysis

Analyses of variance (ANOVA) and Tukey's mean comparison test ($p \leq 0.05$) were performed with R program.

3. Results

Oat hull bleaching was performed with peracetic acid to obtain cellulose. Raw oat hull presented 26% cellulose, 30% hemicellulose and 21% lignin, and these results are in agreement with other authors (Debiagi *et al*., 2015; Gil-Giraldo *et al*., 2021; Paschoal *et al*., 2015). After bleaching with peracetic acid, the obtained sample presented a composition of 78% cellulose, 8% hemicellulose and 3% lignin, and this sample was labeled as cellulose, and it was employed in this study to obtain the modified samples by reactive extrusion with organic acids.

3.1. Degree of substitution (DS)

Esterification implies the substitution of hydroxyl groups of cellulose by less polar ester groups, and the DS is an indicative of the substitution level. DS can be defined as the average number of substituted hydroxyl groups per glucose unit and it has a maximum value of 3 (Nguyen *et al*., 2020; Ratanakamnuan *et al*., 2012; Romeo *et al*., 2020). DS results of cellulose samples modified by reactive extrusion are shown in Table 1, and it is possible to observe that the DS values increased with increase of CA and SA concentration, and also that DS values were not affected by the type of acid employed.

Ratanakamnuan *et al*. (2012) reported similar DS values that the obtained in this study, their values ranged from 2.41 and 2.69 for cotton cellulose esterified with several fatty acids (C4 to C12) under microwave heating, and they stressed that shorter reaction times are required to obtain samples with higher DS when the microwave power increases. Hoang *et al*. (2020) and Ji *et al*. (2019) reported that in esterification of cellulose with citric acid the increased concentration of the acid enhances the interaction between carboxylic groups of citric acid and hydroxyl groups of cellulose. Xin *et al*. (2017) presented DS results ranging from 0.337 to 1.191 in cellulose succinates under a catalyze-free condition. Qin *et al*. (2016) reported that cellulose succinates obtained by reaction with SA performed in a high-energy stirring ball mill presented higher degrees of substitution when subjected to higher SA concentrations.

Table 1. Degree of substitution of all samples.

Sample	**Degree of Substitution**
CA5%	2.36
CA12.5%	2.40
CA20%	3.00
SA5%	2.28
SA12.5%	2.59
SA20%	3.00

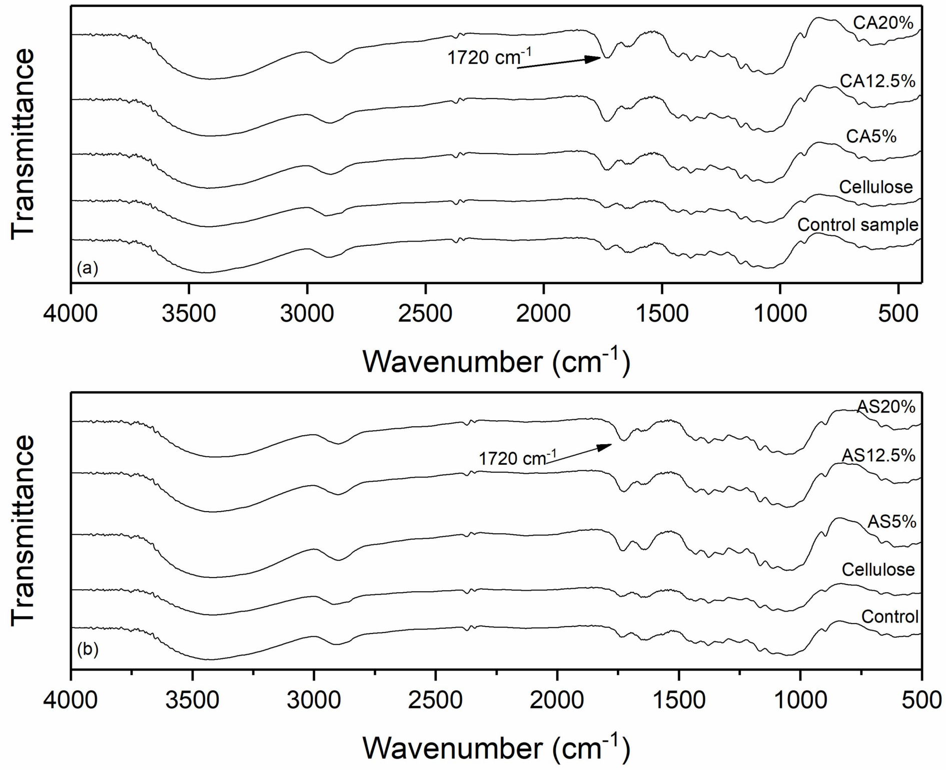
3.2. Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectra of cellulose, control sample and modified cellulose with CA and SA are shown in Figures 1 a. It is possible to see that all samples that were subjected to reactive extrusion with CA and SA presented a pronounced band located between 1720 and 1737 cm^{-1}, which can be associated with the C=O stretching of carbonyl in the ester bonds.

Being an indicative that esterification using CA and SA occurred. These results are similar to those presented by other authors (Cui *et al*., 2020; He *et al*., 2018; Gil-Giraldo *et al*., 2021; Javanmard *et al*., 2020; Ji *et* *al*., 2019; Liu *et al*., 2006; Qin *et al*., 2016; Romeo *et al*., 2020; Xin *et al*., 2017), who also used CA or SA as esterifying agents for cellulose, these authors reported that bands between 1720 to 1750 cm^{-1} can be used to show the success of cellulose esterification.

For cellulose and control sample this band appeared with lower intensity (Figures 1 and 2). Considering that cellulose was extracted from oat hulls, and it remained with 8% of hemicellulose, this band in cellulose and control samples (extruded without organic acids) possible corresponded to the acetyl or uronic groups of the hemicelluloses (Marim *et al*., 2021). Gil-Giraldo *et al*. (2021) reported that this band can also be explained by the thermomechanical treatment applied to cellulose during extrusion, being attributed to the C=O group from the opened terminal glucopyranose rings.

Figure 1. FTIR spectra of cellulose, control sample, and modified cellulose with CA (a) and FTIR spectra of cellulose, control sample, and modified cellulose with SA (b).

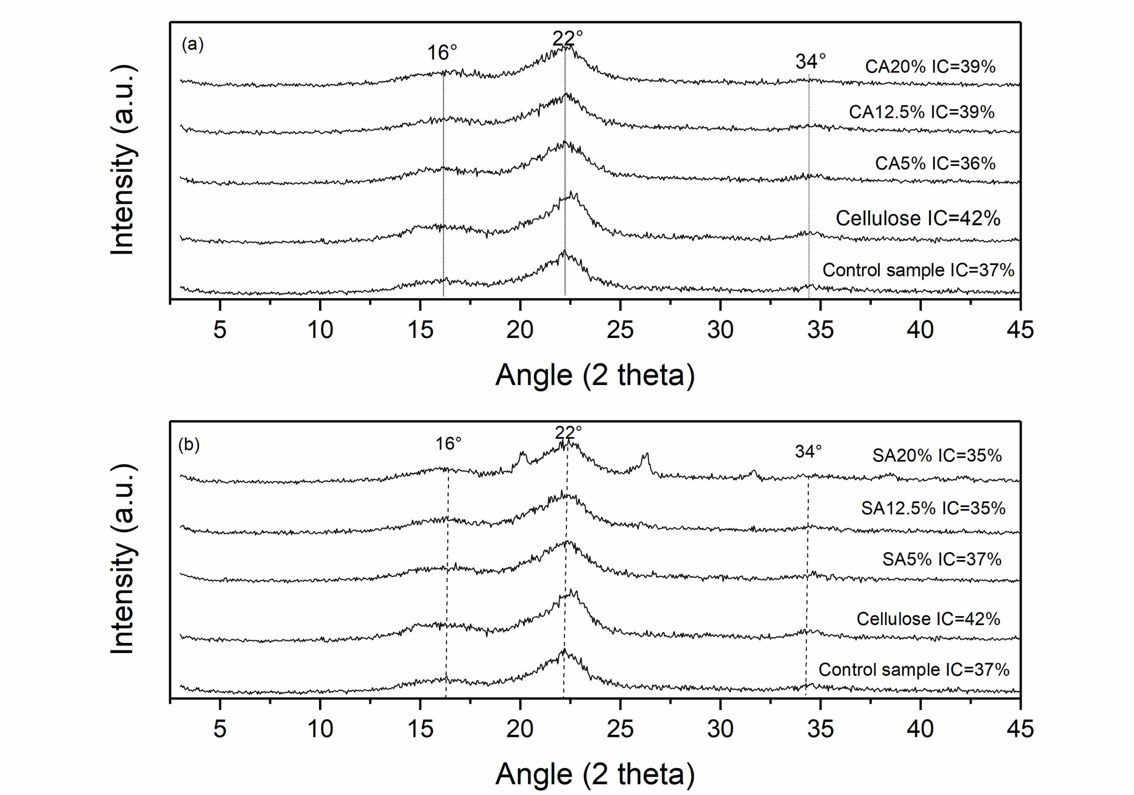


All samples presented a broad band near of 3500 cm^{-1} that can be assigned to O–H stretching groups. The band at 2900 cm^{-1} is due to the C-H stretching (Gil-Giraldo *et al*., 2021; Liu *et al*., 2006). All the bands between 1057 and 1162 cm^{-1} are corresponding to C–O and C–O–C stretching vibration in cellulose (Debiagi *et al*., 2020; Gil-Giraldo *et al*., 2021; Marim *et al*., 2021).

3.3. X-ray Diffraction (XRD)

The X-ray diffractograms and relative crystallinity indexes (IC) of cellulose, control sample, and modified cellulose with CA and SA are shown in Figure 2. It is possible to see that all samples presented characteristic peaks of cellulose type I, at $2\theta = 16, 22$, and 34^{o} (Debiagi *et al*., 2020; Gil-Giraldo *et al*., 2021; Qin *et al*., 2016; Ratanakamnuan *et al*., 2012). The extrusion process with CA and SA employed in this study did not affect the polymorph type of cellulose compared to cellulose sample. Other authors that subjected cellulose extracted from oat hulls to modification by reactive extrusion also reported that this process did not affect the inherent crystalline structure of extruded samples (Debiagi *et al*., 2020; Gil-Giraldo *et al*., 2021). Ji *et al*. (2019) reported that esterification of nanocellulose with CA has no influence on the position of peaks and no changes on the crystalline allomorph of the cellulose. Gil-Giraldo *et al*. (2021) also reported that esterification with CA possible occurred mainly at the cellulose surface, without affecting the inner crystalline structure. According to Qin *et al*. (2016), chemical reagents are difficult to enter into the crystalline region of cellulose because of its stable structure.

Figure 2. X-ray diffractograms of cellulose, control sample, and modified cellulose with citric acid (a), and X-ray diffractograms of cellulose, control sample, and modified cellulose with succinic acid (b).



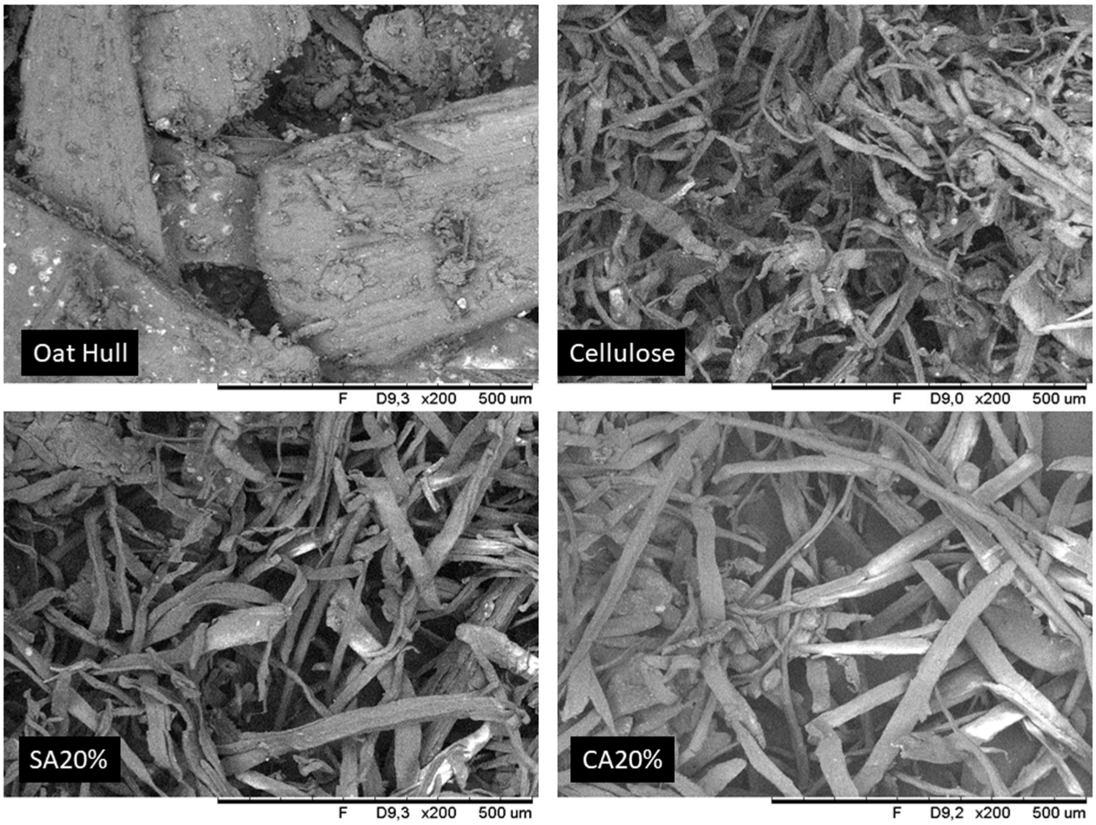
The crystallinity index (CI) of cellulose was 42%, which is typical of a semicrystalline material, and a very close value (43%) was reported by Gil-Giraldo *et al*. (2021) for cellulose from oat hull. The CI for all extruded samples (with and without reagent) slightly decreased, and the CI values ranged from 37 to 39% for samples modified with CA (Figure 4) and for 36 to 37% for samples modified with SA (Figure 5). During the reactive extrusion process, the cellulose fibers were exposed to high temperatures and high shear forces, however, these processing conditions did not affect the crystallinity pattern of the modified samples. These results agreed with other authors that used reactive extrusion to esterify cellulose (Gil-Giraldo *et al*., 2021). Otherwise, Qin *et al*. (2016) reported a decrease in CI of cellulose esterified though reaction with SA in a high-energy stirring ball mill, and they attributed this decrease to the disruption of the intramolecular and intermolecular hydrogen bonds in cellulose, which effectively can destroy its crystalline structure.

3.4. Scanning Electron Microscopy (SEM)

Figure 3 shows the SEM images of oat hull, cellulose and cellulose samples modified with citric acid 20% (CA20% sample) and succinic acid 20% (SA20% sample). It is possible to observe that raw oat hull presented a compact and uniform structure, with the fibers bundled with hemicellulose and lignin components, which are typical for lignocellulosic materials (Gil-Giraldo *et al*., 2021; Liu *et al*., 2006; Marim *et al*., 2021; Setyawan *et al*., 2017). The extraction process with peracetic acid removed the external layer composed mainly by hemicellulose and lignin, as result the cellulose sample showed a different morphology, with long and individualized fibers (Abraham *et al*., 2011; Gil-Giraldo *et al*., 2021; Paschoal *et al*., 2015).

Both CA and SA samples presented long and individualized fibers, showing no difference from the cellulose sample, and these results were consistent with XRD results, that showed that reactive extrusion with the organic acids did not affect the crystallinity pattern of cellulose. Gil-Giraldo *et al*. (2021) subjected cellulose to reactive extrusion with CA employing a higher acid concentration (40%) and also reported that the surface morphology of the cellulosic fibers did not change. He *et al*. (2017) also reported that morphology of nanocellulose was not significantly affected by esterification, suggesting that only the surface of the cellulose was modified during reaction with citric acid. Cui *et al*. (2020) reported a slightly increase in roughness on the cellulose fiber surface after esterification with CA.

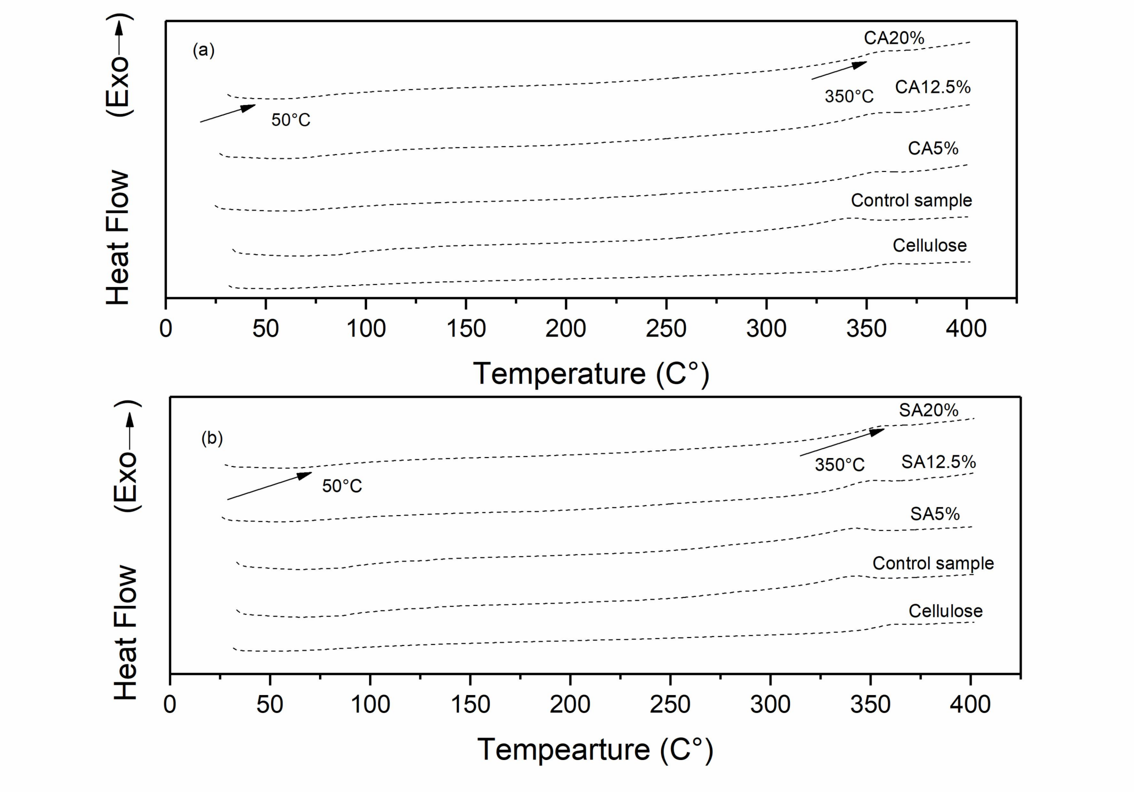
Figura 3. SEM images of oat hull, cellulose, and cellulose modified with CA 20% and SA 20%.



