



**UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE
ALIMENTOS**

**FILMES DE POLI(ÁCIDO LÁTICO) ADICIONADOS DE EXTRATOS DE
CAROTENOIDES – DESENVOLVIMENTO DE MATERIAIS PARA A EMBALAGEM
DE ALIMENTOS SENSÍVEIS À LUZ E AO OXIGÊNIO**

Liana Stoll

Porto Alegre, novembro de 2019

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Tese de Doutorado apresentado ao
Programa de Pós-Graduação em Ciência e
Tecnologia de Alimentos do Instituto de
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Universidade Federal do Rio Grande do Sul.

Orientador: Prof. Dr. Alessandro de Oliveira Rios
Co-orientadora: Profa. Dra. Sonia Marli Bohrz Nachtigall

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ALIMENTOS

Autora: Liana Stoll

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– Desenvolvimento de materiais para a embalagem de alimentos sensíveis à luz e
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“A utopia está lá no horizonte.

Me aproximo dois passos, ela se afasta dois passos.

Caminho dez passos e o horizonte corre dez passos.

Por mais que eu caminhe, jamais alcançarei.

Para que serve a utopia?

Serve para isso: para que eu não deixe de caminhar.”

Eduardo Galeno

RESUMO

A busca por alimentos livres de aditivos artificiais embalados em materiais que não agridam ao meio ambiente é uma tendência. Tendo em vista que o uso de embalagens que protegem os alimentos da luz e do oxigênio permite a extensão do *shelf life* de produtos perecíveis, a incorporação de extratos de carotenoides à matriz polimérica do poli(ácido láctico) (PLA) é uma alternativa para melhorar as propriedades desse polímero e ampliar sua gama de aplicação. Inicialmente, extratos de beta-caroteno, licopeno e bixina foram incorporados à matriz de PLA em filmes produzidos pela técnica de *casting*, onde a bixina apresentou a maior estabilidade de coloração dos filmes e o melhor desempenho na proteção do óleo de girassol frente a reações de oxidação. Os filmes com licopeno e beta-caroteno protegeram o óleo através da barreira à luz, onde a liberação gradual de carotenoides para o óleo teve papel secundário na redução da formação de peróxidos no produto. A cinética de migração dos carotenoides para um líquido simulante foi descrita por um novo modelo matemático, onde a degradação dos compostos liberados foi considerada. Na segunda etapa do estudo, filmes de PLA com bixina foram produzidos através de processamento via fusão, onde o processamento a 160 °C provocou perdas de até 85% dos carotenoides. No entanto, o uso de 0,1% de bixina produziu materiais com excelentes propriedades de barreira à luz UV, a qual foi responsável pela expressiva redução da fotodegradação da riboflavina. As tensões de cisalhamento inerentes ao processamento em câmara de mistura não causaram degradação adicional da bixina, sendo possível manter boa parte do poder corante do carotenoide. Apesar de aumentar a permeabilidade ao oxigênio do material, o uso de plastificante acetil tribul citrato (ATBC) acelerou a migração da bixina para o alimento evitando que a degradação oxidativa do óleo de girassol ocorresse de forma acelerada. O desenvolvimento de filmes a partir de PLA e carotenoides demonstra que é possível produzir embalagens biodegradáveis, de coloração atrativa, mecanicamente resistentes e que protejam os alimentos embalados da fotodegradação. Através do conhecimento gerado, é desejável otimizar o desempenho do PLA como material antioxidante através da modulação da cinética de migração dos carotenoides e do aumento da barreira ao oxigênio do material.

Palavras-chave: bixina; migração; cinética; oxidação; plastificante.

ABSTRACT

Consumers demand for food produced without synthetic and chemical preservatives packaged in environmentally friendly materials is a trend. Considering the shelf life of perishable food, it can be extended by the use of packaging materials that protects it from light and oxygen. The incorporation of carotenoid extracts into the poly (lactic acid) (PLA) matrix is an alternative to improve the properties of this polymer and to broaden its application range. Initially, beta-carotene, lycopene and bixin extracts were used to produce PLA films by the casting technique, where bixin produced films with the highest color stability and the best performance in protecting sunflower oil against oxidation. Lycopene and beta-carotene films protected the oil through a light barrier, where the gradual release of carotenoids to the oil played a secondary role in reducing the formation of peroxides in the product. A new mathematical model described the kinetics of carotenoid migration to a food simulant, where the degradation of the released compounds was considered. In the second part of the study, PLA films with bixin were produced by melt processing at 160 °C, where the heat caused up to 85% of carotenoid degradation. However, the use of 0.1% bixin produced materials with excellent UV-light barrier, which was responsible for significantly protecting riboflavin from photodegradation. The shear stress inherent to the melt mixing process did not cause bixin further degradation, where the coloring properties of the carotenoid in PLA were maintained. Despite increasing the oxygen permeability of the material, the use of acetyl tributyl citrate (ATBC) accelerated bixin migration to the food and prevented the increase of sunflower oil oxidative degradation. The development of PLA materials with carotenoids demonstrates that it is possible to produce colored food packaging that protects packaged food from photodegradation, besides being biodegradable and mechanically resistant. Through the knowledge generated, it is desirable to optimize the performance of PLA as an antioxidant material by modulating the carotenoid migration kinetics and increasing the material's oxygen barrier properties.

Keyword: bixin; migration; kinetic; oxidation; plasticizer.

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1 INTRODUÇÃO

O uso abusivo de materiais plásticos descartáveis tem causado diversos problemas à sociedade e ao meio ambiente, visto que os mesmos são resistentes à degradação e seu acúmulo causa, entre diversos outros problemas, a contaminação de solo e de águas. Ao considerar que embalagens são necessárias para permitir a distribuição e manutenção da qualidade de produtos alimentícios, o desenvolvimento de materiais que sejam biodegradáveis, de grau alimentício e produzidos a partir de fontes renováveis é de suma importância (BARLOW; MORGAN, 2013; PLASTICS EUROPE, 2018)

No Brasil, a produção de polímeros biodegradáveis é ainda incipiente, e apesar dos obstáculos existentes para o estabelecimento de um mercado para estes materiais, há inúmeras possibilidades a serem exploradas. O poli(ácido láctico) (PLA), polímero biodegradável produzido a partir do amido de milho, é um material promissor para o desenvolvimento de embalagens para alimentos, visto que sua biodegradabilidade é aliada a propriedades mecânicas comparáveis a polímeros *commodities* como polipropileno (PP) e poli tereftalato de etila (PET) (DOMENEK; FERNANDES-NASSAR; DUCRUET, 2017).

Até o presente momento, a utilização de PLA em alimentos é limitada à produção de embalagens rígidas, visto que o mesmo apresenta baixa flexibilidade e rigidez a temperatura ambiente (RUELLAN; DUCRUET; DOMENEK, 2014). Por apresentar alta permeabilidade ao oxigênio em comparação a outros polímeros e elevada transparência à luz UV e luz visível, o uso de PLA em alimentos é limitado a produtos não perecíveis ou que não se deteriorem pelo contato com a luz e oxigênio. Com o objetivo de ampliar a gama de aplicação e funcionalidade desse polímero, diversas modificações no PLA têm sido estudadas.

A incorporação de extratos naturais com propriedades antioxidantes e que absorvam radiação ultravioleta-visível (UV-VIS) em materiais poliméricos é uma das possibilidades de inovação sustentável para embalagens de alimentos (GOMEZ-ESTACA et al., 2014). O aumento das propriedades de barreira à luz das embalagens é uma forma de preservar a qualidade nutricional do produto embalado através da proteção de compostos fotossensíveis como ácidos graxos insaturados, vitaminas e compostos bioativos naturalmente presentes nos alimentos. Além disso, a presença de antioxidantes naturais na matriz polimérica é capaz de estender o *shelf-life* dos

alimentos através da migração dos compostos da embalagem para alimentos suscetíveis à oxidação (SAMSUDIN; SOTO-VALDEZ; AURAS, 2014a). A redução da necessidade de aditivação dos alimentos é outra possível vantagem do uso de embalagens contendo antioxidantes, onde a liberação gradual dos compostos para os alimentos pode ser mais efetiva que a incorporação direta de aditivos no produto (LÓPEZ-DE-DICASTILLO et al., 2012). Essa redução é acompanhada da menor exposição dos consumidores a aditivos sintéticos, cuja toxicidade é frequentemente relacionada a danos à saúde e ao meio ambiente (SHAHID; SHAHID-UL-ISLAM; MOHAMMAD, 2013).

Extratos de carotenoides têm sido amplamente utilizados em diferentes matrizes alimentícias, não somente pelo seu poder corante, mas também por seu potencial antioxidante (SHAHID-UL-ISLAM; RATHER; MOHAMMAD, 2016). A incorporação de carotenoides em materiais poliméricos, embora ainda pouco explorada, apresenta resultados positivos relacionados ao aumento da flexibilidade e estabilidade do polímero durante e após o seu processamento (KIRSCHWENG et al., 2017; LÓPEZ-RUBIO; LAGARON, 2010).

A presente tese de doutorado explora as potencialidades do uso de extratos de carotenoides como aditivos em filmes biodegradáveis de PLA para produzir embalagens que protejam os alimentos da oxidação e fotodegradação. Filmes de PLA contendo licopeno, beta-caroteno e bixina foram produzidos por diferentes técnicas, onde a estabilidade dos carotenoides à luz e ao processamento foi avaliada. As características dos filmes e sua interação com alimentos e líquidos simulantes também foi explorada.

A tese está organizada na forma de 4 capítulos. O Capítulo 1 compreende a fundamentação teórica utilizada como embasamento da pesquisa. O capítulo 2 apresenta os artigos científicos produzidos a partir dos resultados obtidos na primeira etapa da pesquisa, realizada no Instituto de Ciência e Tecnologia de Alimentos (ICTA) da UFRGS. O capítulo 3 apresenta os artigos científicos produzidos a partir dos resultados da segunda etapa da pesquisa, realizada na universidade francesa AgroParisTech. A correlação entre os resultados encontrados e a importância do conhecimento gerado para a ciência e inovação em embalagens para alimentos é discutida no Capítulo 4.

2 OBJETIVOS

2.1 Objetivo geral

Desenvolver filmes biodegradáveis de PLA incorporados de extratos de carotenoides que apresentem propriedades adequadas para uso como embalagem de alimentos sensíveis à luz e ao oxigênio.

2.2 Objetivos específicos

- Produzir filmes de PLA incorporados com carotenoides através da técnica de *casting* e do processamento em estado fundido;
- Caracterizar os filmes quanto às propriedades mecânicas, morfológicas, ópticas, térmicas e de barreira;
- Avaliar a influência de diferentes extratos de carotenoides nas propriedades de filmes de PLA;
- Avaliar a estabilidade de coloração dos filmes em diferentes condições de luz e temperatura, através de modelos cinéticos;
- Avaliar a migração dos carotenoides para diferentes alimentos, através do uso de líquidos simulantes;
- Obter um modelo matemático de migração dos carotenoides para diferentes meios, considerando sua degradação após a liberação;
- Compreender os efeitos do uso de altas temperaturas e tensões de cisalhamento - inerentes aos processamentos de polímeros em estado fundido – nas propriedades dos filmes de PLA;
- Compreender a influência do uso de plastificante nas propriedades dos filmes de PLA e carotenoides;
- Avaliar o desempenho dos filmes desenvolvidos na proteção de alimentos sensíveis à luz e ao oxigênio.

CAPÍTULO 1: REVISÃO DE LITERATURA

3 REVISÃO DE LITERATURA

3.1 Polímeros biodegradáveis

Plásticos sintéticos desempenham um papel de extrema importância na vida moderna. Em virtude de sua versatilidade, propriedades mecânicas e resistência química, esses materiais se tornaram indispensáveis em diversas áreas de aplicação, de forma que são utilizados em diversos bens de consumo duráveis e não duráveis. A produção mundial de plásticos, cuja grande maioria deriva-se do petróleo, é exorbitante: segundo dados da Plastics Europe (2018), aproximadamente 348 milhões de toneladas de plástico foram produzidas apenas em 2018. Ao considerar uma população mundial aproximada de 7,6 bilhões de pessoas, 45,8 kg de plástico foram produzidos per capita apenas naquele ano.

Atualmente, a maior fração – aproximadamente 40% - da quantidade de plástico produzida no mundo é destinada à produção de embalagens (PLASTICS EUROPE, 2018). O polímero não-biodegradável polietileno (PE) destaca-se como o material mais utilizado, representando aproximadamente 40% em massa dos materiais sintéticos utilizados em embalagens alimentícias. Polipropileno (PP), poliestireno (PS), poli tereftalato de etila (PET) e policloreto de polivinila (PVC), somados, representam o percentual restante na indústria de embalagens para alimentos (KIRWAN; PLANT; STRAWBRIDGE, 2011).

As características de resistência química que levaram os plásticos convencionais ao sucesso são as mesmas que atualmente os tornam uma ameaça ao meio ambiente. A não biodegradabilidade da maioria dos polímeros derivados do petróleo contribui para o excesso de lixo plástico depositado no meio ambiente, de forma que permanecem praticamente intactos ao longo dos anos e acarretam em sérios problemas ecológicos e ambientais (BRITO et al., 2011; CORNEILLIE; SMET, 2015; DO SUL; COSTA, 2014). Desta forma, o desenvolvimento de polímeros biodegradáveis produzidos a partir de fontes renováveis de energia contribui para a redução da dependência por materiais de origem fóssil, além de reduzir o impacto ambiental causado pelo acúmulo de plásticos convencionais na natureza (ARRIETA et al., 2014a).

Por definição, polímeros biodegradáveis são polímeros nos quais a degradação, inicialmente, resulta da ação de micro-organismos de ocorrência natural, tais como bactérias, fungos e algas (ASTM D6400-19). Este processo, que pode durar semanas ou até meses sob condições favoráveis de biodegradação, depende de fatores relacionados ao ambiente em que são depositados (umidade, atividade microbiana, temperatura, pH, presença de oxigênio) e das características do polímero a ser biodegradado (massa molar, cristalinidade, área de superfície, entre outros fatores) (SAINI, 2017).

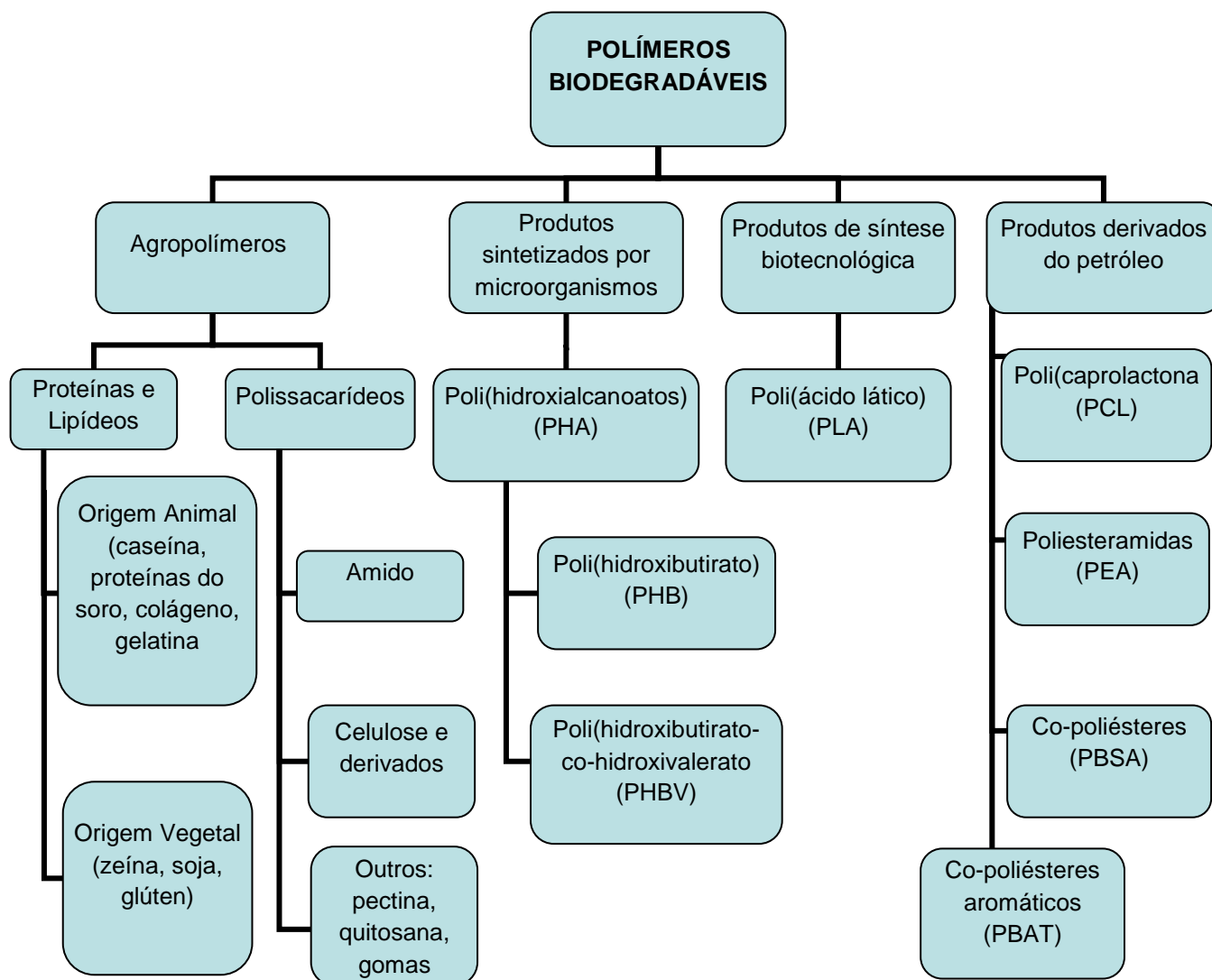
A obtenção de polímeros biodegradáveis pode ocorrer de diversas formas. Estes materiais podem ser produzidos a partir de fontes naturais e renováveis de energia, sejam elas de origem animal como crustáceos, leite ou partes animais (músculos, pele, tendões), ou a partir de fontes vegetais como frutas, hortaliças, tubérculos e madeira. Os açúcares, fibras, proteínas e/ou lipídeos obtidos a partir das fontes citadas podem ser utilizados na sua forma original ou modificados quimicamente. Além disso, os açúcares podem ser fermentados a fim de produzirem monômeros para posterior polimerização, onde a produção de poli(ácido láctico) (PLA) é um exemplo deste mecanismo (BABU; O'CONNOR; SEERAM, 2013; BORDES; POLLET; AVÉROUS, 2009).

Os polímeros biodegradáveis também podem ser sintetizados diretamente por microrganismos, os quais produzem o polímero a partir de substratos de carbono diversos. Como exemplo, podem ser citados os poli(hidroxialcanoatos) (PHA) - uma família de poliésteres produzidos por bactérias. De acordo com o metabolismo dos microrganismos e dos substratos por eles utilizados, diferentes monômeros, polímeros e copolímeros podem ser obtidos. O poli(hidroxibutirato) (PHB) destaca-se como um dos principais polímeros da família PHA (BORDES; POLLET; AVÉROUS, 2009).

Fontes fósseis como o petróleo ou a mistura entre petróleo e biomassa também podem dar origem a polímeros biodegradáveis, onde os materiais mais conhecidos são as poliésteramidas, as policaprolactonas (PCL), os copoliésteres alifáticos e os copoliésteres aromáticos (BRITO et al., 2011).

A Figura 3-1 apresenta um fluxograma com a classificação de alguns polímeros biodegradáveis de acordo com sua fonte de obtenção.

Figura 3.1 Classificação de polímeros biodegradáveis



Fonte: Adaptado de Avérous & Boquillon (2004)

3.1.1 Poli (ácido láctico) – PLA

Polímeros biodegradáveis derivados de fontes renováveis fazem parte de uma nova geração de materiais que desempenha um importante papel no desenvolvimento de economias sustentáveis. Entre esses polímeros, o poli(ácido láctico) (PLA) tem se destacado, pois apresenta boas propriedades mecânicas, alta transparência e fácil

processabilidade em comparação a outros polímeros biodegradáveis (BHARDWAJ; MOHANTY, 2007; CORNEILLIE; SMET, 2015).

O PLA é um polímero termoplástico produzido a partir da polimerização do ácido láctico proveniente da fermentação de açúcares, mais comumente da cana-de-açúcar ou milho. Inicialmente produzido como um polímero de pequena massa molar, o qual foi produzido e relatado pela primeira vez em 1932 e patenteado em 1954 pela DuPont, o poliéster alifático PLA foi ignorado por anos devido à instabilidade frente a reações hidrolíticas que degradavam o material. Na década de 60 o polímero voltou a receber atenção, quando sua estrutura facilmente hidrolisável foi percebida como vantajosa para aplicações no corpo humano. Como exemplo, pode-se citar sua utilização na engenharia de tecidos, onde o polímero substitui temporariamente tecidos lesados (cartilagem, ossos ou pele) e é reabsorvido pelo corpo humano (LOPES; JARDINI; MACIEL FILHO, 2012). Desde o período em que a aplicação do PLA mostrou-se relevante e efetiva em sistemas biológicos, o polímero tem sido aplicado e estudado nas áreas farmacêutica e biomédica (DOMENEK, SANDRA; COURGNEAU, CÉCILE; DUCRUET, 2013).

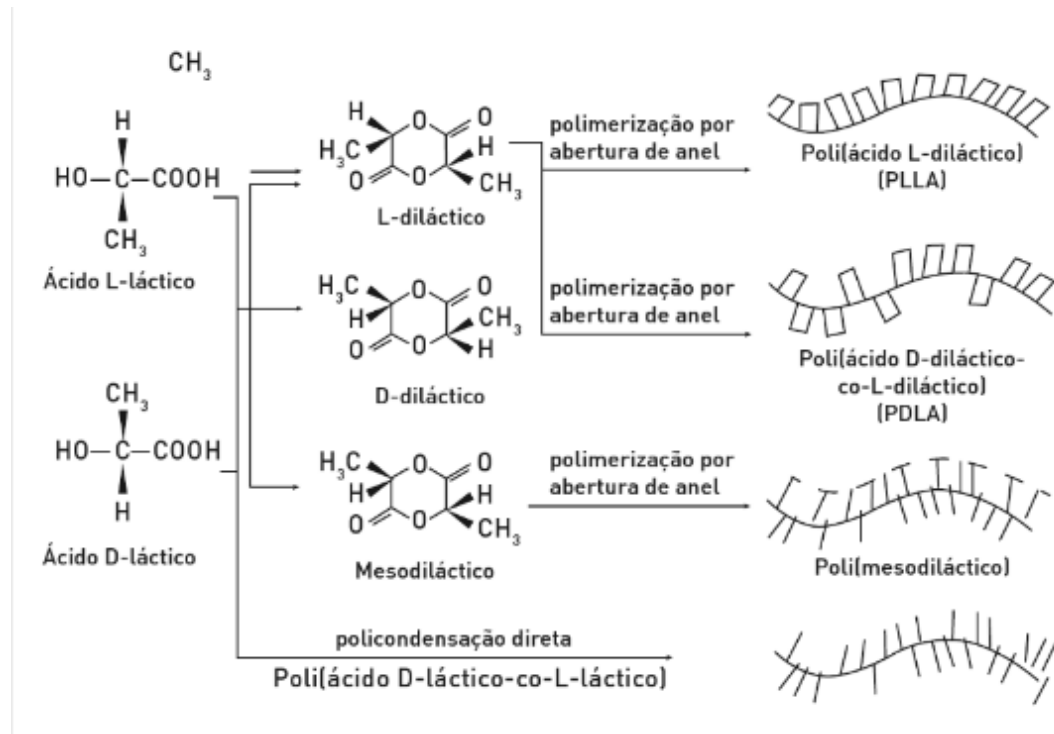
Produzido industrialmente em maior escala desde 2002, o PLA passou a apresentar preços mais competitivos, de forma a ser visto como um possível material para aplicação na indústria têxtil e de embalagens (DOMENEK, SANDRA; COURGNEAU, CÉCILE; DUCRUET, 2013). Atualmente, diversas resinas têm sido desenvolvidas a partir de polímeros de ácido láctico para diferentes aplicações (FARAH; ANDERSON; LANGER, 2016). Ainda que o mercado de polímeros biodegradáveis represente uma pequena parcela dos materiais produzidos atualmente (aproximadamente 1% do mercado), a estimativa é de que a produção de PLA atinja 600 mil toneladas/ano até 2025 (KATHURIA, 2015).

