Universidade Estadual de Londrina

ADRIANA PASSOS DIAS

DESENVOLVIMENTO DE FILMES BIODEGRADÁVEIS MULTICAMADAS PARA EMBALAGENS ATIVAS DE ALIMENTOS

Londrina
2020

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Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Londrina - UEL, como requisito parcial para a obtenção do título de Doutora em Ciência de Alimentos.

Orientador: Prof. Dr. Fabio Yamashita

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“Novos horizontes,
Se não for isso, o que será?
Quem constrói a ponte,
Não conhece o lado de lá.
Quero explodir as grades
E voar...”

(Humberto Gessinger)

DIAS, Adriana Passos. **Desenvolvimento de filmes biodegradáveis multicamadas para embalagens ativas de alimentos.** 2020. 114 f. Tese (Doutorado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2020.

RESUMO

O objetivo do trabalho foi desenvolver filmes biodegradáveis multicamadas contendo pó de chá verde ou hibisco e sua posterior aplicação como embalagem ativa para farinha de amêndoas e lombo suíno. Filmes com três camadas contendo pó de chá verde (GT) na camada do meio foram produzidos por coextrusão sopro em balão, em duas concentrações (2,5 e 5,0%). Os filmes biodegradáveis foram produzidos com amido, poli (butileno adipato-co-tereftalato) e glicerol e caracterizados. A resistência à tração foi em torno de 4,9 MPa, e o alongamento variou de 3 a 5%. A permeabilidade ao vapor de água (WVP) foi de $4,5x10^{-6}$g m^{1} Pa^{-1} d^{-1}. A concentração de compostos fenólicos totais (TPC) nos filmes variou de 76 a 113 mg de ácido gálico / 100 g dos filmes, dependendo da formulação. A retenção de TPC na camada ativa foi de 74% e 54%, nos filmes com 2,5 e 5,0% de GT (GT2.5 e GT5.0), respectivamente. Os filmes contendo GT foram utilizados como embalagem de farinha de amêndoas, durante 121 dias. Em 30 dias de armazenamento o índice de peróxido (IP) da farinha de amêndoas embalada na embalagem GT5.0 foi de 7,5 mEq O_2 / kg de óleo de amêndoas, enquanto as embaladas nas embalagens de polietileno de baixa densidade o IP foi de 38,2 7,5 mEq O_2 / kg de óleo de amêndoas. Os valores de TBARS foram 50% menores para as embalagens com adição de GT, quando comparados com as outras embalagens utilizadas no experimento. As amostras de farinha de amêndoas embaladas com filme GT5.0 apresentou menores índices de acidez e índice de peróxido (IP) e TBARS quando comparado com as embalagens convencionais durante os 121 dias de armazenamento. Filmes biodegradáveis multicamadas também foram produzidos contendo cálices de hibisco em pó (HP), como fonte de compostos fenólicos. Os filmes biodegradáveis multicamadas sem HP ($F_{control}$) e com adição de HP (F_{HP}) tiveram valores de resistência à tração de 2,8 MPa, WVP de 4,7 x 10^{-6} g m^{-1} Pa^{-1} d^{-1}. O filme F_{HP} apresentou TPC de 31,37 mg de equivalente de ácido gálico / 100g filme, sendo que a retenção dos fenólicos do HP na camada ativa dos filmes foi em torno de 90%. A atividade antioxidante avaliada pelo método de DPPH do filme foi de 23% de inibição de radicais livres logo após a extrusão. Quando aplicados como embalagem de lombo suíno, os pHs das carnes embaladas na embalagem F_{HP} foram menores ao longo do armazenamento (5,8 - 6,4) quando comparados com as amostras da embalagem convencional (5,8 – 7,3). Os valores de TBARS para o lombo suíno embalado variou de 0,06 a 0,19 mg de malonaldeído / kg de lombo suíno. Os filmes mantiveram-se íntegros até o fim da armazenagem refrigerada (9 dias) e as amostras de carne das embalagens contendo HP apresentaram menores valores de TBARS até 6 dias. Filmes biodegradáveis multicamada com propriedades antioxidantes são uma alternativa viável para substituição de filmes plásticos não biodegradáveis convencionais para embalagem ativas de alimentos.

Palavras-chave: embalagens ativas, filmes multicamadas, DPPH, compostos fenólicos totais, amêndoas, lombo suíno.

DIAS, Adriana Passos. **Development of multilayer biodegradable films for active food packaging.** 2020. 114 f. Tese (Doutorado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2020.

ABSTRACT

The work's objective was to develop biodegradable multilayer films containing green tea powder or hibiscus and its subsequent application as an active packaging for almond flour and pork loin. Three-layer films containing green tea powder (GT) in the middle layer were produced by blown coextrusion in two concentrations (2.5 and 5.0%). The biodegradable films were produced with starch, poly (butylene adipate-co-terephthalate), and glycerol and characterized. The tensile strength was around 4.9 MPa, and the elongation varied from 3 to 5%. The water vapor permeability (WVP) was $4.5x10^{-6}$ g m^{1} Pa^{-1} d^{-1}. The concentration of total phenolic compounds (TCP) in the films ranged from 76 to 113 mg of gallic acid / 100 g of the films, depending on the formulation. TPC's retention in the active layer was 74% and 54% in films with 2.5 and 5.0% of GT (GT2.5 and GT5.0), respectively. The films containing GT were used as packaging for almond flour for 121 days. After 30 days, the peroxide index (IP) of the almond flour packed in the GT5.0 package was 7.5 mEq O_2 / kg of almond oil, while those packed in low-density polyethylene packages, the IP was 38.2 7.5 mEq O_2 / kg of almond oil. The TBARS values were 50% lower for packaging with GT's addition than the other packaging used in the experiment. The almond flour samples with GT5.0 film showed lower acidity indexes and peroxide index (PI) and TBARS compared with conventional packaging during the 121 days of storage. Multilayer biodegradable films were also produced containing powdered hibiscus (HP) chalices as a source of phenolic compounds. Multilayer biodegradable films without HP ($F_{control}$) and added HP (FHP) had tensile strength values of 2.8 MPa, WVP of 4.7 x 10^{-6} g m^{-1} Pa^{-1} d^{-1}. The FHP film presented a TPC of 31.37 mg of gallic acid equivalent / 100 g film, and the retention of HP's phenolics in the active layer of the films was around 90%. The antioxidant activity evaluated by the film's DPPH method was 23% inhibition of free radicals right after extrusion. When applied as packaging for pork loin, the meat packaged pH in FHP was lower (5.8 - 6.4) than those in conventional packaging (5.8 - 7.3). TBARS values for packaged pork loin ranged from 0.06 - 0.19 mg of malonaldehyde / kg of pork loin. The films remained intact until the end of the cold storage (9 days), and the meat samples from the packages containing HP showed lower TBARS values up to 6 days. Multilayer biodegradable films with antioxidant properties are viable alternatives to replace conventional non-biodegradable plastic films in active food packaging.

Keywords: active packaging, multilayer films, DPPH, total phenolic compounds, almonds, pork loin.

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INTRODUÇÃO

As inovações tecnológicas resultantes das mudanças de hábitos da população mundial nas últimas décadas contribuíram para o aumento da produção de embalagens nos setores industriais e comerciais, destacando-se a indústria de alimentos.

A embalagem é vital para a indústria de alimentos, dado que desempenha um papel importante na preservação dos mesmos, pois atua como barreira física contra impacto, evita a contaminação microbiana, previne a perda ou ganho de umidade pelo produto embalado, aumentando assim a vida útil, além de conter informações importantes sobre o alimento embalado.

Diversos materiais são utilizados na produção de embalagens para alimentos entre eles metais, vidro, celulose e, em grande maioria, diferentes tipos de plásticos. Os vários polímeros têm sido utilizados como materiais de embalagem por muitos anos por causa de seu baixo custo e suas vantagens tecnológicas.

Em 2018 o Brasil produziu aproximadamente 7,5 milhões de toneladas de resinas termoplásticas e 6,2 milhões de toneladas de transformados plásticos, na China os valores giram em torno de 102,3 e 118,9 milhões de toneladas respectivamente. A projeção para o consumo de plásticos para o Brasil em 2023 é em torno de 8,0 milhões de toneladas, uma crescente (ABIPLAST, 2020). Esses polímeros sintéticos têm gerado um considerável volume de resíduos sólidos pois não sofrem ação de microrganismos sendo, portanto, dificilmente degradáveis causando um grande impacto ambiental.

A indústria do plástico no Brasil atravessa um período de transformações, tanto em seu processo produtivo quanto nas utilizações de matérias-primas e aplicações, por conta das novas necessidades do mercado, mediante o impacto ambiental causado, principalmente, pelas embalagens plásticas. Em 2019 a Associação Brasileira da Indústria do Plástico (ABIPLAST) sugeriu que toda a sua concepção fosse questionada e repensada. Porém sugeriu também verificar todos os desafios que envolvem essa questão e os benefícios do plástico de forma sistêmica e criteriosa, a fim de encontrar soluções concretas e viáveis para a sociedade e para o meio ambiente. As mudanças devem envolver a questão da economia circular e os avanços tecnológicos que mostram novas aplicabilidades para os plásticos (ABIPLAST, 2020). Na área alimentos, as chamadas embalagens inteligentes, ativas

e/ou sustentáveis vão ao encontro destes avanços tecnológicos.

Esta nova realidade vem estimulando muitas pesquisas para o desenvolvimento de embalagens biodegradáveis a partir de fontes renováveis. Há algum tempo os polímeros à base de carboidratos se tornaram objeto de estudo para este fim, entre eles o amido, matéria-prima abundante e disponível no mundo todo, além de possuir baixo custo e ser obtido a partir de fontes renováveis (MALI; GROSSMANN; YAMASHITA, 2010; VILPOUX; AVEROUS, 2003). Existem polímeros sintéticos biodegradáveis que são obtidos por processos petroquímicos, fermentativos e tecnológicos, entretanto, possuem elevados custos, quando comparado com os polímeros comerciais não biodegradáveis. O poli (adipato co-tereftalato de butileno) (PBAT) se enquadra nessa categoria, considerado como um poliéster sintético biodegradável com propriedades mecânicas adequadas que permite o uso em blendas com outros materiais de fontes renováveis como o amido e ainda pode ser processado através da extrusão sopro, processo empregado para os plásticos tradicionais (REN et al.,2009).

O processo de coextrusão pode ser definido como a extrusão simultânea de dois ou mais materiais emergindo de uma matriz. A coextrusão em multicamadas tem sido amplamente utilizada nas últimas décadas para combinar as propriedades de dois ou mais polímeros em uma estrutura de múltiplas camadas única, o que englobaria características complementares dos diferentes materiais numa única embalagem (GILES; WAGNER; MOUNT, 2005, YU; DEAN; LI 2006).

De maneira geral, as embalagens convencionais para alimentos têm como principal função manter a qualidade do produto durante o armazenamento, transporte e uso, já que ela previne a perda de qualidade dos produtos resultantes dos possíveis agentes ambientais. Algumas novas funções, além da barreira de proteção, vêm sendo introduzidas às embalagens através das novas tecnologias de embalagens ativas. Em contraste com as embalagens convencionais, que devem ser inertes, as embalagens ativas são elaboradas com o intuito de interação com o seu conteúdo e/ou o ambiente circundante, visando aumento da vida útil além da obtida com as embalagens convencionais (HAN et al., 2014; REALINI; MARCOS, 2014; REIS et al., 2015). Entre essas novas características para as embalagens ativas se encontram o uso de absorvedores de oxigênio e etileno, filmes com atividade antimicrobiana, com atividade antioxidante, com controle de atmosfera e até embalagens comestíveis.

No campo de desenvolvimento das embalagens de alimentos sustentáveis e

ativas, os filmes biodegradáveis com ação antimicrobiana ou outros compostos bioativos são geralmente obtidos usando a técnica de evaporação de solvente (*casting*), ou ainda a modificação da composição de superfície do polímero (imobilização de superfície) (NOBILE et al., 2009). Do ponto de vista industrial, esses métodos não permitem a processabilidade adequada em quantidade e a reprodutibilidade. Portanto, a produção de filmes com propriedades ativas usando processos tecnológicos clássicos, como a extrusão, seria o ideal. Porém, poucos estudos relatam o desenvolvimento de filmes biodegradáveis com propriedades ativas e produzidos por extrusão.

A oxidação é uma das principais causas da deterioração de alimentos e embalagens que contenham antioxidantes faz parte de uma nova geração de materiais que podem liberar compostos com propriedades antioxidantes, com taxas controladas adequadas para inibir ou reduzir o processo de oxidação em uma ampla gama de alimentos (BULOSO; LAGARON, 2015; CARRIZO et al.,2016; LACOSTE et al., 2005; REIS et al., 2015).

Substâncias ativas com diferentes mecanismos de ação têm sido investigadas. As tendências em direção ao uso de aditivos naturais antioxidantes em alimentos têm aumentado (GRANDA-RESTREPO et al., 2009; MARCOS et al., 2014). Extratos de plantas, óleos essenciais, ácidos fenólicos, terpenos, tocoferóis, carotenoides e algumas vitaminas são antioxidantes naturais que têm sido utilizados em embalagens ativas para melhorar a estabilidade à oxidação de lipídios e prolongar a vida útil de alimentos (DICASTILLO et al., 2016; BARBOSA-PEREIRA et al., 2014; ZELIKOVIC; MAKSIMOVI'C, 2012).

O chá verde (*Camellia sinensis*) possui altas concentrações de polifenóis, sendo que a maior parte da atividade antioxidante do chá verde está relacionada aos compostos polifenólicos conhecidos como catequinas. Já os cálices de hibisco (H*ibiscus sabdariffa*) são ricos em polifenóis e flavonoides que melhoram o valor nutricional da planta e ainda estão correlacionados com suas propriedades antioxidante (DA COSTA ROCHA et al., 2014 SIRIPATRAWAN; NOIPHA, 2012; SABAGHI et al., 2015)

Os compostos fenólicos presentes na natureza, são considerados antioxidantes eficazes com capacidade de eliminação de eliminando os radicais reativos de oxigênio e nitrogênio, quelando o ferro e inibindo a peroxidação lipídica (GRAMZA et al., 2006; SABARTHI et al., 2015).

Embora muitos materiais com atividade antioxidante tenham sido testados, percebe-se, através da busca na literatura, a ausência de trabalhos sobre filmes multicamadas biodegradáveis com propriedades ativas.

O objetivo deste trabalho foi o desenvolvimento de filmes biodegradáveis multicamadas com propriedades ativas com adição de folha de chá verde e cálice de hibisco. Os filmes foram produzidos por coextrusão sopro em balão, caracterizado quanto às suas propriedades estruturais e ativas e aplicados como embalagem ativa de alimentos.

Esta tese foi estruturada em capítulos, sendo que os capítulos que abrangem a revisão bibliográfica e a produção dos filmes multicamadas biodegradáveis estão em português. Os capítulos intermediários encontram-se na forma de artigo em inglês. Desta forma, a tese está dividida da seguinte forma:

Capítulo 1 – Revisão Bibliográfica

Revisão bibliográfica sobre materiais biodegradáveis, processamento de filmes biodegradáveis por extrusão sopro em balão, coextrusão multicamadas de filmes, embalagens ativas e compostos naturais ativos (chá verde e hibisco).

Capítulo 2 – Produção dos filmes biodegradáveis

Descrição da produção dos filmes biodegradáveis multicamadas e exemplo de funcionamento do equipamento utilizado, além das condições de processamento dos filmes e a disposição das camadas nos filmes multicamada biodegradáveis produzidos.

Capítulo 3 – Multilayers biodegradable films containing green tea (*Camellia sinensis*)

Produção de filmes multicamadas com a adição de chá verde, com o objetivo de verificar se os filmes incorporaram as propriedades ativas e antioxidantes do composto ativo depois do processamento. Os filmes foram caracterizados quanto às propriedades mecânicas, de barreira, térmicas e estruturais, e as propriedades ativas foram analisadas através da análise de fenólicos totais.

Capítulo 4 – Multilayer biodegradable films containing green tea (*Camellia sinensis*) as active packaging for almond (*Prunus dulcis*) flour.

Utilização dos filmes biodegradáveis multicamadas contendo chá verde como

embalagem com propriedades ativas para farinha de amêndoas. Os filmes foram caracterizados quanto às propriedades mecânicas e de barreira, a vida útil do produto embalado foi acompanhada durante 121 dias através das análises de oxidação: índice de acidez, índice de peróxidos e TBARS. As propriedades ativas dos filmes foram avaliadas no tempo zero e no tempo final de acondicionamento através da análise de fenólicos.

Capítulo 5 – Multilayer biodegradable films with hibiscus flowers (*Hibiscus sabdariffa*) as packaging for fresh pork loin.

Produção e caracterização de filmes biodegradáveis multicamadas contendo pó de cálices de hibisco. Os materiais foram caracterizados quanto às propriedades mecânicas, de barreira, e a caracterizações das possíveis propriedades antioxidantes dos filmes foram avaliadas a partir da quantificação dos fenólicos totais e DPPH. Esse material foi posteriormente utilizado como embalagens ativa de lombo suíno fresco. A avaliação dos lombos embalados durante os 9 dias de vida útil foi feita com análises de cor, pH, TBARS.

CAPÍTULO 1
REVISÃO BIBLIOGRÁFICA

1 MATERIAIS BIODEGRADÁVEIS

Os materiais plásticos derivados de petróleo são amplamente utilizados na produção de embalagens devido as suas boas propriedades mecânicas e de barreira, juntamente com seu baixo custo (FERREIRA et al., 2016; SCAFFARO; SUTERA; BOTTA, 2018), entretanto estes materiais causam problemas ambientais no que diz respeito ao esgotamento de recursos naturais não renováveis e ao acúmulo de materiais não biodegradáveis (CANO et al., 2014; GROSS; KALRA, 2002).

Desta forma, as pesquisas para o desenvolvimento de materiais plásticos compostáveis ou biodegradáveis de origem biológica utilizando polímeros biodegradáveis provenientes de fontes renováveis, tais como polissacarídeos, proteínas e lipídios, tem aumentado nos últimos anos (BONILLA et al, 2014; MALI et al., 2004; MULLER; GONZÁLEZ, CHIRALT, 2017; SANYANG et al., 2016; ZANELLA et al, 2015). Embora muitos destes polímeros não exibam estabilidade térmica e propriedades mecânicas e de barreira semelhantes às dos polímeros convencionais, há diversos estudos com o objetivo de melhorá-los.

O amido é um polímero bastante estudado no desenvolvimento de materiais biodegradáveis, devido ao seu baixo custo abundância, além de ser um recurso renovável (MULLER; GONZALEZ; CHIRALT, 2017; ZANELLA et al., 2015).

Amidos de diferentes fontes têm atraído atenção para o desenvolvimento de filmes biodegradáveis devido a sustentabilidade, biodegradabilidade, disponibilidade e acessibilidade. Eles também apresentam características físicas semelhantes aos filmes plásticos convencionais em termos de aparência, além de serem inodoro e insípido (CANO, et al, 2014; GONZÁLES; IGARZABAL, 2013). Além disso, os filmes à base de amido são relatados como não-tóxicos, o que também contribuiu para sua crescente aceitação como alternativa potencial de embalagem, principalmente de alimentos (CHEN et al.,2008).

Estruturalmente o amido, independente da fonte, contém duas macromoléculas: amilose, que é essencialmente linear; e amilopectina, que é altamente ramificada. Filmes produzidos somente com amido apresentam baixa processabilidade, além de propriedades térmicas, mecânicas e de barreira inferiores

aos dos polímeros convencionais (ERSAN; BABAÇ, 2003; MALI et al., 2004).

O amido não é um termoplástico verdadeiro, mas na presença de agentes plastificantes, calor e cisalhamento, ele acaba perdendo sua propriedade semicristalina, originando o amido termoplástico (ATp) que é um material amorfo com características semelhantes as observadas em polímeros sintéticos (OLIVATO et al., 2014; ZANELLA et al, 2015). A fonte de amido, tipo de plastificante, condições de processamento e armazenamento (temperatura e umidade relativa) influenciam no processo de recristalização polimérica amorfa (ZAMÚDIO-FLORES et al., 2006).

Materiais produzidos somente com ATp apresentam propriedades mecânicas e barreira ao vapor de água inadequadas para produção e aplicação em escala comercial (BRANDELERO et al., 2011). Existem diferentes abordagens para superar as desvantagens desse material, a principal é combinar amido com outros polímeros com propriedades desejáveis ou complementares formando blendas(BRANDELERO; YAMASHITA; GROSSMANN, 2010; BOCCHINI; BATTEGAZZORE; FRACHE, 2010; MULLER; GONZALEZ; CHIRALT, 2017; TANG; ALAVI, 2011; LANDIM et al., 2016).

Os polímeros que melhor se adaptam ao processo de biodegradação completa são os naturais, aqueles hidrolisáveis a CO_2 e H_2O, ou a CH_4 e os polímeros sintéticos que possuem estruturas próximas aos naturais (LANDIM et al., 2016). Alguns polímeros sintéticos biodegradáveis são comercializados atualmente, entre eles o poli (ácido lático) (PLA), poli (vinil álcool) (PVA) e o poli (adipato-co-tereftalato de butileno) (PBAT). Esses materiais têm processabilidade adequada para produção de plásticos com propriedades equiparáveis aos plásticos convencionais, porém com alto custo, o que torna sua utilização com matéria-prima principal de embalagens pouco atrativa para a indústria de embalagem.

A produção de blendas de amido com polímeros sintéticos biodegradáveis, como o poli (adipato co-tereftalato de butileno) (PBAT) (COSTA, 2008; BRANDELERO; YAMASHITA; GROSSMANN, 2010; FLORES et al., 2010; OLIVATO et al., 2012a; OLIVATO et al., 2012b; REIS et al., 2013; SCAPIM, 2009) e o poli (ácido lático) (PLA) (SHIRAI et al., 2013), foram realizadas com resultados satisfatórios.

