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JULIANA FARINASSI MENDES

DESENVOLVIMENTO, OTIMIZAÇÃO E AVALIAÇÃO SENSORIAL DE BIONANOCOMPÓSITOS POLIMÉRICOS COMESTÍVEIS

LAVRAS - MG
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DEVELOPMENT, OPTIMIZATION AND SENSORY ANALYSIS OF EDIBLE POLYMERIC BIONANOCOMPOSITS

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A minha família, meus maiores exemplos para a vida

DEDICO

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RESUMO

A crescente preocupação ambiental desencadeada pela exploração exacerbada de matérias-primas fósseis para a produção de grandes volumes de materiais não biodegradáveis, impulsionada pelo crescimento acelerado da população mundial e da demanda por alimentos, motiva a pesquisa em materiais alternativos oriundos de fontes renováveis. Neste trabalho, biocompósitos ativos baseados em óleo essencial de capim-limão e manteiga de cacau foram produzidos. Para tal, amido (TPS) e fibras de bagaço de malte foram adicionados como agente ligante e de reforço, respectivamente. Antes, porém, os componentes filmogênicos foram extensivamente estudados de forma isolada. Investigaram-se os efeitos da adição de manteiga de cacau, sob a forma de emulsão, do ponto de vista de desempenho hidrofilicidade. As fibras de bagaço de malte foram utilizadas como fonte de preenchimento e reforço mecânico da matriz. A produção das nanoemulsões de óleo essencial de capim-limão deu-se em função da correlação da estrutura do surfactante e da concentração do óleo. Após compreender os efeitos nas propriedades mecânicas, barreira e térmicas dos filmes de manteiga de cacau, compósitos de fibras de bagaço de malte, das nanoemulsões de capim-limão na matriz de TPS, experimentos de misturas quartenários foram conduzidos para estabelecer correlações entre as formulações de biocompósitos e suas propriedades físico-químicas. Esses filmes foram produzidos com a finalidade de se obter (i) biocompósitos biodegradáveis com desempenhos físico-mecânicos adequados à aplicações de embalagens, utilizando emulsão de manteiga de cacau, resíduo de bagaço de malte e nanoemulsão de capim-limão, e (ii) biocompósitos ativos com propriedades nutricionais e sensoriais únicas através da combinação de óleo essencial de capim-limão e TPS. Por fim, a produção destes materiais foi escalonada com sucesso para a escala piloto por meio de uma abordagem contínua de casting. Esses filmes foram avaliados em relação a sua aceitação sensorial aplicados em chocolates e quanto estudos do comportamento do consumidor, utilizando a técnica de hard laddering. Os resultados obtidos nesses estudos demonstraram que os consumidores apresentam percepções distintas em relação aos diferentes tipos de embalagens e que a escolha pelas embalagens bionanocompósitas gerou associações predominantemente positivas, associadas a melhor qualidade para o meio ambiente, saúde, qualidade de vida e bem-estar.

Palavras-chaves: Filmes ativos. Polímeros biodegradáveis. Compósito polimérico. Resíduos agroindustriais. Nanoemulsões.

ABSTRACT

The growing environmental concern triggered by the exacerbated exploitation of fossil raw materials to produce large volumes of non-biodegradable materials, driven by the accelerated growth of the world population and the demand for food, motivates the search for alternative materials from renewable sources. In this work, active biocomposites based on lemon grass essential oil and cocoa butter were produced. For this, starch (TPS) and malt bagasse fibers were added as a binding and reinforcing agent, respectively. Before, however, the filmogenic components were extensively studied in isolation. The effects of adding cocoa butter in the form of an emulsion were investigated from the point of view of hydrophilicity performance. Malt bagasse fibers were used as a source of filling and mechanical reinforcement of the matrix. The production of lemongrass essential oil nanoemulsions was due to the correlation of the surfactant structure and oil concentration. After understanding the effects on the mechanical, barrier and thermal properties of cocoa butter films, composites of malt bagasse fibers, of lemon grass nanoemulsions in the TPS matrix, experiments of four-year mixtures were conducted to establish correlations between the formulations of biocomposites and their physicochemical properties. These films were produced with the purpose of obtaining (i) biodegradable biocomposites with physical-mechanical performances suitable for packaging applications, using cocoa butter emulsion, malt bagasse residue and lemongrass nanoemulsion, and (ii) biocomposites assets with unique nutritional and sensory properties through the combination of lemongrass essential oil and TPS. Finally, the production of these materials has been successfully scaled to the pilot scale through a continuous casting approach. These films were evaluated for their sensory acceptance applied to chocolates and studies of consumer behavior. These films were evaluated in terms of their sensory acceptance applied to chocolates and regarding consumer behavior studies, using the hard laddering technique. The results obtained in these studies demonstrated that consumers have different perceptions in relation to the different types of packaging and that the choice for bio-composite packaging generated predominantly positive associations, associated with better quality for the environment, health, quality of life and well-being.

Keywords: active films. Biodegradable polymers. Polymer composites. Agroindustrial waste. Nanoemulsion.

SUMÁRIO

PRIMEIRA PARTE

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1. Introdução

A globalização e a industrialização exigem cada vez mais sistemas complexos de distribuição de alimentos. Esse cenário leva a períodos mais longos de transporte e armazenamento, durante os quais os produtos alimentícios são suscetíveis à deterioração microbiana, bem como às perdas sensoriais e nutricionais, além do aumento das exigências de embalagem em termos de produção e propriedades. Entre as propriedades, a mecânica e vapor-de-água denotam as mais relevantes para aplicações de embalagens comerciais de alimentos (OTONI et al., 2018).

A iminente limitação e a incerteza sobre as fontes de combustível fósseis, além da crescente preocupação ambiental em relação ao descarte de materiais não biodegradáveis, tem despertado grande interesse da indústria de alimentos em encontrar novos materiais a partir de recursos renováveis capazes de substituir materiais à base de petróleo, para produzir embalagens biodegradáveis com propriedades similares às sintéticas, mas com baixo impacto ambiental e baixos custos de produção (AVELLA et al., 2005; HAERUDIN et al., 2010; OTONI et al., 2018).

Os biopolímeros são originários de vários recursos naturais, como polissacarídeos (amido, celulose, quitosana, dentre outros) e proteínas de origem vegetal e animal (ZHONG et al., 2019). Esses polímeros naturais são alternativas atraentes para materiais de embalagem à base de petróleo ou sintético, devido às suas características ecológicas. Entre vários biopolímeros, o amido é comumente usado nesses casos devido ao seu custo relativamente baixo, alta disponibilidade e facilidade de manuseio (GHASEMLOU et al., 2013; MENDES et al., 2016), além de ser transparente, inodoro, insípido e com boas propriedades de barreiras a gases (dióxido de carbono – CO_2 e oxigênio – O_2) (HERNIOU-JULIEN; MENDIETA; GUTIÉRREZ, 2019; JIANG; NEETOO; CHEN, 2011; NEETOO; YE; CHEN, 2010; TĂNASE et al., 2015).

Os materiais à base de amido, no entanto, apresentam baixa resistência mecânica e a barreira à umidade (CAMPOS; GERSCHENSON; FLORES, 2011). A adição de compostos hidrofóbicos e a produção de compósitos têm sido sugeridas para melhorar as propriedades físicas e funcionais (atividade antimicrobiana e antioxidante) dos filmes de amido. A esse respeito, a melhoria das propriedades de barreira adicionando ácidos graxos, ceras e óleos, em filmes de emulsão ou de bicamada, recebeu um interesse particular devido à alta hidrofobicidade e sustentabilidade dos lipídeos (ALOUI et al., 2019). FERREIRA et al. (2014) adicionaram compostos hidrofóbicos, como óleos e ceras extraídos de bagaço de uva e

adiaram a solubilização de filmes de quitosana na água. A manteiga de cacau, é o componente mais abundante do grão de cacau (SERVENT et al., 2018) e é composto por 97% de glicerolipídeos, principalmente triacilgliceróis, mas também contém vestígios de di- e monoglicerídeos, fosfolipídios e glicolipídios e matéria insaponificável (LIPP et al., 2001). Embora a manteiga de cacau seja amplamente utilizada na indústria de confeitaria, seus efeitos de plastificantes e constituintes hidrofóbicos nas propriedades dos filmes não foram testados, por isso é sugerido que esses constituintes sejam adicionados para otimizar as propriedades mecânicas e de barreira à umidade. Além disso, a substituição do glicerol por um plastificante de maior peso molecular, pode diminuir a difusão e melhorar a estabilidade dos filmes biopoliméricos (KARBOWIAK et al., 2006).

Outros métodos promissores em tecnologias de barreira, e para melhorar a segurança e a qualidade dos alimentos é a incorporação de substâncias bioativas, como agentes antimicrobianos ou antioxidantes em materiais de embalagens (GAIKWAD; LEE, 2017; MAHCENE et al., 2019). Os óleos essenciais (OE) obtidos de plantas aromáticas são líquidos hidrofóbicos concentrados que contêm vários compostos bioativos, como fenólicos e terpenóides. Foi demonstrado que alguns OEs exibem fortes atividades antimicrobianas e antioxidantes, que podem ser servidas como potenciais agentes conservantes para alimentos perecíveis (ATARÉS; CHIRALT, 2016). O óleo essencial de capim-limão (LEO) (*Cymbopogon citratus*) é caracterizado por um forte aroma de limão, devido ao seu alto conteúdo citral, responsável por aproximadamente 75% de sua composição (MOHAMED HANAA et al., 2012) e demonstrou ser um antimicrobiano ativo contra fungos, leveduras e bactérias gram-positivas e negativas (NAIK et al., 2010). Porém, as aplicações diretas dos OEs são limitadas, porque contêm compostos com baixa solubilidade na água; além disso, são necessárias grandes quantidades para induzir seus efeitos inibitórios nas bactérias. Além disso, o impacto dos OEs no sabor dos alimentos pode afetar seu uso. Para resolver esses problemas, os OE podem ser encapsulados em nanoemulsões que demonstraram ser portadores ideais para o fornecimento de substâncias lipídicas (DÁVILA-RODRÍGUEZ et al., 2019). Riquelme et al. (2017) estudaram a adição de LEO encapsulado em filmes à base de alginato produzidos por casting em bancada e descobriram que o tamanho das gotículas de emulsão afetava significativamente as propriedades físicas e a atividade antimicrobiana dos filmes.

Filmes biodegradáveis e comestíveis emulsionados apresentam em geral, propriedades físico-mecânicas insatisfatórias, o que guia a elaboração de biocompósitos a partir da adição de agentes de reforço. A utilização de agentes de reforço obtidos a partir da extração de

produtos valiosos de subprodutos da agricultura e resíduos industriais emergiu como um desafio-chave para combater o impacto ambiental negativo do descarte de resíduo e ao mesmo tempo fornecer resíduos renováveis a um custo aceitável (CHANDEL et al., 2018; LEE et al., 2019; UBANDO; FELIX; CHEN, 2019). Dentre esses resíduos temos o bagaço de malte obtidos das indústrias cervejeiras, representando cerca de 85% do total dos subprodutos gerados. Além disso, o Brasil é o terceiro maior produtor de cerveja do mundo, com uma produção de 13,3 bilhões de litros, atrás apenas da China (46 bilhões de litros) e dos Estados Unidos (22,1 bilhões de litros) (SINDICERV, 2017). Resíduo rico em fibras e proteínas é considerado um material lignocelulósico contendo aproximadamente 17% de celulose, 28% de hemicelulose e 28% de lignina (MUSSATTO; DRAGONE; ROBERTO, 2006).

Diante do exposto, torna-se evidente o potencial dos biocompósitos comestíveis na solução das limitações relacionadas à origem fóssil e à não biodegradabilidade dos polímeros convencionais. Faz-se necessário, contudo, que os desempenhos físico-mecânicos dos materiais tradicionais e inovadores sejam comparáveis, o que motiva a contínua pesquisa por materiais oriundos de fontes renováveis e por combinações originarias entre eles, tais como a formação de biocompósitos comestíveis emulsionados reforçados com fibras vegetais que motivaram o trabalho de pesquisa aqui apresentada. Aliado a isso, ainda não foi verificado na literatura, estudos que avaliassem o efeito combinatório desses materiais, na produção de filmes bionanocompósitos quaternários, e o efeito plastificante e de propriedades de barreira da manteiga de cacau em filmes biopoliméricos. Além disso, torna-se cada vez mais necessário, no entanto, realizar estudos científicos que realmente satisfaçam as necessidades da sociedade e da indústria, ou seja, estudos que forneçam resultados aplicáveis após a escala tecnológica, como por exemplo, utilizando métodos de produção escalonáveis e aplicação desses biocompósitos em matrizes alimentícias, como a técnica de casting contínuo.

De modo geral, objetivou-se a produção de biocompósitos comestíveis inovadores utilizando nanoemulsões de óleo essencial de capim-limão, manteiga de cacau e resíduo da indústria cervejeira, o bagaço de malte como agente de reforço. A concepção da pesquisa envolve enfoques científicos por meio da correlação entre composição, estrutura, propriedade por meio de técnicas de caracterização em níveis micro e macroscópicos; objetivando a otimização das propriedades destes materiais e obtenção dos filmes através do escalonamento de suas produções para escala piloto, com propriedades sensoriais adequadas.

2. Referêncial teórico

2.1.   Embalagens comestíveis

A produção global atual de plásticos é superior a 359 milhões de toneladas, com um crescimento anual de aproximadamente 5%, o que representa o maior campo de aplicação de petróleo bruto (PLASTICSEUROPE, 2019). Até o momento, plásticos à base de petroquímicos, como tereftalato de polietileno (PET), cloreto de polivinil (PVC), polietileno (PE), polipropileno (PP), poliestireno (PS) e poliamida (PA) têm sido cada vez mais utilizados como materiais de embalagem, principalmente devido as suas disponibilidades, custo relativamente baixo e por apresentarem bom desempenho mecânico, como resistência à tração e ao rasgo, boa barreira ao oxigênio, dióxido de carbono, composto de anidrido e aroma, vedação por calor e assim por diante (ZHONG et al., 2019). Porém, atualmente, seus usos devem ser restritos, porque não são totalmente recicláveis, devido à sua heterogeneidade, baixos volumes de mercado, diversas fontes e alto potencial de contaminação de resíduos plásticos, além de não ser economicamente conveniente, e também não são biodegradáveis e/ou compostáveis. Além disso, segundo o regulamento 1935/2004 EC9, estabelecem o uso de plásticos pós-consumo em contato com alimento, desde que os plásticos sejam processados em temperaturas superiores a 200 ºC, como por exemplo, protocolos técnicos existente para o tereftatalo de polietileno (PET) para produção de garrafas de água e refrigerantes e caixas de polipropileno (PP) para frutas e legumes, porém não é suficiente para promover a redução de plásticos descartados no ambiente. Como consequência 93% de materiais plásticos são depositados em aterros e oceanos, criando assim diversos níveis de contaminação. Além disso, aproximadamente 6,2 milhões de toneladas de macro-plásticos (maiores que 5 mm) e 3 milhões de toneladas de micro/nanoplásticos (menores que 3 mm) são depositadas no meio ambiente (RYBERG et al., 2019). Essas partículas possuem a capacidade de atuar como vetores para outros poluentes, o que resultam em partículas inertes e tóxicas (TESSNOW-VON WYSOCKI; LE BILLON, 2019).

Assim, com o crescimento da conscientização ambiental global e pelo aumento da demanda do consumidor por alimentos seguros, convenientes e estáveis impõe-se o desenvolvimento de embalagens comestíveis, biodegradáveis e/ou bioplásticos a partir de recursos renováveis.

As embalagens comestíveis podem ser definidas como sendo uma fina camada comestível revestida em um alimento (revestimento) ou colocada como uma barreira entre o alimento e o ambiente ao redor (filmes) (SAHRAEE et al., 2019). Uma das diferenças entre os filmes e os revestimentos comestíveis, é que os revestimentos, consistem líquidos viscosos aplicados – por pulverização, imersão ou outro método conveniente – diretamente sobre a matriz alimentícia, onde são secos e permanecem para desempenharem suas funções; já os filmes comestíveis são materiais de manipulação independente produzidos em outra superfície para posterior aplicação em alimentos, tanto para revesti-los quanto para separar diferentes componentes (DEHGHANI; HOSSEINI; REGENSTEIN, 2018). Ambos os sistemas devem ser formados por materiais que possam ser ingeridos com ou sem a remoção das camadas aplicadas.

O fato de polímeros comestíveis poderem ser consumidos sem restrições junto com os alimentos, é uma vantagem sobre os polímeros sintéticos tradicionais, uma vez que não há embalagens a serem descartadas. Caso a opção do consumidor seja de não ingerir as embalagens comestíveis, também contribuem para a redução do impacto ambiental por serem produzidas a partir de matérias-primas renováveis (polímeros biodegradáveis e biopolímericos), as quais degradam-se mais rapidamente que as embalagens convencionais, retornando ao ciclo biológico logo após desempenharem suas funções. Além disso, essas embalagens podem ter funções ativas, agindo como carreadores de compostos nutricionais (ZHANG et al., 2019a), antimicrobianos (DHUMAL et al., 2019; KOWALCZYK et al., 2020; ZHENG et al., 2019), antioxidantes (GARCÍA et al., 2020; RUAN et al., 2019; ZHANG et al., 2020b) probióticos (MA et al., 2019; SHAHRAMPOUR et al., 2020) e sensoriais (KULAWIK et al., 2019; OZDEMIR; FLOROS, 2008; ZHAO; MCDANIEL, 2005).

A norma ABNT NBR 15448-1 (2008) define biopolímeros e bioplásticos como "polímeros ou copolímeros produzidos a partir de matérias-primas de fontes renováveis". Esta definição contempla somente a origem dos materiais, podendo estes ser biodegradáveis ou não. Algumas poliolefinas – como polietileno e polipropileno – podem ser polimerizadas a partir de monômeros oriundos de fontes renováveis – como o etanol - em detrimento das fósseis. Independente da origem, estes materiais não atendem o critério de biodegradabilidade previsto na norma ASTM D6400-19 (2019), exige a conversão de pelo menos 90% de seu teor de carbono orgânico em gás carbônico em até 180 dias – em solo com condições específicas de matéria orgânica, microbiota e umidade. Esta norma define, ainda, plásticos

biodegradáveis como aqueles que se degradam completamente ao ataque microbiano, como bactéria, fungos e algas, sob condições ambientais apropriadas. Os polímeros biodegradáveis podem ser classificados em diferentes tipos, de acordo com seus processos e fontes de síntese, descritos na Figura 1. São obtidos diretamente da biomassa (proteínas e polissacarídeos), biopolímeros sintéticos de biomassa (poli(ácido lático) (PLA)) ou petroquímicos (por exemplo, policaprolactona (PCL), poli(ácido glicólico) (PGA), poli (butileno) succinato -co-adipato) (PBSA)) ou aqueles obtidos por fermentação microbiana (por exemplo, poli (hidroxialcanoatos) (PHA), poli (hidroxibutirato) (PHB)).

Figura 1 - Classes de polímeros biodegradáveis de origens renováveis e fósseis
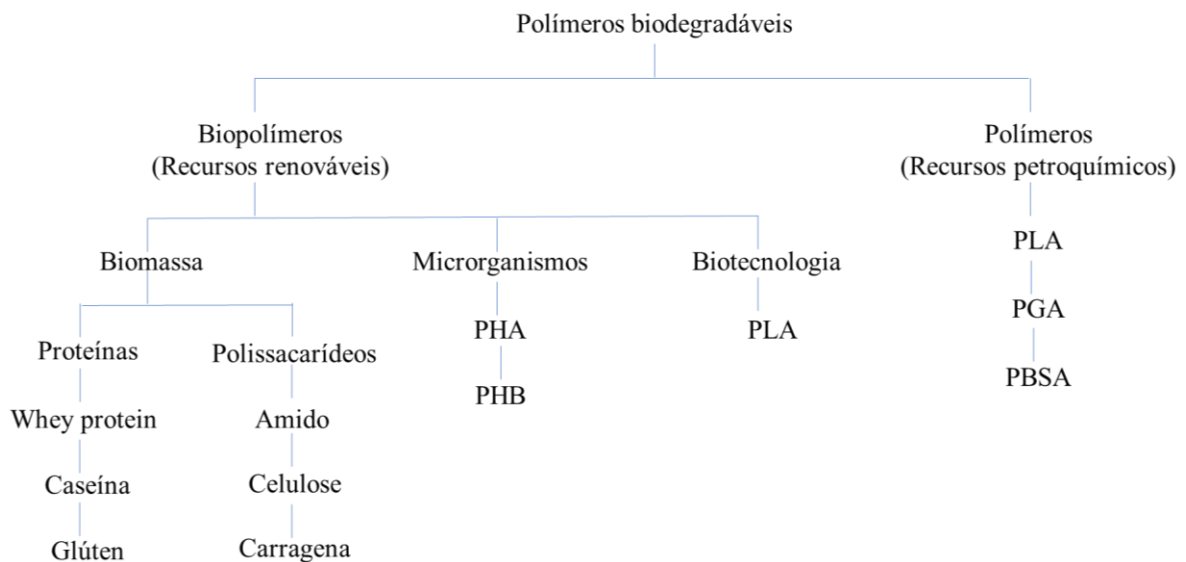
Fonte: Adaptado de ZHONG et al. (2019)

Os biopolímeros que constituem materiais naturais e quimicamente derivados de recursos renováveis estão sendo projetados para apresentar conteúdo de carbono mínima, alto valor de reciclagem e completa biodegradabilidade e compostabilidade (RAMESHKUMAR et al., 2019; WIERCKX et al., 2018). O atributo compostabilidade é muito importante para materiais de biopolímeros porque, embora a reciclagem seja de custos elevados em termos de energia, a compostagem permite o descarte das embalagens no solo, produzindo apenas água, dióxido de carbono e compostos inorgânicos sem resíduos tóxicos (SIRACUSA et al., 2008). Os principais biopolímeros empregados no desenvolvimento de bioplásticos são proteínas e polissacarídeos. Os polissacarídeos estudados até o momento incluem quitosana (DI FILIPPO

et al., 2020; LI et al., 2019; LIU et al., 2019; VÉLEZ-PEÑA et al., 2019; ZHANG et al., 2019c), carboximetilcelulose (DE MELO FIORI et al., 2019; SILVA et al., 2019) e amido (DE SOUSA LEAL et al., 2018; HU et al., 2019; ROY et al., 2020; TAI et al., 2019). A utilização de amido como recurso renovável na produção de bioplástico para embalagens resultou em menor consumo de recursos energéticos não renováveis (~50%) e, portanto, menos emissões de gases de efeito estufa (~60%) quando comparados às embalagens de poliestireno.

2.2.   Amido de mandioca

O amido nativo ou amido é um dos polímeros naturais mais promissores para o desenvolvimento e aplicações em materiais biopoliméricos, devido à sua biodegradabilidade, abundância, renovabilidade e a capacidade de serem processados com equipamentos de processamento de plásticos convencionais (JIANG et al., 2019a). O amido como macromolécula também é atraente por suas propriedades físicas, químicas e funcionais, como facilidade de dissolução e propriedades de retenção de água, gelatinização, comportamento de colagem quando submetido a temperaturas elevadas e facilidade de modificação para otimizar as propriedades funcionais (OGUNSONA; OJOGBO; MEKONNEN, 2018). O amido, além de ser amplamente consumido como alimento ou usado na preparação de alimentos em todo o mundo, pode ser utilizado em diversas aplicações industriais, como por exemplo, aglutinantes, adesivos, têxtil, produção química e outros produtos industriais (WANI et al., 2012).

A maioria dos amidos disponíveis no comércio são isolados de grãos como milho, arroz e trigo, ou de tubérculos como batata e mandioca. A mandioca é uma boa fonte de amido com rendimentos variáveis de amido, dependendo do período de colheita ou da cultivar, podendo atingir 80% do peso seco da raiz (OYEYINKA et al., 2020). A mandioca (Manihot esculenta Crantz: Euphorbiaceae), é um arbusto perene que atualmente é a sexta cultura alimentar mais importante do mundo. Com base nos dados de FAOSTAT (FAO, 2018), a produção mundial de mandioca (raiz) foi estimada em 277 milhões de toneladas (MT). Nos últimos três anos, 2015-2018, os cinco principais países com maior produção de mandioca foram Nigéria (56 MT), Tailândia (27,2 MT), Indonésia (21 MT), Brasil (20,9 MT) e Gana (19,4 MT) (FAO, 2018). Espera-se que a produção mundial de mandioca atinja cerca de 290 milhões de toneladas em 2020, sendo a África o maior produtor responsável por mais de 60% da produção total (FAO, 2018).

A composição do amido de mandioca pode variar, conforme as condições de extração, sendo encontrados valores entre 0,03-0,29% de cinzas, 0,06-0,75% de proteínas, 0,01-1,2% de lipídeos, 0,0029-0,0095% de fósforo e 0,11-1,9% de fibras (TAPPIBAN et al., 2019). Essas diferenças de composição variam de acordo com as fontes botânicas, condições climáticas, localização geográfica para cultivo e tipo de solo (OGUNSONA; OJOGBO; MEKONNEN, 2018; SINGH et al., 2003) e influenciam diretamente na estrutura e nas propriedades físicas e químicas dos amidos.

O amido é um carboidrato polimérico composto por unidades de anidroglucose ligadas por ligações α-1,4 glicosídicas. Os polímeros de amido podem ainda ser subdivididos em duas formas principais: amilose e amilopectina, as quais compõem 98-99% massa seca de amido, formando estruturas cristalinas e amorfas (TAPPIBAN et al., 2019). A amilose (Figura 2a) (25-30%) é essencialmente um polímero solúvel em água, de cadeia praticamente linear, com ligações α-(1,4), cuja massa molar varia de 10^5 a 10^6 g.mol^{-1}, a qual contribuem principalmente para a fase amorfa do grânulo de amido. O conteúdo de amilose é um atributo principal da qualidade do amido e determina diversas propriedades do amido e, eventualmente, o uso final (ZHU, 2017). Pois devido à linearidade, mobilidade e a presença dos grupos hidroxilas, das cadeias de amilose tendem a se orientar, paralelamente, aproximando-se o suficiente para que ligações de hidrogênio entre hidroxilas de polímeros adjacentes. Como resultado dessa associação intermolecular, pastas opacas e filmes resistentes são formados (JONHED, 2006).

Por outro lado, a amilopectina (70-75%) (Figura 2b) é uma estrutura altamente ramificada unidas por ligações α-(1,6) nos pontos de ramificação, localizados a cada 20 ou 30 unidades de glicose. A amilopectina contribui predominantemente para a organização cristalina periférica dos grânulos de amido. Seu tamanho volumoso, causado pelas ramificações, reduz a mobilidade das cadeias e previne os polímeros de amilopectina de se aproximarem o suficiente para que haja a formação de ligações de hidrogênio. Como resultado, as soluções aquosas de amilopectina são transparentes e mais resistentes à retrogradação durante a estocagem (OJOGBO; OGUNSONA; MEKONNEN, 2019). Porém apesar da molécula de amilopectina ser ramificada, a mesma é mais compacta do que em relação à de amilose. Isso implica em uma menor facilidade de penetração de água e de enzimas, sendo, portanto, mais resistente ao processo de hidrólise.

Figura 2 - Estruturas moleculares para (a) Amilose e (b) Amilopectina
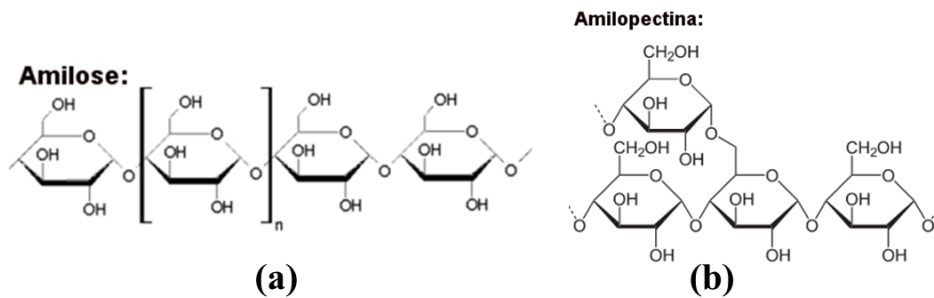
Fonte: Adaptado de VAN SOEST; VLIEGENTHART (1997)

A razão dos componentes moleculares de amilose e amilopectina são naturalmente reunidos na forma de grânulos semicristalinos, com aproximadamente de 20 a 45% de cristalinidade, a qual pode ser influenciada principalmente pelo tipo e idade do amido, e também pelo processamento térmico empregado (JIANG et al., 2019a; SAGNELLI et al., 2016). Normalmente, a proporção de amilose e amilopectina varia juntamente com a distribuição do comprimento da cadeia, tamanho granular e conteúdo de lipídeos. A Tabela 1 mostra várias fontes botânicas de amido e suas proporções amilose-amilopectina.

Tabela 1: Fontes botânicas de amido e sua relação amilose/amilopectina correspondente e cristalinidade

Fonte	Cristalinidade (%)	Amilose (%)	Amilopectina (%)
Arroz	38.	20-30	80-70
Batata	23-53	23-31	77-69
Mandioca	31-59	16-25	84-75
Mandioca ceroso	N/D	0	100
Trigo	36-39	30	70
Milho	43-48	28	72
Sorgo	22-28	24-27	76-73

Fonte: (OGUNSONA; OJOGBO; MEKONNEN, 2018)

O amido granular e nativo não possui características de um material termoplástico, em razão das ligações de hidrogênio intra e intermoleculares entre os grupamentos hidroxilas das cadeias, que representam sua cristalinidade (LIU et al., 2009). Além disso, a temperatura de decomposição do amido é inferior a sua temperatura de fusão, dessa forma, o amido por si só não pode ser utilizado como um polímero termoplástico, principalmente quando processado por extrusão (CHANDRA MOHAN et al., 2018).

Porém uma das características mais atrativas do amido na área de materiais é a sua capacidade de adquirir comportamento termoplástico sob condições específicas de processamento: como por exemplo, o emprego de cisalhamento, energia térmica e plastificante (água, glicerol, sorbitol, ureia e etc), onde envolve várias reações químicas e físicas, por exemplo, difusão de água, expansão de grânulos, gelatinização, decomposição, fusão e cristalização ou retrogradação (JIANG et al., 2019a). Esse amido desestruturado é denominado como amido termoplástico (TPS) e trata-se de um material à base de amido gelatinizado. O conceito bem aceito de "gelatinização" consiste na destruição da estrutura cristalina dos grânulos de amido, que é um processo irreversível. A gelatinização é particularmente importante porque está relacionada às demais etapas do processamento do amido e é a base da conversão do amido em um termoplástico. O processo de gelatinização pode ser realizado empregando tensão cisalhamento, como por exemplo, por meio da extrusão, injeção ou misturador termocinético (FOURATI et al., 2020; OCHOA-YEPES et al., 2019; SIYAMAK; LAYCOCK; LUCKMAN, 2020; VON BORRIES-MEDRANO et al., 2018; ZHANG et al., 2020a), ou sem tensão de cisalhamento. Neste caso, o processo de gelatinização depende principalmente do teor de água e das condições de temperatura, esse método também é conhecido como evaporação de solvente ou casting (DO EVANGELHO et al., 2019; PEIGHAMBARDOUST et al., 2019; ROY et al., 2020; SANDOVAL et al., 2018). Este último será abordado com mais detalhamento nesta contribuição.

Durante o estágio inicial do processamento térmico, à medida que a temperatura aumenta de 20 para 60 ºC ("Temperatura inicial de gelatinização"), acredita-se que a água seja reversivelmente complexada com moléculas de amido, diminuindo sua mobilidade (OLKKU; RHA, 1978). Nesta etapa, os grânulos de amido ainda apresentam birrefringência típica sob luz polarizada (presença de cristalitos intactos), pois os esferulitos ainda estão unidos por forças de van der Waals ou ligações de hidrogênio. Quando a suspensão de amido é aquecida acima da temperatura de gelatinização, as ligações de hidrogênio são interrompidas, resultando em maior inchaço e dissolução dos cristalitos (dissociação das duplas hélices da amilopectina) (LIM; WU; REID, 2000). Assim, para que ocorra a gelatinização completa do amido em condições sem cisalhamento, alguns requisitos devem ser avaliados, já que influenciam diretamente na qualidade da pasta obtida e consequentemente nos filmes obtidos. De acordo com WANG et al. (1991), a gelatinização completa do amido, requer excesso de água, na qual corresponde teores próximos a 63%. No entanto, se a concentração de água for muito alta, os cristalitos do amido podem ser separados

por dilatação, provocando a não fundição deles em altas temperaturas (LAI; KOKINI, 1991), e se a concentração de água for limitada, as forças de dilatação serão muito menos significativas, e a gelatinização completa não ocorrerá na faixa de temperatura usual (LAI; KOKINI, 1991). Outro requisito que deve ser avaliado, é a temperatura, pois à medida que a temperatura aumenta, os grânulos de amido tornam-se progressivamente móveis, vindo a fundir as regiões cristalinas, provocando o comportamento viscoelástico exibido nos termoplásticos fundidos (LIU et al., 2009). Estas etapas são representadas na Figura 3.

Figura 3 - Representação esquemática das transições de fase do amido durante o processamento térmico e o envelhecimento.
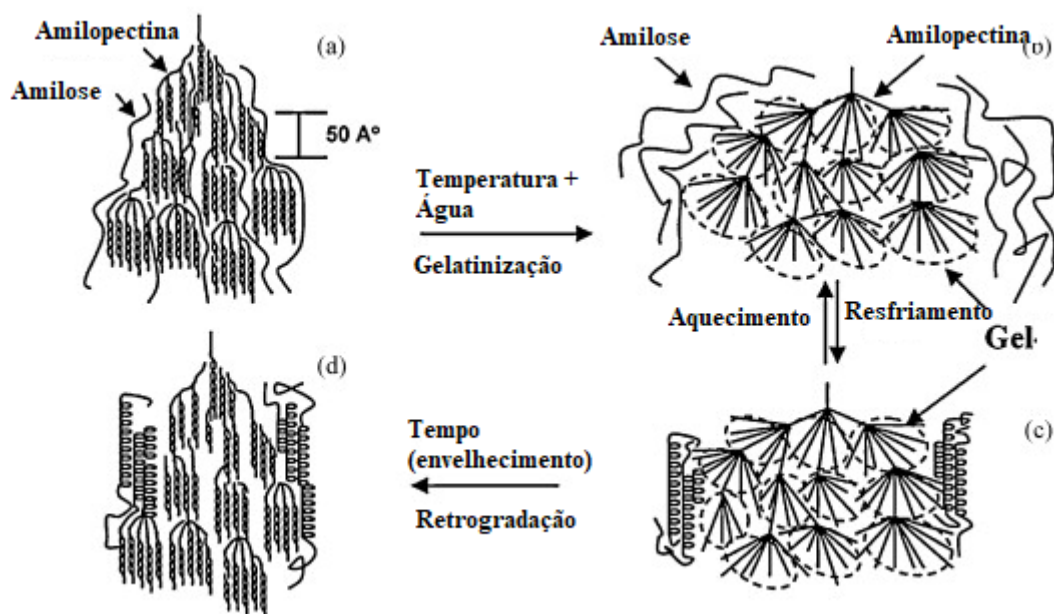
Fonte: (YU; CHRISTIE, 2005)

Após a gelatinização, com o resfriamento as moléculas de amido podem começar a se reassociar através de ligações de hidrogênio, favorecendo a formação de uma estrutura mais ordenada, que sob condições favoráveis pode formar uma estrutura novamente cristalina (diferente da estrutura inicial); a este conjunto de alterações dá-se o nome de retrogradação ou recristalização (ZOBEL; YOUNG; ROCCA, 1988). Este fenômeno trata-se de um processo de recristalização termorreversível sem equilíbrio, com mecanismos consistindo em etapas consecutivas: nucleação, propagação e maturação. Ou seja, com o passar do tempo, as moléculas do amido vão perdendo energia e as ligações de hidrogênio tornam-se mais fortes, assim, as cadeias começam a reassorciar-se num estado mais ordenado. Essa reassociação culmina com a formação de simples e duplas hélices, resultando no enredamento ou na

formação de zonas de junção entre as moléculas, formando áreas cristalinas. Como a área cristalizada altera o índice de refração, o gel vai se tornado mais opaco à medida que a retrogradação se processa (MALIN SJÖÖ LARS NILSSON, 2017). A amilose que se exsuda dos grânulos inchados forma uma rede por meio da associação com cadeias que rodeiam os grânulos gelatinizados. Como consequência, a viscosidade da pasta aumenta, convertendo-se em um sistema viscoelástico turvo ou em concentrações de amido suficientemente altas (>6% p/p) num gel elástico opaco, em que, às vezes, ocorre precipitação de cristais insolúveis de amido levando à separação de fases. A forte interação das cadeias entre si promove a saída da água do sistema, sendo essa expulsão chamada de sinérese.

As características de retrogradação da amilose e amilopectina são cineticamente diferentes. A amilose retrograda mais rapidamente, tendo forte tendência a reassociar-se por meio da formação de pontes de hidrogênio com outras moléculas de amilose adjacentes, formando estruturas cristalinas de duplas hélices quando a solução esfria e se mantém por longo período. A amilose apresenta endoterma de fusão de 140°C a 180°C, e a presença de ácidos graxos livres ou lipídios favorece a formação de complexos de inclusão. Por outro lado, a amilopectina retrograda numa taxa muito menor durante um longo período, e sua endoterma de fusão é menor, aproximadamente, 45°C a 60°C (HSIEN-CHIH; SARKO, 1978).

O grau de retrogradação e a propriedades dos cristais de amido formados são influenciados, não apenas pelo armazenamento, tempo e temperatura, mas também, pela concentração de amido, a origem botânica deste, a cristalinidade, as estruturas e a razão molecular de amilose e amilopectina. A retrogradação também pode ser dependente de fatores inerentes ao grânulo ou seus componentes, entre estes fatores salientam-se: peso molecular e linearidade. Além do mais, o amido de cereais (nativos) tem a maior tendência à retrogradação que amidos de raízes e tubérculos. Tal fato pode estar associado a que os amidos de cereais devido a sua menor dispersibilidade em relação aos amidos de raízes e tubérculos. Portanto, o fenômeno da retrogradação/recristalização leva ao envelhecimento dos filmes de amido, tornando-os mais rígidos e quebradiços, influenciando a cristalinidade dos filmes, e consequentemente no conteúdo de umidade, propriedade de barreira (vapor de água e a gás) e propriedades mecânicas, dentre outras propriedades.

Portanto, a natureza hidrofílica do amido, juntamente com sua fragilidade, retrogradação e degradação térmica, limita seu uso extensivo em aplicações de polímeros industriais que exigem integridade mecânica. Para alcançar essas funcionalidades, vários

métodos de modificação, como químicos, físicos e uma combinação desses métodos, são usados na modificação do amido. No entanto, a maioria dos produtos químicos utilizados na modificação, são sintéticos, podendo gerar resíduos nos alimentos e preocupações de segurança, além de poder apresentar problemas ambientais. A fim de superar esses inconvenientes, o amido pode ser misturado com vários polímeros sintéticos ou naturais. Essas abordagens são: estruturas multicamadas com poliésteres alifáticos, misturas com borracha natural, ou zeína, e compósitos com fibras. Outra abordagem amplamente usada para melhorar as propriedades mecânicas e a processabilidade dos filmes de amido é a adição de plastificantes e compostos hidrofóbicos, como ceras, lipídeos, ácidos graxos e óleos essenciais (CHANDRA MOHAN et al., 2016; CHINACHOTI, 1996; COFFIN; FISHMAN, 1994; DAFE et al., 2017; HUBER; EMBUSCADO, 2009; LIU et al., 2020a; MEDINA JARAMILLO et al., 2016).

2.3.   **Compostos hidrofóbicos**

Os filmes biopoliméricos, a base de polissacarídeos, apesar de fornecer sustentabilidade ambiental, desempenho exclusivo e benefícios econômicos, exibem más propriedades mecânicas e de barreira contra o vapor de água, além de serem sensíveis à umidade. Isso causa um feedback negativo quando aplicado a produtos alimentícios com alta umidade, porque os filmes podem se dissolver, inchar ou desintegrar-se em contato com a água (SUDERMAN; ISA; SARBON, 2018).

Pesquisas atuais sugerem que a adição de agentes plastificantes e outros compostos ativos podem melhorar as propriedades mecânicas e de barreira à água dos polímeros a base de polissacarídeos e proteínas (AZRA et al., 2019; ÖZEREN et al., 2019; RODSAMRAN; SOTHORNVIT, 2019a). Os plastificantes são materiais importantes no desenvolvimento de películas e revestimentos comestíveis, pois aumentam a flexibilidade, reduz a fragilidade e diminui a porosidade e a tendência a rachaduras (DI DONATO et al., 2020).

A utilização de polióis (como, glicerol, sorbitol, manitol) foram relatados como a maioria dos plastificantes compatíveis com o amido (DAS; UPPALURI; DAS, 2019a, 2019b; GAO et al., 2019; KHAN et al., 2019). No entanto, esses plastificantes adicionados são todas moléculas hidrofílicas que causam baixa resistência à água nos filmes, devido a captação de água da estrutura polimérica. Para resolver esse problema, a adição de plastificantes

hidrofóbicos, permite a redução da permeabilidade à umidade, ao fechar micro-vazios dos filmes biopoliméricos (VIEIRA et al., 2011).

Além disso, há um crescente interesse pelo uso de plastificantes naturais, caracterizados por baixa toxicidade e baixa migração (VIEIRA et al., 2011). Este grupo inclui óleos comestíveis (por exemplo, soja, milho, girassol, azeite e óleo de palma) (MA et al., 2020; TIAN et al., 2012, 2018), e ácidos graxos (por exemplo, ácido esteárico, oleico e palmítico) (FANTA et al., 2015; KHANOONKON; YOKSAN; OGALE, 2016a, 2016b; THAKUR et al., 2016). Mais recentemente, uma variedade de outros produtos naturais, incluindo, extratos naturais de plantas, por exemplo, extrato de folha de lótus (FAN et al., 2019; WANG et al., 2018), extrato de chá verde (MEDINA-JARAMILLO et al., 2017; PANRONG; KARBOWIAK; HARNKARNSUJARIT, 2019; SHAH; FERNANDES; SUARES, 2019), extrato de romã, compostos orgânicos (MA et al., 2020) foram investigados como plastificantes e inibidores microbianos.

Essa busca de plastificantes naturais também está relacionada ao interesse dos pesquisadores e indústrias no desenvolvimento de embalagens obtidas de recursos naturais e biodegradáveis, com o potencial de reduzir o uso de produtos plásticos convencionais. É razoável supor que os plastificantes para biopolímeros devem, de preferência, ser também biodegradáveis, preservando a eco-sustentabilidade do produto final (VIEIRA et al., 2011).

