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THOMÁS CORRÊA VIANNA

DESENVOLVIMENTO E CARACTERIZAÇÃO DE FILMES ATIVOS A BASE DE AMIDO, POLI(ÁCIDO ITACÔNICO) E POLI(LIMONENO)

DEVELOPMENT AND CHARACTERIZATION OF ACTIVE FILMS BASED ON STARCH, POLY(ITACONIC ACID) AND POLY(LIMONENE)

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Dissertação de Mestrado apresentada à Faculdade de Engenharia Química da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Mestre em Engenharia Química.

Dissertation presented to the School of Chemical Engineering of the University of Campinas as part of the requirements for the degree of Master in Chemical Engineering.

Orientador: Roniérik Pioli Vieira

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“O real não está na saída nem na chegada: *ele se dispõe para a gente é no meio da travessia*.”

João Guimarães Rosa

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RESUMO

Além da necessidade de aprimorar as propriedades de plásticos à base de amido (S) (principalmente as mecânicas e de barreira), a demanda dos consumidores por alimentos seguros estimularam a pesquisa de novos polímeros e compostos bioativos que possam substituir os materiais mais problemáticos. Nesse sentido, os principais objetivos deste trabalho foram a) melhorar as propriedades mecânicas e de barreira de filmes de amido e b) adicionar uma substância ativa, capaz de conferir propriedades antimicrobianas e antioxidantes ao filme. Para isso, esta pesquisa foi dividida nas etapas sequenciais: 1) desenvolvimento e caracterização de filmes à base de amido com ácido itacônico (IA) e poli(ácido itacônico) (PIA) incorporados em diferentes concentrações (5 – 50 g/100g de amido) e usando diferentes metodologias de preparo (pré-polimerização, polimerização *in situ* e sem polimerização), a fim de se determinar tanto a concentração de IA/PIA quanto a metodologia mais promissora; 2) Após definição da formulação de S/IA a ser utilizada, os filmes foram aditivados com limoneno (LIM) e poli(limoneno) (PL) de baixa massa molar em diferente concentrações (3 – 9 g de LIM ou PL/ 100 g de amido), sendo os filmes demonstraram atividades bioativas completamente caracterizados. Variando-se a concentração de IA, observou-se que a adição de maiores teores conferiu maior cristalinidade e reduziu a permeabilidade ao vapor de água (WVP), mas também reduziu significativamente a resistência à tração (TS) e o alongamento na ruptura (EB). No entanto, o filme com 5 g de IA/100 g apresentou aumento no EB de 18,7 para 45,1 %, sem alterações significativas na TS. A variação dos métodos não afetou TS nem EB, mas influenciou as propriedades de barreira, e para a técnica de pré-polimerização, WVP foi reduzida de 2,5 (controle) para 1,7 x 10^{-10} g m^{-1} s^{-1} Pa^{-1}. Portanto, a concentração de 5 g/100g e o método de pré-polimerização foram empregados na confecção dos filmes da Etapa 2. Os filmes com LIM não apresentaram propriedades ativas, enquanto aqueles com PL foram eficazes contra alguns microorganismos relacionados à deterioração de alimentos (*Bacillus cereus*, *Clostridium perfringens*, *Pseudomonas Aeruginosa* e *Colletotrichum gloeosporioides*) e apresentaram potecial de eliminação de radicais livres (atividade antioxidade) de até 13,4%, demonstrando pela primeira vez o melhor desempenho do PL nesse sentido. A incorporação de PL promoveu uma morfologia geral uniforme do filme, reduziu TS e EB. Nenhuma diferença significativa foi registrada para WVP. A adição de PL também proporcionou um excelente efeito de barreira à luz UV, sugerindo potencial de aplicação dos filmes à base de S/PIA/PL como embalagens ativas.

Palavras chave: poli(ácido itacônico); amido; poli(limoneno); filmes ativos; biopolímeros.

ABSTRACT

In addition to the need to improve the properties of starch (S) - based plastics (particularly mechanical and barrier ones), consumer demand for safe and fresh foods has spurred research into new polymers and the exploration of safer alternatives that can replace problematic materials. In this sense, the main objectives of this work were 1) to improve the mechanical and barrier properties of starch films and 2) to add an active substance, capable of conferring antimicrobial and antioxidant properties. Due to it, this research was divided into sequential steps: 1) development and characterization of S-based films added with itaconic acid (IA) and poly(itaconic acid) (PIA) at different concentrations (5 - 50 g/100g of starch) and using different polymerization methodologies (pre-polymerization, *in situ* polymerization and no polymerization) in order to determine both the optimal concentration of IA/PIA and the methodology; 2) After defining the optimal formulation of S/PIA, limonene (LIM) and low molecular mass poly(limonene) (PL) were added at different concentrations (3 - 9 g of LIM or PL/100g of S), and the films with the most promising bioactive activities were fully characterized. Varying the concentration of IA, it was observed that the addition of higher levels of acid conferred greater crystallinity and improved water vapor permeability (WVP), but significantly reduced tensile strength (TS) and elongation at break (EB). However, the film with 5 g of IA/100 g showed an increase in EB from 18.7 to 45.1 %, without significant changes in TS. The variation of methods did not affect TS or EB, but influenced the barrier properties, and for the pre-polymerization technique, WVP was reduced from 2.5 (control) to 1.7 x 10-10 g m^{-1} s^{-1} Pa^{-1}. Therefore, the concentration of 5 g/100g and the pre-polymerization method were were used in the manufacture of the films of Stage 2. Films with LIM did not show active properties, while those with PL were effective against *B. cereus* , *C. perfringens*, *P. aeruginosa* and *C. gloeosporioides* and showed antioxidant activity of up to 13.4%, demonstrating for the first time the best performance of PL in this regard. The incorporation of PL promoted an overall uniform film morphology, reduced TS and EB and increased moisture content and water solubility. No significant differences were recorded for WVP. The addition of PL also provided an excellent barrier effect to UV light, suggesting great potential for the application of films based on S/PIA/PL as active packaging.

Keywords: poly (itaconic acid); starch; poly(limonene); active films; biopolymers.

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1. CAPÍTULO 1 – INTRODUÇÃO E OBJETIVOS

1.1 MOTIVAÇÃO E RELEVÂNCIA

A necessidade de estimular um desenvolvimento mais sustentável e de equilibrar as demandas econômicas, sociais e ambientais é consenso mundial (RODRIGUEZ-ANTON; ALONSO-ALMEIDA, 2020). Um dos desafios a esse desenvolvimento é mitigar o uso de plásticos de origem fóssil, principalmente aqueles de uso único, bem como o combate do seu desperdício, que criam graves riscos ambientais e problemas de gerenciamento de seus resíduos. Neste contexto, filmes biodegradáveis a base de polímeros de origem renovável, como o amido e poli(ácido itacônico) (PIA) e aditivados com substâncias ativas, como limoneno (LIM) ou poli(limoneno) (PL) de baixa massa molar, surgem como um potencial alternativo para substituir polímeros de origem fóssil na produção de embalagens e diminuir o uso de aditivos potencialmente nocivos na conservação de alimentos.

O amido é considerado um dos polissacarídeos mais promissores no desenvolvimento de filmes biodegradáveis devido a sua abundância, baixo custo-benefício e excelente capacidade de formação de filmes (THAKUR *et al.*, 2019). Embora plásticos à base de amido já estejam sendo comercializados, sua produção e a melhoria de seu desempenho (principalmente propriedades mecânicas e de barreira) ainda representam um grande desafio (SALIU *et al.*, 2019; THAKUR *et al.*, 2019; DIN *et al.*, 2020; SIQUEIRA *et al.*, 2021). A forma mais eficaz de potencializar essas características é a formação de blendas (ROMANI; PRENTICE-HERNÁNDEZ; MARTINS, 2017; DIN *et al.*, 2020). Assim sendo, o presente trabalho partiu da hipótese de que a combinação de amido e IA/PIA aprimoraria as propriedades mecânicas, devido às possíveis interações intermoleculares e, que a adição de LIM/PL conferiria propriedades antimicrobiana e/ou antioxidante ao filme obtido.

O ácido itacônico (IA) é um produto químico promissor, com ampla gama de aplicações, podendo ser obtido em larga escala por processos fermentativos (MAGALHÃES *et al.*, 2017). Sua estrutura permite uma variedade de novas aplicações, sendo que, a possibilidade de modificar a ligação dupla do ácido exibe um alto potencial para desenvolver novos materiais com propriedades sem precedentes (ROBERT; FRIEBEL, 2016). Portanto, um de seus usos mais importantes é a produção de biopolímeros e a possibilidade de formação de plásticos verdes (KUENZ *et al.*, 2012; MAGALHÃES *et al.*, 2017). Nem a preparação de polímeros de IA, nem os produtos desses plásticos são prejudiciais à saúde ou ao meio ambiente (SAHA, 2017).

Apesar de seu enorme potencial para substituir produtos químicos de base petroquímica, seu custo de produção pela fermentação da glicose é fator limitante em sua aplicação industrial em larga escala (TRANSPARENCY MARKET RESEARCH, 2015; SAHA, 2017). O ácido é um exemplo que ilustra os obstáculos e oportunidades de processos biotecnológicos, visto que, embora sua produção biotecnológica já esteja estabelecida em escala, é necessário a otimização (KLEMENT; BÜCHS, 2013). Uma possível alternativa para se produzir filmes de fontes renováveis a partir do ácido itacônico é a formação de blendas ou a copolimerização com outras substâncias.

Ademais, a adição de óleos essenciais (OEs) justifica-se pela presença de fitoconstituintes farmacologicamente ativos (como terpenos, por exemplo), que podem proteger os consumidores dos efeitos da deterioração oxidativa e bacteriana, aumentando a vida útil dos alimentos (AKRAM *et al.*, 2019; JUGREET *et al.*, 2020; CHAWLA; SIVAKUMAR; KAUR, 2021). Como exemplo, OE de laranja possui capacidade inibitória contra as bactérias *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa* e *Shigella dysenteriae* (DO EVANGELHO et al., 2019). LIM é o principal componente de diversos óleos essenciais de cítricos, sendo possível realizar sua extração a partir das cascas, que são subprodutos do processamento de sucos (SENIT *et al.*, 2019; GÜLTEPE, 2020; REN *et al.*, 2020). O Brasil é líder mundial na produção de laranjas e de suco de laranja, o que acarreta acúmulo de enormes quantidades de resíduos, sendo estimado que apenas os da casca representem de 50-60 % da massa total do fruto (MARTINS *et al.*, 2013; OZTURK; WINTERBURN; GONZALEZ-MIQUEL, 2019). A aditivação de embalagens biodegradáveis com LIM, portanto, vai ao encontro dos princípios de economia circular definidos pela Ellen MacArthur Foundation

(2019), que se baseiam na eliminação de resíduos e poluição e na manutenção de produtos e materiais em uso.

Apesar do seu potencial, o LIM é um composto de alta volatilidade e baixa estabilidade térmica e, portanto, pode ser perdido durante o processamento dos filmes (GONZÁLEZ-MAS et al., 2019). Como o poli(limoneno) (PL) (oligômero sintético derivado do limoneno) possui temperatura de degradação máxima de 325 ºC, superior a do seu monômero, de 125 ºC (MARTINS et al., 2011; DE OLIVEIRA; VIEIRA, 2020), o presente trabalho também investigou os efeitos de sua adição nas blendas produzidas.

Ainda que diversos estudos se dediquem à produção de filmes biodegradáveis, não há registro na literatura de blendas a partir de amido e ácido itacônico, aditivados ou não com LIM/PL. Dessa forma, o trabalho em questão foi motivado pela demanda de alternativas sustentáveis ao plástico de fonte fóssil, além da necessidade de melhoria das propriedades dos filmes de amido já produzidos, incorporando substâncias de alto potencial tecnológico.

1.2 OBJETIVOS

De maneira geral, o objetivo do projeto é desenvolver e caracterizar filmes biodegradáveis a base de amido, ácido itacônico/poli(ácido itacônico) aditivados ou não com limoneno ou poli(limoneno) pela técnica de *casting.*

1.2.1 OBJETIVOS ESPECÍFICOS

- Sintetizar poli(ácido itacônico) via polimerização radicalar;
- Avaliar o efeito da adição de diferentes concentrações de ácido itacônico nas propriedades dos filmes formados;
- Avaliar o efeito de três metodologias diferentes na incorporação do ácido itacônico ou de seu polímero sobre as propriedades mecânicas, térmicas, de barreira e estruturais dos filmes formados;
- Sintetizar poli(limoneno) de baixa massa molar via fotopolimerização;
- Comparar as propriedades antioxidante e antimicrobiana de filmes contendo limoneno e poli(limoneno) produzidos;

- Avaliar o efeito da adição de diferentes concentrações da substância ativa mais promissora (LIM ou PL) sobre as propriedades mecânicas, térmicas, de barreira e estruturais dos filmes formados.

1.3 APRESENTAÇÃO DA DISSERTAÇÃO EM CAPÍTULOS

Essa dissertação de mestrado foi estruturada na forma de capítulos, os quais apresentam os resultados de cada uma das etapas da pesquisa realizada. Os resultados foram discutidos por meio de artigos científicos. Os artigos foram apresentados no formato dos periódicos internacionais nos quais foram publicados, submetidos ou em que serão futuramente submetidos.

Os Capítulos 2 e 3 são de revisão de literatura. O Capítulo 2 contém aspectos teóricos gerais sobre o ácido itacônico, limoneno e fotopolimerização. Já o Capítulo 3 foca em uma revisão detalhada, trazendo as publicações mais recentes e relevantes sobre o impacto da incorporação de óleos essenciais em matrizes poliméricas a base de amido.

Os Capítulos 4 e 5 apresentam os resultados obtidos na presente pesquisa. O Capítulo 4 aborda diferentes estratégias de polimerização do ácido itacônico para produção de filmes à base de amido de batata e o Capítulo 5 compara as propriedades ativas dos filmes incorporados com LIM e PL e discute o efeito da incorporação do aditivo mais promissor em blendas de amido/poli(ácido itacônico) para produção de filmes antimicrobianos e antioxidantes. O Capítulo 6 contém as conclusões gerais e sugestões para trabalhos futuros.

2. CAPÍTULO 2: REFERENCIAL TEÓRICO

2.1 ÁCIDO ITACÔNICO

O ácido itacônico (IA, ácido metilenossuccínico ou ácido metileno butanodioico) foi considerado pelo departamento de energia dos EUA um dos doze componentes químicos principais produzidos a partir de açúcares por meio de conversões biológicas ou químicas (WERPY; PETERSEN, 2004). O ácido é composto por dois grupos carboxila e uma ligação dupla conjugada como representado na Figura 2.1 (KUENZ *et al.*, 2012; SAHA, 2017). A diversidade de seus grupos funcionais o torna um intermediário eficaz na fabricação de compostos orgânicos complexos, sendo precursor de várias reações possíveis, como formação de sal com metais, esterificação com álcoois, produção de anidrido, reações de adição e polimerização (KUENZ *et al.*, 2012).

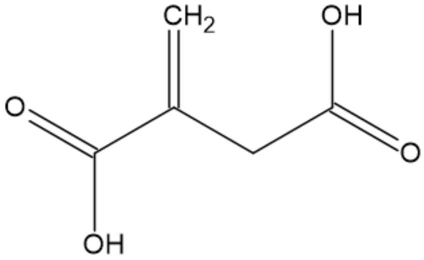

Figura 2.1: Estrutura química do ácido itacônico.

De natureza higroscópica, o ácido tem a forma de um pó branco e apresenta aspecto cristalino no estado sólido (BHARATHIRAJA *et al.*, 2019). Possui massa molecular de 130,099 g/mol, ponto de ebulição de 268ºC, ponto de fusão variando de 165ºC a 168ºC, densidade de 1.632 g/L e sua solubilidade em água é de 83.1 g/L (MAGALHÃES *et al.*, 2017; BHARATHIRAJA *et al.*, 2019; BAMANE *et al.*, 2020).

2.1.1 OBTENÇÃO

O ácido itacônico pode ser produzido por métodos químicos e biológicos, sendo existentes duas rotas principais para a sua produção de base biológica: a fermentação direta em ácido, ou a produção de ácido cítrico seguida de sua conversão em ácido itacônico por pirólise (MAGALHÃES *et al.*, 2017; BHARATHIRAJA *et al.*, 2019).

A conversão da glicose em ácido cítrico por fermentação e depois em ácido itacônico por descarboxilação possui um rendimento global de 59%, enquanto o rendimento teórico máximo para a fermentação direta de glicose é de 72% (MAGALHÃES *et al.*, 2017). A fermentação direta, portanto, é mais eficiente e produz ácido itacônico com concentração de aproximadamente 86 g/L (KUENZ *et al.*, 2012; MAGALHÃES *et al.*, 2017). A rota química não é considerada viável pela quantidade de etapas (WERPY; PETERSEN, 2004).

Desde a década de 1940, os fungos filamentosos *Aspergillus itaconicus* e *Aspergillus terreus* são reconhecidos como excelentes produtores de ácido itacônico, sendo o mesmo produzido industrialmente a partir da fermentação aeróbica de glicose usando *Aspergillus terreus* (WERPY; PETERSEN, 2004; KUENZ *et al.*, 2012; EL-IMAM; DU, 2014; SAHA, 2017). De forma sucinta, este processo, esquematizado na Figura 2.2, envolve a descarboxilação do cis-aconitato intermediário do ciclo do TCA, através da ação da enzima cis-aconitato descarboxilase (CAD) codificada pelo gene CadA em A. *terreus* (SAHA, 2017). As condições ótimas de produção incluem a disponibilidade de fonte de carbono facilmente metabolizável, altos níveis de oxigênio, quantidades limitantes de íons metálicos e uma fonte de nitrogênio (EL-IMAM; DU , 2014). Como pontuado por Klement e Büchs (2013), a biossíntese de ácido itacônico em A. *terreus* e a produção de ácido cítrico em *Aspergillus niger* compartilham importantes características acerca de sua via metabólica, sendo, a produção de IA, em alguns casos, referida como produção de ácido cítrico na presença de um CAD.

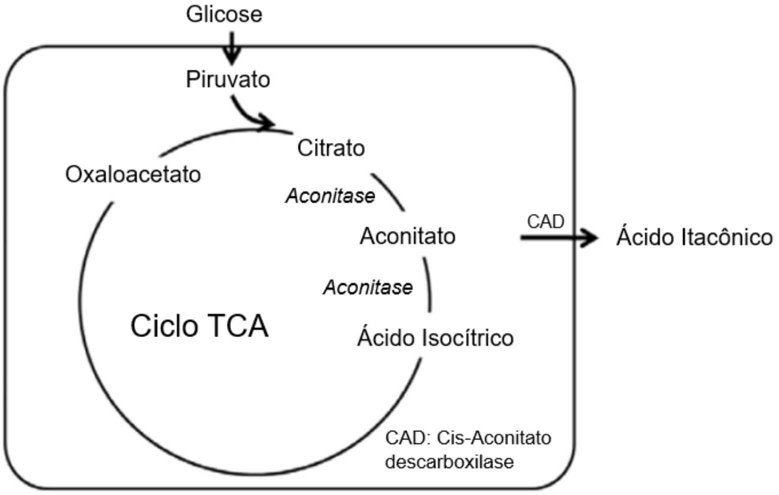

Figura 2.2: via metabólica simplificada para produção de ácido itacônico a partir de glicose. Fonte: adaptado de El-Imam e Du (2014).

Existem três gargalos tecnológicos na conversão de ácido itacônico em um produto comercialmente competitivo: fonte de carbono de baixo custo, fermentação com títulos mais elevados de ácido itacônico e menor custo *downstream* (MAGALHÃES *et al.*, 2019). Uma das soluções é o uso de substratos de menor custo, como resíduos agrícolas. Os principais desafios apresentados pelo uso desses substratos complexos são a composição muito variável, baixos rendimentos, presença de produtos químicos que podem afetar o crescimento e a produção do organismo em fermentação e impurezas no produto final, que aumentam ainda mais o custo com purificação (CHENYU DU, 2014; SAHA, 2017).

Magalhães *et al.* (2019) realizaram simulações para comparar o método atualmente utilizado na indústria para recuperação de ácido itacônico com adsorção, extração reativa e eletrodiálise, e assim avaliar o processo mais vantajoso considerando os custos de produção. Nessas simulações, a adsorção se mostrou o método com maior potencial para substituir a cristalização, seguido pela extração reativa, que apresentou valores próximos aos da cristalização. O único método não competitivo foi a eletrodiálise.

2.1.2 POLIMERIZAÇÃO

O poli(ácido itacônico) pode ser preparado por polimerização de radical livre direto de ácido itacônico, por policondensação e também por métodos como hidrólise de poli(anidrido itacônico) ou poli (itaconatos), sendo a polimerização direta mais comumente utilizada (ROBERT; FRIEBEL, 2016; BEDNARZ *et al.*, 2017). A Figura 2.3 esquematiza de forma simplificada os métodos de polimerização radicalar e policondensação.

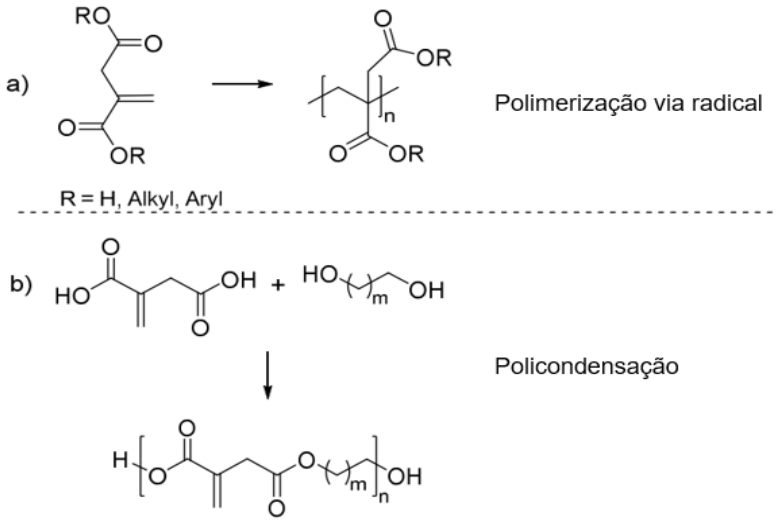

Figura 2.3: Representação esquemática da polimerização do ácido itacônico por policondensação e por radical. Fonte: adaptado de Robert e Friebel (2016).

A Figura 2.4 ilustra o mecanismo da reação do radical iniciador com o monômero de ácido itacônico. Ele ataca o lado CH2 = da ligação dupla por causa do alto impedimento estérico no outro lado da ligação dupla (SOLLKA; LIENKAMP, 2020)**.** E então, a ligação dupla é aberta e forma o radical monômero.

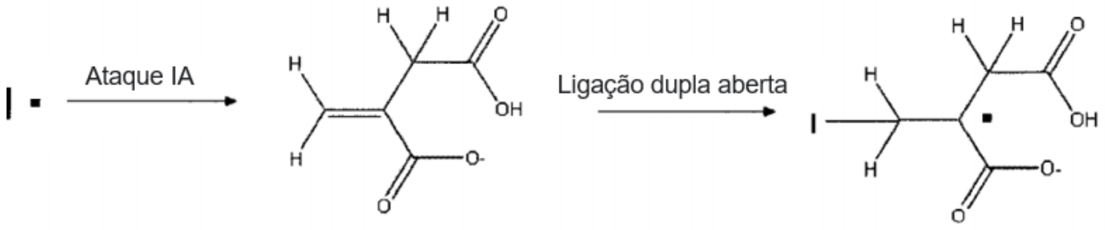

Figura 2.4: Mecanismo da iniciação da polimerização por radical livre. Fonte: adaptado de Cao (2008).

Os principais desafios tecnológicos para a polimerização do ácido itacônico, de acordo com o relatório “Top-12 Platform Chemicals” do Departamento de Energia dos EUA são as baixas taxas de polimerização, dificuldade de controle da massa molar e necessidade de melhora da precisão estrutural dos polímeros resultantes, suprimindo ramificações indesejadas (WERPY; PETERSEN, 2004).

Algumas pesquisas vêm apresentando avanços e Cao (2008) retratou que a neutralização parcial do ácido (entre 50 e 70%) resultava no aumento significativo do rendimento do processo de polimerização. O autor obteve Mn de 10.000 g/mol (resultado superior aos estudos anteriores) e conversão de 100%

à 100ºC e 2h30min, sendo registrado como fator chave o uso de um iniciador de dissociação de alta temperatura, como o hidroperóxido de terc-butila (tBHP).

Bednarz *et al.* (2015) estudaram o mecanismo de polimerização do ácido itacônico iniciada com persulfato em solução aquosa na presença de cloreto de colina. Os autores constaram que os sais de colina ativaram a decomposição do persulfato, aumentando assim a etapa de iniciação da polimerização por radical livre. Além disso, a presença de colina aumentou a solubilidade do ácido itacônico em água e permitiu a síntese de poli (ácido itacônico) com maior massa molecular, mas também com maior dispersidade. Nesse estudo foi atingida Mn máxima de 5.400 g/mol.

2.1.2.1 COPOLIMERIZAÇÃO DO ÁCIDO ITACÔNICO

Dada a dificuldade em homopolimerizar o ácido itacônico, uma alternativa para se produzir filmes é realizar sua copolimerização com outras moléculas.

Bajpai, Bajpai e Jyotishi (2017) produziram blendas a partir de quitosana e poli(acrilamina-co-ácido-itacônico). Os autores registraram como desafio a ser superado o fato de que à medida que o conteúdo de ácido itacônico aumenta no copolímero, a cadeia polimérica em crescimento é mais suscetível ao término do que ao crescimento adicional, sendo que moléculas menores são produzidas resultando na diminuição de sua massa molar média.

Liu *et al.* (2019) desenvolveram por polimerização em emulsão, um tipo de poliacrilato (PIA) modificado com ácido itacônico. Foi registrado que a adição de 2,5% em massa de ácido itacônico aumentou a resistência à tração dos filmes de 8,5 a 11,4 MPa, o módulo de tração de 82 a 582 MPa e a Tg de 6,8 °C para 17,9 °C. Ademais o material foi comprovado sem toxicidade e possuía boa biocompatibilidade.

Ni e Yi (2019) com o objetivo de aumentar a hidrofobicidade e resistência da superfície do papel, estudaram um revestimento produzido a partir da copolimerização via radical livre de álcool polivinílico/ácido itacônico/acrilamida (PVA/IA/AM). Os autores notaram que o copolímero formou uma camada de revestimento hidrofóbico com estrutura de rede espacial na superfície do papel e aumentou o número de ligações de hidrogênio entre o copolímero e a fibra. Os autores também pontuaram que a dupla ligação do ácido itacônico pode ter facilitado a polimerização com monômero AM e na formação de uma estrutura de rede.

2.2 LIMONENO

O d-limoneno ou (R)-(+)-4-isoproprenil-1-metilcicloeseno é o principal constituinte de vários óleos essenciais de citros (limão, laranja, tangerina, lima e toranja), sendo listado no Código de Regulamentação federal como agente aromatizante (LOPRESTO *et al.*, 2014). À temperatura ambiente, a substância se comporta como um óleo incolor com baixa solubilidade em água (13,8 mg/L) com cheiro de laranja doce (CHAIEB *et al.*, 2009; CIRIMINNA *et al.*, 2014; GÜLTEPE, 2020).

