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Aproveitamento de resíduos da indústria alimentícia e nutracêutica no desenvolvimento de ingredientes ativos para aplicação em filmes biodegradáveis

TAINARA DE MORAES CRIZEL

Porto Alegre, 2017.

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APROVEITAMENTO DE RESÍDUOS DA INDÚSTRIA ALIMENTÍCIA E NUTRACÊUTICA NO DESENVOLVIMENTO DE INGREDIENTES ATIVOS PARA APLICAÇÃO EM FILMES BIODEGRADÁVEIS

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Orientador: Profª. Dra. Simone Hickmann Flôres

Co-orientador: Prof. Dr. Alessandro de Oliveira Rios

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Tainara de Moraes Crizel
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TESE

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Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.

Aprovada em: ....../....../......
Pela Comissão Examinadora:

____
Profª. Dra. Simone Hickmann Flôres
Orientadora
Docente do Instituto de Ciência e Tecnologia de Alimentos.
PPGCTA/UFRGS

____
Prof. Dr. Alessandro de Oliveira Rios
Coorientador
Docente do Instituto de Ciência e Tecnologia de Alimentos.
PPGCTA/UFRGS

____
Banca: Profª. Dra. Poliana D. Gurak
Doutora em Ciências de Alimentos
UFCSPA

____
Banca: Profª. Dra. Florência Cladera Olivera
Doutora em Engenharia Química
PPGCTA/UFRGS

____
Banca: Profª. Dra. Jordana Corralo Spada
Doutora em Engenharia Química
UFRGS

Homologada em: ....../....../......
Por:

____
Prof. Dr. Adriano Brandelli
Coordenador do Programa de Pós Graduação em Ciência e Tecnologia de Alimentos
(PPGCTA/UFRGS)

____
Prof. Dr. Vitor Manfroi
Diretor do Instituto de Ciência e Tecnologia de Alimentos
(ICTA/UFRGS)

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RESUMO

Diariamente são descartados no mundo toneladas de resíduos do processamento de frutas que poderiam ser aproveitados pelo seu elevado poder nutricional e funcional, que acabam sendo desperdiçados e podem gerar sérios problemas ao meio ambiente. Outro descarte inadequado que agrava esse problema ambiental é o de embalagens plásticas, que quando não submetidas ao processo de reciclagem trazem enormes danos. Por esses fatores, esse estudo teve como objetivo o aproveitamento de subprodutos da indústria de alimentos para o desenvolvimento de farinhas funcionais e aproveitamento dos resíduos da indústria de capsulas nutracêuticas de gelatina e óleo de chia como matriz para as embalagens biodegradáveis ativas. A quitosana também foi utilizada como matriz no desenvolvimento de filmes aplicados como embalagens. Primeiramente foram avaliados quatro resíduos obtidos de diferentes frutas: resíduo do processamento de suco de mirtilo (bagaço), resíduo do processamento de azeite de oliva (bagaço), cascas de mamão e abacaxi. As propriedades físico químicas, funcionais e antioxidantes desses resíduos foram analisadas, sendo que no geral todos demonstraram alto teor de fibras dietéticas. Em relação às propriedades funcionais a farinha de mamão se destacou pelos elevados valores de capacidade de retenção de água e óleo, pela alta solubilidade e maiores teores de carotenoides (15,56 ± 0,35 mg/100g). A farinha de mirtilo foi a que apresentou o maior poder antioxidante pelo método DPPH (4,62 ± 0,18 IC50 em mg de farinha) e maior teor de compostos fenólicos (23,59 ± 0.85 mg/g GAE), além disso exibiu alto teor de antocianinas. Devido a estas propriedades, a farinha e o extrato do resíduo de mirtilo foram incorporados à gelatina do resíduo do processamento de cápsulas nutracêuticas de óleo de chia para o desenvolvimento de filmes biodegradáveis ativos. Os filmes foram avaliados em relação as suas propriedades mecânicas, de barreira ao vapor da água e luz UV, capacidade antioxidante e aplicação como embalagem em produtos alimentícios. Os resultados sugeriram que a adição de fibras promoveu uma diminuição da resistência à tração e aumento na permeabilidade ao vapor da água. No entanto, a adição de fibra também proporcionou um aumento significativo na barreira de luz UV a 500 nm, sendo eficaz na redução da oxidação lipídica de óleo de girassol. Os filmes com adição de extrato não exibiram alteração nas propriedades mecânicas ou de barreira em comparação com a formulação controle. Além disso, estes filmes exibiram capacidade antioxidante estável por 28 dias. Filmes desenvolvidos com a farinha de mamão e resíduos de gelatina apresentaram comportamento similar aos filmes com resíduos de mirtilo, já que a farinha de mamão também alterou algumas propriedades originais do filme como as propriedades mecânicas e

de barreira, e agregaram poder antioxidante. Com o objetivo de melhorar essas propriedades foram então desenvolvidas micropartículas de farinha de casca de mamão em *spray drying* utilizando o resíduo de gelatina como material de parede. Os resultados indicaram que as micropartículas de casca de mamão ao serem adicionadas na gelatina originaram uma matriz de filme mais contínua e homogênea com aumento da resistência à tração e do módulo de Young. Os filmes com micropartículas (7,5%), quando aplicados como material de embalagem para banha de porco, foram os mais eficientes como barreiras ativas (maior atividade antioxidante), pois um menor teor de peróxidos, dienos e trienos conjugados foram quantificados na amostra após 22 dias. A farinha de resíduos da produção de azeite de oliva também foi utilizada para o desenvolvimento de filmes biodegradáveis, porém o biopolimero utilizado foi a quitosana. A incorporação de farinha de resíduo de oliva na matriz de quitosana também causou alterações na morfologia, tornando o filme mais heterogêneo e áspero. Por esse motivo foram testadas a adição de micropartículas de farinha de oliva nos filmes. A adição de 10% de micropartículas de oliva melhorou significativamente a resistência à tração dos filmes sem alterar as suas propriedades originais. A farinha e as micropartículas de oliva aumentaram a capacidade antioxidante dos filmes, esse aumento foi proporcional à concentração de farinha ou micropartículas adicionadas ao filme. Os filmes com 30% de farinha ou micropartículas foram eficazes como embalagem protetora contra a oxidação de nozes durante 31 dias. A partir dos resultados obtidos neste trabalho fica evidenciado a viabilidade do uso de resíduos da indústria de alimentos e resíduos da indústria de cápsulas nutracêuticas para o desenvolvimento de filmes e uso como embalagens biodegradáveis em diferentes produtos.

