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YURI BURATTO DE FARIAS

Filmes biodegradáveis de alginato de sódio incorporados com sais de norbixina

Orientador: Prof. Dr. Alessandro de Oliveira Rios

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RESUMO

Atualmente o setor de alimentos e bebidas é o principal responsável pela produção de embalagens plásticas. As justificativas do uso excessivo de embalagens pelo setor são a praticidade e a manutenção das qualidades físico-químicas, microbiológicas e sensoriais dos alimentos durante os processos logísticos envolvidos ao longo da cadeia de produção. A falta de conscientização ambiental que resulta no descarte destas embalagens no meio ambiente pode ocasionar seu acúmulo com poluição de solos, água e redução da biodiversidade local. Como alternativa ao uso das embalagens plásticas derivadas do petróleo, o presente trabalho objetivou desenvolver uma embalagem biodegradável a partir de alginato de sódio como polímero, glicerol como plastificante, cloreto de cálcio como agente complexante, e o carotenoide norbixina como antioxidante. Primeiramente, o trabalho avaliou a estabilidade dos sais de norbixina durante o processo de produção dos filmes pelo método de casting. O processo proposto produziu filmes bioativos com 85 % de retenção de sais de norbixina após formulação e secagem, considerando as perdas por lixiviação durante o processo de complexação por imersão em solução de cloreto de cálcio e as perdas por degradação térmica. Todos os filmes bioativos apresentaram coloração amarelo-vermelhado, sendo que com a maior concentração de sais de norbixina, maior foi a intensidade das cores, da barreira a luz UV-visível e ao vapor de água. Contudo, a alta concentração resultou em uma ação prooxidante durante o armazenamento de óleo de girassol. Com isto, as concentrações do carotenoide foram reduzidas e os filmes foram caracterizados quanto as suas propriedades físico-químicas, térmicas e mecânicas. O filme com adição de 0.03 % de sais de norbixina (g sais de norbixina / g alginato de sódio) apresentou capacidade antioxidante no armazenamento de óleo de girassol sob condições aceleradas por até 6 dias. Durante a preparação dos filmes, verificou-se ainda que era possível obter filmes com propriedades diferentes de acordo com o pH de solubilização do alginato de sódio. Deste modo, quando o pigmento foi incorporado em uma solução com pH 11, foram obtidos filmes com maior liberação dos sais de norbixina para meios lipídicos e melhor capacidade antioxidante. Por fim, os resultados obtidos indicaram que é possível obter filmes biodegradáveis de alginato de sódio com propriedades satisfatórias para uso como embalagens de alimentos lipídicos, sendo que a concentração dos sais de norbixina e o pH de formulação dos filmes mostraram-se fatores determinantes em suas propriedades tecnológicas.

Palavras-chaves: carotenoides, embalagem, urucum, antioxidante, óleo.

ABSTRACT

Currently, the food and beverage sector is mainly responsible for the production of plastic packaging. The justifications for the excessive use of packaging by the sector are the practicality and maintenance of the physical-chemical, microbiological and sensorial qualities of the food during the logistic processes involved along the production chain. In addition, the lack of environmental awareness results in the disposal of these packaging in the environment that cause its accumulation with pollution of soils, water, and reduction of local biodiversity. As an alternative to the use of plastic packaging derived from petroleum, the present work aimed to develop a biodegradable packaging from sodium alginate as a polymer, glycerol as a plasticizer, calcium chloride as a complexing agent, and carotenoid norbixin as an antioxidant. Firstly, the work evaluated the stability of the norbixin salts during the process of production of the films by the casting method. The proposed process produced bioactive films with 85% of norbixin salts after formulation and drying, considering the losses by leaching during the complexation process by immersion in calcium chloride solution and the losses by thermal degradation. All bioactive films were yellow-red. The increasing of norbixin concentration increased the color intensity, the UV-visible light barrier, and the water vapor. However, at high concentrations resulted in a prooxidant activity during the storage of sunflower oil. Then, the carotenoid concentrations were reduced, and the films were characterized for their physical-chemical, thermal and mechanical properties. The film containing 0.03% norbixin salts (g norbixin salts / g sodium alginate) showed antioxidant capacity in the storage of sunflower oil under accelerated conditions for up to 6 days. During the preparation of the films, was noticed that it was possible to obtain films having different properties according to the solubilizing pH of the sodium alginate. Thus, when the pigment was incorporated in a solution with pH 11, films with a higher release of the norbixin salts were obtained for lipid media and better antioxidant capacity. Finally, the results indicated that it is possible to obtain biodegradable films of sodium alginate with satisfactory properties for use as the packaging of lipid foods, and the concentration of norbixin salts and the formulation pH of the films showed determinant factors in their properties technological developments.

Keywords: carotenoids;, packing; anatto; antioxidant; oil.

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INTRODUÇÃO

O setor de alimentos é o maior consumidor de embalagens plásticas. Aproximadamente 50 % das embalagens produzidas no mundo são destinadas a este setor e ao incluir o setor de bebidas, este valor aumenta para 69 %. O alto consumo é justificado pela praticidade, conveniência e baixo custo de aquisição destas embalagens (MANALILI; DORADO; OTTERDIJK, 2014).

A grande quantidade de embalagens produzidas a partir de polímeros sintéticos pode representar um problema ao meio ambiente. Tais embalagens sintéticas, como plásticos, não são degradadas a curto e médio prazo, o que resulta em seu acúmulo no ecossistema quando não são devidamente recicladas. Como consequência, pode ocorrer uma redução da biodiversidade local pela contaminação tóxica dos solos e águas.

Além de ser o maior setor consumidor de plásticos, estimasse que o setor de alimentos, aproximadamente, desperdiça um terço dos alimentos e seus recursos produzidos no mundo por ano. Uma das principais razões destas perdas estão relacionadas a utilização de embalagens inadequadas durantes os processos de distribuição e armazenamento que favorecem reações de deterioração que reduzem a vida de prateleira dos produtos e a aceitação dos varejistas e consumidores finais, sendo os produtos descartados antes mesmo de serem adquiridos pelos consumidores (FAO/ONU, 2011).

Diante do exposto, pesquisadores vêm investigando materiais alternativos produzidos a partir de compostos como polissacarídeos, proteínas e lipídeos para a produção de embalagens biodegradáveis que possam prevenir a deterioração dos alimentos e reduzir o consumo de plásticos (NORAJIT; KIM; RYU, 2010; SALGADO et al., 2015).

Os filmes biodegradáveis apresentam propriedades deficientes quando comparados às embalagens sintéticas (ZHANG et al., 2017). Contudo, tais filmes podem apresentar propriedades interessantes para o setor de alimentos como: barreira seletiva à água e ao oxigênio, proteção de compostos lipídicos, atividade antioxidante, entre outras que implicam no prolongamento da qualidade sensorial e microbiológica dos alimentos (ASSIS et al., 2018; BENAVIDES; VILLALOBOS-CARVAJAL; REYES, 2012; CLOUTIER; MANTOVANI; ROSEI, 2015; GRAZIA et al., 2016; JUCK; NEETOO; CHEN, 2010; OUSSALAH et al., 2006;

PAGNO et al., 2016; STOLL et al., 2017, 2018; SUNG et al., 2013; WANG; AUTY; KERRY, 2010).

Os filmes obtidos a partir de polissacarídeos têm ganhado cada vez mais destaque devido ao seu baixo custo em relação aos demais biopolímeros, por serem facilmente maleáveis e transparentes (NORAJIT; KIM; RYU, 2010). Estes filmes, geralmente, apresentam propriedade de barreira efetiva contra permeabilidade de oxigênio e dióxido de carbono. Porém, apresentam baixa resistência à água, devido seu caráter hidrofílico (CAZON et al., 2016; SIRVIÖ et al., 2014). Dentre os polissacarídeos, o alginato de sódio é um composto poliurônico de cadeia linear, encontrado nas paredes celulares de algas marinhas marrons (ALBOOFETILEH et al., 2014). O alginato de sódio apresenta grande afinidade com moléculas de água e é adequado para produção de filmes biodegradáveis, pois apresenta alta biodegrabilidade, não é tóxico e é biocompatível com o organismo humano (ABDOLLAHI et al., 2013; ALBOOFETILEH et al., 2014; LI; NIE, 2016; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016). Além disto, o alginato de sódio pode formar ligações cruzadas com íons de cálcio, formando complexos insolúveis em águas. Essa capacidade de complexação com íons de cálcio vêm sendo usado como uma técnica de impermeabilização de filmes de alginato de sódio para obtenção de filmes resistentes à água e rupturas físicas (BIERHALZ et al., 2014; FENG et al., 2017; OLIVAS; BARBOSA-CÁNOVAS, 2008; PONGJANYAKUL; PUTTIPIPATKHACHORN, 2007; STRACCIA et al., 2015).

Contudo, atualmente, não há registros de filmes de alginato de sódio complexados com íons de cálcio com propriedades antioxidantes capazes de retardar oxidação de alimentos. Uma alterativa para propiciar capacidade antioxidante aos filmes e portanto, prevenir a oxidação, é a incorporação de carotenoides a biopolímeros, uma vez que diversas pesquisas têm relatado tal propriedades em filmes produzidos pela técnica de *casting* (ASSIS et al., 2017, 2018; PAGNO et al., 2016; STOLL et al., 2017, 2018).

Dentre os diversos carotenoides, a norbixina pode representar um pigmento promissor para o desenvolvimento de filmes biodegradáveis, uma vez que apresenta solubilidade em água, sendo possível sua incorporação durante o preparo de filmes de alginato de sódio dispersos em água. Este carotenoide é um ácido carboxílico derivado do processo de saponificação da bixina, sendo este último o principal pigmento encontrado no pericarpo das sementes do urucum (*Bixa orellana* L.). Estima-se que 80% dos pigmentos encontrados na semente do urucum é bixina na forma *cis*, enquanto que o restante são a *trans*-bixina, *cis*-norbixina, *trans*-norbixina, além de compostos derivativos e de degradação (PRESTON; RICKARD, 1980).

A bixina apresenta uma estrutura de ligações insaturadas duplas conjugadas com dois grupos de ácidos carboxílicos nas extremidades que proporciona seu caráter lipofílico. Enquanto que a norbixina apresenta apenas um grupo de ácido carboxílico na extremidade (TAHAM; CABRAL; BARROZO, 2015). A estrutura insaturada de ambos proporciona uma coloração entre o amarelo e o vermelho, além de alta reatividade. Muitos estudos têm relatado o potencial destes carotenoides como antioxidantes (PAGNO et al., 2016; RIOS; ANTUNES; BIANCHI, 2009; RIOS; MERCADANTE; BORSARELLI, 2007), uma vez que podem inativar agentes sintetizadores, oxigênio single e radicais livres (LOBATO et al., 2015; RIOS; MERCADANTE; BORSARELLI, 2007).

Os sais de norbixina, obtidos a partir da reação da bixina em meio álcali (hidróxido de potássio), podem representar uma opção de carotenoide a ser incorporado em matrizes poliméricas, como o alginato de sódio, para o desenvolvimento de filmes biodegradáveis que apresentem propriedades satisfatórias para o uso como embalagens e possam contribuir tanto para a preservação do meio ambiente, como para a manutenção da qualidade dos alimentos.

OBJETIVOS

OBJETIVO GERAL

Desenvolver filmes biodegradáveis de alginato de sódio complexados com íons de cálcio incorporados com sais de norbixina para uso como embalagens para alimentos.

OBJETIVOS ESPECÍFICOS

- Estabelecer um processo e formulação capaz de produzir filmes com textura e aparências similares a embalagens plásticas comerciais
- Determinar uma faixa de concentração ideal de norbixina para incorporação em filmes de alginato de sódio sem comprometer as propriedades físico-química dos filmes
- Avaliar a compatibilidade entre norbixina e alginato de sódio na estrutura filmogênica formada
- Validar a estabilidade da norbixina durante o processo de produção de filmes proposto
- Avaliar a capacidade antioxidante dos filmes e determinar uma faixa de concentração ideal do carotenoide para incorporação
- Caracterizar os filmes de alginato de sódio complexados com íons de cálcio e incorporados com sais de norbixina
- Determinar a concentração de norbixina de maior valor tecnológico dos filmes desenvolvidos
- Avaliar o efeito do pH de solubilização da formulação de alginato de sódio no processo de produção dos filmes sobre suas propriedades
- Avaliar a liberação do pigmento em diferentes meios simulantes de alimentos
- Determinar o melhor tipo de alimento para aplicação da embalagem biodegradável bioativa.

CAPITULO 1 – REVISÃO BIBLIOGRÁFICA

1.1. ALGINATO DE SÓDIO

1.1.1. Obtenção

O alginato é encontrado no material celular das paredes de algas marinhas marrons (ELÇIN, 1995; FUNAMI et al., 2009) complexados com minerais de cálcio, potássio e sódio (GOMEZ et al., 2009). Os alginatos comercializados são obtidos principalmente pela extração de algas marinhas marrons da classe *Phaeophyceae* (RAYMENT et al., 2009; RINAUDO, 2014), sendo as espécies mais utilizadas a *Laminaria hypeborea*, a *Macrocytis pyrifera* e a *Ascophyllum nodosum* (GOMEZ et al., 2009).

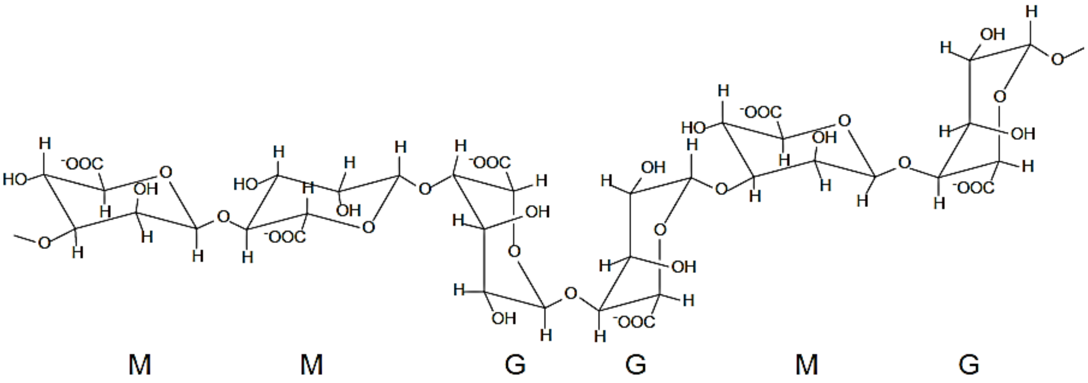
De acordo com Gomez et al. (2009) e Larsen et al. (2003), o alginato é obtido por um processo de extração das algas, na qual estas sofrem uma trituração em soluções alcalinas para liberar esse polissacarídeo das paredes celulares para uma solução aquosa. Após a extração, é formada uma solução espessa, denominada biomassa, a qual é diluída em grandes proporções de água para solubilizar o composto e facilitar a sua separação de resíduos insolúveis através da filtração. O extrato resultante é acidificado em um pH de 4,0 pela adição de ácidos, que hidrolisam o alginato complexado com minerais, formando o ácido algínico. O ácido algínico é neutralizado pela adição de hidróxido de sódio, a um pH próximo de 11,0, para formação do alginato de sódio. O sal obtido é filtrado e passa por um processo de purificação, no qual é solubilizado em água, seguida pela adição de etanol, que irá diminuir sua solubilidade, precipitando-o. O processo de extração, hidrólise, neutralização e purificação ocorre várias vezes conforme o rendimento pretendido, de acordo com o interesse comercial (LARSEN et al., 2003).

Apesar da principal fonte ser de algas marinhas marrons, o alginato também pode ser produzido por bactérias *Pseudomonas* e *Azotobactérias*, as quais utilizam fontes de carbono para sua síntese. Os alginatos obtidos pela *Pseudomonas* apresentam baixa concentração de monômeros de ácido α-L-gulurônico, enquanto que os alginatos obtidos por *Azotobactérias* possuem uma quantidade considerável desses monômeros (LEE; MOONEY, 2012).

1.1.2. Propriedades Físico-químicas

Os alginatos são polissacarídeos heteroglicanos de cadeias lineares, contendo monômeros de ácidos (1,4) $\rightarrow$ β-D-manurônico e (1,4)$\rightarrow$ α-L-gulurônico. Esses monômeros podem ser encontrados em arranjos sequenciais compostos apenas de ácidos D-manurônicos ou ácidos L-gulurônicos, denominados de blocos MM e GG, respectivamente. Também é possível encontrar sequencias alternadas, os blocos heterogêneos MG (BIERHALZ et al., 2014; COMAPOSADA et al., 2015). A Figura 1 ilustra a possível distribuição destes rearranjos ao longo da cadeia polimérica de alginato de sódio.

Figura 1 - Estrutura molecular do alginato de sódio.



Fonte: (LARSEN et al., 2003)

Os blocos MM são ligados entre sí por ligações glicosídicas 1→ 4 do tipo equatorial-equatorial e apresentam uma conformação achatada. Os blocos GG são ligados por uma ligação glicosídica 1→4 do tipo equatorial-axial, a qual proporciona cavidades propicias a formar ligações com íons polivalentes; esta região também é denominada de zona de junção, já que é capaz de formar até 9 ligações com um único íon de cálcio (LARSEN et al., 2003). Devido as diferentes conformações, cada segmento apresenta diferentes propriedades. Os blocos GG são estruturas mais compactas e rígidas, devido ao maior impedimento estérico proporcionado pela ligação axial, e representa a fração insolúvel do alginato de sódio. Os blocos MM representam a fração mais solúvel, por apresentar grupos carboxílicos mais propícios a sofrem ligações com moléculas de água (BIERHALZ et al., 2014; CHAN; LEE; HENG, 2006; FUNAMI et al., 2009; OLIVAS; BARBOSA-CÁNOVAS, 2008).

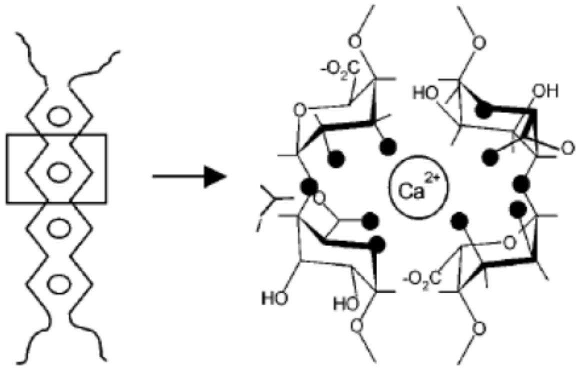
Os alginatos são classificados comercialmente quanto à relação de blocos MM e GG presentes, pois alginatos ricos de blocos GG apresentam habilidades de formar géis mais resistentes e porosos, enquanto os filmes ricos em blocos MM formam géis com viscosidade menores e macios (LARSEN et al., 2003; SHANKAR; WANG; RHIM, 2016). Uma das propriedades interessantes do alginato é de formar géis estruturados na presença de íons divalentes, na qual está relacionada com a cavidade proporcionada pelos blocos GG (TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016).

O alginato de sódio é solúvel em água fria e quente (FUNAMI et al., 2009; GOMEZ et al., 2009; LILING et al., 2016). Quando disperso em água, sua cadeia sofre uma hidratação, sendo assim considerado um hidrocoloide, já que aumenta a viscosidade de soluções aquosas a medida que aumenta sua concentração (YANG; CHEN; FANG, 2009). A capacidade do alginato aumentar a viscosidade está relacionada com o grande número de grupos carboxílicos ao longo da cadeia, capazes de interagir com moléculas de água. Porém, a solubilidade do alginato de sódio pode ser influenciada pelo o pH da solução. As dispersões de alginato de sódio são estáveis em uma grande faixa de pH de 5,0 a 10,0, mas em pH ácidos, entre 3,0 e 4,5, ocorre a protonação dos grupos carboxílicos, diminuindo sua solubilidade, e em pH menor que 3,0 ocorre sua precipitação. Em meios alcalinos, acima de 11, ocorre a despolimerização da cadeia, o que compromete as propriedades de interesse do alginato (HAMBLETON et al., 2009; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016; YANG; CHEN; FANG, 2009).

Além de atuar como espessantes de soluções aquosas e modulador de textura em alimentos, o alginato apresenta a peculiaridade de formar géis com boas propriedades mecânicas e termoresistentes na presença de íons divalentes. Quando o alginato de sódio é solubilizado em uma solução aquosa contendo íons divalentes ocorre a formação de ligações cruzadas entre íons e cadeias poliméricas. À medida que ocorrem as ligações cruzadas, há transição de alginato solúvel para alginato insolúvel, ou seja, forma-se um gel insolúvel e termoresistentes na faixa de 0 a 100 °C (FUNAMI et al., 2009; GALUS; LENART, 2013; LIU et al., 2016).

Os íons divalentes interagem preferencialmente pelos os blocos GG, pois estes apresentam uma conformação axial com cavidades propícias para formação de ligações cruzadas com os íons, ao contrário dos blocos MM. Os cátions bivalentes capazes de formar essas ligações cruzadas são Pb, Cu, Cd, Ba, Sr, Ca e Zn, em ordem decrescente de afinidade. Na área alimentícia, biotecnológica e farmacêutica utilizam-se os íons de cálcio por serem seguros à saúde humana. As ligações cruzadas entre duas cadeias poliméricas de alginato apresentam uma estrutura similar à de uma caixa de ovos (Figura 2). Os íons de cálcio podem formar até nove ligações coordenadas com os oxigênios presente nas cadeias, fato que diminui a solubilidade do alginato (BIERHALZ et al., 2014; GALUS; LENART, 2013; HARPER et al., 2013; LI et al., 2016).

Figura 2- Ligações cruzadas promovidas pelos íons de cálcio entre as cadeias poliméricas de bloco GG do alginato de sódio.

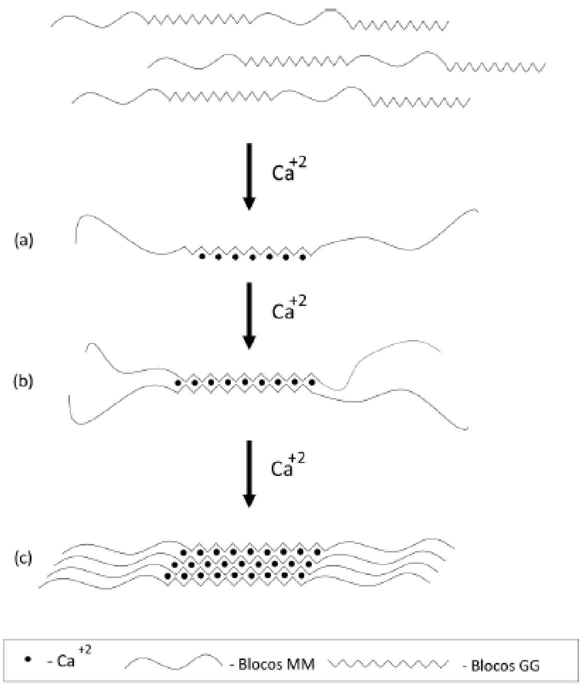


Fonte (DELADINO et al., 2008)

O processo de formação de géis estruturados ocorre em três etapas sucessivas: monocomplexação, dimerização e associação lateral ou multimerização. A Figura 3 ilustra o processo de formação dos géis a partir de íons de cálcio. A primeira etapa ocorre logo após a adição de íons de cálcio em dispersões aquosas contendo alginato de sódio, na qual os íons ligam-se preferencialmente a uma cadeia polimérica de blocos GG, os monocomplexos. Após a formação dos monocomplexos, ocorre a diminuição da repulsão eletrostática entre as cadeias de diferentes polímeros, causando suas aproximações. A aproximação entre as cadeias, proporciona a formação de dímeros pelas ligações cruzadas do tipo “caixa de ovo” entre duas cadeias distintas, com os íons de cálcio entre os interstícios das cadeias, o que forma redes tridimensionais compactas e rígidas. À medida que a reação se desenvolve, a repulsão eletrostática tende a diminuir e formar mais dímeros, até a associação lateral com demais dímeros, com a formação de multímeros (FUNAMI et al., 2009; LI et al., 2016).

Funami et al. (2009), ao estudarem as etapas de gelificação e formação dos géis de alginato, observaram as etapas descritas acima. Em seu estudo, avaliaram a relação entre a concentração de íons cálcio e blocos GG para o início de cada etapa. A etapa de nucleação ou monocomplexação iniciou-se com razões Ca/GG menores de 0,25, considerada uma etapa percussora da etapa seguinte. Em valores maiores de 0,25 e menores que 0,55, os autores observaram a formação das ligações cruzadas entre duas cadeias, os dímeros, aumentando a coordenação molecular das estruturas. Em valores maiores de 0,55, ocorre a formação de multímeros via associação lateral entre demais dímeros. Isto proporciona uma estrutura mais flexível devido ao alongamento.

Figura 3 - Processo de formação da rede tridimensional - (a) etapa de monocomplexação (b) formação de dímeros (c) associação lateral.



Ao compreender a influência dos blocos GG na formação de géis estruturados pela adição de íons de cálcio, torna-se evidente que quanto maior a quantidade de blocos GG na composição de um alginato, mais propenso será a formação de géis rígidos e estáveis. Diversos estudos vêm sendo realizados para melhor entender os fatores que podem influenciar nas propriedades destes géis. Os principais fatores são as concentrações de alginato de sódio, relação de blocos GG e MM, massa molecular, pH, temperatura, presença de sais, tipo e concentração de íons (FERNANDEZ FARRES; DOUAIRE; NORTON, 2013; LI et al., 2016; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016). A composição química e sequência de cada monômeros depende da fonte biológica, estado de maturação da alga e as condições do processo de extração (GALUS; LENART, 2013; HAMBLETON et al., 2009).

Quanto à influência da concentração de cálcio, Liu et al. (2016) verificaram que o aumento da concentração do cálcio aumentava a rigidez do gel. O mesmo resultado foi evidenciado por Farrez et al. (2013). A concentração de cálcio também influencia na porosidade dos géis. De acordo com Jang et al. (2014), quanto maior a concentração de íons maior é as porosidades dos géis produzidos, que apresentaram estruturas mais compactas, porém quebradiças. Jang et al. (2014) e Liu et al. (2016)(LI et al., 2016) observaram que a medida que se aumenta a concentração de íons de cálcio, a associação lateral é favorecida, formando fibrilas (fibras visíveis microscopicamente).

A forma que íon de cálcio é disponibilizado durante a etapa de gelificação proporciona diferentes resultados quanto as propriedades finais dos géis. O processo de gelificação pode ser realizado de dois métodos, o externo e o interno (FUNAMI et al., 2009).

O método externo utiliza íons de cálcio hidrossolúveis, prontos para reagir com os blocos GG (AMARA et al., 2016; GENSKOWSKY et al., 2015; LEE; MOONEY, 2012). Neste processo os íons de cálcio solúveis entram em contato com as dispersões de alginato de sódio. As cadeias de alginato de sódio ao entrar em contato com os íons de cálcio, sofrem o processo de gelificação instantânea; os íons de cálcio difundem de forma heterogênea e irregular ao longo da dispersão de alginato, para formar géis fortes não homogêneos (LEONG et al., 2016; PAQUES et al., 2014). A difusão dos íons de cálcio ocorre através dos espaços intersticiais das cadeias poliméricas. A grande desvantagem deste método é a formação de géis aglomerados, heterógenos e periféricos (não desejável), pois a velocidade de difusão dos íons de cálcio em uma solução aquosa é maior do que em soluções que já sofreram o processo de gelificação. Dessa forma, o processo de gelificação diminui à medida que se formam os géis, devido à resistência mássica imposta pelo processo de gelificação (LEE; MOONEY, 2012; PAQUES et al., 2014).

O método interno utiliza fontes de cálcio parcialmente solúveis ou insolúveis, como por exemplo carbonato de cálcio e sulfato de cálcio. A fonte de cálcio de baixa solubilidade é misturada com a solução de alginato de sódio em condições neutras de forma que não ocorra o processo de gelificação antecipadamente. A dispersão sofre uma redução do pH, o que resulta no aumento da solubilidade dos íons de cálcio e promove sua dissociação interna e homogênea ao longo das cadeias de alginato de sódio. Este processo é o mais adequado pois a gelificação pode ser controlada de forma mais lenta e proporciona a formação de géis mais uniformes (CHAN; LEE; HENG, 2006; LEE; MOONEY, 2012; LEONG et al., 2016; STRACCIA et al., 2015; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016).