3.5. Differential scanning calorimetry (DSC)

Figure 4 present the DSC curves of cellulose, control sample and modified cellulose by CA and SA, respectively. All samples showed an endothermic peak near 50 °C, which can be associated with water loss (Demitri *et al*., 2008; Gil-Giraldo *et al*., 2021). It is possible to observe that all samples showed an exothermic peak near 350 °C which can be attributed to the cellulose depolymerization, this event was also reported by other authors (Agu *et al*., 2017; Gil-Giraldo *et al*., 2021), and it can be observed that the esterification of cellulose by reactive extrusion did not affect the thermal stability observed from DSC curves.

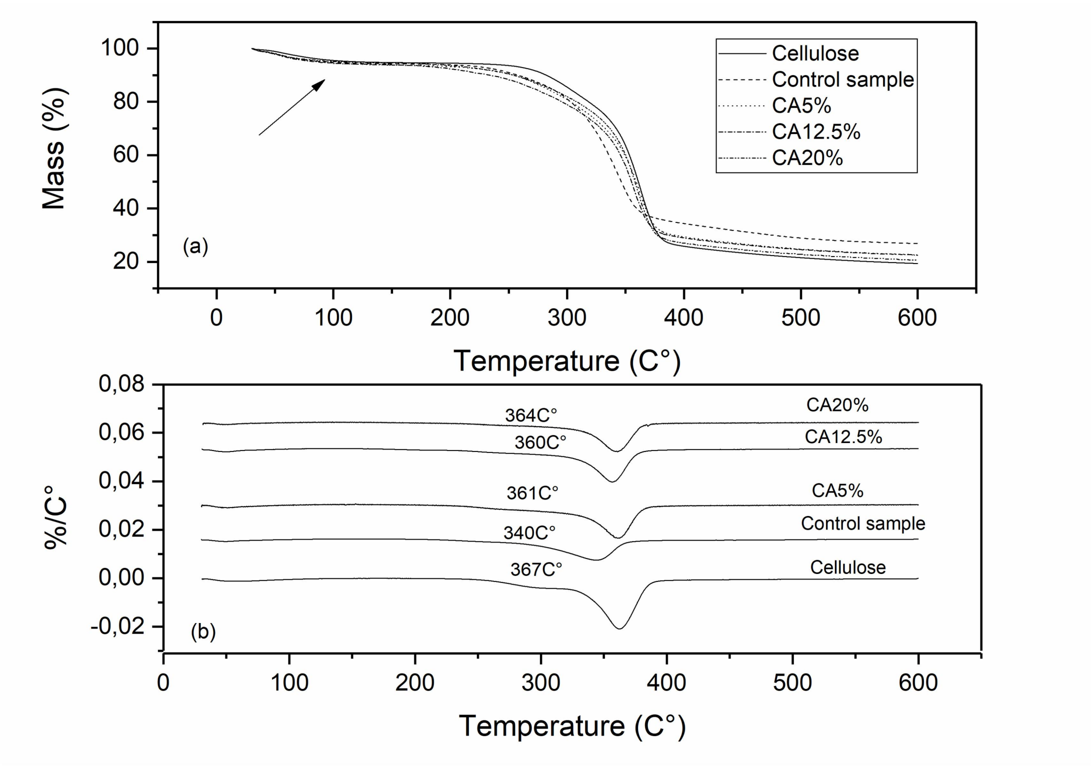
Figure 4. DSC curves of cellulose, control sample and cellulose modified with citric acid (a), and DSC curves of cellulose and succinic acid modified cellulose (b).



3.6. Thermogravimetric analysis (TGA)

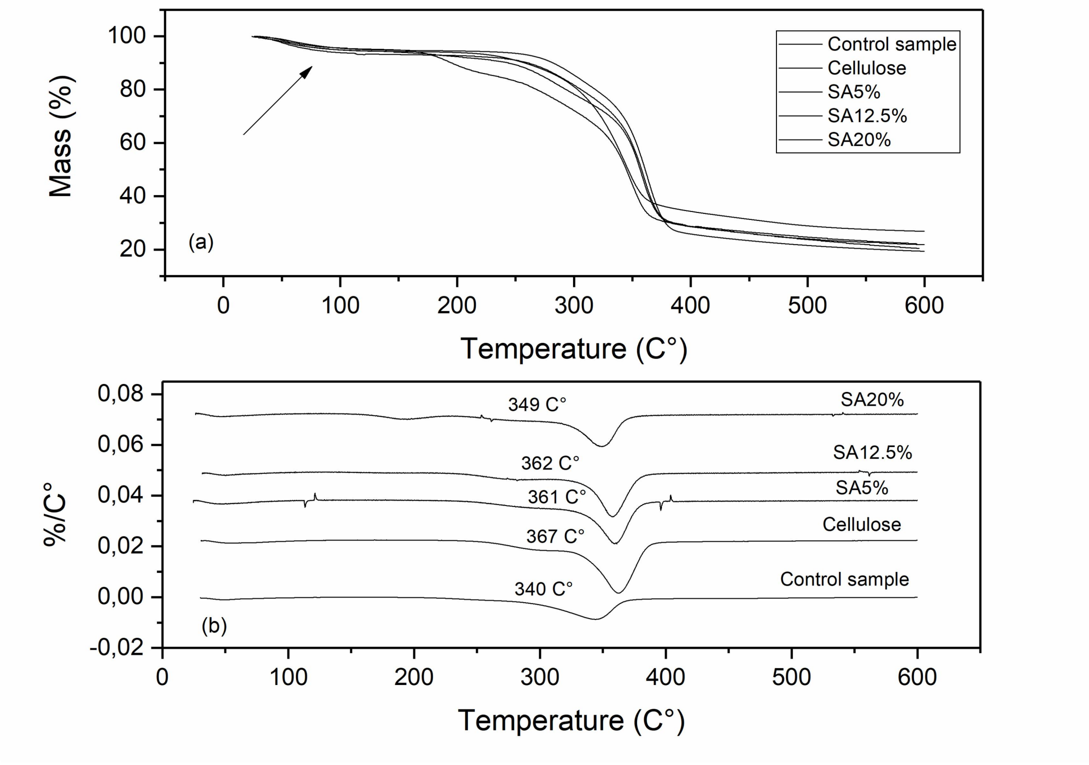
TGA and DTGA curves from cellulose, control samples, and cellulose samples modified with CA and SA are shown in Figures 5 and 6, respectively. All samples presented a small first degradation stage at 50-100 °C (Figures 5a and 6a), which can be attributed to the loss of water of samples (Capanema *et al*., 2018; Gil-Giraldo *et al*., 2021).

Figure 5. TGA (a) and DTGA (b) curves of cellulose, control sample and cellulose modified with citric acid.



All samples started to decompose near 250 °C, and it can be observed that for unmodified cellulose the temperature that correspond to the maximum weight loss rate was 367 °C, and for all modified samples this value decreased; samples modified with CA had the maximum weight loss rate ranging from 360 to 364 °C, and for samples modified with SA these values ranged from 349 to 362 °C (Figures 5b and 6b), which is an indicative of a decrease in thermal stability of the modified samples. Ji *et al*. (2019) also reported that thermal stability of nanocellulose decreased after esterification with CA.

Figure 6. TGA (a) and DTGA (b) curves of cellulose, control sample and cellulose modified with succinic acid.



3.7. Wettability

Figures 7 and 8 shows the wettability results in the two tested systems, water/dichloromethane and water/chloroform, respectively. The wettability property is directly influenced by the polar and non-polar groups presents on the molecules surface (Gil-Giraldo *et al.*, 2021; Hubbe *et al.*, 2015; Shi *et al.*, 2018).

Figure 7. Dispersion of cellulose, control sample, and modified cellulose with CA and SA in a water/dichloromethane system.

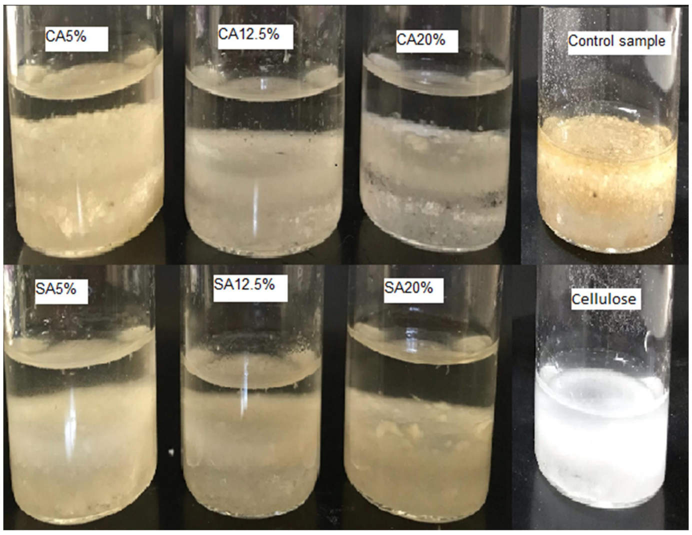
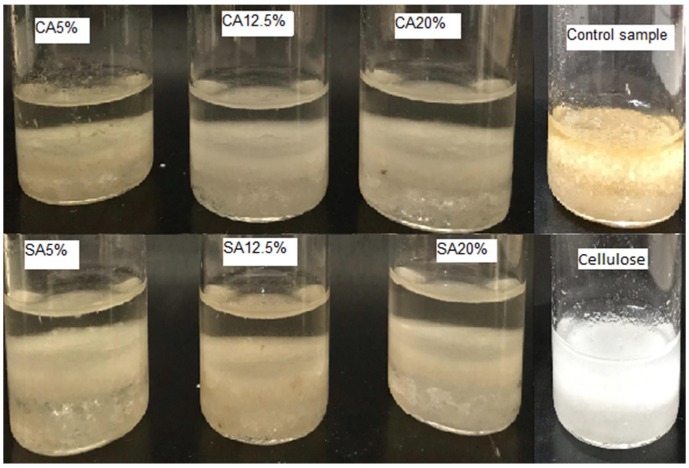


Figure 8. Dispersion of cellulose, control sample and modified cellulose with CA and SA in a in a water/chloroform system.



As can be seen in Figures 7 and 8, cellulose and control samples showed affinity for water (polar solvent), while all the modified samples showed affinity for dichloromethane and chloroform, which are non-polar solvents, indicating that the hygroscopic character of cellulose changed by chemical modification through reaction with CA and SA, with new hydrophobic properties being evident. According to Hubbe *et al*. (2015) the substitution of polar hydroxyl groups in the cellulose surface for less polar groups can affect the wettability results. He *et al*. (2018) reported increases in cellulose hydrophobicity upon esterification with CA. Gil-Giraldo *et al*. (2021) also observed an increased affinity by non-polar solvents when cellulose was esterified by reactive extrusion with CA. According to Adewuyi and Pereira (2017), the poor hydrophobicity of cellulose limits its application, and this limitation can be overcome by surface modification of cellulose, replacing its hydroxyl groups, which can be an effective to improve its hydrophobicity.

3.8. Water absorption capacity (WAC) and oil absorption capacity (OAC)

WAC and OAC results are shown in Table 2. For the modified cellulose, WAC values ranged from 6.46 to 7.13 (g/g). When compared to cellulose (9.27 g/g) and control sample (8.55 g/g), all the modified samples present significantly lower WAC values (Table 2). It is important to observe that the type of acid or the acids concentration did not affect significantly the WAC values of modified samples.

Table 2. Water absorption capacity and oil absorption capacity of samples.

Samples	WAC (g/g)	OAC (g/g)
Cellulose	$9.27^{a} \pm 0.07$	$1.80^{e} \pm 0.01$
Control Sample	$8.55^{a} \pm 0.01$	$4.25^{d} \pm 0.61$
CA5%	$6.46^{f} \pm 0.01$	$7.26^{bc} \pm 1.33$
CA12.5%	$7.13^{bd} \pm 0.01$	$9.07^{a} \pm 0.55$
CA20%	$6.78^{df} \pm 0.05$	$9.94^{a} \pm 0.32$
SA5%	$7.49^{b} \pm 0.03$	$8.88^{ab} \pm 0.79$
SA12.5%	$6.97^{cde} \pm 0.05$	$8.81^{ab} \pm 0.15$
SA20%	$7.35^{bc} \pm 0.02$	$9.10^{a} \pm 0.11$

Different letters on the same line indicate statistical difference by Tukey's test ($p \leq 0.05$).

From the OAC results (Table 2) it is possible to see that the cellulose sample presented the lowest OAC value (1.80 g/g), while the modified samples CA 12.5%, CA 20% and SA 20% showed the highest values, and it can also be observed that the increase of acids concentrations resulted in samples with higher OAC. This increase in hydrophobic capacity of modified samples can be attributed to the decrease of free hydroxyl groups on the cellulose surface by reaction with CA and SA. Adewuyi and Pereira (2017) in their study with modified cellulose through reaction with suberic acid also reported the increase in hydrophobicity of modified samples, which was observed by the decrease in WAC and increase in OAC values. Gil-Giraldo *et al*. (2021) in their study with modified cellulose with CA also described lower WAC and higher OAC values for cellulose modified with CA.

4. Conclusions

All the results showed it was possible to obtain cellulose samples with increased hydrophobicity after employing a reactive extrusion process to perform the esterification of cellulose with two organic acids, citric and succinic acids. FTIR test confirmed the modification for all formulation tested by the appearance of a new band at 1735 cm^{-1}. Regardless of the type of acid used, all modified samples showed higher affinity for non-polar solvents, and increased oil absorption capacity.

The process of modifying cellulose using reactive extrusion and organic acids proved to be possible. When compared to the conventional processes employed to obtain esterified cellulose this is an ecofriendly and efficient alternative for cellulose modification, with some really good advantages such as low effluent generation, short reaction times, lower investment costs and the simplicity of operation due to its continuous nature.

Acknowledgments: The authors would like to thank the State University of Londrina facilities at the Multiuser Laboratories Center (CMLP-UEL FINEP), where X-ray, FT-IR, and microscopy analyses were performed.

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Artigo 2

O artigo 2 foi publicado em 2023.

Pereira, J F. Hydrogels based on gelatin, xanthan gum, and cellulose obtained by reactive extrusion and thermopressing processes. Preparative Biochemistry & Biotechnology, 2023. https://doi.org/10.1080/10826068.2022.2162921

Abstract

Polysaccharides and proteins are compatible macromolecules that can be used to obtain biopolymeric hydrogels through physical interactions. In this study, an environmentally friendly strategy is being proposed to produce gelatin-xanthan gum- cellulose hydrogels, without the addition of chemical synthetic crosslinkers. Xanthan gum was employed as an alternative crosslinking agent, and cellulose was used as a potential reinforcing agent in the polymeric matrix. Firstly, the biopolymers were mixed by the extrusion process, and glycerol was used as a plasticizer. Then, the polymeric mixture was molded by thermopressing to obtain hydrogels as laminated films. All hydrogels formulations resulted in films with smooth surfaces, without pores or cracks, resulting in amorphous polymeric matrices. The obtained hydrogels had a pH-dependent degree of swelling, the highest swelling values were obtained at pH 4 (5.3–7.9 g/g) after 24 h of immersion. Cellulose acted as a reinforcing agent for hydrogels, increasing thermal stability, tensile strength, and Young's modulus of films when employed at the higher level (7%). The strategy employed in this study to obtain nontoxic hydrogels without synthetic crosslinkers was effective, resulting in materials with promising properties to be used as pharmaceutical forms to deliver active compounds in cosmetic or pharmaceutical products.

Keywords: Reactive extrusion, Termopressing, Hydrogel, Biopolymers.

1. Introduction

Advances in biopolymers technology have led to the growing interest in the development of biopolymeric hydrogels, which are a unique class of material that is produced from hydrophilic three-dimensional viscoelastic polymeric networks, allowing the diffusion and fixation of molecules (Cascone *et al*., 2020; Mitura *et al*., 2020). These networks are maintained due to the presence of physical and/or chemical bonds between the polymer chains, presetting an inherent capacity to swell and retain water in their structure, while having resistance to dissolution (Rodriguez *et al*., 2020).

A superabsorbent hydrogel can absorb over 100% of its dry weight in water (Batista *et al*., 2019). The most common commercial hydrogels are based on synthetic polymers, including polyacrylamide, poly (sodium acrylate), poly (acrylic acid), and polyvinylpyrrolidone. These synthetic matrices are bounded by chemical crosslinking, however, the usual crosslinkers may cause some toxicity (Ahmad *et al*., 2022; Batista *et al*., 2019; Berger et al., 2004). Glutaraldehyde and glyoxal are dialdehydes usually employed as crosslinkers; glutaraldehyde is reported as a neurotoxic compound and glyoxal is considered mutagenic (Berger *et al.,* 2004).

Several efforts have been made to increase hydrogel production from biodegradable polymers (Batista *et al*., 2019; Cascone *et al*., 2020; Mitura *et al*., 2020; Rodriguez *et al*., 2020). Additionally, the obtainment of physically crosslinked hydrogels can be considered a solution to overcome the toxicity of chemical crosslinkers, resulting in a biocompatible polymeric structure that gets broken down into nontoxic substances, being able to be use in cosmetic and pharmaceutical sectors (Ahmad *et al*., 2022; Berger *et al.,* 2004; le *et al*., 2013; Le *et al*., 2015; Le *et al*., 2017).