A síntese de PLA inicia pela extração de açúcares de fonte vegetal (milho, batata, beterraba ou cana de açúcar), seguida da conversão em dextrose. A dextrose sofre fermentação bacteriana, de forma a produzir o monômero ácido láctico. O ácido láctico produzido pode ser transformado em lactídeos na presença de um catalisador, os quais são posteriormente purificados via destilação à vácuo. O processo de polimerização (síntese do PLA) pode ser realizado através da policondensação direta do ácido láctico ou

através da polimerização pela formação de lactídeos via abertura de anel do lactídeo dimérico (BAJPAI; SINGH; MADAAN, 2014; RASAL; JANORKAR; HIRT, 2010). A

Figura 3.2 - Rotas de síntese do PLA apresenta, resumidamente, as possíveis rotas de síntese do PLA.

Figura 3.2 - Rotas de síntese do PLA



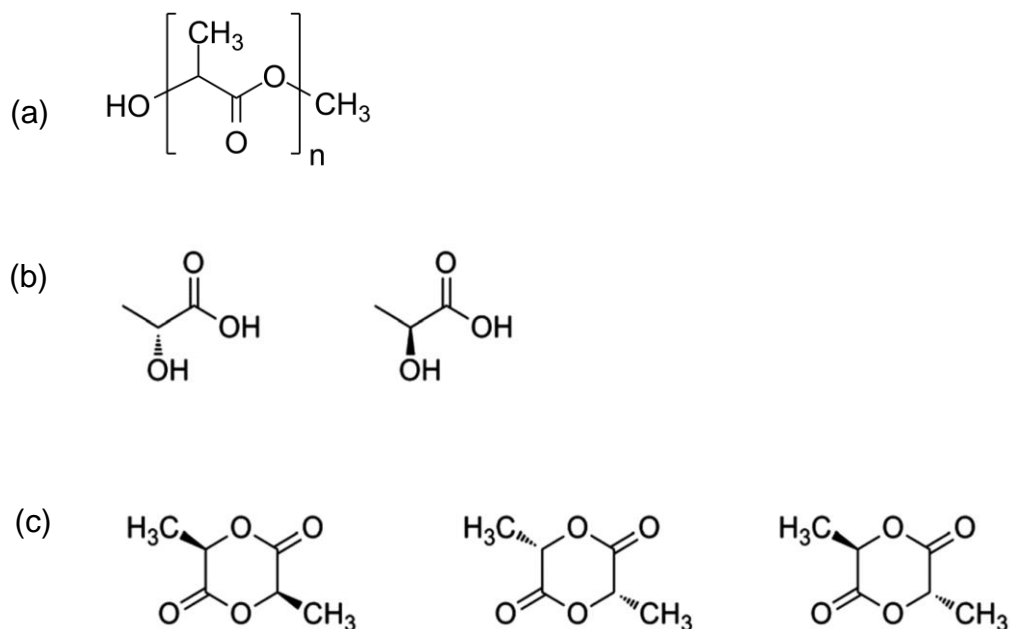
Fonte: Adaptado de Cheng et al. (2009)

A nomenclatura utilizada na literatura para estes polímeros é, por vezes, contraditória: polímeros derivados da policondensação direta do ácido láctico deveriam ser chamados de poli (ácido láctico), enquanto aqueles sintetizados pela abertura do anel de lactídeos seriam chamados de poli (lactídeos). Entretanto, ambos são frequentemente referidos como PLA (DOMENEK, SANDRA; COURGNEAU, CÉCILE; DUCRUET, 2013). A Figura 3.3a apresenta a estrutura molecular do PLA

De uma maneira geral, o PLA se apresenta como um polímero semi-cristalino ou completamente amorfo (BAJPAI; SINGH; MADAAN, 2014). Ao considerar a atividade óptica do ácido láctico, a proporção entre os enantiômeros opticamente ativos (L ou D) e

racêmicos (L e D) que compõe o polímero permite a produção de uma grande variedade de polímeros PLA, as quais são capazes de atender diversos requerimentos de processamento (AURAS; SINGH; SINGH, 2005; LIM; AURAS; RUBINO, 2008; RASAL; JANORKAR; HIRT, 2010). Na **Figura 3.3** estão representados os estereoisômeros de ácido láctico, lactídeos e a estrutura genérica do PLA.

Figura 3.3 - Estrutura molecular do PLA e estereoisômeros de ácido láctico e lactídeos



(a) Estrutura molecular genérica do PLA

(b) Estereoisômeros de ácido láctico: L-ácido láctico (esquerda) e D-ácido láctico (direita);

(c) Estereoisômeros de lactídeos: da esquerda para a direita: L-lactídeo, D-lactídeo e meso-lactídeo.

Fonte: Corneillie & Smet (2015)

As propriedades do PLA dependem de diversos fatores, tais como temperatura de processamento e resfriamento, estereorregularidade, composição de isômeros, entre

outros (NAMPOOTHIRI; NAIR; JOHN, 2010). De uma maneira geral, o PLA produzido comercialmente é um copolímero entre os ácidos D-polilático e L-polilático. A porcentagem de isômeros D e L, também conhecida como pureza óptica, afeta as propriedades do PLA de forma bastante significativa (GARLOTTA, 2002).

Polímeros com altos níveis de L-lactídeos tendem a apresentar maior cristalinidade. Como exemplo pode-se citar o poli-L-lactídeo (PLLA), produto resultante da polimerização do L-lactídeo que possui cristalinidade de aproximadamente 37%, temperatura de transição vítrea entre 50 e 80°C e temperatura de fusão entre 150 e 180°C. Já os polímeros com elevados níveis de D-lactídeos (mais que 15% em sua composição) tendem a ser amorfos, de forma a apresentarem menores temperaturas de transição vítrea e fusão. De maneira geral, polímeros que apresentam estereorregularidade tendem a ser mais cristalinos que aqueles que não a possuem (URAYAMA; MOON; KIMURA, 2003).

A estrutura polimérica e a cristalinidade do PLA são capazes de afetar suas propriedades térmicas, ópticas, mecânicas e de barreira (DOMENEK, SANDRA; COURGNEAU, CÉCILE; DUCRUET, 2013). Consequentemente, essas características terão grande influência no seu desempenho mecânico, seu comportamento frente ao processamento térmico e suas propriedades de biodegradação (AURAS; HARTE; SELKE, 2004).

Quando comparado com polímeros *commodities* à base de petróleo (Tabela 3.1), o PLA apresenta semelhantes propriedades físicas, ópticas e de barreira (LIM; AURAS; RUBINO, 2008). Apresenta tensão na ruptura e módulo de Young maiores que polietileno de alta densidade (PEAD), polipropileno (PP) e poliestireno (PS). No entanto, apesar de sua alta resistência à tração, a elevada temperatura de transição vítrea do PLA (60 °C) faz com que o polímero seja rígido e quebradiço a temperatura ambiente, limitando seu desempenho mecânico (DORGAN; LEHERMEIER; MANG, 2000).

Tabela 3.1 - Propriedades físicas do poli(ácido láctico) (PLA) e dos polímeros *commodities* poliestireno (PS), polipropileno isotático (i-PP) e polietileno tereftalato (PET)

	PLA	PS	i-PP	PET
Densidade relativa	1,24	1,04 – 1,06	0,91	1,37
Claridade	transparente	transparente	translúcido	transparente
PROPRIEDADES MECÂNICAS				
Tração na Ruptura (Mpa)	48 – 110	34 – 46	21 - 37	47
Módulo de Young (GPa)	3,5 – 3,8	2,9 – 3,5	1,1 – 1,5	3,1
Elongação (%)	2,5 – 100	3 – 4	20 - 800	50 - 300
PROPRIEDADES TÉRMICAS				
Temperatura Transição Vítreia (°C)	60	95	0	75
Temperatura de Fusão (°C)	153	NA	163	250
Temperatura Processamento (°C)	210	230	225	275

NA: não aplicável

Fonte: Adaptado de Carrasco et al., 2010.

A fim de melhorar a flexibilidade, reduzir a fragilidade e aumentar as possibilidades de aplicação do PLA, a incorporação de plastificantes tem sido investigada. A adição de plastificantes, porém, apresenta desvantagens como redução da resistência à tração e, em alguns casos, redução da estabilidade térmica do material (RUELLAN; DUCRUET; DOMENEK, 2014).

Quanto às propriedades de barreira, o PLA apresenta coeficientes de permeabilidade ao CO₂, O₂, N₂ e água menores que poliestireno (PS) e maiores que polietileno tereftalato (PET) (AURAS; SINGH; SINGH, 2005; AURAS; HARTE; SELKE, 2004; BAO et al., 2006).

A solubilidade do PLA depende fortemente de sua massa molar e grau de cristalinidade, sendo insolúvel em água, álcool ou éter isopropílico. Para enantiômeros puros de PLA, clorofórmio e outros compostos orgânicos clorados, assim como furano, dioxano, dioxolano e piridina funcionam como bons solventes. Já para os polímeros de PLA que não são enantiomericamente puros, os solventes mais adequados para solubilizar o PLA são acetato de etila, tetra-hidrofurano, acetona, dimetilformamida,

sulfóxido de dimetila, xileno, metil-etil-cetona e lactato de etila (DOMENEK, COURGNEAU, DUCRUET, 2013). Devido ao CH₃ em sua cadeia lateral, o PLA apresenta alta hidrofobicidade quando comparado a outros polímeros (JOHN; THOMAS, 2008).

3.1.1.1 Modificações do PLA

Apesar do PLA apresentar propriedades de resistência à tração, processabilidade e transparência comparáveis aos polímeros *commodities* provenientes do petróleo, sua baixa elasticidade e alta rigidez à temperatura ambiente limita sua aplicabilidade e suas propriedades funcionais. Além disso, o PLA apresenta alta permeabilidade a gases, de forma que diversos estudos têm sido conduzidos a fim de melhorar essas características (DOMENEK; FERNANDES-NASSAR; DUCRUET, 2017).

Entre as possibilidades existentes para alterar as propriedades do PLA destaca-se a produção de misturas com outros materiais (CARMONA et al., 2015; YANG et al., 2015), o uso de plastificantes (CLASEN; MULLER; PIRES, 2015; FERRI et al., 2016; SHIRAI et al., 2016), a incorporação de nanopartículas (ARROYO et al., 2010; YANG et al., 2015), reações de reticulação (*crosslinking*) (SHAYAN et al., 2015; SOARES et al., 2013), uso de agentes compatibilizantes (AKRAMI et al., 2016) e a incorporação de compostos bioativos (CRUZ-ZÚÑIGA et al., 2016; LÓPEZ DE DICASTILLO et al., 2017)). Os objetivos das citadas modificações variam, e vão desde a redução da temperatura de transição vítrea do polímero, com aumento da flexibilidade e resistência ao impacto, até a produção de embalagens com propriedades antioxidantes e/ou antimicrobianas.

3.2 Embalagens antioxidantes produzidas a partir de PLA e antioxidantes naturais

Nas últimas décadas, diversos estudos têm sido conduzidos a fim de desenvolver materiais que supram as necessidades de uma nova geração de embalagens para alimentos. Além de proteger os alimentos do meio externo, também se espera que a embalagem possa prolongar a vida de prateleira dos alimentos e que seu uso cause o menor dano possível ao meio ambiente (DAINELLI et al., 2008; GOMEZ-ESTACA et al., 2014; MELLINAS et al., 2016).

Embalagens ativas são embalagens capazes de interagir com os alimentos através da liberação ou absorção de compostos que mantenham ou até mesmo melhorem suas características químicas e/ou organolépticas (YILDIRIM et al., 2018). Dentro desta categoria, as embalagens com propriedades antioxidantes têm grande importância. Os antioxidantes presentes nestas embalagens, quando em contato com os alimentos embalados, retardam as consequências geradas pela deterioração oxidativa dos produtos, tais como: desenvolvimento de sabores indesejados; alteração de cor; alteração de aroma; degradação de nutrientes; entre outros (GOMEZ-ESTACA et al., 2014). Ao considerar a preocupação com a poluição causada pelo uso de plásticos sintéticos não biodegradáveis, há um crescente interesse no desenvolvimento de embalagens biodegradáveis antioxidantes produzidas a partir do PLA.

O processamento de polímeros via extrusão envolve temperaturas elevadas e tensões de cisalhamento, as quais podem levar a perdas significativas dos antioxidantes ao longo do processo. Logo, a volatilidade e termossensibilidade dos antioxidantes utilizados no processamento de polímeros devem ser observadas (INIGUEZ-FRANCO et al., 2012; MANZANAREZ-LÓPEZ et al., 2011a; SAMSUDIN; SOTO-VALDEZ; AURAS, 2014a). Neste contexto, a maior estabilidade de compostos sintéticos como butil-hidroxitolueno (BHT), butil-hidroxi-anisol (BHA), e terc-butil-hidroquinona (TBHQ) apresenta vantagem quando em comparação aos compostos naturais, que de maneira geral são mais suscetíveis a degradação pelo calor. Entretanto, o potencial toxicológico dos antioxidantes sintéticos gera preocupação em função da possibilidade de migração dos mesmos para o alimento embalado (LÓPEZ-DE-DICASTILLO et al., 2012). Desta forma, estudos que buscam substituir os antioxidantes sintéticos por compostos naturais, com o objetivo de produzir embalagens com propriedades antioxidantes, têm limitado o uso de antioxidantes sintéticos à proteção das reações de oxidação polímero ao longo do processamento (BYUN; KIM; WHITESIDE, 2010).

O desenvolvimento de embalagens antioxidantes produzidas a partir de PLA e antioxidantes naturais tem sido objeto de estudo de diversas pesquisas. A seguir, são apresentados os estudos que realizaram a incorporação de antioxidantes naturais na matriz polimérica de PLA.

3.2.1 Influência da presença de antioxidantes naturais nas propriedades de filmes de PLA

A introdução de antioxidantes naturais à matriz polimérica de filmes de PLA pode acarretar na alteração de diversas propriedades dos materiais. Enquanto que as propriedades mecânicas – fortemente influenciadas pelas propriedades térmicas – são determinantes para o tipo de embalagem a ser produzida (rígidas, semi-rígidas ou flexíveis), as propriedades de barreira a gases e à luz influenciam direta e indiretamente o desempenho dos materiais como embalagem antioxidante.

3.2.1.1 Propriedades térmicas e mecânicas

A redução na temperatura de transição vítrea (T_g) dada pela introdução de antioxidantes naturais em filmes de PLA é observada com frequência, e na maioria das vezes leva ao aumento na flexibilidade e elasticidade dos mesmos. Este efeito plastificante, somado à redução da resistência à tração dos filmes, foi observado pela introdução de tocoferol (0,01% - 5%) e timol (6-8%) em filmes de PLA (BYUN; KIM; WHITESIDE, 2010; DI MAIO et al., 2014; GONÇALVES et al., 2013; RAMOS et al., 2014a, 2014b). Alguns estudos observaram um aumento na elasticidade dada pela adição de palmitato de ascorbila (2%) e tocoferol (1%), porém sem alteração na T_g dos filmes (JAMSHIDIAN et al., 2012a). Uma possível explicação para a diminuição da resistência dos polímeros à deformação, quando introduzidos de compostos de pequeno tamanho molecular, baseia-se na teoria da lubrificação. A teoria propõe que a ação plastificante é resultante da diminuição do atrito intermolecular entre os segmentos poliméricos, facilitando o movimento das macromoléculas umas sobre as outras (PLATZER, 1982).

O aumento na resistência à tração dos materiais, embora menos frequente, também é relatado após a introdução de antioxidantes naturais. O uso combinado de resveratrol e tocoferol (HWANG et al., 2012a, 2013a), além de causar aumento na flexibilidade, também aumentou a força dos filmes (aumento da resistência à tração e módulo de Young). Segundo os autores, a presença de três hidroxilas na molécula de resveratrol possibilitou a formação de pontes de H com a cadeia de PLLA, o que restringe a mobilidade da parte amorfa e aumenta a força dos filmes. Entretanto, nem todas as

concentrações favoreceram as propriedades mecânicas dos filmes, visto que altas concentrações de tocoferol causaram redução na elasticidade e resistência à tração dos filmes.

Já a introdução de extrato de alho e merkén - condimento chileno rico em compostos fenólicos - prejudicaram as propriedades mecânicas de filmes de PLA (LLANA-RUIZ-CABELLO et al., 2015; LÓPEZ DE DICASTILLO et al., 2017). A incorporação dos antioxidantes possivelmente causou o rompimento de ligações intra e intermoleculares, reduzindo o percentual de elasticidade e resistência à tração dos materiais desenvolvidos.

3.2.1.2 Barreira à luz e ao oxigênio

O contato com a luz e oxigênio aceleram a fase de indução da deterioração oxidativa de alimentos, em especial da fração lipídica (GUTIERREZ; BOYLSTON; CLARK, 2018; MICHIELS; PUYVELDE; SELS, 2017), tornando-se desejável que os materiais utilizados na embalagem de alimentos gordurosos ofereçam barreira a estes elementos.

De maneira geral, filmes de PLA apresentam elevada transparência e pouca ou nenhuma barreira à passagem da luz na região do UV-Visível (800-250nm). Estudos demonstram que a incorporação de antioxidantes naturais em filmes de PLA reduz a transparência dos filmes, especialmente na região do visível. A redução da transmitância da luz em filmes adicionados de tocoferol, resveratrol e astaxantina coincide com a região referente ao comprimento de onda de absorbância dos compostos adicionados (HWANG et al., 2012a; MANZANAREZ-LÓPEZ et al., 2011a; SAMSUDIN; SOTO-VALDEZ; AURAS, 2014b; SOTO-VALDEZ; AURAS; PERALTA, 2011).

Em relação à barreira dos filmes ao oxigênio, a permeação de gases em polímeros semi-cristalinos é limitada à região amorfa dos materiais, existindo uma relação direta entre a quantidade de volumes vazios e a permeabilidade ao oxigênio (BYUN et al., 2007). A alteração na permeabilidade a gases em filmes de PLA na presença de antioxidante é fortemente dependente da concentração, estrutura molecular (HWANG et al., 2012b) e interação entre os antioxidantes adicionados com a matriz polimérica (GONÇALVES et al., 2013).

A introdução de astaxantina (0,7%), tocoferol livre (2%) e encapsulado (5%) não apresentou efeitos na permeabilidade ao O₂ em filmes de PLA (DI MAIO et al., 2014; GONÇALVES et al., 2013; SAMSUDIN; SOTO-VALDEZ; AURAS, 2014a). Enquanto isso, a concentração de 0,01% do antioxidante reduziu a barreira ao O₂ em filmes de PLA adicionados de BHT e plastificante polietilenoglicol 400 (BYUN; KIM; WHITESIDE, 2010). O estudo de Byun (2010) atribui o aumento na permeabilidade ao oxigênio ao caráter hidrofóbico do tocoferol, que facilitaria a transferência de oxigênio através do filme, enquanto o uso de plastificante promoveu redução na permeabilidade ao oxigênio pela redução de volumes vazios na matriz do polímero.

3.2.2 Atividade antioxidante e estudos de liberação em filmes de PLA com antioxidantes naturais

No desenvolvimento de embalagens antioxidantes, a liberação dos compostos para diferentes alimentos e a atividade antioxidantes dos filmes desenvolvidos é o enfoque de grande parte dos estudos que introduziram antioxidantes naturais em filmes a base de PLA. Estes estudos estão apresentados na Tabela 3.2, a qual apresenta os principais resultados relacionados a ação antioxidante e estudos de liberação dos filmes desenvolvidos.

Entre os antioxidantes naturais utilizados para produção de embalagens a base de PLA, o composto natural alfa-tocoferol (AT) é um dos mais explorados. Os filmes de PLA e AT apresentaram atividade antioxidante em todas as concentrações estudadas, as quais variaram entre 0,01% e 9,4% (BYUN; KIM; WHITESIDE, 2010; JAMSHIDIAN; TEHRANY; DESOBRY, 2013; MANZANAREZ-LÓPEZ et al., 2011a; MARCOS et al., 2014; VAN AARDT et al., 2007). Em comparação aos antioxidantes sintéticos BHA, BHT, PG e TBHQ, o AT apresentou a migração mais lenta para diversos líquidos simulantes, justificada pelo seu maior tamanho molecular e consequente maior resistência à difusão pela matriz polimérica. Dada a característica hidrofóbica do AT, pouca ou nenhuma migração do antioxidante foi percebida para soluções de maior polaridade, como água e solução hidroalcoólica etanol 10% (JAMSHIDIAN; TEHRANY; DESOBRY, 2013). A velocidade de migração do antioxidante foi mais elevada para etanol 95% (líquido

simulante de alimentos gordurosos), seguido de óleo vegetal (MANZANAREZ-LÓPEZ et al., 2011a).

O tamanho molecular dos compostos teve influência direta na velocidade de migração dos mesmos, onde compostos menores apresentaram os maiores coeficientes de difusão (HWANG et al., 2013a; JAMSHIDIAN; TEHRANY; DESOBRY, 2013). Além disso, o caráter hidrofílico ou hidrofóbico dos antioxidantes incorporados à matriz de PLA foi determinante para sua liberação e ação antioxidante em líquidos de maior ou menor polaridade. Filmes produzidos a partir de extratos vegetais de composição variada, contendo antioxidantes hidrofílicos e lipofílicos, apresentaram ampla atividade antioxidante, capaz de evitar reações de oxidação em diferentes meios (LÓPEZ DE DICASTILLO et al., 2017). Por outro lado, filmes com extratos contendo majoritariamente antioxidantes hidrofílicos, como extrato de tomilho, apresentaram maior atividade antioxidante em meio aquoso (GALLEGO; HAKKARAINEN; ALMAJANO, 2017).

Os testes de liberação dos antioxidantes para diferentes alimentos e líquidos simulantes foram conduzidos em diferentes temperaturas, de 4 à 43 °C, sendo a velocidade de liberação mais elevada para as maiores temperaturas testadas. Apesar da solução hidroalcoólica etanol 95% ser utilizada como líquido simulante para alimentos gordurosos, a migração de catequina e epicatequina para este simulante foi maior que a observada para o óleo vegetal (INIGUEZ-FRANCO et al., 2012). O estudo de Iniguez-Franco (2012) sugere que o etanol possa penetrar na matriz de PLA, interferindo na liberação dos antioxidantes.