Palavras-chave: Compostos antioxidantes; Resíduos de cápsulas de gelatina; Quitosana; Bagaço de mirtilo; Casca de mamão e abacaxi; Resíduos da indústria de azeite de oliva; Filmes biodegradáveis.

ABSTRACT

Every day tons of fruit processing residues are discarded worldwide that could be harnessed for their high nutritional and functional power and that end up being wasted and generating problems for the environment. Another inadequate disposal that aggravates this environmental problem is the plastic packaging, which when not subjected to the recycling process bring huge damages. Due to these factors, this study aims at the utilization of by-products of the food industry for the development of active biodegradable packaging. Firstly, four residues obtained from different fruits, processing residue of blueberry juice (bagasse), processing residue of olive oil (bagasse), peels of papaya and pineapple were evaluated. The physicochemical, functional and antioxidant properties of these residues were analyzed, and in general, all showed high total dietary fiber content. In relation to the functional properties, papaya flour was distinguished by high water and oil retention capacity, high solubility and higher carotenoid content (15.56 ± 0.35 mg / 100g). The blueberry flour had the highest antioxidant power by the DPPH method (4.62 ± 0.18 IC50 in mg of flour) and a higher content of phenolic compounds (23.59 ± 0.85 mg / g GAE), in addition, it exhibited a high content of anthocyanins. Due to these properties, the flour and extract of the blueberry residue were incorporated into the gelatin from the processing residue of chia oleuroceutical capsules for the development of active biodegradable films for packaging. The films were evaluated in relation to their mechanical properties, water vapor barrier, and UV light, antioxidant capacity and application as packaging in food products. The results suggested that fiber addition promoted a decrease in tensile strength and an increase in water vapor permeability. However, the addition of fiber also provided a significant increase in the UV light barrier at 500 nm being effective in reducing the lipid oxidation of sunflower oil. Films with added extract showed no change in mechanical or barrier properties compared to the control formulation. In addition, these films exhibited a stable antioxidant capacity for 28 days. Films developed with papaya flour and gelatin residues showed similar behavior to films with blueberry residues since papaya flour also altered some of the original properties of the film as mechanical and barrier properties, and added antioxidant power. In order to improve these properties microparticles of papaya peel flour were then developed in spray drying using the gelatin residue as the wall material. The results indicated that the microparticles of papaya peel, when added to gelatin, gave a more continuous and homogeneous film matrix increasing tensile strength and Young's modulus. Microparticles films (7.5%), when applied as packaging material for lard, were the most efficient as active barriers (higher antioxidant activity)

because a lower peroxide content was quantified in the sample after 22 days. The residue flour from olive oil production was also used for the development of biodegradable films, but the biopolymer used was chitosan. The incorporation of olive residue flour in the chitosan matrix also caused changes in the morphology, making the film more heterogeneous and rough. For this reason, the addition of olive flour microparticles in the films was tested. The addition of 10% of olive microparticles significantly improved the tensile strength of films without altering their original properties. The flour and the microparticles of olive increased the antioxidant capacity of the films; this increase was proportional to the concentration of flour or micro added to the film. Films with 30% flour or microparticles were effective as protective packaging against Walnut oxidation for 31 days. From the results obtained in this work, it is evident the viability of the use of residues from the food and waste industry of the nutraceutical capsule industry for the development of films and use as biodegradable packaging in different products.

Key words: Antioxidant compounds; Gelatine capsule residues; Chitosan; Blueberry bagasse; Peel of papaya and pineapple; Waste from the olive oil industry; Biodegradable films.

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CAPÍTULO 1

1. Introdução

A crescente demanda do público por conveniência e conscientização dos efeitos benéficos que as frutas exercem sobre a saúde tem levado ao aumento destas na forma de produtos minimamente processados ou de sucos.

Uma grande variedade de resíduos são gerados pelas indústrias de processamento de alimentos, em especial a de processamento de frutas. Na indústria de sucos calcula-se que do total de frutas processadas, sejam gerados de 30-90 % de resíduos, tais como cascas, sementes e polpa. O processamento de frutas como laranja, limão e tangerina podem gerar de 50-60 % de resíduos em relação a massa da fruta, maçã de 25-35 %, banana 30-40 %, abacaxi 50 %, manga 35-60 %, maracujá 65-70 % e açaí 90 % (DELGADO & FLEURI, 2016; O'SHEA et al., 2012; MARTINS et al., 2009) Na indústria de frutas minimamente processadas os resíduos concentram-se basicamente em cascas e sementes de diferentes formas e tamanhos que normalmente não têm mais uso e são comumente descartados (AJILA et al., 2007).

Esses resíduos representam sérios problemas para a indústria, por possuirem limitadas aplicações de uso e serem de fácil contaminação microbiológica (FERNÁNDEZ-LÓPEZ et al., 2004). Neste contexto, o aproveitamento integral das frutas poderia gerar benefícios econômicos aos produtores, além de proporcionar menor desperdício e consequentemente reduzir o impacto desses resíduos sobre o meio ambiente.