O d-limoneno é um monoterpeno monocíclico altamente lipofílico (OZTURK; WINTERBURN; GONZALEZ-MIQUEL, 2019). Quimicamente, terpenos são pequenas moléculas orgânicas com uma diversidade enorme de estruturas, sendo registrados tanto terpenos hidrocarbonetos quanto contendo cadeia, anéis e oxigênio (MC MURRY, 2011). Eles são considerados como a ocorrência da junção cabeça-cauda de cinco carbonos de unidades de isopreno (2-metil-1,3-butadieno) e sua classificação é dada de acordo com o número de unidades de isopreno (FELIPE; BICAS, 2017; MC MURRY, 2011). A estrutura química do limoneno está representada na Figura 2.5.

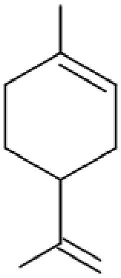

Figura 2.5: Estrutura química do Limoneno.

Como é o composto marjoritário de muitos OEs, o d-limoneno desperta o interesse da indústria alimentícia devido ao seu potencial como agente antimicrobiano, antioxidante ou biopreservativo (GAO *et al.*, 2020b; SHARMA *et al.*, 2020). A atividade antimicrobiana dos OEs e seus componentes principais é explicada pela presença de terpenóides e compostos fenólicos de natureza hidrofóbica, que podem penetrar na camada lipídica das bactérias, resultando em distúrbios da pressão osmótica celular que comprometem a integridade da membrana (AKRAM *et al.*, 2019). Os OEs são mais potentes contra organismos gram-positivos do que contra bactérias gram-negativas, visto que os componentes hidrofóbicos não podem penetrar na membrana celular das bactérias gram-negativas (AKRAM *et al.*, 2019).

2.2.1 OBTENÇÃO

DO EVANGELHO *et al.* (2019) extraíram e caracterizaram o óleo da casca de laranjas da espécie *Citrus sinensis 'Valencia'*. A composição química encontrada pelos autores foi disposta na Tabela 2.1.

Tabela 2.1: Composição química do óleo extraído da casca de laranja da *espécie Citrus sinensis 'Valencia'*. Fonte: Do Evangelho *et al.*, 2019.

Composto	%
d-limoneno	95,96
β-mirceno	2,35
Octanal	0,55
α-pineno	0,53
β-linalol	0,45
Ciclohexeno	0,29
Decanal	0,26

De acordo com a Tabela 2.1, d-limoneno é o composto majoritário do óleo da casca da laranja. Em estudo semelhante, Velázquez-nuñez e colaboradores (2013), também reportaram limoneno (96,62%) como o principal composto do óleo essencial da casca da laranja *Citrus sinensis* variação Valencia. Wolffenbuttel *et al.* (2015) analisaram a composição química de amostras de óleos essenciais cítricos, incluindo *Citrus aurantium Lineu*, *Citrus sinensis Osbeck* e *Citrus reticulata Blanco* obtidas no Brasil. Os resultados mostraram que o principal componente do pericarpo é o limoneno, com valor médio 71,9%.

Estima-se que a produção mundial de d-limoneno seja de aproximadamente 30 a 70 x 10^3 toneladas por ano (MAHATO *et al.*, 2020). Convencionalmente, sua extração em cascas de frutas cítricas se dá por um dos seguintes métodos:

prensagem a frio, separação centrífuga, sistema Soxhlet ou destilação por exposição a água fervente ou vapor (CIRIMINNA *et al.*, 2014; LOPRESTO *et al.*, 2014; AKRAM *et al.*, 2019). Esses métodos convencionais apresentam algumas desvantagens relacionadas aos altos custos de energia e longos tempos de extração (LOPRESTO *et al.*, 2014). Ressalta-se a importância de um método de extração adequado à aplicação final do produto, uma vez que o perfil químico do óleo essencial pode ser afetado (LOPRESTO *et al.*, 2014).

Dentre as técnicas citadas, se destaca a destilação a vapor. Nela, as laranjas são inicialmente espremidas para o suco, sendo o d-limoneno, o óleo de laranja que flutua no topo (CIRIMINNA *et al.*, 2014). Após uma segunda prensagem, as cascas são então transferidas para um extrator de vapor (CIRIMINNA *et al.*, 2014). Em seguida, o d-limoneno é volatizado a 97ºC, em vez de no ponto de ebulição da substância pura (175ºC), o que minimiza sua oxidação (CIRIMINNA *et al.*, 2014). O vapor é condensado formando uma camada de óleo essencial de laranja com cerca de 90 a 95% de d-limoneno puro (CIRIMINNA *et al.*, 2014).

2.2.2 OUTRAS APLICAÇÕES

De acordo com a Renewable Citrus Products Association, o d-limoneno tem perfil ambiental mais sustentável (321 kg CO_2-eq. por 1000 kg de produto) quando comparada à solventes a base de petróleo (CIRIMINNA *et al.*, 2014). Os solventes comumentemente substituídos por d-limoneno incluem metil etil cetona, acetona, tolueno, éteres de glicol e vários solventes fluorados e clorados (AISSOU; CHEMAT-DJENNI; CHEMAT, 2017). Aissou, Chemat-Djenni e Chemat (2017) avaliaram o potencial do d-limoneno como solvente alternativo ao n-hexano na extração de compostos bioativos e obtiveram resultados promissores. Outra vantagem comprovada é que o limoneno tem maior capacidade de ser recuperado e reciclado (90%) quando comparado ao n-hexano (50%) (AISSOU; CHEMAT-DJENNI; CHEMAT, 2017).

Ainda no setor químico, o limoneno foi usado por SINGH *et al.* (2019) como matéria-prima renovável para a produção de produtos químicos valiosos, como xilose, ácido levulínico e lignina. Óxido de limoneno em reações de enetiol com tióis poli-hídricos formam epóxidos multifuncionais de base biológica, que resultam em polímeros de rede com resistência térmica relativamente alta, com possibilidade de aplicação na fabricação de materiais de revestimento (MORINAGA; SAKAMOTO, 2017).

O limoneno também tem potencial de ser usado como plastificante verde, sendo retratado por Brüster *et al.* (2019) como um plastificante eficiente para o Polilactídeo (PLA). Além do uso como plastificante, estudos abrem o potencial de aplicação industrial do polilimoneno (PL) para o processamento de blendas a base de PLA / PL. Sessini *et al.*, (2020) obtiveram PL a partir da polimerização do óxido de limoneno hidrofóbico e com boas propriedades térmicas e de flexibilidade. Também neste sentido, é registrado o uso de d-limoneno como precursor na polimerização por plasma, sendo esta uma alternativa ambientalmente correta, rápida e controlada para a produção de filmes finos que oferece novas aplicações tecnológicas para um produto químico derivado de resíduos industriais (GERCHMAN *et al.*, 2019).

Na área médica, diversas aplicações são registradas, já que é comprovado que o d-limoneno apresenta baixa toxicidade e não apresenta risco mutagênico, carcinogênico ou nefrotóxico para humanos (SUN, 2007). O composto tem sido usado clinicamente para dissolver cálculos biliares contendo colesterol, já que é um excelente solvente do colesterol (SUN, 2007). Também é usado para o alívio da azia devido ao seu efeito neutralizante do ácido gástrico e ao suporte do peristaltismo normal. O composto ainda apresenta atividade quimiopreventiva bem estabelecida contra muitos tipos de câncer (SUN, 2007). Ainda na área médica, de acordo com D'Alessio *et al.* (2013), o d-Limoneno demonstra eficácia como anti-inflamatório, sendo comprovados efeitos protetores sobre a barreira epitelial e diminuição de citocinas.

Na área de medicina veterinária, algumas aplicações são conhecidas, como no combate à mastite bovina (MONTIRONI; CARIDDI; REINOSO, 2016). Além desta, de acordo com Gültepe (2020), dietas suplementadas com d-limoneno podem ser aplicadas como aditivos alimentares e substitutos do imunoestimulante para controlar a doença da boca vermelha entérica na criação de trutas arco-íris. O composto também pode ser usado em lavouras para o manejo de pragas (HEBEISH *et al.*, 2008; ALHMEDI; HAUBRUGE; FRANCIS, 2010).

É apontado por Paggiola, Stempvoort e Bustamante (2016) que o Brasil, por ser o maior produtor de resíduo de laranja (13.568.000 toneladas de casca e polpa anualmente), detém o maior potencial para a transição de base biológica, uma vez que poderia facilmente atender à sua demanda em diversos setores, pelo menos parcialmente (PAGGIOLA; STEMPVOORT; BUSTAMANTE, 2016). Os autores salientam ainda que alguns fatores podem afetar a implementação da economia bioquímica, como a necessidade de investimento de capital, a questão do custo da matéria-prima, a necessidade de superar lacunas de conhecimento e a inércia para mudanças e a adaptação ao ambiente regulatório da região.

2.2.3 FOTOPOLIMERIZAÇÃO

A técnica de polimerização utilizada na síntese de poli(limoneno) de baixa massa molar no presente trabalho foi a fotopolimerização. Em contraste com as sínteses de base térmica, que geralmente requerem temperaturas elevadas, esta pode ser realizada em temperatura ambiente e, oferece a possibilidade de controle topológico e temporal, bem como da estrutura macromolecular (YILMAZ; YAGCI, 2018). A técnica requer um composto fotossensível que converta a energia luminosa em energia química levando à formação de espécies reativas capazes de iniciar a polimerização (YILMAZ; YAGCI, 2018). Desta forma, os monômeros são transformados em seus polímeros correspondentes.

Rodrigues e Neumann (2003) descreveram sucintamente o mecanismo dos processos cinéticos de fotopolimerização em cadeia via radical livre.

Iniciação: produção de radicais livres R· após absorção de luz, seguida de adição a uma primeira molécula de monômero M para produzir as espécies iniciadoras da cadeia, M· (radical iniciador ou radical primário).

$$c + \mathrm{h\nu} \rightarrow \mathrm{R}\cdot \qquad (2.1)$$

$$\mathrm{R}\cdot + \mathrm{M} \rightarrow \mathrm{M}\cdot \qquad (2.2)$$

Propagação: crescimento de M· por adições sucessivas de moléculas do monômero M, que é consumido rapidamente.

$$M_n \cdot \ + \mathrm{M} \ \rightarrow \mathrm{M}_{n+1} \ \cdot \qquad (2.3)$$

Terminação: reação bimolecular entre dois macrorradicais

$$M_n \cdot \ + \mathrm{M_m} \cdot \rightarrow \mathrm{M}_{n+m} \qquad (2.4)$$

$$M_n \cdot \ + \mathrm{M_m} \cdot \rightarrow \mathrm{M}_n \ + \mathrm{M_m} \qquad (2.5)$$

3. CAPÍTULO 3: ÓLEOS ESSENCIAIS COMO ADITIVOS EM FILMES DE EMBALAGEM DE ALIMENTOS À BASE DE AMIDO: UMA REVISÃO

ESSENTIAL OILS AS ADDITIVES IN ACTIVE STARCH-BASED FOOD PACKAGING FILMS: A REVIEW

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Abstract: The production of sustainable food packaging from renewable sources represents a prominent alternative to the use of petrochemical-based plastics. For example, starch remains one of the more closely studied replacement options due to its broad availability, low cost and significant advances in improving properties. In this context, essential oils as additives fulfil a key role in the manufacture of renewable active packaging with superior performances. In this review, a comprehensive summary of the impact of adding essential oils to the starch-based films is provided. After a brief introduction to the fundamental concepts related to starch and essential oils, details on the most recent advances in obtaining active starch-based films are presented. Subsequently, the effects of essential oils addition on the structure-property relationships (from physicochemical to antimicrobial ones) are thoroughly addressed. Finally, applications and challenges to the widespread use of essential oils are critically discussed.

Key-words: starch films; active packaging, antimicrobial activity, antioxidant film.

[1]

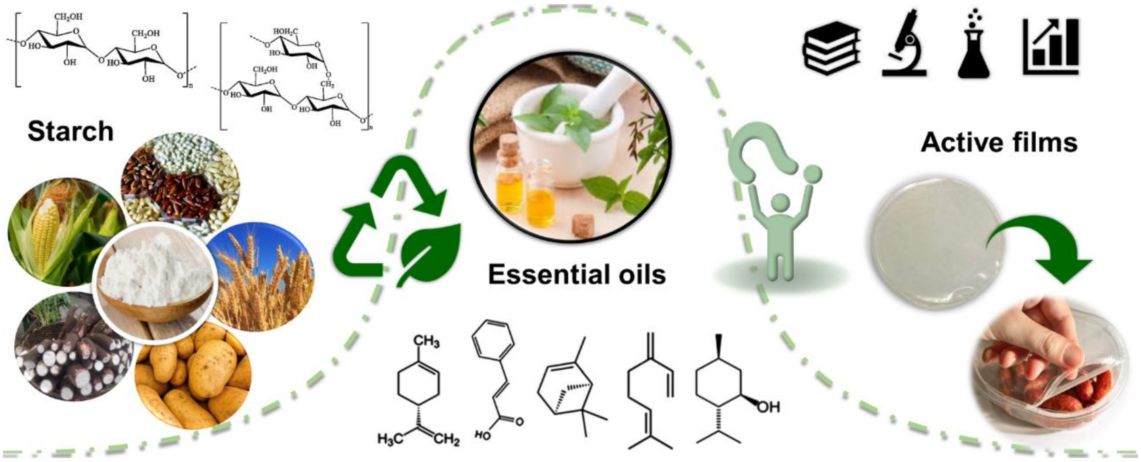

Figura 3.1 - Graphical Abstract

1. Introduction

During the second half of the 20th century, the production of food packaging made of plastics produced from fossil sources increased due to its relatively low cost, mechanical resistance and versatility of application [1]. However, the growing concern over the negative impacts of plastics on the environment has encouraged the development of packaging alternatives that use renewable sources as raw material. For example, natural polymers stand out as outstanding renewable feedstocks to supply the demand for sustainable, active and "intelligent" packaging [2,3]. In this context, polysaccharides, proteins and lipids are well-known in the production of films and coatings for food packaging [4–6].

Alkyl polyesters, such as poly(lactic acid) and polyhydroxyalkates [7–9] and starch-based plastics [10] are primary examples of commercially viable biopolymers used in the production of food packaging films. However, the challenge associated with the application of such a polymeric material is to meet the necessary requirements for superior mechanical and barrier performance in addition to being competitive in the market [11].

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[1][1] Manuscript published in *International Journal of Biological Macromolecules* (2021). DOI: https://doi.org/10.1016/j.ijbiomac.2021.05.170. Reprinted with permission from *Elsevier*. Copyright 2021 (Anexo A).

Starch is present in cereals, roots, tubers, fruits and vegetables. Due to its biocompatibility, biodegradability, and availability, starch has been widely used in the production of edible films and coatings for food packaging. Corn starch, for example, is considered to be the most consolidated bioplastic alternative for this application [12,13]. Considerable improvements in the properties of starch films have been reported with the production of composites, blends, obtaining active and/or intelligent films using additives [14–16], and also by several chemical and physical modifications [17–19].

Edible films and coatings aim to prolong the shelf life of food products, providing microbial protection, better sensory perception, and reduction of antioxidant activity [20–22]. To further enhance these benefits, edible films can also contain active components which not only improve food quality and safety, but can also enhance the physicochemical properties of biopolymer-based films [23,24]. For example, the addition of essential oils in the production of films has resulted in products with better barrier and optical properties, in addition to incorporating antioxidant and antibacterial activities due to the migration of their active compounds [25]. Therefore, essential oils have been gaining more and more attention in this regard.

This review provides a thorough analysis of the impact of essential oils on the main properties of starch-based films for the production of food packaging. Initially, theoretical aspects of starch and essential oils are briefly discussed in order to direct the reader toward the structure-properties relationships that are subsequently addressed. Throughout the paper, a critical analysis of material properties and applications is also presented. The objective is to highlight the significant recent progress on sustainable starch-based films added with essential oils and also help the reader to identify challenges and opportunities related to this topic.

2. Chemical structure and properties of starch

Starch is the most abundant natural, renewable and biodegradable polysaccharide and is also the primary source of carbohydrates, constituting an important energy supply for humans [26–28]. Produced as a reserve carbohydrate, starch is formed by small granules that are present in many constituent parts of plants (seeds, fruits and tubers), and its properties are influenced by botanical origin, cultivation area and climate [28–30]. In the food industry, starch is used to improve the quality of the final products, being able to confer functional properties, modify the texture and consistency of different foods products [27,31].

Starch granules are semicrystalline and are composed predominantly of two polysaccharides: amylose, with a molecular weight ranging from 10^5 to 10^6 Da, and amylopectin, of higher molecular weight (from 10^7 to 10^9 Da) and a large number of short branches [28,32]. With exceptions, amylopectin constitutes the principal mass component of starch, while amylose constitutes 15 to 30 % [28]. **Figure 3.2** outlines the amylose and amylopectin branched chains.

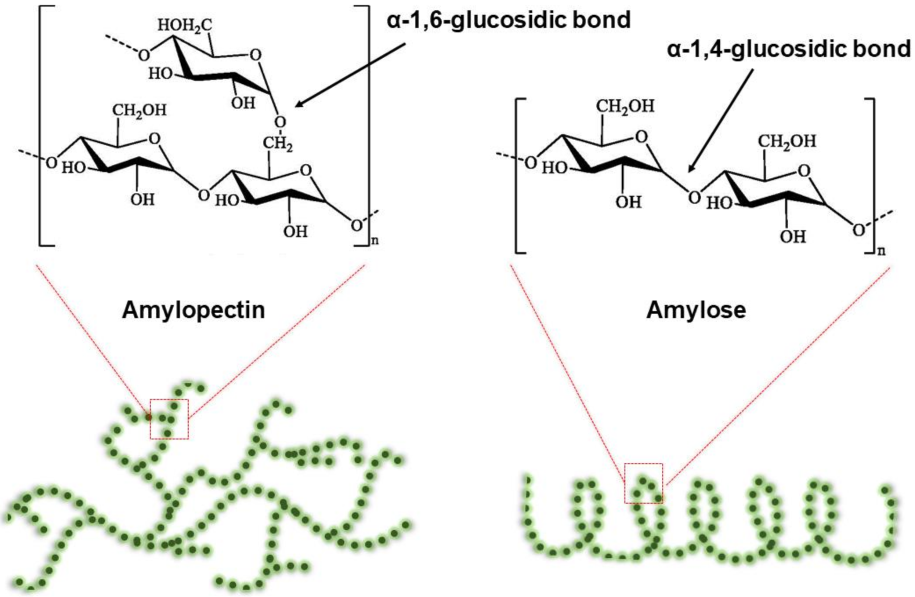

Figure 3.2 – Schematic representation of the components of starch

Amylose is an essentially linear polymer with a helical structure, composed of, on average, 2 to 12×10^3 glucose units that are linked to each other by an α-1,4 glycosidic bond [33]. The average composition of amylopectin is 4 to 35×10^6 glucose units in α-1,4 glycosidic bonds, connected by branching points (corresponding to 5% of the molecule) and linked to α-1,6, resulting in a complex structure [28,29,33]. Amylopectin retains a cluster-like organization, and some of its branches are long enough to form double helices with each other and crystallite clusters [33,34]. The average degree of polymerization of amylose is less than 5000, while that of amylopectin ranges from $5×10^3$ to 10^6 [35].

The molecular structures of amylopectin and amylose, as well as their respective fractions, are critical to the general physicochemical properties of starch [32,34]. In addition to these factors, it has been experimentally proven that the degree of crystallinity equally affects important characteristics of starch such as thermal stability [33] Consequently, it is necessary to study the organization of polymers within the semicrystalline structure. It is recognized that short chains of amylose are located mainly in the crystalline lamellae, the long chains mostly in the amorphous region while the intermediate chains are found in both lamellae [32]. In dimensional terms, the size of starch granules ranges from 1 to 100 μm, while the crystalline and amorphous lamellar structure is approximately 10 nm in size, and the amorphous and semicrystalline growth rings are approximately 0.1 μm [36].

The aforementioned structural characteristics are primarily related to the starch source and therefore can be associated with the properties of the produced film. For example, the properties of starch isolated from various species of rice and corn presented significant variations in amylose moisture content and the solubility index even between species of the same cereal [27]. A comparison between the physical and chemical properties of 7 sweet potato varieties demonstrated a similar result, with significant differences in granule size, crystallinity, amylose content and gelatinization properties among the different varieties [37].

In addition, it was observed that starch that is rich in amylopectin possesses a higher degree of crystallinity (*Indica* rice starch). It is significant to note that, in addition to the composition, the chain length distribution and the number of chains with a certain degree of polymerization are significant determinants of the functional and nutritional properties of starch [38,39]. Considering the rheological properties of three sources of starch (potatoes, rice and corn with amylose concentrations of 34.5%, 26.5% and 24.1%, respectively), all three samples exhibited a notable reduction in the storage module at high voltages and instant recovery at low voltages, with rice starch having higher storage modules and rupture stress followed by potatoes and corn [31]. These differences clearly have a pronounced effect on the characteristics of the film-forming solution, thereby impacting the method of preparing the films.

Moreover, with regard to the effect of the granule size in the structural and physicochemical properties of whole sweet potato starch, the larger the granules, the greater the apparent amylose content and the relative crystallinity and the lower the amounts of ash, lipids and proteins. It was also observed that large granules had less ordered short-range structures. No significant differences were found in the thermal properties of granules of different sizes [40].

Therefore, depending on the source, starch can present significant differences regarding properties, starting from the proportions of amylose and amylopectin, conformation and microstructural arrangement. In this way, in macroscopic terms, the film-forming solution and the obtained films can exhibit a range of properties. As a result, structural factors must be carefully considered in order to establish an exact correlation with the performance of the material. Throughout the subsequent sections of this review, numerous starch-based film properties will be evaluated as the concepts presented in this section are fundamental to understanding the correlations between structure and properties.

3. Starch sources and processing

3.1. Starch sources

The world production of starch is predominantly based on four raw materials: corn, wheat, cassava and potatoes, with over 75% of starch coming from corn [41,42]. In Brazil, the extraction of starch at industrial scale is performed exclusively with cassava and corn [43]. According to ReportLinker, it is estimated that the global market for starch will reach 156.3 million metric tons by 2025 [44].

Some of the most commonly used raw materials were divided according to production prices, namely: raw materials with high prices: sweet potatoes and yams; intermediate prices: potatoes, rice, taro; and with low prices: corn, wheat and cassava, which, together with potatoes, are the most important for the production of starch [45]. The production of rice starch comes essentially from less valued by-products that are not used in the food industry [45]. Commercially isolated starch is frequently used in dry form in order to extend shelf life and to save on transportation and storage costs [46].

3.2. Industrial production

The industrial methods for obtaining the primary commercial starches have been briefly described by Waterschoot (2014). For example, corn starch is commercially isolated by a procedure that involves steeping with sulfur dioxide, followed by wet grinding and centrifugation. On the other hand, the peeled and clean cassava roots go through extractors, centrifugation and drying. To separate the starch from the wheat, the wheat is crushed and moistened and then undergoes centrifugation or sifting. Potatoes are ground after which the starch granules and non-starch polysaccharides are separated from the juice by centrifugation and sieving. Rice starch is isolated by an alkaline procedure since sodium hydroxide solubilizes rice protein and facilitates the isolation of starch during the subsequent wet grinding of the grains.

In the industrial process, the individual plant components are separated. In the first step, simpler physical separation operations such as crushing, sifting and centrifuging are employed [47]. The production methods as well as industrial tools and their costs depend on the botanical origin of the starch [48]. In the second step, drying is typically carried out in a conventional oven, freeze dryer or with an organic solvent. The extracted starch can then be used in its pure form (the so-called “native starch”) or processed in order to modify its performance (“modified starch”) or to obtain sweeteners through the hydrolysis process [47,49].

3.3. Starch modification

As the conservative structure of native starches hinders their applications, a number of modification techniques have been established. According to the changed parameter, the methods can be divided into physical, chemical, enzymatic and genetic modifications [50]. However, the physical and chemical methods are the most frequently used nowadays [51]. In consequence of this market growth, starches with unique characteristics have been developed to supply the peculiarities of each industrial segment [52]. Considering the vastness of the subject, several review articles have been exclusively dedicated to it [30,51–58], and this section attempts to present a brief overview of promising chemical and physical starch modification methods and its effects on films properties.

Physical means to modify starch molecular structure include variables such as temperature, pH, pressure, dry heat, additives etc. [59]. These techniques are generally classified into thermal which includes pre-gelatinization and hydrothermal processing (annealing and heat-moisture treatment and non-thermal modification (high pressure processing, micronization, ultrasonication, pulse electric field etc.) [19]. They are fast, eco-friendly, and, in general, improve starch’s water solubility and reduce its granular size [60,61].

Among the physical methods, pre-gelatinization is the most common. Basically, to prepare a pre-gelatinized starch (PSG), native starch is pre-cooked and dried [19]. In the process, starch is degraded by simultaneously weakening of its granules and shortening of its molecular chain lengths, and so the hydrogen bonds between molecules are broken [62,63]. Comparatively to native starch, PGS has higher swelling capacity, solubility and cold-water dispersion [60].

Additionally, different properties can be achieved by each approach. As an example, Gryszkin *et al.* registered increased in viscosity and in strength of starch gels after thermal modifications (heating followed by freezing and defrosting) [64]. Shahbazi, Majzoobi and Farahnaky observed higher surface hydrophobicity, tensile strength, and improvements on barrier properties on blends of κ-carrageenan with physically modified starch by high-pressure homogenizer than with native starch [65]. These changes were more notable when the starch was modified with high level of homogenization pressure.

Nevertheless, chemical methods are the most explored ones, as a result of the non-destructive nature of the processes and the potential improvements [53]. They occur either with breakdown reactions (hydrolysis or oxidation) or with the introduction of new functional groups using derivatization reactions [59]. The chemical modification is facilitated by the presence of a large number of hydroxyl groups on starch, that provide active sites [66]. The achieved physicochemical properties depend on several factors, namely botanical source, reaction conditions, type and number of substituent as well as its nature [50,66].

The main mechanisms involved in chemical modifications are derivatization via ether or ester formation, oxidation of hydroxyl groups to carbonyl or carboxylic groups, hydrolysis of glycosidic, as well as cross-linking, cationization and acetylation [51,67]. The introduction of functional groups does not change the shape nor the size of molecules, but results in significantly altered physicochemical properties. Even though, these approaches are limited because of conditions such as consumer safety and environmental factors [58].

There is a trend to use combined modifications to create a wide range of starch functionalities [54]. Among the techniques studied by Wang *et al.* (esterification, etherification, crosslinking, grafting and condensing reaction), cross-linking combined with esterification was the method with the greatest potential for further development, due to its superior hydrophobicity and potential to increase the mechanical property of starch-based materials [57].

