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 Ciência e Tecnologia de Alimentos, 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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Autora: Liana Stoll

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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 lático) (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 câmera 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 lático) (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).

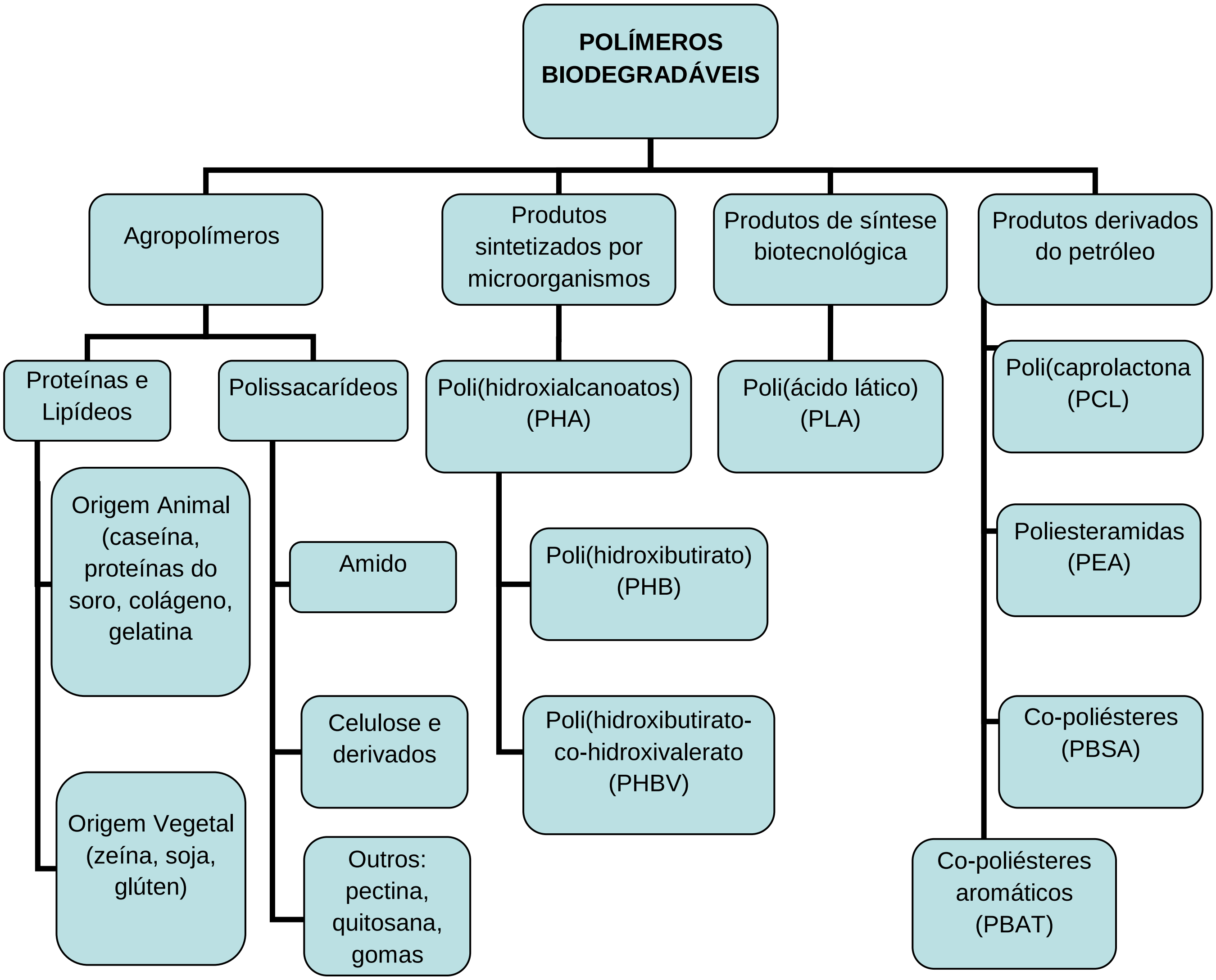
A obtenção de polímeros biodegradáveis pode ocorrer de diversas formas. Estes materiais podem ser produzidos a partir de fontes naturais e renováveis de energia, sejam elas de origem animal como crustáceos, leite ou partes animais (músculos, pele, tendões), ou a partir de fontes vegetais como frutas, 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 lático) (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 poliesteramidas, 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 lático) – 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 lático) (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).

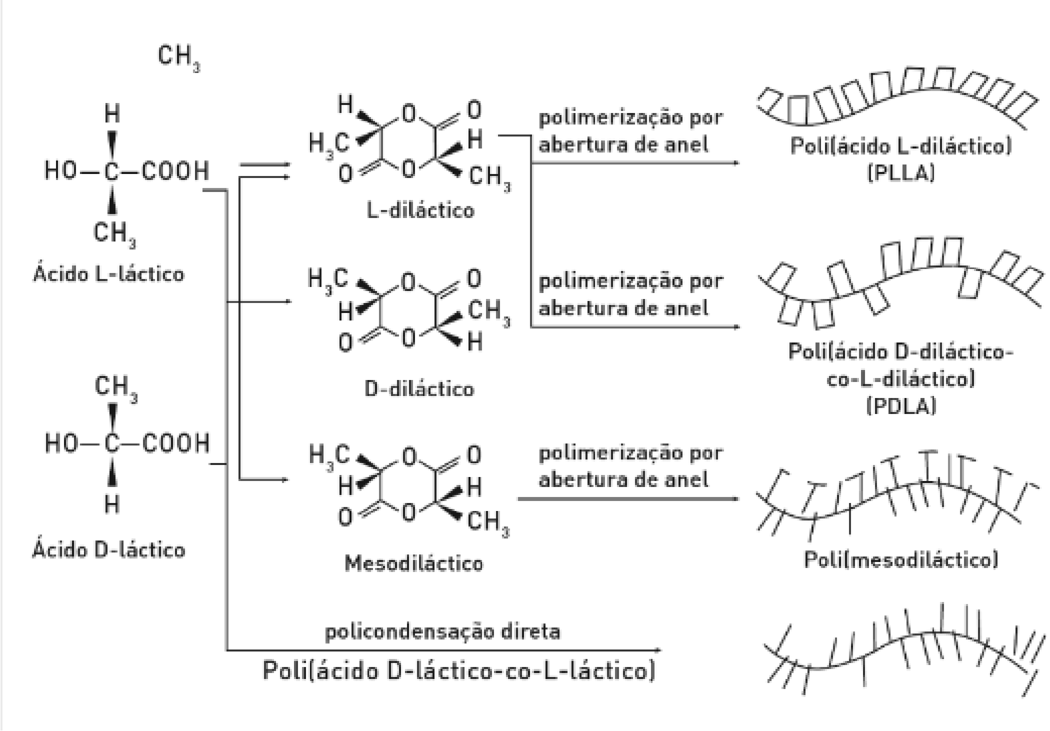
O PLA é um polímero termoplástico produzido a partir da polimerização do ácido láctico proveniente da fermentação de açúcares, mais comumente da cana-de-açúcar ou milho. Inicialmente produzido como um polímero de pequena massa molar, o qual foi produzido e relatado pela primeira vez em 1932 e patenteado 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 lático 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 lático. O ácido lático 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 lático 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

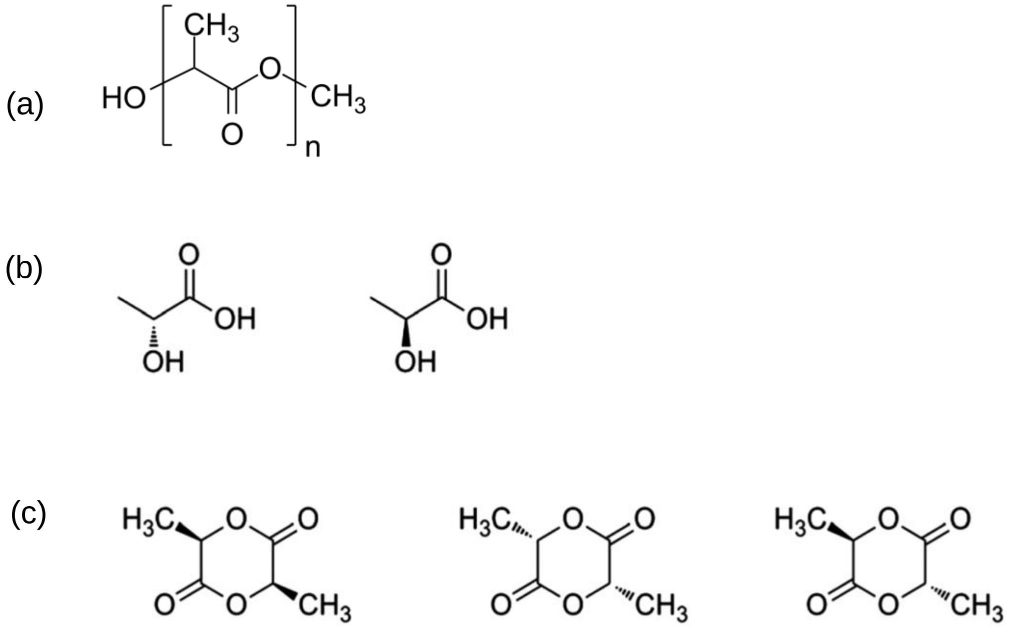


Fonte: Adaptado de Cheng et al. (2009)

A nomenclatura utilizada na literatura para estes polímeros é, por vezes, contraditória: polímeros derivados da policondensação direta do ácido lático deveriam ser chamados de poli (ácido lático), 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 lático, 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 esteroisômeros de ácido lático, lactídeos e a estrutura genérica do PLA.

Figura 3.3 - Estrutura molecular do PLA e esteroisômeros de ácido lático e lactídeos



(a) Estrutura molecular genérica do PLA
(b) Estereoisômeros de ácido lático: L-ácido lático (esquerda) e D-ácido lático (direita);
(c) Estereoisômeros de lactídeos: da esquerda para a direita: L-lactídeo, D-lactídeo e meso-lactideo.

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 lático) (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 Vítrea (°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 CO_2, O_2, N_2 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 CH_3 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-hidroxi-tolueno (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 (Tg) 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 Tg 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 O_2 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 O_2 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 *casting*					
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 leitelho em pó	Água; óleo (Miglyol 812). 4 e 25 °C 8 semanas	• Contato com BHA e BHT reduziu níveis de pentanal em leitelho em pó • α-tocoferol reduziu fomação 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%); epigalocatequina (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)	• 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; HAKKARAINEN; 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	• 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	• 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	• PLA–HA apresentou atividade como *radical scavenging* - 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	• 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	• Maior atividade antioxidante em filme com maior teor de quercetina • Calor promove a deglicosilação de rutina em isoquercitina 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)	• 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 hidrofilicos e hidrofóbicos.	LÓPEZ DE DICASTILLO et al., 2017
PLA	Lignina 1-10%	DPPH (em metanol e etanol 95%)	Etanol 95%. T ambiente 24 h	• Lignina (fonte de polifenois); 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 (*fucus spiralis*) liofilizado (8%) + ácido sórbico (0,5 - 1%)	Estabilidade oxidativa de peixe Linguado-areeiro (peróxidos e grau de fluorescencia)	não realizado	• Leve redução da oxidação da gordura do peixe (menor grau de fluorescência), porém sem diferenças na formação de peróxidos • Extrato da alga *fucus spiralis* – 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/ montmorilo-nita modificada (2,5% e 5%)	Timol (8%)	DPPH do composto AO (timol), extraído com metanol	não realizado	• 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	• Migração dos AOs favorecida pela presença de amido • Presença de dois AOs favoreceu difusão dos compostos em comparação a 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	• 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 *Camelia sinensis* L. (1% e 2%)	DPPH, proteção ao beta-caroteno e estabilidade oxidativa de salmão defumado em contato com o filme	não realizado	• 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 *Allium spp.* (2%, 5% e 6.5%)	DPPH e ABTS em metanol, 25 °C	não realizado	• 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	• Liberação menor em óleo do que em etanol • Maior migração a 40 e 43 °C	MANZANAREZ-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%	• 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	• 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	• 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 *electrospinning*	Ácido gálico (40%)	DPPH em solução salina	Solução salina (NaCl)	• 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	• Ação antioxidante da lignina é aumentada pela presença da celulose (ação sinérgica). • Maior migração de compostos para iso-octano	YANG et al., 2016

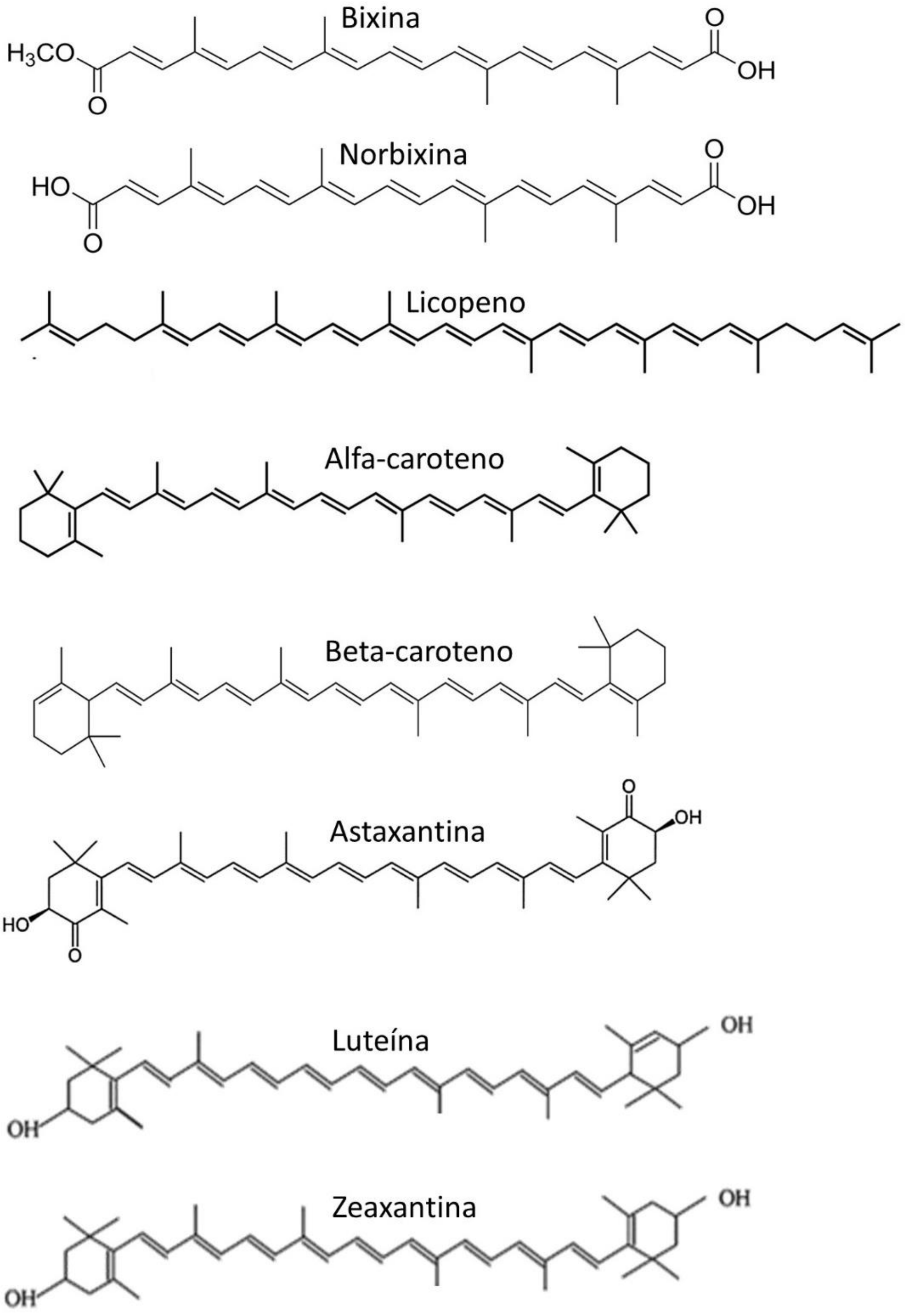
3.3 Carotenoides como aditivos em materiais poliméricos

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, consequentemente, 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 *et al.,* 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-rosca 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-rosca, 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, consequentemente, 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 µg/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 sctructures.(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, anatto 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 µm (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 λ = 466 nm (ε = 150855 L mol^{-1} cm^{-1}); β-carotene at λ = 465 nm ($A_{1\%}$ = 2396), and bixin at λ = 470 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 µg/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 1000×. 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 $CaCl_2$. 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:

$$\mathrm{WVP} = \frac{w\,.\,L}{A\,.\,T\,.\,\Delta p} \qquad (1)$$

Where w is the water weight permeated through the film (g), L is the thickness of the films (m), A is the permeation area (m^2), 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 ºC. The films were cut giving a measurement area of 50 cm^2 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 ºC at 10 ºC/min with an N_2 flow of 50 mL/min, with a heat-cool-heat cycle. The degree of crystallinity (X_c) of films was calculated as follows:

$$Xc = \frac{\Delta H_{m} - \Delta H_{cc}}{\Delta H_{f}\,(1-x)} \times 100 \quad (2)$$

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 ºC and 40 ºC, 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 (*∆E, 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.

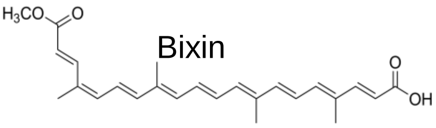
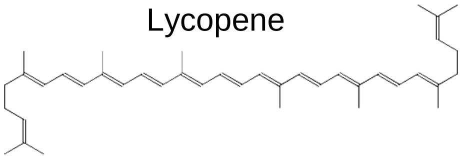
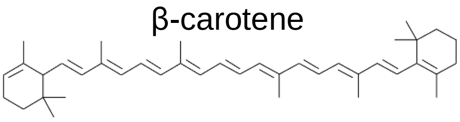
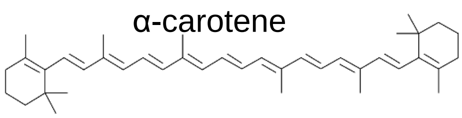
2.7 Statistical Analysis

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.

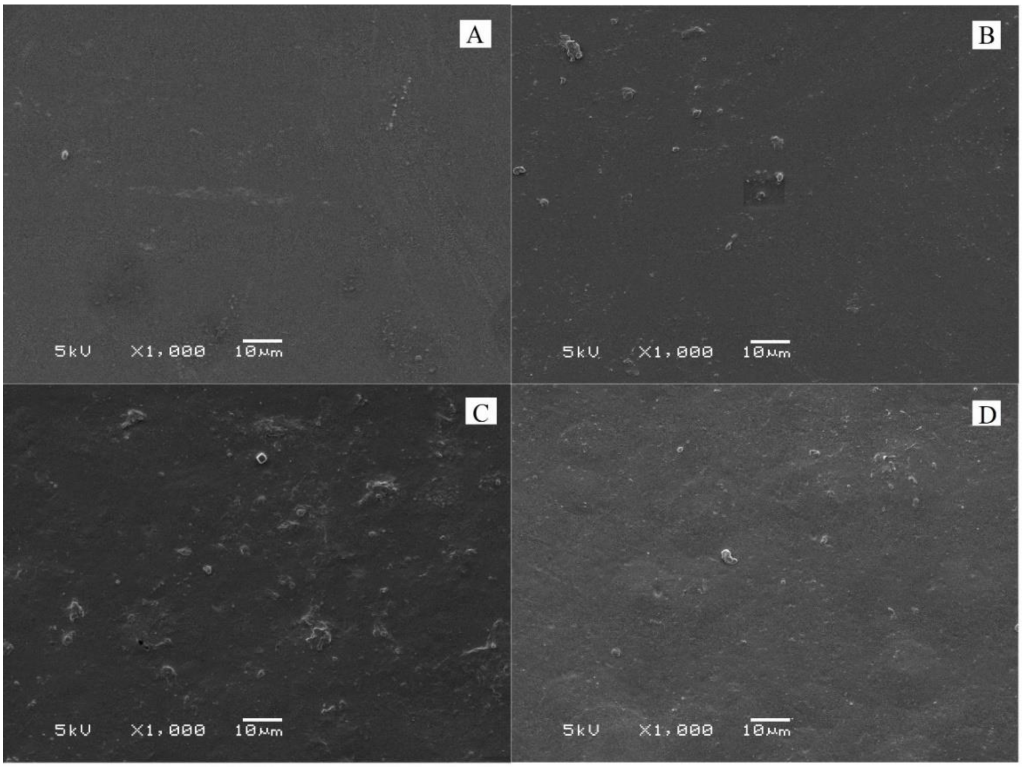
Extract	Composition	Formula	Molecular structure of major carotenoid
Annato seed	Bixin (~94%)	$C_{25}H_{30}O_4$	
Tomato	Lycopene (~89%); β-carotene (~6%); α-carotene (~2%)	$C_{40}H_{56}$	
Carrot	β-carotene (~61%); α-carotene (~34%); Lutein (~2%)	$C_{40}H_{56}$	 

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 cm^{-1}), C=O stretching (1750 cm^{-1}), -CH$_3$ 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 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 µg/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).

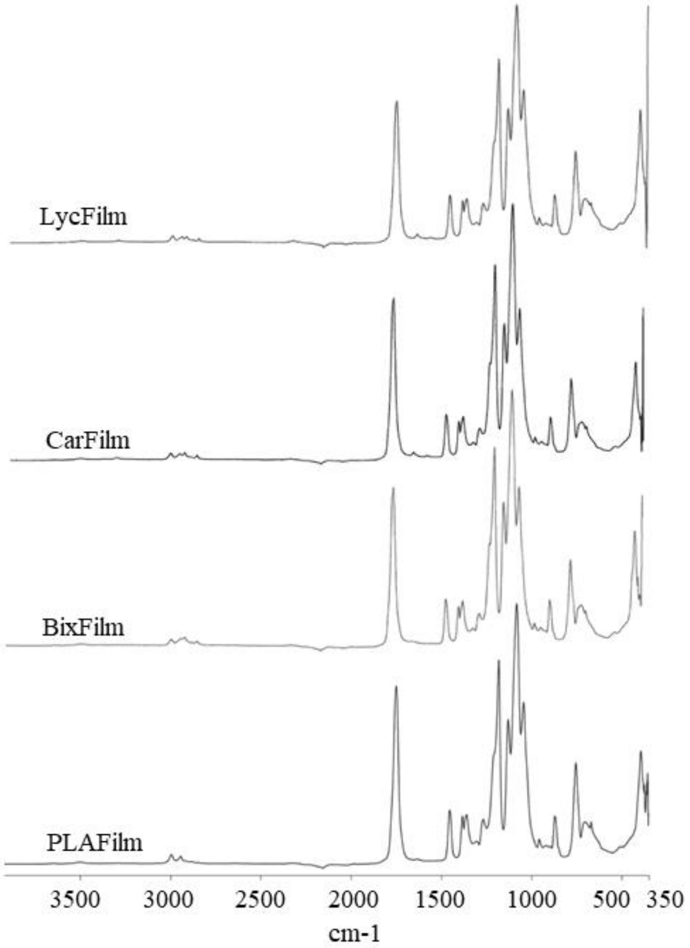


Table 2. Thickness, transparency and mechanical properties (*E%* and *TS*) of PLA films.

	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±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 % □-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 Tg was maintained around 57-58 ºC 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.

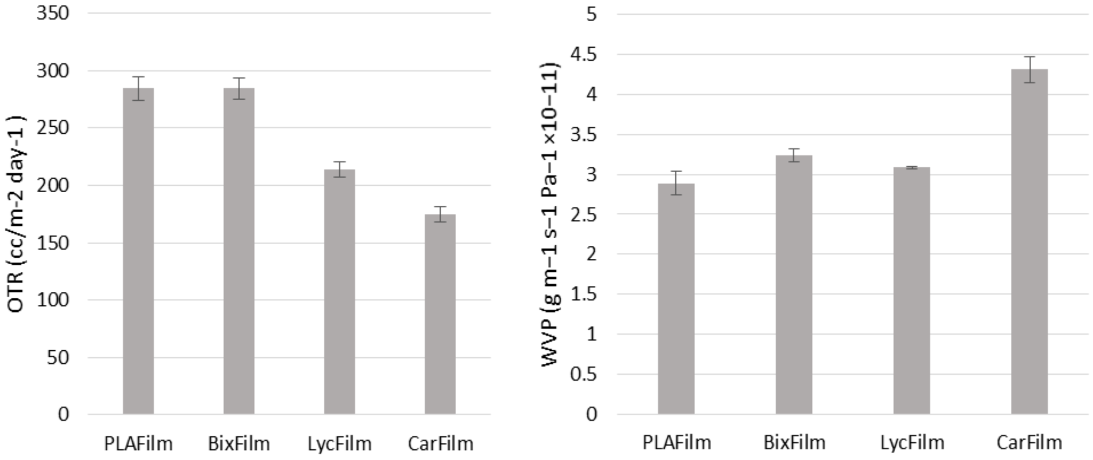
Table 3. Thermal properties obtained from DSC thermograms during the first heating cycle.

	T_g (ºC)	T_m (ºC)	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.

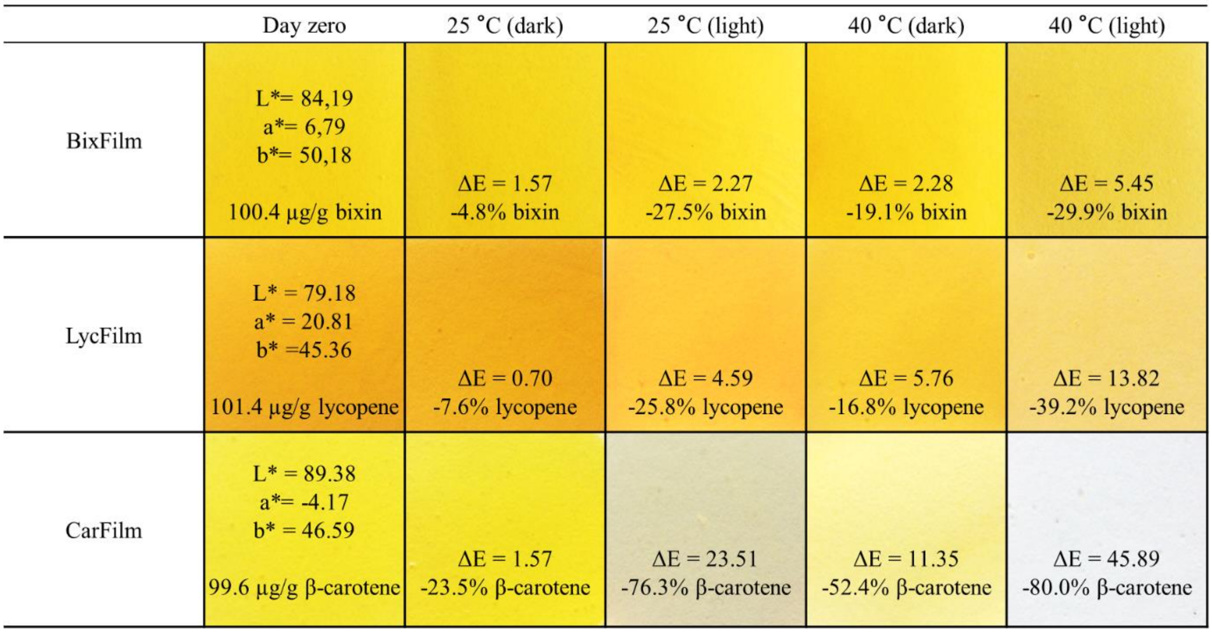
As expected, films exposed to light and temperature of 40 ºC - the least favorable condition tested for color stability - presented the highest reaction rate constants considering both Chroma and ΔE. CarFilm showed the lowest stability in all tested conditions. The light exposure was the most impacting factor on color stability, for all carotenoids. At 40 ºC, 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 (∆*E*) 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	ΔE = 1.57 -4.8% bixin	ΔE = 2.27 -27.5% bixin	ΔE = 2.28 -19.1% bixin	ΔE = 5.45 -29.9% bixin
LycFilm	L* = 79.18 a* = 20.81 b* =45.36 101.4 µg/g lycopene	ΔE = 0.70 -7.6% lycopene	ΔE = 4.59 -25.8% lycopene	ΔE = 5.76 -16.8% lycopene	ΔE = 13.82 -39.2% lycopene
CarFilm	L* = 89.38 a*= -4.17 b* = 46.59 99.6 µg/g β-carotene	ΔE = 1.57 -23.5% β-carotene	ΔE = 23.51 -76.3% β-carotene	ΔE = 11.35 -52.4% β-carotene	ΔE = 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.

						ΔE		Chroma		
			*L**	*a**	*b**	k_0	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	20.8 1 ± 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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6. REFERENCES

ABDEL-RAZIK, E. A. Aspects of degradation and stability of ABS copolymers. I. Effect of β-carotene as antioxidant. **Journal of Polymer Science Part A: Polymer Chemistry**, [s. l.], v. 27, n. 1, p. 343–355, 1989. Disponível em: <http://doi.wiley.com/10.1002/pola.1989.080270129>. Acesso em: 7 nov. 2017.