Comumente os resíduos da indústria de processamento de frutas são utilizados em ração animal, o que acarreta em grande desperdício visto que possuem uma grande quantidade de fibras e ingredientes bioativos como os compostos fenólicos (LÓPEZ-MARCOS et al. 2015).

O uso dos resíduos de frutas é de grande interesse para a indústria, a fim de isolar fitoquímicos específicos para aplicação em suplementos nutracêuticos, aditivos alimentares, novos alimentos, o que contribui para a recuperação de resíduos de processos agro-industriais, com grande impacto ambiental. Além de serem fonte natural de antioxidantes antimicrobianos, aromatizantes, corantes e texturizantes, também são fontes de pectina e fibras alimentares, substâncias consideradas estratégicas para a indústria de alimentos, (O'SHEA et al., 2012; AYALA-ZAVALA et al., 2011; PETKOWICZ et al., 2017).

A adição de farinhas obtida de resíduos de frutas e ricas em fibra dietética proporciona benefícios econômicos para a indústria de alimentos, cosméticos e farmacêutica, já que além dos efeitos benéficos sobre a saúde também mostra algumas propriedades funcionais quando utilizada como aditivo alimentar, tais como a capacidade de retenção de água/óleo, o aumento da viscosidade ou de formação de gel, que são essenciais na formulação de certos produtos alimentares (AYALA-ZAVALA et al., 2011; ELLEUCH et al., 2011). Algumas fibras ou farinhas obtidas de resíduos de frutas podem ser utilizadas como substitutos de gordura em vários alimentos, tais como sorvetes (CRIZEL et al., 2013; CRIZEL et al 2014), pães (STOLL et al., 2015), muffins (MARTÍNEZ-CERVERA et al., 2011), salsichas (GRIGELMO-MIGUEL et al., 1999; GARCÍA et al., 2002; VIUDA-MARTOS et al., 2010), carne de hambúrguer (SELANI et al., 2016) e nuggets de frango (VERMA et al., 2009).

Dentre as frutas que geram grande quantidade de resíduos de alto valor nutricional e tecnológico, porém ainda com poucos estudos quanto seu aproveitamento destaca-se o mirtilo, o mamão, abacaxi e a oliva. Sendo assim, tais resíduos podem ser utilizados para elaboração de farinhas ricas em fibras e compostos antioxidantes.

Essas farinhas ricas em antioxidantes podem ser encapsulados para a proteção contra a oxidação desses pigmentos naturais e suas propriedades bioativas, já que são muito instáveis quimicamente (AZEREDO, 2005). Além disso o processo de microencapsulação, possibilita, tornar esses compostos mais solúveis em água e em outras matrizes e consequentemente melhorar as propriedades tecnológicas dos produtos em que forem adicionados, sejam eles alimentícios ou filmes utilizados como embalagem biodegradável (MATIOLI & RODRIGUEZ-AMAYA, 2003).

Resíduos obtidos da indústria de suco de mirtilo podem ser matérias-primas úteis para elaboração de farinha, já que o mirtilo se destaca entre as frutas pelo seu que alto teor de compostos fenólicos e antocianinas, principalmente em sua casca (SU & SILVA, 2006).

As cascas oriundas do processamento de mamão também são uma boa fonte de fibras dietéticas e compostos antioxidantes (STEFANELLO & ROSA, 2012). Devido a todas essas propriedades, esses resíduos podem ser aproveitados de diversas maneiras, principalmente para agregar valor funcional e/ou antioxidante no desenvolvimento de novos produtos ou embalagens.

Assim como os resíduos do processamento de mirtilo e mamão, os resíduos de abacaxi também podem ser utilizados na produção de farinhas ricas em fibra, já que possuem em média 75% de fibra insolúvel, ou seja, grande quantidade de celulose e hemicelulose,

podendo ser utilizado pela indústria de alimentos como ingrediente, para enriquecer produtos ou melhorar propriedades mecânicas e funcionais de embalagens biodegradáveis (MARTÍNEZ et al., 2012; IAHNKE et al., 2016).

O descarte de resíduos derivados da extração do azeite de oliva representa um grande problema para a indústria, devido seu elevado teor lipídico. Esses resíduos possuem um elevado potencial antioxidante, caracterizado pela presença dos compostos fenólicos em especial o hidroxitirosol (RUBIO-SENENT et al., 2012).

Os extratos de compostos antioxidantes obtidos a partir de resíduos de frutas também podem ter diversas aplicações na produção de ingredientes alimentares. Recentemente pesquisadores têm voltado sua atenção para aplicação destes compostos em embalagens ativas, tais como filmes biodegradáveis. Filmes comestíveis e revestimentos podem ser usados como transportadores de compostos bioativos, agentes antimicrobianos tais como óleos essenciais de frutas ou qualquer outra substância que promova um aumento de vida útil ao alimento revestido (DU et al., 2012).

Considerando tal contexto, é de grande importância avaliar a conversão de resíduos em ingredientes alimentícios e outros materiais de valor agregado. A extração de compostos bioativos e obtenção de farinhas a partir de resíduos agroindustriais representam uma tendência no desenvolvimento de novos produtos funcionais, tendo em vista a elaboração de ingredientes que podem ser utilizados tanto para enriquecer nutricionalmente (fibras e compostos antioxidantes) o alimento como também no desenvolvimento de embalagens ativas e funcionais.

Este trabalho está apresentado na forma de 4 artigos científicos (capítulos 5, 6, 7 e 8), discussão geral (capítulo 9) e conclusão geral (capítulo 10).

CAPÍTULO 2

2. Objetivos

2.1. Objetivo Geral

Estudar o potencial aproveitamento de resíduos da indústria de processamento de frutas na obtenção de farinhas e ingredientes para aplicação em embalagens biodegradáveis.