Enquanto o uso de altas temperaturas durante o processo de extrusão foi danoso para os antioxidantes astaxantina, resveratrol, timol, catequina e epicatequina (INIGUEZ-FRANCO et al., 2012; RAMOS et al., 2014c; SAMSUDIN; SOTO-VALDEZ; AURAS, 2014b; SOTO-VALDEZ; AURAS; PERALTA, 2011), os quais tiveram perdas de até 38% durante o processamento, maiores temperaturas aumentaram a ação antioxidante de filmes produzidos com lignina e rutina. Isso deve à formação de compostos com maiores propriedades antioxidantes durante o processo, tais como reações de liberação de monômeros fenólicos da lignina (DOMENEK et al., 2013) e à deglicosilação de rutina em isoquercitina e quercetina em altas temperaturas (150-160°C) (CRUZ-ZÚÑIGA et al., 2016).

Tabela 3.2 - Filmes produzidos a partir de PLA e antioxidantes naturais: estudos de liberação e ação antioxidante

Matriz polimérica/ aditivos	Composto antioxidante (AO)	Método de análise da atividade antioxidante	Teste de liberação	Principais resultados e observações relacionados à incorporação dos AOs	Referência
Filmes produzidos via técnica de <i>casting</i>					
PLGA (PLA:PGA - 50:50)	BHT (1%) + BHA(1%); α -tocoferol (2%)	Estabilidade oxidativa (níveis de hexanal, pentanal e heptanal) de leite integral em pó e leite em pó	Água; óleo (Miglyol 812). 4 e 25 °C 8 semanas	<ul style="list-style-type: none"> • Contato com BHA e BHT reduziu níveis de pentanal em leite em pó • α-tocoferol reduziu formação de pentanal em leite em pó 	VAN AARDT et al., 2007
PLA	Extrato de tomilho (3%); extrato de alecrim (3%); BHT (1,5%); ácido caféico (1.5%); ácido rosmarínico (1.5%); epigallocatequina (1.5%)	Estabilidade oxidativa (peróxidos, pH, TBARS) de emulsão óleo de girassol em água (10%) coberta com filmes, a 33 °C	Água; etanol 95%. 36 °C 10 dias (avaliado pela perda de massa dos filmes)	<ul style="list-style-type: none"> • Extrato de tomilho e alecrim (fonte de compostos fenólicos): maior solubilidade em água e consequente maior migração dos compostos para água; maior atividade antioxidante que os demais AOs 	GALLEGO; HAKKARAIN EN; ALMAJANO, 2017
PLA / Irgafos	α -tocopherol (2%); AOs sintéticos: palmitato de ascorbila; BHA, BHT, PG, TBHQ (1%)	não realizado	Água; etanol 10%; etanol 50%; etanol 95%. 20 e 40 °C	<ul style="list-style-type: none"> • AOs hidrofóbicos: maior migração para líquidos de menor polaridade (etanol 95%, seguido de etanol 50%) • Não houve liberação de AT e BHT para etanol 10% • AT teve a liberação mais lenta - maior tamanho molecular • Palmitato de ascorbila - totalmente degradado durante o preparo dos filmes 	JAMSHIDIAN ; TEHRANY; DESOBRY, 2013

Matriz polimérica/ aditivos	Composto antioxidante (AO)	Método de análise da atividade antioxidante	Teste de liberação	Principais resultados e observações relacionados à incorporação dos AOs	Referência
Filmes produzidos via extrusão ou câmara de mistura					
PLA-PHB / Acetil tributil citrato (ATBC)	Catequina, 5%	DPPH do líquido simulante - etanol 50%	Etanol 50% 40 °C 20 dias	<ul style="list-style-type: none"> • Atividade antioxidante proporcional à liberação da catequina, a qual foi maior na presença de plastificante. • Plastificante favoreceu a liberação dos AOs 	ARRIETA et al., 2014a
PLA -ácido húmico	Ácido húmico, 0,01%	DPPH e ABTS (reação de 24h, temperatura ambiente)	não realizado	<ul style="list-style-type: none"> • PLA-HA apresentou atividade como <i>radical scavenging</i> - não houve comparação com PLA puro. 	BISHAI et al., 2014
PLA / polietilenoglicol 400 / BHT	α-tocoferol, 0,01%	DPPH (reação filme em metanol, após agitação vigorosa)	não realizado	<ul style="list-style-type: none"> • Aumento da atividade antioxidante relacionado à presença de tocoferol • Filme com BHT: 14% inibição do DPPH; filme com BHT+ tocoferol: 90% inibição do DPPH • Aumento da atividade antioxidante relacionado à presença de tocoferol 	BYUN; KIM; WHITESIDE, 2010
PLLA	Rutina, 0,17-3,96 %, transformada em isoquercetina e quercetina	DPPH (em metanol, após 24h a 40°C)	não realizado	<ul style="list-style-type: none"> • Maior atividade antioxidante em filme com maior teor de quercetina • Calor promove a deglicosilação de rutina em isoquercetina e posteriormente em quercetina. Processamento a 150–160 °C promoveu maior conversão de rutina em quercetina. 	CRUZ-ZÚÑIGA et al., 2016

Matriz polimérica/ aditivos	Composto antioxidante (AO)	Método de análise da atividade antioxidante	Teste de liberação	Principais resultados e observações relacionados à incorporação dos AOs	Referência
PLA	Merkén, 3 e 5%	DPPH, ABTS e FRAP (em etanol, etanol 50%, água)	Etanol 10%; etanol 95%. 40 °C (resposta medida via ABTS)	<ul style="list-style-type: none"> • Merkén, fonte de AOs hidrofílicos e lipofílicos: catequina, miricetina, rutina, ácidos gálico e elágico • Maior ação antioxidante (ABTS) em etanol 50% - maior migração de compostos hidrofílicos e hidrofóbicos. 	LÓPEZ DE DICASTILLO et al., 2017
PLA	Lignina 1-10%	DPPH (em metanol e etanol 95%)	Etanol 95%. T ambiente 24 h	<ul style="list-style-type: none"> • Lignina (fonte de polifenóis); 1% de lignina: filme com a menor taxa de migração e menor atividade antioxidante; 5 e 10% de lignina: inibição similar (50% DPPH). • Quanto maior o tempo de aquecimento da lignina, maior sua atividade antioxidante; aquecimento promove liberação de monômeros fenólicos da lignina. 	DOMENEK et al., 2013
PLA	Extrato de alga (<i>fucus spiralis</i>) liofilizado (8%) + ácido sórbico (0,5 - 1%)	Estabilidade oxidativa de peixe Linguado-areeiro (peróxidos e grau de fluorescência)	não realizado	<ul style="list-style-type: none"> • Leve redução da oxidação da gordura do peixe (menor grau de fluorescência), porém sem diferenças na formação de peróxidos • Extrato da alga <i>fucus spiralis</i> – compostos antimicrobiano e fonte em compostos fenólicos 	GARCÍA-SOTO et al., 2015

Matriz polimérica/ aditivos	Composto antioxidante (AO)	Método de análise da atividade antioxidante	Teste de liberação	Principais resultados e observações relacionados à incorporação dos AOs	Referência
PLA/ montmorilona-nita modificada (2,5% e 5%)	Timol (8%)	DPPH do composto AO (timol), extraído com metanol	não realizado	<ul style="list-style-type: none"> • Perda ~30% do AO durante processamento (evaporação e degradação) • Montmorilonita reduziu a evaporação do AO no processamento 	RAMOS et al., 2014b
PLA (100%); PLA (80%) e amido (20%)	Resveratrol (0-5%); α -tocoferol (0-5%)	não realizado	Etanol 100% 13, 23, 43 °C	<ul style="list-style-type: none"> • Migração dos AOs favorecida pela presença de amido • Presença de dois AOs favoreceu difusão dos compostos em comparação a um único AO. • Difusão do resveratrol foi maior que a do tocoferol (menor tamanho molecular, maior mobilidade). 	HWANG et al., 2013b
PLA	Catequina (1,28%); epicatequina (1,50%)	DPPH, análise em metanol e etanol 95% (20, 30, 40 e 50 °C)	Etanol 95%. 20, 30, 40, 50°C Etanol 50%; água; óleo; emulsão água:óleo (50:50). 40°C	<ul style="list-style-type: none"> • Migração de 17- 19% a 20 °C, e 99% a 50 °C em etanol 95%. Não houve migração para água, óleo e emulsão. Perdas de 22 - 38% dos AOs no processo de extrusão. Possível degradação de AOs nos testes de migração a 40 e 50 °C. • Etanol penetra na matriz de PLA, interferindo na liberação dos AOs, o que não ocorre com o óleo 	IÑIGUEZ-FRANCO et al., 2012

Matriz polimérica/ aditivos	Composto antioxidante (AO)	Método de análise da atividade antioxidante	Teste de liberação	Principais resultados e observações relacionados à incorporação dos AOs	Referência
PLA	Extrato de folhas de <i>Camelia sinensis</i> L. (1% e 2%)	DPPH, proteção ao beta-caroteno e estabilidade oxidativa de salmão defumado em contato com o filme	não realizado	<ul style="list-style-type: none"> Filmes contendo 1% de extrato apresentaram atividade antioxidante, enquanto a presença de 2% teve ação pró-oxidante no salmão após 60 de armazenamento. Redução na permeabilidade ao vapor de água. 	MARTINS et al., 2018
PLA	Extrato de <i>Allium spp.</i> (2%, 5% e 6.5%)	DPPH e ABTS em metanol, 25 °C	não realizado	<ul style="list-style-type: none"> Ausência de atividade antioxidante. Filme com atividade antimicrobiana 	LLANA-RUIZ-CABELLO et al., 2015
PLA	α -tocoferol (2,58 %)	Estabilidade oxidativa (peróxidos) de óleo de girassol em contato com filme imerso, na presença de luz	Óleo de coco 20, 30, 40°C; Etanol 100% 13, 23, 33, 43°C	<ul style="list-style-type: none"> Liberação menor em óleo do que em etanol Maior migração a 40 e 43 °C 	MANZANAR EZ-LÓPEZ et al., 2011
PLA / Ecoflex ©	Extrato de folha de azeitona adicionado de oleuropeína (5,6-7,4%); α -tocoferol (5,6-9,4%)	DPPH em metanol (30 °C)	Etanol 95%	<ul style="list-style-type: none"> Rápida difusão dos AOs para etanol 95% - fraca interação AO/matriz polimérica. Presença do PLA reduziu a migração do tocoferol. Filmes com tocoferol: maior atividade antioxidante. 	MARCOS et al., 2014

Matriz polimérica/ aditivos	Composto antioxidante (AO)	Método de análise da atividade antioxidante	Teste de liberação	Principais resultados e observações relacionados à incorporação dos AOs	Referência
PLA	Extrato de calêndula (2% de astaxantina)	Estabilidade oxidativa de óleo de girassol embalado (peróxidos)	Etanol 95% 30, 40 °C	<ul style="list-style-type: none"> • Lenta migração da astaxantina para etanol 95% • Efeito antioxidante tardio: óleo embalado em filme com AO apresentou menores valores de peróxidos somente após partir de 15 dias de armazenamento. • 25% do extrato perdido no processamento 	SAMSUDIN; SOTO-VALDEZ; AURAS, 2014a
PLA	Resveratrol (0, 1 e 3%)	não realizado	Etanol 100% 9, 23, 33, 43 °C	<ul style="list-style-type: none"> • 23-37% do AO permaneceu no filme a 9 °C, 1-5% do AO permaneceu no filme a 43 °C. • Taxa de difusão semelhante para as diferentes concentrações de AO usadas. • 16-25% do resveratrol perdido no processamento 	SOTO-VALDEZ; AURAS; PERALTA, 2011

Matriz polimérica/ aditivos	Composto antioxidante (AO)	Método de análise da atividade antioxidante	Teste de liberação	Principais resultados e observações relacionados à incorporação dos AOs	Referência
PLA bicamada e multicamada produzido por <i>electrospinning</i>	Ácido gálico (40%)	DPPH em solução salina	Solução salina (NaCl)	<ul style="list-style-type: none"> • Aumento na estabilidade térmica dos filmes • Filmes bicamada apresentaram maior taxa de migração do AO que filmes multicamada. A atividade antioxidante dos filmes foi proporcional à migração de ácido gálico. 	QUILES-CARRILLO et al., 2019
PLA grafitizado glicidil metacrilato/ nanocristais de celulose	Nanopartículas de lignina	DPPH em metanol	Etanol 10%; iso-octano	<ul style="list-style-type: none"> • Ação antioxidante da lignina é aumentada pela presença da celulose (ação sinérgica). • Maior migração de compostos para iso-octano 	YANG et al., 2016

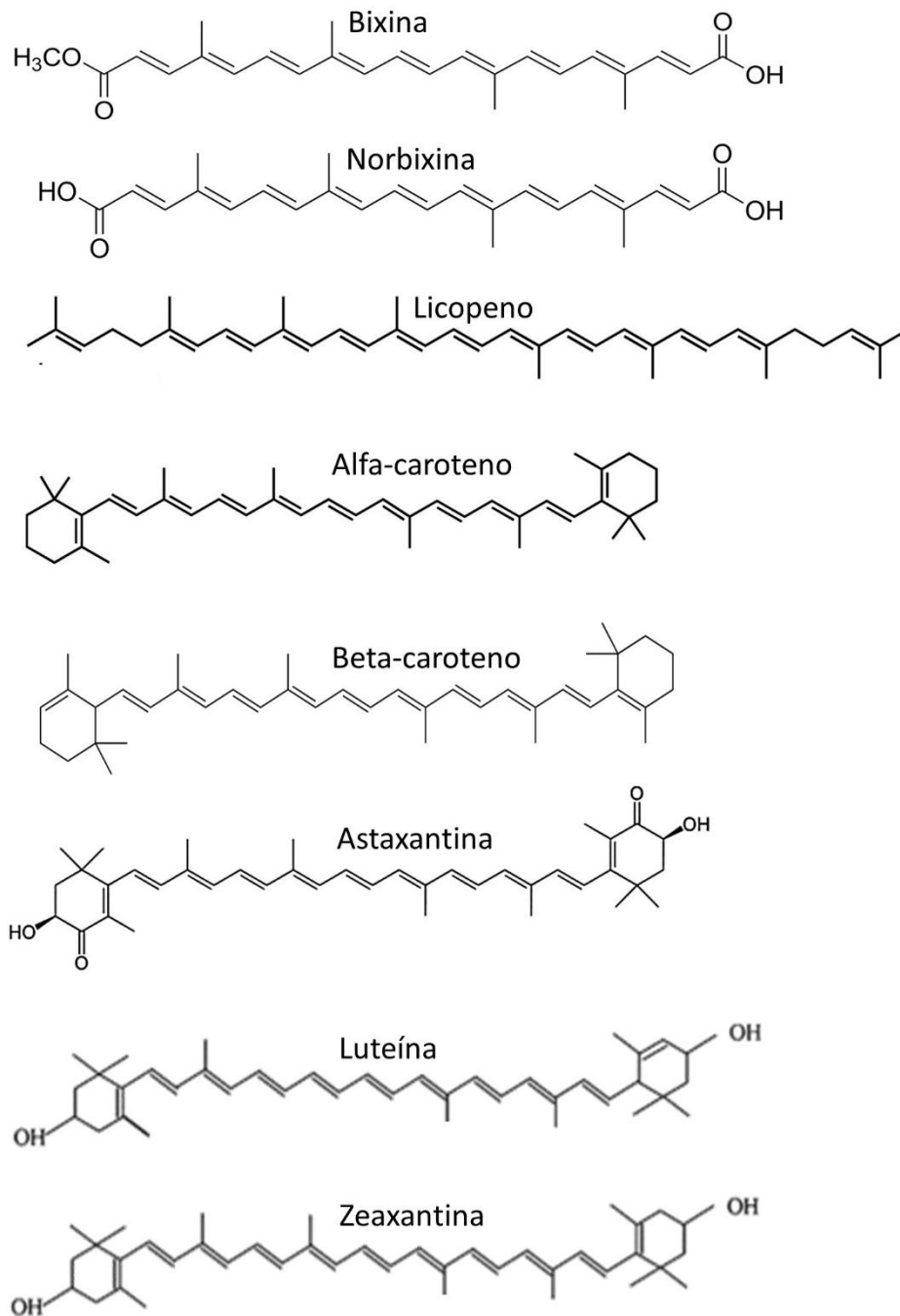
3.3 Carotenoides como aditivos em materiais poliméricos

A indústria de plásticos utiliza diversos aditivos a fim de alterar as características dos materiais poliméricos, onde a melhora na processabilidade e o aumento da estabilidade oxidativa e térmica dos materiais são objetivos recorrentes desta aditivação. Em relação a embalagens para alimentos, a interação entre materiais e produtos embalados é uma preocupação, tendo em vista a possível migração de aditivos para os alimentos. Logo, a substituição de aditivos potencialmente tóxicos por compostos naturais é uma tendência. Neste contexto, o poder corante e antioxidante dos carotenoides apresenta-se como uma alternativa interessante para a aditivação de polímeros.

Os carotenoides são pigmentos naturais e lipossolúveis presentes em plantas superiores, algas, fungos, bactérias e em alguns animais que os adquirem através da alimentação. Estes compostos são geralmente tetraterpenoides de 40 átomos de carbono, e possuem uma cadeia poliênica que pode ter de 3 a 15 duplas ligações conjugadas (Figura 3.4). A quantidade e posição destas duplas ligações influenciam o espectro de absorção dos carotenoides e, conseqüentemente, determinam sua coloração, que vai do amarelo ao vermelho (FRASER; BRAMLEY, 2004).

Além do poder corante, os carotenoides também são conhecidos por sua atividade antioxidante. O sistema de duplas ligações conjugadas em série torna estes compostos suscetíveis à adição de radicais, podendo levar à desativação de espécies reativas como radicais peroxil. Esta estrutura também é capaz de gerar um sistema de ressonância de elétrons que se deslocam ao longo da cadeia de polieno, e assim propiciam o sequestro de uma das formas mais reativas de oxigênio: o oxigênio singlete (EDGE; MCGARVEY; TRUSCOTT, 1997; KIOKIAS; VARZAKAS; OREOPOULOU, 2008; PALOZZA; KRINSKY, 1992; RODRIGUES et al., 2012).

Figura 3.4 - Estrutura molecular de diferentes carotenoides



Fonte: Adaptado de Cooperstone e Schwartz (2016)

A incorporação de carotenoides em materiais poliméricos tem sido realizada com diferentes propósitos. Visto que o processamento necessário para fundir e processar o material causa degradação pelo calor, oxigênio e tensão de cisalhamento,

a ação antioxidante dos carotenoides poderia reduzir o efeito de tais reações (KIRSCHWENG et al., 2017).

Beta-caroteno e licopeno utilizados em diferentes matrizes poliméricas apresentaram efeitos satisfatórios no aumento da estabilidade dos polímeros durante seu processamento e armazenamento (CERRUTTI, P.; MALINCONICO, M.; RYCHLY, J.; MATISOVA-RYCHLA, L.; CARFAGNA, 2009; LÓPEZ-RUBIO; LAGARON, 2010; TÁTRAALJAI, D. MAJOR, L., FÖLDES, E., PUKÁNSZKY, 2014). Além disso, beta caroteno aumentou a estabilidade de PCL, PLA e PHBV à luz UV e agiu como plastificante, aumentando a elasticidades dos materiais (LÓPEZ-RUBIO; LAGARON, 2010). O carotenoide bixina foi utilizado como corante de PHB, onde sua incorporação aumentou a estabilidade do polímero à fotodegradação (PAGNAN et al., 2017a). A Tabela 3.3 sintetiza os principais resultados de estudos que utilizaram carotenoides como aditivos em materiais poliméricos para o desenvolvimento de embalagens ativas, como estabilizante e como corante.

Tabela 3.3 - Carotenoides em polímeros utilizados com diferentes finalidades: desenvolvimento de embalagens ativas, estabilizante e corante.

Carotenoide	Polímero	Resultados	Referências
Desenvolvimento de embalagens ativas			
astaxantina	PEBD/ filme bicamada PEBD-car/PEAD	Os filmes reduziram a formação de peróxidos em óleo de girassol; a difusão de carotenoides dos filmes para etanol 95% (v/v) apresentou comportamento Fickiano; a presença da camada de PEAD nos filmes bicamada retardou a liberação de carotenoides e reduziu a influência da temperatura na liberação.	(COLÍN-CHÁVEZ et al., 2013a; COLÍN-CHÁVEZ; SOTO-VALDEZ; PERALTA, 2014; COLÍN-CHÁVEZ; VICENTE-RAMÍREZ, 2014; COLÍN-CHÁVEZ et al., 2013)
astaxantina	Filme bicamada PEAD-car/PEAD- TiO ₂	A presença de TiO ₂ reduziu a degradação da astaxantina; os filmes aumentaram a estabilidade oxidativa de óleo de soja como resultado da ação sinérgica entre proteção à luz e liberação de carotenoides dos filmes.	COLÍN-CHÁVES <i>et al.</i> , 2014a
astaxantina	Filme bicamada PLA-car/PLA	A astaxantina reduziu a permeabilidade ao vapor de água dos filmes, sem afetar a barreira ao oxigênio; filmes imersos em etanol apresentaram aumento na cristalização, a qual foi induzida pelo contato com o solvente; filmes reduziram a formação de peróxidos em óleo de soja.	(SAMSUDIN; SOTO-VALDEZ; AURAS, 2014b)
extrato comercial de carotenoides	PE	A presença de carotenoides aumentou a permeabilidade ao vapor de água e ao oxigênio, aumentou a elasticidade e reduziu a tensão de ruptura dos filmes.	(MOURA et al., 2018)
Estabilizante			
beta-caroteno	PLA; PCL; PHBV	O carotenoide aumentou a estabilidade à luz UV dos polímeros e agiu como plastificante	(LÓPEZ-RUBIO; LAGARON, 2010)
beta-caroteno	PE	Beta-caroteno reduziu a oxidação do polímero, evitando reações de oxidação em cadeia durante o processamento do material; as condições de armazenamento dos filmes afetaram a interação entre beta-caroteno e o polímero.	(TÁTRAALJAI, D. MAJOR, L., FÖLDES, E., PUKÁNSZKY, 2014)
licopeno	PP	Os carotenoides presentes no extrato de tomate aumentaram a estabilidade térmica do polímero durante o processamento.	(CERRUTTI, P.; MALINCONICO, M.; RYCHLY, J.; MATISOVA-RYCHLA, L.; CARFAGNA, 2009)
Corante			
bixina	PHB	Bixina protegeu o polímero da fotodegradação.	(PAGNAN et al., 2017a)

*car: carotenoides; PCL: poli(caprolactona); PE: polietileno; PEAD: polietileno de alta densidade; PEBD: polietileno de baixa densidade; PHB: poli(hidroxibutirato); PHBV: poli(hidroxibutirato-co-hidroxivalerato); PP: polipropileno

3.4 Extrusão vs técnica de *casting*

3.4.1 Extrusão

O crescimento do uso de materiais plásticos está, em parte, relacionado à facilidade de seu processamento. Nesse contexto, a extrusão é um processo largamente empregado na fabricação destes produtos por viabilizar sua produção contínua e em larga escala, apresentar baixo custo operacional e necessitar de pouco espaço por unidade de produção (ROSATO, 2013).