However, some properties may be negatively affected by modifications. Even though Sharma *et al.* registered that crosslinking (CL) agent improved barrier properties and tensile strength of starch films, elongation at break was reduced [68]. Similar results were obtained by Yıldırım-Yalçın *et al.* [69] and Mehboob *et al.* [70], and the addition of acetic anhydride overcame this issue showing better mechanical properties [70]. Likewise, Hu *et al.* reported that debranching substantially decreased elongation at break and hydroxypropylation decreased tensile strength of starch films. On the other hand, dual modification (debranching and hydroxypropylation) was capable of overcome drawbacks of both single modification [71].

Finally, starch modifications represent great advances in the properties of renewable starch films, and, consequently, in its ability of food preservation. For instance, Punia *et al.* observed that heat treated octenyl succinic anhydride modified starch films were more efficient than starch native films in the preservation of fruits [19]. Therefore, starch films can be boosted by the right modification method, which may contribute to its popularization and large-scale applications.

4. Composition and functional properties of essential oils

Oils, in general, are composed by triacylglycerols, diacylglycerols, monoacylglycerols, fatty acids, phospholipids, free etherols, sterol esters, tocols, triterpene alcohols, hydrocarbons and fat-soluble vitamins when first extracted [72]. They can be obtained from animal source, such as chicken and fish oil, for example; from vegetables such as soybean and canola oil [73]; and from the organs of the plant: roots, stems, leaves and flowers. They can also be obtained from mineral source, consisting of a mixture of paraffinic, naphthenic, aromatic hydrocarbons and other organic compounds. This source is known as non-renewable and with compounds that are hardly biodegradable [74].

Essential oils (EOs) are complex mixtures of small, volatile and lipophilic compounds [75] that are extracted from plants by employing hydro distillation, dry distillation or mechanical extraction methods [76]. The OEs are also known as etheric oil [77,78] or volatility oil because it contains volatile organic compounds at room temperature [78]. The chemical characteristics of EOs vary according to the botanical source, geographic area, production station, extraction method, storage conditions and stability, making it difficult to standardize and compare different essential oils [79]. The composition of EOs depends on which part of the plant is used for extraction: flowers, whole fruit, bark, green parts (leaves and stems), pericarp, seeds, or roots [80,81].

In general, EOs are composed of various components such as phenols, aldehydes, ketones, terpenes, carbohydrates, ethers and alcohols which are responsible for its outstanding biological activities [82]. Among all of these components, terpenes are the most predominant. Terpenes are the secondary metabolites of plants formed by isoprene units and have high volatility. When biochemically modified, terpenes are called terpenoids [83]. The chemical structures of some typical compounds present in EOs are shown in **Figure 3.3**.

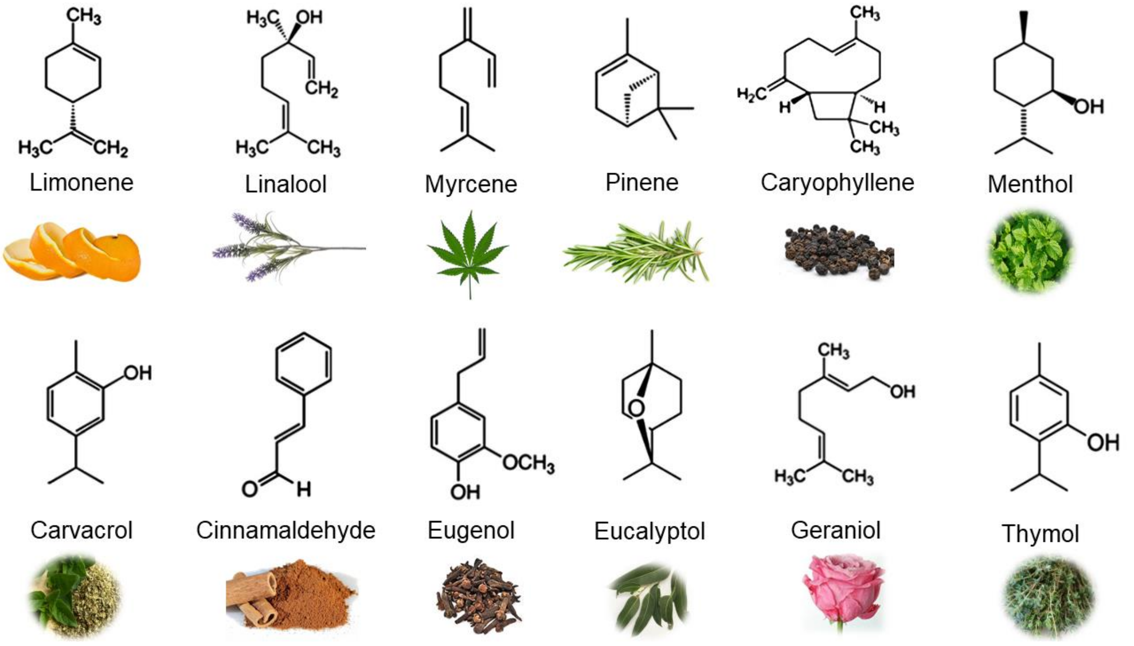

Figure 3.3 – Typical compounds found in several essential oils and examples of the compounds' main sources

EOs have a natural origin, low toxicity and relatively low cost, occupying an important role in the food, pharmaceutical and cosmetic industries [84–86]. Some well-known examples of EOs include jasmine, lavender, ylang-ylang and basil, whose primary components are linalool and benzyl acetate; linalool and linalyl acetate; linalool, thymol and germacrene; estragole and limonene, respectively [87].

Limonene is a well-known monoterpene that is usually obtained from citrus essential oil and other several sources [88–94]. Linalool is a monoterpene alcohol that is also found in a number of essential oils such as lavender, mint, rose, cypress, and lemon [95,96]. Myrcene, or β-myrcene, is a monoterpene with remarkable presence in bay, cannabis, and hops essential oils [97–99]. Other examples of abundant components of essential oils are eugenol, vanillin, cinnamaldehyde, eucalyptol, geraniol, carvacrol, and capsaicin [100].

EOs are widely used because they exhibit antimicrobial activities due to the presence of hydrophilic functional groups and/or lipophilicity [83,101–105]. This characteristic facilitates the penetration EOs compounds through bacterial membranes into the interior of the cell, resulting in inhibitory activity [106,107].

The phenolic nature of EOs also elicits an antimicrobial response against foodborne pathogen bacteria since it disrupts the cell membrane resulting in the inhibition of the functional properties of the cell. This eventually causes leakage of the internal contents resulting in the cell death [106]. Essential oils have been reported to be effective antimicrobials against several foodborne pathogens, such as *Escherichia coli*, *Salmonella Typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter* among others [80,108].

The antimicrobial activity of OEs is more dominant against Gram-positive bacteria than against Gram-negative one, once they have a more sensitive cell wall, which is made of peptidoglycan and offers minimal resistance to the penetration of hydrophobic molecule. The peptidoglycan layer of Gram-negative bacteria is thinner and lies inside an outer membrane that consists of a double layer of phospholipids. This outer membrane contains lipopolysaccharides and prevents the penetration of hydrophobic compounds [101].

In addition, experiments showed that OE of oregano increased the sensitivity of bacterial spores to heat treatment [109] and reduced the number of sporogens and molds [110]; lavender OE showed zones of inhibition against sporogenic bacteria [111], and the constituents cinnamaldehyde, eugenol and carvacrol exhibited inhibitory activity against the germination of bacteria spores [112].

Similar to the performance of essential oils in Gram-positive bacteria, in yeasts and fungi they also damage the cell wall. In yeasts, OEs establish a membrane potential across the cell wall and disrupt the assembly of ATP, damaging the cell wall. In fungi, OEs penetrate and disrupt the cell wall and protoplasm membranes through a permeabilization process, which results in the disintegration of mitochondrial membranes [105].

The antioxidant activity of EOs is manifested through the scavenging mechanism of reactive oxygen species and the capacity of modifying endogenous antioxidant systems [113,114]. Several terpenoids such as terpinolene, α- and γ-terpinene among others found in essential oils have been shown to possess high antioxidant properties [83,102,115]. Other well-known properties of essential oils include antiviral [83,102,105], anti-inflammatory, anti-cancer [83,102], antifungal [102,105], in addition to antimutagenic, antiprotozoal, and antidiabetic activities.

These beneficial characteristics vary according to the chemical composition of the EO [80,102].

In the food industry in particular, there has been an increase in the application of EOs as an additive in the production of food packaging, aiming to replace synthetic additives [25]. For example, when EOs are incorporated in packages, the EOs add their antimicrobial and antioxidant properties through the possibility of migration into food over time. As a result, there is an increase in the shelf-life of the food and an improvement in its nutritional and sensory quality [116]. Despite such significant benefits in food preservation, it is important to note that the difficulty in standardizing EOs implies packaging materials with properties that vary considerably. Therefore, it is essential to assess the impact of adding each type of EO on the mechanical, thermal, barrier and structural polymer properties. These effects will be discussed in detail in the following sections.

5. Starch-based films containing essential oils

Due to its abundance, cost-effectiveness and excellent film-forming capacity, starch has been widely employed for the film production [117]. In the process of developing starch-based films, at least three materials are utilized: a biopolymer, solvent (usually water) and a plasticizer (most frequently glycerol or sorbitol). In addition, in order to improve the film's properties, the mixture of starch with other biopolymers is widely used. Still in this context, the incorporation of nanoparticles, plant extracts and essential oils can include smart or "intelligent" and active functions to films [118]. Antibacterial films are prepared with antibacterial materials and biological polymers substrates with the potential to ensure the quality and safety of food and to extend the shelf life [119]. Despite these benefits, it is necessary to examine the potential of each agent (additive) and their interaction with the matrix used [120].

One of the crucial challenges in the large-scale application of edible starch-based films remains the fact that their properties still demonstrate severe limitations [117,121]. As EOs are excellent sources of pharmacologically active phytochemicals, several studies using essential oils have been developed aiming to optimize and improve the properties of these films [122]. In this sense, in order to develop biopolymer films with characteristics comparable to commercialized packaging, the researchers have developed starch blends containing numerous polymers, EOs and nanoparticles, employing different preparation methods.

5.1. Preparation of starch-based films

Starch-based films are generally processed using two main techniques: casting and extrusion [123]. However, the casting technique is the most commonly used in research due to its ease, economical equipment and the possibility of preparing small film samples [123,124]. In this section, the methodologies for obtaining starch-based films containing essential oils are presented, with emphasis on the casting technique.

The production of starch films using the classic casting method consists of pouring a suspension into small plates, with the thickness of the film being controlled from the mass of the poured suspension [124]. In this process, the starch is gelatinized with excess water, then heated and stirred, ensuring hydration of its molecules [125]. This method guarantees the proper mixing of the formulation components, the absorption and mixing time being significant parameters to guarantee the homogeneous dispersion of the compounds in their polymeric matrix [123]. **Figure 3.4** illustrates the possibility of producing coatings on food products or plastic films by the casting technique [4].

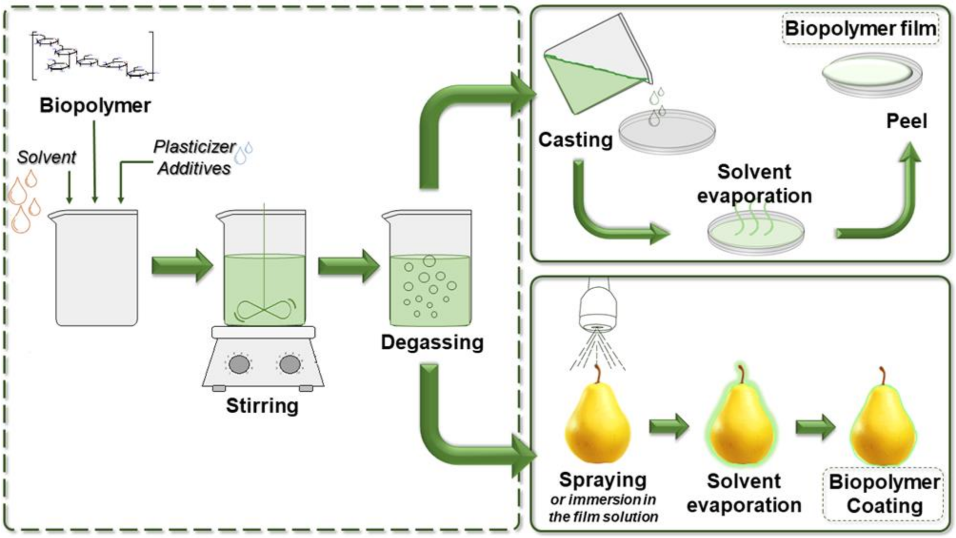

Figure 3.4 – Simplified scheme for biopolymer-based films and coatings production by casting and spraying, respectively. The pear fruit in this case was

used only as an illustration of the application of coatings [4]. Reprinted with permission from Elsevier, Copyright (2020).

The main disadvantages that make the casting technique impractical industrially are related to the difficulty in scaling their production to larger sizes and the demand for long drying times [124]. A variation of the casting technique, called tape casting, allows the spreading of the film-forming suspension on large supports and on continuous conveyor belts. This makes it possible to control its thickness of films with an adjustable blade, a technique already used industrially [124,126]. The composition and some details of film preparation using the casting technique of the films covered in the present work are available in **Table 3.1**.

Even though in most studies starch-based films containing EOs were prepared by casting method, extrusion is the technique that sands out as an alternative to scale-up and achieve industrial-scale outcome [127,128]. This process involves high temperature, pressure, and shear force, and combines different unit operations including mixing, cooking, kneading, shearing, shaping and forming [125,129]. It results in the break of starch bonds, leading to gelatinization, melting and degradation of starch [125].

As extruded films are formed by thermomechanical process, they have different properties from those obtained by casting technique [128]. The main advantages of extrusion include the ability to handle high-viscosity polymers without solvents and the broad range of processing conditions (0–500 atm and 70–500 ºC) [130]. Disadvantages are associated with crystalline destruction, that culminates to difficulty in controlling the texture of the films [125]. There are few commercial starch products on the market, mainly due to the fact that extrusion parameters influence starch processibility, and also because of the little knowledge of plastics companies about large-scale production without having to invest in new equipment [131,132].

Even so, with the increased interest on renewable source packaging, extrusion of starch films has recently become the focus of several researches [127,128,131–139]. However, only a few have investigated the use of this method to prepare active packaging based on starch and EOs.

De Medeiros *et al.* successfully produced starch poly(butylene adipate-co-terephthalate) films incorporated with oregano EO by extrusion, which presented antibacterial activity [140]. In compliance*,* Estevez-Areco *et al.* developed starch films with rosemary extract through extrusion, which granted high antioxidant activity [137]. The results showed the potential of the method to produce active starch films, once these additives could resist to the shear forces and were well dispersed within the matrix. Nevertheless, further research needs to be addressed to this specific topic since some EOs may evaporate under extrusion conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils.

Quantity/percentage of materials used	$\dot{\omega}$[1]	**DP[2]/DG[3]**	m_s[4]	S_e[5]	ξ[6] (µm)	σ_p[7]	**STO[8]**	**References**
Starch (3%) + glycerol (30% relative to dry starch mass) + 0; 0.5; 0.3 e 0.7 µL/g (relative to the film-forming solution mass) of orange EO	14,000 rpm for 10 min	–	20 g	Oven with air circulation at 30 ° C for 16 h	84 - 142	Acrylic	16 °C and 58 % relative humidity	[120]
Corn starch (100g) + glycerol (7,5%) + magnesium stearate (3 %) + 7,5 or 10% (g of EO/g of starch) of oregano or thyme EOs	1,500 rpm for 10 min	-	50 g	160 °C for 10 min and under 60 bar in a mechanical thermopress	240 – 250	Teflon	25 ± 1 °C and 60 % relative humidity for four days	[141]
Corn starch (3%) + glycerol (1.8%) + 1 – 20 mg/mL of *Zataria multiflora* and *Bonium persicum EOs*	2,000 rpm for 2 min	heating at 90°C under agitation for 10 min	25 mL	solution cooled down to approx. 40°C	~ 100	-	Room temperature for 24h	[142]

[1] Agitation; [2] Dispersion; [3] Degassing; [4] Mass/volume of solution; [5] Solvent evaporation method; [6] Thickness; [7] Material of the plate; [8] Storage conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils (continuation).

Quantity/percentage of materials used	$\dot{\omega}^1$	**DP**^{2}/**DG**^{3}	m_s^4	S_e^5	ξ^6 (μm)	σ_p^7	**STO**^{8}	**References**
Starch (5 g) + clay nanoparticle (0.1 g) + cinnamon EO (0.4, 0.6 and 0.8 g) + emulsifier (0.01; 0.015 and 0.020 g) + glycerol (0.75, 1.13 and 1.50 g)	200 rpm	Heating in microwave oven up to 69 ºC	–	Drying at 35ºC from 18 to 24 h	~ 100	Teflon	23 ºC at 75% RH	[143]
Cassava starch + 3 g of glycerol + EO of lemon grass (0 – 4%)	–	Rest at room temperature	–	Drying under laminar cabinet ambient conditions for 3 days	–	Glass	Packed in aluminum foil until evaluation	[144]

[1] Agitation; [2] Dispersion; [3] Degassing; [4] Mass/volume of solution; [5] Solvent evaporation method; [6] Thickness; [7] Material of the plate; [8] Storage conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils (continuation).

Quantity/percentage of materials used	**$\dot{\omega}$[1]**	**DP[2]/DG[3]**	**m_s[4]**	**S_e[5]**	**ξ[6]** (µm)	**σ_p[7]**	**STO[8]**	**References**
Cassava starch (3%) + glycerol (0.85 - 2.55% relative to the solution volume) + oregano EO (0 - 2% relative to the solution volume) + pumpkin extract residue (0 - 6% relative to the solution volume)	–	–	0.28 g/cm^2	Drying at 30 ºC for 16 h	124 – 193	Acrylic	Desiccator with 58% RH for 48 h	[145]
Starch (3.5% w / v) + sorbitol (50% w / w of the starch weight) + Borage seed EO (0.2 - 1% w / v of the film solution) + Tween 80 (10% of the EO weight w / w)	12.000 rpm for 5 min	Heating at 95ºC for 20 min and 260 rpm	–	Drying at 25 ºC, 50% relative humidity for 15 h	–	–	–	[146]

[1] Agitation; [2] Dispersion; [3] Degassing; [4] Mass/volume of solution; [5] Solvent evaporation method; [6] Thickness; [7] Material of the plate; [8] Storage conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils (continuation).

Quantity/percentage of materials used	$\dot{\omega}^1$	DP^2/DG^3	m_s^4	S_e^5	ξ^6 (µm)	σ_p^7	STO^8	**References**
Corn starch (1.5 mg / mL) + glycerol (40% of the starch by mass) + guar-xanthan (0.1% of the starch by mass) + EO of oregano and black cumin (0.5 - 2%)	10,000 rpm for 1 min	Vacuum homogenizer and degasser	50 g	heating at 90°C for 60 min	101 – 111	Petri	Dry air at 25ºC for 5 days	[147]
Corn starch (3% w / v) + glycerol (1.8% v / v) + EO (1% w / w) or nanoemulsions of *Zataria multiflora* EO (1% w / w) + Tween 80 (4.5% w / w)	2,000 rpm for 2 min	–	70 mL	Drying at 25 ºC for 36 - 48 h	256 – 203	Teflon	–	[148]
Cassava starch (8% w / v) + glycerol (20% dry basis w / w) + emulsion containing lemongrass EO (0 - 1% vol%) + pectin + Tween 80	15,500 rpm for 15 min	Vacuum for 20 min	1.5 mm	Oven drying in 2 stages of 90 ºC	50 – 150	Polyester	Desiccator at 52% RH and 25 ºC for 48 h	[149]

[1] Agitation; [2] Dispersion; [3] Degassing; [4] Mass/volume of solution; [5] Solvent evaporation method; [6] Thickness; [7] Material of the plate; [8] Storage conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils (continuation).

Quantity/percentage of materials used	$\dot{\omega}^{1}$	**DP^{2}/DG^{3}**	m_s^{4}	S_e^{5}	ξ^{6} (µm)	σ_p^{7}	**STO^{8}**	**References**
Starch (33,3 g/L) + glycerol (13 mL per L of solution) + Garlic EO (1, 2 and 3%)	–	Heating at 60 ºC for 30 min	40 mL	Drying at 75 ºC for 5 h	120 – 150	Glass	–	[150]
Wheat starch (36 g) + corn starch (24 g) + sorbitol (6 g) + citric acid (25 g) + sodium carboxymethyl cellulose (14 g) + sodium alginate (14 g) + lemon EO (0,5 - 2% v / v) + surfactants (Span 80 and Tween 80 0,1 % v/v)	10,000 rpm for 4 min	Vacuum for 1 h	200 mL	Drying at 25ºC for 16 h	72 – 77	Glass	25 ºC and 53% RH	[151]
3% corn starch (w / v) + glycerol (30% w / w of starch) + citric acid (10% w / w of starch) + thyme EO microcapsules (10 - 30% w / w of starch)	600 rpm for 1 h	Stir under heating for 10 min	–	Drying in an oven with air circulation at 45 ºC for 6 h	146 – 200	Acrylic	16ºC and 58% RH	[119]

[1] Agitation; [2] Dispersion; [3] Degassing; [4] Mass/volume of solution; [5] Solvent evaporation method; [6] Thickness; [7] Material of the plate; [8] Storage conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils (continuation).

Quantity/percentage of materials used	**$\dot{\omega}^1$**	**DP^2/DG^3**	**m_s^4**	**S_e^5**	**ξ^6** (µm)	**σ_p^7**	**STO^8**	**References**
Corn starch (5% w / v) + glycerol (50% w / w of starch) + *Zataria multiflora Boiss* EO or *Mentha pulegium* (1 - 3% v / v) + Tween 80 (0.1 - 0.3% v / v of EO)	20,000 rpm for 3 min	Rest for a few minutes	70 mL	Drying at 20 ºC, 30% relative humidity for 48 h	151 – 247	Teflon	Desiccator at 25ºC	[152]
Corn starch (6% w / v) + glycerol (50% w / w of the amount of starch) + xanthan gum (0.1% w / v) + *Syzygium aromaticum* EO and *Cinnamomum cassia* EO (0.5 - 4% v / v)	–	–	15 g	Oven drying at 30 ºC for 72 h	83 – 343	Plastic	Vacuum desiccator	[153]
Corn starch (3% w/v) + octenyl succinate starch (3% w/v) + glycerol (2% w/v) + soy oil (0 - 0.12% w/v)	400 rpm	–	30 g	Oven drying at 50 ºC for 5 h	–	Plastic	–	[154]

[1] Agitation; [2] Dispersion; [3] Degassing; [4] Mass/volume of solution; [5] Solvent evaporation method; [6] Thickness; [7] Material of the plate; [8] Storage conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils (continuation).

Quantity/percentage of materials used	**$\dot{\omega}$**[1]	**DP**[2]**/DG**[3]	**m_s**[4]	**S_e**[5]	**ξ**[6] (µm)	**σ_p**[7]	**STO**[8]	**References**
Starch (5 % w/v) + glycerol (2% v/v) + gum (0.5 - 1.5%) + *S. officinalis* EO (0.25 - 0.5 ml)	–	Slow shaking for 60 min	100 mL	Drying at 60 ºC for 18 h	199 – 220	Teflon	–	[155]
Cassava starch (5% w / v) + glycerol (2.5% w / w) + cinnamon EO (1.5 - 2.5%)	1,200 rpm	Vacuum oven at 50 ºC for 30 min	–	Drying at 50 ºC for 24 h	290 – 380	–	Desiccators at 30–40% RH	[156]
Starch (3.3% w/v) + glycerol (25% w/w of starch) + Carvacrol EO (3 - 10% w/w of starch) + montmorillonite (4.5 - 15% w/w of EO)	800 rpm for 5 min	–	20 mL	Drying at 35 ºC for 12 h	–	Polystyrene	–	[157]

[1] Agitation; [2] Dispersion; [3] Degassing; [4] Mass/volume of solution; [5] Solvent evaporation method; [6] Thickness; [7] Material of the plate; [8] Storage conditions.

Table 3.1. Compositions and details of the preparation methods of starch-based films added with essential oils (continuation).

Quantity/percentage of materials used	**$\dot{\omega}^1$**	**DP^2/DG^3**	**m_s^4**	**S_e^5**	**ξ^6 (µm)**	**σ_p^7**	**STO^8**	**References**
Starch octenyl succinate (4% w/w) + glycerol (40% w/w of solid content) + sodium alginate (30% w/w content) + emulsion containing cinnamon EO (10 - 50% w / v)	500 rpm por 10 min	Vacuum	–	Drying at 50 ºC for 6 h	112 – 153	Acrylic	25ºC and 50% RH for 48h	[158]
Cassava starch + glycerol + citric acid + poly (butylene adipate-co-terephthalate) + microencapsulated and free oregano EO	*Film produced by the extrusion technique*	–	–	–	122 - 219	–	–	[140]
Sweet potato starch (0 – 55,5 g/L) + grenetine (22,2 g) + glycerol (5,5 g/L) + cinnamon EO (0 - 60 mg/L)	–	–	100 mL	Drying in a convection oven at 55 ºC for 1 h	10 – 80	–	Room temperature for 48 h	[159]

6. Effect of essential oils addition on the properties of starch-based films

6.1. General properties

Among the various properties of biopolymer-based films, thermal and mechanical behavior are paramount. First, because there is a need to evaluate the thermal stability and possible thermal transitions that would assist in the identification of the conditions of use of the material. Second, mechanical properties such as tensile strength and elongation at break allow for an analysis of film packaging performance. Other physical properties such as moisture content, water solubility, contact angle, water vapor, gas and light barrier properties also provide interesting information that can assist in selecting the best formulations for use as food packaging [2]. Particularly, water vapor permeability results can be useful to understand possible mass transfer mechanisms and solute-polymer interactions in edible films [160].

The addition of essential oils to the composition of the films can significantly alter physical-chemical properties as well as provide functional antioxidant and antimicrobial activity that help to maximize the film performance. For example, the incorporation of oregano, cinnamon bark and cloves EOs into blends of gelatin and cassava starch plasticized with glycerol improved its water vapor and oxygen barrier properties, as well as its transparency, resulting in films with enhanced functional properties [161]. Similar results were obtained for starch and gellan blends containing emulsified thyme EO or thyme EO encapsulated in lecithin, which exhibited a high oxygen barrier capacity [162].

Numerous other recent studies are available with very interesting results. Thus, the impact of adding essential oils to each of the aforementioned properties is carefully discussed in this section. It is important to emphasize that, in the vast majority of cases, the results of properties are perfectly correlated with the structure and microstructure. As a result, microscopy is an indispensable tool for assessing the superficial and internal morphology as well as the structural analysis (FT-IR) of films.

6.2. Morphology

The microstructure of films depends primarily on the interactions among the components of the film and may have an influence on the functional properties such as mechanical, barrier and optical properties [163]. In verifying the morphology of the films in question, visual and microscopic analyses employing Scanning Electron Microscopy (SEM) are widely used.

Do Evangelho *et al.* examined the effects of adding orange EO to the morphology of corn starch films. The film produced without EO presented a smooth and uniform surface. However, after the incorporation of EO, reduced the homogeneity of the cross sections (more pronounced effect at more elevated concentrations of EO) with the presence of more concentrated pores on the surface, as reflected in **Figure 3.5** [120]. Similar results were obtained by other authors [145,146,151], who observed an increase in the heterogeneity of the surface of the films after adding borage seed, lemon and oregano EOs, respectively.