2.2. Objetivos específicos

- Obtenção de farinha a partir de resíduos das frutas como, mamão, abacaxi, mirtilo, e oliva provenientes de seu processamento industrial.
- Avaliação físico química, propriedades tecnológicas e capacidade antioxidante das farinhas obtidas.
- Aplicação da farinha e extrato de bagaço de mirtilo em filmes biodegradáveis de resíduos de capsulas nutracêuticas de óleo de chia.
- Verificar a influência da adição dos resíduos de mamão e oliva na forma de farinha e micropartículas em matriz de gelatina (resíduos de capsulas nutracêuticas) e quitosana, respectivamente.
- Aplicação dos filmes biodegradáveis com farinha e com micropartículas de resíduos em produtos alimentícios.

CAPÍTULO 3

3. Revisão Bibliográfica

O processamento industrial de produtos agrícolas no Brasil para extração de sucos, polpas e óleos gera uma grande quantidade de resíduos, como cascas, bagaço e sementes, que possuem elevado valor nutricional e podem ser aproveitadas para obtenção de ingredientes, principalmente se o resíduo for oriundo de frutas e vegetais (MIRABELLA et al., 2014)

3.1. Resíduos da indústria de frutas

O Brasil é o terceiro maior produtor mundial de frutas sendo que em 2013 foram colhidas 43,6 milhões de toneladas de frutas. Além disso, estima-se que no mesmo ano a indústria do processamento consumiu 23,8 milhões de toneladas do total de frutas produzidas (IBRAF, 2015).

A indústria de alimentos gera grandes volumes de resíduos, resultantes do processamento de frutas utilizadas para produção de sucos, produtos minimamente processados, geleias e outros doces (LIMA et al., 2014; TARAZONA-DÍAZ & AGUAYO, 2013). Esses resíduos são compostos por cascas, bagaço, sementes e polpa que são gerados em diferentes etapas do processo industrial e, normalmente, não têm mais uso sendo comumente desperdiçados ou descartados (AJILA et al., 2007).

O bagaço das frutas é um resíduo que constitui cerca de 20-25% do peso da fruta fresca. É tratado como um lixo industrial com pouco valor econômico e é frequentemente utilizado para compostagem. Uma vez que o bagaço contém grande quantidade de água (66,4-78,2%, base úmida) e açúcares fermentáveis (3,6%), seu descarte diretamente no solo cria preocupações ambientais devido à fermentação não controlada e à elevada quantidade de oxigênio gerada durante a sua degradação (PARAMAM et al., 2015).

O aproveitamento integral de resíduos é uma necessidade cada vez maior na indústria moderna em nível mundial, já que a quantidade de resíduos pode chegar a várias toneladas; e agregar valor a esses produtos é de interesse econômico e ambiental necessitando, porém, de investigação científica e tecnológica, que possibilite sua utilização eficiente, econômica e segura (LÓPEZ-MARCOS et al., 2015)

Os resíduos obtidos a partir do processamento de frutas, contém maior de teor de compostos fenólicos e atividade antioxidante do que a polpa e por isso podem ser utilizados como fontes de ingredientes funcionais (TARAZONA-DÍAZ & AGUAYO, 2013; AYALA-ZAVALA et al., 2011;KUNRADI VIEIRA et al., 2009; AJILA et al., 2007). Dentre os resíduos de frutas que se destacam pelo elevado valor nutricional e tecnológico, porém com reduzido aproveitamento, são os resíduos de mirtilo, mamão, abacaxi e oliva.

3.1.1. Mirtilo

Os Estados Unidos são os maiores produtores mundiais de mirtilos, em 2014 um total de aproximadamente 303 milhões kilos de mirtilos foram produzidos (NASS, 2015). No Brasil a produção ainda é pequena e concentra-se no estado do Rio Grande do Sul, que produz 75% da produção nacional de mirtilo.

As frutas são normalmente consumidas frescas porém possuem vida de prateleira relativamente curta, e por isso os mirtilos são frequentemente processados para obtenção de sucos e geléias. Esse processamento gera uma quantidade grande de resíduos que podem ser ser matérias-primas úteis para a criação de novos produtos de valor agregado.

Os resíduos gerados são compostos por casca, bagaço e sementes que podem representar 15-55% da massa original da fruta inteira, esses resíduos contêm maiores concentrações de antocianinas e fenólicos se comparados com a polpa (BENER et al. 2013).

Devido ao alto conteúdo desses antioxidantes o bagaço obtido da extração de mirtilo apresenta grande potencial para recuperação e aproveitamento destes compostos com a finalidade de serem utilizados em diferentes aplicações tecnológicas da indústria de alimentos (MIRABELLA et al., 2014).

3.1.2. Mamão

O mamão (*Carica papaya*) é nativo da América Tropical e sua maior produção está no Brasil, Peru, Venezuela e Filipinas, estando disponível para o consumo ao longo de todo o ano. O mamão tem espaço garantido nas prateleiras das frutas frescas minimamente processadas. Esse tipo de produto tem ganhado destaque devido à crescente demanda do

público por conveniência e praticidade juntamente com a consciência dos benefícios das frutas para a saúde (TARAZONA-DÍAZ & AGUAYO, 2013).

A produção de mamão em cubos gera em torno de 7 % de sementes, 8, % de cascas, 32 % de polpa inutilizável (devido à falta de forma uniforme do cubo), e 53 % de produto final (AYALA-ZAVALA et al., 2010). As cascas oriundas do processamento de mamão também apresentam novas e interessantes aplicações tecnológicas, uma vez que são consideradas boa fonte de fibras dietéticas, proteínas, gordura, minerais e compostos antioxidantes, tais como carotenoides (STEFANELLO & ROSA, 2012).