O processamento via extrusão pode ser descrito como o processo em que o material polimérico fundido é moldado de maneira contínua, fazendo-o passar por uma abertura (matriz) que apresenta a forma aproximada da seção transversal do produto desejado. De uma maneira geral, as funções da extrusora consistem em fundir, misturar, plastificar, transportar o material plastificado, e por fim, forçar sua passagem através de uma matriz (MUCCIO, 1994).

A extrusão de materiais pode ser utilizada por empresas de segunda geração, com a finalidade de desenvolver novos polímeros a partir da mistura de materiais – polímeros e/ou aditivos – e obter *pellets* para posterior transformação; ou então, ser utilizada por indústrias de terceira geração (indústrias transformadoras) com o objetivo de realizar a fusão de um polímero previamente desenvolvido, permitindo diferentes moldagens dependendo da matriz em uso.

Durante o processo, o polímero sólido (usualmente em forma de *pellets*) é arrastado sob altas pressões por uma rosca-sem-fim, a qual localiza-se dentro de um canhão circundado de resistências elétricas capazes de controlar a temperatura do processo. O polímero sofre cisalhamento intenso e é expelido através de uma matriz.

As extrusoras podem apresentar uma, duas, ou até mesmo quatro roscas. Extrusoras dupla-roscas são as mais utilizadas para o preparo e extrusão de blendas termoplásticas, visto que essas permitem uma melhor mistura e maior flexibilidade de operação. Por sua vez, as extrusoras mono-roscas, por serem mais simples e de menor custo, são mais empregadas em empresas de terceira geração, na obtenção de produto final (ROSATO, 2013).

Os parâmetros do processo de extrusão, tais como temperatura nos diferentes compartimentos, teor de umidade, velocidade da rosca, taxa de alimentação, entre

outros, influenciam diretamente nas características do produto final (LI et al., 2011). Como exemplo, é possível obter produtos com diferentes características e aplicações de acordo com a taxa de cisalhamento a qual se expõe o polímero. Estes parâmetros influenciarão na orientação e alinhamento das cadeias poliméricas e, conseqüentemente, na cristalinidade do polímero final (GUERRINI et al., 2004).

Inúmeros estudos utilizaram o processo de extrusão para a obtenção de filmes a partir do PLA. Para o processamento deste polímero, assim como em qualquer outro material polimérico, é preciso respeitar sua temperatura de processamento – a qual deve estar acima da temperatura de fusão e abaixo da temperatura de degradação do material. Por envolver altas temperaturas e pressão elevada, a produção de filmes por extrusão causa perda de compostos termolábeis ao longo do processamento, como, por exemplo, a degradação de antioxidantes naturais (SAMSUDIN; SOTO-VALDEZ; AURAS, 2014a). Entretanto, quando comparados a materiais obtidos por processo de *casting*, o processo de extrusão permite uma melhor distribuição dos aditivos na matriz polimérica (JAMSHIDIAN et al., 2012b).

3.4.2 Casting

A técnica de produção de filmes por evaporação de solvente, ou “*casting*”, é uma das mais antigas técnicas utilizadas para a produção de filmes de baixa espessura. Apesar de sua popularidade ter entrado em declínio na década de 50, quando as técnicas de extrusão tornaram-se dominantes na produção de plásticos, a técnica de *casting* voltou a ganhar força devido a sua capacidade de produzir filmes com elevados padrões de qualidade, espessura uniforme, pureza óptica e baixa turbidez (SIEMANN, 2005).

Simplificadamente, o processo de *casting* envolve a solubilização de um polímero em um solvente adequado, a deposição da solução filmogênica sobre um molde e a evaporação do solvente. O processo inicia pela penetração das moléculas de solvente na estrutura emaranhada do polímero, de forma a romper as ligações de Van der Waals entre as cadeias poliméricas. Calor e agitação mecânica são utilizados para acelerar a dissolução entre polímero e solvente, contudo, estes devem ser usados com cautela a fim de prevenir a degradação do polímero pelo rompimento de ligações covalentes (MILLER-CHOU; KOENIG, 2003; SIEMANN, 2005).

De acordo com Siemann 2005, para que a técnica possa ser utilizada, alguns pré-requisitos são essenciais:

- O polímero deve ser solúvel em um solvente volátil para favorecer a evaporação;
- A dissolução do polímero no solvente deve formar uma solução estável com um teor mínimo de sólidos e de viscosidade;
- A solução deve ser homogênea e o filme formado deve ser passível de ser removido do suporte após a secagem.

Para atingir tais pré-requisitos, alguns artifícios podem ser utilizados. Entre eles destaca-se: utilização de misturas de solventes; dissolução a altas pressões; uso de polímeros ou copolímeros com distribuições de massas molares específicas; uso de plastificantes (BYUN et al., 2012; SIEMANN, 2005; YANG et al., 2015).

Os solventes estudados para realizar a dissolução do PLA durante a técnica de *casting* são clorofórmio, diclorometano, acetonitrila ou a mistura destes. Byun e colaboradores (2012) avaliaram a influência do solvente nas características de filmes de PLA obtidos por *casting*, onde diclorometano foi o solvente que permitiu a obtenção de filmes com maior cristalinidade e estabilidade térmica do que clorofórmio ou a mistura clorofórmio:diclorometano. Os demais estudos encontrados na literatura utilizaram apenas clorofórmio como solvente (JAMSHIDIAN; TEHRANY; DESOBRY, 2013; LIU et al., 2010; RHIM et al., 2006; SÉBASTIEN et al., 2006; YANG et al., 2015). A dissolução do PLA ocorre em temperatura ambiente, sob agitação constante.

O processo de secagem passa por diferentes regimes de difusão. Após a dispersão do polímero no solvente e sua adição sobre uma superfície adequada, quando a concentração de solvente é elevada, a temperatura de transição vítrea do polímero é reduzida e fica abaixo da temperatura de processamento. Como consequência, o coeficiente de difusão do solvente é elevado e a evaporação é alta. A partir do momento em que a concentração de solvente é reduzida (aproximadamente de 3 - 6% de solvente residual, o que dependerá da natureza química da relação polímero-solvente), a temperatura de transição vítrea aumenta, ficando acima da temperatura de processamento, e o processo de secagem torna-se mais lento (BYUN et al., 2012; MILLER-CHOU; KOENIG, 2003; SIEMANN, 2005). Em geral, a secagem de filmes produzidos com PLA ocorre a temperatura ambiente,

podendo-se utilizar vácuo para promover a remoção completa do solvente (BYUN; KIM; WHITESIDE, 2010; JAMSHIDIAN et al., 2012b; YANG et al., 2015).

Uma das limitações da técnica de *casting* é que, após a secagem, os filmes tendem a apresentar diferentes propriedades entre as regiões expostas ao ar e as regiões do filme em contato com a superfície de suporte. Além disso, o método de *casting* é mais lento e dispendioso quando comparado ao processamento via extrusão, o que dificulta o *scale-up* do processo. Entretanto, diferentemente de materiais produzidos via extrusão, a técnica de *casting* permite a obtenção de filmes com elevada pureza óptica, espessura regular, elevada transparência e possibilidade de obtenção de filmes ultrafinos (SIEMANN, 2005; SOTHORNVIT et al., 2007). Pelo fato da técnica dispensar o uso de altas temperaturas, a sua utilização torna-se interessante para a produção de filmes adicionados por substâncias termolábeis.

CAPÍTULO 2

ARTIGOS CIENTÍFICOS ETAPA 1

ARTIGO 1

Carotenoids extracts as natural colorants in poly(lactic acid) films

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ABSTRACT

The use of vivid packaging colors is a strategy to attract consumers' attention and interest. In this context, the awareness of harmful effects caused by the use of synthetic colorants has led to an increasing interest in naturally derived alternatives. The use of carotenoids as colorants for polymeric materials represents an environmentally friendly way of obtaining colored packaging. The incorporation of carotenoids extracts rich in bixin, lycopene, and β -carotene into poly(lactic acid) (PLA) films was evaluated, where the presence of carotenoids (100 $\mu\text{g/g}$) reduced oxygen permeability and presented a lubricant effect, increasing films elasticity up to 50%. No effect on films crystallinity was observed. Carotenoids reduced films transparency and bixin was the most stable carotenoid at dark and light conditions. The color stability of films, evaluated through kinetic parameters of color degradation, presented Chroma half-life times (40 °C/light) of 111, 51 and 5 days for bixin, lycopene, and β -carotene, respectively.

Keywords: Properties and Characterization; Pigments; Biodegradable; Packaging; Kinetics.

1. INTRODUCTION

Packaging plays a significant and strategic role in attracting consumers' attention. It influences the perception on the product quality and on how the product is seen.(KOUTSIMANIS et al., 2012) In this sense, the use of vivid packaging colors is a tool that contributes to catch consumers' attention and interest (AHMED; PARMAR; AMIN, 2014),

Colorants, which are generally classified into dyes and pigments, are components capable of imparting color to products to make them more appealing, and synthetic colorants have been extensively used due to their high stability and low cost.(ALLEN, 1994; SIGURDSON; TANG; GIUSTI, 2017) However, the use of synthetic colorants have been associated to toxic, allergic, carcinogenic, and harmful responses, besides causing detrimental effects on the environment.(SHAHID; SHAHID-UL-ISLAM; MOHAMMAD, 2013) Regarding its application in food packaging materials, the possibility of migration is the most significant process related to public health awareness, since substances that migrate into food could potentially introduce risks to human health.(SILVA et al., 2008) As a consequence, the replacement of synthetic colorants with safer and naturally derived alternatives has been a consumers' demand.(SIGURDSON; TANG; GIUSTI, 2017)

Plant-derived colorants are produced through biochemical pathways within the organism, which results in a vast range of organic compounds with unique physicochemical properties. These colored substances are abundant in nature, completely biodegradable and easily extracted. Plant colorants include a variety of chemical classes, such as porphyrins, anthocyanins, betalains, and carotenoids.(SIGURDSON; TANG; GIUSTI, 2017) Among natural colorants, carotenoids are lipophilic pigments naturally occurring in the chromoplasts of plants and in some other photosynthetic organisms such as algae, some types of fungi and bacteria.(YAHIA; ORNELAS-PAZ, 2009) Applied as natural yellow, orange or red colorants in various foods, carotenoids are not used only because of their coloring properties, but also due to their antioxidant potential and healthcare functions.(COLÍN-CHÁVEZ et al., 2014; FIEDOR; BURDA, 2014; TÁTRAALJAI, D. MAJOR, L., FÖLDES, E., PUKÁNSZKY, 2014)

The long conjugation systems and the π -electrons delocalized along the polyene chain of carotenoids are responsible for their absorbance in the visible spectrum, which results in colors ranging from yellow to orange and red hues. (RIVERA; CANELA-GARAYOA, 2012) Large conjugated systems tend to present redder hues, whereas short double bond chains - as in phytofluene, with five double bonds - show achromaticity. The color of carotenoid is also influenced by cyclization.⁴ γ -Carotene, β -carotene, and lycopene, for instance, show the same number of double bonds. However, they exhibit orange-red, orange, and red colors, respectively, due to the presence of different rings in their structures. (SIGURDSON; TANG; GIUSTI, 2017)

Carotenoids have been widely applied as natural colorants in food and beverages, while their utilization in non-food applications is still scarce. (SHAHID-UL-ISLAM; RATHER; MOHAMMAD, 2016) Few studies concerning the use of carotenoids in polymeric packaging materials are found in literature. Their application have distinct approaches: β -carotene and lycopene have been used as thermal and photo-oxidative stabilizants of polymers (ABDEL-RAZIK, 1989; CERRUTTI, P.; MALINCONICO, M.; RYCHLY, J.; MATISOVA-RYCHLA, L.; CARFAGNA, 2009; LÓPEZ-RUBIO; LAGARON, 2010; MASEK et al., 2015; TÁTRAALJAI, D. MAJOR, L., FÖLDES, E., PUKÁNSZKY, 2014); astaxanthin has been used with the target of producing antioxidant active packaging to food (COLÍN-CHÁVEZ et al., 2014); and bixin has been used to obtain colored films. (PAGNAN et al., 2017a) In general, results show that despite presenting low stability to oxygen, ultraviolet radiation and sunlight, carotenoids produced films with enhanced thermo-oxidative stability.

Studies have reported that poly(lactic acid) (PLA) is an interesting polymer for use as packaging because it is a biodegradable and biobased material. The hydrophobic character of PLA suggests its compatibility with lipophilic colorants such as carotenoids. However, PLA presents elevated transparency and poor oxygen barrier (MANZANAREZ-LÓPEZ et al., 2011b), which hamper its application to fatty-food which are sensitive to oxygen and light. The incorporation of carotenoids, besides imparting color to PLA, could offer a barrier to light, bringing also stability to perishable food due to the antioxidant properties of these natural compounds.

Most of the current studies restrict their analysis on the effect of only one type of carotenoid in different polymeric matrices. There is few information about the

behavior of different carotenoids in the same polymeric structure. Hence, the objective of the present study is to investigate the effect of distinct carotenoids applied as natural colorants additives in PLA films for food packaging. Carotenoids extracts rich in bixin, lycopene, and β -carotene were incorporated into PLA films, which had their mechanical, thermal and barrier properties evaluated. The color stability of the films was analyzed under different conditions of light and temperature, and kinetic parameters of color degradation were obtained.

2. EXPERIMENTAL

2.1 Materials

PLA 2003D, a semi-crystalline biopolymer grade with a D-Lactic acid content around 4 %, was obtained from NatureWorks (Blair-NE, USA). Tomatoes (*Solanum lycopersicum*), carrots (*Daucus carota*) and annatto seeds (*Bixa orellana*) were obtained from a local market. All chemicals used were of analytical grade.

2.2 Carotenoids extracts: obtention and characterization

Tomato, carrot, and annatto seed were used to obtain lycopene, β -carotene, and bixin rich extracts, respectively. Lycopene and β -carotene were extracted from deseeded chopped tomatoes and sliced carrots, with ethyl acetate.(NUNES; MERCADANTE, 2004) To extract bixin, annatto seeds were previously washed with hexane and methanol, and the extraction was performed with chloroform.(RIOS; MERCADANTE, 2004) All the extractions were carried out at ambient temperature and protection from light. Extracts were filtered and concentrated in a Fisatom rotaevaporator (M802, Brazil) at 40 °C until complete removal of solvents.

The composition of extracts was analyzed by High Performance Liquid Chromatography (HPLC). Chromatographic analyses were performed using a Shimadzu HPLC-DAD (Kyoto, Japan) equipped with two pumps, on-line degasser, column oven, connected in series to a diode array detector. Before the injection, samples were pre-filtered using a modified PTFE membrane for aqueous and organic solvents with a pore diameter of 0.45 μ m (Millipore, SP, Brazil).

Carotenoids were identified through the comparison of retention times of external standards, and the UV-vis spectra (λ_{\max} and shape) were compared to literature data. The carotenoids compounds of tomato and carrot extracts were separated using a C30 polymeric column YCM (250 × 4.6 mm i.d.; 3- μm particle size), where the mobile phase gradient consisted of water, methanol, and methyl tert-butyl ether, starting from 5:95:5 v/v and reaching 0:95:5 v/v at 12 min, 0:89:11 v/v at 25 min, 0:75:25 v/v at 40 min and finally 0:50:50 v/v at 80 min. Anatto seed extract analysis was conducted on a C18 Spherisorb ODS-2 column (150 × 4.6 mm i.d.; 3 μm particle size), where acetonitrile/acetic acid (2 %) (63:35, v:v) was used as mobile phase. All HPLC analyses were performed at 33 °C, a flow rate of 1 mL/min and 5 μL of injection volume. ChemStation® software was used to acquire and process data.

In order to verify the presence of other classes of components besides carotenoids, UV-Vis spectra were obtained between 200 and 700 nm and the chromatograms were processed at 280, 320, 360, 450, 470 and 520 nm. No peaks were observed at the mentioned wavelengths except in between 450 and 470 nm, which means extracts were composed solely of carotenoids.

2.3 Carotenoids quantification

To incorporate the carotenoid extracts into PLA solutions, pigments were dissolved in chloroform and analyzed via spectrophotometry UV-Vis (IL-592, Spectrale, Japan). Lycopene was evaluated at $\lambda = 466 \text{ nm}$ ($\epsilon = 150855 \text{ L mol}^{-1} \text{ cm}^{-1}$); β -carotene at $\lambda = 465 \text{ nm}$ ($A_{1\%} = 2396$), and bixin at $\lambda = 470 \text{ nm}$ ($A_{1\%} = 2826$), where the λ_{\max} of each component in chloroform was confirmed after scanning from 200 nm to 700 nm. The results were expressed as total lycopene, total β -carotene, and total bixin equivalent, for tomato, carrot, and annatto seed extracts, respectively.

2.4 PLA films production

Solutions of PLA in chloroform (5 %, w/v) were prepared by continuous mechanical stirring at ambient temperature, up to pellets dissolution. Subsequently, carotenoid extracts were added to PLA solution to achieve a concentration of 100 $\mu\text{g/g}$ – carotenoid mass related to PLA dry matter. Solutions were stirred for 10 min to completely dissolve the carotenoids in the polymer solution and then poured on Teflon recipients at a leveled surface. Pierced plastic caps were used to cover the assemblies

preventing from uneven solvent evaporation. Chloroform was left to evaporate for 24 h at 23 ± 2 °C, in the dark. To guarantee complete solvent evaporation, films were peeled from Teflon recipients and placed in oven with forced air circulation (DeLeo B5AFD) at 40 °C for 6 h.

2.5 Films Characterization

2.5.6 Scanning Electron Microscopy

Films surfaces were closely observed with a scanning electron microscope (SEM) (Model JSM 6060, JEOL) at a magnification of 1000x. The samples were fixed on aluminum stubs, coated with gold, and scanned with an accelerating voltage of 5.0 kV.

2.5.7 Attenuated total reflectance (ATR)-FTIR spectroscopy

The Infrared analysis was performed to study the differences in frequencies of functional groups of PLA, carotenoids, and the connections between them. Infrared spectra between 4000 and 350 cm^{-1} were recorded with a Perkin Elmer Spectrum Two™ Infrared Spectrometer (Perkin Elmer U.K.), equipped with an ATR prism crystal accessory with automatic signal gain, at a resolution of 4 cm^{-1} at 20 °C, collected in 32 scans. The spectra were obtained in triplicate, and the average spectrum was taken for analyses.

2.5.8 Thickness and mechanical properties

The final film thickness was measured with a mechanical micrometer (MDC-25, Mitutoyo Corp, Japan, precision 0.001 mm, resolution/0~25 mm) at ten random points. A texture analyzer (TA.XT2i e Stable Micro Systems, UK) with a load cell of 5 kg and an A/TGT self-tightening roller grips fixture was used to evaluate the tensile strength at break (*TS*) and the percentage of elongation at break (*E* %). Parameters were obtained from stress–strain curves from uniaxial tensile tests and were calculated according to the ASTM D882 standard method.(ASTM INTERNATIONAL, 2012) The strips were mounted individually between the grips of the equipment with an initial grip separation of 50 mm and a test speed of 0.8 mm/s. Ten replications were run for each test sample.

2.5.9 Films transparency

Films transparency was determined by measuring their transmittance spectra, in triplicate, at 500 nm using a UV spectrophotometer (Shimadzu UV-1800, Japan).(TANG et al., 2005) Films were cut into rectangular pieces and were directly placed in a spectrophotometer test cell. An empty quartz test cell was used as a control.

2.5.10 Water Vapor Permeability

Water vapor permeability (WVP) was gravimetrically determined. Films samples were mounted in permeation cells (height 25 mm, inner diameter 63 mm) that were filled with anhydrous CaCl₂. The cells were hermetically sealed and then placed in a glass chamber with saturated sodium chloride solution, providing relative humidity gradients of 0/75 % at 25 °C. The mass gain of the cell permeation was determined after 24 h by weighing the cell on an analytical balance. The analysis was performed in triplicate, and results were calculated through Eq. 1:

$$WVP = \frac{w \cdot L}{A \cdot T \cdot \Delta p} \quad (1)$$

Where w is the water weight permeated through the film (g), L is the thickness of the films (m), A is the permeation area (m²), T is the time of permeation (s), and Δp is the water vapor pressure difference (at 25 °C) between the two sides of the film (Pa).

2.5.11 Oxygen permeability

The oxygen permeability coefficient was evaluated based on the standard method (ASTM INTERNATIONAL, 2015) ASTM D1434 using the manometric gas permeation analyzer Lyssy L100-5000 (Illinois Instruments) at 23 ± 2 °C. The films were cut giving a measurement area of 50 cm² and then mounted inside the testing chamber. The pressure change via oxygen transmission through films was evaluated up to steady state, and films oxygen transmission rate (OTR) were exposed in cc m⁻² day⁻¹.

2.5.12 Thermal analysis by DSC

The glass transition (T_g), cold crystallization (T_{cc}) and melting (T_m) temperature regions, and the enthalpies of cold crystallization (ΔH_{cc}) and melting (ΔH_m), obtained

from the first heating run, were estimated using a DSC, Q2000 TA-Instruments (New Castle, DE, USA). Samples of about 2 mg were heated up from 10 to 200 °C at 10 °C/min with an N₂ flow of 50 mL/min, with a heat-cool-heat cycle. The degree of crystallinity (X_c) of films was calculated as follows:

$$X_c = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_f (1-x)} \times 100 \quad (2)$$

where ΔH_f is the heat of fusion of 100 % crystalline PLA (93.7 J/g)(FISCHER; STERZEL; WEGNER, 1973) and x is the amount of carotenoids in films (0.0001).

2.6 Carotenoids and color stability of films under storage

The PLA films containing carotenoids were stored at 25 °C and 40 °C, in the presence and absence of light. Three white fluorescent lamps were used as luminous source (2.0 ± 0.2 klx). The color measurement was performed using a Minolta model colorimeter (CR-300; Minolta Co, Ltd, Japan) through the CIELab color parameters L^* (luminosity), a^* (red-green), and b^* (yellow–blue). For the analysis, performed in triplicate, films were placed over a white disk (L^* , 97.45; a^* , 5.23; and b^* , -3.68) used as a white standard.

The total color difference ΔE between stored film samples and the films before storage (control) was calculated using Eq 3. The Chroma was calculated through Eq 4.

$$\Delta E = \sqrt{(L^*_{control} - L^*_{sample})^2 + (a^*_{control} - a^*_{sample})^2 + (b^*_{control} - b^*_{sample})^2} \quad (3)$$

$$Chroma = \sqrt{a^*_{sample}{}^2 + b^*_{sample}{}^2} \quad (4)$$

Color changes were evaluated through a chemical kinetics approach, by calculating kinetics parameters of reaction order, reaction rate constants (k_0 and k) and half-life time ($t_{1/2}$), using the following equations

Zero-order reaction:

$$C = C_0 - k_0 \cdot t$$

$$t_{1/2} = \frac{C_0}{2 k_0}$$

First order reaction:

$$\ln\left(\frac{c}{c_0}\right) = -k \cdot t$$

$$t_{1/2} = \frac{\ln 2}{k}$$

where c and c_0 are the values of the properties (ΔE , *Chroma*) determined at the day zero and after t days of storage, respectively.

The carotenoids content of films was analyzed via UV-Vis spectrophotometry after 18 days of storage under different conditions. Photographs were taken to illustrate films visual attributes.