Outro inconveniente que ocorre com os biopolímeros, é o envelhecimento, o que leva à instabilidade pela indução de várias alterações químicas (por exemplo, degradação, agregação) e/ou alterações físicas (por exemplo, migração) de materiais poliméricos, dependendo das condições ambientais, como temperatura e umidade ao longo do tempo (HUNTRAKUL; HARNKARNSUJARIT, 2020). Observa-se que a migração ocorre naturalmente com substâncias de baixo peso molecular, como os plastificantes. A instabilidade dos biopolímeros afeta diretamente as propriedades físicas e barreira dos filmes que influenciam significativamente as aplicações das embalagens de alimentos. Entretanto, apenas alguns estudos abordaram esse tópico em filmes comestíveis e biodegradáveis (CHEN et al., 2019; LIU et al., 2020; LUO et al., 2020; ZHANG et al., 2019b; ZHU; LIU, 2019). A plastificação aumenta a difusão; portanto, a substituição do glicerol por um plastificante de maior peso molecular pode retardar a difusão e melhor a estabilidade dos filmes de biopolímeros (HUNTRAKUL; HARNKARNSUJARIT, 2020). Huntrakul and Harnkarnsujarit (2020) avaliaram o efeito plastificante de glicerol e de sua mistura com xilitol, sorbitol, polietilenoglicol 400 (PEG400) e ácido oleico em blendas de proteína de soro

de leite e carboximetilcelulose. Eles observaram que o xilitol apresentou comportamento de absorção de água semelhante ao sorbitol, mas com maior efeito de plastificação, porém, foi observado uma redução significativa na absorção de água com a adição do PEG 400 e do ácido oleico, que continham componentes não polares. Assim, concluíram que o tamanho da molécula de plastificante e consequentemente mudanças nas estruturas dos filmes, desempenham um papel fundamental na absorção de água e no envelhecimento dos filmes plastificados, estando também relacionado com o número de grupos hidroxilas.

2.3.1. Manteiga de cacau

A manteiga de cacau é um ácido graxo natural obtido de sementes do cacau (*Theobroma cacao*). As principais variedades do cacau são a Forasteiro, Criolo e Trinitário. Apesar da manteiga ser comumente utilizada como um ingrediente majoritário em chocolate e outros produtos de confeitaria com características físicas e químicas especificas (JAHURUL et al., 2013), seu efeito plastificante e nas propriedades de barreira em filmes biopoliméricos não foram avaliados.

A manteiga de cacau é sólida à temperatura ambiente (25ºC) e líquida à 37ºC, tendo seu ponto de fusão entre 27 e 35ºC. É formada majoritariamente pelos seguintes ácidos graxos: ácido palmítico (P), ácido esteárico (S), ácido oleico (O) e ácido linoleico (L), mas também contém alguns outros ácidos graxos em pequenas concentrações como o ácido láurico (La) e o miristico (M). Suas propriedades são ditadas por sua composição triacilglicerólica, e seus principais triacilgliceróis são POS, SOS e POP (SCHENK E PESCHAR, 2004, GALDÁMEZ ET AL., 2009, JAHURUL ET AL., 2013). Os triacilgliceróis (TAGs) monoinsaturados, com ácidos graxos saturados na posição 1 e 3 da molécula de glicerol, contribuem para formação de características peculiares de cristalização, polimorfismo e textura (AFOAKWA ET AL., 2008).

De acordo com os estudos de Bertan et al., (2005) observou-se redução significativa da permeabilidade ao vapor de água, em função da adição de ácido esteárico e palmítico, nos filmes de gelatina. Isso foi observado nos estudos de Pommet et al. (2003) no qual produziram filmes de glúten e ácidos graxos saturados com diferentes comprimentos de cadeia de carbono como plastificantes. Os autores concluíram que a incorporação homogênea de ácidos só ocorreu até um comprimento de cadeia de até 10 carbonos, cadeias mais longas (C12, C14 e C16) sendo apenas parcialmente incorporadas. Apesar de que os ácidos graxos de

cadeias longas estarem incompletamente incorporados, a permeabilidade ao vapor de água diminuiu com o aumento do comprimento da cadeia (C6 a C16).

Os lipídios influenciam as propriedades mecânicas dos filmes de polissacarídeos e proteínas, de acordo com o tipo de lipídio adicionado e sua capacidade de interagir com a matriz. Diversos estudos observaram uma diminuição na tensão máxima (TS) com o aumento da concentração de lipídios (BERTAN et al., 2005; FADINI et al., 2013). A porcentagem de elongação aumentou com a adição de substâncias hidrofóbicas, causada pelo efeito plastificante (BERTAN et al., 2005).

Assim, a incorporação de lipídios e ácidos graxos saturados de cadeia longa, como plastificantes, reduzem as interações com a água e podem aumentar a flexibilidade dos filmes. Várias variáveis precisam ser consideradas para obter resultados satisfatórios. Eles incluem a natureza e concentração de lipídios, o tipo de cristal, o tamanho e distribuição de lipídios e o processamento utilizado (ROCCA-SMITH et al., 2016). No entanto, também foi relatado que a incorporação de certos materiais lipídicos em filmes hidrofílicos tem um efeito adverso sobre as propriedades mecânicas e ópticas dos filmes (FADINI et al., 2013).

2.3.2. Nanoemulsão de capim-limão

Nos últimos anos, a indústria de alimentos demonstra uma demanda crescente por compostos naturais em substituição dos aditivos sintéticos, devido aos potenciais riscos à saúde (DAMMAK; LOURENÇO; SOBRAL, 2019), para desenvolver novos conservantes de alimentos contra microrganismos patogênicos e deteriorantes, bem como para sustentar a inovação nas embalagens de alimentos.

Os óleos essenciais (OEs) são compostos aromáticos de origem natural com atividades biológicas de amplo espectro (PRAKASH et al., 2018). Até o momento, os OEs têm sido explorados como aditivos aromatizantes (RIETJENS et al., 2020), medicamentos (HULLEY et al., 2019; LI et al., 2006; OLIVEIRA DE VERAS et al., 2020) ou cosméticos (VOLPE et al., 2018; YINGNGAM et al., 2019), inseticidas (BACCARI et al., 2020; RIBEIRO et al., 2020), antioxidantes (FALLAH et al., 2020; HIMED et al., 2019), anti-inflamatórios (VALENTE et al., 2015), antialérgicos (VIKHE; NIRMAL, 2018) e anticancerígenos (GALA; MILLER; WILLIAMS, 2020; SALEHI et al., 2020). Porém, muitos OEs, dependendo de sua composição, podem exercer atividades antibacterianas, antivirais e antifúngicas (ILIĆ et al., 2019; JIANG et al., 2019b; VALE et al., 2019), estimulando sua

aplicação também como antimicrobianos naturais em alimentos e bebidas. Além disso, a adição de óleos essenciais, em filmes biopoliméricos, tem sido sugerido para melhorar as suas propriedades físicas e funcionais (JIMÉNEZ et al., 2012; PELISSARI et al., 2009).

Dentre os óleos essenciais, temos óleo de capim-limão (*Cymbopogon citratus*), obtido de uma planta medicinal e aromática, pertencentes à família Poaceae e, embora seja originária da Índia, também pode ser encontrada nas Américas, África e Austrália (FOKOM et al., 2019). O óleo essencial de capim-limão é classificado como composto reconhecido como seguro (GRAS) pela Food and Drug Administration dos Estados Unidos (FDA; $21CFR_{182.20}$) (TAJIDIN, 2012).

É conhecido no Brasil por diversos nomes populares, tais como capim-cidreira, capim-cidró, capim-santo, capim-cheiroso e em países de língua inglesa, como lemongrass (MOHAMED HANAA et al., 2012). Caracteriza-se por um forte odor de limão devido ao seu elevado teor de citral (75% de sua composição) e o mirceno. O citral é constituído pela mistura isomérica de geraniale e neral; além desses, outros aldeídos, como o citronelal, isovaleraldeído e deciladeído, também pode ser encontrado, além de cetonas e álcoois, como geraniol, nerol, metil heptenol e farnesol (PEICHEL et al., 2019). Com isso o óleo de capim limão e reconhecido pelas suas atividades antimicrobianas contra uma gama diversificada de microrganismos, como fungos, leveduras e bactérias Gram (+) e Gram (-) (NAIK et al., 2010), tanto *in vitro* quanto em uma variedade de matrizes alimentares (ARREBOLA et al., 2010; AZARAKHSH et al., 2014). Porém, a eficácia *in vitro* nem sempre foi acompanhada por um respectivo *in situ*, provavelmente devido ao efeito dos componentes dos alimentos, condições de armazenamento ou da forma de aplicação do óleo essencial (HADJILOUKA et al., 2017). Além de se destacar também na capacidade de realçar as características sensoriais dos alimentos, aumentando e/ou acrescentando sabor ao alimento, e, secundariamente, com finalidade de conservação, devido às suas propriedades antimicrobianas e antioxidantes.

No entanto, devido a alta volatilidade, baixa estabilidade, alta suscetibilidade às condições ambientais e fortes características sensoriais, além da hidrofobicidade dos OEs, sua incorporação direta em alimentos e bebidas representam um desafio formidável. Assim, para resolver esse empecilho os OEs podem ser encapsulados em sistemas de entrega compatíveis com as aplicações alimentares (DONSÌ; FERRARI, 2016). Emulsões, lipossomas e nanopartículas lipídicas sólidas, são alguns dos sistemas de encapsulamento (PRAKASH et al., 2018). Entre eles, as emulsões têm sido os sistemas mais populares e amplamente utilizados, pois garantem uma distribuição uniforme de constituintes parcial ou totalmente

hidrofóbicos em uma matriz hidrofílica (TERJUNG et al., 2012). Além disso, emulsões em escala nanométrica ou nanoemulsões (diâmetro menor que 500 nm) (OTONI et al., 2016) oferecem vantagens adicionais, como a estabilidade física, aumento da bioatividade, melhor difusão e minimização dos impactos nas propriedades sensoriais nos produtos alimentícios. Entretanto, apesar das nanoemulsões serem sistemas cineticamente estáveis, eles são termodinamicamente instáveis, cuja produção requer emulsificantes ou surfactantes para estabilizar a fase dispersa – óleo em uma emulsão de óleo em água (O/A) – como gotículas na fase contínua (água ou soluções aquosas em uma emulsão A/O) (MENDES et al., 2020).

A seleção de um emulsificante adequado e sua concentração em relação à fase oleosa deve levar em considerações vários fatores, como (i) a cobertura superficial necessária para a formação de emulsões estáveis, (ii) tensão interfacial (iii) cinética de absorção nas interfaces O/A, bem como o rearranjo molecular no caso de emulsificantes de alto peso molecular, como proteínas e polissacarídeos e (iv) o balanço hidrofílico-lipofílico (HLB) das moléculas do surfactante, ou concentração e localização de porções hidrofóbicas no caso de emulsificantes de alto peso molecular (PRAKASH et al., 2018; TERJUNG et al., 2012).

Segundo Chang e McClments (2014) surfactantes com valores de HLB muito altos (Tween 20 (16,7)) ou baixos (Tween 85 (11) e Span 80 (8,6) são inadequados para a formação de nanoemulsões, ao contrário dos emulsificantes com HLB intermediários, como por exemplo, Tween 40 (15,6) 60 (14,9) e 80 (15), os quais foram capazes de formar nanoemulsões claras, independente do processo de emulsificação. Atualmente, a utilização de biopolímeros naturais (proteínas ou polissacarídeos) ou a combinação com os emulsificantes convencionais estão ganhando relevância pelo seu uso como emulsificantes e espessantes. Além disso, há um interesse crescente por ingredientes mais naturais, tanto de clientes quanto de empresas.

Neste caso, a utilização de polissacarídeos, como a pectina, para estabilizar nanoemulsões, é um potencial material promissor, pois possuí vários benefícios, como estabilizador de emulsão, propriedades gelificantes e capacidade de ligação (ASSADPOUR; JAFARI; MAGHSOUDLOU, 2017; GHAREHBEGLOU et al., 2019; REHMAN et al., 2019). Akhtar et al. (2002) mostraram que a pectina cítrica despolimerizada com um grau de esterificação (DE) de 70% e massa molecular de 70 kDa produziu emulsões de óleo de colza muito estáveis com propriedades comparáveis às emulsões preparadas com goma arábica.

A pectina é um polissacarídeo complexo e consiste em cadeias lineares de ligações α-1,4 de ácido d-galacturônico interrompidas por ligações 1,2 L-rhamonose, obtida principalmente a partir de resíduos da indústria de processamento de alimentos (cascas cítricas ou de maçã) (BERMÚDEZ-ORIA et al., 2017). Portanto, sua produção não compete em terra para a agricultura de alimentos, como bioplásticos obtidos a partir de fermentação de amido ou glicose, cujo crescimento pode até estar associado a consideráveis cargas ambientais. A pectina, quando processada para a obtenção de filmes apresentam boa barreira ao oxigênio e possuem boa dureza e adesividade (BERMÚDEZ-ORIA et al., 2017; RODSAMRAN; SOTHORNVIT, 2019b). Ainda apresentam como vantagem de utilização, baixo custo, disponibilidade, biodegradabilidade e viabilidade de produzir filmes isoladamente ou em combinação com outras matrizes poliméricas (DASH et al., 2019). Também é considerada como ingrediente alimentar seguro (REHMAN et al., 2019). Observa-se que a pectina, devido sua estrutura, possui propriedades anfifílicas que ajudam a reduzir a tensão interfacial entre as fases oleosa e aquosa, tornando-a eficaz na melhoria da estabilidade das nanoemulsões (BURAPAPADH; TAKEUCHI; SRIAMORNSAK, 2012; MUNGURE et al., 2018). Foi relatado que dependendo do grau de metoxilação aumenta a natureza hidrofóbica das moléculas de pectina, dando-lhe um caráter tensoativo, além de maior interação com os compostos hidrofóbicos (BURAPAPADH; TAKEUCHI; SRIAMORNSAK, 2012). Além disso, devido à natureza aniônica da pectina, algumas características desejadas podem ser transmitidas para as nanoemulsões, tais como o comportamento interfacial específica (por exemplo, forças eletrostáticas, de repulsão estérica e reologia) (AKHTAR et al., 2002; ASSADPOUR; JAFARI; MAGHSOUDLOU, 2017; CHANG; MCCLEMENTS, 2014). A aplicação de pectina como único biopolímero para estabilizar as nanoemulsões formam gotículas com diâmetro de aproximadamente 1000 nm, em comparação com as formadas pela combinação de pectina com outros biopolímeros.

Portanto, verifica-se a necessidade de maiores estudos sobre a combinação de pectina com outros biopolímeros, mas principalmente com emulsificantes de baixo peso molecular (por exemplo, Tween 80) para produzir emulsões com propriedades otimizadas. Isso permitiria um mapeamento comportamental completo de diferentes componentes e suas interações nas características da nanoemulsão.

Guerra-Rosas et al. (2016) investigaram a estabilidade a longo prazo (56 dias) das nanoemulsões sintetizadas com óleos essenciais (por exemplo, orégano, tomilho, capim-limão ou tangerina), um surfactante não iônico (Tween 80) e pectina de alto grau de metoxilação.

Embora as formulações preparadas com capim-limão e tangerina fossem transparentes e seus tamanhos de partícula estivessem na faixa de 11 a 18 nm, foi observado um provável aumento (1000 nm) no tamanho de partícula das emulsões fabricadas por orégano e tomilho devido ao amadurecimento de Ostwald. Dependendo do tipo de óleo essencial, o potencial zeta das nanoemulsões foi negativo (na faixa de -6 e -15 mV) devido à natureza aniônica da pectina que foi adsorvida na interface óleo-água. As formulações de nanoemulsão preparada com capim-limão e tangerina tiveram um potencial zeta mais negativo do que as fabricadas por orégano e tomilho, mostrando uma maior adsorção de pectina na superfície do óleo, o que resultou em uma maior estabilidade.

Por fim, a boa interação e compatibilidade dos polímeros de pectina e amido pode facilitar a estabilidade microestrutural e melhorar o desempenho dos filmes de amido (BAGLIOTTI MENEGUIN; STRINGHETTI FERREIRA CURY; EVANGELISTA, 2014; DASH et al., 2019; MENEGUIN et al., 2017; SUCHETA et al., 2019).

2.4.   Fibras lignocelulósicas

O uso combinado de polímeros biodegradáveis de base renovável juntamente com um material de enchimento de fibra natural, obtendo assim um biocompósito, representam uma alternativa sustentável e tecnicamente promissora para substituir os chamados "commodities" de plásticos no setor de embalagens de alimentos (SÁNCHEZ-SAFONT et al., 2018).

A utilização de materiais lignocelulósicos têm sido utilizados na preparação de compósitos desde os tempos históricos, com uma de suas aplicações particulares em aeronaves desde os anos 1940 (SATHISHKUMAR et al., 2013). No entanto, por várias razões, como as propriedades superiores das fibras sintéticas e a indisponibilidade de dados completos sobre diferentes fibras lignocelulósicas, o uso de fibras naturais em compósitos diminuiu até a década de 1980. Desde então, houve um aumento da motivação para o uso de fibras lignocelulósicas, na produção de biocompósitos (BIAN et al., 2019; CHAITANYA; SINGH; SONG, 2019; DHAKAL et al., 2018a; SANCHEZ-OLIVARES et al., 2019).

Em geral, a maior utilização das fibras lignocelulósicas, como carga biodegradável nos biocompósitos, deve-se por serem materiais baratos, abundância natural, menos abrasivas para os equipamentos durante o processamento, baixa densidade, alta rigidez específica, são ótimos isolantes térmicos e acústicos. Além disso, proporcionam menor riscos à saúde e ao meio ambiente do que as fibras sintéticas (GEORGE; CHAE; BRESSLER, 2016; SÁNCHEZ-

SAFONT et al., 2018). No entanto, limitações como baixa estabilidade térmica, absorção de umidade e incompatibilidade com resinas à base de petróleo reduziram as perspectivas e limitaram o potencial dessas fibras (GEORGE; CHAE; BRESSLER, 2016).

As fibras lignocelulósicas são materiais constituídos principalmente de celulose (60 a 80%), hemicelulose e lignina (5 a 20%) (Figura 4). Há também baixos teores de extrativos – gordura, proteínas e sais – e pectina (até 20%), que variam amplamente dependendo do tipo e origem da fibra (BALLA et al., 2019). Cada fibra é formada por um lúmen central cercado por camadas de fibrilas e microfibrilas. O lúmen, responsável por transportar água e nutrientes na planta, é cercado por uma parede primária, três paredes secundárias (S1, S2 e S3) e uma parede intermediária. A parede primária, inicialmente depositada durante o crescimento das células, consiste em um arranjo desordenado de fibrilas de celulose dispostas em uma matriz de pectina, hemicelulose, lignina e proteínas. As paredes secundárias são compostas por microfibrilas de celulose cristalina organizadas em um arranjo espiral. A camada S2 determina as propriedades mecânicas da fibra e é composta por uma série de microfibrilas celulósicas helicoidalmente orientadas no sentido da fibra e formando um ângulo microfibrilar com o eixo vertical. Este ângulo é característico de cada fibra e tem uma grande influência na rigidez dos materiais (BALLA et al., 2019).

Figura 4: Esquema da estrutura de microfibrilas celulósicas
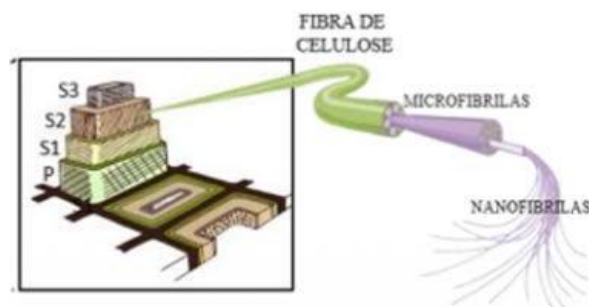
Fonte: Adaptado de BALLA et al. (2019)

As propriedades e características gerais dos biocompósitos produzidos pelas fibras lignocelulósicas são fortemente afetadas por diversos fatores, como por exemplo, características dos constituintes e suas compatibilidades, além da degradabilidade e temperatura máxima permitida ao longo dos processos de fabricação (AL-OQLA; HAYAJNEH; FARES, 2019). Além disso, alguns outros fatores influenciam os produtos finais de base renovável, como fração do volume da fibra, geometria da fibra, comprimento e orientação da fibra (HANAN; JAWAID; MD TAHIR, 2020). No entanto, as características

mecânicas e químicas das fibras naturais são os principais critérios considerados no design dos biocompósitos. Existem fortes relações entre a composição química das fibras naturais e seus desempenhos mecânicos correspondentes (SANYANG et al., 2018). Consequentemente, a maioria dos trabalhos da literatura enfatiza tais variações nas características mecânicas das fibras naturais, pois elas podem determinar o potencial de sua adequação para os compósitos de fibras naturais (ALMAGABLEH; AL-OQLA; OMARI, 2017; FARIS M. AL-OQLA AND MOHD S. SALIT, 2017). As variações dessas propriedades nas fibras naturais geralmente vêm de sua origem, data de cultivo, fertilizantes, solo, idade e clima.

Estudos visando entender o comportamento estrutura-processamento-aplicação dos biocompósitos reforçados com fibras naturais, foram publicados. A maioria deles é baseado em matrizes de biopoliésteres (poliéster biodegradável) (SATHEES KUMAR, 2019). Por exemplo, o polihidroxialcanoatos (PHA) foi combinado com fibras lignocelulósicas (CHAN et al., 2019; DIETRICH et al., 2019; TARRAHI et al., 2019), fibras de juta (KHAN et al., 2016; KHAN; KHAN, 2015), fibras de abacaxi (DILLI BABU et al., 2019; SINGH et al., 2019), fibras de linho (CHENG et al., 2020; MUNIYASAMY et al., 2019) ou farinha lignocelulósica (HASSAINI et al., 2017; YIN et al., 2019). O poli(ácido lático) (PLA) foi associado a resíduos de fibras de papel, farinha de madeira (COLLAZO-BIGLIARDI; ORTEGA-TORO; CHIRALT, 2019; SCAFFARO et al., 2019), kenaf, juta ou fibras de linho. Outras fibras citadas como reforço em biocompósitos são, fibras de bambu (DHAKAL et al., 2018b) e dendezeiro (AMIR et al., 2019; MAHMOOD; HUSSAIN; MOHAMAD, 2019). LE DIGABEL et al.(2004) demonstraram uma boa compatibilidade entre as cargas e a matriz biodegradável sem compatibilizadores ou tratamento especial de cargas. Porém, poucas publicações foram baseadas com matriz de polissacarídeos, como por exemplo, amido plastificados (TPS) ou pectina, justificando assim a elaboração desse trabalho.

2.4.1. Bagaço de malte

Atualmente, ficou provado que as indústrias agro-alimentares são responsáveis pela produção da maior parte do total de resíduos vegetais ou bagaço (cerca de 38 milhões de toneladas) (DI DONATO et al., 2020). Esses resíduos contêm muita água (geralmente acima de 80%), e é facilmente contaminado microbiologicamente. Isso torna o bagaço um produto perecível e implica várias dificuldades em seu armazenamento (NAYAK; BHUSHAN, 2019), principalmente devido ao limitado espaço de armazenamento nas plantas de processamento. Assim, os resíduos das indústrias agro-alimentares parcialmente são reutilizados para

alimentação animal ou por processos de compostagem; porém há maior parte são depositados em aterros ou incinerados, aumentando significativamente a poluição ambiental (CHIANESE et al., 2018; DINOI et al., 2017). Por esse motivo, o desenvolvimento de uma estratégia de reciclagem de resíduos vegetais representa um objetivo muito importante para reduzir a poluição ambiental.

A reciclagem de resíduos lignocelulósicos para produzir produtos comerciais e energia é conhecida como o conceito de bio-refinaria e está rapidamente sendo absorvida na comunidade científica como uma opção sustentável (NAYAK; BHUSHAN, 2019), pois promove a maximização da utilização da biomassa, minimiza os resíduos e agrega valor aos produtos.

As fábricas de cerveja geram o licor de grãos ou bagaço de malte usados como subproduto, juntamente com a produção de itens de cervejaria, como cerveja (SINGH; KAUR; KENNEDY, 2019). O licor de grãos é um resíduo líquido gerado após a separação final do mosto dos grãos gastos. A quantidade de licor de grãos gastos produzida pela indústria cervejeira é uma fonte notável de desperdício.

Segundo Fadel (1999), o resíduo de cervejaria chega a um rendimento de 25% da matéria-prima utilizada na fabricação da cerveja, ou seja, para cada 100 kg de grãos de cevada são gerados 25 kg de matéria seca de mosto ou, de acordo com Portilho (2010), com 100 kg de malte, se produz 120 a 130 kg de bagaço de malte com 80% de umidade. O Brasil é o terceiro maior produtor de cerveja do mundo, com uma produção de 13,3 bilhões de litros, atrás apenas da China (46 bilhões de litros) e dos Estados Unidos (22,1 bilhões de litros) (SINDICERV – NATIONAL SYNDICATE OF BREWING INDUSTRY, 2017).

O bagaço de malte contém materiais orgânicos e sólidos em suspensão. É considerado um material lignocelulósico, contendo aproximadamente 17% de celulose, 28% de lignina (MUSSATTO; DRAGONE; ROBERTO, 2006). Singh, Kaur and Kennedy, (2019) afirmaram em seus estudos que o bagaço de malte consiste em hemicelulose (40% m/v), celulose (12% m/v), amido (2,7% m/v), proteínas (14,2% m/v), lignina (11,5% m/v), lipídios (13% m/v) e cinzas (3,3% m/v). A composição química do bagaço de malte varia de acordo com a variedade de cevada, época da colheita, condições de moagem do malte e a qualidade e tipo de adjuntos (milho, arroz, trigo e sorgo) adicionados no processo de fermentação (SANTOS et al., 2003).

Embora parte do bagaço de malte possa ser usada para processos de biossorção (FONTANA et al., 2016a, 2016b; JUCHEN et al., 2018), o principal destino é na suplementação

nutricional para alimentação animal (AJILA et al., 2012). Porém, o uso incorreto ou sem os devidos cuidados, bem como o armazenamento de forma inadequada, contudo, podem ser responsáveis por quadros de intoxicação por etanol, neurotoxicose por Aspergillus clavatus, acidose ruminal e botulismo (BRUST et al., 2015).

Outra aplicação, que vem ganhando destaque é a utilização do bagaço de malte como fibras para reforço em matriz biopolimérica, como por exemplo, nos estudos de Mello e Mali, (2014), no qual avaliaram a distribuição de fibras de bagaço de malte na matriz de amido de mandioca para a produção de bandejas biodegradáveis e descobriram que a concentração de 15% p/p promoveu a redução inicial da taxa de absorção de água.

2.5.   Processamento de filmes biopoliméricos

Diversas técnicas podem ser empregadas na produção de filmes biodegradáveis, como por exemplo: casting, extrusão, moldagem por injeção ou termoprenssagem, entre outros. A diferença nos métodos de processamento e preparação influencia fortemente as características dos filmes bio-termoplásticos (DE MORAES et al., 2013).

A maioria das pesquisas sobre produção de filmes biodegradáveis e comestíveis utiliza para a produção laboratorial a técnica de casting, que permite a preparação de filmes de pequenas dimensões, e implica em equipamento econômico e espaço limitado (OCHOA-YEPES et al., 2019). A produção de filmes pela técnica clássica de casting (Figura 5) consiste em derramar uma suspensão em pequenas placas (placa de Petri ou poliestireno (PS) ou bandejas de Teflon), controlando a espessura média úmida dos filmes resultantes da massa de suspensão derramada na placa; variações locais são geralmente inevitáveis. Esse processo não é adequado para a formação de filmes muito maiores que 25 a 30 cm, levando a dificuldades de expansão. A maioria dos estudos relata que a secagem com circulação de ar forçada a temperaturas moderadas (30 a 40ºC), exigem tempos de secagem de 10 a 24h (MÜLLER; LAURINDO; YAMASHITA, 2009). Embora o método de casting tenha sido utilizado na maioria das pesquisas sobre filmes à base de amidos, ele apresenta duas desvantagens importantes: (i) a dificuldade em aumentar sua produção para uma maior escala e (ii) os longos tempos de secagem. Essas desvantagens tornam esta técnica impraticável em escala industrial.

Figura 5 - Representação da técnica de casting clássica
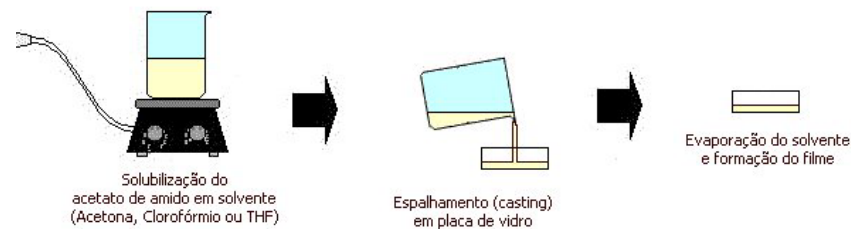
Fonte: Adaptado de Otoni et al. (2017)

Nos últimos anos, a técnica de extrusão (Figura 6) para processamento de filmes biopolímericos começou a ser foco de investigações (DALBHAGAT; MAHATO; MISHRA, 2019). Essa técnica pode representar uma ótima alternativa para satisfazer altas demandas a baixo custo (CHEVALIER et al., 2018; XIE et al., 2017). Na verdade, a extrusão é o processo mais utilizado para obter plásticos industriais em grande volume de produção, pois atinge altas temperaturas em tempos curtos e sua operação é muito simples (VON BORRIES-MEDRANO; JAIME-FONSECA; AGUILAR-MÉNDEZ, 2016). Esse processo envolve energia térmica e mecânica (ALAM; PATHANIA; SHARMA, 2016). As condições operacionais, como perfil de temperatura e velocidade do parafuso, influenciam fortemente nas propriedades finais do material (GONZÁLEZ-SELIGRA et al., 2017). Otoni et al.(2017) usaram extrusão seguida por processo de termocompressão para obter filmes de amido-proteínas com resultados bem-sucedidos. Entretanto, alguns problemas podem ser verificados no processamento e com o filmes final obtido, como por exemplo, altas temperaturas utilizadas durante o processamento podem ocasionar a degradação dos biopolímeros, além disso, é necessário a adição de maior concentração de plastificantes e auxiliares de processamento, proporcionando o aumento da absorção de água e permeabilidade ao vapor de água dos filmes, e por fim, quando ocorre a incorporação de compostos funcionais, como óleos essências e/ou extratos nos filmes, para impedir o crescimento microbiano ou danos oxidativos, a taxa de evaporação desses compostos quando obtidos por extrusão são maiores, reduzindo a eficiência de aplicação desses filmes.

Figura 6 - Representação de uma extrusora
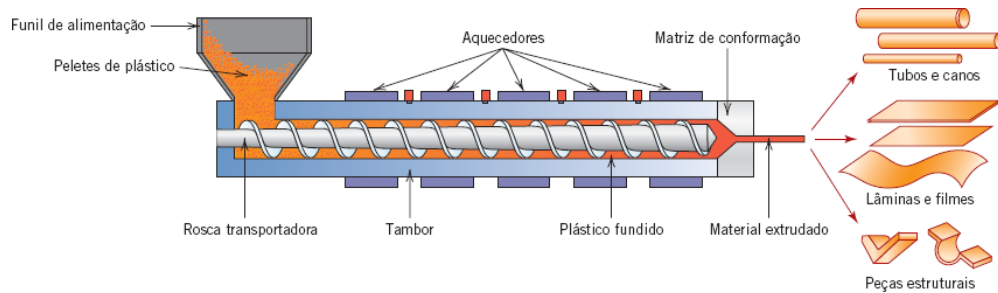
Fonte: Adaptado de Manrich (2005)

Assim, combinando a eficiência do processo de extrusão, em função de alta produção em um curto período com o casting clássico, temos a técnica de casting contínuos (Figura 7), também conhecida como "tape-casting", "spread-casting" ou "knife-coating". A técnica de casting contínuo já é bem conhecida nas indústrias de fabricação de papel, plástico, cerâmica e tinta (TWINAME, RICHARD E. MISTLER, 2000). No processo de casting contínuo, uma suspensão é colocada em um reservatório com uma lâmina, cuja altura pode ser ajustada com parafusos micrométricos (HOTZA, 1997). A suspensão é moldada como uma fina camada em um suporte (fita polimérica), devido ao movimento da fita transportadora (processo contínuo) ou ao movimento da lâmina raspadora (processo em lote) (DE MORAES et al., 2013). O filme formado é seco no suporte, por condução de calor, circulação de ar quente (convecção de calor) e infravermelho, resultando em uma redução de sua espessura. As espessuras dos filmes resultantes geralmente variam entre 20 µm e 1 mm. É possível preparar filmes multicamadas com diferentes suspensões repetindo as etapas de moldagem da fita (TANIMOTO; HAYAKAWA; NEMOTO, 2005). A Figura 7 apresenta um esboço do processo de casting contínuo.

Figura 7 - Representação da técnica casting contínuo
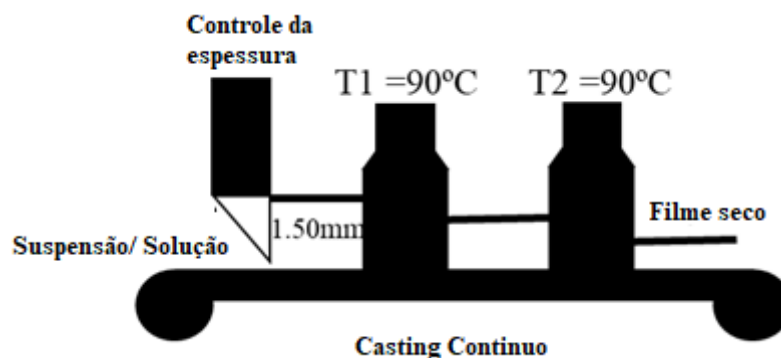
Fonte: Do autor (2020)

Este processo permitiu a redução do tempo de secagem de bioplásticos comestíveis baseados em polpa de tomate para 12 min através do emprego de uma fonte emissora de radiação infravermelha – 4 min – e forno com circulação de ar forçada a 132 ºC – 8 min (DU et al., 2008a). De Moraes et al. (2013) avaliaram a produção de filmes de amido de mandioca pela técnica tape-casting. Eles observaram que os filmes obtidos por tape-casting apresentaram espessura final de 0,118 mm, não eram quebradiços e apresentaram propriedades adequadas, como resistência à tração de aproximadamente 30 MPa. Muller, Yamashita e Laurindo (2008) relataram resultados de filmes de amido preparados por casting clássico, os quais foram secos a 30ºC por 10h e apresentaram à resistência à tração de 20 MPa. Outros trabalhos encontrados na literatura também utilizaram a técnica de casting contínuo para a produção dos bioplásticos, como por exemplo, bioplásticos à base de polpa de maça (DU et al., 2008b), melancia, goiaba e maracujá (MATTOSO, L.H.C.; MOREIRA, F.K.V.; LOREVICE, M.V.; OTONI, C.G.; DE MOURA, M.R.; AZEREDO, 2015), foram produzidos através da mesma abordagem, a qual foi também explorada no procedimento experimental aqui apresentado.

2.6.   Avaliação sensorial de filmes biopoliméricos e aromáticos

A análise sensorial correspondente ao conjunto de respostas transmitidas pelos indivíduos às sensações advindas das reações fisiológicas resultantes de estímulos, gerando a interpretação das propriedades intrínsecas dos produtos (SIDEL; STONE, 1993). Devendo haver, para tanto, contato e interação entre os indivíduos e a matriz que se quer estudar. O estímulo é medido por meio de processos físicos e químicos e as sensações por efeitos psicológicos. Essas sensações produzidas podem dimensionar a intensidade, extensão, duração, qualidade, gosto ou desgosto em relação ao produto avaliado. Nesta avaliação, os indivíduos, por meio dos próprios órgãos sensoriais, numa percepção somato-sensorial, utilizam os sentidos da visão, olfato, audição, tato e paladar (KRISHNA; CIAN; AYDINOĞLU, 2017)

Na última década, houve uma mudança de paradigma para o desenvolvimento de produtos e serviços sensivelmente atraentes e interativos. Geralmente os novos produtos são lançados no mercado após extensas análises sensoriais, nos quais confirma a aceitação e podem determinar compras repetidas. Os estudos demonstram que os consumidores consideram que as características sensoriais são o fator mais importante na escolha dos

alimentos, mas os atributos não sensoriais estão se tornando cada vez mais importante e podem afetar a percepção do sabor. Portanto, a combinação de fatores sensoriais e não sensoriais pode gerar informações mais completas e realistas sobre o comportamento do consumidor nas situações de compra (ASIOLI et al., 2017).

Novas pesquisas ressaltam a importância do "marketing sensorial", no qual incorpora a ideia de que percebemos o mundo por meio dos nossos sentidos, afetando sua percepção, julgamento e comportamento (KRISHNA; SCHWARZ, 2014). De um modo geral, o marketing sensorial pode ser usado para criar noções subconscientes que definem a percepção dos consumidores, julgamento e comportamento. Estrategicamente, o marketing sensorial proporciona uma experiência multi-sensorial aos consumidores com a intenção de criar valor adicional. Os aspectos sensoriais dos produtos e sua apresentação aos consumidores (cheiro, som, toque, gosto ou aparência), individualmente ou por meio de sua interação, moldam a experiência do cliente e a interação entre empresas e consumidores.

Atualmente, várias empresas investem grandes valores no estudo do comportamento de compra dos consumidores e na criação e modificação das embalagens. O objetivo é identificar os atributos das embalagens/rótulos valorizados pelos consumidores, despertar a atenção dos consumidores e maximizar a venda de produtos. O interesse nas pesquisas de embalagens de alimentos aumentou nos últimos anos, devido à sua influência no processo de compra, pois a embalagem funciona como um vendedor silencioso e é o primeiro contato do consumidor com o produto. Avaliar a embalagem é essencial na determinação dos critérios do consumidor para a escolha, decisão de compra e consumo do produto.

A incorporação de aromas – óleos essenciais e/ou extratos – às embalagens melhora a qualidade sensorial do produto (VERMEIREN; DEVLIEGHERE; DEBEVERE, 2002). A liberação controlada dos aromas desejáveis dos materiais plásticos aumenta a percepção do sabor dos alimentos (YILDIRIM et al., 2018). Logo, os compostos voláteis aromáticos têm sido usados pelas indústrias de alimentos como ferramenta para melhorar o aroma e o sabor dos seus produtos, conquistar a preferência dos consumidores e melhorar a imagem da marca.

Soares et al. (2010), objetivando incentivar o consumo de leite fluido, desenvolveram sachês aromáticos, os quais foram incorporados no interior das embalagens de leites integral e desnatados. Os autores avaliaram sensorialmente o novo produto, e os resultados demonstraram maior aceitação para as amostras de leites integral e desnatados com aroma de coco. Silva et al. (2008) desenvolveram e avaliaram embalagens incorporadas com diferentes

níveis (0, 15, 30 e 45%) de aroma de fumaça para salsicha. Na análise sensorial realizada cinco dias após a embalagem das salsichas nos filmes aromáticos foi observada maior aceitação do produto, com relação ao atributo sabor, para as amostras com 30 e 45% de aroma de fumaça. Análise microbiológicas mostraram ainda que os filmes inibiram o desenvolvimento da microbiota do produto, sendo a maior atividade detectada nas concentrações de 15 e 30%. Os autores concluíram que a utilização de embalagem ativa aromática possibilita ao mercado consumidor produtos diferenciados e com qualidade.

Observa-se que a qualidade sensorial dos alimentos armazenados pelos filmes é influenciada pela migração dos componentes ativos presentes na embalagem/filmes para o alimento, assim estudos sobre o potencial de migração e a concentração desses componentes após o processamento é de extrema importância. Para estudar a migração de componentes, é necessários o desenvolvimento e a validação de métodos analíticos que expressem o valor real das medidas qualitativas e quantitativas. A maioria dos estudos envolvendo a determinação de compostos a partir de materiais de embalagem utiliza técnicas instrumentais de separação por cromatografia (RIBEIRO-SANTOS et al., 2017). Sánchez-González et al. (2011) produziram filmes de quitosana com óleo de bergamota e estudaram a liberação de limoneno em simuladores de alimentos (água, etanol 10,50 e 95% v/v). Usando cromatografia, os autores detectaram a presença de limoneno na maioria dos simuladores testados. Também foi observada uma redução da concentração de limoneno no filme durante o armazenamento. Kuorwel et al. (2013) relataram que a temperatura aumentou a taxa de liberação de agentes antimicrobianos de óleo essencial (carvacrol, timol e linalol) do filme à base de amido.

Assim, a migração de componentes ativos encapsulados nos filmes biopoliméricos podem ser influenciados por diversos fatores, como por exemplo, a estrutura alimentar, o nível de hidratação do filme – filmes com elevada absorção de água podem aumentar a taxa de difusão de compostos fenólicos do filme para o alimento (ZINOVIADOU; KOUTSOUMANIS; BILIADERIS, 2009). Além disso, a temperatura é uma variável que influencia significativamente a migração devido a contribuir para uma maior mobilidade das moléculas dos agentes ativos em altas temperaturas (KUORWEL et al., 2013). A migração também pode ser acelerada devido à afinidade/solubilidade química entre o migrante e o simulador/alimento, e devido à espessura do filme (HUANG et al., 2013).

Entretanto, os estudos sobre a aplicação de embalagens aromáticas em alimentos ainda são escassos na literatura, necessitando maiores estudos sobre a forma de aplicação desses filmes. Os consumidores atualmente têm mais opções disponíveis e, portanto, os

produtos devem ser desenvolvidos considerando o que os consumidores querem. Os testes afetivos têm sido referidos como fator chave de sucesso para o desenvolvimento de alimentos mais saudáveis e prioridades para futuras pesquisas. Dessa forma, a utilização destes testes está aumentando entre as empresas que tem conhecimento da sua necessidade para assegurar que sejam atendidas as expectativas do consumidor final (ARES; GIMÉNEZ; GÁMBARO, 2008). Neste contexto, metodologias sensoriais que possam obter respostas diretamente com os consumidores alvos do produto podem ser uma valiosa opção para obtenção de uma caracterização e percepção do produto (CRUZ et al., 2013).

Nos últimos anos a ciência sensorial alcançou considerável progresso devido ao desenvolvimento de novas metodologias e abordagens, principalmente ferramentas complementares aplicadas para identificar atributos relevantes ao consumidor, entender sua percepção e auxiliar na tomada de decisão durante o desenvolvimento de produtos (DECKER; PARK, 2010).

2.6.1. Ferramentas utilizadas em estudo com consumidores

No atual cenário do mercado competitivo, a inovação e a capacidade para gerar novos produtos ou aprimorar os já existentes são fundamentais para as indústrias de alimentos. Não é surpreendente que a compreensão dos mecanismos cognitivos das percepções dos consumidores, especialmente durante os estágios iniciais do desenvolvimento de novos produtos e criação de estratégias de marketing, tem sido extremamente importante na pesquisa de alimentos (CRUZ et al., 2013; DECKER; PARK, 2010; SIDEL; STONE, 1993).