Devido a este forte apelo nutricional, Kang et al. (2010) propuseram nova e interessante utilização de resíduo do processamento de mamão. Os autores utilizaram este resíduo como substrato para crescimento de levedura (*Sacharomyces cerevisae)* e aplicaram no desenvolvimento de ração de alto valor biológico para camarões.

3.1.3. Abacaxi

O abacaxi se destaca entre as frutas brasileiras e está presente no mercado, praticamente, o ano todo. No ano de 2014 foram produzidas mais de 3 milhões de toneladas da fruta no Brasil (IBRAF, 2015).

Os resíduos obtidos do seu processamento industrial representam cerca de 73 % do total da fruta e é composto por casca, talo e coroa (BOTELHO et al., 2002). A casca de abacaxi contém altos níveis de fibras insolúvel, essa fração da fibra tem características que permitem sua aplicação na indústria alimentar (HUANG et al., 2014).

Morais Ribeiro da Silva et al. (2014) quantificaram níveis de vários compostos bioativos de resíduos de frutas tropicais do Brasil tais como abacaxi, mamão, acerola, maça, goiaba e manga e verificaram que os resíduos destas frutas apresentaram maiores níveis de compostos bioativos quando comparados com suas polpas, tendo, portanto, grande potencial para futuras aplicações na indústria de alimentos.

De acordo com um estudo de Martínez et al. (2012) subprodutos de abacaxi (casca e coração) apresentaram elevado teor de fibra dietética total (75,8%), além de mostrar elevada capacidade de retenção de água e potencial antioxidante.

3.1.4. Oliva

A produção de azeite de oliva é uma atividade agroindustrial bastante significativa nos países Mediterrâneos, está associada com a geração de grandes quantidades de resíduos (LOZANO-SÁNCHEZ et al., 2011). Segundo dados fornecidos pela empresa processadora de azeite de oliva “Olivas do Sul”, a quantidade de resíduos gerada pode chegar a 85 % do peso inicial da fruta.

O bagaço de oliva é um dos principais resíduos da indústria de alimentos, sendo que esses são ricos em compostos fenólicos com um grande interesse biológico e farmacêutico, devido as suas propriedades antioxidantes; é um resíduo sólido que contém os fragmentos de pele, polpa, pedaços de caroço e alta quantidade de óleo. Seus principais ingredientes são açúcares, principalmente, na forma de polissacáridos, proteínas, ácidos graxos como o ácido oleico e outros ácidos C2-C7, poliálcoois, polifenois, e outros pigmentos (KARANTONIS et al., 2007).

Entre os principais compostos fenólicos encontrados na oliva estão a oleuropeína e o hidroxitirosol (BOUAZIZ et al., 2008). Estes compostos vêm sendo estudados devido à sua presença nos resíduos de oliva e sua elevada atividade biológica, além de diversas aplicações promissoras em alimentos, cosméticos e medicamento (MIRABELLA et al., 2014). Além disso, a partir do resíduo de oliva pode ser extraída a pectina, de acordo com Cardoso et al. (2003), a capacidade de geleificação do extrato é comparável à da pectina cítrica vendida comercialmente. A pectina pode ser utilizada na indústria alimentar como agentes estabilizantes, gelificantes e emulsionantes, além disso podem também ser utilizadas como material de parede para encapsular compostos tais como ácido fólico e licopeno (RUBIO-SENENT ET AL., 2015; MADZIVA, KAILASAPATHY, & PHILLIPS, 2005; SHU, YU, ZHAO & LIU, 2005).

3.2. Ingredientes obtidos a partir dos resíduos

Muitos são os ingredientes que podem ser obtidos a partir dos resíduos oriundos do processamento de frutas e da indústria farmacêutica. A partir dos resíduos das frutas podem ser obtidos extratos naturais ricos em antioxidantes como os carotenoides e antocianinas, farinhas ricas em fibras e compostos bioativos e micropartículas dessas farinhas ou dos compostos extraídos dos resíduos (CONTRERAS-CALDERÓN et al., 2011; LIMA et al.,

2014; FLORES et al., 2014). Como resíduos aproveitáveis da indústria farmacêutica se destacam os resíduos de gelatina oriundos das capsulas nutracêuticas de óleos de coco, cartamo, chia e linhaça.

3.2.1. Farinhas ricas em fibras

Os resíduos agroindustriais (sementes, cascas, bagaço) podem ser utilizados no processo de fabricação de farinhas como matéria-prima rica em fibras (MARQUES et al., 2014). Isso se torna importante considerando que a fibra dietética está entre os alimentos funcionais mais importantes devido a seus inúmeros benefícios para a saúde (NIBA, 2012).

A fibra dietética como uma classe de compostos inclui uma mistura de polímeros de carboidratos das plantas, substâncias pécticas, gomas, amido resistente, inulina, que podem ser associados com a lignina e outros componentes diferente dos carboidratos (por exemplo, polifenois, ceras, saponinas, cutina, fitatos, proteína resistente) (ELLEUCH et al., 2011).

As fibras dietéticas são tipicamente classificadas como polímeros que são solúveis em água, fibra dietética solúvel (FDS) ou insolúveis em água, fibra dietética insolúvel (FDI). A maioria dos alimentos de origem vegetal contém uma combinação de fibra solúvel e insolúvel (HASSAN et al., 2011).

De acordo com "American Heart Association" (2011) e a Agência Nacional de Vigilância Sanitária (2003) recomenda-se a ingestão mínima de 25 g de fibra dietética por dia. A maioria dos profissionais de nutrição sugerem que cerca de 20 % a 30 % da ingestão diária de fibras deve vir de fibras solúveis (ELLEUCH et al., 2011).