1.1.3. Filmes biodegradáveis de alginato de sódio obtidos pelo método de *casting*

Os alginatos de sódio também são capazes de formar filmes com ótimas propriedades mecânicas. Os filmes de alginato apresentam propriedades mecânicas e de barreira efetiva a gases como oxigênio e dióxido de carbono, o que pode prevenir e reduzir os processos de deterioração dos alimentos (ABDOLLAHI et al., 2013; CAZON et al., 2016; OUSSALAH et al., 2006). Contudo, a característica dos filmes depende do processo utilizado na sua produção. O método de produção por fundição, do inglês *casting*, é o método mais utilizado nos estudos para produção de filmes de alginato. As etapas de formação de filmes pelo método de *casting* ocorre basicamente em 5 etapas: preparado das dispersões do alginato de sódio em água, desaeração das dispersões, moldagem, secagem e padronização (HAN; ARISTIPPOS, 2005; JANJARASSKUL; KROCHTA, 2010; LILING et al., 2016; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016).

No processo de dispersão o alginato de sódio é disperso e dissolvido em água destilada entre 0 a 100 °C, através de agitação mecânica. Nesta etapa, as cadeias poliméricas do alginato são dispersas no meio aquoso e ocorre sua hidratação. Após a agitação, as dispersões de alginato são deixadas sob repouso para a etapa de desaeração, aonde microbolhas formadas durante a agitação são retiradas ao longo do repouso, as quais podem interferir nas propriedades finais dos filmes (ABDOLLAHI et al., 2013; ACEVEDO-FANI et al., 2015; FENG et al., 2017; LILING et al., 2016; MARCOS et al., 2016; WANG; SHANKAR; RHIM, 2017a).

Após a desaeração das dispersões, as mesmas são despejadas em moldes (etapa de moldagem – *casting*). Os moldes contendo as dispersões são submetidos à etapa de secagem. A etapa de secagem consiste basicamente na eliminação da água, ou solvente utilizado, das matrizes poliméricas hidratadas, com evaporação primeiramente das moléculas menos ligadas. As moléculas de água fortemente ligadas às cadeias, atuam como plastificantes, o que aumenta a flexibilidade das cadeias poliméricas e diminui a força iônica entre as cadeias (GAO; POLLET; AVÉROUS, 2017a; JANJARASSKUL; KROCHTA, 2010; PAULA et al., 2015). Após a formação dos filmes, os mesmos são destacados dos moldes e armazenados durante 48 horas (ou mais) em dessecadores com umidade controlada de aproximadamente 58% UR. Essa etapa denominada padronização, serve para equalizar a umidade dos filmes ao longo da matriz polimérica, estabilizando as ligações formadas do polímero com moléculas de água e outros constituintes.

A adição de íons divalentes em filmes é realizada em etapas exclusivas ou em combinação. Estes podem ser adicionados durante a etapa de dispersão do alginato de sódio (ABDOLLAHI et al., 2013; ACEVEDO-FANI et al., 2015; ASHIKIN; WONG; LAW, 2010; CHEN et al., 2016a; HAMBLETON et al., 2009; NG; TAN, 2015; REZVANIAN; AMIN; NG, 2016; WANG; SHANKAR; RHIM, 2017), após a secagem dos filmes via imersão dos filmes em soluções contendo íons divalentes (BIERHALZ; DA SILVA; KIECKBUSCH, 2012; HARPER et al., 2013; LILING et al., 2016; MARCOS et al., 2016; OUSSALAH et al., 2006; SIRVIÖ et al., 2014), ou via aspersão de solução de íons divalentes na superfície dos filmes (CONCHA-MEYER et al., 2011; ZACTITI; KIECKBUSCH, 2006).

A forma como os íons de cálcio é disponibilizada influência nas propriedades finais dos filmes resultante. Podem ser obtidos filmes complexados com íons de cálcio através do método externo e/ou interno com propriedades distintas (CHAN; LEE; HENG, 2006; STRACCIA et al., 2015; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016).

Os filmes produzidos pelo método externo de gelificação, devido à disponibilização imediata de íons bivalentes, têm o processo de gelificação instantâneo, resultando em um processo de gelificação mais rápido que a difusão dos íons ao longo da matriz filmogênica, obtendo regiões periféricas com gelificação intensificadas e indesejáveis na superfície mais externa dos filmes. Filmes obtidos pelo método externo, são mais compactos e apresentam melhor controle na liberação de compostos de interesse, com uma liberação mais lenta que os formados pelo métodos interno (BENAVIDES; VILLALOBOS-CARVAJAL; REYES, 2012; CAZON et al., 2016; ZHANG et al., 2017).

Filmes produzidos através do método interno de gelificação produz uma matriz gelificada homogênea e menos compacta que os filmes obtidos pelo método externo. O método de produção utiliza carbonato de cálcio como fonte de cálcio insolúvel e glucono-delta-lactona (GDL) como acidificante. Primeiramente, são formadas as dispersões contendo alginato de sódio e cálcio insolúvel. Depois, são adicionados GDL nas dispersões, o qual acidificam lentamente a dispersão, aumentando a solubilização dos íons de cálcio, iniciando o processo de gelificação entre o interstício do polímero (LEONG et al., 2016; STRACCIA et al., 2015; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016).

PROPRIEDADES DE FILMES SEM COMPLEXAÇÃO

Os filmes de alginato de sódio produzidos pelo método de fundição, sem a adição de íons bivalentes, assumem características hidrofílicas do polissacarídeo, o que resulta em filmes com alta solubilidade em água, baixa propriedade mecânicas e alta permeabilidade a vapor de água. Outra característica destes filmes é que são altamente sensíveis em ambientes úmidos, resultando na absorção de umidade em sua matriz polimérica, com redução das propriedades mecânicas e o aumento da permeabilidade a vapor de água e gases (REZVANIAN; AMIN; NG, 2016).

Há uma grande variabilidade nas formulações e condições de processo durante a formação destes filmes, mas basicamente consiste na dissolução de alginato de sódio e glicerol em água, auxiliado por uma agitação intensa, seguidos pelas as etapas de desaeração, moldagem, secagem e padronização. A Tabela 1 apresenta algumas formulações e condições de processos para formação de filmes de alginato de sódio sem complexão.

Os filmes de alginato sem uma etapa de complexação apresentam propriedades mecânicas baixas e altas permeabilidades a vapor de água. Para melhorar as propriedades mecânicas e de permeabilidade dos filmes de alginato de sódio, alguns estudos têm adicionado outros polímeros, como compostos de celulose (WANG; SHANKAR; RHIM, 2017), pectina (REZVANIAN; AMIN; NG, 2016), gelatina (NG; TAN, 2015) e goma carragena (PAULA et al., 2015). Também vem sendo adicionados compostos com propriedades antimicrobianas como óleos essenciais (ACEVEDO-FANI et al., 2015; HAMBLETON et al., 2009) e partículas de prata (SHANKAR; WANG; RHIM, 2016) que embora sejam adicionadas com outros propósitos, melhoraram a permeabilidade e propriedades mecânicas dos filmes de alginato.

Tabela 1 - Formulações e condições de processo de filmes de alginato de sódio sem complexação

Formulação		Etapa de dispersão	Etapa de secagem	Fonte
Alginato de sódio	Glicerol			
1 % (p/v)	0,25 % (p/v)	Agitação a 70 °C durante 30 min	Secagem a 40 °C durante 24 h	(ABDOLLAHI et al., 2013)
3% (p/v)	2 % (p/v)	Agitação de 17.500 rpm a 70 °C durante 2 min	Secagem a 25 °C durante 24 h	(ACEVEDO-FANI et al., 2015)*
2% (p/v)	Sem plastificante	Agitação de 300 rpm a 25 °C durante 2 horas	Secagem a 40, 60 e 80 °C durante 48 h	(ASHIKIN; WONG; LAW, 2010)
2,5% (p/v)	Sem plastificante	Agitação de 450 rpm a 50 °C durante 30 min	Secagem a 70 °C durante 40 min	(CHEN et al., 2016b)
6 % (p/v)	1,8 % (p/v)	Agitação de 24.000 rpm a 60 °C durante 15 min	Secagem a 30 °C durante 8 h	(HAMBLETON et al., 2009)
6 % (p/v)	2 % (p/v)	Não informado	Secagem a 30 °C durante 24 h	(NG; TAN, 2015)*
2,5 % (p/v)	0,75 % (p/v)	Agitação de 15.000 rpm a 25 °C durante 15 min	Secagem a 24 °C durante 24 h	(PAULA et al., 2015)*
5 % (p/v)	2,5 % (p/v)	Agitação de 1.000 rpm a 40 °C durante 2 h	Secagem a 45 °C durante 48 h	(REZVANIAN; AMIN; NG, 2016)*
2% (p/v)	0,8 % (p/v)	Agitação a 90 °C durante 30 min	Secagem a 22 °C durante 24 h	(WANG; SHANKAR; RHIM, 2017)*
0,8 % (p/v)	0,24 % (p/v)	Agitação a 90 °C durante 30 min	Secagem a 23 °C durante 48 h	(SHANKAR; WANG; RHIM, 2016)*

* Filmes os quais foram utilizados outros polímeros ou compostos em sua composição.

Pongjanyakul & Puttipipatkhachorn (2007), ao desenvolver filmes de alginato de sódio adicionados de silicato de magnésio de alumínio, avaliaram a influência da adição de glicerina ou PEG-400, plastificantes, nas propriedades finais dos filmes. Como resultado, a incorporação de glicerina e PEG-400 (nas concentrações entre 10 a 30% v/v) causou uma diminuição da permeabilidade de vapor de água. A tensão de ruptura de filmes diminuiu com o aumento de plastificante glicerol e PEG-400, sendo que o glicerol afetou mais a tensão do que o PEG-400, resultado esperado com o uso de plastificantes, sendo que o glicerol apresentou o melhor efeito. Tal resultado está relacionado com o fato do tamanho da molécula de glicerol ser menor que a de PEG-400, sendo, portanto, melhor incorporada ao longa das cadeias de alginato de sódio.

Valero et al. (2013) avaliaram os efeitos da aplicação de filmes comestíveis de alginato de sódio, sem complexação, sobre as qualidades de 4 tipos de ameixas durante as condições de pós colheitas. Os autores verificaram que a aplicação dos filmes comestíveis reduziu significativamente a produção de etileno, a perda de peso (perda de água), sendo a concentração de 3 % (p/p) de alginato de sódio com efeitos maiores. Os filmes apresentaram permeabilidade alta de oxigênio e baixa de dióxido de carbono.

Ng & Tan (2015), ao desenvolverem filmes monocamada e bicamada a partir de alginato de sódio e gelatina, avaliaram a influência da adição de diferentes tipos de plastificantes e a adição de hidroxitirosol como antioxidante. Os autores relataram que a incorporação do composto antioxidante resultou na redução da resistência a ruptura e aumento da capacidade de alongamento. A resistência à tração é um reflexo da intensidade das forças intermoleculares entre as cadeias poliméricas, desta forma, sugeriu-se que o composto antioxidante poderia ter formado ligações de hidrogênio e covalentes com as cadeias laterais dos polímeros, diminuindo a força intermolecular dos filmes. Também verificaram que os filmes bicamada apresentaram melhor controle de liberação do composto em questão, tendo uma liberação mais lenta que os filmes monocamada. Além disto, os filmes monocamada de alginato e pectina em 24 horas sofreram uma hidratação de 82 %, o qual foi justificado pelo grande número de grupos carboxílicos do alginato.

Acevedo-fani et al. (2015), ao produzirem filmes a base de alginato de sódio, sem complexação, contendo óleo essenciais com propriedades antimicrobianas de tomilho, limão-grama e sálvia, verificaram que os filmes de alginato não apresentaram capacidade antimicrobianas. Dos resultados obtidos, o mais interessante foi uma redução da permeabilidade a vapor de água com a adição dos óleos, o qual indicou que a presença de compostos lipofílicos na matriz polimérica melhora as propriedades de barreira de água devido ao preenchimento das porosidades dos filmes.

Paula et al. (2015) ao avaliarem as propriedades físicas e de barreira de filmes compostos de k-carragena, l-carragena e alginato de sódio, em variadas proporções, produzidos através do método de *casting*, sem etapas de complexação, verificaram que a adição de k-carragena resultou em filmes com caráter mais hidrofílicos, com permeabilidade a vapor de água superiores aos demais filmes com ausência de k-carragena. Estes filmes, também apresentaram maior transparência e menor resistência a tração.

Shankar et al. (2016) desenvolveram filmes compostos de alginato de sódio e partículas de prata, com propriedades antimicrobianas, através do método de *casting* sem complexação. De forma geral, a resistência mecânica e as propriedades de barreira ao vapor de água da película de alginato foram melhoradas após a adição de compósitos de prata. As partículas de prata incluídas nos filmes apresentaram atividade antibacteriana contra duas bactérias patogênicas representativas, *E. coli* e *L. monocytogenes*.

Wang et al. (2017) avaliaram a influência da adição de fibras de celulose e nanocompósitos de celulose em filmes de alginato de sódio, produzidos pelo método de *casting*, sem complexação. Entre os resultados encontrados, destaca-se o aumento da permeabilidade pelo o aumento da concentração de celulose, tanto em fibra quanto em nanopartículas. A adição de fibra de celulose diminuiu as propriedades mecânicas e à medida que aumentava a concentração, menor foi resistência à tração e o alongamento. Os filmes com nanopartículas apresentaram maior resistência à tração em comparação aos filmes compostos apenas de alginato de sódio.

PROPRIEDADES DE FILMES COM COMPLEXAÇÃO DURANTE A DISPERSÃO DE ALGINATO

O método de formação de filmes de alginato de sódio com complexação durante a dispersão, diferentemente do método descrito anteriormente possui uma etapa de complexação, ou seja, a adição de íons de cálcio na etapa de dispersão de alginato de sódio.

O processo de complexação aumenta as propriedades mecânicas e diminui a permeabilidade de água, as quais são características de grande interesse para embalagens de alimentos (MARCOS et al., 2016; OLIVAS; BARBOSA-CÁNOVAS, 2008; ZHANG et al., 2017). Trabalhar com formulações diferentes de plastificantes, agentes complexantes e alginato de sódio permite o desenvolvimento de inúmeras embalagens com propriedades distintas. Ao contrário do método sem complexação, não há necessidade de incorporação de outros polímeros em sua formulação para melhorar propriedades como resistência física ou permeabilidade a vapor da água, pois a adição de íons pode-se modular tais propriedades. A adição de outros polímeros pode, inclusive, interferir no processo de complexação e reduzir propriedades mecânicas e aumentar o caráter hidrofílico dos filmes, como a pectina (GALUS; LENART, 2013). Bierhalz et al. (2012) verificaram que o aumento da concentração de pectina em filmes de alginato reduziu a permeabilidade a vapor da agua e aumentou a resistência física dos filmes.

O método de complexação durante a dispersão é o único que permite a produção de filmes pelo processo de gelificação interna, o qual é descrito por Benavides et al. (2012). Primeiramente, deve-se dissolver o alginato de sódio e glicerol em água, seguidos pela adição de íons de carbonato de cálcio (insolúvel). Após a mistura da dispersão é adicionado glucono-delta-lactona (GDL) que vai se dissociando na solução aquosa, com a liberação de íons H^+ que acidificam o meio. Consequentemente, o carbonato de cálcio sofre um aumento gradual da sua solubilidade, com uma lenta liberação dos íons de cálcio ao longo da dispersão de alginato, o que promove um processo de gelificação lento, controlado e homogêneo.

Chan et al. (2006) avaliaram a influência dos processos de gelificação interno e externo nas propriedades finais de filmes de alginato de sódio, produzidos pelo método de *casting*, complexados durante a dissolução do polissacarídeo. Os autores verificaram que filmes produzidos através do método externo apresentaram resistência à tração maior que filmes obtidos pelo método interno. Filmes sem complexação foram solubilizados em água a 37 °C em minutos, enquanto filmes complexados com carbonato de cálcio foram praticamente insolúveis em água, menos que 8 % (p/p) em um período de 6 horas. Também foi verificado que com o aumento da concentração de íons de cálcio, menor foi o índice de hidratação dos filmes. Os filmes obtidos pelo método externo apresentaram capacidade de hidratação maior que os do método interno, assim como a permeabilidade ao vapor de água. Deste modo, os filmes externos apresentaram uma impermeabilização intensificada na superfície e menos reticulada no interior, sendo mais intensificada com o aumento da concentração de íons.

PROPRIEDADES DE FILMES COM COMPLEXAÇÃO POR IMERSÃO OU ASPERSÃO

O método de complexação por imersão dos filmes ou aspersão de íons bivalentes é método mais utilizado. Por imersão o processo impermeabiliza de forma mais eficiente os filmes de alginato de sódio, atuando mais na superfície do filme do que ao longo de toda a matriz polimérica, como um processo de selagem. O processo de aspersão, atua da mesma forma que o de imersão, porém não garante uma impermeabilização homogênea em toda a superfície do filme. A desvantagem destes métodos está no fato de que após a etapa de complexação, seja por imersão ou por aspersão de uma solução aquosa contendo íons de cálcio, ocorre a umidificação da superfície dos filmes e obrigatoriamente a necessidade de mais uma etapa de secagem.

O método inicia-se pela dissolução de alginato de sódio e glicerol em água destilada, com concentrações na faixa de 0,4 a 10 % (p/p) e 0,6 a 3 % (p/p), respectivamente. A temperatura de dissolução varia de 25 a 70 °C, com agitação entre 500 a 11.000 rpm, durante tempos entre 30 a 60 minutos. Após a dissolução, recomenda-se manter a dispersão em repouso até a eliminação das microbolhas incorporadas durante o processo de agitação. Posteriormente, a dispersão é vertida em moldes, muitas vezes placas de petri, com gramaturas entre 0,003 a 5,264 g/cm², na qual irá determinar o tempo de secagem, espessura e propriedades finais dos filmes. As placas são submetidas a temperaturas de secagem entre 25 a 60 °C, entre períodos de 3 horas a 5 dias. Após secos, os filmes são destacados das placas e submetidos ao processo de complexação por imersão ou aspersão em soluções aquosas de cloretos de cálcio com concentrações entre 2 a 10 % (p/v), durante tempos de imersão entre 30 segundos a 60 minutos. Por aspersão, ocorre apenas uma borrificação na superfície. Em alguns casos, os filmes ainda são levados para uma secagem em estufa em temperaturas moderadas ou então são secos naturalmente nas condições ambiente. Após a secagem, os filmes devem passar por uma etapa de estabilização, na qual são armazenados em recipientes com umidade relativa controlada entre 25 a 52 %, para estabilização da umidade e peso dos filmes (BIERHALZ; DA SILVA; KIECKBUSCH, 2012; HARPER et al., 2013; LILING et al., 2016; MARCOS et al., 2016; NESIC et al., 2016; OLIVAS; BARBOSA-CÁNOVAS, 2008; OUSSALAH et al., 2006; SILVA; BIERHALZ; KIECKBUSCH, 2009; SIRVIÖ et al., 2014; ZACTITI; KIECKBUSCH, 2006).

A incorporação de outros compostos pelo método de complexação durante a dissolução de alginato pode interferir na interação alginato-cálcio, o que impede a formação de uma estrutura mais coesa, conforme já relatado (GALUS; LENART, 2013). Porém, estudos mostram que a interferência não é significativa pelo método de imersão, ou seja, não comprometem as propriedades gerais do filme, podendo até melhorar algumas propriedades de interesse, como acontece com a incorporação de pectina (BIERHALZ; DA SILVA; KIECKBUSCH, 2012; NESIC et al., 2016) e compostos de celulose (SIRVIÖ et al., 2014). O uso de proteínas, como gelatina, *whey protein* e proteína de soja, podem melhorar propriedades de alongamento, porém podem reduzir significativamente a resistência a ruptura dos filmes (HARPER et al., 2013).

Oussalah et al. (2006) desenvolveram filmes biodegradáveis a partir de alginato de sódio adicionados de óleos essenciais de canela, orégano e salgado, com estabilizantes poli-prolactona, produzidos pelo método de *casting* e complexados após secagem por meio de imersão em solução de cloreto de cálcio. Verificou-se que filmes com concentrações de 2 % (p/p) de cloreto de cálcio, apresentaram melhor atividade antimicrobiana dos compostos bioativos (óleos essenciais) do que filmes com concentrações de 20 % (p/p) de cloreto de cálcio. A razão foi justificada com possíveis interações de íons de cálcio sobre os compostos e obstrução física destes.

Olivas & Barbosa (2008) avaliariam a influência do tipo de plastificantes (PEG-8.000, frutose, glicerol e sorbitol), proporções de blocos M/G e algumas condições de processo nas propriedades de filmes biodegradáveis de alginato de sódio, produzidos pelo método de *casting*, complexados após a secagem por meio de imersão. Os autores verificaram que os filmes produzidos com alginato de sódio contendo alta concentração de blocos G (M/G de 0,45) apresentaram permeabilidade a vapor de água três vezes menores que filmes com baixa concentração de blocos G (M/G de 1,5). Além disto, observaram que quanto maior o tempo de imersão, menores eram as permeabilidades a vapor de água de todos filmes; porém, entre 1 e 3 minutos de imersão, os resultados de permeabilidades não mostraram diferenças significativas. Também, observaram que filmes expostos em condições de alta umidade relativa (UR ~100 %) apresentaram permeabilidades a vapor de água maiores que filmes em condições de baixa humidade relativa (UR ~76%), mostrando a ação plastificante da água. Quanto ao tipo de plastificante, filmes com plastificante frutose ou sorbitol apresentaram menores permeabilidade de vapor de água quando comparado com filmes com PEG-8.000 ou glicerol. Porém, filmes com PEG-8.000 apresentaram resistência a tração e alongamento menores que os filmes com outros plastificantes. Como resultado final, o plastificante glicerol mostrou melhor compatibilidade com o alginato de sódio, assim como os melhores resultados.

Silva et al. (2009a) desenvolveram filmes de alginato de sódio com duas etapas de complexação, uma durante a etapa de dispersão e outra após a secagem por meio de imersão com cloreto de cálcio. Verificaram que a adição e aumento da concentração de plastificante na solução da etapa de imersão com cloreto de cálcio resultou no aumento da umidade e solubilidade dos filmes de alginato de sódio. Também observaram que o aumento da concentração de íons de cálcio na solução de imersão resultou em filmes com espessuras maiores, devido à maior presença de sólidos e massa. O uso de glicerol na etapa de imersão, em concentrações abaixo de 3 % (p/p) resultou em filmes frágeis e com concentrações acima de 10 % (p/p) observou-se a separação de fases do plastificante.

Nojarit et al. (2010) desenvolveram filmes biodegradáveis de alginato de sódio, adicionados de óleos essenciais de ginseng, produzidos pelo método de *casting*, com complexação por meio de imersão com cloreto de cálcio. A adição de óleos essenciais aumentou a solubilidade de filmes de alginato de cálcio. Também, verificou-se que o aumento da adição de glicerol aumentou a solubilidade dos filmes devido seu perfil hidrofílico.

Bierhalz et al. (2012) avaliaram a influência da adição de antimicrobiano natamicina nas características físicas, mecânicas e de barreira de filmes biodegradáveis de alginato de sódio e pectina, produzidos pelo método de *casting*, com duas etapas de complexação com cloreto de cálcio, uma durante a dissolução de alginato de sódio e uma segunda por imersão. Também foi avaliado a proporção de pectina e alginato de sódio na composição dos filmes sob as respectivas propriedades dos filmes. Verificou-se que os filmes com maiores concentrações de alginato de sódio apresentaram maiores resistências a tração e alongamento e baixas propriedades de barreira a vapor de água, quando comparados com os filmes com concentrações mais elevadas de pectina. Os autores também verificaram que a adição de natamicina aumentou a solubilidade dos filmes biodegradáveis de alginato de sódio e pectina, e a razão disto foi relacionada com a alta solubilidade e liberação do antimicrobiano na água. Além disto, observaram que filmes com a adição de natamicina, apresentaram caráter mais hidrofílico, com maior permeabilidade a vapor de água, resistência a tração e alongamento quando comparados com os filmes sem adição do antimicrobiano.

Bierhalz et al. (2014) avaliaram o efeito do tipo de cátion bivalente, cálcio e bário sobre as propriedades físicas de filmes biodegradáveis de alginato de sódio carregados de natamicina, produzidos pelo método de *casting*, com duas etapas de complexação: uma durante a dissolução do alginato e outra por imersão após a secagem dos filmes. Os pesquisadores relataram que filmes complexados superficialmente com bário, obtiveram solubilidade, umidade, índice de inchamento menores que filmes complexados superficialmente com íons de cálcio. Além disto, também verificaram que estes primeiros filmes tinham a permeabilidade a vapor de oxigênio, opacidade e rugosidade maiores que os complexados superficialmente com íons de cálcio. Tais resultados, foram justificados pelo fato de que filmes com íons bário apresentaram ligações cruzadas mais fracas que com íons de cálcio, o que proporcionou aos filmes uma menor resistência a tração. Filmes complexados com cálcio apresentaram uma liberação lenta e controlada de natamicina devido à maior reticulação da matriz filmogênica.

Sirvio et al. (2014) desenvolveram filmes biodegradáveis de alginato de sódio, por método de *casting* e complexados por meio de imersão em solução de cloreto de cálcio, adicionados de compostos celuliticos e derivados. Primeiramente, o alginato foi lentamente dissolvido em água deionizada e depois foi realizada a adição de celulose ou derivados. Os autores avaliaram a sinergia da celulose e do alginato. Quanto menores foram as fibras celulósicas, maior foi a interação entre as matrizes poliméricas de ambos os polissacarídeos, o que resultou na melhoria da resistência física dos filmes.

Comaposada et al. (2015) avaliaram as propriedades físicas de filmes de alginato de sódio complexados com cloreto de cálcio durante a dissolução de alginato de sódio e imersão, para aplicação em linhas de produção de co-extrusão destinados a produtos cárneos, como a salsicha. Foi verificado que o aumento da concentração de alginato aumentou significativamente a resistência a tração, resistência a perfuração e o alongamento.

Shao et al. (2015) desenvolveram filmes com celulose e alginato de sódio adicionados de sulfadiazina de prata como agente antimicrobiano. Os filmes foram produzidos através do método de *casting* com duas etapas de complexação com cloreto de cálcio, uma durante a dissolução dos biopolímeros e a segunda após secagem por meio de imersão. Durante o experimento observaram que a capacidade de inchamento resultante de filmes complexados, por meio de imersão em soluções de cloreto de cálcio com pH de 2,5 foi menor quando comparado aos resultados de filmes imergidos em soluções de 11,5. Os filmes mostraram alta capacidade de inchamento e hidratação das cadeias em meios alcalinos. Tal resultado foi relacionado com o fato do alginato de sódio ter suas cadeias de grupos carboxílicos desprotonadas em meios alcalinos, o qual resulta no aumento da repulsão eletrostática que pode interferir na dilatação das cadeias poliméricas. Assim, como as moléculas de prata também possuem uma carga negativa, observou-se que sua concentração estava diretamente relacionada com o aumento da capacidade de inchamento, ou seja, com o aumento da concentração de prata houve um aumento da carga negativa, o que promoveu maior repulsão eletrostática e assim maior inchamento da cadeia.

Marcos et al. (2016a) avaliaram a influência das condições de processo nas propriedades de filmes de alginato de sódio, produzidos pelo método de *casting*, complexados após a secagem por meio de imersão com cloreto de cálcio. Os autores relataram que a redução do pH na solução de imersão com cálcio reduziu as propriedades mecânicas dos filmes significativamente, sendo o pH mais adequado o de 6,7. Observaram que quanto menor a temperatura do banho de imersão, maiores foram as propriedades mecânicas dos filmes. Tal fato ocorreu devido a redução da reatividade dos íons de cálcio durante o processo de ligação cruzada, o que reduz de forma mais controlada as reações, produzindo filmes mais homogêneos. Verificou-se também que o aumento da concentração de íons de cálcio na solução de imersão, produziu filmes com menor umidade. A adição de sais influenciou nas propriedades finais dos filmes, com redução de suas propriedades mecânicas. O sódio atua como quelante, competindo com o cálcio pelas ligações dentro das zonas de junções, o que produz géis mais fracos.

Nesic et al. (2016) desenvolveram filmes biodegradáveis de pectina com adição de alginato de sódio e plastificante, complexados com íons de zinco após secagem por meio de imersão. Os autores avaliaram as propriedades físicas, mecânicas e de barreira destes filmes. Verificou-se que a adição de plastificante aumentou a permeabilidade a vapor de água e diminuiu a resistência a tração dos filmes não complexados e complexados com íons de zinco, sendo este efeito mais significativo em filmes sem cálcio. Também foi observado que a adição de alginato de sódio melhorou as propriedades mecânicas, apresentando uma sinergia com a pectina.