Para desenvolver e lançar um produto e/ou serviço no mercado que alcance o propósito do marketing de atender e satisfazer as necessidades e desejos dos consumidores, é indispensável compreender o comportamento do consumidor, bem como suas razões, influências, intenções e o processo de compra (GARCIA; GONZALEZ; MAUAD, 2010). Existem dois tipos de insights do consumidor que são importantes para o desenvolvimento de novos produtos. O primeiro tipo refere-se à percepção do produto e intenção de compra antes do primeiro teste de compra. Nesta fase, os consumidores não possuem nenhuma experiência sensorial do produto, e dependerão exclusivamente das suas percepções e expectativas para direcionar a decisão de compra. O segundo tipo de insight refere-se ao ajuste do consumidor em relação à percepção da qualidade e intenção de compra após ele experimentarem o produto. O entendimento do primeiro tipo de insight é extremamente importante porque a primeira compra experimental será o primeiro obstáculo do processo de desenvolvimento de

produtos (GRUNERT; GRUNERT, 1995). Além disso, estudos anteriores evidenciaram que o envolvimento dos consumidores como co-criadores no desenvolvimento de novos produtos é positivamente relacionado com a redução de tempo e custos nas etapas de desenvolvimento e comercialização(CRUZ et al., 2013; DECKER; PARK, 2010; GARCIA; GONZALEZ; MAUAD, 2010; GRUNERT; GRUNERT, 1995; SIDEL; STONE, 1993).

Cada vez mais, os pesquisadores sugerem que processos automáticos ou não conscientes podem operar o comportamento do consumidor. Existem diversas abordagens que utilizadas individualmente e/ou combinadas podem auxiliar na compreensão da percepção dos consumidores em relação a produtos alimentícios. Tanto os desenvolvedores quanto os profissionais de marketing podem se beneficiar das interações das metodologias de consumo, mas de diferentes maneiras. Além disso, através do emprego de técnicas sensoriais e de marketing em conjunto, é possível reunir informações mais precisas sobre diferenças individuais e acelerar o processo de desenvolvimento de novos produtos (ASIOLI et al., 2017a).

2.6.2. Técnicas qualitativas

Uma possível abordagem para compreender a percepção e as decisões de compra de produtos alimentícios é através da aplicação de técnicas qualitativas (ROININEN; ARVOLA; LÄHTEENMÄKI, 2006). Essas técnicas envolvem tarefas menos estruturadas do que as abordagens quantitativas, que são baseadas em questionários fixos, permitindo assim uma sondagem profunda do comportamento do consumidor. Estas abordagens são apropriadas para identificar oportunidades de mercado, gerar ideias, explorar e desenvolver novos conceitos e compreender as perspectivas dos consumidores antes da pesquisa quantitativa (VIANA; DOS SANTOS SILVA; TRINDADE, 2014).

Os métodos qualitativos são mais adaptáveis a múltiplas realidades, mais flexíveis às diversas influências e aos diferentes valores que podem ser encontrados e consequentemente, expõem com mais clareza a natureza da troca entre investigador e respondente. Devido a estas características, estes métodos podem ser empregados em uma série de atividades na ciência sensorial e marketing, tais como: avaliação de conceitos de novos produtos e impacto de uma ideia, identificação de valores e linguagem do público-alvo, escolha de nome e embalagem de produtos, identificação de hábitos, atitudes e expectativas em relação à compra de um produto, avaliação de motivações e avaliação de outros fatores não explicitados em métodos estruturados (ASIOLI et al., 2017a).

As técnicas qualitativas têm sido utilizadas principalmente nos primeiros estágios do desenvolvimento de um produto. Quando se trata de uma inovação ou conceito, os métodos qualitativos apresentam-se como ferramentas adequadas e de grande importância para revelar como os consumidores veem e percebem essa inovação ou conceito, pois uma série de preferências e crenças dos consumidores não são verbalizadas em outros tipos de pesquisas (RUGG; MCGEORGE, 1995).

A utilização de vários métodos em conjunto visa gerar uma melhor compreensão do consumidor, já que cada um dos métodos traz as informações acessíveis de uma forma distinta. Como por exemplo de técnicas qualitativas, podem ser citadas as entrevistas de profundidade, grupos focais, técnicas projetivas e pesquisas observacionais (ASIOLI et al., 2017a; CRUZ et al., 2013; DECKER; PARK, 2010; GARCIA; GONZALEZ; MAUAD, 2010).

2.6.3. Entrevista em profundidade: laddering (encadeamento)

A conceitualização da metodologia laddering foi introduzida no campo do marketing e pesquisa de consumidor por Reynolds e Gutman em 1988. O laddering é uma técnica de entrevista em profundidade, utilizada para compreender a forma como os consumidores percebem atributos de produtos em associações significativas a eles próprios (PHILLIPS; REYNOLDS, 2009).

Esta metodologia é baseada na teoria da cadeia de meios-fim, que descreve ligações entre atributos de produtos, com as consequências fornecidas por esses atributos para os consumidores e com os valores pessoais que essas consequências reforçam (REYNOLDS, T. J., & GUTMAN, 1984).

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ajuda-los a conseguir os valores desejados através das consequências ou benefícios do uso do produto (REYNOLDS, T. J., & GUTMAN, 1984).

Esta técnica envolve uma entrevista detalhada e individualizada, cujo objetivo é obter uma rede hierárquica de significados, através de um processo ascendente de questionamentos que começa com perguntas sobre atributos e termina com perguntas para descobrir os níveis mais altos de valores dos consumidores (PHILLIPS; REYNOLDS, 2009). Esta metodologia sugere a existência de passos intermediários que devem ser levados em consideração na explicação de como os valores pessoais se refletem frente à decisão de compra (CARDOSO SOBRINHO et al., 2010). Portanto, o objetivo principal da técnica laddering, é especificar o conteúdo e sequência (estrutura) de resultados originados do comportamento de escolha dos atributos inerentes ao objeto/produto (PHILLIPS; REYNOLDS, 2009; REYNOLDS, T. J., & GUTMAN, 1984; SANTOSA; GUINARD, 2011).

A proposição principal é que o conhecimento dos consumidores quanto ao produto é hierarquicamente organizado por níveis de abstração. Na análise de esquemas mentais, cada nível básico de abstração pode ser dividido em subníveis, levando à formação de diferentes categorias de abstração pelo tomador de decisão (BARRENA; SÁNCHEZ, 2010). Quanto maior o nível de abstração, mais forte e mais direta é a relação entre consumidor-produto (BARRENA; SÁNCHEZ, 2010). Através do entendimento dos links subjetivos entre atributos, consequências e valores, é possível a obtenção de informações sobre quais atributos do produto os consumidores preferem e porque eles os preferem, onde estas associações entre todos os elementos podem ser usadas para criar conteúdo publicitários persuasivos que são significativos para os consumidores (PHILLIPS; REYNOLDS, 2009).

Há duas alternativas principais para aplicação da técnica laddering, o soft e o hard laddering. Enquanto o soft laddering é baseado em uma entrevista pessoal realizada por um entrevistador treinado, o hard laddering baseia-se em um questionário estruturado (RUSSELL et al., 2004).

A soft laddering utiliza o sistema de entrevista semiestruturada e face-a-face. Esta técnica permite uma conversa de fluxo natural, sem muitas interferências e restrições, cujo objetivo é extrair dos entrevistados os elementos que retratam a teoria da cadeia de meios-fim (RUSSELL et al., 2004). Esse tipo de entrevista demanda profissionais extremamente capacitados e treinados, para que sejam evitados alguns problemas que possam surgir durante

a entrevista, tais como: perda do foco e bloqueios. Além de demandar entrevistadores bem treinados, a aplicação da soft laddering é demorada e dispendiosa (RUSSELL et al., 2004).

O termo hard laddering foi usado primeiramente por Grunert e Grunert (1995), que estudaram uma forma de quantificar uma pesquisa de caráter qualitativo, mantendo os fundamentos da teoria da cadeia de meios-fim e da técnica de entrevista tradicional (soft laddering). Esta técnica baseia-se em um questionário estruturado, que foi desenvolvido como uma técnica alternativa para descobrir quantitativamente a cadeia de meios-fim (RUSSELL et al., 2004). O hard laddering foi proposto com o objetivo de superar algumas limitações do soft laddering, como redução de tempo, custo de administração e também para minimizar a influência do entrevistador. Embora o hard laddering apresente menor tempo e custo de administração, em alguns casos os resultados obtidos através do soft laddering podem ser mais precisos (BARRENA; SÁNCHEZ, 2010; CARDOSO SOBRINHO et al., 2010; RUSSELL et al., 2004).

(RUSSELL et al., 2004) ao compararem se a forma de administração do laddering (hard e soft) tem algum efeito nos resultados de pesquisa com consumidores, relataram que ambas as construções resultantes da administração dos dois tipos de laddering foram semelhantes, no entanto o soft laddering forneceu um mapa hierárquico de valor com uma maior riqueza de detalhes. Estes pesquisadores sugeriram que a escolha do tipo de técnica dependerá dos objetivos específicos do pesquisador, e que quanto o objetivo da pesquisa não é explicar um fenômeno complexo de forma detalhada, o hard laddering pode ser considerado uma forma apropriada para entender as motivações dos consumidores na escolha de produtos.

3. Considerações finais

A tendência atual de embalagens sustentáveis de alimentos é baseada no uso de materiais renováveis e recicláveis, redução no consumo de matérias-primas (redução da demanda por combustíveis fósseis) e redução de resíduos, que aumentaram o interesse em embalagens biodegradáveis de alimentos. Propriedades aprimoradas, funcionalidade e opções de custo mais baixo, sem dúvida, aumentarão a aceitação de embalagens biodegradáveis nos atuais mercado.

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SECOND PART – ARTICLE

ARTICLE 1 - Characterization of pectin films integrated with cocoa butter by continuous casting: Physical, thermal and barrier properties
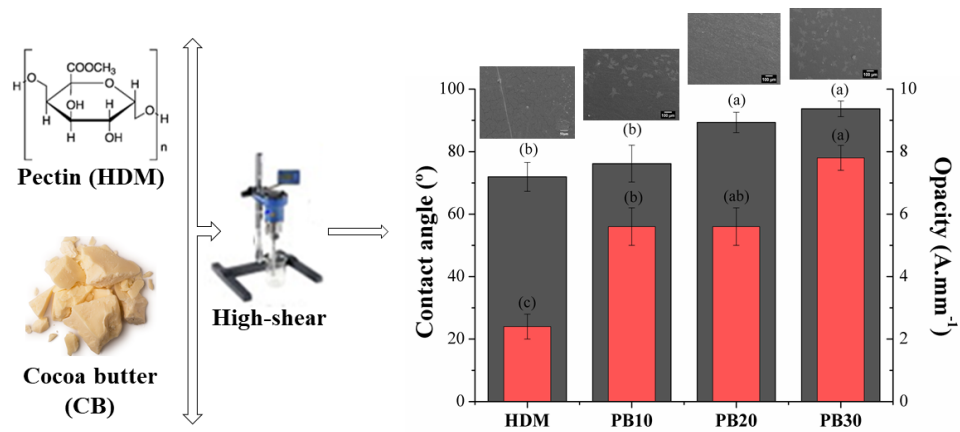
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"Versão Preliminar"

Characterization of pectin films integrated with cocoa butter by continuous *casting*: Physical, thermal and barrier properties

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Abstract

The properties of the mixtures of fatty acids obtained by adding different concentrations of cocoa butter (CB) to a pectin matrix (HDM) were studied. The influence of the CB concentration on the optical properties, mechanical properties, barrier properties, surface properties, microstructure and thermal stability of pectin polysaccharide films were determined. The addition of the CB affected the main properties of the films, with the mechanical strength, transparency and water absorption being reduced. The addition of cocoa butter led to opaque films with a heterogeneous microstructure, resulting in an increase in the film opacity. The surface hydrophilicity of the pectin films was reduced following the addition of the CB. The degree of the pectin crystallinity increased with the addition of the butter, making the HDM/CB films more thermally stable and shiny. The new materials obtained present great potential for the production of edible films or coatings for moisture-sensitive food applications.

Keywords: Edible emulsified films; Cocoa Butter; Barrier properties; Contact angle.

1. **Introduction**

Food industries regularly face challenges related to preserving the properties of processed products. One way to preserve the characteristics of food products is to develop protection systems such as packaging, film and coating and is an ongoing study area (Thakhiew et al., 2010).

Edible coating may be prepared from protein, polysaccharides, lipids and combination of edible constituents. Films based on polysaccharides, such as pectin, exhibit good mechanical properties and act as an effective barrier against gases, increasing the shelf life of food without creating anaerobic conditions (Jiménez et al., 2010). Due to its low cost, availability, biodegradability, and feasibility to film production alone or in combination with other polymeric matrixes, pectin incorporated edible films are an environmentally friendly and an alternative to petroleum-based polymers (Dash et al., 2019). However, as a consequence of their hydrophilic and hygroscopic nature, polysaccharide-based films constitute a poor moisture barrier (Jiménez et al., 2010; Liu et al., 2016). Improving water resistance properties of edible films has been of relevance to numerous researches. Yang and Paulson (2000) produced pectin–soy flour films in the presence of the enzyme transglutaminase, which exhibited a permeability to oxygen and carbon dioxide even lower than that possessed by polyethylene films. Batista et al. (2005) produced pectin-based films added with 6, 12 and 18% of lauric, palmitic and stearic acids and the mixture of these. They observed that the WVP in general increased with increasing amount of fatty acid, thus the films were unable to form a cohesive and continuous matrix.

Among the lipid materials investigated, waxes and long-chain saturated fatty acids and fatty alcohols were most able to effectively improve the moisture barrier properties of hydrophilic films. Cocoa kernels are typically consumed in the human diet and used

industrially; indeed, they is often used as ingredients in a wide variety of processed foods,(Battegazzore et al., 2014) for example in obtaining cocoa butter. Cocoa butter (CB) is edible fat of naturally occurring, it consists of a mixture of triacylglycerides (TAGs) and is one of the major ingredients of chocolate. Although CB is widely used in the confectionery industry, its effects of plasticizers and hydrophobic constituents on film properties have not been tested, so it is suggested that these constituents be added to optimize mechanical and moisture barrier properties. In addition, replacing glycerol with a higher molecular weight plasticizer, such as cocoa butter, may slow diffusion (Karbowiak et al., 2006) and improve the stability of biopolymer films.

CB lipophilic, but it is not expected to form tough, free-standing films. Such shortcoming of cocoa butter may be overcome by the addition of pectin – a water soluble polysaccharide – which is largely know for its excellent film-forming properties (Espitia et al., 2014). In this context, the present study analysed the influence of the fatty acid composition, obtained by the incorporation of CB, on the microstructural properties of film dispersions relative to pectin control films, as well as its impact on the relevant properties of the film, which ensure their suitability for coating and/or food packaging, such as water vapor barrier, mechanical, thermal and optical properties.

2. Materials and methods

2.1. Materials

HDM pectin (DM > 50% and Mw = 130.000 g.mol^{-1}) was kindly provided by CP Kelco (Limeira, Brazil). The lipid phase was CB that was obtained from a local market. The films were plasticized by vegetable glycerine from Synth (Rio de Janeiro, Brazil) and ultrapure water from a Milli-Q system (Millipore Inc., USA) was used.

2.2. Preparation of films: formulation and processing

HDM and HDM / CB films were prepared by an aqueous dispersion of pectin (6% w / v), glycerol (20% by weight) and CB (10-30% w / v), respectively. The previously prepared solution was homogenized (7000 rpm / 45 min) (Lara-Espinoza et al., 2018; Lorevice et al., 2016; Otoni et al., 2014; Peretto et al., 2014; Ravishankar et al., 2009; Rojas-Graü et al., 2006; Viana et al., 2018) degassed and film-cast on a KTF-SB continuous lamination system (Mathis, Switzerland). Dry films were equilibrated at $52 \pm 3\%$ relative humidity (RH) and $25 \pm 2\,^\circ C$ and characterized as described previously (Mendes et al., 2020).

2.3. Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR measurements were obtained using a FITR model Vertex 70 Bruker spectrophotometer (Bruker, Germany) equipped with a single reflection diamond crystal ATR module. Spectra were recorded at a spectral range between 400 and 4000 cm^{-1} at a scan rate of 32 scans and a spectral resolution of 4 cm^{-1}.

2.4. X-ray diffraction (XRD)

The crystal structures of the pectin and pectin/cocoa butter were analysed from diffraction patterns obtained on a model XRD-6000 Shimadzu X-ray diffractometer (Shimadzu, Kyoto, Japan). Samples were scanned from 5 to 40 (2Ө) using a scan rate of $1^\circ.min^{-1}$.

2.5. Scanning electron microscopy (SEM) analyses

A qualitative evaluation of the degree of mixing (distribution and dispersion of the CB phase in pectin) was performed by using a model JSM 6510 JEOL SEM (Japan) operating at 5 kV. Samples were mounted with carbon tape on aluminium stubs. All specimens were sputter-coated with gold in a sputter coater (Balzer, SCD 050).

2.6.   Thermogravimetry

The film samples (5-6 mg) were accurately weighed in a platinum pan and heated from 25 to 600ºC at a rate of 10ºC.min^{-1} within an atmosphere comprising synthetic air (21% O_2) flowing at 40 mL.min^{-1}.

2.7.   Mechanical properties

The tensile strength, maximum elongation at break and elastic modulus were measured using a model DL3000 universal testing machine (EMIC, São Paulo, Brazil). Tests were carried out according to ASTM D882-09.("ASTM D882-09, Standard Test Method for Tensile Properties of Thin Plastic Sheeting.," 2009) Test samples with a mid-section 15 mm wide, 100 mm long and 0.8 mm thick were cut from the casting films. At least ten samples were tested for each composition. The clamp-to-clamp distance, test speed and load cell capacity were 100 mm, 10 mm.min^{-1} and 10 kgf, respectively. The tensile strength (σ_{max}) was calculated by dividing the maximum force by the cross-sectional area, and the percent elongation (ε) was calculated as follows:

$$e\left(\%\right) = \frac{d - d_0}{d_0} \cdot 100 \qquad (1)$$

where d is the final displacement and d_0 is the initial displacement (clamp-to-clamp distance). The elastic modulus (E) was determined from the linear slope of the stress *versus* strain curves. The film thickness was measured using a digital micrometer IP65 MITUTOYO (Japan) at five random positions around the film.

2.8.   Water vapor permeability (WVP)

A gravimetric method obtained by the modification of the ASTM E96-16(ASTM E96, 2016) and Otoni.(Otoni et al., 2014) Eight weights were periodically taken for each test cell

after steady state was reached. Five replications were used to calculate water vapor permeability (*WVP*) and water vapor transmission rate (*WVTR*).

2.9. Contact angle measurements

The contact angle determinations were performed with 5 replicates as per ASTM D5725-99 (2008).(ASTM, 2008) The contact angle was determined by a CAM 101 Optical Contact Angle Meter (KSV Instruments) equipped with a CCD KSV-5000 digital camera.

2.10. Optical properties - colors

Luminosity (*L**) and the chromatic coordinates *a** and *b** were determined and used to calculate total color difference (*ΔE**), yellowness index (*YI*), and whiteness index (*WI*) were determined with a Konica Minolta CM-5 (Minolta Camera Co., Ltd, Osaka, Japan), the accord in Mendes.(Mendes et al., 2020)

2.11. Statistical analysis

The data were submitted to analysis of variance (ANOVA) and Tukey's test, both at 5% significance (p = 0.05), in the Sisvar® software, version 5.4.

3. Results and discussion

3.1. Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectroscopy was used to elucidate possible interactions among the film-forming components. The spectra are shown in Figure 1.
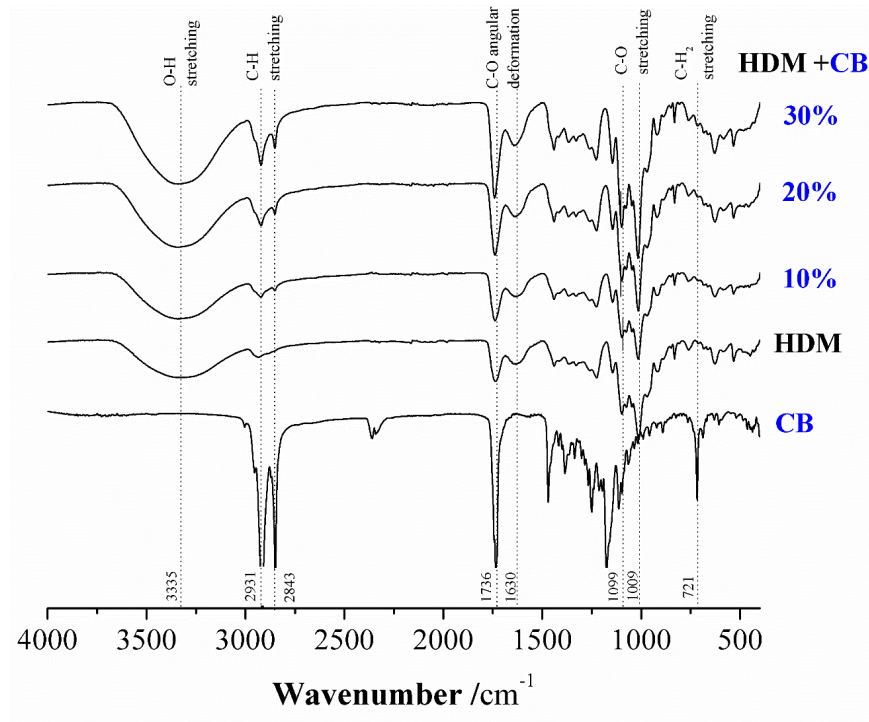
Figure 1: Attenuated total reflectance Fourier-transform infrared spectra of films comprising pectin (HDM), and cocoa butter (CB; 10-30 wt.%). Y-axis: reflectance/a.u.

The FTIR spectrum of CB (Fig.1) shows absorption bands at 2931 and 2843 cm^{-1}, corresponding to the stretching vibrations of symmetric and asymmetric $–CH_2$ groups, respectively. The band at 1747 cm^{-1} corresponds to the stretching vibration of the carbonyl groups (C=O) of triglyceride esters. The band at 1466 cm^{-1} refers to the bending vibration of $–CH_2$ and $–CH_3$ aliphatic groups. The band at 1385 cm^{-1} corresponds to the symmetrical bending vibration of $–CH_3$. The bands at 1254 and 1163 cm^{-1} refer to the stretching of the $–CO$ groups of esters. The band at 1093 cm^{-1} refers to the change in fatty acids by the bending vibration of the $–CH$ groups. Lastly, the band at 711 cm^{-1} corresponds to the oscillation of the $–CH_2$ groups and olefins. These results are in agreement with Goodacre and Anklam (2001), Lo et al. (2013) and Sri Rahayu et al. (2015).

The HDM has a broad absorption band at *ca.* 3335 cm^{-1}, related to the stretching vibration of –OH (Nisar et al., 2018). The band at *ca.* 2931 cm^{-1} refers to the vibrations of the –CH methylene groups of the polymer chains and the methyl group of the methyl ester (Pasini Cabello et al., 2015). In addition, the bands at 1737 and 1223 cm^{-1} are attributed to the -CO groups and -CO of ester bonds, respectively. The bands at 1113 and 1012 cm^{-1} are attributed to the -COC stretching vibrations of the structure. Additionally, two bands at 1635 and 1441 cm^{-1} are attributed to the asymmetric and symmetric stretching vibrations, respectively, of the carboxylic groups present in the pectin (Nisar et al., 2018; Pasini Cabello et al., 2015).

The spectral differences between the different film samples were largely attributed to the changes in the conformation and orientation of the polypeptide chains, affected by the incorporation of CB, as observed by the decrease in the band of –OH stretching (in the 3500-3200 cm^{-1} range), which is related to the reduction of the hydroxyl groups of the HDM after reaction with the anhydrides of fatty acids. The other bands of the films with CB added were similar to those of the HDM film, but the band ranges varied (Fig. 1).

3.2. X-ray diffraction (XRD)

The X-ray diffraction patterns of the HDM-CB films with different concentrations of CB are shown in Figure 2.
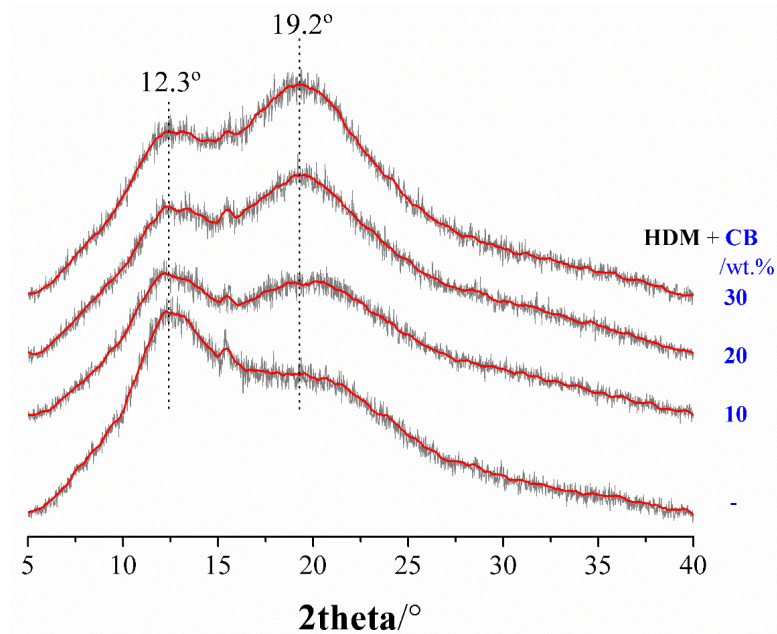
Figure 2. X-ray diffraction patterns of pectin (HMD)-based films containing cocoa butter (CB; 10-30 wt.%). Y-axis: intensity/a.u.; gray curve: actual data – used for calculations; red curve: smoothed – used for representation only.

Peaks were reported at 12.7° and 20.5° (2Θ), associated with the crystallinity of HDM (Nisar et al., 2018). In the present study, the pectin films exhibited a defined peak at 12.3° (2Θ), confirming the presence of a crystalline structure (Chaichi et al., 2017) (Fig. 2). The inclusion of CB in the HDM films did not significantly change the peak positions in the XRD pattern. All the HDM/CB films preserved this XRD (Fig. 2) suggesting that the modification occurred predominantly on the outmost and amorphous regions without affecting the internal structure of the films (Tomé et al., 2011).

The diffractograms of these films suggest a semi-crystalline structure and indicate a combination of crystalline peaks with an amorphous halo (Chaichi et al., 2017). The illustrated diffractograms of the films propose good compatibility between the pectin and cocoa butter because the basic crystalline fraction of the pectin matrix was not decreased (Chaichi et al., 2017).

3.3. SEM characterization

The microstructure of the films HDM containing CB was studied to obtain a clarification of the organization of the butter droplets along the matrix of the biopolymer film and its possible influences on the mechanical and barrier properties of the films (Nisar et al., 2018). Figure 3 show micrographs corresponding to the surfaces and cross-sections of the films.
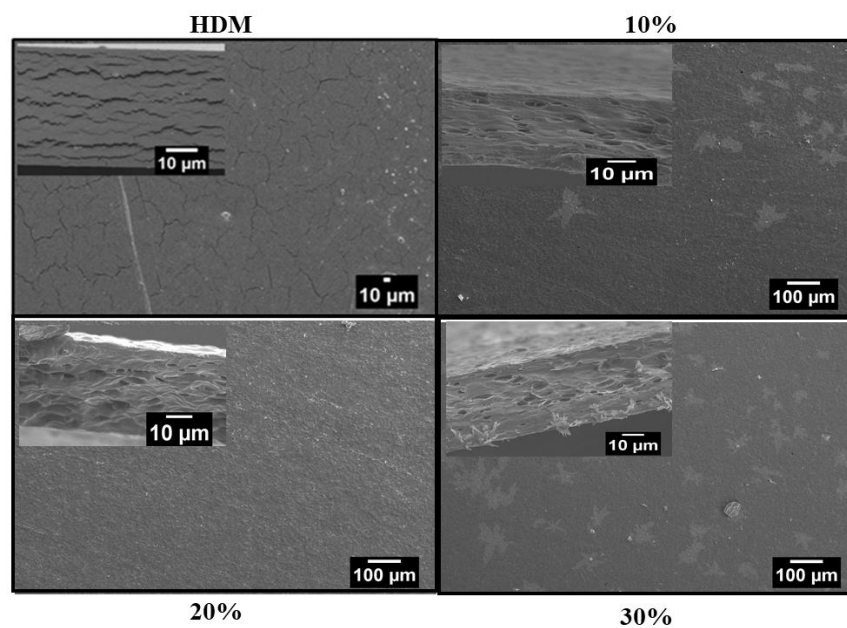

Figure 3. SEM micrographs of the surface and cross-sections of the films: pectin (HDM) and films containing different percentages of cocoa butter (10-30% CB).

In the HDM, a homogeneous aspect was observed, but the matrix did not present a continuous phase, presenting cracks. Compared with the pure pectin film the HDM/CB films showed remarkable differences in surface microstructures, showing discontinuities of different shapes and sizes due to the presence of the fatty acids. An increase in the CB content in the film led to appreciable changes in surface topography, revealing a different amount of lipid droplets on the surface and, particularly for the PB30 film, a larger droplet size, which coincides with the higher lipid concentration. In addition, at the concentration of 20% w/w of

CB the lipids appear finely distributed in the polymer matrix, although the formation of two distinct phases was evident (Batista et al., 2005). This finding seems to indicate that specific polymer-lipid interactions occur that inhibit lipid micellar growth, both in the initial solutions, promoting the dispersion of CB by the action of Turrax and high rotation which provided sufficient energy for the formation of small globules invisible to the naked eye at the concentrations studied e and during film drying carried out in a continuous manner, which probably avoided a greater separation of phases (Manrich et al., 2017). A similar result was observed by Jiménez et al. (2010) however the separation and non-incorporation of the lipids into the filmogenic matrix homogeneously was observed by Batista et al. (2005) in which the drying took place by batch casting.

Figure 3 shows SEM micrographs of the cross-sections of the films with the presence of lipid droplets (or their voids that originated during the cryogenic fracture of the film). A higher proportion of drops can be seen in the upper part of the films than in the lower part (higher number of voids), and this may be due to the occurrence of a possible creaming formation during film drying (Nisar et al., 2018; Vargas et al., 2009). In addition, the cocoa butter droplets dispersed homogeneously on the matrix surface, forming a nearly continuous layer, which may explain the exceptional brightness of these films and the reduction in the contact angle values.

3.4. Thermogravimetry

Thermogravimetric (TG) curves and their first derivatives (DTG) for HDM-based films are shown in Figure 4. The temperatures corresponding to the onset (T_{onset}) and offset (T_{offset}) of the thermal degradation are shown in Table 1.
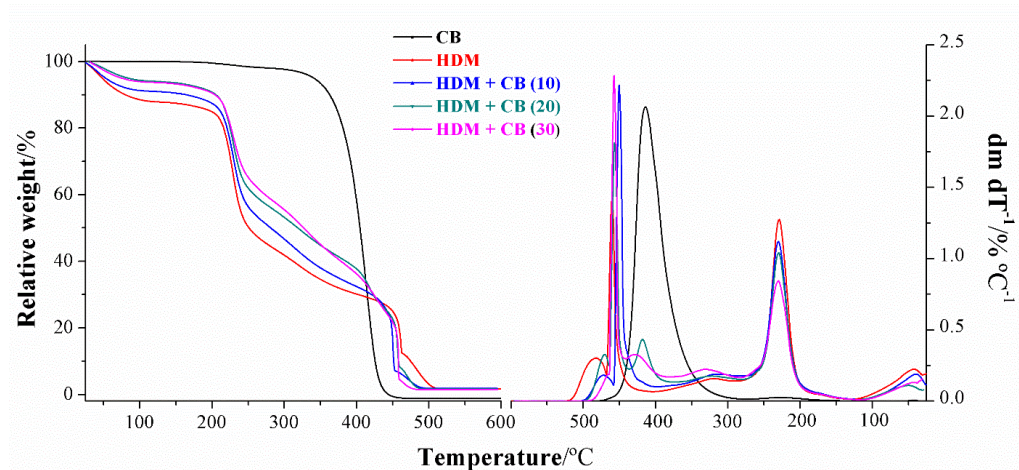
Figure 4. Thermogravimetric (TG; left) and derivative TG (right) profiles of films comprising pectin (HDM) and cocoa butter (CB). Numbers in parentheses correspond to weight percentages of CB.

Table 1. Thermogravimetric analysis parameters of plasticized pectin and CB/pectin films.

Sample	MC (%)	Tonset1 ºC	Tpeak1 ºC	Tonset2 ºC	Tpeak2 ºC	(%)Residue at 600 °C
HDM	26	212	228	457	460	2.2
PB10	24	210	230	451	451	2.3
PB20	6	220	230	458	458	2.3
PB30	6	216	230	457	458	1.3

Based on the analysis of the curves related to the HDM and HDM/CB films, there are four different well-defined stages of mass loss. The initial stage that appears in all TG curves, from room temperature to approximately 150 ºC, can be attributed to the elimination of water adsorbed onto the matrix of the films (Bodin et al., 2007; Chaichi et al., 2017; Manrich et al., 2017). However, it was observed that with the increase in the percentage of CB added to the

matrix, there was a reduction in the hydrophilicity of the films, as shown in Table 1 and by the contact angle values (Fig. 5). This reduction in the amount of adsorbed water in the films is due to the reduction in the number of hydroxyl groups due to the interaction with CB, which is a lipid. These results are in accordance with the ATR-FTIR analysis previously described in Fig. 1.

The HDM/CB ratio significantly influenced the Tonset values of the films. This parameter corresponds to the beginning of the thermal degradation of the pectin fraction and of the CB (Fig. 4(a)).

Figure 4 showed that the intensity of the third stage of mass loss (Tonset2) at 360-480 ℃ did not alter the degradation with the increase in the HDM/CB ratio. The other thermal degradation stages that occurred at higher temperatures (T > 485 ℃) represent combustion reactions triggered by oxygen.

In general, the data presented in Figure 4 and Table 1 suggest that the addition of CB had an influence on the thermal profile of the hydrophobic films, conferring better thermal stability to the films.

3.5. Mechanical properties

The HDM/CB films were characterized with regard to their mechanical properties. The results summarized in Table 2 correspond to the thickness (t), tensile strength (σ_T), elastic modulus (E) and elongation at break (ε_B).

Table 2. Thickness (t), mechanical (σ_T: tensile strength, ε_B: elongation at break, E: elastic modulus) and barrier properties (water vapor permeability - WVP and water vapor transmission rate - WVTR) and relative humidity (RH) of CB/pectin films.

Sample	T (mm)	σ_T (MPa)	ε_B (%)	E (GPa)	RH (%)	WVP (g.mm.KPa^{-1}.h^{-1}.m^{-2})	WVTR (g h^{-1}m^{-2})
HDM	0.04[b]	37±2[a]	2.1±0.3[a]	0.9±0.3[a]	28.3±3.2[NS]	2.0±0.2 [a]	87±8 [a]
PB10	0.04[b]	20±2[b]	2.2±0.4[a]	1.2±0.2[a]	27.8±2.2	1.7±0.3 [a]	87±6 [a]
PB20	0.05[a]	19±4[b]	1.9±0.6[a]	1.1±0.1[a]	27.8±2.2	1.4±0.4 [ab]	77±12 [a]
PB30	0.06[a]	17±1[b]	2.2±0.2[a]	1.1±0.1[a]	26.7±2.2	0.7±0,1 [b]	41±4 [a]

[a-b] Mean values ± standard deviations followed by equal superscript letters within the same column are not different ($p>0.05$).

[NS] No significant difference ($p>0.05$) was observed within the same column.

The addition of CB to the polymer matrix at concentrations above 10% w/w significantly affected ($p<0.05$) the film thickness (Table 2). This is attributable to the increase in the content of solids in the solutions forming the films, due to the addition of CB, leading to thicker dry films. The same effect was observed by Lorevice et al. (2016). The films with added CB presented mechanical behaviour similar to that of HDM films. In the range studied, the cocoa butter presented a slight trend towards reduced σ_T, mainly when the CB was added at concentrations higher than 20%. Thus, the addition of fatty acids resulted in less rigid structures due to the weakening of the intermolecular forces between the chains of macromolecules, increasing the free volume and causing a reduction in the mechanical strength (Fadini et al., 2013; Fakhouri et al., 2018b). Moreover, the addition of fatty acids caused a decrease in the elasticity and tensile strength due to the introduction of

discontinuities into the polymer matrix, as seen in the SEM images (Fig. 3). A similar effect was observed when other lipid components (essential oil) were added to the polysaccharide films (Sánchez-González et al., 2009).

Although the results of tensile strength (σT) obtained in this study were lower than those reported in the literature for pectin-based films,(Manrich et al., 2017; Otoni et al., 2014) it has been found that these values are superior to studies that produced biodegradable films incorporated with fatty acids and waxes, such as Dangaran and Krochta (2007) that produced serum protein films. Fadini et al. (2013) produced collagen films with sucrose plasticized cocoa butter exhibited a tensile strength ranging from 2-4 MPa. Al-Hassan and Norziah (2012) reported that edible sago-gelatin edible films plasticized with glycerol exhibited a tensile strength ranging from 1.28 to 1.67 MPa.

However, no significant differences were observed between the ε_B and E values. This is because the CB did not present a sufficient plasticizing behaviour to promote an increase in the film deformation, as it is a long-chain hydrocarbon (Andreuccetti et al., 2009).

Overall, the σ_T of films obtained in this study was comparable to the σ_T of some commercial packagings such as PVC (19MPa) (Al Naim et al., 2017).

3.6. Water vapor permeability (WVP)

The WVP values of the HDM and HDM/CB films are presented in Table 2. No change ($p>0.05$) was observed in the RH of the films (Table 2). The migration of water vapor through a film is driven by the difference in RH between the two sides, so it is important to keep the RH differentials as uniform as possible to compare the WVP values of different films. In all samples, water vapor migrated from an environment with the same RH ($p>0.05$) (Table 2) to the same environment (0% RH), ensuring that the driving forces for the migration of water vapor were constant, allowing adequate comparison of the WVP values.

The WVP values ranged from 0.7 and 2.0 g.mm.kPa^{-1}.h^{-1}.m^{-2}. WVP of composite films comprised of biopolymers and lipids strongly depend on the type, structure and quantity of the lipids (Yang and Paulson, 2000).

The incorporation of CB caused a reduction in WVP values of pectin films. This reduction was more pronounced with 30 % w/w CB, as CB has low affinity for water. Second Rezvani et al. (2013) water vapor permeates through the hydrophilic portion of the films; Depending on the ratio of hydrophobic to hydrophilic fraction, the presence of fatty acids in the film may decrease WVP. In addition to the low water affinity of CB, its homogeneous distribution in the HDM matrix reduced water diffusion rates (Table 2) and increased film water resistance. WVP of these films based on fatty acids can be compared with those obtained for gellan and palmitic acid and beeswax films (Yang and Paulson, 2000) whose values are 1.6 and 1.4 gmm / m²h.kPa, respectively.

3.7. Contact angle measurements

Figure 5 shows the mean contact angle values for films produced by continuous casting.
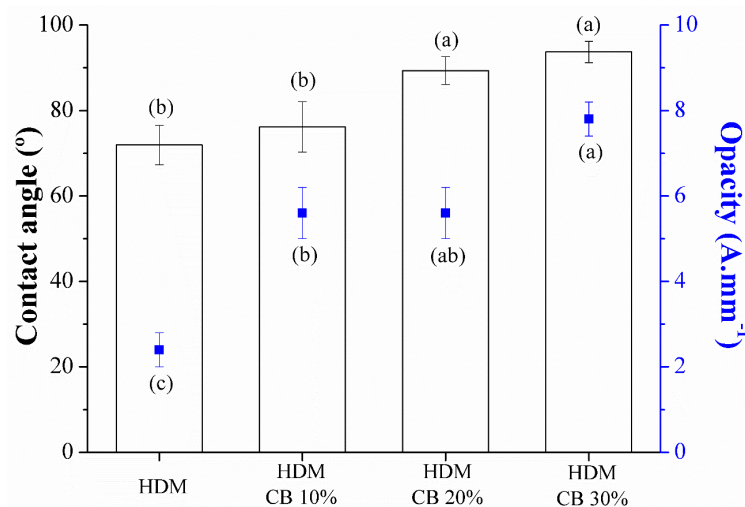

Figure 5. Contact angle and opacity of films based on pectin (HDM), either added with cocoa butter (CB). Contents are weight percentages. Equal letters in parentheses indicate equal

contact angle (above) and opacity (below) values (p>0.05). Samples conditioned at 50% RH and 25 ° ± 2 ° C.

The contact angle is an evaluation of the affinity between the solvent and the surface of the material, which is generally used to assess the wettability of the films. Normally, the film surface is considered hydrophilic when the contact angle is below 65° (Shankar et al., 2016; Vogler, 1998). Therefore, it is expected that higher CB in the HDM lead to higher contact angles between water drops and film surfaces, being hydrophobicity driven by film composition (Pereda et al., 2012). Ferreira et al. (2014) verified increased contact angle values when higher amounts of hydrophobic compounds (grape pomace wax and oil) were incorporated into chitosan films, whereas decreased values were observed when a polar (polysaccharide-based) extract was added. Similar results were reported by Ferreira et al. (2014) when casting chitosan films incorporated by cinnamon essential oil.

The angle of the HDM film was 71.9°, which increased significantly to 93.4° after increasing the concentration of CB. According to Fig. 6, the addition of CB at concentrations of 10%, 20% and 30% caused increases in the contact angle of approximately 5%, 20% and 29%, respectively, which is due to the hydrophobic nature of the added lipid. Therefore, it is observed that higher cocoa butter contents in the HDM matrix led to higher contact angles between the water droplets and the surface of the films, making the films hydrophobic, with the hydrophobicity guided by the composition of the film (Manrich et al., 2017).

These results are in agreement with the SEM (Fig. 3), ATR-FTIR (Fig. 1) and WVP (Table 2) analyses, which revealed a homogeneous distribution and a uniform CB dispersion inside the film matrix. This led to high contact angle values found on the surfaces of films. Similar behaviours were found by Ferreira et al. (2014) who observed increased contact angle values when higher amounts and hydrophobic compounds (grape marc wax and oil) were incorporated into chitosan films, while lower values were observed when a polar

(polysaccharide-based) extract was added. Other studies found similar results, such as in films prepared with the incorporation of cutin extracted from tomatoes,(Manrich et al., 2017) olive oil (Pereda et al., 2012) and cinnamaldehyde essential oil (Ojagh et al., 2010).

3.8. Optical properties

The colour parameters and absorbance measurements normalized by the thickness of the HDM films with and without CB are shown in Table 3 and Figure 5.

Table 3. Optical properties of pectin and CB/pectin films.