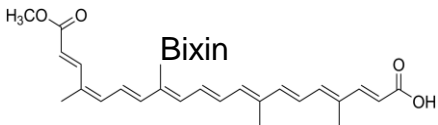
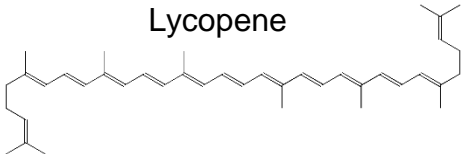
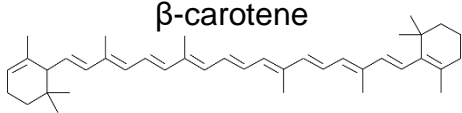
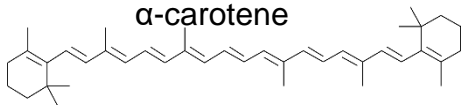
2.7 Statistical Analysis

The results were evaluated by analysis of variance (ANOVA) and a Tukey test at significance level of 5 % ($p < 0.05$) using the software Statistica 7.1. (STATSOFT Inc.), corresponding to the average of three replicates ($n = 3$).

3. RESULTS AND DISCUSSION

The composition of carotenoids extracts is presented in Table 1. Once annatto seed extract was composed of 94 % of bixin, carrot extract presented 95 % of carotenes (61 % β -carotene; 34 % γ -carotene), and tomato extract had 89 % of lycopene, no subsequent purification step was performed. Films were named according to the major carotenoid contained in its formulation: BixFilm, LycFilm, and CarFilm to films produced with annatto seed, tomato and carrot extracts, respectively, while the film without carotenoids was named PLAFilm.

Table 1. Carotenoids extracts composition and molecular characteristics of major carotenoids found.

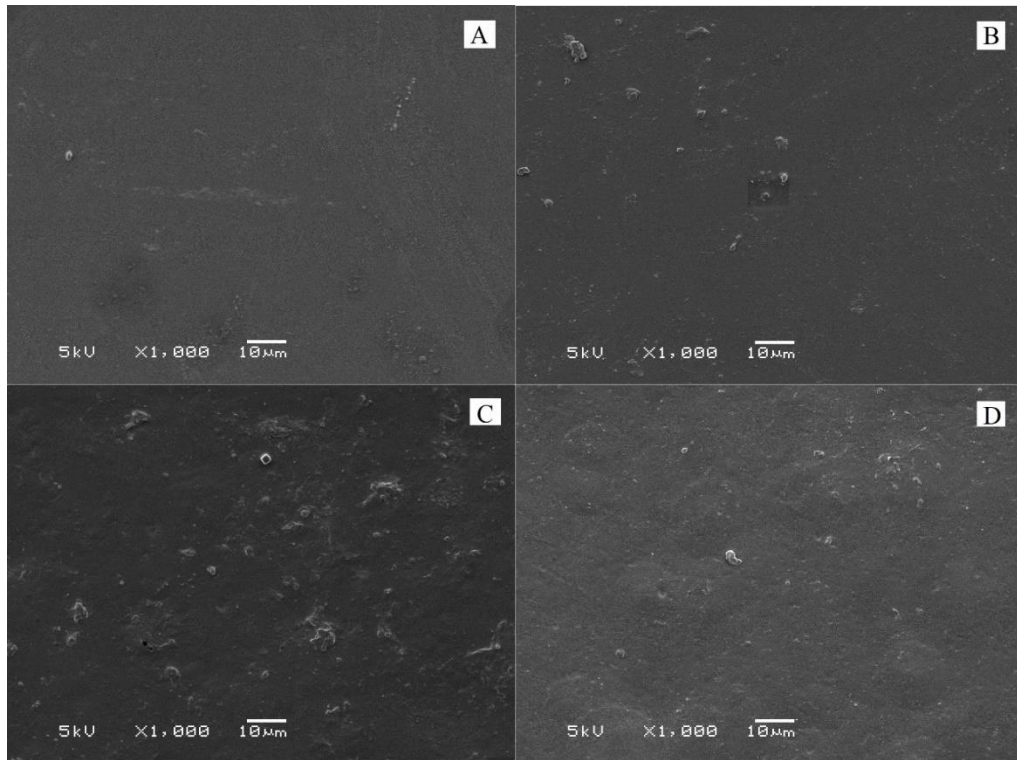
Extract	Composition	Formula	Molecular structure of major carotenoid
Annato seed	Bixin (~94%)	$C_{25}H_{30}O_4$	 <p style="text-align: center;">Bixin</p>
Tomato	Lycopene (~89%); β -carotene (~6%); α -carotene (~2%)	$C_{40}H_{56}$	 <p style="text-align: center;">Lycopene</p>
Carrot	β -carotene (~61%); α -carotene (~34%); Lutein (~2%)	$C_{40}H_{56}$	 <p style="text-align: center;">β-carotene</p>  <p style="text-align: center;">α-carotene</p>

3.1 Films Characterization

3.1.1 SEM

The surface morphology of the PLA films was evaluated through SEM (Figure 1). CarFilm and LycFilm were similar to the PLAFilm, exhibiting a relatively smooth and homogeneous surface. Despite presenting no wrinkles, some small visual discontinuities were revealed in PLA colored films, where BixFilm presented more evident spots of discontinuity with a slightly rough surface. This roughness could explain BixFilm lower transparency (Table 2), since higher surface roughness predicts that less light could pass through the material. (LIN et al., 2007) The small prominent particles correspond to carotenoids aggregates throughout the polymer matrix. It should be noted that through a macroscopic observation of films with the naked eye, films presented a completely smooth and homogeneous surface.

Figure 1. SEM micrographs of PLAFilm (A), LycFilm (B), BixFilm (C) and CarFilm(D).



3.1.2 FTIR Spectroscopy

As shown in Figure 2, the FTIR spectra of all the films showed the typical bands of PLA: saturated -CH- stretching ($2996 - 2851 \text{ cm}^{-1}$), C=O stretching (1750 cm^{-1}), -CH₃ bending (1453 cm^{-1}), symmetric and asymmetric -CH- bending (1384 and 1361 cm^{-1}), C=O bending (1265 cm^{-1}) and a set of bands in the range $1190 - 1080 \text{ cm}^{-1}$ related to -C-O- stretching vibrations (GARLOTTA, 2002; LÓPEZ DE DICASTILLO et al., 2017). No difference was found among the IR spectra of the samples – neither in position, intensity nor proportion of peaks. This is due to the small amount of carotenoids added ($100 \text{ } \mu\text{g/g}$) which did not allow to detect its functional groups through FTIR analysis.

Figure 2. FTIR spectra of PLAFilm and PLA added with lycopene (LycFilm), beta-carotene (CarFilm), and bixin (BixFilm).

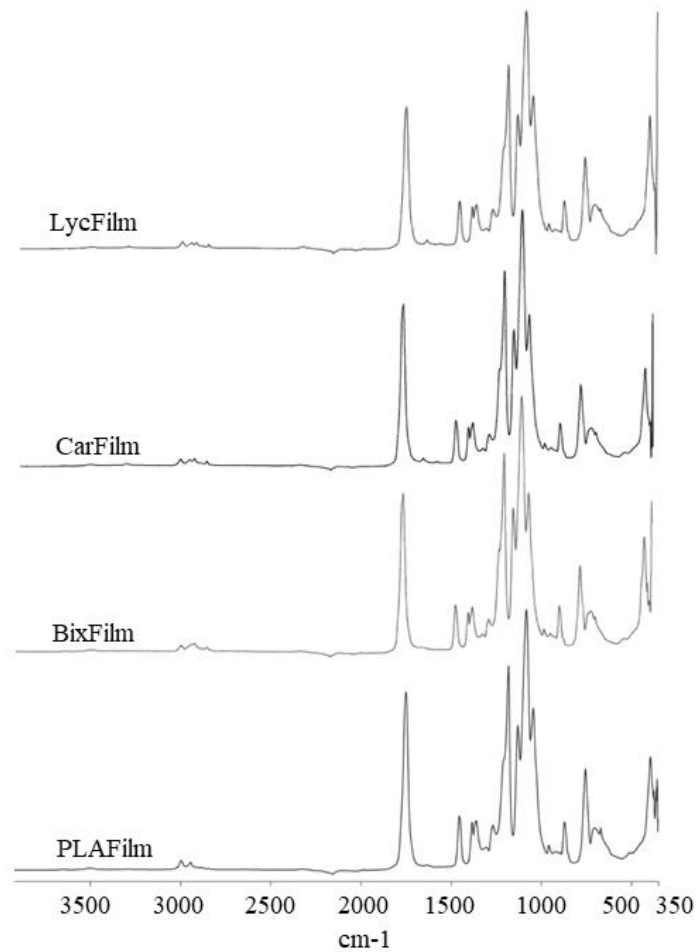


Table 2. Thickness, transparency and mechanical properties ($E\%$ and TS) of PLA films.

	Thickness (μm)	Transparency (%)	$E\%$	TS (MPa)
PLAFilm	138 ± 39 a	85.72 ± 2.53 a	246 ± 14 c	22.4 ± 1.3 c
BixFilm	121 ± 27 a	31.89 ± 3.49 c	296 ± 12 b	29.9 ± 1.2 a
LycFilm	123 ± 27 a	51.93 ± 3.82 b	374 ± 13 a	26.4 ± 1.2 b
CarFilm	122 ± 27 a	42.69 ± 5.02 b	302 ± 12 b	27.8 ± 1.2 b

The analyses were performed in at least three replicated samples. Values expressed as the mean \pm standard deviation. Different letters in the same column indicate statistically significant differences ($p < 0.05$) between the treatments.

3.1.3 Transparency and mechanical properties

The addition of carotenoids extracts significantly reduced PLA films transparency (Table 2), which implies in a protective barrier against light. These results are of great importance when films are used to package light-sensitive products such as fatty-foods.

The carotenoids incorporation into PLA films did not affect their thickness. This result was already expected considering the low amount of carotenoids added. However, such small quantity of carotenoids was sufficient to promote considerable changes in the mechanical properties of the materials. The films elongation at break ($E\%$) was improved through the addition of carotenoids extracts, where LycFilm presented the highest deformation with 50 % of increase compared to PLAFilm. The greater elongation obtained indicated that the presence of carotenoids made the chains slippage easier under the tensile force, acting as lubricants. Similar behavior was observed in the study of Lopez-Rubio (2010), where the addition of 0.4 % α -carotene (w/w) improved the elasticity of PLA, polycaprolactone (PCL) and polyhydroxybutyrate-co-valerate (PHBV) films. According to the lubricity theory, low molecular weight components may facilitate the movement of polymer chains over each other by reducing their intermolecular friction. (PLATZER, 1982)

Among the carotenoids, lycopene imparted the highest elongation at break for the PLA films. This result is probably related to the different molecular structures of the additives and needs more studies to fully understand the behavior. One explanation could be the highest flexibility of the lycopene chains, which is an acyclic open-chain molecule with some sp^3 carbon atoms, with no end groups - differently from β -carotene (which has β -rings) and bixin (which has a free carboxyl and an esterified carboxyl end groups). The absence of functional and bulky end groups in lycopene could have facilitated PLA chain slipping and thus decreased the rigidity of the material.

PLA toughening has become the focus of numerous investigations in order to broaden its application, which is still limited due to PLA poor impact strength and brittle character. (WANG et al., 2017) In this sense, these results mean significant improvements, once films elongation was substantially increased by the incorporation of carotenoids. Besides that, the improvement on elongation was accompanied by an increase in tensile strength, which is an interesting phenomena taking into

consideration the common trend - a decrease in tensile strength, while elongation increases.(BAIARDO et al., 2003) Hence, the reduced intermolecular friction caused by the presence of carotenoids may have allowed rearrangements of the polymer chains, enhancing the strength of PLA films through the mechanism of strain hardening.

3.1.4 Thermal properties and crystallinity

The thermal properties of films are presented in Table 3. No cold crystallization peak was observed in the first heating run, indicating that the drying method - performed at room temperature - enabled the maximum possible organization of the polymer chains.

An increment on polymers flexibility is frequently accompanied by a reduction in its glass transition temperature.(FARAH; ANDERSON; LANGER, 2016) Nevertheless, no substantial changes in films thermal properties were observed, where T_g was maintained around 57-58 °C and crystallinity (X_c) around 22 %. Although improvements in films tensile strength and barrier properties may be associated with an increase in films crystallinity (GUINAULT et al., 2012; HARRIS, A. M., ELLEN, 2008), it is not a requirement, once crystallinity is not the sole influencing factor. The addition of α -tocopherol into PLA have produced films with lower, the same, and even higher levels of crystallinity, meanwhile a decrease in tensile strength was observed in all circumstances.(GONÇALVES et al., 2013; JAMSHIDIAN et al., 2012b) The addition of α -tocopherol (3 %), BHT (1.5 %), and marigold flower extract (1.5 %) to PLA have not caused significant changes in the thermal properties of PLA films(MANZANAREZ-LÓPEZ et al., 2011b; ORTIZ-VAZQUEZ et al., 2011; SAMSUDIN; SOTO-VALDEZ; AURAS, 2014b), despite affecting their barrier and mechanical properties.

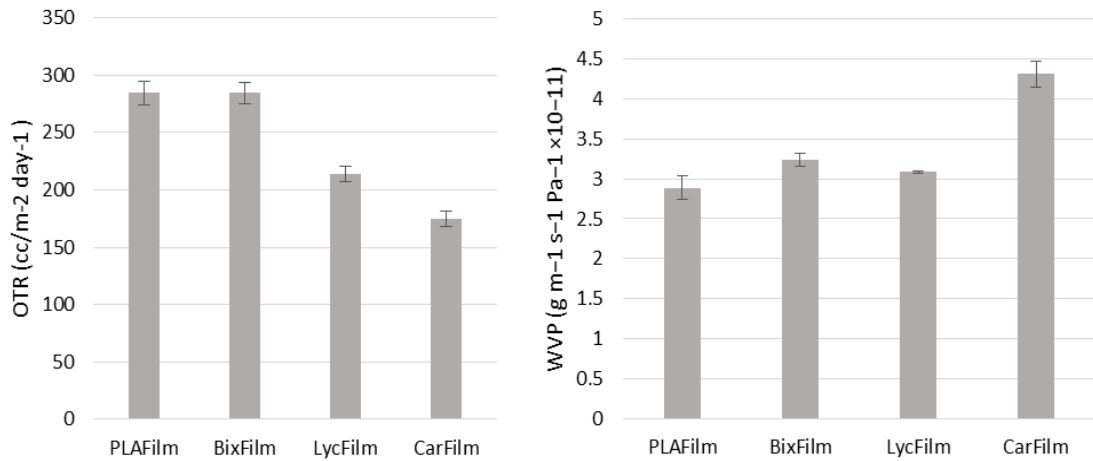
Table 3. Thermal properties obtained from DSC thermograms during the first heating cycle.

	T_g (°C)	T_m (°C)	H_m (J/g)	X_c (%)
PLAFilm	56.8	150.2	20.6	22.2
BixFilm	58.4	150.6	20.6	22.2
LycFilm	57.6	150.7	21.3	22.9
CarFilm	58.1	151.0	20.7	22.2

3.1.5 Barrier properties

The barrier or permeability against the transfer of gases, water vapor, and aroma molecules is considered one of the most important factors in food packaging polymers.(FARAH; ANDERSON; LANGER, 2016) Increasing the oxygen barrier performance of films is of great importance as food deterioration is mainly governed by oxidation processes.(BARDEN; DECKER, 2013) The incorporation of β -carotene and lycopene reduced the OTR of PLA films in 38 % for CarFilm and 25 % for LycFilm, while the presence of bixin did not affect films permeability to oxygen when compared to PLAFilm (Figure 3). This behavior could be explained by considering that OTR is governed by sorption and diffusion mechanisms.(SIRACUSA, 2012) The presence of polar groups (carboxylic structures) in the bixin structure reduces oxygen adsorption, facilitating oxygen diffusion through the PLA polymer matrix. In contrast to bixin, the absence of such groups in lycopene and β -carotene molecular structures may have enhanced oxygen adsorption, consequently reducing oxygen diffusion. In terms of permeability to water vapor, the presence of carotenoids increased WVP in values to up to 49 % (CarFilm) in comparison with PLAFilm. Although the incorporation of hydrophobic compounds is known to lower the water permeability of materials, it did not occur through the addition of the tested carotenoids in the present research.

Figure 3. Oxygen transmission rate (OTR) and water vapor permeability (WVP) of PLA films.



3.2 Carotenoids and color stability

Carotenoids stability in films is important not only to maintain their visual attributes but also have consequences on films functionality due to potential changes on films UV-barrier properties.

The colored films, which presented yellow-reddish colors, had their red and yellow components of color (a^* and b^*) strongly affected during the stability test. Table 4 presents the L^* , a^* and b^* parameters, reaction rate constants and half-life time, where Chroma degradation was successfully described by a first order kinetics, while changes in ΔE followed zero order kinetics.

As expected, films exposed to light and temperature of 40 °C - the least favorable condition tested for color stability - presented the highest reaction rate constants considering both Chroma and ΔE . CarFilm showed the lowest stability in all tested conditions. The light exposure was the most impacting factor on color stability, for all carotenoids. At 40 °C, the Chroma half-life time of CarFilm decreased from 41 days under darkness conditions to 5 days under the exposure of light. Meanwhile, the Chroma of BixFilm and LycFilm were much more stable, presenting half-life times of 341 and 280 days at dark, that were reduced to 111 and 51 days under the exposure of light, respectively. As expected, the loss in color was accompanied by a decrease

in carotenoids concentration. The color visual attributes of films under different storage conditions are shown in Figure 4.

The color stability of carotenoids depends on many factors and it is strongly correlated with the stability of conjugated C=C double bonds in the molecule, which serve as chromophore moieties and are responsible for its light absorption capacity.(MORABITO et al., 2012) Being highly unsaturated, carotenoids easily overcome oxidation and isomerization, where the presence of light, oxygen, and heat, among other factors, stimulates oxidation and promote isomerization of *all-trans* carotenoids (their usual configuration) to their *cis* forms. At least seven conjugated double bonds are needed for a carotenoid to have a perceptible color. (RODRIGUEZ, 2001)

The differences in color and on carotenoids stability between the films could be comprehended through the carotenoids' structure. In terms of molecular structure, lycopene differs slightly from β -carotene in the conjugated double bond system and in the absence of ring structures at the ends of the molecule. These differences may account for differences in degradation rates between the carotenoids. The results obtained in this study are similar to those of the study of Pesek and Warthesen(PESEK; WARTHESEN, 1987) where lycopene photodegradation was about one-fifth that of β -carotene. Bixin, the colorant that produced the most stable films in terms of color and carotenoids concentration, is known to present better stability to light and heat during food processing than many other carotenoids colors(SHAHID-UL-ISLAM; RATHER; MOHAMMAD, 2016), and this behavior is mainly related to its apocarotenoid nature.

Carotenoids' oxidation under dark conditions does not follow a photodegradative route (with the production of singlet oxygen), but a radical pathway that can be induced by temperature. Instead, in the presence of light, it can be supposed that two parallel reactions take place: (1) the oxidation of radicals, and (2) the photooxidation, where the excited triplet sensitizer might react with the triplet oxygen to form singlet oxygen and singlet sensitize.(LIMBO; TORRI; PIERGIOVANNI, 2007; MIN; BOFF, 2002) The rate of singlet oxidation is generally much higher than that of atmospheric triplet oxidation, which could lead to an accelerated degradation of carotenoids and losses in color. However, at the same time carotenoids take part of the above mentioned oxidation reactions, these antioxidant molecules are potent

singlet oxygen quenchers and exert significant light protection, simultaneously operating as UV absorbers.(YAHIA; ORNELAS-PAZ, 2009) As a consequence, the incorporation of carotenoids into PLA films could help to increase the photostability of the packed product and of the biopolymer itself.(LÓPEZ-RUBIO; LAGARON, 2010)

Figure 4. Color-visual attributes, total color difference (ΔE) and carotenoids lost (%) of colored films after 18 days under different storage conditions.

	Day zero	25 °C (dark)	25 °C (light)	40 °C (dark)	40 °C (light)
BixFilm	L* = 84,19 a* = 6,79 b* = 50,18 100.4 µg/g bixin	$\Delta E = 1.57$ -4.8% bixin	$\Delta E = 2.27$ -27.5% bixin	$\Delta E = 2.28$ -19.1% bixin	$\Delta E = 5.45$ -29.9% bixin
LycFilm	L* = 79.18 a* = 20.81 b* = 45.36 101.4 µg/g lycopene	$\Delta E = 0.70$ -7.6% lycopene	$\Delta E = 4.59$ -25.8% lycopene	$\Delta E = 5.76$ -16.8% lycopene	$\Delta E = 13.82$ -39.2% lycopene
CarFilm	L* = 89.38 a* = -4.17 b* = 46.59 99.6 µg/g β -carotene	$\Delta E = 1.57$ -23.5% β -carotene	$\Delta E = 23.51$ -76.3% β -carotene	$\Delta E = 11.35$ -52.4% β -carotene	$\Delta E = 45.89$ -80.0% β -carotene

Table 4. Carotenoids content and CIELab color parameters after 18 days of storage, ΔE zero order kinetic reaction coefficient (k_0), Chroma first-order kinetic reaction coefficient (k), and half-life times ($t_{1/2}$) of films under different storage conditions.

		L^*	a^*	b^*	k_0	ΔE		Chroma		
						R^2	k	R^2	$t_{1/2}$ (days)	
BixFilm	Time zero	100.4 ± 0.6	84.27 ± 0.83	6.79 ± 0.56	50.18 ± 1.64					
	25°C, dark	95.6 ± 0.2	84.12 ± 0.70	6.90 ± 0.34	48.64 ± 0.70	0.0180	0.950	0.00031	0.921	2236.0
	40°C, dark	81.2 ± 1.5	85.07 ± 0.40	4.86 ± 0.19	49.49 ± 0.55	0.119	0.947	0.00202	0.979	334.1
	25°C, light	72.8 ± 1.4	84.86 ± 0.30	5.70 ± 0.11	48.32 ± 0.17	0.136	0.941	0.00245	0.912	282.9
	40°C, light	70.7 ± 2.2	86.00 ± 0.17	3.60 ± 0.18	45.16 ± 0.33	0.327	0.941	0.00625	0.976	110.9
LycFilm	Time zero	101.4 ± 1.7	79.18 ± 0.25	20.81 ± 0.19	45.36 ± 0.67					
	25°C, dark	93.6 ± 3.2	79.12 ± 0.03	20.29 ± 0.01	45.48 ± 0.28	0.0253	0.992	0.000714	0.981	970.8
	40°C, dark	84.4 ± 2.5	81.86 ± 0.23	15.86 ± 0.51	44.28 ± 0.55	0.315	0.991	0.00247	0.95	280.6
	25°C, light	75.8 ± 3.0	81.13 ± 0.34	17.31 ± 0.46	43.14 ± 0.04	0.288	0.945	0.00456	0.944	152.0
	40°C, light	61.7 ± 2.5	84.63 ± 0.18	10.83 ± 0.56	37.95 ± 0.37	0.799	0.989	0.0137	0.994	50.6
CarFilm	Time zero	99.6 ± 1.5	89.38 ± 1.48	-4.17 ± 0.35	46.59 ± 0.30					
	25°C, dark	76.2 ± 2.5	90.20 ± 0.09	-5.15 ± 0.28	45.51 ± 0.07	0.0672	0.991	0.00115	0.982	602.7
	40°C, dark	47.4 ± 2.00	91.63 ± 0.40	-4.38 ± 0.05	35.80 ± 1.28	0.667	0.974	0.017	0.995	40.8
	25°C, light	26.6 ± 0.3	92.10 ± 0.39	-1.87 ± 0.53	23.35 ± 2.24	1.342	0.992	0.0367	0.99	18.9
	40°C, light	20.0 ± 1.1	94.86 ± 0.20	3.70 ± 0.26	1.72 ± 0.72	2.796	0.934	0.145	0.975	4.8

4. CONCLUSIONS

More than solely colorants, carotenoids acted as lubricants allowing the obtention of less rigid PLA films with improved mechanical properties. The presence of plant-derived carotenoids extracts reduced the oxygen transmission rate and lowered films transparency. Therefore, the colored PLA films could offer higher protection against oxygen and light action in terms of product packaging, which turns the polymer more suitable to protect oxidation-sensitive products. Light exposure, the most impacting factor on carotenoids color stability, caused β -carotene rapid degradation. The very low stability of β -carotene could be used in the development of smart packaging materials as an indicator monitoring the conditions in which the product was kept. The elevated stability of bixin turns it into a potential natural orange-red colorant to polymeric materials for food packaging.