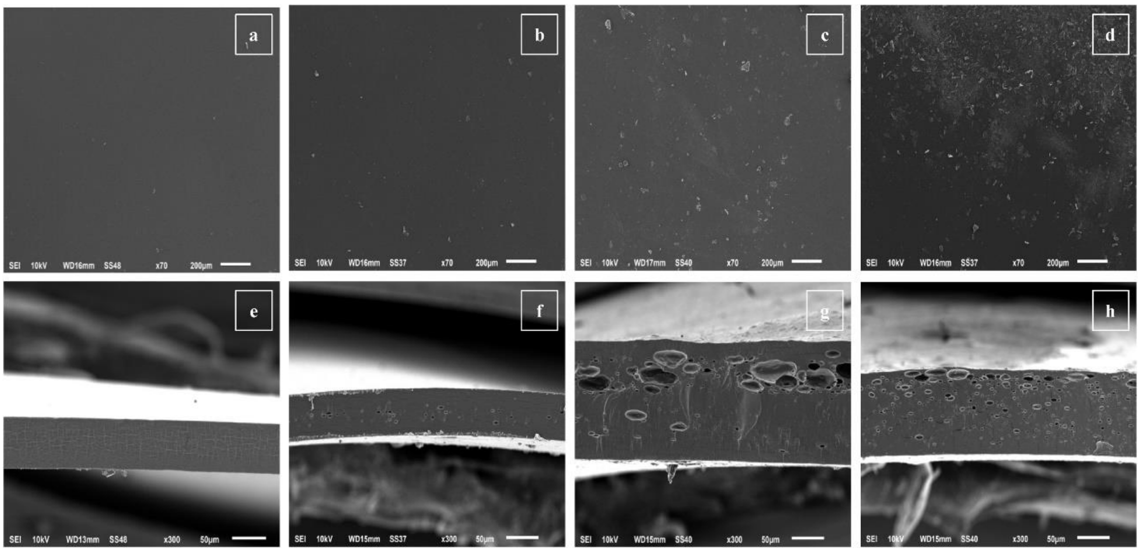

Figure 3.5 – SEM images of the surface (a, b, c, d) and cross sections (e, f, g, h) of corn starch films with 0.0, 0.3, 0.5 and 0.7 μL g^{-1} of EO, respectively [120]. Reprinted with permission from Elsevier, Copyright (2019).

The surfaces of films with oregano EO microcapsules showed more significant heterogeneity than the surfaces of control films and those containing free EO, indicating that films without microcapsules retained more dense internal structures. In addition, it was noted that films with free EO presented a rougher structure than the control films, pointing to more plastic behavior (Medeiros *et al.*, 2019). In another study, the structure of films with the addition of emulsions containing cinnamon EO were evaluated. The authors in that study observed that, in comparison with the control film, those with emulsion possessed a compact and relatively smooth structure, indicating uniform distribution of the emulsion droplets. However, as the EO concentration increased, the structure became thicker and less compact [158].

In this context, the use of emulsions (on a nano and micro scale) has represented an advance in improving the performance of food packaging based on biopolymers, particularly with regard to the successful incorporation of new compounds and functionalities in conventional films and coatings. These emulsions are designed to protect, encapsulate and deliver hydrophobic, bioactive and functional compounds [164]. According to the results highlighted in this section, with the use of emulsifying agents, the surface morphology tends to be improved, which positively impacts the other properties.

6.3. Optical properties

The optical properties (color and transparency) of food packaging play a crucial role in the appearance and acceptance of packaging by consumers [149]. Essential oils can be colorless or have a color ranging from light tones to quite intense ones. The color of EOs in some cases provides interesting properties as a barrier to certain wavelengths, which can maximize the preservation of packaged foods. Thus, the evaluation of this property becomes essential.

The addition of thyme [119], oregano [145,147], lemon [151], and black cumin EOs [147] significantly reduced the transparency of starch films. Regarding control samples, a decrease of 15.3%, 29.4% and 44.6% in transparency was observed after adding 0.1, 0.2 and 0.3% (in relation to the starch mass) of thyme EO to the films [119]. Similar results were obtained by Caetano *et al.* who registered significantly higher opacity when the oregano EO concentrations increased [145]. This effect is probably related to the alteration in the crystalline structure, as well as to an increase in the light scattering induced by oil droplets in the film matrix [149,151,152].

There are also numerous reports of statistically non-significant variations in relation to the optical properties of starch-based films containing essential oils. For example, although the total color difference increases with the concentration of lemongrass EOs [149], *Zataria multiflora Boiss* EOs and *Mentha pulegium* EOs [152], the differences in appearance between the films containing EOs and the control sample were minimal. This result can be considered quite satisfactory with regard to the commercial appeal of the film, in which the absence of opacity and coloring is considered to be advantageous.

6.4. Moisture content

Most studies found that the addition of EO decreased the moisture content of the films, for example oregano and black cumin, *Zataria multiflora Boiss, Mentha pulegium* EOs [147,152,155]. This would seem obvious, given that the incorporation of a hydrophobic additive throughout the polysaccharide matrix tends to reduce possible interactions between water and functional groups of the biopolymer. However, there were cases in which the moisture content was increased [120], or was significantly unchanged, for example, for pumpkin residue extract (0 to 6%) and oregano EO [145].

The incorporation of 0.7 μL/g of orange EO in starch films increased the moisture content from 18.8% (control) to 21.9% [120]. This phenomenon was attributed to the formation of a porous structure that facilitated the movement of water molecules between the polymer chains. However, divergent results were obtained by Ghasemlou *et al.*, whose films incorporated with 3% of *Zataria multiflora Boiss* EO had their moisture content reduced from 21.9% to 14% and with the addition of 3% *Mentha pulegium* EO to 13 % [152]. Šuput *et al.* observed a reduction from 14.1% (control) to 12.2% with the addition of 2% of black cumin EO and to 12.0% with 2% of oregano EO [147], thus corroborating the theory of the hydrophobic nature of EOs [146,155].

6.5. Water solubility

Water solubility is an important factor when choosing a film based on biopolymers, since it can direct the application as packaging for food products [120,152,155]. The addition of EOs significantly changed this property, as exemplified by cases of increase [119,120] and reduction in the water solubility of starch films [146,151,155].

Films containing orange EO, regardless of concentration, showed greater solubility than the control sample, and the addition of 0.7 μL/g of EO changed the solubility from 15.2% to 18.6% [120]. A similar result was obtained in another study in which 45 g of thyme EO was added, and the authors noticed an increase in solubility from 23.5% to 36.5% [119]. Both authors observed that the variation in solubility varies according to the hydrophilicity of the added EO.

Song *et al.* observed a reduction in water solubility with an increase in the concentration of lemon EO, which varied from 46.2% (control) to 38.7% and 33.4% with the addition of 1 and 2% of EO, respectively [151]. This result was attributed to a decrease in the hydrophilic nature of the films, as well as to the interaction between EO components and hydroxyl groups. The addition of 1% borage seed EO changed the solubility in water from 38.7% to 31.1%, with a reduction in the diffusion of soluble substances in water due to the formation of an insoluble outer layer of starch granules [146]. Pirouzifard *et al.* achieved similar results and also noticed a more marked decrease with an increase in the content of EO *Salvia officinalis* [155].

6.6. Thermal behavior

Two primary techniques are used for a complete thermal characterization of biopolymer based-films: thermogravimetric analysis (TGA) to identify the effect of essential oil addition on thermal stability, and the differential scanning calorimetry (DSC) to analyze the impact on thermal transitions, as shown in **Table 3.2**. In general, in the TGA analyses, it was observed that the addition of EOs decreased, even if minimally, the thermal stability of the formed films, and the higher the concentration of EO, the greater the loss in thermal stability [119,149].

DSC analysis showed that the presence of EO alters the thermal behavior of the film due to low degradation temperatures and changes in the glass transition temperature (T_g) and crystalline melting temperatures (T_m), as well as in the enthalpy variation (ΔH), which would suggest greater or lesser ordering of the components in the films. It is noted that, depending on the starch source and the types and concentrations of EOs added, the values of T_g and T_m can vary widely in the literature. This may be one of the main reasons for the quite different results described in **Table 3.2**. In general, the inclusion of EO in the composition of the films can influence these properties by occupying the spaces between the helical amyloses, reducing the interactions between the starch polymers and weakening the film matrix [146,147,157].

Table 3.2. Effect of adding essential oils on the thermal properties of starch films.

Quantity/percentage of materials used	Conclusions	Reference
Starch + glycerol + carvacrol EO (3 - 10% wt%)	The incorporation of EO in the films decreased the T_{onset} from 120.8 ºC to 49.0 ºC, the T_m from 144.8 ºC to 90.8 ºC and increased the ΔH from 77.4 J/g to 204.1 J/g.	[157]
Starch (3.5% w / v) + sorbitol (50% w / w of the starch weight) + Borage seed EO (0.2 - 1% w / v of the film solution) + Tween 80 (10% of the EO weight w / w)	The incorporation of EO in the films decreased the T_m from 122.4 ºC to 110.6 ºC and the ΔH from 148.6 J/g to 116.6 J/g.	[146]
Corn starch (1.5 mg / mL) + glycerol (40% of the starch by mass) + guar-xanthan (0.1% of the starch by mass) + EO of oregano and black cumin (0.5 - 2%)	The incorporation of EO in the films decreased the T_m from 199 ºC to 107 ºC, the ΔH from 141 J/g to 78 J/g and increased the T_g from 14.8 ºC to 18.2 ºC.	[147]
Cassava starch (8% w / v) + glycerol (20% dry basis w / w) + emulsion containing lemongrass	The incorporation of EO in the films decreased the thermal stability of the films from 308 ºC to 293 ºC.	[149]

EO (0 - 1% vol%) + pectin + Tween 80		
Corn starch + glycerol + thyme EO microcapsules (10-30% based on starch mass w/w) and citric acid	The incorporation of EO in the films did not cause marked differences in thermal stability. The maximum decomposition temperature occurred between 320 °C and 330 °C.	[119]
Cassava starch (3%) + glycerol (0.85 - 2.55% relative to the solution volume) + oregano EO (0 - 2% relative to the solution volume) + pumpkin extract residue (0 - 6% relative to the solution volume)	TGA analysis showed that the addition of EO and pumpkin extract residue did not influence the thermal stability of the films, which was approximately 300 °C.	[145]

6.7. Mechanical properties

The primary mechanical parameters of biopolymer-based films are measured mainly in terms of tensile strength (TS) and elongation at break (EB). TS represents the film's resistance to tension forces, and EB determines its stretching capacity, indicating flexibility [145,148]. The elasticity modulus can also be an interesting evaluation parameter, since it provides quantitative values of the film stiffness. The effect of adding EOs on the mechanical properties of starch-based films is described in **Table 3.3.**

The addition of EOs significantly changed the mechanical behavior of starch films, since the structure and the intra and intermolecular bonds of starch were changed [120,141,146,150]. In addition, the effect on TS and EB is highly dependent on the type of EO used, as well as on the composition of the film and the addition or not of other formulation components [144].

In addition to these factors, the different behaviors of starch films can be attributed to the difference in filmogenic materials, as well as to the different interactions with the essential oil components [151]. As previously described, the structural characteristics of starch, which are strongly related to its source and processing method, can be factors that differentiate the mechanical properties of the control films [165]. These factors plus the wide compositional variability of the different essential oils also explain the marked differences in the values of the mechanical properties summarized in **Table 3.3**.

In general, there is a marked reduction in TS with the gradual increase in the concentration of EOs. On the other hand, many EB values have undergone a proportional increase, which would indicate a plasticizing effect related to the inclusion of the additive. The present compounds of low molar mass are positioned between the macromolecules of the starch, causing a separation and, consequently, reducing the intensity of the secondary bonds. This mechanism obviously results in a reduction in the film resistance and an increase in macromolecular mobility, which macroscopically responds with an increase in EB.

Table 3.3. Mechanical properties of starch films containing EOs.

Film material	Thickness (µm)	Tensile Strength (TS) (MPa)	Elongation at break (EB) (%)	Conclusion	Reference
Starch (3%) + glycerol (30% relative to dry starch mass) + 0; 0.5; 0.3 e 0.7 µL/g (relative to the film-forming solution mass) of orange EO	84 - 131	5.1 – 2.4	64.6 – 15.2	The addition of EO gradually reduced TS and EB of the films. EB of the film containing 0.3 µL/g of EO was lower than that of the films containing 0.5 and 0.7. The addition of EO caused stronger polysaccharide intermolecular interactions to be partially replaced by weaker polysaccharide-EO interactions, generating more flexible domains within the film.	[120]
Corn starch (100g) + glycerol (7,5%) + magnesium stearate (3 %) + 7,5 or 10% (g of essential oil/g of starch) of oregano or thyme Eos	24 - 25	1.1 – 0.4	1.6 – 0.9	Addition of EOs reduced TS and EB. Foams containing oregano EO showed higher TS and lower EB values than those containing thyme EO. The reductions in TS and EB may be associated with the plasticizer and crosslinking effects of EOs.	[141]
Starch (5 g) + clay nanoparticle (0.1 g) + cinnamon EO (0.4, 0.6 and 0.8 g) + emulsifier	~ 100	3.7 – 1.0	128.8 – 191.3	In addition to EO, the presence of glycerol and emulsifier also significantly decreased TS and increased EB. The introduction of EO reduced the intermolecular interaction between polymer chains.	[143]

(0.01; 0.015 and 0.020 g) + glycerol (0, 75, 1.13 and 1.50 g)					
Cassava starch (3%) + glycerol (0.85 - 2.55% relative to the solution volume) + oregano EO (0 - 2% relative to the solution volume) + pumpkin extract residue (0 - 6% relative to the solution volume)	124 - 193	1.7 – 0.3	136 – 247	The addition of EO decreased TS and Young's Module and increased EB. Glycerol also contributed to the reduction of these properties, since it modifies weakening the structure of the starch.	[145]
Starch (3.5% w / v) + sorbitol (50% w / w of the starch weight) + Borage seed EO (0.2 - 1% w / v of the film solution) + Tween 80 (10% of the EO weight w / w)	-	9.5 – 4.7	82.5 – 103.8	TS decreased and EB gradually increased as the concentration of EO increased. EO fatty acid molecules reduced interactions between starch chains and weakened the film matrix.	[146]

Corn starch (1.5 mg/mL) + glycerol (40% of the starch by mass) + guar-xanthan (0.1% of the starch by mass) + EO of oregano and black cumin (0.5 - 2%)	101 - 111	14.3 – 2.1	32.0 – 61.0	Both EOs decreased the TS values and increased the EB values. The reduction in the TS value indicates loss of macromolecular mobility and is attributed to the plasticization capacity of the EOs.	[147]
Corn starch (3% w / v) + glycerol (1.8% v / v) + *Zataria multiflora* EO (1% w / w) or nanoemulsions of *Zataria multiflora* EO (1% w / w) + Tween 80 (4.5% w / w)	256 - 203	2.9 – 0.2	128.6 – 141.9	The incorporation of EO reduced the value of TS and increased EB. Films containing EO in the form of nanomulsion showed lower TS and higher EB values, which may be related to the fact that the reduction in particle size results in a more significant plasticizing effect.	[148]
Cassava starch + glycerol + emulsion containing lemongrass EO (0 - 1% vol) + pectin + Tween 80	50 – 150	21.0 – 16.0	2.4 – 4.7	The addition of pectin/EO emulsions increased TS and EB of the films in all formulations, except for 2/1 pectin/EO wt%. It is believed that the starch-glycerol interactions were partially replaced by the starch-pectin/EO interactions that strengthened the associative network, thus improving the tensile strength of the emulsified films.	[149]

Starch (33,3 g/L) + glycerol (13 mL per L of solution) + Garlic EO (1, 2 and 3%)	120 - 150	10.5 – 6.2	9.3 – 5.7	TS and EB gradually decreased with increasing concentration of EO. The lipid molecules filled the protein matrix and interactions between the lipid and polar molecules occurred, making the structure weaker.	[150]
wheat starch + corn starch + sorbitol + citric acid + sodium carboxymethylcellulose + sodium alginate + lemon EO (0.5 - 2% v/v) + emulsifiers (Span 80 or Tween 80)	72 - 77	15.5 – 11.2	30 – 36.3	The increase in EO concentration decreased TS and increased EB. In addition, the addition of surfactants also reduced TS and EB, particularly Tween 80, which is a hydrophilic surfactant and whose interaction with water or starch weakens intermolecular hydrogen bonds.	[151]
Corn starch + glycerol + citric acid + thyme EO microcapsules (10-30% of starch w/w)	146 - 200	2.1 – 3.4	56.8 – 23.5	The increase in EO concentration gradually increased TS and decreased EB. This fact was attributed to the presence of extensive hydrogen bonds between the hydroxyl groups of the starch matrix and microcapsules, thus increasing the network structure of the film.	[119]

Starch + glycerol + gum + *S. officinalis* EO (0.25 - 0.5 mL)	199 - 220	4.7 – 1.6	99.0 – 34.2	By adding gum to the films, EB and TS were significantly reduced, as the interactions between amylose and amylopectin were reduced, destroying hydrogen bonds. The addition of EOs to the film also reduced EB and TS, and the increase in EO concentration further pronounced this reduction. The presence of EO in the film structure causes structural condensation and decreases the resistance to force.	[155]
starch octenyl succinate + sodium glycerol alginate + emulsion containing cinnamon EO (10 - 50% w/v)	112 - 153	21.6 – 8.6	13.2 – 53.2	TS decreased significantly and gradually with increased concentration of EO, while EB increased. The decrease in TS may be due to the effect of EO plasticization. Increase in EB may be associated with facilitating the sliding of the polymeric chains during stretching, caused by the emulsion.	[158]

6.8. Barrier properties

Barrier properties, such as water vapor permeability (WVP), are important factors in the quality of food packaging materials, since they must be able to prevent, or at least reduce the transfer of water between the environment and food [156,166,167]. WVP can be defined as the product of diffusivity and solubility only when Fick and Henry laws fully apply. Water vapor strongly interacts with the polymer structure of the film, which results in diffusion and solubility coefficients that are dependent on the driving force [168].

Some more recent WVP results for starch films with the addition of different essential oils are shown in **Table 3.4**, in which quite contrasting values of WVP can be observed. It is clear that the addition of EOs contributed as much to the increase [120,143,145] as to the decrease in WVP [119,146,150,152,156]**.**

The increase in WVP could be related to the formation of cavities caused by the addition of EO that increased the amount of free spaces in the polymeric network [169]. These cavities promote preferential ways of diffusing water vapor, substantially reducing the film barrier. Conversely, the WVP reduction of EO starch films may be due to the hydrogen and covalent interactions between the starch network and the polyphenolic compound. These interactions probably minimize the accessibility of hydrogen groups to form a bond with water, which reduces the affinity of the film [152].

It is important to note that although the hydrophobic compounds can play an important role in the physical-chemical structure of the film, the water permeability of starch-based films is also influenced by other factors such as temperature, the thickness of the film, relative humidity gradients and the content of the additives [168]. Therefore, the benefits of adding EOs to the barrier properties must be evaluated within the context of a broad spectrum of analysis.

Table 3.4. Water vapor permeability (WVP) and oxygen permeability coefficient (PO_2) of starch films containing EOs.

Film material	Thickness (µm)	WVP (g mm m^{-2} day^{-1} kPa^{-1})	PO_2 (cm^3 m^{-1} d^{-1} kPa^{-1})	Conclusion	References
Starch (3%) + glycerol (30% relative to dry starch mass) + 0; 0.5; 0.3 e 0.7 µL/g (relative to the film-forming solution mass) of orange EO	84 – 131	2.8 to 4.5	-	The increase in the WVP of the films with the addition of EO is related to the formation of cavities that caused changes in the structural integrity of the films, increasing the amount of free spaces in the polymeric network and facilitating the passage of water vapor.	[120]
Starch (5 g) + clay nanoparticle (0.1 g) + cinnamon EO (0.4, 0.6 and 0.8 g) + emulsifier (0.01; 0.015 and 0.020 g) + glycerol (0, 75, 1.13 and 1.50 g)	~ 100	3.6 to 14.8	21.5 to 143.5	Increased content of glycerol, emulsifier and EO caused an increase in WPV. However, this variation is not credited to the EO, but to the emulsifier, for being hydrophilic.	[143]

Cassava starch (3%) + glycerol (0.85 - 2.55% relative to the solution volume) + oregano EO (0 - 2% relative to the solution volume) + pumpkin extract residue (0 - 6% relative to the solution volume)	124 - 193	8.6 to 15.8	-	In general, the increase in glycerol led to higher WVP values and, the incorporation of EO, decreased WVP. The addition of EO to the filmogenic matrix reduced the water absorption by the films, since these substances are complex and highly hydrophobic mixtures.	[145]
Starch (3.5% w / v) + sorbitol (50% w / w of the starch weight) + Borage seed EO (0.2 - 1% w / v of the film solution) + Tween 80 (10% of the OE weight w / w)	-	383.6 to 315.4	-	The incorporation of EO decreased the WVP, indicating the formation of hydrophobic layers around the starch granules, which prevented the permeation of water vapor through the films.	[146]

Starch (33,3 g/L) + glycerol (13 mL per L of solution) + Garlic EO (1, 2 and 3%)	120 - 150	6.9 to 12.5	19.7 to 28.9	The WVP of the films increased proportionally with the concentration of EO. EO modifies the performance of the barrier, which is associated with starch and oil compatibility, leading to the permeation of gas molecules through the films.	[150]
Corn starch + glycerol + thyme EO microcapsules (10-30% of starch w/w) and citric acid	146 – 200	60.0 to 45.6	-	WPV decreased significantly with increasing EO concentration.	[119]
Corn starch + glycerol + EO from *Zataria multiflora Boiss* or *Mentha pulegium* (1 - 3%) v/v + Tween 80	151 - 247	7.9 to 3.0	12.1 to 15.5	The addition of EOs improved the barrier properties by up to 50% compared to the control sample. The reduction in WVP of films containing EO is due to covalent and hydrogen interactions between the starch network and polyphenolic compounds that limit the availability of hydrogen groups to form hydrophilic bonds with water.	[152]
Cassava starch + glycerol +	290 - 380	137.0 to 107.7	-	The WVP of the films decreased with an increase in the concentration of EO, a fact attributed to the hydrophobicity of EO.	[156]

cinnamon EO (1.5 - 2.5%)					
Starch + glycerol + gum + *S. officinalis* EO (0.25 - 0.5 ml)	199 - 220	8.23 to 4.82	-	The WPV decreased only with the addition of gum, but the simultaneous addition of gum and OE reduced even more.	[155]

6.9. Antioxidant properties

Recently, packaging materials based on biopolymers with functional properties such as antioxidant capacity have been gaining a lot of attention. They can delay undesirable reactions such as oxidation, affecting the quality of food. In addition, the use of natural active compounds, such as essential oils, can help reduce dependence on artificial additives [170,171].

In this regard, phenolic compounds such as carvacrol, thymol and eugenol that are present in EOs can inhibit the formation of free radicals, which react with oxygen during the self-oxidation process [148,172]. These compounds are thus the main contributors to the antioxidant properties of plant extracts [173]. In addition, the phenolic compounds act by providing a hydrogen atom from their -OH groups, with the resultant phenolic radical being thermodynamically stable due to a resonant structure [174].

Researchers concluded that the incorporation of EOs in starch films conferred antioxidant properties which increased with increasing oil concentration [145,146,148,150,153,155,158]. For example, an antioxidant activity of up to 79.88% in barnyard millet starch films containing borage seed oil were reported [146]. In addition, antioxidant activity up to 15.96% using 3% of clove essential oil was observed in millet starch films [150].

6.10. Antimicrobial properties

Active antimicrobial films are developed with the aim of extending the shelf life of food products and maintaining their safety [2,175,176]. The controlled release of antimicrobials from packaging materials represents an important innovation in active packaging [175]. In this context, the use of natural antimicrobials incorporated into biopolymer films is a new way of obtaining safe products and reducing the amount of additives and artificial preservatives [176].

Thus, research has been carried out to evaluate the inhibition of pathogenic microorganisms after the incorporation of different EOs in starch films. For example, the incorporation of EO can be effective against *Listeria monocytogenes* [120,141,145], *Staphylococcus aureus* [120,140,142,149,150,152,156], *Salmonella enterica* [141,147,156], *Escherichia* *coli* [142,145,147,149,150,152,156], *Eurotium amstelodami* [143]; *P. commune* [143]; *Trichoderma harzianum* [144,150], *Penicillium expansum.* (Supardan *et al.*, 2016), *Bacillus cereus* [150], *Enterobacter s.* [150], *Pseudomonas aeruginosa* [150], *Colletotrichum gloeosporioides Penz* [119], *Botryodiplodia theobromae Pat.* [119], *Leuconostoc mesenteroides* [153], *Pseudomonas fluorescens* [153] and *Shewanella putrifaciens* [153].

In addition, in most cases, the inhibitory capacity increases with increasing concentration of EO. As a result, changes in other properties such as mechanical properties which may restrict the use of films as packaging, must be analyzed concurrently. As recorded by Souza *et al.*, films have become increasingly fragile, making it necessary to vary the plasticizer content according to the increase in the essential oil content in the formulation [143].

It is also important to note that the type of bacteria should be considered when assessing the antimicrobial activity of films with essential oil, that is, whether the bacteria is Gram positive or Gram negative. In general, Gram positive bacteria are more sensitive to EO inhibitory activity [140–142,147,152,158]. The greater antimicrobial effect in Gram-positive bacteria is explained by the absence of the outer membrane around the cell wall, which facilitates the diffusion of hydrophobic compounds such as EOs [141,142]. In Gram-negative bacteria, phenolic compounds present in EO are responsible for damaging the microbial phospholipid cell membrane, which increases cell permeability and causes leakage of the cytoplasm content [145].

In addition, in order not to lose the antimicrobial activity of the films, care must be taken during preparation and storage of the film. In these stages, EO evaporation can occur, as confirmed by Souza and collaborators (2020), who did not register antimicrobial activities even with high concentrations of EOs.

7. Food applications

The consumer demand for healthy and safe food products led the researchers to find more natural alternative approaches to enhance the quality and safety of foods. Since essential oils are approved as additives by EC and FDA, they are now most likely to be used in and on the food products instead of synthetic preservatives [25]. Considering the application of this materials in industrial level, some patents providing the beneficial properties of essential oils in the food packaging are already available [116].

As examples, the patent number WO 2013084175A1 states that the incorporation of *Rosmarinus officinalis*, *Citrus limon* and *Vitis vinifer* EOs into packaging materials controlled the development of biogenic amines in fresh produce [177]; and the patent US20160325911A1 showed the development of an antimicrobial compositions for food packaging consisting of salicylaldehyde and carvacrol, thymol or their mixture [178]. These examples illustrate well some cases of research already focused on practical industrial applications. However, some challenges still hinder the widespread use of these materials, as discussed in the final section of this review article.

Following the development and characterization of active and intelligent starch films with essential oil, it is necessary to evaluate the potential application of these materials in the packaging of different types of food. In this context, this section reviews the application of starch films with essential oils in fruits & vegetables and meat products.