Polysaccharides and proteins are compatible biomacromolecules that can be used to obtain hydrogels by physical interactions, including electrostatic interactions when these biopolymers carry net opposite electric, hydrogen interactions, hydrophobic or Van der Waals forces (Alavarse *et al*., 2022; Batista *et al*., 2019; Le *et al*., 2017). In general, polysaccharides and proteins mixed systems can result in hydrogels with high water absorption capacity, which is related to the presence of hydrophilic groups present in their chemical structure, such as amine (NH_2), hydroxyl (–

OH), amide (–CONH–, –$CONH_2$), carboxyl (–COOH), among others (Le *et al*., 2013; Le *et al*., 2015; Le *et al*., 2017).

Gelatin is a low-cost protein obtained from partial hydrolysis of collagen from skin, bones or connective tissue of animals, being classified as type A (acid hydrolysis) or type B gelatin (alkaline hydrolysis). Gelatin exhibits an amphoteric behavior due to the presence of both basic and acidic groups, presenting in its composition a large number of glycine, proline and hydroxyl-proline residues. It is widely used in food, cosmetic and pharmaceutical industries (Laffleur *et al*., 2019; Rodriguez *et al*., 2020). It is easily soluble in water at an average temperature of 40 °C, forming a viscous solution by chain association and threedimensional network formation, resulting in gels and films employed for several applications (Laffleur *et al*., 2019; Wang *et al*., 2016). Xanthan gum is the most important commercial bacterial polysaccharide, and it is mostly produced by Gram-negative Xanthomonas campestris (Petri *et al*., 2015).

Xanthan gum is a negatively charged heteropolysaccharide with a main chain consisting of β(1→4)linked D-glucose residues, and a trisaccharide side chain that contains a D-glucuronic acid unit between two D-mannose units attached to alternate D-glucose residues (Sethi *et al*., 2015). One-half of the terminal D-mannose contains a linked pyruvic acid, and the D-mannose unit linked to the main chain can contain a linked acetyl group (Sethi *et al*., 2015; Simões *et al*., 2020).

Cellulose is a linear homopolysaccharide formed by the repetition of β -D-glucose units joined by β(1→4) bonds, with three free hydroxyl groups per monosaccharide unit (Gil-Giraldo *et al*., 2021). Cellulose esterification is one of the most studied modifications, which can be performed with nontoxic and renewable source reagents, such as organic polycarboxylic acids (Alavarse *et al*., 2022; Gil-Giraldo *et al*., 2021; Pereira *et al*., 2022), resulting in hydrophobic materials that can be efficiently used to reinforce biopolymeric matrices. Cellulose and cellulose derivatives can be successfully used in biodegradable hydrogel formulations as reinforcing agents (Cagnin *et al*., 2021; Capanema *et al*., 2018; Fu *et al*., 2019; Javanbakt *et al*., 2018; Koneru *et al*., 2020). The use of polymeric mixtures to obtain new hydrogels is a simple and viable strategy to combine the advantages of different materials, improving their properties (Antonioli *et al*., 2018).

The use of protein and polysaccharides mixtures in aqueous systems has been reported in the literature, including gelatin-xanthan gum (Lii *et al*., 2002; Harirah *et al*., 2016; Wang *et al*., 2016; Wang *et al*., 2017) and β -lactoglobulin-xanthan gum mixtures (Le *et al*., 2013; Le *et al*., 2015). Other studies reported the production of films and hydrogels from gelatin, xanthan gum, and carboxymethyl cellulose (Harirah *et al*., 2016), and gelatin-xanthan gum mixtures by casting method (Demir *et al*., 2022; Guo *et al*., 2014; Shawan *et al*., 2019). Hazirah et al. (2016) reported that xanthan gum can be used as an alternative crosslinking agent in gelatin-carboxymethyl films, resulting in a compatible blend, however, a major concern of the use of xanthan gum is its dispersion in high solids concentrations (Sereno *et al.,* 2007; Zeng *et al*., 2021).

Biodegradable hydrogel production by the casting method is extensively reported in the literature, on other hand, the use of extrusion and thermopressing processes is little exploited. According to Simões *et al*. (2020) extrusion and thermopressing subject the materials to thermal and mechanical combined energies, promoting chemical and physical reactions between polymers.

Additionally, extrusion can be considered an eco-friendly technological solution to obtain polymeric mixtures with higher solids contents (Sereno *et al*., 2007; Zeng *et al*., 2021). This process combines thermal and mechanical energies to promote the mixture and physicochemical modification of materials in a single step, with some advantages that include a continuous process easily adaptable to large industrial scales, short reaction times (2- 3 min), and low effluents generation (Gil-Giraldo *et al*., 2021; Simões *et al*., 2020; Pereira *et al*., 2022).

Guerrero *et al*. (2014) reported that proteins denature during extrusion, which allows intermolecular interactions between proteins and polysaccharides by hydrogen interactions. Sereno *et al*. (2007) and Zeng *et al*. (2021) reported that extrusion of xanthan gum can favor its dispersion at higher solids contents; they stressed that during extrusion, xanthan gum loses its helical ordered conformation, and after hydration extruded xanthan can forms particles that behave as superswelling polyelectrolyte gels.

In this study, a simple and environmentally friendly strategy is being proposed to produce biobased hydrogels without addition of chemical synthetic crosslinkers. Xanthan gum was employed to act as an alternative crosslinking agent, and cellulose was used as potential reinforcing agent for the polymer matrix, resulting in a gelatin-xanthan gumcellulose mixture. In a first step, the biopolymers were mixed by the extrusion process, and glycerol was used as a plasticizer. Then, the polymeric mixture was molded by thermopressing to obtain hydrogels in the form of laminated films, which were characterized in terms of their physicochemical, mechanical, and thermal properties. The use of this strategy allowed the mixing of three biopolymers at higher solids contents, which would be unfeasible in aqueous systems given the difficulty of dissolving them in high concentrations. Additionally, in this study we utilized renewable source reagents and polymers to obtain the biobased hydrogels, aiming its future use as a potential pharmaceutical form to deliver active compounds in cosmetic or pharmaceutical products.

2. Materials and methods

2.1 Materials

Oat hull was kindly donated by a local industry (SL Alimentos - Mauá da Serra-PR, Brazil). Succinic acid (SA) and xanthan gum of analytical grade were purchased from Synthlab (Synth, Brazil). Gelatin (type B) was purchased from Biotec (Brazil). All reagents employed in this study were of analytical grade.

2.2. Preparation of Hydrogels

Hydrogels formulations were prepared according the methodology from Simões *et al*. (2020). The hydrogels films were prepared in three stages, according to the formulations shown in Table I.

Table 1- Formulations used in the preparation of 100 g of hydrogels formulation used to prepare the hydrogels.

Samples	**Formulation 1 (g/100g)**	**Formulation 2 (g/100g)**	**Formulation 3 (g/100g)**	**Formulation 4 (g/100g)**
Gelatin	51	51	51	51
Native cellulose	5	-	7	-
Modified cellulose	-	5	-	7
Xanthan gum	7	7	7	7
Glycerol	35	35	35	35

In the first stage, for the pellets production, cellulose, gelatin, and xanthan gum in different concentrations were dissolved in glycerol and then manually mixed. The final mixtures were processed using a laboratory single-screw extruder (model EL-25, BGM, São Paulo, Brazil) with a screw diameter (D) of 25 mm and screw length of 26D, through a die with six circular 2 mm 5 diameter holes at temperatures of 90/100/110/100 °C, from the feeding zone to the die zone, and a screw speed of 35 rpm. Then, the obtained profiles were pelletized. In the second stage, the pellets were reprocessed in the same extruder, using a flat die with a 5 mm x 130 mm (thickness and width, respectively) gap, at temperatures of 90/100/110/100 °C and screw speed of 35 rpm, resulting in partially homogeneous sheets. Finally, to obtain the hydrogel in films, approximately 8 g of the extruded pieces were placed between two sheets of cellulose acetate and pressed in a hydraulic press (JOMAQ, model PHB 200, Franca, Brazil) at a temperature of 100 °C for 10 min.

2.3. Thickness and apparent opacity

The thickness was determined using a micrometer (Mitutoyo, Japan). The measurements were repeated at teen random positions on each hydrogel formulation. The apparent opacity was determined using a colorimeter 123 (BYK Gardner, Germany) as described by Garcia *et al*. (2018).

2.3. X-ray Diffraction (XRD)

Samples were powdered and the analysis was performed in a

PANalytical X’Pert PRO MPD diffractometer (Almelo, The Netherlands) with copper Ka radiation under the operational conditions of 40 kV and 30 mA. All the assays were performed with a ramp rate of 0.05 °C/min .

2.4. Fourier Transform-Infrared Spectroscopy (FTIR)

The pulverized and dried samples were then mixed with potassium bromide and compressed into tablets. The FT-IR analyses were carried out with a Shimadzu FT-IR-8300 (Kyoto, Japan), which has a spectral resolution of 4 cm^{-1} and a spectral range of 4000–500 cm^{-1}.

2.5. Scanning Electron Microscopy (SEM)

SEM analyses were performed on the FEI Quanta 200 equipment (OR, USA). Samples were incubated in an air circulation oven (Marconi MA 035, Piracicaba, Brazil) at 60 °C for 3 h and then kept in desiccators containing anhydrous calcium chloride for one week. The dry samples were assembled for viewing on bronze stumps using double-sided tape. Afterward, the samples were covered with a thin layer of gold (40–50 nm) and an accelerated voltage of 20 kV was used for all samples.

2.6. Thermogravimetric Analysis (TGA)

TGA of the samples will be performed using the Shimadzu TGA-50 (Japan) equipment. The scans will be performed at room temperature up to 600 °C with a heating rate of 20 °C /min under a nitrogen flow of 20 mL/min.

2.7. Degree of swelling

Degree of swelling was determined using a gravimetric method described by Pereira *et al*. (2022). Samples (2 x 2 cm) of each formulation were carefully weighed and placed inside previously weighed cylindrical devices (3.0 x 3.5 cm) containing at one end a mesh with a nominal aperture of 138-75 mm, and the devices were immersed in 10 mL of different pH buffered solutions (4, 7 and 9). At pre-determined times (5, 10 and 20 min, and 24 h), the devices containing the hydrogels films were removed from the solutions, the excess was removed using filter paper, and then the films were weighed again. From the obtained value, the degree of swelling of each sample was calculated in relation to its initial dry weight. Analyses were performed in triplicate.

2.8. Water Sorption Isotherms

Samples of the materials (0.5 g) were dried for 15 days over anhydrous calcium chloride, and then they were placed over saturated salt solutions in separate desiccators, each with a specific level of relative humidity (RH) (11, 33, 58, 75 and 90%) and held at 25 °C. Each sample was weighed at regular intervals, and when two consecutive equal measurements had been recorded, it was assumed that the equilibrium weight had been reached. The equilibrium moisture content was calculated as the mass increase of the dried sample at equilibration for each RH.

The GAB (Guggenheim– Anderson-de Boer) model was used to fit the data from the sorption isotherms, and the monolayer values were calculated from the GAB isotherm model (Capanema *et al.*, 2018). $M= m0CKaw/(1-Kaw)\ (1-Kaw+CKaw)$, where M is the equilibrium moisture content at a given water activity (aw), aw is RH/100, m0 is the monolayer value (g water/g solids), and C and K are GAB constants. All tests were conducted in triplicate.

2.9. Water vapor permeability (WVP)

WVP was determined gravimetrically according to ASTM E96/E96M-16. Each film sample was placed in a 60 mm circular opening of a permeation cell, which was placed in a desiccator at 25 °C, and to maintain a 75% RH gradient across the film, anhydrous calcium chloride (0% RH) was placed inside the permeation cell, and a saturated sodium chloride solution (75% RH) was placed in the desiccator. After an equilibration period of 3 h, the cells were weighed at regular intervals until a constant rate of mass gain was obtained. Changes in the weight of the cell were recorded to the nearest 0.0001 g and plotted as a function of time. The angular coefficient of each line was calculated by linear regression at a constant regime ($r^2 > 0.99$), and the water vapor permeability (WVP) was calculated according to Equation 1:

$$WVP = \frac{(TWVP*e)}{\left(Ps\ x\left(\frac{URd-U}{100}\right)\right)} \quad (1)$$

Where WVP is the water vapor permeability (g/msPa), TWVP is the water vapor permeability rate (g/m^2 s), e is the film thickness, Ps is the water vapor saturation pressure at 25 °C, and URd and URc are the relative humidity in the desiccator and capsule, respectively. Analyses were performed in triplicate.

2.10. Mechanical properties

The tensile properties were determined using the texture analyzer model TA.XT 147 plus (Stable Micro Systems, Surrey, UK), according to Garcia *et al*. (2020) based on the 148 ASTM-D882-02 (2002) standard method.

2.11. Statistical Analysis

Analyses of variance (ANOVA) and Tukey's mean comparison test ($p \leq 0.05$) were performed with R program.

3 Results and Discussion

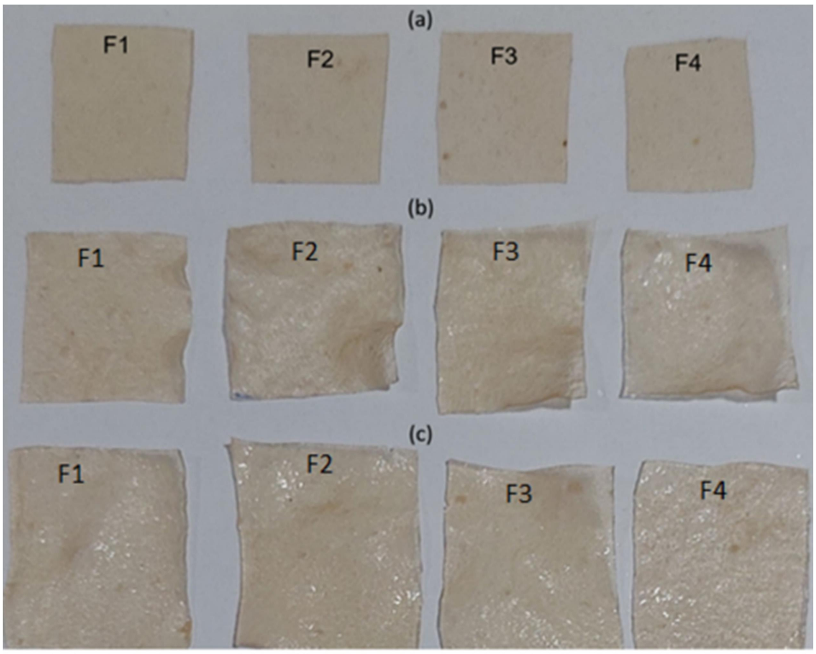
Oat hull was bleached with peracetic acid, resulting in a material with 78% cellulose, 8% hemicellulose, and 3% lignin, and this sample was labeled as native cellulose. Native cellulose was employed to obtain the surface-modified cellulose by reactive extrusion with succinic acid, and the degree of substitution of was 2.28. The degree of substitution can be defined as the average number of substituted hydroxyl groups per glucose unit, and it has a maximum value of 3 (Romeo *et al.,* 2020). Native or modified cellulose were employed as reinforcing agents in hydrogels.

3.1 Appearance, apparent opacity, thickness

All hydrogel formulations (Table 1) were easily processed by extrusion, indicating that the employed process can be considered as a viable alternative to obtain mixtures of gelatin, xanthan gum and cellulose. Four formulations were prepared varying the type of cellulose (native or modified) and the cellulose concentration (5 or 7%). Gelatin, xanthan gum and glycerol proportions were fixed as the quantities that resulted in films that were homogeneous, continuous and easy to handle. A major concern of the use of xanthan gum is its dispersion in high concentrations, and the extrusion of this gum results in a good dispersion of xanthan gum (Severo *et al*., 2007; Zeng *et al*., 2021). In previous reports from other authors that produced gelatin films by casting (Harizah *et al*., 2016), xanthan gum was added in concentrations ranging from 5 to 25% in relation to the total mass of gelatin, which means that in a polymeric matrix produced in an aqueous system with 5% of solids content, for each 1.00 g of gelatin, it was employed the maximum of 0.25 g of xanthan gum (Harizah *et al*., 2016). In this study, 100 g of each formulation were extruded employing 51–53 g of gelatin, 7 g of xanthan gum and 5–7 g of cellulose, resulting in a simple and rapid process when compared to the casting method.

Figure 1a presents the visual appearance of hydrogels films obtained from gelatin, xanthan gum and cellulose mixtures; it can be observed that all formulations resulted in laminated films with a smooth surface, without pores or cracks, with good resistance to handling, folding and crushing, which suggests that the extrusion process was effective in obtainment of a stable polymeric network. In this study, we employed two polysaccharides (cellulose and xanthan gum) and one protein (gelatin), which is compatible macromolecules that can establish intermolecular interactions by hydrogen bonding, and also by electrostatic interactions between xanthan gum, which is an anionic polymer, and gelatin, a protein with positively charged reactive groups, which probably was favored by the extrusion process. According to Guo *et al*. (2014) addition of xanthan gum to gelatin edible films can result in interactions between these biopolymers by electrostatic forces, hydrogen bonding and entanglements between them.

Figure 1. Visual appearance of hydrogels films (a); visual appearance of hydrogels films after 20 min (b) and after 24 h immersed (c) in buffered solution at pH 7.



Hazirah *et al.* (2016) reported that xanthan gum can be used as an alternative crosslinking agent in gelatin/carboxymethil cellulose films, resulting in a compatible blend of composite film. Some brown spots were sparsely distributed on hydrogels samples, and may possibly result from the Maillard reactions. Su *et al.* (2012) reported the occurrence of Maillard reactions between proteins and polysaccharides in films obtained by casting. The carbonyl group of the polysaccharides reacts with the amino group of the proteins, resulting in reddish-brown materials. All hydrogels presented a homogeneous yellowish color, which can be related to the use of xanthan gum; this was also observed by Simões *et al*. (2020) who developed hydrogels based on cassava starch and xanthan gum by a similar process. Cellulose was employed as a reinforcing agent (Cagnin *et al*., 2019; Capanema *et al*., 2018; Fu *et al*., 2019; Javanbakht *et al.,* 2018; Koneru *et al*., 2020), and the variation in cellulose contents and the type of cellulose did not affect the extrusion of mixtures or the films appearance and handling after thermopressing (Figure 1a).