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ARTIGO 2

Poly(acid lactic) films with carotenoids extracts: release study and effect on sunflower oil preservation

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ABSTRACT

Oxidative deterioration is one of the most important factors limiting shelf-life of fatty food products, where the use of active packaging produced with natural antioxidants is a strategy to minimize these reactions. Poly(acid lactic) (PLA) films were produced with carotenoids extracts rich in beta-carotene, lycopene, and bixin. The kinetics of carotenoids' release to a fat simulant was evaluated and successfully explained by a new mathematical release model which considered the degradation of the migrants after their release. In the presence of light, films with lycopene and beta-carotene protected sunflower oil mainly by their light barrier properties, and secondly by their barrier to oxygen and antioxidant gradual releasing. The films produced with bixin presented the best performance as antioxidant active packaging to sunflower oil, where differences found in films barrier properties and in the transfer coefficients among the carotenoids were influenced by their molecular structures.

Keywords: beta-carotene; lycopene; bixin; active packaging; kinetic; oxidation.

1 INTRODUCTION

Oxidative deterioration is one of the most important factors limiting shelf-life of edible oils and lipid-rich foods. It rises the formation of toxic aldehydes through the degradation of polyunsaturated fatty acids, reducing the nutritional value of food products (GUILLEN; GOICOECHEA, 2008). Lipid oxidation also leads to significant changes in food sensory properties, such as undesirable flavors, color, and texture. On account of the considerable economic impact of these phenomena, the food industry has constantly been looking for strategies to reduce the effects of oxidation, and it has long recognized the importance of using antioxidants for this purpose (SANCHES-SILVA et al., 2014).

The direct addition of antioxidants and the design of suitable packaging technologies are the two most common options for reducing the oxidative deterioration in food products (LÓPEZ-DE-DICASTILLO et al., 2012). The presence of oxygen can be limited by vacuum or modified atmosphere packaging combined with a high-barrier packaging material, though it can rarely be completely eliminated since the oxygen dissolved in the food matrix cannot be evacuated. The incorporation of antioxidant compounds into a polymeric structure, producing the so-called antioxidant active packaging, is a valid alternative to the above-mentioned protection systems (GOMEZ-ESTACA et al., 2014). One of the main advantages of antioxidant active packaging is the extension of the antioxidant effect as a result of the gradual migration from the film to the food matrix.

Nowadays, there is a consumers' demand for natural antioxidants rather than artificial substances, which have often been related to possible toxicological side-effects. The addition of natural antioxidants to the packaging material may reduce the need of synthetic antioxidants, minimizing the risk of potential toxicity by migration (LÓPEZ-DE-DICASTILLO et al., 2012) as well as reducing the use of additives in the packed food.

The use of carotenoids as antioxidants for the protection of lipid-rich food systems has gained attention in the last decades, where their dosage and manipulation are reported not to be easy. Carotenoids' molecular structure present an extensive system of conjugated double bonds, which makes them very susceptible to radical addition (EDGE; MCGARVEY; TRUSCOTT, 1997). Scientific evidences suggests that

carotenoids antioxidant activity is related to their ability to scavenge and deactivate free radicals, including peroxy radicals (KIOKIAS; VARZAKAS; OREOPOULOU, 2008), and also through the quenching of singlet oxygen (PALOZZA; KRINSKY, 1992), both *in vivo* and *in vitro*.

The incorporation of carotenoids into packaging materials has been carried out with different purposes (Table 1). The heat processing of polymers causes material degradation by heat, shear, oxygen and/or UV light, and the presence of beta-carotene and lycopene-rich extracts in different polymeric matrices presented satisfactory effects on increasing the stability of polymers during its processing and storage (Kirschweng, Tátraaljai, Földes, & Pukánszky, 2017; López-Rubio & Lagaron, 2010).

The increased stability of polymers is frequently associated with the antioxidant activity and UV absorbing potential of carotenoids. Moreover, bixin-rich extracts have been used as a coloring and antioxidant additives in packaging materials (PAGNAN et al., 2017b), where the carotenoid was shown as a potential natural dye for food packaging. The use of carotenoids as antioxidants to produce active packaging has also been evaluated and is mainly intended to extend the shelf-life of fatty food products. Tocopherol, astaxanthin, beta-carotene, and lycopene were introduced into different polymers to produce antioxidant packaging materials (Colín-chávez, Soto-valdez, Peralta, Lizardi-mendoza, & Balandrán-quintana, 2013; Colín-Chávez & Vicente-ramírez, 2014; Manzanarez-López, Soto-Valdez, Auras, & Peralta, 2011; Samsudin, Soto-Valdez, & Auras, 2014)

Table 1. Application of carotenoids in polymeric matrices

Carotenoid	Polymer	Main results	Reference
Development of antioxidant active packaging			
astaxanthin	LDPE; bilayer LDPE- car/HDPE	Films reduced the peroxides formation in soybean oil; carotenoids diffusion from films into ethanol 95% exhibited typical Fick's behavior; the presence of HDPE in bilayer films layer delayed the release of carotenoids and reduced the temperature effect on carotenoids' release.	Colín-Chávez <i>et al.</i> , 2012; 2013; 2014a; 2014b
astaxanthin	bilayer HDPE- car/HDPE-TiO ₂	TiO ₂ reduced astaxanthin degradation; films improved soybean oil stability as a result of the synergic effect of light protection by TiO ₂ and carotenoids release.	Colín-Chávez <i>et al.</i> , 2014a
astaxanthin	bilayer PLA-car/PLA	Astaxanthin reduced films water vapor permeability and did not affect films O ₂ permeability; films in contact with ethanol 95% suffered solvent-induced crystallization; films reduced peroxides formation in soybean oil.	Samsudin <i>et al.</i> , 2014
α-tocopherol	PLA	The diffusion of α-tocopherol to oil was slower than to ethanol 95%; films delayed the induction period of soybean oil oxidation.	Manzanarez-López <i>et al.</i> , 2011
Protect polymers from oxidation			
beta-carotene	PLA; PCL; PHBV	Carotenoid increased the UV stability of the three biopolyesters and acted as a plasticizer.	López-Rubio <i>et al.</i> , 2011
beta-carotene	PE	Beta-carotene hindered the oxidation of polyethylene and did not increase the chain extension reactions during polymer processing; the reactions between the polymer and beta-carotene were strongly affected by the storage conditions.	Tátraaljai <i>et al.</i> , 2014
lycopene	PP	Carotenoids in tomato extract acted as a thermal and processing stabilizer.	Cerutti <i>et al.</i> , 2009
Colorants to polymers			
bixin	PHB	Bixin acted as a hiding agent, reducing the photodegradation processes.	Pagnan <i>et al.</i> , 2017
bixin; lycopene; beta-carotene	PLA	Carotenoids reduced the oxygen permeability, increased the elasticity and reduced the transparency of films; bixin was the most stable carotenoid at dark and light storage conditions.	Stoll <i>et al.</i> , 2018

*car: carotenoids; HDPE: high-density polyethylene; LDPE: low-density polyethylene; PCL: polycaprolactone; PE: polyethylene; PHB: polyhydroxybutyrate; PHBV: polyhydroxybutyrate-co-valerate; PP: polypropylene.

2 MATERIALS AND METHODS

2.1 Materials

PLA 2003D (4% D-Lactic acid) was acquired from NatureWorks (Blair-NE, USA). Annatto seeds (*Bixa orellana*), carrots (*Daucus carota*), tomatoes (*Solanum lycopersicum*) and sunflower oil were obtained from a local market at Porto Alegre, Brazil. Beta-carotene standard was obtained from Sigma-Aldrich (CAS 7235-40-7, purity of 93.0%), lycopene standard (purity of 94.4%) was obtained following the methodology of extraction and crystallization of Nunes and Mercadante (2004), and bixin standard (purity of 99.9%) was obtained after bixin extraction and crystallization methodology described by Rios & Mercadante (2004). All chemicals used were of analytical grade.

2.2 Beta-carotene, lycopene and bixin extracts

Beta-carotene and lycopene were extracted from sliced carrots (500 g) and deseeded chopped tomatoes (500 g), respectively, using approximately 1 L of ethyl acetate for each extraction (NUNES AND MERCADANTE, 2004). After 2 hours of mechanical stirring, the extracts were filtered and transferred into a separating funnel to perform the water removal. Bixin was extracted from annatto seeds following the methodology of Rios and Mercadante (2004): after washing 25 g of annatto seeds with hexane and methanol, the bixin extraction occurred in two phases of 30 min each, with 50 ml portions of chloroform. All the extractions were performed at dark and ambient temperature. After filtration with Whatman No.1 filter paper, the extracts of beta-carotene, lycopene and bixin were concentrated in a Fisatom rotaevaporator (M802, Brazil) at 40 °C.

The purity of the carotenoids extracts was confirmed by High-Performance Liquid Chromatography (HPLC), following the methodology described in our previous study (STOLL *et al.*, 2018). Carotenoids were identified through the comparison of peaks retention times to external standards, and the carotenoids UV-vis spectra (λ max and shape) were compared to literature data. The extracts purity, calculated through the relation between the main carotenoid peak area to the sum of peaks, exceeded 89% of the total peak area ($\geq 89\%$ of purity) (STOLL *et al.*, 2018). The

analyses were conducted using a Shimadzu HPLC-DAD (Kyoto, Japan) chromatographer equipped with two pumps, on-line degasser, column oven, connected in series to a diode array detector. The beta-carotene and lycopene extracts were analysed using a C30 polymeric column YCM (250 × 4.6 mm i.d.; 3 µm particle size). The mobile phase gradient consisted of water, methanol, and methyl tert-butyl ether, starting from 5:95:5 v/v and reaching 0:95:5 v/v at 12 min, 0:89:11 v/v at 25 min, 0:75:25 v/v at 40 min and finally 0:50:50 v/v at 80 min. The bixin extract analysis was conducted on a column C18 Spherisorb ODS-2 (150 × 4.6 mm i.d.; 3 µm particle size), and acetonitrile/acetic acid (2 %) (63:35, v:v) was used as mobile phase. All analyses were performed at 33 °C, with a flow rate of 1 mL/min and 5 µL of injection volume. ChemStation® software was used to acquire and process data.

2.2.1 Carotenoids quantification

The carotenoids quantification previously to their incorporation into PLA solutions followed the methodology of Stoll *et al.* (2018). Chloroform, the solvent used to dissolve the PLA polymer, was also used to dissolve and quantify the carotenoids via spectrophotometry UV-Vis (IL-592, Spectrale, Japan). Beta-carotene was evaluated at $\lambda = 465\text{nm}$ ($A_{1\%} = 2396$), lycopene at $\lambda = 466\text{nm}$ ($\epsilon = 150855\text{ L mol}^{-1}\text{ cm}^{-1}$), and bixin at $\lambda = 470\text{nm}$ ($A_{1\%} = 2826$). The λ_{max} of each carotenoid in chloroform was confirmed after scanning (200 nm - 700 nm). The results were expressed as total beta-carotene, total lycopene, and total bixin equivalent, for carrot, tomato, and annatto seed extracts, respectively.

2.3 Production of PLA films with carotenoids

The PLA films with carotenoids were prepared following the methodology of Stoll *et al.* (2018). Solutions of PLA in chloroform (5 % w/v) were obtained through continuous mechanical stirring at ambient temperature. After that, a known amount of carotenoids dissolved in chloroform was added to PLA solutions in order to achieve a concentration of $100\text{ }\mu\text{g g}^{-1}$ (carotenoid/PLA dry matter). The solutions were stirred for 10 min and then poured on Teflon recipients placed at a leveled surface, in a sufficient quantity to produce films with $125 \pm 5\text{ }\mu\text{m}$ of thickness. Chloroform was left to evaporate at ambient temperature, for 24 h in the dark. Finally, films were peeled from the recipients and placed in an oven at 40 °C, with forced air circulation, for 6 h. Pure PLA

films and films added with beta-carotene, lycopene and bixin were named as F.PLA, F.Beta, F.Lyco and F.Bixin, respectively.

2.4 Films optical properties

The UV-Vis light barrier properties of the films were measured by wavelength scanning the samples from 200 to 700 nm using a UV spectrophotometer (Shimadzu UV-1800, Japan), and results were expressed as percentage transmittance. The films were placed in a spectrophotometer test cell and an empty quartz test cell was used as the reference. The films transparency was determined as the percentage of transmittance at 500 nm (TANG et al., 2005).

2.5 Oxygen Permeability

The oxygen permeation rate of the films was determined according to the ASTM D-3985 at 23 °C, with an Oxygen Permeability Tester, Mocon Oxtran MS 2/21 (Lippke, Neuwied, Germany). The transmission values were determined every 45 min until constant and results were expressed as oxygen transfer rate per film thickness (OTR.t), in $\text{cm}^3 \text{ mm m}^{-2} \text{ day}^{-1}$. Thickness was measured using a micrometer (Digimes, IP40, Brazil; 0.001 mm precision and 0–25 mm resolution) from 10 readings taken at random positions of the films.

2.6 Release study

To evaluate the kinetics of carotenoids release to a fatty food, a solution of 95% ethanol (v/v) in distilled water was used as a food simulant. Even though the Regulation (EU) No 10/2011 proposes the use of vegetable oil as a food simulant for fatty food, the natural composition of vegetable oil and the formation of oxidation products (390–550 nm) hampers carotenoids quantification by spectrophotometry. For this reason, ethanol 95 % v/v, approved as a fatty food simulant substitute, was used as a simulant.

Films (6 cm × 2.5 cm ~125 μm) were placed in 12 mL amber flasks and 10 mL of food simulant was added, somehow completely immersing film samples. The flasks were tightly sealed with plastic caps and stored at 40 °C for 60 days, in the dark. To minimize mass transfer resistance of carotenoids from the films, the flasks were shaken manually, three times a week. The carotenoids release was periodically

determined by spectrophotometry (UV-Vis, IL-592, Spectrale, Japan) at $\lambda = 449$ nm, 480 nm, and 457 nm for bixin, lycopene and beta-carotene, respectively. Aliquots of 2 mL of each flask were used to measure total carotenoid content in the simulant, being the liquid returned to flask after the analysis.

2.6.1 Carotenoids quantification in food simulant

For the quantification of carotenoids in the food simulant (C), an analytical method was performed and validated through the construction of calibration curves using absorption versus nominal concentrations of each carotenoid in ethanol 95%. Seven standard solutions, each one in triplicate, were obtained by diluting carotenoids standard stock solutions in ethanol 95%. The standard curves of beta-carotene, lycopene, and bixin in ethanol 95 % were constructed in the following concentration ranges: beta-carotene ($\lambda_{\max} = 449$ nm), from 2.2 to 105.4 $\mu\text{g mL}^{-1}$ ($R^2 = 0.999$); lycopene ($\lambda_{\max} = 480$ nm), from 0.3 to 13.9 $\mu\text{g mL}^{-1}$ ($R^2 = 0.999$); bixin ($\lambda_{\max} = 457$ nm), from 0.3 to 5.3 $\mu\text{g mL}^{-1}$ ($R^2 = 0.996$). The lower and upper limits of detection (LOD) and quantification (LOQ) for carotenoids dissolved in ethanol 95 % were 0.008 and 0.025 $\mu\text{g mL}^{-1}$ for beta-carotene, 0.004 and 0.012 $\mu\text{g mL}^{-1}$ for lycopene, and 0.002 and 0.008 $\mu\text{g mL}^{-1}$ for bixin, respectively. The LOD and LOQ were calculated as 3.3 and 10 σ/S , respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve, as defined by International Conference on Harmonization (2005).

2.7 Degradation kinetics of carotenoids in the food simulant

Solutions of known initial concentration of beta-carotene, lycopene, and bixin in ethanol 95 % had its concentration evaluated over time, in the same storage conditions of the release study. The carotenoids degradation in the food simulant was evaluated by quantifying carotenoids by the methodology already described in section 2.6.1. The carotenoids' kinetics of degradation in the food simulant (k_d) has been found to follow a first-order reaction model and was calculated according to equation 1.

$$\frac{dC}{dt} = -k_d C \quad (\text{Eq.1})$$

where C is the carotenoid concentration in the food simulant ($\mu\text{g mL}^{-1}$), k_d is the first-order kinetic degradation coefficient (s^{-1}), and t is the exposure time (s). The parameter k_d was estimated from the linear regression of $\ln C$ versus t using MS Excel.

2.8 Mathematical release model

The migration process involves the transfer of a substance from the packaging film into a packaged product, where the diffusion coefficient is frequently used to describe this process based on the Fick's second law (GAVRIIL; KANAVOURAS; COUTELIERIS, 2017). However, this approach neglects the external mass transfer resistance and can lead to an underestimate of the real diffusion coefficient of the migrant in the solid phase (VITRAC; HAYERT, 2006). A novel approach was proposed by Samsudin *et al.*, (2018) to evaluate simultaneously the diffusion coefficient in the film (D), the partition coefficient (K_f) and the external convective mass transfer coefficient (k). The model equals the mass balances in the film and in the surroundings (food simulant), but does not consider the chemicals reaction, *i.e.*, the possible degradation of migrants after their release, which is of foremost importance regarding natural and sensitive compounds such as carotenoids. In addition, the simultaneous evaluation of k and D is hampered by the high correlation between these parameters.

In this work, we used the mass balance in the food simulant to evaluate the overall mass transfer resistance (K), taking into account the carotenoid degradation in the food simulant, as shown in equation 2.

$$\frac{dC}{dt} = K \frac{A}{V} (C^* - C) - k_d C \quad (\text{Eq. 2})$$

where C is the carotenoid concentration in the food simulant ($\mu\text{g mL}^{-1}$), K is the overall mass transfer coefficient (m s^{-1}), A is the surface area of the film considering the migration occurs in both sides (0.003 m^2), V is the volume of the food simulant solution (10^{-5} m^3), C^* is the carotenoid concentration ($\mu\text{g mL}^{-1}$) in the inner part of the film ($x = L/2$), and k_d is the first-order kinetic degradation coefficient (s^{-1}) (Figure 1).

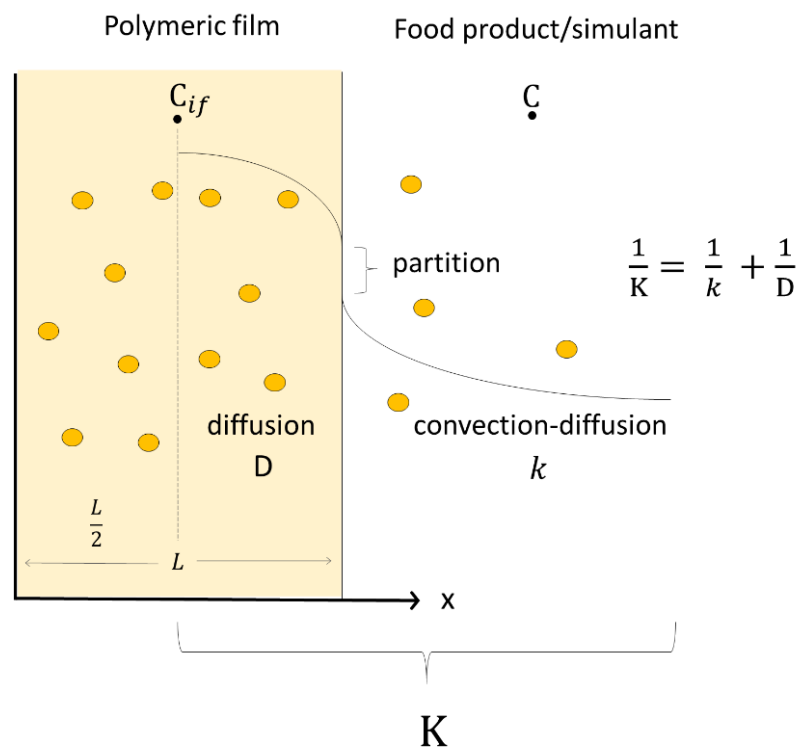
The overall mass transfer coefficient takes into account the sum of the resistances of mass transfer by diffusion inside the polymeric film, the migration through the film-food simulant interface, and the mass transfer in the food simulant, as follows:

$$\frac{1}{K} = \frac{1}{k} + \frac{L/2}{D} \quad (\text{Eq. 3})$$

where k is the mass transfer convection coefficient in the food simulant and D is the diffusion coefficient in the PLA.

The model is based on the following assumptions: 1) carotenoids were uniformly distributed in the PLA matrix at the beginning of the process; 2) the initial carotenoid concentration in the simulant is zero ($C(0) = 0$); 3) the overall mass transfer coefficient (K) is not affected by the carotenoids concentration; 4) the carotenoid concentration in the inner part of the film (C^*) is constant during the entire experiment. The last assumption was proven true by the good fitting of the model and by the difficulty of reliable parameter estimation when using a different approach. The parameter k_d was evaluated previously by an independent experiment (“Degradation kinetics of carotenoids in the food simulant”). The parameter K was evaluated from the experimental data k_d and C (section 2.6.1) using the software EMSO (Environment for Modeling, Simulation and Optimization).

Figure 1. Graphical representation of the overall mass transfer coefficient (K)



2.9 Partition Coefficient

The partition coefficient (K_{part}), which is defined as the ratio of migrant equilibrium concentration in the polymeric material (C_p) to its equilibrium concentration in the food phase/simulant (C_s), was calculated according to equation 4. The C_p was measured by film dissolution in chloroform followed by the quantification via spectrophotometry (section 2.2.1).

$$K_{part} = \frac{C_p}{C_s} \quad (\text{Eq. 4})$$

2.10 Antioxidant activity of films and carotenoids in sunflower oil

The antioxidant capacity of the films and carotenoids on protecting sunflower oil from oxidation was evaluated by different manners: Treatment 1 – Immersion of the films in the oil; Treatment 2 – Direct addition of the carotenoids in the oil; Treatment 3 – Packing oil samples in film sachets stored in the dark; Treatment 4 - Packing oil samples in film sachets stored in the light. For treatment 1, PLA films (5 cm × 10.5 cm) were double-sided immersed in sunflower stored in amber glass flasks, where oil in contact with the F.PLA was used as a control. To compare the effect of carotenoids gradual release from films with the direct incorporation of the carotenoids into the oil, carotenoids were directly added to sunflower oil (16.4 $\mu\text{g mL}^{-1}$) and stored in amber glass flasks (treatment 2). The concentration of 16.4 $\mu\text{g mL}^{-1}$ is the amount of carotenoids that the oil from treatment 1 would achieve in the case of total migration of the carotenoids contained in films.