O poli (adipato co-tereftalato de butileno) (PBAT) é um co-poliéster alifático-aromático de natureza hidrofóbica, biodegradável e compostável, comercializado pela empresa alemã BASF com o nome comercial de Ecoflex®. Materiais produzidos com PBAT são resistentes à água e à gordura, tornando-o apropriado para o uso como embalagem descartável (KOLYBABA et al., 2003). Para produção dos poliésteres

alifático-aromáticos, como o PBAT, é utilizada a técnica de policondensação padrão, onde três diferentes dióis são utilizados como precursores, além de dois ácidos dicarboxílicos alifáticos e o ácido tereftálico, utilizados como componente aromático (MULLER et al., 1998; WITT et al., 2001).

Segundo a BASF (2004), o PBAT é um polímero versátil, que permite o processamento de filmes extrudados, soprados, termomoldados e injetados, para embalar alimentos. É flexível e apresenta temperatura de fusão entre 110-115 °C (AVEROUS; FRIGANT, 2001). Este material é amplamente degradado em poucas semanas com o auxílio de enzimas que ocorrem naturalmente, superando ainda as desvantagens de muitos materiais alifáticos, como melhor resistência máxima à tração e ao alongamento, quando comparado ao polietileno de baixa densidade (PEBD) (WITT et al., 2001). Este co-poliéster alifático-aromático possui potencial para ser combinado com amido e ainda outros polímeros biodegradáveis na formação de blendas poliméricas, sendo processado através de extrusão-sopro em balão ou calandragem, processo empregado para plásticos tradicionais (VILPOUX; AVEROUS, 2003; RAQUEZ et al., 2008).

2 PROCESSAMENTO DE FILMES BIODEGRADÁVEIS

Casting, extrusão, moldagem por injeção, calandragem são algumas das técnicas que podem ser empregadas na produção de filmes biodegradáveis. Entre estas, a mais citada é a técnica de *casting*, que consiste na produção de filmes por evaporação do solvente de uma solução filmogênica. Porém apresenta-se inviável pelo gasto energético demandado pelo tempo necessário para a secagem, além da dificuldade de implantação em escala industrial. Para a produção dos filmes biodegradáveis é desejável que seja utilizada uma técnica que combine alta produtividade, eficiência energética e que seja possível o emprego industrial (THUNWALL et al., 2008).

A maior parte da produção de filmes plásticos convencionais é feita a partir da extrusão, já que essa tecnologia oferece as vantagens associadas ao sistema contínuo de produção, incluindo versatilidade, baixo custo operacional e necessidade de menor espaço por utilidade de operação (SOTHORNVIT et al., 2007). Com o intuito de facilitar a viabilização da produção de plástico a partir de polímeros biodegradáveis, o ideal seria utilizar a mesma tecnologia disponível atualmente para os materiais

convencionais.

2.1 EXTRUSÃO TERMOPLÁSTICA E COEXTRUSÃO MULTICAMADAS

A extrusão termoplástica é um processo contínuo e térmico, que é capaz de provocar mudanças substanciais nas matérias-primas, alterando formas, estruturas e características, por meio da combinação de temperatura e cisalhamento. Durante o processo, ocorrem diversas operações simultâneas como mistura, cisalhamento, plastificação cozimento e modelamento por meio de uma rosca-sem-fim A matéria-prima extrudada é submetida a várias mudanças durante o processo, como, por exemplo, a plastificação do material processado (EL-DASH, 1981; FELLOWS, 2000; ZHANG et al., 2001, WOLF, 2010), sendo muito difundido para o processamento de polímeros pelas seguintes vantagens: ampla flexibilidade de operação, habilidade de processamento de polímeros com elevada viscosidade na ausência de solvente, viabilidade de injeções múltiplas e controle de tempo de residência e grau de mistura (LIU et al., 2009; THUNWALL et al., 2008).

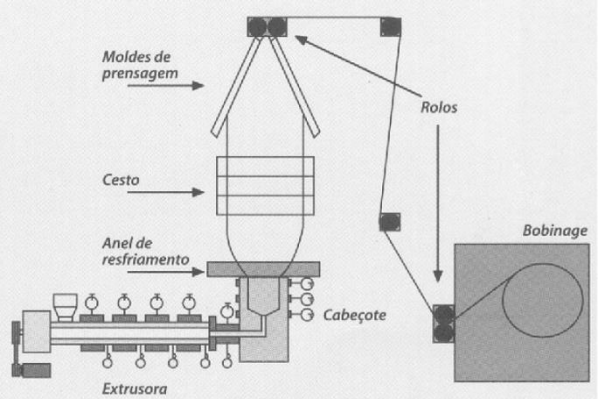
O processo de extrusão por sopro pode ser usado para produzir filmes biodegradáveis de amido. Durante a extrusão há o rompimento dos grânulos de amido (fusão), produzindo o amido termoplástico (ATp). O ATp, expandido por sopro em uma matriz ou forma tubular, produz filmes rígidos, que requerem a adição de plastificantes, por exemplo, glicerol, para diminuir a temperatura de fusão do amido e aumentar a flexibilidade, mas os filmes resultantes são frágeis (BRANDELERO et al., 2011; ZULLO; IANNACE, 2009).

Existem diversos estudos sobre produção de filmes biodegradáveis por extrusão-sopro em balão a partir de blendas de amido com diferentes polímeros sintéticos. Raquéz et al. (2008) avaliaram a compatibilização de blendas contendo amido termoplástico e PBAT por extrusão reativa através da produção de filmes de alto teor de amido termoplástico de mandioca com PBAT. Olivato et al. (2012a) estudaram a utilização de compatibilizantes como anidrido maleico e ácido cítrico na extrusão reativa de blendas de amido e PBAT para a produção de filmes biodegradáveis. Brandelero, Grossmann e Yamashita (2010) estudaram o efeito dos parâmetros de processo em filmes biodegradáveis produzidos por extrusão-sopro em balão. Souza, Soares-Junior e Yamashita (2013) realizaram a produção de filmes biodegradáveis soprados a partir de blendas de farinha de arroz com PBAT. Além

destes, muitos outros estudos foram realizados utilizando blendas de amido com poliésteres biodegradáveis (BILCK; GROSSMANN; YAMASHITA, 2010; COSTA, 2008; NOBREGA et al., 2012; REIS, 2013; SCAPIM, 2009).

A Figura 1, mostra o processo de formação de filme tubular por sopro em balão.

Figura 1 – Representação do processo de produção de filmes por extrusão-sopro em balão



Fonte: desconhecida

Filmes multicamadas são utilizados para atender as necessidades dos produtos a serem embalados. Neste caso, cada camada proporciona uma utilização final especifica como, por exemplo, barreira ao oxigênio e à umidade, termoformagem, atributos mecânicos e capacidade de selagem. Filmes multicamadas com diversas combinações podem ser aplicadas em embalagens de alimentos, laminação com papel e usos decorativos. Esses materiais são fabricados através do processo de coextrusão tubular sopro em balão, ou ainda por extrusão plana com calandragem, sendo que as camadas ainda podem ser dispostas por adesão, com uso de lubrificantes (ANTURKAR, PAPANASTASIOU, WILKES, 1990).

O processo de coextrusão pode ser definido como a extrusão simultânea de dois ou mais materiais emergindo de uma matriz. A coextrusão em multicamadas tem sido amplamente utilizada nas últimas décadas para combinar as propriedades de dois ou mais polímeros em uma estrutura de múltiplas camadas única (GILES; WAGNER; MOUNT, 2005, YU; DEAN; LI 2006).

A coextrusão pode produzir materiais multicamadas constituídos de dois ou mais polímeros diferentes. YU et al. (2006) produziram filmes multicamadas biodegradáveis, através da extrusão plana, onde a camada interna era composta de

amido termoplástico e as duas camadas externas de outros polímeros biodegradáveis como o poli (ácido lático) (PLA) e o poli (hidróxido butirato) (PHB). Os autores obtiveram resultados satisfatórios quando comparados aos materiais elaborados exclusivamente de amido termoplástico, nas propriedades mecânicas e de barreira.

A produção de materiais multicamadas feitas através da adesão de diferentes materiais é dificultada pela instabilidade interfacial entre as camadas (GILES; WAGNER; MOUNT, 2005; ZHANG; AJJI; JEAN-MARIE, 2001), por exemplo, a adição de uma camada individual de poli (etileno-co-álcool vinílico) (EVOH) em filme multicamada de polietileno de alta densidade (PEAD) não foi suficiente para produzir novo filme de estrutura multicamada composto por esses dois materiais, pela falta de adesão entre eles. Portanto, a aplicação de uma camada de ligação em filmes multicamadas têm sido relatada (ZHANG; AJJI; JEAN-MARIE, 2001).

Scaffaro e colaboradores (2018) afirmam que benefícios do filme de duas camadas, desenvolvidos a partir da combinação de PLA (poliácido láctico) e MaterBi, foram demonstrados por uma comparação sistemática das propriedades do filme bicamada com aqueles de filmes de camada única soprados.

Polímeros de origem natural, como amido e proteínas, são alternativas aos polímeros à base de petróleo para diversas aplicações. Um problema inerente com a utilização desses polímeros (por exemplo, o amido termoplástico) como materiais biodegradáveis é a sua sensibilidade à umidade. Uma abordagem para resolução dessas desvantagens é o processo de coextrusão de amidos termoplásticos com polímeros biodegradáveis menos hidrofílicos.

Não foram relatados na literatura estudos relativos à produção de filmes biodegradáveis com três camadas por coextrusão termoplástica, o que sugere um nicho a ser explorado para o desenvolvimento de embalagens mais sustentáveis para aplicação em alimentos.

3 EMBALAGENS ATIVAS PARA ALIMENTOS

As embalagens convencionais para alimentos, de maneira geral, têm como principal função manter a qualidade do produto durante o armazenamento, transporte e uso. A embalagem previne a perda de qualidade dos produtos oriunda dos possíveis agentes ambientais.

Novas funções, além da barreira de proteção, vêm sendo introduzidas às

embalagens através das chamadas "embalagens ativas". Em contraste com as embalagens convencionais, que devem ser inertes, as embalagens ativas interagem com o seu conteúdo e/ou o ambiente circundante, visando aumento da vida útil além das obtidas com as embalagens convencionais, ou ainda em materiais de embalagem mais sustentáveis. As embalagens ativas podem apresentar atividade antimicrobiana, atividade antioxidante, controlar a atmosfera através de absorvedores de oxigênio e etileno e liberadores de dióxido de carbono e etanol, dentre outras funções.

Na embalagem ativa os aditivos são incorporados ou imobilizados e liberados controladamente para o alimento, desta forma estão presentes em pequenas quantidades e apenas em sua superfície, não sendo necessária sua incorporação direta ao produto (ARAB-TEHRANY; DESOBRY, 2012; AZEREDO; FARIA; AZEREDO, 2000; BOLUMAR; ANDERSEN; ORLIEN, 2011; KERRY, GRADY, HOGAN, 2006; SANDHYA, 2010; JAMSHIDIAN).

Embalagens ativas biodegradáveis com ação antimicrobiana ou outros compostos bioativos, são geralmente obtidos nível laboratorial usando a técnica de evaporação de solvente (*casting*), ou ainda a modificação da composição de superfície do polímero (imobilização de superfície) (NOBILE et al., 2009). Do ponto de vista industrial, esses métodos não permitem a processabilidade adequada em quantidade e reprodutibilidade. Portanto, a produção de filmes com propriedades ativas usando processos tecnológicos clássicos, como a extrusão, seria o ideal. Porém poucos estudos relatam o desenvolvimento de filmes biodegradáveis com propriedades ativas e produzidos por extrusão.

3.1 Embalagens Ativas com Propriedades Antioxidantes

A oxidação lipídica é um fenômeno espontâneo responsável pelo desenvolvimento do ranço, sendo uma das principais causas de perda de qualidade em produtos cárneos, responsável pela perda de cor, alteração de sabor, modificação da textura e formação de compostos tóxicos, podendo ter sua velocidade de reação acelerada por ação de microrganismos (MATHIAS et al., 2010).

O fenômeno de oxidação e estabilidade dos lipídios dependem de vários fatores como do número e a natureza das insaturações presentes, o tipo de interface entre os lipídios e o oxigênio, a exposição à luz e ao calor, a presença de pró-oxidantes ou de antioxidantes (SILVA, BORGES, FERREIRA, 1998).

De acordo com a FDA (*Food and Drug Administration*) antioxidantes são compostos adicionados, em pequenas quantidades, aos alimentos para retardar sua deterioração por rancidez e descoloração causadas pela oxidação de óleos e gorduras. Devem ser eficientes em baixa concentração, possuir afinidade com a matriz do alimento, aceitação sensorial, ser atóxico e não interferir nas propriedades físicas dos alimentos.

Antioxidantes podem aumentar a vida útil dos produtos alimentícios diminuindo oxidação lipídica, um processo típico da cadeia de radicais livres (MARTINÉZ et al., 2013).

Os antioxidantes sintéticos mais aplicados à alimentos são o BHA (butil-hidroxi-anisol), BHT (butil-hidroxi-tolueno), TBHQ (butil-hidroquinona terciária) e PG (galato de propila) (SHIRAHIGUE, 2008). O BHA é um antioxidante efetivo frente a oxidação de gorduras de origem animal e possui eficiência limitada em óleos insaturados de vegetais ou sementes, baixa estabilidade a altas temperaturas e controle de oxidação de ácidos graxos de cadeia curta, além de agir como sequestrante de radicais peróxidos. No Brasil, o uso de BHA em alimentos é permitido, sendo controlado pelo Ministério da Saúde, podendo ser adicionados em alimentos em até 200 mg/kg (RAMALHO, JORGE, 2005).

O desenvolvimento de sistemas de embalagens ativas com propriedades antioxidantes está atraindo considerável atenção como uma tecnologia emergente para reduzir a oxidação lipídica em alimentos (CARRIZO, et al.,2013; ECHEGOYEN; NERÍN, 2015; GOMÉS-ESTACA et al., 2014). Estas embalagens podem eliminar os radicais livres do alimento embalado ou da atmosfera interna. Nos dois casos, esse tipo de embalagem não requer contato direto com o alimento para exibir propriedades antioxidantes (NERÍN et al., 2006).

A eficiência da atividade antioxidante de materiais com propriedades ativas é dependente, principalmente, da liberação dos componentes ativos que foram adicionados, além da interação entre os componentes ativos e polímeros e a microestrutura dos filmes (PIÑEROS-HERNANDEZ et al., 2016; ZHANG et al., 2019).

Substâncias bioativas com diferentes mecanismos de ação têm sido investigadas e as tendências atuais vão em direção ao uso de aditivos naturais em alimentos (GRANDA-RESTREPO et al., 2009; MARCOS et al., 2014). Extratos de plantas, óleos essenciais, ácidos fenólicos, terpenos, tocoferóis, carotenoides e algumas vitaminas são antioxidantes naturais que têm sido utilizados em embalagens

ativas para melhorar a estabilidade à oxidação de lipídios e prolongar a vida útil de alimentos (BARBOSA-PEREIRA et al., 2014; CAVAR; MAKSIMOVI'C, 2012; DICASTILLO et al., 2016;). Embora muitos materiais com atividade antioxidante tenham sido testados, poucos estudos têm utilizado compostos naturais, e ainda menos estudos avaliaram como estes compostos podem ser incorporados aos filmes biodegradáveis multicamadas extrudados e com comprovação de eficiência e se os mesmos possuiriam ação sob essa matriz.

4 FONTES NATURAIS DE COMPOSTOS FENÓLICOS

Os compostos bioativos de fontes naturais são cada vez mais populares entre os consumidores, devido às preocupações com a saúde associadas a compostos sintéticos. Óleos essenciais e polifenóis são os dois principais tipos de compostos bioativos naturais usados no revestimento comestível ou embalagens para melhorar a conservação dos alimentos (XIONG et al., 2020; ZAMBRANO-ZARAGOZA, 2018)

Os compostos fenólicos são metabólitos vegetais secundários que estão envolvidos em uma ampla gama de funções fisiológicas especializadas, tais como desenvolvimento e mecanismos de defesa das plantas. Estes compostos são capazes de modular a atividade de diversas enzimas (DI CARLO et al., 1999), sugerindo seu envolvimento em processos bioquímicos e fisiológicos, não apenas em plantas, mas também em animais e humanos (RUSAK et al., 2008).

Os compostos fenólicos são considerados antioxidantes eficazes, pois eliminam os radicais reativos, quelam o ferro, inibindo assim a peroxidação lipídica (YU et al., 2002). Compostos fenólicos geralmente se referem a compostos produzidos pela substituição de átomos de hidrogênio no anel benzeno de hidrocarbonetos aromáticos por grupos hidroxila, e podem ser divididos em monofenóis e polifenóis de acordo com o número de grupos hidroxila contidos em suas moléculas. Quimicamente, de acordo com o número e os elementos estruturais dos grupos fenólicos, os polifenóis podem ser divididos em quatro categorias: flavonoides, estilbenos, lignanas e ácidos fenólicos (GE et al., 2021; MANACH et al., 2004)

4.1 CHÁ VERDE (*CAMELLIA SINENSIS*)

O chá verde (*Camellia sinensis*) é a bebida mais consumida globalmente. Sua popularidade é creditada ao atraente aroma, sabor e presença de antioxidantes por conta da alta concentração de compostos fenólicos (DAS et al.., 2019). Muitos estudos já comprovaram as propriedades antioxidantes dos polifenóis derivados do chá verde (GRAMZA et al., 2006; SABARTHI et al., 2015)

Os principais compostos fenólicos presentes nas folhas do chá (C*amellia sinensis*) são os flavan-3-ols (também conhecidos como catequinas), que constituem até 30% de sua massa seca (RUSAK et al., 2008). Embora as catequinas sejam os compostos fenólicos dominantes (KILMARTIN; HSU, 2003), vários flavonóis (até 4%) e flavonas (em traços) também estão presentes nas folhas do chá. Os principais flavonóis do chá são conjugados de quercetina e kaempferol com a porção de conjugação variando de mono- a di- e triglicosídeos (DEL RIO et al., 2004). Outros compostos relacionados encontrados no chá são os ácidos gálico, cumárico e cafeico, bem como os alcaloides purínicos, teobromina e cafeína (RUSAK et al., 2008).

A maior parte da atividade antioxidante do chá verde está relacionada aos compostos polifenólicos conhecidos como catequinas sendo as epicatequinas e as galato epicatequina com maior atividade antioxidante (GRAMZA et al., 2006; LEE et al., 2007; SABARTHI, et al., 2015).

A literatura sugere que os compostos fenólicos do extrato de chá verde atuam como antioxidante através da prevenção de iniciação de cadeia radical, ligação de catalisadores de íons metálicos de transição e interação com os radicais livres para inibir a oxidação lipídica (FARHOOSH; GOLMOVAHHED; KHODAPARAS, 2007; PERUMALLA; HETTIARACHCHY, 2011; SIRIPATRAWAN; NOIPHA, 2012; SABAGHI et al., 2015). Essas catequinas presentes podem atuar como antioxidantes por meio da doação de um átomo de hidrogênio, exercendo um papel de aceitador de radicais livres, interrompendo a reação em cadeia da oxidação lipídica, (GRAMZA et al., 2009).

Há também a sugestão de que a anexação de grupos hidróxidos as moléculas de catequinas é provavelmente o principal fator que fazem delas compostos com fortes propriedades antioxidantes em óleos quando comparado com antioxidantes como BHT, BHA e tocoferol (SIRIPATRAWAN; NOIPHA, 2012; SABAGHI et al., 2015).

Os compostos fenólicos podem eliminar espécies reativas de oxigênio e

nitrogênio. Assim, o chá verde tem sido utilizado para prevenir a oxidação lipídica em diversos produtos alimentícios (CARRIZO et al., 2015; CHAN; LIM; CHEW, 2007; FABRA et al., 2018; SIRIPATRAWAN; HARTE, 2010). Além disso têm sido utilizados em embalagens ativas para melhorar a estabilidade à oxidação de lipídios e prolongar a vida útil de alimentos (BARBOSA-PEREIRA et al., 2014; CARRIZO et al., 2015; CAVAR; MAKSIMOVI'C, 2012; DICASTILLO et al., 2016; SABAGHI et al., 2015; SIRIPATRAWAN; HARTE, 2010).

4.2 HIBISCO (*HIBISCUS SABDARIFFA*)

Os cálices das flores de hibisco (H*ibiscus sabdariffa*) são ricos em carboidratos, fibras alimentares, proteínas, vitaminas, minerais e compostos bioativos (BERGMEIER et al., 2014; PERALTA et al., 2019) e a cor vermelha brilhante e o sabor único o tornam um produto alimentar valioso.

H. sabdariffa possui atividades antitumorais, antioxidantes e anti-hiperlipêmicas (CHEN et al., 2003; DE MOURA et al., 2019; FERNANDEZ-ARROYO et al., 2011; MOHD-ESA et al., 2010), pois seus cálices são ricos em polifenóis e flavonoides que melhoram a valor nutricional da planta e ainda estão correlacionados com suas propriedades antioxidante. O conteúdo fenólico na planta consiste principalmente em antocianinas como delfinidina-3-glucósido, sambubiosídeo e cianidina-3-sambubiosídeo e outros flavonoides como a gossipetina, hibiscetina e seus respectivos glicosídeos; ácido protocatecuico, eugenol, e esterol como β-sitosterol e ergosterol. Dentre esses componentes, a delfinidina-3-sambubiosídeo e a cianidina-3-sambubiosídeo são as substâncias com maior atividade antioxidantes e são fortemente hidrofílicos (DA COSTA ROCHA et al., 2014; DE MOURA et al., 2019; FERNANDEZ-ARROYO et al., 2011; RIAZ, CHOPRA, 2018).