AHMED, R. R.; PARMAR, V.; AMIN, M. A. Impact of Product Packaging on Consumer’s Buying Behavior. **European Journal of Scientific Research202X European Journal of Scientific Research**, [s. l.], v. 120, n. 2, p. 1450–216, 1450. Disponível em: <www.europeanjournalofscientificresearch.com>

AKRAMI, M. et al. A new approach in compatibilization of the poly (lactic acid)/thermoplastic starch (PLA/TPS) blends. **Carbohydrate polymers**, [s. l.], v. 144, p. 254–262, 2016.

ALLEN, N. S. Photofading and light stability of dyed and pigmented polymers. **Polymer Degradation and Stability**, [s. l.], v. 44, n. 3, p. 357–374, 1994.

ANDERSON, K. S.; SCHRECK, K. M.; HILLMYER, M. A. Toughening polylactide. **Polymer Reviews**, [s. l.], v. 48, n. 1, p. 85–108, 2008.

ARRIETA, M. P. et al. Ternary PLA-PHB-Limonene blends intended for biodegradable food packaging applications. **European Polymer Journal**, [s. l.], v. 50, n. 1, p. 255–270, 2014. a.

ARRIETA, M. P. et al. Plasticized poly(lactic acid)-poly(hydroxybutyrate) (PLA-PHB) blends incorporated with catechin intended for active food-packaging applications. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 62, n. 41, p. 10170–10180, 2014. b.

ARRIETA, M. P. et al. Effect of D-Limonene on the Stabilization of Poly (Lactic Acid). [s. l.], p. 719–726, 2015.

ARROYO, O. H. et al. Processing and properties of PLA/thermoplastic starch/montmorillonite nanocomposites. **Polymer Composites**, [s. l.], v. 31, n. 1, p. 114–127, 2010.

ASTM INTERNATIONAL. **ASTM D882 - 12 Standard Test Method for Tensile Properties of Thin Plastic SheetingASTM D882-12, Standard Test Method for Tensile Properties of Thin Plastic Sheeting**. West Conshohocken, PA. Disponível em: <www.astm.org>.

ASTM INTERNATIONAL. **ASTM D1434 - 82 Standard Test Method for Determining Gas Permeability Characteristics of Plastic Film and Sheeting**. West Conshohocken, PA. Disponível em: <www.astm.org>.

AURAS, R. A.; SINGH, S. P.; SINGH, J. J. Evaluation of oriented poly (lactide) polymers vs. existing PET and oriented PS for fresh food service containers. **Packaging technology and science**, [s. l.], v. 18, n. 4, p. 207–216, 2005.

AURAS, R.; HARTE, B.; SELKE, S. An overview of polylactides as packaging materials. **Macromolecular bioscience**, [s. l.], v. 4, n. 9, p. 835–864, 2004.

AVEROUS, L.; BOQUILLON, N. Biocomposites based on plasticized starch: thermal and mechanical behaviours. **Carbohydrate Polymers**, [s. l.], v. 56, n. 2, p. 111–122, 2004.

BABU, R. P.; O’CONNOR, K.; SEERAM, R. Current progress on bio-based polymers and their future trends. **Progress in Biomaterials**, [s. l.], v. 2, n. 1, p. 8, 2013.

BAIARDO, M. et al. Thermal and Mechanical Properties of Plasticized Poly(L-lactic acid). **J Appl Polym Sci**, [s. l.], v. 90, p. 1731–1738, 2003.

BAJPAI, P. K.; SINGH, I.; MADAAN, J. Development and characterization of PLA-based green composites. **Journal of Thermoplastic Composite Materials**, [s. l.], v. 27, n. 1, p. 52–81, 2014. Disponível em: <http://journals.sagepub.com/doi/10.1177/0892705712439571>

BAO, L. et al. Gas permeation properties of poly(lactic acid) revisited. **Journal of Membrane Science**, [s. l.], v. 285, n. 1–2, p. 166–172, 2006.

BARDEN, L.; DECKER, E. A. Lipid oxidation in low-moisture food: A review. **Critical Reviews in Food Science and Nutrition**, [s. l.], v. 56, n. 15, p. 2467–2482, 2013.

BARLOW, C. Y.; MORGAN, D. C. Polymer film packaging for food: An environmental assessment. **Resources, Conservation and Recycling**, [s. l.], v. 78, p. 74–80, 2013. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0921344913001432>

BHARDWAJ, R.; MOHANTY, A. K. Advances in the properties of polylactides based materials: a review. **Journal of Biobased Materials and Bioenergy**, [s. l.], v. 1, n. 2, p. 191–209, 2007.

BISHAI, M. et al. A comprehensive study on enhanced characteristics of modified polylactic acid based versatile biopolymer. **European Polymer Journal**, [s. l.], v. 54, p. 52–61, 2014.

BORDES, P.; POLLET, E.; AVÉROUS, L. Nano-biocomposites: biodegradable polyester/nanoclay systems. **Progress in Polymer Science**, [s. l.], v. 34, n. 2, p. 125–155, 2009.

BRITO, G. F. et al. Biopolímeros, polímeros biodegradáveis e polímeros verdes. **Revista Eletrônica de Materiais e Processos**, [s. l.], v. 6, n. 2, p. 127–139, 2011.

BYUN, Y. et al. The effect of solvent mixture on the properties of solvent cast polylactic acid (PLA) film. **Journal of applied polymer science**, [s. l.], v. 124, n. 5, p. 3577–3582, 2012.

BYUN, Y. J. et al. Physical and chemical properties of γ-irradiated EVOH film. **Radiation Physics and Chemistry**, [s. l.], v. 76, n. 6, p. 974–981, 2007.

BYUN, Y.; KIM, Y. T.; WHITESIDE, S. Characterization of an antioxidant polylactic acid (PLA) film prepared with α-tocopherol, BHT and polyethylene glycol using film cast extruder. **Journal of Food Engineering**, [s. l.], v. 100, n. 2, p. 239–244, 2010. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2010.04.005>

CARMONA, V. B. et al. Properties of a biodegradable ternary blend of thermoplastic starch (TPS), poly (ε-Caprolactone)(PCL) and poly (Lactic Acid)(PLA). **Journal of Polymers and the Environment**, [s. l.], v. 23, n. 1, p. 83–89, 2015.

CARRASCO, F. et al. Processing of poly(lactic acid): Characterization of chemical structure, thermal stability and mechanical properties. **Polymer Degradation and Stability**, [s. l.], v. 95, n. 2, p. 116–125, 2010.

CARVALHO, W. et al. Estimativa indireta de teores de licopeno em frutos de genótipos de tomateiro via análise colorimétrica. **Horticultura Brasileira**, [s. l.], v. 23, n. 3, p. 819–825, 2005.

CASTRO-AGUIRRE, E. et al. Poly(lactic acid)—Mass production, processing, industrial applications, and end of life. **Advanced Drug Delivery Reviews**, [s. l.], v. 107, n. April, p. 333–366, 2016. Disponível em: <http://dx.doi.org/10.1016/j.addr.2016.03.010>

CERRUTTI, P.; MALINCONICO, M.; RYCHLY, J.; MATISOVA-RYCHLA, L.; CARFAGNA, C. Effect of natural antioxidants on the stability of polypropylene films. **Polymer Degradation and Stability**, [s. l.], v. 94, n. 11, p. 2095–2100, 2009. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0141391009002687>. Acesso em: 8 nov. 2017.

CHALIER, P. et al. Moisture and temperature triggered release of a volatile active agent from soy protein coated paper: Effect of glass transition phenomena on carvacrol diffusion coefficient. **Journal of agricultural and food chemistry**, [s. l.], v. 57, n. 2, p. 658–665, 2008.

CHAN, T. C.; LI, H. T.; LI, K. Y. Effects of Shapes of Solute Molecules on Diffusion: A Study of Dependences on Solute Size, Solvent, and Temperature. **Journal of Physical Chemistry B**, [s. l.], v. 119, n. 51, p. 15718–15728, 2015.

CHENG, Y. et al. Polylactic acid (PLA) synthesis and modifications: a review. **Frontiers of chemistry in China**, [s. l.], v. 4, n. 3, p. 259–264, 2009.

CHOE, E.; MIN, D. B. Comprehensive Reviews in Food Science and Food Safety Mechanisms and Factors for Edible Oil Oxidation. **Comprehensive Reviews in**

Food Science and Food Safety, [s. l.], v. 5, p. 169–186, 2006.

CLASEN, S. H.; MULLER, C. M. O.; PIRES, A. T. N. Maleic Anhydride as a Compatibilizer and Plasticizer in TPS/PLA Blends. **Journal of the Brazilian Chemical Society**, [s. l.], v. 26, n. 8, p. 1583–1590, 2015.

CODEX ALIMENTARIUS, C. Codex alimentarius: Fats, oils and related products (Vol. 8). **Rome: FAO**, [s. l.], 2001.

COLES, R.; MCDOWELL, D.; KIRWAN, M. J. **Food packaging technology**. [s.l.] : CRC Press, 2003. v. 5

COLÍN-CHÁVEZ, C. et al. Diffusion of natural astaxanthin from polyethylene active packaging films into a fatty food simulant. **Food research international**, [s. l.], v. 54, n. 1, p. 873–880, 2013. a.

COLÍN-CHÁVEZ, C. et al. The Release of Carotenoids from a Light-Protected Antioxidant Active Packaging Designed to Improve the Stability of Soybean Oil. **Food and Bioprocess Technology**, [s. l.], v. 7, n. 12, p. 3504–3515, 2014.

COLÍN-CHÁVEZ, C.; SOTO-VALDEZ, H.; PERALTA, E. Diffusion of carotenoids from mono and bilayer polyethylene active packaging into soybean oil. **Food Packaging and Shelf Life**, [s. l.], v. 1, n. 2, p. 170–178, 2014.

COLÍN-CHÁVEZ, C.; VICENTE-RAMÍREZ, E. B. The Release of Carotenoids from a Light-Protected Antioxidant Active Packaging Designed to Improve the Stability of Soybean Oil. [s. l.], p. 3504–3515, 2014.

COLÍN-CHÁVEZ, C. et al. Fabrication and properties of antioxidant polyethylene-based films containing marigold (Tagetes erecta) extract and application on soybean oil stability. **Packaging Technology and Science**, [s. l.], v. 26, n. 5, p. 267–280, 2013.

CORNEILLIE, S.; SMET, M. PLA architectures: the role of branching. **Polym. Chem.**, [s. l.], v. 6, n. 6, p. 850–867, 2015. Disponível em: <http://xlink.rsc.org/?DOI=C4PY01572J>

COURGNEAU, C. et al. Analysis of the Structure-Properties Relationships of Different Multiphase Systems Based on Plasticized Poly (Lactic Acid) Analysis of the Structure-Properties Relationships of Different Multiphase Systems Based on Plasticized Poly (Lactic Acid). [s. l.], n. May 2016, 2011.

COURGNEAU, C. et al. Effect of crystallization on barrier properties of formulated polylactide. **Polymer International**, [s. l.], v. 61, n. 2, p. 180–189, 2012.

CRUZ-ZÚÑIGA, J. M. et al. Development of an antioxidant biomaterial by promoting the deglycosylation of rutin to isoquercetin and quercetin. **Food Chemistry**, [s. l.], v. 204, p. 420–426, 2016.

DAINELLI, D. et al. Active and intelligent food packaging: legal aspects and safety concerns. **Trends in Food Science & Technology**, [s. l.], v. 19, p. S103–S112, 2008.

DI MAIO, L. et al. Preparation and characterization of biodegradable active PLA film for food packaging. **AIP Conference Proceedings**, [s. l.], v. 1593, n. 2014, p. 338–341, 2014.

DIMAKOU, C.; OREOPOULOU, V. Antioxidant activity of carotenoids against the oxidative destabilization of sunflower oil-in-water emulsions. **LWT - Food Science and Technology**, [s. l.], v. 46, n. 2, p. 393–400, 2012. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2011.12.013>

DO SUL, J. A. I.; COSTA, M. F. The present and future of microplastic pollution in the marine environment. **Environmental pollution**, [s. l.], v. 185, p. 352–364, 2014.

DOMENEK, SANDRA; COURGNEAU, CÉCILE; DUCRUET, V. Characteristics and Applications of Poly(lactide). In: **Biopolymers: Biomédical and Environmental Applications**. [s.l: s.n.]. p. 183–224.

DOMENEK, S. et al. Potential of Lignins as Antioxidant Additive in Active Biodegradable Packaging Materials. **Journal of Polymers and the Environment**, [s. l.], v. 21, n. 3, p. 692–701, 2013.

DOMENEK, S.; FERNANDES-NASSAR, S.; DUCRUET, V. Rheology, Mechanical Properties, and Barrier Properties of Poly (lactic acid). In: **Synthesis, Structure and Properties of Poly (lactic acid)**. [s.l.] : Springer, 2017. p. 303–341.

DORGAN, J. R.; LEHERMEIER, H.; MANG, M. Thermal and rheological properties of commercial-grade poly (lactic acid) s. **Journal of Polymers and the Environment**, [s. l.], v. 8, n. 1, p. 1–9, 2000.

DUNCAN, S. E.; CHANG, H. H. **Implications of Light Energy on Food Quality and Packaging Selection**. 1. ed. [s.l.] : Elsevier Inc., 2012. v. 67

EDGE, R.; MCGARVEY, D. J.; TRUSCOTT, T. G. The carotenoids as anti-oxidants—a review. **Journal of Photochemistry and Photobiology B: Biology**, [s. l.], v. 41, n. 3, p. 189–200, 1997.

FARAH, S.; ANDERSON, D. G.; LANGER, R. Physical and mechanical properties of PLA, and their functions in widespread applications — A comprehensive review. **Advanced Drug Delivery Reviews**, [s. l.], v. 107, p. 367–392, 2016. Disponível em: <http://dx.doi.org/10.1016/j.addr.2016.06.012>

FERRI, J. M. et al. The effect of maleinized linseed oil (MLO) on mechanical performance of poly (lactic acid)-thermoplastic starch (PLA-TPS) blends. **Carbohydrate polymers**, [s. l.], v. 147, p. 60–68, 2016.

FIEDOR, J.; BURDA, K. Shahid-ul-Islam. **Nutrients**, [s. l.], v. 6, n. 2, p. 466–488, 2014.

FIKSELOVÁ, M. et al. Extraction of carrot (Daucus carota L.) carotenes under different conditions. **Czech Journal of Food Sciences**, [s. l.], v. 26, n. 4, p. 268–274, 2008.

FISCHER, E. W.; STERZEL, H. J.; WEGNER, G. Investigation of the structure of solution grown crystals of lactide copolymers by means of chemical reactions. **Kolloid-Zeitschrift und Zeitschrift für Polymere**, [s. l.], v. 251, n. 11, p. 980–990, 1973. Disponível em: <http://link.springer.com/10.1007/BF01498927>. Acesso em: 13 nov. 2017.

FRASER, P. D.; BRAMLEY, P. M. The biosynthesis and nutritional uses of carotenoids. **Progress in lipid research**, [s. l.], v. 43, n. 3, p. 228–265, 2004.

GALLEGO, G.; HAKKARAINEN, M.; ALMAJANO, M. P. Stability of O/W emulsions packed with PLA film with incorporated rosemary and thyme. **European Food Research and Technology**, [s. l.], v. 243, n. 7, p. 1249–1259, 2017.

GARCÍA-SOTO, B. et al. Effect of biodegradable film (lyophilised alga Fucus spiralis and sorbic acid) on quality properties of refrigerated megrim (Lepidorhombus whiffiagonis). **International Journal of Food Science and Technology**, [s. l.], v. 50, n. 8, p. 1891–1900, 2015.

GARLOTTA, D. A Literature Review of Poly (Lactic Acid). **Journal of Polymers and the Environment**, [s. l.], v. 9, n. 2, p. 63–84, 2002. Disponível em: <http://link.springer.com/10.1023/A:1020200822435%5Cnhttp://www.springerlink.com/index/X15J565570160G41.pdf>

GAVRIIL, G.; KANAVOURAS, A.; COUTELIERIS, F. A. Food-packaging migration models: A critical discussion. **Critical Reviews in Food Science and Nutrition**, [s. l.], v. 8398, n. June, p. 1–11, 2017. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/10408398.2017.1317630>

GEROMETTA, M. et al. **Physical and Chemical Stability of PLA in Food Packaging**. [s.l.] : Elsevier, 2019.

GEUEKE, B.; GROH, K.; MUNCKE, J. Food packaging in the circular economy: Overview of chemical safety aspects for commonly used materials. **Journal of Cleaner Production**, [s. l.], v. 193, p. 491–505, 2018. Disponível em: <https://doi.org/10.1016/j.jclepro.2018.05.005>

GIMENEZ-ROTA, C. et al. SC. **The Journal of Supercritical Fluids**, [s. l.], 2019.

GOMEZ-ESTACA, J. et al. Advances in antioxidant active food packaging. **Trends in Food Science and Technology**, [s. l.], v. 35, n. 1, p. 42–51, 2014.

GONÇALVES, C. M. B. et al. Effect of natural and synthetic antioxidants incorporation on the gas permeation properties of poly(lactic acid) films. **Journal of Food Engineering**, [s. l.], v. 116, n. 2, p. 562–571, 2013.

GUERRINI, L. M. et al. **Correlação entre as propriedades reológicas, óticas e a morfologia de filmes soprados de LLDPE/LDPE** , scielo , 2004.

GUILLEN, M. D.; GOICOECHEA, E. Formation of oxygenated α,β-unsaturated aldehydes and other toxic compounds in sunflower oil oxidation at room temperature in closed receptacles. **Food Chemistry**, [s. l.], v. 111, n. 1, p. 157–164, 2008. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0308814608003476>

GUINAULT, A. et al. Impact of crystallinity of poly(lactide) on helium and oxygen barrier properties. **European Polymer Journal**, [s. l.], v. 48, n. 4, p. 779–788, 2012.

GUTIERREZ, A. M.; BOYLSTON, T. D.; CLARK, S. Effects of Pro-Oxidants and Antioxidants on the Total Antioxidant Capacity and Lipid Oxidation Products of Milk During Refrigerated Storage. **Journal of food science**, [s. l.], v. 83, n. 2, p. 275–283, 2018.

HAILA, K. M.; LIEVONEN, S. M.; HEINONEN, M. I. Effects of Lutein, Lycopene, Annatto, and +�-Tocopherol on Autoxidation of Triglycerides. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 44, n. 8, p. 2096–2100, 1996. Disponível em: <http://www.scopus.com/inward/record.url?eid=2-s2.0-0000203065&partnerID=40&md5=4fe89f39d59c9e95d0a888d65c92d353>

HARRIS, A. M., ELLEN, C. L. Improving Mechanical Performance of Injection Molded PLA by Controlling Crystallinity. **Journal of Applied Polymer Science**, [s. l.], v. 107, p. 2246–2255, 2008.

HRÁDKOVÁ, I. et al. Antioxidant effect of mono-and dihydroxyphenols in sunflower oil with different levels of naturally present tocopherols. **European Journal of Lipid Science and Technology**, [s. l.], v. 115, n. 7, p. 747–755, 2013.

HWANG, S. W. et al. Poly(L-lactic acid) with added α-tocopherol and resveratrol: Optical, physical, thermal and mechanical properties. **Polymer International**, [s. l.], v. 61, n. 3, p. 418–425, 2012. a.

HWANG, S. W. et al. Migration of α-tocopherol and resveratrol from poly(L-lactic acid)/starch blends films into ethanol. **Journal of Food Engineering**, [s. l.], v. 116, n. 4, p. 814–828, 2013. a. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2013.01.032>

IÑIGUEZ-FRANCO, F. et al. Antioxidant activity and diffusion of catechin and epicatechin from antioxidant active films made of poly(l-lactic acid). **Journal of Agricultural and Food Chemistry**, [s. l.], v. 60, n. 26, p. 6515–6523, 2012.

IÑIGUEZ-FRANCO, F. et al. Concurrent solvent induced crystallization and hydrolytic degradation of PLA by water-ethanol solutions. **Polymer (United Kingdom)**, [s. l.], v. 99, n. July, p. 315–323, 2016. Disponível em: <http://dx.doi.org/10.1016/j.polymer.2016.07.018>

JAMSHIDIAN, M. et al. Structural, mechanical and barrier properties of active PLA-antioxidant films. **Journal of Food Engineering**, [s. l.], v. 110, n. 3, p. 380–389, 2012. a. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2011.12.034>

JAMSHIDIAN, M. et al. Effects of synthetic phenolic antioxidants on physical, structural, mechanical and barrier properties of poly lactic acid film. **Carbohydrate Polymers**, [s. l.], v. 87, n. 2, p. 1763–1773, 2012. b. Disponível em: <http://dx.doi.org/10.1016/j.carbpol.2011.09.089>

JAMSHIDIAN, M.; TEHRANY, E. A.; DESOBRY, S. Antioxidants Release from Solvent-Cast PLA Film: Investigation of PLA Antioxidant-Active Packaging. **Food and Bioprocess Technology**, [s. l.], v. 6, n. 6, p. 1450–1463, 2013.

JOHN, M. J.; THOMAS, S. Biofibres and biocomposites. **Carbohydrate polymers**, [s. l.], v. 71, n. 3, p. 343–364, 2008.

KAMALY, N. et al. Degradable Controlled-Release Polymers and Polymeric Nanoparticles : Mechanisms of Controlling Drug Release. [s. l.], 2016.

KAUR, D.; SOGI, D. S.; WANI, A. A. Oxidative stability of soybean triacylglycerol using carotenoids and y-tocopherol. **International Journal of Food Properties**, [s. l.], v. 18, n. 12, p. 2605–2613, 2015. Disponível em: <http://dx.doi.org/10.1080/10942912.2013.803118>

KIM, N.; CHOE, E. Contribution of minor compounds to the singlet oxygen-related photooxidation of olive and perilla oil blend. **Food Science and Biotechnology**, [s. l.], v. 22, n. 2, p. 315–321, 2013.

KIOKIAS, S.; DIMAKOU, C.; OREOPOULOU, V. Activity of natural carotenoid preparations against the autoxidative deterioration of sunflower oil-in-water emulsions. **Food Chemistry**, [s. l.], v. 114, n. 4, p. 1278–1284, 2009. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2008.10.087>

KIOKIAS, S.; OREOPOULOU, V. Antioxidant properties of natural carotenoid extracts against the AAPH-initiated oxidation of food emulsions. **Innovative food science & emerging technologies**, [s. l.], v. 7, n. 1–2, p. 132–139, 2006.

KIOKIAS, S.; VARZAKAS, T.; OREOPOULOU, V. In vitro activity of vitamins, flavonoids, and natural phenolic antioxidants against the oxidative deterioration of oil-based systems. **Critical reviews in food science and nutrition**, [s. l.], v. 48, n. 1, p. 78–93, 2008.

KIRSCHWENG, B. et al. Natural antioxidants as stabilizers for polymers. **Polymer Degradation and Stability**, [s. l.], 2017. Disponível em: <http://dx.doi.org/10.1016/j.polymdegradstab.2017.07.012>

KIRWAN, M. J.; PLANT, S.; STRAWBRIDGE, J. W. Plastics in food packaging. **Food and Beverage Packaging Technology, Second Edition**, [s. l.], p. 157–212, [s.d.].

KOUTSIMANIS, G. et al. Influences of packaging attributes on consumer purchase decisions for fresh produce. **Appetite**, [s. l.], v. 59, n. 2, p. 270–280, 2012.

LACOSTE, A. et al. Advancing controlled release packaging through smart blending. **Packaging Technology and Science**, [s. l.], v. 18, n. 2, p. 77–87, 2005.

LEVY, L. W.; RIVADENEIRA, D. M. Annatto. In: IFT BASIC SYMPOSIUM SERIES 2000, **Anais**... [s.l: s.n.]

LI, M. et al. Extrusion processing and characterization of edible starch films with different amylose contents. **Journal of Food Engineering**, [s. l.], v. 106, n. 1, p. 95–101, 2011.

LIM, L.-T.; AURAS, R.; RUBINO, M. Processing technologies for poly(lactic acid). **Progress in Polymer Science**, [s. l.], v. 33, n. 8, p. 820–852, 2008. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0079670008000373>

LIMBO, S.; TORRI, L.; PIERGIOVANNI, L. Light-induced changes in an aqueous β-carotene system stored under halogen and fluorescent lamps, affected by two oxygen partial pressures. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 55, n. 13, p. 5238–5245, 2007.

LIN, Y. J. et al. Surface roughness and light transmission of biaxially oriented polypropylene films. **Polymer Engineering & Science**, [s. l.], v. 47, n. 10, p. 1658–1665, 2007. Disponível em: <http://doi.wiley.com/10.1002/pen.20850>. Acesso em: 7 nov. 2017.

LIU, D. Y. et al. Characterisation of solution cast cellulose nanofibre - Reinforced poly(lactic acid). **Express Polymer Letters**, [s. l.], v. 4, n. 1, p. 26–31, 2010.

LLANA-RUIZ-CABELLO, M. et al. Characterisation and evaluation of PLA films containing an extract of Allium spp. to be used in the packaging of ready-to-eat salads under controlled atmospheres. **LWT - Food Science and Technology**, [s. l.], v. 64, n. 2, p. 1354–1361, 2015.

LOPES, M. S.; JARDINI, A. L.; MACIEL FILHO, R. Poly (lactic acid) production for tissue engineering applications. **Procedia Engineering**, [s. l.], v. 42, p. 1402–1413, 2012.

LÓPEZ-DE-DICASTILLO, C. et al. Reducing Oxidation of Foods Through Antioxidant Active Packaging Based on Ethyl Vinyl Alcohol and Natural Flavonoids. **Packaging Technology and Science**, [s. l.], v. 25, n. 8, p. 457–466, 2012. Disponível em: <http://doi.wiley.com/10.1002/pts.992>. Acesso em: 1 mar. 2018.

LÓPEZ-RUBIO, A.; LAGARON, J. M. Improvement of UV stability and mechanical properties of biopolyesters through the addition of β-carotene. **Polymer Degradation and Stability**, [s. l.], v. 95, n. 11, p. 2162–2168, 2010. Disponível em: <http://dx.doi.org/10.1016/j.polymdegradstab.2010.03.002>

LÓPEZ DE DICASTILLO, C. et al. A traditional aboriginal condiment as an antioxidant agent in the development of biodegradable active packaging. **Journal of Applied Polymer Science**, [s. l.], v. 134, n. 15, p. 1–10, 2017.

MANZANAREZ-LÓPEZ, F. et al. Release of α-Tocopherol from Poly(lactic acid) films, and its effect on the oxidative stability of soybean oil. **Journal of Food Engineering**, [s. l.], v. 104, n. 4, p. 508–517, 2011. a.

MARCET, I. et al. PRODUCTION AND CHARACTERISATION OF BIODEGRADABLE PLA NANOPARTICLES LOADED WITH THYMOL TO IMPROVE ITS ANTIMICROBIAL EFFECT. **Journal of Food Engineering**, [s. l.], 2018.

MARCOS, B. et al. Development of biodegradable films with antioxidant properties based on polyesters containing α-tocopherol and olive leaf extract for food packaging applications. **Food Packaging and Shelf Life**, [s. l.], v. 1, n. 2, p. 140–150, 2014.

MARTINS, C. et al. Active polylactic acid film incorporated with green tea extract: Development, characterization and effectiveness. **Industrial Crops and Products**, [s. l.], v. 123, p. 100–110, 2018.

MASEK, A. et al. Application of β-carotene, a natural flavonoid dye, to polymeric materials as a natural antioxidant and determination of its characteristics using cyclic voltammetry and FTIR spectroscopy. **International Journal of Electrochemical Science**, [s. l.], v. 10, n. 4, p. 3372–3386, 2015.

MASTROMATTEO, M. et al. Advances in controlled release devices for food packaging applications. **Trends in Food Science & Technology**, [s. l.], v. 21, n. 12, p. 591–598, 2010.

MELLINAS, C. et al. Active edible films: Current state and future trends. **Journal of Applied Polymer Science**, [s. l.], v. 133, n. 2, 2016.

MICHIELS, Y.; PUYVELDE, P. Van; SELS, B. Barriers and Chemistry in a Bottle: Mechanisms in Today's Oxygen Barriers for Tomorrow's Materials. **Applied Sciences**, [s. l.], v. 7, n. 7, p. 665, 2017.

MILLER-CHOU, B. A.; KOENIG, J. L. A review of polymer dissolution. **Progress in Polymer Science**, [s. l.], v. 28, n. 8, p. 1223–1270, 2003.

MIN, D. B.; BOFF, J. M. Chemistry and Reaction of Singlet Oxygen in Foods. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 1, n. 2, p. 58–72, 2002. Disponível em: <http://doi.wiley.com/10.1111/j.1541-4337.2002.tb00007.x>

MORABITO, K. et al. Proximal effects of ultraviolet light absorbers and polymer matrix in the photostability of ??-carotene. **Dyes and Pigments**, [s. l.], v. 92, n. 1, p. 509–516, 2012. Disponível em: <http://dx.doi.org/10.1016/j.dyepig.2011.06.031>

MOURA, L. E. et al. Bioactive efficacy of low-density polyethylene films with natural additives. **Journal of Applied Polymer Science**, [s. l.], v. 135, n. 34, 2018.

MUCCIO, E. A. **Plastics processing technology**. [s.l.] : ASM international, 1994.

NAMPOOTHIRI, K. M.; NAIR, N. R.; JOHN, R. P. An overview of the recent developments in polylactide (PLA) research. **Bioresource technology**, [s. l.], v. 101, n. 22, p. 8493–8501, 2010.