The flasks were tightly sealed with plastic caps and stored at 40 °C, in the dark. Concurrently, sachets (7 cm × 6 cm) were produced by heat sealing the PLA films. Sunflower oil was poured into the sachets, air bubbles were removed, and sachets were sealed avoiding the presence of headspace. The oil sachets were stored at 40 °C in the presence (treatment 3) and the absence (treatment 4) of light. A sachet produced with F.PLA was used as a control. Treatments 1, 2 and 3 presented the same surface contact area of film (cm^2)/oil volume (mL) ratio (~10.5).

The peroxide value (PV) of the oil samples was periodically determined according to the IUPAC method. The analysis of conjugated dienes, given by the K_{232}

extinction coefficient, followed the analytical method described in European Regulation EC 2568/91 and was measured using a UV spectrophotometer (UV-1800, Shimadzu, Japan). The samples were evaluated in triplicate.

2.11 Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and a Tukey test at significance level of 5 % ($p < 0.05$) using the software Statistica 13.0 (STATSOFT Inc.), corresponding to the average of three replicates ($n = 3$).

3 RESULTS AND DISCUSSION

3.1 Barrier properties

One of the desired characteristics of a fatty food packaging material is that it should offer a barrier against oxygen and light. Studies have shown that PLA, like other biopolymers, has lower oxygen barrier performance than petrochemical polymers such as PET, but higher than low oxygen barrier films such as LDPE and PS (Domenek, Fernandes-Nassar, Ducruet, 2017). The measurements of oxygen transfer rate (OTR.t), given in [10^{-18} kg m m⁻² s⁻¹ Pa⁻¹] were 4.56 ± 0.07 (F.PLA), 3.06 ± 0.15 (F.Beta), 3.19 ± 0.17 (F.Lyco), and 4.00 ± 0.21 (F.Bixin).

The presence of lycopene and beta-carotene reduced the OTR.t of PLA films, while bixin has not effectively affected films permeability to oxygen when compared to F.PLA. The carotenoids incorporation into PLA films did not affect their thickness ($p > 0.05$), which varied from 135 ± 39 μm (F.PLA) to 122 ± 27 μm (F.Bixin). Considering that OTR.t is governed by sorption and diffusion mechanisms (SIRACUSA, 2012), the bixin's carboxylic group – which have a polar character - may have reduced oxygen adsorption, thus facilitating its diffusion through the PLA polymer matrix (Stoll *et al.*, 2018). Inversely, the absence of such groups in beta-carotene and lycopene molecular structures might have increased oxygen adsorption, therefore reducing the diffusion of oxygen.

The light transmission properties of the films were assessed at wavelengths ranging from 200 to 700 nm and the percentage light transmittance was recorded (Table 2). Since light is a powerful lipid-oxidizing agent in food systems, a low light

transmittance is desired. Films with carotenoids exhibited lower UV/Vis light transmission than pure PLA films, demonstrating that carotenoids extracts endowed the films with a barrier to ultraviolet (200-300 nm) and visible light (300-700 nm). F.Beta, when analyzed soon after its production (time zero), presented the better results for UV light barrier, while F.Bixin showed the lowest transparency before and after storage. Changes in films' light transmittance occurred after storage under light - possibly due to carotenoids photodegradation – where an increase in the transparency of the film was observed. The transparency of F.Beta increased the most, which is reasonable considering the photostability of beta-carotene is reported to be lower than lycopene and bixin (Shahid-ul-Islam, Rather, & Mohammad, 2016; Stoll *et al.*, 2018). The color stability of carotenoids in PLA films was evaluated in a previous study, where bixin was the most stable carotenoid under dark and light conditions of storage, followed by lycopene and beta-carotene (Stoll *et al.*, 2018).

3.2 Release study

The kinetic model proposed by the present study showed >96% confidence between the experimental and theoretical migration data (Table 3, Figure 2a), demonstrating the importance of considering the degradation coefficient of sensitive migrants after their release from active packaging. The migration of active compounds from a controlled release packaging film involves the molecular diffusion within the film toward to the film/food interface, the mass transfer across the interface, and the dispersion into the bulk food. In most cases, diffusion is the slowest or rate controlling step (LACOSTE *et al.*, 2005). Mathematical release model is a tool to estimate the concentration of the migrant in the food after contact with the packaging material during the shelf-life of a product (GAVRIIL; KANAVOURAS; COUTELIERIS, 2017).

Table 2. UV and visible light transmittance (%) and transparency (%) of fresh films (time zero) and films stored for 21 days at 40 °C under light.

Wavelength (nm)	Time zero				After storage		
	F.PLA	F.Beta	F.Lyco	F.Bixin	F.Beta	F.Lyco	F.Bixin
200	0.03±0.01	0.02±0.02	0.02±0.03	0.02±0.02	0.02±0.02	0.11±0.0	0.04±0.01
280	67.93±6.20	34.19±7.27	53.25±5.08	55.65±3.39	28.81±0.68	43.33±1.26	43.24±0.26
300	74.39±5.21	42.29±7.57	53.98±4.86	62.51±3.17	37.82±0.65	46.54±1.48	50.39±0.00
400	83.82±3.15	52.92±7.66	65.02±4.41	56.95±3.52	64.14±0.41	58.55±2.18	56.61±0.29
500*	85.72±2.53	42.69±8.27	51.93±3.81	31.89±3.49	73.95±0.33	56.89±2.10	40.41±0.10
600	86.83±2.13	81.86±7.05	81.11±3.71	81.77±2.61	79.73±0.33	79.96±3.47	80.93±0.48
700	87.65±1.90	83.12±6.80	82.43±3.50	83.05±2.53	83.14±0.34	82.46±3.62	83.07±0.55

*Films transparency. Values are given as mean ± SD (n = 3)

Table 3. Experimental results for kinetic rate of degradation (k_d) and partition coefficient (K_{part}), and parameters estimated using the experimental data through the mathematical release model (K and C^*).

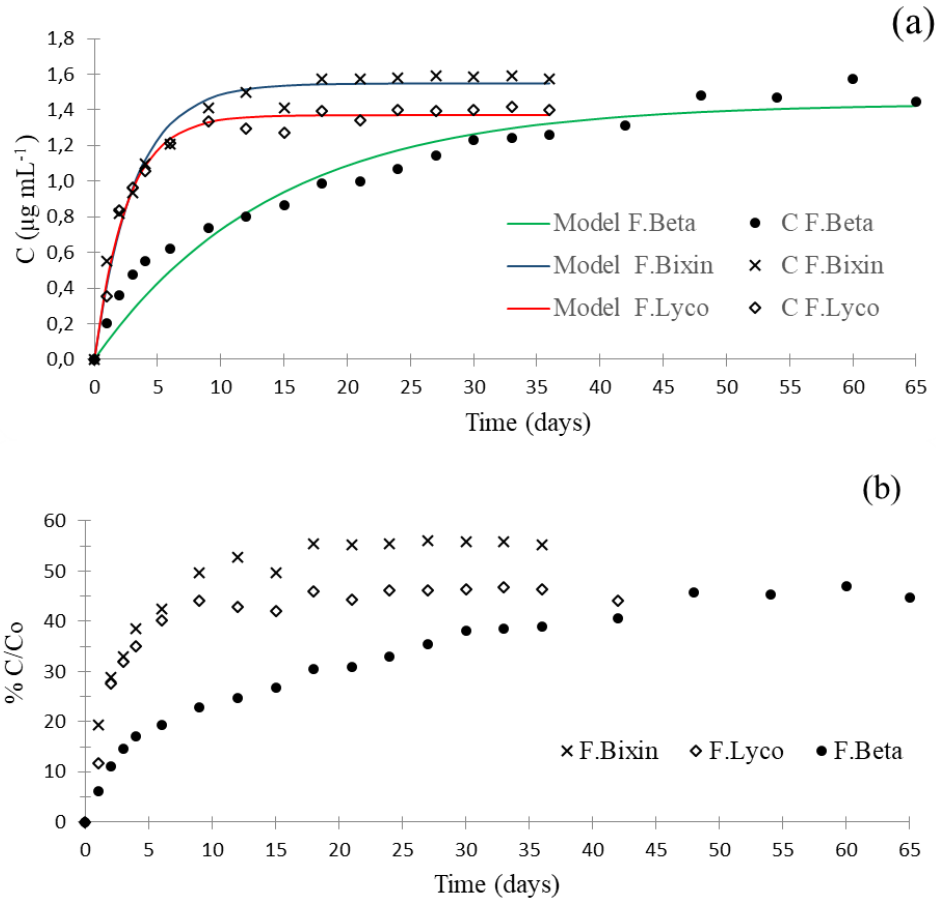
	F.Beta	F.Lyco	F.Bixin
K_{part}	3.29 a	3.14 a	3.45 a
k_d ($s^{-1} \times 10^{-7}$)	1.089	1.134	2.155
$R^2 k_d$	0.93	0.96	0.94
K ($m s^{-1} \times 10^{-8}$)	0.237	1.462	1.157
$R^2 K$	0.96	0.97	0.98
C^* ($\mu g ml^{-1}$ simulant)	1.66	1.54	1.64

The three tested carotenoids were gradually released to the food simulant, reaching ~45% release of beta-carotene and lycopene, and ~55% release of bixin (Figure 2b). However, beta-carotene migrated at a much lower rate than lycopene and bixin, given by its lower overall mass transfer coefficient (K). While F.Lyco and F.Bixin reached equilibrium after approximately 15 days of contact with the simulant, F.Beta took almost 60 days to reach the equilibrium state. It is important to emphasize that tests were performed at 40 °C, and according to an accelerated testing protocol recommended by FDA, a temperature of 40 °C during 10 days has migration levels roughly equivalent for tests performed at 20°C during 6-12 months. Hence, considering edible oils have a shelf-life of approximately 18 months, the release rate of beta-carotene may be considered very low.

The molecular size and shape of compounds are considered to affect their diffusion process. Larger molecules tend to present lower diffusion coefficients than smaller ones (CHAN; LI; LI, 2015) and compounds of bulky structure are supposed to migrate at slower rates (SAMSUDIN; SOTO-VALDEZ; AURAS, 2014a). Besides that, the higher the number of hydroxyl groups in the structure, the slower might be the rate of diffusion of compounds from PLA matrix into ethanol (Iñiguez-Franco, Soto-Valdez, Peralta, Ayala-Zavala, F., Auras & Gámez-Meza, 2012), which might be related to the stronger interaction between the migrant compound and the PLA polymeric chain.

In the present study, bixin presented an overall mass transfer coefficient (K) relatively similar to that of lycopene and significantly higher than beta-carotene. Hence, the above-mentioned effects were counterbalanced in F.Bixin, which is the smaller carotenoid molecule tested and the only one with a hydroxyl group in its structure. The differences found in the coefficient K between beta-carotene and lycopene might be understood by their molecular structures: while lycopene is a flexible acyclic open-chain molecule, beta-carotene has two rings at the ends which confer more rigidity. Hence, despite presenting the same molecular formula ($C_{40}H_{56}$), the higher flexibility of lycopene chains may have reduced the intermolecular friction with PLA (Stoll *et al.*, 2018), facilitating lycopene release to the food simulant.

Figure 2. (a) Experimental data of carotenoids concentration (C) in ethanol 95% with the models prediction curves of carotenoids release from PLA films; (b) Percent ratio of released carotenoids in the food simulant related to the initial carotenoid amount in PLA films ($\% C/C_0$).



Meanwhile the overall mass transfer coefficient (K) determines how fast is the carotenoid transfer rate from the film into the simulant, the partition coefficient (K_{part}) is a measure of the relative chemical affinity of the migrant towards the film and the food simulant, indicating the physical process between the parts. The K_{part} did not differ ($p > 0.05$) among the carotenoids (Table 3), and its value ($K_{part} > 1$) represents a higher affinity of the carotenoids towards the PLA film matrix with respect to the food simulant. However, the release of carotenoids reached distinct percent ratio among the films, where bixin presented a higher percent ratio of release than lycopene and beta-carotene (Figure 2b). At the same time bixin presented the highest k_d among the carotenoids, representing its lower stability in ethanol 95%, it was shown to be the most stable carotenoid in the PLA matrix compared to lycopene and beta-carotene (Stoll *et*

al., 2018) . Hence, the lack of stability of antioxidants such as carotenoids might affect their final concentration after release studies (CRUZ-ZÚÑIGA *et al.*, 2016), both in the polymer and in the simulant, interfering in the K_{part} value and in release percent ratio ($\% C/C_0$) of the compounds.

3.3 Antioxidant effect of films on sunflower oil

The development of new materials that present a controlled release of active compounds has been hindered by the lack of information dealing with the real benefits they present compared to direct addition of the active compounds (MASTROMATTEO *et al.*, 2010). The results obtained by keeping sunflower oil in contact with immersed PLA films (treatment 1) and directly adding carotenoids into the oil (treatment 2) are shown in Table 4. In the present study, the direct incorporation of beta-carotene and lycopene presented a pro-oxidant effect on sunflower oil, while bixin retarded the formation of peroxides during 21 days of storage. On the other hand, the gradual release of carotenoids into the oil - provided by the contact with immersed active films – did not present effects on peroxides formation, but effectively retarded the formation of dienes during the storage ($p < 0.05$). The comparison between the controls of treatment 1 and 2 indicates that F.PLA presented an antioxidant effect itself. This was also observed in other studies (CRUZ-ZÚÑIGA *et al.*, 2016) and indicates the presence of antioxidants in the PLA resin, as reported by Iñiguez-Franco *et al.* (2012). (2012)

Other studies have demonstrated a pro-oxidant effect caused by the direct incorporation of lycopene and beta-carotene into vegetable oils, where the effect of carotenoids on lipid oxidation was found to be concentration dependent (Haila, Lievonen, & Heinonen, 1996; Yanishlieva, Aitzetmüller, & Raneva, 1998; Zeb & Murkovic, 2013). Carotenoids are suggested to inhibit lipid oxidation primarily by trapping peroxy radicals, and their antioxidative behavior is closely related to carotenoids own oxidation. However, the autoxidation of carotenoids produces a complex mixture of products with epoxy, hydroxy, and carbonyl groups (HAILA; LIEVONEN; HEINONEN, 1996), which might enhance the propagation stage of the oxidation reaction by supplying the system with more oxidizable substrates (Stenson & Min, 2000). Hence, the presence of carotenoids oxidized products generated by the

direct introduction of beta-carotene and lycopene into sunflower oil might be risky, considering those compounds may accelerate the oxidation of oils.

Meanwhile the immersion of the developed films in sunflower oil shows their antioxidant potential related to the release of carotenoids, the film's performance on protecting the oil from oxidation can only be entirely evaluated through the packing of oil with the films. Oxygen and light play an important role on accelerating lipid oxidation, so the barrier offered to those elements is crucial for films antioxidant activity as a packaging material. After 14 days of storage in the dark (treatment 3), the sachets containing carotenoids protected the oil from the formation of peroxides, when the oil packed in F.PLA sachets had already exceeded the limit of peroxides (10 meq/kg) established by the Codex Alimentarius) to oil commercialization. F.Bixin was the only film sachet which effectively maintained a lower level of peroxides after 21 days when compared to the F.PLA (Table 5). In the absence of light, the oil oxidation rate is affected mainly by autoxidation, which occurs due to the reaction between the oxygen and unsaturated fatty acids (KIOKIAS; VARZAKAS; OREOPOULOU, 2008). Hence, the differences between the oxidative stability of treatment 1 and treatment 3 to the same carotenoid are mainly related to the availability of oxygen. Under this perspective, it could be inferred that F.Beta and F.Lyco presented lower PV results than F.PLA due to their lower oxygen permeability, once the immersed films F.Beta and F.Lyco (treatment 1) - on which the availability of oxygen was the same to all treatments - did not present any antioxidant or pro-oxidant effect.

In the presence of light (treatment 4) the films with carotenoids effectively protected the sunflower oil from peroxides formation (Table 5), presenting PV levels lower than the oil packed in F.PLA during the whole period analyzed. The level of secondary products of oxidation, given by dienes values, was lower to oils packed in carotenoids rich films up to the second day of storage, but did not differ from F.PLA sachet ($p > 0.05$) after 4 days. Hence, the presence of carotenoids acted in the initiation stage of oxidation, trapping peroxy radicals probably by its addition to the carotenoid polyene chain, although not avoiding the propagation of the reaction.

Table 4. Peroxides (meq O₂/kg) and conjugated dienes (K₂₃₂) of sunflower oil packed in amber glass flasks: oil in contact with immersed PLA films (Treatment 1) and oil directly added with carotenoids (Treatment 2), stored at 40 °C, in the dark.

	Control	F.Beta	F.Lyco	F.Bixin
Treatment 1				
Peroxides				
0 days	2.20 ± 0.06 a C	2.20 ± 0.06 a C	2.20 ± 0.06 a C	2.20 ± 0.06 a C
7 days	7.75 ± 0.31 a B	6.84 ± 0.68 ab B	5.55 ± 0.26 b B	6.19 ± 0.07 ab B
14 days	8.93 ± 0.98 ab AB	12.12 ± 1.12 a A	7.08 ± 0.30 b A	7.09 ± 0.98 b B
21 days	9.16 ± 0.02 a A	9.83 ± 1.42 a A	7.11 ± 0.32 a A	9.83 ± 0.99 a A
Conjugated Dienes				
0 days	2.35 ± 0.12 a C	2.35 ± 0.12 a C	2.35 ± 0.12 a D	2.35 ± 0.12 a D
7 days	3.72 ± 0.51 a B	3.71 ± 0.06 a B	3.68 ± 0.07 a C	3.51 ± 0.06 a C
14 days	4.26 ± 0.14 b B	5.13 ± 0.08 a A	4.25 ± 0.06 b B	4.16 ± 0.02 b B
21 days	7.52 ± 0.02 a A	4.85 ± 0.31 c A	6.09 ± 0.19 b A	5.77 ± 0.03 bc A
Treatment 2				
Peroxides				
0 days	2.20 ± 0.06 a D	2.20 ± 0.06 a D	2.20 ± 0.06 a C	2.20 ± 0.06 a D
7 days	10.48 ± 0.72 c C	15.32 ± 1.01 a C	12.71 ± 0.41 b B	12.81 ± 1.02 b C
14 days	17.04 ± 0.06 a B	11.89 ± 0.22 c B	14.01 ± 0.38 b B	16.78 ± 1.06 a B
21 days	26.45 ± 0.08 d A	36.47 ± 0.54 b A	48.51 ± 0.91 a A	24.60 ± 0.45 c A
Conjugated Dienes				
0 days	2.35 ± 0.12 a BC	2.35 ± 0.12 a C	2.35 ± 0.12 a B	2.35 ± 0.12 a C
7 days	2.33 ± 1.09 a C	3.43 ± 0.62 a C	2.21 ± 0.12 a B	1.75 ± 0.36 a C
14 days	4.58 ± 0.56 b B	4.88 ± 0.35 b B	6.12 ± 0.12 a A	4.73 ± 0.17 b B
21 days	8.67 ± 1.52 a A	8.75 ± 0.11 a A	6.63 ± 0.52 a A	7.51 ± 0.66 a C

Values are given as mean ± SD (n = 3). Different lowercase letters in the same line indicate significant differences (p < 0.05) among the treatments. Different uppercase letters in the same column indicate significant differences (p < 0.05) among the days of a treatment.

Table 5. Peroxides (meq O₂/kg) and conjugated dienes (K₂₃₂) of sunflower oil packed in film sachets stored in the absence (Treatment 3) and the presence of light (Treatment 4).

	F.PLA	F.Beta	F.Lyco	F.Bixin
Treatment 3				
Peroxides				
0 days	2.20 ± 0.06 a B	2.20 ± 0.06 a C	2.20 ± 0.06 a C	2.20 ± 0.06 a C
7 days	2.78 ± 0.21 a B	2.51 ± 0.03 a C	2.75 ± 0.03 a C	2.86 ± 0.16 a C
14 days	13.01 ± 0.78 a A	9.61 ± 0.10 b B	5.40 ± 0.03 c B	6.10 ± 0.21 c B
21 days	13.5 ± 0.76 a A	12.54 ± 0.43 ab A	12.99 ± 0.36 ab A	11.77 ± 0.50 b A
Conjugated Dienes				
0 days	2.35 ± 0.12 a C	2.35 ± 0.12 a C	2.35 ± 0.12 a C	2.35 ± 0.12 a C
7 days	2.94 ± 0.27 a B	3.02 ± 0.02 a B	3.00 ± 0.07 a B	2.90 ± 0.23 a B
14 days	3.64 ± 0.18 a A	3.28 ± 0.05 ab B	3.04 ± 0.18 b B	3.32 ± 0.12 ab B
21 days	4.06 ± 0.19 a A	4.57 ± 0.33 a A	4.46 ± 0.07 a A	4.68 ± 0.04 a A
Treatment 4				
Peroxides				
0 days	2.20 ± 0.06 a D	2.20 ± 0.06 a E	2.20 ± 0.06 a D	2.20 ± 0.06 a E
1 day	8.14 ± 0.31 a C	4.95 ± 0.04 b D	4.03 ± 0.12 bc D	3.72 ± 0.08 c D
2 days	14.99 ± 0.31 a B	11.22 ± 0.33 b C	10.40 ± 0.14 bc C	7.74 ± 0.03 c C
3 days	19.24 ± 0.70 a B	15.33 ± 0.57 b B	15.31 ± 1.03 ab B	10.07 ± 0.11 c B
4 days	36.55 ± 3.83 a A	29.00 ± 1.14 b A	23.66 ± 1.53 bc A	19.86 ± 0.14 c A
Conjugated Dienes				
0 days	2.35 ± 0.12 a C	2.35 ± 0.12 a BC	2.35 ± 0.12 a A	2.35 ± 0.12 a BC
1 day	2.08 ± 0.06 a C	1.87 ± 0.09 a C	2.06 ± 0.24 a B	1.89 ± 0.09 a C
2 days	4.08 ± 0.06 a A	2.89 ± 0.30 b AB	3.36 ± 0.20 b A	3.26 ± 0.09 b A
3 days	4.41 ± 0.10 a A	3.63 ± 0.93 ab A	3.28 ± 0.44 b A	3.33 ± 0.43 ab A
4 days	3.71 ± 0.39 a B	3.66 ± 0.46 a A	3.43 ± 0.39 a A	3.13 ± 0.47 a AB

Values are given as mean ± SD (n = 3). Different lowercase letters in the same line indicate significant differences (p < 0.05) among the treatments. Different uppercase letters in the same column indicate significant differences (p < 0.05) among the days of a treatment.

At the end of storage under light exposure, the oils packed in F.Beta and F.Lyco presented approximately 21% and 35% lower PV levels, respectively, than the oil packed in F.PLA. However, after 21 days of storage in the dark (treatment 3), these oils presented no differences in PV and dienes values ($p>0.05$) comparing to the oil packed in F.PLA, which infers that lycopene and beta-carotene protected the oil mainly by their light barrier properties.

The oxidation of edible oils is influenced by an energy input such as light or heat, where the light incidence present the most detrimental effects on the oil stability due to $^1\text{O}_2$ oxidation (CHOE; MIN, 2006; KIM; CHOE, 2013). F.Bixin, the film with the lowest transparency and the one which presented the highest percent ratio of release to the food simulant, presented the highest protection on sunflower oil under light. Besides that, bixin is reported to have higher light stability than beta-carotene and lycopene in PLA films (Stoll *et al.*, 2018). During the light storage, the presence of carotenoids in PLA matrix protected the oil from oxidation mainly by its light barrier properties, while the carotenoids released to the oil may have acted as $^1\text{O}_2$ quenchers, free radical scavengers, and also as filters for the active wavelengths of light during irradiation, protecting the oils by light absorption (CHOE; MIN, 2006).