A estabilidade desses componentes depende do pH, temperatura, presença de enzima, luz, presença de outros flavonoides, ácidos fenólicos e metais (RIAZ, CHOPRA, 2018).

Estudos que relatam mudanças de cor em filmes poliméricos contendo antocianinas de fontes distintas, quando expostos em diferentes valores de pH, sugerem a importância e relevância deste pigmento em novas abordagens. (PERALTA et al., 2019; YOSHIDA et al., 2014).

Mohd-Esa e colaboradores (2010) utilizaram extratos de *H. sabdariffa* na

conservação de produtos cárneos e relataram que as sementes possuem compostos bioativos antioxidantes mais poderosos do que os antioxidantes comerciais (BHT e α-tocoferol).

As propriedades antioxidantes do hibisco acabaram estimulando pesquisas em relação ao seu comportamento em matrizes poliméricas e no desenvolvimento de embalagens com propriedades antioxidantes. Idhan e colaboradores (2012) avaliaram o efeito termal de antocianinas encapsuladas em diferentes matrizes poliméricas. Zhang et al. (2019) e Peralta et al. (2019) utilizaram antocianinas do hibisco como indicador biodegradável em um sistema de embalagem inteligente, obtendo resultados satisfatórios por conta da sensibilidade destes compostos em diferentes pHs.

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CAPÍTULO 2
PRODUÇÃO DOS FILMES BIODEGRADÁVEIS MULTICAMADAS

O Capítulo descreve as extrusoras utilizadas neste trabalho, a disposição e a estrutura das camadas na formação do balão, além das condições de processamento gerais dos filmes. Todos os filmes biodegradáveis multicamadas desta tese foram produzidos por coextrusão sopro em balão no mesmo equipamento e nas mesmas condições de processamento.

1 EQUIPAMENTO DE EXTRUSÃO MULTICAMADA

O equipamento utilizado neste trabalho foi uma coextrusora piloto de três camadas (modelo AX-16L / D26, AX Plásticos, Brasil) (Figura 1).

Figura 1 – Coextrusora piloto de três camadas modelo AX-16L/D26

Fonte: o autor.

Este modelo é composto por três extrusoras, cada uma delas possui um parafuso com 16 mm de diâmetro (D), comprimento do parafuso (L) de 416 mm (razão L / D de 26) e a velocidade dos parafusos pode variar entre 12 e 120 RPM. O equipamento possui oito zonas de aquecimento, no *manifold* e 01 no cabeçote, e o

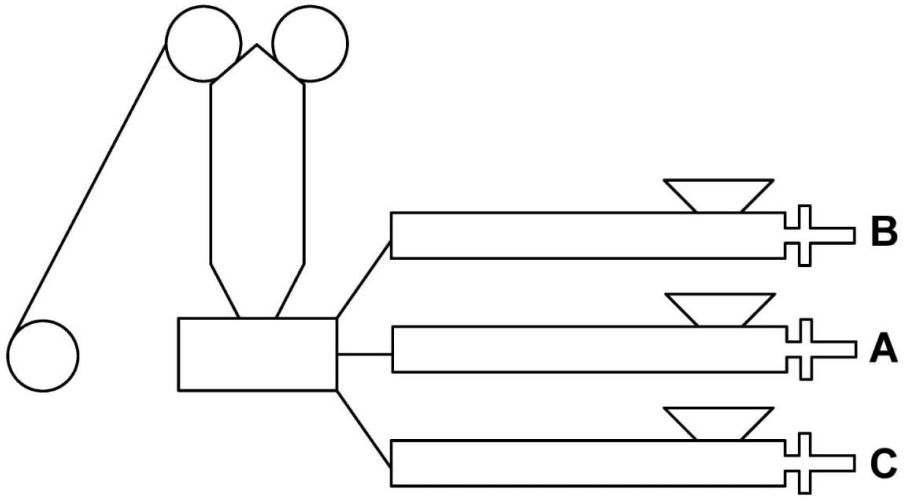
perfil de temperatura pode ser estabelecido conforme o material utilizado, a temperatura pode chegar a 160 °C para as todas as zonas de aquecimento. A matriz (cabeçote) do equipamento foi projetada para produzir filmes tubulares de três camadas.

1.1 Esquema de Funcionamento da Extrusora e Disposição das Camadas nos Filmes

Uma das principais vantagens desse equipamento é a possibilidade de utilização de diferentes disposições de camadas num mesmo filme, por esse motivo a importância de entender a disposição das camadas durante o processamento desses materiais no equipamento.

A Figura 2 contém uma ilustração esquemática de como as três extrusoras estão dispostas neste modelo de coextrusora em camadas observada na Figura 1.

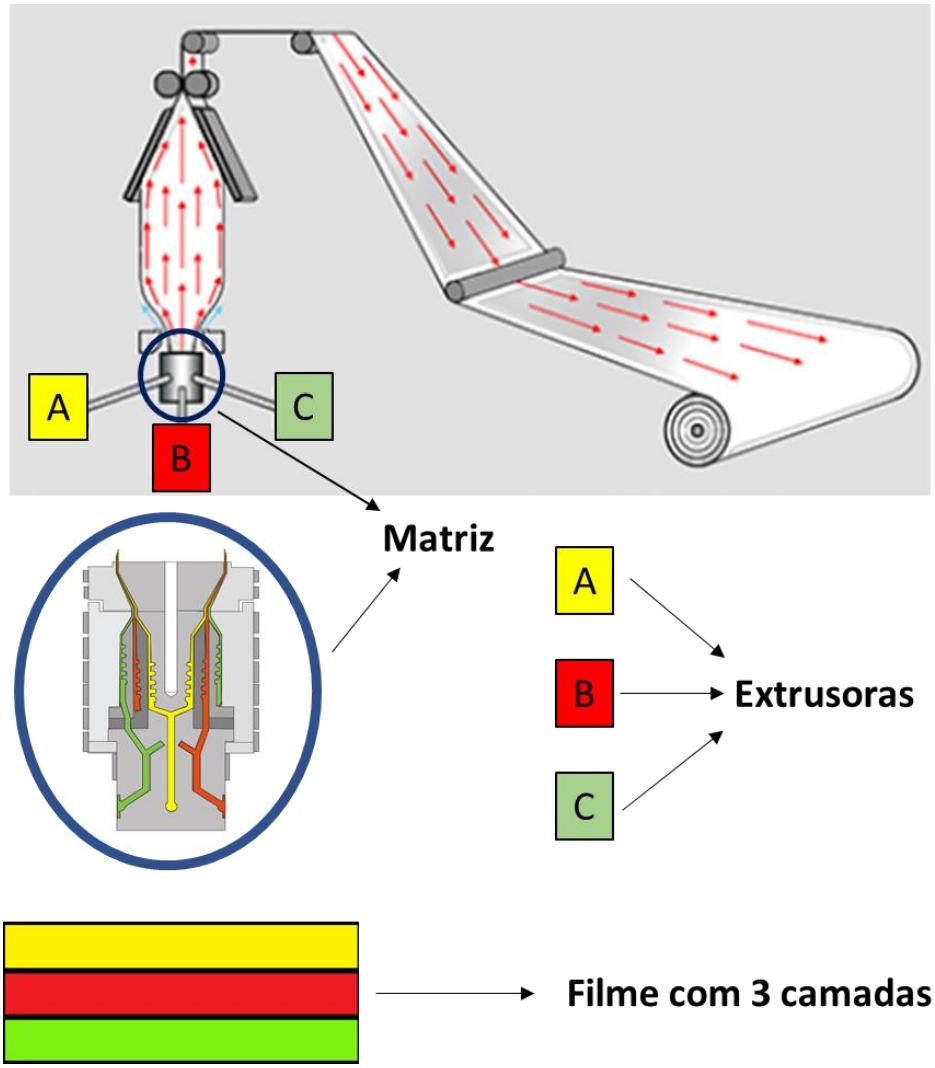
Figura 2 – Ilustração esquemática da coextrusora piloto de três camadas



Fonte: o autor.

A partir desse esquema é possível observar a disposição das camadas do filme multicamada durante a extrusão, sendo que a extrusora **B** forma a camada interna do balão, **C** a camada externa e a extrusora **A** a camada intermediária.

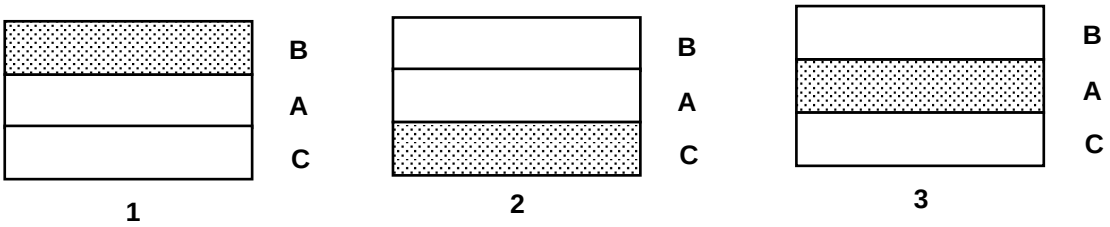
Figura 3 – Ilustração esquemática do funcionamento da coextrusora piloto de três camadas



Fonte: o autor.

Durante a formação do balão a extrusora dispõe as três camadas da forma representada esquematicamente pela Figura 3. Caso a necessidade seja de um material diferente na camada interna do balão, deve-se adicionar esta formulação na extrusora B (1), caso o material seja necessário na camada externa a adição do mesmo deve ser na extrusora C (2), já na camada intermediária do balão, na extrusora A (3) (Figura 4).

Figura 4 – Distribuição das camadas nos filmes biodegradáveis multicamadas



Fonte: o autor.

A disposição das camadas ativas dos filmes produzidos para cada parte do trabalho será apresentada em seu respectivo capítulo com uma ilustração esquemática para facilitar a leitura assim como as formulações utilizadas para cada camada.

2 CONDIÇÕES DE PROCESSAMENTO PARA PELLETS E FILMES

Os ingredientes de cada formulação foram manualmente misturados e extrudados em uma extrusora piloto monorrosca (BGM, modelo EL-25, Brasil) para produção de pellets, necessários para a segunda extrusão na formação do balão. Essa extrusora possui um diâmetro de parafuso (D) de 25 mm, comprimento de parafuso (L) de 750 mm (razão L / D de 30), cinco zonas de aquecimento e uma matriz com seis furos de 2 mm e o perfil de temperatura do barril foi definido em 90/120/120/125 °C com uma velocidade de parafuso de 35 RPM.

Os pellets produzidos na primeira extrusão foram extrusados em uma coextrusora piloto (modelo AX-16L / D26, AX Plasticos, Brasil) (Figura 1) para produzir filmes de três camadas por extrusão por sopro em balão. O perfil de temperatura usado foi o mesmo da primeira fase da extrusão, em 90/120/120/125 °C com uma velocidade de parafuso de 50 RPM para as três extrusoras.

CAPÍTULO 3

MULTILAYER BIODEGRADABLE FILM CONTAINING GREEN TEA POWDER (*Camellia sinensis*)

1 INTRODUCTION

Plastics generate a considerable volume of wastes causing significant environmental impact (LANDIM et al., 2016; WEI et al., 2015), and stimulates the development of biodegradable materials from renewable sources (FOURATI et al., 2018; MALI; GROSSMANN; YAMASHITA, 2010) like starch, due to its availability, biodegradability, and low cost (FOURATI et al., 2018; PARRA et al., 2004; ZULLO; IANNACE, 2009).

The advantages of the extrusion process for the development of biodegradable materials are the speed and lower production costs (FLORES et al., 2010; LIU et al., 2009; MALI; GROSSMANN; YAMASHITA, 2010; RAQUÉZ et al., 2008; THUNWALL et al., 2008; ZHANG et al., 2001). Biodegradable materials produced with starch/PBAT blends by blow extrusion had excellent processability and mechanical properties (BRANDELERO; GROSSMANN; YAMASHITA, 2012; OLIVATO et al., 2012; WEI et al., 2015). The coextrusion process can be defined as the simultaneous extrusion of two or more materials emerging from a matrix. Multilayer coextrusion has been widely used in recent decades to combine the properties of two or more polymers in a single multiple-layer structure (GILE; WAGNER; MOUNT, 2005; GONZÁLEZ; IGARZABAL, 2013; YU; DEAN; LI, 2006; ZHANG et al., 2001).

Biodegradable materials produced with starch are fragile and sensitive to environmental conditions (MALI; GROSSMANN; YAMASHITA, 2010; SHIRAI et al., 2013; YAVUZ; BABAÇ, 2003; ZANELA et al., 2015). To overcome these drawbacks, several researchers have focused on the development of blends of starch with biodegradable polyesters, such as poly (butylene adipate-co-terephthalate) (PBAT) (BRANDELERO; YAMASHITA; GROSSMANN, 2010; FOURATI et al., 2018; MALI; GROSSMANN; YAMASHITA, 2010; OLIVATO et al., 2011; OLIVATO et al., 2012; OLIVATO et al., 2013; RAQUEZ et al., 2008; SANTOS et al., 2014; WEI et al., 2015; ZHANG et al., 2009). There are no studies on the coextrusion of these materials available in the literature.

Besides the protection barrier, new functions have been introduced to the packaging through the new technologies of active packaging. In contrast to conventional packaging, which must be inert, the active packaging is designed for interaction with their contents and the surrounding environment, aiming to increase shelf life beyond those obtained with conventional packaging. (MARCOS et al., 2014; REALINI; MARCOS, 2014; REIS et al., 2015; ZHANG et al., 2015)

Oxidation is one of the most common mechanisms of degradation in food products and can limit shelf life (PERAZZO et al., 2014). Packaging containing antioxidants can release active compounds with controlled rates to inhibit or reduce the oxidation process in a wide range of foods (BULOSO; LAGARON, 2015; CARRIZO et al., 2016; LACOSTE et al., 2005; REIS et al., 2015).

Biodegradable films with antioxidant action or antioxidant compounds are generally obtained using the technique of solvent evaporation (casting) or modification of the polymer surface composition (surface immobilization) (ADILAH et al., 2018; ADILAH; JAMILAH; NUNHANANI, 2018; FABRA et al., 2018; JARAMILLO et al., 2016 NOBILE et al., 2009; PERAZZO et al., 2014; REIS et al., 2015; SRIPATRAWAN; HART, 2010; WANG et al., 2013; YANG et al., 2016). From an industrial point of view, these methods do not have adequate processability. Therefore, the production of films with active properties using industrial processes, such as extrusion, would be ideal. However, few studies report the development of active biodegradable films by extrusion (CARDOSO et al., 2017; CARRIZO et al., 2015; MARTINS et al., 2018; WRONA et al., 2017).

The most popular antioxidants in food products are beta-butylated hydroxyanisole (BHA) and butylhydroxytoluene (BHT) because of their stability, and low cost, but there are concerns about their toxicological aspects (ADILAH et al., 2018; MOUDACHE et al., 2016; SIRIPATRAWAN; HARTE, 2010; SIRIPATRAWAN; NOIPHA, 2012).

Plant extracts, essential oils, phenolic compounds, flavonoids, terpenes, tocopherols, carotenoids, and some vitamins are natural antioxidants that were used in active packaging to improve lipid oxidation stability and to prolong the shelf life of food products (DICASTILLO et al., 2016; CHAN; LIM; CHEW, 2007; JONGJAREONRAK et al., 2008; PERAZZO et al., 2014; SIRIPATRAWAN; HARTE, 2010).

Green tea (*Camellia sinensis*) is a non-fermented tea product, and it has a potent antioxidant property as it contains about 36% of polyphenolic compounds (CABRERA; GIMÉNEZ; LÓPES, 2003; RUSAK et al., 2008; SIRIPATRAWAN; NOIPHA, 2012; SIRIPATRAWAN; HARTE, 2010). The major phenolic compounds in tea belong to the catechin family, also known as flavan-3-ols, which constitute up to 30% (w/w) of the tea solids, while various flavonols are also present (up to 4%). The phenolic compounds can scavenge reactive oxygen and nitrogen species. Thus, green tea has been used to prevent lipid oxidation in various food products (CARRIZO et al., 2015; CHAN; LIM; CHEW, 2007; FABRA et al., 2018; SIRIPATRAWAN; HARTE, 2010).

This study aimed to develop biodegradable multilayer films with an antioxidant activity using cassava starch, PBAT, and green tea leaves powder.

2 EXPERIMENTAL

2.1 MATERIAL

The biodegradable films were produced with cassava starch (Yoki, Brazil), poly (adipate co-terephthalate butyl) (PBAT) (Ecoflex®, BASF, Germany), glycerol, and citric acid, both technical grade from Dinamica (Brazil). Gallic acid and Folin-Ciocalteu (F–C) reagent were from Sigma Aldrich (USA), sodium carbonate from Anidrol (Brazil), and acetone P.A. from Synth (Brazil). The green tea dry leaves (*Camellia sinensis*) were purchased in the local market (Nutribom, Brazil).

2.2 PRODUCTION OF THE MULTILAYER BIODEGRADABLE FILMS

The green tea leaves powder (GT) was obtained by milling the dried leaves without the stems in a knife mill (IKA A11 Basic, Germany) and sieved (60 mesh sieve). The moisture content of the GT was around 12%.

The formulations used for pellet production and subsequent film production were determined by preliminary tests using different compositions of native cassava starch (ST), poly (butylene adipate-co-terephthalate) (PBAT), glycerol (GLY) as a plasticizer, green tea leaves powder (GT) as the active agent and citric acid (CA) as a compatibilizer. The maximum concentration of PBAT in the blends was 30 g 100 g^{-1} of

mixture to reduce the material cost. The GT content ranged from 2.5 to 5 g 100 g^{-1} of mixture, and the citric acid used as a compatibilizer was 0.03 g 100 g^{-1} of the mixture (Table 1).

Table 1 Pellet formulations

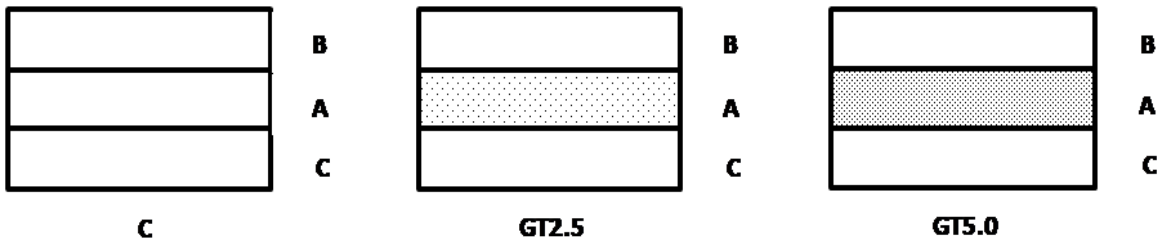
Formulation	ST*	GLY*	PBAT*	GT*	CA*
C	48.97	21.00	30.00	0.00	0.03
GT2.5	46.47	21.00	30.00	2.50	0.03
GT5.0	43.97	21.00	30.0	5.00	0.03

ST - starch, PBAT - poly (butylene adipate-co-terephthalate), GLY - glycerol, GT - green tea leaves powder, CA - citric acid
*g 100 g^{-1} of mixture

The ingredients (Table 1) were manually mixed and extruded under the conditions specified in Chapter 2 of this thesis.

As shown in Figure 2, the three formulations were arranged for the formation of a multilayer film. The films are called C for control film (three layers of control formulation), GT2.5, and GT5.0 for the films with GT2.5 and GT5.0 formulations.

Figure 1 - Layers in each formulation of the films produced



Source: the author.

The formulation containing the active compound (GT) was at the middle layer to prevent its exposure to the external ambient, improve its stability, and avoid direct contact with the product to be packaged and avoid sensorial changes to the product.

2.3 CHARACTERIZATION OF THE FILMS

2.3.1 Opacity and Color Analyses

The opacity was determined, according to Olivato *et al*. (2017), using a colorimeter (BYK Gardner, Germany) with illuminant D65 and a visual angle of 10°. Opacity was determined as the ratio of sample luminosity on the standard black (Yp) and the white standard (Yb) (0 - 100%) (Equation 1). The data were expressed as CIELab L* a* b* values, and the analyses were performed in triplicate

$$Y = \left(\frac{Yb}{Yw}\right) \times 100 \quad (1)$$

The color difference was calculated according to Equation 2, and the analyses were performed in triplicate.

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

Where: ΔL*, Δa*, and Δb* are the differences between the sample measured under the white standard and the white standard alone.

2.3.2 Thickness, Density, and Grammage

The film's thickness was measured with a micrometer (± 0.001 mm) (Digimess, Brazil) at fifteen different points. Samples of 20 mm x 20 mm were cut and were kept in a desiccator with anhydrous calcium chloride (0% RH) for 7 days at 0% RH. Then, the samples were weighed, measured (length, width, and thickness), and the density and grammage were calculated in g cm^{-3} and g m^{-2}, respectively. The analyses were performed in triplicate according to the procedure described by Muller *et al*. (2011).

2.3.3 Mechanical Properties

2.3.3.1 Tensile test

The tensile strength (MPa), elongation at break (%) and Young's modulus (MPa) of the materials were determined according to ASTM method D882-02 (2002a) with some modifications using a texture analyzer (model TA.XT2i, Stable Micro Systems, England) fitted with a 50-kg load cell. Ten specimens of each formulation were cut in the longitudinal direction (50 mm x 20 mm), and they were conditioned at 23 ± 2 °C

and 53 ± 2% RH for at least 7 days. The crosshead speed was set at 0.8 mm/s, and the initial distance between the grips was 30 mm.