NARAYANAN, A. M.; LOGANATHAN, S.; BABU, R. UV Protective Poly(lactic acid)/Rosin Films for Sustainable Packaging. **International Journal of Biological Macromolecules**, [s. l.], 2017.

NASSAR, S. F. et al. Multi-scale analysis of the impact of polylactide morphology on gas barrier properties. **Polymer**, [s. l.], v. 108, p. 163–172, 2017.

NIU, X. et al. Highly Transparent, Strong, and Flexible Films with Modified Cellulose Nanofiber Bearing UV Shielding Property. **Biomacromolecules**, [s. l.], v. 19, n. 12, p. 4565–4575, 2018.

NUNES, I. L.; MERCADANTE, A. Z. Obtenção de cristais de licopeno a partir de descarte de tomate. **Ciência e Tecnologia de Alimentos**, [s. l.], v. 24, n. 3, p. 440–447, 2004.

OLIVEIRA, M. et al. The role of shear and stabilizer on PLA degradation. **Polymer Testing**, [s. l.], v. 51, n. August 2019, p. 109–116, 2016. Disponível em: <http://dx.doi.org/10.1016/j.polymertesting.2016.03.005>

ORTIZ-VAZQUEZ, H. et al. Release of butylated hydroxytoluene (BHT) from Poly(lactic acid) films. **Polymer Testing**, [s. l.], v. 30, n. 5, p. 463–471, 2011. Disponível em: <http://dx.doi.org/10.1016/j.polymertesting.2011.03.006>

PAGNAN, C. S. et al. Annatto-colored Poly(3-hydroxybutyrate): A Comprehensive Study on Photodegradation. **Journal of Polymers and the Environment**, [s. l.], v. 0, n. 0, p. 1–10, 2017. a.

PAGNO, C. H. et al. Synthesis of biodegradable films with antioxidant properties based on cassava starch containing bixin nanocapsules. **Journal of Food Science and**

Technology, [s. l.], v. 53, n. 8, p. 3197–3205, 2016.

PALOZZA, P.; KRINSKY, N. I. [38] Antioxidant effects of carotenoids in Vivo and in Vitro: An overview. In: **Methods in enzymology**. [s.l.] : Elsevier, 1992. v. 213p. 403–420.

PESEK, C. A.; WARTHESEN, J. J. Photodegradation of Carotenoids in a Vegetable Juice System. **Journal of Food Science**, [s. l.], v. 52, n. 3, p. 744–746, 1987.

PLATZER, N. The technology of plasticizers, J. Kern Sears and Joseph R. Darby, SPE Monograph Series, Wiley, New York, 1982, 1166 pp. Price: $130.00. **Journal of Polymer Science: Polymer Letters Edition**, [s. l.], v. 20, n. 8, p. 459–459, 1982. Disponível em: <http://doi.wiley.com/10.1002/pol.1982.130200810>. Acesso em: 7 nov. 2017.

POSPÍŠIL, J.; NEŠPŮREK, S. Polymer additives. **Plastic Packaging: Interactions with Food and Pharmaceuticals**, [s. l.], p. 63–88, 2008.

PRABHAKARA RAO, P. G. et al. Effect of processing conditions on the stability of annatto (Bixa orellana L.) dye incorporated into some foods. **LWT - Food Science and Technology**, [s. l.], v. 38, n. 7, p. 779–784, 2005.

PRESTON, H. D.; RICKARD, M. D. Extraction and chemistry of annatto. **Food Chemistry**, [s. l.], v. 5, n. 1, p. 47–56, 1980.

QUILES-CARRILLO, L. et al. Bioactive Multilayer Polylactide Films with Controlled Release Capacity of Gallic Acid Accomplished by Incorporating Electrospun Nanostructured Coatings and Interlayers. **Applied Sciences**, [s. l.], v. 9, n. 3, p. 533, 2019.

RAMOS, M. et al. Development of novel nano-biocomposite antioxidant films based on poly (lactic acid) and thymol for active packaging. **Food Chemistry**, [s. l.], v. 162, p. 149–155, 2014. a. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2014.04.026>

RAMOS, M. et al. Influence of thymol and silver nanoparticles on the degradation of poly (lactic acid) based nanocomposites: thermal and morphological properties. **Polymer Degradation and Stability**, [s. l.], v. 108, p. 158–165, 2014. b.

RASAL, R. M.; JANORKAR, A. V; HIRT, D. E. Poly (lactic acid) modifications. **Progress in polymer science**, [s. l.], v. 35, n. 3, p. 338–356, 2010.

REGULATION, C. No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. **Official Journal L**, [s. l.], v. 12, 2011.

RHIM, J. W. et al. Effect of the processing methods on the performance of polylactide films: Thermocompression versus solvent casting. **Journal of Applied Polymer Science**, [s. l.], v. 101, n. 6, p. 3736–3742, 2006.

RIOS, A. D. O.; MERCADANTE, A. Z. Optimization of the conditions to obtain bixin crystals and for extraction and saponification to quantify bixin in extruded snacks by HPLC. **Alimentos e Nutrição**, [s. l.], v. 15, n. 3, p. 203–213, 2004.

RIVERA, S. M.; CANELA-GARAYOA, R. Analytical tools for the analysis of carotenoids in diverse materials. **Journal of Chromatography A**, [s. l.], v. 1224, n. Supplement C, p. 1–10, 2012. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0021967311018097>

RODRIGUES, E. et al. Development of a novel micro-assay for evaluation of peroxyl radical scavenger capacity: Application to carotenoids and structure–activity relationship. **Food chemistry**, [s. l.], v. 135, n. 3, p. 2103–2111, 2012.

RODRIGUEZ-AMAYA, D. B. Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of food carotenoids-A review. **Journal of**

Food Composition and Analysis, [s. l.], v. 23, n. 7, p. 726–740, 2010.

RODRIGUEZ, D. **A Guide to Carotenoid Analysis in Foods**. [s.l: s.n.].

ROSATO, D. V. **Extruding plastics: a practical processing handbook**. [s.l.] : Springer Science & Business Media, 2013.

ROUSSAKI, M. et al. Encapsulation of the natural antioxidant aureusidin in biodegradable PLA nanoparticles. **Polymer Degradation and Stability**, [s. l.], v. 108, p. 182–187, 2014.

RUELLAN, A. et al. Solubility factors as screening tools of biodegradable toughening agents of polylactide. **Journal of Applied Polymer Science**, [s. l.], v. 132, n. 48, 2015.

RUELLAN, A.; DUCRUET, V.; DOMENEK, S. Plasticization of Poly (lactide). In: **Poly (lactic acid) Science and Technology**. [s.l: s.n.]. p. 124–170.

SAINI, R. D. Biodegradable Polymers. **International Journal of Applied Chemistry**, [s. l.], v. 13, n. 2, p. 179–196, 2017.

SAMSUDIN, H. et al. Migration of antioxidants from polylactic acid films, a parameter estimation approach: Part I – A model including convective mass transfer coefficient. [s.l.] : Elsevier Ltd, 2018. v. 105 Disponível em: <http://dx.doi.org/10.1016/j.foodres.2017.11.065>

SAMSUDIN, H.; SOTO-VALDEZ, H.; AURAS, R. Poly(lactic acid) film incorporated with marigold flower extract (Tagetes erecta) intended for fatty-food application. **Food Control**, [s. l.], v. 46, p. 55–66, 2014. a. Disponível em: <http://dx.doi.org/10.1016/j.foodcont.2014.04.045>

SANCHES-SILVA, A. et al. Trends in the use of natural antioxidants in active food packaging: a review. **Food Additives & Contaminants: Part A**, [s. l.], v. 31, n. 3, p. 374–395, 2014. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/19440049.2013.879215>

SCHMIDT, H. de O. et al. Vitamin and bioactive compound diversity of seven fruit species from south Brazil. **Journal of the Science of Food and Agriculture**, [s. l.], v. 99, n. 7, p. 3307–3317, 2019.

SCOTTER, M. The chemistry and analysis of annatto food colouring: a review. **Food Additives and Contaminants**, [s. l.], v. 26, n. 8, p. 1123–1145, 2009.

SCOTTER, M. J.; CASTLE, L.; APPLETON, G. P. Kinetics and yields for the formation of coloured and aromatic thermal degradation products of annatto in foods. **Food chemistry**, [s. l.], v. 74, n. 3, p. 365–375, 2001.

SÉBASTIEN, F. et al. Novel biodegradable films made from chitosan and poly(lactic acid) with antifungal properties against mycotoxinogen strains. **Carbohydrate Polymers**, [s. l.], v. 65, n. 2, p. 185–193, 2006.

SHAHID-UL-ISLAM; RATHER, L. J.; MOHAMMAD, F. Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications - A review. **Journal of Advanced Research**, [s. l.], v. 7, n. 3, p. 499–514, 2016. Disponível em: <http://dx.doi.org/10.1016/j.jare.2015.11.002>

SHAHID, M.; SHAHID-UL-ISLAM; MOHAMMAD, F. Recent advancements in natural dye applications: A review. **Journal of Cleaner Production**, [s. l.], v. 53, p. 310–331, 2013. Disponível em: <http://dx.doi.org/10.1016/j.jclepro.2013.03.031>

SHAYAN, M. et al. Effect of modified starch and nanoclay particles on biodegradability and mechanical properties of cross-linked poly lactic acid. **Carbohydrate polymers**, [s. l.], v. 124, p. 237–244, 2015.

SHIRAI, M. A. et al. Poly(lactic acid)/thermoplastic starch sheets: effect of adipate esters on the morphological, mechanical and barrier properties. **Polímeros**, [s. l.], v. 26, n. 1, p. 66–73, 2016. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-14282016000100010&lng=en&nrm=iso&tlng=en>

SIEMANN, U. Solvent cast technology - A versatile tool for thin film production. **Progress in Colloid and Polymer Science**, [s. l.], v. 130, n. June, p. 1–14, 2005.

SIGURDSON, G. T.; TANG, P.; GIUSTI, M. M. Natural Colorants: Food Colorants from Natural Sources. **Annual Review of Food Science and Technology**, [s. l.], v. 8, n. 1, p. 261–280, 2017. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev-food-030216-025923>

SILVA, A. S. et al. Mass transport studies of model migrants within dry foodstuffs. **Journal of Cereal Science**, [s. l.], v. 48, n. 3, p. 662–669, 2008. Disponível em: <http://dx.doi.org/10.1016/j.jcs.2008.02.006>

SIRACUSA, V. Food packaging permeability behaviour: A report. **International Journal of Polymer Science**, [s. l.], v. 2012, n. i, 2012.

SOARES, F. C. et al. Thermoplastic starch/poly(lactic acid) sheets coated with cross-linked chitosan. **Polymer Testing**, [s. l.], v. 32, n. 1, p. 94–98, 2013. Disponível em: <http://dx.doi.org/10.1016/j.polymertesting.2012.09.005>

SOTHORNVIT, R. et al. Tensile properties of compression-molded whey protein sheets: Determination of molding condition and glycerol-content effects and comparison with solution-cast films. **Journal of Food Engineering**, [s. l.], v. 78, n. 3, p. 855–860, 2007.

SOTO-VALDEZ, H.; AURAS, R.; PERALTA, E. Fabrication of poly (lactic acid) films with resveratrol and the diffusion of resveratrol into ethanol. **Journal of Applied Polymer Science**, [s. l.], v. 121, n. 2, p. 970–978, 2011.

SOUKOULIS, C.; BOHN, T. Critical Reviews in Food Science and Nutrition A Comprehensive Overview on the Micro- and Nano-technological Encapsulation Advances for Enhancing the Chemical Stability and Bioavailability of Encapsulation of carotenoids. [s. l.], n. June, p. 37–41, 2015.

STANCIK, C. M. et al. Accelerated light protection performance measurement technology validated for dairy milk packaging design. **Packaging Technology and Science**, [s. l.], v. 30, n. 12, p. 771–780, 2017.

STEENSON, D. F.; MIN, D. B. Effects of β-carotene and lycopene thermal degradation products on the oxidative stability of soybean oil. **JAOCS, Journal of the American Oil Chemists' Society**, [s. l.], v. 77, n. 11, p. 1153–1160, 2000.

STOCLET, G. et al. In-situ SAXS study of the plastic deformation behavior of polylactide upon cold-drawing. **Polymer**, [s. l.], v. 55, n. 7, p. 1817–1828, 2014.

STOLL, L. et al. Carotenoids extracts as natural colorants in poly(lactic acid) films. **Journal of Applied Polymer Science**, [s. l.], 2018.

STOLL, L. et al. Poly (acid lactic) films with carotenoids extracts: Release study and effect on sunflower oil preservation. **Food chemistry**, [s. l.], v. 281, p. 213–221, 2019.

TANG, C. H. et al. Effect of transglutaminase treatment on the properties of cast films of soy protein isolates. **Journal of Biotechnology**, [s. l.], v. 120, n. 3, p. 296–307, 2005.

TÁTRAALJAI, D. MAJOR, L., FÖLDES, E., PUKÁNSZKY, B. Effect of carotene on the processing stability of polyethylene and the characteristics of films under storage conditions1. Tátraaljai, D. Major, L., Földes, E., Pukánszky, B. Effect of carotene on the processing stability of polyethylene and the character. [s.l: s.n.]. v. 102

THOMPSON, K. A. et al. Cultivar, maturity, and heat treatment on lycopene content in tomatoes. **Journal of Food Science**, [s. l.], v. 65, n. 5, p. 791–795, 2000.

TSUCHIHASHI, H. et al. Action of β-carotene as an antioxidant against lipid peroxidation. **Archives of Biochemistry and Biophysics**, [s. l.], v. 323, n. 1, p. 137–147, 1995.

URAYAMA, H.; MOON, S.; KIMURA, Y. Microstructure and thermal properties of polylactides with different L-and D-unit sequences: importance of the Helical nature of the L-sequenced segments. **Macromolecular Materials and Engineering**, [s. l.], v. 288, n. 2, p. 137–143, 2003.

VAN AARDT, M. et al. Release of antioxidants from poly(lactide-co-glycolide) films into dry milk products and food simulating liquids. **International Journal of Food Science and Technology**, [s. l.], v. 42, n. 11, p. 1327–1337, 2007.

VITRAC, O.; HAYERT, M. Identification of diffusion transport properties from desorption/sorption kinetics: An analysis based on a new approximation of fick equation during solid-liquid contact. **Industrial and Engineering Chemistry Research**, [s. l.], v. 45, n. 23, p. 7941–7956, 2006.

WANG, M. et al. Progress in Toughening Poly(Lactic Acid) with Renewable Polymers. **Polymer Reviews**, [s. l.], v. 57, n. 4, p. 557–593, 2017. Disponível em: <http://dx.doi.org/10.1080/15583724.2017.1287726>

WANG, N. et al. Influence of citric acid on the properties of glycerol-plasticized dry starch (DTPS) and DTPS/poly(lactic acid) blends. **Starch/Staerke**, [s. l.], v. 59, n. 9, p. 409–417, 2007.

YAHIA, E. M.; ORNELAS-PAZ, J. de J. **Chemistry, Stability, and Biological Actions of Carotenoids**. [s.l: s.n.].

YANG, W. et al. Effect of processing conditions and lignin content on thermal, mechanical and degradative behavior of lignin nanoparticles/polylactic (acid) bionanocomposites prepared by melt extrusion and solvent casting. **European Polymer Journal**, [s. l.], v. 71, p. 126–139, 2015. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0014305715003997>

YANG, W. et al. Effect of cellulose and lignin on disintegration, antimicrobial and antioxidant properties of PLA active films. **International Journal of Biological Macromolecules**, [s. l.], v. 89, p. 360–368, 2016. Disponível em: <http://dx.doi.org/10.1016/j.ijbiomac.2016.04.068>

YANISHLIEVA, N. V.; AITZETMÜLLER, K.; RANEVA, V. β-Carotene and lipid oxidation. **Lipid - Fett**, [s. l.], v. 100, n. 10, p. 444–462, 1998. Disponível em: <http://doi.wiley.com/10.1002/(SICI)1521-4133(199810)100:10%3C444::AID-LIPI444%3E3.0.CO;2-A>

YILDIRIM, S. et al. Active Packaging Applications for Food. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 17, n. 1, p. 165–199, 2018.

ZEB, A.; MURKOVIC, M. Pro-oxidant effects of β-carotene during thermal oxidation of edible oils. **JAOCS, Journal of the American Oil Chemists' Society**, [s. l.], v. 90, n. 6, p. 881–889, 2013.

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 peroxyl 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-Cháves *et al.,* 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 TiO2 and carotenoids release.	Colín-Cháves *et al.,* 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 *et al.,* 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 *et al.,* 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 *et al.,* 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 *et al.,* 2014
lycopene	PP	Carotenoids in tomato extract acted as a thermal and processing stabilizer.	Cerutti *et al*., 2009
Colorants to polymers			
bixin	PHB	Bixin acted as a hiding agent, reducing the photodegradation processes.	Pagnan *et al.,* 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 *at al.,* 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 (≥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 λ = 465nm ($A_{1\%}$ = 2396), lycopene at λ = 466nm (ε = 150855 L mol^{-1} cm^{-1}), and bixin at λ = 470nm ($A_{1\%}$ = 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 µg 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 ± 5 µm 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 cm^3 mm m^{-2} day^{-1}. Thickness was measured using a micrometer (Digimess, 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 λ = 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 (λ_{max} = 449 nm), from 2.2 to 105.4 µg mL^{-1} (R^2 = 0.999); lycopene (λ_{max} = 480 nm), from 0.3 to 13.9 µg mL^{-1} (R^2 = 0.999); bixin (λ_{max} = 457 nm), from 0.3 to 5.3 µg 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 µg mL^{-1} for beta-carotene, 0.004 and 0.012 µg mL^{-1} for lycopene, and 0.002 and 0.008 µg 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.

$$\frac{dC}{dt} = -k_{\mathrm{d}}C \qquad \text{(Eq.1)}$$

where C is the carotenoid concentration in the food simulant (µg 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.

$$\frac{dC}{dt} = K\frac{A}{V}(C^* - C) - k_\mathrm{d}C \qquad \text{(Eq. 2)}$$

where C is the carotenoid concentration in the food simulant (µg 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 (µg 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).

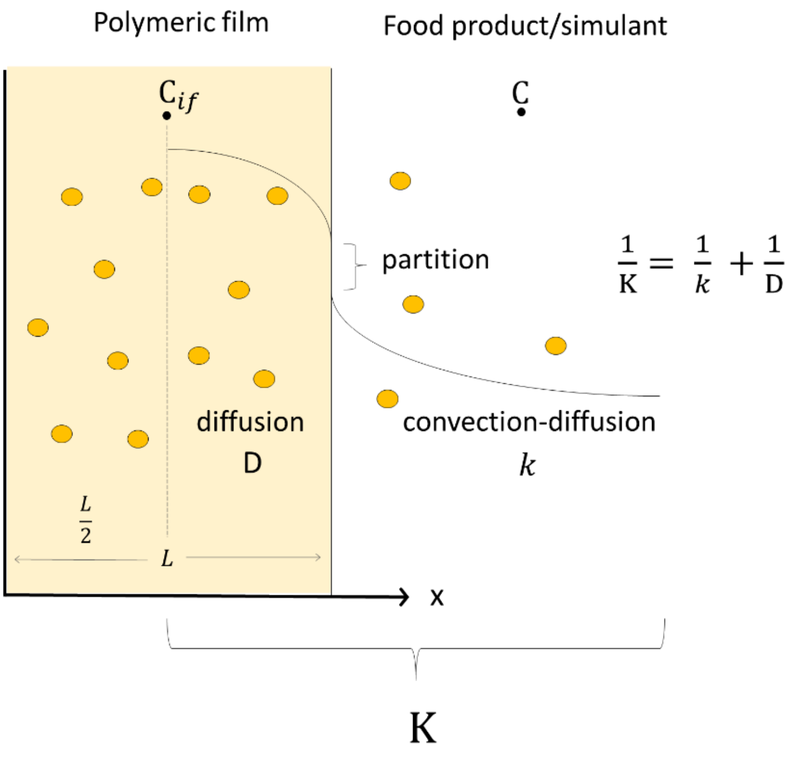
The overall mass transfer coefficient takes into account the sum of the resistances of mass transfer by diffusion inside the polymeric film, the migration through the film-food simulant interface, and the mass transfer in the food simulant, as follows:

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

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).

$$K_{part} = \frac{C_P}{C_S} \qquad \text{(Eq. 4)}$$

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 µg mL^{-1}) and stored in amber glass flasks (treatment 2). The concentration of 16.4 µg 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 ºC 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 then 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	Time zero				After storage		
(nm)	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^*).

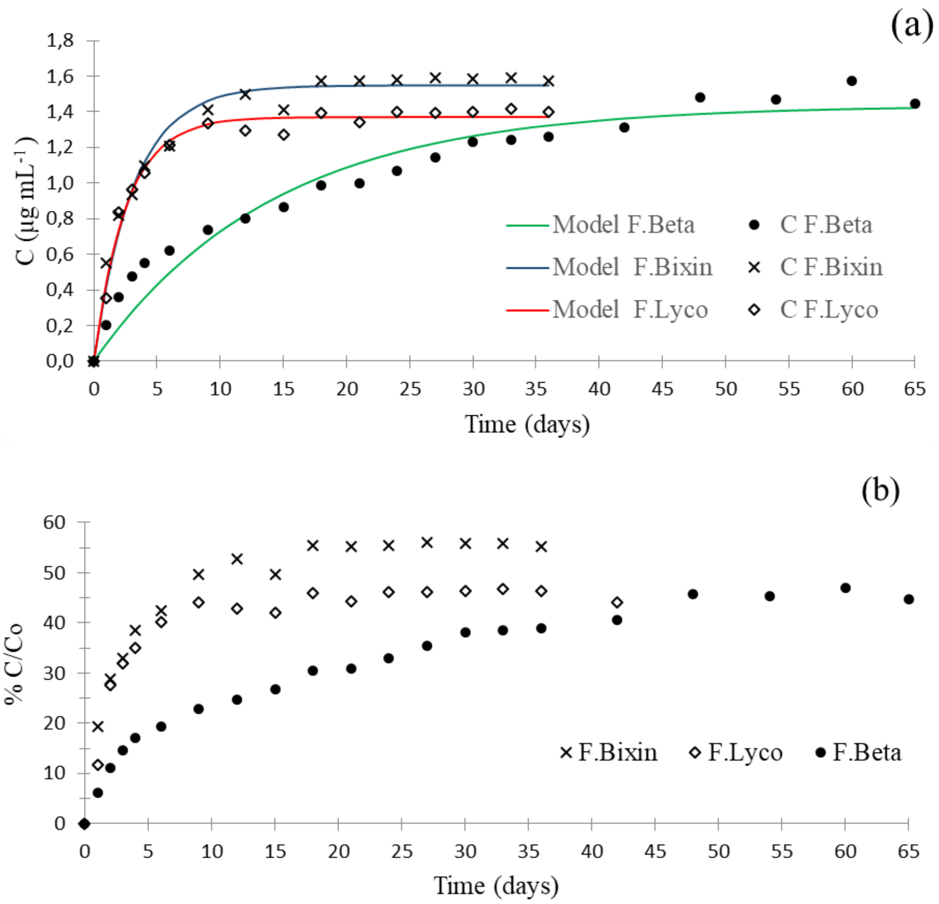
	F.Beta	F.Lyco	F.Bixin
K_{part}	3.29 a	3.14 a	3.45 a
k_d ($s^{-1}\times10^{-7}$)	1.089	1.134	2.155
R^2 k_d	0.93	0.96	0.94
K($m\ s^{-1}\times10^{-8}$)	0.237	1.462	1.157
R^2 K	0.96	0.97	0.98
C^* (μg ml^{-1}simulant)	1.66	1.54	1.64

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 peroxyl 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 (Steenson & 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 peroxyl radicals probably by its addition to the carotenoid polyene chain, although not avoiding the propagation of the reaction.

Table 4. Peroxides (meq O_2/kg) and conjugated dienes (K_{232}) 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 ± 00.17 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 O_2/kg) and conjugated dienes (K_{232}) 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 $^1O_2$ 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 $^1O_2$ 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 plastificants), 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.

REFERENCES

CERRUTTI, P.; MALINCONICO, M.; RYCHLY, J.; MATISOVA-RYCHLA, L.; CARFAGNA, C. Effect of natural antioxidants on the stability of polypropylene films. **Polymer Degradation and Stability**, 94(11), 2095–2100, 2009

CHAN, T. C.; LI, H. T.; LI, K. Y. Effects of Shapes of Solute Molecules on Diffusion: A Study of Dependences on Solute Size, Solvent, and Temperature. **Journal of Physical Chemistry B**, [s. l.], v. 119, n. 51, p. 15718–15728, 2015.

CHOE, E.; MIN, D. B. Comprehensive Reviews in Food Science and Food Safety Mechanisms and Factors for Edible Oil Oxidation. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 5, p. 169–186, 2006.

CODEX ALIMENTARIUS, C. Codex alimentarius: Fats, oils and related products (Vol. 8). **Rome: FAO**, [s. l.], 2001.

COLÍN-CHÁVEZ, C. et al. Diffusion of natural astaxanthin from polyethylene active packaging films into a fatty food simulant. **Food Research International**, [s. l.], v. 54, n. 1, p. 873–880, 2013. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2013.08.021>

COLÍN-CHÁVEZ, C.; VICENTE-RAMÍREZ, E. B. The Release of Carotenoids from a Light-Protected Antioxidant Active Packaging Designed to Improve the Stability of Soybean Oil. [s. l.], p. 3504–3515, 2014.

CRUZ-ZÚÑIGA, J. M. et al. Development of an antioxidant biomaterial by promoting the deglycosylation of rutin to isoquercetin and quercetin. **Food Chemistry**, [s. l.], v. 204, p. 420–426, 2016.

DIMAKOU, C.; OREOPOULOU, V. Antioxidant activity of carotenoids against the oxidative destabilization of sunflower oil-in-water emulsions. **LWT - Food Science and Technology**, [s. l.], v. 46, n. 2, p. 393–400, 2012. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2011.12.013>

EDGE, R.; MCGARVEY, D. J.; TRUSCOTT, T. G. The carotenoids as anti-oxidants—a review. **Journal of Photochemistry and Photobiology B: Biology**, [s. l.], v. 41, n. 3, p. 189–200, 1997.

GAVRIIL, G.; KANAVOURAS, A.; COUTELIERIS, F. A. Food-packaging migration models: A critical discussion. **Critical Reviews in Food Science and Nutrition**, [s. l.], v. 8398, n. June, p. 1–11, 2017. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/10408398.2017.1317630>

GOMEZ-ESTACA, J. et al. Advances in antioxidant active food packaging. **Trends in Food Science and Technology**, [s. l.], v. 35, n. 1, p. 42–51, 2014.

GUILLEN, M. D.; GOICOECHEA, E. Formation of oxygenated α,β-unsaturated aldehydes and other toxic compounds in sunflower oil oxidation at room temperature in closed receptacles. **Food Chemistry**, [s. l.], v. 111, n. 1, p. 157–164, 2008. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0308814608003476>

HAILA, K. M.; LIEVONEN, S. M.; HEINONEN, M. I. Effects of Lutein, Lycopene, Annatto, and +alfa-Tocopherol on Autoxidation of Triglycerides. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 44, n. 8, p. 2096–2100, 1996. Disponível em: <http://www.scopus.com/inward/record.url?eid=2-s2.0-0000203065&partnerID=40&md5=4fe89f39d59c9e95d0a888d65c92d353>

INIGUEZ-FRANCO, F. et al. Antioxidant Activity and Di ff usion of Catechin and Epicatechin from Antioxidant Active Films Made of Poly(. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 60, p. 6515−6523, 2012.

JAMSHIDIAN, M.; TEHRANY, E. A.; DESOBRY, S. Antioxidants Release from Solvent-Cast PLA Film: Investigation of PLA Antioxidant-Active Packaging. **Food and Bioprocess Technology**, [s. l.], v. 6, n. 6, p. 1450–1463, 2013.

KAUR, D.; SOGI, D. S.; WANI, A. A. Oxidative stability of soybean triacylglycerol using carotenoids and y-tocopherol. **International Journal of Food Properties**, [s. l.], v. 18, n. 12, p. 2605–2613, 2015. Disponível em: <http://dx.doi.org/10.1080/10942912.2013.803118>

KIM, N.; CHOE, E. Contribution of minor compounds to the singlet oxygen-related photooxidation of olive and perilla oil blend. **Food Science and Biotechnology**, [s. l.], v. 22, n. 2, p. 315–321, 2013.

KIOKIAS, S.; DIMAKOU, C.; OREOPOULOU, V. Activity of natural carotenoid preparations against the autoxidative deterioration of sunflower oil-in-water emulsions. **Food Chemistry**, [s. l.], v. 114, n. 4, p. 1278–1284, 2009. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2008.10.087>

KIOKIAS, S.; OREOPOULOU, V. Antioxidant properties of natural carotenoid extracts against the AAPH-initiated oxidation of food emulsions. **Innovative food science & emerging technologies**, [s. l.], v. 7, n. 1–2, p. 132–139, 2006.

KIOKIAS, S.; VARZAKAS, T.; OREOPOULOU, V. In vitro activity of vitamins, flavonoids, and natural phenolic antioxidants against the oxidative deterioration of oil-based systems. **Critical reviews in food science and nutrition**, [s. l.], v. 48, n. 1, p. 78–93, 2008.