Despite having higher permeability to oxygen, bixin was the carotenoid that presented the best performance on protecting the sunflower oil from oxidation, reducing the formation of peroxides under dark and light storage conditions. Regarding the antioxidant activity of lycopene, beta-carotene and bixin in the oil, the results of the present study are in accordance with Kiokias *et al.* (2006) and Kiokias *et al.* (2009) where annatto extracts (rich in bixin) were more effective than beta-carotene and a tomato extract (lycopene-rich) on protecting oil-in-water emulsions from oxidation.

The higher antioxidant activity of bixin, considered a polar carotenoid, has been attributed to the electron withdrawing character of the polar groups, which stabilizes the carotenoid derived radical and reduces its ability to participate in propagating reactions. Authors also suggest that carotenoids with higher hydrophilicity provide a better interaction with hydroperoxides, thus increasing protection against lipid oxidation (YANISHLIEVA; AITZETMÜLLER; RANEVA, 1998). Besides that, polar carotenoids may act through hydrogen donation to free radicals from their hydroxyl or carboxyl groups, while hydrogen donation from the conjugated polyene chain seems more

difficult (Dimakou & Oreopoulou, 2012; Kiokias, Dimakou, Oreopoulou, 2009; Kiokias & Oreopoulou, 2006).

Considering the antioxidative performance of bixin in sunflower oil, its affinity with PLA polymeric matrix and with the fatty food simulant, the production of PLA added with bixin extract has shown to be a promising alternative on the acquisition of antioxidant packaging intended to protect lipid-rich food.

4 CONCLUSIONS

The films and the carotenoids antioxidant performances on protecting sunflower oil from oxidation, evaluated by different manners, allows the assertion that the antioxidant activity of films performed differently among the carotenoids. When exposed to light, the films produced with lycopene and beta-carotene protected the oil mainly by their light barrier properties, and secondly by their oxygen barrier and antioxidant releasing. Bixin and lycopene presented a similar overall mass transfer coefficient (K) from films to the fatty food simulant, and beta-carotene presented the lowest K value. The films produced with bixin presented the best performance as antioxidant active packaging to sunflower oil, where bixin effectively retarded the formation of peroxides under dark and light storage conditions. However, since the level of peroxides and dienes in the packed oil was not greatly reduced by the presence of carotenoids, their release rate could be increased (*e.g.* by the use of plasticants), allowing the carotenoids to effectively scavenge and/or deactivate the free radicals formed during all stages of lipid oxidation.

The differences found in films barrier properties and in the transfer coefficients between the tested carotenoids are mainly related by their molecular structures, their affinity with the polymeric matrix and with the food simulant. Concerning the release study, the good fitting of the newly proposed model exalts the importance of considering the instability of some natural antioxidants after their release from active packaging.

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CAPÍTULO 3

ARTIGOS CIENTÍFICOS ETAPA 2

CAPÍTULO 4
DISCUSSÃO GERAL E CONCLUSÕES

4 DISCUSSÃO GERAL

O presente trabalho atuou no desenvolvimento de filmes biodegradáveis de poli(ácido láctico) (PLA) adicionados de beta-caroteno, licopeno e bixina. A incorporação de carotenoides à matriz polimérica do PLA apresenta-se como uma alternativa para fornecer cor e propriedades de barreira ao material. As alterações causadas pela inserção de carotenoides podem ampliar a gama de aplicação do polímero, o que permite seu uso na embalagem de alimentos sensíveis a ação da luz e oxigênio.

O estudo e desenvolvimento de materiais com características ideais para a embalagem de alimentos é um campo que merece atenção. A possibilidade de migração de aditivos químicos tóxicos que contaminem os produtos embalados é uma preocupação crescente, fazendo com que o conceito de 'migração' envolvendo embalagens para alimentos seja, sobretudo, visto de modo negativo. Logo, o estudo dos fenômenos de interação entre os materiais e os alimentos é de extrema importância do ponto de vista da saúde e da segurança dos alimentos. A substituição de aditivos químicos sintéticos por compostos naturais com propriedades antioxidantes, além de reduzir a possibilidade de contaminação de alimentos causada pelo contato com a embalagem, é uma forma de auxiliar na manutenção da qualidade do produto ao longo do tempo de prateleira (LÓPEZ-DE-DICASTILLO et al., 2012).

Um dos fatores que limitam a utilização de compostos naturais - seja no desenvolvimento de alimentos ou polímeros - é a menor estabilidade à luz, ao calor e ao oxigênio quando comparado aos compostos sintéticos. O Artigo 1, que explora a propriedade corante de beta-caroteno, licopeno e bixina, avaliou as características de filmes de PLA com carotenoides e sua estabilidade em diferentes condições de luz e temperatura. Os filmes desenvolvidos pela técnica de *casting* apresentaram estabilidade de coloração significativamente diferentes entre si. Enquanto filmes produzidos com bixina mantiveram coloração estável por 18 dias em condições aceleradas de degradação (luz e calor), os filmes com beta-caroteno perderam a coloração rapidamente. Apesar da similaridade entre as estruturas moleculares de compostos da mesma classe, o estudo demonstra que o uso de diferentes carotenoides influenciou fortemente a estabilidade de coloração dos filmes. Além disso, como apresentado no Artigo 2, filmes que diferem entre si exclusivamente pelo tipo de carotenoide apresentaram diferentes propriedades de barreira, cinéticas de

migração e ação antioxidante, o que demonstra que compostos da mesma classe têm comportamentos diversos em uma mesma matriz polimérica.

É importante ressaltar que a estabilidade dos carotenoides no polímero afeta o material e sua performance como embalagem para alimentos de diversas formas: uma menor estabilidade tem efeitos negativos nas propriedades de barreira à luz, a qual reduz a medida que os carotenoides são degradados e perdem cor; afeta o aspecto visual das embalagens, visto que estas têm a coloração alterada ao longo do tempo; altera o potencial antioxidante dos carotenoides, onde a degradação reduz a proteção que estes poderiam oferecer ao alimento frente a reações de oxidação.

A fim de compreender o efeito dos materiais desenvolvidos na proteção de alimentos, o estudo apresentado no Artigo 2 explorou a performance dos filmes produzidos na extensão do *shelf life* de óleo de girassol, um produto sensível à luz e ao oxigênio. Ao avaliar a estabilidade oxidativa de óleo de girassol embalado nos materiais desenvolvidos, simulando as mesmas condições dos testes de migração, poderia-se relacionar a taxa de migração de bixina, beta-caroteno e licopeno ao efeito antioxidante dos filmes na proteção do óleo. No entanto, tendo em vista que os carotenoides apresentaram diferentes cinéticas de migração, as diferenças encontradas estão relacionadas tanto à velocidade com que os carotenoides migram dos filmes para o óleo, quanto à capacidade antioxidante intrínseca de cada composto. Para avaliar efetivamente o efeito da taxa de migração do carotenoide no retardo das reações oxidativas do óleo, faz-se necessário produzir filmes que apresentem a mesma composição (mesmo carotenoide), apenas diferindo-se na cinética de migração. A partir desta percepção que os estudos do Artigo 3 e 4 foram conduzidos, onde bixina foi o carotenoide escolhido.

A bixina produziu filmes de coloração mais estável que aqueles produzidos com licopeno e beta-caroteno. Apesar dos últimos causarem a redução na permeabilidade ao oxigênio dos filmes – o que é altamente desejável para embalagens de alimentos - os filmes produzidos com bixina foram os únicos que ofereceram proteção ao óleo de girassol na ausência de luz. Em outras palavras, a bixina foi o único carotenoide cuja ação antioxidante foi demonstrada independentemente dos seus efeitos de barreira à luz, sendo o contato da bixina com o óleo efetivo para retardar a formação de peróxidos no óleo de girassol.

A comparação entre os diferentes carotenoides é importante do ponto de vista da ciência básica, pois ajuda na compreensão da influência de estruturas moleculares semelhantes nas propriedades do polímero e na interação dos compostos com o produto embalado. No entanto, o conhecimento produzido também tem valia na tomada de decisão em relação à seleção do carotenoide utilizado na produção de filmes biodegradáveis a partir do PLA. Além de ter demonstrado maior estabilidade e efetividade como antioxidante nesse estudo, a escolha da bixina como carotenoide para a continuidade da pesquisa atende a fatores importantes. No tocante à acessibilidade, as sementes de urucum apresentam elevado teor desse carotenoide, chegando a 60 mg por grama de semente. Esse valor é muito maior que os carotenoides encontrados na cenoura (0,6-3,0 mg beta-caroteno/g) e no tomate (0,02-0,06 mg licopeno/g). Ainda que os carotenoides possam ser obtidos a partir de outras fontes como algas ou através da produção biotecnológica, a valorização do carotenoide do urucum é de alta relevância, tendo em vista que o Brasil é o maior produtor, consumidor e exportador mundial de corantes e sementes desta planta (CARVALHO et al., 2005; FIKSELOVÁ et al., 2008; THOMPSON et al., 2000).

Ao considerar que o processamento de polímeros em escala industrial exige a fusão do material, o andamento da pesquisa foi conduzido no ímpeto de avaliar a estabilidade e comportamento da bixina na matriz de PLA em condições semelhantes às exigidas para o processamento via extrusão. Para tanto, os filmes foram submetidos à prensagem térmica sob elevada temperatura (Artigo 3) e, adicionalmente, a condições de alta temperatura e tensões de cisalhamento provocadas pelo processamento em câmara de mistura (Artigo 4). Prevendo a degradação do carotenoide causada pelo uso de calor, maiores concentrações de bixina foram utilizadas, chegando a 0,1%.

Apesar da elevada degradação térmica do carotenoide, o estudo demonstrou que a incorporação de 0,1% de bixina alterou as propriedades de barreira à luz do PLA de forma significativa. Falar das outras concentrações.... Deste modo, a bixina restante somada aos compostos de degradação formados durante o processamento a 160 °C são suficientes para reduzir a passagem da luz UV pelo material, protegendo a riboflavina da degradação e reduzindo reações de oxidação em óleo de girassol (Artigo 4).

O calor e as tensões de cisalhamento utilizadas no processo, apesar de causarem perdas de até 85% dos carotenoides, mantiveram boa parte do poder corante da bixina, onde as forças de cisalhamento não provocaram aumento na degradação do composto. Os resultados do artigo 4 demonstram que os filmes produzidos em câmara de mistura - processo que envolve calor e tensões de cisalhamento - apresentaram maiores taxas de migração que aqueles produzidos por *casting*. Tal fato pode ser decorrente da cisão das cadeias poliméricas de PLA durante o processamento, o que facilita a difusão da bixina no polímero.

O uso de aditivos plastificantes no processamento polímeros é muito comum. Ao diminuir a intensidade de ligação entre as moléculas do polímero, o uso desse aditivo aumenta a flexibilidade do material e facilita seu processamento. Ao considerar a elevada temperatura de transição vítrea do PLA (60 °C) – que torna o polímero rígido a temperatura ambiente – o uso de plastificante permite a obtenção de embalagens mais maleáveis e menos suscetíveis a fissuras causadas pelo impacto (RUELLAN; DUCRUET; DOMENEK, 2014). Além de melhorar as propriedades mecânicas dos filmes de PLA, o uso de plastificante no presente estudo teve também a intenção de alterar a cinética de migração da bixina para o alimento, avaliando assim os efeitos da liberação acelerada do antioxidante na estabilidade oxidativa do óleo embalado. O plastificante acetil tributil citrato (ATBC) foi escolhido por ser classificado como GRAS (Geralmente Reconhecido como Seguro) e apresentar compatibilidade satisfatória com o PLA (ARRIETA et al., 2014b).

A presença do ATBC nos filmes de PLA apresentou efeitos controversos. Apesar de aumentar a permeabilidade do material ao oxigênio e reduzir direta ou indiretamente a estabilidade térmica da bixina na matriz, o plastificante reduziu a temperatura de transição vítrea do polímero, tornando-o mais maleável, e aumentou a taxa de migração da bixina para o líquido simulante. Os resultados demonstram que o uso combinado de ATBC e bixina permitem a incorporação do plastificante sem causar prejuízo nas propriedades do polímero de forma significativa. Isso é, ainda que o aumento na permeabilidade ao oxigênio do material tenha sido observado, a presença da bixina não permitiu que as reações de oxidação ocorressem de forma mais pronunciada nos óleos embalados no material produzido com plastificante. Tais resultados são muito relevantes, tendo em vista que o uso de plastificante tem significativa importância no processamento de embalagens.

Outro aspecto que merece ser destacado é que a presença de antioxidantes na embalagem possibilita a redução da necessidade de aditivação dos alimentos, onde a migração gradativa dos compostos para o produto embalado pode ser mais efetiva no retardo das reações de oxidação que a adição direta no produto, como demonstrado no Artigo 2. No entanto, para que seja possível eliminar os aditivos ou substituí-los parcialmente devido à presença de compostos na embalagem, a predição da migração dos compostos ao longo do tempo faz-se necessária. O modelo matemático sugerido pelo Artigo 2, que prevê a concentração de carotenoides em alimentos gordurosos após o tempo de contato, contempla a baixa estabilidade dos carotenoides através do uso de coeficientes de degradação no líquido simulante. No entanto, ao utilizar os coeficientes de migração encontrados no Artigo 2 e seu modelo matemático para prever a concentração de carotenoides em óleos, deve-se considerar que os valores encontrados são possivelmente superestimados. Essa observação se baseia em estudos demonstram que o etanol 95%(v/v), por conter água, provoca a hidrólise da cadeia polimérica de PLA e a cristalização induzida ao longo do tempo (IÑIGUEZ-FRANCO et al., 2016), fazendo com que a migração de compostos para o líquido simulante possa ocorrer de forma mais acelerada quando comparado à taxa de migração de compostos para o óleo.

O tipo de PLA utilizado na primeira etapa do estudo (PLA-2002D; NatureWorks) - cujos resultados estão contemplados nos Artigos 1 e 2 - apresenta cristalinidade de 22% e é classificado como semi-cristalino. Já o tipo utilizado na segunda etapa (PLE001; NaturePlast) é considerado amorfo, apresentando percentual de cristalinidade inferior a 3% (Artigos 3 e 4). O grau de cristalinidade do material afeta suas propriedades de diversas formas. Além de influenciar as propriedades mecânicas do polímero, a presença de regiões cristalinas limita a difusão de moléculas (COURGNEAU et al., 2012), conseqüentemente interferindo na permeabilidade do polímero e na taxa de migração de compostos. Tendo em vista que os estudos realizados na primeira e segunda etapa diferem-se tanto pelas técnicas de produção de filmes quanto pelos tipos de PLA utilizados, a comparação entre materiais desenvolvidos nas diferentes etapas torna-se complexa. Ainda que não seja possível fazer afirmações conclusivas acerca da razão das diferenças encontradas, as características dos filmes vão ao encontro dos dados da literatura. O filme de PLA puro produzido a partir de polímero amorfo apresentou permeabilidade ao oxigênio

aproximadamente 10 vezes maior que aquele produzido com PLA semi-cristalino: $2,3 \times 10^{-19} \text{ m}^3 \text{ m}^{-2} \text{ Pa}^{-1} \text{ s}^{-1}$ para o PLA semi-cristalino (Artigo 2) e $3,3 \times 10^{-18} \text{ m}^3 \text{ m}^{-2} \text{ Pa}^{-1} \text{ s}^{-1}$ para o PLA amorfo (Artigo 3). Além disso, o coeficiente de migração de bixina para o líquido simulante foi mais elevado para os filmes produzidos com polímero amorfo ($K = 1,8 \times 10^{-7} \text{ m s}^{-1}$, artigo 4), comparado ao filme de PLA e bixina do Artigo 2 ($K=1,57 \times 10^{-8} \text{ m s}^{-1}$).

As propriedades mecânicas entre os filmes de diferentes tipos de PLA diferiram fortemente, com destaque para a elasticidade dos materiais. No entanto, a maior elasticidade dos filmes produzidos sem aplicação de calor (Artigo 1 e 2) está possivelmente relacionada a resquícios de solvente na matriz dos filmes. A elasticidade apresentada pelos materiais chegou a 300%, valor muito acima do observado na literatura para o PLA puro, seja ele amorfo ou semi-cristalino. Já os filmes submetidos à prensagem térmica (Artigos 3 e 4) apresentaram elasticidade entre 3 e 4%, valores que são condizentes com a literatura (Domenek, Fernandes-Nassar, Ducruet 2018).

É sabido que a presença de solventes como cloroformio e diclorometano atua como plastificante na matriz de PLA (Rhym et al., 2006), sendo necessários longos períodos de secagem para que o solvente seja eliminado por completo de filmes produzidos por *casting*. Ao considerar a aplicação dos filmes em embalagens de alimentos, a remoção completa do solvente faz-se necessária para assegurar a inocuidade do material. Cabe ressaltar que o clorofórmio, solvente utilizado na produção dos filmes de PLA nos estudos contemplados pelo Artigo 1 e 2, foi substituído na segunda etapa. Enquanto o clorofórmio é extremamente tóxico e sua presença não é tolerada em alimentos, o diclorometano apresenta menor toxicidade. Como exemplo, tem-se o regulamento técnico para água envasada e gelo, que estabelece um limite máximo tolerável de até 20 micrograma/L de diclorometano na água embalada (BRASIL, 2005).

Considerando que a difusividade de oxigênio através do PLA tem ordem de grandeza 10^3 vezes maior que a difusividade de carotenoides (Colin Chaves et al., 2014), o aumento na barreira ao oxigênio do material é uma forma eficiente de reduzir as reações de oxidação no produto. Logo, paralelamente à incorporação de compostos que retardam a oxidação dos alimentos, a busca pelo incremento nas

propriedades de barreira do PLA faz-se necessária para o sucesso de sua aplicação em embalagens alimentícias.

5 CONCLUSÕES

O desenvolvimento de filmes a partir de PLA e carotenoides realizado no presente estudo demonstra que é possível obter embalagens biodegradáveis, de coloração atrativa, mecanicamente resistentes e que protejam os alimentos embalados da fotodegradação. Apesar da elevada degradação da bixina no processamento via fusão, a incorporação de 0,1% do carotenoide permitiu a obtenção de um material com excelente propriedade de barreira à luz ultravioleta-visível (UV-VIS). Enquanto o beta-caroteno apresentou baixa estabilidade na matriz polimérica de PLA, a bixina mostrou-se estável à ação da luz, demonstrando capacidade de proteger vitaminas no alimento embalado e retardar a oxidação em óleo de girassol.

O modelo matemático desenvolvido para descrever a cinética de migração dos carotenoides para o líquido simulante, o qual permite prever a concentração dos compostos em alimentos gordurosos, auxiliará no desenvolvimento de formulações de alimentos com teor reduzido de aditivos antioxidantes. Tendo em vista que a cinética de migração dos carotenoides influencia na performance antioxidante do material, a taxa de migração pode ser modulada através do uso de plastificante e do processamento térmico, onde o uso de ATBC e/ou aplicação de tensões de cisalhamento podem aumentar a velocidade de migração dos compostos. Apesar do aumento na permeabilidade ao oxigênio causado pelo uso de plastificante, a presença da bixina foi capaz de reduzir a formação de peróxidos em óleo embalado no material plastificado.

Futuramente, através do conhecimento gerado no presente estudo, é desejável otimizar o desempenho do PLA como material antioxidante em alimentos gordurosos através da modulação da cinética de migração dos carotenoides e do aumento da barreira ao oxigênio do material.

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ANEXO I

Figura 4. Filmes de PLA produzidos pela técnica de casting contendo 0,01% de carotenoides: (a) beta-caroteno; (b) licopeno; (c) bixina

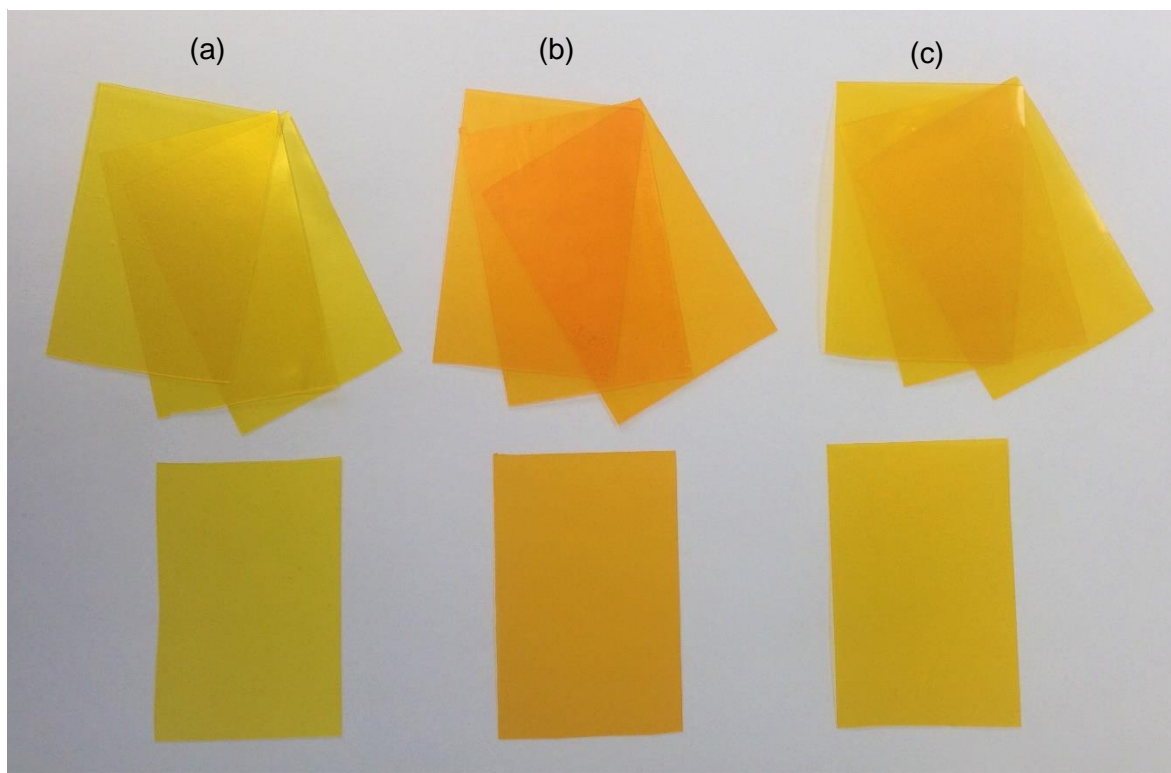


Figura 5. Filmes de PLA produzidos pela técnica de casting com diferentes concentrações de bixina



Figura 6. Master-batch de 1% bixina em PLA utilizado na produção de filmes por fusão em câmara de mistura



Figura 7. Polímero obtido por fusão em câmara de mistura antes (esquerda) e após a termo-compressão (direita)



Figura 8. (a) Filme de PLA e bixina (0,01%) obtido pela técnica de casting – Artigo 1 e 2. (b) Filme de PLA e bixina (0,1%) produzido através da fusão do material em câmara de mistura (160 °C) seguido por termo-compressão (160 °C) – Artigos 3 e 4.

(a)

(b)