Films	L^*	a^*	b^*	ΔE^*	Opacity (A.mm^{-1})
HDM	91.5±0.3[c]	-0.6±0.02 [a]	4,5±0.6[b]	7.9±0.6[a]	2.4±0.4[c]
PB10	92.8±0.1[b]	-0.4±0.01[d]	4.5±0.2[b]	6.9±0.2[ab]	5.6±0.6[b]
PB20	93.5±0.5[a]	-0.4±0.01[c]	5.4±0.4[b]	7.1±0.6[b]	5.6±0.6[ab]
PB30	92.5±0.2[b]	-0.5±0.02[b]	7.1±0.4[a]	9.1±0.4[a]	7.8±0.4[a]

[a-b] Mean values ± standard deviations followed by equal superscript letters within the same column are not different (p>0.05).

The HDM films were lighter and more transparent. The addition of CB significantly affected the colour and opacity of the HDM films (Table 3). These results corroborate the studies of Ferreira et al. (2014) and Silva et al. (2018) who found that the brightness is directly related to the surface roughness, while the transparency depends on the internal microstructure of the matrix and the distribution of the components.

Some authors have indicated that the addition of lipids enhances the opacity of films, making them less transparent. Opacity is particularly important if the film is to be used as a food coating or food packaging. Gontard, Nathalie Guilbert, Stéphane Cuq, (1992) Low opacity values indicate a transparent film. Silva et al. (2018) observed that film opacity depends on film thickness; film opacity increases with increasing thickness, and in fact, the

sample with the lowest opacity was the sample with the lowest thickness (Table 2 and 3). In addition, the increase in film opacity probably arose from light scattering from lipid droplets which were dispersed in the emulsion and continuously distributed throughout the polymer network after the film formed, as can be seen in SEM images (Fig, 3)(Yang and Paulson, 2000). The increase in opacity in a film favors its barrier to prevent light-induced oxidative deterioration when applied in food products, avoiding nutrient losses, discoloration, and off-flavors (Souza et al., 2015).

The colour measurements of the films are provided in Table 3. The film colour was evaluated in terms of the L^*, a and b factors as well as the colour difference (ΔE^*). The L^* values for the pectin films and their composite films ranged from 91.5 to 92.5 (Table 3). The incorporation of cocoa butter in all combinations significantly increased the L^* value. The a^* values became significantly negative when the fatty acid was added, which means that the CB decreases the redness. The b^* and ΔE^* values were higher in comparison to those of the pure pectin film (p<0.05), which means that the films became more yellowish, presenting opaque characteristics (Table 3).

4. Conclusion

The properties of the HDM films were strongly affected by the incorporation of CB, and a close correlation was observed between the microstructure developed in the films and the physical properties. This microstructure was, in turn, greatly affected by the size/shape of the lipid aggregates during film drying. The incorporation of CB improved the moisture barrier properties and the thermal stability of the films but resulted in more fragile films with increased brightness and opacity. Therefore, the pectin films with CB exhibited properties suitable for food coating or packaging.

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ARTICLE 2 - Development, physical-chemical properties and photodegradation of pectin film reinforced with malt bagasse fibers by continuous casting

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Development, physical-chemical properties and photodegradation of pectin film reinforced with malt bagasse fibers by continuous *casting*

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Abstract

This work aims to develop composite films from renewable sources and to use a residue from the brewing industry, malt bagasse, as a reinforcing agent. The composites were prepared with 0 to 15% w/w of malt bagasse, using the continuous *casting* process. All films were characterized in terms of their microstructure, physicochemical, mechanical and photodegradation properties. The properties of the films were influenced by the concentration variation of the malt bagasse fiber, allowing the deformation of the films to increase by approximately 57%. The incorporation of 15% w/w of malt bagasse provided a reduction in the water vapor transmission rate (WVTR) and in the hygroscopicity of the pectin films. However, the fibers acted as UV light blockers, making it difficult to photodegradate the films. Therefore, the composite films based on pectin and malt bagasse presented adequate properties to be used in the packaging industry.

Keywords: biodegradable films; by-product of the brewing industry; continuous *casting*.

1. Introduction

Several biopolymers have been explored to develop environmentally friendly packaging materials, [1–3] mainly due to interest in minimizing the ecological impact of the use of synthetic packaging materials.

Biodegradability, abundance and renewable in naturally occurring sources and of low toxicity are positive features found in pectin, a biopolymer frequently used to produce packaging materials.[4–6] Pectin is a plant cell wall polysaccharide rich in d-galacturonic acid, which is mainly obtained from food processing industry waste (citric or apple peels).[7] Therefore, its production does not compete on land for food farming, such as bioplastics obtained from the fermentation of starch or glucose, which growth can even be associated with considerable environmental burdens. Even though presenting inferior mechanical properties than other polymers of renewable origin, pectin is easily biodegradable, unlike polylactic acid (PLA), for example, which requires specific composting conditions.[8] Pectin films act as a good oxygen barrier and have good hardness and adhesiveness.[7,9] Due to its low cost, availability, biodegradability, and feasibility to film production alone or in combination with other polymeric matrixes, pectin incorporated edible films are an environmentally friendly and an alternative to petroleum-based polymers.[10] However, some limitations need to be solved, such as brittleness, rigidity, high water sensitivity and poor water barrier of pectin films.[2,3,11] A blend of pectin and other ingredients may improve the pectin-based film properties and thus be more suitable for food application.

The use of lignocellulosic fibers obtained of agroindustrial residues for reinforcement in biodegradable pectin matrices is interesting because it may reduce rigidity, improve gas barriers, tensile strength and biodegradability of the material and minimize environmental impacts, besides adding value to the agroindustrial chain.[12–14] Although several studies have

shown that the use of natural fibers improves the physical and mechanical properties of packaging based on starch,[15–17] polylactic acid (PLA),[18,19] poly(ε-caprolactone) (PCL),[19,20] or polyvinyl alcohol (PVA),[21] no studies of natural fibers based on agroindustrial residues and with pectin as a material for the composite matrix have been reported. According to Chaichi[22] the pectin films added of natural fibers can show improvements in their properties due to the structural similarity, which enables strong interaction between the polymer matrix and the cellulose reinforcement (including carboxyl and hydroxyl groups) through interfacial hydrogen and ionic interactions. Cellulose consists of β-1,4-linked glucan chains and is organized into microfibrils that are 3-5 nm in diameter in the primary wall [23] and pectin is the gel-forming component in primary cell wall sand intercellular regions of higher plants. It is a structural heteropolysaccharide containing partially methyl-esterified homogalacturonan, rhamnogalacturonan I and II.[24] Biochemical evidences of cellulose to pectin conformity has been reported. Thus pectin, which are rich in neutral sugar side chains such as arabinans and galactans, showed high bincing affinity to cellulose through hydrogen bondings.[23] Furthermore Chaichi[22] observed in their studies that with the increase of the of corn husk fibers (1-8%) in the pectin matrix the mechanical properties of the films (tensile strength) generally decreased, except for the concentration of 5%, where a significantly increase of the tensile strength was observed, thus the particle distribution at this concentration enabled the formation of a stiff continuous network of cellulosic fibers linked through hydrogen bonding, and appeared to be ideal for the mechanical strength due to the length of the fibers that behaved as reinforcing filler material, making the film stronger.

Brazil is one of the countries with the greatest abundance of natural and renewable resources and generates significant amounts of biomass residues in activities arising from harvesting and processing of agricultural products, such as rice, cotton, corn, soybeans that can be used as matrix and reinforcement components in biodegradable polymeric

materials.[25,26] Other useful agroindustrial residues include sugarcane bagasse, cassava bagasse, and malt bagasse. The malt bagasse is a by-product of brewing and consists mainly of leftover shells, malt pulp and grains, and some additives such as rice, corn, and wheat.[27] In addition, Brazil is the third-largest producer of beer in the world, with a production of 13.3 billion litres, behind only China (46 billion litres) and the United States (22.1 billion litres).[28] Though some part of malt bagasse can be used for biosorption processes[29–31] and nutritional supplement for animal feed,[32] there are still large quantities leftover or even discarded into the wild. Mello and Mali[3] evaluated the distribution of malt bagasse fibers in cassava starch matrix for the production of biodegradable trays and found that the concentration of 15% w/w promoted the initial reduction of the water absorption rate.

In line with the environmental concerns raised above, as well as with the trend towards the maximum use of natural resources, this contribution aims to produce pectin- and malt bagasse-based biodegradable biocomposites obtained in pilot scale, using continuous *casting* to evaluate the effects of malt bagasse addition on the composite morphology, mechanical, thermal properties and photodegradation properties, aiming at large scale production of films for industrial.

In this context, the aim of this study was to analyse the influence of malt bagasse as reinforcing agent in composites. In addition, in line with the environmental concerns raised previously, as well as the trend towards the use of industry residue for aggregation values, in composite packaging, this contribution also to produce pectin-based biodegradable films comprising malt bagasse. To the best of our knowledge, this is the very first report on the solidification of malt bagasse/pectin (HM) into biodegradable films, which were herein produced in a continuous fashion using a pilot-scale lamination unit. Finally, the roles played by components of malt bagasse on the key properties of the produced films, aiming at large-

scale production of active films for food packaging applications, were measured by structure-processing-performance relationships.

2. Experimental

2.1. Materials

The high methoxylation pectin (HM) (DM> 50% and Mw = 130,000 g.mol^{-1}, CP Kelco, Limeira, Brazil) was used as the matrix. The reinforcing agent was used the malt bagasse obtained from the Joia Mesquita Brewery (Lavras-MG, Brazil). The malt bagasse was dried in a forced air circulation oven at 50 ºC for approximately 12 h. It was then ground in a knife mill until it reached a particle size of 0.2 mm. Glycerol was obtained from Synth (Rio de Janeiro-RJ, Brazil).

2.2. Film-forming protocol

Pectin (HM) films were produced by dissolving 6% w/v HM in ultra-pure water, with approximately 15% w/w glycerol. The HM solutions were added to the concentrations of malt bagasse (5%, 10% and 15% w/w). All solutions were shaken with a mechanical stirrer at 7000 rpm for 45 min and degassed under vacuum for about 1 hour. The films were then dried in a continuous *casting* machine (Mathis® KTF-S-B, Germany) operating at 80 °C in both furnaces of the equipment and at a transport velocity of 0.10 mm.min^{-1}. The thickness of the wet layer was adjusted to 1.5 mm. Dried films were allowed to equilibrate in a desiccator at 52 ± 3 % relative humidity (RH) and 25 ± 2 ºC for at least 48 h before further steps.

2.3. Malt bagasse chemical composition analysis

The chemical composition of the malt bagasse fibers were estimated according to the procedures: protein – AOAC Official Method 934.01;[33] lipid – AOAC Official Method 925.04;[34] ash – AOAC Official Method 942.05;[35] lignin – ABNT;[36] holocellulose (cellulose +

hemicellulose) – Rebenfeld;[36] cellulose – White.[37] Hemicellulose contents were calculated from the difference between holocellulose and cellulose values and finally the water content was determined by gravimetric loss of weight.

2.4. Infrared spectroscopy

ATR-FTIR of biocomposites were determined using a FITR model Vertex 70 Bruker spectrophotometer (Bruker, Germany) equipped with a single reflection diamond crystal ATR module. Spectra were recorded from 4000 to 400 cm^{-1} at 32-scan rate and 4 cm^{-1} spectral resolution using an attenuated total reflectance (ATR) module.

2.5.   Morphology analyzes (SEM)

The surfaces morphology analyzes of the biocomposites were performed using a JSM 6510 JEOL SEM (Japan) model. Samples were coated with a layer of gold and analyzed using an acceleration voltage of 5 kV.

2.6.   Thermogravimetry

Thermogravimetric analysis was carried out using TGA Q500 (TA Instruments, USA), to evaluate the stability of composite films. The samples (5-6 mg) were placed in a platinum pan and heated at 25 to 600 ºC at 10 ºC.min^{-1}, within a synthetic air atmosphere. The initial (T_{onset}) and final (T_{offset}) temperature of the film degradation were determined by evaluating by extrapolating the beginning of the mass variation and the lowest temperature indicating that the process responsible for the mass variation was completed, respectively.

2.7.   Mechanical properties

Elastic modulus (MPa), tensile strength (MPa), and elongation at break (%) were measured according ASTM D882-09, [38] using universal testing machine EMIC (Model DL3000, São Paulo, Brazil), equipped with a 10 kN load cell. Films (15 x 100 x 0.04-0.12

mm) were stretched at speed 10 mm.min^{-1}. The test was performed with six samples for each treatment. The elastic modulus (*EM*) was determined from the linear slope of the stress *versus* strains curves. The tensile strength (σ_{max}) was calculated by dividing the maximum force by the initial cross-sectional area. Elongation at break (ε) was calculated by Eq. 1, wherein *d* is the final displacement and d_0 is the initial clamp-to-clamp distance:

$$\varepsilon(\%) = \frac{d - d_0}{d_0} \; x \; 100 \tag{1}$$

The test puncture was performed in texturometer (Stable Microsystems, model TATX2i, England) equipped with a central opening (1_0 = 2.1 cm diameter) and by a spherical probe (Probe A/TG). Six samples for each film with 9 cm² was drilled at a constant speed of 0.8 mm.s^{-1} . The test puncture was using to determine puncturing force (PS, Puncture strength/thickness) and puncturing deformation (PD, %). The thickness values (e) of the films were obtained with an IP65 digital micrometer (Mitutoyo Manufacturing, Japan).

2.8. Water vapor permeability (WVP)

The water barrier properties of the films were determined gravimetrically using a modification of the ASTM E 96-00 Standard Method[39] and according to the work of Cazón.[40] The samples were cut into circles and sealed on the top of permeation cells containing dried silica gel (for 7 days at 200 °C) to provide constant relative humidity (RH). This system sets the water activity to 0 in the atmosphere in contact with the film lower face. Then, the cells were stored in a desiccator containing distilled water (100% RH; 2.337 x 10^3 Pa vapor pressure at 20 °C) and monitored by a Minipa MT 240 Thermo-hygrometer. The mass of water absorbed by the silica (and thus transferred through the film) was determined by weighing the measuring cell every 1.5 h for 12 h. *WVP* (g.mm.kPa^{-1}.h^{-1}.m^{-2}) was calculated by Equation 2:

$$WVP = \frac{W \times \delta}{A.t.\Delta P} \quad (2)$$

Where W is change in cell weight [g], δ is average film thickness [m], and A, t, and ΔP are exposed film area [m^2], time [s], and partial water vapor pressure differential [Pa], respectively. The average WVP value for each sample was obtained in triplicates. Control capsules were prepared containing only the films without active silica gel to verify only the mass variation of the films.

2.9. Moisture content of film

The moisture content was determined according with Ashoush[41] and Mahajan[42]. Four replications of each film treatment were used for calculating the moisture content (Equation 3):

$$Moisture(\%) = \left(\frac{Mi - Mf}{Mi}\right) x100 \quad (3)$$

Where Mi is the initial moisture and Mf is the moisture after time t.

2.10. Optical Properties

Film color and opacity were determined using a Konica Minolta CM-5 colorimeter (Minolta Camera Co., Ltd, Osaka, Japan). A white standard color plate ($L = 97.76$, $a = -0.26$, $b = -0.44$) was used for calibration and as background before luminosity (L^*) as well as the chromatic coordinates a^* and b^* were determined by reflectance according to the CIE L^*, a^*, and b^* scale. The color parameters were used to calculate total color difference (ΔE^*, Equation 4):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

2.11. Photocatalytic degradation of films

The HM films and the HM/malt bagasse composites were exposed to UV irradiation in a photodegradation chamber. In the chamber (40 x 60 cm), the lamps (16 W) were 20 cm

from the surface of the films and were irradiated continuously for 168 h. The assay was performed at constant temperature (25 °C) and RH 65%. The degradation of the film was evaluated directly by the weight loss and ATR-FTIR analyzes. The weight loss of HM and HM/malt bagasse films were obtained from the difference in weights of the films before and after exposure to lamps (and under dark condition) for a specific period. The extent of oxidation was determined by measuring the intensity of carbonyl groups (1700-1800 cm^{-1}) absorbance, and the carbonyl index (CI) was calculated using the following Equation 5:

$$CI = \frac{A_{1700-1800\,cm^{-1}}}{A_{1375\mp1380\,cm^{-1}}} \qquad (5)$$

Where $A_{1700\text{-}1800}$ cm^{-1} is the average absorbance of carbonyl (C=O) peak at 1700 -1800 cm^{-1} and $A_{1375\text{-}1380}$ cm^{-1} is the absorbance of methylene (CH$_2$) scissoring peak at 1375 – 1380 cm^{-1}.

2.12.   Statistical analysis

The results were submitted to analysis of variance (ANOVA) and Tukey test, both at 5% significance (p <0.05), using the statistical software Sisvar® (Version 5.4).

3. Results and discussion

3.1. Characterization of malt bagasse

The chemical composition of the malt bagasse fibers is shown in Table 1:

Table 1: Malt bagasse fibers chemical characterization

Analysis	Malt bagasse fibers
Moisture (%)	0.5±0.1
Lipids (%)	2.3±0.3
Proteins (%)	14.7±1.3
Carbohydrates (%)	62.0±1.5
Ashes (%)	2.5±0.1
Lignin (%)	26.0±0.9
Cellulose (%)	28.4±0.9
Hemicellulose (%)	32.6±2.1
Total dietary fibers	17.6 ± 0.6%

According to Table 1, malt bagasse presents characteristics of fibers derived from secondary sources of cellulose, due to the low concentration of cellulose,[43] although high concentration of hemicellulose. Similar composition has been reported by Mello and Mali.[3]
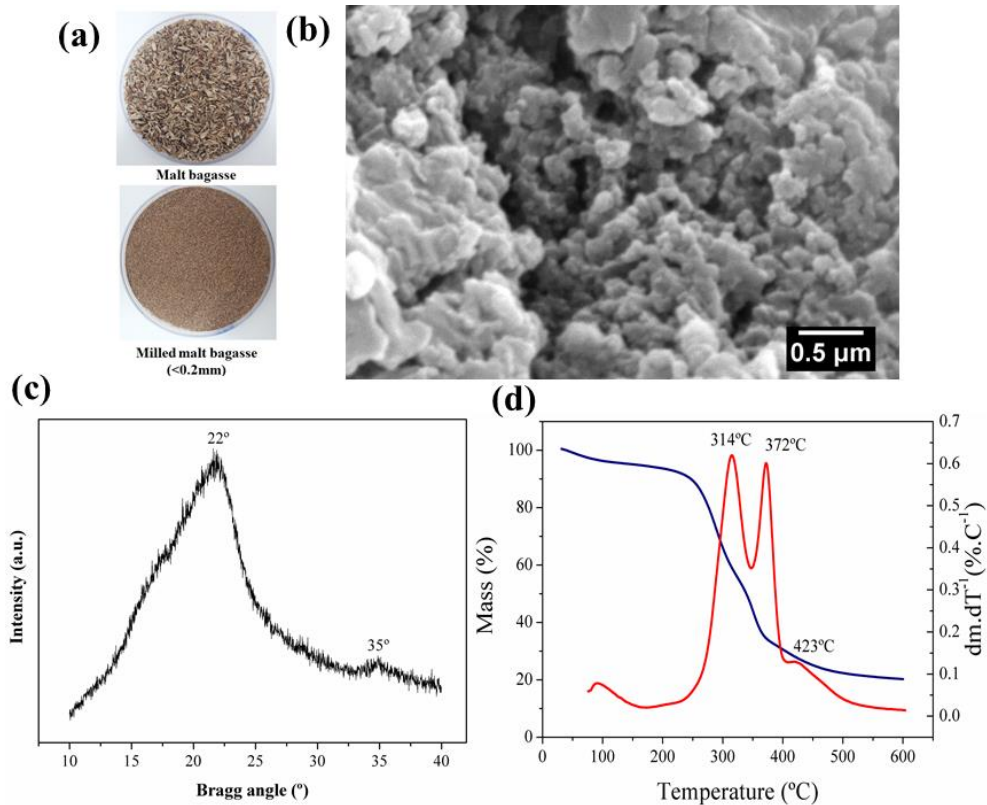
Figure 1: (a) Schematic illustration of malt bagasse. (b) SEM micrograph (30000x, scale = 0.5µm) of malt bagasse. (c) XRD diffractogram of malt bagasse, (d) TG (blue) and DTG (red) curves of malt bagasse (heating rate of 10 ºC.min^{-1}, oxidizing atmosphere (O$_2$ 20% and N$_2$ 80%)).

SEM and XRD were performed to characterize malt bagasse fibers (Figure 1b and 1c). Figure 1b shows a fibrous and porous surface appearance with micrometric fibers. The presence porous and roughness surface may improve the interaction and adhesion between the reinforcement material and the matrix, increasing improvement of mechanical properties of the composite.[44]

In addition, the XRD pattern in Figure 1c, confirms the presence of cellulose, even if masked by the other components, hemicellulose, lignin and proteins, which diminish the crystallinity feature of the lignocellulosic material, making it predominantly amorphous. This can be verified by the XRD profile, which has a broad hallo.[45] The cellulose peaks are

denoted by the peaks at 16º, 22º, and 35º 2ɵ, which correspond to the (110), (200) and (040) crystalline plans of cellulose I, respectively. These peaks are present, although not evident (sharp), as by cellulose-rich fibers, such as cotton.[46–48] These results show that malt bagasse present typical profile of a lignocellulosic material.[49]

To establish the processing conditions for continuous *casting*, the thermal stability of the malt bagasse was evaluated under an oxidative atmosphere. The TG curve in Figure 1d and the DTG curve in the inset show that, at approximately 50-100 °C, malt bagasse fibers undergo 6% weight loss due to the volatilization of absorbed water.[48,50–52] The maximum processing temperature may be established at 230-250 °C because of negligible weight loss (~ 6% by weight).[51] Cordeiro[27] states in its malt bagasse fiber thermal analysis study that temperatures between 250 and 350 ºC represent for the release of many organic extractive e.g., fats, waxes, alkaloids, glycosides, etc. At temperatures of 314 ºC and 372 ºC checks that the decomposition of hemicellulose and cellulose, respectively. In these phases, dehydration, decarboxylation and decarbonylation reactions occur, in which involve the breakdown of C-H, C-O and C-C bonds. According Amutio[53] from 300 °C, differences are perceptible in the curves obtained for different atmospheres. The degradation of proteins and lignin, from this temperature, occurs in two stages when in oxidative atmosphere. Initially there is oxidative degradation and then the volatilization of degradation products occurs. Lignin begins to decompose at approximately 400-450 °C due to the cleavage of aromatic rings and produces various by-products such as water, methane, carbon monoxide, and carbon dioxide.[52] This process occurs over a wide temperature range due to the difficult degradation of lignin in relation to that of cellulose and hemicellulose because lignin has a cross-linked structure.[54]

3.2. Infrared spectroscopy

The ATR-FTIR spectra of the malt bagasse fibers and the HDM/malt bagasse composite films were performed to study the interactions between the functional groups of the materials, as shown in Figure 2.
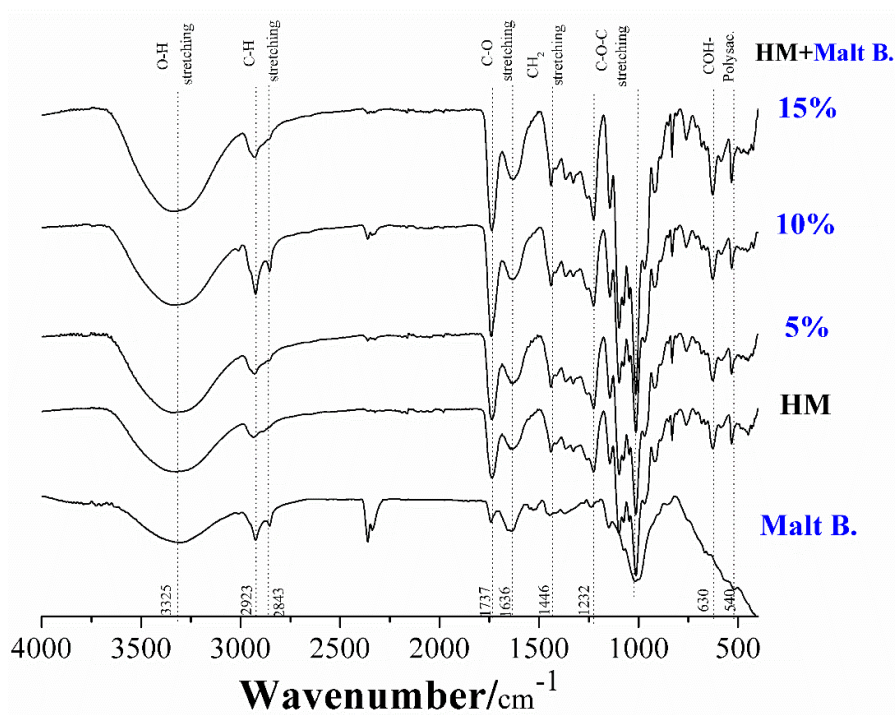

Figure 2: Attenuated total reflectance Fourier-transform infrared spectra of films pectin and malt bagasse (5-15 % p/p). Y-axis: reflectance/a.u.

HM-containing films presented a broad peak centred at *ca.*3335 cm^{-1} associated with the stretching of hydroxyl groups that are abundant in pectin, glycerol, and water (both free and adsorbed).[55] The peak at 2843 and 2923 cm^{-1} are attributed to asymmetric and symmetrical stretching vibrations of the -CH bonds and are characteristic of pectin -CH$_2$ groups.[56] In addition, peaks between 1737 and 1444 cm^{-1} are attributed to -C-O- and -C=O- angular deformations. Peaks corresponding to -C-O-C- groups were identified at 1113 cm^{-1}. The sharp peaks detected at 1010 cm^{-1} in all films is attributed to –C–O–C– glycosidic bonds.[57] The band peaks between 1727-1636 cm^{-1} and 1234-1022 cm^{-1} for malt bagasse

spectrum may indicate the presence of lignocellulosic materials because its main constituents are cellulose, hemicellulose and lignin. Similar results were observed by Lessa.[58] The sharp peaks detected at 1010 cm^{-1} result from –C-OH bonds typical of polysaccharides.[58]

FTIR spectra of HM/malt bagasse encompass typical peaks of all components, however, did not present evidence of substantial interactions. The spectra of HM/malt bagasse films showed slight intensity increases compared with the control film (HM), especially in the bands 2923 and 2843 cm^{-1} (groups –CH$_2$ and –CH$_3$) present in the cellulose, hemicellulose and lignin of the constituents.

3.3. Morphology analyses

Surface and cross-section images of films were observed by SEM and the imagens were shown in Figure 3.
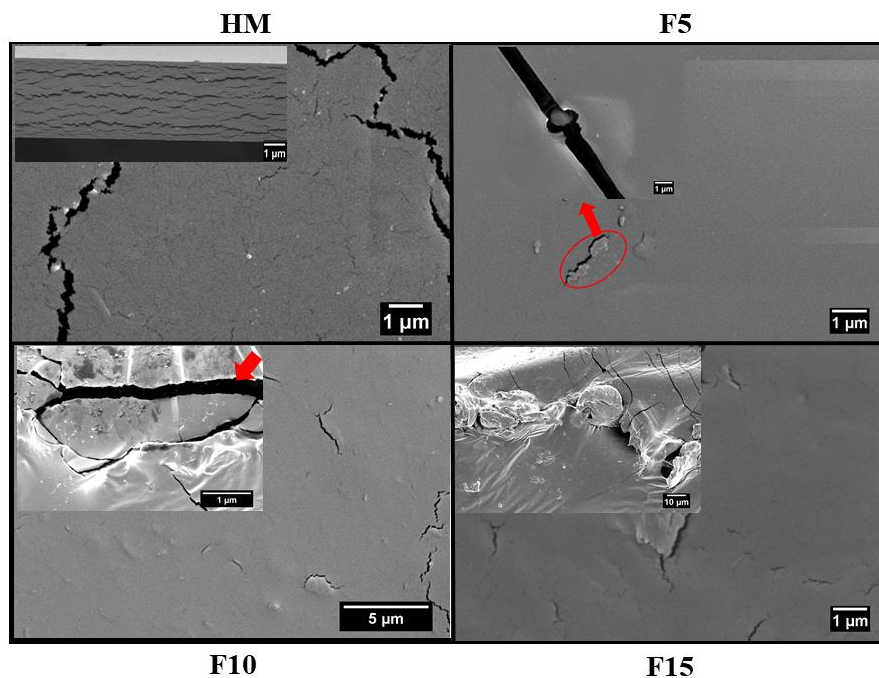

Figure 3: Surface and cross-section (insets) scanning electron micrographs of films de pectin (HM) and HM/malt bagasse composites.

A homogeneous aspect was observed in the HM films; however, the matrix did not present a continuous phase and had cracks (Fig. 3a). In contrast, the HM/malt bagasse composites (Fig. 3b-d) presented a uniform distribution of cellulosic fibers in the pectin matrix and no clusters of cellulose fibers were present in the matrix when the concentration of incorporated fiber was low. In turn, with increasing fibers concentration inside the matrix, the presence of small clusters (Fig. 3d) was observed. This can be observed in the micrographs of the fractured samples. Sample F10 shows some fractures (indicated by the red arrows) and the fibers cross the fracture lines. However, at concentrations above 15% w/w malt pulp fibers were well consolidated in the film matrix, despite the presence of small fractures. In general, these results therefore confirm the results of mechanical properties. The mechanical properties with 10% w/w in the composite films were adversely affected by these fractures and voids or trapped air presents on the fiber-matrix interface, on the other hand, composites incorporated with 15% w/w of fibers showed an increasing tendency in their mechanical properties. This suggest the formation of an interconnected fiber network, allowing greater interaction between the matrix fiber. Sánchez-Safont[14] have reported similar observations.

3.4.   Thermogravimetry

The TG curves and their first derivatives (DTG) for HM and HM/malt bagasse films are shown in Figure 4. The temperatures corresponding to the onset (T_{onset}) and offset (T_{offset}) of thermal degradation are presented in Table 2.
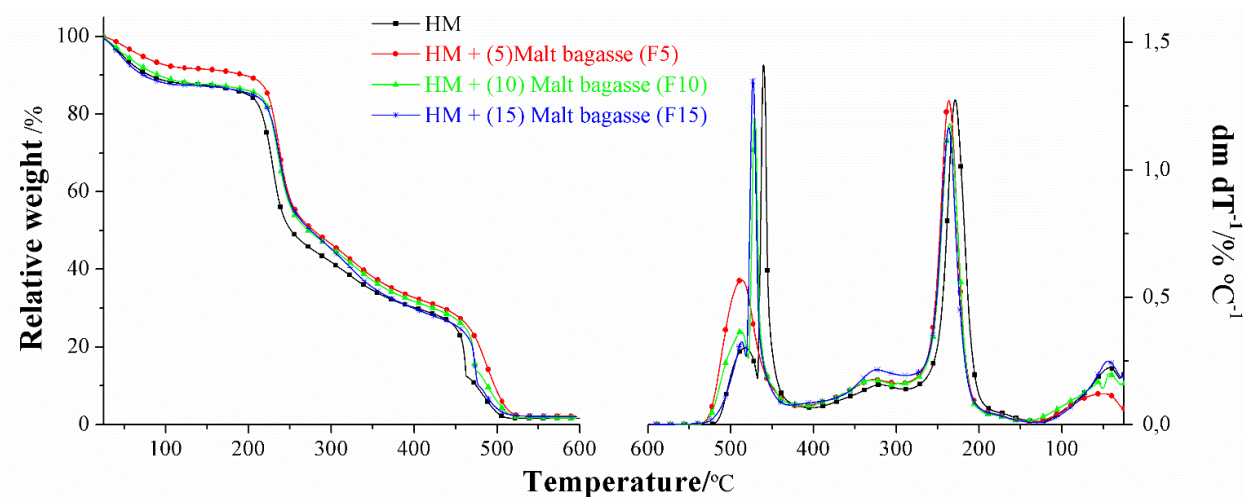
Figure 4: Thermogravimetric (TG; left) and derivative TG (right) profiles of pectin films (HM), and HM/malt bagasse composites. Number in parentheses correspond to weight percentages of malt bagasse and sample code, respectively.

The thermal decomposition of the produced films occurred in three main stages, similarly to other HM-based films. The first mass loss ranging from *ca.* 25 °C to approximately 150 °C is attributed the physical desorption of water and/or other volatiles. The second mass loss stage (*ca.* 180 - 230 °C) is attributed to the process of sample degradation and depolymerization pectin components. The temperature ca. 300 °C (Fig. 3b) present in the HM/malt bagasse composite films is related to the decomposition of hemicellulose, followed by cellulose. Temperatures above 400 ºC can be related to the decomposition of the lignin present in the fiber, with the maximum DTG curve around 470 ºC.

Table 2: Thermal parameters – initial (Tonset) and final (Toffset) degradation temperatures and residual mass at 600 ℃ – of different HM composites reinforced by the malt bagasse fibers.

Sample	MC /%	Tonset / ℃	Toffset / ℃	R_{600} /%
HM	26	212	460	2.2
F5	19	225	475	0.8
F10	17	223	475	0.4
F15	18	227	475	0.5

For the composites films, the pectin-rich phase started to decompose at a slightly higher temperature (Table 2). HM was thermally stable up to 212 ℃, whereas composites films were thermally stable up to 225 ℃. The higher of onset degradation temperature (T_{onset}) for composites films suggest to the presence of abundant hydrophilic groups that formed inter-chain hydrogen bonds in the protein chains.[59] Finally, regardless of composition, all films had a remarkably low residual mass at 600 °C (Table 2).

3.5. Mechanical properties

The effect of different malt bagasse concentrations on composites mechanical properties (tensile and puncture) was investigated and the results are found in Table 3. Malt bagasse and HM factors interaction was significant ($p<0.05$) for tensile strength (TS), elastic modulus (EM), elongation at break (ε), puncture strength/thickness (PS) and puncture deformation (PD).

Table 3: Tensile attributes – thickness (e), tensile strength (TS), elongation at break (ε), elastic modulus (EM) and puncture before and after photodegradation – puncture strength (PS and PS$_{photo}$), puncture deformation (PD and PD$_{photo}$), respectively - of HM and composites films (F5, F10 and F15)

Samples	e /mm	TS /MPa	ε /%	EM /MPa	PS /N.mm^{-2}	PD /mm	PS $_{photo.}$ /N.mm^{-2}	PD$_{photo.}$ /mm
HM	0.04±0.01^c	37±2 ^{a}	2.1±0.3 ^{b}	900±30 ^{c}	326±14 $^{a(a)}$	2.0±0.1 $^{c(a)}$	302±57 $^{a(a)}$	2.5±0.5 $^{c(a)}$
F5	0.09±0.01 ^{b}	15±7 ^{c}	3.2±0.3 ^{a}	531±71 ^{b}	67±11 $^{b(a)}$	3.4±0.5 $^{a(a)}$	69±17 $^{b(a)}$	3.4±0.2 $^{a(a)}$
F10	0.10±0.00 ^{b}	6±1 ^{d}	2.9±0.5 ^{a}	289±46 ^{a}	49±6 $^{c(a)}$	3.6±0.2 $^{a(a)}$	54±9 $^{b(a)}$	3.6±0.3 $^{a(a)}$
F15	0.09±0.04 ^{b}	18±1^b	3.3±0.3 ^{a}	692±84 ^{a}	32±6 $^{d(a)}$	2.6±0.2 $^{b(a)}$	33±11 $^{c(a)}$	2.5±0.2 $^{b(a)}$

Values correspond to the mean and standard deviation of the mechanical properties. Two consecutive letters of the same type indicate that the values are not statistically significant (p < 0.05) using the Tukey test. Different letters indicate that mean values are significantly different at the same level of significance (p < 0.05).

As shown in Table 3, with incorporation of concentrations variations of malt bagasse, the thicknesses of the composites films showed an increase 0.04 to 0.10 mm. Although the films were made from established thicknesses (1.5 mm humidi), significant differences ($p <$ 0.05) were observed between the films (Table 3). However, the total solid content of the samples increased as malt bagasse concentration increased, leading to the formation of thicker films, due to conformational changes of the pectin chain by the addition of malt bagasse.[60] The observed findings could be attributed to the intermolecular interaction between the fiber and the polymer matrix. It is possible that, due to increase in hydrophilicity of films, a sorption process occurred, altering the films thickness when compared to the control film.[61] Similar results were observed about the effect of fiber loading on the composites film thickness when cornstarch biocomposites was filled with corn husk fiber.[62]

The effect of malt bagasse fibers concentration on TS and EM values of HM/malt bagasse films is show in Table 3. The TS and EM of pectin films reduced from 37 and 900 MPa for the control film to 6 and 289 MPa, respectively with 10% wt.% addition of malt bagasse, then increased with further increase of malt bagasse fibers concentration (up to 15%). For 5 and 10% of the malt bagasse fiber content, the decrease in breaking strength, and therefore in failure resistance (Table 3), may probably indicate a low number of particles distributed in the HM matrix behaved as follows. heterogeneity and, therefore, points of failure for the polymer matrix, as observed in cross-section micrographs (Fig. 3). In addition, this increase in TS and MS values at concentration of 15% wt.%, suggests that's the optimum concentration of fiber in the matrix was achieved, thus allowing the formation of a continuous and rigid network of malt bagasse fibers. Another possible mechanism is the similar chemical structure of cellulose and pectin, as verified by FTIR results (Figure 2). The same was verified by Bernhardt.[13]

Contrary to what was expected, ε increased significantly (p <0.05) with the addition of malt bagasse fiber (Table 3). The ε values of the composites increased approximately 57% in relation to the control film. Thus, we infer that the incorporation of malt bagasse in the composites allowed the plasticization of the same, due to the fiber hydrophilicity and composition (Table 1), which allows high moisture absorption, resulting in increased deformation, corroborating the composite thickness results. This was also verified when pectin films where incorporated with corn husk fiber.[13] Thus, water molecules plasticize the pectin matrix.[63] Huntrakul[64] states in his studies water highly plasticizes hydrophilic phases which affects the physico-chemical properties and possibly leads to changes in material properties. In addition, as malt bagasse is a by-product of the brewery production process, consisting mainly of malt husk and pulp leftovers, grains, but also of substitutes such as rice, maize and wheat, the presence of lipids in its composition. may have provided this increased deformation, as found in other studies.[65–67]

The same was verified in the puncture test data (Table 3). It was observed a decrease in PS and an increase in DP, influenced by the effect of the addition of the malt bagasse concentration, indicating that the malt bagasse presented a plasticizing action in the HM matrix, resulting in less resistant and more elastic films.

3.6. Water vapor permeability (WVP)

The water barrier properties of the HM-based films added or not by malt bagasse were investigated through WVP measurements, and the results are shown in Table 4.

Table 4: Water vapor rates (WVTR), Water vapor permeability (WVP) and color parameters of control (HM) and composites (HM/malt bagasse) films.

Sample	WVTR (g.m^{-2}.day^{-1})	WVPx10^{-5} (g.mm^{-1}.day^{-1}m^{-2}.KPa^{-1})	L^*	a^*	b^*	ΔE^*
HM	36.1±0.6[a]	0.66±0.08 [b]	91.5±0.3[a]	-0.60±0.01[a]	4.5±0.6[d]	8.0±0.6[d]
F5	36.8±1.3 [a]	1.35±0.02 [a]	90.2±0.1[b]	-0.20±0.01[bc]	7.4±0.2[c]	11.0±0.2[c]
F10	36.8±0.6 [a]	1.45±0.02 [a]	88.6±0.3[d]	-0.10±0.1[c]	10.6±0.6[a]	14.3±0.6[a]
F15	37.6±1.7 [a]	1.13±0.03 [c]	89.5±0.4[c]	-0.30±0.01[b]	8.9±0.6[b]	12.5±0.7[b]

The means observed in the column with the same letter do not differ statistically ($p < 0.05$).

WVPs increased in the malt bagasse fibers concentrations ranges used. The WVP of the HM film (0.66 g.mm^{-1}.day^{-1}.m^{-2}.kPa) was lower than the values for pectin films in previous studies (9.91 g.mm^{-1}.day^{-1}.m^{-2}.kPa) by Salazar.[63] Differences in WVP for the same type of film may be due to different contents of material and plasticizer. The WVP of composite films of HM-malt bagasse increased compared with that of the control film, ranging from 1.13 to 1.45 g.mm^{-1}.day^{-1}.m^{-2}.kPa, differing statistically ($p < 0.05$). This fact may be associated with the concentration of fibers added to the matrix, which ranged from 0 to 15%, unlike the other studies, in which the maximum fibers added were 10% w/w [22,68] and nanofibers.[22,69] The same was observed in the studies by Sanchez-Garcia[68] and Chaichi[22], where WVP increased in the films with higher concentrations of crystalline nanocellulose filler. We speculate that this behaviour is related to a possible reduction in homogeneity and cohesion of the matrix due to fibers aggregation, which leads to preferred penetration paths. Moreover, this increase in the WVP values of the composite films is believed to be related to

the number of available polar groups the polymer contains[70] and according to the mechanical properties results (Section 3.5) the fibers were able to plasticize the HM matrix, the plasticizing effect may increase the diffusion of water molecules.[64] Despite this slight increase in permeability values, the obtained WVP levels appear to be appropriate for these films to be used as moisture barriers in food packaging, similar to cellulose films.[71] Bedane[71] found WVTR of nano-fibrillated celulose and regenerated cellulose films (grammage of 51-84 $g.m^{-2}$) in between 18-450 $g.m^{-2}.day^{-1}$ at T = 25 ºC and ΔRH = 10-100 %. This result is greater than our measurement, and the reason could be due to the difference in the thickness of the film. LDPE polymer, often used in the production of plastic bags and in the agricultural sector, presents a WVP of only 0.11 $g.mm.m^{-2}.day.kPa$ (40 ºC, 75 % RH), but exhibits low barrier properties to oxygen and to solvents, apart from not being biodegradable.[72] PLA, a biodegradable polymer with similar properties to polystyrene, could be used in the field of plastic bags and agricultural sector, if it does not exhibit a low deformation and high cost compared to the polymers employed for this purpose.[73,74] PLA presents a WVTR of 12.6 $g.m^{-2}$. day (23ºC, 50 % RH).[75]

3.7. Moisture absorption

Figure 5 shows the moisture content the biocomposite films of pectin (HM) and HM/malt bagasse.
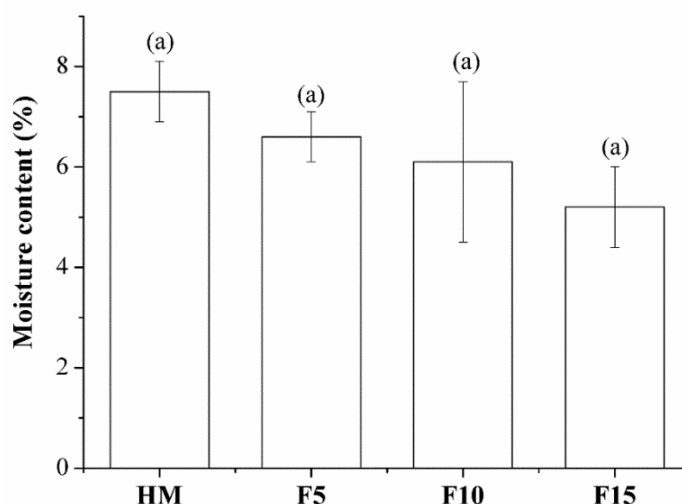
Figure 5: Moisture content of pure HM films and composites with different concentrations malt bagasse fibers at 75% RH and room temperature.