Os resíduos agroindustriais são ricos em fibras dietéticas (FD) com efeitos positivos para a saúde. A adição de fibra dietética fornece benefícios econômicos para as indústrias de alimentos, cosméticos e farmacêuticos (AJILA et al., 2010). Além dos efeitos benéficos sobre a saúde, as FD demonstram algumas propriedades funcionais, como a capacidade de retenção de água, capacidade de inchaço, aumento da viscosidade ou formação de gel que são essenciais na formulação de certos produtos alimentícios (AYALA-ZAVALA et al., 2011).

Resíduos ricos em fibra alimentar são interessantes para os processadores de alimentos, especialmente porque os consumidores preferem ingredientes naturais, temendo que compostos sintéticos possam ser fonte de toxicidade. Além disso, a fibra dietética possui notáveis benefícios nutricionais e efeitos protetores sobre a saúde humana, tais como

prevenção do câncer de cólon e de diversos tipos de doenças cardiovasculares (PALAFOX-CARLOS et al., 2010; SPILLER, 2005).

A incorporação de resíduos ricos em fibras, como farelo de trigo, em cereais matinais, farelo de arroz, bagaço de cana, farelo de trigo em pão e fibra dietética de pêssego em geleia foram investigados por Elleuch et al., (2011). FD de diferentes fontes foram incluídas em diferentes alimentos funcionais, tais como barras de frutas, pão, bebidas e outros alimentos processados (BERTAGNOLLI et al., 2014; MARQUES et al., 2014; BHOL et al., 2016).

Propriedades texturais e estabilizadoras são resultado das propriedades de hidratação das fibras. Os mecanismos diferem de acordo com a solubilidade das fibras. As propriedades de espessamento (por exemplo, goma xantana) e de geleificação (por exemplo, carragena e pectinas) e a capacidade de retenção de água contribuiram para a estabilização da estrutura dos alimentos (dispersões, emulsões e espumas), modificando as propriedades reológicas da fase contínua. Devido à sua capacidade de retenção da água e propriedades de inchaço, as fibras insolúveis podem influenciar na textura dos alimentos (THEBAUDIN et al., 1997). A indústria de alimentos pode se beneficiar das propriedades físico-químicas da fibra para melhorar a viscosidade, textura e características sensoriais de seus produtos (ELLEUCH et al., 2011).

Além disso, os resíduos ricos em fibras podem ser incorporados em produtos alimentícios como agentes espessantes de baixo custo e não-calóricos para parcial substituição de farinha, gordura ou açúcar, como potenciadores de retenção de água e óleo e para melhorar a emulsão ou a estabilidade oxidativa (ELLEUCH et al., 2011). No entanto, o porcentual de fibras que pode ser adicionado aos alimentos é limitante, pois pode causar alterações indesejáveis na cor, sabor e na textura dos alimentos (ELLEUCH et al. 2011).

A literatura contém muitos relatos sobre adição de fibra dietética para produtos alimentares, tais como produtos de panificação, bebidas, produtos de confeitaria, produtos lácteos, sorvetes, embutidos cárneos, massas e sopas. Mais comumente, as fibras dietéticas são incorporadas em produtos de padaria para prolongar o frescor desses produtos, devido à sua capacidade de retenção de água (AYALA-ZAVALA et al., 2011).

As fibras ou farinhas ricas em fibras também podem ser adicionadas em filmes biodegradáveis para aumentar o poder antioxidante e/ou melhorar propriedades estruturais.

Nascimento et al. (2012) caracterizaram filmes flexíveis à base de amido e farinha de mesocarpo de maracujá (FMM) com nanopartículas de argila. Filmes produzidos a partir de FMM foram mais hidrofílicos em comparação com filmes de amido. Quanto às propriedades

mecânicas, os filmes feitos a partir de FMM revelaram-se mais resistentes, mais fortes e menos flexíveis já a formulação com base numa mistura de FMM e amido resultou em filmes menos rígidos e menos resistentes à tensão, em comparação com filmes baseados apenas em FMM.

Fibras de cana-de açúcar foram adicionadas em filmes de amido desenvolvidos por Gilfillan et al. (2012). A adição de fibra de bagaço em ambos os tipos de amido, preparada por prensagem a quente, reduziu a absorção de umidade em até 30% a 58% de umidade relativa. A adição de 5% de fibra aumentou a resistência à tração e o módulo de Young em 16% e 24%, respectivamente.

Schettini et al. (2013) usaram fibras naturais de cascas de tomate e de fibras de cânhamo para reforçar as propriedades mecânicas de potes biodegradáveis e demonstrou que a estrutura reticulada do filme foi capaz de prender fisicamente as fibras de reforço por meios de ligação de hidrogênio, como evidenciado por microscopia eletrônica de varredura (MEV) e energia dispersiva análise por espectroscopia de raios-X (EDS). A fibra de banana tem sido empregada com a mesma finalidade em filmes de goma de sementes de tamarindo (KIRUTHIKA et al., 2012).

Resíduos compostos por cascas e bagaço de raízes de mandioca foram utilizados como matéria-prima para o desenvolvimento de filmes. A adição dos resíduos aumentou a capacidade de barreira UV e a opacidade dos filmes, e manteve os valores da permeabilidade ao vapor de água. Ambos os resíduos reforçam a matriz dos filmes mesmo quando foram utilizadas baixas concentrações de resíduos. A adição de bagaço (1,5%) aumentou 260% de módulo de elasticidade e 128% de tensão máxima dos filmes (VERSINO et al., 2015).

Encalada et al. (2016) adicionaram fibras de cenoura com diferentes tamanhos de micropartículas em filmes de pectina com o objetivo de aumentar o poder antioxidante. Verificaram que os carotenoides presentes na fibra não deterioraram durante o armazenamento e que as fibras não modificaram a permeabilidade ao vapor da água dos filmes.

Com o objetivo de elevar o poder antioxidante de filmes biodegradáveis de gelatina Iahnke et al. (2015) e Iahnke et al. (2016) adicionaram resíduos do processamento mínimo de cenoura e beterraba na matriz dos filmes e observaram um aumento significativo na atividade antioxidante pelo método Dpph.