KIRSCHWENG, B. et al. Natural antioxidants as stabilizers for polymers. **Polymer Degradation and Stability**, [s. l.], 2017. Disponível em: <http://dx.doi.org/10.1016/j.polymdegradstab.2017.07.012>

LACOSTE, A. et al. Advancing controlled release packaging through smart blending. **Packaging Technology and Science**, [s. l.], v. 18, n. 2, p. 77–87, 2005.

LÓPEZ-DE-DICASTILLO, C. et al. Reducing Oxidation of Foods Through Antioxidant Active Packaging Based on Ethyl Vinyl Alcohol and Natural Flavonoids. **Packaging Technology and Science**, [s. l.], v. 25, n. 8, p. 457–466, 2012. Disponível em: <http://doi.wiley.com/10.1002/pts.992>. Acesso em: 1 mar. 2018.

LÓPEZ-RUBIO, A.; LAGARON, J. M. Improvement of UV stability and mechanical properties of biopolyesters through the addition of β-carotene. **Polymer Degradation and Stability**, [s. l.], v. 95, n. 11, p. 2162–2168, 2010. Disponível em: <http://dx.doi.org/10.1016/j.polymdegradstab.2010.03.002>

MANZANAREZ-LÓPEZ, F. et al. Release of α-Tocopherol from Poly(lactic acid) films, and its effect on the oxidative stability of soybean oil. **Journal of Food Engineering**, [s. l.], v. 104, n. 4, p. 508–517, 2011.

MASTROMATTEO, M. et al. Advances in controlled release devices for food packaging applications. **Trends in Food Science & Technology**, [s. l.], v. 21, n. 12, p. 591–598, 2010.

PAGNAN, C. S. et al. Annatto-colored Poly (3-hydroxybutyrate): A Comprehensive Study on Photodegradation. **Journal of Polymers and the Environment**, [s. l.], p. 1–10, 2017.

PALOZZA, P.; KRINSKY, N. I. [38] Antioxidant effects of carotenoids in Vivo and in Vitro: An overview. In: **Methods in enzymology**. [s.l.] : Elsevier, 1992. v. 213p. 403–420.

REGULATION, C. No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. **Official Journal L**, [s. l.], v. 12, 2011.

RIOS, A. D. O.; MERCADANTE, A. Z. Optimization of the conditions to obtain bixin crystals and for extraction and saponification to quantify bixin in extruded snacks by HPLC. **Alimentos e Nutrição**, [s. l.], v. 15, n. 3, p. 203–213, 2004.

SAMSUDIN, H. et al. **Migration of antioxidants from polylactic acid films, a parameter estimation approach: Part I – A model including convective mass transfer coefficient**. [s.l.] : Elsevier Ltd, 2018. v. 105 Disponível em: <http://dx.doi.org/10.1016/j.foodres.2017.11.065>

SAMSUDIN, H.; SOTO-VALDEZ, H.; AURAS, R. Poly(lactic acid) film incorporated with marigold flower extract (Tagetes erecta) intended for fatty-food application. **Food Control**, [s. l.], v. 46, p. 55–66, 2014. Disponível em: <http://dx.doi.org/10.1016/j.foodcont.2014.04.045>

SANCHES-SILVA, A. et al. Trends in the use of natural antioxidants in active food packaging: a review. **Food Additives & Contaminants: Part A**, [s. l.], v. 31, n. 3, p. 374–395, 2014. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/19440049.2013.879215>

SHAHID-UL-ISLAM; RATHER, L. J.; MOHAMMAD, F. Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications - A review. **Journal of Advanced Research**, [s. l.], v. 7, n. 3, p. 499–514, 2016. Disponível em: <http://dx.doi.org/10.1016/j.jare.2015.11.002>

SIRACUSA, V. Food packaging permeability behaviour: A report. **International Journal of Polymer Science**, [s. l.], v. 2012, n. i, 2012.

STEENSON, D. F.; MIN, D. B. Effects of β-carotene and lycopene thermal degradation products on the oxidative stability of soybean oil. **JAOCS, Journal of the American Oil Chemists' Society**, [s. l.], v. 77, n. 11, p. 1153–1160, 2000.

STOLL, L. et al. Carotenoids extracts as natural colorants in poly(lactic acid) films. **Journal of Applied Polymer Science**, [s. l.], v. 135, n. 33, 2018.

TANG, C. H. et al. Effect of transglutaminase treatment on the properties of cast films of soy protein isolates. **Journal of Biotechnology**, [s. l.], v. 120, n. 3, p. 296–

307, 2005.

TSUCHIHASHI, H. et al. Action of β-carotene as an antioxidant against lipid peroxidation. **Archives of Biochemistry and Biophysics**, [s. l.], v. 323, n. 1, p. 137–147, 1995.

VITRAC, O.; HAYERT, M. Identification of diffusion transport properties from desorption/sorption kinetics: An analysis based on a new approximation of fick equation during solid-liquid contact. **Industrial and Engineering Chemistry Research**, [s. l.], v. 45, n. 23, p. 7941–7956, 2006.

YANISHLIEVA, N. V.; AITZETMÜLLER, K.; RANEVA, V. β-Carotene and lipid oxidation. **Lipid - Fett**, [s. l.], v. 100, n. 10, p. 444–462, 1998. Disponível em: <http://doi.wiley.com/10.1002/(SICI)1521-4133(199810)100:10%3C444::AID-LIPI444%3E3.0.CO;2-A>

ZEB, A.; MURKOVIC, M. Pro-oxidant effects of β-carotene during thermal oxidation of edible oils. **JAOCS, Journal of the American Oil Chemists' Society**, [s. l.], v. 90, n. 6, p. 881–889, 2013.

CAPÍTULO 3

ARTIGOS CIENTÍFICOS ETAPA 2

ARTIGO 3

Polylactide films produced with bixin and acetyl tributyl citrate: properties evaluation for active packaging development

ABSTRACT

The materials used as food packaging are determinant to preserve the quality and nutritional value of products. Polylactide (PLA) is a biodegradable polymer with satisfactory mechanical properties, but its poor barrier properties hampers PLA application to pack oxygen- and light-sensitive products. Bixin, a carotenoid with coloring and antioxidant character, was used to improve the light barrier of PLA films. Despite more than 74% of bixin was degraded after polymer processing (160 °C) films presented a bright yellow-orange color, blocking up to 95% of UVA transmission. Bixin increased films tensile strength without affecting films thermal and oxygen barrier properties. ATBC increased films elasticity, reduced films oxygen barrier, and accelerated bixin migration to a food simulant, where the partial replacement of PLA by the plasticizer reduced the bixin stability in films. The observed modifications in PLA films structure may positively affect the polymer performance as an active packaging material, where ATBC can be used to modulate the release of bixin.

Keywords: PLA; casting; thermal degradation; antioxidant, migration

1. INTRODUCTION

The success of the food industry is highly dependent on the quality of the packaging materials used to wraps food products and food ingredients. The fundamental role of a food packaging is to contain the food, to protect it from outside influences and damage, and to provide consumers with ingredient and nutritional information (COLES; MCDOWELL; KIRWAN, 2003). In spite of the undoubtable importance of food packaging, most of the polymers used to produce such materials are petroleum-based and present serious environmental issues due to their non biodegradability. Poly(lactic acid) (PLA), a biopolymer produced from annually renewable resources such as sugar beet or corn starch, is currently the most used biodegradable material for short term applications (DOMENEK; FERNANDES-NASSAR; DUCRUET, 2017).

PLA is easy to process, presents satisfactory mechanical properties, and high transparency. However, the poor gas and light barrier properties restrict its commercial application in the food packaging sector (CASTRO-AGUIRRE et al., 2016). When dealing with lipid-rich foods, for example, the product shelf life is highly dependent on its oxidative stability. Indeed, the packaging barrier to light and oxygen is crucial to avoid the degradation of polyunsaturated fatty acids and maintain the food quality for extended periods. The preservation of vitamins and bioactive compounds is another example where packaging barrier properties is a concern since light radiation is known to present a detrimental effect on such substances (DUNCAN; CHANG, 2012). Considering the increasing demand on the production of healthier food products, which quality and health benefits are directly related to their vitamins and bioactive compounds stability, the use of packaging materials that protect these substances from light transmission should be a priority.

The incorporation of UV blockers/absorbers are often added to polymers as a mean of protecting the polymer itself from UV degradation, but also to protect the product from UV light. Commonly used UV blockers include benzophenone and its derivatives, benzotriazoles, oxamide, and alfa-cyanocrylate (NIU et al., 2018; POSPÍŠIL; NEŠPŮREK, 2008). Such agents have shown excellent light barrier performances; however, there is a concern related to their migration from packaging to food products and their adverse effects on human health (ANDERSON; SCHRECK;

HILLMYER, 2008; NIU et al., 2018). In this sense, the use of natural antioxidants with UV-Vis absorbing properties could be a simple alternative on the acquisition of light barrier materials, where its migration from packaging to food could avoid oxidative reactions, extending the shelf life of perishable goods.

Bixin, the main carotenoid present in annatto seeds (*Bixa Orellana* L), is a natural coloring agent with antioxidant properties that could be explored for its light absorbing character in packaging materials. Annatto extracts are permitted as a food additive in the European Union and elsewhere and are currently used for the coloring of many commodities food products (SCOTTER, 2009). Studies about its application into polymeric packaging materials are still scarce. In previous studies of our group, bixin-rich extracts were used as a coloring and antioxidant additives in PLA casted films. Bixin was shown to be stable in PLA matrix after films obtention, endowing films with light barrier properties and protecting sunflower oil from oxidation reactions (STOLL et al., 2018b, 2019). When introduced into PHB films, annatto extracts protected the polymer against thermal degradation and erosion caused by light incidence (PAGNAN et al., 2017b).

Once polymers melt processing - method industrially employed for the manufacturing of packaging films – requires the use of elevated temperatures to melt the material, the stability and thermal degradation of bixin within the polymeric matrix is of great relevance and should be considered.

This study aimed to produce PLA films with increased light barrier properties through the use of different concentrations of bixin. To plasticize the PLA, acetyl tributyl citrate (ATBC) was chosen based on literature data, where the monomeric plasticizer has shown to efficiently decrease the glass transition temperature and improve the mechanical properties of PLA, in addition of being approved for food contact by the US Food and Drug Administration (ARRIETA et al., 2015; COURGNEAU et al., 2011). The bixin thermal stability in films and its migration to a food simulant was evaluated, where films were thermal-pressed after casting to mimic the heat damage caused during polymers processing.

2. MATERIALS AND METHODS

2.1 Materials

L-Lactic acid type polylactide (PLA) of extrusion grade “PLE001’’ was supplied from NaturePlast (France). According to its technical data sheet, it melts between 155°-160°C and degrades at 220 °C, with a density of 1.24 g/cm^3. Acetyl tributyl citrate (ATBC) was supplied by Sigma-Aldrich (France). Annatto seeds were acquired from the Ivory Coast. All chemicals used were of analytical grade.

2.2 Bixin Extraction

Annatto seeds were washed with hexane and methanol, and bixin was extracted from the seeds by mechanical stirring with dichloromethane for 30 min, at ambient temperature and protection from light. The purity of bixin extracts (>97%) was confirmed by Ultra High Performance Liquid Chromatography (UHPLC). Mobile phases included phase A - water with 0.1% (v/v) formic acid, and phase B - acetonitrile with 0.1 % (v/v) formic acid (both supplied by Carlo Erba, Val de Reuil, France). An isocratic elution with phases A/B 25/75 (v/v) was used at a 0.6ml/min flow rate, on a Ethylene Bridged Hybrid (BEH) C18 column (100 x 2.1 mm, 1.7 µm particle diameter, 130 Å) and pre-column Vanguard BEH C18 5 x 2.1 mm ; 1.7μm (Waters, St Quentin en Yvelines, France). An injection volume of 2 μL was used, and the auto-sampler temperature was maintained at 10 °C. The bixin was quantified before the extract incorporation into PLA solutions with a UV–vis spectrophotometer (Jasco V-750, Japan), at λ_{max} ($A_{1\%}$) =470 nm (2826) in quartz cells using dichloromethane as a blank.

2.3 Film production

PLA pellets were dried at 80 °C for 6 h to remove residual moisture, and PLA was dissolved in dichloromethane (5%w/v) by mechanical stirring at ambient temperature. Subsequently to the polymer dissolution, the plasticizer ATBC and the bixin extract were poured into PLA solutions, achieving ATBC concentration of 0.13 g/g and bixin concentrations of 0.01, 0.05, and 0.1 mg/g - mass related to the sum PLA and ATBC total content. The solutions were stirred for 10 min and then poured into Teflon recipients at a leveled surface. After solvent evaporation, the casted films were peeled and placed in an oven with forced air circulation at 40 °C for 6 hours. The formulations of films are presented in Table 1.

Table 1. Formulations of PLA films produced with different bixin concentrations, with and without the plasticizer ATBC.

	PLA (%)	ATBC (%)	BIXIN* (%)
Films without ATBC			
FP	100	0	0
F1	100	0	0.01
F5	100	0	0.05
F10	100	0	0.1
Films with ATBC			
FPA	87	13	0
F1A	87	13	0.01
F5A	87	13	0.05
F10A	87	13	0.1

*Bixin percentage based on the total content of PLA and ATBC.

In order evaluate the bixin thermal stability when exposed to polymer melt processing temperatures, the casted films were thermal-pressed (Scamex, France) at 160 °C during 4 and 6 min. For that, film samples were placed between two Teflon sheets and two steel plates. Films thermal-pressed for 4 min were kept in the press for 2 min without pressure (P=0) followed by 2 min with P=5 tons. Films thermal pressed during 6 min were kept in the press for 4 min at P=0 and 2 min at P=5 tons. Afterwards, the press was quickly opened, and films were quenched at room temperature at a cold surface. Films were stored in desiccators protected from light, at room temperature

2.4 Bixin quantification in PLA films

The bixin content of casted and thermal treated PLA films was evaluated by dissolving the films in dichloromethane with the aid of an ultrasonic bath (STOLL et al., 2018b). The bixin quantification was performed in quartz cells in a spectrophotometer (Jasco V-750, Japan) UV-vis (dichloromethane): λ_{max} ($A_{1\%}$) =470 nm (2826). The results were expressed as total bixin equivalent.

2.5 Thermal analysis – DSC

The analysis of the glass transition temperature (T_g) and the crystallinity degree (X_c) of the film samples were carried out using a Q100 DSC (TA Instruments), equipped with an intercooler. Calibration was performed with the help of an indium standard, with purge N_2 gas flow of 50 mL min^{-1}. Samples (3–5 mg, loaded in aluminum crucibles) were heated at a constant heating rate of 10 °C min^{-1} from 10 to 180 °C, keeping the temperature at 180 °C for 5 min, with a heat-cool-heat cycle. The degree of crystallinity of the films was calculated using the enthalpy peaks from the second heating, through the following equation:

$$Xc = \frac{\Delta H_{m-} \Delta H_{cc}}{\Delta H_f\ (1-x)} \times 100 \qquad \text{Eq. 1}$$

where ΔH_m is the enthalpy of melting, ΔH_{cc} is the enthalpy of cold crystallization, ΔH_f is the heat of fusion of 100% crystalline PLA (93.7 J/g), and *x* is the fraction sum of platicizer and carotenoids in films.

2.6 Mechanical properties

A texture analyzer (TA.XT2i e Stable Micro Systems, UK) was used to evaluate the maximum tensile strength (TS) and the percentage of elongation at break (E). Analyses were perfomed at 25 °C with a load cell of 5 kg and an A/TGT self-tightening roller grips fixture. Ten replications were run for each test sample, where 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. Parameters were obtained from stress–strain curves from uniaxial tensile tests and were calculated according to the ASTM D882 standard method.

2.7 Oxygen barrier properties

The barrier properties of films to oxygen were determined using a manometric method on a permeability testing apparatus (GDP-C permeameter, Brugger

Feinmechanik GmbH, Munich, Germany), working at 23 °C. The gas permeation system was initially outgassed under vacuum for 15 h when an O_2 flow of 50 $cm^3\ min^{-1}$ started on one side of the film sample. The pressure increase was recorded over time, and Permeability (P) was determined from the steady state. The Sorption (S) and Diffusion (D) coefficients were calculated with the help of the time-lag method, which was obtained by calculating the intercept on the abscissa of the cumulated flux curve. D was found by its relationship with t_{lag} (Eq 2) and S was obtained with by its correlation with P and D (Eq 3). Films thickness (L) was measured in at least 5 random points of films surface, with a Digimatic micrometer (Mitutoyo, Japan).

$$t_{lag} = \frac{L^2}{6D} \quad \text{(Eq. 2)}$$

$$P = D \times S \quad \text{(Eq. 3)}$$

2.8 Light transmittance of PLA films

The films barrier to ultraviolet (UV) and visible light was evaluated using a UV-Vis spectrophotometer (Jasco V-750, Japan). The percentage of light transmittance through the films was measured in triplicate at selected wavelengths from 200 to 800 nm, and an empty quartz test cell was used as control.

2.9 Color analysis

The color of films was evaluated with a colorimeter (Hunter Lab system, model Miniscan XE, USA) using the CIELab color parameters L*(luminosity), a* (red-green), and b* (yellow–blue). The films (4 cm x 4 cm) were placed over a white disk (L*, 97.45; a*, 5.23; and b*, -3.68), which was used as a white standard, and measurements were performed in three different parts of films' surfaces, in triplicate. The Chroma value was calculated through Eq 4, and ΔChroma was obtained by the difference between the parameter of casted and heat treated (HT) films. The total color differences (ΔE) between the HT and the casted films was calculated using Eq 5.

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

$$\Delta E = \sqrt{(L*_{HT} - L*_{casted})^2 + (a*_{HT} - a*_{casted})^2 + (b*_{HT} - b*_{casted})^2} \quad \text{(Eq. 5)}$$

2.10 Bixin release kinetics

The kinetic of bixin release from films to a fatty food simulant was evaluated following the methodology of our previous study (STOLL et al., 2019). Ethanol 95% (v/v), approved as a fatty food simulant substitute by the Regulation No 10/2011) was used as a simulant. For that, 20 mg (~1 cm^2) of film was immersed in 6 mL of ethanol 95% (v/v) contained in 12 mL amber flasks. The flasks were sealed with plastic caps and stored at 40 °C for 65 days, in the dark, being the flasks shaken regularly to minimize mass transfer resistance. The concentration of bixin (C) was periodically determined by spectrophotometry (UV-Vis Jasco V-750, Japan), at λ=457 nm, and tests were interrupted when bixin concentration in food simulant was steady (C_{eq}). The simulant aliquots were returned to flasks after the analyses. A calibration curve of bixin in ethanol 95% was constructed from 0.3 to 5.3 μg mL^{-1} (R^2=0.996), where the limits of detection (LOD) and quantification (LOQ) were 0.002 and 0.008 μg mL−1, respectively. The LOD and LOQ were calculated following the methodology of the International Conference on Harmonization (2005).

To evaluate the overall mass transfer resistance (K) of bixin from the films to the liquid, a mass balance was used taking into account bixin degradation in the food simulant, as shown in Eq 6.

$$\frac{dC}{dt} = K\frac{A}{V}(C^{*} - C) - k_{\mathrm{d}}C \qquad \text{(Eq. 6)}$$

where C is the bixin concentration in the food simulant (μg 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 (0.002 m^2), V is the volume of the food simulant solution (6 x 10^{-4} m^3), C^{*} is the bixin concentration (μg mL^{-1}) in the inner part of the film and k_d is the first-order kinetic degradation coefficient of bixin in ethanol 95% at 40 °C (2.155 s^{-1}×10^{-7}) (STOLL et al., 2019). As described in our previous work, K takes into account the sum of diffusion mass resistances inside the polymeric film structure, the migration through the film-food simulant interface, and the mass transfer of the migrant into the food simulant (Stoll et al., 2019). The parameter K was obtained using the experimental data C through the software EMSO (Environment for Modeling, Simulation and Optimization).

3. RESULTS AND DISCUSSION

3.1 Thermal stability of bixin during film processing

The fabrication of plastics packaging involves thermal treatment of the materials during their processing. In the aim to produce packaging films with light barrier and antioxidant properties, the easiest procedure is to introduce the compounds in the polymer melt. The thermal stability of bixin at amorphous PLA extrusion temperatures and during different processing times was tested in simulated conditions by the means of a heating press. The Table 2 reports the degradation of bixin at 160 °C over time.

Table 2. Bixin content of casting films and bixin degradation after 4 and 6 min of heat treatment (HT) at 160 °C

	PLA	ATBC	Bixin*	Bixin (µg g^{-1})	Bixin degradation (%) at 160 °C	
sample	(wt%)	(wt%)	(wt%)	casting	HT - 4 min	HT - 6 min
FP	100	0	0	-	-	-
F1	100	0	0.01	99.3 ± 0.3 ^{c}	62.9 ± 0.5 ^{c}	82.3 ± 2.1 ^{bc}
F5	100	0	0.05	481 ± 16 ^{b}	62.7 ± 1.2 ^{c}	82.6 ± 0.5 ^{bc}
F10	100	0	0.1	930 ± 22 ^{a}	60.5 ± 0.1 ^{c}	74.5 ± 1.9 ^{c}
FPA	87	13	0	-	-	-
F1A	87	13	0.01	114 ± 14 ^{c}	83.2 ± 1.4 ^{a}	88.9 ± 3.3 ^{a}
F5A	87	13	0.05	416 ± 5 ^{b}	74.6 ± 0.2 ^{b}	93.0 ± 0.1 ^{a}
F10A	87	13	0.1	896 ± 17 ^{a}	75.3 ± 0.1 ^{b}	85.3 ± 0.3 ^{ab}

*Bixin percentage based on the total content of PLA and ATBC. Results are given as mean ± SD (n=3). Different letters in the same column indicate significant differences ($p < 0.05$) among the films.

The concentration of bixin in the cast PLA films was verified. At the higher use of bixin addition the effective concentration was somewhat lower than the target value. The degradation of bixin was substantial, reaching up to 93% of bixin loss in films containing ATBC. Heat, oxygen, and light are known to accelerate the oxidation and *cis*-/*trans*-isomerization of the highly unsaturated polyene chain of bixin (SCOTTER, 2009). The main annatto coloring agent, 9-*cis*-bixin, undergoes a series of complex degradation reactions at temperatures above 70 °C producing a range of products, including *trans* and other mono- and di-*cis* isomers of bixin (PRESTON; RICKARD, 1980; SCOTTER; CASTLE; APPLETON, 2001). The degradation of bixin under high temperatures was studied by Rao (2005), where almost 50% of bixin solubilized in vegetable oil was lost after exposure to 160 °C for 2 min, being completely degraded at 180 °C for the same period. However, only 23% of bixin loss was observed in biscuit dough baked at 150 °C for 30 min, showing that the matrix presents a considerable influence on the carotenoid stability. Many experiments about the encapsulation of carotenoids by polymers in the aim to enhance their thermal stability were already reported, and PLA is a widely used polymer in the field (GIMENEZ-ROTA et al., 2019; MARCET et al., 2018; ROUSSAKI et al., 2014). Our system might produce an effect similar to matrix encapsulation, where the bioactive compound is uniformly distributed over the formed biopolymer carrier system (SOUKOULIS; BOHN, 2015). PLA might offer a protection towards oxygen and heat. The PLA/ATBC system is more fluid at a given temperature with higher oxygen solubility, which might be a reason for the higher bixin degradation in the plasticized matrix.

The impact of the more severe heat treatment (HT: 160 °C, 6 min) on color, mechanical and barrier properties, and bixin kinetic of migration was investigated in the following.

3.2 Impact of thermal treatment of PLA/bixin films on film properties

3.2.1 Color changes due to heat treatment

The degradation of bixin due to the heat treatment of the films resulted in color changes. Bixin gave its typical yellow to red color to PLA. which can be observed in Table 3. The conjugated C=C double bonds in the molecule serve as the light-absorbing chromophore. It is important to mention that at least seven conjugated double bonds are required for a carotenoid to have a perceptible color (RODRIGUEZ-AMAYA, 2010). The color stability of bixin is therefore strongly correlated with the stability of those conjugated double bonds. When bixin is heated, isomerization and degradation reactions occur producing a range of pale-yellow to orange products, where the temperature and the duration of the heating govern the red/yellow balance (PRESTON; RICKARD, 1980).

Table 3. Visual color attributes, color parameters *L, a*, b*,* and Chroma of cast PLA films and color changes (Δa, Δb, ΔE, Δ Chroma) after the heat treatment.

	ATBC (%)	Bixin (%)	Cast film	HT film	L	a*	b*	Chroma	Δa*	Δb*	ΔE	ΔChroma
FP	0	0	⬜	⬜	94.6	-0.4	2.4	2.6	0.1	0.0	0.2	0.4
FPA	13	0	⬜	⬜	94.4	-0.5	2.7	2.7	0.0	0.3	0.3	0.3
F1	0	0.01	🟨	⬜	93.0	-4.0	19.2	20.0	2.6	-43.8	43.8	-43.4
F1A	13	0.01	🟨	⬜	91.5	-4.5	21.9	22.3	1.1	-44.7	44.9	-44.5
F5	0	0.05	🟧	🟨	88.4	-7.7	84.7	85.1	-7.6	-24.1	25.5	-23.7
F5A	13	0.05	🟧	🟨	88.2	-7.5	70.8	72.3	-9.3	-34.7	35.2	-33.3
F10	0	0.1	🟧	🟨	83.7	2.9	105.5	105.5	-10.3	18.4	21.6	17.5
F10A	13	0.1	🟧	🟨	84.9	-1.5	103.0	103.0	-11.0	13.7	18.2	13.2

HT: heat treatment - 160 °C, 6 min

Results demonstrates that the higher the concentration of bixin in the films, the lower were the color changes in *b*,* Chroma, and total color differences (Table 3). The reduction in **b* and **a* values are related to the loss of yellowness and redness, respectively, while changes in Chroma are related to an alteration on color intensity. When bixin was added in lower concentrations, a significant decrease in film yellowness and color intensity was observed after thermal treatement, while the opposite was observed on formulations with 0.1 % of bixin (F10 and F10A). Over 74 % of bixin was degraded after 6 min of thermal treatement for the different film formulations (Table 2), where the final content of bixin in films ranged between ~20 µg g^{-1} (F1 and F1A) and 200 µg g^{-1} (F10 and F10A). This result may explain the differences in their behavior concerning color changes: the highest the bixin initial content, the highest is the concentration of yellow degradation products responsible for an increase on yellow color intensity given by an increase in b* and Chroma. The presence of ATBC did not affect the films color neither their color changes. In the absence of bixin, the color of films was stable.

3.2.2 Glass transition and mechanical properties of heat-treated PLA/bixin films

The thermal and mechanical properties of the different PLA blends (Table 4) were analyzed in the aim to study the impact of the plasticizer, bixin and bixin degradation products in film properties. The concentration of 13 % ATBC was chosen in accordance with literature (Courgneau et al. 2012, Ruellan et al. 2015) as being the minimum concentration to yield brittle to ductile transition of PLA. At this concentration the Tg of PLA is shifted below 40 °C, in which case its glass transition region extends near the mechanical testing temperature, hence PLA has a ductile behavior at room temperature (Courgneau 2012, Ruellan 2015).

The addition of bixin to the films had no impact on the Tg. The thermal treatment of the films caused an increase in Tg, which might be attributed the loss of residual solvent from the casting process (Table 4). The Tg of the heat-treated films without ATBC corresponded to the literature values (GEROMETTA et al., 2019) and the one of the PLA specification sheet of the fabricant. The Tg values of PLA/ATBC were also coherent with literature (Ruellan, A., Ducruet, V., & Domenek, S. (2015), reaching up to 306 % of elongation at break. Indeed, dichloromethane has a very strong plasticizing effect on PLA (Rhym et al., 2006) and very rigourous drying and heating is needed to evaporated it. All PLA films were amorphous (crystallinity degree < 3 %).

Films produced without ATBC were stiff, tearing before the yield point. According to Stoclet et al. (2014), in the brittle regime, PLA failure generally occurs before yielding because the yield stress is higher than the critical stress. The highest concentration of carotenoid (0.1%) anticipated films disruption in the presence of ATBC, significantly reducing the elongation at break for F10A. The maximum tensile strength was augmented in the presence of bixin both with and without ATBC, which may be due to a denser chain packing formation.

3.3 Functional properties of heat-treated PLA/ bixin films for food preservation

The oxidative stabilization of food products can be achieved by exclusion of oxygen from the pack, which removes the reaction partner. For that, the packaging polymer needs to present adapted barrier properties to oxygen. Table 5 reports the oxygen permeability of the different heat-treated samples. The permeability values, diffusion and solubility coefficients of PLA corresponded to the upper values which are commonly reported (Sonchaeng et al., 2018), which might be a result of the film preparation procedure by casting. The presence of ATBC reduces the barrier properties of PLA because of the additional free volume inside the polymer (Sonchaeng et al., 2018, Ruellan et al., 2015). This phenomenon was largely described in literature and increased permeability of PLA/ATBC was already reported (Courgneau et al., 2012). The presence of bixin did not affect the oxygen barrier of PLA films, which is in agreement with the results found in our first study (Stoll et al., 2018).