2.3.3.2 Slow rate penetration resistance (SRPR)

The slow rate penetration resistance (SRPR) of the films was performed according to F1306-90 (98) (2002b) standard method, with modifications, using the same equipment used for tensile, with a 3.2 mm diameter hemispherical probe. Ten specimens of each formulation were cut in squares (30 mm x30 mm), and they were conditioned at 23 ± 2 °C and 53 ± 2% RH for at least 7 days. The force and the elongation to perforation were determined at 25 mm/min (velocity of the probe).

2.3.4 Water Vapor Permeability (WVP)

Water vapor permeability was determined gravimetrically according to the ASTM E96 00 (2009) standard, with some modifications. The measurements were performed in triplicate using a relative humidity gradient of 33 - 64%. This gradient was chosen because the standard covers relative humidity that the food does not reach. Before analysis, the samples were conditioned at 23 ± 2 °C and 53 ± 2% RH for at least 7 days.

2.3.5 Solubilization capacity in water (SCW)

The analysis of solubilization capacity in water (SCW) was performed according to Reis *et al*. (2018). The samples of each formulation were conditioned for 7 days in a desiccator containing anhydrous $CaCl_2$ (0% RH), and then they were weighed (M_i), immersed in distilled water (30:1 water/sample w/w) for 48 h at 25 °C, dried at 105 °C for 4 h. The weight of the specimen after drying (M_f) was used to calculate the mass solubilized in water (SCW); according to Equation 3, and the analysis was performed in triplicate.

$$SCW(\%) = \left[\frac{Mi - Mf}{Mi}\right] \times 100 \quad (3)$$

2.3.6 Scanning Electron Microscopy (SEM)

Scanning electron micrographs were recorded using a scanning electron microscope (FEI Quanta 200, USA). The films were fractured in liquid nitrogen, attached to aluminum supports, and coated with gold (BAL-TEC SCD 050 sputter coater, Leica Microsystems, Germany) (40-50 nm in thickness) at 25 °C and a pressure of 2.105 Torr for 180 seconds. Before coating with a gold layer, the samples were conditioned for 7 days in a desiccator containing anhydrous $CaCl_2$ (0% RH). The surface and the fracture surface of the films were analyzed at a magnification of 200x and 800x.

2.3.7 Thermogravimetric analysis (TGA)

The films' thermal stability was determined using a thermogravimetric analyzer (TGA-50, Shimadzu, Japan) with a detector TA-60WS. The samples were conditioned over anhydrous $CaCl_2$ (0% RH) for 7 days and then analyzed. Approximately 5 mg of sample was scanned from 25 °C to 600 °C with a 10 °Cmin^{-1} heating rate under a nitrogen atmosphere (20 mLmin^{-1}). The thermal stability of the films was evaluated from the curves of TG and DTG.

2.3.9 X-ray diffraction (XRD)

X-ray patterns of the samples were obtained using a diffractometer XPert PRO (Panalytical, Philips, Netherlands) using copper Kα radiation (λ=1.5406 Å) operating at room temperature, 20 mA, and 30 kV. The specimens were ground and conditioned in a desiccator under 0% RH for 7 days at 25 °C. The scanned 2θ region ranged from 2.0° to 60.0° with 0.05 ° step and 0.05°/s speed. The relative crystallinity index (RCI) was calculated as the ratio between the crystalline regions (CR) area and the sum of CR and amorphous regions (AR) area, according to Equation 4 (adapted from Müller et al. (2011)).

$$RCI = \frac{CR}{(CR+AR)} \quad (4)$$

2.3.10 Fourier Transform Infrared Spectroscopy (FTIR)

The specimens were ground and conditioned over anhydrous $CaCl_2$ (0% RH) for 7 days in a desiccator at 25 °C and compressed into tablets with potassium bromide. The analyses were performed with a Fourier transform infrared spectrophotometer (FT-IR) (IR Prestige-21, Shimadzu, Japan), with a spectral range of 4000 - 400 cm^{-1} and a spectral resolution of 2 cm.

2.3.11 Total Polyphenol Content

Total polyphenol content (TPC) of the films and the green tea powder (GT) were determined by the Folin-Ciocalteau colorimetric method adapted from Kumazawa; Hamasaka; Nakayama (2004). The TPC analysis was performed only for the films containing green tea powder (GT2.5 and GT5.0).

The extraction of the phenolic compounds from the films and the GT were performed using aqueous acetone (70% acetone and 30% distilled water v/v) as a solvent. The film (0.25 g) was infused in 10 mL of solvent (1:40), the green tea powder (0.05 g) in 200 mL of solvent, and they were shaken at 150 rpm for one hour and then filtered. The extraction conditions were determined through preliminary tests, where the most efficient one was chosen (data not shown).

The extraction solution (0.5 mL) was mixed with 0.5 mL of Folin-Ciocalteau reagent (Sigma Aldrich, USA) and 0.5 mL of 10% $NaCO_3$ solution and stored at room temperature for 1 h. The absorbance of the solutions was measured at 765 nm using a spectrophotometer Libra S22 (Biochrom, United Kingdom). TPC was expressed as mg/g of gallic acid equivalent. The TPC analysis was performed in triplicate.

After quantifying the TPC, the retention of the phenolic compounds in the active layer (%TPC) after the extrusion process was calculated by Equation 5.

$$\%\,TPC = \left(\frac{TPC_{film}}{TPC_{GT}}\right) x\ 100 \qquad (5)$$

Where: TPC_{film} is the total polyphenolics content in the film, resulting from the active layer, TPC_{GT} is the total polyphenolics content in the green tea powder used to produce the film.

2.3.12 Statistical Analysis

The data were analyzed using STATISTICA 10.0 software (StatSoft, USA), with analysis of variance (ANOVA) and Tukey's test at a 5% significance.

3 RESULTS AND DISCUSSION

3.1 FILMS PRODUCTION

All the films produced were flexible and easy to handle, and they can be observed in Figure 2.

Figure 2 – Multilayer biodegradable films

C - control film; GT2.5 - film with 2.5 g GT 100 g^{-1} film; GT5.0 - film with 5 g^{-1} GT 100 g^{-1} film
Source: the author.

The samples had good processability during the extrusion, and the different concentrations of GT did not interfere in the processability. The thickness ranged from 150 to 283 μm for all the formulations (C, GT.2.5, and GT5.0) films, and this wide range was due to the higher expansion of the balloon for more plasticized materials; consequently, the film has a smaller thickness.

3.2 OPACITY AND COLOR ANALYSIS

The multilayer biodegradable films' opacity and color parameters were determined, and the results are in Table 2.

Table 2–Opacity and color parameters of the multilayer biodegradable films

Samples	Opacity (%)	b*	ΔE*
C	64 ±1.1[a]	8.55 ±0.48[c]	8.17 ±0.70[c]
GT2.5	58 ±0.2[a]	24.70 ±0.63[b]	24.55 ±0.69[b]
GT5.0	61 ±6.7[a]	30.13±4.10[a]	31.50 ±5.39[a]

C - control film; GT2.5 - film with 2.5 g GT 100 g^{-1} film; GT5.0 - film with 5 g^{-1} GT 100 g^{-1} film
Results expressed as mean ± standard deviation.
[a,b,c] Different letters in the same column indicate significant differences (Tukey test $p \leq 0.05$).

The films were opaque, from 58 to 64% of opacity, and the GT content did not influence the opacity because they did not present significant differences ($p < 0.05$). Opacity was already expected for these materials, and extruded starch / PBAT films are generally opaque due to apparent crystallization due to processing.

The addition of green tea powder (GT) in different concentrations changed the films' color parameters compared to the control film (Table 2).

The films containing GT were yellowish than the control films(C) (higher b* parameter), and the GT5.0 films were the yellower (b*=30.13). The red-green color coordinate (a *) were not significant and ranged from -0.33 to -1.64.

The compounds present in the green tea powder (GT) influenced the color parameters of the films. The parameters a* and b* of the GT were 0.72 ± 0.08 and 17.49 ± 0.20, respectively.

Green tea refers substantially to poorly fermented tea leaves (CABRERA; GIMÉNEZ; LOPÉZ, 2003). This fermentation process refers to the hydrolytic and oxidative process that tea undergoes when specific endogenous enzymes and substrates are assembled, for example, by mechanical disruption of the cells by maceration of the leaves. During this process, colorless catechins in the leaves are converted into a complex mixture of yellow and orange to dark brown (POU, 2016). Therefore, the longer the fermentation time of the leaves of *Camellia sinensis*, the higher the color parameters' modification, ranging from green to brown, which is characteristic of black tea, for example.

The ΔE* of the films ranged from 8.17 to 31.5 (Table 1), and the higher the GT content, the higher the ΔE*. As previously discussed, the GT changes the films' color parameters and, consequently, the ΔE* values.

3.3 DENSITY AND GRAMMAGE

The density and grammage of the multilayer biodegradable films are shown in Table 3.

Table 3 – Density and grammage of the multilayer biodegradable films

Sample	Density ($g\ cm^{-3}$)	Grammage ($g\ m^{-2}$)
C	$1.2 \pm 0.1^{a,b}$	239 ± 39^{b}
GT2.5	1.1 ± 0.1^{b}	246 ± 54^{b}
GT5.0	1.3 ± 0.08^{a}	367 ± 42^{a}

C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g
Results expressed as mean ± standard deviation.
[a,b] Different letters in the same column indicate significant differences (Tukey test $p \leq 0.05$).

The grammage of the biodegradable multilayer films produced varied from 239 to 367 g m^{-2}, and the films with higher GT content (GT5.0) presented the higher values. The density had a small variation among the formulations, from 1.1 to 1.3 g m^{-3}, and the GT5.0 films' density was higher than the GT2.5 ones, but they did not differ from the control film.

The significant differences in grammage presented by the film sample with the highest GT content (GT5.0) can be justified by the significant difference in these samples' thicknesses since the calculus of these parameters are thickness dependent.

The higher the density and grammage of the films, the higher the amount of material used to produce them, increasing the material cost. Kormin et al. (2017) produce injected materials of LDPE (density of 0.92 g cm^{-3}) with two different starch sources and evaluated those materials' densities. According to the authors, the density of materials increased with increasing the starch ratio in the blend, probably due to the weak bonding between the starch and LDPE, because the thermoplastic starch is hydrophilic, and the LDPE is hydrophobic, and the same behavior can be suggested for films with PBAT and starch.

Brandelero *et al.* (2012) produced films by blown extrusion, and the control formulation had a similar composition with this study (starch/PBAT 65/35 w/w). The average thickness was 200 µm, and the average density of 1.34 g cm^{-3}.

3.4 MECHANICAL PROPERTIES

3.4.1 Tensile Testing and Slow Rate Penetration Resistance (SRPR)

The tensile strength of the films ranged from 4.8 to 4.9 MPa (Table 4), with no significant differences ($p < 0.05$) between them, i.e., the addition of GT did not impair the tensile strength of the films. These results are auspicious because the addition of new compounds to the formulation usually impair such property.

Table 4– Tensile testing and slow rate penetration resistance of the multilayer biodegradable films

Sample	σ (MPa)	ε (%)	MY (MPa)	FP (N)	EP (mm)
C	4.9 ±0.2[a]	318 ±109[b]	36.8 ±5.3[a]	13.4 ±1.7[a]	3.2 ±0.3[b]
GT2.5	4.8 ±0.2[a]	482 ±131[a]	26.3 ±2.1[b]	13.7 ±3.9[a]	3.5 ±0.4[a,b]
GT5.0	4.9 ±0.4[a]	465 ±123[a]	23.2 ±2.1[b]	14.4 ±1.5[a]	3.9 ±0.2[a]

C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g
σ: tensile strength; ε: elongation at break; MY: Young's modulus
FP: force to perforation; EP: elongation to perforation
Results expressed as mean (± standard deviation).
[a,b] Different letters in the same column indicate significant differences (Tukey test $p \leq 0.05$).

The impact of adding new components to a film formulation can impair the mechanical properties of the material, so the milling and sieving of green tea have been performed so that it has a starch-like grain size.

The elongation was higher for the materials containing the formulation GT in the multilayer film intermediate layer (GT.2.5 and GT5.0) (Table 4). The Young's modulus of the control film was significantly higher than the other films, i.e., the control film was more rigid because the tensile strength was similar.

The addition of GT in one of the films' layers improved the elongation at break without reducing the tensile strength because of a possible additional plasticizer action.

Even though the matrix of biodegradable multilayer films is at least 95% the same, and the GT did not have reactive characteristics to change the blend's behavior, different materials' behavior was visually observed during the analysis. The films containing GT and the intermediate layer ruptured, unlike the multilayer film that

contained only the control formulation, it was possible to perceive the formation of threads, like a gum, that did not happen with the C films that broke dryly.

Brandelero, Grossmann, Yamashita (2011) produced biodegradable films by blown extrusion with blends of thermoplastic starch and PBAT, with similar proportions used in this work, and had tensile strength around 4 MPa and elongation at break around 50%. Olivato et al. (2011) had values of 6 MPa for tensile strength, elongation of 150%, and MY around 55 MPa for films with blends of thermoplastic starch and PBAT (55/45 w/w) produced by blown extrusion.

The films' perforation force ranged from 13.4 to 14.4 N, and the films had no significant differences between them.

The films with higher GT content had the highest flexibility, and they stretched around 3.9 mm, and the films C had the lowest elongation, around 3.2 mm. The films with an average concentration of GT (GT2.5) did not differ significantly from the others.

3.5 Water Vapor Permeability (WVP) and Solubilization Capacity in Water (SCW)

The WVP of the films ranged from 4.2 to 4.5 x 10^{-6} g m^{-1} Pa^{-1} d^{-1} under 33-64% RH gradient (Table 5), and there were no significant differences between them. Thus, the GT did not influence the WVP of the films. It probably did not influence PVA changes because of the change in formulation, and GT addition happened only for the intermediate layer of the multilayer biodegradable film.

Table 5 – Water vapor permeability (WVP) and solubilization capacity in water (SCW) of multilayer biodegradable films

Sample	***WVP(x10^6)** (g m^{-1} Pa^{-1} d^{-1})	**SCW** (%)
C	4.5 ±0.2[a]	0.7 ± 0.1[a]
GT2.5	4.2 ±0.1[a]	0.6 ± 0.1[a]
GT5.0	4.2 ±0.1[a]	0.6 ±0.1[a]

C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g
*Results expressed as mean (± standard deviation) x $10^{-6}$for WVP
[a,b] Different letters in the same column indicate significant differences (Tukey test $p \leq 0.05$).

Brandelero, Grossmann, and Yamashita (2011) reported WVP from 0.70 to $7.35x10^{-7}$ m^{-1} Pa^{-1} d^{-1} and Muller, Yamashita, and Laurindo (2008) 8.2 10^{-6} g m^{-1} Pa^{-1} d^{-1} for films formulated with PBAT and starch in proportions similar to the present work.

The SCW ranged from 0.6 to 0.7%, and there were no significant differences ($p<0.05$) between them (Table 5), despite the differences between the thickness of the control film and the films with GT. These materials could be considered relatively poorly soluble in water, and that it is an attractive property of these materials since starch-based materials are highly hygroscopic (MALI et al., 2005).

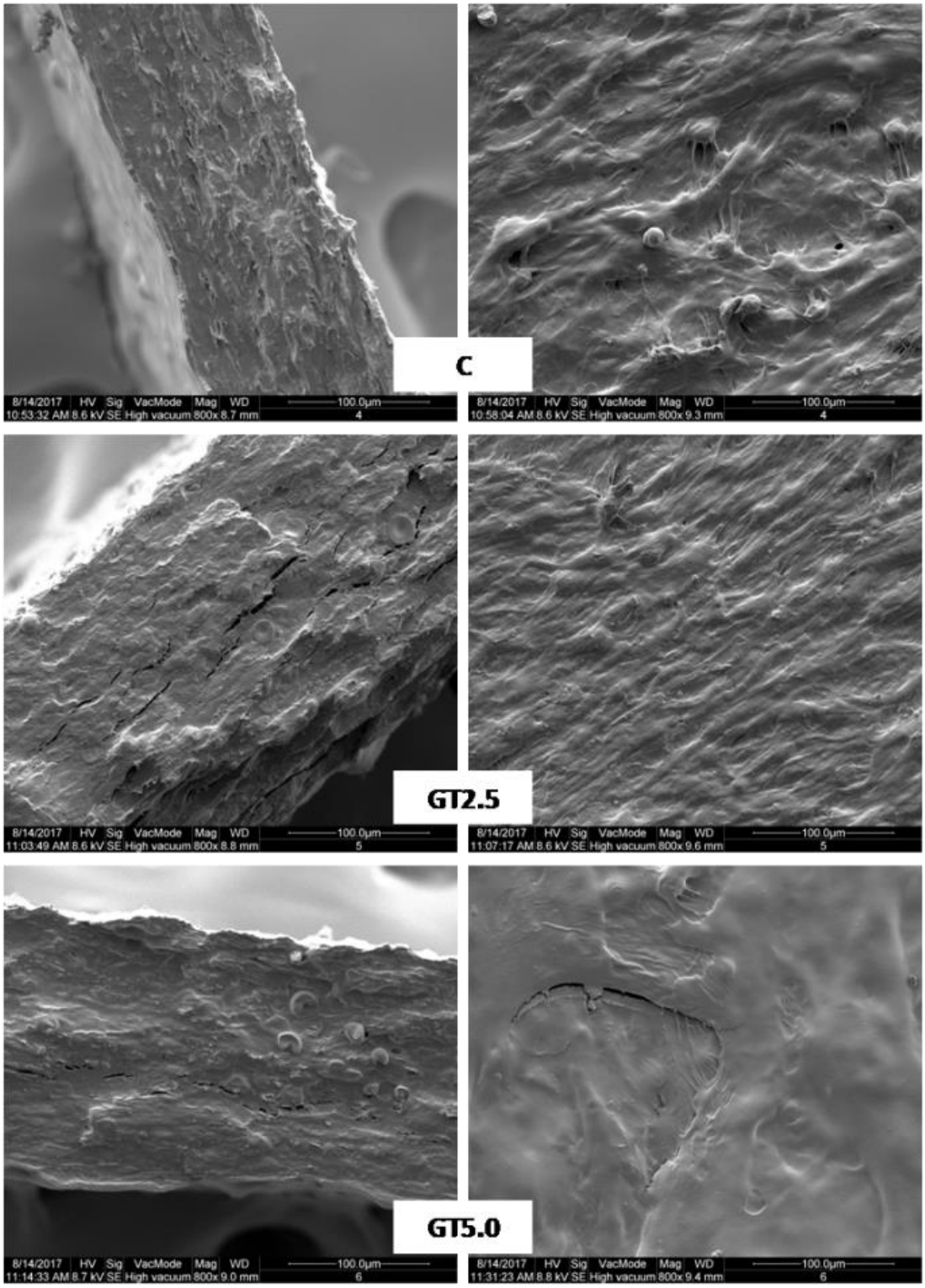
3.7 SCANNING ELECTRON MICROSCOPY (SEM)

SEM micrographs of the fractures (800× magnification) and surfaces (800× magnification) of the films are shown in Fig. 3.

The images of the films' fracture show a cohesive structure, without discernible granules of starch, cracks, voids, or even other substances present in the GT, and there was no visible phase separation between the three layers.

The films' microstructural characteristics showed that processing conditions were satisfactory, with proper incorporation of the GT into the polymeric matrix.

Figure 3 – SEM images of the cross-section (left) and surface (right) of the multilayer biodegradable films.

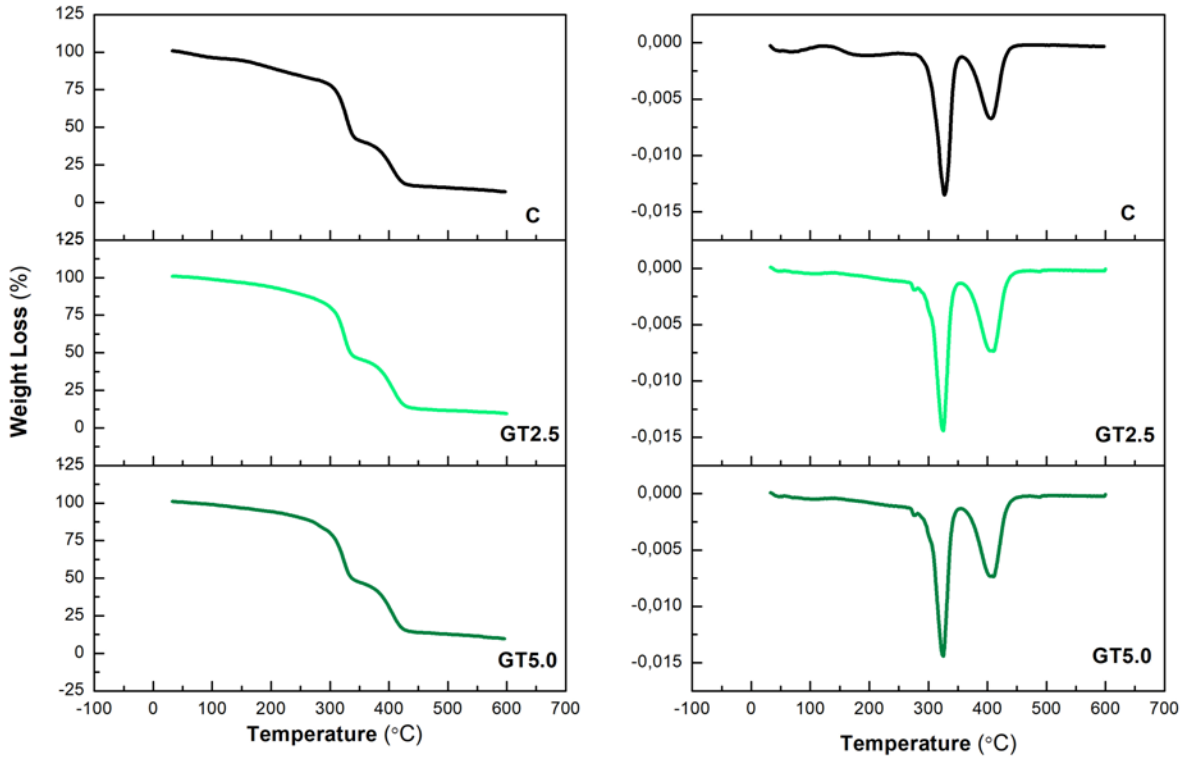


C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g
Source: the author, 2018.