Todos esses estudos destacam o potencial tecnológico e antioxidante que as farinhas podem trazer aos alimentos ou aos filmes biodegradáveis em que são adicionadas.

3.2.2. Extrato

Os extratos concentrados obtidos a partir dos resíduos da indústria de alimentos, principalmente de frutas e vegetais, apresentam elevado poder antioxidante, principalmente quando extraídos do resíduo in natura e a frio, podendo ser utilizados como ingredientes pela indústria.

Os extratos naturais têm sido incorporados como antioxidantes naturais, em substituição total ou parcial aos aditivos ou antioxidante sintéticos, que podem apresentar toxicidade. Devido à multiplicidade das espécies químicas de componentes bioativos, como os compostos fenólicos, observam-se diferentes mecanismos de ação e grande potencial para sinergismos, que podem aumentar sua ação nos sistemas a serem incorporados, em comparação com o uso de substâncias isoladas (GRUZ et al., 2013).

Muitos desses extratos ricos em compostos bioativos também podem ser chamados ser utilizados como corantes naturais, principalmente extratos obtidos a partir de resíduos ricos em carotenoides ou antocianinas, por serem bastante pigmentados. Estudo desenvolvido por Vargas et al.(2016b) utilizou resíduos do processamento de polpa de pêssego para obtenção de um extrato etanólico de carotenoides, para aplicação como corante natural em alimentos. Resíduos industriais de polpa de amora também foram utilizados como matéria-prima para extração de corantes de naturais (VARGAS et al., 2015).

Para que esses extratos ou corantes sejam utilizados em alimentos ou em embalagens que interajam com alimentos eles devem seguir a legislação. Segundo resolução 44 da Anvisa (Brasil, 1977) permite a utilização de água, açúcares, álcool etílico, amidos, cloreto de sódio, dextrina, gelatina, glicerol, óleos e gorduras comestíveis como solventes para elaboração de corantes naturais. Além desses solventes muitas também podem ser as técnicas utilizadas para extração sólido: líquido de compostos antioxidantes, como lixiviação, imersão, agitação, micro-ondas, pulsos elétricos, alta pressão hidrostática, extração com fluido supercrítico, etc. (PALENZUELA ET AL., 2004; LEE AND WROLSTAD, 2004; NICOUE ET AL., 2007; (CORRALES ET AL. 2008; ZOU ET AL., 2012; SILVA ET AL., 2014). Muitos fatores devem ser considerados na escolha do solvente e do método a ser utilizado, porém Gruz et al. (2013) mostrou que a extração de compostos do bagaço de uva pela extração hidroetanólica apresentou resultados superiores quando comparada a extração enzimática. De acordo com os resultados apresentados, a extração com etanol:água (30:70, v/v) conduzida a 50 ºC, com pH 4,0, e razão solvente/substrato 3:1, foi a condição mais favorável para a obtenção de extrato bioativo do bagaço de uva rosada.

3.2.3. Microcápsulas

Muitos dos compostos extraídos a partir de resíduos, como os antioxidantes podem ser bastante sensíveis a oxidação pela luz, calor, oxigênio e humidade. A microencapsulação é uma alternativa para manter a estabilidade dos compostos durante o processamento e também consiste em tornar os ingredientes mais solúveis na matriz em que são incorporados.

As microcápsulas são resultado do método de encapsulação que é um processo de empacotamento de partículas (ex: compostos de sabor, pigmentos, acidulantes, nutrientes, enzimas, conservantes) em cápsulas comestíveis (Figura 1). Essa tecnologia pode embalar materiais sólidos, líquidos e gasosos em pequenas cápsulas que liberam seus conteúdos em taxas controladas por longos períodos de tempo (CHAMPAGNE & FUSTIER, 2007; AZEREDO, 2005). O material encapsulado pode ser denominado de recheio ou núcleo, e o material que forma a cápsula, encapsulante, cobertura ou parede. As cápsulas podem ser classificadas de acordo com seu tamanho em 3 categorias: macro- (>5000μm), micro- (0,2-5000 μm) e nanocápsulas (<0,2 μm). As cápsulas podem ser divididas em dois grupos: aquelas nas quais o núcleo é nitidamente concentrado na região central, circundado por um filme definido e contínuo do material de parede, e aquelas nas quais o núcleo é uniformemente disperso em uma matriz. O primeiro grupo pode ser classificado como sistema do tipo reservatório, e caracteriza as "verdadeiras" microcápsulas; e o segundo, classificado como sistema matricial, resulta nas chamadas microesferas. A principal diferença entre as microcápsulas e as microesferas está no fato de que, nas microesferas, uma pequena fração do material "encapsulado" permanece exposta na superfície, o que é evitado pela verdadeira encapsulação (AZEREDO, 2005).

A microencapsulação pode ser realizada com vários objetivos como facilitar a dose de determinados ingredientes, aditivos ou compostos ativos com problemas de volatilidade, viscosidade ou baixa solubilidade em água, ou para liberar gradativamente compostos bioativos (FANG & BHANDARI, 2010). Na indústria de alimentos, o encapsulamento pode melhorar as características organolépticas de um produto, ao mascarar indesejáveis sabores, odores e cores, além de prolongar a vida de prateleira (VOS et al., 2010). As microcápsulas formadas também protegem os componentes bioativos contra condições ambientais adversas durante o processamento e armazenamento no produto que serve como veículo para os componentes bioativos e contra as condições nocivas durante a sua passagem através do trato gastrointestinal (VOS et al., 2010).