All films were translucent, and the apparent opacity results of the hydrogels ranged from 1.54 to 1.92%µm^{-1} (Table 2). The samples with the modified cellulose (F2 and F4) were responsible for the highest apparent opacity results; however, this difference was not enough to generate a change in the visual transparency of the films as observed in Figure 1a. Su *et al*. (2012) reported that higher transparency indicates higher compatibility between the polymers in a film matrix, and possibly, in this study free hydroxyls groups of native cellulose were more accessible for interaction with the other polymers than modified cellulose.

The average thickness (Table 2) of the hydrogels ranged from 23.33 to 27.66 µm F2 and F4 formulations had the lower values, these samples were produced with modified cellulose. Possibly, when cellulose was subjected to reaction with succinic acid by extrusion, some degree of acid hydrolysis may have occurred, resulting in materials with lower viscosity and fluidity inside the extruder than the materials produced with native cellulose (F1 and F3), generating films with lower thicknesses. According to Simões *et al*. (2020) the reaction with citric acid can result in acid hydrolysis of starch polymeric chains during reactive extrusion, reducing the viscosity of the molten material, resulting in hydrogels films with lower thickness and larger surface.

Table 2- Apparent opacity, thickness, and mechanical properties of all samples.

Samples	**Y(%µm^{-1})**	**Thinckeness (µm)**	**σ (MPa)**	**ε (%)**	**E (Mpa)**
F.1	1.56^{b}± 0.04	27.66^{a}± 2.42	2.01^{b}± 0.63	59.43^{a}±12.28	5.34^{b} ±0.95
F. 2	1.92^{a}± 0.04	23.33^{b} ± 1.80	1.86^{b}± 0.14	57.03^{a} ± 9.30	5.66^{b} ±0.57
F. 3	1.54^{b}± 0.17	26.66^{a}± 2.07	2.90^{a}± 0.37	58.03^{a} ± 7.84	9.50^{a} ±1.81
F. 4	1.86^{a}± 0.05	23.43^{b}± 2.07	2.11^{b}± 0.35	51.56^{a}±10.55	7.55^{ab}±0.65

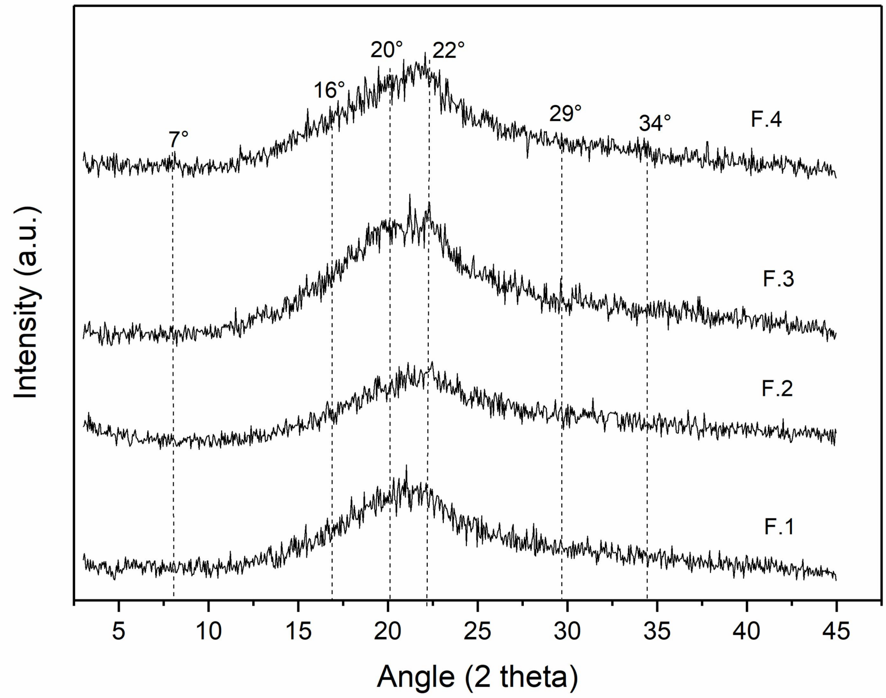
Y (apparent opacity); σ (tensile strength); ε (strain at break); E (Young's modulus). Different letters in the same column indicate significant differences ($p \leq 0.05$) according to Tukey's test.

The mechanical properties results are shown in table 2. The tensile strength results ranged from 1.86 to 2.90σ. The F.3 was responsible for the highest result, followed by the F.4 formulation. In the strain at break results there were no statistic difference between them. The Young's modulus results the F.3 formulation was responsible for the highest value of 9.50 Mpa, followed by the F.4 formulation of 7.55 Mpa. The highest results observed in F.3 and F.4 formulation can be due to the higher concentration of cellulose used in this formulation (Belay *et al*., 20120; Simões *et al*., 2020).

3.2. X-ray Diffraction (XRD)

XRD is an important technique for the characterization of semi-crystalline polymers, revealing important information about their molecular organization, their crystallinity index and how the processing can affect their native structure (Warren *et al*., 2016). The X-ray diffractograms of hydrogels films are shown in Figure 2. It is possible to see that all samples presented an amorphous pattern, without pronounced peaks.

Figure 2. X-ray diffractograms of all hydrogels films.



A peak located at 2θ=22° discretely appeared in all samples, this peak is attributed to cellulose type I (Debiagi *et al.,* 2020; Gil-Giraldo *et al.,* 2021; Marim *et al.,* 2021; Pereira *et al.,* 2022). According to Luciano et al. (2021), peaks near 2θ=7 and 20° refer to the formation of triple helix structure of gelatin, and these peaks were not observed in this study (Figure 2). A peak at

2θ=29° has been attributed to the double helix conformation of xanthan gum (Balasubramanian *et al.,* 2018), and it was not observed in this study.

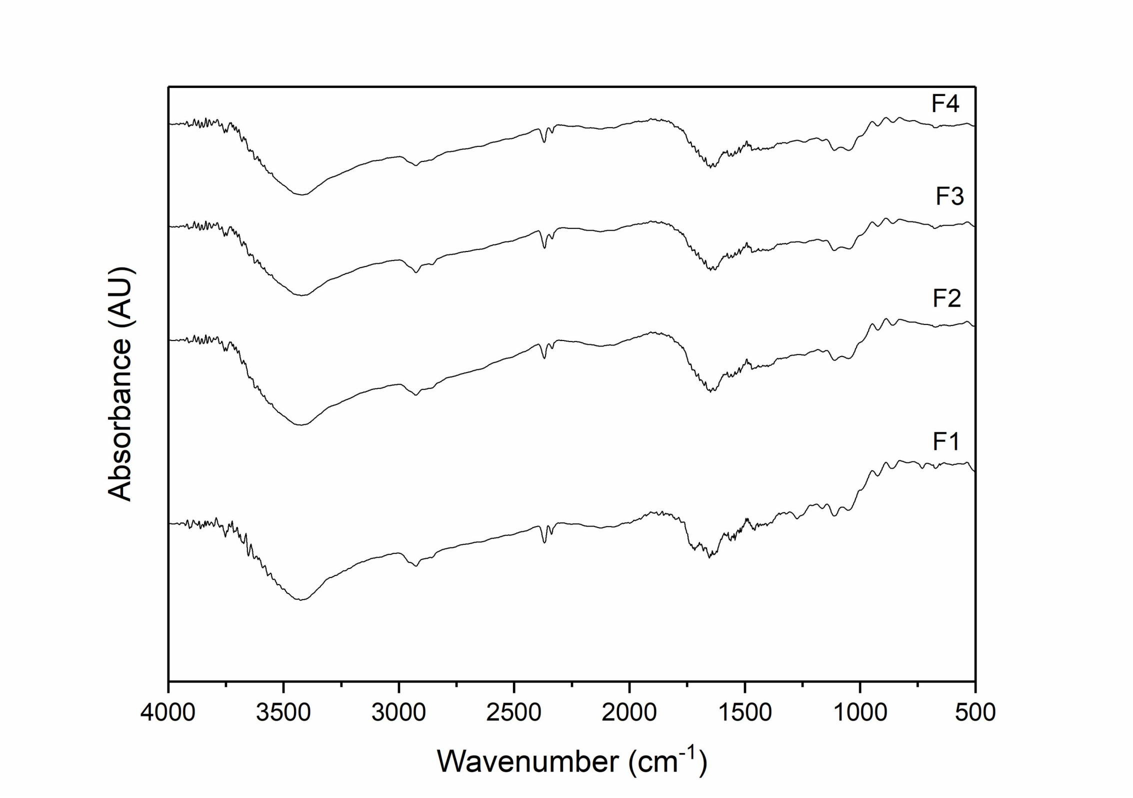
Extrusion process is a combination of physical and thermomechanical treatments, which can affect the proportion of amorphous and crystalline fraction of polymers (Debiagi *et al.,* 2020). In this study, the extrusion process followed by thermopressing resulted in amorphous materials. Hazirah *et al.* (2016) reported that the incorporation of xanthan gum to gelatin-carboxymethyl cellulose films prepared by casting also resulted in amorphous materials.

3.3. Fourier Transform-Infrared Spectroscopy (FTIR)

FTIR is a useful technique to provide information about the hydrogels network structure and their chemical groups (Severo *et al*., 2007; Warren *et al*., 2016). FTIR spectra of hydrogel films are shown in Figure 3. It is possible to see that all formulation presented similar spectra, and this occurred because all hydrogels formulations were obtained from materials with similar chemical groups. There were no changes in the characteristic bands of the formulations made with modified cellulose, which can be due to the presence of cellulose (native or modified) which represents only 5 or 7% of the total of each formulation. Pereira *et al.* (2022) observed that there were no changes in the polymer characteristic FTIR bands after 0.5–10% of antioxidant addition in starch-gelatin films, and they attributed this to the small concentration of antioxidants used in the films formulations.

All formulation presented a wide band near 3400 and 3500 cm^{-1}, which can be attributed to the O–H stretching of hydrogen bonds, and also a band located near 2900 cm^{-1} that was attributed to the presence of C–H stretching (Debiagi *et al.,* 2020; Gil-Giraldo *et al*., 2021; Hazirah *et al.,* 2016; Pereira *et al.,* 2022; Su *et al.,* 2012). Bands located between 1423 and 1325 cm^{-1} can be attributed to the presence of CH2 group, C–OH vibration and C–H stretch (Wannaphatchaiyong *et al.,* 2019). Bands located near 1640, 1540 and 1241 cm^{-1} corresponded to amine I, amine II and amine III, these bands appeared in all samples, and they are characteristics bands of gelatin (Wang *et al.,* 2017).

Figure 3. Fourier Transform-Infrared Spectroscopy of all hydrogel films.

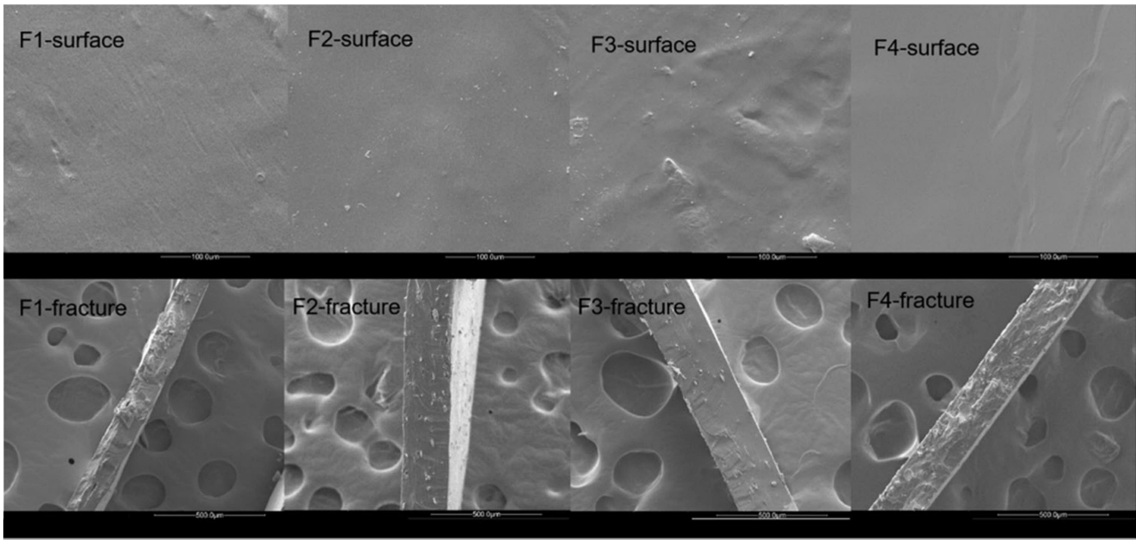


All the bands located between 1057 and 1162 cm^{-1} appeared in all samples, and they corresponded to the presence of C–O and C–O–C stretching vibration in cellulose, xanthan gum and gelatin (Debiagi *et al.,* 2020; Gil-Giraldo *et al*., 2021; Pereira *et al.,* 2022; Wang *et al.,* 2017).

3.4. Scanning electron microscopy (SEM)

Scanning electron microscopy allows obtaining high resolution images from the materials, providing relevant information on surface topography of the specimen (Merrett *et al.,* 2002), and also about compatibility between polymers in polymeric mixtures (Cagin *et al.,* 2021).

Figure 4. SEM imagens of hydrogels films.



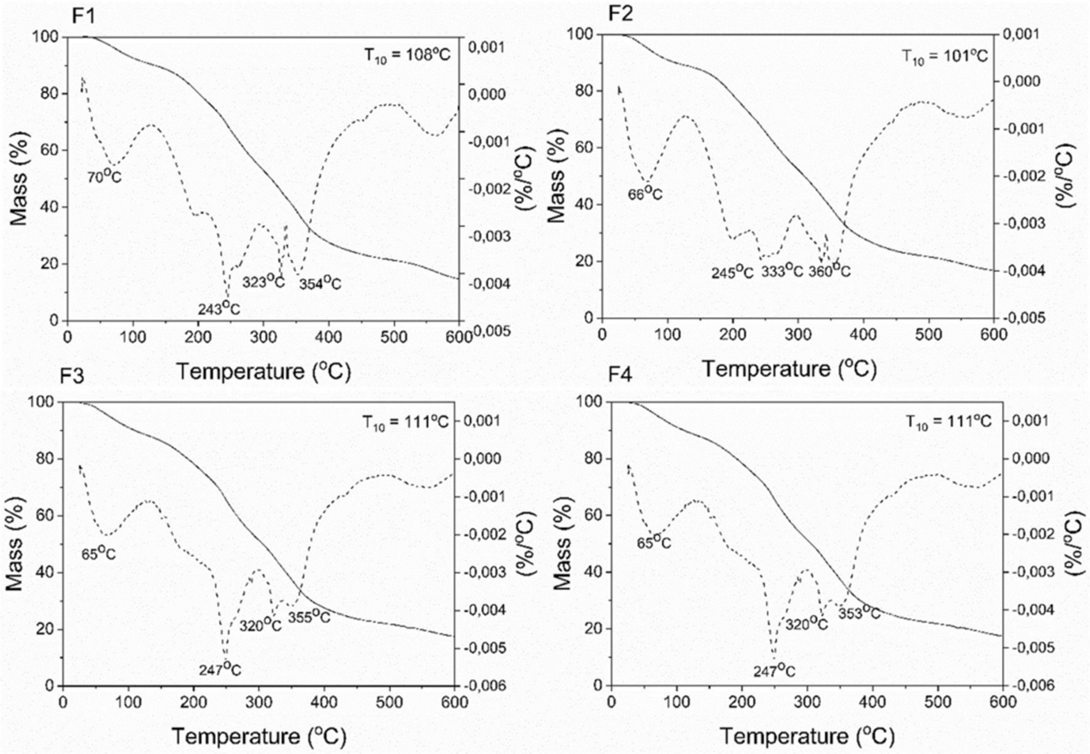
In Figure 4 it can be seen the SEM images of surface and fracture of all hydrogels films. It is possible to see from the surface images that all formulation presented a smooth appearance, without pores or cracks. This fact can be associated to the thermopressing process, as reported by Simões *et al.* (2020) they also obtained homogeneous and smooth hydrogel films of starch and xanthan gum by reactive extrusion followed by thermoprocessing. The fracture images also showed the formation of a compact structure, and according to Cagnin *et al.* (2021) this is an indication of a good interaction between the biopolymers employed to obtain the hydrogel network.

3.5. Thermogravimetric Analysis (TGA)

TGA of hydrogels is performed to determine weight changes as a function of temperature in order to study their thermal stability (Demir *et al.,* 2022; Laffleur *et al.,* 2019). TGA and DTGA curves from the curves formulations are shown in Figure 5. It is possible to see that all formulations presented the same pattern of decomposition, in all samples it can be observed a similar pattern in thermograms (Figure 5), an initial weight loss was found in the range from 65 to 70 °C for all samples, and according to Pereira *et al.* (2022) and Guo *et al.* (2014) this first stage (temperatures below 200 °C) corresponds to the evaporation of water or low molecular weight compounds. It was observed for all samples a second degradation stage between 243 and 247 °C (Figure 5), and a third degradation stage that showed a double-peak distribution at 320–333 °C and 353–360 °C (Figure 4), and these peaks could be related to gelatin, xanthan gum and cellulose degradation.

Guo *et al.* (2014) reported that degradation of xanthan gum and gelatin occurs between 290 and 310 °C. Gil-Giraldo *et al.* (2021) reported that the maximum weight loss rate of native cellulose from oat hull occurs at 360 °C. All formulations presented 10% of mass loss (T10) at temperatures between 101 and 111 °C, and it can be observed that F3 and F4 formulations, which were prepared with the highest concentration of cellulose (7%), had the higher T10 values (Figure 5), indicating a higher thermal stability when cellulose was added at the higher level.