7.1. Fruits and vegetables

Nowadays, consumers are demanding high quality vegetables and fruits, and this demand has resulted in a challenge to improve food preservation techniques. In this sense, scientists have been working on the development and application of natural and biodegradable polymers in order to increase the time to maintain the quality and safety of these products [179].

Based on these premises, the impact of cassava starch and chitosan coatings with EO of *Lippia gracilis Schauer* on the shelf life of guavas (*Psidium guajava* L.) during storage at room temperature for 10 days was evaluated by [180]. The results showed that blends of cassava starch with chitosan and EO were more effective at inhibiting the growth of most bacteria. On the 10th day of storage, the total number of mesophilic aerobic bacteria and the counts of fungi and yeasts were statistically lower in the coated fruits. In addition, the coated fruits did not show significant changes in the content of total soluble solids.

Starch films containing thyme EO microcapsules were evaluated with regard to the quality and physicochemical properties of mango by [119]. Starch films with EO showed a better preservation effect compared to the film without EO. The shelf life of mangoes treated with EO starch films was 10 days at 25 °C, which showed the potential of this film for mango preservation.

The application of sweet potato starch films activated with thyme EO in spinach leaves was investigated by [181]. The incorporation of EO in the film reduced the population of *Salmonella Typhimurium* and *Escherichia coli* in fresh spinach leaves to levels below those detectable in five days, while control samples without essential oil maintained approximately 4.5 Log (CFU)/g. The sensory scores for spinach samples wrapped in films containing EO were higher than those of the control, suggesting that this packaging material can lead to extension in the shelf life of spinach.

7.2. Meat products

Films and coatings based on biopolymers with active components, such as essential oils are suitable for the preservation of meat products. These packaging approaches may present a promising future, as they increase the shelf life of these products by preventing moisture loss and delaying microbial deterioration and lipid oxidation [182].

The effect of edible films of corn starch with spices oils (clove and cinnamon) on the stability of raw beef during refrigerated storage (4 °C) for 15 days was investigated by [153]. It was observed that the active films reduced the microbial populations of the meat samples and improved the color stability at the end of the storage period. These results showed that this material has potential application in meat products as an additional barrier to control microbial deterioration and delay lipid oxidation.

Cassava starch films with oregano essential oil showed satisfactory antioxidant and antimicrobial activity when tested in vitro and were evaluated for protection against ground beef oxidation by [145]. The films resulted in protection of the ground meat against lipid oxidation until the third day of storage under refrigeration. The application of cassava starch films with lemongrass EO also led to a reduction in microbial meat count during storage [144].

The effect of corn starch films containing EO from *Zataria multiflora* and cinnamaldehyde on the physicochemical and sensory characteristics of ground beef hamburgers stored for 20 days at 4 ± 1 ° C was evaluated by [148]. The film containing EO showed the best results in the physical-chemical and sensory parameters at the end of storage compared to the film without EO, demonstrating the potential of applying this material as a packaging for meat products.

8. Conclusions and prospects

The primary objective of this review was to describe the most recent and breakthrough advances in the preparation of starch-based films containing essential oils. Obtaining these materials is reasonably simple, and the particular highlight is the significant increase in antimicrobial and antioxidant characteristics which can contribute to food safety and preservation. However, the limitations associated with reduced mechanical strength, water vapor barrier and thermal stability are still challenging, and need to be overcome so that starch-based films can be competitive in the food packaging market.

The simultaneous addition of essential oils and ceramic nanoparticles (e.g., silicates and carbonaceous materials) represents viable strategies that can partially compensate for the loss of properties by the addition of essential oils. In this context, considering the composite films already produced, investigations into ways to improve the compatibility between nanoparticles and the biopolymer matrix can equally contribute to the improvement in the results. However, recent research is lacking in this type of analysis, which leaves this as a possible path to be followed in the future.

Another attractive point to be considered is the fact that there are no comprehensive studies on the stability of essential oil components in the polymeric matrix. For example, what is the maximum time that such antioxidant and antimicrobial activities remain in the film? This was one of the questions that came up during this review and that needs to be addressed in future research.

Moreover, a standardization of the essential oils used in the film's preparation has thus far been little explored affecting the reproducibility of materials.

It is well-known that essential oil components are volatile and degrade easily. Accordingly, effective strategies for the preservation of these constituents are crucial. The first strategy, a more consolidated one, refers to the encapsulation of oils and the use of micro and nano-emulsions. In addition to enabling greater compatibility between the additive and the biopolymer (improving physical-chemical properties), emulsified EOs can also maximize the stability of the functional compounds in the matrix. Therefore, advances in this area are indispensable, as accessible and scalable methodologies are currently lacking.

A second strategy that may gain prominence in the future in order to extend the presence of essential oil components in these films for longer time frames could be the polymerization of the components. For example, a recent study reported the controlled polymerization of pinene generated stable and reproductible oligomers with antioxidant activities superior to the terpene used as a precursor [183]. The authors suggested the use of the polypinene as an additive in biodegradable films. However, to the best of authors' knowledge, up to now, no work has been encountered in this area. Is this the future of active packaging?

Although innumerable properties for human health have been attributed to essential oils, information regarding the biological activities of their films (studies of cytotoxicity, anti-inflammatory and anti-cancer activities) is still scarce. In this regard, it is believed that directing research to this area may further expand the potential for application of films beyond the area of food packaging. An example would be in the medical field. Finally, the evaluation of productive processes on a larger scale such as continuous casting and extrusion as well as biodegradation studies are also needed in order to consolidate the widespread use of starch-based films with added essential oils.

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4. CAPÍTULO 4 - METODOLOGIA

A realização deste projeto envolveu etapas subsequentes e dependentes entre si, como esquematizado na Figura 4.1.

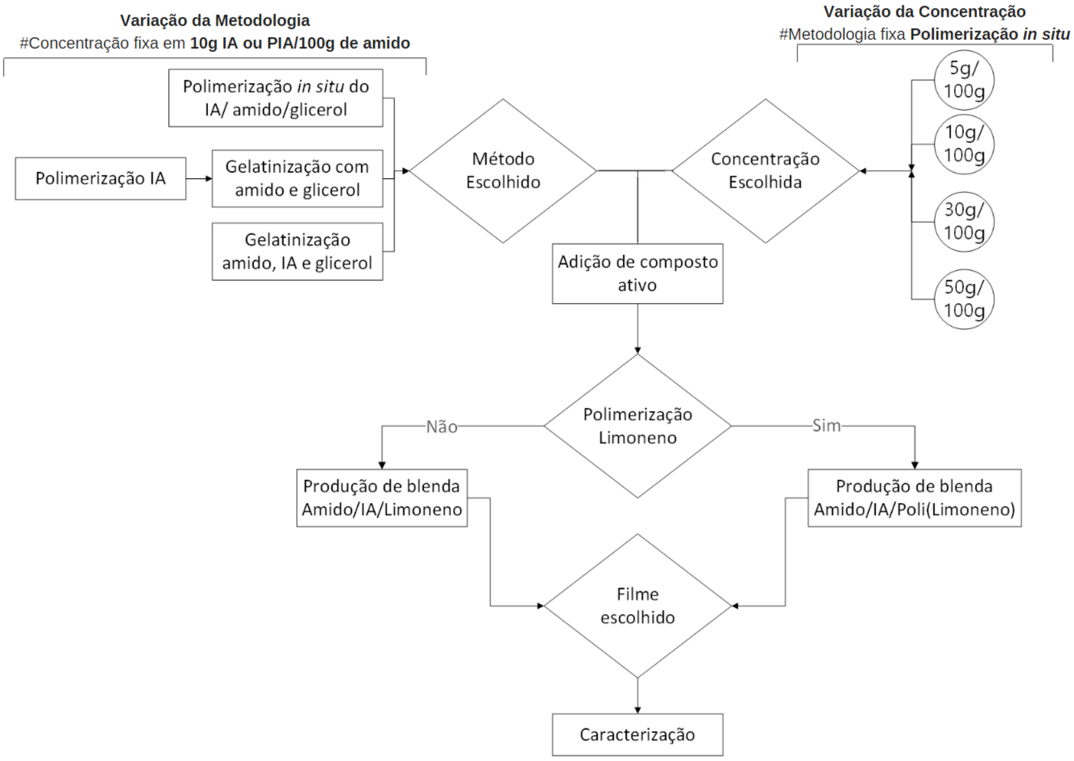

Figura 4.1 – Representação esquemática das etapas e tomadas de decisão durante o desenvolvimento da pesquisa.

Por se tratar de um estudo inédito, foi realizado inicialmente uma análise exploratória. Na primeira etapa dessa análise exploratória, fixou-se uma concentração: 10g de IA ou PIA/100g de amido e foram explorados 3 métodos diferentes para produzir dos filmes:

1) Polimerização *in situ*, em que o tempo de polimerização foi o mesmo necessário para amido para atingir a gelatinização (cerca de 20 min);
2) Pré-polimerização do ácido itacônico, sendo o polímero sintetizado adicionado à solução para gelatinizar com amido;
3) Sem polimerização, com o intuito de investigar como o monômero mudaria a propriedades dos filmes.

Concomitantemente, o método polimerização *in situ* foi fixado e foram produzidos filmes com diferentes concentrações de ácido itacônico: 5, 10, 30 e 50g/100g de amido.

Esse primeiro passo objetivou definir a concentração e metodologia mais promissoras, sendo que todos os filmes produzidos nessa etapa foram caracterizados quanto às suas propriedades químicas, físicas, mecânicas, térmicas e de barreira.

Em seguida foi realizada a segunda etapa desse trabalho que envolveu a adição de composto ativo. Foram produzidos filmes contendo limoneno e poli(limoneno) de baixa massa molecular, adicionados nas concentrações de 3, 6 e 9 g/100g de amido. Tais concentrações foram definidas com base em estudos anteriores do grupo de pesquisa, ainda não publicados. As propriedades antimicrobianas e antioxidades desses filmes foram comparadas e apenas os filmes com o composto ativo mais promissor foram caracterizados quanto às suas propriedades químicas, físicas, mecânicas, térmicas e de barreira.

5. CAPÍTULO 5: ESTRATÉGIAS DE POLIMERIZAÇÃO DO ÁCIDO ITACÔNICO PARA PRODUÇÃO DE FILMES À BASE DE AMIDO DE BATATA

ITACONIC ACID POLYMERIZATION STRATEGIES FOR POTATO STARCH-BASED FILM PRODUCTION

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ABSTRACT

To enhance starch-based film properties, itaconic acid (IA) was added at different concentrations (5-50 g of IA/100 g starch) and by different techniques as *in-situ* polymerization, pre-polymerization and no polymerization. Varying the concentration, it was observed that the addition of higher IA contents conferred greater crystallinity and decreased the water vapor permeability (WVP) but significantly reduced the tensile strength (TS) and elongation at break (EB). However, the film with 5 g of IA/100 g of S showed an increase in EB from 18.7 to 45.1 %, with no significant changes in TS. Different methods did not affect TS or EB but greatly influenced the barrier properties, and for the pre-polymerization technique, WVP was reduced from 2.5 (control) to 1.7×10^{-10} g m^{-1} s^{-1} Pa^{-1}. These results suggest that potato starch and IA can be considered interesting alternatives to packaging materials.

Key words: Sustainable film; polysaccharide; biopolymer; starch; itaconic acid.

1. Introduction

A global consensus is the need to encourage more sustainable development and balance economic, social and environmental demands (Rodriguez-Anton & Alonso-Almeida, 2020). One of the challenges to this development is to mitigate the use of plastics of fossil origin, especially those of single-use, as well as to fight against their waste, which causes serious environmental risks. In this context, the production of sustainable food packaging from renewable sources represents a prominent alternative to the use of petrochemical-based plastics (Vianna *et al.*, 2021), and biobased polymers, such as starch and poly(itaconic acid), appear to be potential alternatives.

Starch is considered one of the most promising polysaccharides in the development of biodegradable films due to its abundance, low cost-effectiveness, and excellent film-forming capacity (Thakur *et al.*, 2019). Although starch-based plastics are already being commercialized, their production and improvement of their performance (mainly mechanical and barrier properties) still represent a great challenge (Din *et al.*, 2020; do Val Siqueira *et al.*, 2021; Saliu *et al.*, 2019; Thakur *et al.*, 2019). Therefore, one of the most effective ways to enhance these characteristics is the elaboration of blends (Din *et al.*, 2020; Romani *et al.*, 2017).

Itaconic acid (IA) is a promising organic acid considered by the U.S. Department of Energy as one of the 12 biotechnological products with the greatest potential for industrial use (Werpy & Petersen, 2004). Since the 1940s, the filamentous *fungi Aspergillus itaconicus* and *Aspergillus terreus* have been recognized as excellent producers of itaconic acid, but industrially it is obtained by aerobic fermentation of glucose using *Aspergillus terreus* (Chenyu Du, 2014; Kuenz *et al.*, 2012; Saha, 2017; Werpy & Petersen, 2004). Its structure allows a variety of novel applications (Robert & Friebel, 2016), highlighting its use as a superabsorbent polymer for personal care or agriculture, unsaturated polyester resin for the transportation industry and synthesis of poly(methyl methacrylate) for electronic devices (Cunha da Cruz *et al.*, 2018).

Likewise, one of its potential uses involves the production of biopolymers and the possibility of forming green plastics with good properties (Kuenz *et al.*, 2012; Magalhães *et al.*, 2017). As an example, in the formation of IA-modified polyacrylate, the addition of 2.5 g of itaconic acid/100 g of the solution increased the films' tensile strength from 8.5 MPa to 11.4 MPa, the Young modulus from 82 MPa to 582 MPa and the glass transition temperature from 6.8 °C to 17.9 °C (Liu *et al.*, 2019). Neither the preparation of IA polymers nor the products of these plastics are harmful to health or the environment (Saha, 2017). Thus, due to the great potential of itaconic acid market, evaluated at $ 86.8 million in 2020, it is projected to reach $ 126.2 million by 2028, with an annual growth rate of 4 % between 2021 and 2028 (RESEARCH, 2021).

Therefore, this research is based on the hypothesis that the combination of starch and itaconic acid might improve the mechanical and barrier properties of potato starch films as a result of possible intermolecular interactions. To the best of our knowledge, this is the first report of a starch-IA blend, and for a more complete understanding and evaluation of the novel bonds, this work presents its combination under several IA contents, as well as prepared by different methods.

This exploratory analysis aimed to determine both the best concentration and the best preparation method. Hence, first, we set a method, *in situ* polymerization, and the proportion of added IA varied from 5 g to 50 g/100 g of starch. Then, a concentration (10 g/100 g of starch) was chosen and 3 methods were evaluated: pre-polymerization of IA, the addition of monomer (no polymerization), and *in situ* polymerization.

2. Materials and Methods

2.1 Materials

Commercial potato starch (S) (CAS 9005-84-9) (product code 110, Dinâmica Química Contemporânea Ltda., Brazil), itaconic acid (purity ≥ 99 %) (product code 102043629), 70% aqueous tert-butyl hydroperoxide solution (product code

102182329, Sigma Aldrich, Brazil), and glycerol (Ecibra, Brazil) were used as received.

2.2 Films Production

2.2.1 Method 1 – pre-polymerization (PreP)

At first, poly(itaconic acid) was synthesized by IA radical polymerization, following a modified procedure of Cao (2008). In a glass vial, itaconic acid was added in the proportion of 5 g of IA:31.2 µL of initiator (tert-butyl hydroperoxide):4 mL of water. The solution was deoxygenated with N_2 for 7 min and then heated with stirring at 100 °C for 150 min. After the synthesis process, the polymer was precipitated with acetone, vacuum filtered, and then placed in an oven for drying for 24 h at 50 °C.

The films were then produced using the casting technique, according to Antosik *et al.* (2017) with some modifications. The film-forming solutions were prepared in Milli-Q water with 5 g of starch per 100 mL of solution, 30 g of glycerol/100 g of starch, and poly(itaconic acid) (10 g/100 g of starch). The film-forming solutions were heated at 70 °C for 20 min. After cooling, the solutions were placed under ultrasound (Dekel/TS-218) for 30 min. Then, 50 mL of each solution was spread on polystyrene plates (diameter of 14 cm) and dried in an oven (MMM Group/Venticell) at 45 °C for 48 h.

2.2.2 Method 2 – in situ polymerization (I.S.)

The *in-situ* polymerization method is similar to that described in Section 2.2.1, but in this method, monomer and initiator ratio of 5 g of AI: 31.2 µL of tert-butyl hydroperoxide, respectively, were directly added to the starch and glycerol solution. Films with 5, 10, 30 and 50 g of IA/100 g of starch were produced using this method. The polymerization time was 20 min, the time spent for starch gelatinization.

2.2.3 Method 3 - without initiator (no polymerization occurring) (N.P.)

This method was added to evaluate the individual effect of the monomer (10 g/100 g) on the properties of starch films. The procedure is similar to that described in Section 2.2.2; however, no initiator was added; hence, polymerization does not occur. Films without IA were also prepared and considered as control.

2.3 Characterization of the films

2.3.1 Film Morphology

The morphology of the surface and transverse sections of the films was evaluated using scanning electron microscopy (SEM) (Leo 440i, LEO Electron Microscopy/Oxford, Cambridge, England) at an acceleration voltage of 15 kV and a current of 50 pA. The films were fixed on a metallic support with the aid of double-sided carbon tape and covered with a thin layer of gold in a sputter coater (SC7620, VG Microtech, Kent, United Kingdom). For the cross-section analysis, the films were fractured with liquid nitrogen. The surfaces and cross-sections of the films were analyzed at 1,000× and 2,000× magnifications, respectively.

2.3.2 Molecular Changes

Infrared spectra of the films were obtained using a Fourier transform infrared (FT-IR) spectrometer Thermo Scientific spectrophotometer (Nicolet Continuum, Madison, USA). Spectra were recorded in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 128 scans per point.

2.3.3 Crystallinity by X-ray diffraction

The crystallinity of the film was obtained by measurements of the XRD pattern ($2\theta = 5–60°$) using an X-ray analyzer (X'Pert-MPD, Philips, Almelo, Netherlands) with Cu Kα radiation ($\lambda = 1.54056$) at a scan rate of 0.033333°/s (step = 0.04 ° and time per step = 1.2 s) operated at a generator voltage of 40 kV and current of 40 mA.

2.3.4 Thermal Stability

Thermogravimetric analysis (TGA) was carried out using a TGA device (Mettler Toledo, TGA/DSC1, Schwerzenbach, Switzerland) under airflow (50 mL/min) to analyze the thermal stability of the films. Samples of approximately 10 mg were heated from 20 to 700 °C at a heating rate of 20 °C min^{-1}. The TG/DTG curves were recorded.

2.3.5 Physical Properties

Thickness

The average thickness (*t*) of the films was determined using a system composed of a digital micrometer (Mitutoyo Co., Kawasaki-Shi, Japan) and a flat granite base with a resolution of 0.1 µm. Measurements were performed using five random points from five samples of each formulation (ISO-4593, 1993), and the results were expressed in µm.

Moisture content and water solubility

The moisture content (*MC*) of the prepared films was determined in triplicate according to ASTM D644-04 (2004). The films were cut into squares (2 × 2 cm), weighed, and dried for 24 h at 105 °C. The moisture content at equilibrium was calculated with Equation (1):

$$MC(\%) = \frac{m_i - m_f}{m_i} \times 100 \tag{1}$$

wherein m_i and m_f are the initial and final masses of the samples, respectively.

To measure the water solubility (*WS*), the dried films were cut into squares (2 × 2 cm) and weighed. The dried films were then put into Milli-Q water at 25 °C for 24 h. Subsequently, the films were dried overnight at 105 °C to reach a constant weight. The *WS* of the samples was calculated with Equation (2):

$$WS(\%) = \frac{W_i - W_f}{W_i} \times 100 \tag{2}$$

where W_i and W_f are the initial and final masses of the film, respectively. Each sample was measured in triplicate.

Swelling ratio

The swelling ratio (*SR*) of the films was determined in triplicate according to Bajpai, Bajpai and Jyotishi (2016). A pre-weighed piece of film was placed in a phosphate buffer solution of pH 7.4, and its mass was measured at definite time intervals until the film attained constant weight. The swelling ratio (SR) was determined when equilibrium was reached using Equation (4).

$$SR(\%) = \frac{m_f - m_i}{m_i} \times 100 \tag{4}$$

where m_i is the initial dry mass and m_f is the swollen mass at equilibrium.

2.3.6 Mechanical Properties

To determine mechanical properties, samples of the films were cut with a width of 15 mm in high precision equipment (RDS-100-C, Chem Instruments, OH, USA). Then, they were stored for 48 h at 25 ± 2 °C and 75 ± 5 % RH. Tensile strength (MPa) and elongation at break (%) were determined using a universal testing machine (Instron, 5966-E2, USA), from the stress versus strain curve. The tests were performed with a 100 N load cell with a speed of 12 mm min^{-1} and an initial distance between the grips of 50 mm (ASTM-D882, 2018). The mechanical properties of each film were evaluated in 5 replicates.

2.3.7 Water vapor permeability

The water vapor transmission rate (WVTR) was determined by a gravimetric method based on ASTM-E96/E96M (2016). Capsules with a permeation area of 50 cm^2 and an analytical balance (Mettler Toledo, Columbus, USA) with a resolution of 10^{-4} g were used. The tests were carried out at 25 °C and 75 % RH in an air-conditioned chamber (Weiss Technik, Reiskirchen, Germany) with anhydrous calcium chloride desiccant. The WVTR (g m^{-2} day^{-1}) was determined from the slope of the curve "mass change vs. time". The water vapor permeability coefficient (g m^{-1} s^{-1} Pa^{-1}), WVP, of the films was calculated in four replicates for each sample, according to Equation (3):

$$WVP = \frac{WVTR \times t}{p_s \times RH} \tag{3}$$

wherein t is average thickness (m) of the sample, p_s is the water vapor saturation pressure (3167.20 Pa at 25 °C), and RH is the relative humidity of the chamber (75 % = factor 0.75) since the relative humidity inside the capsule is considered zero.

2.3.8 Light Transmission

The light transmission (%) of each film was determined with a dual-beam UV–visible spectrophotometer (Analytik Jena - Specord 210) using a scanning speed of 120 nm min^{-1} and a scan range from 200 to 800 nm (ASTM-E-1348, 2015). The light transmission of the films was measured in triplicate.

2.4 Statistical analysis

The results were statistically evaluated employing analysis of variance (ANOVA) and the Tukey's test, conducted in Minitab® Software, to compare the average results ($p < 0.05$).

3. Results and Discussion

3.1 Film Morphology

Figures 5.1 shows the SEM micrographs of the surface and cross-sectional area of potato starch films incorporated with different IA contents, and Figure 5.2 compares the films prepared by different methods. The pure starch film (Fig. 5.1a) showed a reduction in starch granules after processing, indicating that heating during casting caused the gelatinization of the native starch granules, as expected.

Additionally, as observed in the cross-sectional micrographs of Figure 5.1, the increase in the IA content reduced the uniformity of the films, with the appearance of surface ripples and few grains on the surface. On the surface micrographs, smaller grains are observed in the films with higher concentrations of IA, which is probably non-polymerized IA, as the same pattern is seen for the film prepared by the no polymerization method (Fig. 5.2a).

Moreover, as observed in the micrographs of Figure 5.2, the different methods provided distinct surface patterns. It is noteworthy that Fig. 5.2b contains the same SEM image of Figure 1c to facilitate the comparison between the different methods of film preparation containing 10 g IA/100 g of S. It is possible to note larger grains for *in-situ* polymerization (Fig. 5.2b) than for the no polymerization technique (Fig. 5.2a). However, when comparing the *in-situ* polymerization (Fig. 5.2b) and pre-polymerization (Fig. 5.2c) methods, it is possible to conclude that a pre-polymerization provided a more homogeneous structure since the presence of polymer on the surface and phase separation were not observed.

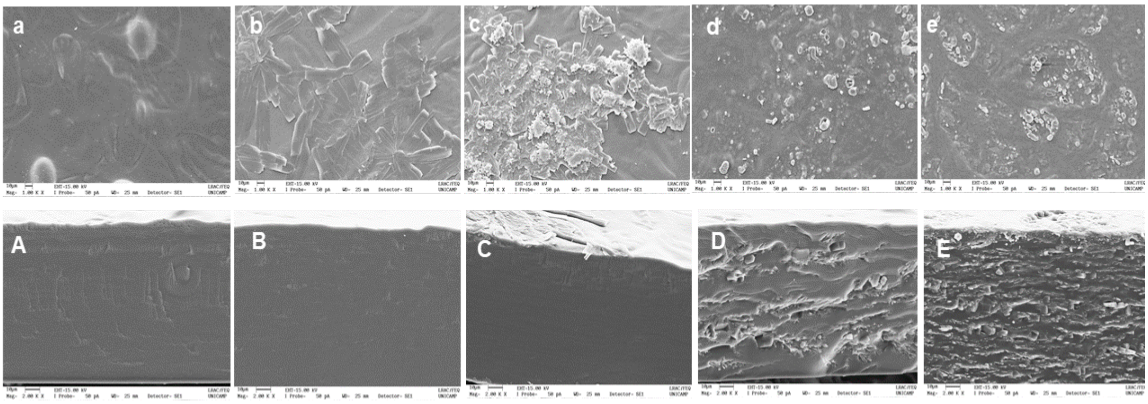

Figure 5.1. Surface micrographs (a, b, c, d, e) and cross-sections (A, B, C, D, E) of the starch films with 0, 5, 10, 30 and 50 g of IA/100 g of S, respectively.

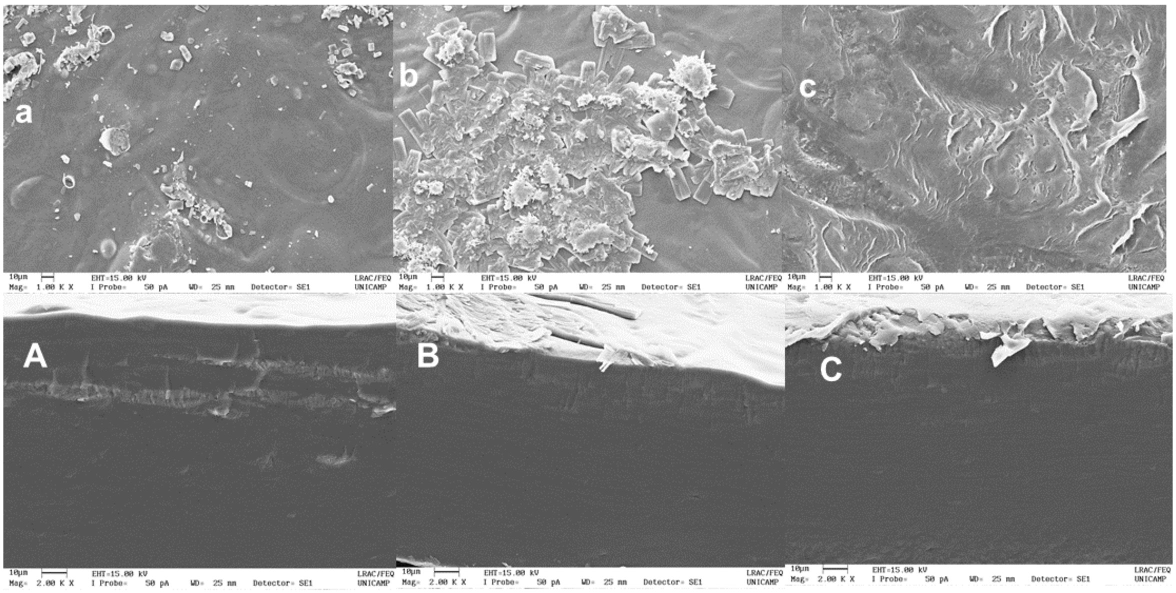

Figure 5.2. Surface micrographs (lowercase letters) and cross-sections (uppercase letters) of the starch films prepared by the following methods: (a, A) no polymerization, with 10 g of IA/100 g of S; (b, B) *in situ* polymerization, with 10 g of IA/100 g of S; and (c, C) pre-polymerization with 10 g of PIA/100 g of S. The SEM images for the *in-situ* polymerization method are the same as in Fig.1 to facilitate the comparison among morphologies from different methods.