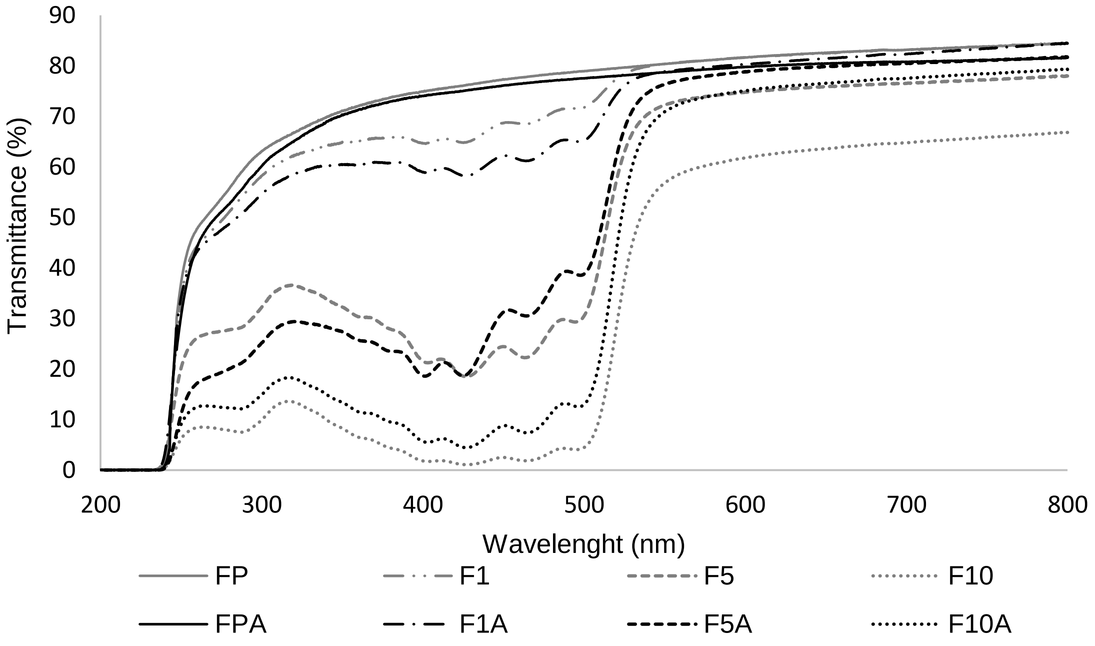
Table 4. Thermal properties of casted and heat-treated (HT) films, and mechanical properties of the HT films.

	ATBC (%)	Bixin (%)	Thickness (µm)	Tg (°C)		X_{c} (%)		Tensile strength at yied (MPa)	Elongation at break (%)
				cast film	HT film	cast film	HT film	HT film	HT film
FP	0	0	113 ± 7 a	48 ± 1	59 ± 1	1 ± 1	2 ± 1	48 ± 6 Bd	3 ± 1Ad
F1	0	0.01	121 ± 4 a	49 ± 4	60 ± 2	1 ± 1	2 ± 1	91 ± 4 Aa	3 ± 1Ad
F5	0	0.05	114 ± 3 a	48 ± 1	60 ± 2	2 ± 1	2 ± 1	87 ± 5 Aa	4 ± 1Ad
F10	0	0.1	122 ± 10 a	50 ± 3	60 ± 1	2 ± 1	1 ± 1	98 ± 11 Aa	4 ± 1 Ad
FPA	13	0	128 ± 12 a	32± 1	38 ± 1	1 ± 1	3 ± 1	42 ± 3 Cd	306 ± 22 Aa
F1A	13	0.01	127 ± 7 a	28 ± 1	39 ± 1	1 ± 1	1 ± 1	65 ± 5 Acd	219 ± 156 Aab
F5A	13	0.05	125 ± 12 a	30± 1	35 ± 2	1 ± 1	1 ± 1	51 ± 2 Bbc	274 ± 94 Aab
F10A	13	0.1	113 ± 6 a	32 ± 8	37 ± 1	1 ± 1	1 ± 1	70 ± 5 Ab	59 ± 13 Bc

Tg: glass transition temperature; X_{c}: crystallinity degree. HT: heat treatement 160°C - 6 min. Values are given as mean ± SD (n=3). Different lowercase letters in the same column indicate significant differences ($p < 0.05$) among the 8 film formulations. Different uppercase letters in the same column indicate significant differences ($p < 0.05$) between formulations of each group: films without ATBC and films with ATBC.

Photo-oxidation reactions are a real concern to the food industry, leading to the degradation of vitamins and bioactive compounds, to the development of rancidity in fatty food products, and hence to the decrease in nutritional and sensory quality of food products (DUNCAN; CHANG, 2012; HRÁDKOVÁ et al., 2013). Since the amount of energy carried by the light rays is inversely proportional to their wavelength, UV rays possess more energy than visible light, inducing a higher rate of photochemical oxidation processes (NARAYANAN; LOGANATHAN; BABU, 2017). Stablilization against photo-oxidative degradation of food can be achieved by UV-shielding. In the case of polymer packaging films, UV absorbers are often included in the polymer formulation.

Figure 1. Light transmittance (%) spectra from 200 to 800 nm of heat-treated PLA films blended with ATBC and bixin. Films without ATBC: FP (0% bixin); F1 (0.01% bixin); F5 (0.05% bixin); F10 (0.10% bixin); Films with ATBC: FPA (0% bixin); F1A (0.01% bixin); F5A (0.05% bixin); F10A (0.10% bixin).



The UV light absorption capacity of bixin is with this respect of great importance in the development of food packaging materials. Neat PLA films, especially when they are amorphous, are colorless and highly transparent to UV (280-400 nm) and visible light (400 – 800 nm) (Figure 1). The blending of PLA with or without plasticizer with bixin did not greatly affect films barrier to visible light, despite of the strong and vibrant yellow-orange color of thermal treated films (Table 3). In the Table 5 the quantification of the light transmittance at different wavelengths is reported. A substantial decrease of UVA (315-400nm) and UVB (280-315nm) light transmission was observed with increasing bixin content. The films formulated with 0.1% (F10) of bixin reached UVA transmission values below 10% and UVB light transmittance below 15 %. The presence of ATBC in PLA films did not seem to alter the light transmission properties. We can conclude that bixin, even if it is partially degraded during heat treatment, could provide an interesting barrier against photo-degradation of packed foods.

Table 5. Light transmittance at UV-B (300 nm), UV-A (380 nm) and Visible (700 nm) light regions and oxygen barrier properties of PLA films

			Light transmittance (%)			$P \times 10^{18}$	$D \times 10^{12}$	$S \times 10^{6}$
	ATBC (%)	Bixin (%)	UV-B	UV-A	Visible	[m^3 m m^{-2} Pa^{-1} s^{-1}]	[m^2 s^{-1}]	[m^3 m^{-3} Pa^{-1}]
FP	0	0	63.1 a	73.8 a	83.2 a	3.31	3.3	1.0
FPA	13	0	60.2 a	73.0 a	80.8 a	5.06	4.9	1.0
F1	0	0.01	58.2 ab	65.8 b	83.1 a	4.14	3.5	1.2
F1A	13	0.01	54.6 b	60.8 b	82.3 a	4.55	4.3	1.0
F5	0	0.05	32.2 c	27.9 c	76.5 b	3.04	2.8	1.1
F5A	13	0.05	25.2 d	23.5 c	80.5 a	5.08	5.2	1.1
F10	0	0.1	10.0 e	4.4 d	64.8 c	3.59	3.2	1.1
F10A	13	0.1	14.9 de	9.5 d	77.5 b	5.04	4.2	1.2

P: Oxygen permeability; D: Oxygen diffusivity; S: Oxygen solubility. Values are given as mean ± SD (n=3). Different letters in the same column indicate significant differences ($p < 0.05$) among the films.

3.4 Bixin release from active films

In our previous work (Stoll 2019) we showed that the release of bixin from semi-crystalline PLA films allowed the decrease of oxidation reactions in edible oil. However, the release rates were too slow for active protection of the oil at short times, i.e. in the starting phase of the degradation. Therefore, an amorphous PLA was used and plasticized, which should result in increased release kinetics. Furthermore, the thermal degradation of bixin during the processing was taken into account. The release kinetics of bixin into a food simulant are plotted in Figure 2 and the mass transport parameters, calculated with our previously developed model (eq.5) are shown in Table 6. The model fit was satisfying. In a previous study of our group, where 0.01% of bixin was added in PLA films with higher degree of crystallinity (Xc=22%), the migration rate of bixin to ethanol 95%(v/v) was 1.2×10^{-8} m s^{-1}. This value is about 5 fold lower than the one observed for F1 film in Table 6 (Stoll et al., 2018; Stoll et al., 2019). This result was expected because crystallites induce tortuosity to the diffusive pathway of molecules and decrease thereby transport rates (Michaels and Bixler 1961). The relationship between PLA crystallinity and the release of active substances from polymeric matrices has been more frequently study in the pharmaceutic field, where drugs release is shown to migrate faster in amorphous structures (KAMALY et al., 2016). The overall mass transfer coefficient (K) of bixin to the food simulant increased 6 fold thanks to PLA blending with ATBC (Table 6). The lower glass transition temperature of plasticized films – which ranged between 35 °C and 38 °C – implied that films were in a rubbery state at the temperature conditions of the migration test (40 °C), hence facilitating bixin release from the PLA matrix (CHALIER et al., 2008), (Sonchaeng et al. 2018).

The bixin concentration in PLA films did not affect its migration rate (Table 4). Bixin equilibrium concentration (C_{eq}) in the food simulant was reached after 150 h and 600 h for films with and without ATBC, respectively (Figure 2). Comparing films produced with the same bixin initial content, the differences found in C_{eq} might be related to the higher bixin stability in the absence of ATBC. These differences were not observed for F1 and F1A, where the equilibrium concentration in the end of release test was statistically the same – probably due to films low concentration of bixin, reducing the precision of its quantification. Considering the degradation of bixin might occur in the films both before and after its release to the media - inside and outside of the film matrix - the partition coefficient was not calculated.

Figure 2. Experimental data of bixin concentration (C) in ethanol 95% with the models prediction curves of bixin release from PLA films (F1: 0.01% bixin; F1A: 0.01% bixin, 13% ATBC; F5: 0.05% bixin; F5A: 0.05%, 13% ATBC; F10: 0.1% bixin; F10A: 0.1% bixin, 13% ATBC).

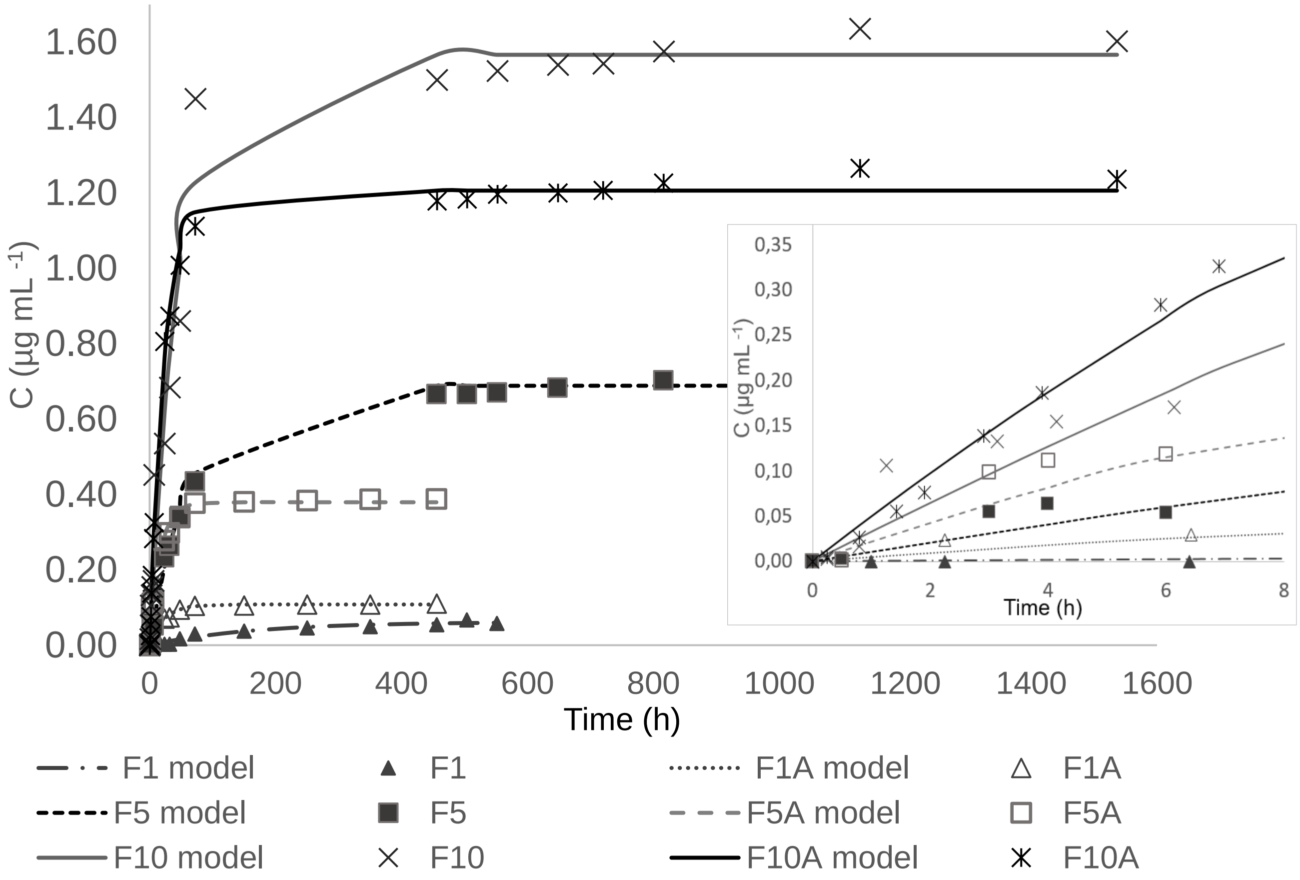


Table 6. Release kinetics of bixin from heat-treated PLA films blended or not with ATBC.

	K (m s^{-1} × 10^{-7})	C^* (μg mL^{-1})	R^2	C_{eq} (μg mL−1)
FP	-	-	-	-
FPA	-		-	-
F1	0.5 c	0.07 c	0.96	0.08 e
F1A	3.5 a	0.11 c	0.98	0.11 e
F5	1.3 c	0.69 b	0.96	0.72 c
F5A	5.3 a	0.37 bc	0.98	0.39 d
F10	1.7 bc	1.63 a	0.98	1.60 a
F10A	3.4 ab	1.23 a	0.99	1.24 b

K: overall mass transfer coefficient (K), C^*: film inner concentration estimated using the experimental data by the mathematical release model eq. 5, C_{eq}: bixin concentration in food simulant after reaching the equilibrium. Different letters in the same column indicate significant differences ($p < 0.05$) among the films.

4. CONCLUSIONS

In this study, we analyzed the properties of active packaging films based on neat and plasticized PLA using bixin, a carotenoid of known antioxidant and UV shielding properties. The effect of a heat treatment of the films - simulating polymer processing temperatures on the degradation of bixin - was evaluated, also as the impact of its degradation on UV shielding, oxygen barrier properties and bixin release kinetics. The stability of bixin was higher in the absence of ATBC, where neat PLA had a positive influence on the carotenoid stability. Despite of the substantial loss of bixin caused by the thermal treatment, it was possible to produce transparent films with bright color and which endowed a satisfactory barrier to UVA and UVB transmission. The use of the plasticizer ATBC caused a decrease on the oxygen barrier properties of the films, but had a largely positive impact on the release kinetics of bixin to food simulants. The overall mass transfer coefficients were accelerated about 6 times. Applicative studies are underway to investigate the effect of this acceleration on the oxidative protection of food oils. As an additive for PLA, the bixin could allow its application on light-sensitive products packaging, extending their shelf life through the protection of food lipids, vitamins and bioactive compounds from light exposure. Besides the UV protection, the release of bixin could avoid oxidation reactions in perishable goods.

REFERENCES

ANDERSON, K. S.; SCHRECK, K. M.; HILLMYER, M. A. Toughening polylactide. **Polymer Reviews**, [s. l.], v. 48, n. 1, p. 85–108, 2008.

ARRIETA, M. P. et al. Effect of D-Limonene on the Stabilization of Poly (Lactic Acid). [s. l.], p. 719–726, 2015.

CASTRO-AGUIRRE, E. et al. Poly(lactic acid)—Mass production, processing, industrial applications, and end of life. **Advanced Drug Delivery Reviews**, [s. l.], v. 107, n. April, p. 333–366, 2016. Disponível em: <http://dx.doi.org/10.1016/j.addr.2016.03.010>

CHALIER, P. et al. Moisture and temperature triggered release of a volatile active agent from soy protein coated paper: Effect of glass transition phenomena on carvacrol diffusion coefficient. **Journal of agricultural and food chemistry**, [s. l.], v. 57, n. 2, p. 658–665, 2008.

COLES, R.; MCDOWELL, D.; KIRWAN, M. J. **Food packaging technology**. [s.l.] : CRC Press, 2003. v. 5

COURGNEAU, C. et al. Analysis of the Structure-Properties Relationships of Different Multiphase Systems Based on Plasticized Poly (Lactic Acid) Analysis of the Structure-Properties Relationships of Different Multiphase Systems Based on Plasticized Poly (Lactic Acid). [s. l.], n. May 2016, 2011.

DOMENEK, S.; FERNANDES-NASSAR, S.; DUCRUET, V. Rheology, Mechanical Properties, and Barrier Properties of Poly (lactic acid). In: **Synthesis, Structure and Properties of Poly (lactic acid)**. [s.l.] : Springer, 2017. p. 303–341.

DUNCAN, S. E.; CHANG, H. H. **Implications of Light Energy on Food Quality and Packaging Selection**. 1. ed. [s.l.] : Elsevier Inc., 2012. v. 67

GEROMETTA, M. et al. **Physical and Chemical Stability of PLA in Food Packaging**. [s.l.] : Elsevier, 2019.

GIMENEZ-ROTA, C. et al. SC. **The Journal of Supercritical Fluids**, [s. l.], 2019.

HRÁDKOVÁ, I. et al. Antioxidant effect of mono- and dihydroxyphenols in sunflower oil with different levels of naturally present tocopherols. **European Journal of**

Lipid Science and Technology, [s. l.], v. 115, n. 7, p. 747–755, 2013.

KAMALY, N. et al. Degradable Controlled-Release Polymers and Polymeric Nanoparticles : Mechanisms of Controlling Drug Release. [s. l.], 2016.

MARCET, I. et al. PRODUCTION AND CHARACTERISATION OF BIODEGRADABLE PLA NANOPARTICLES LOADED WITH THYMOL TO IMPROVE ITS ANTIMICROBIAL EFFECT. **Journal of Food Engineering**, [s. l.], 2018.

NARAYANAN, A. M.; LOGANATHAN, S.; BABU, R. UV Protective Poly(lactic acid)/Rosin Films for Sustainable Packaging. **International Journal of Biological Macromolecules**, [s. l.], 2017.

NIU, X. et al. Highly Transparent, Strong, and Flexible Films with Modified Cellulose Nanofiber Bearing UV Shielding Property. **Biomacromolecules**, [s. l.], v. 19, n. 12, p. 4565–4575, 2018.

PAGNAN, C. S. et al. Annatto-colored Poly (3-hydroxybutyrate): A Comprehensive Study on Photodegradation. **Journal of Polymers and the Environment**, [s. l.], p. 1–10, 2017.

POSPÍŠIL, J.; NEŠPŮREK, S. Polymer additives. **Plastic Packaging: Interactions with Food and Pharmaceuticals**, [s. l.], p. 63–88, 2008.

PRABHAKARA RAO, P. G. et al. Effect of processing conditions on the stability of annatto (Bixa orellana L.) dye incorporated into some foods. **LWT - Food Science and Technology**, [s. l.], v. 38, n. 7, p. 779–784, 2005.

PRESTON, H. D.; RICKARD, M. D. Extraction and chemistry of annatto. **Food Chemistry**, [s. l.], v. 5, n. 1, p. 47–56, 1980.

REGULATION, C. No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. **Official Journal L**, [s. l.], v. 12, 2011.

RODRIGUEZ-AMAYA, D. B. Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of food carotenoids-A review. **Journal of Food Composition and Analysis**, [s. l.], v. 23, n. 7, p. 726–740, 2010.

ROUSSAKI, M. et al. Encapsulation of the natural antioxidant aureusidin in biodegradable PLA nanoparticles. **Polymer Degradation and Stability**, [s. l.], v. 108, p. 182–187, 2014.

SCOTTER, M. The chemistry and analysis of annatto food colouring: a review. **Food Additives and Contaminants**, [s. l.], v. 26, n. 8, p. 1123–1145, 2009.

SCOTTER, M. J.; CASTLE, L.; APPLETON, G. P. Kinetics and yields for the formation of coloured and aromatic thermal degradation products of annatto in foods. **Food chemistry**, [s. l.], v. 74, n. 3, p. 365–375, 2001.

SOUKOULIS, C.; BOHN, T. Critical Reviews in Food Science and Nutrition A Comprehensive Overview on the Micro- and Nano-technological Encapsulation Advances for Enhancing the Chemical Stability and Bioavailability of Encapsulation of carotenoids. [s. l.], n. June, p. 37–41, 2015.

STOCLET, G. et al. In-situ SAXS study of the plastic deformation behavior of polylactide upon cold-drawing. **Polymer**, [s. l.], v. 55, n. 7, p. 1817–1828, 2014.

STOLL, L. et al. Carotenoids extracts as natural colorants in poly(lactic acid) films.

Journal of Applied Polymer Science, [s. l.], 2018.

STOLL, L. et al. Poly (acid lactic) films with carotenoids extracts: Release study and effect on sunflower oil preservation. **Food chemistry**, [s. l.], v. 281, p. 213–221, 2019.

ARTIGO 4

Bixin and acetyl tributyl citrate in polylactide films produced by casting and melt processing - stability and protection performance on food products

ABSTRACT

Poly(lactic acid) (PLA) is one of the most used biodegradable materials for short term applications. However, different additives are required to fulfill desirable polymer properties for food packaging application. In this study, a carotenoid with coloring and antioxidant properties named bixin was used to increase PLA light barrier, where bixin migration to the packed food could protect it from oxidative reactions. PLA films containing 0.1% bixin were produced by casting and melt processing methods and bixin stability to heat and shear stress was evaluated. The melting process promoted more than 75% of bixin loss, where shear forces did not increase the carotenoid thermal degradation. The developed films presented satisfactory light barrier properties, protecting riboflavin, and reducing the oxidation reactions of sunflower oil. The melt processing and the use of acetyl tributyl citrate (ATBC) increased bixin transfer coefficient to ethanol 95%, where the combined incorporation of bixin and ATBC into the polymer broaden PLA application to oxygen- and light-sensitive food products.

Keywords: PLA; thermal degradation; shear; carotenoids; acetyl tributyl citrate.

1. INTRODUCTION

Food packaging facilitates its handling, preservation, and distribution. Considering food is usually required at different places and times from its growth and processing, packaging becomes a connecting link between production and consumption in urbanized societies. However, the negative environmental effect caused by plastic – the main material used to pack food – is a growing concern. Around 40% of the plastic production is used for packaging, from which 60% is applied for the packaging of food and beverages (PLASTICS EUROPE, 2018).

Recycling is one of the main strategies to reduce plastics environmental impact. However, the use of recycled materials to produce new food packaging presents particular challenges concerning food safety, increasing in number and level the sources of contamination of the packed products (GEUEKE; GROH; MUNCKE, 2018). Under this perspective, the use of biodegradable materials for food packaging represents a promising alternative to reduce the societal waste disposal problem.

Poly(lactic acid) (PLA) is a biopolymer produced from renewable sources such as corn starch and is currently the most used biodegradable material for short term applications (DOMENEK; FERNANDES-NASSAR; DUCRUET, 2017). PLA can be blended with other resins and compounded with different fillers such as fibers, and micro- and nanoparticles to fulfill additional desirable polymer properties (CASTRO-AGUIRRE et al., 2016). The incorporation of carotenoids into PLA was shown to improve the light and oxygen barrier properties of films - characteristics that hamper the polymer application to light/oxygen-sensitive foods (STOLL et al., 2018b).

Bixin is a carotenoid abundant in annatto seeds (*Bixa orellana* L.) that presented satisfactory light stability in PLA casted films and the best performance on protecting sunflower oil from oxidation when compared to beta-carotene and lycopene (STOLL et al., 2018b, 2019). Besides of its coloring properties, bixin could be used on the development of active packaging, where its migration to the packed food could extend food shelf life by reducing oxidative reactions (PAGNO et al., 2016; STOLL et al., 2019).

Considering that melt processing - characterized by heating the material above its melting temperature, shaping the molten polymer into desired shapes, and finally cooling to stabilize its final dimensions (CASTRO-AGUIRRE et al., 2016) - is required to obtain packaging films at an industrial scale, the knowledge about the stability of additives during PLA processing is a need. The extrusion is the first step before any further processing of PLA (i.e., injection, thermoforming and spinning) and involves heat and shear forces, which might impact on the PLA structure and on its additives (FARAH; ANDERSON; LANGER, 2016).

Among PLA additives, bixin is a promising light-barrier compound and acetyl tributyl citrate (ATBC) a common plasticizer. The plasticizers facilitate PLA processing through the reduction of polymer melt viscosity besides reducing PLA brittle fracture (RUELLAN; DUCRUET; DOMENEK, 2014). In the light of this, our study aimed to evaluate bixin stability to heat and shear during PLA melt processing. The effect of bixin and ATBC on the food-preserving properties of PLA was also evaluated.

2. MATERIALS AND METHODS

2.1 Bixin extraction

Bixin was extracted from annatto seeds with dichloromethane, following the methodology described by Stoll et al. (2019). The extracts were evaluated by Ultra High Performance Liquid Chromatography (UHPLC) and presented >97% of bixin purity. The chromatographic conditions are described below at section “Chromatographic analysis.”

2.2 PLA materials and films production

The PLA-bixin materials were obtained by casting and melt-mixing, producing Casting (Cast) and Melt-Processing (MP) films, respectively, as illustrated in Figure 1. The films’ formulations are presented in Table 1.

Figure 1. Casting and Melt Processing PLA films production scheme

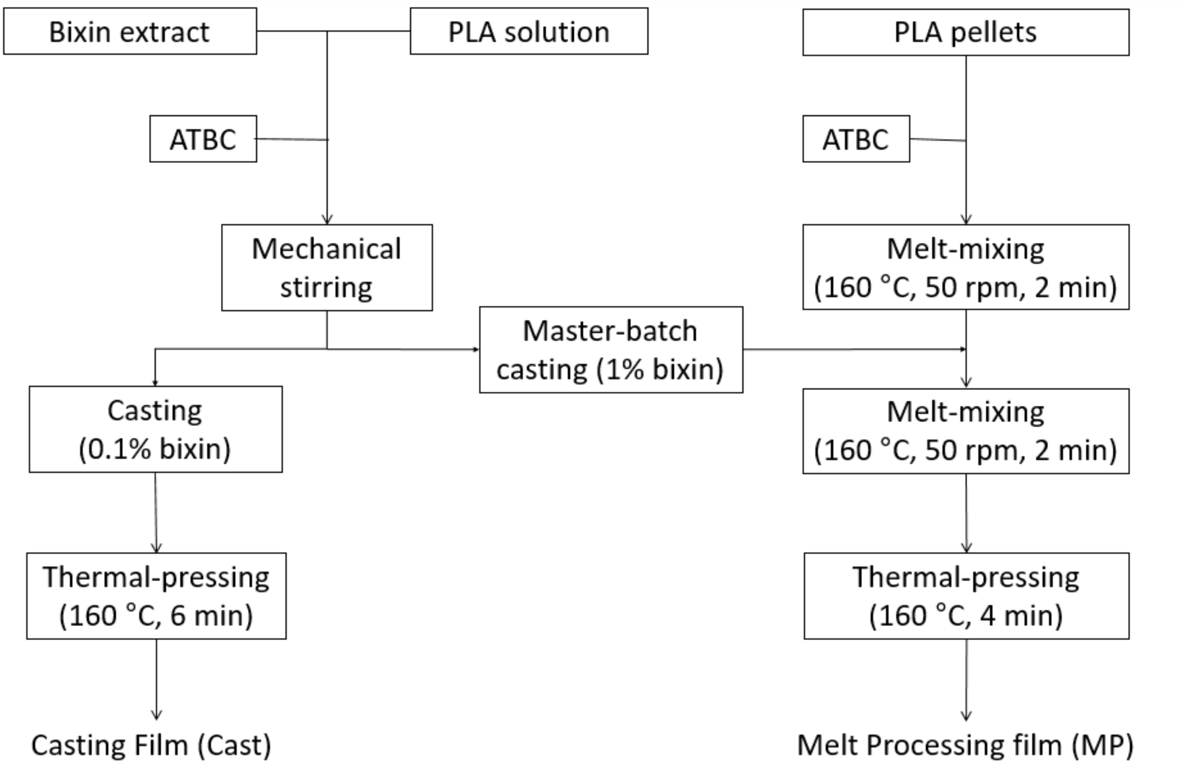


Table 1. Films' formulation and processing methods

Films designation*	Processing method	Film formulation			Heat processing method***	
		PLA (%)	ATBC (%)	Bixin** (%)	Melt Processing	Thermal-Pressing
Cast.Control	Cast	100	-	-	-	6 min
Cast.Control.P	Cast	87	13%	-	-	6 min
Cast.Bix	Cast	100	-	0.1	-	6 min
Cast.Bix.P	Cast	87	13%	0.1	-	6 min
MP.Control	MP	100	-	-	2 min	4 min
MP.Control.P	MP	87	13%	-	2 min	4 min
MP.Bix	MP	100	-	0.1	2 min	4 min
MP.Bix.P	MP	87	13%	0.1	2 min	4 min

*Abbreviations: Cast - films produced by casting; MP - films produced by melt processing; P – film produced with plasticizer; Control - films without bixin; Bix - films with bixin.
**Bixin percentage based on the total content of PLA and plasticizer ATBC. ***Period of bixin exposure to heat (160 °C) during films production.