Liling et al. (2016) avaliaram as propriedades físicas, mecânicas e de barreira de filmes de alginato de sódio, produzidos pelo método de *casting*, complexados com diferentes tipos de íons, cloreto de cálcio, zinco e magnésio. Verificou-se que o processo de complexação aumentou a resistência a tração e o alongamento dos filmes, sendo até 40 % maior nos filmes com cloreto de cálcio. Também, verificou-se que o aumentou da concentração de cálcio resultou na redução da permeabilidade a vapor de água e de luz transmissível, porém aumentou significativamente a resistência a tração até a concentração de 2 % (p/p). O aumento no tempo de 1 para 6 minutos de imersão, resultou no aumento da permeabilidade a vapor de água e propriedades mecânicas. Todos os resultados foram relacionados com o aumento da reticulação das matrizes poliméricas com os íons de cálcio.

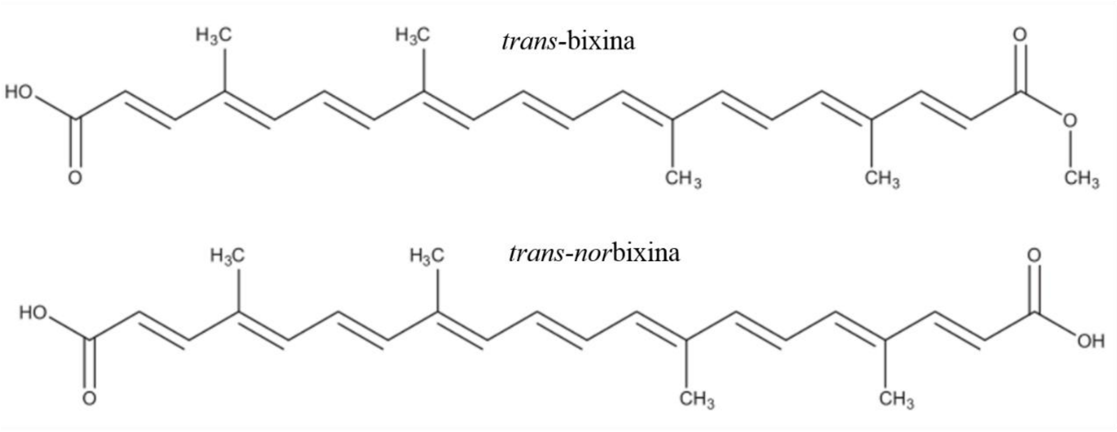
Zhang et al. (2017) avaliaram a concentração de glicerol entre 10 e 80 % (p/p), cloreto de cálcio de 1 a 10 % (p/p), temperatura de secagem entre 30 a 70 °C e tempo de imersão entre 1 a 10 minutos na resistência a água de filmes de alginato de sódio com goma gelana, produzidos pelo método de *casting*, complexados por meio de imersão com cloreto de cálcio. Verificou-se que a concentração de íons de cálcio reduziu significativamente a permeabilidade a vapor de água e índice de inchamento dos filmes, até uma concentração de 5 % (p/p), resultando em estruturas mais reticuladas e ordenadas. Os filmes produzidos com tempo de imersão entre 5 e 8 minutos obtiveram as melhores propriedades mecânicas e sem diferença significativa entre si. O efeito da temperatura de secagem reduziu o índice de inchamento e a permeabilidade a vapor de agua, acima disto houve um aumento significativo, sendo a temperatura mais indicada a de 50 °C. Altas temperaturas resulta em processos excessivos de evaporação, formando estruturas desordenadas, o que aumenta o índice de inchamento e a permeabilidade ao vapor de agua. Estes parâmetros foram maiores nos filmes com maiores concentrações de goma gelana em comparação aos de alginato de sódio. Ao verificar a sinergia entre os dois polímeros, verificou-se que a mistura de ambos reduziu as propriedades mecânicas e de barreira, o que indicou baixa compatibilidade entre si.

1.2. CAROTENOIDES DE URUCUM

O urucuzeiro (*Bixa orellana* L.) é um arbusto de pequeno porte (altura inferior a 6 metros) e com diâmetro de caule entre 15 e 20 cm. Apresenta uma copa ampla, abundantemente ramificada, sendo que o tronco é reto e de casca lisa de coloração cinza clara. A planta nativa do clima tropical é encontrada no Brasil e apresenta como fruto o urucum que apresenta aparência de cápsulas em formato ovoide, com sementes ricas em pigmentos de coloração amarela e vermelha, denominados carotenoides (SILVA, 2007). Os principais pigmentos presentes no pericarpo são os apocarotenoides bixina (metil hidrogênio 9’-*cis*-6,6*-diapocaroteno-6,6*-dioato) e norbixina (9-*cis*-6,6’-diapocaroteno-6,6’-dioato). (LANCASTER; LAWRENCE, 1996; SCOTTER, 1995). O processo de síntese da bixina ainda é desconhecido, contudo acredita-se que sua estrutura é derivada da estrutura inicial do β-caroteno, devido a sua similaridade e coloração (GARCIA et al., 2012).

A bixina apresenta uma estrutura com uma cadeia central de nove ligações duplas conjugadas, com um grupo éster metílico e ácido carboxílico nas extremidades. Essa estrutura possibilita a formação de isômeros *cis* e *trans*. Aproximadamente 80 % dos pigmentos presentes no pericarpo são *cis*-bixina e o restante *trans*-bixina, *cis*-norbixina, *trans*-norbixina e compostos de degradação. A formação de isômeros *trans* (Figura 4) é favorecida quando a *cis*-bixina é aquecida ou expostas a solventes orgânicos (LANCASTER; LAWRENCE, 1996). Quando o grupo éster metílico da bixina é hidrolisado, ocorre a formação de uma estrutura mais polar e solúvel em água, denominada de norbixina (LANCASTER; LAWRENCE, 1996; PRESTON; RICKARD, 1980).

Figura 4 - Estrutura química da trans-bixina e trans-norbixina.



Fonte (SCHEPPINGEN; BOOGERS; DUCHATEAU, 2012).

A ingestão de bixina e norbixina não são tóxicas e carcinogênicas ao metabolismo humano. De acordo com o JECFA, os pigmentos de urucum são considerados seguros, com um IDA de 12 mg/kg de peso corpóreo para bixina e 0,60 mg/kg de peso corpóreo para a norbixina (FAO/WHO, 2005). A bixina e norbixina são metabolizada principalmente no fígado e absorvidas no intestino (HAGIWARA et al., 2003), as quais apresentam uma biodisponibilidade de aproximadamente 60 e 30 %, respectivamente (KOPEC; FAILLA, 2017; POLAR-CABRERA et al., 2010). Além disto, a ingestão destes pigmentos podem prevenir doenças cardiovasculares pela regulação da concentração de colesterol na corrente sanguínea (SANTOS et al., 2002) e pela redução de reações oxidativas às células do organismo humano (OUYANG et al., 2008; RIOS; ANTUNES; BIANCHI, 2009).

1.2.1. Processos de extração dos pigmentos do urucum

O processo de extração da bixina, a partir das sementes de urucum, pode ser realizado de várias formas, como sua imersão em óleo vegetal quente, diluição em água alcalina ou extração com solventes (BALASWAMY et al., 2006; TAHAM; CABRAL; BARROZO, 2015). O método de suspensão em óleo é o método mais tradicional, pois consiste apenas na imersão das sementes de urucum em óleos quentes, formando uma suspensão avermelhada (TAHAM; CABRAL; BARROZO, 2015). De acordo com Scotter (1995), esse método obtém extratos em que o principal pigmento é a bixina, a qual é lipossolúvel. O aquecimento do óleo com as sementes permite maior extração e solubilidade do pigmento, com a obtenção de uma suspensão com concentrações entre 0,2 e 1,5 % de bixina. Além de um extrato de baixa concentração, este método proporciona a degradação dos pigmentos e isomerização da forma *cis*-bixina para *trans*-bixina (RAO; SATYANARAYANA; RAO, 2002; SILVA, 2007).

A utilização de solventes orgânicos no processo de extração de bixina e norbixina é muito utilizado devido à alta solubilidade dos pigmentos nestes solventes, o que proporciona rendimentos e concentrações superiores aos demais métodos. O rendimento deste método pode chegar até 3,6% (g bixina / g sementes) (GARCIA et al., 2012). Porém, os extratos secos costumam apresentar resíduos tóxicos dos solventes. Devido a esse problema, tecnologias emergentes como extração supercrítica surgem como solução (BARROZO; SANTOS; CUNHA, 2013; STOLL et al., 2018; TAHAM; CABRAL; BARROZO, 2015).

Rios e Mercadante (2004) extraíram a bixina de sementes de urucum através de três lavagens sucessivas com os solventes orgânicos hexano, metanol e acetato de etila. Os dois primeiros foram utilizados para remover compostos polares de baixo interesse presentes nas sementes. Após a limpeza das sementes, as mesmas foram lixiviadas com acetato de etila para extração especifica da bixina, sendo que o solvente foi eliminado por rotaevaporação. Esse método mostrou-se ser de fácil replicação e produziu um extrato com 94 % de *cis*-bixina. Além disto, os autores realizaram posteriormente o processo de cristalização para obter cristais de bixina puros. O extrato seco foi diluído em diclorometano, com adição lenta de etanol absoluto na proporção de 1:4, sendo a solução submetida a banho de gelo para conduzir a precipitação da bixina em cristais pela redução repentina de solubilidade do carotenoide. O processo obteve cristais de bixina com 98 % de pureza.

Industrialmente, o processo mais utilizado é a extração aquosa alcalina com hidróxidos de sódio ou potássio, pelo seu baixo custo. As sementes de urucum são imersas em soluções aquosos em pH acima de 10,0, o que permite a conversão da bixina em norbixina, e sua extração sucessivamente. Os extratos obtidos por esse método apresentam maior concentração de norbixina do que de bixina. Como são utilizados hidróxidos de sódio ou potássio, após a hidrólise e formação da norbixina, ocorre a neutralização da sua forma ácida para a forma de sal. Como o método utiliza água como solvente, o processo é fácil de ser conduzido e apresenta baixo custo (SILVA, 2007; TAHAM; CABRAL; BARROZO, 2015).

O método de extração alcalina resulta em extratos com baixa concentração de pigmento e baixa pureza. Como alternativa pode-se extrair a bixina das sementes através do método que utiliza solventes orgânicos, para obter extratos mais concentrados e puros do carotenoide e, posteriormente, converter o pigmento para norbixina pelo processo de saponificação. De acordo com Rios e Mercadante (2004), o processo pode ser conduzido a partir de cristais de bixina pela diluição dos mesmos em uma solução de 10 % de hidróxido de potássio metanólica, sendo esta mantida em repouso durante 12 horas em condições ambientes e protegidas da luz. A exposição da bixina nas condições alcalinas promove a hidrólise do grupo carboxílico formando a norbixina. Após um processo extenso de lavagem e purificação, a norbixina pode ser obtida por esse método, alcançando uma pureza de 93 %. Contudo, a pureza dos cristais de norbixina depende diretamente da pureza dos cristais de bixina inicialmente utilizados.

Silva e Nachtigall (2009) avaliaram os fatores que influenciam o processo de saponificação da bixina para norbixina. O estudo imergiu sementes de urucum diretamente em soluções aquosas de hidróxido de sódio com concentrações até 10 % (p/v). Os autores relataram que o aumento da concentração de hidróxido de potássio aumentou a taxa de conversão de bixina para norbixina. De acordo com os autores, ao aumentar a concentração de 1 % para 5% de hidróxido de sódio o conteúdo de norbixina aumentou 3 vezes. No mesmo estudo os autores verificaram que em baixas concentrações de 0,6%, os extratos apresentavam um perfil de 97,1% de bixina e 0% de norbixina. Ao aumentar a concentração para 5%, o perfil do extrato foi de 9,2 % de bixina e 84,3 % de norbixina.

1.2.2. Propriedades dos pigmentos de urucum

A propriedade mais explorada dos pigmentos de urucum é sua capacidade de colorir alimentos. Desta forma, a utilização destes carotenoides durante muitos anos foi aplicada em alimentos lipídicos, como manteiga e queijo, com o objetivo de desenvolver colorações mais atrativas (LANCASTER; LAWRENCE, 1996). Como os pigmentos de urucum são capazes de proporcionar o desenvolvimento de cores entre amarelo e vermelho, esses pigmentos mostraram ser um potencial substituto do corante sintético Tartrazina, o qual pode apresentar toxicidade, sendo proibido em alguns países (TAHAM; CABRAL; BARROZO, 2015). A baixa solubilidade em água, alta reatividade, absorção de raios de luz, capacidade antioxidante e pode corante são relacionada a estrutura insaturada característica de carotenoides (PAGNO et al., 2016). A escolha de qual pigmento (bixina ou norbixina) utilizar depende da matriz alimentícia em questão. Quando são alimentos lipídicos, como manteiga e margarinas, recomenda-se a utilização de extratos ricos em bixina para promover maior solubilização e dispersão do pigmento ao longo da matriz alimentícia. Quando são alimentos com alto teor de umidade, como queijos e bebidas, recomenda-se a utilização da norbixina por ser hidrossolúvel (FERREIRA et al., 1999; PRESTON; RICKARD, 1980; SILVA; NACHTIGALL, 2009).

Satyanarayana et at. (2010) avaliaram a influência do método de extração de pigmentos de urucum nas propriedades colorimétricas dos extratos. Os extratos obtidos por sistemas aquosos alcalinos e suspensão em óleos aquecidos apresentaram colorações localizadas entre os quadrantes amarelo (b^*) e vermelho (a^*) da escala CIE-LAB. A bixina em óleo apresentou o parâmetro amarelo (b^*) maior que o parâmetro (a^*) em todas concentrações analisadas, indicando a predominância de uma coloração amarela. A norbixina dissolvida em água apresentou com coloração mais clara (L^* maior) com o valor a^* e b^* 3 e 4 vezes maior que a bixina, respectivamente; indicando um poder colorante superior que a bixina. O estudo também verificou que ao aumentar a concentração de norbixina ocorreu o aumento de a^* e queda do b^*, desenvolvendo um extrato de cor avermelhada.

A solubilidade da bixina e norbixina são diferentes devido a polaridade de suas estruturas. A bixina apresenta um caráter lipofílico devido a estrutura apolar, com isto sua aplicação se destina em alimentos lipídicos. De acordo com Zhang e Zhong (2013), a bixina apresenta uma solubilidade em água próximo de 0,0034 mg/mL. Enquanto que etanol sua solubilidade aumenta para 0,12 mg/mL. Com um ácido carboxílico em sua estrutura, a norbixina apresenta uma polaridade maior, o que possibilita sua solubilidade parcial em água alcalina (LANCASTER; LAWRENCE, 1996; RIOS; MERCADANTE, 2004). Devido à isomerização, a solubilidade dos isômeros apresenta sutis diferenças entrei si. De acordo com Garcia et al. (2012), os isômeros *cis* apresentam uma estrutura mais polar e com isto menor solubilidade em óleos e solventes orgânicos que os isômeros *trans*.

Ao se tratar de norbixina, sua solubilidade em água é influenciada pelo pH da solução aquosa. Em pH menores que 3,5, o pigmento precipita, devendo, assim, utilizar um emulsificante para melhorar a estabilidade do corante. Sua precipitação é devido a desprotonação da sua estrutura central, o que equilibra as cargas eletrostáticas da molécula e reduz sua afinidade pelas moléculas de água (SILVA, 2007). Zhang e Zhong (2013) verificaram que em pH baixos (ácidos) as soluções ficam mais avermelhadas e turvas devido a precipitação do pigmento. Quando solubilizados em pH altos (alcalinos), as soluções desenvolvem uma coloração amarelo intenso.

Rao et al. (2002) adicionaram extratos de norbixina em bebidas acidificadas prontas para consumo. Em água destilada neutra foi possível dissolver 224 mg de norbixina/ L. Enquanto que a máxima concentração dissolvida de norbixina nas bebidas acidificadas (pH 2,89) prontas para consumo foi de 30,6 mg/L. De acordo com os autores, em pH ácidos ocorre a desprotonação e redução da polaridade da norbixina, o que causa sua precipitação devido a solubilidade 7 vezes menor que em uma solução aquosa de pH neutro.

1.2.3. Capacidade antioxidante da bixina e norbixina

A utilização de carotenoides em alimentos começou primeiramente como o objetivo de desenvolver cores especificas em alimentos. Ao longo dos anos, descobriu-se a capacidade antioxidantes benéficas para a saúde humana, como também para alimentos (KIOKIAS; OREOPOULOU, 2006).

Na área de alimentos, os lipídeos sofrem oxidação da sua estrutura insaturada quando são expostos ao oxigênio, luz, e altas temperaturas, normais em processos industriais. Quanto mais insaturado são os óleos, mais propensos são para a formação de radicais livres. A bixina e norbixina, assim como outros carotenoides, apresentam capacidade antioxidantes devido a sua estrutura insaturada altamente reativa, que pode reagir preferencialmente com outras moléculas reativas presentes em alimentos ou em sistemas celulares. Os carotenoides têm uma estrutura com energia de ativação menor que os lipídeos ou demais células, sofrendo reações mais rápido e preferencialmente por radicais livres, peróxidos, oxigênio singlete ou qualquer outra molécula excitada por agente sintetizadores (LOBATO et al., 2015; OUYANG et al., 2008; RIOS; MERCADANTE; BORSARELLI, 2007).

De acordo com Kiokias e Oreopoulou (2006), os carotenoides protegem outras moléculas insaturadas e células contra processos de oxidação. Os carotenoides podem reagir preferencialmente com moléculas reativas, como radicais livre, evitando que estes oxidassem lipídeos e células. Outra forma que os carotenoides atuam como antioxidante é através de inativação de agentes sintetizadores, como a clorofila presentes em óleos. Essas substancias quando expostas à luz, absorvem energia da luz e transmitem a energia para moléculas de oxigênio triplete (atmosférico) tornando mais reativo. Essa molécula de oxigênio poderia oxidar diretamente lipídeos e moléculas de DNA, ou com outras moléculas como radicais livres formando estrutura ainda mais reativas. Além disto, os carotenoides também podem inativar diretamente as moléculas de oxigênio singlete, absorvendo sua energia.

Kiokias e Oreopoulou (2006) avaliaram a formação de dienos conjugados em emulsões contendo extratos de carotenoides (β-caroteno, luteína, bixina e norbixina) quando submetidas a 6 °C durante 4 horas. As emulsões de óleo de girassol (50%), água (49%) e tween 20 (0,5%) e extratos de carotenoides (0,5%) apresentaram concentrações de dienos menores que a emulsão sem carotenoides. As emulsões de bixina e norbixina formaram 3 vezes menos dienos conjugados que a emulsão sem carotenoide. Ao comparar o desempenho entre os carotenoides, a norbixina e bixina apresentaram os melhores desempenhos antioxidante, seguidos pela luteína e β -caroteno. O desempenho superior foi relacionado maior solubilidade da bixina e norbixina na emulsão preparada, o que permitiu aos pigmentos maior atividade. Nos estágios terminais da oxidação de lipídeos os autores verificaram a formação de aldeídos voláteis como hexanal (40%), 2-heptanal (50%), e 2-6 nonadienal (10%). O estudo verificou que a bixina e reduziu em 70 % a formação de 2-heptenal, reduziu 5 vezes a hexanal e 2 vezes a 2,6-nonadienal.

Rios et al. (2007) quantificaram os níveis de energia da bixina através de calorimetria fotoacústica com auxílio de fotossintetizadores para formação de oxigênio singlete. De acordo com os autores, a bixina triplete apresenta uma energia de ativação (18 kcal/mol) menor e suficiente para inativar moléculas de oxigênio singletes (22,5 kcal/mol) formados por fotossintetizadores, podendo prevenir reações de oxidação em alimentos e células de DNA.

Rios et al. (2009) avaliaram a influência da bixina e licopeno na formação de espécies reativas de oxigênio (ROS) produzidas pela interação cisplatina e DNA humano em um modelo experimental de células. Os ROS (peróxidos e outros radicais livres) apresentam propriedades citotóxicas e nefrotóxicas maléficas a saúde humana. O trabalho verificou que os carotenoides podem prevenir a formação destes compostos em condições *in vitro*. Os resultados encontrados sugerem que o $O_2{}^1$ pode ser extinguido pelo licopeno e pela bixina devido à sua capacidade de doar elétrons, neutralizando-os. O licopeno e a bixina inibiram a formação de superóxido em

90% e 82%, respectivamente. Enquanto que a formação total de ROS reduziu 44% e 42%, respectivamente.

1.2.4. Estabilidade da bixina e norbixina

As propriedades da bixina e norbixina pode ser comprometida quando sua estrutura é degradada, podendo formar compostos de degradação de colorações amarelas (LANCASTER; LAWRENCE, 1996; SCOTTER, 1995). A estrutura de bixina é instável na presença de fatores, tais como oxigênio, sintetizadores e alta temperatura (LOBATO, 2013).

Por exemplo, Scotter (1995) mapeou através do método de CLAE o perfil de compostos de degradação formados durante tratamentos térmicos de extratos de urucum. Em seu estudo, o autor verificou que compostos de degradação com cadeias insaturadas intensificava a formação da coloração amarela nos extratos. Foi também verificada a formação de uma estrutura de 17 carbonos (ácido-4,8,dimetil-tetra-deca-hexaenedoico), m-xileno e traços de tolueno. O autor relata que a estrutura *trans* é mais estável que a estrutura *cis*-bixina, uma vez que configuração eletrônica da molécula *cis* é mais propensa a clivagem em situações de excitação (menor energia de ativação), formando mais compostos de degradação.

Scheppingen et al. (2012) avaliaram os produtos de decomposição da norbixina em condições de branqueamento em água e em uma matriz de soro de leite. Na água, vários aldeídos e cetonas insaturados de ácidos carboxílicos foram identificados com UPLC-UV / MS e técnicas de espectrometria de massa de alta resolução. Os autores identificaram 5 compostos de degradação derivados da estrutura inicial da norbixina. Quanto menor a estrutura central menor o comprimento de onda para identificar o composto, uma vez que para um menor número de ligações duplas, menor é a energia absorvida pela estrutura.

Balaswamy et al. (2006) avaliaram a estabilidade da bixina quando armazenadas em resinas obtidos pelo próprio pericarpo das sementes de urucum ou em pó. Para a bixina em resina após 1 ano armazenada no escuro e 5 °C houve degradação de 1%. Ao aumentar a temperatura para 25 °C, ocorreu o aumento da degradação para 8 %. Quando exposta a luz e 25 °C a bixina em resina degradou 13 %. A bixina em pó armazenada a 25 °C e a luz apresentou perda de aproximadamente 60%, quase 5 vezes mais que a resina. De acordo com os autores, ocorreu uma interação entre a resina e as ligações insaturadas da bixina, o que propiciou uma proteção em torno da estrutura insaturada do carotenoide. Além de mostraram que o armazenamento da bixina em resina proporciona mais estabilidade da estrutura, ficou evidente que o aumento da temperatura resultou em um aumento na degradação da bixina de 7 %, no caso da resina.

Rao et al. (2002) avaliaram a estabilidade de extratos hidrossolúveis de urucum - ricos em norbixina - em bebidas acidificadas prontas para servir armazenadas em diferentes condições durante 5 meses. Os autores verificaram que os pigmentos secos armazenados a 7 ° C sem luz após 150 dias degradaram apenas 5%. Ao diluí-los em água destilada e armazená-los sob as mesmas condições, a degradação aumentou para 22 %. Os autores verificaram que ao armazenar as soluções aquosas em temperatura ambiente (25 °C) ausente de luz e com luz, as soluções apresentaram uma degradação de 82 e 100%, respectivamente. Ao avaliar a estabilidade da norbixina nas bebidas acidificadas (pH 2,89), verificou-se um aumento da degradação em todas as condições, indicando uma instabilidade da estrutura em meios ácidos.

Apesar da sua aplicação ser difundida na indústria de alimentos, os carotenoides de urucum têm suas propriedades facilmente comprometidas quando expostos a altas temperaturas, oxigênio singlete e a luz durante os processos industrias, como já comentado acima. Essa exposição degrada as estruturas dos pigmentos, e com isto há redução das suas propriedades de cor e de atividade antioxidante. Para prevenir essa perda de valor tecnológico, soluções vem sendo encontradas na encapsulação dos compostos em micro e nanoestruturas. Esses processos mostraram aumentar a estabilidade térmica e a exposição a luz dos carotenoides (LOBATO et al., 2013, 2015; TUPUNA et al., 2018; ZHANG; ZHONG, 2013).

1.2.5. Filmes biodegradáveis incorporados com carotenoides do urucum

Além da aplicação em alimentos e bebidas com o intuito de colori-los, os carotenoides de urucum também podem ser incorporados em filmes biodegradáveis para atuar como embalagens com atividade antioxidantes em alimentos lipídicos (PAGNO et al., 2016; STOLL et al., 2018).

Pagno et al. (2016) incorporaram nanocapsulas de bixina em filmes biodegradáveis de amido e glicerol. Os filmes bioativos retardaram o processo oxidativo em óleo de girassol em 13 dias de armazenagem em condições aceleradas de oxidação. De acordo com os autores, o óleo de girassol embalado e armazenado em condições aceleradas em filmes de amido com nanocapsulas de bixina apresentaram menor concentração de peróxido que o filme padrão de amido e embalagem convencional. A bixina atuou como antioxidante contra os radicais livres, oxigênio singlete e agentes sintetizadores presentes no óleo. Além disto, os filmes apresentaram uma proteção contra a luz UV (210 nm) de 41% e luz visível (500 nm) de 22 %. Contudo, a capacidade de retardar a formação de peróxidos no óleo reduziu na maior concentração de bixina, indicando um potencial prooxidante do carotenoide em altas concentrações. A adição das nanocapsulas de bixina desenvolveu filmes com colorações entre amarelo-vermelho, com maior presença do tom amarelo (*b** foi maior que *a**). O aumento da concentração favoreceu a formação de cores mais amarelas (aumento do parâmetro *b**).

Stoll et al. (2018) desenvolveram filmes biodegradáveis de ácido poli láctico incorporados de extratos de bixina. Os filmes apresentaram uma barreira a luz visível (500 nm) de 70 %, podendo proteger alimentos fotossensíveis durante o armazenamento. De acordo com os autores, os filmes apresentaram uma coloração amarela atrativa com parâmetros colorimétricos *b** superior ao *a**. Esta coloração foi relacionada a estrutura insaturada característica da bixina que também proporciona a maior retenção de raios de luz através da estrutura filmogênica. Os autores também verificaram que a bixina fixada na estrutura filmogênica perdeu sua coloração quando submetidos a aquecimento e exposição a luz, indicando a degradação do composto bioativo. Após 18 dias a 25 e 40 °C no escuro, a bixina presente nos filmes degradou 5 e 20 %, respectivamente. Ao expô-los nas mesmas condições, porém na presença de luz, apresentaram uma degradação de 28 e 30 %.

Na literatura consultada não foram encontrados trabalhos publicados com a incorporação de norbixina em filmes biodegradáveis.

CAPITULO 2 – ARTIGOS

Development of biodegradable sodium alginate films incorporated with carotenoid norbixin

Yuri Buratto de Farias, Anderson Kaiszewski Coutinho, Alessandro de Oliveira Rios*

Institute of Food Science and Technology, Federal University of Rio Grande do Sul (UFRGS). Avenue Bento Gonçalves, 9500, Prédio 43.212, Campus do Vale, Porto Alegre, RS, CEP 91501-970, Brazil. Phone: +55 51 3308 9787 *E-mail: alessandro.rios@ufrgs.br

ABSTRACT

In this study was developed a process for the production of biodegradable sodium alginate films incorporated with norbixin at concentrations of 0.05, 0.10 and 0.50% (g norbixin/g alginate). Were also evaluated carotenoid degradation, loss of pigment along the process and barrier properties, colorimetric and antioxidant properties of all the films. The results showed that the proposed process resulted in a low percentage of degradation and leaching losses of norbixin. The increase norbixin concentration increased film opacity on visible and ultraviolet light and increased red-yellow coloration. Also, it was observed that the polymer matrix retarded thermal degradations of norbixin in the respective process. The increase of the norbixin concentration did not influence the water vapor permeability of the films until the concentration of 0.50% and provided the prooxidant activity when used as packaging in the storage of sunflower oil.

Keywords: permeability; opacity; stability; peroxides.