3.2 Molecular Changes

FTIR spectroscopy was used to assess the intermolecular interactions and structural changes in starch films prepared by different methods (Fig. 5.3.A) or upon incorporation of IA at different concentrations (Fig. 5.3. B). Overall, the FTIR spectra of the films indicated the same major peaks, but their amplitudes differed depending on the concentration and method.

All films exhibited an absorption peak in the spectral region at approximately 3338 cm^{-1} corresponding to the stretching vibration of the free hydroxyl group (O-H). Films also presented characteristic peak at 2926 cm^{-1} (C–H) and wavenumbers of 1150, 1080, and 1010 cm^{-1} for C–O–C stretching (a triplet peak of starch) (Lanthong *et al.*, 2006).

Peaks are observed at approximately 1650 and 1701 cm^{-1} for IA and PIA, respectively, as well as for films with concentrations greater than 10 g of IA or PIA/100 g of S. The peak at 1701 cm^{-1} evidenced the presence of the carbonyl groups of the carboxylic acid groups. The band at 1650 cm^{-1} is characteristic of a double bond, and its presence in pure PIA spectra indicated that there is still a residual monomer together with the polymer, so the separation method used was not completely efficient. Meantime, this peak is more intense for IA than for PIA.

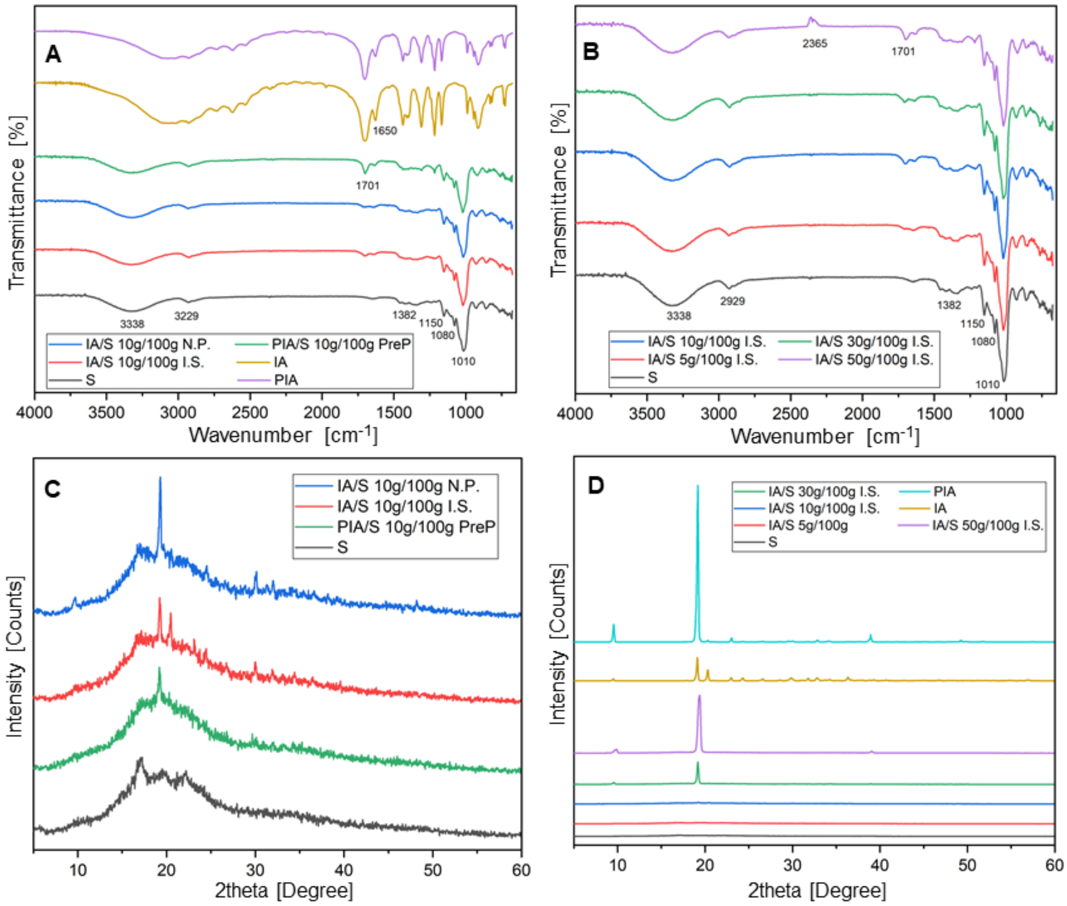

Figure 5.3. IR spectra of pure IA and PIA, of the control film (S) and of the films produced by different methods, with the concentration of IA or PIA fixed at 10 g /100 g of S (A); IR spectra of the control film (S) and of the films produced by *in situ* polymerization at different concentrations (B), XRD analysis of the control film (S) and of the films produced by different methods, with the concentration of IA or PIA fixed at 10 g/100 g of S (C), XRD analysis of pure IA and PIA, of the control film (S) and of the films produced by *in situ* polymerization at different concentrations (D). Legend: S: starch, IA itaconic acid, PIA: poly(itaconic acid), N.P.: no polymerization; I.S.: *in situ* polymerization; PreP: pre-polymerization.

3.3 Crystallinity by XRD

The crystallinity of starch is related to linear chains, which give different X-ray diffraction patterns depending on the starch source (Buléon *et al.*, 1997). Pure starch-glycerol films showed peaks at approximately 2θ = 17.12, 19.6 and 22.15° (Fig. 5.3C and 5.3D), which correspond to the B-type crystalline structure typical of potato starch (Dome *et al.*, 2020). For the control sample and films with 5 and 10 g of IA/100 g of S, the XRD patterns showed semicrystalline characteristics (presence of amorphous and crystalline zones), and the addition of IA caused peak shifts, indicating changes in the crystallization of the amylopectin chain. Similar results were found by Soto *et al.* (2016), who modified starch by IA grafting polymerization to form hydrogels. They observed that the relative percentage of crystallinity slightly decreased with the addition of IA, giving rise to an increase in the amorphous phase. These authors suggested that the hydrogen bonds between molecules are weakened, and thus, the thermoplasticity of IA-grafted starches increased.

Furthermore, the IA and PIA patterns (Fig. 5.3D) showed a common peak at 19.15°, which is more pronounced in the polymer. This peak was also observed in the films prepared by the studied methods, and it became more intense with the increase in IA concentration. Additionally, the peak at 9.5°, observed for PIA, was also present in films with 30 and 50 g of IA/100 g of S. For lower IA values, the intensity of crystallinity peaks was reduced, while for the highest values, it was increased. This behavior may be associated with a possible agglomeration of AI along the polymeric matrices of higher concentration, being identified in the XRD pattern.

3.4 Thermal Stability

Table 5.1 presents the onset (T_{onset}) and maximum degradation (T_{max}) temperature values of the films, while Figure 4.4 shows the TGA and DTG curves from 25 to 700 °C. The DTG curve showed 2 main thermal decomposition peaks for the control and for the film with 5 g IA/100 g S added. The first step, with T_{max} between 94.5 (S) and 120.3 °C (50 g IA/100 g S), is attributed to the evaporation of water absorbed by starch, glycerol and IA (Pelissari *et al.*, 2009). In the second stage (T_{max} approximately 300 °C), starch was completely degraded (dos Santos Caetano *et al.*, 2018).

For films with 10 (*in-situ* polymerization), 30 and 50 g of IA/100 g of S, an additional peak was noticed, with values ranging from T_{onset} 144.2 to T_{max} 239.7 °C. At this stage, the degradation of itaconic acid occurred, which is consistent with the findings of Cao (2008), who observed over 90 % weight loss at 210 °C for itaconic acid. Further heating results in weight loss due to decarboxylation and chain scission (Bajpai *et al.*, 2016). Although it was expected that films with 10 g /100 g S (Fig. 5D), regardless of the preparation method, would show the second stage of degradation, it did not occur for pre-polymerization and no-polymerization techniques.

Overall, the addition of IA slightly changed the thermal profile of S-based films, increasing the values of the T_{onset} (Table 5.1) for the second thermal event, as well as adding another thermal event for the films with higher IA concentrations.

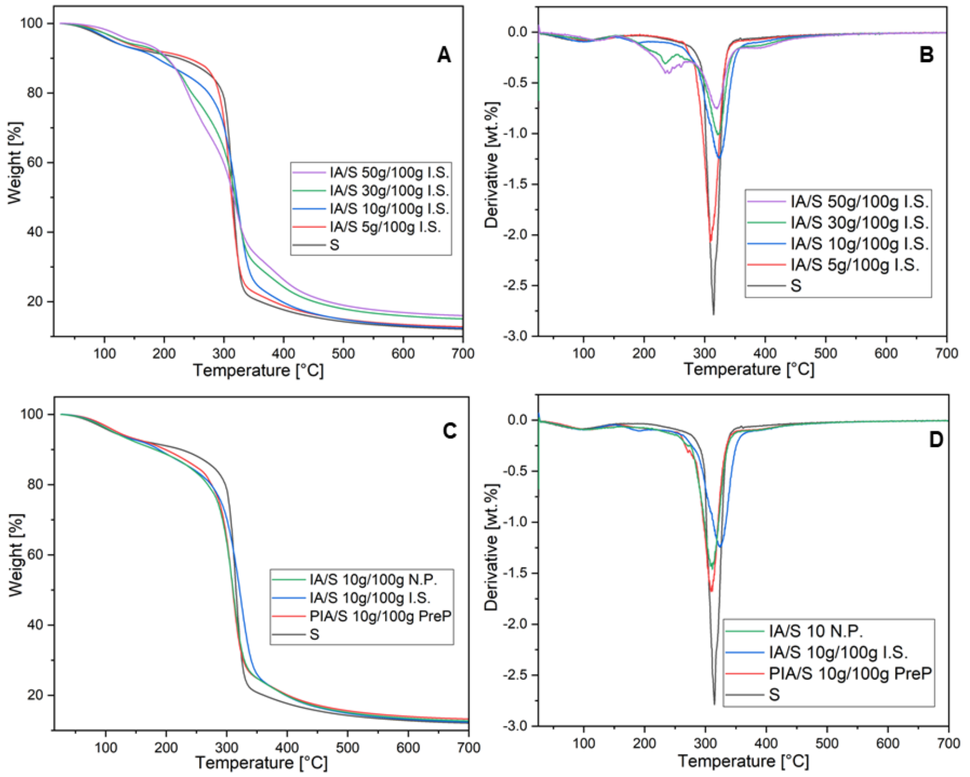

Figure 5.4. A and B, respectively: TG and DG curves of the control film (S) and of films produced by *in situ* polymerization at different concentrations of IA. C and D, respectively: TG and DG curves of films produced by different methods, with the concentration of IA or PIA fixed at 10 g/100 g of S. Legend: S: starch; IA: itaconic acid; PIA: poly (itaconic acid); NP: no polymerization; I.S.: in situ polymerization; PreP: pre-polymerization.

Table 5.1: Onset temperature (T_{onset}) and maximum degradation temperature (T_{max}) of the control film (S) and films with IA or PIA added. S: starch; IA: itaconic acid; PIA: poly (itaconic acid); N.P.: no polymerization; I.S.: in situ polymerization; PreP: pre-polymerization.

	First thermal event		**Second thermal event**		**Third thermal event**	
Film	**T_{onset} (°C)**	**T_{max} (°C)**	**T_{onset} (°C)**	**T_{max} (°C)**	**T_{onset} (°C)**	**T_{max} (°C)**
S	33.5	94.5	-	-	211.6	313.9
IA/S 5 g/100 g I.S.	38.6	109.5	-	-	203.5	308.9
PIA/S 10 g/100 g PreP	33.5	104.4	-	-	251.4	310.6
IA/S 10 g/100 g I.S.	36.8	99.6	144.2	187.1	231.4	318.8
IA/S 10 g/100 g N.P.	35.3	101.2	-	-	208.3	308.9
IA/S 30 g/100 g I.S.	28.7	116.1	158.9	234.6	256.2	323.8
IA/S 50 g/100 g I.S.	31.9	120.3	155.6	239.7	272.6	320.5

3.5 Thickness and Mechanical properties

The thickness of the films (Table 5.2) increased as a function of the concentration of IA due to the increase in the free volume of starch matrix. No significant difference in thickness was observed by changing the preparation method (Table 5.3).

The values of *TS* and *EB* of the control film were similar to those obtained in a study with potato starch and glycerol as plasticizers (Talja *et al.*, 2007), both at the same proportion used in the present study. The addition of IA in proportions of 5 g and 10 g of IA/100 g of S increased the *EB* of the films from 18.7 % (S) to 45.1 and 42.9 %, respectively, suggesting a plasticizing effect at low IA concentrations, that avoided crystallinity of the system, imposing chains flexibility. However, when the proportion of 5 g/100 g of starch was used, there was no significant change in *TS* (Table 4.4), which is an interesting result, since, in general, plasticizers reduce the resistance of films, as they act by weakening the intermolecular bonds of the polymeric matrix chains (Sun *et al.*, 2020).

At concentrations above 5 g IA/100 g S, TS decreased, a behavior that can be associated with the replacement of stronger starch chain-to-chain interactions with weaker starch–itaconic acid interactions, causing the material to break with less effort. The decrease in *EB* at higher concentrations might be due to the increase in crystallinity, as observed in the intense peaks in Figure 5.3d for the films with 30 and 50 g of IA/100 g of S. Among the methods, no significant difference was observed in *EB* or *TS*, which increased and decreased, respectively, as expected for the concentration of 10 g/100 g S.

Table 5.2: Thickness (*t*), tensile strength (*TS*), elongation at break (*EB*), moisture content (*MC*), water solubility (*WS*), water vapor transmission rate (*WVTR*), water vapor permeability (*WVP*), and swelling ratio (*SR*) of the control film (S) and of the films prepared by *in situ* polymerization, according to the IA concentration. S: starch (control); IA: itaconic acid.

Property	S	IA/S 5 g/100 g I.S.	IA/S 10 g/100 g I.S.	IA/S 30 g/100 g I.S.	IA/S 50 g/100 g I.S.
t (µm)	130.8 ± 13.6^{b}	130.8 ± 8.9^{b}	137.5 ± 12.4^{b}	155.8 ± 17.8^{a}	165.0 ± 13.9^{a}
TS (MPa)	7.2 ± 0.9^{a}	6.6 ± 0.7^{ab}	4.6 ± 0.6^{c}	5.8 ± 0.5^{bc}	3.1 ± 0.4^{d}
EB (%)	18.7 ± 0.9^{b}	45.1 ± 3.5^{a}	42.9 ± 1.8^{a}	10.9 ± 1.2^{c}	8.5 ± 4.2^{c}
MC (g/100g)	12.3 ± 0.7^{a}	11.7 ± 0.4^{a}	11.1 ± 1.4^{a}	12.9 ± 0.9^{a}	11.1 ± 0.5^{a}
WS (%)	19.9 ± 1.9^{c}	19.0 ± 0.4^{c}	21.5 ± 0.7^{c}	27.6 ± 0.5^{b}	36.4 ± 1.0^{a}
WVTR (g m^{-2} day^{-1})	373.6 ± 38.3^{a}	393.6 ± 25.1^{a}	371.9 ± 10.46^{a}	252.3 ± 20.4^{b}	199.1 ± 11.4^{b}
WVP ($\times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1})	2.5 ± 0.2^{a}	2.4 ± 0.1^{a}	2.5 ± 0.3^{a}	1.8 ± 0.1^{b}	1.6 ± 0.1^{b}
SR (%)	74.1 ± 3.2^{a}	77.1 ± 0.9^{a}	61.4 ± 0.6^{b}	61.2 ± 4.4^{b}	48.1 ± 1.3^{c}

The results are expressed as an average ± standard deviation.

$^{a, b, c...}$ *Values in a given line followed by the same letter do not differ at the 95% confidence level ($p > 0.05$).*

Table 5.3: Thickness (*t*), tensile strength (*TS*), elongation at break (*EB*), moisture content (*MC*), water solubility (*WS*), water vapor transmission rate (*WVTR*), water vapor permeability (*WVP*), and swelling ratio (*SR*) of the control film (S) and of the films prepared from different methods with a concentration of 10 g of IA or PIA/100 g S.

Property	S	pre-polymerization (10 g PIA/100 g S)	*In-situ* polymerization (10 g IA/100 g S)	no polymerization (10 g IA/100 g S)
t (µm)	130.8 ± 13.6^{b}	141.9 ± 8.2^{a}	137.5 ± 12.4^{ba}	138.9 ± 11.1^{ba}
TS (MPa)	7.2 ± 0.9^{a}	3.5 ± 0.5^{bc}	4.6 ± 0.6 ^{b}	3.3 ± 0.3^{c}
EB (%)	18.7 ± 0.9^{b}	46.7 ± 13.5^{a}	42.9 ± 1.9^{a}	38.0 ± 5.9^{a}
MC (g/100g)	12.3 ± 0.7^{a}	11.8 ± 0.4^{a}	11.1 ± 1.4^{a}	11.1 ± 0.4^{a}
WS (%)	19.9 ± 1.9^{a}	20.4 ± 0.5^{a}	21.5 ± 0.7^{a}	22.2 ± 0.3^{a}
WVTR (g m^{-2} day^{-1})	373.6 ± 38.3^{a}	247.9 ± 10.8^{b}	371.9 ± 10.5^{a}	427.4 ± 21.7^{a}
WVP (×10^{-10} g m^{-1} s^{-1} Pa^{-1})	2.5 ± 0.2^{a}	1.7 ± 0.09^{b}	2.5 ± 0.3^{a}	2.6 ± 0.3^{a}
SR (%)	74.1 ± 3.2^{b}	134.9 ± 1.6^{a}	61.4 ± 0.6^{c}	65.4 ± 2.3^{c}

The results are expressed as an average ± standard deviation.
$^{a, b, c...}$ *The values in a given line followed by the same letter do not differ at the 95% confidence level (p > 0.05).*

3.6 Moisture Content, Water Solubility, Swelling Ratio and Barrier Properties

Solubility is a factor that directs the application of the film as packaging for food products (do Evangelho *et al.*, 2019). As observed in Tables 5.2 and 5.3, the WS of the films presented values from 19.9 (S) to 36.4 % (50 g IA/100 g S). Once IA is hydrophilic, its addition increases the solubility in water; therefore, the higher the IA content is, the greater the solubility. The *WS* of the films produced by different methods did not present significant differences ($p > 0.05$). The moisture content (*MC*) did not change with the addition of IA or PIA.

The swelling ratio (*SR*) decreased with IA load. Similarly, Bajpai, Bajpai, and Jyotishi (2016) observed a reduction in the *SR* with increase in IA content in chitosan and [poly(acrylamide-co-itaconic acid] films. These authors attribute this to the unionized –COOH groups of IA (second carboxylic groups) that may produce inter- and intramolecular H-bonding interactions and cause a slight decrease in water absorption. Another explanation is that the gain of crystallinity in films with a high concentration of IA (confirmed by X-ray diffraction) leads to a decrease in the amorphous region, which is hydrophilic (Afolabi, 2012).

Nevertheless, it was noticed that for the film prepared by pre-polymerization, the *SR* increased from 74.1 (S) to 134.9 % (Table 5.3). This result is consistent with the findings of Lanthong, Nuisin and Kiatkamjornwong (2006) and Soto *et al.* (2016), who prepared IA-starch-based hydrogels.

Regarding *WVTR* and *WVP*, there were no significant difference between the control and films with 5 and 10 g of IA/100 g of S prepared by the *in-situ* polymerization technique (Table 5.2). On the other hand, at higher concentrations, for films with the addition of 30 g and 50 g of IA/100 g S, the *WVTR* decreased from 373.6 (control) to 252.3 and 199.1 g m^{-2} day^{-1}, and the *WVP* decreased from 2.5 (control) to 1.8 and 1.6×10^{-10} g m^{-1} s^{-1} Pa^{-1}, respectively. At these concentrations, the incorporation of larger particles (IA and PIA) into the starch matrix formed films without severe defects (Fig 5.1D and 5.1E), causing an increase in film crystallinity.

As a result, the tortuous path hindered water diffusion, explaining the reduction in *WVP*, which is closely related to these microstructural aspects. Furthermore, it is important to note that the significant increase in film thickness, at the mentioned concentrations, may also have influenced the reduction in the diffusion of water vapor, which was assessed by the reduction in *WVTR*.

Moreover, when comparing the 3 preparation methods, the pre-polymerization highlights the improvement of the water vapor barrier properties. For this technique, the *WVTR* decreased from 373.6 to 247.9 g m^{-2} $day^{-1,}$ and the *WVP* decreased from 2.5 to 1.7×10^{-10} g m^{-1} s^{-1} Pa^{-1}. It is necessary to emphasize that when comparing the results of *WVTR* with *WVP*, the film with 50 g of IA/100 g S (*in-situ* polymerization) showed similar performance to the film with 10 g PIA/100 g S pre-polymerized, so that pre-polymerization can supply the addition of high concentrations of acid without compromising the mechanical properties.

3.7 Light Transmission

Transparency is a functional film property with an important impact on food preservation as well as on appearance (Cai *et al.*, 2020). Figure 5.5 shows the transmittance as a function of wavelength for starch-based films with itaconic acid. The addition of IA, regardless of the employed method or the concentration, conferred some UV protection when compared to the control.

For wavelengths between 200 and 240 nm (in the UV-C range), for example, the transmittance of the control film was 14 %, while for all the films with IA it was close to zero. Since UV light tends to generate free radicals, which are the main causes of food oxidation, this result is very interesting to enhance the shelf life of foods that in the future may be packaged with starch films supplemented with IA. However, for 350 nm, the light transmittance was 90 % (S) and 53 % (50 g IA/100 g S), indicating that this UV-light blocking effect still occurs in the UV-A range, but the reduction is not in a highly desirable manner. As shown in Figure 5.5B, the transmittance decreased with increasing concentration, and when comparing the distinct methods (Figure 5.5A), the difference in transmittance is not marked. Furthermore, it is relevant to detach that thickness may also influence, since the thicker the film, the less transmittance observed.

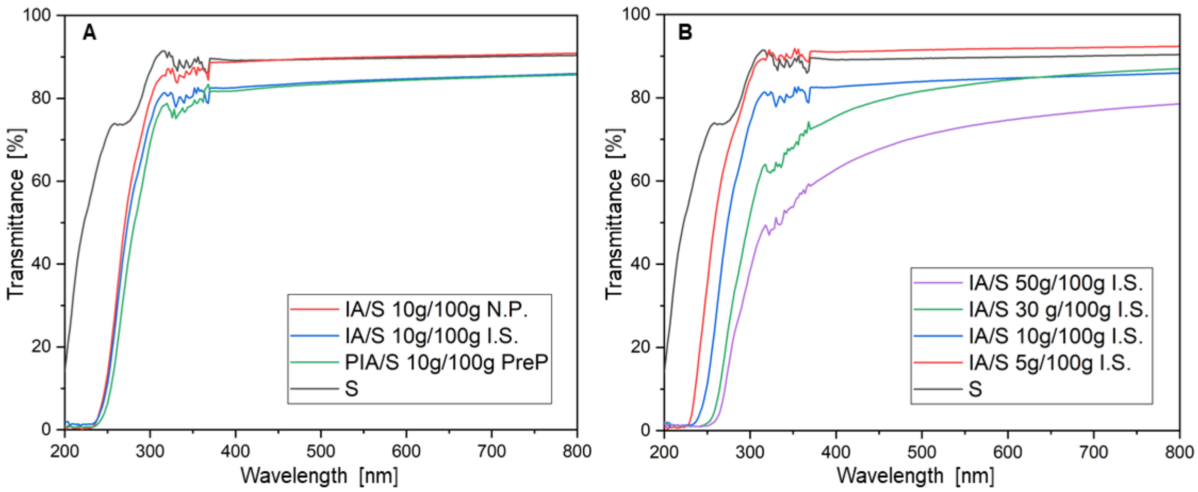

Figure 5.5. A: UV–vis transmittance spectra of control film (S) and of the films produced by different methods, with the concentration of itaconic acid or its polymer fixed at 10 g/100 g. B: UV–vis transmittance spectra of the control film (S) and of the films produced by *in situ* polymerization at different concentrations. Legend: S: starch; IA: itaconic acid and PIA: poly (itaconic acid).

4. Conclusion

The variation of the IA concentration and methods influenced the barrier, mechanical, and physical properties of the films in different ways. The addition of higher concentrations of IA produced by the *in situ* polymerization method conferred greater crystallinity and decreased *WVTR* and *WVP*, beyond significantly reduced *TS* and *EB*. On the other hand, films with 5 and 10 g of IA/100 g of starch showed an increase in *EB* from 18.7 (control) to 43.8 and 42.9 %, respectively. Furthermore, in the proportion of 5 g/100 g S, there was no significant change in *TS*, which is an interesting result, since in general the plasticizer agents reduce the resistance of films.

The use of different methods did not statistically affect the mechanical properties of the films but greatly influenced the barrier properties, and for the pre-polymerization technique, the *WVTR* was reduced from 373.6 (control) to 247.9 g m^{-2} day^{-1} and the *WVP* from 2.5 (control) to 1.7×10^{-10} g m^{-1} s^{-1} Pa^{-1}. Comparing these results together, the film with 50 g of IA/100 g S (*in situ* polymerization) showed a similar performance to the film with 10 g of PIA/100 g S pre-polymerized, so that pre-polymerization can supply the addition of high concentrations of acid without compromising the mechanical properties.