The results show that the moisture content was not significantly influenced by the malt bagasse content, despite the observed tendency of reduction of the moisture absorption of the films. The HM film composed of 15% mass of malt bagasse presented a lower moisture content (5.2 ± 0.8%) than the HM film (7.5 ± 0.6%). The higher moisture resistance was attributed to better dispersion of the fibers in HM matrix. The fibers form hydrogen bonds with the matrix and decrease the number of free -OH groups. This reduces the diffusion force on the water molecules drawing them into the film. The decrease in MC of the biocomposite film is consistent with the FTIR pattern showing a higher value at peak of the -OH stretching (Fig. 2) with fiber loading. Similar result was observed by Shankar, [49] where even though the values of the moisture content of the films decreased with the addition of the fibers, the values (15-20%) were higher than those in the present study. Other similar results have also been reported in previous studies that showed an increase in fibers content in the matrix resulting in a more hydrophobic film.[76–79]

3.8.   Optical Properties

The effect of malt bagasse on the colorimetric parameters of HM films is presented in Table 3.

According to the values observed for the parameters a^*, b * and L^* (Table 3), the colour of the pectin films and the HM/malt bagasse composites was between green and yellow and lighter shades ($L^* > 50$). Lower values of L^* were obtained for the films of composites with increasing concentration of malt bagasse fibers. The composite films presented higher values of a^* and b^* than the control film (HM). The a^* values of the composite films increased with the increase of the malt bagasse fibers content; the same was verified with the values of b^*, which indicated a paler yellow appearance due to the nature and amount of fibers in the composite films. The changes in the colour values of the HM/malt bagasse composite films were mainly attributed to malt bagasse fibers, which have a brown coloration, as verified in Figure 1c. Consequently, the total colour difference (ΔE) of the HM/malt bagasse composite films (11-14.3) increased notably compared with that of the pure HM film (8.0). The film with a higher value of ΔE was F10 (10% w/w malt bagasse fibers), which presented an increase of approximately 81% in the value of ΔE when compared with the film without fibers (HM) (Table 3). The results corroborate with Shankar[49] and dos Santos Caetano.[80] Akhtar[81] state that colored films - white, red and yellow- can reduce photo-oxidation the level of samples in darkness.

3.9.   Photocatalytic degradation of films

Figure 6 lists the observed infrared bands for the HM films and for the HM/malt bagasse composites, along with the corresponding identified functional groups of unaged films and films subjected to UV accelerated ageing for up to 7 days.
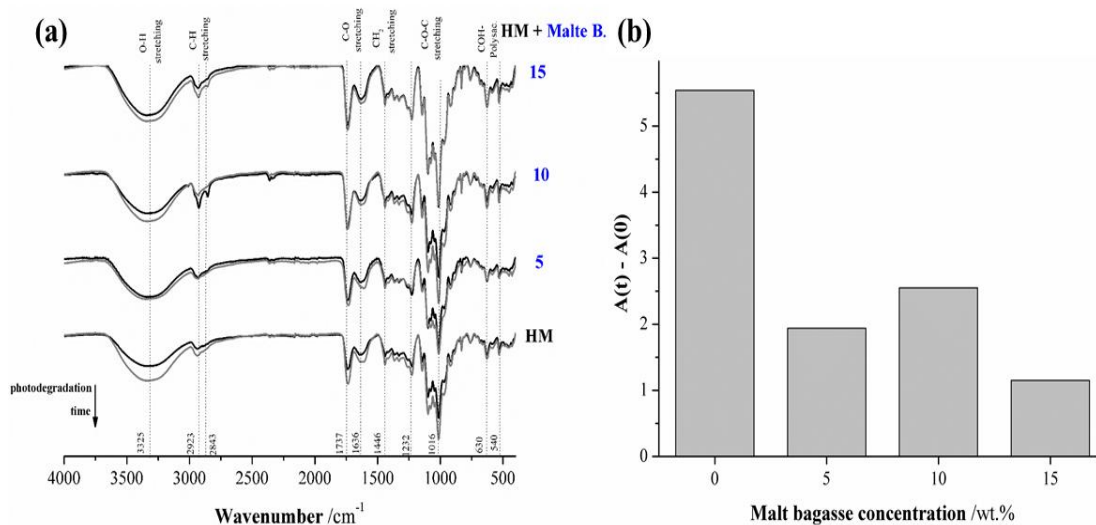
Figure 6: (a) Visible light photodegradation performances of pectin (HM) and HM/malt bagasse films with different malt bagasse contents. Y-axis: reflectance/a.u. Arrow indicate the change direction with time. (b) Amount of carbonyl groups in pectin (HM) and HM/malt bagasse films versus malt bagasse concentration (wt.%).

The infrared bands of the films showed slight displacements compared with those of the films that did not undergo UV-induced degradation. Second Kaczmarek[82] the biggest alterations in pectin films were observed in the first period of exposure (1-5h); while prolonged irradiation (>5h) resulted in a smaller effect. An example of such changes in spectra is shown in Fig. 6. The intensity of the carbonyl band increases slightly, besides verifying the development of the shoulder in the band in 1636 cm^{-1}. Increased intensity of other bands in the region of 1000-1500 cm^{-1} suggests possible development of macromolecular order induced by UV radiation. The same was observed in studies of Kaczmarek.[82]

The course of photochemical reactions in HM and HM/malt bagasse composites depends on their composition. Taking into account the fact that systematic changes were observed in all the absorption bands, it was not possible to choose and use as the reference-band any one peak of those usually applied for normalization. Thus, for the quantitative

analysis, the relative changes of carbonyl band's integral intensities in the range of 1636-1736 cm^{-1} were calculated from the spectra of samples having the same thickness. The obtained Δ(C=O) values were plotted versus malt bagasse concentration (wt.%) (Fig. 6b). It is visible on the plot (Fig.6b) that HM undergoes the most efficient photooxidation, whereas HM/malt bagasse composites is rather resistant to such a process. This was also verified in the mechanical properties of puncture (Table 3) of HM films and composites. In which the puncture streght (PS) and puncture deformation (PD) values did not show significant differences (p> 0.05) after being exposed to UV radiation in relation to the films without being photodegraded. Using this approach, we can verify that the bagasse fibers provided a protective effect against UV-induced degradation. This protective effect may be associated with the malt bagasse fiber composition because it is mainly composed of cellulose. According to the study by Vercelheze,[83] cellulose is not a good light absorber. However, lignin (~ 24%) is a good light absorber due to the presence of chromophoric groups, such as phenolic groups, hydroxyls, carbonyls and double bonds, and the ability to form free radicals as intermediates.[84] Therefore, we can verify that the energy of the photon absorbed by the cellulose is probably transferred to the lignin.

4. **Conclusion**

In this contribution, we demonstrated that the addition of malt bagasse in different concentrations to HM-based films affected their matrices, while preserving or even improving their physical-mechanical properties, as a high chemical affinity occurred between polymer matrix and fibers. Barrier, optical, and thermal properties were the most affected by malt bagasse addition. In particular, addition of malt bagasse at 5 to 15% w/w acted as a plasticizer for the HM films, due probably to moisture incorporation resulted from fiber hygroscopy, which increased the elongation at break by approximately 57%. Finally, the HM/malt bagasse films showed protective effect against UV-induced degradation. In summary, the addition of

malt bagasse into HM-based films provided these with relevant properties, adding values to the waste industry, further increasing its potential application in the food industry.

Acknowledgments

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ARTICLE 3 - Correlating emulsion characteristics with the properties of active starch films loaded with lemongrass essential oil
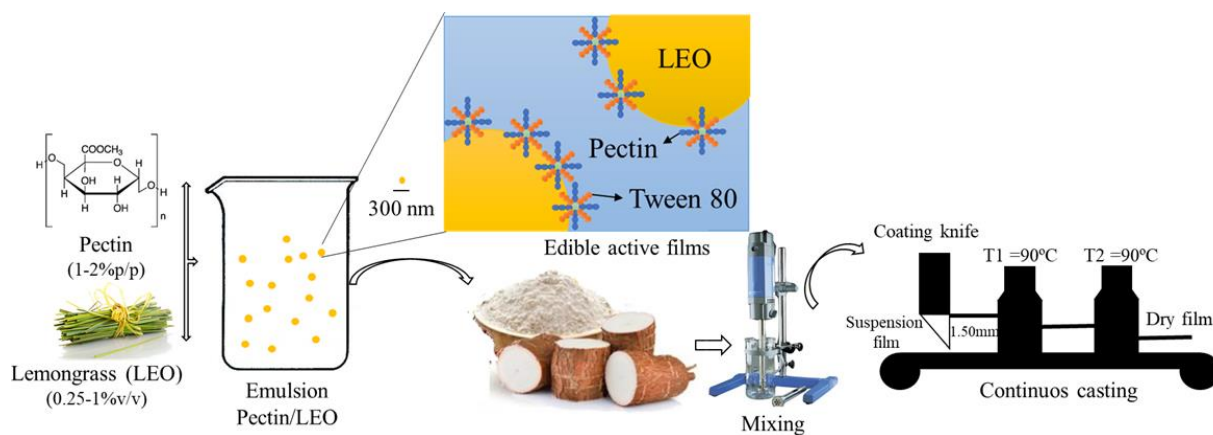
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Correlating emulsion characteristics with the properties of active starch films loaded with lemongrass essential oil

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Abstract

Edible films supplemented with active ingredients are promising for food packaging and preservation. Numerous plant essential oils present antimicrobial activity against pathogenic microorganisms, but their low solubility in water limits food-related applications. Emulsification is herein exploited as a means of improving the dispersion of such active substances in water, imparting increased functionality and reduced degradation. Lemongrass essential oil (LEO) was directly emulsified as dispersed phase into aqueous solutions of pectin, which was used together with Tween 80 as polymeric emulsifiers. LEO nano- and micro-sized droplets were added to film-forming formulations based on glycerol-plasticized cassava starch (TPS) and then dried into films by continuous casting. LEO contents in films relied upon microbiological assays to ensure antimicrobial activity. Films' chemical composition, morphological, thermal, mechanical, and barrier properties, in addition to biodegradability in vegetal compost, were comprehensively studied. In general, emulsions were suitably dispersed within the TPS matrix, both phases showing good interaction and compatibility. Emulsification caused important changes in the TPS-based films, such as improved colorimetric attributes, thermal stability, barrier to moisture, and mechanical properties. Another key observation was the maintenance of the biodegradation profile in soil of TPS after the addition of LEO emulsions. Overall, the emulsification approach was successful in providing TPS films with multifunctionality by the supplementation of an active substance without negatively affecting its fundamental properties for food packaging applications.

1. Introduction

Due to environmental concerns caused by excess waste from petroleum-derived packaging materials, researchers have been intensively focusing on the development of environmentally friendly packaging materials using natural and biodegradable (macro)molecules (Arcan & Yemenicioğlu, 2011; Li, Ye, Lei, & Zhao, 2018), such as proteins (Kowalczyk & Baraniak, 2014; Pires et al., 2013; Seydim & Sarikus, 2006), lipids (Fabra, Talens, Gavara, & Chiralt, 2012), and polysaccharides (Ghasemlou et al., 2013). Starch is commonly used in these cases due to its relatively low cost, high availability, and ease of handling (Ghasemlou et al., 2013; Mendes et al., 2016), as well as being transparent, odorless, tasteless, and presenting a good CO_2 and O_2 barriers (Jiang, Neetoo, & Chen, 2011; Neetoo, Ye, & Chen, 2010).

Starch-based materials, nonetheless, present poor resistance and barrier to moisture (Campos, Gerschenson, & Flores, 2011). The addition of hydrophobic compounds has been suggested in order to improve the physical and functional properties of starch films (Jiménez, Fabra, Talens, & Chiralt, 2012a; Pelissari et al., 2009). The low compatibility and stability of systems comprising both hydrophilic and hydrophobic components, however, denote another technical obstacle. Hydrophobic substances, such as essential oils, can be dispersed as emulsions within film matrix. In this sense, the active lipophilic compounds can be properly distributed within the bulk, potentially leading to active films containing a wide range of food additives, including antimicrobials (Otoni, Avena-Bustillos, Olsen, Bilbao-Sáinz, & McHugh, 2016a; Pranoto, Salokhe, & Rakshit, 2005).

Lemongrass (*Cymbopogon citratus*) is a herb that has been cultivated for medicinal purposes in several countries (Naik, Fomda, Jaykumar, & Bhat, 2010; Riquelme, Herrera, & Matiacevich, 2017). It is characterized by a strong lemon aroma due to its high citral contents, accounting for *ca.* 75% of its composition (Mohamed Hanaa, Sallam, El-Leithy, & Aly,

2012). Lemongrass essential oil (LEO) has been shown to be antimicrobially active against fungi, yeasts, and Gram-positive and -negative bacteria (Naik et al., 2010). Riquelme et al. (2017) studied the addition of encapsulated LEO into alginate-based films produced by bench casting and found that emulsion droplet size significantly affected the physical properties and antimicrobial activity of the films.

Nanoemulsions have recently been described as colloidal dispersions of droplets with particle diameters smaller than 500 nm (Otoni et al., 2016a). Nanoemulsions are kinetically stable, but thermodynamically unstable systems, whose production requires emulsifiers to stabilize the dispersed phase – oil in an oil-in-water (O/W) emulsion – as droplets within the continuous phase (water or aqueous solutions in an O/W emulsion). Surfactants, particularly, are capable of adsorbing at the oil/water interface, reducing the interfacial tension, facilitating the emulsification process, and preventing destabilization phenomena (Kralova & Sjöblom, 2009). Although through mechanisms that may not involve reductions on the interfacial tension as in the case of surfactants, natural biopolymers are gaining relevance for their use as emulsifiers and thickeners. Besides, there is an increasing interest for more natural ingredients from both costumers and companies. In this context, pectin stands out as an interesting naturally occurring plant polysaccharide. The emulsifying activity of pectin is mostly attributed to its ability in increasing the viscosity of the aqueous phase (Dickinson, 2003). In addition, it may confer negative electrical charge (at pH > *ca.* 3.5) to the surroundings of the oil droplets due to its anionic nature, contributing also to the electrostatic stability of O/W emulsions (Alba & Kontogiorgos, 2017; Artiga-Artigas, Guerra-Rosas, Morales-Castro, Salvia-Trujillo, & Martín-Belloso, 2018; Chan, Choo, Young, & Loh, 2017; Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016; Morris, 2000; Ozturk & McClements, 2016). It has been recently suggested that pectin might also act as surface-active emulsifier, *i.e.*, as a true surfactant (Acevedo-Fani, Salvia-Trujillo, Rojas-Graü,

& Martín-Belloso, 2015), although the actual adsorption mechanism remains unclear, as well as possible interactions between pectin and conventional emulsifiers. Artiga-Artigas et al. (2018) elucidated, through pseudo-ternary phase diagrams, the role of pectin and Tween 80 as nonionic emulsifiers for formulations containing essential oils. Only 1 wt.% pectin and 1.8 wt.% Tween 80 allowed the formation of submicron-sized emulsion droplets.

Even though much effort has been devoted for (nano)emulsion-containing films lately, as reviewed elsewhere (Espitia, Fuenmayor, & Otoni, 2019), research is lacking on the combination of pectin and low-molecular weight surfactants (*e.g.*, Tween 80) to produce emulsions featuring optimized properties. This would allow a complete behavioral mapping of different components and their interactions on nanoemulsion characteristics.

In this context, the aim of this study was to analyze the influence of combining Tween 80 and pectin as emulsifiers for food-grade emulsions of LEO. In addition, in line with the environmental concerns raised previously, as well as the trend towards the use of antimicrobial agents in active packaging, this contribution also aimed to produce starch-based biodegradable films comprising LEO emulsions. To the best of our knowledge, this is the very first report on the solidification of lipid/thermoplastic starch (TPS) into active films, which were herein produced in a continuous fashion using a pilot-scale lamination unit. Finally, the roles played by emulsion components and droplet size on the key properties of the produced films, aiming at large-scale production of active films for food packaging applications, were addressed by structure-processing-performance relationships.

2. Materials and Methods

2.1. Materials

Cassava starch (amylose content: 17-20 wt.%) was supplied by Fécula da Amazonia (Moju-PA, Brazil). High methyl ester pectin (DM > 50%; $Mw = 130,000$ g mol^{-1}) was provided by CP Kelco (Limeira-SP, Brazil). LEO was purchased from Ferquima (Vargem

Grande Paulista-SP, Brazil). Glycerol and Tween 80 were obtained from Synth (Rio de Janeiro-RJ, Brazil). *Escherichia coli*, strain INCQS 00181 (EPC; CDC O55), was kindly donated by the Oswaldo Cruz Foundation (Fiocruz, Rio de Janeiro-RJ, Brazil), while *Staphylococcus aureus* (GL4348) was provided by Embrapa Dairy Cattle (Juiz de Fora-MG, Brazil).

2.2. Essential oil characterization

LEO composition was determined on a gas chromatograph (Shimadzu CG-17a) coupled to a mass spectrometer (QP 5000). The interphase, ion source, and mass selective detector were kept at 243, 230, and 150 ºC, respectively. Helium was used as carrier gas at 1 mL min^{-1}. The oven temperature was increased from 40 to 240 °C at 3° C min^{-1}. The active compounds from LEO were identified by comparing their retention indexes and mass spectra with the data from the literature.

2.3. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations

MIC and MBC were determined using the microdilution method in 96-well polystyrene plates according with the standard methodology M7-A6 of the National Committee for Clinical Laboratory Standards (NCCLS, 2003). Different LEO concentrations (0.015 to 2 vol.%) as well as 0.5 vol. of Tween 80 were added to Tryptone Soy Broth (TSB), homogenized, and 150-µL aliquots were added to the wells after prior the inoculation of 10 µL of standard cultures previously standardized t 10^8 CFU mL^{-1}. The microplates were sealed and incubated at 37 °C for 24 h. The cultures were plated onto solidified Tryptone Soy Agar (TSA) and incubated likewise. Bacterium-free TSB (*i.e.*, comprising only Tween 80 and LEO) was used as negative control, whereas LEO-free TSB (*i.e.*, comprising only Tween 80 and standardized inoculum) was used as positive control. The lowest LEO concentrations capable of inhibiting bacterial growth were taken as the MIC, and the lowest LEO

concentrations that led to complete bacterial inactivation were taken as MBC. The experiments were performed in triplicates.

2.4. Emulsification

The emulsions were prepared following the method described by López-Mata et al. (2018), with modifications. Pectin was dissolved in 20 mL of distilled water at 1 or 2 wt.% and added by glycerol (500 µL g^{-1}, dry basis) and the solution was homogenized for 4 min at 15,500 rpm on a Yellowline DI 25 basic disperser (IKA, Staufen, Germany). Subsequently, Tween 80 (at 0.1 vol.%) and LEO (at 0, 0.25, 0.50, and 1.0 vol.%) were added and the system was homogenized likewise.

2.5. Droplet size, size distribution, and short-term stability

Droplet diameter (indicated by z-average) and diameter distribution (indicated by polydispersity index, PdI) were determined by dynamic light scattering on a Zetasizer Nano ZS (Malvern Instruments Inc., Worcestershire, U.K.). Distilled water was used as dispersant to avoid multiple scattering as well as inter-droplet dispersion and interaction effects. Measurements were carried out soon after emulsification as well as after 24 h sitting at room conditions to assess stability during a typical film-forming period.

2.6. Film-forming protocol

Cassava starch was suspended in ultrapure water at 8% (w/v), homogenized at 15,000 rpm, and was heated at 95 °C for until complete gelatinization (*ca.* 25 min). Glycerol was then added at 20 wt.% (dry basis) under further stirring for 15 min to obtain TPS. The mixture was cooled down to 40 °C, combined at 1:1 (weight ratio) with the previously prepared emulsions (0.5-1% w/v), homogenized at 15,500 rpm for 15 min on the Yellowline DI 25 basic disperser, and vacuum-degassed for 20 min. Finally, the TPS and TPS/emulsion formulations were dried into films in a continuous fashion on a KTF-S-B lamination system

(Mathis, Germany). The film-forming formulations were deposited onto a polyester conveyor moving at 0.10 mm min^{-1}. The slurry was forced across a gap and spread into a uniform 1.5-mm-thick wet layer that was driven through two oven-drying stages at 90 ℃, conditions which had been reported to allow complete drying (Lorevice, Otoni, Moura, & Mattoso, 2016; Manrich et al., 2017a; Otoni, Avena-Bustillos, Olsen, Bilbao-Sáinz, & McHugh, 2016b; Otoni, Lorevice, Moura, & Mattoso, 2018). Dried films were allowed to equilibrate in a desiccator at $52 \pm 3\%$ RH and 25 ± 2 °C for at least 48 h before further steps.

2.7. Infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) measurements were obtained using a Vertex 70 spectrophotometer (Bruker, Germany). Spectra were recorded from 4000 to 400 cm^{-1} at 32-scan rate and 4 cm^{-1} spectral resolution using an attenuated total reflectance (ATR) module.

2.8. X-ray diffraction (XRD)

The crystalline structures of the films were analyzed through diffraction patterns obtained on a XRD-6000 X-ray diffractometer (Shimadzu, Kyoto, Japan). Samples were scanned from 5 to 40 ° (2θ) at 1 ° min[-1]. Crystallinity index (CI) was determined based on the areas under the Gaussian-deconvoluted crystalline and amorphous peaks after baseline correction. CI for TPS was estimated as a function of B and Vh crystals, according with Hulleman, Kalisvaart, Janssen, Feil, & Vliegenthart, (1999).

2.9. Thermogravimetry

The thermal degradation profiles of the samples (*ca.* 6 mg) were obtained on a TGA Q500 (TA Instruments, USA) from 25 to 600 ℃ at 10 ℃ min[-1], within a synthetic air atmosphere.

2.10. Optical properties

Film color and opacity were determined using a Konica Minolta CM-5 colorimeter (Minolta Camera Co., Ltd, Osaka, Japan). A white standard color plate (L = 97.76, a = -0.26, b = -0.44) was used for calibration and as background before luminosity (L^*) as well as the chromatic coordinates a^* and b^* were determined by reflectance according to the CIE L^*, a^*, and b^* scale. The color parameters were used to calculate total color difference (ΔE^*, Eq. 1), yellowness index (YI, Eq. 2), and whiteness index (WI, Eq. 3):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$$YI = \frac{142.86 \, x \, b}{L} \quad (2)$$

$$WI = 100 - \sqrt{(100 - L)^2 + (a)^2 + (b)^2} \quad (3)$$

2.11. Mechanical properties

Films were submitted to uniaxial tensile assay on a DL3000 universal testing machine (EMIC, São Paulo-SP, Brazil). Tests were carried out according with ASTM D822-09 (ASTM, 2009) and other studies (Abral et al., 2019; Gouveia, Biernacki, Castro, Gonçalves, & Souza, 2019; Saricaoglu, Tural, Gul, & Turhan, 2018). At least six 15-mm wide, 100-mm long, and $ca.$ 0.8-mm thick (measured using an IP65 Mitutoyo digital micrometer at five random positions) specimens per treatment were stretched at 25 mm min^{-1} using an initial clamp-to-clamp distance of 50 mm and a 50-kgf load cell. The elastic modulus (E) was determined from the linear slope of the stress $versus$ strains curves. The tensile strength (σ_{max}) was calculated by dividing the maximum force by the initial cross-sectional area. Elongation at break (ε) was calculated by Eq. 4, wherein d is the final displacement and d_0 is the initial clamp-to-clamp distance:

$$\varepsilon(\%) = \frac{d-d_0}{d_0} \; x \; 100 \qquad (4)$$

Through the values obtained in the stress versus strain graphs, the toughness of the films was calculated for the area below the curve.

2.12. Water vapor permeability (WVP)

The water barrier properties of the films were determined using a modified ASTM method E96/16 (ASTM E96, 2016). The sample were cut into circles and sealed on the top of permeation cells containing dried silica gel to provide constant RH. The cells were then stored in a desiccator containing distilled water (100% RH; 2.337 x 10^3 Pa vapor pressure at 20 ℃). Afterwards, the amount of water vapor transferred through the film was quantified gravimetrically over 8 h. WVP (g.mm.kPa^{-1}.h^{-1}.m^{-2}) was calculated by Eq. 5:

$$WVP = \frac{W \; x \; \delta}{A.t.\Delta P} \qquad (5)$$

where W is change in cell weight [g], δ is average film thickness [m], and A, t, and ΔP are exposed film area [m^2], time [s], and partial water vapor pressure differential [Pa], respectively. The average WVP value for each sample was obtained in triplicates.

2.13. Biodegradability in vegetable compost

Biodegradability was assessed as described by Dalev, Patil, Mark, Vassileva, & Fakirov (2000), with some modifications. Briefly, vegetal compost (soil) was poured into a plastic tray (10 x 20 x 5 cm^3) up to a height of $ca.$ 40 mm. The samples (20 x 20 mm^2) were individually weighed and then buried in the soil to a $ca.$ 10-mm depth. The systems were kept at room temperature and water was sprayed twice daily to sustain moisture. The samples were carefully taken out after 0, 7, 15, and 30 days, dried in a vacuum oven at 50 ℃, and weighed. High-density polyethylene (HDPE) was tested likewise for comparison purposes.

2.14. Statistical analysis

The results were submitted to analysis of variance (ANOVA) and multiple comparison test Tukey test, both at 5% significance ($p < 0.05$), using the statistical software Sisvar® (Version 5.4).

3. Results and discussion

3.1. LEO composition and antimicrobial efficiency

A total of 21 components were identified in LEO, including monoterpene hydrocarbons (3.8%), oxygenated monoterpenes (86%), sesquiterpene hydrocarbons (6%), and oxygenated sesquiterpenes (4.8%). The detailed composition profile is provided in Table S1 (Supporting Information). The major LEO constituents were geranial (35%), neral (29%), geraniol (8%), geranyl acetate (5.5%), caryophyllene (4%), t-cadiene (2.5%), camphene (2%), linalool (2%), and caryophyllene oxide (1%). A similar LEO composition has been reported by Tyagi, Gottardi, Malik, & Guerzoni, (2014).

The antimicrobial performance of LEO itself was herein assessed against *S. aureus* and *E. coli* (Acevedo-Fani et al., 2015; Ma et al., 2016) bacteria that are frequently associated with foodborne outbreaks and diseases. In this sense, MIC and MBC determinations were performed to shed light on the minimum LEO content in cassava starch-based films for the materials to display suitable antimicrobial performance. Images of wells and plates used in MIC and MBC determinations are depicted in Figure S1 (Supporting Information). As expected, LEO exhibited a concentration-dependent inhibition activity, with MIC values being 0.025% for *S. aureus* and 0.5% for *E. coli* and MBC values being 0.5% for the Gram-positive bacterium and 1.0% for its Gram-negative counterpart. In line with the extensive literature on antimicrobial essential oils (Acevedo-Fani et al., 2015; Amit Kumar Tyagi,

Davide Gottardi, Anushree Malik, 2014; Jouki, Yazdi, Mortazavi, & Koocheki, 2014) the Gram-positive bacterium was more sensitive to LEO than the Gram-negative one.

Aligiannis, Kalpoutzakis, Mitaku, & Chinou, (2001) suggested a classification for the antimicrobial activity of plants based on MIC values: strong (MIC ≤ 0.5 μL mL^{-1}), moderate (0.5 < MIC ≤ and 1.5 μL mL^{-1}), and weak inhibitors (MIC > 1.5 μL mL^{-1}). In this sense, LEO presented a strong inhibition against the tested strains. This was attributed to the significant content of oxygenated monoterpenes (86%), mainly represented by citral compounds, *i.e.*, the isomers geranial and neral, the major constituents of this LEO (Table S1) and that are known to have a remarkable antimicrobial capacity (Amit Kumar Tyagi, Davide Gottardi, Anushree Malik, 2014; Tyagi & Malik, 2010; Tzortzakis & Economakis, 2007). The low LEO contents capable of inhibiting bacterial growth and inactivating bacteria not only confirm the promising antibacterial efficiency of such essential oil, reason why it has been investigated herein, but also enable its application as a food additive (Amit Kumar Tyagi, Davide Gottardi, Anushree Malik, 2014).

3.2. Emulsion morphology and stability

LEO-in-water emulsions were stabilized by two polymeric emulsifiers, namely pectin, as an anionic surface-active macromolecule, and Tween 80, as a classical nonionic surfactant (Lorenzo, Zaritzky, & Califano, 2018; McClements & Gumus, 2016). To investigate the effect of formulation on emulsion droplet size, size distribution, and stability, LEO and pectin contents were varied from 0 to 1 wt.% and 1 to 2 wt.%, respectively, and the outcome is presented in Table 1.

Table 1. Average diameter (z-average), polydispersity index (PdI), and Zeta potential of lemongrass essential oil (LEO)/pectin emulsions determined right after and one day after production through low-shear homogenization (15000 rpm, 4 min).

Pectin /wt.%	LEO /wt.%	Zeta potential/mV		Diameter/nm		PdI	
		0 h	24 h	0 h	24 h	0 h	24 h
1	0	-	-	-	-	-	-
1	0.25	-16.2±0.4 $^{a(a)}$	-16±2 $^{a(a)}$	1222±30 $^{d(a)}$	1280±33 $^{e(a)}$	0.27±0.05 $^{a(a)}$	0.33±0.02 $^{a(a)}$
1	0.5	-14±1 $^{ab(a)}$	-14±1 $^{ab(a)}$	301±4 $^{a(a)}$	398±8 $^{a(b)}$	0.32±0.04 $^{a(a)}$	0.47±0.02 $^{ab(b)}$
1	1	-6.9±0.4 $^{c(b)}$	-9.3±0.4 $^{c(a)}$	372±5 $^{e(a)}$	380±6 $^{b(a)}$	0.29±0.01 $^{a(a)}$	0.41±0.02 $^{c(b)}$
2	0	-	-	-	-	-	-
2	0.25	-16±1 $^{a(a)}$	-14±1 $^{a(a)}$	753±6 $^{c(a)}$	1060±33 $^{d(b)}$	0.50±0.02 $^{b(a)}$	0.57±0.01 $^{bc(b)}$
2	0.5	-12±1 $^{b(a)}$	-11.7±0.5 $^{b(a)}$	420±9 $^{b(a)}$	433±2 $^{a(a)}$	0.99±0.02 $^{c(a)}$	0.98±0.03 $^{d(a)}$
2	1	-9±2 $^{c(a)}$	-9.3±0.5 $^{c(a)}$	743±32 $^{c(b)}$	720±29 $^{c(a)}$	0.55±0.02 $^{b(a)}$	0.6±0.1 $^{c(a)}$

Values are reported as average values ± standard deviations. Different letters within the same column (comparing treatments) and row (in parentheses, comparing times) indicate statistically different (p>0.05).

Interestingly, higher LEO contents led to smaller droplets, suggesting that some amphiphilic components of LEO (Table S1) may contribute to droplet size reduction (Bonilla, Atarés, Vargas, & Chiralt, 2012). LEO at 0.5 and 1 wt.% together with pectin at 1-2 wt.% led to droplet diameters in the nanoscale (1% pectin) and submicron-scale (2% pectin). Particles are susceptible to gravitational forces regardless of their dimensions, but nanodroplet sedimentation is less likely to occur compared to conventional droplets since the Brownian motion of smaller droplets is greater than the gravity-induced creaming rate (Huang, Yu, & Ru, 2010). This is in accordance with the observation that droplets smaller than 1 μm are normally kinetically stable (Yi, Li, Zhong, & Yokoyama, 2014). Concerning our emulsions, even if the droplet size of some formulations changed ($p<0.05$) after 24 h of emulsification (Table 1), stability was not lost by coalescence because the drying conditions used allowed dry films to be obtained within a few minutes, solidifying the emulsions (Manrich et al., 2017b).

Overall, the electrical charge of droplets is controlled by the load of adsorbed surfactants around oil droplets, molecules which may be anionic, cationic, or nonionic in nature. The emulsions prepared in this study contained nonionic emulsifiers (Tween 80 and pectin). A *quasi*-zero electrical charge would therefore be expected. The charge did not change significantly at 1 and 2 wt.% pectin. Overall, the zeta-potential did not change drastically during the storage period (Table 1), keeping the system stable. Choi, Kim, Cho, Hwang, & Kim, (2011) observed negative electrical charge on multilayer nanoemulsions containing capsaicin and Tween 80 when they added the oil-surfactant phase into alginate solutions. The electric charge of droplets plays an important role in nanoemulsions stability and when the charge is sufficiently large, the droplets are prevented from aggregating due to electrostatic repulsion between them. Taking into consideration both droplet diameter and

emulsion stability, the formulations chosen for producing biodegradable, active films comprised 1-2 wt.% pectin and 0.5-1 wt.% LEO.

3.3. Infrared spectroscopy

ATR-FTIR spectroscopy was carried out to elucidate possible interactions between TPS and LEO. The spectra are shown in Figure 1.
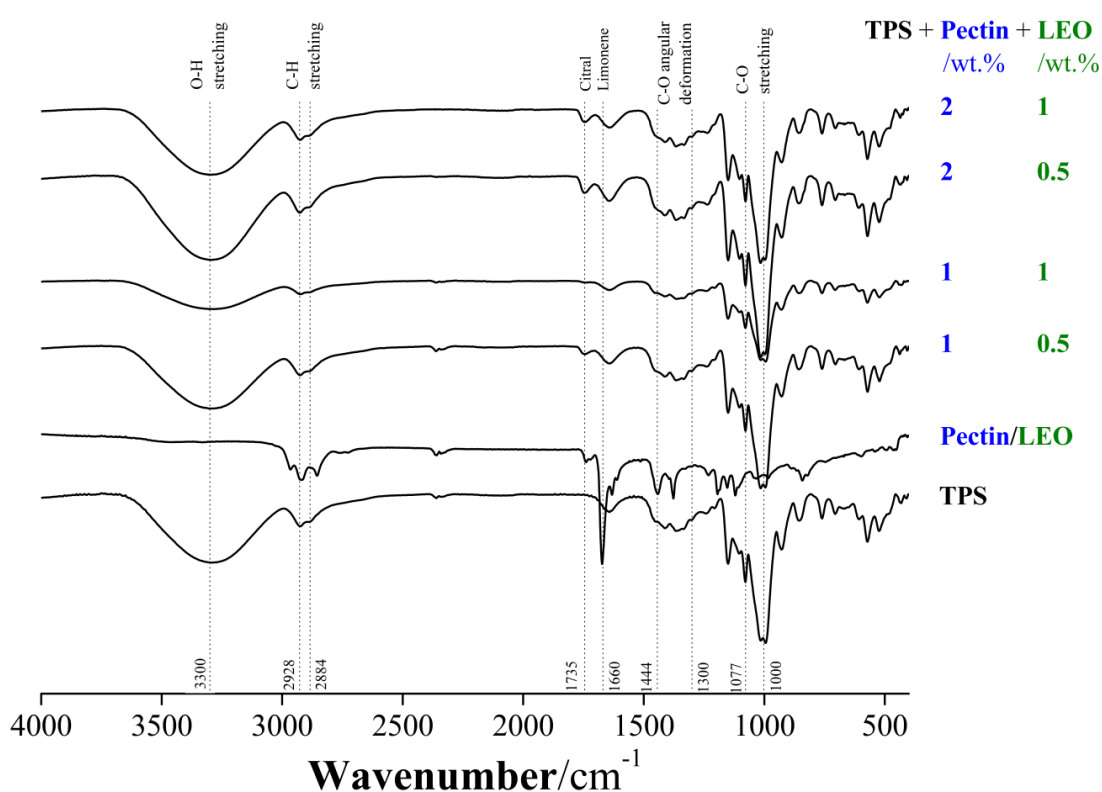

Figure 1. Attenuated total reflectance Fourier-transform infrared spectra of films comprising thermoplastic starch (TPS), pectin (0-2 wt.%), and lemongrass essential oil (LEO; 0.1 wt.%), in addition to TPS-free pectin/LEO sample. Y-axis: reflectance/a.u.

TPS-containing films presented a broad peak centered at *ca.* 3300 cm^{-1} associated with the stretching of hydroxyl groups that are abundant in starch, glycerol, and water (both free and adsorbed) molecules (Bourtoom & Chinnan, 2008; Dang & Yoksan, 2015; Herniou-Julien, Mendieta, & Gutiérrez, 2019; Pereira, de Arruda, & Stefani, 2015; Shi et al., 2007). The peaks at 2884 and 2928 cm^{-1} are attributed to asymmetric and symmetrical stretching

vibrations of the –CH bonds and are characteristic of starch CH_2 groups (Dang & Yoksan, 2015; Gutiérrez, 2017). In addition, bands between 1300 and 1444 cm^{-1} are attributed to –C–O– angular deformations (Gutiérrez & Alvarez, 2017). Peaks corresponding to –C=O groups were identified at 1077 cm^{-1}. The sharp peaks detected at 1000 cm^{-1} in all films is attributed to –C–O–C– glycosidic bonds (Gutiérrez, 2018). The peak at 1735 cm^{-1} for pectin/LEO spectrum may indicate the presence of citral, a highly therapeutic compound found in LEO, while that at 1660 cm^{-1} is attributed to limonene (Natrajan, Srinivasan, Sundar, & Ravindran, 2015). Alternatively, the peak at 1735 cm^{-1} could be associated with C=O stretching vibrations of the methyl esterified carboxyl groups at position C6 within pectin chain (*e.g.*, in pectin/LEO emulsions), since higher intensities were observed for higher pectin contents in the films.

FTIR spectra of TPS/pectin/LEO films encompass typical peaks of all components, as a merge of TPS-only and pectin/LEO-only spectra. Specifically, adding LEO caused the appearance of a peak at 1735 cm^{-1} (citral) as well as the increase in the intensity of that at *ca.* 1660 cm^{-1} (limonene), thus suggesting that the films retained the components that provide the antimicrobial action to LEO. Similar results were observed by Pelissari et al. (2009). Additionally, the band associated with the hydroxyl stretching vibration (3700-3000 cm^{-1}) was narrower upon LEO addition, possibly indicating the decrease in film hydrophilicity due to the presence of such a hydrophobic component. As further discussed in this text, this is in accordance with WVP results.

3.4. X-ray diffraction

XRD was used to analyze the crystalline structure and evaluate the compatibility of TPS and LEO. Figure 2 shows the XRD patterns for pure and pectin/LEO-containing TPS
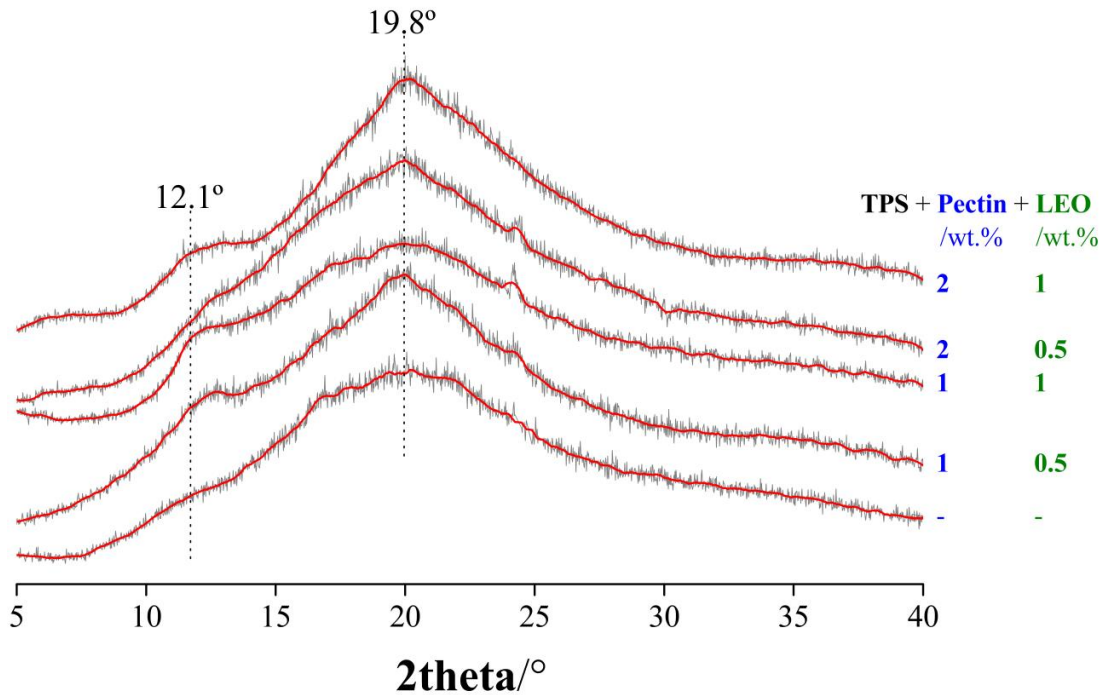
films.

Figure 2. X-ray diffraction patterns of thermoplastic starch (TPS)-based films containing pectin (0-2 wt.%) and lemongrass essential oil (LEO; 0-1 wt.%). Y-axis: intensity/a.u.; gray curve: actual data – used for calculations; red curve: smoothed – used for representation only.

The XRD pattern of the control (*i.e.*, TPS-only) film evidenced a semicrystalline pattern with peaks centered at 12.1 and 19.8 °, indicating the collapse of starch A-type crystal, prevailing the Vh-type crystal structure (Chanjarujit, Hongsprabhas, & Chaiseri, 2018; Corradini, de Morais, Demarquette, Agnelli, & Mattoso, 2007; Medina Jaramillo, Gutiérrez, Goyanes, Bernal, & Famá, 2016; van Soest, Hulleman, de Wit, & Vliegenthart, 1996). The presence of B structures arising mainly from starch retrogradation or non-gelatinized starch granules, typically fingerprinted by peaks at 14.9, 16.8, and 22.1 ° (Estevez-Areco, Guz, Famá, Candal, & Goyanes, 2019), was not verified herein. Thus, the starch films were fully gelatinized (see Figure 2) and did not undergo retrogradation, regardless of the presence of

emulsion. According to Denardin, C.C. & Silva (2009), A-type polymorph is a more stable, highly condensed, and organized structure. Yang, Yu, & Ma, (2006) suggested that Vh-type crystallinity is induced by thermal processing and arises from the replacement of the strong hydrogen bonding network of starch molecules by new hydrogen bonds formed between plasticizer and starch during thermoplastic processing. LEO addition, in turn, narrowed such diffraction peak as well as led to another peak at $2\theta = 12.1^\circ$. These changes could be associated with the inclusion of some pectin/LEO compound in the crystalline structure, which would modify the inter-planar spacing and deform the lattice and is in accordance with FTIR spectra (Figure 1). Besides, the peak at *ca.* 20 ° recorded in films containing pectin/LEO emulsions showed higher intensity with respect to the control. The increased relative height indicates a different structure factor, which could be associated with the presence of another molecule in the Vh crystals. Similar results were reported by Estevez-Areco, Guz, Famá, Candal, & Goyanes (2019).