2.2.1 Casting materials

Solutions of PLA were produced by stirring dry PLA pellets in dichloromethane (5% w/v) at ambient temperature. After polymer dissolution, the bixin extract and the plasticizer ATBC were added to PLA solutions, achieving ATBC concentration of 0.13 g/g and bixin concentrations of 0.1 mg/g – bixin mass related to the sum PLA and ATBC total content. The concentration of bixin was based on a previous study, where the use of 0.1 % of bixin was shown to effectively increase the light barrier properties of PLA films produced by casting (data not published). Bixin quantification was performed before its incorporation into PLA matrix using a UV–vis spectrophotometer (Jasco V-750, Japan) at λ_{max} =470 nm, in quartz cells, using dichloromethane as a blank. The PLA/ATBC/bixin solutions were poured into Teflon recipients at a leveled surface. The casted films were peeled off from Teflon after solvent evaporation, being placed in an oven (40 °C – 6 h) with forced air circulation.

2.2.2 Melt-mixing materials

The melt of PLA was performed in a HAAKE™ Rheomix Internal-Mixer at 160 °C with a 50 rpm screw rotation rate (Figure 1). Dried PLA pellets were manually mixed with the plasticizer ATBC and then were melted in the internal mixer. After 2 min processing, the masterbatch containing 1 % bixin (produced by casting) was introduced in the mixer and the system PLA/ATBC/bixin was processed for 2 min more. The processed material containing 0.1 % bixin was collected from the internal mixer and crushed using liquid nitrogen and a grinder.

2.3 Films production

After obtaining materials through casting and melt mixing techniques, the materials were thermal-pressed at 160 °C to complete 6 min of heat treatment (Table 1; Figure 1). The films production by Casting (Cast) aims to evaluate the bixin stability to heat, while films production by Melt Processing (MP) evaluates bixin stability to both heat and shear forces.

The thermal pressing was performed at 160 °C by placing PLA samples between two Teflon sheets and two steel plates in the press (Darragon, France). Casting materials were pressed for 6 min (Cast films) and Melt Processed materials for 4 min (MP films). The press was opened and films were quenched at room temperature at a cold surface. Films were stored in a desiccator at room temperature, in the dark.

2.4 Bixin quantification

The bixin content of Cast and MP films was evaluated following the methodology of Stoll et al., 2018, by the spectrophotometric method. For that, films were dissolved in dichloromethane with the aid of an ultrasonic bath. Solutions were poured into quartz cells, and their absorbance was measured at 470 nm in a spectrophotometer (Jasco V-750, Japan). The results were expressed as total bixin equivalent.

2.5 Release study

The bixin release to a food simulant was periodically evaluated under accelerated conditions of migration (40 °C) following the methodology of Stoll et al. (2018). Ethanol 95% (v/v) - approved as a fatty food simulant substitute by the Regulation No 10/ 2011 - was used as a simulant to immerse the produced films and evaluate the bixin migration. After the periodical quantification of bixin in ethanol, the overall mass transfer coefficient (K) was obtained through the following equation

$$\frac{dC}{dt} = K\frac{A}{V}(C^{*} - C) - k_{\mathrm{d}}C \qquad \text{(Eq. 1)}$$

where C is the carotenoid concentration in the food simulant (μg mL^{-1}), A is the surface area of the film in contact with the liquid (0.003 m^2), V is the volume of the food simulant solution ($10^{-5}m^3$), $C*$ is the carotenoid concentration (μg mL^{-1}) in the inner part of the film, and k_d is the first-order kinetic degradation coefficient of bixin in ethanol 95% (2.155 s^{-1}×$10^{-7}s^{-1}$ – Stoll *et al.* (2018).

2.6 Chromatographic analysis

The identification of compounds in the bixin extract and films migrants to ethanol 95% was performed by UHPLC. The mobile phases included phase A - water with 0.1% (v/v) formic acid, and phase B - acetonitrile with 0.1 % (v/v) formic acid (both supplied by Carlo Erba, Val de Reuil, France). An isocratic elution with phases A/B 25/75 (v/v) was used at a 0.6ml/min flow rate, using an Ethylene Bridged Hybrid (BEH) C18 column (100 x 2.1 mm, 1.7 μm particle diameter, 130 Å) and pre-column Vanguard BEH C18 5 x 2.1 mm; 1.7μm (Waters, St Quentin en Yvelines, France). An injection volume of 2 μL was used, and the auto-sampler temperature was maintained at 10 °C.

2.7 Films characterization

2.7.1 DSC

The analysis of films crystallinity and glass transition temperature (T_g) were carried out using a Q100 DSC (TA Instruments) equipped with an intercooler. An indium standard was used for equipment calibration, with purge N_2 gas flow of 50 mL.min^{-1}. The films samples (3–5 mg, loaded in aluminum crucibles) were heated (10 °C – 180 °C), cooled (180 °C – 10 °C) and heated again (10 °C to 180 °C) at a constant rate (10 °C min^{-1}), keeping the temperature at 180 °C for 5 min. The degree of crystallinity of the films was calculated by the second heating enthalpy peaks through the following equation:

$$Xc = \frac{\Delta H_{m} - \Delta H_{cc}}{\Delta H_{f}\,(1-x)} \times 100 \qquad \text{(Eq. 2)}$$

where ΔH_m is the enthalpy of melting, ΔH_{cc} is the enthalpy of cold crystallization, ΔH_f is the heat of fusion of 100% crystalline PLA (93.7 J/g), and x is the sum of carotenoids and plasticizer in films.

2.7.2 Oxygen permeability

The oxygen permeability of films was determined through the manometric method on a permeability testing apparatus (GDP-C permeameter, Brugger Feinmechanik GmbH, Munich, Germany), at 23 °C. After outgassing the system for 15 h under vacuum, an O_2 flow of 50 cm^3 min^{-1} was released on one side of the film sample. The oxygen Permeability (P) was determined from the pressure steady state. Films thickness was measured in at least 5 random points of films surface, with a Digimatic micrometer (Mitutoyo, Japan).

2.7.3 UV-light transmission of PLA films

The UV-A and UV-B light barrier properties of films were measured by placing the films in a quartz test cell and scanning the samples from 200 to 800 nm in a UV-Vis spectrophotometer (Jasco V-750, Japan). An empty test cell was used as reference, and results were expressed as transmittance percentage.

2.8 Application of Melt Processing films in light- and oxygen-sensitive food products and food simulants

2.8.1 Riboflavin protection

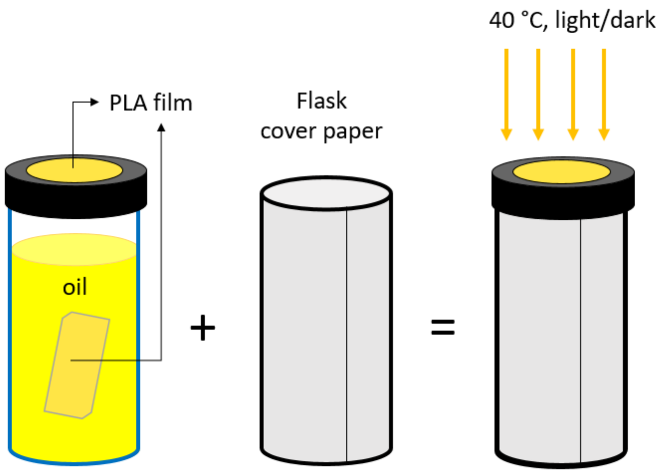
The light protection performance of Melt Processing films was evaluated over riboflavin stability. An accelerated test was performed following the validated methodology of Stancik et al. (2017) with some modifications. The methodology, which rapidly predicts materials light protection in dairy products, evaluates the ability of a package to preserve riboflavin, a light-sensitive nutrient present in milk. For the analysis, 8 mL of riboflavin aqueous solution (30 mg/mL, pH 6.4 potassium phosphate buffer) was kept in 12 mL open glass vials carefully involved with the PLA films, from the bottom to the top, in the outer side. The samples were stored at 5 °C and were positioned 15 cm away from a light source comprised of three white lamps (4700 ± 200 lux). Every 90 min, 0.5 mL of riboflavin solution was collected, and its riboflavin content was quantified by HPLC. The test was interrupted when >95% of riboflavin was degraded.

The quantification of riboflavin was performed on an Agilent Chromatograph, 1100 series (Santa Clara, CA, USA), using a C18 ODS Phenomenex HyperCloneTM, Allcrom (120 A, 250 x 4.6 mm, 5μm) column. A mixture of potassium phosphate buffer pH 6.4 with dimethylformamide 85:15 (v/v) was used as mobile phase at isocratic flow (1 mL min^{-1}), 25° C and injection volume of 10 µL. The detection was done by fluorescence with excitation of 450 nm and emission of 530 nm, where riboflavin quantification was performed after the obtention of a standard curve ($R^2 = 0.99$) made with a standard from Sigma-Aldrich (SCHMIDT et al., 2019).

2.8.2 Films effect over sunflower oil oxidative stability

The capacity of MP films on protecting sunflower oil from oxidation was evaluated at 40 °C, under light and dark storage conditions. To mimic the packaging performance of films, glass vials produced with films lids were used to store the oil. The films' lid evaluated the light transmittance and oxygen permeability effect in oil stability, while a piece of film was immersed in the oil allowing the bixin to migrate and eventually protect the oil. The vials were placed under white lamps and were side covered by thick paper (Figure 2). The peroxide value of the oil samples was periodically determined according to the IUPAC method.

Figure 2. Evaluation of MP films performance over sunflower oil oxidative stability



3. RESULTS AND DISCUSSION

The thermal processing of PLA films caused a substantial loss of bixin and its derivatives. Between 75 and 85% of bixin degradation was observed (Table 2), where no statistical differences were found between MP films with and without plasticizer. However, the presence of ATBC increased 10% the bixin loss in Cast films. PLA is frequently used in the encapsulation of diverse bioactive compounds, presenting a protective effect. Hence, its replacement by ATBC might have reduced bixin stability in films matrix.

The bixin degradation was statistically the same for the films produced with plasticizer MP.Bix.P and Cast.Bix.P, where both present the same formulation but differ by the processing method (Table 2). This suggests that the shear forces, which occurred in MP films and were absent in the Cast films, did not cause any additional degradation on the carotenoid when the plasticizer was used. Considering that in the absence of ATBC the degradation of bixin was higher for MP films, the facilitated chains slippage caused by plasticizer addition may have reduced bixin fracturing during the shearing process.

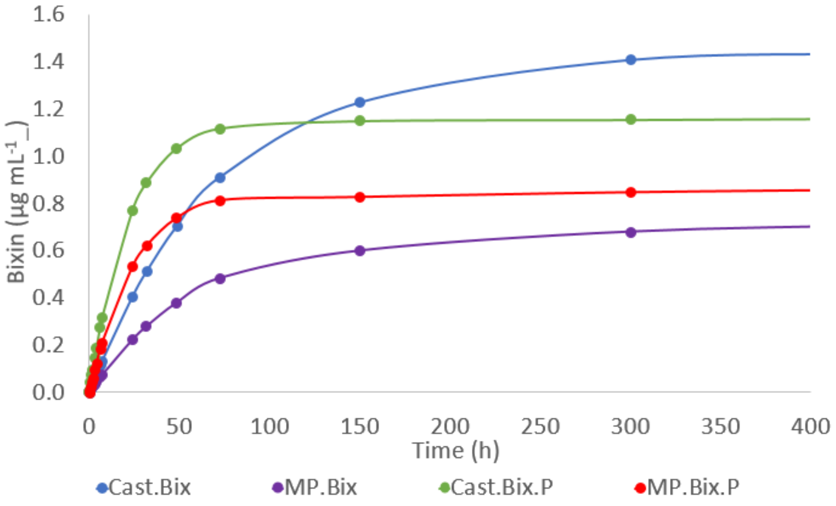
Table 2. Bixin thermal degradation in PLA films, its migration coefficients to the food simulant (ethanol 95% v/v), and migrants composition after 25 days of contact with PLA films

	Bixin* degradation	Bixin* migration coefficients			Films' migrants composition*** (%)			
	(%)**	K mig	C inf	R2	Comp 1	Comp 2	Comp 3	Comp 4
Bixin extract	-	-	-	-	0	0	0	100
Cast.Bix	74.9 ± 1.9 c	1.8E-07 c	1.64 a	0..98	49.9	5.0	8.0	37.2
Cast.Bix.P	85.3 ± 0.3 a	3.7E-07 b	1.03 b	0..99	72.7	<loq	<loq	27.3
MP.Bix	79.9 ± 1.1 b	5.9E-07 a	0.87 c	0.98	66.4	<loq	<loq	33.6
MP.Bix.P	82.4 ± 4.5 ab	5.8E-07 a	0.99 b	0.99	54.3	6.0	11.0	28.7

*Bixin and bixin derivatives, quantified at 460 nm by spectrophotometry. **Degradation after films thermal processing considering a bixin initial content of 0.1% (mass of bixin/mass of PLA and plasticizer ATBC). ***Comp: compounds identified and quantified by UHPLC-MS, according to Table 3.

The migration rate of bixin and its derivatives to the food simulant was up to 4 times higher for MP films when comparing to Cast films (Table 2, Figure 3). Concerning the migration of active compounds from polymeric materials, diffusion is known as the slowest or rate-controlling step. Besides the molecular diffusion toward the film/food interface, the migration involves the mass transfer across the interface and the dispersion into the bulk food (LACOSTE et al., 2005). The faster release of bixin to ethanol 95% in MP films might be related to the PLA chain scission during the process, which may have facilitated bixin diffusion through the polymeric matrix. Melting processes, such as extrusion and melt mixing, were shown to cause PLA thermo-mechanical degradation due to high temperature, oxygen and shear (FARAH; ANDERSON; LANGER, 2016; OLIVEIRA et al., 2016). The study of Oliveira (2016) demonstrated that PLA is more sensitive to thermo-mechanical degradation than thermo-oxidative degradation, which was related to temperature-shear combination during melt mixing.

Figure 3. Bixin migration from PLA films to ethanol 95% (v/v)



Compounds 1, 2, 3 and 4 were the film migrants identified by UHPLC-MS (Table 3). The results demonstrated that 9'-*cis*-bixin - the principal coloring component of annatto and the solely compound present in bixin extract in this study - isomerized to *trans*-bixin and other bixin derivatives. It is well known that the polyene chain is responsible for carotenoids instability and susceptibility to oxidation, where *cis/trans* isomerization occurs due to various factors such as temperature and light (SCOTTER; CASTLE; APPLETON, 2001). A series of complex degradation reactions occur to 9'-*cis*-bixin at elevated temperatures producing pale yellow to orange components (LEVY; RIVADENEIRA, 2000). Films color changed from orange to a bright yellow color after thermal processing, which is in agreement with the literature in terms of bixin isomerization and thermal degradation products color.

Table 3. UHPLC-MS identification of films migrants after 25 days of contact between films and the food simulant ethanol 95 % (v/v)

	Retention Time (min)	UV spectrum (nm)	MS Signal (m/z)	Identification
Compound 1	*1.22*	464 (M); 493 (m); 436 (s); 285 (m); 274(s)	**[M+H]+ = 395.2211 (maj);** [M•]+ = 394.2142; [M+H-H2O]+ = 377.2112; [M+H-CH3OH] = 363.1953	*trans*-bixin
Compound 2	*1.32*	455 (M); 481 (m); 430 (s); 404 (s) + 285 (m); 274 (s)	low signal, poor resolution	bixin degradation product
Compound 3	*1.38*	455 (M); 481 (m); 285 (m); 274 (s)	low signal, poor resolution	bixin degradation product
Compound 4	*1.61*	460 (M); 485 (m); 433 (s)	**[M+H]+ = 395.2207 (maj);** [M•]+ = 394.2136; [M+H-H2O]+ = 377.2117; [M+H-CH3OH]+ = 363.1960	9'-*cis*-bixin

Table 4 presents the oxygen-light barrier and thermal properties of the developed films. Meanwhile Control films allowed more than 41% of UV-A light transmittance, the incorporation of 0.1% of bixin in the polymer substantially increased the light barrier of PLA, blocking more than 85 and 98% of light passage in the Cast and MP films produced with bixin. The barrier improvement is due to the presence of conjugated C=C double bonds in bixin molecule, which serve as chromophore moieties and are responsible for its light absorption capacity (MORABITO et al., 2012). These results are of great importance, demonstrating that the heat and shear forces required for polymers processing, despite causing a considerable loss of the carotenoid content, preserved the light absorbance capacity of the remaining bixin and its derivatives and degradation products. Differences between the Cast and MP films are related to their thickness, where MP films presented higher values than Cast films.

Table 4. PLA films oxygen barrier properties, light transmittance (%) at UV-B (300 nm) and UV-A (380 nm) light, thickness, and thermal properties

	$P.10^{18}$	Light transmittance (%)		Thickness	Tg
	$[m^3\ m\ m^{-2}\ Pa^{-1}\ s^{-1}]$	UV-B	UV-A	µm	°C
Cast.Control	3.3 b	63.1 a	73.8 a	113 c	59 a
Cast.Control.P	5.1 a	60.2 a	73.0 a	128 c	32 b
MP.Control	3.1 b	41.9 b	54.9 b	200 a	60 a
MP.Control.P	5.0 a	43.2 b	56.4 b	170 b	38 b
Cast.Bix	3.6 b	10.0 c	4.4 c	122 c	60 a
Cast.Bix.P	5.0 a	14.9 c	9.5 c	113 c	37 b
MP.Bix	3.0 b	0.7 d	0.1 d	217 a	59 a
MP.Bix.P	3.8 a	1.8 d	0.3 d	166 b	38 b

Different letters in the same column indicate significant differences ($p < 0.05$) among the films.

*Abbreviations: Cast - films produced by casting; MP - films produced by melt processing; P – film produced with plasticizer; Control - films without bixin; Bix - films with bixin.

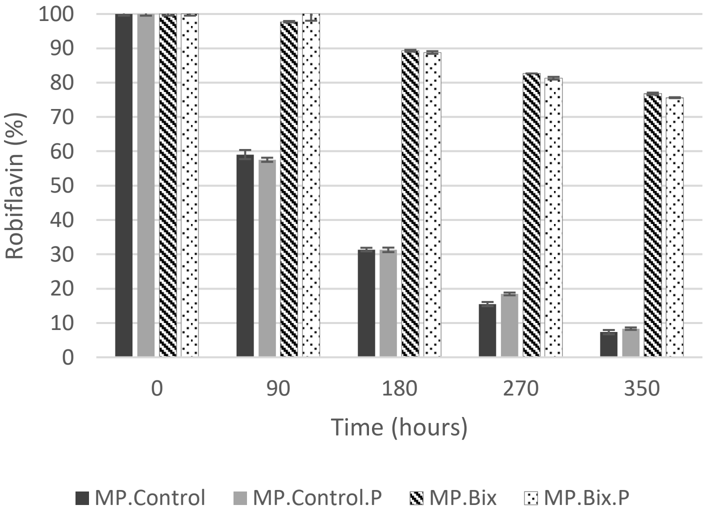
As expected and observed by many studies, the presence of ATBC in PLA films considerably reduced the polymer glass transition temperature (Tg) (Table 4). The decrease in Tg was followed by a minor reduction in films thickness in MP films, which might be related to the reduced melt viscosity of plasticized materials (RUELLAN; DUCRUET; DOMENEK, 2014), allowing the melted polymer to easily flow under the pressure and heat offered by the thermal press.

The incorporation of bixin into the PLA matrix did not affect oxygen permeability meanwhile the use of ATBC increased the oxygen permeability (Table 4). Plasticizers are known to create free volumes in polymeric matrices and hence decrease materials barrier properties (RUELLAN et al., 2015). The increase in oxygen permeability is an undesirable effect on the development of food packaging materials, specially when dealing with oxygen-sensitive goods such as fatty food products. Crystalline zones in polymer matrices tend to force a tortuous diffusive pathway of permeants and to reduce the available volume for permeant sorption (NASSAR et al., 2017). The PLA films amorphous structure – with a degree of crystallinity lower than 3% (data not shown) -

may have facilitated oxygen permeation when compared to our previous work, where a PLA grade of higher crystallinity was used (Stoll et al., 2018).

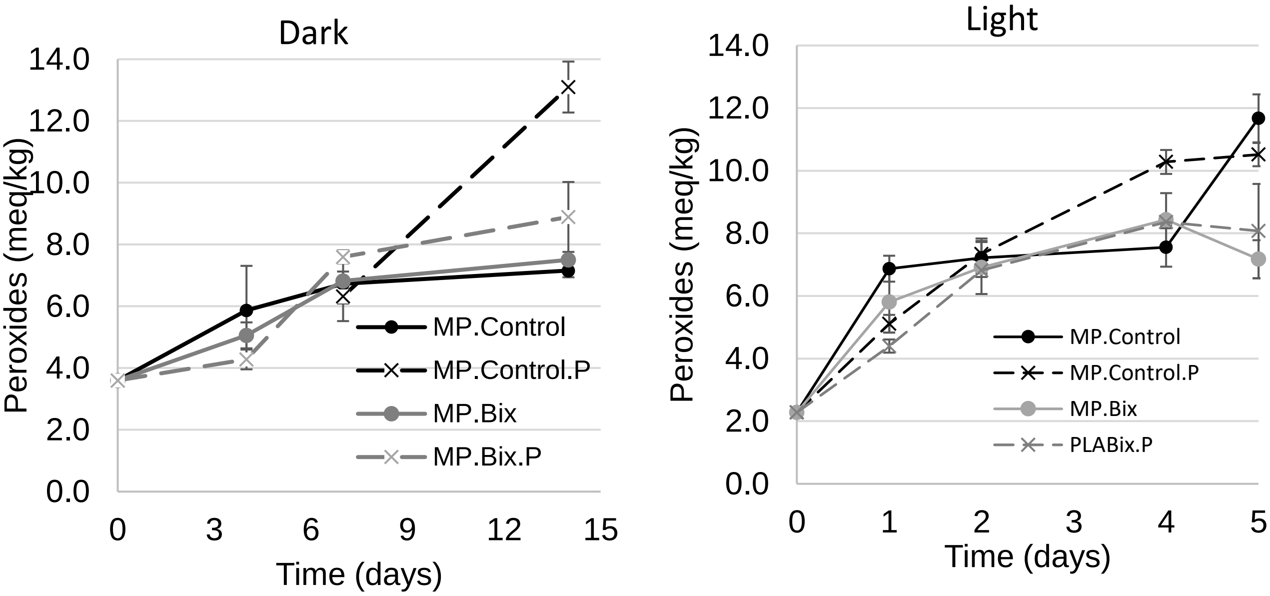
The light barrier offered by the packaging material is a key factor for the preservation of food vitamins and other light-sensitive products such as fatty foods. Riboflavin is a light-sensitive micronutrient (vitamin B2) that acts as a photosensitizer. At specific wavelengths, the light energy leads to the photochemical excitation of riboflavin, promoting photochemical reactions that may lead to the degradation of nutritive food components, such as vitamins, lipids, and proteins (MIN; BOFF, 2002). The PLA light barrier improvement was shown to protect riboflavin from photodegradation, where MP films containing bixin preserved the vitamin in milk simulant solutions. After 350 h of storage under intense light exposure, the solutions covered by MP.Bix and MP.Bix.P preserved nearly 80% of the riboflavin, while solutions covered by Control and MP.Control.P films have lost more than 95% of the vitamin after the same period (Figure 4).

Figure 4. Riboflavin stability in milk simulant solutions prepared with 30µg/mL riboflavin covered by PLA films under light exposure at refrigeration temperature (5 °C)



According to the results shown in Figure 5a, PLA films used to package the oil did not completely suppress the degradation of this oil when it was aged in the dark. The use of a plasticizer gave mobility to the polymer chains, decreasing the Tg and allowing higher oxygen permeation. Because of this, films prepared with the plasticizer (MP.Control.P and MP.Bix.P) allowed higher degradation, with increased level of peroxide formation. Under dark storage, the oil packed in MP.Control.P presented the highest levels of peroxides after 14 days, overpassing the limit of commercialization established by Codex. In the specific case of the films containing plasticizer, the antioxidant effect of bixin was clear, significantly decreasing the level of peroxide formation at the end of the experiment.

Figure 5. Effect of MP films packaging performance on the oxidative stability of sunflower oil stored at dark and light exposure conditions at 40 °C, measured by peroxides formation.



The rapid increase of peroxides level in oil stored under light (Figure 5b) when compared to the oil stored under dark conditions (Figure 5a) confirms the powerful lipid-oxidizing effect of light in food systems. Oil stored under light oxidizes through both photosensitized and autoxidation reactions, where singlet oxygen ($^1O_2$) and triplet oxygen ($^3O_2$) react with the oil, respectively (CHOE; MIN, 2006). In our study, we observed that the films containing bixin reduced the oxidation of sunflower oil under light, since they kept the peroxides level under the limit of commercialization (10 meq/kg) for 5 days under accelerated storage conditions. In addition to the protection against autoxidation it became clear that bixin was also effective in protecting against photochemical degradation.

Considering the experimental results we can conclude that bixin is able to prevent both autoxidation and photochemical oxidation of foods when added to packaging films. In this way, it shows to be an interesting natural additive to improve the performance of commercial packaging materials, preserving the quality of diversal food products.

4. CONCLUSION

PLA films containing bixin were prepared after melt mixing or solution casting to obtain new packaging materials for light-sensitive food products. It was observed that the melt mixing process promoted around 85% of bixin loss. Even so, the developed films presented satisfactory light barrier properties, protecting the milk vitamin riboflavin from degradation and reducing the oxidation of sunflower oil with the incorporation of only 0.1 % of bixin.

The mixing shear forces during melt mixing might have caused the cleavage of PLA long chains, facilitating the diffusion of bixin and increasing its transfer coefficient to the food simulant. Cis/trans isomerization and other degradation reactions of bixin occured during melt mixing.

The incorporation of 0.1% bixin increased the light and oxygen barrier properties of PLA, preventing packaged oil photochemical and autoxidation reactions. However, besides decreasing the glass transition temperature, the incorporation of ATBC decreased theoxygen barrier properties and reduced bixin stability. The negative effects of ATBC on the oil degradation was counterbalanced by the presence of bixin.

The combined incorporation of bixin and ATBC into PLA matrix allowed the obtention of plasticized films with outstanding light barrier properties, expanding the PLA application range to light-sensitive food products.

REFERENCES

CASTRO-AGUIRRE, E. et al. Poly(lactic acid)—Mass production, processing, industrial applications, and end of life. **Advanced Drug Delivery Reviews**, [s. l.], v. 107, n. April, p. 333–366, 2016. Disponível em: <http://dx.doi.org/10.1016/j.addr.2016.03.010>

CHOE, E.; MIN, D. B. Comprehensive Reviews in Food Science and Food Safety Mechanisms and Factors for Edible Oil Oxidation. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 5, p. 169–186, 2006.

DOMENEK, S.; FERNANDES-NASSAR, S.; DUCRUET, V. Rheology, Mechanical Properties, and Barrier Properties of Poly (lactic acid). In: **Synthesis, Structure and Properties of Poly (lactic acid)**. [s.l.] : Springer, 2017. p. 303–

341.

FARAH, S.; ANDERSON, D. G.; LANGER, R. Physical and mechanical properties of PLA, and their functions in widespread applications — A comprehensive review. **Advanced Drug Delivery Reviews**, [s. l.], v. 107, p. 367–392, 2016. Disponível em: <http://dx.doi.org/10.1016/j.addr.2016.06.012>

GEUEKE, B.; GROH, K.; MUNCKE, J. Food packaging in the circular economy: Overview of chemical safety aspects for commonly used materials. **Journal of Cleaner Production**, [s. l.], v. 193, p. 491–505, 2018. Disponível em: <https://doi.org/10.1016/j.jclepro.2018.05.005>

LACOSTE, A. et al. Advancing controlled release packaging through smart blending. **Packaging Technology and Science**, [s. l.], v. 18, n. 2, p. 77–87, 2005.

LEVY, L. W.; RIVADENEIRA, D. M. Annatto. In: IFT BASIC SYMPOSIUM SERIES 2000, **Anais**... [s.l: s.n.]

MIN, D. B.; BOFF, J. M. Chemistry and Reaction of Singlet Oxygen in Foods. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 1, n. 2, p. 58–72, 2002. Disponível em: <http://doi.wiley.com/10.1111/j.1541-4337.2002.tb00007.x>

MORABITO, K. et al. Proximal effects of ultraviolet light absorbers and polymer matrix in the photostability of ??-carotene. **Dyes and Pigments**, [s. l.], v. 92, n. 1, p. 509–516, 2012. Disponível em: <http://dx.doi.org/10.1016/j.dyepig.2011.06.031>

NASSAR, S. F. et al. Multi-scale analysis of the impact of polylactide morphology on gas barrier properties. **Polymer**, [s. l.], v. 108, p. 163–172, 2017.