3.8 THERMOGRAVIMETRIC ANALYSIS (TGA)

The thermograms (TG and dTG) of the multilayer biodegradable films are presented in Figure 4, and all the films showed three weight loss peaks at the dTG thermogram.

Figure 4 – Thermogravimetric analysis of the multilayer biodegradable films.



C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g

The degradation temperatures and the residual weight of the samples are in Table 6. The first degradation step of the film C was related to the loss of water, a peak that was more subtle in the other samples (Figure 4 and Table 6). The second step of thermal degradation (275 and 280 °C) for both films containing GT (GT2.5 and GT5.0) was attributed to the volatilization of glycerol, a peak that was not identified in the control film. For all the films, the second step (~320 °C) was attributed to the thermal degradation of starch, and the peak at around 405 °C was related to the thermal degradation of PBAT (OLIVATO *et al*., 2015).

Table 6–Degradation temperatures for the films obtained by TGA analysis

Sample	Degradation temperature (°C)						Residue Weight (%)
	Water	Glycerol	Amylose	Starch	Amylopectin	PBAT	
*Neat PBAT	-	-	-	-	-	439	-
*Neat TPS	174	282	314	-	351	-	-
*80/20	-	-	-	353.1	-	435.2	-
C	177	-	-	327	-	405	9.8
GT2.5	-	275	-	325	-	405	11.9
GT5.0	-	284	-	322	-	407	12.4

*Data from OLIVATO et al. (2015) for neat PBAT, neat TPS and TPS/PBAT films (80/20 w/w).
C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g

Film C had 9.8% of residues, and films GT2.5 and GT5.0 around 11.9 and 12.4%, respectively, because of the higher mineral content in GT.

According to the TGA, green tea powder's addition did not change the materials' thermal stability due to possible interactions occurring between the components of the mixture during the extrusion processes.

3.10 X-RAY DIFFRACTION (XRD)

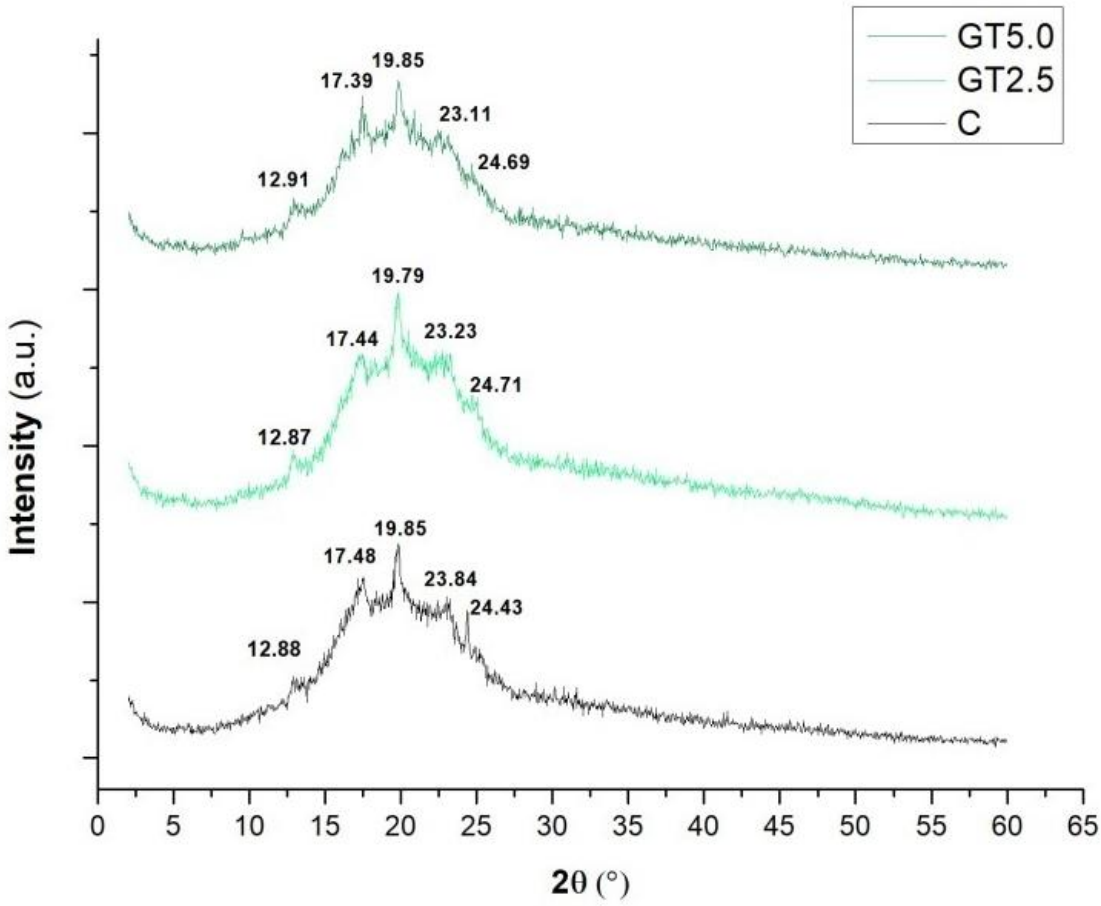
Figure 5 shows the diffractogram of the films C, GT2.5, and GT5.0, and all the films presented similar XDR patterns.

All the films presented crystallinity peaks at 2θ = 12.88°, 17.48°, 19.85°, 23.84°, and 24.43°. The peaks at 2θ = 13.1° and 19.8° were due to the crystallization of the starch induced by processing (type V_H) (OLIVATO *et al*. 2013; OLIVATO *et al*., 2015; RAQUEZ *et al*., 2008), and the peaks at 2θ = 17.6° and 23.2° were related to the PBAT crystallinity in thermoplastic starch/PBAT blends (RAQUEZ *et al*., 2008; OLIVATO *et al*., 2013).

The samples' relative crystallinity indexes were 6.91, 6.86, and 5.40 % for the films C, GT2.5, and GT5, respectively.

GT's addition decreased the crystallinity index of the materials as the ratio increased; consequently, the film with higher GT content presented a lower value of the index (GT5.0). The films' crystallinity probably was due to the starch and PBAT contents that were in higher concentration.

Figure 5 – XRD of the biodegradable multilayer films



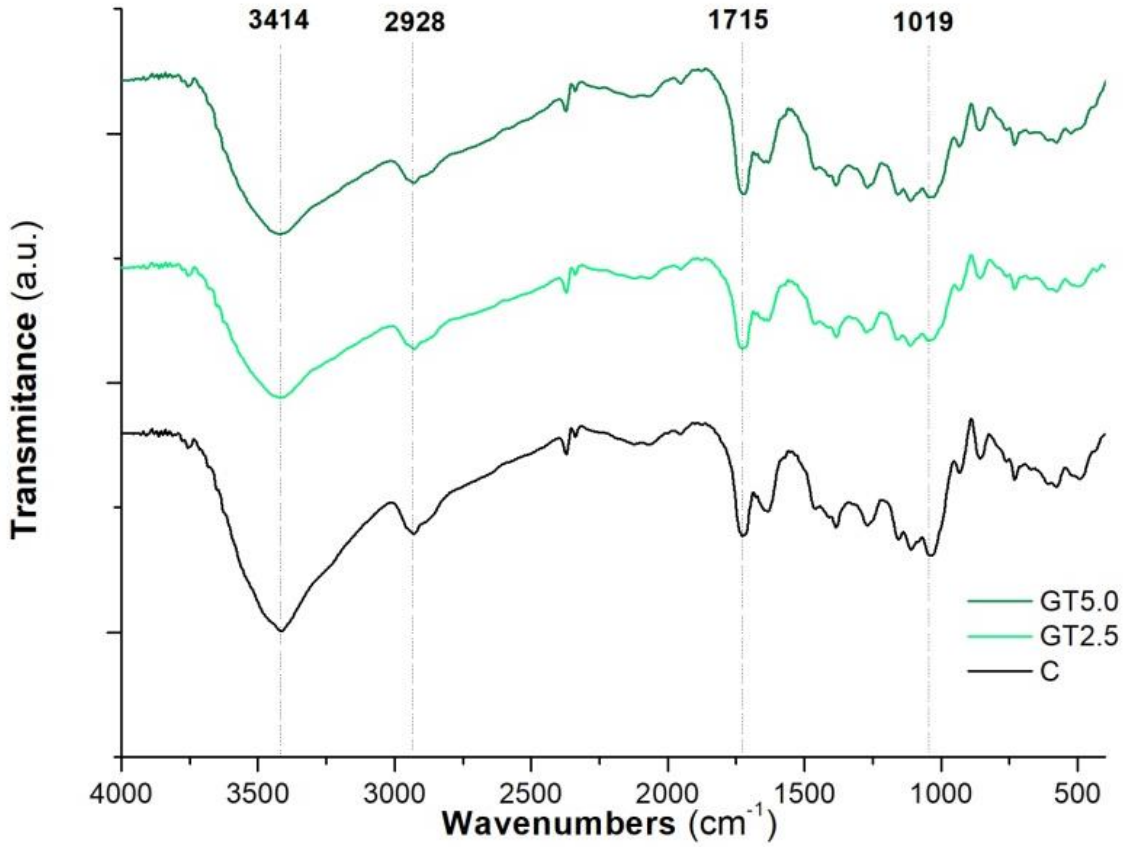
C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g
Source: the author, 2018.

3.11 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The FTIR spectra of the control film and those incorporated with 2.5 and 5% of GT are shown in Figure 6, and the spectra of the three films (C, GT2.5, and GT5.0) showed similar behavior.

The peaks between 1019 and 1015 cm^{-1} were attributed to the stretching vibration of C-O in C-O-C bonds, mostly present in the glycosidic linkages, and the peaks between 3300 and 3400 cm^{-1} were due to hydroxyl groups, which are present in large numbers in the starch chains (BRANDELERO, GROSSMANN, YAMASHITA, 2011).

Figure 6– FTIR spectra of multilayer biodegradable films



C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g
Source: the author, 2018.

Peaks around 1712 cm^{-1} were due to C=O from the ester of PBAT structure, and from the esterification (monoester or cross-linking) since all formulations had citric acid in their composition, used as a compatibilizer.

Other researchers reported similar FTIR spectra for similar biodegradable materials (BRANDELERO, GROSSMANN, YAMASHITA, 2012; BRANDELERO, GROSSMANN, YAMASHITA, 2011; OLIVATO *et al*. 2011).

Films containing GT (GT2.5 and GT5.0) did not show peaks specifically related to the green tea compounds.

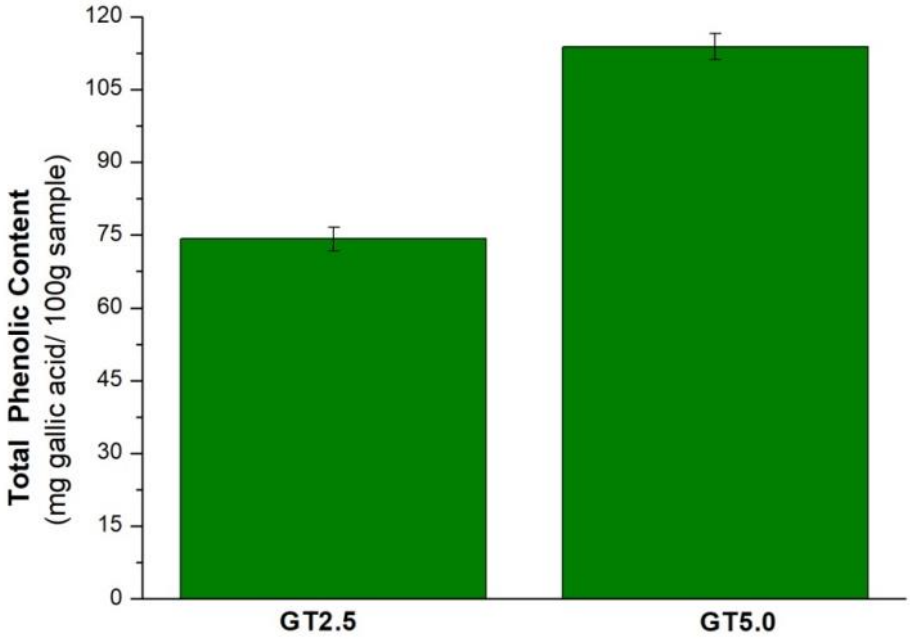
3.12 TOTAL POLYPHENOL CONTENT OF THE FILMS

The green tea leaf powder (GT) used to produce GT2.5 and GT5.0 films had a total polyphenol content (TPC) of 12.500 mg of gallic acid equivalent/100 g of GT. Chan, Lim, and Chew (2007) reported 7.280 and 5.836 mg/100 g for young and mature

green tea leaves, respectively. The difference with our data was probably due to differences in cultivation, climate, and tea variety.

The TCP in GT2.5 and GT5.0 films were 76 (±2) and 113(±2) mg/100 g of the films, respectively (Figure 7). Films made with chitosan, glycerol, and green tea extract produced by casting method had TCP around 600 mg/100 g with 2% GT and 1000 mg/100 g with 5% GT (SIRIPATRAWAN; HARTE, 2010). The higher quantity of TPC was probably because of the production process (*casting*), which is milder than extrusion, besides the different phenolic quantification conditions that can contribute significantly to the difference of these results. Also, they used GT extract, which contributed to the higher availability of phenolic compounds or the method of extraction.

Figure 7– Total polyphenolic content of biodegradable multilayer films incorporated with GT



GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g

The active layer of GT2.5 multilayer biodegradable film had % $TPC_{retention}$ of 74%, and the retention for the active layer of the GT5.0 films was 54%, due to the phenolic compound losses during the extrusion processes, since the formulations were exposed to high temperatures and pressures.

Some studies reported that the extraction of catechins or theaflavins from teas is higher with stirring, a longer infusion time, and higher water temperature, but the use

of higher temperature infusion can also lead to the loss of labile polyphenolic components after standing for 10–15 min (KILMARTIN, HSU, 2003).

Although the phenolic compounds were not stable at high temperature, these components had good retention in the matrix of the films, mainly in GT2.5 films (74%), probably due to the protective action of the polymeric matrix, once GT was only at the middle layer of the three-layer biodegradable films, and the short residence-time at the extruder, despite the extreme processing conditions,

These materials these materials probably have antioxidant activity and can be used as active packaging for food. It is important to emphasize that this active property was achieved using only ground green tea leaves and incorporated into the polymer matrix, i.e., it was not necessary to produce liquid or solid extracts.

4 CONCLUSIONS

The multilayer biodegradable films produced by blown extrusion using cassava starch, PBAT, and green tea leaves powder (GT) had antioxidant activity. They are an alternative for conventional plastic films because of their excellent processability, good mechanical and barrier properties for specific uses.

The multilayer biodegradable films can be used as active packaging for food products because of the active middle layer containing antioxidant agent (GT). The middle layer is protected from the environment and does not have direct contact with the product.

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CAPÍTULO 4

MULTILAYERS BIODEGRADABLE FILMS CONTAINING GREEN TEA POWDER (*Camellia sinensis*) AS ACTIVE PACKAGING FOR ALMOND (*Prunus dulcis*) FLOUR

1 INTRODUCTION

The regular consumption of almonds nuts (*Prunus dulcis*) brings many health benefits (MEXIS; BADEKA KONTOMINAS, 2009; SALCEDO; NAZARENO, 2015). Almonds are consumed whole or peeled, raw or roasted, whole or ground, or as an ingredient in many food products, such as bakery and confectionery, and as a flavoring agent in beverages and ice cream (MEXIS et al., 2009; MEXIS; BADEKA; KONTOMINAS, 2009).

Almonds are known as a lipid source (48-67%), and the main fatty acid is oleic, representing 50-70% of the total fatty acid content (GALLIER; GORDON; SINGH, 2012; RABADÁN et al., 2017). Linoleic, palmitic, and stearic acids are present at levels of 10 to 26%, 5 to 9%, and 1.5 to 4%, respectively (RABADÁN et al., 2017; OZCAN et al., 2011). The species *Prunus dulcis* was evaluated by Salcedo, Mishima, and Nazareno (2010), which obtained around 57% of lipid content.

Lipid oxidation is the leading cause of unpleasant taste development in almonds due to its high content of unsaturated fatty acids and the presence of riboflavin, which acts as a photosensitizer in photooxidation (RAISI et al., 2015; VENKATACHALAM; SATHE, 2006). Oxygen concentration is one of the most important extrinsic factors affecting the lipid oxidation of nuts; oxidation can be enhanced by light exposure (photo-oxidation) and high storage temperatures (MEXIS; BADEKA KONTOMINAS, 2009).

These nuts are also rich in antioxidant substances such as polyphenols, mainly concentrated in brown skins (BS) or seed husks (SALCEDO; NAZARENO, 2015). The BS that surrounds *Prunus dulcis* almonds has phenolic compounds that play the role of natural antioxidants. According to Salcedo et al. (2010), the total phenolic compounds in almonds are 1.4 µg gallic acid/mg nut in different solvents (methanol and water), and in the brown shells approximately 7.9 µg gallic acid/mg nut, when the solvent used was methanol and 12 µg gallic acid/mg walnut in water.

Almond flour is a food product highly susceptible to oxidation due to the high content of polyunsaturated lipids, more than the whole almond, requiring the use of

appropriate packaging to maintain the product's sensory and nutritional characteristics.

Several studies reported new packaging systems using biodegradable matrices, like starch and natural additives, and they are so-called active biodegradable packaging. (JU; SONG, 2019; MARSH; BUGUSU, 2007; NERÍN; TOVAR; SALAFRANCA, 2008.). Starch-based films have been extensively studied recently, but their physical properties have yet to be improved (JU; SONG, 2019; MALI et al., 2004; OLIVATO et al., 2015; ROMPOTHI et al., 2017; YOUSSEF; EL-SAYED, 2018).

Among the active packaging, those containing antioxidants are considered very important for the industry. Oxidation is one of the most critical degradation reactions in foods, limiting shelf lives (FENG et al., 2018; NERÍN; TOVAR; SALAFRANCA, 2008). These packaging may contain natural or synthetic antioxidants for this purpose (VEIGA-SANTOS et al., 2018). However, due to consumer concerns about the safety of synthetic antioxidants, the use of natural antioxidants derived from plant extracts is highlighted (CERRUTI et al., 2009; FENG et al., 2018; JU; SONG, 2019; NAVIKAITE-SNIPAITIENE et al., 2018; OUDJEDI et al., 2019).

Tea leaves (*Camellia sinensis* L.) contain polyphenols, alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluorides, minerals and trace elements, and other undefined compounds (CABRERA; GIMÉNEZ; LÓPES 2003; DAS et al., 2019). The main phenolic compounds of tea belonging to the catechin family, also known as flavan-3-ols, make up to 30% of tea solids by weight, while various flavonols are also present (up to 4%) (KILMARTIN; HSU, 2003).

Green tea was included in the formulation of some products to increase the overall antioxidant activity or technological purposes (LORENZO; MUNEKATA, 2016; RUSAK et al., 2008).

According to the literature, the phenolic compounds in green tea extract act as an antioxidant through the prevention of radical chain initiation, binding of transition metal ion catalysts, and interaction with free radicals to inhibit lipid oxidation (FARHOOSH et al., 2007; PERUMALLA; HETTIARACHCHY, 2011; SIRIPATRAWAN; NOIPHA, 2012; SABAGHI et al., 2015). The attachment of hydroxide groups to catechin molecules is probably the main factor that makes them potent antioxidants in oils compared to antioxidants such as BHT, BHA, and tocopherol (GRAMZA et al., 2006; WANASUNDARA; SHADINI, 1996).

This work's objective was to develop a multilayered biodegradable packaging

with active properties containing green tea and use it as packaging for almond flour to evaluate the change in product quality in this type of package during storage.

2. EXPERIMENTAL

2.1 MATERIAL

The biodegradable films were produced with cassava starch (Yoki, Brazil), poly (butylene adipate-co-terephthalate) (PBAT) (Ecoflex®, BASF, Germany), glycerol, and citric acid, both technical grade from Dinamica (Brazil). Gallic acid and Folin-Ciocalteu (F–C) reagent were from Sigma Aldrich (USA), sodium carbonate from Anidrol (Brazil), and acetone P.A. from Synth (Brazil). The green tea dry leaves (*Camellia sinensis L*.) and almonds used for flour production, was purchased in local commerce (Nutribom, Brazil).

2.2 PRODUCTION OF MULTILAYER BIODEGRADABLE FILMS.

The films were produced according to Item 2.2 (Chapter 3 - Production of Multilayer Biodegradable Films). The three formulations previously described were used as flour packaging, and the green tea powder (GT) was added to provide antioxidant properties to the films.

2.2.1 Characterization of the Films

The films produced were characterized by their mechanical properties, water vapor permeability, solubilization capacity in water, opacity, and content of phenolic compounds.

2.2.1.1 Tensile testing

The tensile strength (MPa), elongation at break (%) and Young's modulus (MPa) of the materials were determined according to ASTM method D882-02 (2002a) with some modifications using a texture analyzer (model TA.XT2i, Stable Micro Systems, England) fitted with a 50-kg load cell. Ten specimens of each formulation were cut in

the longitudinal direction (50 mm x 20 mm), and they were conditioned at 23 ± 2 °C and 53 ± 2% RH for at least 7 days. The crosshead speed was set at 0.8 mm/s, and the initial distance between the grips was 30 mm.