1. Introduction

The large number of packages produced from synthetic polymers represents a problem for the environment, due to they are not easily degraded, which can result in their accumulation in the ecosystem (WANG; AUTY; KERRY, 2010). According to Manalili, Dorado, & Otterdijk (2014), 50% of all packaging produced in the world is consumed by the food sector and by including the beverage sector, this number rise up to 69%. In this way, researchers seek to change synthetic packaging for ecological packaging (CAZON et al., 2016; CHEN et al., 2016a). The use of alternative materials such as polysaccharides, proteins and lipids has been studied for the production of packaging susceptible to degradation under environmental conditions (BIERHALZ et al., 2014; NORAJIT; KIM; RYU, 2010; SALGADO et al., 2015; WANG; AUTY; KERRY, 2010; ZACTITI; KIECKBUSCH, 2006).

Alginate has a straight chain polyuronic polysaccharide found in the cell walls from brown seaweed. This compound is able for the production of biodegradable films and they are not toxic for the human organism. A peculiarity of alginate is its ability to form complexes in the presence of polyvalent cations, such as calcium. This characteristic results in specific and strong interactions that present a molecular conformation called the "egg box"(ALBOOFETILEH et al., 2014; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016).

Sodium alginate films produced by the casting method are physically resistant and have low water resistance. However, alginate films complexed with calcium ions have better mechanical properties, lower water vapor permeability and lower solubility in water (BIERHALZ et al., 2014; PONGJANYAKUL; PUTTIPIPATKHACHORN, 2007; STRACCIA et al., 2015; ZHANG et al., 2017).

In addition, the use of inadequate packaging during the food distribution and storage processes stimulates the degradation process, causing food losses before even bought by final clients (GUSTAVSSON et al., 2011). The present study suggests the incorporation of norbixin as an anti-oxidant agent capable to retarding food oxidation processes during the food storage.

The norbixin is an apocarotenoid (9-*cis*-6,6'-diapocarotene-6,6'-dioic acid) obtained by the process of alkali demethylation of the carotenoid bixin, the main pigment present in the seeds of the fruit of *Bixa orellana*. Norbixin is extracted in its acid form and neutralized to norbixin salts form, which presents partial solubility in water (SILVA; NACHTIGALL, 2009; ZHANG;

ZHONG, 2013). Some studies showed that bixin and norbixin have coloring and antioxidant properties (BALASWAMY et al., 2006; BARBOSA et al., 2012; KIOKIAS; GORDON, 2003; PAGNO et al., 2016; RIOS; ANTUNES; BIANCHI, 2009).

Thus, the present work developed a method for the production of sodium alginate films complexed with calcium and incorporated with norbixin salts. The influence of the incorporation and increase of the norbixin salts concentration in the films on barrier properties, colorimetric properties and antioxidant or pro-oxidant performance were also evaluated through the analysis of the peroxide index in sunflower oil.

2. Material and methods

2.1. Materials

Anatto seeds and sunflower oil were purchased at the local market in Porto Alegre, Rio Grande do Sul, Brazil. The sodium alginate (PA) had a purity of 90% (g / g) (Dynamics® da Química Contemporânea Ltda.) The calcium chloride (PA) was pentahydrate with 99.5% (g / g) purity (Nuclear®). Glycerin (Exodus cientifica®) was used as a plasticizer for films. The entire experiment was conducted using distilled water to avoid cross-contamination with calcium ions.

2.2. Norbixin extraction

The bixin extraction was performed according to Rios and Mercadante (2004), followed by modifications. For each 25 g of annatto seeds, two successive washes were performed with 50 mL of hexane for 15 minutes under magnetic stirring. The seeds were separated by filtration. Thereafter, two successive washes were performed with 50 mL of methanol for 15 minutes under magnetic stirring, and the seeds were again filtered. The bixin presents in the anatto seeds were extracted with two successive ethyl acetate extraction steps with magnetic stirring for 15 min and the extracts were concentrated on a rotary evaporator.

The resulting bixin powder was converted to norbixin by the saponification process as described by Rios and Mercadante (2004), followed by modifications. The dried bixin extract was diluted with 50 mL of 10% (w / v) potassium hydroxide methanol and with ultrasound, The bixin was dissolved in the methanolic solution for 15 minutes and then held for 12 hours for total conversion into norbixin salts.

The obtained norbixin extract was recovered in a separatory funnel with 150 mL of ethyl acetate / ethyl ether / water (1: 1: 1) and acetic acid was added until the extract reached a pH of about 3.5. Then, it was washed with water until the norbixin extract pH reached 6.0 value. After, the extract was separated of the lipophilic phase. Finally, the separated norbixin extract was dried on a rotary evaporator and then stored at -18° C. The purity of the dried norbixin sals was evaluated by High Performance Liquid Chromatography (HPLC) using a wavelength of 450 nm (TUPUNA et al., 2018).

2.3. Preparation of the sodium alginate dispersions

An aqueous solution containing norbixin salts at the concentrations of 0.05, 0.10 and 0.50% (g norbixin/g alginate) was prepared. The pH of the water was previously adjusted to 11.0 with a solution of potassium hydroxide (0.5% w/v) and were added sodium alginate (1% w/w) and glycerin (2% w/w). The solutions were adjusted to pH 7.0 with a solution of acetic acid (0.5% v/v). The solutions of norbixin with alginate were dispersed using a mechanical stirrer (Fisatom, 713D, Brazil) with propellant for 30 minutes at 20 °C with a speed of 1000 rpm. After stirring, the dispersions were kept stand, protected from light for 12 hours for deaeration of microbubbles incorporated into the dispersion.

2.4. Film preparation

The dispersions were poured into petri dishes (0.48 g/cm²) and dried in oven (De Leo) at 55 ° C for 13 hours. After the first drying step, 50 mL of calcium chloride (1% w / v) was poured on each plate for the complexing process for 5 minutes by immersion complexation. After complexation, the remaining surface liquid present in the plates were discarded and the petri dishes were returned to the oven at 55 ° C for more 45 minutes. After drying, the films were detached from the plates and kept in a vat of 58% relative humidity, controlled by sodium bromide, for 48 hours.

2.5. Leaching losses

As the norbixin salts is soluble in water, possible leach losses in the liquid discarded after the complexation process could occur. Aliquots of these liquids were collected in triplicate for each concentration, and the amount of norbixin present in each aliquot was evaluated by High Performance Liquid Chromatography (HPLC) using a wavelength of 450 nm as described by Tupuna et al., (2018), with injection volume adjusted to 100 μL. The result was expressed as a percentage of norbixin leached, calculated according to equation (1):

$$\%Leaching = \frac{\mu g\ of\ Norbixin\ released}{\mu g\ of\ Initial\ Norbixin} \tag{1}$$

2.6. Thermal stability of norbixin

The thermal stability of norbixin was evaluated after the drying process of the films (55 ° C for 13 h and 45 minutes). Each film, immediately after the drying process was placed in a beaker with 20 mL of potassium hydroxide (0.5% w / v) and triturated in a Turrax (IKA® T25 digital) for 5 minutes, 16,000 rpm for pigment extraction. The liquid was vacuum filtered and the sodium alginate was precipitated with absolute ethyl alcohol in the ratio of 1: 3 (extract: ethanol). Subsequently a second filtration was carried out to remove the precipitate for injection in the chromatograph.

Carotenoids were evaluated by High Performance Liquid Chromatography (HPLC) as described by Tupuna et al. (2018). The results were expressed according to equation (2) and performed in triplicate:

$$\%\ \text{degradation compounds} = \frac{\text{total area of degradation compounds}}{\text{total area}} \tag{2}$$

It was possible to identify seven different peaks under the conditions used (total area). The first five peaks were identified as degradation compounds from norbixin isomers (total area of degradation compounds), while the last two peaks were identified as *cis* and *trans* norbixin.

For comparison purposes, the formation of norbixin salts degradation compounds under the same conditions was also evaluated in free water with pH 7.0 and 2% (w / v) glycerol, but without alginate. The solutions were added in petri dishes in the same proportions as the alginate dispersions to avoid interference.

2.7. Films barrier properties

The water vapor permeability was determined gravimetrically at 25 ° C (ASTM, 2000), followed by modifications described by Assis et al., (2017). The analysis was conducted in triplicate and calculated according to equation (3):

$$WVP = \frac{w.L}{\mathrm{A.t.\Delta p}} \tag{3}$$

Where w is the weight in grams of water permeated into the capsule through the film, L is the average film thickness in mm, A is the permeation surface area in m², t is the permeation time in hours, and Δp is the water vapor pressure difference in kPa between the film surfaces.

The transmittance of the films was evaluated by a UV (Shimadzu UV-1800) spectrophotometer at wavelengths of 300 and 600 nm, representing invisible and visible light, respectively. Each film was cut into rectangles and placed in a quartz cuvette. The empty cuvette was used as a blank (PAGNO et al., 2016).

2.8. Films colorimetric characteristics

The colorimetric parameters of the films were measured instrumentally by the colorimeter (Hunter Lab system, model Miniscan XE, USA) using CIELab color parameters. The parameters *L* * (brightness), *a* * (red-green) and *b* * (yellow-blue) were determined.

The color stability of the respective films was evaluated after 3 and 7 days under continuous and controlled light conditions (intensity of 900-1000 Lux, Luxometer VA Instrument, MS6610, China), temperature 30 ° C and relative humidity 70%. In order to better represent colors, the color parameters were synthesized by Corel Draw X6® software.

2.9. Evaluation of the antioxidant or pro-oxidant effect of norbixin films

The oxidative stability of sunflower oil was used to evaluate the antioxidant or pro-oxidant potential of the films as described by Assis et al. (2017), with modifications. The films were folded into envelopes forms, where sunflower oil (13 mL) was added into the films. All samples were stored in a chamber (Tecnal, TE-402, Brazil) under the incidence of light with an intensity of 900-1000 Lux (Luxometer VA Instrument, MS6610, China) at 30 ° C and relative humidity of 70 %. Sunflower oil samples were collected after 3 and 7 days of storage for peroxide index (PI) determination (AOCS, 1993) - standard oil oxidation indicator.

2.10. Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and Tukey's test at a significance level of 5% using Statistica 12.0 software (StatSoft, Inc., Tulsa, USA).

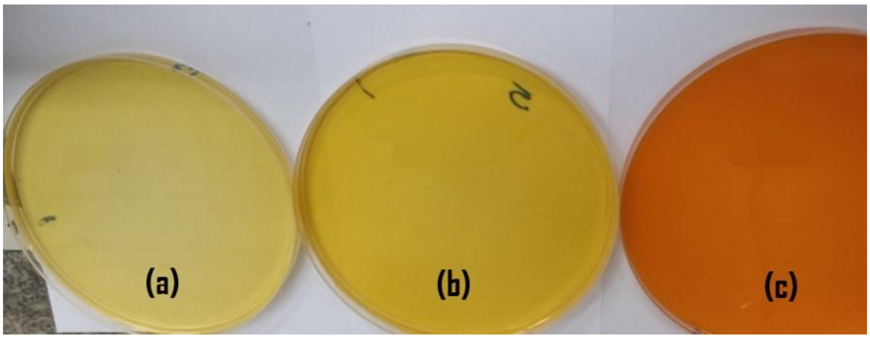
3. Results and discussion

3.1. *Norbixin salts and films*

The norbixin salts obtained in the extraction process from the annatto seeds had a purity of 96.82%. Tupuna et al. (2018) used the same method and obtained a norbixin salts with purity of 100%, higher than the present work. The reason of higher value is the additional step of purification (crystallization from the norbixin extract) used that resulted in pure crystals. In order to objective of the present work the value found (96%) was considered satisfactory, not being necessary a purification step.

The obtained films presented an attractive appearance, with vivid colors and close to the yellow-red (Figure 1). The texture of the films was smooth and with a plastic texture.

Figure 1 - biodegradable sodium alginate films complexed with 1% calcium incorporated with norbixin (a) 0.05%, (b) 0.10% and (c) 0.50% salts.



3.2. *Leaching losses and thermal degradation of norbixin*

During the complexation process it was verified that the solutions used after the immersion developed yellow colorations, an indicative of leaching of the norbixin pigment in the films. Table 1 shows the results of the leaching losses occurring during the process of complexing the films. It was observed that the films with higher concentrations presented the highest release ($p < 0.05$), in absolute terms (μg of Norbixin released/film), a result already expected in order to norbixin solubility in water. While the relative losses (%) was observed that the 0.10% film obtained the highest leaching loss. This result may be associated with some interaction between norbixin and the alginate polymer matrix, which affected the release of the matrix pigment. However, it is important to note that leaching losses were minimal, not influencing the formation process of the films.

Table 1- Leaching loss of norbixin by during the complexation process in the formation of incorporated sodium alginate films of norbixin salts

Film	μg of Norbixin released/film	% Leaching
0.05%	4.15 ± 0.01^{c}	0.98 ± 0.01^{b}
0.10%	12.54 ± 0.63^{b}	1.48 ± 0.07^{a}
0.50%	15.68 ± 0.32^{a}	0.37 ± 0.01^{c}

The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$).

In the present work, the methodology used to norbixin obtained was realized by 3 steps: extraction, hydrolysis and conversion of *cis*-bixin from anatto seeds to the *cis*-norbixin salt. During the hydrolysis process, the formation of the carboxylic acid norbixin occurs, which has liposoluble properties. The conversion process, by the addition of potassium hydroxide, neutralize the acid and occurs salt formation stabilized by potassium, obtaining an alkaline water soluble salt, as reported by other authors (BARBOSA et al., 2012; GARCIA et al., 2012; HAGIWARA et al., 2003; SILVA, 2007). Thus, the salt incorporated in the films presents alkaline water solubility, which influenced the low values of norbixin release in aqueous solution at pH 7.0 used during the process of immersion of the films in calcium chloride solutions.

The *Table 2* shows the percentage amount of degradation compounds formed during the drying process from norbixin.

Table 2- Percentual of degradation compounds of norbixin formed during the drying process.

	% Degradation compounds of Norbixin	
Film	Norbixin film	Free Norbixin
0.05 %	15.02 ± 0.43^a	23.08 ± 2.15^b
0.10%	11.90 ± 1.83^b	24.84 ± 0.47^b
0.50%	5.89 ± 2.14^c	36.37 ± 0.76^a

The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences (p <0.05).

The process of film production proposed in this work formed degradation products of norbixin in the worst situations of 15.02%, for films incorporated with lower amounts of norbixin (0.05%). The increases norbixin concentration significantly reduced the formation of degradation compounds, but more studies should be realize to understand the influence of norbixin concentration on its own degradation.

The unsaturated conjugated structure of the central chain of norbixin are thermosensitive to temperatures higher than 40 °C. Although, some studies showed that bixin and norbixin are more stable than other carotenoids (KIOKIAS; GORDON, 2003; LEVY; RIVADENEIRA, 2000). The thermal stability of norbixin has also been studied by Ferreira et al. (1999), that observed that norbixin aqueous solution color, after 450 minutes of heating at temperatures of 90, 100, 120 and 140 °C was reduced, indicating the degradation of the carotenoid promoted by the exposure at elevated temperatures.

Tupuna et al. (2018) observed that the thermal stability of norbixin dissolved in water with ethanol (1: 1), at temperatures of 60, 90 and 98 ° C, presented expressive losses of approximately 15, 35 and 45%, after 300 minutes of heating respectively. When comparing the percentage of degradation of 60 ° C temperature curve of this work with the results found in this study, it was verified that the norbixin present in the biodegradable films showed a lower percentage of degradation, even when submitted 2.75 times longer than the work quoted. This conclusion indicates that the presence of sodium alginate exerts a protective function on norbixin molecules in high temperature processes. The formation of degradation compounds between norbixin incorporated in the film-forming matrix and free norbixin in water with glycerol, showed that the polymeric matrix of sodium alginate delayed the thermal degradation process of the carotenoid. Free norbixin molecules in water are more exposed and susceptible to the action of external oxidative agents such as temperature, oxygen and light, while in the polymer matrix thay are enveloped and protected by the alginate chains.

Balaswamy et al. (2006) reported that bixin, precursor of norbixin, when stored in oleorisins from annatto seeds, presented superior degradation stability to light and oxygen when stored as a powder. The result was related to the interaction of the lipophilic molecules of the resin with the unsaturated chains of bixin, enveloping them and protecting them from the evil action of light and oxygen.

3.3. *Films barriers properties*

Table 3 shows film barrier properties to water vapor permeability and light transmission. Such results are important to films with potential application in food, since the passage of water and light can compromise the final quality of the packaged product, accelerating degradative processes.

Table 3 - Water vapor permeability and transmissible light from biodegradable sodium laginate films incorporated with norbixin salts complexed with calcium chloride 1%

Film	Water permeability vapor (g.mm/m².h.kPa)	Light transmission (%) at	
		300 nm	600 nm
0.00%	0.5145 ± 0.0432^{a}	34.82 ± 1.78^{a}	62.64 ± 2.45^{a}
0.05%	0.3644 ± 0.0710^{b}	32.60 ± 4.16^{a}	60.08 ± 1.92^{a}
0.10%	0.3505 ± 0.0169^{b}	16.90 ± 0.16^{b}	52.84 ± 8.83^{a}
0.50%	0.2712 ± 0.0366^{c}	1.84 ± 0.37^{c}	35.36 ± 2.06^{b}

The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences (p <0.05).

It was observed that addition of norbixin in the sodium alginate films reduced the water vapor permeability. The addition of 0.50% of the carotenoid resulted a more significant reduction ($p < 0.05$) of this parameter. The increase of the norbixin concentration may have increased the ionic strengths of the bonds between the polymer chains of the sodium alginate, forming a more compact network, with shorter connections and with smaller interstices, making it impossible to pass water molecules between the hydrophobic chains of the sodium alginate. In addition, the norbixin may have contributed to the filling of the interstices formed in the alginate polymer network, reducing the passage of water molecules.

Olivas e Barbosa-Cánovas (2008) evaluated the influence of the proportion of G and M blocks of sodium alginate on the barrier properties and mechanical properties of biodegradable films complexed with calcium chloride verified that films after 5 minutes of immersion in solutions of calcium chloride of 10%. The study showed water vapor permeability of 2.52 and 5.4 g.mm / m².h.kPa for the proportions of 0.45 M / G and 1.5 M / G. The 0.5% norbixin film had a permeability of 10 and 20 times lower than the films of 0.45 M/G and 1.5 M/G, respectively, even using a 10-fold lower calcium chloride concentration in the complexation step.

Zactiti e Kieckbusch (2006) evaluated the influence of calcium chloride concentration during the complexation step on the properties of sodium alginate films. The water vapor permeability was 0.58 g.mm/m².h.kPa for films not complexed with calcium chloride, 0.46 g.mm/m².h.kPa for films complexed with 2% chloride of calcium and 0.29 g.mm/m².h.kPa for complexed films of 3 to 7% of calcium chloride. This result shows that the complexation step is essential for obtaining films with low permeability. Even the range of permeability found contemplates the values found in this work.

According to Olivas e Barbosa-Cánovas (2008), several factors have a significant impact on the water vapor permeability of alginate films. Among them are the ratio of G and M blocks, concentration and type of plasticizer, sodium alginate concentration, concentration and type of calcium source, as well as calcium immersion time.

Table 3 shows the results of transmissible light from the films. It was observed that the addition of norbixin in the standard alginate film increased the light barrier property of the films at the two wavelengths kind. This fact was related with the increased color of the films, caused by the addition of norbixin dye, in which it visually increased their opacity.

For the wavelength of 300 nm, the addition up to 0.05% of norbixin did not increase the opacity significantly (p> 0.05), however for the concentrations of 0.10% and 0.50%, the increase resulted in reduction in the light passage of two and seventeen times, respectively. For the 600nm length, the increase in norbixin concentration also increased the opacity of the films. Up to the 0.10% concentration, the increase was not significant (p> 0.05), while at the concentration of 0.50 % was an approximately two time more in the opacity of the alginate film.

Sodium alginate films without the complexation process are generally transparent with low opacity (BIERHALZ et al., 2014; GALUS; LENART, 2013). The process of complexation with bivalent cations influences the transparency and transmissible light of films, but varies with the type of cation used. For example, in a study by Liling et al., (2016), the addition of calcium ions up to 2% (w / v) showed no significant difference in relation to films without complexing, while films with concentrations of 5 % (w / v), there was a reduction in light transmissibility. The reason for this is due to the development of a reticulated and compact structure promoted by calcium ions

The sodium alginate films complexed with calcium ions performed by Liling et al. (2016) presented a light transmittance higher than that present work. The reason could be the immersion time that was 2 minutes instead of 5 minutes, as used in the present study. During the complexation process a change in opacity of the films over the immersion time was noticeable. The films becomes whiter and less transparent. However, in the work of Liling et al. (2016), when evaluating the change in opacity over the immersion time, the authors did not find statistical differences.

In addition, sodium alginate films synthesized by other authors also showed a transmissible light superior to that of this (NORAJIT; KIM; RYU, 2010; WANG; SHANKAR; RHIM, 2017), indicating that the film resulting from the process presented good barrier property to light.

In general, food packaging must have a high water and light barrier, since both can stimulate reactions of food degradation and oxidation, respectively. Then, it is evidenced that the addition of norbixin improved properties of the standard film of sodium alginate, in which the concentration of 0.50% presented the best barrier results.

3.4. *Films colorimetric characteristics*

Table 4 shows the colorimetric parameters *L* *, *a* * and *b* * of the films after 0, 3, and 7 days under controlled humidity, temperature and light conditions.

Table 4 - Colorimetric parameters of sodium alginate films incorporated of salts norbixina complexed with 1% calcium chloride after 0, 3 and 7 days under controlled conditions

	Day 0		Day 3		Day 7	
0.00%	$L^* =$	95.82 ± 0.38^{aA}	$L^* =$	96.08 ± 0.52^{aA}	$L^* =$	96.45 ± 0.72^{aA}
	$a^* =$	4.96 ± 0.15^{cB}	$a^* =$	5.11 ± 0.07^{bA}	$a^* =$	5.15 ± 0.05^{aA}
	$b^* =$	-1.87 ± 0.26^{dA}	$b^* =$	-2.71 ± 0.39^{dB}	$b^* =$	-2.51 ± 0.32^{bB}
0.05%	$L^* =$	91.17 ± 2.10^{abB}	$L^* =$	95.85 ± 0.48^{aA}	$L^* =$	96.22 ± 0.54^{aA}
	$a^* =$	3.23 ± 0.46^{dC}	$a^* =$	4.46 ± 0.25^{bB}	$a^* =$	5.09 ± 0.07^{aA}
	$b^* =$	42.71 ± 5.68^{cA}	$b^* =$	2.52 ± 0.37^{cB}	$b^* =$	-2.27 ± 0.30^{bB}
0.10%	$L^* =$	87.78 ± 1.20^{bC}	$L^* =$	93.54 ± 1.28^{aB}	$L^* =$	95.63 ± 0.70^{aA}
	$a^* =$	7.02 ± 0.87^{bA}	$a^* =$	3.04 ± 0.32^{cC}	$a^* =$	5.01 ± 0.38^{aB}
	$b^* =$	$58.25 \pm 5.4b^{cA}$	$b^* =$	19.36 ± 2.42^{bB}	$b^* =$	-1.71 ± 0.11^{bC}
0.50%	$L^* =$	76.42 ± 7.00^{cC}	$L^* =$	84.53 ± 6.59^{bB}	$L^* =$	92.31 ± 3.38^{bA}
	$a^* =$	36.10 ± 2.13^{aA}	$a^* =$	17.46 ± 1.40^{aB}	$a^* =$	3.88 ± 0.52^{bC}
	$b^* =$	77.24 ± 11.88^{aB}	$b^* =$	81.80 ± 2.69^{aA}	$b^* =$	28.26 ± 8.73^{aC}

The colors filled in the tables represent the colors resulted from the colorimetric parameters obtained. The values are represented as mean ± standard deviation. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) among the same days. Different uppercase letters in the same line indicate significant differences ($p < 0.05$) among the days of a treatment.

After the production of the films (day zero), it was observed that the increased concentration of norbixin resulted in films with darker colors due to the significant reduction ($p < 0.05$) of the *L* * parameter. It was also observed that the increase in norbixin concentration resulted in films with red-yellow quadrant staining, due to the significant increase ($p < 0.05$) in the parameter *a* * and *b* *.

On day 3, there was a tendency for the films to lose their original color, approaching the staining of the standard film (0.00%), increasing the *L* * parameter and reducing the *b* * parameter. Significant increases in the *L* * parameter and significant reduction of the *b* * parameter were also observed between day 0 and 3 in all bioactive films, indicating the loss of dark and yellow color due to the degradation of norbixin. At day 3, only the 0.10 and 0.50% films continued to have a red-yellow color.

On day 7, all bioactive films, except for the 0.50% film, showed similar color parameters to the standard film, indicating representative degradations of norbixin incorporated in the films. It was observed a increase of the parameter *L* * and reduction of the parameter *b* * significant in all the films after degradation, demonstrating the great instability of the color of the films in exposure to artificial light and temperatures of 30 °C.

Galus and Lenart (2013), when evaluating the color parameters of sodium alginate films obtained by a similar method of casting, found similar values for *L* * and values of *a* * and *b* * close to zero. The difference in the parameters *a* * and *b* * with those of the present work may be related to the higher concentration of glycerol used by the authors and the fact that the film was not complexed with calcium.

The results of the present study show the coloring power of norbixin described by Tupuna et al. (2018), the addition and increase of concentration resulted in significant changes ($p < 0.05$) in *L* *, *a* * and *b* *. It was verified that the increase of the concentration reduces significantly ($p < 0,05$) the parameter *L* *, obtaining films less translucent. The increase in concentration also increased significantly ($p < 0.05$) the parameter *a* * and *b* *, indicating an increase of red and yellow color, respectively.

According to Satyanarayana et al. (2010), when evaluating the colorimetric characteristics of anatto extracts in different formulations of bixin and norbixin dissolved in both aqueous and oily media, the development of the yellow-red color was observed. In the same study, the authors observed that the increase in concentration decreased the parameters *L* * and *b* *, and increased the parameter *a* *, the same results found in the present work when there was an increase of norbixin concentration in the films.

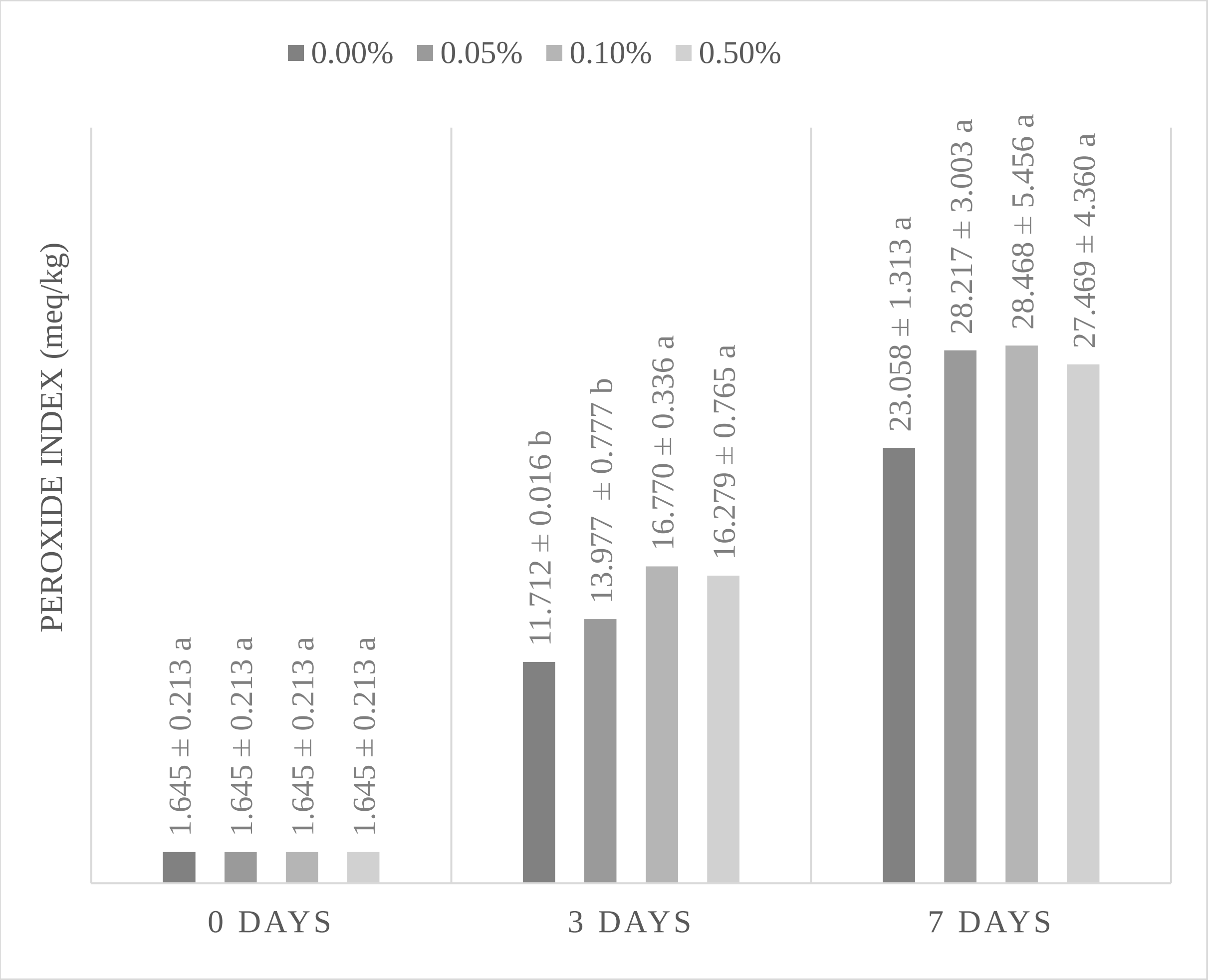
The antioxidant performance analysis of the films was conducted with sunflower oil, a unsaturated fatty acids prone to lipid oxidation processes under accelerated conditions (DAMODARAN; PARKIN; FENNEMA, 2008). The high temperature of 30 ° C and exposure to light were also used to stimulate the lipid oxidation process and evidence the antioxidant/prooxidant effect of the bioactive compounds incorporated in the films.