OLIVEIRA, M. et al. The role of shear and stabilizer on PLA degradation. **Polymer Testing**, [s. l.], v. 51, n. August 2019, p. 109–116, 2016. Disponível em: <http://dx.doi.org/10.1016/j.polymertesting.2016.03.005>

PAGNO, C. H. et al. Synthesis of biodegradable films with antioxidant properties based on cassava starch containing bixin nanocapsules. **Journal of Food Science and Technology**, [s. l.], v. 53, n. 8, p. 3197–3205, 2016.

PLASTICS EUROPE 2018. An Analysis of European Plastics Production, Demand and Waste Data**, Plastics Europe-Association of Plastics Manufacturers** (2018) Disponível em >http://www.plasticseurope.org/>.

REGULATION, C. No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. **Official Journal L**, [s. l.], v. 12, 2011.

RUELLAN, A. et al. Solubility factors as screening tools of biodegradable toughening agents of polylactide. **Journal of Applied Polymer Science**, [s. l.], v. 132, n. 48, 2015.

RUELLAN, A.; DUCRUET, V.; DOMENEK, S. Plasticization of Poly (lactide). In: **Poly (lactic acid) Science and Technology**. [s.l: s.n.]. p. 124–170.

SCHMIDT, H. de O. et al. Vitamin and bioactive compound diversity of seven fruit species from south Brazil. **Journal of the Science of Food and Agriculture**, [s. l.], v. 99, n. 7, p. 3307–3317, 2019.

SCOTTER, M. J.; CASTLE, L.; APPLETON, G. P. Kinetics and yields for the formation of coloured and aromatic thermal degradation products of annatto in foods. **Food chemistry**, [s. l.], v. 74, n. 3, p. 365–375, 2001.

STANCIK, C. M. et al. Accelerated light protection performance measurement technology validated for dairy milk packaging design. **Packaging Technology and Science**, [s. l.], v. 30, n. 12, p. 771–780, 2017.

STOLL, L. et al. Carotenoids extracts as natural colorants in poly(lactic acid) films.

Journal of Applied Polymer Science, [s. l.], 2018.

STOLL, L. et al. Poly (acid lactic) films with carotenoids extracts: Release study and effect on sunflower oil preservation. **Food chemistry**, [s. l.], v. 281, p. 213–221, 2019.

CAPÍTULO 4

DISCUSSÃO GERAL E CONCLUSÕES

4 DISCUSSÃO GERAL

O presente trabalho atuou no desenvolvimento de filmes biodegradáveis de poli(ácido lático) (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 câmera 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), consequentemente 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 x 10^{-19} m^3 m m^{-2} Pa^{-1} s^{-1} para o PLA semi-cristalino (Artigo 2) e 3,3 x 10^{-18} m^3 m m^{-2} Pa^{-1} 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 x 10^{-7} m s^{-1}, artigo 4), comparado ao filme de PLA e bixina do Artigo 2 (K=1,57 x 10^{-8} 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.

6 REFERÊNCIAS

ABDEL-RAZIK, E. A. Aspects of degradation and stability of ABS copolymers. I. Effect of β-carotene as antioxidant. **Journal of Polymer Science Part A: Polymer Chemistry**, [s. l.], v. 27, n. 1, p. 343–355, 1989. Disponível em: <http://doi.wiley.com/10.1002/pola.1989.080270129>. Acesso em: 7 nov. 2017.

AHMED, R. R.; PARMAR, V.; AMIN, M. A. Impact of Product Packaging on Consumer’s Buying Behavior. **European Journal of Scientific Research202X European Journal of Scientific Research**, [s. l.], v. 120, n. 2, p. 1450–216, 1450. Disponível em: <www.europeanjournalofscientificresearch.com>

AKRAMI, M. et al. A new approach in compatibilization of the poly (lactic acid)/thermoplastic starch (PLA/TPS) blends. **Carbohydrate polymers**, [s. l.], v. 144, p. 254–262, 2016.

ALLEN, N. S. Photofading and light stability of dyed and pigmented polymers. **Polymer Degradation and Stability**, [s. l.], v. 44, n. 3, p. 357–374, 1994.

ANDERSON, K. S.; SCHRECK, K. M.; HILLMYER, M. A. Toughening polylactide. **Polymer Reviews**, [s. l.], v. 48, n. 1, p. 85–108, 2008.

ARRIETA, M. P. et al. Ternary PLA-PHB-Limonene blends intended for biodegradable food packaging applications. **European Polymer Journal**, [s. l.], v. 50, n. 1, p. 255–270, 2014. a.

ARRIETA, M. P. et al. Plasticized poly(lactic acid)-poly(hydroxybutyrate) (PLA-PHB) blends incorporated with catechin intended for active food-packaging applications. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 62, n. 41, p. 10170–10180, 2014. b.

ARRIETA, M. P. et al. Effect of D-Limonene on the Stabilization of Poly (Lactic Acid). [s. l.], p. 719–726, 2015.

ARROYO, O. H. et al. Processing and properties of PLA/thermoplastic starch/montmorillonite nanocomposites. **Polymer Composites**, [s. l.], v. 31, n. 1, p. 114–127, 2010.

ASTM INTERNATIONAL. **ASTM D882 - 12 Standard Test Method for Tensile Properties of Thin Plastic SheetingASTM D882-12, Standard Test Method for Tensile Properties of Thin Plastic Sheeting**. West Conshohocken, PA. Disponível em: <www.astm.org>.

ASTM INTERNATIONAL. **ASTM D1434 - 82 Standard Test Method for Determining Gas Permeability Characteristics of Plastic Film and Sheeting**. West Conshohocken, PA. Disponível em: <www.astm.org>.

AURAS, R. A.; SINGH, S. P.; SINGH, J. J. Evaluation of oriented poly (lactide) polymers vs. existing PET and oriented PS for fresh food service containers. **Packaging technology and science**, [s. l.], v. 18, n. 4, p. 207–216, 2005.

AURAS, R.; HARTE, B.; SELKE, S. An overview of polylactides as packaging materials. **Macromolecular bioscience**, [s. l.], v. 4, n. 9, p. 835–864, 2004.

AVEROUS, L.; BOQUILLON, N. Biocomposites based on plasticized starch: thermal and mechanical behaviours. **Carbohydrate Polymers**, [s. l.], v. 56, n. 2, p. 111–122, 2004.

BABU, R. P.; O’CONNOR, K.; SEERAM, R. Current progress on bio-based polymers and their future trends. **Progress in Biomaterials**, [s. l.], v. 2, n. 1, p. 8, 2013.

BAIARDO, M. et al. Thermal and Mechanical Properties of Plasticized Poly(L-lactic acid). **J Appl Polym Sci**, [s. l.], v. 90, p. 1731–1738, 2003.

BAJPAI, P. K.; SINGH, I.; MADAAN, J. Development and characterization of PLA-based green composites. **Journal of Thermoplastic Composite Materials**, [s.

l.], v. 27, n. 1, p. 52–81, 2014. Disponível em: <http://journals.sagepub.com/doi/10.1177/0892705712439571>

BAO, L. et al. Gas permeation properties of poly(lactic acid) revisited. **Journal of Membrane Science**, [s. l.], v. 285, n. 1–2, p. 166–172, 2006.

BARDEN, L.; DECKER, E. A. Lipid oxidation in low-moisture food: A review. **Critical Reviews in Food Science and Nutrition**, [s. l.], v. 56, n. 15, p. 2467–2482, 2013.

BARLOW, C. Y.; MORGAN, D. C. Polymer film packaging for food: An environmental assessment. **Resources, Conservation and Recycling**, [s. l.], v. 78, p. 74–80, 2013. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0921344913001432>

BHARDWAJ, R.; MOHANTY, A. K. Advances in the properties of polylactides based materials: a review. **Journal of Biobased Materials and Bioenergy**, [s. l.], v. 1, n. 2, p. 191–209, 2007.

BISHAI, M. et al. A comprehensive study on enhanced characteristics of modified polylactic acid based versatile biopolymer. **European Polymer Journal**, [s. l.], v. 54, p. 52–61, 2014.

BORDES, P.; POLLET, E.; AVÉROUS, L. Nano-biocomposites: biodegradable polyester/nanoclay systems. **Progress in Polymer Science**, [s. l.], v. 34, n. 2, p. 125–155, 2009.

BRITO, G. F. et al. Biopolímeros, polímeros biodegradáveis e polímeros verdes. **Revista Eletrônica de Materiais e Processos**, [s. l.], v. 6, n. 2, p. 127–139, 2011.

BYUN, Y. et al. The effect of solvent mixture on the properties of solvent cast polylactic acid (PLA) film. **Journal of applied polymer science**, [s. l.], v. 124, n. 5, p. 3577–3582, 2012.

BYUN, Y. J. et al. Physical and chemical properties of γ-irradiated EVOH film. **Radiation Physics and Chemistry**, [s. l.], v. 76, n. 6, p. 974–981, 2007.

BYUN, Y.; KIM, Y. T.; WHITESIDE, S. Characterization of an antioxidant polylactic acid (PLA) film prepared with α-tocopherol, BHT and polyethylene glycol using film cast extruder. **Journal of Food Engineering**, [s. l.], v. 100, n. 2, p. 239–244, 2010. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2010.04.005>

CARMONA, V. B. et al. Properties of a biodegradable ternary blend of thermoplastic starch (TPS), poly (ε-Caprolactone)(PCL) and poly (Lactic Acid)(PLA). **Journal of Polymers and the Environment**, [s. l.], v. 23, n. 1, p. 83–89, 2015.

CARRASCO, F. et al. Processing of poly(lactic acid): Characterization of chemical structure, thermal stability and mechanical properties. **Polymer Degradation and Stability**, [s. l.], v. 95, n. 2, p. 116–125, 2010.

CARVALHO, W. et al. Estimativa indireta de teores de licopeno em frutos de genótipos de tomateiro via análise colorimétrica. **Horticultura Brasileira**, [s. l.], v. 23, n. 3, p. 819–825, 2005.

CASTRO-AGUIRRE, E. et al. Poly(lactic acid)—Mass production, processing, industrial applications, and end of life. **Advanced Drug Delivery Reviews**, [s. l.], v. 107, n. April, p. 333–366, 2016. Disponível em: <http://dx.doi.org/10.1016/j.addr.2016.03.010>

CERRUTTI, P.; MALINCONICO, M.; RYCHLY, J.; MATISOVA-RYCHLA, L.; CARFAGNA, C. Effect of natural antioxidants on the stability of polypropylene films. **Polymer Degradation and Stability**, [s. l.], v. 94, n. 11, p. 2095–2100, 2009. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0141391009002687>. Acesso em: 8 nov. 2017.

CHALIER, P. et al. Moisture and temperature triggered release of a volatile active agent from soy protein coated paper: Effect of glass transition phenomena on carvacrol diffusion coefficient. **Journal of agricultural and food chemistry**, [s. l.], v. 57, n. 2, p. 658–665, 2008.

CHAN, T. C.; LI, H. T.; LI, K. Y. Effects of Shapes of Solute Molecules on Diffusion: A Study of Dependences on Solute Size, Solvent, and Temperature. **Journal of Physical Chemistry B**, [s. l.], v. 119, n. 51, p. 15718–15728, 2015.

CHENG, Y. et al. Polylactic acid (PLA) synthesis and modifications: a review. **Frontiers of chemistry in China**, [s. l.], v. 4, n. 3, p. 259–264, 2009.

CHOE, E.; MIN, D. B. Comprehensive Reviews in Food Science and Food Safety Mechanisms and Factors for Edible Oil Oxidation. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 5, p. 169–186, 2006.

CLASEN, S. H.; MULLER, C. M. O.; PIRES, A. T. N. Maleic Anhydride as a Compatibilizer and Plasticizer in TPS/PLA Blends. **Journal of the Brazilian Chemical Society**, [s. l.], v. 26, n. 8, p. 1583–1590, 2015.

CODEX ALIMENTARIUS, C. Codex alimentarius: Fats, oils and related products (Vol. 8). **Rome: FAO**, [s. l.], 2001.

COLES, R.; MCDOWELL, D.; KIRWAN, M. J. **Food packaging technology**. [s.l.] : CRC Press, 2003. v. 5

COLÍN-CHÁVEZ, C. et al. Diffusion of natural astaxanthin from polyethylene active packaging films into a fatty food simulant. **Food research international**, [s. l.], v. 54, n. 1, p. 873–880, 2013. a.

COLÍN-CHÁVEZ, C. et al. Diffusion of natural astaxanthin from polyethylene active packaging films into a fatty food simulant. **Food Research International**, [s. l.], v. 54, n. 1, p. 873–880, 2013. b. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2013.08.021>

COLÍN-CHÁVEZ, C. et al. The Release of Carotenoids from a Light-Protected Antioxidant Active Packaging Designed to Improve the Stability of Soybean Oil. **Food and Bioprocess Technology**, [s. l.], v. 7, n. 12, p. 3504–3515, 2014.

COLÍN-CHÁVEZ, C.; SOTO-VALDEZ, H.; PERALTA, E. Diffusion of carotenoids from mono and bilayer polyethylene active packaging into soybean oil. **Food Packaging and Shelf Life**, [s. l.], v. 1, n. 2, p. 170–178, 2014.

COLÍN-CHÁVEZ, C.; VICENTE-RAMÍREZ, E. B. The Release of Carotenoids from a Light-Protected Antioxidant Active Packaging Designed to Improve the Stability of Soybean Oil. [s. l.], p. 3504–3515, 2014.

COLÍN-CHÁVEZ, C. et al. Fabrication and properties of antioxidant polyethylene-based films containing marigold (Tagetes erecta) extract and application on soybean oil stability. **Packaging Technology and Science**, [s. l.], v. 26, n. 5, p. 267–280, 2013.

CORNEILLIE, S.; SMET, M. PLA architectures: the role of branching. **Polym. Chem.**, [s. l.], v. 6, n. 6, p. 850–867, 2015. Disponível em: <http://xlink.rsc.org/?DOI=C4PY01572J>

COURGNEAU, C. et al. Analysis of the Structure-Properties Relationships of Different Multiphase Systems Based on Plasticized Poly (Lactic Acid) Analysis of the Structure-Properties Relationships of Different Multiphase Systems Based on Plasticized Poly (Lactic Acid). [s. l.], n. May 2016, 2011.

COURGNEAU, C. et al. Effect of crystallization on barrier properties of formulated polylactide. **Polymer International**, [s. l.], v. 61, n. 2, p. 180–189, 2012.

CRUZ-ZÚÑIGA, J. M. et al. Development of an antioxidant biomaterial by promoting the deglycosylation of rutin to isoquercetin and quercetin. **Food Chemistry**, [s.

l.], v. 204, p. 420–426, 2016.

DAINELLI, D. et al. Active and intelligent food packaging: legal aspects and safety concerns. **Trends in Food Science & Technology**, [s. l.], v. 19, p. S103–S112, 2008.

DI MAIO, L. et al. Preparation and characterization of biodegradable active PLA film for food packaging. **AIP Conference Proceedings**, [s. l.], v. 1593, n. 2014, p. 338–341, 2014.

DIMAKOU, C.; OREOPOULOU, V. Antioxidant activity of carotenoids against the oxidative destabilization of sunflower oil-in-water emulsions. **LWT - Food Science and Technology**, [s. l.], v. 46, n. 2, p. 393–400, 2012. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2011.12.013>

DO SUL, J. A. I.; COSTA, M. F. The present and future of microplastic pollution in the marine environment. **Environmental pollution**, [s. l.], v. 185, p. 352–364, 2014.

DOMENEK, SANDRA; COURGNEAU, CÉCILE; DUCRUET, V. Characteristics and Applications of Poly(lactide). In: **Biopolymers: Biomédical and Environmental Applications**. [s.l: s.n.]. p. 183–224.

DOMENEK, S. et al. Potential of Lignins as Antioxidant Additive in Active Biodegradable Packaging Materials. **Journal of Polymers and the Environment**, [s. l.], v. 21, n. 3, p. 692–701, 2013.

DOMENEK, S.; FERNANDES-NASSAR, S.; DUCRUET, V. Rheology, Mechanical Properties, and Barrier Properties of Poly (lactic acid). In: **Synthesis, Structure and Properties of Poly (lactic acid)**. [s.l.] : Springer, 2017. p. 303–341.

DORGAN, J. R.; LEHERMEIER, H.; MANG, M. Thermal and rheological properties of commercial-grade poly (lactic acid) s. **Journal of Polymers and the Environment**, [s. l.], v. 8, n. 1, p. 1–9, 2000.

DUNCAN, S. E.; CHANG, H. H. **Implications of Light Energy on Food Quality and Packaging Selection**. 1. ed. [s.l.] : Elsevier Inc., 2012. v. 67

EDGE, R.; MCGARVEY, D. J.; TRUSCOTT, T. G. The carotenoids as anti-oxidants—a review. **Journal of Photochemistry and Photobiology B: Biology**, [s. l.], v. 41, n. 3, p. 189–200, 1997.

FARAH, S.; ANDERSON, D. G.; LANGER, R. Physical and mechanical properties of PLA, and their functions in widespread applications — A comprehensive review. **Advanced Drug Delivery Reviews**, [s. l.], v. 107, p. 367–392, 2016. Disponível em: <http://dx.doi.org/10.1016/j.addr.2016.06.012>

FERRI, J. M. et al. The effect of maleinized linseed oil (MLO) on mechanical performance of poly (lactic acid)-thermoplastic starch (PLA-TPS) blends. **Carbohydrate polymers**, [s. l.], v. 147, p. 60–68, 2016.

FIEDOR, J.; BURDA, K. Shahid-ul-Islam. **Nutrients**, [s. l.], v. 6, n. 2, p. 466–488, 2014.

FIKSELOVÁ, M. et al. Extraction of carrot (Daucus carota L.) carotenes under different conditions. **Czech Journal of Food Sciences**, [s. l.], v. 26, n. 4, p. 268–274, 2008.

FISCHER, E. W.; STERZEL, H. J.; WEGNER, G. Investigation of the structure of solution grown crystals of lactide copolymers by means of chemical reactions. **Kolloid-Zeitschrift und Zeitschrift für Polymere**, [s. l.], v. 251, n. 11, p. 980–990, 1973. Disponível em: <http://link.springer.com/10.1007/BF01498927>. Acesso em: 13 nov. 2017.

FRASER, P. D.; BRAMLEY, P. M. The biosynthesis and nutritional uses of carotenoids. **Progress in lipid research**, [s. l.], v. 43, n. 3, p. 228–265, 2004.

GALLEGO, G.; HAKKARAINEN, M.; ALMAJANO, M. P. Stability of O/W emulsions packed with PLA film with incorporated rosemary and thyme. **European Food**

Research and Technology, [s. l.], v. 243, n. 7, p. 1249–1259, 2017.

GARCÍA-SOTO, B. et al. Effect of biodegradable film (lyophilised alga Fucus spiralis and sorbic acid) on quality properties of refrigerated megrim (Lepidorhombus whiffiagonis). **International Journal of Food Science and Technology**, [s. l.], v. 50, n. 8, p. 1891–1900, 2015.

GARLOTTA, D. A Literature Review of Poly (Lactic Acid). **Journal of Polymers and the Environment**, [s. l.], v. 9, n. 2, p. 63–84, 2002. Disponível em: <http://link.springer.com/10.1023/A:1020200822435%5Cnhttp://www.springerlink.com/index/X15J565570160G41.pdf>

GAVRIIL, G.; KANAVOURAS, A.; COUTELIERIS, F. A. Food-packaging migration models: A critical discussion. **Critical Reviews in Food Science and Nutrition**, [s. l.], v. 8398, n. June, p. 1–11, 2017. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/10408398.2017.1317630>

GEROMETTA, M. et al. **Physical and Chemical Stability of PLA in Food Packaging**. [s.l.] : Elsevier, 2019.

GEUEKE, B.; GROH, K.; MUNCKE, J. Food packaging in the circular economy: Overview of chemical safety aspects for commonly used materials. **Journal of Cleaner Production**, [s. l.], v. 193, p. 491–505, 2018. Disponível em: <https://doi.org/10.1016/j.jclepro.2018.05.005>

GIMENEZ-ROTA, C. et al. SC. **The Journal of Supercritical Fluids**, [s. l.], 2019.

GOMEZ-ESTACA, J. et al. Advances in antioxidant active food packaging. **Trends in Food Science and Technology**, [s. l.], v. 35, n. 1, p. 42–51, 2014.

GONÇALVES, C. M. B. et al. Effect of natural and synthetic antioxidants incorporation on the gas permeation properties of poly(lactic acid) films. **Journal of Food Engineering**, [s. l.], v. 116, n. 2, p. 562–571, 2013.

GUERRINI, L. M. et al. **Correlação entre as propriedades reológicas, óticas e a morfologia de filmes soprados de LLDPE/LDPE** , scielo , 2004.

GUILLEN, M. D.; GOICOECHEA, E. Formation of oxygenated α,β-unsaturated aldehydes and other toxic compounds in sunflower oil oxidation at room temperature in closed receptacles. **Food Chemistry**, [s. l.], v. 111, n. 1, p. 157–164, 2008. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0308814608003476>

GUINAULT, A. et al. Impact of crystallinity of poly(lactide) on helium and oxygen barrier properties. **European Polymer Journal**, [s. l.], v. 48, n. 4, p. 779–788, 2012.

GUTIERREZ, A. M.; BOYLSTON, T. D.; CLARK, S. Effects of Pro-Oxidants and Antioxidants on the Total Antioxidant Capacity and Lipid Oxidation Products of Milk During Refrigerated Storage. **Journal of food science**, [s. l.], v. 83, n. 2, p. 275–283, 2018.

HAILA, K. M.; LIEVONEN, S. M.; HEINONEN, M. I. Effects of Lutein, Lycopene, Annatto, and +�-Tocopherol on Autoxidation of Triglycerides. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 44, n. 8, p. 2096–2100, 1996. Disponível em: <http://www.scopus.com/inward/record.url?eid=2-s2.0-0000203065&partnerID=40&md5=4fe89f39d59c9e95d0a888d65c92d353>

HARRIS, A. M., ELLEN, C. L. Improving Mechanical Performance of Injection Molded PLA by Controlling Crystallinity. **Journal of Applied Polymer Science**, [s. l.], v. 107, p. 2246–2255, 2008.

HRÁDKOVÁ, I. et al. Antioxidant effect of mono-and dihydroxyphenols in sunflower oil with different levels of naturally present tocopherols. **European Journal of Lipid Science and Technology**, [s. l.], v. 115, n. 7, p. 747–755, 2013.

HWANG, S. W. et al. Poly(L-lactic acid) with added α-tocopherol and resveratrol:

Optical, physical, thermal and mechanical properties. **Polymer International**, [s. l.], v. 61, n. 3, p. 418–425, 2012. a.

HWANG, S. W. et al. Poly (L-lactic acid) with added α-tocopherol and resveratrol: optical, physical, thermal and mechanical properties. **Polymer International**, [s. l.], v. 61, n. 3, p. 418–425, 2012. b.

HWANG, S. W. et al. Migration of α-tocopherol and resveratrol from poly(L-lactic acid)/starch blends films into ethanol. **Journal of Food Engineering**, [s. l.], v. 116, n. 4, p. 814–828, 2013. a. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2013.01.032>

HWANG, S. W. et al. Migration of α-tocopherol and resveratrol from poly (L-lactic acid)/starch blends films into ethanol. **Journal of Food Engineering**, [s. l.], v. 116, n. 4, p. 814–828, 2013. b.

INIGUEZ-FRANCO, F. et al. Antioxidant Activity and Di ff usion of Catechin and Epicatechin from Antioxidant Active Films Made of Poly(. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 60, p. 6515−6523, 2012.

IÑIGUEZ-FRANCO, F. et al. Antioxidant activity and diffusion of catechin and epicatechin from antioxidant active films made of poly(l-lactic acid). **Journal of Agricultural and Food Chemistry**, [s. l.], v. 60, n. 26, p. 6515–6523, 2012.

IÑIGUEZ-FRANCO, F. et al. Concurrent solvent induced crystallization and hydrolytic degradation of PLA by water-ethanol solutions. **Polymer (United Kingdom)**, [s. l.], v. 99, n. July, p. 315–323, 2016. Disponível em: <http://dx.doi.org/10.1016/j.polymer.2016.07.018>

JAMSHIDIAN, M. et al. Structural, mechanical and barrier properties of active PLA-antioxidant films. **Journal of Food Engineering**, [s. l.], v. 110, n. 3, p. 380–389, 2012. a. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2011.12.034>

JAMSHIDIAN, M. et al. Effects of synthetic phenolic antioxidants on physical, structural, mechanical and barrier properties of poly lactic acid film. **Carbohydrate Polymers**, [s. l.], v. 87, n. 2, p. 1763–1773, 2012. b. Disponível em: <http://dx.doi.org/10.1016/j.carbpol.2011.09.089>

JAMSHIDIAN, M.; TEHRANY, E. A.; DESOBRY, S. Antioxidants Release from Solvent-Cast PLA Film: Investigation of PLA Antioxidant-Active Packaging. **Food and Bioprocess Technology**, [s. l.], v. 6, n. 6, p. 1450–1463, 2013.

JOHN, M. J.; THOMAS, S. Biofibres and biocomposites. **Carbohydrate polymers**, [s. l.], v. 71, n. 3, p. 343–364, 2008.

KAMALY, N. et al. Degradable Controlled-Release Polymers and Polymeric Nanoparticles : Mechanisms of Controlling Drug Release. [s. l.], 2016.

KAUR, D.; SOGI, D. S.; WANI, A. A. Oxidative stability of soybean triacylglycerol using carotenoids and y-tocopherol. **International Journal of Food Properties**, [s. l.], v. 18, n. 12, p. 2605–2613, 2015. Disponível em: <http://dx.doi.org/10.1080/10942912.2013.803118>

KIM, N.; CHOE, E. Contribution of minor compounds to the singlet oxygen-related photooxidation of olive and perilla oil blend. **Food Science and Biotechnology**, [s. l.], v. 22, n. 2, p. 315–321, 2013.

KIOKIAS, S.; DIMAKOU, C.; OREOPOULOU, V. Activity of natural carotenoid preparations against the autoxidative deterioration of sunflower oil-in-water emulsions. **Food Chemistry**, [s. l.], v. 114, n. 4, p. 1278–1284, 2009. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2008.10.087>

KIOKIAS, S.; OREOPOULOU, V. Antioxidant properties of natural carotenoid extracts against the AAPH-initiated oxidation of food emulsions. **Innovative food science & emerging technologies**, [s. l.], v. 7, n. 1–2, p. 132–139, 2006.

KIOKIAS, S.; VARZAKAS, T.; OREOPOULOU, V. In vitro activity of vitamins, flavonoids, and natural phenolic antioxidants against the oxidative deterioration of oil-based systems. **Critical reviews in food science and nutrition**, [s. l.], v. 48, n. 1, p. 78–93, 2008.

KIRSCHWENG, B. et al. Natural antioxidants as stabilizers for polymers. **Polymer Degradation and Stability**, [s. l.], 2017. Disponível em: <http://dx.doi.org/10.1016/j.polymdegradstab.2017.07.012>

KIRWAN, M. J.; PLANT, S.; STRAWBRIDGE, J. W. Plastics in food packaging. **Food and Beverage Packaging Technology, Second Edition**, [s. l.], p. 157–212, [s.d.].

KOUTSIMANIS, G. et al. Influences of packaging attributes on consumer purchase decisions for fresh produce. **Appetite**, [s. l.], v. 59, n. 2, p. 270–280, 2012.

LACOSTE, A. et al. Advancing controlled release packaging through smart blending. **Packaging Technology and Science**, [s. l.], v. 18, n. 2, p. 77–87, 2005.

LEVY, L. W.; RIVADENEIRA, D. M. Annatto. In: IFT BASIC SYMPOSIUM SERIES 2000, **Anais**... [s.l: s.n.]

LI, M. et al. Extrusion processing and characterization of edible starch films with different amylose contents. **Journal of Food Engineering**, [s. l.], v. 106, n. 1, p. 95–101, 2011.

LIM, L.-T.; AURAS, R.; RUBINO, M. Processing technologies for poly(lactic acid). **Progress in Polymer Science**, [s. l.], v. 33, n. 8, p. 820–852, 2008. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0079670008000373>

LIMBO, S.; TORRI, L.; PIERGIOVANNI, L. Light-induced changes in an aqueous β-carotene system stored under halogen and fluorescent lamps, affected by two oxygen partial pressures. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 55, n. 13, p. 5238–5245, 2007.

LIN, Y. J. et al. Surface roughness and light transmission of biaxially oriented polypropylene films. **Polymer Engineering & Science**, [s. l.], v. 47, n. 10, p. 1658–1665, 2007. Disponível em: <http://doi.wiley.com/10.1002/pen.20850>. Acesso em: 7 nov. 2017.

LIU, D. Y. et al. Characterisation of solution cast cellulose nanofibre - Reinforced poly(lactic acid). **Express Polymer Letters**, [s. l.], v. 4, n. 1, p. 26–31, 2010.