Regarding the optimal formulation and method of S/IA blends, the best proportion of IA was shown to be 5 g/100 g of starch, and the best-rated method was pre-polymerization. The results of this paper establish that blends based on plasticized potato starch and IA can be considered an interesting alternative as biocomposites for use in food packaging.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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6. CAPÍTULO 6: INCORPORAÇÃO DE OLIGOMERO DE LIMONENO EM BLENDS DE AMIDO/POLI(ÁCIDO ITACÔNICO) PARA PRODUÇÃO DE FILMES ANTIMICROBIANOS E ANTIOXIDANTES

INCORPORATION OF LIMONENE OLIGOMER IN STARCH/POLY(ITACONIC ACID) BLENDS FOR ANTIMICROBIAL AND ANTIOXIDANT FILMS PRODUCTION

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ABSTRACT

Starch/poly(itaconic acid)-based films (PIA/S) incorporated with limonene (LIM) and with low molar mass poly(limonene) (PL) were prepared at different concentrations (3 - 9 g/100 g of starch), and their antimicrobial and antioxidant activities were evaluated. The films with the most promising bioactive properties were fully characterized. Films with LIM did not present active properties, while those with PL were effective against *Bacillus cereus*, *Clostridium perfringens*, *Pseudomonas Aeruginosa* and *Colletotrichum gloeosporioides*, and showed DPPH radical scavenging activity up to 13.4%, demonstrating for the first time the enhanced performance of PL in this regard. The PL incorporation promoted a general uniform film morphology, reduced the tensile strength and elongation at break, and increased the moisture content and water solubility. No significant difference was registered for the water vapor barrier properties. PL addition also provided an outstanding UV-light barrier effect, suggesting that S/PIA/PL films could be employed in the future as active packaging films.

Keywords: Sustainable film, Biopolymer, Biological activities, Poly(limonene), blend.

1. Introduction

In recent years, considerable attention has shifted from petroleum-based polymeric materials toward renewable and biodegradable natural polymers, such as starch, chitosan, and cellulose (ARFAT et al., 2017). Likewise, environmental concerns and consumer demands for safe and fresh food have stimulated research on new polymers and the exploration of safer alternatives that can replace the most problematic materials (SUN et al., 2020). In this fashion, there is significant interest in substituting synthetic food preservatives and synthetic antioxidants for natural substances (SINGH et al., 2014).

Starch (S) remains one of the more closely studied replacement options of petrochemical-based plastics due to its broad availability, low cost, and recent advances in improving its properties (VIANNA et al., 2021). However, some features, such as low shear stress resistance, thermal decomposition at relatively low temperatures, high retrogradation and the poor mechanical and barrier properties of starch-based films, limit their use in industrial applications (BELLO-PÉREZ et al., 2000; SIQUEIRA et al., 2021). To overcome this limitation, our previous work showed that poly (itaconic acid) (PIA) is a promising component for the formulation of S-based blend films, as its addition significantly improved the elongation at break and water vapor barrier of the films, as shown in Chapter 4. PIA derives from itaconic acid, which is a promising organic acid obtained on a large scale by fermentative processes and considered by the U.S. Department of Energy as one of the 12 biotechnological products with the greatest potential for industrial use (WERPY; PETERSEN, 2004; MAGALHÃES et al., 2017). Once our final objective is to develop a biodegradable material with suitable properties and antimicrobial and antioxidant activities, the incorporation of bioactive ingredients in the formulation of S/PIA blends is the focus of the present study.

Limonene (LIM), the main component of several citrus essential oils and the major one in orange peel, is a monocyclic terpene with excellent market potential in food, pharmaceuticals, and cosmetics (GÜLTEPE, 2020; REN *et al.*, 2020; SENIT *et al.*, 2019). Its addition to S-based films is justified by its intrinsic bioactive properties, which can protect consumers from the effects of oxidative and bacterial deterioration, increasing the shelf life of food (AKRAM *et al.*, 2019; JUGREET *et al.*, 2020; CHAWLA; SIVAKUMAR; KAUR, 2021). Some studies have demonstrated that orange essential oil has inhibitory capacity against *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa, Agrobacterium tumefaciens, Dickeya solani, Erwinia amylovora*, and *Shigella dysenteriae* bacteria (DO EVANGELHO et al., 2019; OKLA et al., 2019). Brazil is the world leader in producing oranges and orange juice, which leads to huge amounts of byproducts that can be used as sources of LIM (MARTINS *et al.*, 2013; OZTURK; WINTERBURN; GONZALEZ-MIQUEL, 2019).

Despite its potential, LIM is a volatile compound (GONZÁLEZ-MAS et al., 2019) that may evaporate under heating during film preparation or storage. An exciting alternative is the use of LIM oligomer derivatives, as they are nonvolatile and present higher thermal stability (DE OLIVEIRA; VIEIRA, 2020; DE OLIVEIRA; DA SILVA; VIEIRA, 2021). As a result, better antimicrobial and antioxidant capabilities could be observed for the oligomers compared to their precursors, as reported for poly(β-pinene) (RODRIGUES; GONÇALVES; VIEIRA, 2021). Recently, our group reported the synthesis of low molar mass poly(limonene) (PL) employing a light-induced controlled polymerization approach (DE OLIVEIRA; VIEIRA, 2020). This promising technique allows control of the polymer characteristics, such as dispersity and molar mass. Moreover, it can be conducted under mild reaction conditions (~40 °C) and without using toxic metal catalysts (DE ÁVILA GONÇALVES; R. RODRIGUES; PIOLI VIEIRA, 2021).

Although several studies have investigated the production of biodegradable films, to the best of our knowledge, this is the first research involving blends prepared from S and PIA incorporating LIM/PL. The aim of this study was to explore the potential of PL as a high-performance bioactive compound for the preparation of active films. First, films with added LIM or PL were produced at three different concentrations, and their antimicrobial and antioxidant activities were comparatively evaluated. The films with the most promising bioactive properties were then fully characterized regarding their morphological, structural, mechanical, thermal, solubility, water vapor and light barrier properties.

2. Material and Methods

2.1 Materials

Commercial potato starch (S) (CAS 9005-84-9) (product code 110, Dinâmica Química Contemporânea Ltda., Campinas/SP, Brazil), Itaconic Acid (IA) (purity ≥ 99%) (product code 102043629), D-limonene (LIM) (purity ≥ 93%), tert-butyl hydroperoxide solution (70% in H_2O), thioxanten-9-one, N,N,N′,N″,N″pentamethyldiethylenetriamine (PMDETA), 2,2,2-tribromoethanol (TBE) (Sigma Aldrich, São Paulo, Brazil), glycerol (Ecibra, São Paulo/SP, Brazil), dimethyl acetamide (DMA) (Vetec, São Paulo, Brazil), and ethanol (Product code 319076, Nuclear, Diadema, São Paulo, Brazil) were used as received

2.2 Development of films

2.2.1 Itaconic Acid Polymerization

Poly(itaconic acid) (PIA) was synthesized through free radical polymerization according to the procedure described by Cao (2008), with some modifications. In brief, the molar proportion of monomer (itaconic acid) and initiator (tert-butyl hydroperoxide) was 100:5.927 in a 5:4 (w/w) proportion of monomer and solvent (water). All reagents were added to a 20-mL glass vial. The solution was deoxygenated with N_2 for 7 min and then heated under stirring at 100 °C for 2.5 h. Then, the polymer was precipitated with acetone, vacuum filtered, and dried for 24 h at 50 °C in an oven.

2.2.2 Limonene Polymerization

The poly(limonene) (PL) synthesis was carried out by controlled photopolymerization as described by Rogério et al.(2021). In brief, the molar ratio of monomer (limonene), alkyl halide initiator (2,2,2-tribromoethanol), catalyst (thioxanten-9-one, and electron-donor amine (N,N,N′,N″,N″pentamethyldiethylenetriamine) employed was 100:1.7:1.7:8.5 in a 1:1 (v/v) ratio for monomer and solvent (dimethyl acetamide). After adding the reagents to the 20 mL glass vial, the solution was deoxygenated with N_2 for 10 min under vigorous stirring. Then, the vials were placed in the photoreactor equipped with 4 UV lamps (250 nm, 3800 lx), and the polymerization occurred under isothermal stirring at 40 °C for 6 h. Finally, the solution was transferred to Petri dishes and dried in an oven at 45 °C for 48 h for evaporation of residual monomer and other volatile reagents.

2.2.3 Film preparation

Each film-forming solution was prepared in Milli-Q water with 5 g of S per 100 mL of solution, 1.5 g of glycerol (30 g of glycerol/100 g of S), and 0.25g of PIA (5 g of PIA/100 g of S). PL and LIM were added in the proportions of 3, 6, and 9 g/100 g of S. PL was previously solubilized in ethanol (0.102 g of poly(limonene)/mL of ethanol) and added to gelatinize together with starch solution while LIM was added after cooling. The film-forming solutions were heated at 70 °C for 20 min. After cooling, the solution was placed under ultrasound (Dekel/TS-218) for 30 min. Then, 50 mL of each solution was spread on polystyrene dishes (14 cm in diameter) and dried in an oven (MMM Group/Venticell) at 45 °C for 48 h.

2.3 Active properties of the films

2.3.1 Antioxidant activity

The antioxidant activities of the films were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The analysis was performed in triplicate. First, 30 mg of the film was mixed with 2 mL of a 0.1 mmol L^{-1} ethanolic DPPH solution. The mixture was incubated for 30 minutes in the dark, and then the absorbance was measured in a spectrophotometer (Ionlab, IL-592-LC-BI, Brazil) at 517 nm (WANG; CHEN; HOU, 2019). The percentage of DPPH free radical scavenging activity was determined using Equation (1):

$$DPPH\ scavenging\ effect\ (\%) = \frac{Abs_{DPPH} - Abs_{extract}}{Abs_{DPPH}} * 100 \quad (1)$$

in which Abs_{DPPH} is the absorbance value at 517 nm of the ethanolic solution of DPPH, and $Abs_{extract}$ is the absorbance value at 517 nm for the sample extracts.

2.3.2 Antimicrobial activity

Four bacterial strains, *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 35281, *Clostridium perfringens* ATCC 13124, and the filamentous fungus *Colletotrichum gloeosporioides* ISO024, were obtained from the culture collection of the Microbiology Laboratory of the Human at the Federal University of Paraíba (Bananeiras – Paraíba, Brazil). All bacteria were cultured on Mueller-Hinton agar (MH) at 37 °C, and the filamentous fungi were cultured on potato dextrose agar (PDA) at 25 °C.

The antimicrobial activities of the films were evaluated by the disc diffusion method on agar (ROSS et al., 2014). Aliquots of 100 μL of microbial suspensions at 10^8 CFU mL^{-1} were inoculated onto the surface of the plates containing MH agar for the bacteria and PDA for the fungal strains. For the antimicrobial activity determination of the composite films, 1 × 1 cm of each film (previously disinfected under UV light for 30 min) was applied to the agar plates and allowed to stand on them for 30 min at 25 °C to favor the diffusion of the bioactive compounds from the films to the media. The plates were incubated at 37 °C for 24 h for the bacteria and at 25 °C for 120 h for the fungi. After the incubation period, the diameter of the growth-inhibition zones was measured using a digital caliper.

2.4 Characterization of the films

The films with the most promising bioactive properties were characterized for morphology, molecular structure, crystallinity, thermal stability, thickness, water content, solubility, mechanical properties, water vapor barrier, and light barrier, according to the methods described below.

2.4.1 Film morphology

The morphology of the surface and cross-sections of the films was evaluated using scanning electron microscopy (SEM) (Leo 440i, LEO Electron Microscopy/Oxford, Cambridge, England) at an acceleration voltage of 15 kV and 50 pA current. Samples of the films were fixed in metallic support with the aid of double-sided carbon tape and covered with a thin layer of gold in a sputter coater (SC7620, VG Microtech, Kent, United Kingdom). For the cross-section analysis, the films were fractured with liquid nitrogen. The surfaces and cross-sections of the films were analyzed at 1,000× and 2,000× magnifications, respectively.

2.4.2 Fourier Transform Infrared spectroscopy

The molecular structure of the films was investigated using a Fourier transform infrared (FT-IR) spectrometer (Thermo Scientific, Nicolet Continuum, Madison, USA). The spectra were recorded in the spectral range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}, 128 scans per point, and the attenuated total reflection (ATR) module.

2.4.3 Crystallinity by X-ray diffraction

The crystallinity of the film was obtained by X-ray diffraction (XRD) pattern measurements ($2\theta = 5 - 15°$), using an X-ray analyzer (X'Pert-MPD, Philips, Almelo, Netherlands) with Cu Kα radiation (λ = 1,54056 Å) at a scan rate of 0.033333 °/s (step = 0.04 ° and time per step = 1.2 s) operated at a generator voltage of 40 kV and current of 40 mA.

2.4.4 Thermal stability

Thermogravimetric analysis (TGA) was carried out using a TGA device (Mettler Toledo, TGA/DSC1, Schwerzenbach, Switzerland) under N_2 flow (50 mL min^{-1}) to analyze the thermal stability of the films. Samples of approximately 10 mg were heated from 20 to 400 °C at a rate of 20 °C min^{-1}. The TG/DTG curves were recorded.

2.4.5 Physical Properties

Thickness

The average thickness (T) of the films was determined using a system composed of a digital micrometer (Mitutoyo Co., Kawasaki-Shi, Japan) and a flat granite base with a resolution of 0.1 µm. Measurements were performed using five random points from five samples of each formulation (ISO-4593, 1993).

Moisture content and water solubility

The moisture content (MC) of the films was determined in triplicate, according to ASTM D644-04 (2004). The films were cut into squares (2 × 2 cm), weighed, and dried for 24 h at 105 °C. The moisture content at equilibrium was calculated by Equation (2):

$$MC\ (\%) = \frac{W_0 - W_1}{W_0} \times 100 \quad (2)$$

where W_0 and W_1 are the initial and final masses of the samples, respectively.

The water solubility (WS) was also determined gravimetrically. The dried films were cut into squares (2 × 2 cm), weighed, and put into Milli-Q water at 25 °C for

24 h. Afterward, the films were dried overnight at 105 °C to reach a constant mass. The WS of the samples was calculated using Equation (3):

$$WS\ (\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (3)$$

in which W_1 and W_2 are the initial and final mass of the film, respectively. Each sample was measured three times.

2.4.6 Mechanical properties

The samples of the films were cut with a width of 15 mm in high-precision equipment (RDS-100-C, ChemInstruments, OH, USA). Then, they were stored for 48 hours at 25 ± 2 °C and 75 ± 5% RH. Tensile strength (MPa) and elongation at break (%) were measured using a universal testing machine (Instron, 5966-E2, Norwood, USA). The tests were performed with a 100 N load cell with a speed of 12 mm min^{-1} and an initial distance between the grips of 50 mm (ASTM-D882, 2018). The mechanical properties of each film were evaluated in 5 replicates.

2.4.7 Water vapor permeability

The water vapor transmission rate (WVTR) was determined by a gravimetric method based on ASTM-E96/E96M (2016). Capsules with a permeation area of 50 cm^2 and an analytical balance (Mettler Toledo, Columbus, Ohio, USA) with a resolution of 10^{-4} g were used. The tests were carried out at 25 °C and 75% RH in an air-conditioned chamber (Weiss Technik, Reiskirchen, Germany) with anhydrous calcium chloride desiccant. The WVTR (g m^{-2} day^{-1}) was determined from the slope of the curve of mass change vs. time. The water vapor permeability coefficient (g m^{-1} s^{-1} Pa^{-1}), WVP, of the films was calculated in four replicates for each sample, according to Equation (4):

$$WVP = \frac{WVTR \times e}{p_s \times RH_1} \quad (4)$$

where *e* is the average thickness of the samples (µm), p_s is the water vapor saturation pressure (23.756 mmHg at 25 °C), and RH_1 is the relative humidity of the chamber (75% = factor 0.75) since the relative humidity inside the capsule is considered to be zero.

2.4.8 Light transmission

The light transmission (%) of each film was determined with a dual-beam UV–visible spectrophotometer (Analytik Jena - Specord 210) using a scanning speed of 120 nm min^{-1} and a scan range of 200 to 800 nm (ASTM-E-1348, 2015). The light transmission of the films was measured in triplicate.

2.5 Statistical analysis

The results were statistically evaluated employing analysis of variance (ANOVA) and the Tukey test, conducted in Minitab® Software, to compare the average results ($p < 0.05$).

3 Results and discussion

S/PIA films containing additives were prepared by adding 3, 6 and 9 g of LIM or PL/100g of S. The S/PIA films appeared transparent, with the color gradually turning yellow with increasing PL load, while the films with LIM appeared whiter with increasing concentration. Both films presented a characteristic smell. The films that demonstrated antimicrobial and antioxidant properties were submitted to thermal, chemical, and physical characterizations to evaluate their performances.

3.1 Antimicrobial and antioxidant properties

The obtained results of the antioxidant and antimicrobial activities of biodegradable S/PIA films impregnated with LIM or PL are presented in Table 6.1. The addition of LIM did not significantly increase the radical inhibition percentage or confer antimicrobial activity. Similarly, Antosik et al. (2017) studied the efficiency of incorporating LIM (40 g LIM/100 g S) in starch-based films against *E. coli*, *Candida albicans* and *Aspergillus niger*. They did not observe any activity, even though pure LIM has shown inhibitory power against the same fungus and bacteria. These results may be related to LIM evaporation in some preparation steps, such as film-forming solution heating, film drying or storage conditions. As an example, it has been reported that the LIM losses during chitosan-based film processing steps can be higher than 60% (SÁNCHEZ-GONZÁLEZ et al., 2011), drastically decreasing the process efficiency and target performance.

On the other hand, when PL was added at the same low concentrations, both antimicrobial and antioxidant properties were verified. In addition, a higher concentration of PL provided both better inhibition zones and antioxidant activity. Films produced from the S/PIA blend incorporated with PL revealed DPPH radical scavenging activity ranging from 1.3 (control) to 13.4% (PL/PIA/S 9 g/5 g/100 g) and antimicrobial activity against *B. cereus*, *C. perfringens, P. aeruginosa,* and *C. gloeosporioides*. The antimicrobial activity can be explained by the lipophilic nature of the low molar mass PL, which can permeate through the lipid layer, compromising the microbial cell membrane (AKRAM et al., 2019). The antioxidant capacity of terpenes is mainly due to their redox properties, which allow them to act as hydrogen donors, reducing agents, singlet oxygen quenchers, and metal chelators. Since the films incorporated with PL presented the best activity properties, they were chosen to be fully characterized.

Table 6.1: Antimicrobial and antioxidant activity of starch-poly(itaconic acid) with limonene (LIM) or poly(limonene) (PL), both at 3, 6 and 9 g of active compound/100 g of starch. Legend: S: starch and PIA: poly (itaconic acid), n.d.: not detected.

		Zone of inhibition (mm)				
Film	DPPH radical scavenging activity (%)	*B. cereus*	*C. perfringens*	*P. aeruginosa*	*E. coli*	*C. gloeosporioides*
PIA/S	1.3 ± 0.4^{c}	n.d.	n.d.	n.d.	n.d.	n.d.
LIM/PIA/S 3g/5g/100 g	0.7 ± 0.2^{c}	n.d.	n.d.	n.d.	n.d.	n.d.
LIM/PIA/S 6g/5g/100 g	1.6 ± 0.2^{c}	n.d.	n.d.	n.d.	n.d.	n.d.
LIM/PIA/S 9g/5g/100 g	1.1 ± 0.6^{c}	n.d.	n.d.	n.d.	n.d.	n.d.
PL/PIA/S 3g/5g/100 g	1.3 ± 1.9^{c}	4.2 ± 0.1^{b}	3.5 ± 0.6^{b}	3.6 ± 0.3^{c}	n.d.	n.d.
PL/PIA/S 6g/5g/100 g	7.5 ± 2.0^{b}	5.9 ± 1.0^{a}	4.8 ± 1.1^{ab}	4.4 ± 0.2^{a}	n.d.	2.1 ± 0.1^{b}
PL/PIA/S 9g/5g/100 g	13.4 ± 0.9^{a}	6.9 ± 0.4^{a}	6.6 ± 1.1^{a}	5.3 ± 0.2^{b}	n.d.	2.7 ± 0.3^{a}

The results are expressed as an average ± standard deviation.

$^{a,\, b,\, c...}$ *The values in a given line followed by the same letter do not differ at the 95% confidence level (p > 0.05).*

3.2 Film morphology

Figure 6.1 shows SEM micrographs of the surface and cross-section of the S/PIA blends with and without PL. The surface of the control film exhibited a smooth and uniform texture (Figure 5.1Aa), as also reported in our previous study, as shown in Chapter 4. Even though the PL addition modified the control film microstructure, the micrographs showed a uniform distribution of the oligomer, indicating good compatibility with these matrices, without oil droplet separation. Nevertheless, the homogeneity of the cross-sections was slightly reduced at higher concentrations of PL (PL/PIA/S 6 g/5 g/100 g and PL/PIA/S 9 g/5 g/100 g).

Similar to our results, the addition of poly(β-pinene), another terpene oligomer, to chitosan films, at the proportion of 10 g of polymer /100g of chitosan also affected the microstructure of the films, which was evidenced mainly through the cross-section micrographs (RODRIGUES et al., 2021). Higher concentrations of poly(β-pinene) (30 and 50 g of polymer /100 g of chitosan) severely disrupted the film matrix by forming droplets and pores (RODRIGUES et al., 2021).

Besides, the incorporation of orange essential oil (EO/S 0.0252 g - 0.0588 g/100 g) (DO EVANGELHO et al., 2019), oregano essential oil (EO/S 25 – 50 g/100 g) (DOS SANTOS CAETANO et al., 2018), and cinnamon essential oil (EO/S 10 – 10 g/100 g) (SUN et al., 2020) also caused loss of homogeneity in S-based films. These heterogeneities may contribute to the antibacterial property of the films, considering that they facilitate the diffusion process of the EO from the interior of the polymer matrix to the surface (DO EVANGELHO et al., 2019).

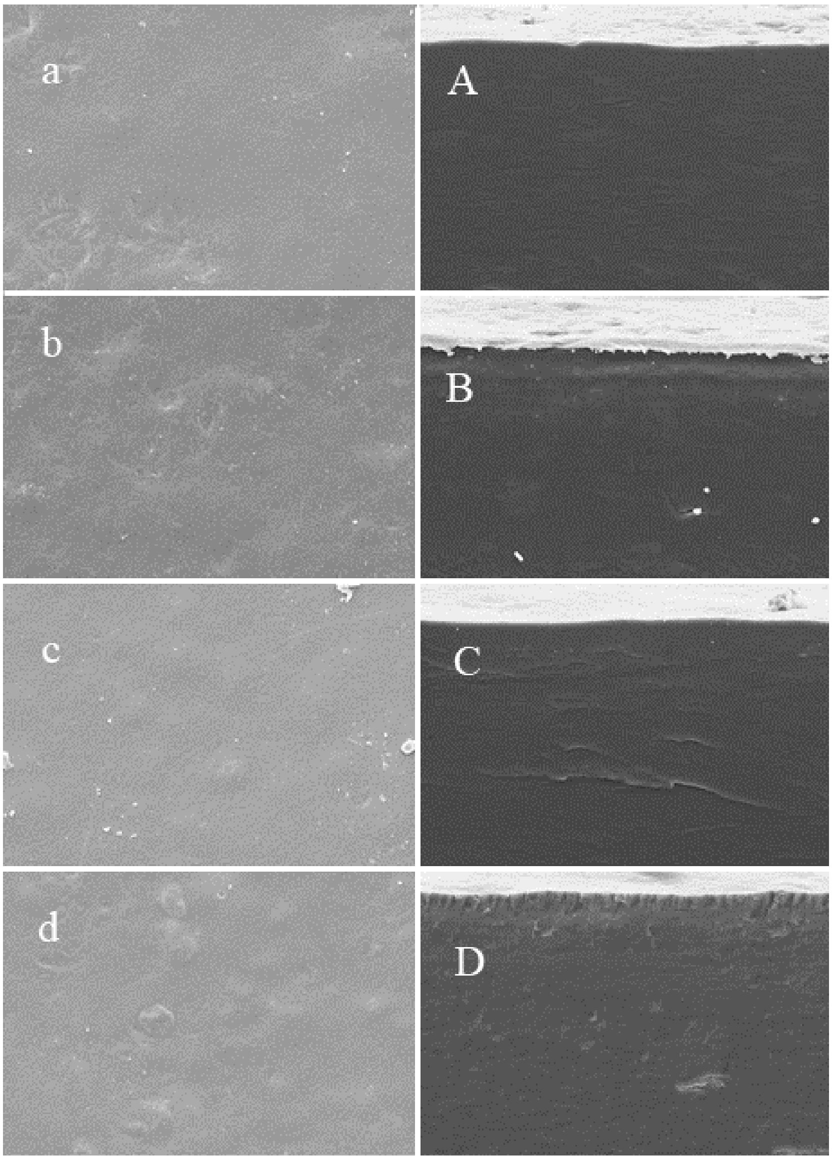

Figure 6.1: Surface micrographs (a, b, c, d) and cross sections (A, B, C, D) of the starch–poly (itaconic acid) films containing 0 (a and A), 3 (b and B), 6 (c and C), and 9 (d and D) g of poly(limonene)/100 g of starch, respectively.

3.3 Fourier Transform Infrared spectroscopy

FTIR spectroscopy was carried out to elucidate possible interactions between PL and S/PIA-based films. The amount of PL added did not generate new peaks (Figure 6.2) or show band intensity changes/dislocations, which may be related to the low concentrations of additive employed in this study.

The spectra of all the produced films displayed a broad absorption band at approximately 3339 cm^{-1} due to O-H bond stretching. This band is also related to the intermolecular hydrogen interactions between the biopolymer and film constituents, such as water, PIA and glycerol.

Films also presented characteristic bands at 2926 cm^{-1} (C–H), as well as at 1150, 1080, and 1010 cm^{-1} for C–O–C stretching (the triplet peak of starch) (LANTHONG; NUISIN; KIATKAMJORNWONG, 2006). The band at approximately 1671 cm^{-1} indicates an ester bond, which is attributed to the poly(itaconic acid) structure, as shown in Chapter 4. Bands at approximately 1408 cm^{-1} can be assigned to –C–O– angular deformations (GUTIÉRREZ; ALVAREZ, 2017). In addition, at low wavenumbers (below 800 cm^{-1}), starch-based films exhibited complex vibrational modes due to the skeletal mode vibrations of the glucose pyranose ring (SOTO et al., 2016).

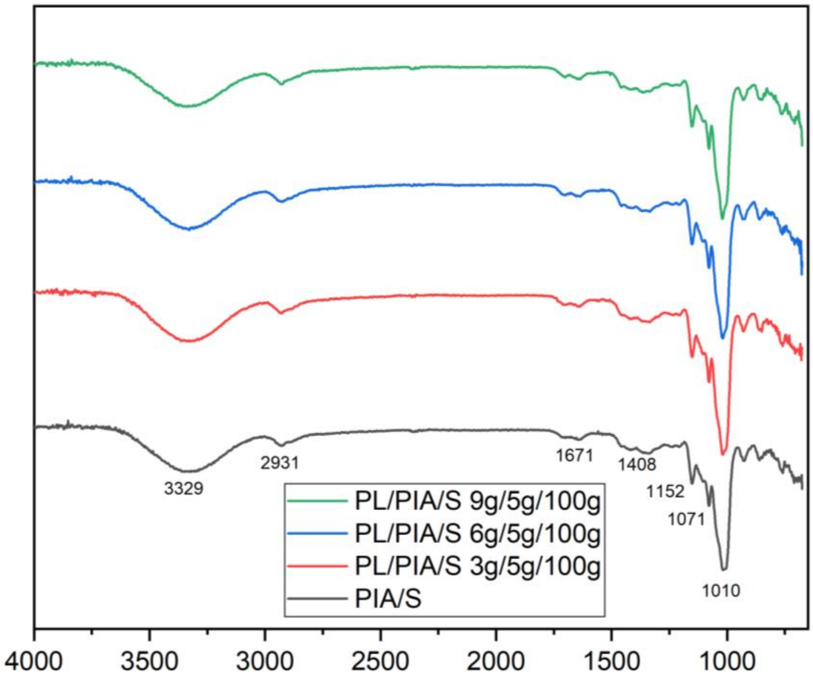

Figure 6.2: FT-IR spectra of the control film (S/PIA) and the films containing 3, 6, and 9 g of PL/100 g of starch.