Figure 5. Thermogravimetric Analysis of all hydrogel films.



3.6. Degree of swelling

The swelling process of a hydrogel is governed by physical and chemical forces that induce the entry of liquid into the polymeric matrix, by capillarity and diffusion. The swelling process of hydrogels can be controlled by varying their composition, by the amount of crosslinking and also by external factors, such as the presence of salts, pH of the medium and temperature

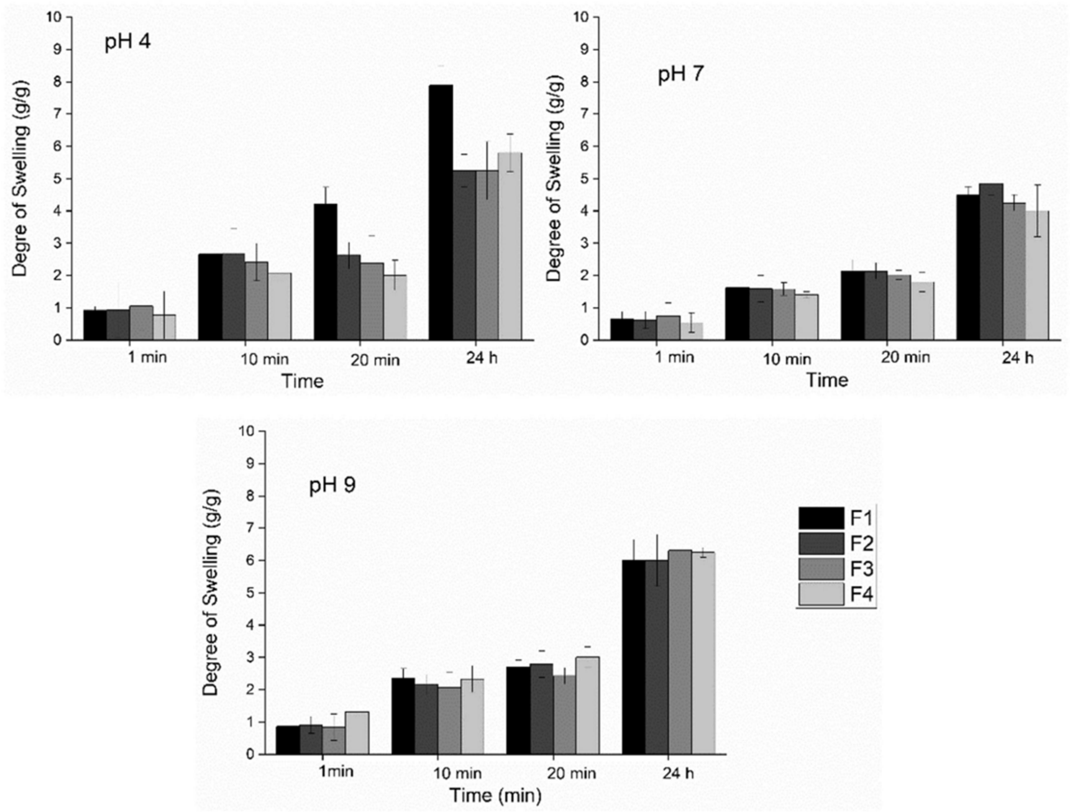
(Batista *et al*., 2015; Bukhari *et al*., 2015; Eman *et al*., 2022; Simões *et al*., 2020; Shafagh *et al*., 2018). The degrees of swelling of the hydrogels are shown in the Figure 6.

According to Batista *et al.* (2015) the stability during swelling of biodegradable hydrogels is one the main technological challenges in developing these materials. Depending on the hydrogel application, different swelling times and stabilities are required. Additionally, the swelling profile can help understanding the release behavior when these materials are employed as drug delivery systems (Eman *et al*., 2022; Pereira *et al*., 2022).

For all samples and pH conditions, with increase of time from 1 min to 24 h, the degree of swelling increased. It can be observed that the obtained hydrogels had a pH-dependent swelling; the highest swelling values were obtained at pH 4 for all samples, followed by samples at pH 9, and the lowest values were obtained at pH 7 (Figure 6). At pH 4, the swelling ranged from 5.3 to 7.9 g/g after 24 h of immersion; at pH 9, the swelling degree ranged from 6.0 to 6.3 g/g, and at pH 7, the values ranged from 4.0 to 6.3 g/g (Figure 6). At higher pH, the hydrogels swell due to the ionic repulsion of protonated carboxylic groups in xanthan and gelatin (Bukhari *et al*., 2015), while at lower pH, protonated aminic groups on gelatin molecule also resulted in repulsion of these charged groups (Shafagi *et al*., 2018), and in both cases, these repulsion forces could be responsible for the increase of swelling in hydrogels.

Shafagh *et al.* (2018) reported that hydrogels based on gelatin, citric acid and silver nanocomposite had higher degree of swelling at acidic pH, and they attributed this to the presence of a large number of electrostatic repulsive forces between the positively charged groups in gelatin, resulting in higher swelling degrees. The F1 formulation presented the higher degree of swelling in pH 4, increasing from 0.92 g/g to 7.90 g/g (92–790%) after 1 min and 24 h of immersion in water, respectively, and at pH 7 and 9, degree of swelling was not affected by the different formulations (Figure 6).

Figure 6. Degree of swelling of hydrogels films.



According to Batista *et al.* (2019) when the hydrogel has the ability of absorb water more than 100% of its total weight, these hydrogels can be considered superabsorbent hydrogels, therefore, the hydrogels obtained in this study can be considered as superabsorbent hydrogels.

The swelling values obtained in this study were close to the values reported by Simões *et al.* (2020) who reported values from 5.0 to 9.0 g/g for biodegradable hydrogels from starch and xanthan gum after immersion in water for 48 h. Demir *et al.* (2022) reported swelling degrees between 4.0 and 6.0 g/g for gelatin-xanthan gum hydrogels after 24 h of immersion, and Farris *et al.* (2011) observed a swelling degree of 3.0 g/g for a gelatin-pectin physical hydrogel, while a gelatin hydrogel chemically crosslinked with a synthetic crosslinker (glutaraldehyde 0.3%) presented a swelling of 2.15 g/g after 24 h of immersion. On the other hand, the values were lower than values reported by Eman and Shaken (2022), who reported maximum swelling degrees ranging from 21.6 to 24.8 g/g after 24 h at pH 6 for a hydrogel made from a synthetic polymeric matrix based on poly(N-isopropylacrylamide) and reinforced with nanocellulose esterified with succinic anhydride. All hydrogels remained intact after 20 min (Figure 1b) and after 24 h (Figure 1c) immersed in a pH 7 buffered solution, which demonstrated that they have the capacity of swelling without losing their structural integrity, which is an important feature considering their use as a potential polymeric matrix in drug delivery systems.

3.7. Water Sorption Isotherms

Moisture sorption isotherm of a material describes the equilibrium relationship between water activity and moisture content at constant temperature, being useful to give insights about the moisture-binding characteristic of the studied material (Kartniz *et al.,* 2007; Marim *et al.,* 2021; Merci *et al.,* 2015). Moisture sorption isotherms and GAB model parameters are shown in Figure 7 and Table 3, respectively. As can be seeing from Figure 7, all samples showed similar patterns, with an increase close to linear in the equilibrium moisture content with increase of aw, which is typical of hydrophilic materials. It can be attributed to the water adsorption that are partially bound in the samples (Gil-Giraldo *et al.,* 2021).

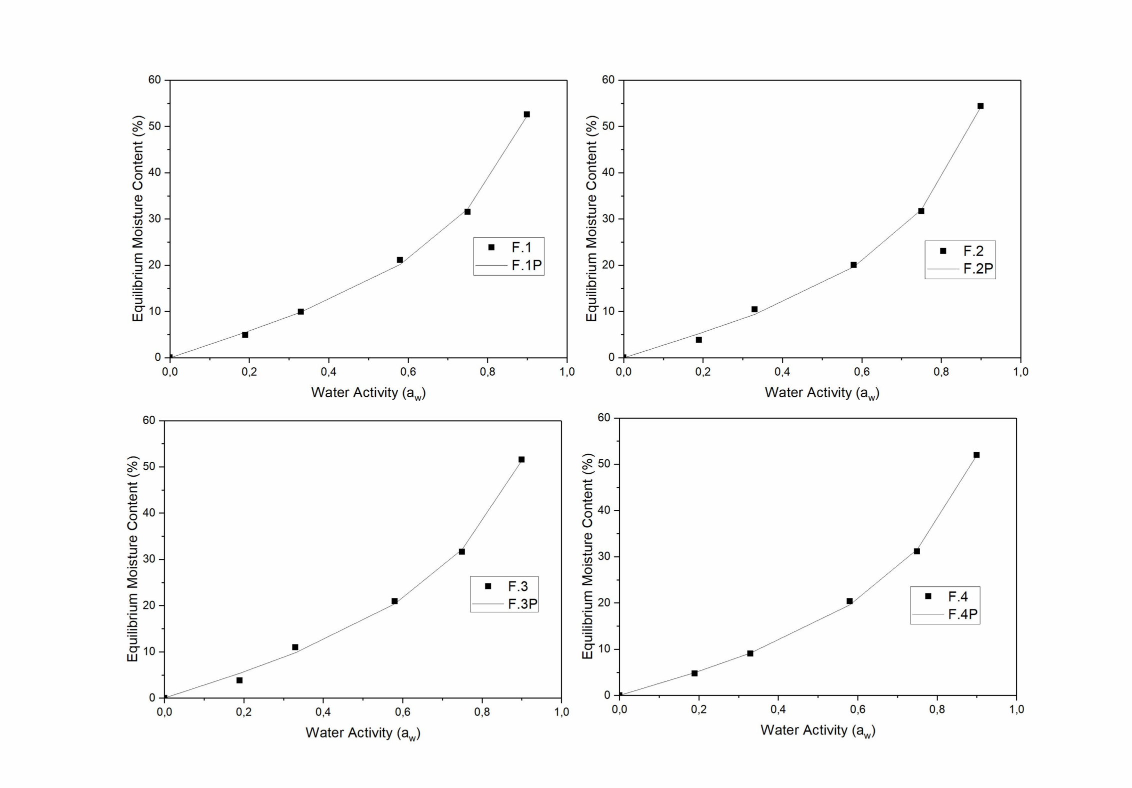
The GAB model was efficient to describe the moisture sorption isotherms of the hydrogel samples, and the monolayer value (m0) is an indicator of the maximum amount of absorbed water of a single layer per gram of dry matter, being an indicative of how hydrophilic the samples are (Gil-Giraldo *et al.,* 2021; Marim *et al.,* 2021; Merci *et al.,* 2015). According to the results of m0 presented in Table 3, the m_0 increases by increasing the cellulose concentration in the hydrogel films, it is possibly because of the increase of hydroxyl groups presented on hydrogels matrix, which were able to interact with water by hydrogen bonds.

Table 3 - GAB model parameters of all samples.

Samples	**m_0**	**C**	**K**	**r2**
F.1	15.63	2.28	0.82	0.99
F.2	14.84	2.17	0.84	0.99
F.3	17.07	2.04	0.80	0.99
F.4	16.23	1.91	0.82	0.99

$M = m_0CKaw/(1 - Kaw)\ (1 - Kaw + CKaw)$, where M is the equilibrium moisture content at a water activity (aw), m0 is the monolayer value (g water/100 solids), and C and K are the GAB constants.

Figure 7. Moisture sorption isotherms of all hydrogels. Lines are derived from GAB models.



According to Cagnin *et al.* (2021) in carboxymethyl cellulose hydrogels crosslinked with sodium trimetaphosphate the free hydroxyls and carboxyl groups can interact with water by hydrogen bonds.

3.8. Water vapor permeability (WVP)

Water vapor permeability measures the passage of water vapor through the hydrogel film (Demir *et al.,* 2022). Water vapor permeability values are presented in Table 4. It can be observed that the highest value was from F4 formulation, while the lowest value was observed for F1, which is the formulation with the lower concentration of native cellulose. Only the formulation F1 and F4 showed statistic difference between each other, and it can be due to the concentration of cellulose used in each one, increasing the WVP as the cellulose concentration increased. This result agrees with the result of moisture sorption isotherms presented in this study, where the m0 values increased with the increase in cellulose content, possibly because of the higher hydroxyl groups from native cellulose.

Demir *et al.* (2022) studied the production of xanthan-gelatin and xanthan-gelatin-keratin wound dressings for local delivery of vitamin C, and they reported that hydrogels with lower WVP can causes the accumulation of exudates, increasing the risk of bacterial growth, and higher WVP results in faster drying and scar formation, making it more suitable for exudate wounds.

3.9. Mechanical properties

According to Cagnin *et al.* (2022) the requirement for the mechanical properties of hydrogels films depends on the applications for which they are intended, however, it is important enough tensile strength to withstand processing and handling. The mechanical properties results are shown in Table 4. The tensile strength values ranged from 1.86 to 2.90 MPa, and F3 formulation resulted in the film with the highest result. Elongation of samples ranged from 52 to 59%, and the different formulations did not present statistical differences between each other. Simões *et al.* (2020) reported similar values of tensile strength (1.04–3.83 MPa) and higher values for elongation (63.31–138.64%) for hydrogels based on cassava starch and xanthan gum.

Table 4. Water vapor permeability (WVP) and mechanical properties of hydrogels.

Samples	WVP x 10^8 (g/msPa)	Mechanical Properties		
		Tensile Strength (MPa)	Elongation (%)	Young Modulus (MPa)
F1	1.73^b ±0.31	2.01^b ± 0.63	59^a ±12	5.34^b ±0.95
F2	2.05^{ab} ±0.04	1.86^b ± 0.14	57^a ± 9	5.66^b ±0.57
F3	2.45^a ±0.55	2.90^a ± 0.37	58^a ± 7	9.50^a ±1.81
F4	2.77^a ±0.31	2.11^b ± 0.35	52^a ±10	7.55^{ab} ±0.65

Different letters indicate significant differences ($p \leq 0.05$) according to Tukey's test. F1 (53% gelatin, 7% xanthan gum, 5% native cellulose and 35% glycerol); F2 (53% gelatin, 7% xanthan gum, 5% modified cellulose and 35% glycerol); F3 (51% gelatin, 7% xanthan gum, 7% native cellulose and 35% glycerol); F4 (51% gelatin, 7% xanthan gum, 7% modified cellulose and 35% glycerol).

F3 formulation also presented the highest Young modulus (9.50 MPa), followed by the F4 formulation (7.95 MPa). The highest results observed in F3 and F4 formulations can be due to the higher concentration of cellulose used in these formulations, which acted as a reinforcing agent in the polymeric matrix. Cagnin *et al.* (2022) reported that the reinforcement in a hydrogel network results from interactions between the polymers via hydrogen bonds (physical crosslinking).

4. Conclusions

The strategy employed in this study to obtain nontoxic hydrogels without the use of synthetic crosslinkers was effective, resulting in a gelatin-xanthan gum-cellulose hydrogel with promising properties to be used as a modified pharmaceutical form to deliver active compounds in cosmetic or pharmaceutical products. Xanthan gum acted as an alternative crosslinking agent, resulting in hydrogels that had a pH-dependent swelling, the highest swelling values were obtained at pH 4, which values ranging from 5.3 to 7.9 g/g. Cellulose acted as a reinforcing agent for hydrogels, increasing thermal stability, tensile strength and Young's modulus of films when employed at the higher level (7%). Higher cellulose content also resulted in higher water adsorption capacity and water vapor permeability of hydrogels.

Acknowledgment: The authors wish to thank the Laboratory of X-ray Diffraction (LARX), Laboratory of Spectroscopy (ESPEC) and Laboratory of Electronic Microscopy and Microanalysis (LMEM) of the State University of Londrina.

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Artigo 3 – Este artigo foi desenvolvido durante o período de Doutorado Sanduíche, dentro do Programa PDSE (Programa 2022) da CAPES, no Instituto de Agroquímica y Tecnologia de Alimentos (Valencia – Espanha), sob a supervisão de María José Fabra e Amparo Lopez Rubio.

The potential of lignocellulosic hemp fibers to develop compostable packaging materials

Jéssica Fernanda Pereira[ab].

[a]Food Safety and Preservation Department, Institute of Agrochemistry and Food Technology (IATA-CSIC), Valencia, Spain;

[b]Department of Biochemistry and Biotechnology, CCE, State University of Londrina, Londrina, PR, Brazil.

Abstract

The ability of natural hemp fibers (HF) to be use as reinforcement in biodegradable packaging films has been evaluated by mixing them with commercially- available biodegradable polyesters, aiming at incorporating the maximum amount of HP in a more cost-efficient and environmentally friendly way. A blend of poly(hydroxy-butyrate) (PHBV) and poly(butylene adipate-co-terephthalate) (PBAT), was used as a continuous matrix. HP was minimally processed by melt-blending and compression molding, and the effect of HP loadings on the final performance of the films was investigated. HP fibers were mainly composed of lignin (~22%) and carbohydrates (~62-67%) but significant amounts of proteins (~8.77%) were also detected. Interestingly, up of 40 % of HF could be incorporated into the biopolymer matrix. The incorporation of HP resulted in films with higher water vapor permeability than pure biopolyesters. In order to improve its hydrophilic nature, food-grade beeswax or carnauba wax were incorporated into the biopolymer matrix and the resulting films showed similar mechanical properties than their counterparts prepared without wax but displayed significantly higher contact angle and better water vapor barrier performance.

Key words: Biodegradable, PHBV, PBAT, Hemp fiber, Biocomposite.

1. Introduction

Advancements in biodegradable materials play a crucial role in sustainability and offer an effective contribution to reduce the dependence on fuels fossils. Currently, most of the efforts in this field are focused on the use of biodegradable polymers derived from renewable sources and also the integration of diverse natural fibers, such as wood fibers, jute, miscanthus, sisal, hemp, and flax (Pal *et al.,* 2020; 2023; Wu *et al.,* 2018). The use of natural fibers contributes to enhance properties of the biobased material, including mechanical and barrier performance, and also it results in a decrease in the final cost (Dixit *et al.,* 2022).