2.2.1.2 Water Vapor Permeability (WVP)

Water vapor permeability was determined gravimetrically according to the ASTM E96 00 (2009) standard, with some modifications. The measurements were performed in triplicate using a relative humidity gradient of 33 - 64%. Before analysis, the samples were conditioned at 23 ± 2 °C and 53 ± 2% RH for at least 7 days.

2.2.1.3 Solubilization capacity in water (SCW)

The analysis of solubilization capacity in water (SCW) was performed according to Reis *et al*. (2018). The samples of each formulation were conditioned for 7 days in a desiccator containing anhydrous $CaCl_2$ (0% RH), and then they were weighed (M_i), immersed in distilled water (30:1 water/sample w/w) for 48 h at 25 °C, dried at 105 °C for 4 h. The weight of the specimen after drying (M_f) was used to calculate the mass solubilized in water (SCW); according to Equation 3, the analysis was performed in triplicate.

$$SCW(\%) = \left[\frac{Mi - Mf}{Mi}\right] \times 100 \quad (3)$$

2.2.1.4 Opacity

The opacity of the films was determined according to Olivato *et al*. (2017) using a colorimeter (BYK Gardner, Germany) with illuminant D65 and a visual angle of 10 °. The opacity (Y) was determined as the ratio of the luminosity of the sample on the black (Y_p) and white (Y_b) standards (Equation 1). The analyses were performed in triplicate.

$$Y = \left(\frac{Y_b}{Y_w}\right) \times 100 \quad (1)$$

2.2.1. 5 Total Polyphenol Content of the Films and Active Layer % Retention

Total polyphenol content (TPC) of the films was determined by the Folin-Ciocalteau colorimetric method adapted from Kumazawa; Hamasaka; Nakayama (2004). The TPC analysis was performed only for the films containing green tea powder (GT2.5 and GT5.0).

The extraction of the phenolic compounds from the films and the GT were performed using aqueous acetone (70% acetone and 30% distilled water v/v) as a solvent. The film (0.25 g) was infused in 10 mL of solvent (1:40), and they were shaken at 150 rpm for one hour and then filtered.

The extraction solution (0.5 mL) was mixed with 0.5 mL of Folin-Ciocalteau reagent (Kanto Chemicals, Tokyo, Japan) and 0.5 mL of 10% $NaCO_3$ solution and stored at room temperature for 1 h. The absorbance of the solutions was measured at 765 nm using a spectrophotometer Libra S22 (Biochrom, United Kingdom). TPC was expressed as mg/g of gallic acid equivalent. The TPC analysis was performed in triplicate.

The quantification of phenolic compounds in the films was performed after their production, considering them the initial TCP concentration. Packaging containing the active compound was also evaluated after 121 days of almond flour storage.

2.3 PRODUCTION OF THE ALMOND FLOUR

About 5 kg of raw unpeeled *Prunus dulcis* almonds were ground in a knife mill (IKA A11 Basic, Germany) to produce the whole flour. The brown skins were not removed before grinding because the skin has antioxidants, enhancing the flour's nutritional value.

2.4 PRODUCTION OF PACKAGING AND STORAGE CONDITIONS

The multilayer biodegradable films' formulations were the same as those described in Chapter 4, one control film (C), and two films with different green tea concentrations (GT2.5 and GT5.0). The almond flour was also packaged in aluminum foil and low-density polyethylene bags to simulate commercial packaging.

The films were cut into squares of approximately 30 x 30 cm and sealed to produce the bags. About 40 g of the almond flour was put in the bags and then sealed in a vacuum sealer.

The packed almond flours were stored in a temperature-controlled chamber at 25 °C, and they were analyzed in triplicate after 0, 15, 30, 45, 67, 89, 107, and 121 days of storage.

Figure 1 – Almond flour packed in five different packages

AL - aluminum film; LDPE - low-density polyethylene film; C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g
Source: the author.

The samples were coded according to the types of materials used for their production; AL for aluminum foil bag, LDPE for low-density polyethylene bag. For biodegradable bags, C for control film (without green tea powder), GT2.5 for the bag containing 2.5 g of GT / 100 g of film at the middle layer, and GT5.0 for the bag containing 5 g of GT / 100 g of film at the middle layer.

2.5 ANALYSES OF ALMOND FLOUR DURING STORAGE

Lipid oxidation of almond flour packaged in five different packaging was evaluated by measuring the values of a) Free Fatty Acids and Peroxide Value for

primary oxidation products and b) Determination of secondary products by TBARS method to evaluate the formation of secondary oxidation products.

These analyses were performed after 0, 15, 30, 45, 67, 88, 107, and 121 days of storage, along with aw analysis.

2.5.1 Free Fatty Acids

The acidity was determined by titrating the sample with a standard sodium hydroxide solution (AOCS Ca 5a-40) and expressed as the percentage of free fatty acids (FFA) when it comes from vegetable oils or as acidity index (AI = 0.503% x FFA) (Weiss, 1983). The acidity index was expressed as the NaOH milligrams required to neutralize FFA in 1 g of fat (Swern 1964). This analysis was performed in triplicate with the raw flour samples.

2.5.2 Oil Extraction and Peroxide Value

The extraction of oil from the nut samples was carried out using hexane, as described by Raise et al. (2015), with some modifications. The powdered almond (25 g) was stirred for 6 h at room temperature (25 °C) with hexane (165 mL) in the dark, and they were shaken at 150 rpm for 24 h and then filtered. The resulting oil in hexane mixture was filtered through a filter paper via a Buchner funnel under vacuum. The solvent was removed by rotary evaporation (Model RV O5 BASIC, IKA, USA) at 35 °C and the residue was kept in a screw cap bottle until used for chemical analysis. The peroxide value of oil derived from almond flour was evaluated according to the AOAC method (AOAC, 1995) and the methodology described by KANG et al. (2013). Tests were conducted in triplicate for each sample.

2.5.3 Determination of Secondary Products by TBARS Method

Extraction of 2-thiobarbituric acid reactive substances was performed according to Salcedo, Nazareno (2015), with some modifications, 1.8 g of almond flour was transferred to 50 mL (Falcon type) polyethylene tubes, and 15.00 mL of 7.5% (w/v) trichloroacetic acid (TCA) solution was added. Each mixture was homogenized in a vortex for 30 s (7000 rpm), centrifuged for 15 min (10000 rpm, 25 °C), and filtered in

filter paper. The extract was used for derivatization reaction with 2-thiobarbituric acid (TBA), according to Tarladgis, Pearson, and Dugan (1964), in which 5.00 mL of 20.0 mmol L^{-1} TBA solution was added to 5.00 mL of the extract, and vigorously vortexed and placed in a boiling water bath for 35 min. The standard used was 1,1,3,3-tetraethoxypropane (TEP). After cooling both samples and standard, the absorbance was read at 532 nm, and the results were expressed in mg of malonaldehyde/kg of sample. Tests were conducted in triplicate for each sample.

2.6.4 Water Activity (Aw)

The almond flour's water activity was determined using an AquaLab 4TE water activity meter (Decagon Devices Inc., USA).

2.7 Statistical analysis

The data were analyzed using STATISTICA 10.0 software (StatSoft, USA), with analysis of variance (ANOVA) and Tukey's test at a 5% significance.

3 RESULTS AND DISCUSSION

3.1 Films Production and Characterization

All the biodegradable films produced were flexible and easy to handle. They have excellent tensile properties since they are made up of 70% of TPS, as can be seen in the previous chapter, where the structural properties of these materials were discussed. The water vapor permeability values (WVP) of the films were around $4.0x10^{-6}$ g m^{-1} Pa^{-1} d^{-1}, and the solubilization capacity in water (SCW) was low compared to similar materials. The coextrusion process probably reduced the SCW because of the excellent adhesion between layers and the high thickness of the films. The relatively low solubility, in the case of materials with high starch content, is desirable.

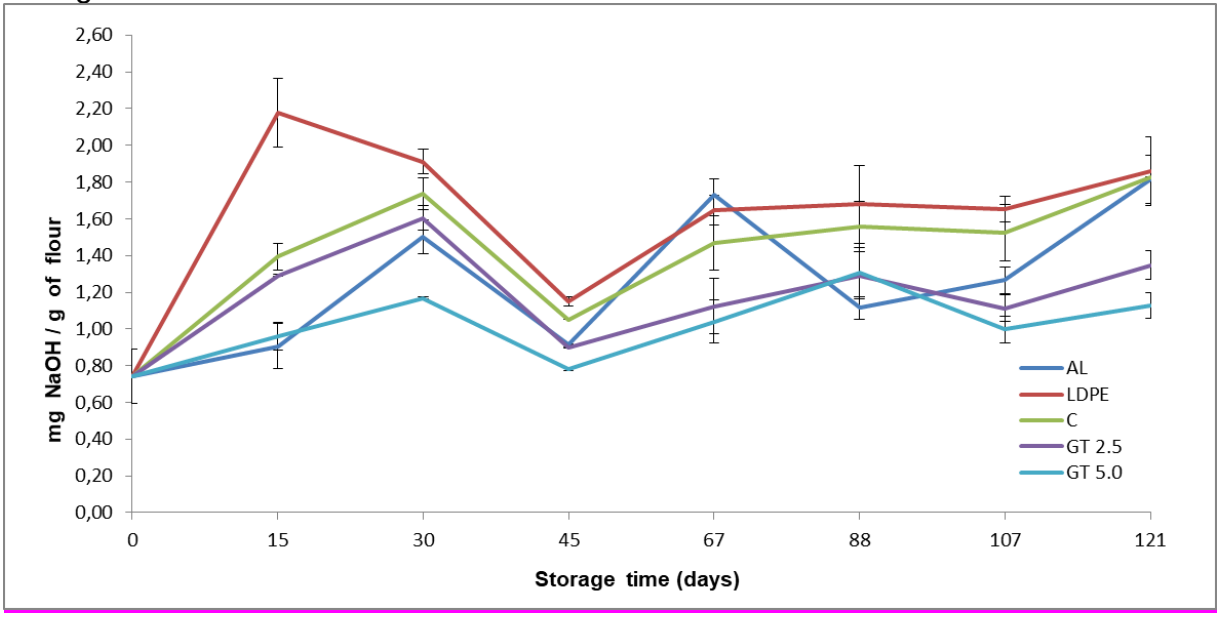
The addition of green tea leaves powder did not impair the films' mechanical properties or their homogeneity, became the films darker and consequently more opaque, reducing the catalytic oxidation effect by partially blocking the light.

Therefore, these films that were developed to be tested as active packaging with antioxidant properties have advantageous characteristics, such as high opacity and the dark color due to the green tea that would prevent photo-oxidation. The low water solubility can be considered an advantage as it becomes resistant to contact with external humidity. The relatively high WVP of the biodegradable films did not reduce the almond flour's shelf life because it is not hygroscopic due to its high lipid content.

3.2 ACIDITY INDEX, PEROXIDE VALUE, AND TBARS OF ALMOND FLOUR DURING STORAGE

The almond flour was subjected to analyzes of acidity index (AI), peroxide value (PV), and substances reactive to thiobarbituric acid (TBARS) during storage, as the product has a high lipid content (60% for this species), being 90% of them are unsaturated (YADA; LAPSLEY; HUANG, 2011), which are more susceptible to lipid oxidation (MCCLEMENTS; DECKER, 2010).

Figure 2 - Acidity index of almond flour packaged in different types of packaging during storage



AL - aluminum film; LDPE - low-density polyethylene film; C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g

The initial AI was 0.72 mg NaOH / g of almond flour (Figure 2), and no comparative values were found for this parameter in the literature.

The initial PV was 3.26 Meq O_2 / kg almond oil (Figure 3). Mexis, Bateka, Kontominas (2009) evaluated the PV of ground almonds without skin, reported values

around 0.17 Meq O_2 / kg almond oil, while Nawab et al. (2017) obtained 1.38 Meq O_2 / kg almond oil for the same raw material. Some authors suggest that almonds should have a PV lower than 2.0 Meq O_2 / kg almond oil to be considered fresh (BURANSOMBOP et al., 2003).

The higher PV of almond flour in our study was probably due to the more advanced state of oxidation of the sample, as the flour has a higher oxidation rate due to the higher contact surface compared to crushed almonds and also due to differences in variety, local and cultural treatment (YADA; LAPSLEY; HUANG, 2011, PISCOPO et al., 2010; PADEHBAN; ANSARI; KOSHANI, 2018).

Based on current studies, the ideal conditions for packaging and storage of ground almonds were using an O_2 absorber combined with a high barrier material, regardless of the lighting and storage temperature. Under such conditions, the shelf life is 12 months (less 2.0 Meq O_2 / kg almond oil) (MEXIS; BATEKA; KONTOMINAS, 2009; MEXIS; KONTOMINAS, 2010).

The oxidation by-products' initial values for almond flour, quantified by the TBARS analysis, averaged 0.11 mg malonaldehyde / kg of almond flour (Figure 4). Salcedo and Nazero (2015) found values of 0.68 mg malonaldehyde / kg of almond in almonds with skin in the initial time, while Nawab et al. (2017) found mean TBARS values of 0.08 mg malonaldehyde / kg of almond for raw almonds.

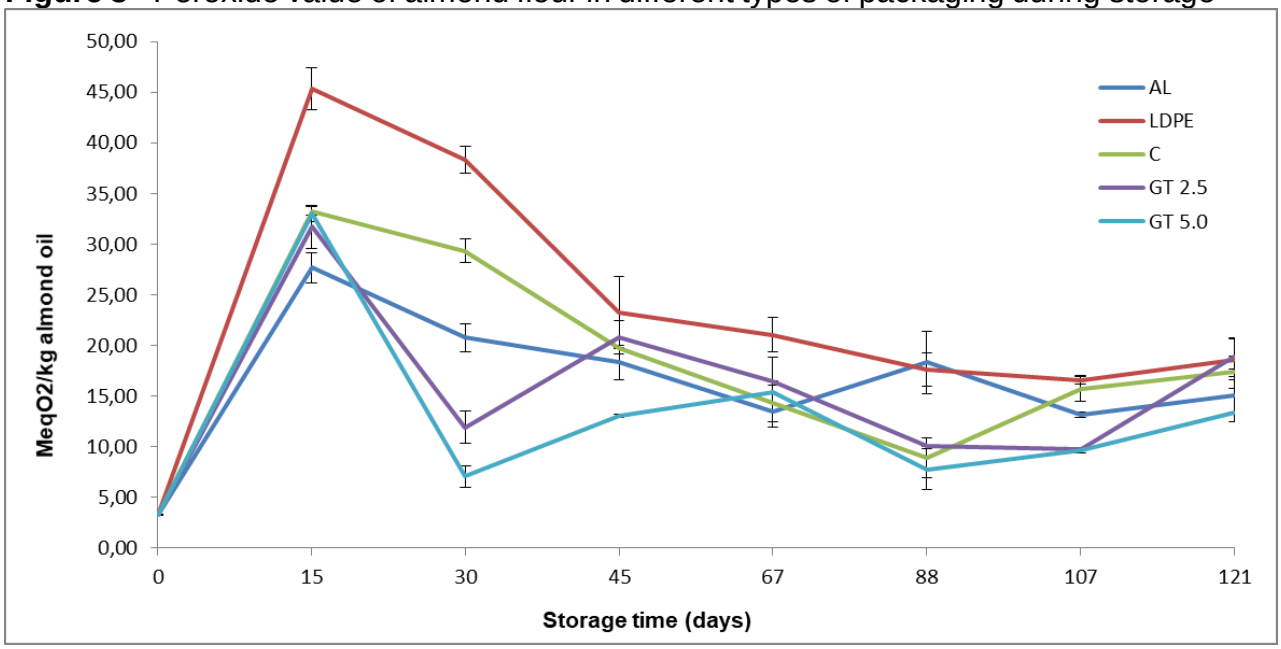
According to the results of PV and the TBARS, the almonds flour in our study were oxidized. However, these results did not impair the study since the objective was to compare the different packaging's efficiency.

After 15 days, the flours had a significant increase ($p > 0.05$) of AI for practically all packaging, except aluminum (AL). The rapid increase of AI was due to the crushing of the raw material to obtain the flour, which accelerated oxidative reactions and favored enzymatic action on the food components, such as the hydrolysis of the triacylglycerols by the action of lipases (MCCLEMENTS; DECKER, 2010).

The protective behavior of aluminum packaging was due to the high light barrier and low gas permeability offered by this material (LAMBERTI; ESCHER, 2007). The flour packaged in LDPE had a higher increase in AI (301%) because LDPE did not offer protection against light and O_2. The control and GT2.5 packaging presented intermediate AI after 15 days because they were opaque (apparent opacity above 60%). Although the AI of the flour in GT5.0 packaging had increased, over the analysis period, there was no significant difference ($p<0.05$) between the flour packaged in

aluminum (AL), probably due to the high antioxidant content.

Figure 3 - Peroxide value of almond flour in different types of packaging during storage



AL - aluminum film; LDPE - low-density polyethylene film; C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g

After 15 days of storage, the peroxide value (Figure 3) of the flours increased significantly ($p>0.05$) for all packages, and the TBARS values (Figure 4) remained the same ($p<0.05$). This behavior corroborates the explanation that crushing favored oxidative reactions involving lipids, as there was a significant increase in the formation of hydroperoxides, determined by the PV analysis, which are primary products of lipid oxidation (SILVA; BORGES; FERREIRA, 1998). The decomposition of hydroperoxides in secondary oxidation products, such as aldehydes and ketones, was insufficient to represent a significant increase in TBARS values (SALCEDO, MISHIMA; NAZARENO, 2010; SILVA, BORGES, FERREIRA, 1998).

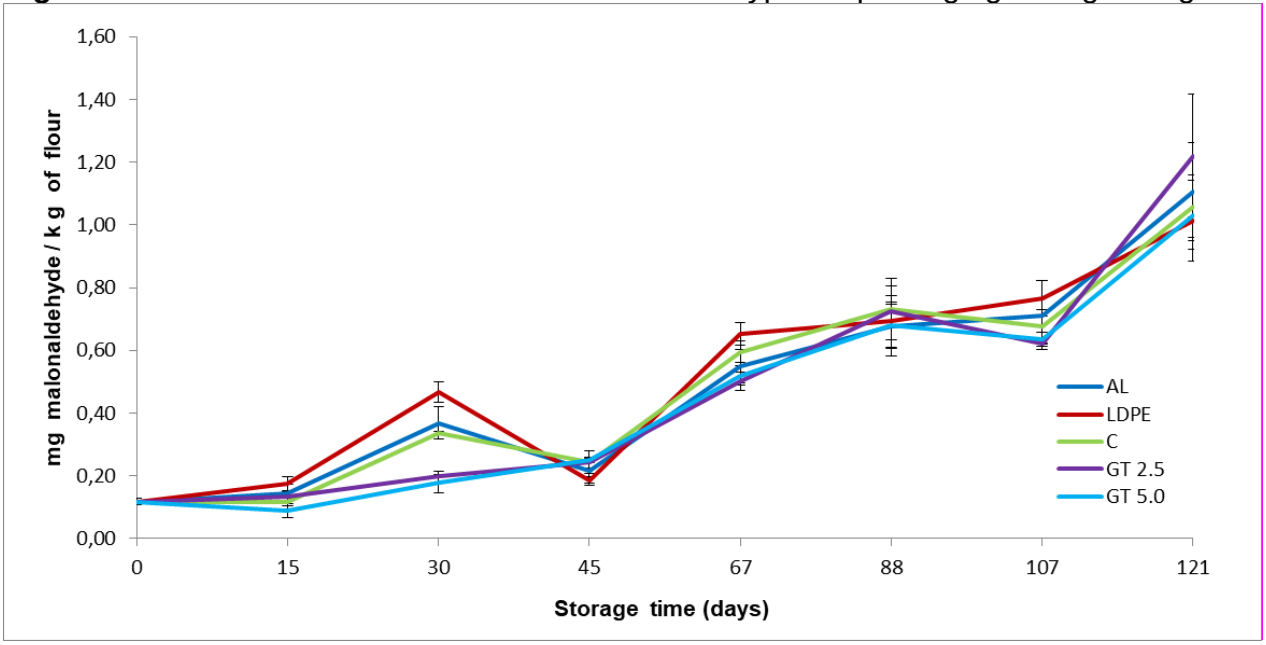
After 30 and 45 days of storage, the flours packaged in GT5.0 had AI 1.5 times lower than those stored in LDPE (Figure 2). After 30 days, the PV of the flours in LDPE was 5 times greater than those in GT5.0, indicating that oxidative processes, which lead to loss quality of almond flours, occurred more quickly in conventional packaging than in packaging containing green tea, which contains a high content of antioxidant compounds (SABAGHI et al., 2015; DICASTILLO et al., 2012; CARRIZO et al., 2016).

After 67 days of storage, samples packaged in GT2.5 and GT5.0 had the lowest AI values, and samples packed in LPDE showed the highest AI values, and similar

behavior occurred for the PV. After 88 days, this behavior was maintained for the PV, and the control and packaged samples in LDPE and C showed the highest AI value, while the others did not differ from each other ($p<0.05$).

When evaluating the performance of the multilayer biodegradable packaging (C, GT2.5, and GT5.0), it was observed that the packaging containing GT maintained lower values in AI and PV during the 121 days of storage. After 15 days of storage, TBARS values were higher for flours in LDPE and GT2.5 packaging and lower for AL and GT5.0.

Figure 4 - TBARS values of almond flour in different types of packaging during storage



AL - aluminum film; LDPE - low-density polyethylene film; C - control film; GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g

After 30 days of storage, the flours in GT.2.5 and GT5.0 packaging had TBARS values 44% and 52% lower when those packaged in aluminum and 56% and 63% for flours in LDPE, which suggests that until that time, oxidation has been milder, especially for flour stored in the GT5.0 packaging. After 45 days of storage, none of the samples differed significantly ($p<0.05$) (Figure 4).