3.5. Thermogravimetry

The thermogravimetric (TG) curves and their first derivatives (DTG) for TPS-based films are shown in Figure 3. The temperatures corresponding to the onset (T_{onset}) and offset (T_{offset}) of thermal degradation are presented in Table 3.
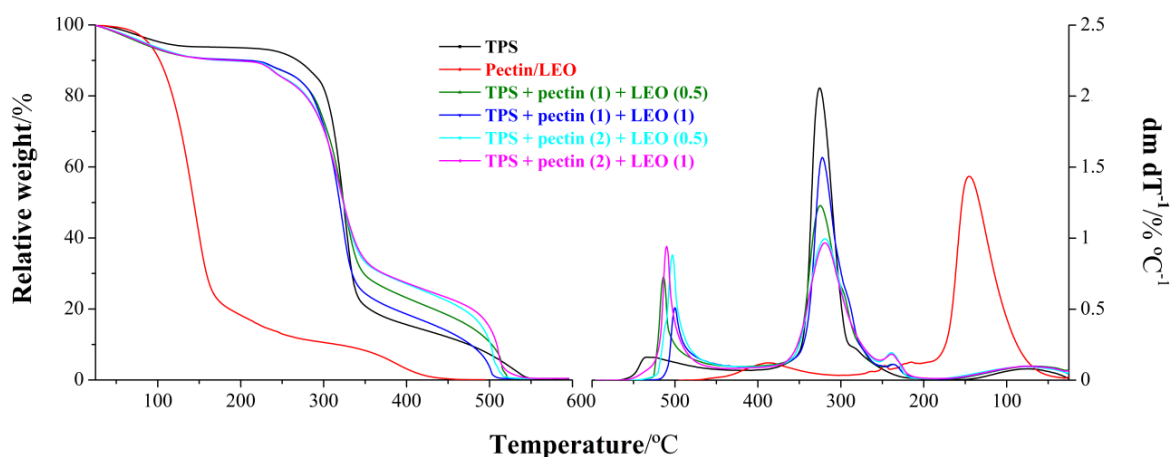
Figure 3. Thermogravimetric (TG; left) and derivative TG (right) profiles of films comprising thermoplastic starch (TPS), pectin, and/or lemongrass essential oil (LEO). Numbers in parentheses correspond to weight percentages of pectin and LEO.

The thermal decomposition of the produced films occurred in three main stages, similarly to other TPS-based films (García, Famá, Dufresne, Aranguren, & Goyanes, 2009). In the TPS-containing film, the first stage (from room temperature to *ca.* 150 ºC) refers to the physical desorption of water and/or other volatiles. The second mass loss stage (*ca.* 180-260 ºC) is related to complex processes including the dehydration of the saccharide rings, depolymerization (Basiak, Lenart, & Debeaufort, 2017; Mathew & Dufresne, 2002), and glycerol evaporation (boiling point = 182 ºC). Finally, the third stage (between 230 and 326 ºC) refers to the degradation of starch components (Liu, Xie, Yu, Chen, & Li, 2009; Wilhelm, Sierakowski, Souza, & Wypych, 2003). Two mass loss events were observed for TPS/pectin/LEO films, the first one at around 240 ºC, related to pectin depolymerization (Meneguin et al., 2017), and the second one at 325 ºC, attributed to TPS degradation.

For the emulsified films, the starch-rich phase started to decompose at a slightly lower temperature (Table 3). TPS was thermally stable up to 308 °C, whereas emulsified TPS films were thermally stable up to 293 °C. The reduced onset degradation temperature (T_{onset}) for emulsified films suggests that adding emulsion may have decreased the molecular interactions

among adjacent starch chains as well as strengthened the hydrogen bonding between the hydroxyl groups of starch and of the polar compounds (polyphenols) found in the pectin/LEO emulsion, as indicated by FTIR (Figure 1) and XRD (Figure 2). A decrease in T_{onset} in thermoplastics due to the addition of emulsions or extracts with antioxidant capacity has been previously reported (Cerruti et al., 2011; Medina Jaramillo et al., 2016; Sanyang, Sapuan, Jawaid, Ishak, & Sahari, 2016). This was also observed in polymer-based films with greater plasticization (Curvelo, 2001; Sanyang et al., 2016). Therefore, the pectin/LEO emulsions not only act as an antioxidant, but also as a plasticizer, as per the results presented in Table 3. This is of particular relevance because films supplemented with low-molecular weight plasticizers (*e.g.*, glycerol) are known to be susceptible to exudation throughout storage or usage due to the migration of such towards film surface. This would eventually lead to increased brittleness, decreased extensibility, and appearance of a glossy aspect, which are overall undesirable for packaging materials. Even though we did not investigate such properties on a time course, one may expect exudation to be remarkably lower in the herein produced materials, provided the polymeric nature of the plasticizer-encapsulating agent. Finally, regardless of composition, all films had a remarkably low residual mass at 600 °C (Table 3). Overall, components comprising aromatic rings in their chemical structures are stable up to 600 °C (Medina Jaramillo et al., 2016; Patel et al., 2010).

3.6. Optical properties

Color and transparency of food packaging materials play an important role in their appearance and consumers acceptance. The effect of pectin/LEO on the colorimetric parameters of TSP films is presented in Table 2.

Table 2. Luminosity (*L**), chromatic coordinates (*a** and *b**), and colorimetric parameters (*ΔE*, total color difference; *YI*, yellowness index; *WI*, whiteness index) of thermoplastic starch films as influenced by different concentrations of lemongrass essential oil (LEO) and pectin.

Pectin/LEO /wt.%	*L**	*a**	*b**	*ΔE*	*YI*	*WI*
0	93±1 [a]	-0.38±0.01 [c]	0.32±0.05 [c]	-	0.5±0.1 [c]	92±1 [a]
1/0.5	93.3±0.1 [a]	-0.52±0.01 [b]	1.77±0.03 [b]	0.4±0.03	2.7±0.05 [b]	93.0±0.9 [a]
1/1	93.6±0.4 [a]	-0.53±0.01 [b]	1.7±0.3 [b]	0.6±0.3	2.7±0.4 [b]	93.3±0.5 [a]
2/0.5	93.1±0.1 [a]	-0.57±0.01 [b]	2.3±0.1 [b]	0.3±0.1	3.5±0.1 [b]	92.7±0.2 [a]
2/1	93.4±0.2 [a]	-0.76±0.04 [a]	3±1 [a]	0.7±0.2	5.2±0.9 [a]	92.5±0.2 [a]

[a-c] Different letters in the same column indicate a significant difference (p<0.05)

 L* represents the luminosity (ranging from black to white) in the color system, low *L** values corresponding to dark and high *L** values referring to bright. The parameters a* and b* are the chromatic coordinates, where + *a** and -*a** are in the red and green directions, respectively, and + *b** and -*b** are in the yellow and blue directions, respectively. The values of *a** and *b** approach zero for neutral colors and increase when color turns more chromatic and saturated. The luminance (*L**) and red-green (*a**) parameters increased significantly by increasing pectin/LEO concentration compared to the control film. The same occurred for the blue-yellow (b*) parameter as LEO and emulsifier concentrations increased. The values of total color difference (*ΔE*) were calculated considering cassava starch film as standard. All films presented high lightness (*i.e.*, *L** values close to 100), which is recurrent for edible films (Araujo-Farro, Podadera, Sobral, & Menegalli, 2010; Basiak, Debeaufort, & Lenart, 2016; Galus & Kadzińska, 2016). In addition, by increasing the pectin/LEO concentration in the films, the *YI* increased significantly. Most of the investigated films presented *ΔE* < 2, which means that the differences in appearance among these films and the control are slight. The same was reported by Pająk, Przetaczek-Rożnowska, & Juszczak (2019). On the other hand, the *WI* did not change with increasing LEO concentration. The results corroborate with those

reported by Hasheminya et al. (2019), Hasheminya et al. (2019), and Atef, Rezaei, & Behrooz (2015). In this sense, the color of emulsified films is directly dependent on the essential oil type and concentration, as well as on the presence of additives (*e.g.*, pectin to increase emulsion stability).

3.7. Mechanical properties

The effect of different pectin/LEO concentrations on the mechanical properties of TPS-based films was investigated and the results are presented in Figure S3 and Table 3. The interaction among pectin/LEO and TPS was significant ($p<0.05$) for the elastic modulus (EM), tensile strength (TS), elongation at break (ε), and toughness.

Table 3. Tensile attributes – elastic modulus (EM), tensile strength (TS), elongation at break (ε) and tenacity (T) – as well as thermal parameters – initial (T_{onset}) and final (T_{offset}) degradation temperatures and residual mass at 600 ℃ – of thermoplastic starch (TPS) films as influenced by different concentrations of lemongrass essential oil (LEO) and pectin (P).

P/LEO /wt.%	Thickness /mm	TS /MPa	ε /%	EM /MPa	T /J.mm^{-3}	T_{onset} /℃	T_{offset} /℃	R_{600} /%
0	0.05±0.01 [c]	21±2 [b]	2.4±0.2 [c]	975±123 [c]	22±7	308	486	0.2
1/0.5	0.12±0.02 [a]	24±1 [b]	2.8±0.1 [bc]	1326±25 [a]	29±4	175	353	1.1
1/1	0.1±0.1 [a]	25±4 [b]	2.82±0.02 [b]	1361±73 [a]	36±14	293	507	0.5
2/0.5	0.12±0.02 [a]	31±2 [a]	4.7±0.4 [a]	1439±68 [a]	67±20	293	488	0.5
2/1	0.15±0.04 [b]	16±4 [c]	3.0±0.3 [bc]	1189±88 [b]	12±6	285	495	0.4

Mechanical attributes are reported as average values and standard deviations. The same letters on the same column indicate that the values are not statistically significant ($p>0.05$) using Tukey's test.

The incorporation of pectin/LEO emulsion increased the thickness of the films (Table 3).As expected, the addition of pectin/LEO emulsions, regardless of LEO content and droplet size, increased the extensibility of TPS films, as indicated by increased ($p<0.05$) elongation at break values by 16 and 47% for nano- and micro-sized droplets, respectively (Table 3). The same was observed for toughness. This suggests that LEO acted as a plasticizer by increasing the flexibility of polymer chains, which is in accordance to similar systems comprising different essential oils and biopolymer matrices (Otoni et al., 2014; Prodpran, Benjakul, & Artharn, 2007; Souza, Goto, Mainardi, Coelho, & Tadini, 2013).

Contrastingly, our emulsions also increased ($p<0.05$) the TS (47%) and EM (48%) of TPS-only films (Table 3). Although the exact mechanism remains unclear, a range of factors may be contributing, concurrently or separately, to this observation. XRD patterns (Figure 2), for instance, indicated higher crystallinity (Figure 4) when emulsions were added, characteristics that are in line with more mechanically resistant and tougher films. Also, interactions between starch and emulsion components (*e.g.*, pectin), as suggested by FTIR (Figure 1), may also take place and lead to increased crystallinity (Figure 4) and therefore strength. Droplet size may have influenced these properties, as smaller droplets have higher surface areas available to increase the level of interaction with the TPS matrix (Figure 1), whereas larger particles may disturb film formation. These theories are supported by reports of de Moura et al. (2009) and Pereira, et al(2007). It is believed that TPS-TPS interactions were partially replaced by TPS-pectin/LEO interactions that strengthened the associative network, thus improving the TS of the emulsified films. Indeed, according to Ahmad, Benjakul, Prodpran, & Agustini (2012), essential oils at appropriate levels can strengthen protein-based film by increasing the interaction among adjacent chains, due to hydrogen bond-based cross-link formation between small mobile plasticizer molecules and the polymer side chain (Roussenova, Murith, Alam, & Ubbink, 2010). Behavior that can also be explained by the anti-plasticizing acting of essential

oil in biopolymer matrices at certain conditions, as can be seen in other studies (Luís, Pereira, Domingues, & Ramos, 2019). Fishman, Coffin, Konstance, & Onwulata (2000), state in their studies that starch and pectin have strong interactions, enabling highly compatible film formations in which they have a wide range of good mechanical properties (Coffin, D.R.; Fishman, 1994; Fishman, Coffin, Unruh, & Ly, 1996). Regardless of the mechanism, the emulsified films presented TS values comparable to HDPE (typically 22-23 MPa) and low-density polyethylene (LDPE; typically 19-44 MPa), materials which are commonly used in commercial packaging. The same was observed for EM values (Table 3) and could also be associated with the lower content of adsorbed water – known to plasticize biopolymer-based films – in films containing nanoemulsions (Figure 4) because of the hydrophobic nature of essential oils.

3.8. Water vapor permeability (WVP)

The water barrier properties of the TPS-based films added or not by LEO emulsions were investigated through WVP measurements, and the results are shown in Figure 4.
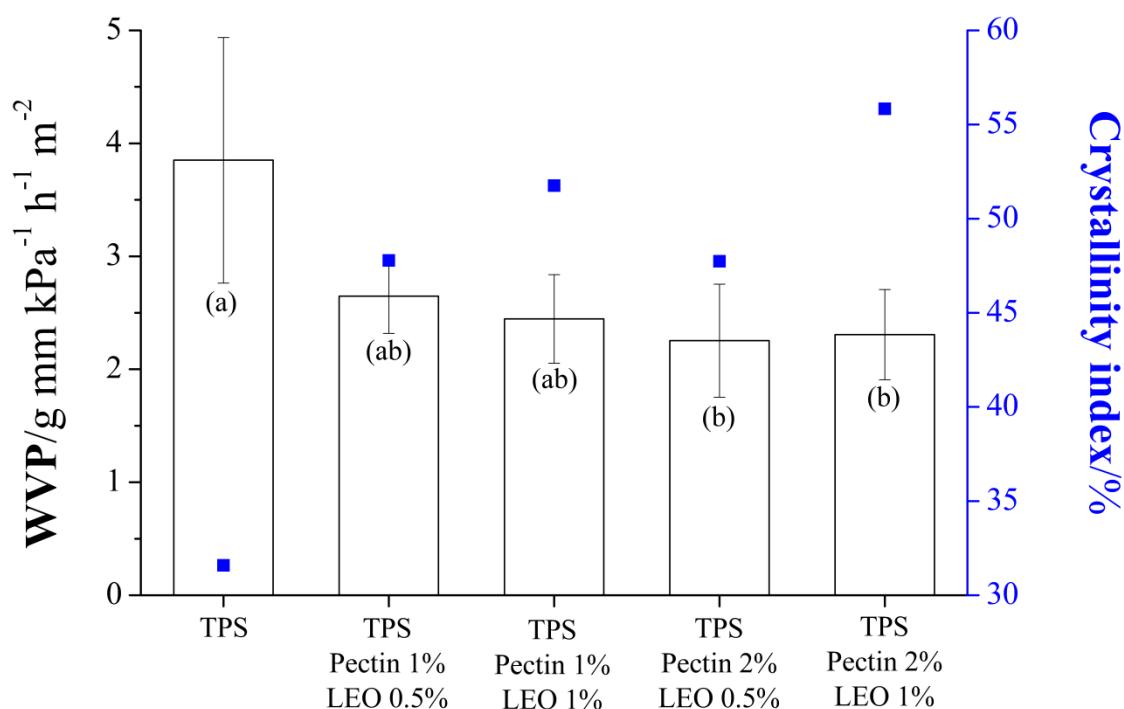
Figure 4. Water vapor permeability (WVP) and crystallinity index of films based on thermoplastic starch (TPS), either added or not with pectin and lemongrass essential oil (LEO). Contents are weight percentages. Equal letters in parentheses indicate equal WVP values ($p > 0.05$).

The WVP of TPS films was significantly reduced ($p < 0.05$) by *ca.* 70% upon emulsification, but all pectin/LEO-containing films showed the same ($p > 0.05$) WVP, indicating that droplet size did not influence the barrier properties. It is worth mentioning that WVP is directly related to the quantity of polar (*e.g.*, hydroxyl) groups in the molecular assembly (Huber & Embuscado, 2009). Pectin/LEO addition provided TPS films with increased crystallinity (Figures 2 and 4) due to the increased mobility and rearrangement of starch chains, decreasing the availability of hydroxyl groups responsible for polysaccharide-water interactions, which resulted from the formation of hydrogen bonds between pectin/LEO and hydroxyl groups of starch (Hosseini, Razavi, & Mousaviousavi, 2009; Möller, Grelier, Pardon, & Coma, 2004; Park & Zhao, 2004). The uniform and interconnected matrix (Figure

S2) of film added with emulsions favored the reduced water vapor diffusivity at film interface. According to Oliveira et al. (2016), the use of pectin as an emulsifier increased film hydrophobicity, as well as the addition of essential oil emulsions into polysaccharide-based films improved their moisture barrier (Lorevice et al., 2016). A similar effect was reported by Nisar et al.(2018) for pectin-based films supplemented with clove bud essential oil. Considering the WVP levels, the herein produced films are appropriate for use as moisture barriers in food packaging (Bedane, Eić, Farmahini-Farahani, & Xiao, 2015; Jiménez, Fabra, Talens, & Chiralt, 2012b; Park & Zhao, 2004).

3.9. Biodegradability in vegetal compost

In order to evaluate film biodegradability, a qualitative degradation assay was carried out in soil. A quantitative approach by means of sample weight over time was not considered because residual soil impaired accurate weighing. Film images throughout a 30-day period are shown in Figure 5.
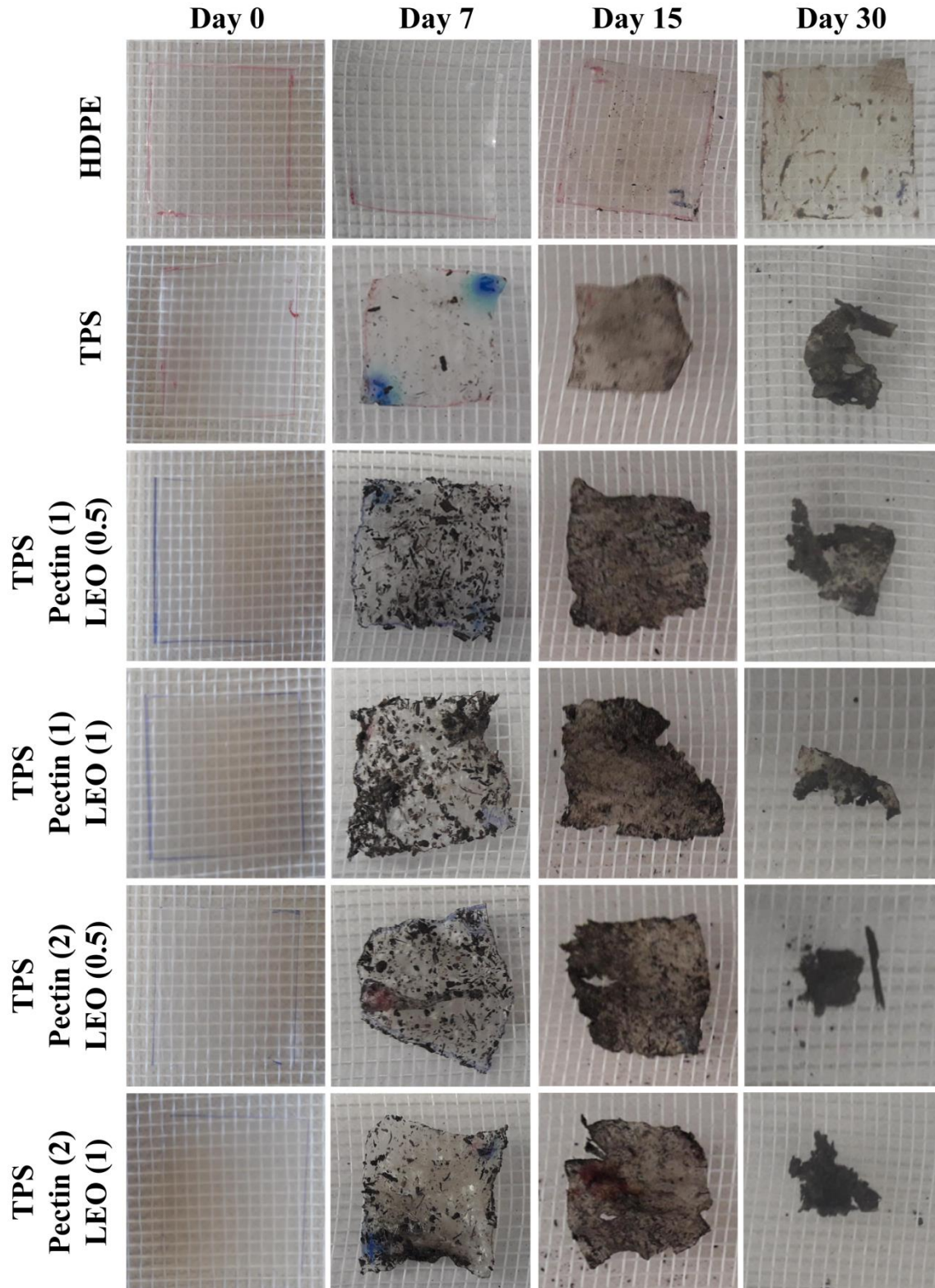
Figure 5. Pictures of films based on thermoplastic starch (TPS), pectin, and/or lemongrass essential oil (LEO) during biodegradation in soil for 30 days. High-density polyethylene (HDPE) film was used for comparison. Numbers in parentheses correspond to weight percentages of pectin and LEO in film-forming formulation.

After a 7-day storage, all TPS-based films presented altered in their tonality and breakdowns, indicating hydrolysis reactions that occur during early degradation (Cinelli et al., 2014; Medina Jaramillo et al., 2016) Except HDPE, all films were almost entirely degraded within a month. Indeed, HDPE was used as a negative control provided its widely known lack of biodegradation capacity in short periods. Starch, in turn, has been extensively demonstrated to degrade in vegetal compost (Medina Jaramillo et al., 2016; Torres, Troncoso, Torres, Díaz, & Amaya, 2011; Xiong, Tang, Tang, & Zou, 2008). Xiong et al. (2008) observed that starch films biodegraded within 100 days. Medina Jaramillo et al. (2016) noted complete film degradation after 6 days. Torres et al. (2011) observed a weight loss of 99.5% after 31 days. The degradation profile of starch, and particularly TPS, was corroborated here. Interestingly, this was not qualitatively affected by the addition of LEO emulsions, suggesting that this component did not impair TPS biodegradability. Therefore, film disintegration meets ISO 20200 (Plastics – Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test) specifications for 90+% disintegration within 30 days, as seen in Figure 5. This outcome is relevant because it indicates that it was possible to produce new polymer-based materials featuring active properties but still biodegradable.

4. Conclusion

In this contribution, we demonstrated that the addition of LEO emulsions by direct emulsification with two emulsifiers (pectin and Tween 80) to TPS-based films affected their matrices, providing these with active functionality while preserving or even improving their physical-mechanical properties. Barrier, optical, and thermal properties were the most affected by emulsion addition. In particular, emulsification improved film mechanical properties, increasing extensibility, resistance, and stiffness. Droplet size also played a role,

being mainly attributed to the hydrophobic and plasticizing natures of the dispersed phase, which were boosted upon the increase in surface area by means of miniaturization. Finally, the TPS/pectin/LEO films showed suitable degradation in vegetal compost, securing their complete biodegradation in a short time. In summary, the addition of nano- and micro-sized emulsions into TPS-based films provided these with relevant functional properties, further increasing its potential application in the food industry.

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Supporting Information for

Correlating emulsion characteristics with the properties of active starch films loaded with lemongrass essential oil

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Table S1. Chemical composition of lemongrass essential oil determined by gas chromatography-mass spectrometry (GC/MS).

Component	Retention time[a] /min	Content /%	Refractive index
Tricyclene	5.57	0.26	923.29
α-Pinene	5.83	0.37	932.78
Camphene	6.29	1.85	949.53
6-Methyl-5-hepten-2-one	7.23	1.47	983.43
D-Limonene	8.77	0.42	1038.77
3.7-dimethyl-1.3.7-octatriene	8.95	0.35	1045.31
β-Ocimene	9.35	0.24	1059.60
4-Nonane	10.27	1.20	1092.92
Linalool	11.47	1.79	1102.36
2.3.6-Trimethyl-1.6-heptadiene	13.01	0.27	1144.19
trans-Chrysanthemal	13.44	0.55	1155.94
3,7-Dimethyl-6-octenal (Citronellal)	13.55	0.33	1158.79
Verbenol	13.96	1.15	1169.97
trans-4,5-epoxy-Carane	14.74	1.88	1139.27
Neral	17.52	29.08	1222.59
Geraniol	18.07	8.05	1228.97
Geranial	18.89	34.79	1238.46
Geranyl acetate	23.27	5.54	1340.02
Caryophyllene	24.97	3.66	1360.35
t-cadinene	28.82	2.48	1457.68

Caryophyllene oxide	31.52	1.13	1541.44
Monoterpene hydrocarbons	3.8		
Oxygenated monoterpene	85.8		
Oxygenated sesquiterpenes	4.8		
Others aliphatic hydrocarbons	6.1		
Total identified compounds	96.9		

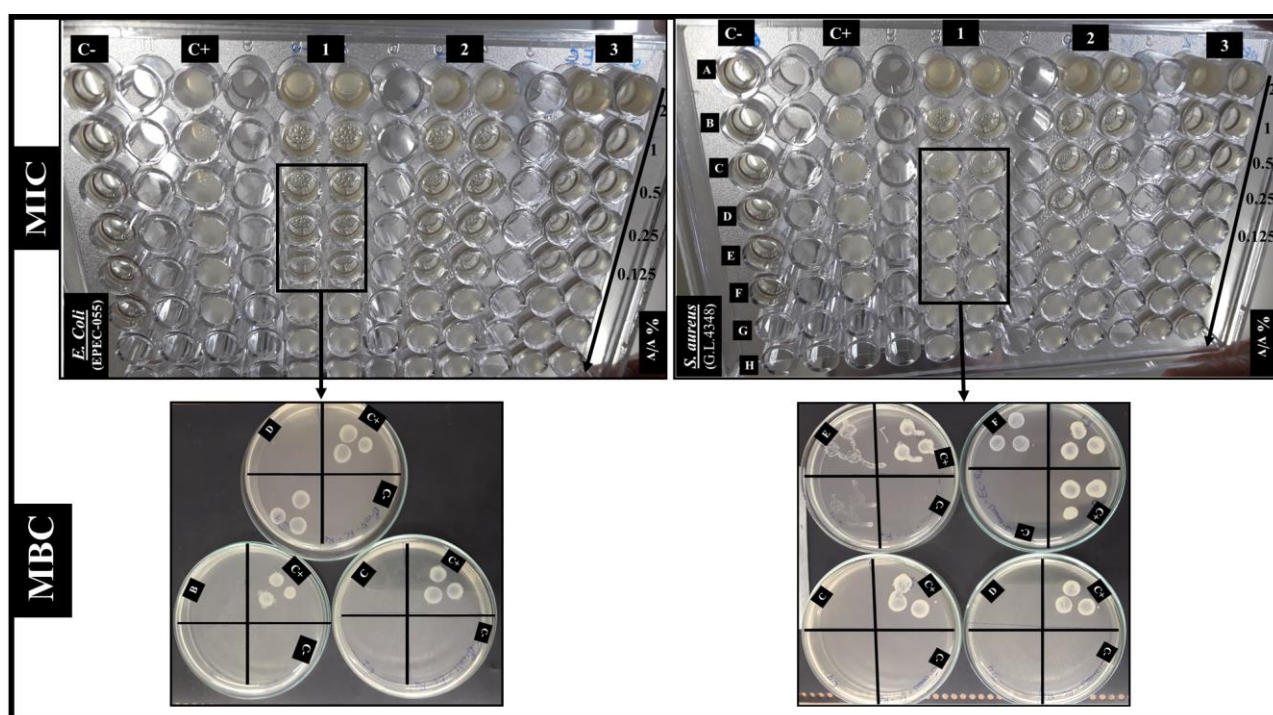[a] MS acquisition started 4 min after injection.

Figure S1. Minimum inhibitory (MIC) and bacteriostatic (MBC) concentrations of lemongrass essential oil (LEO) at different concentrations (0.015-2 vol.%) against *Escherichia coli* and *Staphylococcus aureus*. C- and C+ correspond to negative (bacterium-free) and positive (LEO-free) controls, respectively; 1-3 correspond to three repetitions of the same treatment.
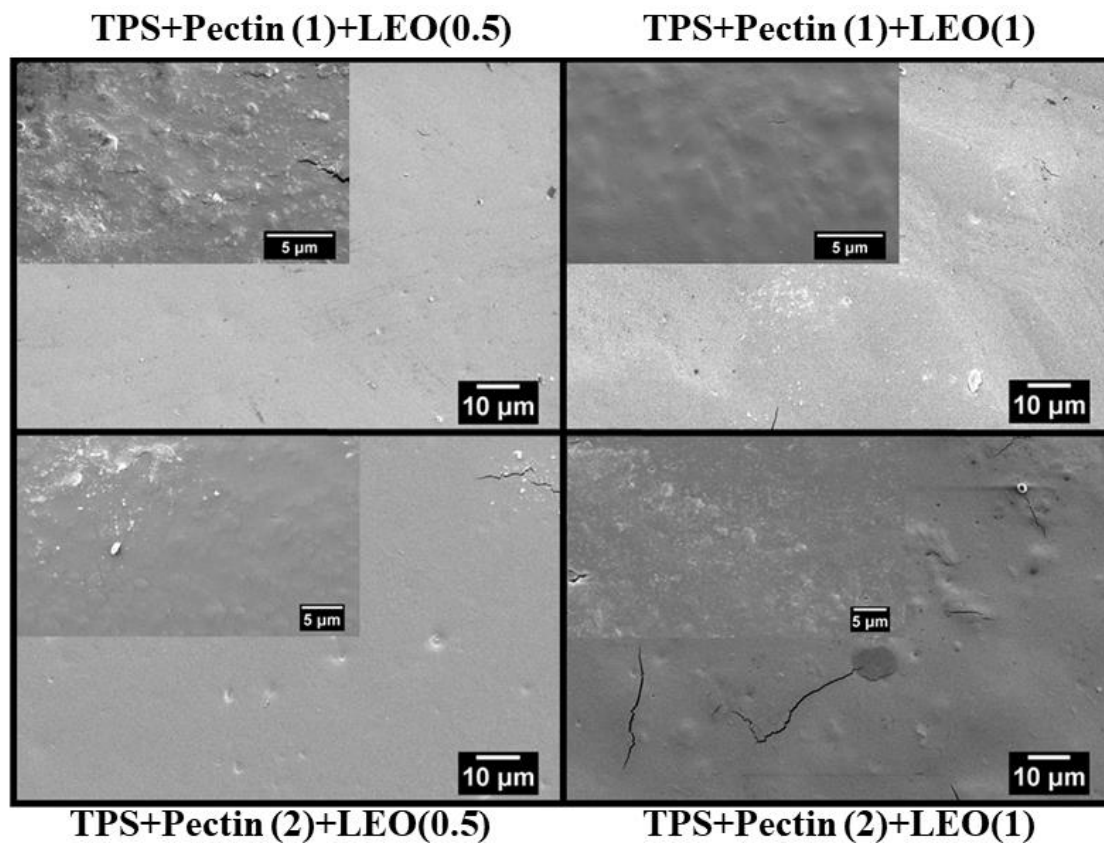
Figure S2. Scanning electron micrographs of the surfaces and cross sections of films based on thermoplastic starch (TPS), pectin, and lemongrass essential oil (LEO). Insets represent higher magnification images of the same samples. Numbers in parentheses correspond to weight percentages of pectin and LEO.
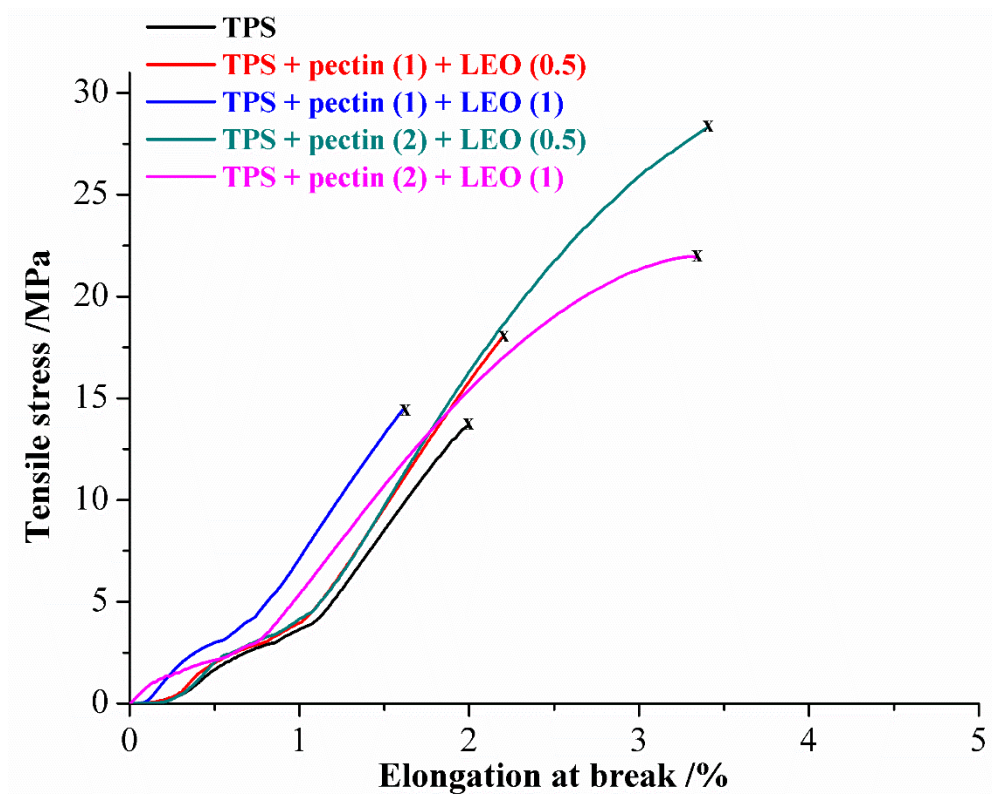
Figure S3. Stress-strain curves of films based on thermoplastic starch (TPS), pectin, and lemongrass essential oil (LEO). Numbers in parentheses correspond to weight percentages of pectin and LEO. x present in stress versus strain curve indicates rupture.

ARTICLE 4 - On the development and sensory perception of quaternary nanocomposites made up of cassava starch, cocoa butter, lemongrass essential oil nanoemulsion, and malt bagasse fibers
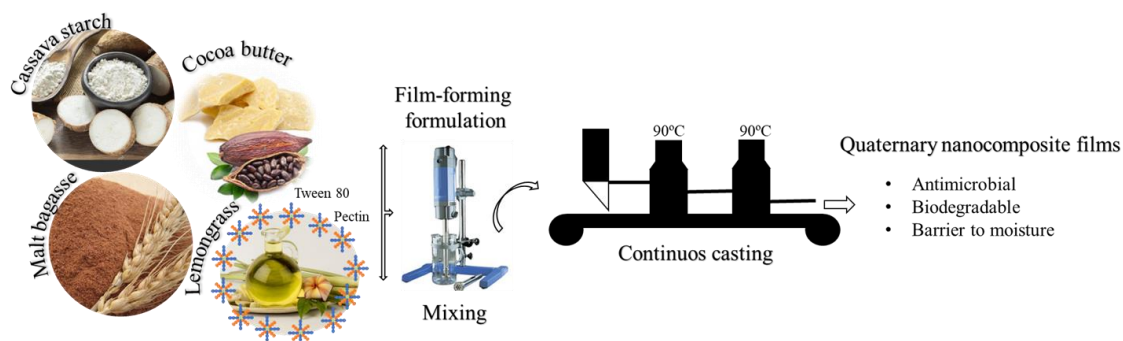
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On the development and sensory perception of quaternary nanocomposites made up of cassava starch, cocoa butter, lemongrass essential oil nanoemulsion, and malt bagasse fibers

Mendes, J.F.[a*], Norcino, L.B.[b], Martins, H.H.A.[a], Manrich, A.[c], Otoni, C.G.[c], Carvalho, E.E.N.[d], Piccoli, R.H.[d], Oliveira, J.E.[e], Pinheiro, A.C.M.[d], Mattoso, L.H.C.[c]

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Abstract

We report on the large-scale production of novel quaternary nanocomposite films based on cassava starch, cocoa butter, (CB), and lemongrass essential oil (LEO) nanoemulsions reinforced with different concentrations of malt bagasse (MB) fibers. The addition of CB, LEO, and MB caused relevant changes in the starch-based films, such as increased extensibility (from 2.4 to 9.4%) and improved barrier to moisture. Contrastingly, the thermal stability of the starch film was slightly decreased. The biodegradability of the herein developed quaternary nanocomposite films was the same as that of thermoplastic starch films, eliminating concerns on the supplementation with active ingredients that are expected to have some biocidal effect. Overall, this new approach towards quaternary active, biodegradable films produced in a pilot-scale lamination unit was successful in either improving or at least maintaining the essential properties of starch-based films for food packaging applications, while providing them with unique features and functionalities.

1. Introduction

The greater awareness of consumers towards food safety and environmental issues has increased the number of studies on the replacement traditional fossil-derived materials with active biodegradable films to either extend food shelf life or to improve their sensory properties (Bahrami, Rezaei Mokarram, Sowti Khiabani, Ghanbarzadeh, & Salehi, 2019; Zhuang et al., 2018). Active biodegradable films are usually produced from polysaccharides, proteins, lipids, and combinations among these (Xu et al., 2019) that are to be optimized in an effort to achieve suitable biodegradability, biocompatibility, and potential to carry active additives such as nutritional, antimicrobial, or antioxidant agents.

Natural additives such as essential oils (EOs), are extracted from aromatic plants and exhibit well-documented antimicrobial activities and are hydrophobic (Ojagh, Rezaei, Razavi, & Hosseini, 2010; Xu et al., 2019). This capability is thanks to their structure, compositions, and functional groups. When added as cargo into packaging materials, the retention and release profiles of EOs are to be optimized in order to maintain consistently effective doses during long-term storage (Ojagh et al., 2010; Xu et al., 2018). Considering the (potentially adverse) sensory effects of directly adding EOs into food matrices, as well as their relatively high costs, incorporating these compounds into polymer matrices in the emulsified state may address several limitations related to miscibility and stability, further enabling practical applicability. The EO from lemongrass (*Cymbopogon citratus*), also known as Melissa, encompasses neral, citral, and geranial as main active components, giving it a strong lemony aroma and promising functional properties (Mohamed Hanaa, Sallam, El-Leithy, & Aly, 2012). In recent years, the use of nanoemulsions has been intensively investigated for improving the performance of films intended food packaging (Amit Kumar Tyagi, Davide Gottardi, Anushree Malik, 2014; Espitia, Fuenmayor, & Otoni, 2019; Lei, Wang, Li, & Wang, 2019). Indeed, these systems benefit from their nano-sized droplets to perform differently

from conventional emulsions due to the larger surface area per mass or volume, feature which allows for a slower and more controlled release of active ingredients from film matrices (Acevedo-Fani, Soliva-Fortuny, & Martín-Belloso, 2017).

Another promising approach for the production of sustainable materials relies on the biorefinery concept of converting waste or byproducts of the food industry into energy or other products for our daily activities. Lignocellulosic residues containing high (hemi)cellulose and lignin loads represent a major waste from food processing. Among such, malt bagasse (MB) stands out as a beer brewing byproduct consisting mainly of bark, pulp, malt grains, and some additives such as rice, corn, and wheat (Cordeiro et al., 2013). According to Mello & Mali (2014), MB has the potential to increase the mechanical properties of starch-based materials. However, the low moisture barrier starch/MB composite films. A means of overcoming this practical limitation as far as food packaging applications is their combination with lipids. Cocoa butter (CB), for instance, may prevent moisture migration due to its hydrophobic nature and tightly packed crystal structure (Bravin, Peressini, & Sensidoni, 2004).

In this context, the aim of this study was to develop new multifunctional quaternary films using thermoplastic starch (TPS), MB, CB, and LEO nanoemulsions and application as primary packaging in chocolates. This is, as far as it is known, the first study on the continuous development of quaternary TPS-based films through a pilot-scale lamination unit. Ultimately, the roles played by the reinforcing components and lipid emulsions in the key properties of the produced films were elucidated, aiming at the large-scale production of biodegradable films for food packaging applications.

2. Materials and methods

2.1. Materials

Cassava starch (amylose content: 17-20% wt%) was purchased from Fécula da Amazônia (Moju-PA, Brazil). High methyl ester pectin (degree of methyl esterification > 50%; M_w = 130,000 g mol^{-1}) was supplied by CP Kelco (Limeira-SP, Brazil). Glycerol and Tween 80 were obtained from Synth (Rio de Janeiro-RJ, Brazil). LEO was obtained from Ferquima (Vargem Grande Paulista-SP, Brazil). CB was purchased from the local trade. MB (cellulose, hemicellulose, and lignin contents: 28, 32, and 26 wt%, respectively; 0.07-mm-long fibers) was kindly provided by Cervejaria Joia Mesquita (Lavras-MG, Brazil). *Staphylococcus aureus* (ATCC6538) was obtained from Embrapa Gado de Leite (Juiz de Fora-MG, Brazil).

2.2. Emulsification production and characterization

The emulsion was prepared in accordance with the method proposed by López-Mata et al. (2018) and modified by us, as described in a previous publication (Mendes et al. (2020). The final formulation contained pectin (1 wt%), glycerol (500 µg g^{-1}, dry basis), Tween 80 (0.1 vol%, wet basis), and LEO (1.0 vol%, wet basis). Droplet size (z-average), polydispersity index (PdI), and short-term kinetic stability were determined by dynamic light scattering, as described previously Mendes et al. (2020).

2.3. Film-forming protocol and characterization techniques

Films were produced as detailed in our previous paper Mendes et al. (2020), except for the addition of CB and MB. Briefly, an aqueous dispersion of cassava starch (8% w/v), glycerol (20 wt%), CB (30 wt%), and MB (5 or 10 wt%) – weight percentages are relative to starch dry mass – was combined at 1:1 (weight ratio) with the previously prepared emulsion, homogenized, degassed, and cast into films on a KTF-S-B continuous lamination system (Mathis, Switzerland). Dried films were equilibrated at $52 \pm 3\%$ relative humidity (RH) and

$25 \pm 2\,°C$ and characterized, as described previously Mendes et al. (2020), as far as their *optical properties* – luminosity (*L**) and the chromatic coordinates *a** and *b** were determined and used to calculate total color difference (*ΔE**), yellowness index (*YI*), and whiteness index (*WI*); *thermal properties* – by thermogravimetry; *mechanical properties* – through tensile assays to determine tensile strength (*TS*), elongation at break (*EB*), and elastic modulus (*EM*); *barrier properties* – *via* water vapor transmission rate (*WVTR*) water vapor permeability (*WVP*) determinations; and *biodegradability in vegetable compost*. LEO compounds were identified and quantified in the herein produced films by gas chromatography-mass spectrometry and Fourier-transform infrared (FTIR) spectroscopy, also as reported previously Mendes et al. (2020). Finally, in addition to this comprehensive set of characterizations, the quaternary films were characterized through sensory and antimicrobial evaluations, as follows.