LLANA-RUIZ-CABELLO, M. et al. Characterisation and evaluation of PLA films containing an extract of Allium spp. to be used in the packaging of ready-to-eat salads under controlled atmospheres. **LWT - Food Science and Technology**, [s. l.], v. 64, n. 2, p. 1354–1361, 2015.

LOPES, M. S.; JARDINI, A. L.; MACIEL FILHO, R. Poly (lactic acid) production for tissue engineering applications. **Procedia Engineering**, [s. l.], v. 42, p. 1402–1413, 2012.

LÓPEZ-DE-DICASTILLO, C. et al. Reducing Oxidation of Foods Through Antioxidant Active Packaging Based on Ethyl Vinyl Alcohol and Natural Flavonoids. **Packaging Technology and Science**, [s. l.], v. 25, n. 8, p. 457–466, 2012. Disponível em: <http://doi.wiley.com/10.1002/pts.992>. Acesso em: 1 mar. 2018.

LÓPEZ-RUBIO, A.; LAGARON, J. M. Improvement of UV stability and mechanical properties of biopolyesters through the addition of β-carotene. **Polymer Degradation and Stability**, [s. l.], v. 95, n. 11, p. 2162–2168, 2010. Disponível em: <http://dx.doi.org/10.1016/j.polymdegradstab.2010.03.002>

LÓPEZ DE DICASTILLO, C. et al. A traditional aboriginal condiment as an antioxidant agent in the development of biodegradable active packaging. **Journal of Applied Polymer Science**, [s. l.], v. 134, n. 15, p. 1–10, 2017.

MANZANAREZ-LÓPEZ, F. et al. Release of α-Tocopherol from Poly(lactic acid) films, and its effect on the oxidative stability of soybean oil. **Journal of Food Engineering**, [s. l.], v. 104, n. 4, p. 508–517, 2011. a.

MANZANAREZ-LÓPEZ, F. et al. Release of ??-Tocopherol from Poly(lactic acid) films, and its effect on the oxidative stability of soybean oil. **Journal of Food Engineering**, [s. l.], v. 104, n. 4, p. 508–517, 2011. b.

MARCET, I. et al. PRODUCTION AND CHARACTERISATION OF BIODEGRADABLE PLA NANOPARTICLES LOADED WITH THYMOL TO IMPROVE ITS ANTIMICROBIAL EFFECT. **Journal of Food Engineering**, [s. l.], 2018.

MARCOS, B. et al. Development of biodegradable films with antioxidant properties based on polyesters containing α-tocopherol and olive leaf extract for food packaging applications. **Food Packaging and Shelf Life**, [s. l.], v. 1, n. 2, p. 140–150, 2014.

MARTINS, C. et al. Active polylactic acid film incorporated with green tea extract: Development, characterization and effectiveness. **Industrial Crops and Products**, [s. l.], v. 123, p. 100–110, 2018.

MASEK, A. et al. Application of β-carotene, a natural flavonoid dye, to polymeric materials as a natural antioxidant and determination of its characteristics using cyclic voltammetry and FTIR spectroscopy. **International Journal of Electrochemical Science**, [s. l.], v. 10, n. 4, p. 3372–3386, 2015.

MASTROMATTEO, M. et al. Advances in controlled release devices for food packaging applications. **Trends in Food Science & Technology**, [s. l.], v. 21, n. 12, p. 591–598, 2010.

MELLINAS, C. et al. Active edible films: Current state and future trends. **Journal of Applied Polymer Science**, [s. l.], v. 133, n. 2, 2016.

MICHIELS, Y.; PUYVELDE, P. Van; SELS, B. Barriers and Chemistry in a Bottle: Mechanisms in Today’s Oxygen Barriers for Tomorrow’s Materials. **Applied Sciences**, [s. l.], v. 7, n. 7, p. 665, 2017.

MILLER-CHOU, B. A.; KOENIG, J. L. A review of polymer dissolution. **Progress in Polymer Science**, [s. l.], v. 28, n. 8, p. 1223–1270, 2003.

MIN, D. B.; BOFF, J. M. Chemistry and Reaction of Singlet Oxygen in Foods. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 1, n. 2, p. 58–72, 2002. Disponível em: <http://doi.wiley.com/10.1111/j.1541-4337.2002.tb00007.x>

MORABITO, K. et al. Proximal effects of ultraviolet light absorbers and polymer matrix in the photostability of ??-carotene. **Dyes and Pigments**, [s. l.], v. 92, n. 1, p. 509–516, 2012. Disponível em: <http://dx.doi.org/10.1016/j.dyepig.2011.06.031>

MOURA, L. E. et al. Bioactive efficacy of low-density polyethylene films with natural additives. **Journal of Applied Polymer Science**, [s. l.], v. 135, n. 34, 2018.

MUCCIO, E. A. **Plastics processing technology**. [s.l.] : ASM international, 1994.

NAMPOOTHIRI, K. M.; NAIR, N. R.; JOHN, R. P. An overview of the recent developments in polylactide (PLA) research. **Bioresource technology**, [s. l.], v. 101, n. 22, p. 8493–8501, 2010.

NARAYANAN, A. M.; LOGANATHAN, S.; BABU, R. UV Protective Poly(lactic acid)/Rosin Films for Sustainable Packaging. **International Journal of Biological Macromolecules**, [s. l.], 2017.

NASSAR, S. F. et al. Multi-scale analysis of the impact of polylactide morphology on gas barrier properties. **Polymer**, [s. l.], v. 108, p. 163–172, 2017.

NIU, X. et al. Highly Transparent, Strong, and Flexible Films with Modified Cellulose Nanofiber Bearing UV Shielding Property. **Biomacromolecules**, [s. l.], v. 19, n.

12, p. 4565–4575, 2018.

NUNES, I. L.; MERCADANTE, A. Z. Obtenção de cristais de licopeno a partir de descarte de tomate. **Ciência e Tecnologia de Alimentos**, [s. l.], v. 24, n. 3, p. 440–447, 2004.

OLIVEIRA, M. et al. The role of shear and stabilizer on PLA degradation. **Polymer Testing**, [s. l.], v. 51, n. August 2019, p. 109–116, 2016. Disponível em: <http://dx.doi.org/10.1016/j.polymertesting.2016.03.005>

ORTIZ-VAZQUEZ, H. et al. Release of butylated hydroxytoluene (BHT) from Poly(lactic acid) films. **Polymer Testing**, [s. l.], v. 30, n. 5, p. 463–471, 2011. Disponível em: <http://dx.doi.org/10.1016/j.polymertesting.2011.03.006>

PAGNAN, C. S. et al. Annatto-colored Poly(3-hydroxybutyrate): A Comprehensive Study on Photodegradation. **Journal of Polymers and the Environment**, [s. l.], v. 0, n. 0, p. 1–10, 2017. a.

PAGNAN, C. S. et al. Annatto-colored Poly (3-hydroxybutyrate): A Comprehensive Study on Photodegradation. **Journal of Polymers and the Environment**, [s. l.], p. 1–10, 2017. b.

PAGNO, C. H. et al. Synthesis of biodegradable films with antioxidant properties based on cassava starch containing bixin nanocapsules. **Journal of Food Science and Technology**, [s. l.], v. 53, n. 8, p. 3197–3205, 2016.

PALOZZA, P.; KRINSKY, N. I. [38] Antioxidant effects of carotenoids in Vivo and in Vitro: An overview. In: **Methods in enzymology**. [s.l.] : Elsevier, 1992. v. 213p. 403–420.

PESEK, C. A.; WARTHESEN, J. J. Photodegradation of Carotenoids in a Vegetable Juice System. **Journal of Food Science**, [s. l.], v. 52, n. 3, p. 744–746, 1987.

PLATZER, N. The technology of plasticizers, J. Kern Sears and Joseph R. Darby, SPE Monograph Series, Wiley, New York, 1982, 1166 pp. Price: $130.00. **Journal of Polymer Science: Polymer Letters Edition**, [s. l.], v. 20, n. 8, p. 459–459, 1982. Disponível em: <http://doi.wiley.com/10.1002/pol.1982.130200810>. Acesso em: 7 nov. 2017.

POSPÍŠIL, J.; NEŠPŮREK, S. Polymer additives. **Plastic Packaging: Interactions with Food and Pharmaceuticals**, [s. l.], p. 63–88, 2008.

PRABHAKARA RAO, P. G. et al. Effect of processing conditions on the stability of annatto (Bixa orellana L.) dye incorporated into some foods. **LWT - Food Science and Technology**, [s. l.], v. 38, n. 7, p. 779–784, 2005.

PRESTON, H. D.; RICKARD, M. D. Extraction and chemistry of annatto. **Food Chemistry**, [s. l.], v. 5, n. 1, p. 47–56, 1980.

QUILES-CARRILLO, L. et al. Bioactive Multilayer Polylactide Films with Controlled Release Capacity of Gallic Acid Accomplished by Incorporating Electrospun Nanostructured Coatings and Interlayers. **Applied Sciences**, [s. l.], v. 9, n. 3, p. 533, 2019.

RAMOS, M. et al. Development of novel nano-biocomposite antioxidant films based on poly (lactic acid) and thymol for active packaging. **Food Chemistry**, [s. l.], v. 162, p. 149–155, 2014. a. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2014.04.026>

RAMOS, M. et al. Influence of thymol and silver nanoparticles on the degradation of poly (lactic acid) based nanocomposites: thermal and morphological properties. **Polymer Degradation and Stability**, [s. l.], v. 108, p. 158–165, 2014. b.

RAMOS, M. et al. Development of novel nano-biocomposite antioxidant films based on poly (lactic acid) and thymol for active packaging. **Food chemistry**, [s. l.], v. 162, p. 149–155, 2014. c.

RASAL, R. M.; JANORKAR, A. V; HIRT, D. E. Poly (lactic acid) modifications. **Progress in polymer science**, [s. l.], v. 35, n. 3, p. 338–356, 2010.

REGULATION, C. No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. **Official Journal L**, [s. l.], v. 12, 2011.

RHIM, J. W. et al. Effect of the processing methods on the performance of polylactide films: Thermocompression versus solvent casting. **Journal of Applied Polymer Science**, [s. l.], v. 101, n. 6, p. 3736–3742, 2006.

RIOS, A. D. O.; MERCADANTE, A. Z. Optimization of the conditions to obtain bixin crystals and for extraction and saponification to quantify bixin in extruded snacks by HPLC. **Alimentos e Nutrição**, [s. l.], v. 15, n. 3, p. 203–213, 2004.

RIVERA, S. M.; CANELA-GARAYOA, R. Analytical tools for the analysis of carotenoids in diverse materials. **Journal of Chromatography A**, [s. l.], v. 1224, n. Supplement C, p. 1–10, 2012. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0021967311018097>

RODRIGUES, E. et al. Development of a novel micro-assay for evaluation of peroxyl radical scavenger capacity: Application to carotenoids and structure–activity relationship. **Food chemistry**, [s. l.], v. 135, n. 3, p. 2103–2111, 2012.

RODRIGUEZ-AMAYA, D. B. Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of food carotenoids-A review. **Journal of Food Composition and Analysis**, [s. l.], v. 23, n. 7, p. 726–740, 2010.

RODRIGUEZ, D. **A Guide to Carotenoid Analysis in Foods**. [s.l: s.n.].

ROSATO, D. V. **Extruding plastics: a practical processing handbook**. [s.l.] : Springer Science & Business Media, 2013.

ROUSSAKI, M. et al. Encapsulation of the natural antioxidant aureusidin in biodegradable PLA nanoparticles. **Polymer Degradation and Stability**, [s. l.], v. 108, p. 182–187, 2014.

RUELLAN, A. et al. Solubility factors as screening tools of biodegradable toughening agents of polylactide. **Journal of Applied Polymer Science**, [s. l.], v. 132, n. 48, 2015.

RUELLAN, A.; DUCRUET, V.; DOMENEK, S. Plasticization of Poly (lactide). In: **Poly (lactic acid) Science and Technology**. [s.l: s.n.]. p. 124–170.

SAINI, R. D. Biodegradable Polymers. **International Journal of Applied Chemistry**, [s. l.], v. 13, n. 2, p. 179–196, 2017.

SAMSUDIN, H. et al. **Migration of antioxidants from polylactic acid films, a parameter estimation approach: Part I – A model including convective mass transfer coefficient**. [s.l.] : Elsevier Ltd, 2018. v. 105 Disponível em: <http://dx.doi.org/10.1016/j.foodres.2017.11.065>

SAMSUDIN, H.; SOTO-VALDEZ, H.; AURAS, R. Poly(lactic acid) film incorporated with marigold flower extract (Tagetes erecta) intended for fatty-food application. **Food Control**, [s. l.], v. 46, p. 55–66, 2014. a. Disponível em: <http://dx.doi.org/10.1016/j.foodcont.2014.04.045>

SAMSUDIN, H.; SOTO-VALDEZ, H.; AURAS, R. Poly(lactic acid) film incorporated with marigold flower extract (Tagetes erecta) intended for fatty-food application. **Food Control**, [s. l.], v. 46, p. 55–66, 2014. b. Disponível em: <http://dx.doi.org/10.1016/j.foodcont.2014.04.045>

SANCHES-SILVA, A. et al. Trends in the use of natural antioxidants in active food packaging: a review. **Food Additives & Contaminants: Part A**, [s. l.], v. 31, n. 3, p. 374–395, 2014. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/19440049.2013.879215>

SCHMIDT, H. de O. et al. Vitamin and bioactive compound diversity of seven fruit species from south Brazil. **Journal of the Science of Food and Agriculture**, [s. l.], v. 99, n. 7, p. 3307–3317, 2019.

SCOTTER, M. The chemistry and analysis of annatto food colouring: a review. **Food Additives and Contaminants**, [s. l.], v. 26, n. 8, p. 1123–1145, 2009.

SCOTTER, M. J.; CASTLE, L.; APPLETON, G. P. Kinetics and yields for the formation of coloured and aromatic thermal degradation products of annatto in foods. **Food chemistry**, [s. l.], v. 74, n. 3, p. 365–375, 2001.

SÉBASTIEN, F. et al. Novel biodegradable films made from chitosan and poly(lactic acid) with antifungal properties against mycotoxinogen strains. **Carbohydrate Polymers**, [s. l.], v. 65, n. 2, p. 185–193, 2006.

SHAHID-UL-ISLAM; RATHER, L. J.; MOHAMMAD, F. Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications - A review. **Journal of Advanced Research**, [s. l.], v. 7, n. 3, p. 499–514, 2016. Disponível em: <http://dx.doi.org/10.1016/j.jare.2015.11.002>

SHAHID, M.; SHAHID-UL-ISLAM; MOHAMMAD, F. Recent advancements in natural dye applications: A review. **Journal of Cleaner Production**, [s. l.], v. 53, p. 310–331, 2013. Disponível em: <http://dx.doi.org/10.1016/j.jclepro.2013.03.031>

SHAYAN, M. et al. Effect of modified starch and nanoclay particles on biodegradability and mechanical properties of cross-linked poly lactic acid. **Carbohydrate polymers**, [s. l.], v. 124, p. 237–244, 2015.

SHIRAI, M. A. et al. Poly(lactic acid)/thermoplastic starch sheets: effect of adipate esters on the morphological, mechanical and barrier properties. **Polímeros**, [s. l.], v. 26, n. 1, p. 66–73, 2016. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-14282016000100010&lng=en&nrm=iso&tlng=en>

SIEMANN, U. Solvent cast technology - A versatile tool for thin film production. **Progress in Colloid and Polymer Science**, [s. l.], v. 130, n. June, p. 1–14, 2005.

SIGURDSON, G. T.; TANG, P.; GIUSTI, M. M. Natural Colorants: Food Colorants from Natural Sources. **Annual Review of Food Science and Technology**, [s. l.], v. 8, n. 1, p. 261–280, 2017. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev-food-030216-025923>

SILVA, A. S. et al. Mass transport studies of model migrants within dry foodstuffs. **Journal of Cereal Science**, [s. l.], v. 48, n. 3, p. 662–669, 2008. Disponível em: <http://dx.doi.org/10.1016/j.jcs.2008.02.006>

SIRACUSA, V. Food packaging permeability behaviour: A report. **International Journal of Polymer Science**, [s. l.], v. 2012, n. i, 2012.

SOARES, F. C. et al. Thermoplastic starch/poly(lactic acid) sheets coated with cross-linked chitosan. **Polymer Testing**, [s. l.], v. 32, n. 1, p. 94–98, 2013. Disponível em: <http://dx.doi.org/10.1016/j.polymertesting.2012.09.005>

SOTHORNVIT, R. et al. Tensile properties of compression-molded whey protein sheets: Determination of molding condition and glycerol-content effects and comparison with solution-cast films. **Journal of Food Engineering**, [s. l.], v. 78, n. 3, p. 855–860, 2007.

SOTO-VALDEZ, H.; AURAS, R.; PERALTA, E. Fabrication of poly (lactic acid) films with resveratrol and the diffusion of resveratrol into ethanol. **Journal of Applied Polymer Science**, [s. l.], v. 121, n. 2, p. 970–978, 2011.

SOUKOULIS, C.; BOHN, T. Critical Reviews in Food Science and Nutrition A Comprehensive Overview on the Micro- and Nano-technological Encapsulation Advances for Enhancing the Chemical Stability and Bioavailability of Encapsulation of carotenoids. [s. l.], n. June, p. 37–41, 2015.

STANCIK, C. M. et al. Accelerated light protection performance measurement technology validated for dairy milk packaging design. **Packaging Technology and Science**, [s. l.], v. 30, n. 12, p. 771–780, 2017.

STEENSON, D. F.; MIN, D. B. Effects of β-carotene and lycopene thermal degradation products on the oxidative stability of soybean oil. **JAOCS, Journal of the American Oil Chemists' Society**, [s. l.], v. 77, n. 11, p. 1153–1160, 2000.

STOCLET, G. et al. In-situ SAXS study of the plastic deformation behavior of polylactide upon cold-drawing. **Polymer**, [s. l.], v. 55, n. 7, p. 1817–1828, 2014.

STOLL, L. et al. Carotenoids extracts as natural colorants in poly(lactic acid) films. **Journal of Applied Polymer Science**, [s. l.], v. 135, n. 33, 2018. a.

STOLL, L. et al. Poly (acid lactic) films with carotenoids extracts: Release study and effect on sunflower oil preservation. **Food chemistry**, [s. l.], v. 281, p. 213–221, 2019.

TANG, C. H. et al. Effect of transglutaminase treatment on the properties of cast films of soy protein isolates. **Journal of Biotechnology**, [s. l.], v. 120, n. 3, p. 296–307, 2005.

TÁTRAALJAI, D. MAJOR, L., FÖLDES, E., PUKÁNSZKY, B. **Effect of carotene on the processing stability of polyethylene and the characteristics of films under storage conditions1. Tátraaljai, D. Major, L., Földes, E., Pukánszky, B. Effect of carotene on the processing stability of polyethylene and the character**. [s.l: s.n.]. v. 102

THOMPSON, K. A. et al. Cultivar, maturity, and heat treatment on lycopene content in tomatoes. **Journal of Food Science**, [s. l.], v. 65, n. 5, p. 791–795, 2000.

TSUCHIHASHI, H. et al. Action of β-carotene as an antioxidant against lipid peroxidation. **Archives of Biochemistry and Biophysics**, [s. l.], v. 323, n. 1, p. 137–147, 1995.

URAYAMA, H.; MOON, S.; KIMURA, Y. Microstructure and thermal properties of polylactides with different L-and D-unit sequences: importance of the Helical nature of the L-sequenced segments. **Macromolecular Materials and Engineering**, [s. l.], v. 288, n. 2, p. 137–143, 2003.

VAN AARDT, M. et al. Release of antioxidants from poly(lactide-co-glycolide) films into dry milk products and food simulating liquids. **International Journal of Food Science and Technology**, [s. l.], v. 42, n. 11, p. 1327–1337, 2007.

VITRAC, O.; HAYERT, M. Identification of diffusion transport properties from desorption/sorption kinetics: An analysis based on a new approximation of fick equation during solid-liquid contact. **Industrial and Engineering Chemistry Research**, [s. l.], v. 45, n. 23, p. 7941–7956, 2006.

WANG, M. et al. Progress in Toughening Poly(Lactic Acid) with Renewable Polymers. **Polymer Reviews**, [s. l.], v. 57, n. 4, p. 557–593, 2017. Disponível em: <http://dx.doi.org/10.1080/15583724.2017.1287726>

WANG, N. et al. Influence of citric acid on the properties of glycerol-plasticized dry starch (DTPS) and DTPS/poly(lactic acid) blends. **Starch/Staerke**, [s. l.], v. 59, n. 9, p. 409–417, 2007.

YAHIA, E. M.; ORNELAS-PAZ, J. de J. **Chemistry, Stability, and Biological Actions of Carotenoids**. [s.l: s.n.].

YANG, W. et al. Effect of processing conditions and lignin content on thermal, mechanical and degradative behavior of lignin nanoparticles/polylactic (acid) bionanocomposites prepared by melt extrusion and solvent casting. **European Polymer Journal**, [s. l.], v. 71, p. 126–139, 2015. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0014305715003997>

YANG, W. et al. Effect of cellulose and lignin on disintegration, antimicrobial and antioxidant properties of PLA active films. **International Journal of Biological Macromolecules**, [s. l.], v. 89, p. 360–368, 2016. Disponível em: <http://dx.doi.org/10.1016/j.ijbiomac.2016.04.068>

YANISHLIEVA, N. V.; AITZETMÜLLER, K.; RANEVA, V. β-Carotene and lipid oxidation. **Lipid - Fett**, [s. l.], v. 100, n. 10, p. 444–462, 1998. Disponível em: <http://doi.wiley.com/10.1002/(SICI)1521-4133(199810)100:10%3C444::AID-LIPI444%3E3.0.CO;2-A>

YILDIRIM, S. et al. Active Packaging Applications for Food. **Comprehensive Reviews in Food Science and Food Safety**, [s. l.], v. 17, n. 1, p. 165–199, 2018.

ZEB, A.; MURKOVIC, M. Pro-oxidant effects of β-carotene during thermal oxidation of edible oils. **JAOCS, Journal of the American Oil Chemists' Society**, [s. l.], v. 90, n. 6, p. 881–889, 2013.

ANEXO I

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

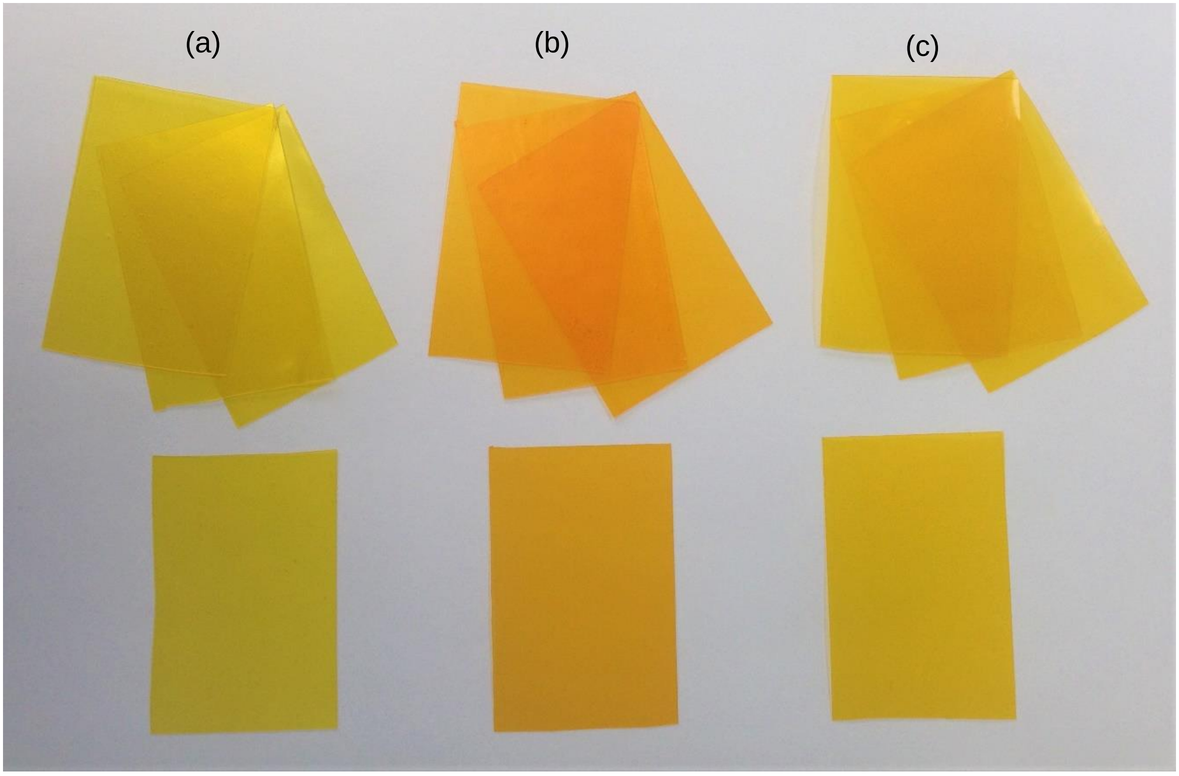


Figura 5. Filmes de PLA produzidos pela técnica de casting com diferentes concentrações de bixina

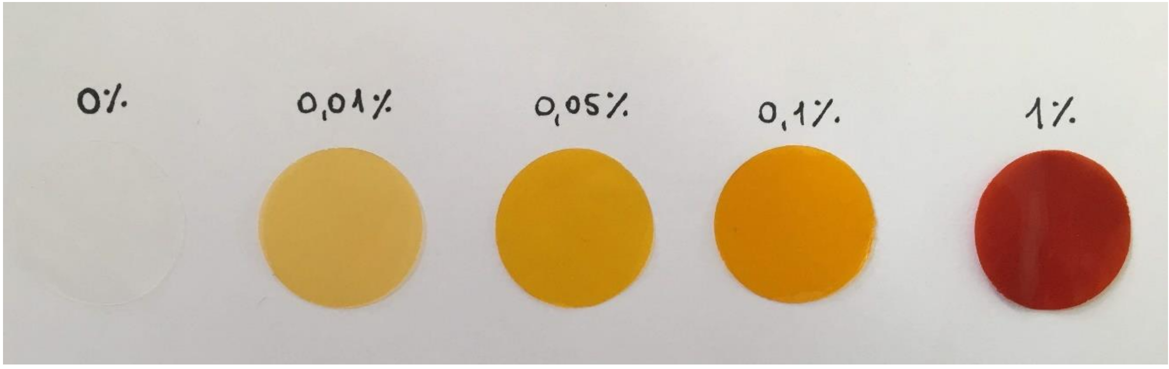
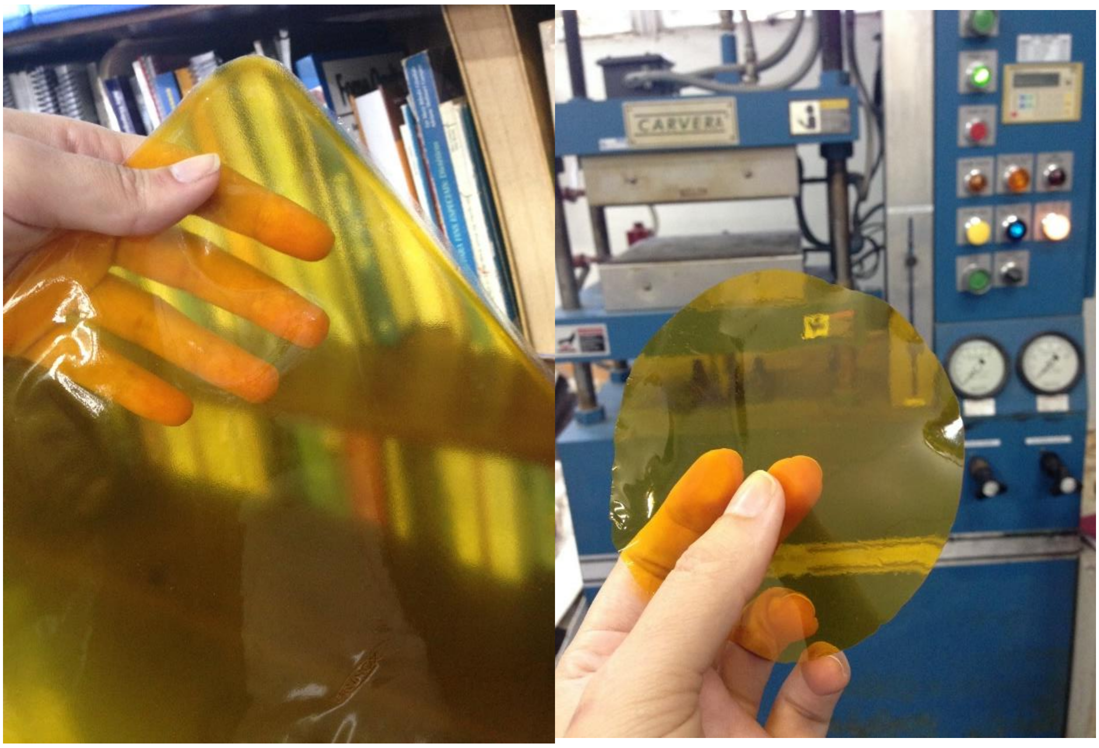


Figura 6. Master-batch de 1% bixina em PLA utilizado na produção de filmes por fusão em câmera de mistura

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)