Flours in the control packaging (C) had more accelerated oxidation than flours in the GT2.5 and GT5.0 packaging. Thence the inclusion of green tea as an active compound improved the protection of flour against oxidation.

Biodegradable packaging with high starch concentration produced by extrusion has high opacities (FAKHOURY et al., 2012), reducing the passage of light and,

consequently, the oxidative processes. The GT2.5 and GT5.0 packaging were opaque, and with high phenolic compounds content, that reduced the oxidative processes of the almond flours.

The AI increased considerably in the first 15 days for all samples, but after this period, its values decreased (30 and 45 days) until it stabilized during the storage (Figure 2). Samples GT2.5 and GT5.0 after 107 days showed significantly lower values of these parameters, together with the sample stored in AL, however only samples GT2.5 and GT5.0 maintained this behavior until the end of storage, and the active compounds in green tea were responsible for the protective effect.

TBARS values have increased over time since lipid oxidation reactions are radical, and in the case of TBARS, they depend on the formation of primary oxidation compounds, hydroperoxides, for the propagation and termination phases, which result in secondary products such as aldehydes and ketones (SALCEDO; MISHIMA; NAZARENO, 2010). This behavior can be confirmed by Figure 1, where it is observed that after 45 days of storage, the PV values start to decrease until remaining constant after 88 days, while the TBARS values increase progressively until the end of the experiment.

The behavior of AI, PV, and TBARS was different between the packaging and the rate and extent of reaction, as already discussed. The GT5.0 packaging stood out because the almond flours had lower levels of AI and PV, lower values of TBARS after 30 days of storage, and the packaging remained intact during the 121 days of storage.

3.3 Water Activity of the Almond Flour During Storage

Flour water activity (Aw) ranged from 0.46 to 0.67 during the experiment (Table 2), and these Aw values contributed to the product stability since Aw below 0.6 and higher than 0.2 ensure less probability of the occurrence of lipid oxidation to foods (REID; FENNEMA, 2010).

Table 2 - Water activity of packaged almond flours stored at 25 °C

Storage Time (day)	WATER ACTIVITY Packaging				
	AL	LDPE	C	GT2.5	GT5.0
0	0.63±0.00^{a,A,B}	0.63±0.00^{a,A}	0.63±0.00^{a,A,B}	0.63±0.00^{a,B}	0.63±0.00^{a,B}
15	0.58±0.06^{a,B}	0.59±0.00^{a,B}	0.578±0.00^{a,C}	0.57±0.00^{a,D}	0.58±0,00^{a,B}
30	0.58±0.00^{a,B}	0.55±0.00^{a,B,C}	0.53±0.00^{b,D}	0.52±0.00^{b,E}	0.53±0.00^{b,D}
45	0.59±0.00^{a,A,B}	0.52±0.02^{b,C}	0.47±0.00^{c,E}	0.48±0.00^{c,F}	0.48±0.00^{c,F}
67	0.60±0.00^{a,A,B}	0.54±0.02^{b,C}	0.52±0.00^{b,D}	0.52±0.00^{b,E}	0.52±0.00^{b,H}
88	0.56±0.01^{a,B}	0.48±0.00^{b,D}	0.47±0.00^{b,E}	0.47±0.00^{b,F}	0.46±0.00^{b,G}
107	0.66±0.04^{a,A}	0.67±0.00^{a,A}	0.66±0.00^{a,A}	0.66±0.00^{a,A}	0.67±0.00^{a,A}
121	0.59±0.00^{b,B}	0.58±0.00^{b,B}	0.60±0.00^{a,B,C}	0.61±0.00^{a,C}	0.62±0.00^{a,C}

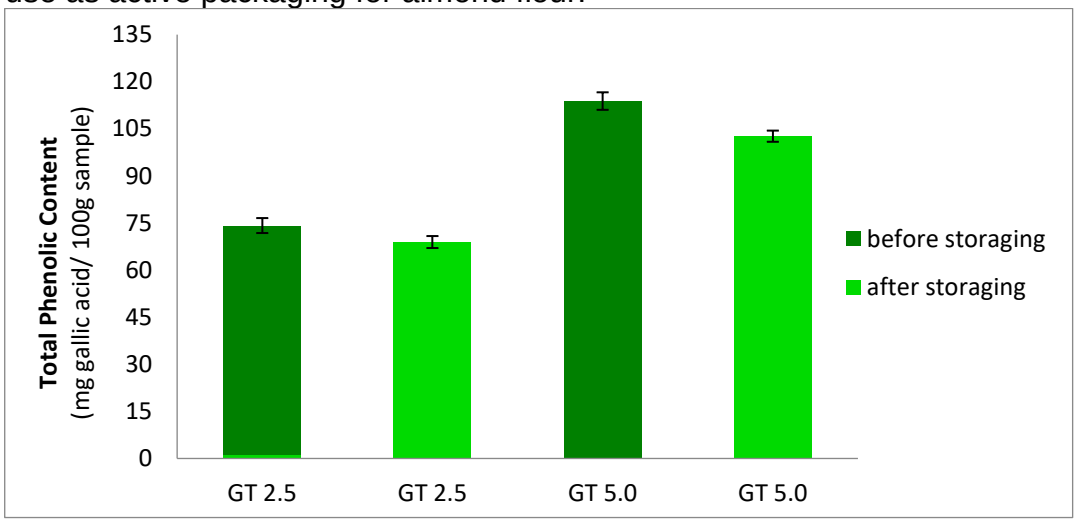
AL:- aluminum film; LDPE: low-density polyethylene film; C: control film; GT2.5: film with 2.5 g of GT/100 g of film; GT5.0: film with 5 g of GT/100 g of film
* Results expressed as mean (± standard deviation)
^{a,b} Different small letters in the same row indicate significant differences (Tukey test $p \leq 0.05$)
^{A,B} Different capital letters in the same column indicate significant differences (Tukey test $p \leq 0.05$)

Aw's erratic behavior during the storage was probably due to the uneven initial Aw of the samples because even the samples packaged in AL had a wide Aw variation and ranged from 0.56 to 0.66. The Aw of the flour in C, GT2.5, and GT5.0 packaging ranged from 0.47 to 0.67, and the relative humidity (RH) within the BOD chamber ranged from 39 to 78% during the storage. These packaging had a higher water vapor permeability than LDPE, but the Aw of the LDPE samples also ranged from 0.48 to 0.67.

3.4 Active Properties of the Active Multilayer Films Before and After Storage

The total phenolic content (TPC) of the biodegradable multilayer films (GT2.5 and GT5.0) was quantified before and after use as active packaging for almond flour after 121 days, shown in Figure 5.

Figure 5 – Total phenolic content of multilayer biodegradable films before and after their use as active packaging for almond flour.



GT2.5 - film with 2.5 g of GT /100 g of film; GT5.0 - film with 5 g of GT/100 g

The initial TCP of the GT2.5 and GT5.0 films were 76 (± 2) and 113 (± 2) mg of GAE/100 g of film, respectively, and after 121 d, the TCP reduced to 68 (± 2) and 102 (± 2) mg of GAE/100 g of film, respectively, only 10% reduction after 121 d being used as almond flour packaging.

The green tea powder (GT) provided to the film antioxidant activity, as previously discussed, and the fact that the GT was in the middle layer prevented or reduced GT compounds' migration to the sample and preserved the antioxidant activity of the phenolic compounds.

The low degradation of the GT compounds ensured no relevant changes in the packaging's appearance, which could discolor if the material had low stability, making it possible to obtain an active, biodegradable packaging with antioxidant characteristics and stable for at least 121 days.

Sabarthi et al. (2015) concluded that the addition of 5% GT in edible chitosan coating was considered the best active coating among all evaluated in the study, as it reduced oxidative activity and undesirable effects on the sensory properties of coated nuts with the material.

4 CONCLUSION

Multilayer biodegradable film containing green tea powder produced by coextrusion has antioxidant activity, and as almond flour packaging, it delays the lipid

oxidation of the product, characterizing it as active packaging.

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CAPÍTULO 5

MULTILAYER BIODEGRADABLE FILMS WITH HIBISCUS POWDER *(Hibiscus sabdariffa*) AS PACKAGING FOR FRESH PORK LOIN

1 INTRODUCTION

For biodegradable packaging to be competitive, it must have performance and cost comparable to conventional packaging. However, films made from biopolymers have some drawbacks like poor mechanical, thermal, and barrier properties (MATSUDA et al., 2013; SCAFAFFARO, BOTTA, GALLO, 2012). Strategies for developing new materials with properties that transform them into possible substitutes for traditional polymers have attracted research interest. Among the several strategies, there are the blends of biodegradable polymers such as PBAT and starch (BRANDELERO; YAMASHITA; GROSSMANN, 2010; FLORES et al., 2010; OLIVATO et al., 2012; OLIVATO et al., 2014); the development of multilayer films represents a strategy to obtain successful properties and improve the performance of these materials (SCAFFARO; BOTTA; GALLO, 2012; SCAFFARO; SUTERA; BOTTA, 2018). Extrusion is a technical tool for the industrial manufacture of films from these materials and can also be used to produce multilayered biodegradable films (MESSIN et al., 2017).

Previous research has indicated that lipid oxidation and microbial growth in meat products can be controlled or minimized using synthetic or natural food additives (JAYAWARDANA et al., 2015; MIELNIK, AABY, SKREDE, 2003). In general, pork is popularly consumed, but there is a problem with oxidation lipids during storage due to its high content of polyunsaturated fatty acids, which causes a decrease in quality and shelf life (HUANG et al., 2014; XIONG et al., 2020).

Food industries commonly use synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutyl hydroquinone (TBHQ). However, some concerns about the safety of these synthetic additives have led many meat processors to seek alternative "natural" antioxidants (JAYAWARDANA et al., 2015; XIONG et al., 2020). Antioxidants can attenuate tissue damage indirectly, improving natural cell defenses, and directly eliminating free radical species. Therefore, increasing attention was paid to these phytochemicals characterized as natural antioxidant products (VERMA et al. 2009).

The search for antioxidants from plant sources and evaluating the antioxidant properties of plant extracts have become the focus of many studies (BENMEDDOUR et al., 2013; SENEVIRATNE; KOTUWEGEDARA, 2009; SINGH, NEGI, RADHA, 2013).

Several studies confirm that hibiscus (*Hibiscus sabdariffa*) has great antioxidant potential (MOHD-ESA et al., 2010). Rosella chalices are rich in polyphenols, especially anthocyanins such as delphinidin-3-sambubioside and cyanidin-3-sambubioside, which are potent hydrophilic antioxidants (FERNANDEZ-ARROYO et al., 2011; RIAZ, CHOPRA, 2018).

H. sabdariffa petals are a good source of antioxidant agents such as anthocyanins (SEGURA-CARRETERO et al., 2008). The antioxidant properties of *H. sabdariffa* and other hibiscus species have been widely studied along with antimicrobial properties (BÜYÜKBALCI; EL, 2008; OBOH; ROCHA, 2008; VANKAR; SRIVASTAVA, 2008; FERNANDEZ-ARROYO et al., 2011). The phenolic content in the plant consists mainly of anthocyanins such as delphinidin-3-glucoside, sambubioside, and cyanidin-3-sambubioside, and other flavonoids such as gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols such as β-sitosterol and ergosterol, and their stability depends on pH, temperature, presence of enzyme, light, and structure, presence of other flavonoids, phenolic acids and metals (IDHAM et al., 2011; RIAZ, CHOPRA, 2018). In addition to the high concentration of anthocyanins, hibiscus contains considerable amounts of ascorbic acid and tannins that have antioxidant activity and do not act as primary antioxidants; i.e., they are reducing agents and capture singlet oxygen (AKARCA, 2020; RIAZ, CHOPRA, 2018).

Several packaging with natural active compounds for fresh meat and meat products are reported (CARDOSO et al., 2017; LÓPEZ-DICASTILHO et al., 2012; SIRIPATRAWAN, HART, 2012; YANG et al., 2016). The pork loin is a cut of meat with high added value; it has a homogeneous fat composition throughout the piece and has unsaturated fatty acids (MA; SUN, 2020).

MOUDACHE et al. (2016) showed the potent antioxidant properties of olive leaf extract in multilayer films made of polyethylene and paper. According to the authors, this material acts as a free radical scavenger and does not permit the extract to migrate to the product, being a promising packaging for fresh meat (MOUDACHE et al., 2017).

This work's objective was to produce by blown coextrusion biodegradable

multilayer material containing hibiscus leaf powder (HP) and use them as active packaging for fresh pork loin to extend their shelf life.

2 EXPERIMENTAL

2.1 MATERIAL

The biodegradable films were produced with cassava starch (Yoki, Brazil), poly (butylene adipate co-terephthalate) (PBAT) (Ecoflex®, BASF, Germany), glycerol and citric acid, both technical grade from Dinamica (Brazil).

The dried hibiscus (*Hibiscus sabdariffa*) flowers/calyces and the pork loin used were purchased in the local market (Londrina, Brazil),

2.2 REAGENT

Gallic acid and Folin-Ciocalteu (F–C) reagent were from Sigma Aldrich (USA), sodium carbonate from Anidrol (Brazil), acetone PA, and ethanol from Synth (Brazil). The 1,1,3,3-tetraethoxypropane (TEP), thiobarbituric acid (TBA) and trichloroacetic (TCA) from Sigma (Argentina). The DPPH standard (2,2-Diphenyl-1-picrylhydrazyl) used was from Sigma (Germany).

2.2 PRODUCTION OF MULTILAYER BIODEGRADABLE FILMS

The dried hibiscus flowers/calyces without the stems were milled in a knife mill (IKA A11 Basic, Germany) and sieved (60 mesh sieve) to produce a powder with a moisture content of around 10%.

The formulations of the pellets were determined in preliminary tests using native cassava starch (ST), poly (butylene adipate-co-terephthalate) (PBAT), glycerol (GLY) as a plasticizer, hibiscus flowers/calyces powder (HP) as the active agent, and citric acid (CA) as a compatibilizer (Table 1). The ingredients were manually mixed and extruded under conditions specified in Chapter 2 to produce the pellets.

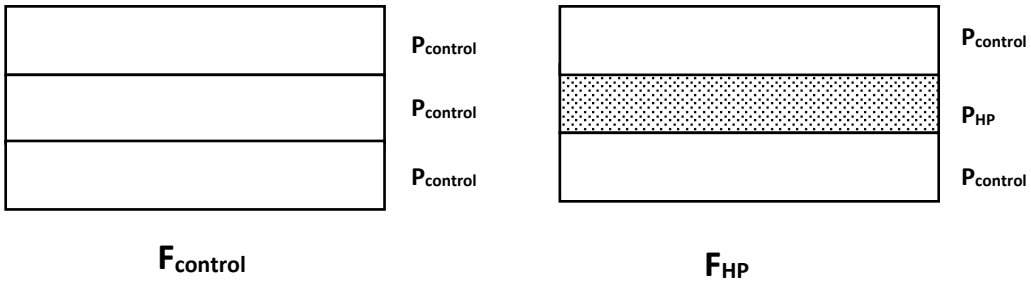
Table 1 - Formulation of the pellets

Formulation	ST*	GLY*	PBAT*	HP*	CA*
$P_{control}$	48.97	21.00	30.00	0.00	0.03
P_{HP}	43.97	21.00	30.0	5.00	0.03

ST - starch, PBAT - poly (butylene adipate-co-terephthalate), GLY - glycerol, HP- hibiscus flower powder, CA - citric acid
*g 100 g^{-1} of mixture

The two pellet formulations were coextruded under de conditions specified in Chapter 2 to produce the multilayer films: biodegradable control film ($F_{control}$), and the biodegradable film containing hibiscus powder (HP) in the intermediate layer (F_{HP}) (Figure 1).

Figure 1 - Composition of the multilayer biodegradable films



Source: the author.

The formulation containing the active compound (GT) was at the middle layer to prevent its exposure to the external ambient, improve its stability, and avoid direct contact with the product to be packaged and avoid sensorial changes to the product.

2.3 Characterization of Biodegradable Multilayer Films

2.3.1 Mechanical Properties

The tensile strength (MPa), elongation at break (%), and Young's modulus (MPa) of the materials were determined according to ASTM method D882-02 (2002) with some modifications using a texture analyzer (model TA.XT2i, Stable Micro Systems, England) fitted with a 50-kg load cell. Ten specimens of each formulation were cut in the longitudinal direction (50 mm x 20 mm), and they were conditioned at 23 ± 2 °C and 53 ± 2% RH for at least 7 d. The crosshead speed was set at 0.8 mm/s,

and the initial distance between the grips was 30 mm; ten repetitions were performed for each sample analyzed

2.3.2 Water Vapor Permeability (WVP)

Water vapor permeability was determined gravimetrically according to the ASTM E96 00 (2009) standard, with some modifications. The measurements were performed in triplicate using a relative humidity gradient of 33 - 64%. Before analysis, the samples were conditioned at 23 ± 2 °C and 53 ± 2% RH for at least 7 d. The analysis was carried out in triplicate.

2.3.3 Color and Opacity of the Films Produced

The opacity of the films was determined according to Olivato *et al*. (2017) using a colorimeter (BYK Gardner, Germany) with illuminant D65 and a visual angle of 10°. Opacity was determined as the ratio of the luminosity on the standard black (Yp) and luminosity of the sample on the white standard (Yb) (0 - 100%) (Equation 1), and the analyses were performed in triplicate.

$$Y = \left(\frac{Yb}{Yw}\right) \times 100 \quad (1)$$

The color was measured with a colorimeter (BYK Gardner, Germany) with an illuminant D65 and a visual angle of 10°. The color was determined on the samples placed on top of a white pattern and expressed as CIELab L* a* b* values.

2.3.4 Total Polyphenol Content (TPC)

Total polyphenol content (TPC) of the films was determined by the Folin-Ciocalteau colorimetric method adapted from Kumazawa; Hamasaka; Nakayama (2004). The TPC analysis was performed for the film containing hibiscus flower/calyces powder (F_{HP}) and the powder (HP) itself.

The phenolic compounds extraction from both the film and the HP were performed using aqueous acetone (70% acetone and 30% distilled water v/v) as a

solvent. The film (0.25 g) was infused in 10 mL of solvent (1:40), and they were shaken at 150 rpm for 1 h and then filtered. The HP (0.05 g) was infused in 200 mL of solvent (1:4000), and they were shaken at 150 rpm for one hour and then filtered. The extraction conditions were determined through preliminary tests, where the most efficient one was chosen (data not shown).

The extraction solution (0.5 mL) was mixed with 0.5 mL of Folin-Ciocalteau reagent (Kanto Chemicals, Japan) and 0.5 mL of 10% $NaCO_3$ solution and stored at room temperature for 1 h. The absorbance of the solutions was measured at 765 nm using a spectrophotometer Libra S22 (Biochrom, United Kingdom). TPC was expressed as mg g^{-1} of gallic acid equivalent. The TPC analysis was performed in triplicate.

The retention of the phenolic compounds in the active layer after the extrusion process (%$TCP_{retention}$) was calculated by Equation 5.

$$\%\, TPC_{\ retention} = \left(\frac{TPC_{film}}{TPC_{HP}}\right) x\ 100 \quad (3)$$

Where: TPC_{film} is the total polyphenolics content in the film, TPC_{HP} is the total polyphenolics content in the hibiscus flowers/calyces powder used to produce the film.

The quantification of phenolic compounds was performed shortly after the film's production, i.e., before their application as packaging. The films that contained the active compound (F_{HP}) were re-evaluated after 9 d of being used as meat packaging.

2.3.5 Antioxidant Activity by DPPH Assay

The extracts from the films and powder (HP) used in the analysis of the antioxidant activity for those were obtained in the proportion of 1: 5 and 1: 200 for HP, in solutions of 80% (v/v) ethanol, 70% (v/v) acetone, completing with distilled water according to Rufino et al. (2007), in triplicate.

The determination of total antioxidant activity by capturing the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was carried out according to the methodology described by Brand-Williams, Cuvelier, and Berset (1995) modified by Rufino et al. (2007). 0.1 mL of extract, in three different dilutions, were transferred to test tubes

containing 3.9 mL of DPPH radical (DPPH solution 60 µmol L^{-1}, diluted in ethyl alcohol). 0.1 mL of control solution with 3.9 mL of the DPPH radical was used. Then, readings were performed on a spectrophotometer (Libra S22, Biochrom, England) at 515 nm. Ethyl alcohol was used as white to calibrate the spectrophotometer. The results were expressed as % inhibition, according to Sepahpour et al. (2018), following the equation below:

$$\%\ inhibition = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) x\ 100 \quad (4)$$

Where: $A_{control}$ is the absorbance of DPPH without extract, and A_{sample} is the absorbance of the DPPH after adding the extracts.

2.3 MEAT PACKAGING

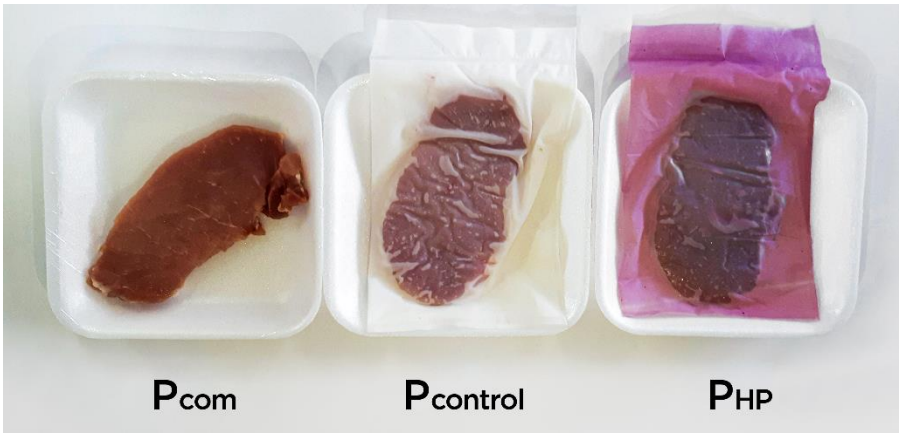
The biodegradable films $F_{control}$ and F_{HP} (Figure 1) were used as fresh boneless pork loin steaks packaging.