2.4.   Antimicrobial activity

The antibacterial activity of the films was assessed using the agar disc diffusion method described by Martucci, Gende, Neira, & Ruseckaite (2015) and Pereda, Ponce, Marcovich, Ruseckaite, & Martucci (2011). Films were tested both 24 h and 1 year after they were produced to investigate the long-term maintenance of its activity. Film samples were aseptically cut into 10-mm-diameter discs and then placed onto agar plates previously seeded with 100 µL of an inoculum containing 10^8 *S. aureus* colony-forming units (CFU) per mL. The plates were then incubated at 37 ºC for 24 h and the diameters of the growth-free (inhibition) zones surrounding the film discs were accurately measured with a caliper (Mitutoyo, Japan) from the center of the film. Each assay was performed in triplicate on two separate experimental runs.

2.5.   Acceptance test

The herein developed quaternary films were used as primary packaging for dark and white chocolate. They were subsequently wrapped with aluminum foil and stored for 7 days. After that period, the packaging was removed and the chocolates were evaluated for acceptability of chocolate aroma, taste and global impression by potential consumers. A total of 120 chocolate consumers was recruited, with a regular frequency, in addition to consuming chocolate and having healthy eating habits, of which 52% were male and 48% female. Consumers and chocolate lovers, both black and white were between 18 and 50 years old, of which 66% were between 18 and 25 years old, 33% were between 26 and 45 years old and 1% were between 46 and 50 years old, respectively. The evaluations were performed in individual booths under artificial daylight at temperatures ranging between 22 and 24 ℃ with air circulation. The samples were evaluated in a monadic manner in a complete and balanced block, the evaluation being carried out in two sessions to avoid sensory fatigue. Each participant evaluated all six samples (three white and three dark chocolates), with a control for each type of chocolate according to a balanced and randomized experimental design. The samples (one unit at a time) were served in disposable cups coded with randomized 3-digit numbers. A 9-point structured hedonic scale was used for quantifying acceptability, which ranged from "(1) extremely disliked" to "(9) extremely liked" (Ferreira Saraiva et al., 2016). The study plan was previously evaluated by the Research Ethics Committee of the Federal University of Lavras (Lavras-MG, Brazil; under resolution # 89319918.9.0000.5148), which endorsed the development of this study (protocol # 2746637).

2.6.   Statistical analysis

The data from the physical and the consumer test were submitted to analysis of variance (ANOVA) and Tukey's test, both at 5% significance (p = 0.05), in the Sisvar® software, version 5.4.

The sensory results (aroma, flavor and global impression) were analyzed by the t-Test, to verify differences between white and black chocolates. For PARAFAC the consumer acceptance data sets were arranged in a matrix of i lines (samples) and j columns (consumers) for the attribute of overall liking; and in a matrix of I lines (samples) and j columns (attributes) by calculating the average of consumer assessments. Data were auto scaled and the PARAFAC were carried out using SensoMaker software -version 1.7 (Cruz et al., 2013; Pinheiro et al., 2013). Plots of scores and loadings were built form the first 2 principal components (Matera et al., 2014; Gaze et al., 2015).

3. Results and discussions

3.1.   Emulsion morphology and kinetic stability

As expected, the mechanical energy input and the presence of surfactants (pectin and Tween 80) led to emulsions comprising droplets with z-average diameters of 372±5 nm and narrow size distribution (PdI values no greater than 0.29±0.01). Put other way: this combination of components – continuous (water) and dispersed (LEO) phases plus the surfactants – and processing conditions provided homogeneous nanoemulsions, each of these playing important roles: while on one hand, high energy inputs create deforming forces that overcome Laplace pressure and miniaturize the dispersed phase (Fernandez, André, Rieger, & Kühnle, 2004), on the other hand this top-down approach is likely to be suppressed by thermodynamically driven coalescence unless surface active substances (*e.g.*, surfactants) can slow down or even prevent this process.

In addition to the stabilizing role of surfactants, droplet size is also expected to affect kinetic stability. Particles are susceptible to gravitational forces regardless of their size, but nanosized droplets are less likely to sediment than to larger droplets because the Brownian motion of smaller droplets may be greater than the gravity-induced creaming rate (Donsì & Ferrari, 2016; Huang, Yu, & Ru, 2010; Otoni, Avena-Bustillos, Olsen, Bilbao-Sáinz, & McHugh, 2016; Otoni et al., 2014). Indeed, the average droplet size did not change (p > 0.05) during 24 h (380 ± 6 nm), suggesting that kinetic stability would not be lost during film drying if these nanoemulsions are to be incorporated into films produced by solvent casting. One-day drying can be taken as an overestimation, as increasing solid contents upon drying would increase the viscosity of the continuous phase and further boost kinetic stability, and drying films in a continuous fashion require only a few minutes (Manrich et al., 2017).

3.2. Qualitative and quantitative analysis of LEO compounds in quaternary films

Five LEO components were identified in the films, including oxygenated monoterpenes (97%) and oxygenated sesquiterpenes (3%). The major constituents of films comprising 5 and 10 wt% of MB were geranial or α-citral (*ca.* 50%), neral (*ca.* 40%), geraniol (*ca.* 6%), geranyl acetate (5.6 and 3.4%), and caryophyllene (3.4 and 2.64%), noting a slight variation in these concentrations due to the added MB. These compounds have been identified in LEO (Mendes et al., 2020; Tyagi et al., 2014). The presence of significant amounts (97%) of oxygenated monoterpenes, represented by citral compounds, suggests a strong antimicrobial potential of the films (Tyagi & Malik, 2010; Tyagi et al., 2014).

In addition, ATR-FTIR spectroscopy was used to elucidate possible interactions among the film-forming components. The spectra are shown in Fig. 1.
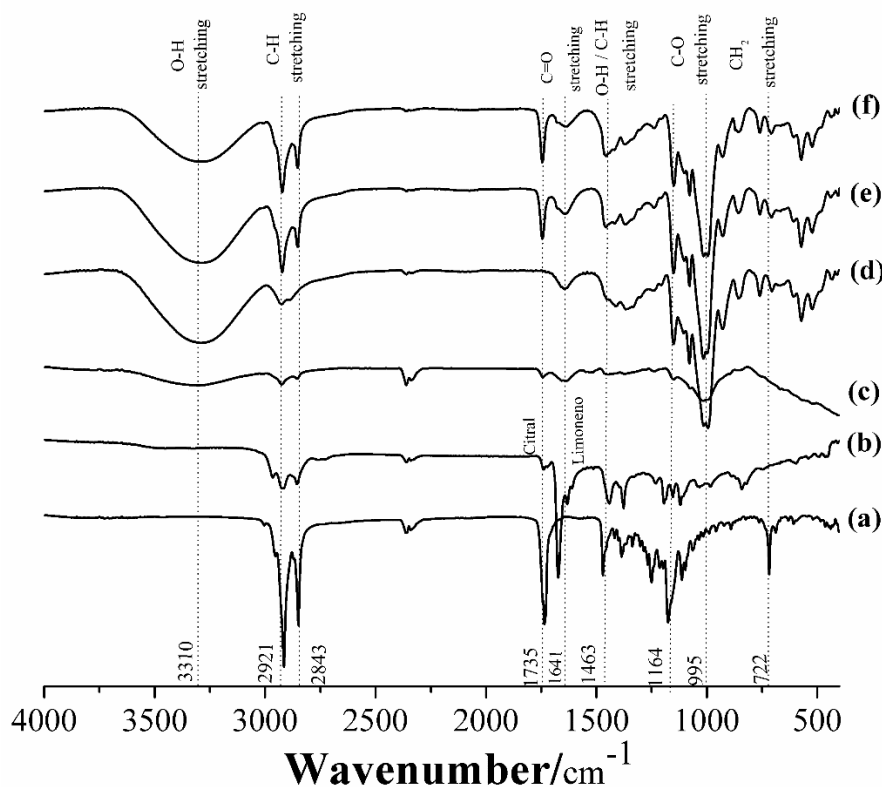
Figure 1: Attenuated total reflectance Fourier-transform infrared spectra of film components (namely: cocoa butter (a), pectin (1%)/lemongrass essential oil nanoemulsion (b), malt bagasse (MB; (c), and thermoplastic starch (d)) separately or combined into quaternary biocomposites comprising 5 (e) or 10 wt% (f) of MB. Y-axis: reflectance/a.u.

The intensity of the peaks at 2921and 2843 cm^{-1}, which refer to asymmetric and symmetrical vibrations, increased after the addition of CB and MB into the biocomposites, suggesting decreased film hydrophilicity due to the presence of these hydrophobic components. This is in line with the WVP results, as it is to be discussed later. The addition of LEO, specifically, caused a peak to appear at 1735 cm^{-1} (citral), as well as that at 1660 cm^{-1} (limonene) to increase in intensity, confirming that the films retained the components that confer antimicrobial action to LEO. Similar results were reported by Pelissari et al. (2009) and can be attributed to interactions between the nanoemulsion and TPS.

1.1. Thermogravimetry

Thermogravimetric (TG) curves and their first derivatives (DTG) for TPS-based films are shown in Fig. 2. The temperatures corresponding to the onset (T_{onset}) and offset (T_{offset}) of the thermal degradation are shown in Table 4.
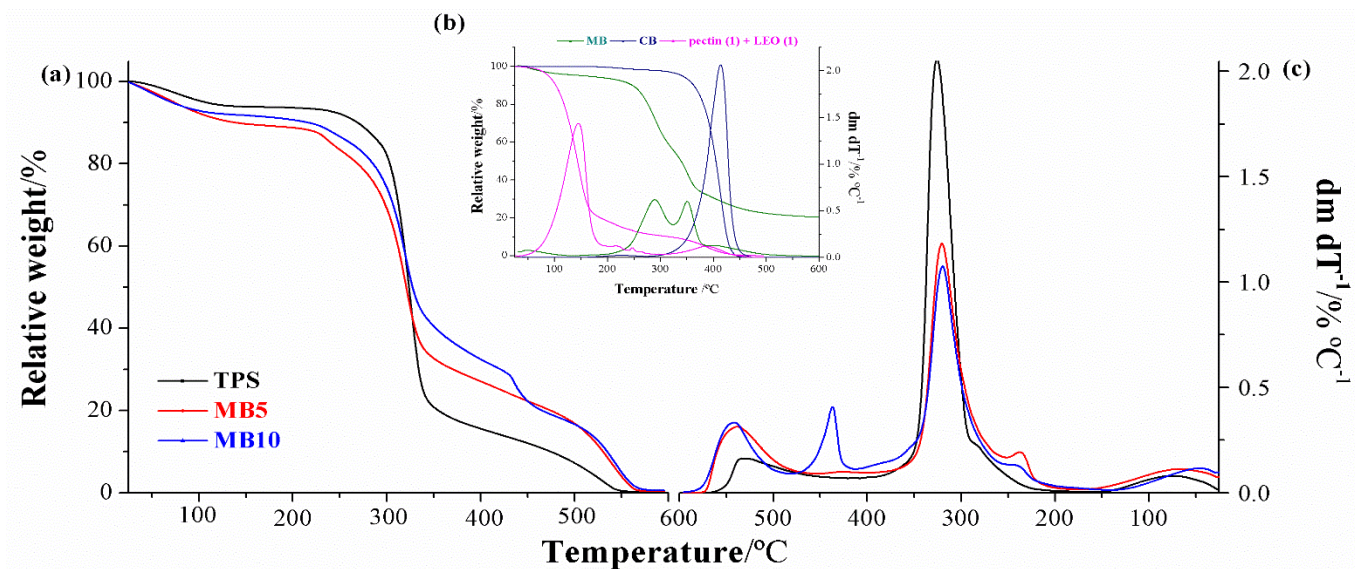

Figure 2: Thermogravimetric (TG; left) and derivative TG (right) profiles of thermoplastic starch (TPS), cocoa butter (CB), pectin/lemongrass essential oil (LEO) nanoemulsion, and malt bagasse (MB), as well as of quaternary bicomposite films comprising 5 or 10 wt% of MB.

Table 2: T_{onset} degradation temperatures and Toffset of thermoplastic starch (TPS), malt bagasse (MB), cocoa butter (CB), lemongrass essential oil (LEO), and TPS/CB/LEO/MB films comprising 5 (MB5) or 10 wt% (MB10) of MB.

Sample	T_{onset} /°C	T_{offset} /°C	Residual mass at 600 °C /%
TPS	291	340	0.2
MB	257	435	19
CB	375	438	0.2
LEO	174	354	1.1
MB5	282	337	0.5
MB10	288	527	0.5

Three main weight loss stages were observed here and elsewhere for glycerol-plasticized starch-based films (García, Famá, Dufresne, Aranguren, & Goyanes, 2009). The first stages were related to water desorption and/or other volatiles (below 150 °C).

The third stage (between 250 and 350 °C) is related to the thermal decomposition of starch components (Liu, Xie, Yu, Chen, & Li, 2009; Wilhelm, Sierakowski, Souza, & Wypych, 2003), mainly due to dehydration of hydroxyl groups and the subsequent formation of unsaturated and aliphatic low molecular weight carbon species (Sin, Rahman, Rahmat, & Mokhtar, 2011). The last stage of thermal degradation (500-550°C) is generally carbonization (Shi et al., 2011). The temperatures at which the weight loss rate was maximum for the films are presented in Table 2. The quaternary biocomposite films exhibited two additional events in the DTG curve (Figure 3c), the first one occurring at about 240 °C and being related to the depolymerization of pectin chains (Meneguin et al., 2017), whereas the second one at 325 °C

is attributed to TPS degradation (Correa, Carmona, Simão, Capparelli Mattoso, & Marconcini, 2017). The addition of MB, CB, and LEO nanoemulsions induced changes in the thermal stability of the TPS-based films. All quaternary biocomposites presented all the thermal degradation stages of the TPS and the other components used but moved to lower temperatures by adding nanoemulsions, which can be attributed to the lower thermal stability of LEO when compared to starch, CB, and MB (Table 2). However, quaternary biocomposite films showed higher thermal stability than TPS film at higher temperatures (350-600 ℃) despite their initial lower resistance to thermal degradation, resulting in lower weight loss (Fig.2).

3.3. Optical properties

Both color and transparency of food packaging materials play an important role in their appearance and acceptance by consumers. The effects of CB, LEO, and MB additions on the color parameters of the films are shown in Table 3.

Table 3: Colorimetric parameters of thermoplastic starch (TPS)-only and TPS/cocoa butter/lemongrass essential oil/malt bagasse (MB) films comprising 5 (MB5) or 10% (MB10) of MB.

Sample	L^*	a^*	b^*	ΔE^*	YI	WI
TPS	93.0±0.6[a]	-0.38±0.01[c]	0.32±0.05[c]	4.82±0.28[c]	0.49±0.1[c]	92.3±0.6[a]
MB5	89.2±0.3[b]	0.23±0.05[b]	11.8±0.2[b]	14.9±0.3[b]	18.8±0.4[b]	84.1±0.3[b]
MB10	81.4±0.8[c]	3.3±0.3[a]	25.7±0.6[a]	30.9±0.9[a]	44.7±1.4[a]	68.3±0.9[c]

Different letters in the same column indicate a significant difference (p < 0.05)

The TPS film had a higher (p < 0.05) luminosity than those containing CB, LEO, and MB fibers. Increases in MB content resulted in further lower L^* values. This is in accordance

with other reports on the darkening of starch biocomposites upon the addition of fibers (Ačkar, Babić, Šubarić, Kopjar, & Miličević, 2010; Famá, Gerschenson, & Goyanes, 2009; Mali, Debiagi, Grossmann, & Yamashita, 2010). The film components also affected (p < 0.05) $a*$ and $b*$ values: the redness (indicated by $a*$) and yellowness (indicated by $b*$ and YI) were increased by the addition of MB, which indeed presents such hue probably due to lignin. The total color difference of the composite films was slightly higher than that of TPS-only films. These values are in agreement with the results reported by other authors, who observed greater yellow coloration in fiber-containing starch composites (Famá et al., 2009; Mali et al., 2010). These characteristics may be related to non-enzymatic browning reactions (*e.g.*, caramelization and Maillard reaction), which occur due to the high levels of carbohydrates (particularly reducing sugars) and proteins in the starting materials as well as the high temperatures used (Mello & Mali, 2014).

3.4. Mechanical properties

The effects of adding CB, LEO nanoemulsions, and different MB concentrations on the mechanical properties of TPS-based films were investigated, and the results are presented in Table 4. The TPS/CB/LEO/MB interaction was significant (p < 0.05) for tensile strength (TS), elongation at break (EB) and elastic modulus (EM).

Table 4: Thickness, tensile strength (TS), elongation at break (EB), elastic modulus (EM), water vapor transmission rate (WVTR), and water vapor permeability (WVP) of thermoplastic starch (TPS)-only and TPS/cocoa butter/lemongrass essential oil/malt bagasse (MB) films comprising 5 (MB5) or 10% (MB10) of MB.

Sample	Thickness /μm	TS /MPa	EB /%	EM /MPa	WVTR /g h^{-1}m^{-2}	WVP /g mm (kPa h)$^{-1}$ m^{-2}
TPS	50.2±0.1	21±1[a]	2.4±0.2[b]	8±1[b]	62±17[a]	4±1[a]
MB5	142.5±7.9	12±1[b]	2.8±0.4[b]	13±2[a]	45±1[ab]	2.9±0.2[a]
MB10	230.4±27.1	3.6±0.3[c]	9.4±0.8[a]	1.2±0.9[c]	30±2[b]	2.4±0.2

Values correspond to average and standard deviation. The same letters on the column show that values were not statistically significant ($p > 0.05$) according to Tukey test.

The TPS-based films incorporated with CB, LEO, and MB were stiffer and more extensible, but weaker than the TPS-only film. Furthermore, all mechanical attributes showed to be concentration-dependent as far as MB. The herein observed decrease in TS has been observed for other biopolymer-based films added by lipophilic species (*e.g.*, essential oils or fatty acids), including gelatin (Limpisophon, Tanaka, & Osako, 2010), chitosan (Martins, Cerqueira, & Vicente, 2012; Rubilar et al., 2013), and whey protein (Soazo, Rubiolo, & Verdini, 2011). This behavior has been attributed to the inability of lipids to form continuous and cohesive matrices (Péroval, Debeaufort, Despré, & Voilley, 2002). Overall, the mechanical properties of biocomposites are closely associated with the distribution and density of inter- and intramolecular interactions between polymer chains in the film matrix (Bahrami et al., 2019). The decreased TS of composite films may also result from impaired matrix compatibility with the filler, which would lead to a strong interaction otherwise

(Shankar, Wang, & Rhim, 2016). Despite this reduction in TS (which is measured at high strains), the EM (determined at low deformations) of MB5 was higher than that of control films, highlighting the stiffening effect of MB fibers at this concentration. On the contrary, MB10 films had lower EM than the control, behavior that is typical of poorly dispersed fillers within biopolymer matrices, as evidenced by SEM images (Fig. 3S). The increased extensibility of films added with LEO nanoemulsions, CB, and MB fibers is mainly attributed to the widely reported plasticizing role of essential oils and lipids (Pérez-Córdoba et al., 2018; Tongnuanchan, Benjakul, & Prodpran, 2012), as well as to the presence of polyphenols (Orsuwan, Shankar, Wang, Sothornvit, & Rhim, 2016), fibers, proteins, and carbohydrates (Mello & Mali, 2014), resulting in a more continuous matrix that is able to present ductile behavior. Similar changes in TS and EB were reported elsewhere (Noshirvani, Ghanbarzadeh, Mokarram, Hashemi, & Coma, 2017; Orsuwan et al., 2016).

3.5.   Barrier to moisture

The barrier properties of a biocomposite film should be considered to determine its capacity in enhancing food safety and prolonging food shelf life. The water barrier properties are particularly important for both fresh and processed food items that are susceptible to dehydration and moisture uptake from the environment (Bahrami et al., 2019; Cazón, Velazquez, Ramírez, & Vázquez, 2017), making low levels of WVTR and WVP desirable to reduce the transfer of moisture. The values of these parameters for the herein produced quaternary biocomposite films are shown in Table 4. The WVTR and WVP values of the control film were decreased ($p < 0.05$) after adding the other components, especially at the higher MB content. This positive barrier effect was expected because of i) the hydrophobic nature of CB (according to data reported in Table 3S) and LEO as well as ii) more tortuous diffusion pathway that water vapor has to go throughout the matrix, introduced by the virtually impermeable MB fibers. The presence of MB fibers as a discontinuous phase also

reduces the mobility of biopolymer chains, which, according to Bahrami et al. (2019), may decrease WVP of the composite films. The WVP values of the herein produced biocomposites are comparable to those seen in biocomposite films based on Tragacanth/hydroxypropyl methylcellulose/beeswax/silver nanoparticles (Bahrami et al., 2019) and starch/ZnO/chitosan nanoparticles (Hu, Jia, Zhi, Jin, & Miao, 2019).

3.6. Antimicrobial activity

Fig. 4 shows the antimicrobial activity against *S. aureus* of the herein produced biocomposite films both one year and 24 h after their productions.
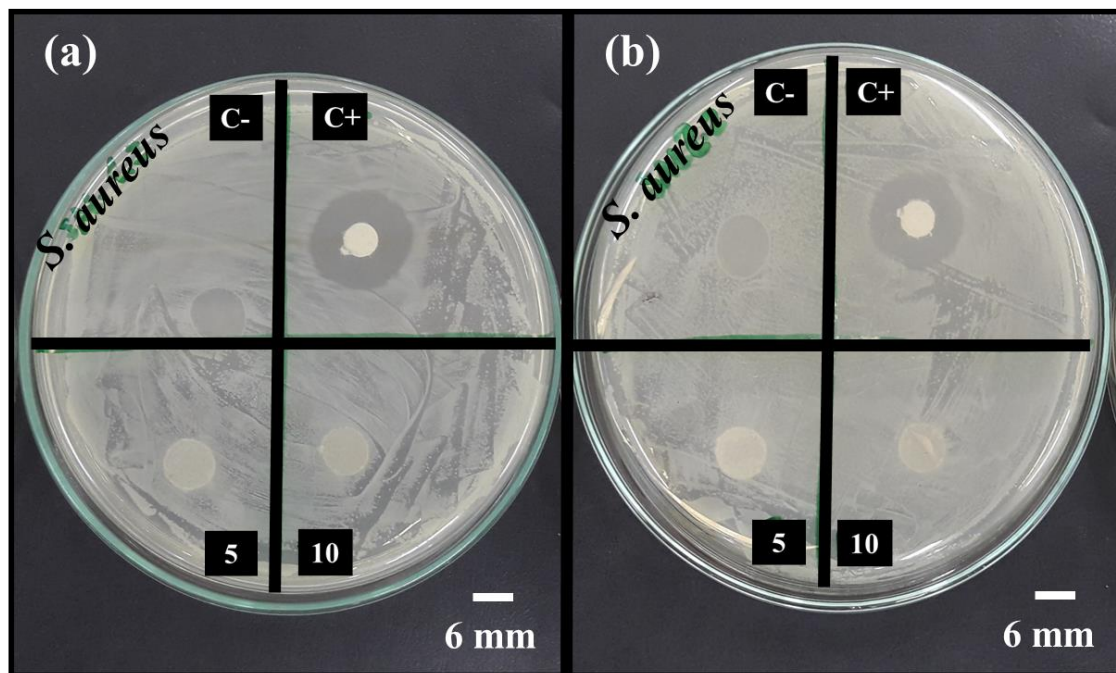

Figure 4: Antimicrobial activities against *Staphylococcus aureus* of thermoplastic starch-based based films containing lemongrass essential oil (LEO) emulsions, cocoa butter, and malt bagasse at 5 or 10 wt%. Films tested one year (a) and 24 h (b) after production. C- and C+ correspond to negative (thermoplastic starch film) and positive (Chloramphenicol) controls, respectively.

Despite verifying the entrapment of essential oil within the resulting polymer matrix (Tables S1 and S2; Fig.1) and emulsion stability, no bacterial growth inhibition was noticed,

indicating no antimicrobial activity. The absence of antimicrobial effect in the films could be related to the entrapment of LEO compounds during emulsification and later in the films. This could be confirmed with antimicrobial testing in quaternary nanocomposite films produced 24 h prior to testing (Figure 4b). This result is in line with the studies by Xu et al. (2018), Xu et al. (2019), and Perdones, Chiralt, & Vargas (2016), who reported that a good encapsulation of essential oils in film matrices may limit their release and impair the antimicrobial performance of the films.

3.7. Sensory evaluation

The sensory properties of white (W) and dark (D) chocolate coated and uncoated with the quaternary nanocomposite films after seven days of storage are shown in Table 5 and Fig. 5.

Table 5: Sensory acceptances of the aroma and flavor of white and dark chocolates wrapped or not (W sand D, respectively) by thermoplastic starch/cocoa butter/lemongrass essential oil/malt bagasse (MB) composite films comprising 5 (WMB5 and DMB5, respectively) or 10 wt% (WMB10 and DMB10) of MB.

Samples	W	WMB5	WMB10	D	DMB5	DMB10
Aroma	**7.8±0.9**[ab]	**7.6±1.2**[abc]	7.0±1.3[d]	**8.0±1.0**[a]	7.5±1.2[bcd]	7.3±1.0[cd]
Taste	**8.1±0.9**[a]	**7.6±1.0**[bc]	7.2±1.3[c]	**8.0±0.9**[ab]	7.3±1.4[c]	7.1±1.6[c]
Global impression	7,8±1.1[ab]	**8,1±1.4**[a]	7.5±1.2[b]	7.8±1.5[ab]	7.7±1.6[ab]	7.5±1.2[b]

Data are reported as average ± standard deviation values. No difference by t-Test. Higher average values mean greater product acceptance.

When comparing the results of white and black chocolates, in relation to the attributes, aroma (0.91), flavor (0.49) and global impression (0.91), by the t-test, it appears that there is

no significant difference (p> 0.05), among the evaluated samples, indicating that the films can be used to package both chocolates.

However, when analyzing, the effect of the different films (MB5 and MB10) on the acceptance of white and black chocolates, it appears that there is a significant difference (p <0.05) between the samples and the evaluated attributes. The three highest values are highlighted in bold and indicate the favorite products. Both white and dark control (unwrapped) chocolates obtained the highest scores, with dark chocolate having greater acceptance, suggesting consumer preference for the latter. This was not observed when the samples were packaged in the composite films, corroborating the greater acceptance for the samples packaged with MB5 and for white chocolate.

The PARAFAC map is used to evaluate and plot the correlation between the variables. PARAFAC were performed separately for each variable, resulting in three maps that are shown in Figure 5.
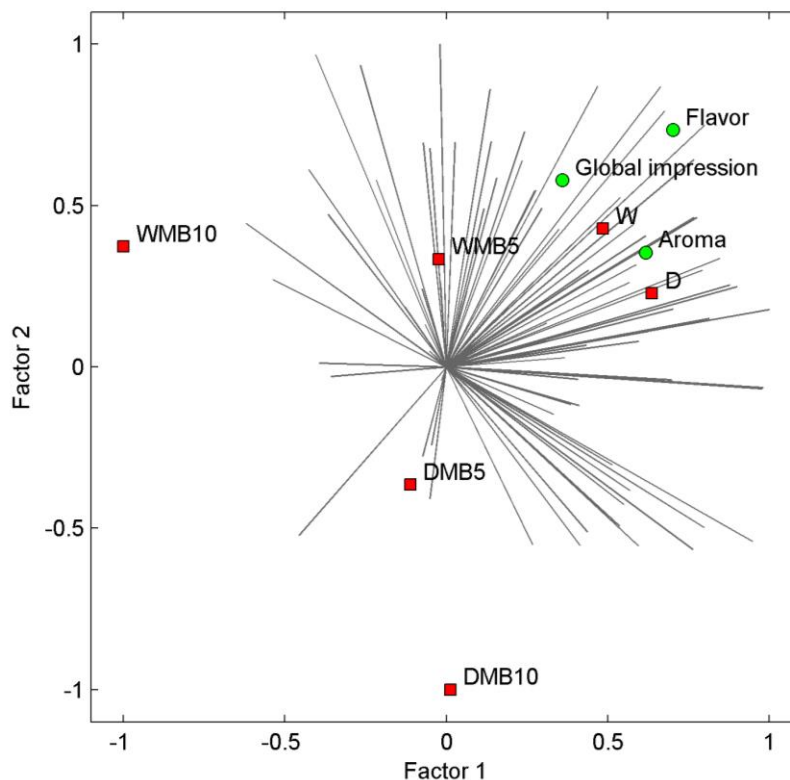
Figure 5: PARAFAC of chocolate samples packaged with two biodegradable films. The dots represent the products, while the vectors represent the consumers. The direction of consumption vectors expresses the course of consumer acceptance aroma, taste, and global impression. The results were based on responses from n = 120 consumers. Product names express *W- white chocolate; WMB5- white chocolate with -MB5 film; WMB10-white chocolate with MB10 film; D-dark chocolate; DMB5-dark chocolate with MB5 film, and DMB10-dark chocolate with MB10 film.

The PARAFAC map confirmed the results presented in Table 5, in which the largest number of vectors were directed to the control samples (W and D) and samples packed with quaternary nanocomposite films, and among them, samples WMB5. The aroma and flavor attributes were responsible for consumer preferences, both the control samples and the WMB5. It is concluded that consumers appreciate the lemongrass aroma in chocolate. Several factors may have contributed to the aroma acceptance, such as the physicochemical characteristics of the aromatic compounds (polarity, shape, and size), chemical composition of the product, concentration and mixing, as well as external factors such as storage time (Benbettaïeb et al., 2019). Besides that, suggesting that consumers accepted the lemongrass flavor transferred from the starch-based composite films. This greater acceptance could be due to the sensation of freshness provided by the active components of LEO (Table S1). In addition, the film with 5% malt bagasse fiber (MB5) offered greater acceptance for WMB5 and DMB5 samples. Thus, one may infer that, depending on the concentration, the added fibers could cause the migration of the active components found from the films to the product. According to Hossain et al. (2019), the surface wettability of fibers allows the controlled release of active compounds. Similar results were reported by Salmieri et al. (2014). Thus, the purchase intention (Fig. 5) showed great discrepancies between the samples, clearly showing high acceptance for products containing lemongrass aroma/flavor released by the films.

3.8.   Biodegradability in vegetal compost

Film biodegradation was assessed by qualitative evaluation of soil degradation. Sample weight could not be checked throughout the test since residual soil impaired an accurate weighing. Film images taken over a 30-d period are shown in Fig. 6. High-density polyethylene (HDPE) was used as a negative control for comparison purposes because of its widely known long-term biodurability in soil.
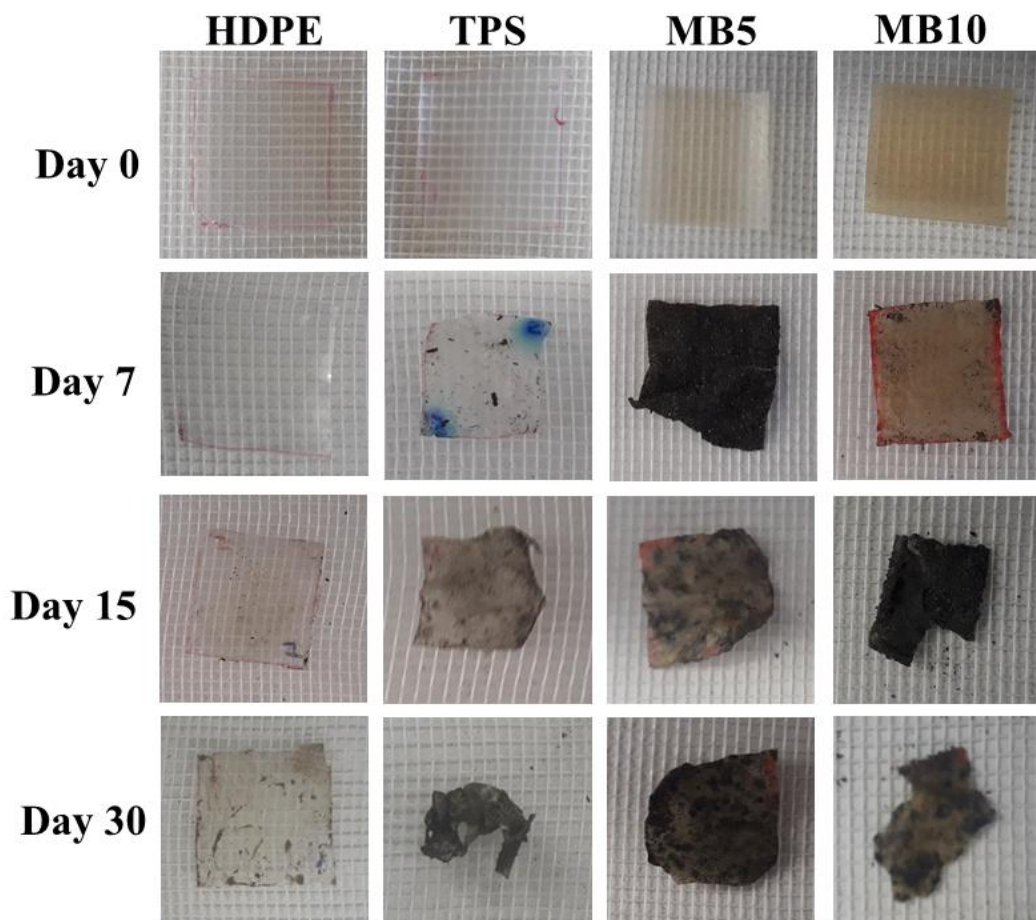

Figure 6: Pictures of films based on thermoplastic starch (TPS), emulsion, and malt bagasse fibers during biodegradation in soil for 30 d. High-density polyethylene (HDPE) film was used for comparison.

The TPS-based films lost their original appearances and structural integrity after 7 d of testing, indicating a clear and rapid degradation. According to Carissimi, Flôres, & Rech (2018), the biodegradability of films is directly related to their solubility and permeability, since the presence of hydroxyl groups fosters a more hygroscopic film, making them available to microorganisms. The reduction in the nanocomposite films permeability in relation to TPS film (Table 4), due to the addition of CB and LEO emulsion nanodroplets, did not influence the biodegradation of the film. When comparing the biodegradability of TPS-based films with that of HDPE, the formers were almost fully degraded after one month, whereas the latter persisted as expected.

4. Conclusions

For the first time, quaternary nanocomposite films made up of thermoplastic starch, cocoa butter, lemongrass essential oil nanoemulsions, and malt bagasse were prepared. This contribution was successful in demonstrating not only their production in a pilot scale, which eliminates the need for further scale up from the traditional bench casting film-forming protocol, but also the effect of film formulation on the key properties of such materials as far as their application as packaging for foodstuff. More specifically, MB provided TPS films with a concentration-dependent yellow hue, while its combination with CB and LEO nanoemulsions led to improved thermal stability and mechanical properties. Overall, the TPS/CB/LEO/MB films showed good acceptance by potential consumers and suitable degradation in vegetal compost, ensuring their complete short-term biodegradation. In summary, the production of quaternary nanocomposite films provided with relevant functional properties is demonstrated and envisaged for application in the food industry, particularly as packaging material.

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Zhuang, C., Jiang, Y., Zhong, Y., Zhao, Y., Deng, Y., Yue, J., … Mu, H. (2018). Development and characterization of nano-bilayer films composed of polyvinyl alcohol, chitosan and alginate. *Food Control*, *86*, 191–199. https://doi.org/10.1016/j.foodcont.2017.11.024

Supporting Information for

On the development of quaternary nanocomposites made up of cassava starch, cocoa butter, lemongrass essential oil nanoemulsion, and malt bagasse fibers

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Table S1: Chemical composition of nanocomposite films made up of thermoplastic starch, cocoa butter, lemongrass essential oil, and malt bagasse at 5%, determined by gas chromatography-mass spectrometry.

Component	Retention time[a] /min	Content /%	Refractive index
Neral	17.3	39.7	1246.35
Geraniol	17.9	3.4	1251.26
Geranial	18.6	48.0	1256.53
Geranyl acetate	23.3	5.5	1378.21
Caryophyllene	25.0	3.4	1418.21
Monoterpene hydrocarbons		**-**	
Oxygenated monoterpenes		**96.6**	
Oxygenated sesquiterpenes		**3.4**	
Other aliphatic hydrocarbons		**-**	
Total identified compounds/%		**100**	

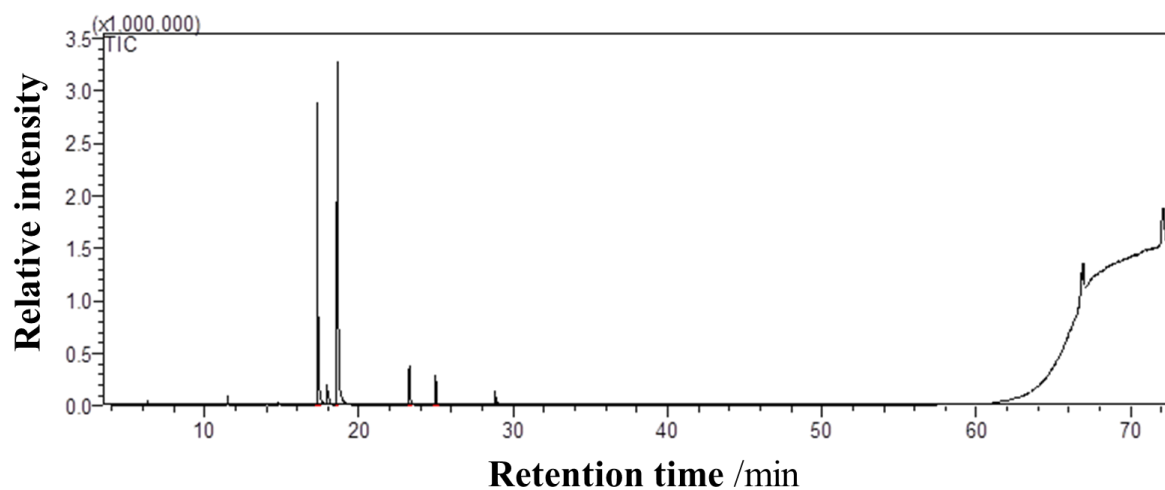[a] MS acquisition started 4 min after injection.

Figure S1: GC-FID patterns of quaternary bicomposite films comprising 5 wt% of MB.

Table S2: Chemical composition of nanocomposite films made up of thermoplastic starch, cocoa butter, lemongrass essential oil, and malt bagasse at 10%, determined by gas chromatography-mass spectrometry.

| Chemical components	Retention time[a] /min	Content /%	Refractive index
Neral	17.3	39.2	1246.35
Geraniol	17.9	5.6	1251.26
Geranial	18.6	49.1	1256.53
Geranyl acetate	23.3	3.5	1378.21
Caryophyllene	25.0	2.6	1418.21
Monoterpene hydrocarbons		**-**	
Oxygenated monoterpene		**97.4**	
Oxygenated sesquiterpene		**2.6**	
Others aliphatic hydrocarbons		**-**	
Total identified compounds/%		**100**	

[a] MS acquisition started 4 min after injection.
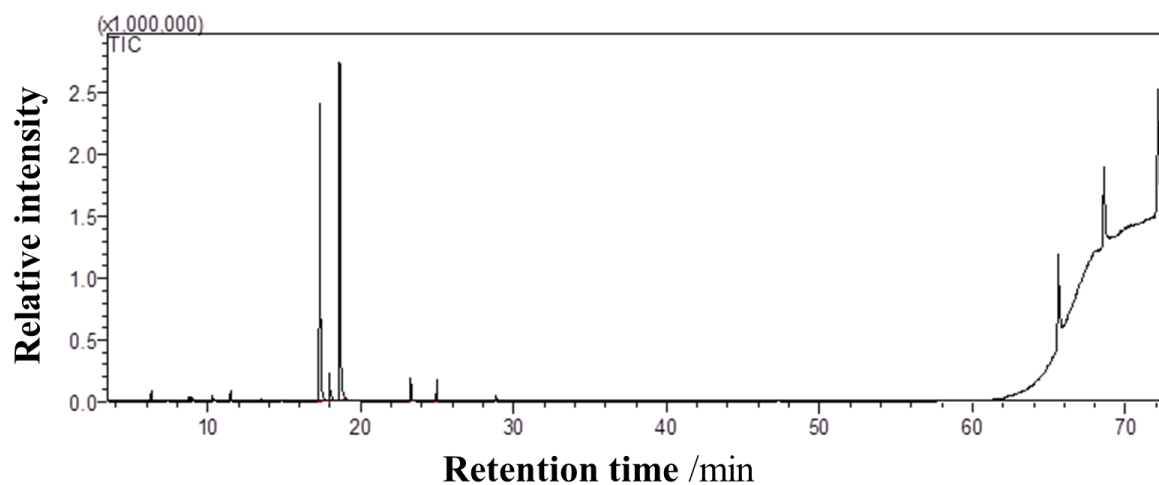
Figure S2: GC-FID patterns of quaternary bicomposite films comprising 10 wt% of MB.
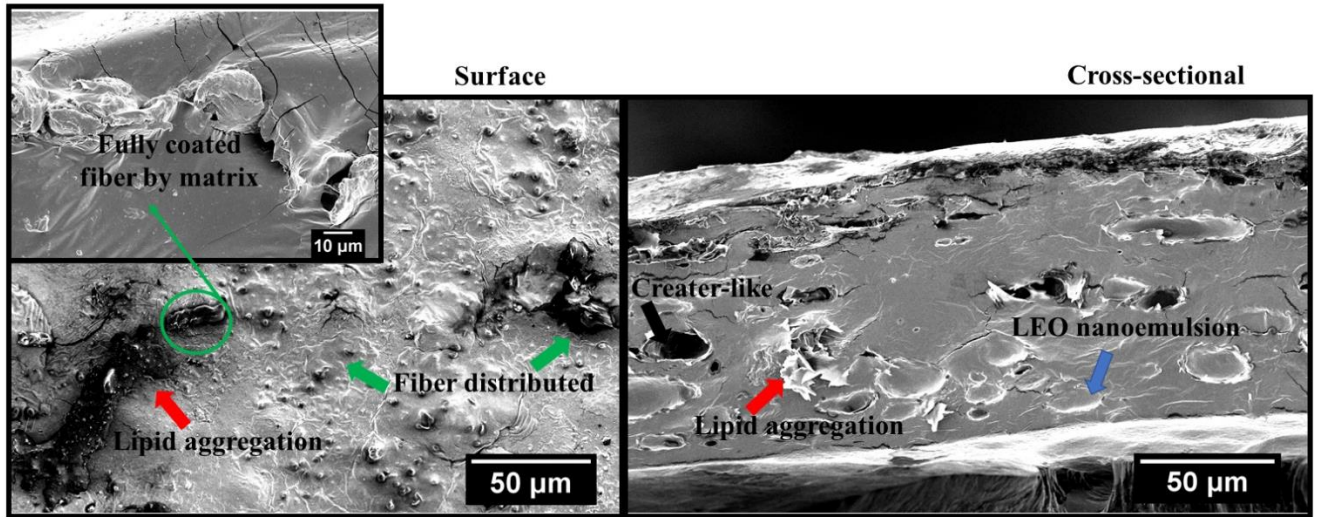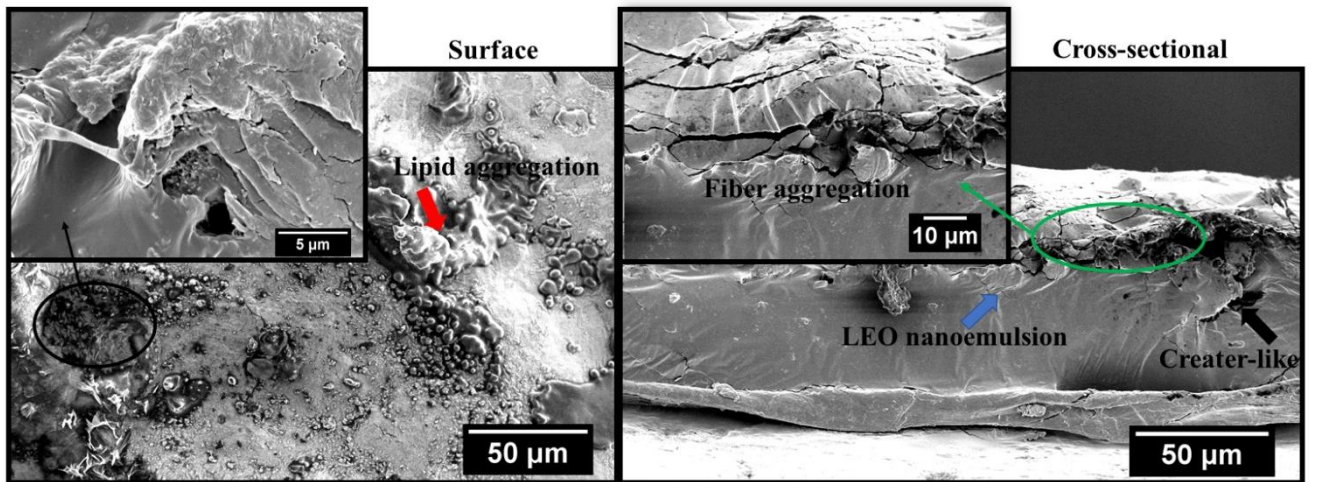
Figure 3S: Surface and cross-sectional scanning electron micrographs of films comprising 5 (MB5) or 10 wt% (MB10) of malt bagasse.