Among all natural fibers, hemp has the greatest Young's modulus values and has one of the strongest cellulose fibers. Additionally, HF possess high aspect ratio (length/width ratio), which is another characteristic that make them suitable for their use as fillers in biocomposite materials (Promhuad *et al.,* 2020). HF is a natural fiber that presents crystalline cellulose fibrils and lengthy fibers, which have a potential material to be used as fillers in biocomposites materials (Pal *et al.,* 2020; Wu *et al.,* 2018). The hemp is highly used as an industrial crop in both Europe and China, being a great source of cheap lignocellulosic material that is originated from waste biomass (Wu *et al.,* 2018).

In the last decades the world is facing a huge problem related to environmental pollution; this situation has led to the increasing in the study and research involving the use of more environment friendly materials for this application (Jian *et al.,* 2020; Moreno *et al.,* 2020; Pal *et al.,* 2020). Biodegradable polymers are considered a great alternative for conventional plastics (Lamsaf *et al.,* 2023; Pal *et al.,* 2023). Among them, polyhydroxyalkanoates (PHAs) are biodegradable and non-toxic biopolyesters, which are produced by different microorganisms. Within the PHAs group, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) has gained considerable attention due to its favorable performance features and lower price, as compared to other PHAs polymers. PHBV is a copolymer, produced by the addition of poly(3-hydroxyvalerate) (PHV) to the PHB molecule (Gupta *et al.,* 2022; Meereboer *et al.,* 2020; Pal *et al.,* 2023). Nonetheless, PHBV has a few inherent drawbacks, including brittleness, and higher expenses compared to other biodegradable and traditional polymers. To overcome this problem, blending PHBV with others biodegradable polymers and natural fibers obtained from waste biomass can be a good strategy to improve the mechanical property and to decrease production costs. On the other hand, poly(butylene adipate-co-terephthalate) (PBAT) is an amorphous copolyester, with an aliphatic-aromatic chain, made by a polycondensation of 1,4-butanediol with the adipic and terephthalic acids (Jian *et al.,* 2020; Pal *et al.,* 2020). Although PBAT is not a natural polymer, its biodegradable capacity makes it a good alternative to be used in the production biodegradable materials.

To the best of our knowledge, little information is available on literature regarding the use of hemp fibers as reinforcing agents for composites, and they were mainly reported for conventional plastics such as polyethylene and polypropylene (Dixit *et al.,* 2022; Wu *et al.,* 2018). Gupta *et al.* (2022) stated that the use of hemp fibers in biodegradable matrices, such as PHBV, enhanced the overall performance of the resulting material. Considering these aspects, this work is aimed at evaluating the suitability of hemp fibers to develop biodegradable packaging materials based on PHBV and PBAT, by means of extrusion processing, optimizing the highest amount of natural fibers that can be incorporated in the biopolymer matrix. Then, food-grade natural waxes (beeswax and carnauba wax) were incorporated into the biopolymer, with the aim of improving overall properties of the resulting film.

2. Material and Methods

2.1. Materials

The hemp fibers (HF) were obtained from hemp stems (*Cannabis sativa*), that was grown in Spain (Burgos) and provided by a local converter. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and polybutylene adipate-co-terephthalate (PBAT) were purchased from NaturePlast SAS (France). The beeswax was purchased from WWR (BDH Chemicals). The carnauba wax was donated from Agrakem (Barcelona, Spain).

2.2 Physicochemical analysis of the hemp fibers

The stalk of the HF were washed, dried, and either used as whole, or separated into pulp and cortex. Then it was ground and sieved to a size of 2 mm before the characterization. The protein content of the hemp fiber was obtained based on the nitrogen content estimated with the Dumas combustion method, multiplied by a factor 6.25 (Wiles *et al.,* 1998). The lignin conten was analysed following the TAPPI 222 (lignin insoluble) and 250 (lignin soluble) standards. The lipid content was determined using the Soxhlet 8000 extraction with hexane according to AOCC method 2003.06. The moisture content was determined by gravimetry by drying the samples overnight in a convection oven at 60 °C. The ash content was determined by thermogravimetric curves using a Setaram TG/ DTA92 (SETARAM Instrumentation, France). The monosaccharide composition was determined after acid methanolysis, using a high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) with a ICS-3000 system (Dionex) equipped with a CarboPac PA1 column (4 × 250 mm, Dionex) at 30 °C and a flow rate of 1 mL min^{-1}.

2.3. Development of the biocomposite films

Before the production of the films, the polymers and HF were dried in a convection oven at 60°C for 2 h, to ensure elimination of non-bound water. Later on, the different components from each formulation were manually mixed, before feeding them into the compounding machine. The masterbatch formulations were prepared using a co-rotative twin-screw mini extruder (Xplore Instruments, The Netherlands) at a temperature of 190 °C for 2 min and 100 rpm. To obtain the films, the extruded materials were pressed in a hydraulic press (Carver 4122, USA) at a temperature of 190 °C for 2 min with a pressure of 12 tonnes. Different percentages of the polymers (PHBV and PBAT) and the hemp fibers (HF) were used to obtain the masterbatch formulation, as described in Table 1. The different material samples were named as “xPHBV-yPBAT_zHF” where ‘x’and ‘y’ refers to the PHBV and PBAT percentage in the biopolymer, respectively, and z the percentage of HF respect to the biopolymer matrix. In the ones containing 5% of beeswax (BW) or carnauba wax (CW), the nomenclature was included BW or CW, respectively.

Table 1- Formulations of the biodegradable films.

Formulation	**Mass(%)**				
	PHBV	**PBAT**	**HF**	**BW**	**CW**
50PHBV/50PBAT	50.0	50.0	0	0	0
50PHBV/50PBAT_15HF	42.5	42.2	15.0	0	0
50PHBV/50PBAT_30 HF	35.0	35.0	30.0	0	0
50PHBV/50PBAT_40HF	30.0	30.0	40.0	0	0
50PHBV/50PBAT_40HF/5BW	27.5	27.5	40.0	5.0	0
50PHBV/50PBAT_40HF/5CW	27.5	27.5	40.0	0	5.0
60PHBV/40PBAT	60.0	40.0	0	0	0
60PHBV/40PBAT_15HF	51.0	34.0	15.0	0	0
60PHBV/40PBAT_30HF	42.0	28.0	30.0	0	0
70PHBV/30PBAT	70.0	30.0	0	0	0
70PHBV/30PBAT_15HF	59.5	25.5	15.0	0	0
70PHBV/30PBAT_30HF	49.0	21.0	30.0	0	0
80PHBV/20PBAT	80.0	20.0	0	0	0
80PHBV/20PBAT_15HF	68.0	17.0	15.0	0	0
80PHBV/20PBAT_30 HF	56.0	14.0	30.0	0	0

2.4. Mechanical Properties

A universal testing machine (Instron, USA) was used to determine the tensile strength (TS), elastic modulus (E) and elongation at break (EAB) of the films, according to ASTM standard method D882.10 (ASTM, 2001). Pre-conditioned specimens at 53% RH (1 cm wide by 7 cm long) were mounted in film-extension grips of the testing machine and stretched at 50 mm min^{-1} until breaking. The tensile parameters were determined from the stress-strain curves, estimated from the force-distance data obtained for the different films. At least 4 replicates per film formulation were obtained.

2.5. Contact angle measurements

The hydrophobicity of the surface as well as the wettability of the hemp-based films were assessed by measuring the contact angle on a

DSA25 (Kruss- Germany) equipment equipped with the AD4021 image analysis software under ambient conditions. A precision syringe deposited a drop of water on the surface of the film. The contact angle values were obtained by analyzing the shape of the drop of distilled water after placing it on the film for 10 s. Four replicates per film formulation were analyzed.

2.6. Water vapor permeability (WVP)

WVP was determined from the difference of the weight gain versus time curves at 25 °C, using the ASTM 2010 gravimetric method. Permeability cups containing silica were positioned inside a stabilized cabinet at 75% relative humidity, which was achieved by utilizing a saturated chloride salt solution.

2.7. Scanning electron microscopy (SEM)

SEM images were obtained by a Hitachi microscope (Hitachi S-4800) at an accelerating voltage of 10 kV. The samples were cryo-fractured after immersion in liquid nitrogen and randomly broken to investigate the cross-section of the samples. Samples were fixed on M4 Aluminum Specimen Mount and sputtered with a gold-palladium mixture under vacuum before their morphology was examined.

2.8. Statistical analysis

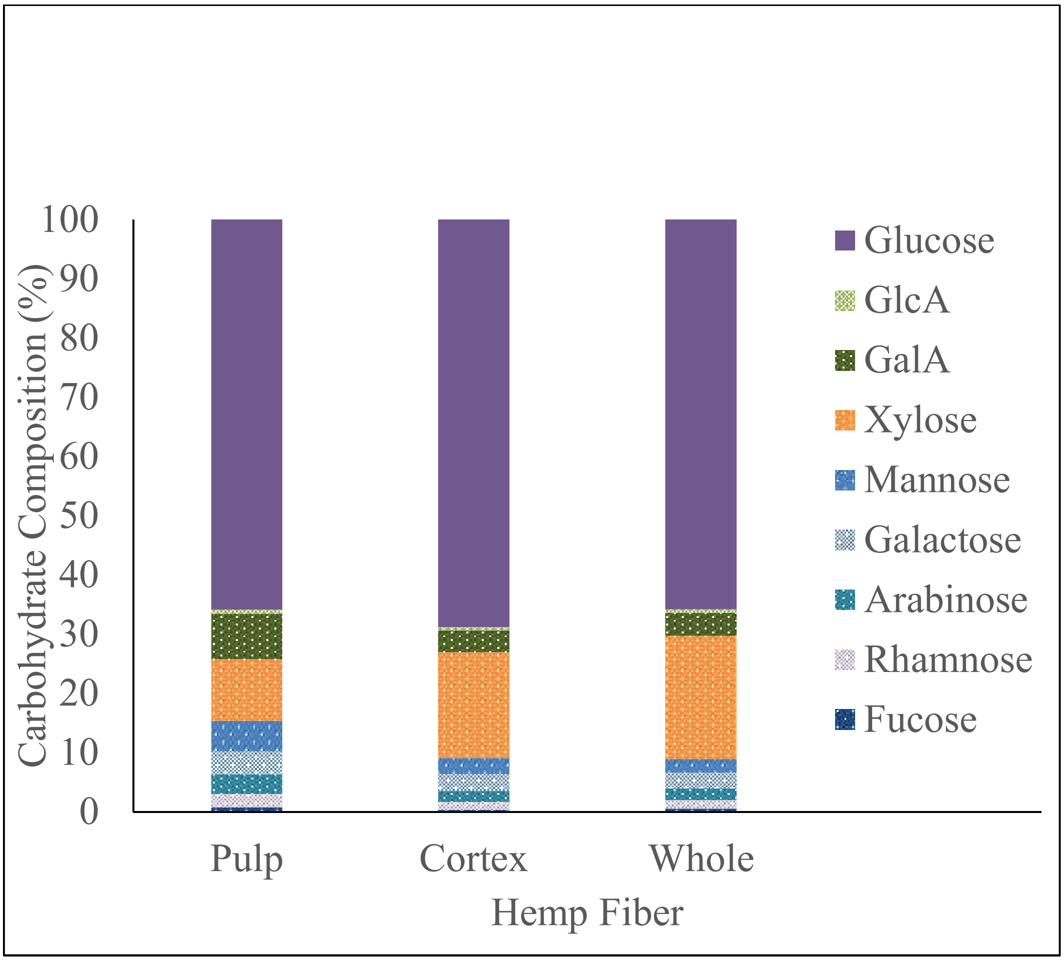
Analysis of variance (ANOVA) and Tukey's mean comparison test ($p \leq 0.05$) were performed with R program.

3. Results and Discussion

3.1. Compositional characterization of the hemp fibers

Hemp was valorized in this study to evaluate the possibility of using it to develop biodegradable packaging materials. The composition of the whole hemp, pulp and cortex was characterized and the results are compiled in Table 2. The results evidenced no significant differences between whole hemp and cortex, being mainly composed of structural carbohydrates. As expected, lignin was the second major component representing ca. 22-23% of the biomass, in line with previous works (Baltazar-y-Jimenez *et al.,* 2007; Bismarck *et al.,* 2006; Kostic *et al.,* 2008). Only small amount of lipids (ca. 1.2%), proteins (ca. 7-8%) and ashes (ca-5%) were detected in both whole hemp and the cortex. In contrast, the pulp had slightly higher protein content (ca. 10%) and ashes (ca. 9%) and, as expected, significantly lowers lignin content. The carbohydrate composition (Figure 1) consisted mainly of glucose, ascribed to cellulose. Xylose was also present being two-fold higher in the cortex and whole hemp than in the pulp, and representing ca. 20% and 10% for the cortex and pulp, respectively. Apart from glucose and xylose, all the samples contained typical pectin components, mainly glucuronic acid, as well as low amounts arabinose, rhamnose and galactose, being higher in the pulp. In fact, galacturonic acid, represented ca. 7% of the carbohydrate content in the pulp, being twice higher than its content in the cortex and whole biomass.

Figure. 1- Monosaccharide composition of the pulp, cortex and whole hemp fiber.



Based on the similar composition between the whole biomass and the cortex as well as the tedious work and low pulp yields obtained to completely separate the pulp from the cortex, the whole hemp was selected to develop biodegradable packaging materials. Furthermore, the presence of hemicelluloses (such as pectin) has been reported to have a positive impact on packaging performance (Cebrián-Lloret *et al.,* 2022).

Table 2 - Composition of the hemp fiber.

Samples	**Protein (%)**	**Fat (%)**	**Lignin (%)**	**Total carbohydrates (%)**	**Ashes (%)**	**Moisture content (%)**
Pulp	10.60±0.04^{a}	1.42±0.06^{a}	11.63±1.54^{b}	67.50±4.7	8.88^{a}	7.41±0.06^{a}
Cortex	7.72±0.02^{c}	1.28±0.01^{a}	23.93±62.50^{a}	61.70±1.2	5.38^{b}	6.76±0.08^{b}
Whole	8.01±0.05^{b}	1.34±0.01^{a}	22.06±29.64^{a}	62.64±3.8	5.95^{b}	7.30±0.01^{a}

Values with different letters are significantly different ($p \leq 0.05$).

3.2. Optimization of the maximum hemp loading in the film.

In this work, hemp biomass in different percentages were mixed with PHA and PBAT , aiming the maximum possible amount of HF addition in the films; the material was processed by melt mixing and compression molding to prepare packaging materials. Adjusting the PHBV:PBAT ratio in the formulations was crucial to guarantee the maximum amount of hemp biomass in the biobased packaging, in order to obtain homogeneous materials with good mechanical integrity. Based on screening results, PBAT was needed to guarantee a good compatibility between PHBV and hemp biomass. Therefore, the effect PHBV: PBAT ratio on the mechanical performance of the films was assessed by means of tensile testing and representative parameters obtained from the stress-strain curves compiled in Table 3. Pure PHBV films showed poorer tensile properties than the well-known thermoplastic starch or polylactic acid (PLA) films (Gupta *et al.,* 2022; Meereboer *et al.,* 2022).

In general, the incorporation of PBAT had a plasticizing effect (reducing the elastic modulus, while increasing the elongation at break). This effect was more evident as the PBAT concentration increased, reaching 3-fold increase in the EAB values with 50% PBAT. On the other hand, tensile strength was not significantly improved at higher PBAT concentration.

It is well-know that the interfacial adhesion between the fibers and the matrix directly influence the mechanical response of the biocomposites. Therefore, the effect of HF concentration (15 and 30% respect to the biopolymer matrix) was also evaluated in all the films prepared with PHBV: PBAT mixtures and the results are also presented in Table 3. As observed, the mechanical properties of the films were largely affected by the hemp addition showing lower elongation at break and tensile strength but higher elastic modulus than their counterparts prepared without hemp biomass. This agrees with literature since fibers usually decrease the chain mobility in the respective biopolymer matrix which ultimately enhances the elastic modulus (Gitari *et al.,* 2019).

Considering that the elastic modulus is a property at low deformation which should not be affected to interfacial adhesion (Pukánszly, 1990), the higher E values obtained in hemp containing films can be ascribed to the higher intrinsic rigidity of the fibers as compared to the biopolymer matrix. Similarly, Gupta *et al.* (2022) reported a decrease in EAB values for PHBV/PBAT films containing maleic acid and hemp. Robledo-Ortiz *et al.* (2021) also observed a decreased in TS values of PHBV films containing 30 or 40% of sugarcane straw. The presence of lipids and other non-cellulosic compounds (lignin, hemicellulose and pectin) on the surface of hemp fibers could reduce the adhesion with the matrix, thus reducing TS values, as it was reported for other natural fiber reinforced composites (Ku *et al.,* 2011). However, there was not a clear effect of the hemp concentration, except when it was incorporated in the mixture containing the higher amount of PHBV (80PHBV-20PBAT), effect that can be ascribed to a partial degradation of PHBV. Thus, the presence of hemp in PHBV-PBAT mixture led to more rigid films and less elongable but not significant differences were observed between 15 and 30% loadings, except in those containing the higher amount of PHBV.

Therefore, considering the overall mechanical performance and aiming at optimizing the maximum hemp loading in the film, the one prepared with 50% of each biopolymer was selected to evaluate the effect of a higher amount of hemp fibers, with the additional advantage that the lower amount of PHBV in the resulting films will be also important for the economical perspective.

Furthermore, increasing the amount of the natural fiber in the resulting biocomposite material will be of great interest due to its low cost, eco-friendly and biodegradability properties. Thus, the amount of hemp fibers in the 50PHBV-50PBAT film was optimized to maximize its content in the biocomposite. A maximum amount of 40% of hemp fibers could be incorporated in the biopolymer matrix, since a higher amount could not be properly processed in the mini-extruder. The results, also shown in Table 3, did not show significant differences in the elongation at break and tensile strength values, although a higher elastic modulus was observed, evidencing the higher intrinsic tenacity of the hemp fibers.