3.4 X-ray diffraction

Figure 6.3 illustrates the X-ray diffraction patterns of the S/PIA control film and those incorporated with PL. The peaks at approximately 2θ = 17, 19.7 and 21.9° of the control film coincide with the B-type crystalline structure typical of potato starch (DOME et al., 2020). All the films presented peaks centered at the same 2θ values. However, with the addition of PL, these peaks became wider and less intense as a function of additive concentration. This result suggests a reduction in the films' crystallinity by the presence of PL molecules between starch polymer chains.

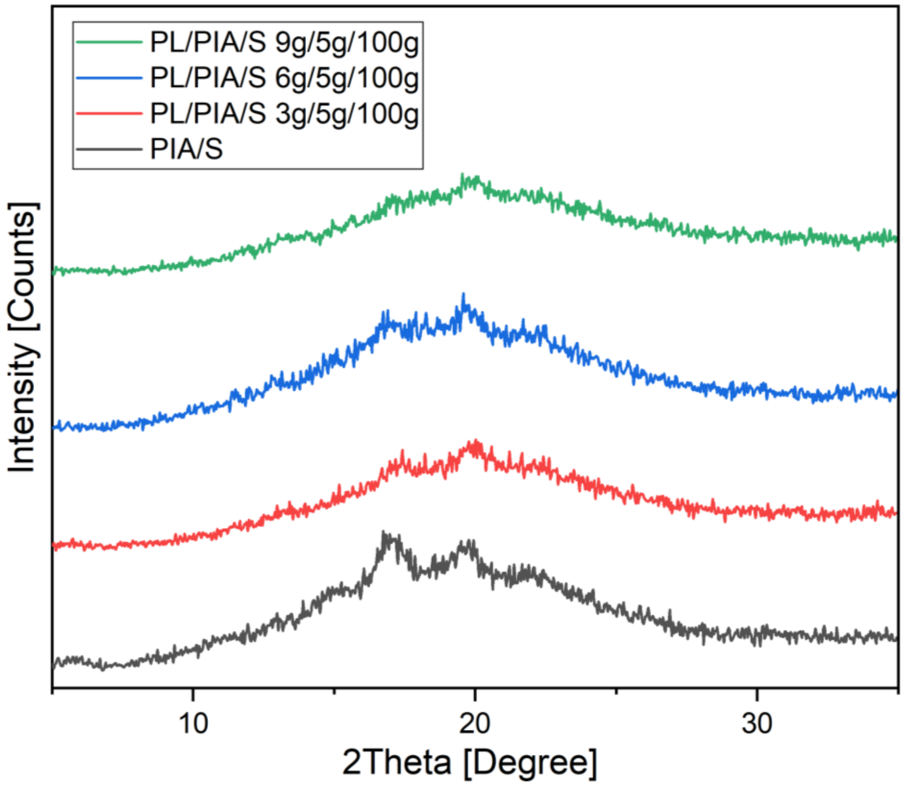

Figure 6.3*:* X-ray diffraction patterns of the control film (S/PIA) and the films containing 3, 6, and 9 g of PL/100 g of S.

3.5 Thermal stability

The thermogravimetric (TG) curves and their first derivatives (DTG) for S/PIA blend films incorporated with PL are shown in Figure 6.4. The temperatures corresponding to initial degradation (T_{onset}) and maximum decomposition (T_{max}) are presented in Table 6.2.

All the films showed two main thermal events. As shown in Table 6.2, the moisture content of the films ranged from 13.4 to 15.2%, and the first mass loss (38 – 112 °C) can be associated with its evaporation from the polymeric matrix (KAYA et al., 2018). In the following stage (205 – 309 °C), glycerol and PL were degraded, and the depolymerization of amylose might have occurred (BHAT et al., 2013; DE OLIVEIRA; VIEIRA, 2020; XU et al., 2021). As shown in Figure 6.4, the temperature of the maximum decomposition occurred between 287 °C and 309 °C, without remarkable differences between film samples, although films with PL showed a slight shift toward a lower degradation temperature.

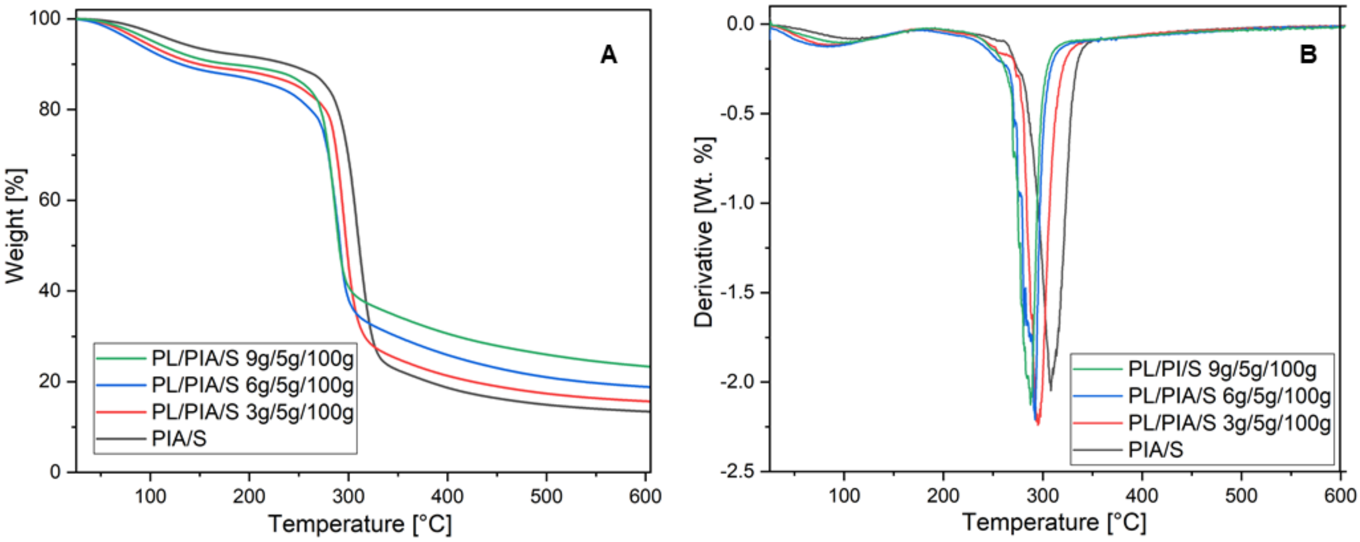

Figure 6.4: TGA (a) and DTGA (b) of the control film (S/PIA) and the films containing 3, 6, and 9 g of poly(limonene)/100 g of starch, respectively.

Table 6.2: Onset temperature (T_{onset}) and maximum degradation temperature (T_{max}) of the control film (S/PIA) and the films containing 3, 6, and 9 g of PL/100 g of S.

	First thermal event		Second thermal event	
Film	T_{onset} (°C)	T_{max} (°C)	T_{onset} (°C)	T_{max} (°C)
PIA/S	44.83	112.39	217.54	309.84
PL/PIA/S 3g/5g/100 g	38.22	86.22	215.16	296.62
PL/PIA/S 6g/5g/100 g	36.24	85.43	209.73	291.06
PL/PIA/S 9g/5g/100 g	38.09	94.02	205.90	287.09

3.6 Thickness and mechanical properties

Thickness (*T*), tensile strength (*TS*), and elongation at break (*EB*) values are presented in Table 6.3. The *T* of the films increased with PL addition at higher concentrations (PL/PIA/S 6 g/5 g/100 g and PL/PIA/S 9 g/5 g/100 g). This result can be attributed to the increase in the amount of polymer in the sample. In parallel, the reduction in crystallinity and consequent increase in free volume between starch macromolecules may also have contributed to this more pronounced increase in thickness at higher concentrations of PL.

The *TS* and *EB* of the films ranged from 6.5 MPa to 3.4 MPa, and from 41.8% to 28.4%, respectively. Studies in the literature have demonstrated a decrease in TS upon essential oil addition (SOUZA et al., 2013; DOS SANTOS CAETANO et al., 2018; DO EVANGELHO et al., 2019; AL-HASHIMI et al., 2020; CRUZ-TIRADO et al., 2020). Analogously, the incorporation of PL in the present study, even at low concentrations, may have contributed to partially replacing the stronger starch-starch interactions with the weaker polysaccharide-PL interactions (LI et al., 2018), resulting in low *TS*. This behavior can also be correlated to the reduction in film crystallinity observed in the XRD patterns (Figure 6.3).

The *EB* of the films incorporated with the highest concentration of PL (PL/PIA/S 9g/5g/100 g) was significantly lower than that of the control, with the same pattern being observed in corn S-based films supplemented with orange essential oil (DO EVANGELHO et al., 2019). Although the incorporation of PL reduced the crystallinity of the films, this reduction in *EB* may be attributed to the slight increase in microstructural defects (Figure 6.1d), which may have acted as stress concentrators, contributing to the premature rupture of the films.

Table 6.3: Thickness (*T*), tensile strength (*TS*) and elongation at break (*EB*) of the control film (S/PIA) and those with PL incorporated.

Film	*T* (µm)	*TS* (MPa)	*EB* (%)
PIA/S	140.5 ± 16.9^{b}	6.5 ± 0.4^{a}	41.8 ± 4.6^{a}
PL/PIA/S 3 g/5g/100 g	140.8 ± 11.6^{b}	3.4 ± 0.2^{c}	43. 9± 6.2^{a}
PL/PIA/S 6 g/5g/100 g	160.1 ± 9.1^{a}	3.4 ± 0.4^{c}	40.1 ± 3.8^{ab}
PL/PIA/S 9 g/5g/100 g	156.7 ± 7.7^{a}	4.6 ± 0.9^{b}	28.4 ± 11.1^{b}

The results are expressed as an average ± standard deviation.
$^{a,\ b,\ c\ldots}$ The values in a given line followed by the same letter do not differ at the 95% confidence level (p > 0.05).

3.7 Moisture content, water solubility, and barrier properties

PL incorporation into the S/PIA-based film significantly increased *MC* from 13.4 to 15.2% (Table 6.4). This result is consistent with the findings of Do Evangelho (2019), who observed that the addition of 0.0588 g of orange EO/100 g of S changed the *MC* of starch films from 18.81 to 21.9%. Additionally, Table 6.4 also shows that *WS* ranged from 19.8 (control) to 28.6% (PL/PIA/S 9 g/5 g/100 g). Similarly, the addition of orange EO (DO EVANGELHO et al., 2019) and thyme EO microcapsules (CAI et al., 2020) increased the *WS* of starch films. However, based on general literature reports, it is expected that the incorporation of a hydrophobic additive would reduce the *MC* and *WS* (VIANNA et al., 2021). The opposite trend identified in the present study can also be correlated with what was observed in the XRD patterns (Figure 6.4). The lower the crystallinity of the films, the greater the free volume. Despite the inclusion of a hydrophobic additive such as PL, the slight increase in free volume could have contributed to the increase in *WS* and *MC*.

Moreover, despite the mean values showing an apparent tendency to increase, the PL addition did not significantly change the *WVTR* and *WVP* of the films. S-based film studies have reported either an increase (DOS SANTOS CAETANO et al., 2018; DO EVANGELHO et al., 2019; AL-HASHIMI et al., 2020) or a decrease (GHASEMLOU et al., 2013; CAO; YANG; SONG, 2017; IAMAREERAT et al., 2018; CAI et al., 2020) in the moisture barrier properties of films containing EOs. Although there was a reduction in the crystallinity of the films from the incorporation of PL, this reduction did not occur so drastically as to impair the water vapor barrier properties, which is a beneficial result. Furthermore, as seen in Figure 6.1, the addition of PL did not lead to the formation of morphological defects that could contribute to the increase of the-permeability.

Table 6.4: Moisture Content (*MC*), Water Solubility (*WS*), Water Vapor Transmission Rate (*WVTR*), and Water Vapor Permeability (*WVP*) of the control film (S/PIA) and those incorporated with PL.

Film	*MC* (g/100g)	*WS* (%)	*WVTR* (g m^{-2} day^{-1})	*WVP* (×10^{-10} g m^{-1} s^{-1} Pa^{-1})
PIA/S	13.4 ± 0.5^{b}	19.8 ± 0.5^{c}	282.8 ± 11.9^{a}	1.9 ± 0.1^{a}
PL/PIA/S 3g/5g/100 g	13.8 ± 0.4^{ab}	24.6 ± 1.9^{b}	298.8 ± 9.3^{a}	2.3 ± 0.3^{a}
PL/PIA/S 6g/5g/100 g	14.5 ± 0.7^{ab}	24.0 ± 0.3^{b}	296.5 ± 51.1^{a}	2.3 ± 0.1^{a}
PL/PIA/S 9g/5g/100 g	15.2 ± 0.2^{a}	28.6 ± 0.5^{a}	299.0 ± 21.4^{a}	2.3 ± 0.1^{a}

The results are expressed as an average ± standard deviation.
$^{a, b, c...}$ *The values in a given line followed by the same letter do not differ at the 95% confidence level (p > 0.05).*

3.8 Light transmission

Figure 6.5 shows the light transmittance as a function of wavelength for PIA/S blends incorporated with PL. The addition of PL conferred some light barrier, especially for ultraviolet (UV) radiation. In the full range of UV light (from 200 to 400 nm), the samples PL/PIA/S 6g/5g/100g and PL/PIA/S 9g/5g/100g presented transmittance close to 0. This interesting UV-blocking behavior can probably be explained by the presence of endocyclic double bonds in the PL structure. This molecular configuration is well known to be highly susceptible to light absorption at low wavelengths (BARBOSA et al., 2022). Similar results were observed with the addition of LIM to chitosan-based films (BARBOSA et al., 2022).

At wavelenghts between 400 and 500 nm, the films with PL have also delivered some light protection, especially the PL/PIA/S 9g/5g/100g film, which presented transmittance of 5.2 and 28.2%, respectivetely. This particular light range has been identified as the main catalyst of oxidative reactions in many foods products like as lipid oxidation reactions, the oxidation of vitamins and the photodegradation of pigments that alter the nutritional value and color of the product, respectively. Another example arising from photodegradation is the development of off-taste and odor in dairy products (ANDERSEN; SKIBSTED, 2010)

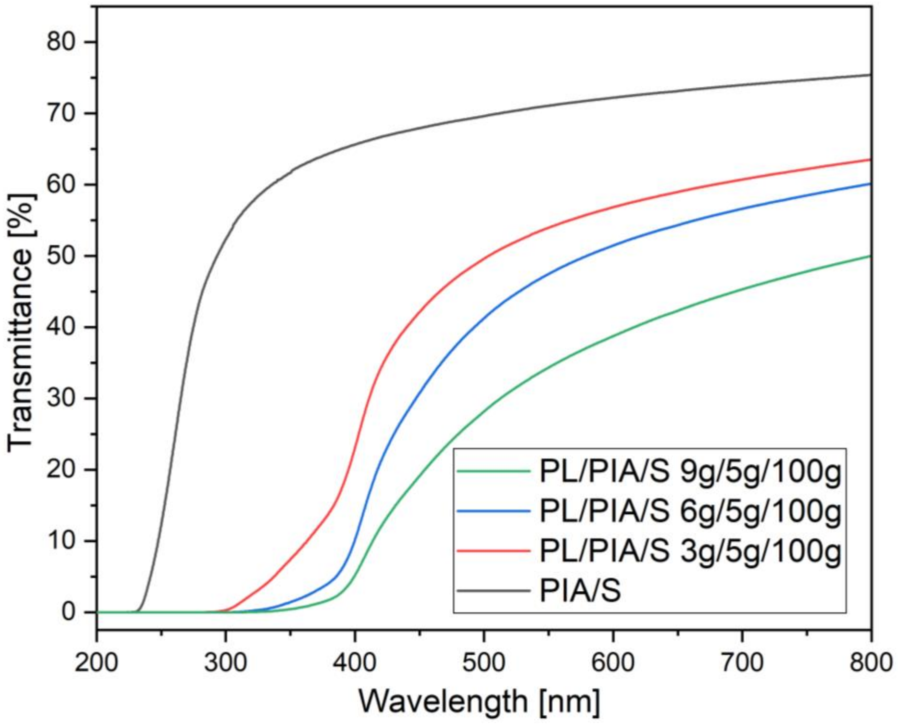

Figure 6.5: UV–vis transmittance spectra of the control film (S/PIA) and the films containing 3, 6 and 9 g of PL/100 g of starch.

4 Conclusion

Considering the additive concentration range employed in this study, it was demonstrated that films with limonene (LIM) did not present antimicrobial or antioxidant properties, while those with poly limonene (PL) were effective against *B. cereus*, *C. perfringens*, *P. aeruginosa* and *C. gloeosporioides* and showed DPPH radical scavenging activity (%) of up to 13.4%. In addition, PL incorporation in S/PIA films significantly affected their properties. TS and EB were reduced as long as the moisture content and water solubility increased. No significant difference was registered in the WVTR and WVP of the films. Furthermore, an impressive UV-blocking effect was observed as a function of PL concentration. These results suggest that S/PIA films incorporated with PL have the potential for use as bioactive films. Further investigation may include the application of these films in the conservation of food.

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7. CAPÍTULO 7 – RESULTADOS PRINCIPAIS E DISCUSSÃO

Em suma, os principais resultados do presente trabalho foram:

- Filmes com 5 e 10 g de IA/100 g de amido apresentaram aumento de EB de 18,7 (controle) para 43,8 e 42,9 %, respectivamente, sugerindo um efeito plastificante. Além disso, na proporção de 5 g/100 g de amido, não houve alteração significativa em TS, o que é um resultado interessante, pois, em geral, os agentes plastificantes reduzem a resistência dos filmes.
- A adição de altas concentrações de IA conferiu maior cristalinidade e diminuição de WVTR e WVP, além de reduzir significativamente TS e EB.
- O uso de diferentes métodos não afetou estatisticamente as propriedades mecânicas dos filmes, mas influenciou consideravelmente nas propriedades de barreira, sendo que para a técnica de pré-polimerização, WVTR foi reduzida de 373,6 (controle) para 247,9 g m^{-2} dia^{-1} e WVP de 2,5 (controle) a 1,7 x 10^{-10} g m^{-1} s $^{-1}$ Pa^{-1}. Além disso, o filme com 50 g de IA/100 g de amido (polimerização *in situ*) apresentou desempenho semelhante ao filme com 10 g/100 g com PIA pré-polimerizado, de modo que a pré-polimerização pode substituir a adição de altas concentrações de ácido sem comprometer as propriedades mecânicas.
- Em relação à concentração e método avaliados das blendas S/IA, a proporção 5 g de IA/100 g de amido gerou filme com a performance mais promissora, e o método mais bem avaliado foi a pré-polimerização.
- Os filmes com LIM não apresentaram propriedades antimicrobianas nem antioxidantes, provavelmente devido à perdas significativas do aditivo nas etapas que evolvem aquecimento (*casting* e secagem);
- Filmes com PL foram eficazes contra *B. cereus*, *C. perfringens*, *P. aeruginosa* e *C. gloeosporioides* e apresentaram atividade antioxidante de até 13,4%.
- A adição de PL em filmes S/PIA afetou significativamente suas propriedades. TS e EB foram reduzidas à medida que o teor de umidade e a solubilidade em água aumentaram. Não foi registrada diferença estatística em WVTR e WVP.

- Foi observado efeito de bloqueio à luz UV-Vis em função da concentração de PL., o que é explicado pela presença de duplas ligações endocíclicas na estrutura PL. Esta configuração molecular é bem conhecida por ser altamente suscetível à absorção de luz.

8. CAPÍTULO 8: CONCLUSÕES GERAIS E SUGESTÕES PARA TRABALHOS FUTUROS

No presente trabalho foram desenvolvidos filmes à base de amido, incorporados com ácido itacônico (IA), poli(ácido itacônico) (PIA), limoneno (LIM) e poli(limoneno) (PL) de baixa massa molar.

A hipótese de que a adição de ácido itacônico ou poli(ácido itacônico) melhoraria as propriedades mecânicas e de barreira dos filmes de amido foi confirmada. Em baixas concentrações (5 e 10g de IA/100g de amido), IA atuou como agente plastificante aumentando consideravelmente o alongamento na ruptura dos filmes de amido produzidos. Esse comportamento foi explicado pela substituição parcial das ligações fortes amido-amido por ligações mais fracas amido-ácido itacônico, sendo também observado um perfil semi-cristalino nos padrões DRX. Além disso, o filme com 5g IA/100g de amido apresentou resistência à tração estatisticamente igual ao filme controle, indicando que nesta concentração o efeito plastificante do IA não comprometeu a resistência à tração. Dessa forma, 5g/100g foi a concentração mais promissora dentre as analisadas.

A adição de poli(ácido itacônico) na concentração de 10g/100g de amido reduziu significativamente a taxa de transmissão de vapor d'água e a permeabilidade ao vapor d'água dos filmes de amido. Tal comportamento pode ser explicado pela incorporação de partículas relativamente grandes na matriz polimérica de amido, sem causar defeitos graves. Além disso, a adição de IA em altas concentrações (30 e 50g IA/100 amido) também reduziu os valores de permeabilidade, o que pode estar relacionado ao aumento de cristalinidade, observando-se intensos picos de cristalinidade nos padrões DRX nestas concentrações. É necessário ressaltar que ao comparar os resultados de WVTR e WVP em conjunto, o filme com 50 g de IA/100 amido (polimerização in situ) apresentou desempenho semelhante ao filme com 10 g PIA/100 g S pré-polimerizado, indicando que a pré-polimerização supriu a necessidade de adição de altas concentrações de IA, que comprometem as propriedades mecânicas.

Na etapa de adição do composto ativo, limoneno não apresentou atividade antioxidante e nem antimicrobiana contra nenhum dos microrganismos testados, o que foi atribuído à sua evaporação durante as etapas de secagem e armazenamento. Já o poli(limoneno), que possui maior resistência térmica que seu monômero, apresentou atividade antioxidante de até 13.4% e atividade contra *B. cereus*, *C. perfringens, P. aeruginosa e C. gloeosporioides*. A adição de poli(limoneno) também reduziu a resistência a tração e o alongamento na ruptura dos filmes e não impactou nas propriedades de barreira.

Adicionalmente, tanto a adição de IA quanto PL conferiram considerável barreira à luz, o que é um resultado promissor em termos de conservação de alimentos. No geral, os resultados sugerem que os filmes S/PIA incorporados ao PL têm grande potencial para uso como filmes ativos

Sugestões para trabalhos futuros:

- Investigar novas técnicas de separação de IA/PIA, uma vez que análises de FT-IR mostraram que houve monômero residual junto ao polímero: sugere-se a realização de uma filtração adicional ou testar novos solventes;
- Estudar o efeito da adição de PIA nas propriedades de barreira ao O_2 e ao CO_2 ;
- Produzir blendas S/PIA por meio de extrusão e avaliar as propriedades dos filmes;
- Investigar a eficácia dos filmes contendo PL na preservação de alimentos.

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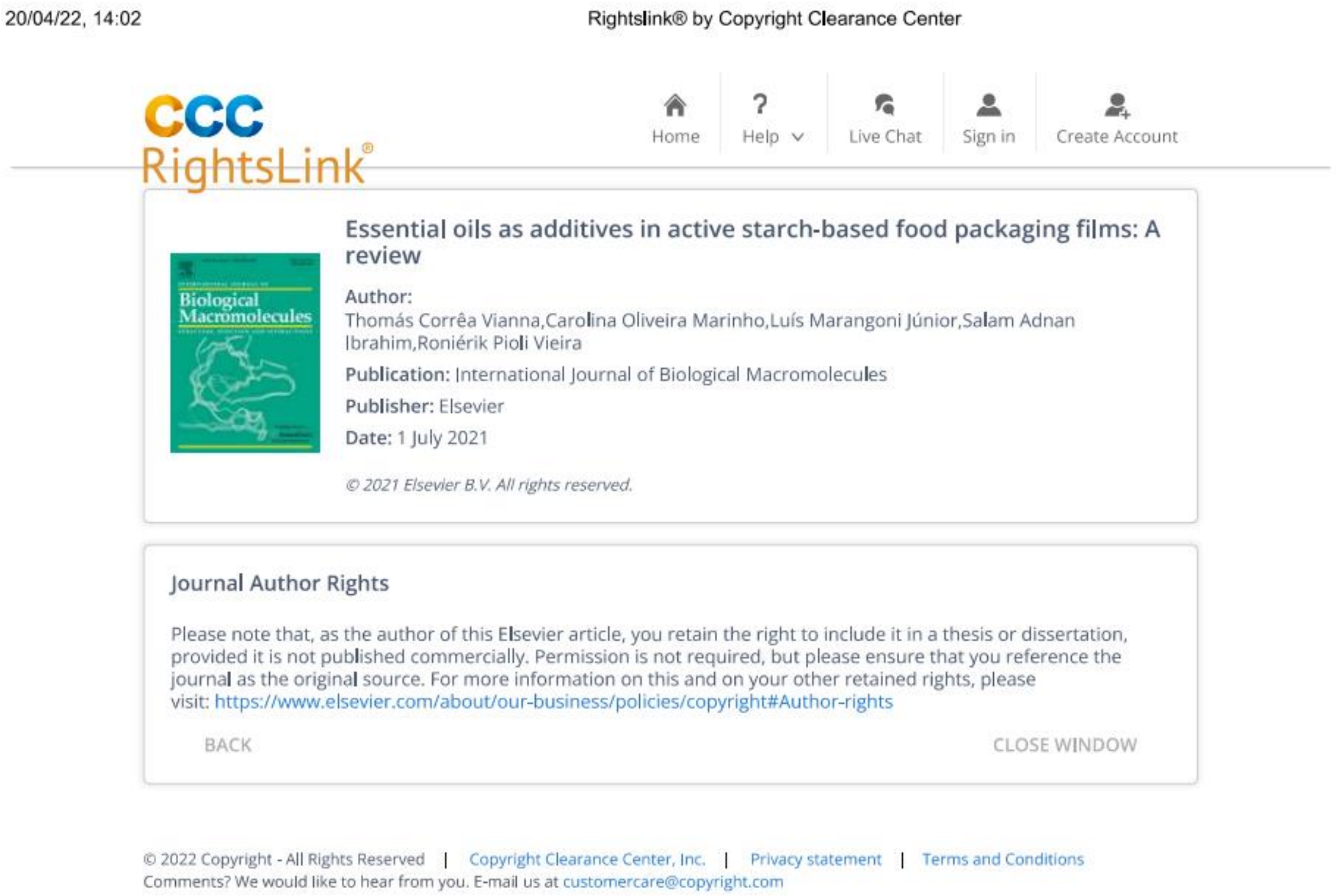

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Essential oils as additives in active starch-based food packaging films: A review

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