The films were cut and sealed to produce bags of 200 x 260 mm, and steaks of approximately 100 g were packaged in the bags and placed on polystyrene trays. Steaks packaged on polystyrene trays wrapped with PVC film were used to simulate a commercial packaging (P_{com}). It is important to note that only one piece of pork loin was used in the whole experiment to avoid variability inherent in the sample.

The packaged steaks were stored at 4 °C, simulating the retail sale conditions. All the steaks were from the loin of a single animal.

The packaged steaks are in Figure 2, where the P_{com} is the commercial packaging, $P_{control}$ is the packaging produced with film $F_{control}$, and P_{HP} is the packaging containing the active compound in the intermediate layer, produced with film F_{HP}.

Figure 2 - Packaging of fresh pork loin steaks



P_{com}: commercial packaging; $P_{control}$: packaging produced with film $F_{control}$; $P_{HP:}$ packaging containing HP
Source: the author.

The fresh pork loin steaks in different packaging were evaluated for color, pH, and TBARS after 0, 3, 6, and 9 days of storage at 7 °C, following the methodologies.

2.3.1 Color of the fresh pork loin steaks

The color parameters CIE L*a*b* (illuminant D65 and 0° viewing angle) of the packaged fresh pork loin steaks over the storage period were determinate with a colorimeter Chroma Meter CR-400 (Konika Minolta, Japan). The color was measured at the surface of the unpacked steaks, avoiding the borders with fat. The measurements were performed in triplicate for each sample.

2.3.2 pH

The pH measurements were performed in duplicate at the central part of the steaks, using an insertion pot (Testo 205) method described by Boulianne and King (1995), adapted for pork.

2.3.3 Assay of Thiobarbituric Acid Reactive Substances (TBARS)

The extraction of substances reactive to 2-thiobarbituric acid was carried out according to the methodology proposed by Mendes, Cardoso, and Pestana (2009), with adaptations. Portions of 10.00 g of pork loin were transferred to 50 mL

polyethylene tubes (Falcon type), and 15.00 ml of 7.5% TCA solution (w/v) was added. Each mixture was homogenized (Ultra-Turrax, IKA, USA) for 1 min at 7000 rpm, centrifuged for 10 min (6000 rpm, 20 °C), and then filtered through filter paper. The extract was used for derivatization reaction with TBA, followed by the method proposed by Tarladgis, Pearson, and Dugan (1964), in which 5.00 mL of 20.0 mmol L^{-1} TBA solution was added to 5.00 mL of the extract, homogenized vigorously in a vortex and placed in boiling water bath for 35 min. The standard used was 1,1,3,3-tetraethoxypropane (TEP). After cooling the samples and the standard, the absorbance was read on a spectrophotometer at 532 nm. The results were expressed in mg of malonaldehyde kg^{-1} of meat, and the samples were analyzed in triplicate.

2.4 STATISTICAL ANALYSIS

Data were analyzed statistically using the STATISTICA 10.0 software, performing analysis of variance (ANOVA), and Tukey's test at the level of 5% significance.

3 RESULTS AND DISCUSSION

3.1 FILMS PRODUCTION, MECHANICAL CHARACTERIZATION AND BARRIER PROPERTIES OF FILMS PRODUCED

The processability of the multilayered biodegradable films that contained powdered hibiscus flowers (HP) in the active layer (F_{HP}) was worse than that of the control films, as the balloon had holes caused by HP (Figure 3), although the flowers were separated from the stems, milled and sieved twice in a 60 mesh sieve.

Figure 3 - Extrusion of the multilayer biodegradable film with HP in the active layer (F_{HP}).

F_{HP} - film with 5 g of HP/100 g in the middle layer
Source: the author, 2019.

The maximum tensile strength, elongation at break, Young's modulus, water vapor permeability, and film opacity were evaluated (Table 2).

Table 2 – Mechanical properties, water vapor permeability (WVP) and opacity of the multilayer biodegradable films

Samples	**σ** (MPa)	**ε** (%)	**E_0** (MPa)	***WVP(x10^6)** (g m^{-1} Pa^{-1} d^{-1})	**Opacity** (%)
$F_{control}$	2.9±0.4a	54.7±11b	29.7±5.4a	4.7±0.4a	62.9±1,3b
F_{HP}	2.7±0.3a	78.1±23a	28.5±9.7a	4.7±1.0a	67.4±0,6a

$F_{control}$ - control film; F_{HP}- film with 5 g of HP/100 g in the middle layer
σ: tensile strength; ε: elongation at break; E0: Young's modulus
Results expressed as mean (± standard deviation).
*Results expressed as mean (± standard deviation) x $10^{-6}$for WVP
[a,b] Different letters in the same column indicate significant differences (Tukey test $p \leq 0.05$).

Monolayer biodegradable films made of PBAT / starch with formulations similar to the present study, with the addition of curcumin produced by De Campos et al. (2019) presented Young's modulus values from 23 to 33 MPa, elongation from 27 to 39%, maximum tensile strength from 4.1 to 6.6 MPa, and for all parameters the

formulation with the highest concentration of the active component showed higher values than the control. The authors suggest that the increase in mechanical properties can be attributed to the lower degree of crosslinking of starch in extrusion processes. Therefore, the addition of components with active properties improved biodegradable films' properties between mixtures of starch with extruded PBAT, as in our case.

Zhai et al. (2020) produced extruded biodegradable films with PBAT / starch / nanoclays and reported stress values around 4 MPa, 350% elongation at break, and Young's module around 80 MPa. The higher elongation and MY than our results were probably due to the nanoclay, which acted as a compatibilizer since PBAT / starch proportion was similar in both studies.

The barrier properties of biodegradable films are essential for food packaging, as they are parameters with direct effects on product conservation. The water vapor permeability of multilayer biodegradable films ($F_{CONTROL}$ and F_{HP}) was 4.7 x 10^{-6} g m^{-1} Pa^{-1} d^{-1}, for both films (Table 2), under a gradient of 33-64 % RH. HP addition as an active component did not influence this property, and these values are similar to those reported in the literature. Zhai et al. (2020) reported WVP values of 8.0 x 10^{-6} g m^{-1} Pa^{-1} d^{-1} for films extruded in the same PBAT / starch proportion. However, the permeability depends on environmental factors (temperature, relative humidity) and the material's characteristics, such as its composition and its manufacturing process characteristics.

Consumer acceptance of biodegradable films as food packaging can be affected by its optical properties (SHOJAEE-ALIABADI et al., 2013; CARDOSO et al., 2017). The apparent opacity of biodegradable films was relatively high and varied between 62.9% ($F_{control}$) and 67.4% (F_{HP}) (Table 1), i.e., the HP increased the opacity of the film. High opacity is a characteristic of films that contain starch in their formulation and are produced by extrusion (FAKHOURY et al., 2012).

Anthocyanins are very sensitive to high temperatures and pH changes, and the films were produced by extrusion in two stages; consequently, the HP used as a source of phenolic compounds (in particular anthocyanins) was exposed to high temperatures (between 90-120 °C) twice, that could have substantially impaired the active properties of the material. For this reason, after extrusions, the F_{HP} film formulation was evaluated for the total phenolics content (TCP), retention of total phenolics in the active layer, and DPPH.

The F_{HP} multilayer biodegradable film had a TPC of 31.37 ± 1.59 mg of gallic acid equivalent / 100 g film, with the retention of HP's phenolics in the active layer of

the films being around 90 ± 4%. As for DPPH, the film showed 23.4 ± 0.1% inhibition of free radicals right after extrusion. The extrusion process did not affect HP's active properties, and 90% of the phenolic compounds remained in the intermediate layer of the multilayer films, enabling the use of these films as active biodegradable multilayer packaging.

3.2 pH, TBARS AND COLOR PARAMETERS (L*, a*, b*) OF PIG LOIN PACKAGED IN DIFFERENT PACKAGES FOR 9 DAYS OF STORAGE

There were visual changes in the packaging and meat during the refrigerated storage for 9 d. After 6 d of storage, the samples packaged in P_{com} were visibly deteriorated, with yellowish exudate.

After 3 d of storage, the multilayer biodegradable packaging containing hibiscus powder (P_{HP}) began to change color (changes that will be discussed in item 3.2) and the control multilayer biodegradable packaging ($P_{control}$) dried out, making it difficult to unpack the samples.

Packaged fresh pork loin fillets were evaluated for pH and TBARS during 0, 3, 6, and 9 d of cold storage (Table 3).

The pH values of pork tenderloin ranged from 5.82 to 7.32, and the highest values were from samples packaged in P_{com} at all time of analysis (3, 6, and 9 d). The pH of the samples packaged in multilayered biodegradable packaging ($P_{control}$ and P_{HP}) did not differ during the storage.

The P_{com} and P_{HP} samples did not change their pH value between 0 and 3 d, and the pH of the $P_{control}$ samples after 3 d was higher than the initial one. The highest pH values are found after 6 and 9 d, with no difference between all packaged samples.

In the process of meat deterioration, protein degradation occurs, which generates volatile organic amines, such as trimethylamine, increasing the pH of the meat (ZHANG et al., 2019; XIAOBO, 2008). Zhang et al. (2019) found TVB-N values that indicated that pork proteins begin to degrade and produce amines after 48 h of storage at 4 °C.

Table 3- pH, TBARS of fresh pork loin stored in three different packaging

Storage Time (day)	**pH**		
	Packaging		
	P_{com}	$P_{control}$	P_{HP}
0	5.82±0.02^{aB}	5.82±0.02^{aC}	5.82±0.02^{aB}
3	6.39±0.15^{aB}	6.11±0.02^{bB}	6.01±0.02^{bB}
6	7.28±0.35^{aA}	6.41±0.09^{bA}	6.36±0.10^{bA}
9	7.32±0.20^{aA}	6.37±0.12^{bA}	6.41±0.09^{bA}
Storage Time (day)	**TBARS (mg of malonaldehyde/kg of pork loin)**		
	Packaging		
	P_{com}	$P_{control}$	P_{HP}
0	0.06±0.01^{aC}	0.06±0.01^{aC}	0.06±0.01^{aC}
3	0.10±0.01^{aB}	0.09±0.01^{aBC}	0.07±0.01^{bC}
6	0.12±0.01^{aB}	0.12±0.01^{aAB}	0.09±0.00^{bB}
9	0.19±0.03^{aA}	0.16±0.03^{aA}	0.13±0.01^{aA}

P_{com}: commercial packaging; $P_{control}$: packaging produced with film $F_{control}$; P_{HP}: packaging containing HP
Results expressed as mean (± standard deviation)
^{a,b} Different small letters in the same row indicate significant differences (Tukey test $p \leq 0.05$)
$^{A, B}$ Different capital letters in the same column indicate significant differences (Tukey test $p \leq 0.05$), for each analysis

Meat samples stored in conventional packaging (P_{com}) had an increase in pH during storage, indicating a loss in product quality when compared to biodegradable packaging ($P_{control}$, P_{HP}). Some authors suggest that the pH of fresh pork should range from 5.4 to 5.9 (WARNER; KAUFFMAN; GREASER, 1997; ZHANG et al., 2019), but the pH exceeded these values, indicating a beginning of deterioration, either by protein degradation and / or by the action of microorganisms (HOLMER et al., 2009; XIONG et al., 2020).

TBARS values of stored meat ranged from 0.06 to 0.12 mg of malonaldehyde/kg of meat after 9 d of cold storage (Table 3). Similar TBARS values have been reported for pork by Yang et al. (2016) and by Xiong et al. (2020), where the initial value of fresh pork samples was 0.12 mg MDA / kg and subsequently increasing.

After 6 d of storage, the PHP package's meat had a lower value of TBARS than the meat in the P_{com} and $P_{control}$ packaging. After 9 d, the meat's TBARS values were approximately 0.12 mg of malonaldehyde/kg of meat, and there was no significant difference between packages.

Yang et al. (2016) packaged diced pork in green tea / soluble protein films and

reported TBARS values ranging from 0.97 to 0.64 mg of malonaldehyde/kg of meat after 10 d of storage at 4 °C.

Siripatrawan and Noipha (2012) reported that sausage packaged with chitosan film containing green tea extract had lower TBARS values than the control after 20 d of storage, and Qin et al. (2013) reported that pork products packaged with chitosan film containing polyphenols had lower TBARS values than the control after 12 d of storage.

The CIE-Lab color parameters of the packaged meat during storage are shown in Table 4. The initial color parameters of the fresh tenderloin samples were L* = 50.95, a* = 6.26, b* = -0.76. Pork loin samples were evaluated by Xiong and collaborators (2020) for color parameters, and the reported values were L* = 52.73, a* = 4.28, b* = 5.45.

The meat's L* values gradually decreased over the storage period, regardless of the packaging; after 3 d of storage, the luminosity (L*) of the meat packaged in P_{HP}.0 and P_{com} had higher values than $P_{control}$. In the following analysis times (6 and 9 d), the PHP samples and $P_{control}$ had lower values of L* than P_{com}.

The red color is an important indicator for consumers to evaluate the freshness of the meat (XIONG et al., 2020), and after 3, 6, and 9 d of refrigerated storage, the meat samples from the $P_{control}$ packaging had the highest values of parameter a*, which is related to the intensity of red. The a* values of the samples packaged in P_{HP} remained constant during the 9 d of cold storage.

The color parameter b* of the samples in the P_{com} packaging (Table 4) increased over time, which did not happen for the multilayer biodegradable packaging ($P_{control}$ and P_{HP}). The increase of the b* values during storage is related to lipid oxidation, as high b* values are associated with greater yellow intensity and high lipid oxidation (JOUKI; KHAZAEI, 2012) and may also be associated with the formation of metmyoglobin, which is the form of oxidized myoglobin, which is brown (WARNER, 2014; XIONG et al., 2020).

Table 4- Color parameters L*, a*, b* of fresh pork loin stored in three different packages

Storage Time (day)	**L***		
	Packaging		
	P_{com}	$P_{control}$	P_{HP}
0	50.95±0.77aA	50.95±0.77aA	50.95±0.77aA
3	47.35±2.31aAB	39.30±2.53bB	46.27±2.54aB
6	45.56±3.93aB	38.65±2.41bB	37.69±4.09bC
9	46.16±3.02aB	38.09±2.29bB	38.63±2.21bC
Storage Time (day)	**a***		
	Packaging		
	P_{com}	$P_{control}$	P_{HP}
0	6.26±0.92aA	6.26±0.92aA	6.26±0.92aA
3	6.35±0.72bA	8.59±2.25aAB	6.34±2.20bA
6	2.49±1.15cC	10.11±3.27aB	6.26±2.19bA
9	4.50±1.29bB	9.96±1.57aB	5.71±1.24bA
Storage Time (day)	**b***		
	Packaging		
	P_{com}	$P_{control}$	P_{HP}
0	-0.76±0.55aA	-0.76±0.55aA	-0.76±0.55aA
3	-0.72±1.21abA	1.29±2.97aA	-0.96±0.37bA
6	2.54±1.58aB	0.98±2.48abA	-0.75±1.35bA
9	0.85±1.16aA	-0.73±1.21bA	-1.29±0.48Ba

P_{com}: commercial packaging; $P_{control}$: packaging produced with film $F_{control}$; P_{HP}: packaging containing HP
Results expressed as mean (± standard deviation)
[a,b] Different small letters in the same row indicate significant differences (Tukey test $p \leq 0.05$)
[A, B] Different capital letters in the same column indicate significant differences (Tukey test $p \leq 0.05$), for each analysis

3.3 TOTAL AMOUNT OF PHENOLIC COMPOUNDS, ANTIOXIDANT CAPACITY, AND COLOR PARAMETERS OF P_{HP} PACKAGES

The active packaging containing powdered hibiscus (P_{HP}) was evaluated before and after its use as a package of pork tenderloin stored for 9 d under refrigeration. The total amount of phenolic compounds (TPC), the antioxidant capacity by DPPH (free radical capture), and the color parameters CIE-LAB (Table 4) were determined on the packaging.

Table 5 - Properties of biodegradable multilayer packaging containing hibiscus (P_{HP}) during storage of pork loin

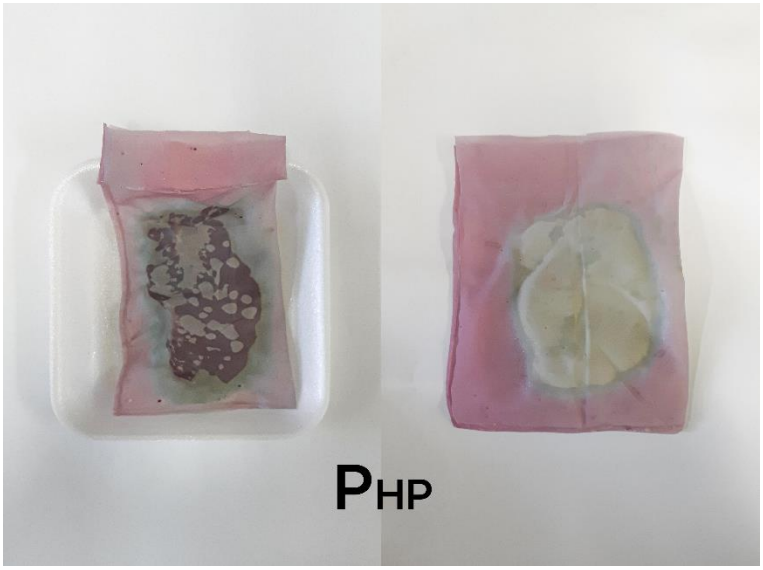
Analysis	Storage Time (d)	
	Initial time	Final time (9 d)
TPC (mg of GAE /100 g sample)	37.37±1.59a	29.45±0.68b
DPPH (% inhibition)	23.43±0.00a	16.43±0.02b
L*	74.01±1.32a	67.70±2.73b
a*	22.72±2.01a	-2.56±0.39b
b*	-0.92±0.40b	3.58±0.34a

$P_{HP:}$ packaging containing the active compound

The TPC of the P_{HP} packaging decreased by 21% over 9 d of meat storage, decreasing from 37.37 to 29.45 mg of GAE / 100 of film. The phenolic compounds that scavenge free radicals acted as antioxidants, based on the TBARS results discussed previously, where meat samples in P_{HP} packages showed less oxidation up to 6 d. The inhibition of free radicals decreased by 30% after being used as packaging (Table 5) due to the phenolic compounds that acted as antioxidants, as discussed previously.

After 3 d of storage, the packaging in direct contact with the meat changed their color. The PHP packaging image at the beginning of the storage is in Figure 1, and the same packaging after 9 d of pork storage is in Figure 4.

Figure 4 - Color changes of the packaging containing the active compound after 9 d of storage.



$P_{HP:}$ packaging containing the active compound in the intermediate layer

After 9 d of storage, the L* value decreased from 74.01 to 67.70, an increase in opacity as a function of time, probably due to the packaging's aging. The parameter a*

decreased from 22.72 to -2.56, i.e., the packaging had a decrease in the redness, tending to green (negative values of a*). The parameter b* increased from -0.92 to 3.58, i.e., it changed from blue (negative values of b*) to yellow (positive values of b*).

The color change of the packaging in contact with the meat was probably due to the reaction of the phenolic compounds that acted as antioxidants, and the color of several phenolic compounds change, depending on the pH. At pH 2.0-3.0, anthocyanins in solution appear red; at pH 4.0-6.0, their structures gradually change, and the red decreases; at pH 7.0-9.0, its structures turn into a colorless pseudo-base, and the color gradually turns blue. When the pH value is higher than 9.0, anthocyanins degrade in a strongly alkaline environment, and the color changes to yellow-green (GRAJEDA-IGLESIAS et al., 2017; ZHANG et al., 2019).

The color change of the P_{HP} packaging was due to its antioxidant activity and the contact with the fresh meat that changed its pH during storage; therefore, it can be used as active packaging and an indicator of meat freshness.

4 CONCLUSION

Multilayer biodegradable film produced by blown coextrusion containing hibiscus powder (HP) has good processability and excellent phenolic compounds retention after coextrusion.

This film, used as packaging for fresh pork, delays the lipid oxidation of the product and changes its color depending on the meat's pH.

The biodegradable multilayer film containing HP can be used as active packaging for fresh meat and as an indicator of meat freshness.

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CONSIDERAÇÕES GERAIS

Foi possível produzir filmes biodegradáveis multicamadas com amido, poli (adipato co-tereftalato de butileno) e glicerol através da coextrusão sopro em balão com adição de compostos ativos em forma de pó derivados das folhas de chá verde e dos cálices do hibisco.

Os materiais multicamadas biodegradáveis produzidos apresentram propriedades adequadas e são pouco solúveis em água, o que pode ser considerado uma propriedade atrativa desses materiais, uma vez que materiais à base de amido são altamente higroscópicos, fazendo com que seja possível sua utilização como materiais de embalagens para alimentos.

Os filmes apresentaram uma alta porcentagem de retenção de compostos fenólicos totais (TPC) na camada intermediária ativa, após o processo de coextrusão. O filme produzido com adição de HP apresentou 90% de retenção dos compostos fenólicos.

As embalagens multicamadas biodegradáveis, que continham compostos fenólicos na camada ativa, retardaram o início da oxidação nos alimentos analisados neste trabalho nas condições de armazenamento propostas. De acordo com os resultados obtidos no presente trabalho, os filmes biodegradáveis multicamadas podem ser considerados embalagens ativas e devem ser considerados como uma alternativa relevante as embalagens convencionais.