Table 3 - Mechanical properties of the films.

Formulation	**EAB (%)**	**TS (MPa)**	**E (MPa)**
50PHBV/50PBAT	$5.40^{a}\pm0.55$	$20.83^{a}\pm1.52$	$913.68^{f}\pm77.02$
50PHBV/50PBAT_15HF	$1.71^{cd}\pm0.52$	$9.34^{bc}\pm2.23$	$1083.79^{ef}\pm94.86$
50PHBV/50PBAT_30 HF	$1.69^{cd}\pm0.20$	$9.38^{bc}\pm0.77$	$1195.18^{df}\pm107.90$
50PHBV/50PBAT_40HF	$1.02^{cd}\pm0.35$	$8.85^{bc}\pm2.89$	$1523.71^{bd}\pm200.72$
60PHBV/40PBAT	$3.43^{b}\pm0.52$	$22.05^{a}\pm2.02$	$169.76^{ef}\pm144.95$
60PHBV/40PBAT_15HF	$1.65^{cd}\pm0.32$	$9.05^{bg}\pm1.6$	$1043.94^{ef}\pm57.85$
60PHBV/40PBAT_30HF	$0.98^{d}\pm0.26$	6.21c±1.6	$1204.70^{df}\pm138.14$
70PHBV/30PBAT	$3.01^{b}\pm0.45$	$23.64^{a}\pm2.21$	$1341.69^{cde}\pm113.99$
70PHBV/30PBAT_15HF	$1.11^{cd}\pm0.50$	$10.84^{bc}\pm3.03$	$1352.00^{cde}\pm124.46$
70PHBV/30PBAT_30HF	$1.12c^{d}\pm0.24$	$8.20^{bc}\pm0.71$	$1371.26^{bde}\pm175.41$
80PHBV/20PBAT	$1.76^{cd}\pm0.32$	$20.14^{a}\pm3.48$	$1712.84^{ab}\pm169.92$
80PHBV/20PBAT_15HF	$1.92^{c}\pm0.08$	$21.32^{a}\pm1.72$	$2026.14^{a}\pm188.35$
80PHBV/20PBAT_30 HF	$1.52^{cd}\pm0.15$	$11.74^{b}\pm1.16$	$1574.27^{bc}\pm76.71$

TS: tensile strength; EAB: elongation at break; E: Young's modulus. Values with different letters are significantly different ($p \leq 0.05$).

3.3 Effect of wax addition

A potential undesirable effect of incorporating natural lignocellulosic fibers, such as HF, in a more hydrophobic biopolymer matrix (PHBV/PBAT) is the higher water sensibility of the resulting biocomposites which could affect their potential application due to the high-water activity of fresh foods and an accelerated degradation of the film' components caused by the presence of water molecules. As a strategy to limit the moisture uptake of these materials is the incorporation of natural waxes to improve the permeability resistance of the biocomposites. Table 4 gathers the mechanical and barrier properties of biocomposites films containing 5% of BW or CW. The amount of wax was optimized based on the processing conditions in the mini-extruder.

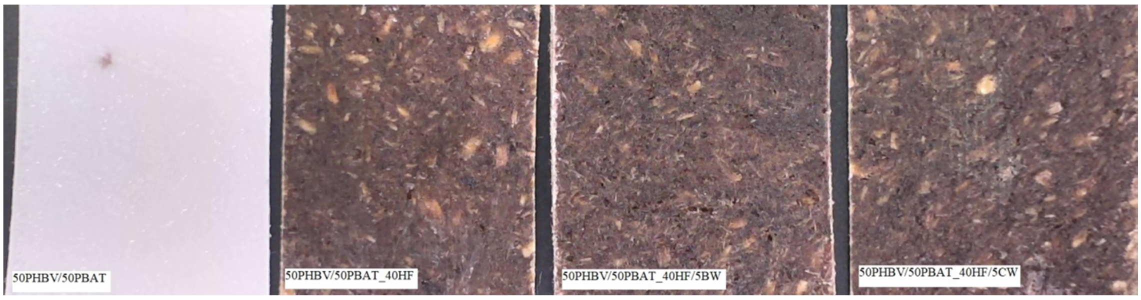
Table 4 - Mechanical properties and water vapor permeability results of the films.

Formulation	**EAB (%)**	**TS (MPa)**	**E (MPa)**	**WVP $*10^{15}$ (Kg-m/Pa-s-m^{2})**
50PHBV/50PBAT	$5.40^{a}\pm0.55$	$20.83^{a}\pm1.52$	$913.68^{b}\pm77.02$	3.69 ± 0.03^{b}
50PHBV/50PBAT_40HF	$1.02^{b}\pm0.35$	$8.85^{b}\pm2.89$	$1523.71^{a}\pm200.72$	9.39 ± 0.31^{a}
50PHBV/50PBAT_40HF/5BW	$1.20^{a}\pm0.08$	$6.23^{b}\pm0.42$	$893.90^{b}\pm57.93$	8.77 ± 0.15^{a}
50PHBV/50PBAT_40HF/5CW	$0.84^{a}\pm0.05$	$6.10^{b}\pm0.43$	$1115.92^{b}\pm61.41$	7.85 ± 0.02^{a}

TS: tensile strength; EAB: elongation at break; E: Young's modulus. Values with different letters are significantly different ($p \leq 0.05$).

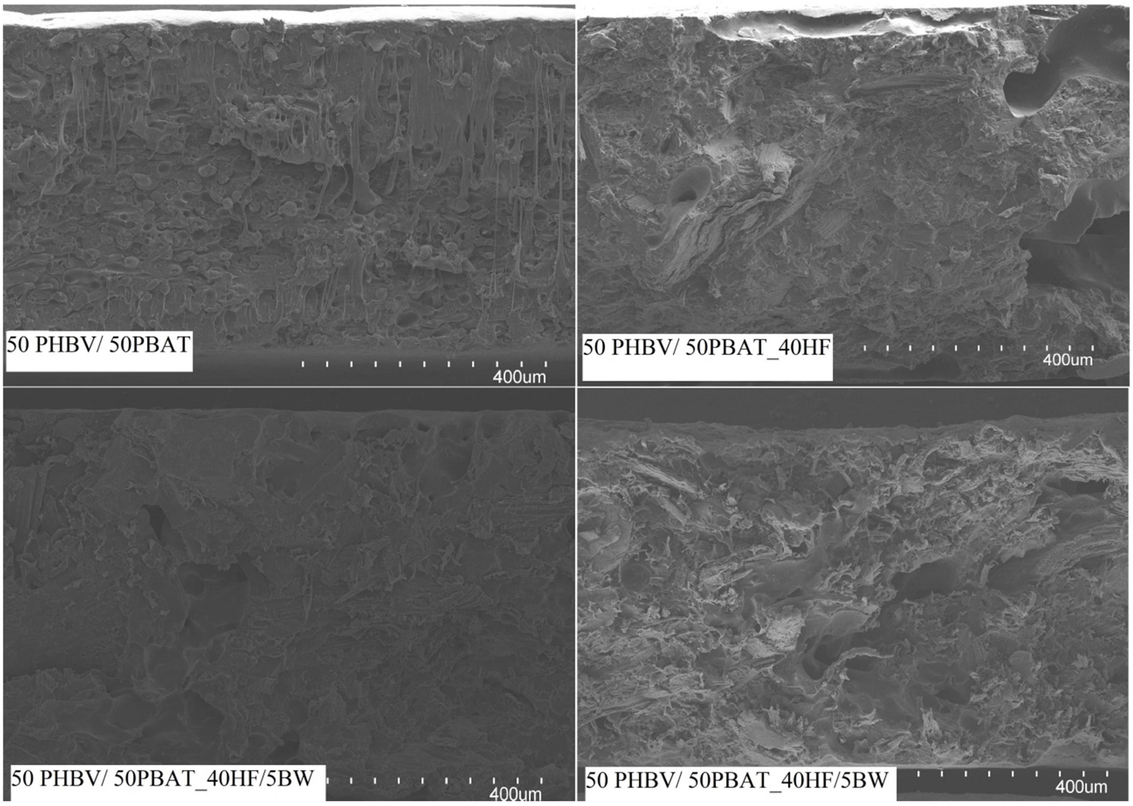
Figure 2 shows the contact transparency images of the obtained films, with and without waxes, which were very similar. SEM micrographs of the cross-section of the films (with and without HF or waxes) are given in Figure 3.

Figure. 2- Images of the obtained films.



The incorporation of HF and waxes gave rise to a more homogenous film matrix than the neat PHBV/PBAT film, but showing some micro-voids at the filler/matrix interphase which could have an impact on the barrier properties, as it will be detailed below. The degree of heterogeneity depended on the nature of the wax, since BW appeared better integrated in the matrix than CW.

Figure 3 – SEM images from the fracture of the formulation.

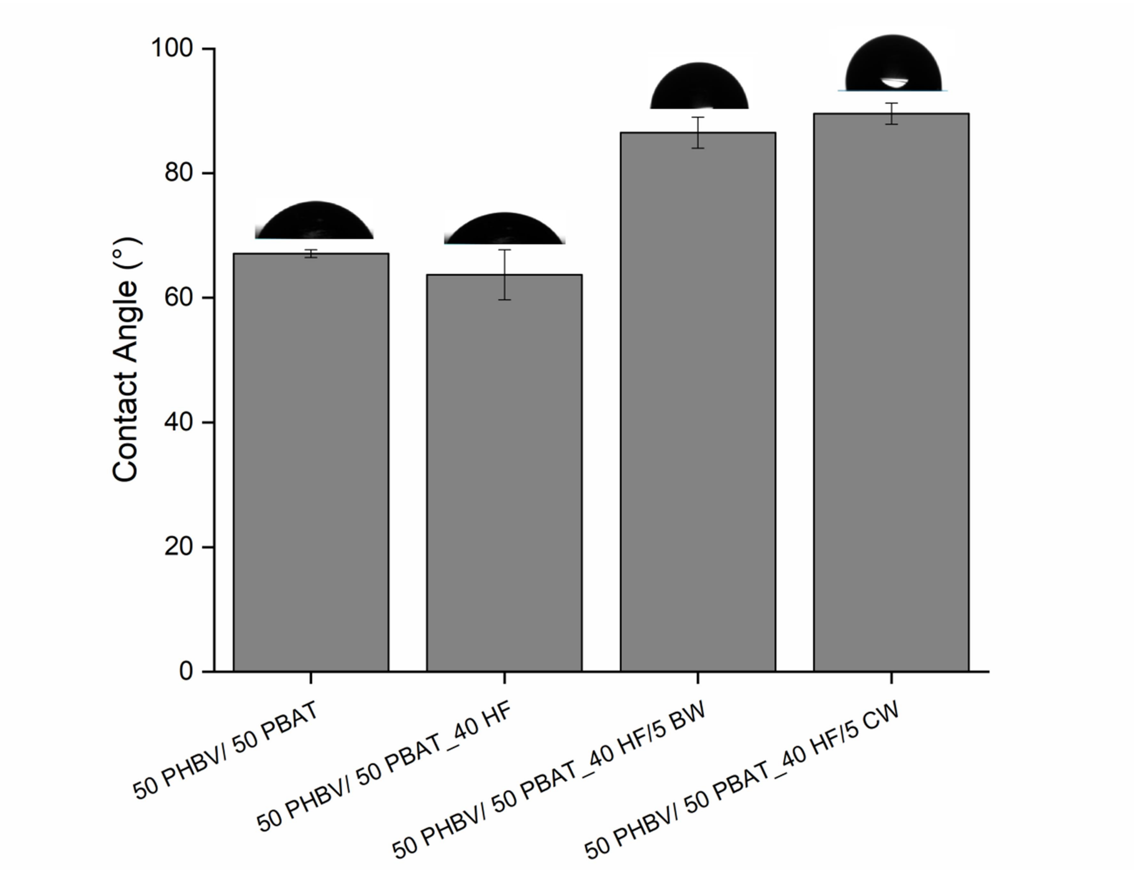


When comparing the films, the first thing to highlight is that, as expected, the presence of hemp fibers significantly decreased the water vapor barrier properties of biocomposites compared to the neat 50PHBV-50PBAT, mainly ascribed to the hydrophilic character of the HF due to hydroxyl groups of cellulose (Espert *et al.,* 2004). The decrease of the WVP after the addition of HP was also reported by other authors; Zhang *et al.* (2020) worked with PVA and HF to produce films by the casting technique, the WVP results of the pure PVA film was 0.98 × 10^{-13} /(Kg-m/Pa-s-m^2), while the result of the film with 10% of HF increased to 1.14 × 10^{-13}(Kg-m/Pa-s-m^2). Dixit *et al.* (2022) in his study with polyethylene, polypropylene, natural and alkali treated HF observed an improvement in the WVP value with the treated HF when compared to the results of natural HF.

The incorporation of waxes reduced the average water vapor permeability value compared to biocomposite containing 40% HP, but showing no significant differences between the samples. As expected the most hydrophobic nature of BW and CW containing films was confirmed by its higher contact angle values reported for these films (figure 4), which were around 89º. This improvement has been previously reported in nanocomposites and multilayer films containing lipids (El Mouzahim *et al.,* 2023; Fabra, López-Rubio, Ambrosio-Martín, Lagaron, 2016; Pérez-Bassart *et al.,* 2023). In a recent work, Dixit *et al.* (2022) reported higher contact angle values for films prepared with natural and alkali HF films which were incorporated in polyethylene and polypropylene matrices.

Regarding the mechanical properties, either BW or CW provided less rigid and more ductile films with lower TS and E values but they were not more deformable. This could be expected since if lipids are well-dispersed in the matrix, they could act as a lubricant for the polymer chain slipping during the film stretching, thus inducting an increase in stretchability and ductility.

Figure 4 – Contact angle results and representative images of water droplets formed after 10 minutes of contact.



4. Conclusion

In this work, biodegradable packaging films were produced by melt-blending and compression molding. The concentration of the biopolymers used (PHBV and PBAT) played an important role in the amount of the hemp fiber that could be incorporated. The biocomposites produced using 40% of HF showed an improvement in the interaction between the film´s components, as shows the SEM images although the water vapor permeability was increased. The incorporation of waxes increased the hydrophobicity of the resulting films, as deduced from the contact angle results.

Acknowledgment: This research is part of the CSIC program for the Spanish Recovery, Transformation and Resilience Plan funded by the Recovery and Resilience Facility of the European Union, established by the Regulation (EU) 2020/2094. CSIC Interdisciplinary Thematic Platform (PTI+) Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy+. (PTI-SusPlast+).

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CONSIDERAÇÕES FINAIS

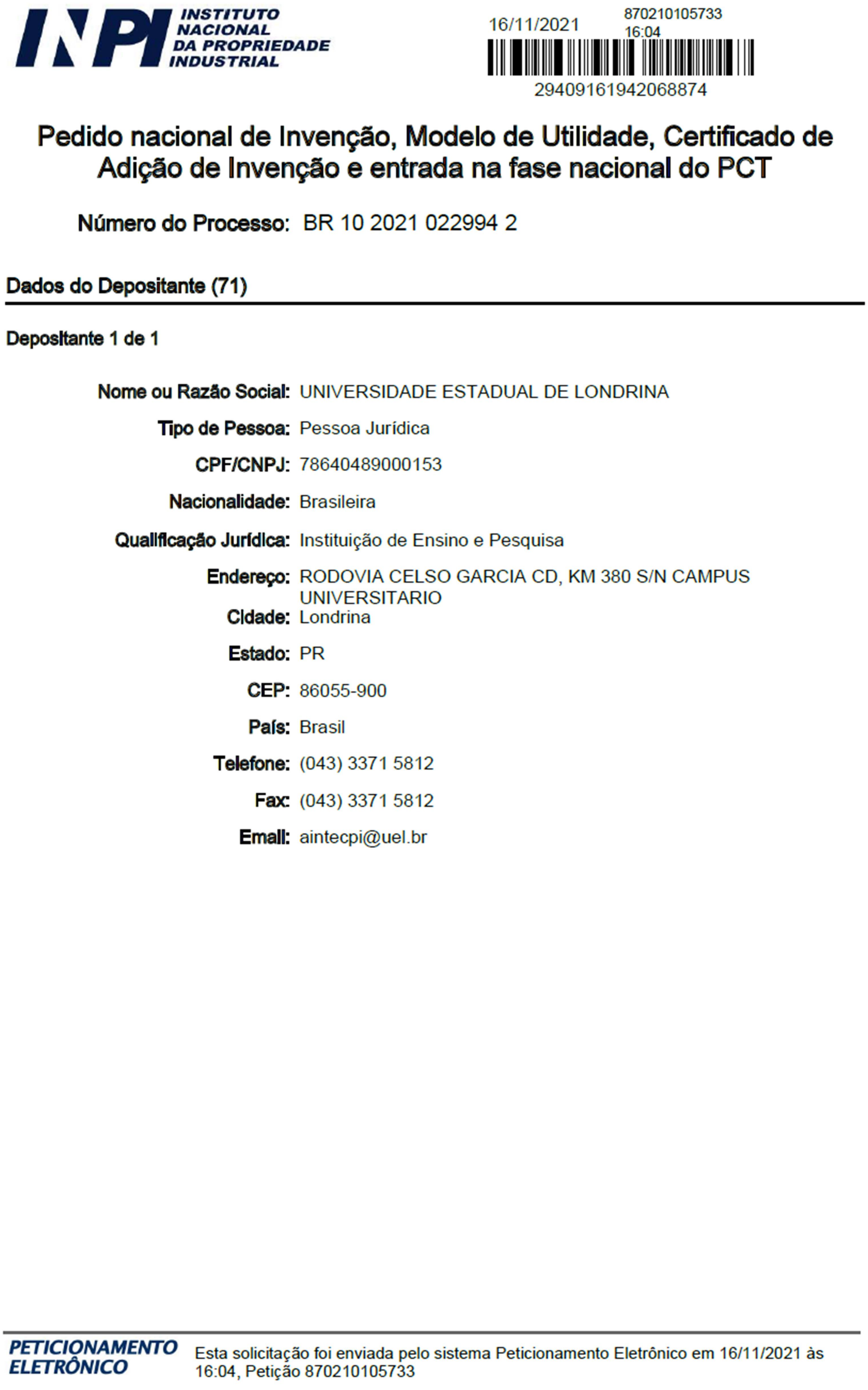
Celulose extraída da casa de aveia foi modificada com os ácidos cítrico e succínico, nas concentrações de 5, 12.5 e 20%, por processo de extrusão reativa em extrusora monorosca. A comprovação da ocorrência de modificação química foi verificada por espectroscopia FT-IR com o aparecimento de uma nova banda em 1720 e 1737 cm^{-1} em todas as formulações produzidas. Todas as formulações com celulose modificada apresentaram alta afinidade para solventes não polares e aumento na sua capacidade de absorção de óleo. O processo de obtenção de celulose por extrusão reativa pode ser considerado um método ecologicamente correto, com baixa geração de efluentes, tempo curto de reação, baixos custos de investimento, simplicidade de operação, e é escalonável para uso em larga escala.