When light and sensitive compounds are present, the activation of singlet oxygen in atmospheric air can act as an important factor in inducing oxidative deterioration of oils. In addition, the spontaneous reaction of atmospheric oxygen with lipids, known as auto-oxidation, is the most common process leading to oxidative deterioration (DAMODARAN; PARKIN; FENNEMA, 2008).

3.5. *Peroxide index of sunflower oil stored by biodegradable films*

The results of the peroxide index after 0, 3 and 7 days storage of sunflower oil are presented in Figure 2. First, it was observed that the oil used had a low value of 1.645 ± 0.213 mEq/kg of peroxides.

Figure 2 - Peroxide content of biodegradable sodium alginate films



The values are represented as mean ± standard deviation. Different letters within the same area indicate significant differences (p <0.05) among the same day.

The results of peroxide indices after 3 days showed that the addition of norbixin at 0.05% concentration did not differ significantly (p >0.05) from pure alginate film (0%). However, at concentrations higher than 0.10 and 0.50% resulted in a significant increase (p

>0.05) in the peroxide indices, indicating that the increase in norbixin concentration had a prooxidant effect on the oil at short times.

After 7 days the incorporation of norbixin into the sodium alginate film did not result in a significant increase (p <0.05) in the peroxide index. The increase in concentration also did not result in significant differences between the three bioactive concentrations. Although there was no statistical difference.

The pro-oxidant effect of carotenoids on biodegradable films was also reported by Pagno et al. (2016), in which films with concentrations of 10% (w/w) nanocapsules of bixin crystals incorporated in starch films resulted in the peroxide index higher than at lower concentrations after 3, 6, 9 and 13 days of experiment under controlled light and temperature conditions (35 °C).

There are few studies in the literature evidencing the prooxidant effect of carotenoids applied in oils. However, some studies have studied the pro-oxidant effect of carotenoids applied in other areas. Lowe et al. (1999) observed that β-carotene and lycopene could cause breakdown of DNA molecules and there is an optimal concentration which the carotenoids protected the cells from oxidation. However, with the increased carotenoid dose, the ability to protect the cell was gradually lost and resulting in increased damage, indicating a pro-oxidant effect. According to Lowe et al. (2003), many studies aim to evaluate the ability of carotenoids to combat oxidant molecules using their own molecule as a mechanism. However, because the carotenoid molecule has a reactive structure, after degradation, it can form epoxides and prooxidant small chain compounds.

Subagio and Morita (2001) when evaluating the effect of concentration on the antioxidant performance of β-carotene and lutein carotenoids found that the increased concentration of these carotenoids promoted the formation of peroxides and conjugated dienes in corn oils. The b-carotene presented concentrations with antioxidant effect for the lowest concentrations, while lutein oxidized the oil more than the oil without carotenoid. In addition to the concentration, Palozza (1998) found that b-carotene also had a prooxidant effect on certain conditions such as high concentration of oxygen and redox media.

According to the results obtained in this research, a factor to be considered in the incorporation of carotenoids in biodegradable films refers to the concentration of the pigment as well as characteristic of the packaged product. An adverse effect (pro-oxidant) to the expected (antioxidant capacity) can be obtained at short time (3 days), therefore it is necessary to evaluate the type of product to be stored. However, such films may not be suitable for fatty foods and to avoid lipid oxidation in foods, but may be applied for packaging products with low moisture content and not prone to the oxidation process.

4. Conclusion

The developed films presented visual appearance and plastic texture similar to commercial packaging. In addition, the films presented a smooth, compact and cohesive structure, with an attractive reddish yellow color and with great flexibility, facilitating their handling. Norbixin showed excellent compatibility with sodium alginate, low leaching during the complexation process and some degradation during the drying process.

It was verified that the increased norbixin concentration improved the vapor barrier properties of water and the reduction of the visible and UV light barrier, but did not present antioxidant capacity in the conservation of sunflower oil. The importance of the evaluation of the mechanical and physical properties of films as well as the antioxidant/prooxidant potential of carotenoids in the incorporation in polymer matrices is emphasized.

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Biodegradable sodium alginate films incorporated with norbixin salts

Yuri Buratto de Farias, Anderson Kaiszewski Coutinho, Renato Queiroz Assis, Alessandro de Oliveira Rios*

Institute of Food Science and Technology, Federal University of Rio Grande do Sul (UFRGS). Avenue Bento Gonçalves, 9500, Agronomia, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil.

*Corresponding author.

E-mail address: alessandro.rios@ufrgs.br (A. de O. Rios). Phone: +55 51 3308 9787

ABSTRACT

Plastic packaging derived from petroleum has a low cost of acquisition and high physical stability, which results in an economical alternative and widely used by the food and beverage sector. However, such packaging may have a negative impact on the environment when it is not properly recycled. Some studies present the use of biodegradable packages developed from natural polymers as a sustainable alternative, for example, sodium alginate. In addition, the food sector presents losses of production in the storage stage; thus, the present work aimed to develop biodegradable films of sodium alginate with antioxidant properties by the incorporation of carotenoid norbixin. Norbixin salts at the concentration of 0.01, 0.03 and 0.05% were added to the sodium alginate films complexed with calcium ions. Carotenoid incorporation did not significantly interfere with the moisture content and the swelling capacity of the films. The thickness, water activity, and solubility were higher in the films with 0.05% addition of norbixin salts. The presence of the carotenoid improved the thermal stability of the films, as well as the water vapor barrier properties and ultraviolet-visible light. The film with 0.03% concentration of norbixin salts showed antioxidant activity, prolonging the shelf life of sunflower oil according to FAO parameters.

KEYWORDS: carotenoids, oxidation, sunflower oil, antioxidant, packaging.

1. INTRODUCTION

Synthetic packaging, derived from petroleum, represents a problem for the environment since they are not biodegradable; which may result in their accumulation in the ecosystem when they are not adequately recycled (WANG; AUTY; KERRY, 2010). An alternative is the biodegradable packaging with technological properties suitable for use in food and beverages has been developed from natural materials such as lipids, proteins, and polysaccharides (BIERHALZ et al., 2014; CAZON et al., 2016; SALGADO et al., 2015; WANG; AUTY; KERRY, 2010).

A polysaccharide widely used by the food industries is sodium alginate, which is found in the cell walls of brown seaweed of the *Phaeophyceae* class. Besides its possible to form complexes of sodium alginate in the presence of polyvalent cations like calcium ions, result of specific and strong interactions that present a molecular conformation called the "egg box"(ALBOOFETILEH et al., 2014; LI et al., 2016; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016). The films produced with this polymer are biodegradable, non-toxic to the human organism, and when produced by the casting method and complexed with calcium ions, are physically resistant, have low water vapor permeability and water solubility, which makes it possible to apply them in food (BIERHALZ et al., 2014; PONGJANYAKUL; PUTTIPIPATKHACHORN, 2007; STRACCIA et al., 2015; ZHANG et al., 2017).

Besides the environmental issue, the food industry faces problems of losses and wastes along its productive chain. According to Gustavsson et al. (2011), the industry wasted approximately 1.3 billion tons of food in 2011, equivalent to one-third of all world production. In developing countries, approximately 40% of the losses occur in the post-harvest and processing stages, including the storage and transport of the products to the final consumer. An alternative to prevent losses is the use of appropriate packaging that can prolong the shelf life of food.

In addition to the use of biodegradable packaging, studies have been carried out to enable the development of active packaging (PAGNO et al., 2016; ASSIS et al., 2017). These packs may be added to a bioactive compound that exhibits antioxidant activity, as carotenoids. Among these compounds, there is the norbixin (9-*cis*-6,6'-diapocarotene-6,6'-dioic acid), a compound derived from bixin, the primary pigment present in the seeds of *Bixa orellana L fruit*.

Norbixin may be obtained by hydrolysis of a bixin extract with an aqueous alkali solution (saponification reaction), that produce a sodium or potassium salts of norbixin, a dye soluble in aqueous solution (SILVA; NACHTIGALL, 2009; ZHANG; ZHONG, 2013).

Norbixin has food coloring properties, capable of developing colorations from yellow to red colors (SATYANARAYANA; RAO; RAO, 2010), which is applied in meat products (GARCIA et al., 2012), butter and cheeses (LANCASTER; LAWRENCE, 1996), baked goods (RAO et al., 2005) and others. In addition to this property, norbixin presents antioxidant properties in order to its structure rich in conjugated double bonds, which provides a reactive structure passive to react with free radicals, that avoid lipid oxidation reactions (KIOKIAS; GORDON, 2003; KIOKIAS; OREOPOULOU, 2006). Ingestion of norbixin salts via food is considered safe by JEFCA (FAO/WHO, 2005), academic studies suggest that ingesting norbixin does not cause damage to DNA molecules (FERNANDES et al., 2002) and its structure is stable and bioavailable during the human digestive process. (POLAR-CABRERA et al., 2010)

Thus, the present work intends to integrate the physicochemical properties of sodium alginate films with the antioxidant properties of carotenoid norbixin. For this, films of sodium alginate complexed with calcium ions and incorporated with different concentrations of the pigments in the form of norbixin salts were developed, and all the films were evaluated the physical-chemical properties and antioxidant capacity.

2. MATERIAL AND METHODS

2.1. Materials

Annatto seeds and sunflower oil were purchased at the local market in Porto Alegre, Rio Grande do Sul, Brazil. Sodium alginate (PA) with a purity of 90% (g/g) (Dynamics® da Química Contemporânea Ltda.) and calcium chloride (PA) pentahydrate with 99.5% (g / g) purity (Nuclear®) were used. Glycerin (Exodus cientifica®) was also used as a plasticizer for films. The entire experiment was conducted using distilled water to avoid cross-contamination with calcium ions.

2.2. Obtaining norbixin extract

Bixin extraction and conversion to norbixin by the saponification process was performed according to Rios and Mercadante (2004), with some modifications. For each 25 g of annatto seeds, 2 successive washes were performed with 50 mL of hexane for 15 minutes under magnetic stirring. The seeds were separated by filtration. After that, 2 successive washes were performed with 50 mL of methanol for 15 minutes under magnetic stirring, and the seeds were again filtered. The bixin present in the anatto seeds were obtained with 2 successive extraction steps with ethyl acetate with magnetic stirring for 15 min, and the extracts were concentrated in a rotary evaporator (Fisatom, M802, Brazil). The dry bixin extract was diluted with 50 mL of methanolic potassium hydroxide (10% w / v) on ultrasound (Unique, USC 1650 A, Brazil) and maintained over 12 hours in the dark (*overnight*) for complete conversion into norbixin salts. The obtained norbixin extract was recovered in the funnel with 150 mL of ethyl acetate / ethyl ether / water (1: 1: 1), added acetic acid to a pH of 3.5 and washed with water to pH 6.0. Finally, the norbixin salts were dried on a rotary evaporator (Fisatom, M802, Brazil) and stored at -18 °C. The purity of the norbixin salts, was evaluated by High-Performance Liquid Chromatography (HPLC) using a wavelength of 450 nm (TUPUNA et al., 2018).

2.3. Preparation of the sodium alginate films by the casting method

Previously an aqueous solution of norbixin salts was prepared and adjusted to pH 11 with a solution of potassium hydroxide (0.5% w / v). Dispersions of sodium alginate (1% w / w), glycerin (2% w / w) and norbixin salts at concentrations of 0.01, 0.03 and 0.05 % (g salts of norbixin / g alginate) were prepared using a mechanical stirrer (Fisatom, 713D, Brazil) with propellant for 30 minutes,20 °C at a speed of 1000 rpm. After stirring, the filmogenic solution was protected from light and kept stand by for deaeration (12h). The solution was poured into petri dishes (0.48 g/cm^2) and dried in an oven (De Leo, TLK48, Brazil) at 55 °C for 13 h. After this period, the films obtained were subjected to the complexation process for 5 min by the addition of 50 ml of calcium chloride (1% w / v) in each plate. The remaining liquid was discarded and the films were subjected to drying for 45 min. The films were stored for 48 hours under controlled humidity (58 %, saturated solution of sodium bromide) before the characterization analyzes.

2.4. Determination of physicochemical films properties

The water activity of the films was measured instrumentally (Rotronic instruments, HP23, United Kindom). Circles with a diameter of 2.5 cm were trimmed and left 5 minutes inside the measuring chamber of the equipment until stabilization. The analysis was carried out in quadripartite.

For the determination of the moisture, samples with 2 cm in diameter were weighed (Wi), and dried for 24 h at 105 °C in an oven dried (DeLeo, TLK48, Brazil). The dry films were weighed (Wd) and the moisture was calculated according to equation (1), as described by Assis et al. (2017). The experiment was carried out in quadripartite.

(1) $Moisture\ (\%) = \frac{(Wi - Wd)}{Wd} x\ 100$

The solubility of films was performed as described and adapted by Assis et al. (2017) The previously dried films were weighed (Wd), added to the distilled water (30 mL) and shaken during 24h in a water bath with a controlled temperature of 25 °C (Nova Tecnica, NT145, Brazil). After the solubilization, the samples were filtered, dried at 105 °C for 24 h and weighed (Wf). The solubility was calculated according to equation (2) and the experiment was carried out in quadripartite.

(2) $Solubility\ (\%) = \frac{(Wd - Wf)}{Wd} x\ 100$

Swelling capacity was performed as described by Silva et al. 2009), followed by modifications. Squares were cut off from the films (2 cm x 2 cm) and putted into aluminum grids, weighted (Wi), immersed in 30 mL of water and subjected to continuous shaking at a controlled temperature of 25 ºC during 2 h to stabilize the weight gain. After 2 hours, the grids with the films were surface dried with paper towel and weighted (Wf). The swelling capacity was calculated according to equation (3). The experiment was carried out in quadripartite.

(3) $Swelling\ Capacity\ (\%) = \frac{(Wi - Wf)}{Wi} x\ 100$

2.5. Scanning electron microscopic analysis (SEM) of films

The structures of the films were analyzed by scanning electron microscopy (JEOL, JSM-6060®, Japan) with a voltage between 5 and 15 kV. The samples were previously vacuum metallized by gold and carbon (BAL TEC, SCD 050, Brazil) to aid in the reading of the images.

2.6. Thermogravimetric analysis of films

The analysis was performed between 25 and 850 °C in a thermogravimetric (Perkin Elmer, Pyris 1 TGA®, USA) with a nitrogen gas flow rate of 20 mL/min and heat rate of 20 °C/min. The analyzes were performed in duplicate. The curves were processed by Pyris Manager ® software.

2.7. Colorimetric characteristics of films

The colorimetric parameters of the films were measured by the colorimeter (Hunter Lab, Miniscan XE, USA) using the CIE Lab color parameters: L * (brightness), a * (red-green) and b * (yellow-blue). In order to better represent colors, the color parameters were synthesized by Corel Draw X6® software.

2.8. Barrier properties of films: water vapor permeability and light transmission

The water vapor permeability (WVP) was determined gravimetrically at 25 °C (ASTM, 2000), followed by modifications described by Assis et al. (2017). The analysis was conducted in triplicate and calculated according to equation (4):

$$(4)\ WVP = \frac{w.L}{\mathrm{A.t.\Delta p}}$$

Where w is the weight in grams of water permeated into the capsule through the film, L is the average film thickness in mm, A is the permeation surface area in m², t is the permeation time in hours, and Δp is the water vapor pressure difference in kPa between the film surfaces.

The transmittance of the films was evaluated by a UV spectrophotometer (Shimadzu, UV-1800, Japan) at wavelengths of 300 and 600 nm, representing invisible and visible light, respectively. Each film was cut into rectangles and placed in a quartz cuvette. The empty cuvette was used as standard (PAGNO et al., 2016).

2.9. Mechanical properties of films: traction and elongation

The mechanical properties of the films were evaluated by tensile strength (TS) and elongation at break (E). Previously, 10 strips of each film were cut into rectangular to form test pieces of 70 mm x 20 mm. The thickness was measured with a digital micrometer (Digimess, IP40, Brazil) in three different points. Subsequently, the lowest thickness was used in the device software to calculate TS, E, and MY. The analyzes were performed in a texturometer (Stable Micro Systems, TA-XT2, United Kindom), at 25 ºC, with an initial separation of the 50 mm claws and probe velocity of 80 mm / minute.

2.10. Evaluation of the antioxidant effect of films

The oxidative stability of sunflower oil was used to evaluate the antioxidant or pro-oxidant potential of the films as described by Assis et al. (2018). The films were folded into envelopes form, where sunflower oil without addition of synthetic antioxidants (13 mL) was added. The oil was stored in an glass jar as standard for comparation of biodegradable films. All samples were stored in a chamber (Tecnal, TE-402, Brazil) under the constant light incidence of 900-1000 Lux (Luxometer VA Instrument, MS6610, China), with a temperature of 30 ºC and a relative humidity of 70%. After 0, 3, 6, 12 and 15 days of storage, the oils were submitted for of peroxide index (AOCS, 1993) and conjugated dienes analysis (ASSIS et al., 2017). The experiment was conducted in quad-plot each day and for each concentration.

2.11. Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and Tukey's test at a significance level of 5% using Statistica 12.0 software (StatSoft, Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Physical-chemical properties: thickness, water activity, moisture content and swelling capacity of films

Table 5 presents the results of thickness, water activity, moisture content, solubility and swelling capacity of sodium alginate films with different concentrations of norbixin salts (0.00, 0.01, 0.03 and 0.05%).

Table 5 - Physicochemical properties (thickness, Aw, moisture, solubility, and swelling capacity) of biodegradable sodium alginate films complexed with calcium chlorates and incorporated with norbixin salts.

Films	Thickness (mm)	Aw	Moisture (%)	Solubility (%)	Swelling Capacity (%)
0.00 %	0.075 ± 0.008^{b}	0.659 ± 0.014^{b}	74.23 ± 4.91^{a}	54.98 ± 2.65^{b}	20.28 ± 3.58^{a}
0.01 %	0.083 ± 0.008^{b}	0.679 ± 0.005^{b}	77.30 ± 5.67^{a}	54.68 ± 1.61^{b}	19.47 ± 2.55^{a}
0.03 %	0.076 ± 0.010^{b}	0.674 ± 0.010^{b}	64.48 ± 10.37^{a}	59.35 ± 1.50^{b}	19.86 ± 2.91^{a}
0.05 %	0.099 ± 0.010^{a}	0.739 ± 0.030^{a}	58.40 ± 10.18^{a}	66.73 ± 4.18^{a}	17.99 ± 3.19^{a}

The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$).

The incorporation of norbixin salts in the films did not influence the moisture content and swelling capacity of the films. Although the thickness, water activity, and solubility did not show a significant difference ($p > 0.05$) for incorporated films with up to 0.03% concentration of norbixin salts, the increase of norbixin salts concentration to 0.05% (w/w) resulted in a significant increase ($p < 0.05$).

The 0.05% films showed greater free water content (Aw), behavior related to norbixin salts that may have significantly saturated the sodium alginate hydroxyl bonds, reducing the formation of hydrogen bonds between molecules of water and hydroxyls of the polymer, providing only weak interactions with the surface of the films. Galus and Lenart (2013), developed and characterized films of sodium alginates, and reported water vapor adsorption isotherms of sodium alginate films to have a hydrophilic profile with high humidity absorption capacity. The result was related to the polymer hydrophilic characteristics due to the hydroxyls present along its chain. Regarding the moisture content, all films had values above 58%, but without significant differences ($p > 0.05$).

The addition of norbixin salts significantly increased the solubility of biodegradable films only at the 0.05% concentration. The solubility increase occurred due to of the hydrophilic characteristics of the norbixin salt. According to Rios (2004), the solubility of norbixin salts in water is a result of the alkaline saponification process of bixin, in which the methyl ester group is hydrolyzed from the main chain of the diapocarotenoid, forming the *cis*-norbixin carboxylic acid, which is neutralized, forming salts with polarities near the water. When analyzing the influence of pH on the bixin extraction process and conversion to norbixin, Silva et al. (2009) reported that the *cis*-norbixin, carboxylic acid formed after the saponification step, has a higher polarity than *cis*-bixin and it has lipophilic character. However, after neutralization of the acid, the salt formed develops a hydrophilic character with partial solubility in aqueous solutions.

Although the hydrophilic character of sodium alginate and norbixin salts, less soluble films were expected, once the films were submitted to the complexion process with calcium ions, which partially converts sodium alginate to calcium alginate, forming insoluble complexes in water. The process of complexation can occur in several ways, and in this work, the external diffusion process was used. According to Leong et al. (2016), in this method, the complexation occurs instantaneously by immersion of the filmogenic matrix of sodium alginate in a medium containing calcium. The diffusion of the calcium ions occurs from the outside to the inside area, and the formation of complexes through the polymer chains depends mainly on the calcium concentration and the immersion time. The solubility of the films produced could be reduced by increasing the calcium ions concentration or by increasing the immersion time. However, in preliminary tests, it was found that increasing the concentration to 2% of calcium chloride or increasing the immersion time to 6 minutes resulted in brittle films showing negative influences on other parameters, such as mechanical properties.

Silva et al. (2009) developed films of sodium alginate complexed with calcium ions with solubility between 15 to 20%. Although the concentration of glycerol and calcium chloride were the same, the lower solubility of the films was due to the longer complexation time (30 min). Probably, with the longer exposure time, more insoluble complexes were formed in the filmogenic matrix. Bierhalz et al. (2012) developed sodium alginate films, complexed with calcium ions and incorporates natamycin with solubility between 17 and 20%. The lower solubility of these films about the films of the present work may be related to the use of a 5% (w/v) calcium chloride solution and a 20 minute complexation step, providing a more strong complexation.

The addition of norbixin salts did not significantly influence the swelling capacity of the films. The ability of a filmogenic matrix to absorb water is related to the hydrophilic character of the polymer used. How higher the number of hydrophilic compounds, the greater the number of bonds formed with water molecules. These bonds hydrate the restricted and rigid chains of the filmogenic matrix, reducing ionic forces and making the bonds more flexible, as well as expanding the structure (HAN; ARISTIPPOS, 2005). In this way, many factors can interfere with this property. For example, Roger et al. (2006) while evaluating the effect of calcium ion concentration on the formation and swelling capacity of sodium alginate films, reported that films complexed with higher concentrations of calcium ions or with longer immersion times obtained lower hydration capacity. This fact was related to the reduction of the available hydroxyls to water molecules.

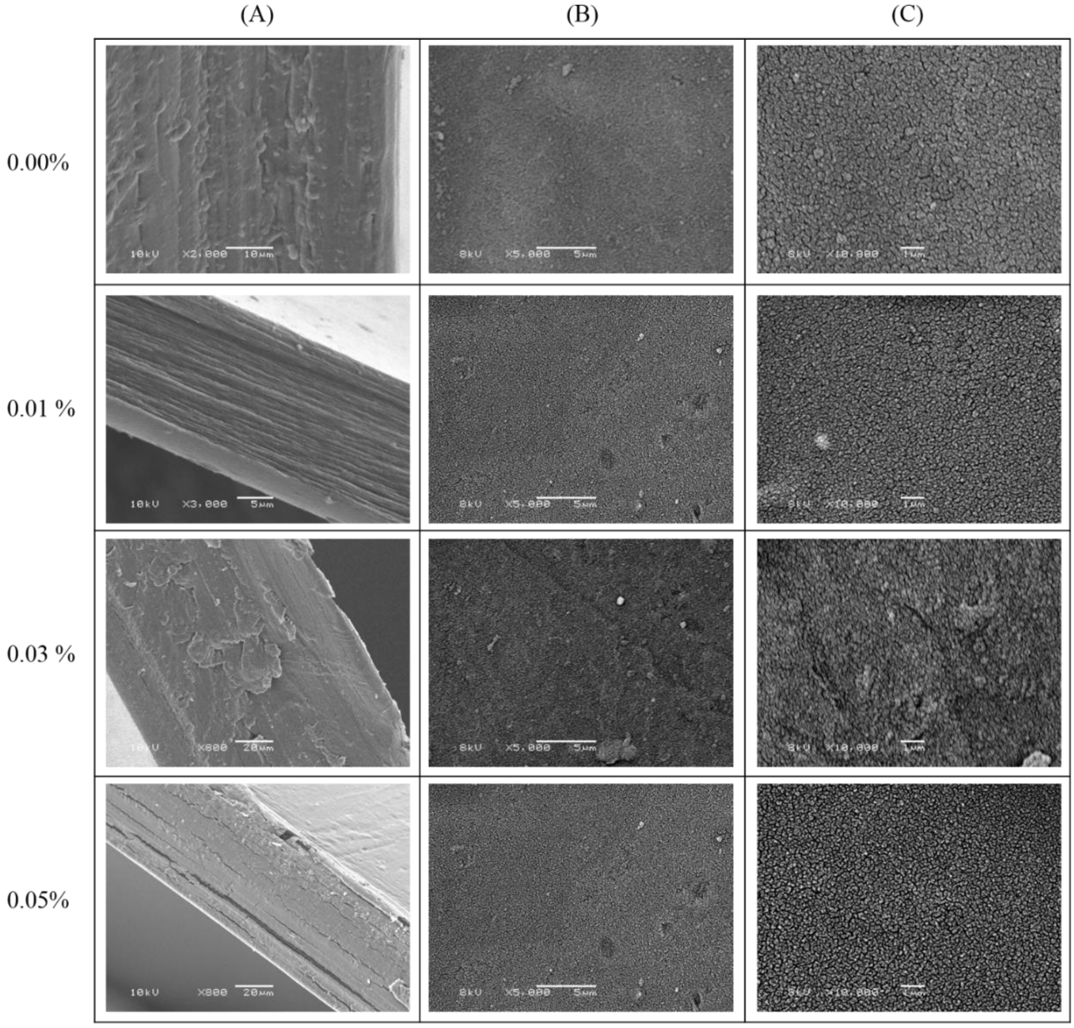
3.2. Scanning electron microscopy

Figure 3 shows the scanning electron microscopy of the cross-section of the films, wide and approximate top view of the films.

The first column (A) shows the cross sections of the films, that is possible to verify a cohesive structure, without porosities. The 0.05 % film show some cross-fissures, being the bioactive films with the lowest cohesiveness. Also, it was possible to visualize the layer overlap caused by the slow drying process performed in this experiment. The top view images of the films (B) show a smooth surface, without porosity or agglomeration. At the 10,000x (C) zoom, it is possible to visualize the crosslinking of the polymer matrix developed between sodium alginate and norbixin salts, showing a similar and regular surface.

The smooth, cohesive and homogeneous surface found in this work was also evidenced in sodium alginate films complexed with calcium ions from other works (BIERHALZ et al., 2014; BIERHALZ; DA SILVA; KIECKBUSCH, 2012; NESIC et al., 2016; NORAJIT; KIM; RYU, 2010; ZHANG et al., 2015a). According to Zhang et al. (2015b), the smooth surface indicates that the interaction between the polymer and the plasticizer is adequate, allowing the addition of other compounds to fill the interstices of the films without forming agglomerates, reducing the porosity of the films. According to Tavassoli-Kafrani et al. (2016), the addition of plasticizer reduces the ionic strengths of the bonds, giving them sufficient flexibility to extend, increasing the space between the chains.