Table S3: Thickness, water vapor transmission rate (WVTR), and water vapor permeability (WVP) of pectin (HM)-only and HM/cocoa butter (CB) blends with differences concentrations of CB (10-30% w/w).

Sample	Thickness /μm	WVTR /g h^{-1}m^{-2}	WVP /g mm (kPa h)$^{-1}$ m^{-2}
HM	40.1±0.1	87±8[a]	2.0±0.2 [a]
CB10	39.1±0.1	87±6 [a]	1.7±0.3 [a]
CB20	40.2±0.8	77±12 [a]	1.4±0.4 [ab]
CB30	47.3±0.5	41±4 [a]	0.7±0.1 [b]

ARTICLE 5 - Estudo da percepção dos consumidores sobre as embalagens ativas e biodegradáveis

Artigo redigido conforme normas da revista Food Quality and Preference "Versão Preliminar"

Estudo da percepção dos consumidores sobre as embalagens ativas e biodegradáveis

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Resumo

Este estudo avaliou a percepção dos consumidores em relação a diferentes embalagens de alimentos (convencional, biodegradável, ativa e ativa-biodegradável), a fim de identificar as barreiras existentes à compra e as possíveis medidas para superá-las, por meio de técnicas de hard laddering e testes de aceitação. A técnica de hard laddering permitiu identificar consequências e valores relacionados aos atributos do produto e aos vínculos entre eles, o que pode ser útil para entender as escolhas dos consumidores. As percepções dos consumidores sobre as embalagens ecologicamente corretas estavam relacionadas à conscientização ambiental, conservação dos alimentos, saúde, bem-estar e qualidade de vida. A tradicionalidade, princípios, praticidade e o fato de serem recicláveis caracterizaram os grupos de embalagens convencionais. Quanto ao teste de aceitação das embalagens apresentaram resultados semelhantes ao hard laddering, sendo as percepções dos consumidores sobre as embalagens ativa-biodegradável relacionadas ao aroma, aos materiais utilizados na produção das embalagens, o qual influenciavam na cor, aparência e textura dessas embalagens. Portanto, os consumidores estão dispostos a substituir as embalagens convencionais por uma opção mais sustentável. Entre as perspectivas mais promissoras para o mercado, estão as embalagens ativas-biodegradáveis.

Palavras-chaves: atitude dos consumidores; hard-laddering; embalagens ecologicamente corretas

1. Introdução

O setor de embalagens experimentou vários avanços da história para melhorar a qualidade e a segurança do produto. As embalagens convencionais, como por exemplo, as plásticas, são consideradas uma solução econômica pelas indústrias para entregar produtos com segurança e qualidade, mas tanto em termos de produção de embalagens quanto de resíduos impacta negativamente o meio ambiente devido à sua natureza não biodegradável, existindo um paradoxo entre os impactos e os benefícios das opções de embalagem. Assim, estão sendo feitos esforços para desenvolver os materiais de embalagem a partir de fontes renováveis (Latos-Brozio & Masek, 2020; Piscopo et al., 2019; Zhao, Lyu, Lee, Cui, & Chen, 2019; Zhong, Godwin, Jin, & Xiao, 2019).

O polímero biodegradável pode servir todas as funções de uma embalagem e se desintegra em produtos ecológicos no final de sua vida útil. Além disso, a demanda por tecnologias de embalagem inovadoras, como embalagens ativas, cresceu rapidamente nos últimos anos devido a novos requisitos do mercado de alimentos em termos de extensão do prazo de validade, melhoria da qualidade, segurança e redução de resíduos (van Birgelen, Semeijn, & Keicher, 2009; Wilson, Harte, & Almenar, 2018).

A embalagem ativa pode ser definida como a tecnologia de embalagem na qual certos aditivos, conhecidos como "compostos ativos" são incorporados ao material da embalagem, a fim de interagir diretamente com o produto e / ou seu ambiente para aumentar sua qualidade e qualidade / ou segurança (Wilson et al., 2018). No entanto, estudos que investigam a percepção do consumidor por embalagens ativas e biodegradáveis são escassos e limitados.

Apesar de verificar aumento significativo na difusão de conhecimento sobre essas embalagens, segundo estudos de Nordin & Selke (2010), as percepções dos consumidores revelam uma falta de conhecimento sobre o conceito de sustentabilidade, além de apresentar

uma presença significativa de lacunas terminológicas e inconsistência em suas atitudes em relação a embalagens sustentáveis. O que motiva indústrias e pesquisadores a entender profundamente como os consumidores percebem essas embalagens, como suas necessidades são moldadas e influenciadas e como fazem as escolhas de produtos com base nelas (Wilson et al., 2018). Rokka & Uusitalo (2008) descobriram que os consumidores favoreciam as embalagens rotuladas ambientalmente como o critério mais importante na escolha do produto. Os resultados de van Birgelen, Semeijn, & Keicher (2009) indicam que decisões de compra e descarte ecológicos estão relacionadas ao nível de conscientização ambiental e atitude ecológica dos consumidores.

Técnicas qualitativas, como questionário de atitude, associação de palavras e laddering, são úteis nesse estágio de desenvolvimento de novos produtos. Técnicas de laddering foram aplicadas para entender as percepções dos consumidores sobre produtos alimentares e suas motivações para suas escolhas (Lee, Lusk, Mirosa, & Oey, 2014). O laddering pode ser avaliado sob duas alternativas, soft-laddering e hard-laddering. Enquanto o soft laddering se baseia sem uma entrevista pessoal realizada por um entrevistador treinado, o hard laddering depende de um questionário estruturado (Botschen & Thelen, 1998). O hard-laddering, escolhido para este estudo, fornece resultados semelhante ao soft laddering, mas supera algumas limitações do primeiro, como redução de tempo e custo de administração, além de minimizar a influência do entrevistador (Masson, Delarue, Bouillot, Sieffermann, & Blumenthal, 2016). Polizer Rocha, de Noronha, & Trindade (2019) afirmaram que a técnica de hard laddering geram conceitos e associações relacionadas principalmente às preferências e razões dos consumidores por trás da escolha.

Portanto, o presente estudo tem por objetivos coletar informações sobre a percepção, atitude e aceitação dos consumidores sobre as embalagens convencionais e sustentáveis (biodegradáveis, ativa e ativa-biodegradável).

2. Material e métodos

2.1. Análise sensorial

O presente estudo foi realizado no Laboratório de Análise Sensorial da Universidade Federal de Lavras (Lavras, Brasil) e por meio de um questionário eletrônico "Estudo sobre a percepção dos consumidores em relação a embalagem ativa e biodegradável" no google drive. Os participantes eram voluntários adultos que foram informados sobre o escopo do estudo e forneceram seu consentimento informado. Este estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Lavras (UFLA, Lavras-MG, Brasil), segundo o número do parecer: 2.746.637 (CAAE: 89319918.9.0000.5148).

2.2. Obtenção dos filmes biodegradáveis

Os filmes foram produzidos conforme detalhado em nosso artigo anterior Mendes et al. (2020), exceto pela adição de manteiga de cacau (CB) e fibras de bagaço de malte (MB). Resumidamente, uma dispersão aquosa de amido de mandioca (8% p / v), glicerol (20% em peso), CB (30% em peso) e MB (5 ou 10% em peso) - as porcentagens em peso são relativas à massa seca de amido - / foi combinado a 1: 1 (razão de peso) com a emulsão previamente preparada, homogeneizada, desgaseificada e moldada em filmes em um sistema de laminação contínua KTF-SB (Mathis, Suíça). As amostras finais receberam o nome de F5 (5% de MB) e F10 (10%MB).

2.3. Estímulos

Os estímulos utilizados neste estudo foram descrições de vários tipos de embalagens. Estas incluíam a embalagem tradicional (estímulo A); embalagem biodegradável (estímulo B); embalagem ativa (estímulo C) e embalagem ativa e biodegradável produzida conforme seção 2.2. (estímulo D). Cada descrição foi apresentada aos consumidores com uma imagem das embalagens com as respectivas informações.
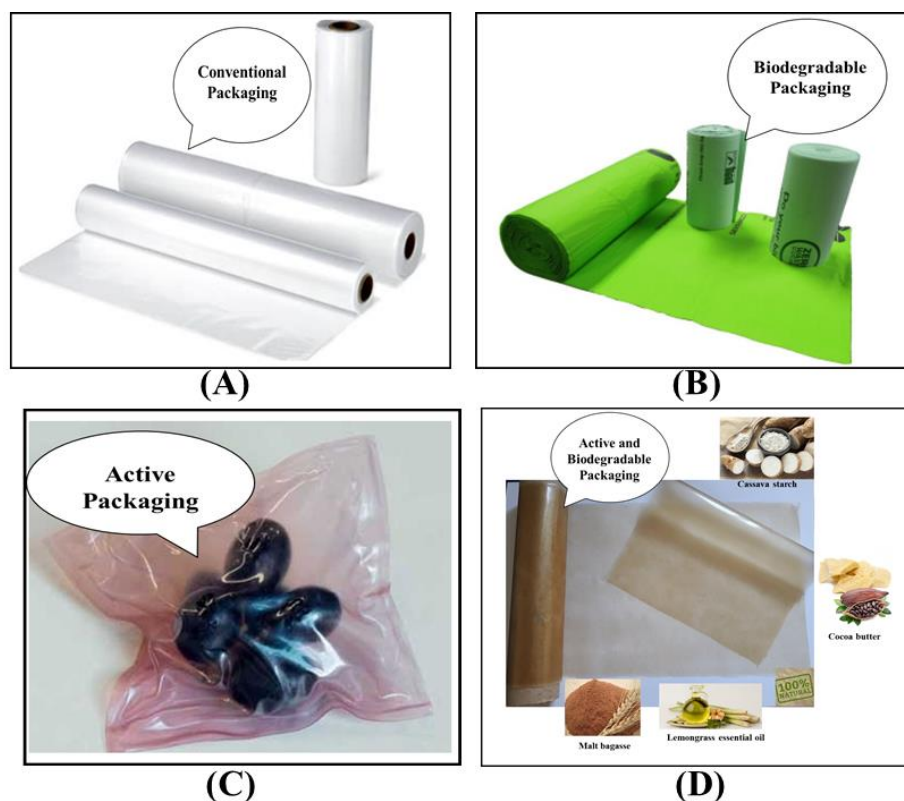
Figure 1: Exemplos de imagens para o estudo

2.4. Questionário de atitude

As respostas foram coletadas de um total de 262 participantes. Os dados da Tabela 2 apresentam a distribuição por gênero de nossos entrevistados, que era de 83 homens (31%) e 182 mulheres (69%). Os participantes tinham idade entre 18 e 61 anos, dos quais 30,5% eram de 18 a 24 anos, 49,6% eram de 25 a 34 anos e 11,5% tinham entre 35 e 49 anos, respectivamente. Em relação ao grau de escolaridade, observou-se que 54,3% possuíam pós-graduação, 28% possuíam ensino universitário incompleto, 15% possuíam diploma universitário e 2,3% possuíam ensino médio completo.

Os participantes foram solicitados a responder um questionário sobre suas atitudes em relação às embalagens convencional, ativas, biodegradáveis e nanotecnologia. Os participantes tiveram que endossar seu grau de concordância com 9 declarações usando uma

escala de Linkert de cinco com "Discordo totalmente" à esquerda e "Concordo totalmente" à direita. O questionário é mostrado na Tabela 1. As respostas a este questionário serviram para identificar grupos de consumidores que têm interesses e preocupações diferentes sobre meio ambiente e conservação de alimentos, usando para isso embalagens.

Tabela 1: Itens do questionário atitudinal sobre embalagens convencional, ativas, biodegradáveis e nanotecnologia

Afirmações
1- **Embalagens produzidas a partir de fontes naturais e renováveis**
2- **Embalagens produzidas com compostos bioativos (antioxidantes e antimicrobianas)**
3- **Embalagens com aromatizantes encapsulados aumenta a sua propriedade sensorial (sabor, aroma e gosto)**
4- **Embalagens produzidas com óleos essenciais com propriedades antimicrobianas**
5- **Produção de embalagens com nanopartículas**
6- **A nanotecnologia possibilita maior frescor aos alimentos**
7- **A nanotecnologia possibilita menor risco de contaminação**
8- **A nanotecnologia possibilita maior vida de prateleira (durabilidade)**
9- **A nanotecnologia possibilita maior valor nutricional**

O questionário atitudinal foi desenvolvido por meio de trabalhos já publicado na literatura (Ares et al., 2008). Uma escala não estruturada de 7 pontos foi usada para avaliar a importância de embalagens antimicrobianas, antioxidante, aromatizantes e por fim, quanto a

sustentabilidade e aparência das embalagens (variando de 1 = pouco importante a 7 = extremamente importante). No mesmo questionário, os consumidores também verificaram a probabilidade de consumo das embalagens. Para isso, uma escala não-estruturada de 5 pontos foi aplicada (variando de 1 = certamente não compraria a 5 = certamente compraria, para estas descrições).

2.5.   Hard laddering

A técnica de hard laddering sugerida por Ares, Giménez, & Gámbaro (2008), Polizer Rocha, de Noronha, & Trindade (2019) e Roininen, Arvola, & Lähteenmäki (2006) foi aplicada por meio de um questionário on-line. Os mesmos participantes que responderam o "Questionário de atitude" (Item 2.4) foram instruídos a avaliar quatro estímulos e depois responder à seguinte pergunta: "Se você optasse por comprar um alimento com umas dessas embalagens, qual delas compraria?". Posteriormente, os consumidores foram questionados sobre as razões de sua escolha, utilizando uma série de questões iniciadas com "por quê" (Fig. 2). O questionário apresentado aos participantes foi elaborado para identificar os atributos das embalagens que influenciam os consumidores e por que esses atributos foram importantes na percepção dos consumidores sobre as consequências do consumo e princípios ou crenças que representam objetivos ou estados finais desejados (valores pessoais).
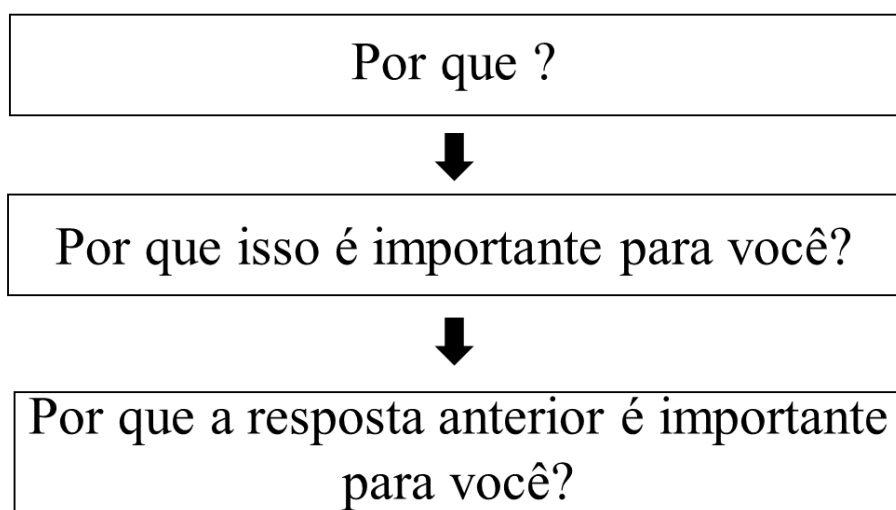
Figure 2: Questionário usado para a técnica hard laddering.

2.6. **Aceitação Sensorial das embalagens ativas produzidas**

As embalagens ativas-biodegradáveis produzidas também foram avaliadas quanto a aceitação dos consumidores, quanto a sua cor, textura, aroma e impressão global. Uma embalagem de papel, de coloração parda foi apresentada como controle. O teste de aceitação foi realizado com e sem informações da composição das embalagens. Um total de 120 consumidores foram recrutados. As avaliações foram realizadas em cabines individuais sob luz artificial a uma temperatura entre 22 e 24ºC e circulação de ar. Inicialmente as amostras sem nenhum tipo de informação foram servidas em bandejas transparentes, codificadas com números aleatórios de 3 dígitos, em uma ordem balanceada de apresentação (Wakeling & MacFie, 1995). Em seguida, esse consumidor era solicitado avaliar as mesmas amostras, porém com suas respectivas definições e composições, conforme a Figura 3.
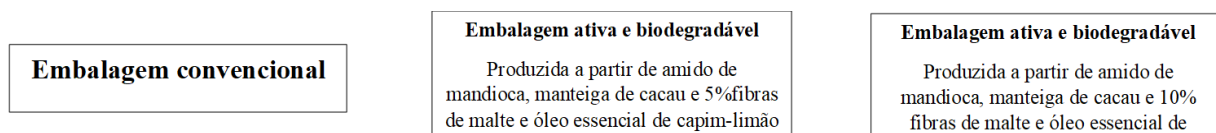
| **Embalagem convencional** | **Embalagem ativa e biodegradável**
Produzida a partir de amido de mandioca, manteiga de cacau e 5%fibras de malte e óleo essencial de capim-limão | **Embalagem ativa e biodegradável**
Produzida a partir de amido de mandioca, manteiga de cacau e 10% fibras de malte e óleo essencial de |

Figura 3: Definições das embalagens controle (convencional) e da embalagem ativa e biodegradável.

Uma escala hedônica estruturada de 9 pontos foi usada no teste de aceitação com extremos de "(1) desgostei extremamente" e "(9) gostei extremamente" (Ferreira Saraiva et al., 2016).

2.7. Análise estatística

2.7.1. Questionário de atitude

A partir das escalas de atitude, os dados foram extraídos (média e desvio) das respostas do consumidor e estatisticamente avaliadas pelo Test t para determinar se houve diferenças significativas ($p < 0,05$) entre as atitudes homens e mulheres usando a escala Likert. Essas condições foram estabelecidas com base em trabalhos anteriores relatados por Bosten et al. (2017), Esmerino et al. (2017) e Pacheco et al. (2018). Utilizou-se o software Sisvar® (Version 5.4).

2.7.2. Hard laddering

Os dados obtidos foram analisados conforme proposto por Reynolds e Gutman, (1988) e Ares et al. (2008). O conteúdo do hard laddering de cada entrevistado foi analisado. Atributos, consequências e valores com o mesmo significado foram agrupados e rotulados. Mapas de valores hierárquicos (HVM) foram construídos usando o software usando o software LadderUX (2017).

2.7.3. Análise de correspondência

A análise de correspondência foi aplicada nos dados obtidos de por meio do hard laddering, para visualizar a relação entre os conceitos de atributos e consequências levantados pelos participantes para cada embalagem analisada. A análise de correspondência é uma técnica descritiva / exploratória projetada para analisar tabelas de contingência bidirecionais simples, contendo alguma medida de correspondência entre as linhas e colunas. Usando esta técnica, as variáveis de linha e coluna foram representadas espacialmente, o que permitiu uma representação visual dos dados (Greenacre, 1984, dez Kleij e Musters, 2003). Todas as análises estatísticas foram realizadas usando o software R.

2.7.4. Aceitação das embalagens ativas e biodegradáveis produzidas

Os resultados foram submetidos à análise de variância (ANOVA) e teste de Tukey para comparações de médias, considerando um nível de significância de 5%. Utilizou-se o software Sisvar® (Version 5.4).

3. Resultados e discussão

3.1. Questionário de atitude

A Tabela 2 apresenta as pontuações médias, desvio padrão e cargas fatoriais dos atributos avaliados em relação à atitude do consumidor relacionados à embalagens ecologicamente corretas, ativas, e nanotecnologia. Além disso, apresenta o grau de importância dos consumidores as embalagens avaliadas e conceitos de sustentabilidade.

Tabela 2: Pontuações médias e desvio padrão (SD) dos atributos avaliados em relação à atitude do consumidor em relação à embalagem, considerando a escala Likert.

Atributos	Masculino		Feminino		t-Test
	Média	SD	Média	SD	
Embalagens produzidas a partir de fontes naturais e renováveis	4.6	0.9	4.7	0.9	0.2
Embalagens produzidas com compostos bioativos (antioxidantes e antimicrobianas)	4.4	1.2	4.7	0.9	**0.03**
Embalagens com aromatizantes encapsulados aumenta a sua propriedade sensorial (sabor, aroma e gosto)	3.7	1.4	3.8	1.4	0.22
Embalagens produzidas com óleos essenciais com propriedades antimicrobianas	3.9	1.3	4.3	1.1	**0.02**
Pagaria a mais por uma embalagem biodegradável	1.7	04	1.8	0.4	**0.05**
Produção de embalagens com nanopartículas (propriedades)	4.3	1.2	4.5	1.0	0.06

Nanotecnologia proporciona maior frescor nos alimentos	3.5	1.0 3.7	1.2	0.16
Nanotecnologia proporciona menor risco de contaminação	3.8	1.1 3.9	1.1	0.31
Os alimentos produzidos por nanotecnologia apresentam maior vida de prateleira	3.9	1.1 4.2	1.2	0.06
A nanotecnologia produz alimentos com maior valor nutricional	3.2	1.2 3.6	1.2	**0.009**
Embalagem antimicrobiana	$6,2\pm1,2^c$			
Embalagem antioxidante	$5,8\pm1,4^b$			
Embalagem aromatizante	$3,9\pm1,8^d$			
Sustentabilidade de embalagem	$6,5\pm0,9^a$			
Aparência da embalagem	$5,3\pm1,7^c$			

[*]número em negrito significa que os valores se diferem estatisticamente ($p \geq 0,05$) pelo Teste-t.

Os resultados apresentados na Tabela 2, indicam que os valores de obtenção de embalagens a partir de fontes naturais e renováveis, não apresentaram diferenças significativas ($p > 0,05$) entres os consumidores do sexo masculino e feminino. Isso significa que os consumidores, independente do sexo, estão preocupados com a questão ambiental, aceitando a substituição das embalagens convencionais, vindas do petróleo, pelas obtidas de materiais de fontes naturais. Além disso, subentende-se que por esse estudo ser realizado com consumidores altamente instruídos, podem apresentar uma maior consciência ambiental, não apresentando diferença entre os consumidores.

Isso pode ser confirmado pelas respostas dos consumidores à escala Likert de importância (1-7, menos e mais importante, respectivamente) onde mostraram uma maior importância para embalagens produzidas a partir de fontes sustentáveis (7, extremamente importante). Segundo Prakash et al. (2019), nos últimos tempos, os consumidores têm se concentrado cada vez mais na preocupação ambiental, provocando mudanças em seus

comportamentos de compra e aceitação dos produtos. Verdurme, Viaene, & Gellynck (2003) em seus estudos avaliaram a aceitação do consumidor a novas tecnologias de uma perspectiva geral. Nos estudos de Koutsimanis, Getter, Behe, Harte, & Almenar (2012) verificaram que o apelo à compra de embalagens ecologicamente correto foi classificado como alto para a população total; porém, quando os participantes foram solicitados a identificar as matérias-primas usada para produzir essas embalagens, apenas 54,5% deles conseguiram identificar corretamente os materiais.

Quanto as afirmações, de obtenção de embalagens a partir da adição de aromatizantes para aumentar as propriedades sensoriais (sabor, aroma e gosto) dos alimentos, embalagens com adição de nanopartículas, e questões sobre nanotecnologia e suas possíveis interações com o alimento (maior frescor, menor risco de contaminação, vida útil do alimento), também não apresentaram diferencias significativas ($p > 0,05$). Tal fato pode estar relacionado com nível de escolaridade deste estudo, pois mais da metade dos consumidores possuíam pós-graduação. Dessa forma, esses temas estão presentes na vida cotidiana desses consumidores, relacionando a todo tipo de associação, ideias e definições sobre consequências comportamentais ou compromissos sociais.

Entretanto, observa-se que as respostas dos consumidores (masculino e feminino) se diferenciaram estatisticamente ($p \geq 0,05$), em relação as embalagens produzidas a partir de adição de compostos bioativos, como por exemplo, óleo essencial, quanto ao valor a ser pago por embalagens sustentáveis e o seu valor nutricional. A obtenção de uma embalagem ativa, pela adição de compostos bioativos, como os óleos essenciais, para aplicação em alimentos, ainda causa certa rejeição por parte dos consumidores, devido ao forte aroma e sabor desses óleos, conforme evidenciado em diversos estudos (do Evangelho et al., 2019; Lian, Shi, Zhang, & Peng, 2020; Wang et al., 2018, 2019), sendo que um comportamento mais tradicional também possa ser esperado devido à existência de consumidores conservadores

com fortes raízes regionais ou culturais, o que justifica os resultados obtidos pela escala de importância (Tabela 2), no qual 27% dos consumidores afirmaram que as embalagens aromatizantes, não eram nem mais/menos importantes. Pacheco et al. (2018) sugerem que as mulheres são mais sensíveis na detecção, identificação de produtos químicos, odores, que os homens, justificando assim, a diferença encontrada para esse atributo.

Quanto a disponibilidade dos consumidores a pagar um preço maior para produtos ecologicamente corretos, Kimura et al. (2012), afirmam em seus estudos há existência de uma lacuna de comportamento e atitude, que nesse caso, significa que os consumidores dizem que estão dispostos a pagar mais por produtos sustentáveis, mas eventualmente não pagam.

Além disso, pode-se observar que os consumidores do sexo feminino, possuem uma tendência maior a aceitação de produtos embalados ecologicamente, com adição de compostos bioativos e os efeitos da nanotecnologia sobre os alimentos. Portanto, verifica-se que a aceitação de embalagens biodegradáveis com nanotecnologia, podem ser significativamente influenciadas tanto pela preocupação ambiental geral como pelas avaliações racionais dos benefícios.

3.2.   Hard laddering

Durante a aplicação da técnica hard-laddering, os participantes escolheram o tipo de embalagem (convencional, biodegradável, ativa e biodegradável-ativa) com maior probabilidade de comprar. Houve uma alta probabilidade de comprar as embalagens ativas e biodegradáveis (74,6%) e a embalagem biodegradável (20,5%). Porcentagens intermediárias foram apresentadas para as embalagens convencionais (3,4%), enquanto os consumidores eram menos propensos a comprar as embalagens ativas (1,5%).

O mapa de valores hierárquicos, representado na Figura 3, foi utilizado para entender os motivos das escolhas dos consumidores.
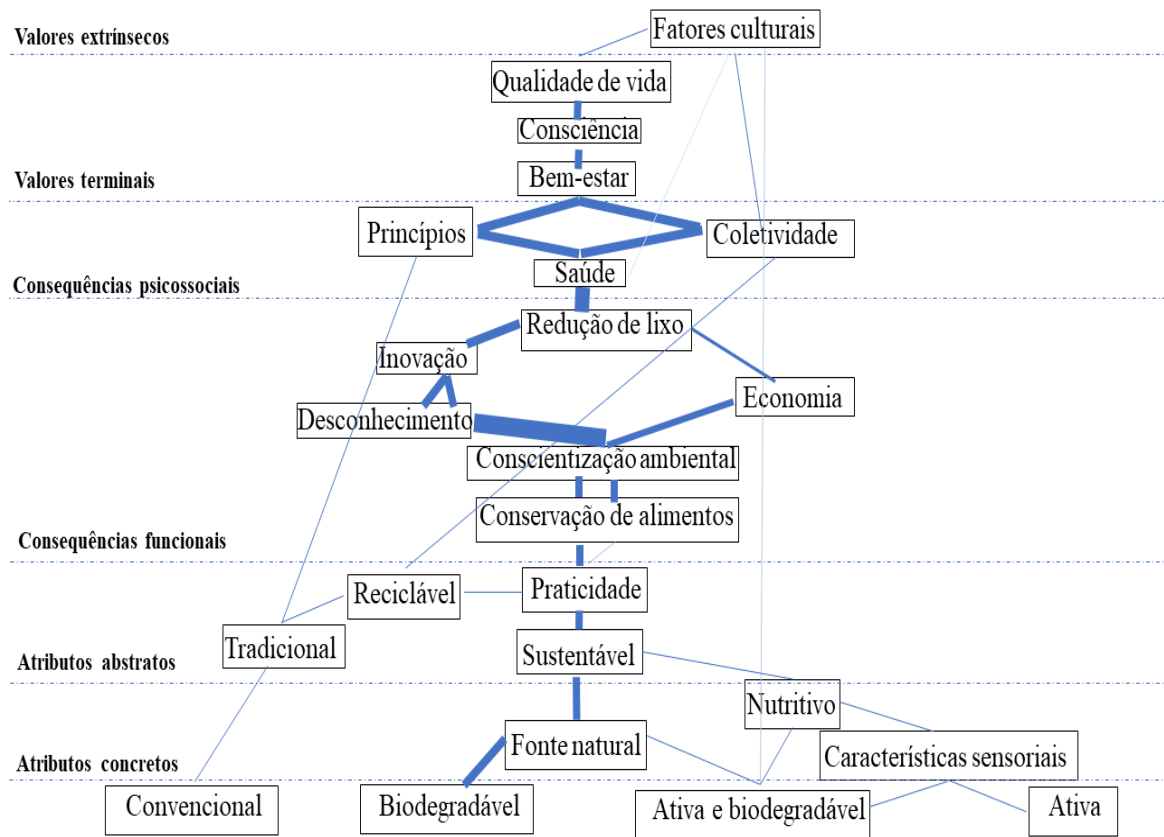
Figura 3: Mapa de valores hierárquicos

As consequências mencionadas pelos consumidores que racionalizaram a escolha da embalagem ativa-biodegradável foram por serem materiais sustentáveis, quanto a sua praticidade, conservação dos alimentos, conscientização ambiental, economia (redução do desperdício de alimento), inovação, redução de lixo, saúde, bem-estar, consciência e qualidade de vida, conforme apresentado nas Figuras 3 e 4. As categorias resultantes da escolha das embalagens biodegradáveis e ativas são semelhantes das mencionadas acima, com exceção, do desconhecimento dos consumidores em relação as embalagens ativas, o que justifica a baixa frequência de sua escolha (Tabela 3). Outro fator que deve ser considerado é a escolha do estímulo (Figura 1c) para representar as embalagens ativas. Pesquisas mostram que os atributos representados nas imagens ancoram os julgamentos dos consumidores, afetando a forma como o produto é avaliado e percebido, e que sua escolha é influenciada por essa percepção e experiência fundamentada (Gil-Pérez, Rebollar, & Lidón, 2020). Além

disso, como já discutido (Seção 3.1), o conhecimento do consumidor relacionado à presença de compostos bioativos, como a adição de óleo essencial, ainda é limitado. Por fim, os consumidores consideram que o estímulo (embalagem convencional) esteja relacionado com a tradicionalidade e reciclabilidade desses materiais, o que favorece a sua praticidade. De acordo com Sijtsema et al. (2016), os participantes que não estavam familiarizados com o termo "biodegradáveis" ou "ecologicamente correto" questionaram se essas embalagens realmente eram inovadoras, ecológicas e saudável, optando pelas embalagens sintéticas.

Os valores pessoais que as consequências reforçaram foram a consciência, bem-estar e qualidade de vida, sugerindo que esses foram os principais motivos (valores dos consumidores) que justificaram as escolhas de embalagens ativa-biodegradável e biodegradável. Para as embalagens ativa-biodegradável, a conscientização de estar contribuindo para um planeta com melhores condições de vida para seus habitantes, reforçaram principalmente a escolha deste produto. Esse achado indica que os consumidores percebem as embalagens ecologicamente corretas como benéficas não apenas para o meio ambiente, mas também para gerar benefícios à saúde. O mesmo foi verificado nos estudos de Scott & Vigar-Ellis (2014) no qual os consumidores afirmaram que as embalagens ecológicas melhorariam a sustentabilidade e salvaria o planeta, que isso lhes dava uma boa sensação, reduziriam o desperdício e que achavam que essa embalagem é boa para a saúde e evitariam doenças. Verma, Chandra, & Kumar (2019), enfatizaram que boa saúde e melhor qualidade de vida, poderiam motivar os indivíduos a demonstrar um comportamento ecologicamente correto. Ademais, corrobora com os resultados obtidos pelos princípios da cadeia de meios-fins, onde todas as escolhas foram justificadas pela categoria conscientização ambiental, conservação de alimentos, princípios, bem-estar e qualidade de vida.

Para complementar os resultados de laddering, verificando as consequências que diretamente influenciaram na escolha da embalagem (estímulo), uma análise de correspondência foi elabora e apresentada na Figura 4.
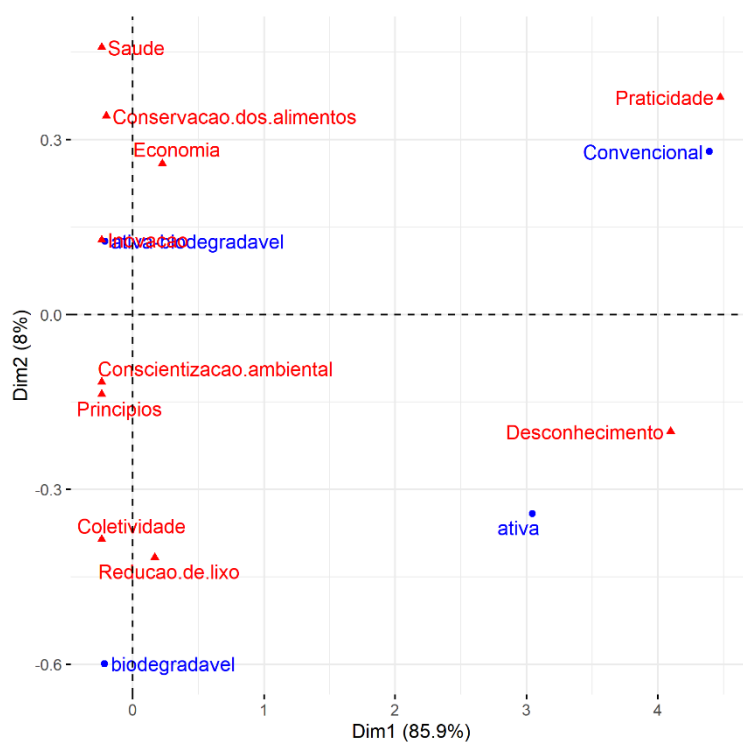

Figura 4: Configuração dos estímulos (vários tipos de embalagens) obtidos pela análise de correspondência.

A representação dos quatro grupos diferentes de embalagens (estímulos) no gráfico (Figura 4) quanto suas consequências, indica grandes diferenças percebidas pelos consumidores entre os tipos de conceitos das embalagens avaliadas, confirmando os resultados obtidos no laddering.

3.3. Aceitação sensorial das embalagens ativas-biodegradáveis produzidas

As respostas dos consumidores em relação a aceitabilidade das embalagens utilizadas, quanto a sua cor, aroma, textura e impressão global são apresentadas na Tabela 4. Também

são comparadas as médias de aceitação dos consumidores ao analisarem as embalagens controle e ativas-biodegradáveis (5% e 10% m/m de fibras de bagaço de malte – F5 e F10, respectivamente) sem e com informações das respectivas composições. Dentre as embalagens avaliadas, a aceitação foi maior ($p<0,05$) para as embalagens ativas e biodegradáveis, independentemente de sua composição (Tabela 4). Verificando um aumento na aceitação dessas embalagens com a informação das suas composições, isso não foi verificado com a embalagem convencional. Milagres et al. (2014) concluíram que o leite contendo informações sobre a alta concentração de melatonina e seus benefícios à saúde teve maior aceitação sensorial em comparação com um teste cego.

A cor é um atributo muito importante para a aceitabilidade do consumidor e para caracterização dos filmes. Assim as amostras F5 (6,9) e F10 (6,8) obtiveram maior média. Pode-se relatar que tal aceitação é devido a melhor visualização do produto que está embalado do que o filme controle, apesar de verificar que esses filmes apresentavam uma coloração amarelada, em função das fibras de bagaço de malte adicionadas. Para o atributo aroma, foi detectado que as amostras de filmes ativas-biodegradáveis (F5 e F10) diferiram significativamente ($p < 0,05$) da amostra padrão, inclusive ganhando nota superior (6,8 e 6,9, respectivamente), o que indica maior aceitabilidade nesse atributo. Balcerzak, Gibka, Sikora, Kula, & Strub (2019) afirmaram em seus estudos que as moléculas de baixo peso molecular possuem atributos sensoriais e possui um limiar olfativo mais alto que os demais compostos presentes nos óleos, tais moléculas são pertencentes principalmente aos grupos cinalmadeído e citral. Tais compostos estão fortemente presentes no óleo de capim-limão, conforme evidenciado nos estudos de Mendes et al. (2020). Esses grupos de compostos podem ter proporcionado maior aceitação dos consumidores. Isso foi verificado em outros estudos (Pavinatto et al., 2019; Riquelme, Herrera, & Matiacevich, 2017). Considerando o conjunto

de características e atributos analisados, a presença de fibras, cor, aroma e uniformidade, foram fatores importantes na aceitação global dos filmes biodegradáveis.

O fato de as notas dos filmes ativo-biodegradáveis serem maiores após o fornecimento de informações sobre suas composições, significa que os consumidores favorecem as embalagens que são rotuladas ambientalmente, sendo isso importante para o marketing desse produto. Rokka & Uusitalo (2008) descobriram que os consumidores favoreciam as embalagens rotuladas ambientalmente como o critério mais importante na escolha do produto.

Tabela 5: Aceitações sensoriais de cor, aroma, textura e impressão global das embalagens controle e filmes composto de amido termoplástico/ manteiga de cacau / óleo essencial de capim-limão / bagaço de malte (MB) compreendendo 5% ou 10% de MB, sem ou com informações.

Amostras	Sem informação				Com informação			
	Cor	Aroma	Textura	Impressão Global	Cor	Aroma	Textura	Impressão Global
Controle	$5.9 \pm 1.9^{b(a)}$	$5.8 \pm 1.7^{b(a)}$	$6.2 \pm 1.8^{c(a)}$	$6.3 \pm 1.6^{b(a)}$	$3.6 \pm 2.0^{b(b)}$	$5.3 \pm 1.8^{b(b)}$	$5.8 \pm 1.8^{b(b)}$	$5.7 \pm 1.7^{b(b)}$
F5	$6.9 \pm 1.5^{a(b)}$	$6.8 \pm 1.9^{a(b)}$	$7.3 \pm 1.3^{a(b)}$	$7.3 \pm 1.1^{a(b)}$	$7.6 \pm 1.2^{a(a)}$	$7.2 \pm 1.8^{a(a)}$	$7.5 \pm 1.3^{a(a)}$	$7.7 \pm 1.0^{a(a)}$
F10	$6.8 \pm 1.7^{a(b)}$	$6.5 \pm 1.9^{a(b)}$	$6.8 \pm 1.8^{b(b)}$	$7.0 \pm 1.3^{a(b)}$	$7.2 \pm 1.3^{a(b)}$	$7.2 \pm 1.8^{a(a)}$	$7.4 \pm 1.2^{a(a)}$	$7.5 \pm 1.2^{a(a)}$

Values are reported as average values ± standard deviations. Different letters within the same column (comparing treatments) and row (in parentheses, comparing times) indicate statistically different (p>0.05).

4. Conclusion

Os resultados do presente estudo mostraram que os consumidores têm percepções distintas sobre os diferentes tipos de embalagens (conceitos). A utilização de materiais provenientes de fontes naturais e sustentáveis proporcionaram o apelo mais positivo ao consumidor. Assim, quando comparados aos outros atributos de conscientização ambiental e conservação de alimentos estudados (com embalagens biodegradáveis, ativas e ativas-biodegradáveis) esses atributos influenciaram favoravelmente a probabilidade de compra dos produtos e a percepção do consumidor sobre saúde. Além disso, no presente estudo, foi possível reconhecer o conhecimento limitado do consumidor sobre o uso de compostos bioativos, como óleos essenciais e o uso da nanotecnologia para melhorar o valor nutricional dos alimentos. Pode-se sugerir que dentre as opções avaliadas, as que parecem promissoras para o desenvolvimento e possível comercialização na indústria são as embalagens biodegradáveis e as ativas-biodegradáveis. Quanto a aceitabilidade dessas embalagens, em especial das ativas-biodegradáveis, desenvolvidas nesse estudo (formulação 5% de bagaço de malte (F5) e 10% de bagaço de malte (F10)), verifica que a formulação F5 apresenta melhor aceitação, verificando aumento significativo da aceitação, conforme as informações da composição das embalagens foram fornecidas. Portanto, esses resultados indicaram a importância de realizar investigações contextuais, para avaliar a percepção do consumidor, com o objetivo de viabilizar o desenvolvimento e a possível comercialização desses produtos no mercado consumidor.

Acknowledgments

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