A formulação de celulose modificada com 5% de ácido succínico foi escolhida para a produção de hidrogéis. Filmes laminados de hidrogéis foram produzidos utilizando celulose *in natura* e modificada, gelatina e goma xantana, por processo de extrusão reativa seguido de termoprenssagem. Todas as formulações apresentaram boa aparência e homogeneidade. No teste de intumescimento foi comprovado que todos os filmes de hidrogéis apresentam elevado intumescimento em diferentes faixas de pH, sendo os melhores resultado obtidos em pH 4. Todas as formulações apresentam elevada estabilidade quando expostas a altas temperaturas. O processo de produção de filmes de hidrogéis utilizando a combinação de extrusão reativa e termoprenssagem se mostrou eficiente, sendo uma alternativa promissora para a produção de um material com excelente custo benefício.

Fibras de cânhamo foram caracterizadas, e posteriormente foram mescladas com PBHV e PBAT por processo de extrusão, seguido de termoprenssagem para a produção de filmes biodegradáveis. Para melhorar a hidrofobicidade dos filmes produzidos, dois aditivos foram incorporados, a cera de abelha e a cera de carnaúba. Todas as formulações apresentaram boa aparência e homogeneidade. O teste de ângulo de contato comprovou que a incorporação das ceras aumentou a hidrofobicidade das amostras. O processo utilizado para a produção de filmes biodegradáveis utilizando uma alta concentração de fibras de cânhamo se mostrou eficiente, sendo uma possível alternativa para a produção de um material ecoamigável com possíveis aplicações em embalagens alimentícias.

ANEXO 1

Patente depositada



INSTITUTO
NACIONAL
DA PROPRIEDADE
INDUSTRIAL

16/11/2021
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16:04
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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

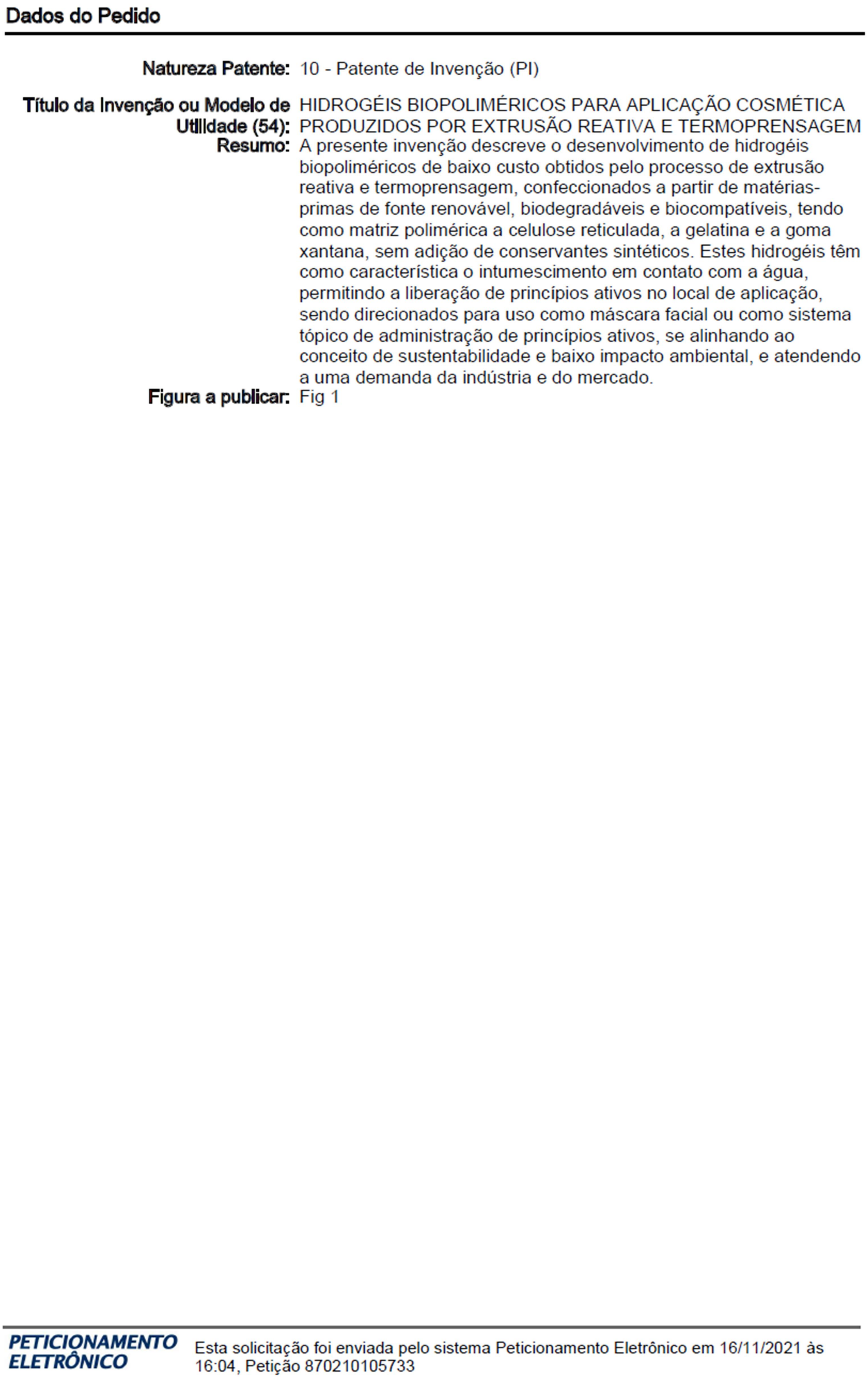
Número do Processo: BR 10 2021 022994 2

Dados do Depositante (71)

Depositante 1 de 1

Nome ou Razão Social: UNIVERSIDADE ESTADUAL DE LONDRINA
Tipo de Pessoa: Pessoa Jurídica
CPF/CNPJ: 78640489000153
Nacionalidade: Brasileira
Qualificação Jurídica: Instituição de Ensino e Pesquisa
Endereço: RODOVIA CELSO GARCIA CD, KM 380 S/N CAMPUS UNIVERSITARIO
Cidade: Londrina
Estado: PR
CEP: 86055-900
País: Brasil
Telefone: (043) 3371 5812
Fax: (043) 3371 5812
Email: aintecpi@uel.br

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 16/11/2021 às 16:04, Petição 870210105733



Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): HIDROGÉIS BIOPOLIMÉRICOS PARA APLICAÇÃO COSMÉTICA PRODUZIDOS POR EXTRUSÃO REATIVA E TERMOPRENSAGEM

Resumo: A presente invenção descreve o desenvolvimento de hidrogéis biopoliméricos de baixo custo obtidos pelo processo de extrusão reativa e termoprensagem, confeccionados a partir de matérias-primas de fonte renovável, biodegradáveis e biocompatíveis, tendo como matriz polimérica a celulose reticulada, a gelatina e a goma xantana, sem adição de conservantes sintéticos. Estes hidrogéis têm como característica o intumescimento em contato com a água, permitindo a liberação de princípios ativos no local de aplicação, sendo direcionados para uso como máscara facial ou como sistema tópico de administração de princípios ativos, se alinhando ao conceito de sustentabilidade e baixo impacto ambiental, e atendendo a uma demanda da indústria e do mercado.

Figura a publicar: Fig 1

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 16/11/2021 às 16:04, Petição 870210105733

Dados do Inventor (72)

Inventor 1 de 5

Nome: SUZANA MALI DE OLIVEIRA
CPF: 57998523115
Nacionalidade: Brasileira
Qualificação Física: Professor do ensino superior
Endereço: Rua João Huss, 199, ap. 602
Cidade: Londrina
Estado: PR
CEP: 86050-490
País: BRASIL
Telefone: (43) 337 15812
Fax:
Email: aintecsec@uel.br

Inventor 2 de 5

Nome: JÉSSICA FERNANDA PEREIRA
CPF: 38708603839
Nacionalidade: Brasileira
Qualificação Física: Estudante de Pós Graduação
Endereço: AV. ARTHUR THOMAS, 1475 - AP 0302 BL D
Cidade: Londrina
Estado: PR
CEP: 86065-000
País: BRASIL
Telefone: (43) 337 15812
Fax:
Email: aintecsec@uel.br

Inventor 3 de 5

Nome: BEATRIZ MARJORIE MARIM
CPF: 08612035937
Nacionalidade: Brasileira
Qualificação Física: Estudante de Pós Graduação
Endereço: AV VINICIUS DE MORAES, 510, AP 404 C.
Cidade: Londrina
Estado: PR
CEP: 86075-000
País: BRASIL
Telefone: (43) 337 15812
Fax:
Email: aintecsec@uel.br

Inventor 4 de 5

Nome: FABIO YAMASHITA
CPF: 09698351833
Nacionalidade: Brasileira
Qualificação Física: Professor do ensino superior
Endereço: Rua SERGIPE, 01720, APT 6
Cidade: Londrina
Estado: PR
CEP: 86010-380
País: BRASIL
Telefone: (43) 337 15812
Fax:
Email: aintecsec@uel.br

Inventor 5 de 5

ANEXO 2

Artigo publicado

polysaccharides

MDPI

Article

Chemical Modification of Cellulose Using a Green Route by Reactive Extrusion with Citric and Succinic Acids

Jéssica Fernanda Pereira, Beatriz Marjorie Marim and Suzana Mali *

Department of Biochemistry and Biotechnology, State University of Londrina—UEL,
Londrina 86057-970, PR, Brazil; jeh-fernanda@hotmail.com (J.F.P.); beatrizmarjorie1@gmail.com (B.M.M.)
* Correspondence: smali@uel.br; Tel.: +55-43-3371-4270; Fax: +55-43-3371-5470

Abstract: Cellulose is a natural, unbranched, and fibrous homopolymer that is a major component in several agroindustrial residues. The aim of this study was to extract cellulose from oat hulls and then to modify it using a green route to obtain esterified cellulose through reaction with organic acids employing the reactive extrusion process, which is a process that presents some advantages, including low effluent generation, short reaction times, and it is scalable for large scale use. Citric (CA) and succinic (SA) acids were employed as esterifying agents in different concentrations (0, 5, 12.5, and 20%). Modified cellulose samples were characterized by their degree of substitution (DS), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (DRX), scanning electron microscopy (SEM), wettability, oil and water absorption capacities, and thermal stability. DS of modified samples ranged from 2.28 to 3.00, and FTIR results showed that the esterification occurred in all samples for both acids by observation of important bands at 1720 and 1737 cm^{-1} for samples modified with CA and SA, respectively. All modified samples presented increased hydrophobicity. The modification did not have an influence on the morphological structure or crystallinity pattern of all samples. This study proved to be possible to modify cellulose using a simple and ecofriendly process based on reactive extrusion with organic acids.

Keywords: reactive extrusion; esterification; lignocellulosic residues; oat hull

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Citation: Pereira, J.F.; Marim, B.M.; Mali, S. Chemical Modification of Cellulose Using a Green Route by Reactive Extrusion with Citric and Succinic Acids. *Polysaccharides* **2022**, *3*, 292–305. https://doi.org/10.3390/polysaccharides3010017

Academic Editors: Ricardo J. B. Pinto and Alessandra Braga Ribeiro

Received: 28 January 2022
Accepted: 11 March 2022
Published: 13 March 2022

1. Introduction

Cellulose is one of the most abundant natural, renewable, and biodegradable polymers; it is an unbranched and fibrous homopolymer that can be obtained from plants or synthesized by bacteria. The cellulose chain consists of β-D-glucose units repetedly joined by β (1-4) glycosidic bonds, with three hydroxyl groups per monosaccharide unit [1–3], which makes cellulose an excellent platform for chemical modifications [4]. So, cellulose can be modified to be used in the cosmetic, pharmaceutic, and food industries and in agricultural systems, among others [5–7].

Agroindustrial residues can be considered interesting sources for cellulose extraction, and in the last few years, an increased interest in the obtainment of cellulose from these materials using different approaches was seen, which can be considered a promising alternative for the production of sustainable products at affordable prices to reduce the dependency on petroleum-based products [8]. The use of agroindustrial residues to obtain new products is inserted into the concept of biorefineries, meeting the vision of a sustainable economy using biological resources, maximizing benefits and profits through strategies to add value to the plant biomass chain [9,10].

Oat hull is a by-product from oat grain milling and represents 25 to 30% of oat grain weight, with approximately 28–35% of cellulose, 18–28% of hemicellulose, and 18–22% of lignin [4,11,12], being considered an interesting raw material for cellulose obtainment.

Modifications in the cellulose structure are largely studied to enhance its properties. Among all the possible modification types, esterification is one of the most reported

ANEXO 3

Artigo publicado

PREPARATIVE BIOCHEMISTRY & BIOTECHNOLOGY
https://doi.org/10.1080/10826068.2022.2162921

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Hydrogels based on gelatin, xanthan gum, and cellulose obtained by reactive extrusion and thermopressing processes

Jéssica F. Pereira[a], Beatriz M. Marim[a], Bruno M. Simões[b], Fabio Yamashita[b], and Suzana Mali[a]

[a]Department of Biochemistry and Biotechnology, CCE, State University of Londrina, Londrina, PR, Brazil; [b]Department of Food Science and Technology, Center for Agricultural Sciences, State University of Londrina, Londrina, PR, Brazil

ABSTRACT
Polysaccharides and proteins are compatible macromolecules that can be used to obtain biopolymeric hydrogels through physical interactions. In this study, an environmentally friendly strategy is being proposed to produce gelatin-xanthan gum- cellulose hydrogels, without the addition of chemical synthetic crosslinkers. Xanthan gum was employed as an alternative crosslinking agent, and cellulose was used as a potential reinforcing agent in the polymeric matrix. Firstly, the biopolymers were mixed by the extrusion process, and glycerol was used as a plasticizer. Then, the polymeric mixture was molded by thermopressing to obtain hydrogels as laminated films. All hydrogels formulations resulted in films with smooth surfaces, without pores or cracks, resulting in amorphous polymeric matrices. The obtained hydrogels had a pH-dependent degree of swelling, the highest swelling values were obtained at pH 4 (5.3–7.9 g/g) after 24 h of immersion. Cellulose acted as a reinforcing agent for hydrogels, increasing thermal stability, tensile strength, and Young's modulus of films when employed at the higher level (7%). The strategy employed in this study to obtain nontoxic hydrogels without synthetic crosslinkers was effective, resulting in materials with promising properties to be used as pharmaceutical forms to deliver active compounds in cosmetic or pharmaceutical products.

KEYWORDS
pH-dependent swelling;
polymeric mixtures;
superabsorbent hydrogels

Introduction

Advances in biopolymers technology have led to the growing interest in the development of biopolymeric hydrogels, which are a unique class of material that is produced from hydrophilic three-dimensional viscoelastic polymeric networks, allowing the diffusion and fixation of molecules.[1–3] These networks are maintained due to the presence of physical and/or chemical bonds between the polymer chains, presenting an inherent capacity to swell and retain water in their structure, while having resistance to dissolution.[3]

A superabsorbent hydrogel can absorb over 100% of its dry weight in water.[4] The most common commercial hydrogels are based on synthetic polymers, including polyacrylamide, poly (sodium acrylate), poly (acrylic acid), and polyvinylpyrrolidone. These synthetic matrices are bounded by chemical crosslinking, however, the usual crosslinkers may cause some toxicity.[4–6] Glutaraldehyde and glyoxal are dialdehydes usually employed as crosslinkers; glutaraldehyde is reported as a neurotoxic compound and glyoxal is considered mutagenic.[5]

Several efforts have been made to increase hydrogel production from biodegradable polymers.[1–6] Additionally, the obtainment of physically crosslinked hydrogels can be considered a solution to overcome the toxicity of chemical crosslinkers, resulting in a biocompatible polymeric structure that gets broken down into nontoxic substances, being able to be use in cosmetic and pharmaceutical sectors.[5–9]

Polysaccharides and proteins are compatible biomacromolecules that can be used to obtain hydrogels by physical interactions, including electrostatic interactions when these biopolymers carry net opposite electric, hydrogen interactions, hydrophobic or Van der Waals forces.[4,7–10] In general, polysaccharides and proteins mixed systems can result in hydrogels with high water absorption capacity, which is related to the presence of hydrophilic groups present in their chemical structure, such as amine (NH_2), hydroxyl ($-OH$), amide ($-CONH-$, $-CONH_2$), carboxyl ($-COOH$), among others.[7–9]

Gelatin is a low-cost protein obtained from partial hydrolysis of collagen from skin, bones or connective tissue of animals, being classified as type A (acid hydrolysis) or type B gelatin (alkaline hydrolysis). Gelatin exhibits an amphoteric behavior due to the presence of both basic and acidic groups, presenting in its composition a large number of glycine, proline and hydroxyl-proline residues. It is widely used in food, cosmetic and pharmaceutical industries.[3,11] It is easily soluble in water at an average temperature of 40 °C, forming a viscous solution by chain association and three-dimensional network formation, resulting in gels and films employed for several applications.[11,12]

CONTACT Suzana Mali smali@uel.br Department of Biochemistry and Biotechnology, CCE, State University of Londrina, Londrina, PR, Brazil