Figure *3 - Microstructure of biodegradable sodium alginate films incorporated with norbixin salts in cross sections (A) and a top view at zoom of 5,000 (B) and 10,000 (C).*

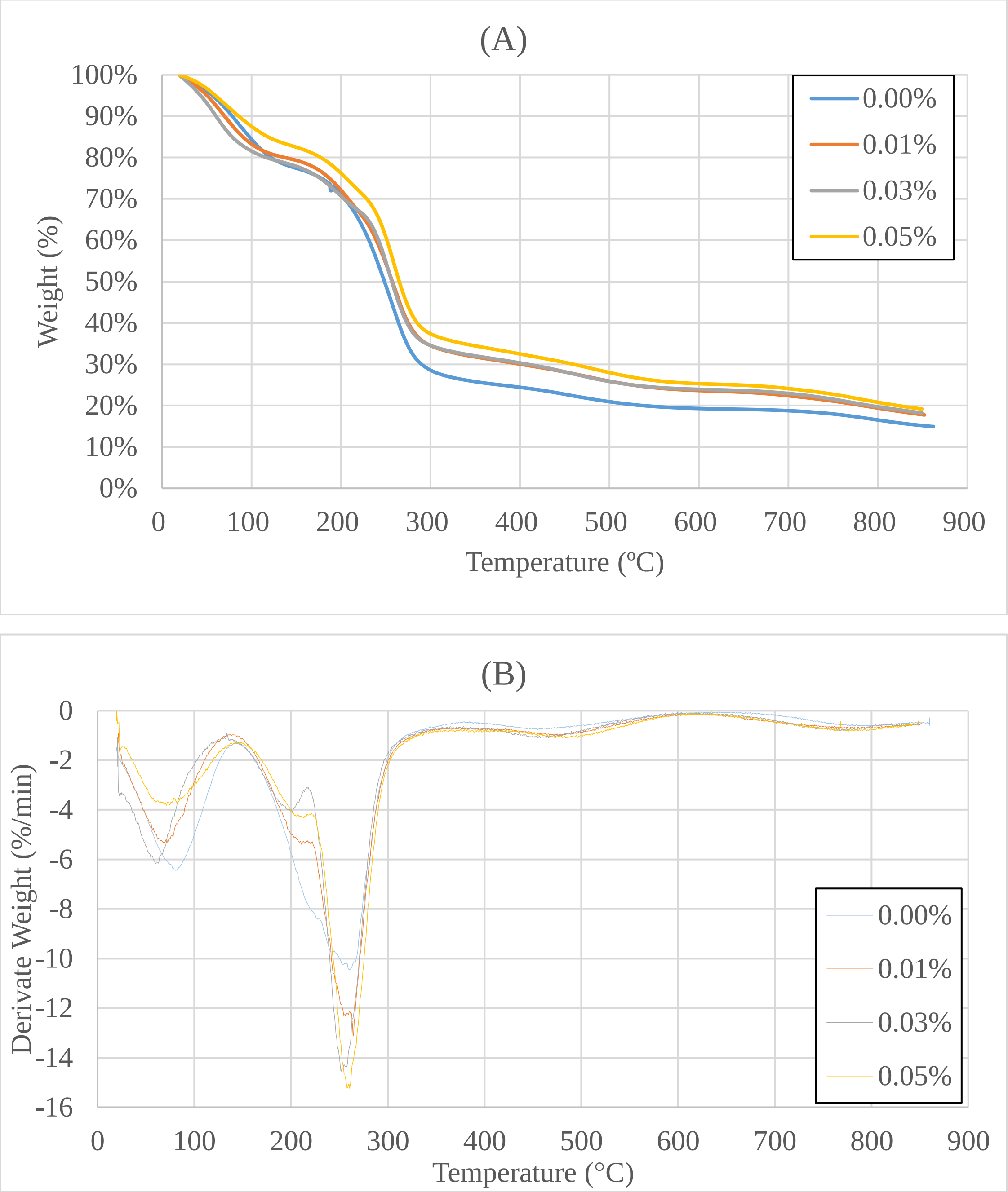


Another important observation is the combination of the concentration of sodium alginate, glycerol, calcium chloride and norbixin salts used in this work. The concentration of glycerol used allowed the flexibilization of the sodium alginate chains, forming pores with sufficient size to be filled by particles of norbixin salts, and the norbixina could made significant bond with sodium alginate. Then, after the addition of calcium chloride at 1 % concentration was sufficient to compact the filmogenic structure, adjusting the pores of polymer matrix around the molecules of norbixin salts, forming the smooth and tight structure (Figure 3).

3.3. Thermogravimetric analysis

Figure 4 shows the thermal degradation curves TGA (Figure 4A) and DTG (Figure 4B) of the biodegradable sodium alginate films incorporated with norbixin salts.

Figure 4 - TGA (A) and DTG (B) curves of sodium alginate films incorporated with norbixin salts.



It was possible to identify 3 thermal degradation events in the films. The first event occurred between 40 and 115 °C, causing mass losses between 15 and 19%, related to the process of evaporation of unbound water from the films. The second event occurred between 172 to 280 ºC, with mass losses between 44 to 48%. According to the DTA curves, between 200 and 300 °C, a series of thermal degradation events occurred because of the blend formed between glycerol, water, norbixin salts, calcium chloride, and sodium alginate. In this second event, in the temperature of 250 ºC, films with higher concentrations of salts of norbixin shows greater losses. The third event occurred between 440 and 560 °C, with mass losses between 8 and 9%, related to the oxidation of sodium alginate. Thermal events were also found by Zhao et al. (2015b), Shankar et al. (2016) and Gao et al. (2017b) when evaluating the thermo-degradation of sodium alginate films.

3.4. Colorimetric characteristics and barrier properties of light and water vapor

Table 6 shows the colorimetric parameters, water vapor permeability (WVP) and light transmission of the biodegradable sodium alginate films.

Table 6 - Colorimetric characteristics and barrier properties against water vapor and light of biodegradable sodium alginate films incorporated with norbixin salts

Film	Colorimetric parameters	WVP (g.mm/m^2.h.kPa)	Light transmission (%) at	
			300 nm	600 nm
0.00 %	$L^* = 96.34 \pm 0.43^a$ $a^* = 5.08 \pm 0.05^a$ $b^* = -2.28 \pm 0.36^d$	0.389 ± 0.006^a	57.14 ± 9.08^a	73.49 ± 8.22^a
0.01 %	$L^* = 96.10 \pm 0.28^a$ $a^* = 4.69 \pm 0.08^b$ $b^* = 2.90 \pm 0.67^c$	0.212 ± 0.014^c	43.41 ± 8.16^a	68.82 ± 4.16^a
0.03 %	$L^* = 93.48 \pm 0.98^b$ $a^* = 3.97 \pm 0.34^c$ $b^* = 20.91 \pm 0.20^b$	0.266 ± 0.014^a	42.81 ± 1.10^a	69.59 ± 0.01^a
0.05 %	$L^* = 92.76 \pm 1.34^b$ $a^* = 3.68 \pm 0.42^c$ $b^* = 23.90 \pm 0.26^a$	0.304 ± 0.014^a	31.04 ± 3.49^b	66.08 ± 3.06^a

The colors filled in the tables represent the colors resulted from the colorimetric parameters obtained. The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$).

The addition of norbixin salts reduced the L^* parameter ($p < 0.05$), resulting in a darker coloration in the 0.03 and 0.05% films. Addition of norbixin significantly reduced the a^* parameter, reducing the reddish coloration of the films. The addition of norbixin salts also resulted in a significant increase in the parameter b^*, responsible for the yellow coloration. At the background of the table, it is possible to verify the colors resulting from the interaction of the colorimetric parameters, and the more intense yellow coloration in the films with higher concentrations. The development of the reddish-yellow coloration in biodegradable films is due to norbixin, a pigment capable of acting as a dye.

Ferreira et al. (1999), when evaluating the kinetics of degradation of norbixin extracts, observed the formation of degradation compounds with yellow coloration when submitted to thermal processes during long periods. In this way, degradation compounds may have been formed during the drying process of the films. Beside this, the increase of norbixina concentration may have increased the degradation compounds proportionally and thus the enhancement of the yellow coloration.

In table 6 it is also possible to check the water vapor permeability of films. The addition of norbixin salts significantly reduced ($p < 0.05$) the water vapor permeability when compared to the control film. The addition of norbixin salts can increase the ionic strengths of the alginate chains forming a more compact structure with smaller molecular interstices. The increasing of concentration to 0.03 and 0.05% may have reduced this interaction, forming weaker bonds and expanding the interstices.

According to Benavides et al. (2012), when developing sodium alginate films incorporated with oregano essential oils, reported that the increase of the essential oil reduced the permeability because it filled the interstices of the films, reducing the passage of water molecules. Zactiti and Kieckbusch (2006) developed sodium alginate films complexed with calcium ions and incorporated with potassium sorbate with water vapor permeability 1.5 times higher than the films of the present study, even using a 2 times higher concentration of chloride of calcium. However, due to the immersion time of 1 minute, the complexation process should not have occurred as intensely as this work, forming a less compact structure with a larger interstice. According to Liling et al. (2016), the immersion time also influences the permeability, the longer the immersion time, the higher the complexation and compaction of the film-forming structure, reducing the permeability.

Zactiti and Kieckbusch (2006) also observed that the increase in calcium chloride concentration significantly reduced the water vapor permeability of the films, a concentration of 7% of calcium chloride was required to obtain films with permeability similar to that of present work, a result also reported by Liling et al. (2016). The water vapor permeability the films of this work was 3 times lower than the uncomplexed sodium alginate films like reported by Paula et al. (2015). In this study the researchers even using 5 times more sodium alginate and 2 times less glycerol plasticizer, indicating that the complexion effect with calcium ions develops a more compact structure than films with higher concentrations of sodium alginate or lower concentrations of glycerol.

The films presented greater light protection capacity in the ultraviolet region (300 nm) than in the visible (600 nm) region (Table 6). Although the films presented a transparent texture, the addition of norbixin developed a yellow coloration that gave the films a light protection capacity, the opacity. The addition of norbixin salts significantly reduced ($p > 0.05$) the passage of light from the 0.05% concentration to non-visible light (300 nm), behavior not observed for visible light (600 nm).

The sodium alginate film without the addition of norbixin salts (control film) showed a lower light transmittance than alginate films of Liling et al. (2016) , Harper et al. (2013) and Norajit et al. (2010). On average, the films of these works cited presented a light transmittance from 80% at 300 to 700 nm. The difference should be related to the different concentrations of sodium alginate, plasticizer and calcium chloride.

Assis et al. (2017) reported that addition of the carotenoid lycopene reduced the passage of ultraviolet-visible light through the cassava starch films. Stoll et al. (2018) found that the addition of bixin, beta-carotene and lycopene carotenoids significantly reduced the light transmittance of poly (lactic acid) films.

3.5. Mechanical properties: tensile strength and elongation

Table 7 shows the mechanical properties of biodegradable sodium alginate films.

Table 7 – Tensile strength and elongation of the biodegradable sodium alginate films incorporated with salts norbixin.

Film	TS (MPa)	E (%)
0.00 %	19.84 ± 2.45^{a}	21.26 ± 0.03^{a}
0.01 %	15.93 ± 1.82^{b}	19.98 ± 0.05^{b}
0.03 %	16.77 ± 0.68^{ab}	18.95 ± 0.04^{b}
0.05 %	18.42 ± 2.40^{ab}	18.18 ± 0.03^{b}

The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$).

The addition of norbixin salts at a concentration of 0.01% significantly reduced ($p < 0.05$) the tensile strength of the sodium alginate films in relation to the control film. However, as the carotenoid concentration increased, tensile strength increased, equivalent to the tensile strength of film without the addition of norbixin salts (0.00%). It was also possible to verify that the addition of norbixin significantly reduced ($p < 0.05$) the elongation capacity of the sodium alginate films, with no significant difference between the antioxidant films.

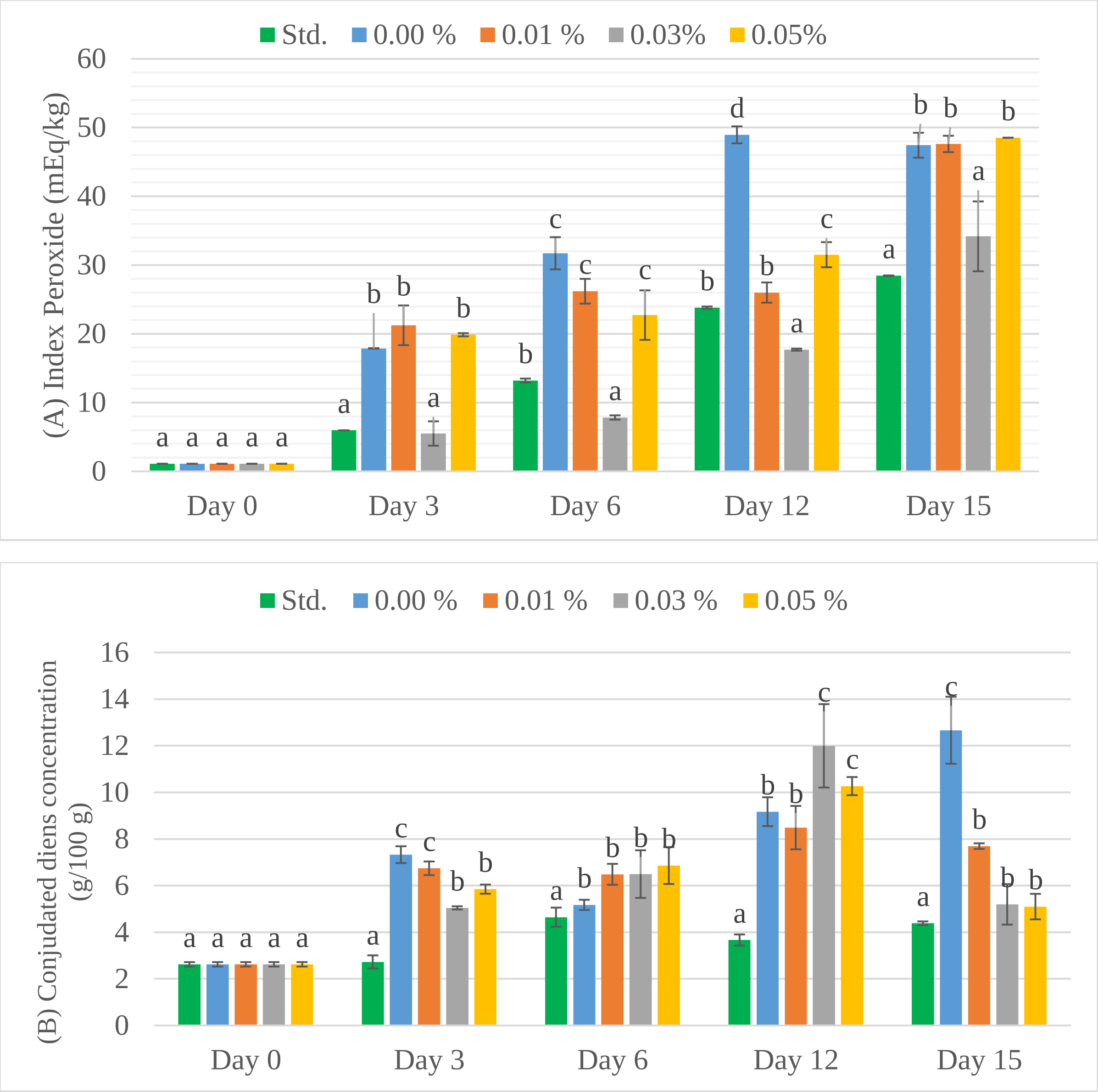
The films of the present work demonstrated lower tensile strength and higher elongation than the sodium alginate films complexed with 2% calcium ions from Zactiti and Kieckbusch. (2006). Probably, the use of 2 times higher of the calcium concentration during the complexation process formed a more reticulated filmogenic structure, with stronger and shorter bonds. Even in this same work, the authors showed that the increased calcium chloride concentration increases the tensile strength of the films and reduces the elongation capacity due to the formation of more rigid bonds.

Olivas and Barbosa-Cánovas (2008) evaluated the effect of plasticizers on the properties of sodium alginate films complexed with calcium ions, found films with tensile strength resistance 4 times higher than the present work. However, those films showed an elongation 5 times smaller. The divergence of the result was probably due to the use of a higher concentration of calcium chloride used by the authors. Liling et al. (2016), when evaluating the effect of complexing factors on the mechanical properties of sodium alginate films, reported a tensile resistance approximately 2 times greater than the present work, as well as an elongation capacity approximately 7 times smaller.

3.6. Evaluation of the antioxidant effect of biodegradable sodium alginate films incorporated with norbixin salts in sunflower oil

The antioxidant properties of bioactive packages is by determining the stability of polyunsaturated oils (oxidation prone foods) under accelerated storage conditions and by monitoring the formation of degradation products (peroxides and conjugated dienes), which are formed mainly in the initiation and propagation step in lipid oxidation (ABIDI; RENNICK, 2003; PEGG, 2002). Figure 5 shows the results of peroxide indices (Figure 5A) and the concentration of conjugated dienes (Figure 5B) of sunflower oil packed with the biodegradable sodium alginate films incorporated with norbixin salts.

Figure 5 - (A) Peroxides Index and (B) Conjugated dienes concentration from sunflower oils stored with sodium alginate films incorporated with norbixin salts during 15 days under controlled conditions.



The values are represented as mean ± standard deviation. Different letters within the same day indicate significant differences (p <0.05). Std is a standard packing used as control (oil in a closed glass jar).

It was observed that the standard oil (stored in glass, protected from exposure to light and oxygen) oxidized over the 15 days, and at day 6 already had a peroxide index (13.21 ± 0.29 meq/kg) higher than recommended by Codex Alimentarius/FAO (2015) (10 meq/kg). When comparing the results with the biodegradable films, it was verified that all the films presented higher results (p <0.05) to the standard in all storage days, except the film with 0.03%. The 0.03% film showed the lowest peroxide index at day 3 (5.51 ± 1.77 meq/kg), 6 (7.84 ± 0.31

meq/kg) and 12 (17.69 ± 0.15 meq/kg). Also, it extended the shelf life of the oil in 3 days, once after 6 days the oil still had a peroxide index below the limit indicated by the Codex Alimentarius/FAO (2015), while the standard oil was no longer fit for consumption.

The incorporation of norbixin salts at the concentration of 0.03% delayed the formation of peroxides during the 15 days. As the analysis was conducted under accelerated conditions, the protection benefit of norbixin salts film with 0.03 % on sunflower oil could be higher under ambient conditions. The lower antioxidant performance of the film from 0.01% to 0.03% film, may be related to the low antioxidant capacity against the free radicals formed during the oxidation of the oil. However, the 0.05% film showed higher protection of ultraviolet-visible light and higher carotenoids, the increase of the concentration to 0.05% showed higher peroxide indices in relation to the concentration of 0.03% in all storage days. The lower performance may be the result of a pro-oxidant effect of norbixin; once the molecule has a structure with double and reactive bonds; its molecule may have been excited by the presence of light and singlet oxygen under the conditions of the experiment, promoting its decomposition to reactive free radicals capable of oxidizing the oil.

According to Kiokias and Oreopoulou (2006), the antioxidant capacity of norbixin is because of its structure of conjugated double bonds that can prevent the oxidation of oils through the neutralization of excited photosynthesizers or direct reaction with free radicals. When analyzing the antioxidant capacity of different carotenoids at a concentration of 1 g / L in olive oils, they reported that norbixin delayed the formation of peroxides and dienes in oils in higher proportion than the carotenoids bixin and beta-carotene. In this same study, the authors validated that norbixin delayed the formation of peroxides by 50% and the formation of conjugated dienes by 40%.

Pagno et al. (2016), when developing incorporated cassava starch films of nanoencapsulated bixin, reported an antioxidant capacity of bixin incorporated into the films, where the sunflower oils packed with the respective films formed fewer peroxides than oils packaged with commercial packages. The authors also reported that the film with a higher concentration of bixin promoted the formation of peroxide, different from the other films with lower concentrations, indicating a prooxidant potential at higher concentrations.

In Figure 5 (B) it was observed that in the standard oil the smaller amount of conjugated dienes ($p < 0.05$) were formed during the 15 days, in relation to the oil packed with biodegradable films. The low formation of conjugated dienes indicates a low occurrence of cleavage of the lipid unsaturated chains, the origin of the formation of such compounds. Although the peroxide indices of the standard oil were higher than the film oil of 0.03% by day 12, this result indicates that the 0.03% film prevented only the formation of more peroxides than the standard oil; however, the peroxides formed in the film oil of 0.03% were more reactive and thus provided a more pronounced cleavage in the oil.

Among the oils packed in the biodegradable films, on day 3 the cleavage of the unsaturated chains was more pronounced in the oils of the films 0.00 (7.33 ± 0.36) and 0.01% (6.74 ± 0.29), indicating that the films 0.03 (5.04 ± 0.07) and 0.05% (5.84 ± 0.19) delayed decomposition. On day 6, there was no difference between the packaged oils. On day 12, oils packed with 0.00 (9.17 ± 0.24) and 0.01% (8.49 ± 0.62) films presented better antioxidant performance, preventing the degradation of lymphatic chains from 12 days. However, on the last day, it was found that the concentration of dienes reduced significantly from the previous day. According to Pegg (2002), this can occur in oils in advanced stages of oxidation, where the dienes undergo an aggressive decomposition of their conjugated structure into smaller and volatile molecules due to the large number of peroxides formed, reducing their concentration.

The formation of more reactive peroxides in the oils of biodegradable films may be related to several factors. It can be considered that first, the standard oil was protected the exposure of light and oxygen, with this, the formed peroxides presented a regular reactivity. Meanwhile, the oils packed by the films were exposed to light and partially to oxygen molecules. Thus, the triplet oxygen permeated through the films must have been excited by photosensitizers present in the oil or by norbixin itself, forming the singlet oxygen, which has a great oxidizing capacity of the lipid unsaturated chains, forming more conjugated compounds significantly.

Rao et al. (2002), when assessing the aqueous stability extracts containing different norbixin storage conditions, reported that norbixin is easily degraded by the presence of light, need to be stored in light-protected media. Alwis et al. (2015) reported that norbixin and other carotenoids can be easily oxidized, forming intermediate species of free radicals, dienes, epoxides, and peroxides. Probably the norbixin present in the films suffered degradation by light absorption of the experiment, forming highly reactive degradation compounds or absorbing and transmitting light energy for singlet oxygen formation.

According to Abidi and Rennick (2003) and Frankel (1998) the alkyl radicals formed at the beginning of the oxidation, in the presence of triplet (atmospheric) oxygen, form low energy radicals incapable of directly cleaving lipid chains. If the triplet oxygen is exposed to light, photosensitizers present in the oil can absorb and transmit light energy to the oxygen molecules, forming singlet oxygen, which is more electrophilic than the triplet and which reacts directly with double chains. According to Min and Boff (2002), it is estimated that singlet oxygen can form lipid hydroperoxides 1,500 times faster than the triplet.

Another factor that may have contributed to the increased concentration of conjugated dienes in the oil packed by bioactive films was reported by Scheppingen et al. (2012). According to the authors, norbixin, when degraded, can form numerous degradation compounds with fragmented structures of the central carotenoid structure, including conjugated dienes capable of overestimating the result of the above experiment.

4. CONCLUSION

There was a compatibility between the sodium alginate polymer and norbixin salts for the development of biodegradable films. The incorporation of norbixin salts at 0.03% concentration in sodium alginate films, complexed with calcium ions, did not significantly interfere in the physical-chemical, mechanical and structural properties of the standard film. In addition, the incorporation of the carotenoids in the water-soluble form improved the thermal stability of the filmogenic blend, as well as the water vapor barrier and UV-Visible light properties, relative to the film without the addition of the bioactive compound. At the concentration of 0.03 % the film presented an antioxidant capacity with the protection of the sunflower oil under the conditions evaluated. Thus, biodegradable sodium alginate films, complexed with calcium ions, and incorporated with norbixin salts may represent a sustainable and viable alternative for the replacement of synthetic packaging used in food lipids.

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Influence of pH the film formulation of sodium alginate incorporated with norbixin salts

Yuri Buratto de Farias, Renato Queiroz Assis, Alessandro de Oliveira Rios*

Institute of Food Science and Technology, Federal University of Rio Grande do Sul (UFRGS). Avenue Bento Gonçalves, 9500, Prédio 43.212, Campus do Vale, Porto Alegre, RS, CEP 91501-970, Brazil. Phone: +55 51 3308 9787 *E-mail: alessandro.rios@ufrgs.br

ABSTRACT: Plastic packaging has been widely used by the food industry because of its low cost of acquisition. However some environmental problems can occur when the plastic is not recycled. The objective of this work was to evaluate the influence of pH solubilization (3.0, 7.0 and 11.0) on proprieties of biodegradable sodium alginate films with the addition of norbixin salts by determining the physicochemical, thermal, colorimetric properties, mechanical, barrier and antioxidant. The pH did not influence water solubility, hydration capacity, colorimetric parameters, thermal properties and the migration of the pigments into acidic aqueous solutions. The pH 3.0 precipitated sodium alginate, forming films with fissures in the cross structure, reduced water permeability and increased transmission to UV-visible light. The film prepared with pH 11.0 presented a more cohesive structure, resulting from the better interaction between sodium alginate and norbixin. In addition, it showed lower water activity, higher tensile strength, higher pigment migration in the lipid medium and higher antioxidant activity on sunflower oil stored for up to 3 days. Whereas after seven days of storage of sunflower oil the film prepared at pH 7.0 showed the highest antioxidant activity. The incorporation of norbixin developed antioxidant properties, since the film without norbixin had the lowest protective effect under oil stability. The results showed that the solubilization pH of sodium alginate could modulates some properties of interest for food application as the antioxidant and carotenoid migration activity of the films.

KEYWORDS: carotenoids; peroxide index; TGA; oxidation; sunflower oil; antioxidant; packaging; DSC.

1. INTRODUCTION

Packaging is an important material and is practically indispensable for the commercialization of many products. Among the various sectors that use packaging, with the most multiple functions, are consumed by the food and beverage industry 69% of all material produced in the world with this goal (MANALILI; DORADO; OTTERDIJK, 2014). The problems generated by the excessive consumption of plastic packaging are associated with the improper disposal of this material, which for the most part is not directed to collection points for recycling, which can accumulate and generate impacts to the environment (WANG; AUTY; KERRY, 2010). Thus, researchers have developed sustainable, biodegradable *packaging*. However, the low costs of the plastic packaging derived from petroleum made it's competitive and predominant in the market. On the other hand, consumers are increasingly concerned about acquiring food healthy and are conscious about the importance of preserving the environment. Thus, researchers evaluate the use of biopolymers to obtain biodegradable packaging, combining the addition of natural antioxidant compounds to obtain a material with added value and more attractive (ASSIS et al., 2017; PAGNO et al., 2016; STOLL et al., 2017).

Biodegradables films can be produced by sodium alginate and, the polysaccharide may be found in the cell walls of brown seaweed of the *Phaeophyceae* class, a biodegradable polymer and non-toxic to the human organism. Besides this, the sodium alginate may form complexes in the presence of calcium ions and this property results in specific and strong interactions that present a molecular conformation called the "egg box" (ALBOOFETILEH et al., 2014; LI et al., 2016; TAVASSOLI-KAFRANI; SHEKARCHIZADEH; MASOUDPOUR-BEHABADI, 2016). Sodium alginate films complexed with calcium ions, produced by the casting method, are physically resistant, have low water vapor permeability and water solubility, and may be used as food packaging (BIERHALZ et al., 2014; PONGJANYAKUL; PUTTIPIPATKHACHORN, 2007; STRACCIA et al., 2015; ZHANG et al., 2017). The production of sodium alginate films can also be influenced by the pH that the polymer is solubilized, such as obtaining brittle films by decarboxylation of their chain or precipitation at pH below 3.5, while its structure is stable at pH between 3.5 and 10.0, with films with structure that is more cohesive and more elongated bonds. (JANG et al., 2014; LI et al., 2016; LI; NIE, 2016).

Considering the importance of adding value in films by the incorporation of bioactive compounds, it is important to find compounds that have compatibility with the polymer used.

Among the carotenoids, norbixin and its salts are pigments that have a solubility in water in the same pH range as sodium alginate. The norbixin is an apocarotenoid (9-*cis*-6,6'-diapocarotene-6,6'-dioic acid) obtained by the process of alkali demethylation of the carotenoid bixin, the main pigment present in the seeds of the fruit of *Bixa orellana*. The norbixin is extracted in its acid form, which can be neutralized to norbixin salts, and presents partial solubility in water(SILVA; NACHTIGALL, 2009; ZHANG; ZHONG, 2013). Besides, some studies showed that bixin and norbixin have coloring and antioxidant properties (LOBATO et al., 2013, 2015; PAGNO et al., 2016; RIOS; ANTUNES; BIANCHI, 2009; RIOS; MERCADANTE; BORSARELLI, 2007). Like alginate, the solubility of norbixin salts is influenced by the pH. Consequently, their coloring ability and antioxidant capacity may be changed according to the environment. At more alkaline pHs, norbixin salts are more soluble and may exhibit higher color intensity and better antioxidant activity.

As sodium alginate and norbixin have properties influenced by the pH of solubilization, the conditions used during the dispersion preparation could substantially affect the final properties of incorporated biodegradable films of this carotenoid. This work aimed to evaluate the influence of pH the film formulation of sodium alginate incorporated with norbixin salts about its physicochemical, mechanical, colorimetric and antioxidant properties.

2. MATERIAL AND METHODS

2.1. Materials

Anatto seeds (*Bixa orellana)* and sunflower oil were purchased at the local market in Porto Alegre, Rio Grande do Sul, Brazil. To obtain the films were used sodium alginate (PA) with a purity of 90% (g / g) (Dynamics® da Química Contemporânea Ltda.), calcium chloride (PA) pentahydrate with 99.5% (g / g) purity (Nuclear®) and glycerin (Exodus cientifica®) as a plasticizer. The entire experiment was conducted using distilled water to avoid cross-contamination with calcium ions.

2.2. Obtaining norbixin extract

Bixin extraction was performed according to Rios e Mercadante (2004), with some modifications. For each 25 g of anatto seeds, two successive washes were performed with 50 mL of hexane for 15 minutes under magnetic stirring. The seeds were separated by filtration. After that, two successive washes were performed with 50 mL of methanol for 15 minutes under magnetic stirring, and the seeds were again filtered. The bixin present in the anatto seeds were obtained with two successive extraction steps with ethyl acetate with magnetic stirring for 15 min, and the extracts were concentrated in a rotary evaporator (Fisatom, M802, Brazil).

The resulting bixin powder was converted to norbixin by the saponification process as described by Rios and Mercante (2004), followed by modifications. The dry bixin extract was diluted with 50 mL of methanolic potassium hydroxide (10% w / v) on ultrasound (Unique, USC 1650 A, Brazil) and maintained over 12 hours in the dark (overnight) for complete conversion into norbixin salts. The obtained extract was recovered with 150 mL of ethyl acetate/ethyl ether/water (1: 1: 1), and acetic acid was added to a pH of 3.5. The extract was washed with water to pH 6.0. Finally, the norbixin salts were dried on a rotary evaporator (Fisatom, M802, Brazil) and stored at -18 °C. The purity of the norbixin salts was evaluated by High-Performance Liquid Chromatography (HPLC) using a wavelength of 450 nm (TUPUNA et al., 2018). A 96% purity norbixin salts were obtained.

2.3. Preparation of the sodium alginate dispersions

A dispersion was prepared with 1% (w / v) sodium alginate and 2% (w / v) glycerol with pH adjusted to 7.0. Then, the same dispersions were prepared with 0.03% of norbixin (g / g sodium alginate) and adjusted to pH of 3.0, 7.0 and 11.0. A magnetic stirrer (Fisatom, 713D, Brazil) stirred each dispersion at 1000 rpm for 30 min at 20 °C. After stirring, the dispersions were rested protected from light for 12 hours to remove microbubbles incorporated during stirring.

The dispersions were poured into Petri dishes (0.48 g/cm^2) and dried and in an oven at 55 °C, with forced air circulation for 13 hours (De Leo, TLK48, Brazil). After drying, 50 mL of a solution of calcium chloride (1% w/v) was added to each plate and kept for 5 minutes for the desired complexation. After 5 minutes, the liquids were discarded and dried again at 55 °C for 45 minutes. Subsequently, the films were kept for 48 hours in a desiccator at 58% relative humidity at 25 °C. The films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0 were identified as A, B, C, and D, respectively.

2.4. Determination of physicochemical films properties

The water activity of the films was measured instrumentally (Rotronic instruments, HP23, United Kindom). Circular films with a diameter of 2.5 cm were a cutter, and left 5 minutes inside the measuring chamber of the equipment until stabilization. The experiment was carried out in quadruplicate.

Water solubility (WS) was performed gravimetrically, following the method described by Assis et al. (2017). Films samples were immersed in 30 mL of distilled water and softly stirred by a shaker (Novatecnica,209 NT145, Brazil) for 24 h. After the solubilization of the films, the water was discarded, and the samples were dried at 105 °C for 24 h. The water solubility was calculated according to equation (1). The experiment was carried out in quadruplicate. Where Wd is the total mass films and Wf is the insoluble mass films.

$$(5)\ \mathrm{WS}\ (\%) = \frac{(Wd - Wf)}{Wd} x\ 100$$

Swelling index was performed as described by Silva et al. (2009), followed by modifications. Films samples (2 cm x 2 cm) were weighted (Wi) and immersed into aluminum capsules containing 30 mL of distilled water and were stirred for 2 hours at a temperature of 25 ° C. After; the films were surface dried with paper towel and weight (Wf). The swelling index of biodegradable films was calculated according to equation (2). The experiment was carried out in quadruplicate.

$$(6)\ Swelling\ Index\ (SI)\ = \frac{(Wf - Wi)}{(Wi)}$$

The water vapor permeability was determined gravimetrically at 25 °C (ASTM, 2000), followed by modifications described by Assis et al. (2017). The analysis was conducted in triplicate and calculated according to equation (3):

$$(7)\ WVP = \frac{w.L}{\mathrm{A.t.\Delta p}}$$

Where *w* is the weight in grams of water permeated into the capsule through the film, *L* is the average film thickness (mm).measured by a high precision digital micrometer (Digimess, IP40, Brazil). *A* is the permeation surface area in m², t is the permeation time in hours, and Δp is the water vapor pressure difference in kPa between the film surfaces. The experiment was carried out in quadripartite.

2.5. Colorimetric characteristics of films

The colorimetric parameters of the films were measured instrumentally by the colorimeter (Hunter Lab, Miniscan XE, USA) using the CIELab color parameters. The parameters *L** (brightness), *a** (red-green) and *b** (yellow-blue) were determined. To better represent colors, the color parameters were synthesized by Corel Draw X6® software.

The transmittance of the films was evaluated by a UV spectrophotometer (Shimadzu, UV-1800, Japan) at wavelengths of 300 and 600 nm, representing invisible and visible light, respectively. Each film was cut into rectangles and placed in a quartz cuvette. The empty cuvette was used as white (PAGNO et al., 2016).

2.6. Mechanical properties of films: tensile strength and elongation

The mechanical properties of films biodegradables were determined by tensile tests (tensile strength TS), elongation at break (E), and elastic modulus using texturometer (Stable Micro Systems, TA-XT2, United Kindom), at 25 °C. The films were attached between the grips with an initial separation of 50 mm, and the crosshead speed was set at 80 mm/minute. Ten strips of each film were evaluated, and the average value for TSwas expressed in megapascal and % E in percentage (%).

2.7. Thermogravimetric analysis

The analysis was performed in a thermogravimetric (Perkin Elmer, Pyris 1 TGA®, USA) with a nitrogen gas flow rate of 20 mL/min, at a heating rate of 40 °C/min, between 25 and 850 °C to TGA analysis and between 25 and 350 °C to DSC analysis. The analyses were performed in duplicate with 2 mg of weight each sample. The thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) curves were processed by Pyris Manager ® software.

2.8. Scanning electron microscopic analysis (SEM)

The structures of the films were analyzed by scanning electron microscopy (JEOL, JSM-6060®, Japan) with a voltage of 8 kV at a zoom of 5.000 in top view and 1.000 to cross session view. The surfaces of the films were previously vacuum metalized by gold and carbon (BAL TEC, SCD 050, Brazil) to aid in the reading of the images.

2.9. The release of norbixin salts from sodium alginate films

Were evaluate of norbixin salts release to different liquid food simulants. The distilled water (SA) was used to simulate foods with higher water content and with pH above 4.5. An aqueous acetic acid solution (SB) with 3 % (v/v) was used to simulate acid foods with higher water content and pH bellow of 4.5. Ethanol absolute (95 %) (SC) was used to simulated lipid foods.

The films were placed into glass tubes with 15 mL of each simulating solvents. Each sample was carried out in quadruplicate to three different solutions. The tubes were kept at 40 ° C during ten days and without light exposure. After, the norbixin concentration was evaluated by High-Performance Liquid Chromatography (HPLC), as described by Tupuna et al. (2018). The results were expressed according to equation (4):

$$(8)\ \%\ \mathit{Norbixin\ released\ from\ film\ to\ solvents} = \frac{C \times V}{Wn} x\ 100$$

Where C (µg norbixin released / mL solvents) is the norbixin concentration measured by HPLC, V is the volume of 15 mL, and *Wn* is the norbixin contents added into each sodium alginate films (254.46 µg).

2.10. Effect of antioxidant film on sunflower oil storage under accelerated conditions

The oxidative stability of sunflower oil was used to evaluate the antioxidant films as described by Assis et al. (2017). The films were folded into rectangles (110 mm x 60 mm) to forming bags, 13 mL of sunflower oil was transferred into each packaging biodegradable and sealed on top. Sunflower oil samples were packaged in al sodium alginate films with or without norbixin. As controls, sunflower oil was packaged with closed glass bottles. All samples were stored in a chamber (Tecnal, TE-402, Brazil) under the incidence of light with an intensity of 900–1000 Lux (Luxometer VA Instrument, MS6610, China), at 30 °C and relative humidity of about 70 %. The samples were collected after 0, 3 and seven days storage for the determination of peroxide value (AOCS, 1993). Each sample was evaluated in quadruplicate.

2.11. Statistical analysis

All results were evaluated by statistic analysis of variance (ANOVA) and Tukey's test at a significance level of 5% using Statistica 12.0 software (StatSoft, Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Physical-chemical properties of films: water activity, swelling capacity, water vapor permeability

Table 8 shows the physical-chemical properties results from biodegradable sodium alginate films with or without norbixin salts (0.03 %) at different pH solubilization.

The free water may be water molecules not bound or weakly linked to the hydroxyl groups of sodium alginate well as the norbixin salts. Norbixin is a carboxylic acid with apolar structure with protonated groups throughout their structure. When solubilized in alkaline media the protonated structure is neutralized, increasing its polarity and consequently its solubility in water. Similarly, the protonated structure of sodium alginate become deprotonate, increasing its affinity for water molecules. Probably, the alkaline solubilization increased the films affinity for water molecules, forming the closest bonds and significantly reducing ($p < 0.05$) the activity and the water. In addition, it was found that the films prepared with norbixin solubilized at pH 11 had a lower water activity ($p < 0.05$) than the other films

Table 8 - Physicochemical properties (water activity - Aw, water solubility - WS, swelling capacity – SI, and water vapor permeability - WVP) of biodegradable films of sodium alginate complexed with calcium ions and incorporated with norbixin salts with different pH solubilization.

Film	Aw	WS (%)	SI	WVP (g.mm/m^2.h.kPa)
A	0.659 ± 0.014^{a}	54.98 ± 2.65^{a}	4.40 ± 0.07^{b}	0.389 ± 0.006^{a}
B	0.539 ± 0.015^{b}	54.25 ± 7.01^{a}	5.32 ± 0.17^{a}	0.227 ± 0.021^{b}
C	0.508 ± 0.012^{b}	56.72 ± 8.44^{a}	5.31 ± 0.43^{a}	0.397 ± 0.060^{a}
D	0.465 ± 0.021^{c}	57.80 ± 4.48^{a}	5.41 ± 1.19^{a}	0.386 ± 0.043^{a}

The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$). A, B, C, and D: films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0, respectively.

Pawar e Edgar (2012) studied the compounds derived from sodium alginate and observed its stable between pH 5 and 10. At pH above ten the cleavage of the glycosidic bonds of the polymer occurs, depolymerizing the monomers, supporting the dispersion and solubilization of polymer by area contact increase with water molecules, forming a greater number of bonds.

Silva e Nachtigall (2009) evaluated the influence of pH on the extraction and conversion of bixin to norbixin in anatto seeds. According to the authors, when bixin is converted to norbixin by the saponification process, it undergoes hydrolysis of the ester group, forming a carboxylic acid structure, which has a protonated structure with low affinity for water molecules. With the addition of alkaline solutions, neutralization of structure occurs, forming norbixin salts, which have a deprotonated structure with higher affinity for water molecules.

For the water solubility, there was no difference between the different formulations of the films ($p > 0.05$). Several factors may influence the solubility of sodium alginate films, such as the ratio of M and G blocks, sodium alginate and glycerol concentration, bivalent cation type, solubility and calcium concentration, immersion time, pH and temperature.

Pongjanyakul e Puttipipatkhachorn (2007) evaluated the effect of plasticizers on the properties of sodium alginate films incorporated with magnesium and aluminum silica. The authors reported that in pH below 3.5, the solubility of the films reduced. According to the authors, in acid pH occurs the formation of alginic acid that is hydrophobic due to protonation of the polymer structure. The carboxylic groups protonation was also evidenced by Tsai et al. (2017). Other studies have also found a reduction in the solubility of sodium alginate in an acidic environment (DRAGET; SKJAK-BRÆK; SMIDSRØD, 1997; RAYMENT et al., 2009; XU; BARTLEY; JOHNSON, 2003; YANG; CHEN; FANG, 2009).

Bierhalz et al. (2014) developed sodium alginate films incorporated with natamycin and complexed twice with calcium and barium ions with approximately 20% solubility. Probably the double complexation step must have formed more insoluble complexes than the present study. Bierhalz et al. (2012) developed sodium alginate and pectin complexes with calcium ions and incorporated natamycin with solubility three times lower than the present work. The solubility was lower because the complexing process was more intense due to the calcium chloride concentration five times higher this study.

Zhang et al. (2017) evaluated the factors involved in the resistance of the film composed of sodium alginate and gellan gum to be applied internally in paper cups for hot drinks. The authors reported that complexation time and concentration are the factors that most influence on the solubility reduction, where the immersion time of 7 minutes in a 5% (w / v) calcium chloride solution provided films with the lowest possible solubility. However, the authors emphasized how importance is to validating the influence of these parameters on other properties.

Table 8 also shows the index of swelling after 2 hours of hydration of the films. The film without norbixin (film A) hydrated less than norbixin films ($p < 0.05$). The addition of norbixin increased the hydrophilic character of the films, because increased the number of molecules capable of forming bonds with water molecules, increasing the hydration capacity of sodium alginate films and in general, retaining more water internally in the structure of the films. In addition, the pH of the solubilization did not significantly interfere with the hydration capacity of films.

Pongjanyakul e Puttipipatkhachorn (2007) evaluated the effect of plasticizers on the properties of sodium alginate films incorporated in magnesium and aluminum silica. The films after 30 minutes immersed in distilled water presented faster hydration due to the fact that the films were not complexed. The films of the present work, because of a complexation process on the surfacepromoted a surface waterproofing, preventing the penetration of the water along the matrix for a longer time.

The water vapor permeability (WVP) results are shows in table 8. The film B showed the highest ($p < 0.05$) water vapor barrier of all films. Sodium alginate when immersed in aqueous solutions undergoes hydration along the hydroxyl-rich polymer chain. However, when dispersed in acid media, sodium alginate undergoes excessive protonation along the chain, increasing the electrostatic repulsion of the hydroxyls groups, reducing their solubility and consequently precipitating it. The dispersion of sodium alginate prepared in water at pH 3.0 probably formed a more reticulated and compact structure than the other films, forming smaller interstices between alginate chains. The solubilization at pH 7.0 and 11.0 with norbixin salts did not influence the WVP.

Zactiti e Kieckbusch (2006) verified that the permeability of sodium alginate films complexed twice with calcium ions and incorporated with the antimicrobial potassium sorbate reduce with the concentration rising of calcium ions until 2% (w/w). Above this concentration, the permeability of films was kept constant. According to the study, above 3% (w/w) occurred the saturation of the active sites of the sodium alginate chains, not forming more complex. In addition, they found that the complexation with 2% of calcium chloride reduced 20% of the WVP of the non-complexed film.

Paula et al. (2015) developed non-complexed sodium alginate films and mixed with carrageenan with WVP approximately three times higher than this work. The study of Liling et al. (2016) evaluated the effect of the complexation in the WVP of sodium alginate films complexed with calcium ions. The films presented WVP 3 times smaller than the present work because were used ten times more sodium alginate than the present work, forming a more cohesive structure.

3.2. Colorimetric and light barriers properties of films

Table 9 shows the results of the colorimetric parameters of the biodegradable sodium alginate films and the light barrier properties to ultraviolet (300 nm) and visible (600 nm) light.

Table 9 - Colorimetric characteristics and light barrier properties of biodegradable films of sodium alginate complexed with calcium chlorates and incorporated with norbixin salts with different pH solubilization.

Film	Colorimetric parameters			Light transmission (%)	
	L*	a*	b*	300 nm	600 nm
A	96.34 ± 0.43^{a}	5.08 ± 0.05^{a}	-2.28 ± 0.36^{b}	57.14 ± 9.08^{a}	73.49 ± 8.22^{a}
B	94.27 ± 1.00^{b}	3.06 ± 0.23^{b}	20.10 ± 2.83^{a}	34.97 ± 0.40^{c}	52.82 ± 0.69^{b}
C	93.90 ± 1.11^{b}	3.12 ± 0.37^{b}	21.00 ± 0.84^{a}	42.23 ± 0.76^{b}	71.19 ± 1.91^{a}
D	93.71 ± 0.22^{b}	2.92 ± 0.19^{b}	21.36 ± 1.56^{a}	53.71 ± 2.65^{a}	74.53 ± 7.43^{a}

The colors filled in the tables represent the colors resulted from the colorimetric parameters obtained. The values are represented as mean ± standard deviation. Different letters within the same column indicate significant differences (p <0.05). A, B, C, and D: films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0, respectively.

The addition of norbixin salts developed a yellow coloration in the sodium alginate films. However the dispersion pH did not influence the color. The incorporation of norbixin salt, independent of the pH of solubilization, reduced the parameter *L** ($p < 0.05$), indicating the development of darker color. The addition also reduced the parameter *a** ($p <0.05$), meaning in the reduction of red colors. However, the parameter *b** increased ($p < 0.05$) approximately 10 times, resulting in films with yellow shade. During the dispersion of the sodium alginate with norbixin at pH 3.0, the dispersions showed a reddish shade while the dispersions at pH 11.0 showed intense yellow shade. However, after the drying process, the active films developed the same colorations.

The norbixin has never been studied into biodegradable films. However, bixin has already been used as an antioxidant carotenoid in film formulation. Zhang e Zhong (2013) when encapsulated bixin in sodium caseinate, the bixin developed different shades in solutions when they changed the pH values. The authors verified that in acidic pH solutions the bixin presented the parameter *a** higher, while bixin in alkaline solutions presented the parameter *b** greater. Pagno et al. (2016) has been nanoencapsulated bixin and incorporated into cassava starch films. The incorporation developed a yellow coloration in the films and the concentration increased the *b** parameter. Stoll et al. (2018) reported that the incorporation of bixin in films in poly-lactic acid developed a reddish-yellow coloration. Where parameter *b** was greater than parameter *a**, indicating the prevalence of yellow coloration, resulting in similar to this work.

The addition of norbixin significantly reduced ($p < 0.05$) the passage of light through the films at 300 nm, with the exception of films D (Table 2). The film C presented greater protection for non-visible light than light visible. The film had a more reticulate that contributes to an opaque structure due to the precipitation of the sodium alginate provided by the pH reduction. The more compact structure results in a cluster of particles/area, presenting more particles to absorb light. In addition, in acidic solution, the norbixin has solubility reduced and like sodium alginate, then they undergo to precipitation process, forming a more turbid dispersion. The two factors evidently contributed to superior performance to the other films at 300 and 600 nm. It is also interesting to note that the film D presented a similar transmittance ($p > 0.05$) to the film without norbixin (film A). This fact is explained by the solubility of norbixin at alkaline pH. The solubilization of the salts was efficient enough that the films presented significant transparency, where the salts were completely dispersed throughout the filmogenic structure.

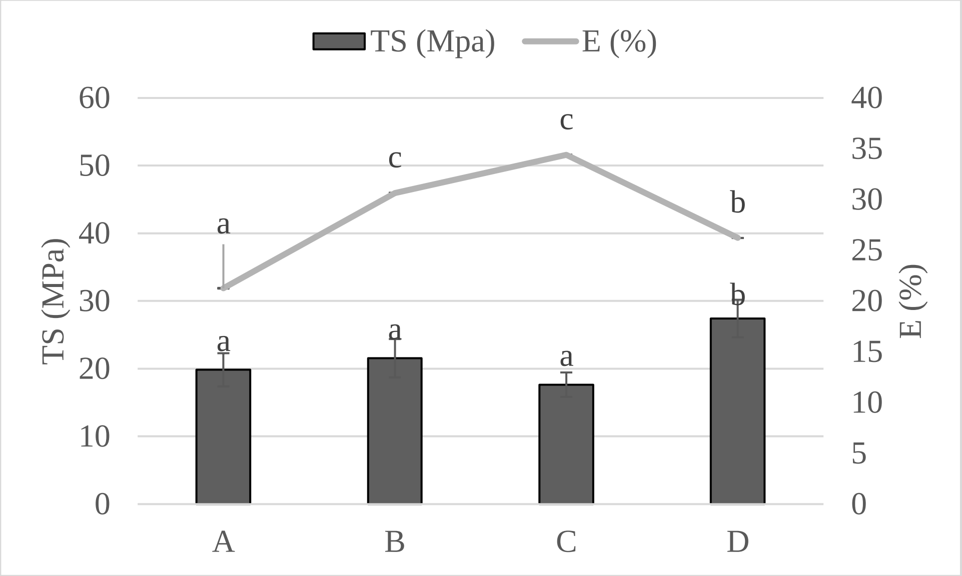
Liling et al. (2016) showed that the complexation process could interfere to light barrier of alginate films. As more intense the complexation, more reticulated the film-forming structure it is, with a structure that retains more light. The authors reported that the increase of calcium ions concentration reduced by 2% of the light passage of films. The films showed a light transmittance of about 80% and 90% for the lengths of 300 and 600 nm, respectively. Norajit et al. (2010) developed sodium alginate complexed with calcium chloride com light transmittance of 57% and 87% at 300 and 600 nm, respectively.

The effect of the pH of solubilization on the light barrier of the films is directly related to the solubility of sodium alginate (RAYMENT et al., 2009; SHAO et al., 2015; YANG; CHEN; FANG, 2009) and norbixin (SILVA; NACHTIGALL, 2009). In acidic pH, occurs the protonation of chains of sodium alginate and norbixin that reduces the electrostatic repulsive forces, causing the precipitation of the particles, forming solutions and films more turbid.

3.3. Mechanical properties of films

The *Figure* 6 shows the results of tensile strength (TS) and elongation (E) of the films. The addition of norbixin did not significantly interfere in the tensile strength of the alginate films. However, the pH of solubilization interferes with tensile strength. The film D presented the highest result (27.42 ± 2.77 MPa) than the other films. Due to the greater dispersion and solubilization of norbixin salts along the filmogenic matrix, forming a less reticulated structure, with smaller points of imperfection (weak points). It's possible that the film D suffered a favored complexation because of the primary structure, formed in the first drying (before complexation), where it was crystallized during drying with alginate chains in maximum deprotonation and repulsion with each other, forming structure more open and promising to penetration and complexation of calcium ions. Besides, the alkaline pH also provided the better dispersion of sodium alginate, improving the distribution of forces applied to the film throughout its structure and thereby withstand tensile strength.

Figure 6 - Tensile strength (TS) and elongation (E) of biodegradable films of sodium alginate complexed with calcium chlorates and incorporated with norbixin salts with different pH solubilization.



A, B, C, and D: films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0, respectively.

The films A (21.26 ± 3.43%) and D (226.21 ± 0.76 %) presented the smaller ($p < 0.05$) elongation capacity, presenting a structure with shorter and rigid connections than other films. The films B (30.66 ± 2.83%) and C (34.40 ± 1.80%) showed the most flexible structure ($p <0 .05$), indicating that the addition of norbixin increased the electrostatic repulsion of the sodium alginate polymers, forming a more elastic structure.

Zactiti e Kieckbusch (2006) developed sodium alginate films complexed with calcium ions in two stages and incorporated with the antimicrobial potassium sorbate with tensile strength two times higher than the films of the present work. However, the films presented a lower elongation that prepared in this work, showing a more rigid and brittle structure. Bierhalz et al. (2014) prepared sodium alginate films incorporated with natamycin and complexed with calcium and barium ions with resistance to attraction approximately five times larger than the present work and elongation approximately six times smaller than this work. Paula et al. (2015) developed non-complexed sodium alginate films and mixed with carrageenan with mechanical properties of approximately 5 MPa of tensile strength and 5% of elongation.

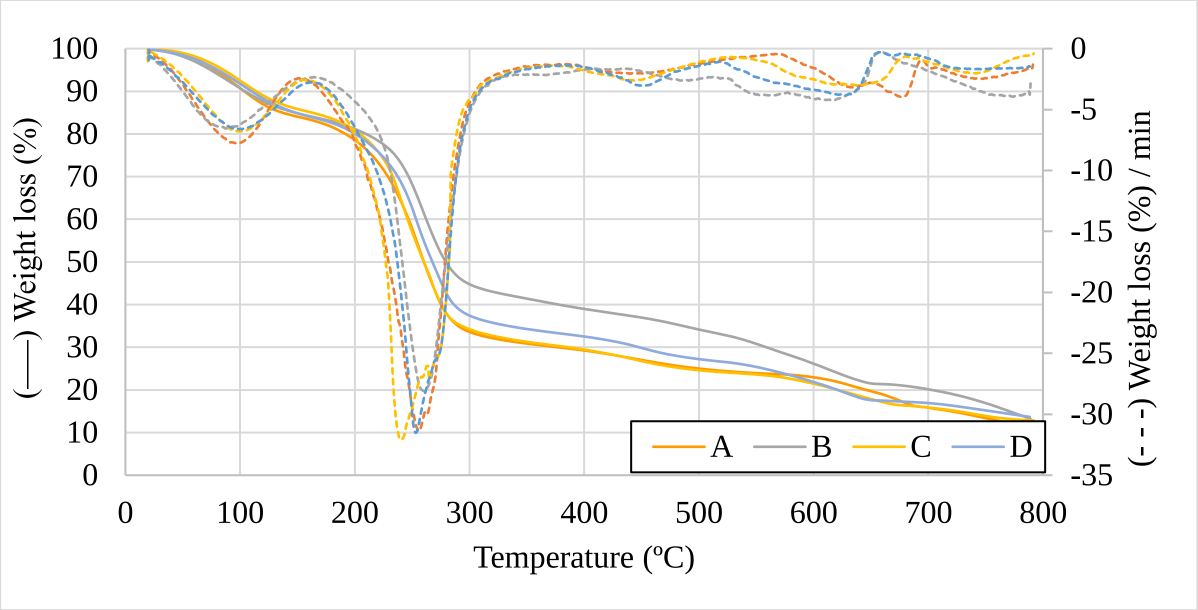
The calcium alginate complex has a shorter and stiffer bond than sodium alginate. The calcium ion is centralized between two G block chain of sodium alginate, reducing the repulsive forces and approaching the two different chains to each other, forming a reticulated structure called the "egg box" (Zheng 1997). As more intense the complexation occurs, more rigid are the films. The process of complexation was better studied by Liling et al. (2016) who verified that the immersion time and concentration of calcium chloride were determinant to mechanical properties of sodium alginate films. According to the authors, the complexation process with 2% (w / w) of calcium chloride and 2 minutes of immersion increased three times the tensile strength of uncomplexed sodium alginate films.

3.4. Thermal properties of films

The films presented three thermal degradation events (Figure 7). The first event occurred between 87 and 97 ° C with a mass loss of approximately 14 to 16% was related to the free water loss of the films. The film B presented the highest loss (15.96%), a result that is in agreement with higher water activity of this film, indicating higher loss of free water.

In the second thermal event, between the temperatures of 239 and 260 ° C, there were the greatest mass losses (41 to 53%). In this temperature range, occurs the mainly degradation process of the hydroxyl groups of the sodium alginate. The B film presented greater stability, with the lowest mass loss (41.65%). From 400 ° C, a series of simultaneous thermal degradation events occurred, possibly related to the oxidation of the remaining central structures of sodium alginate, glycerol, and norbixin.

Figure 7 - Thermogravimetric analysis (TGA ——) and differential thermal analysis (DTA - - - -) of biodegradable films of sodium alginate complexed with calcium chlorates and incorporated with norbixin salts with different pH solubilization.



A, B, C, and D: films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0, respectively. TGA (——) and DTA (- - - -) curves.

Nesic et al. (2016) reported the same thermal events in films composed of sodium alginate and pectin complexed with zinc ions. According to the authors, the first stage of mass loss occurred between the temperatures of 80 and 100 º C, being related to the free water loss of the films. The second thermal event occurred between 100 and 200 º C, is related to the release of bound water and the degradation of alginate carboxyl groups. From 200 º C a series of reactions related to the oxidation of the glycosidic bonds of the polymer began.

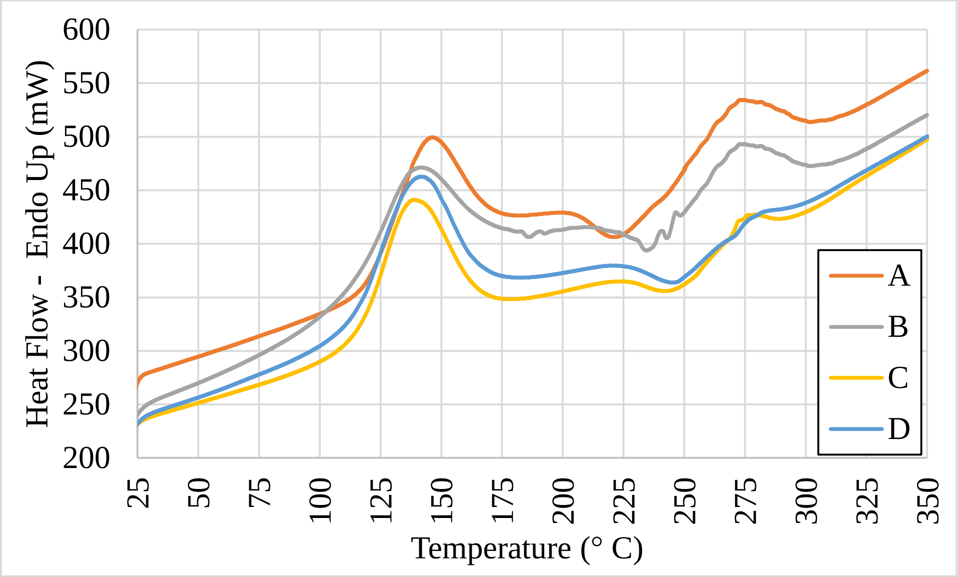
Shankar et al. (2016) reported that sodium alginate films with silver particles had a mass loss between 60 and 110 ° C, which was related to the evaporation of water. The most intense thermal event occurred between 200 and 300 ° C, related to the volatilization of glycerol and the thermal degradation of the polymer. A third thermal event, from 400 ° C, was associated with the decomposition of the complexes formed between alginate and silver particles.

Gao et al. (2017a) developed sodium alginate films through thermal presses and also reported three thermal events of degradation. The first occurred before 200 º C, related to the evaporation of the water, the second occurred between 216 and 300 ° C, is related to the decomposition of the sodium alginate chain. The process was long, with several intermediate peaks related to the breakdown of the glycosidic bonds and dehydration of the polymer saccharide rings. The third stage occurred from 550 º C, being associated with the degradation of the compounds formed in the second stage.

The Figure 7 shows the calorimetric properties of the films. According to the graph, the proposed filmogenic blend showed good compatibility. Sodium alginate films showed two endothermic peaks, the first being between 138 and 145 ° C, indicating the melting point of the crystalline region formed during drying of the monomer of mannuronic acid, which has a more flexible and elongated structure and, consequently, a lower melting temperature. Similarly, the second endothermic peak at 270 to 280 ° C represents the melting point of the crystalline structure of the guluronic acid monomer, which has a more rigid and compact structure, which provides a higher melt temperature than the first peak. The temperature difference between the two regions may be related to the complexation step with calcium ions, which form special bonds with the guluronic acids, forming stronger and more rigid structures with higher melting temperatures. In addition, an exothermic peak found between 200 and 250 ° C was related to the initial oxidation of the sodium alginate structure.

It was not possible to identify interferences in thermal events by the addition of norbixin. According to Zhang e Zhong (2013), the bixin powder has an endothermic peak at the 195 ° C temperature, related to the loss of the crystalline structure of the carotenoid. Since norbixin has a structure similar to that of bixin and because it is in a moisture polymer matrix, the melting temperature may have increased, overlapping by the second endothermic event.

Figure 8 - Differential scanning calorimetry curves (DSC) of biodegradable films of sodium alginate complexed with calcium chlorates and incorporated with norbixin salts with different pH solubilization.



A, B, C, and D: films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0, respectively.

3.5. Scanning electron microscopy

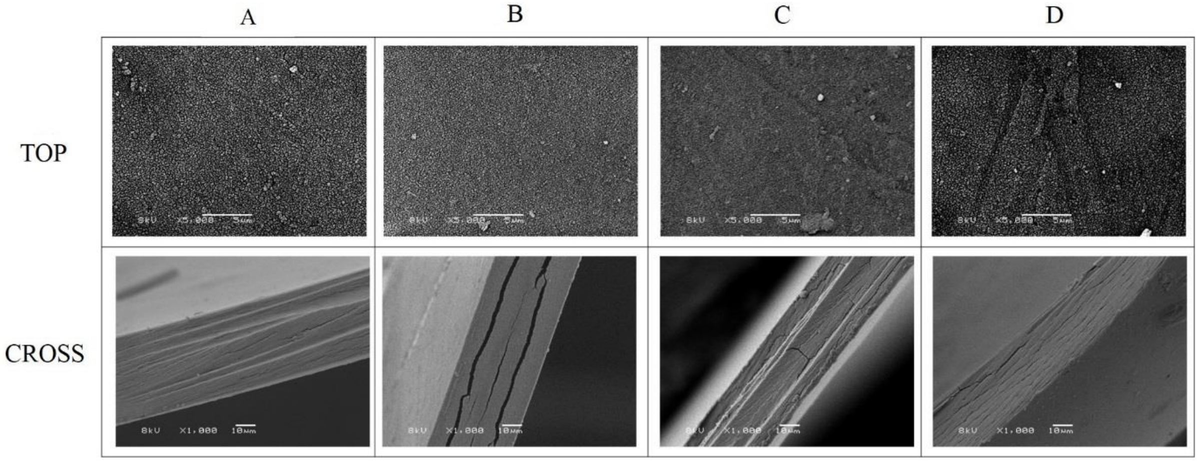
Figure 9 shows the microstructure of the top and cross-sectional (cross-section) films. The films did not present significant visual differences in the surface area, with homogeneous structure, without cracking or formation of agglomerates, resulting from the cohesiveness of the formulation used. A homogeneous and smooth surface is characteristic of the sodium alginate films added with plasticizers and complexed with low concentrations of calcium chloride (AMARA et al., 2016; GALUS; LENART, 2013; GAO; POLLET; AVÉROUS, 2017a; NESIC et al., 2016; PAULA et al., 2015; SHANKAR; WANG; RHIM, 2016). In relation to the cross-section, the films A and D presented a homogeneous structure, indicating a good dispersion/interaction between the compounds. While films B and C showed cracks in the surface not visible to the naked eye.

The film B presented the structure with more cracks, because in pH 3.0 there was precipitation of the alginate with formation of a contracted structure that after the drying process was intensified by the removal of the water, forming transversal cracks. The cracks can allow the higher passage of fluids through the film, as well as decrease the mechanical resistance.

However, film B presented the lowest WVP, and such cracks occurred only in the interior, maintaining the integrated surface structure, without compromising permeability properties.

The incorporation of other compounds into the filmogenic matrixes of the alginate can lead to a loss of film structure. Bierhalz et al. (2014) reported that the addition of natamycin in sodium alginate films complexed with calcium or barium ions resulted in the formation of a heterogeneous structure due to the low miscibility of the crystals of natamycin in the mixture with the polymer. After drying, the solubility of the natamycin crystals occurred, causing their precipitation on the surface of the films.

Figure 9 - Microstructure seen from the top and the cross section of sodium alginate complexed with calcium chlorates and incorporated with norbixin salts with different pH solubilization



A, B, C, and D: films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0, respectively.

3.6. The release of norbixin salts from sodium alginate films

Figure 10 shows the release of norbixin in liquids used to simulate different types of foods. The process of releasing norbixin from the films was identified in 3 steps, as already seen in other works (NESIC et al., 2016; TURBIANI; KIECHBUSCH, 2011). First, the contact of the film with the medium occurs, causing the partial hydration of the water-soluble mannuronic groups (M) and the guluronic groups (G) not complexed with the calcium ions of the sodium alginate polymer network. Second, swelling and expansion of the chain by water molecules occur, increasing the interstices of the films. Third, the release of norbixin into the medium through a leaching process. The differences in results between the films and the liquid could be explained by their influences at each stage of the release process identified above.

First, all films released less than 1.5% of norbixin salts, regardless of the simulant liquid used. The films showed the higher release of the norbixin salts in distilled water (SA) when compared to the acid medium (SB) and the apolar medium (SC). This suggests that the application of the films in foods rich in water with pH higher than 4.5, because the films could release more norbixin salts to act as an antioxidant compound in these foods. The higher release of norbixin in distilled water is due to the affinity of the hydroxyl groups present in the alginate central chain for water molecules. This affinity increases the penetration of water in the interstices of the film facilitating the process of leaching from the pigment to the simulant liquid.

Among the films formulated under different pHs during polymer solubilization, film C released the highest amounts of norbixin salts in distilled water (1.46 ± 0.06%), followed by film D (1.46 ± 0.02%) and B (0.79 ± 0.05%). Since the films showed no significant difference in hydration capacity (SI) in water, the difference in the release of norbixin salts may be related to the structure and pH of the carotenoid solubilization during the preparation of the films.

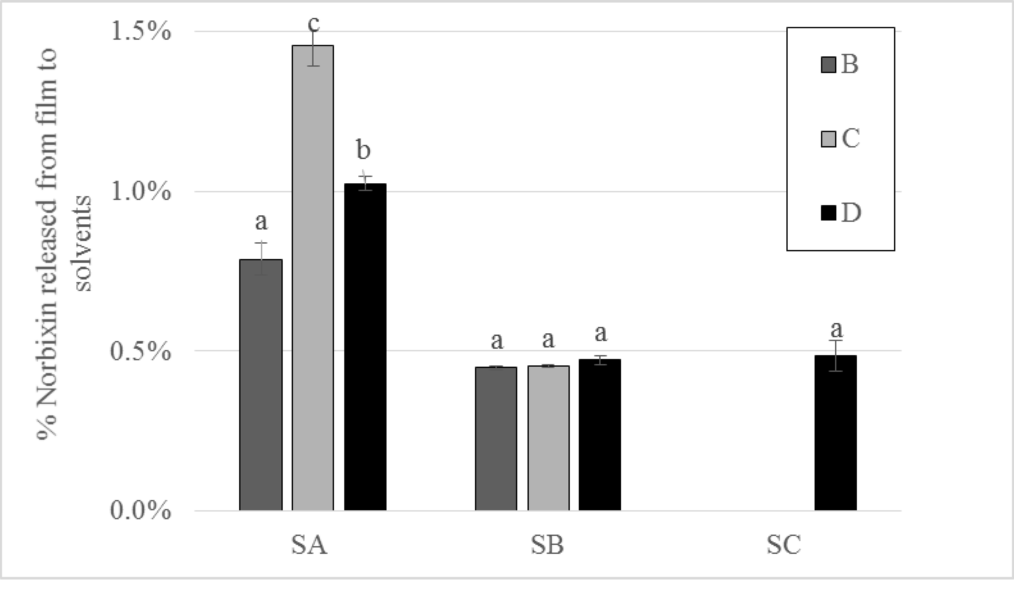
According to the micrographs, it was possible to observe that the film C presented transversal cracks, which can favor the leaching of the pigment. In turn, the film D presented an intact structure, which reduces the contact area of the medium with carotenoid to occur leaching.

Although film B (the least release of norbixin salts in water) showed a structure with more cracks, it is verified that the pH of solubilization interfered in the formation of the film. The salts of norbixin at pH 3.0 undergo dissociation of the potassium salts, forming the norbixin free acid. During drying, saturation of norbixin acid concentration may occur, causing its precipitation in the acid form. Norbixin in the acid form is insoluble in water, and therefore, the films showed a lower release. Furthermore, there may have been sodium alginate precipitation at pH 3.0, with the formation of a more insoluble structure, which reduces the penetration of water inside the films and possibly the release occurs more intensely at the surface of the films.

In the acid simulant medium (SB) there was a reduction in the release of norbixin salts. At pH below 3.5, sodium alginate undergoes a decarboxylation of the polymer chain, which reduces its hydration capacity and partially avoids the entry of water into the film-forming matrix. Therefore, due to the lower availability of water, the norbixin salts were not adequately solubilized. Thus, possibly the released norbixin salts were present on the surface of the film.

Only in film D, there was the release of norbixin salts in ethanol (SC). Probably, the dispersion of norbixin at different pH formed structures with different electrostatic charges, as well as their solubility. The film D formed a structure more able to be hydrated and suffers leaching. Although ethanol is considered the preferred solvent for lipid food simulation, it precipitates sodium alginate, which reduces its hydration (HERMANSSON et al., 2016). When the films were immersed ethanol, instantaneous precipitation of the sodium alginate present on the surface occurred. This change in the surface of the film can prevent the penetration of water inside, as well as the leaching of norbixin. In this way, the leaching process of the pigment from the film occurred only at the surface and the film D with more norbixin dispersion could suffer more hydration action.

Figure 10 - Norbixin released from biodegradable sodium alginate films to different solvents



Solvent SA is destilated water, solvent SB is acetic acid 3 % (v/v) in destilated water and solvent SC is ethanol 95 % (v/v). B, C, and D: films with norbixin at pH 3.0, 7.0 and 11.0, respectively.

3.7. Evaluation of the antioxidant effect of biodegradable sodium alginate films incorporated with norbixin salts with different pH solubilization salts in sunflower oil

Figure 11 shows the peroxide indices (mEq/kg) of sunflower oil packed with the films and stored under light, temperature and controlled humidity. The oil had an initial peroxide value of 2.54 ± 0.10 mEq/kg and was also stored in a standard vessel (closed glass) as a control sample (Standard).

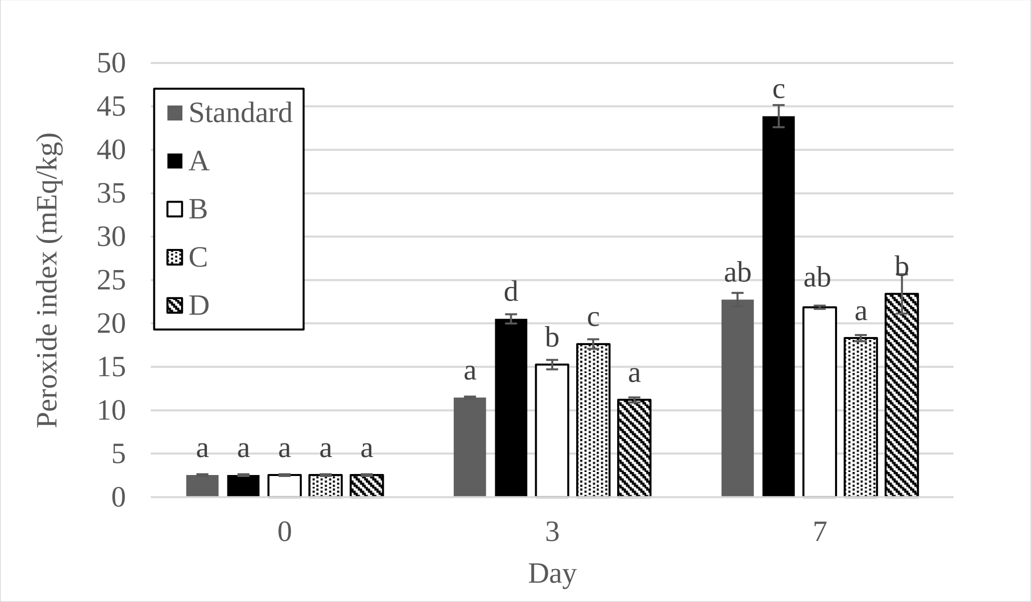
After three days of storage, the oil packed with the D film had a lower peroxide index (11.19 ± 0.29 mEq/kg), followed by the control sample (11.46 ± 0.12 mEq/kg). The incorporation of norbixin salts allowed greater protection of the sunflower oil since the oil packed in the film without norbixin salts had the highest peroxide index (20.53 ± 0.53 mEq/kg). This result is in agreement with the release analysis since the D film was unique that showed the release of norbixin salts in ethanol, a solvent that simulates lipid foods. The B film presented the second best performance in the protection of sunflower oil (15.26 ± 0.54 mEq/kg), probably promoted by its greater backlight protection capacity

After seven days of storage, film C (18.31 ± 0.35 mEq/kg) showed protection of the oil, but with no statistical difference of the Standard (22.74 ± 1.27 mEq/kg) and film B (21.87 ± 0.35 mEq/kg). The film D presented the lowest antioxidant performance when compared to the other bioactive films, as there may have been a more rapid release of the norbixin salts to the oil, exposing them to the accelerated conditions of degradation.

According to Kiokias e Oreopoulou (2006), the antioxidant capacity of norbixin is due to its structure of conjugated double bonds that can prevent the oxidation of oils through the neutralization of excited photosynthesizers or direct reaction with free radicals. The authors, when analyzing the antioxidant capacity of different carotenoids at a concentration of 1 g / L in olive oils, reported that norbixin delayed the formation of peroxides and dienes in oils. In this same study, the authors validated that norbixin delayed the formation of peroxides in 50% and 40% of conjugated dienes.

Pagno et al. (2016), in the development of incorporated cassava starch films of nanoencapsulated bixin, reported an antioxidant capacity of bixin, since the sunflower oils packed with the respective films formed fewer peroxides than oils packed in commercial packages. The authors also reported that the film with a higher concentration of bixin promoted the peroxide formation, different from the other films with lower concentrations, indicating a pro-oxidant potential in higher concentrations.

Figure 11 - Peroxides Index from sunflower oils stored with sodium alginate complexed with calcium chlorates and incorporated with norbixin salts with different pH solubilization



A, B, C, and D: films without norbixin at pH 7.0, with norbixin at pH 3.0, 7.0 and 11.0, respectively. Standard = sunflower oil stored in closed glass packing

CONCLUSION

The addition of norbixin, a natural antioxidant, may add technological value in the development of biodegradable films obtained from sodium alginate complexed with calcium ions. The pH changing during the solubilization of the biopolymer can contribute to get films with specific characteristics, such as better matrix cohesiveness and carotenoid: biopolymer interaction. The adjustment of pH 11 showed better results, with films with higher tensile strength and better pigment release in simulant medium for lipid foods. Behavior that aided in greater stability to the oxidation of sunflower oil in up to three days under controlled storage conditions. However, after seven days, the film prepared with pH 7 presented the best antioxidant activity to sunflower oil. However, all films presented good thermal stability, and the addition of norbixin gave higher color intensity and a better barrier to the light transmission when compared to film without the addition of carotenoid.

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CAPITULO 3 – DISCUSSÃO GERAL E CONCLUSÕES

3. DISCUSSÃO GERAL

Na primeira etapa deste trabalho, foram realizados vários testes preliminares quanto a formulação e condições de processos para a obtenção de um filme biodegradável com aparência, transparência e maleabilidade próxima de uma embalagem plástica convencional. Assim, foi verificado que o aumento da concentração de alginato de sódio resultava em filmes espessos, opacos e com textura “emborrachada”. A concentração de 1 % (p/p) de alginato de sódio, adicionada do plastificante glicerol (2% p/p), mostrou ser adequada para produção dos filmes com boa estabilidade térmica, os quais nesta etapa eram formulados sem a inclusão de sais de norbixina e sem a complexação com cloreto de cálcio.

Embora os filmes não complexados com cloreto de cálcio tenham apresentado aparência e transparência semelhante as embalagens plásticas comercializadas atualmente, os mesmos apresentaram uma baixa resistência física e dificuldade de manuseio. Assim, foi realizado o processo de complexação com diferentes concentrações de cloreto de cálcio (0,5, 1,0, 1,5 e 2,0 % p/p) e diferentes tempos de imersão (1, 5 e 8 minutos) para verificar a melhor condição para obtenção de um filme com características próximas as embalagens plásticas.

Foi verificado que baixas concentrações de cloreto de cálcio e menores tempos de imersão resultavam em filmes flexíveis, porém com alto caráter hidrofílico. Concentrações e tempo de imersão maiores que 1 % e 5 minutos respectivamente, resultaram em filmes quebradiços.

Assim, foi definido a preparação dos filmes a partir de dispersões com 1% de alginato de sódio (p/p) e 2% de glicerol (p/p) vertidas em placas de Petri (0,48 g / cm²) e secas em estufa a 55 ° C por 13 horas. Após esta etapa de secagem, foram adicionados 50 mL de cloreto de cálcio (1% p / v) em cada placa durante 5 minutos para o processo de complexação. Após complexação, o líquido de superfície presente nas placas foi descartado, e as placas de Petri foram retornadas ao forno a 55 ° C por mais 45 minutos. Após a secagem, os filmes foram retirados das placas e mantidos em uma cuba de umidade relativa de 58%, controlada por brometo de sódio, por 48 horas.

A partir destes resultados iniciou-se o primeiro artigo com dois objetivos gerais bem definidos: avaliar o comportamento e estabilidade dos sais de norbixina incorporados nos filmes ao longo do processo proposto; e validar se a norbixina remanescente nos filmes era suficiente para exercer uma atividade antioxidante durante o armazenamento de óleo de girassol sob condições aceleradas.

Ao estudar diferentes concentrações de sais de norbixina (0,05; 0,10 e 0,50% - g de norbixina / g alginato) incorporada aos filmes, foi verificado que a adição do carotenoide desenvolveu uma coloração intensa e atrativa amarelo-vermelhada, que durante o processo de complexação proposto ocorreu lixiviação dos pigmentos em uma concentração máxima de 1,48 %. Ao avaliar a estabilidade da norbixina após o processo, ocorreu a máxima degradação do carotenoide de 15 % em relação ao total adicionado. Além disto, a matriz filmogênica exerceu proteção dos sais de norbixina durante o processo térmico, pois em um controle realizado com o carotenoide diluído em água houve uma degradação até 6 vezes maior.

Em relação à concentração dos sais de norbixina adicionados aos filmes, os resultados indicaram que o aumento desta concentração (0,05, 0,10 e 0,50 %) proporcionou uma redução da permeabilidade a vapor de água e um aumento da barreira a luz UV-visível, sendo que o filme com maior concentração bloqueou em até 98 % a passagem de luz UV (300 nm).

Quanto à estabilidade, as análises colorimétricas indicaram uma perda total do pigmento no filme de 0,05% após 3 dias de armazenamento sob condições aceleradas. Após 7 dias de armazenamento, apenas o filme de maior concentração (0,50%) apresentava coloração amarelada. Contudo, os resultados também indicaram que em tais concentrações, os filmes apresentaram um efeito prooxidante sob óleo de girassol, o que ocasionou a formação de peróxidos quando estes foram armazenados sob condições aceleradas de temperatura e luz. Deste modo, foi fundamental avaliar menores concentrações de sais de norbixina (0,01, 0,03 e 0,05 %) para adição nos filmes de alginato de sódio, de modo que os mesmos pudessem apresentar capacidade antioxidante durante o armazenamento de alimentos. Nesta etapa da pesquisa, os filmes produzidos com menores concentrações do carotenoide apresentaram caráter hidrofílico, com solubilidade e capacidade de hidratação em água indicadas para alimentos lipofílicos.

Nas concentrações estudadas, os filmes apresentaram compatibilidade com a matriz filmogênica formando estrutura homogênea e lisa. A adição de norbixina, além de aumentar o caráter hidrofílico dos filmes, aumentou a estabilidade térmica, reduziu a passagem de luz UV-visível, aumentou a coloração amarelo avermelhada e reduziu a flexibilidade dos filmes. Contudo nas concentrações de 0,03 e 0,05 % de sais norbixina, houve um aumento da permeabilidade a vapor de água dos filmes. Ao avaliar a capacidade antioxidante dos filmes pela determinação da formação de peróxidos no óleo de girassol, foi confirmado que maiores concentrações do carotenoide exerce uma ação prooxidante. O estudo verificou que o filme de 0,03 % de sais de norbixina preveniu a oxidação do óleo de girassol em condições aceleradas por até 6 dias.

Durante o desenvolvimento da pesquisa, também foi verificado que o pH de dissolução do alginato de sódio e dos sais de norbixina, durante o preparo dos filmes, podia influenciar em suas propriedades finais. Em testes preliminares, verificou-se que o pH ácido precipitava o alginato de sódio e o carotenoide, havendo a formação de filmes com colorações mais avermelhados e mais opacos, além de uma estrutura mais quebradiça. Deste modo, foi avaliado as possíveis modificações dos filmes de alginato de sódio incorporados com 0,03 % de sais de norbixina, quando a formulação era produzida sob diferentes pHs (3,0; 7,0 e 11,0). De acordo com os resultados, pH ácidos resultaram em filmes com uma estrutura mais quebradiça, mais compacta e com menor permeabilidade ao vapor de água. O pH não interferiu no caráter hidrofílicos dos filmes (solubilidade e capacidade de hidratação em água). Os resultados colorimétricos indicaram que o pH não influenciou na composição de cor dos filmes. Contudo, o pH 3,0 resultou na formação de filmes mais opacos devido a precipitação do alginato de sódio e do carotenoide. O pH alcalino (11,0) auxiliou na dispersão do alginato de sódio e principalmente dos sais de norbixina, formando filmes com transparência equivalente ao filme sem a incorporação do pigmento. A variação do pH não interferiu nas propriedades térmicas dos filmes.

Nesta etapa da pesquisa, também foi possível avaliar a liberação do pigmento para líquidos simulantes de alimentos. Foi verificado que pela alteração do pH foi possível obter filmes para aplicações em diferentes alimentos. Os filmes preparados com pH 7,0 da formulação são recomendados para alimentos aquosos, com pH acima de 4,5, pois liberam mais sais de norbixina em água neutra, podendo exercer maior atividade antioxidante. O filme preparado em pH 11,0 foi o único capaz de liberar o pigmento em meios lipídicos (simulados através do líquido simulante etanol). Ao avaliar a capacidade antioxidante dos filmes no armazenamento sob condições aceleradas de óleo de girassol, foi verificado que o filme formulado em pH 11 apresentou melhor capacidade antioxidante, provavelmente devido a maior liberação do pigmento para o meio lipídico.

4. CONCLUSÃO

A formulação de filmes proposta neste trabalho, pode propiciar a obtenção de embalagens biodegradáveis com características antioxidantes para o armazenamento de alimentos lipídicos.

Os filmes desenvolvidos apresentaram propriedades compatíveis com o objetivo da presente pesquisa, principalmente devido a compatibilidade entre o carotenoide e blenda filmogênica proposta. Os filmes apresentaram alto caráter hidrofílico, sendo que a adição de sais de norbixina melhorou algumas propriedades de interesse tecnológico e pode agregar valor comercial as embalagens biodegradáveis de alginato de sódio.

O filme formulado em pH 11,0 com 1 % de alginato de sódio, 2 % de glicerol, 0,03% de sais de norbixina e complexado com 1 % de cloreto de cálcio durante o processo de imersão de 5 minutos, apresentou a melhor textura e transparência, com capacidade antioxidante no armazenamento sob condições aceleradas de temperatura e luz de óleo de girassol, o que possibilitou o desenvolvimento de uma embalagem biodegradável, indicada para alimentos lipídicos, o que pode propiciar a redução do consumo de embalagens plásticas derivadas do petróleo e a perda de alimentos.

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