Uma das tecnologias industriais mais utilizados para encapsulamento é o *spray-drying*. *Spray-drying* é uma operação unitária através do qual um produto líquido é atomizado em uma corrente de gás quente para instantaneamente se obter um pó. O gás usado é geralmente ar ou, mais raramente, um gás inerte como o nitrogênio. A alimentação líquida inicial do pulverizador pode ser uma solução, uma emulsão ou uma suspensão. A secagem por pulverização produz um pó muito fino (10-50 μm) ou partículas de grande tamanho (2-3 mm). O princípio da secagem por *spray-drying* é a dissolução do núcleo em uma dispersão de um material (matriz) escolhido. A dispersão é subsequentemente atomizada em ar aquecido, o que promove a remoção rápida do solvente. As partículas em pó são então separadas do ar de secagem na saída a uma temperatura mais baixa (Fig. 1) (VOS et al., 2010).

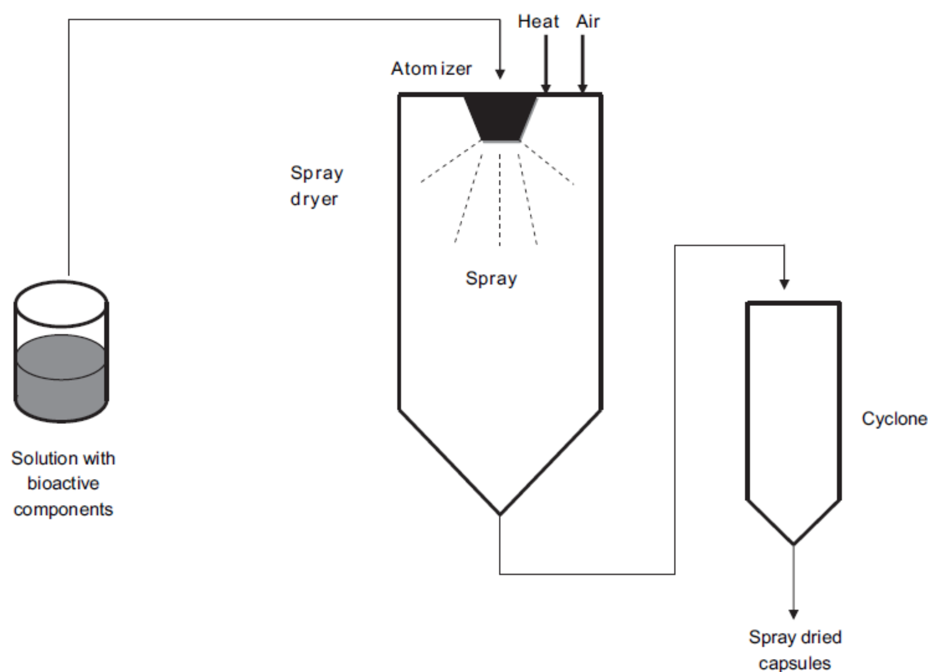

Figura 1. Representação esquemática do processo de secagem por pulverização.

Fonte: (VOS et al., 2010).

A relativa facilidade e também o baixo custo são as principais razões para a ampla aplicação do *spray-drying* em ambientes industriais. A principal vantagem da atomização em *spray-drying* é a possibilidade de utilizar materiais termolábeis e o pequeno tamanho das partículas geradas (geralmente menores que 100 μm), resultando assim em um produto altamente solúvel (AZEREDO, 2005).

Certas classes de compostos fenólicos tais como antocianinas de cenoura preta (ERSUS & YURDAGEL, 2007), procianidinas de sementes de uva (ZHANG et al., 2007), e

polifenóis de groselha preta (BAKOWSKA-BARCZAK & KOLODZIEJCZYK, 2011) foram encapsulados por *spray-drying* e a estabilidade dos compostos foi melhorada.

Agentes encapsulantes

A escolha de um material de parede para a microencapsulação é muito importante para a eficiência da encapsulação e estabilidade da microcápsula. Os critérios para a seleção de um material de parede baseiam-se, principalmente, sobre as propriedades físico-químicas tais como a solubilidade; peso molecular; fusão de transição; cristalinidade; difusibilidade; formação de película e propriedades emulsificantes. Além disso, os custos também devem ser considerados (GHARSALLAOUI et al., 2007). As propriedades físicas e químicas das micropartículas são determinadas pelo material de revestimento selecionado que deve ser compatível com o núcleo e com o material onde será inserida. Este material deve também ser capaz de formar uma película coesiva com o material do núcleo (AGUIAR et al., 2016). O material de parede é projetado para proteger material do núcleo de fatores que podem causar a sua deterioração, assim evitando uma interação antecipada entre o material do núcleo e outros ingredientes, para limitar perdas de matérias voláteis e também para permitir a liberação seja controlada ou prolongada sob condições desejadas (SHAHIDI & HAN, 1993).

Os materiais de parede, utilizados na indústria de alimentos são geralmente considerados como biomoléculas, classificadas em três categorias principais, ou seja, polímeros de carboidratos, proteínas e lipídios. Os carboidratos são os mais abundantemente utilizados como materiais de parede. Polímeros de carboidratos são classificados em cinco subcategorias, ou seja, derivados de amido, derivados de celulose, exsudatos de plantas e extratos, extratos marinhos e microbianos e polissacárideos de origem animal (MURUGESAN & ORSAT, 2012). Geralmente são usados como materiais de parede a maltodextrina e a goma arábica (WANDREY et al. 2010).

As proteínas geralmente possuem boa emulsão, porém algumas são relativamente caras e potencialmente alergênicas. Os carboidratos tendem a ter preços mais baixos e baixa viscosidade (maltodextrina e goma arábica) (DESAI & PARK, 2005). Em alguns casos, os materiais de parede devem ser associados a outros.

As micropartículas também podem ter como material de parede a gelatina, micropartículas de gelatina contendo extrato de própolis foram preparadas por técnica de secagem por pulverização. A eficiência de encapsulação para as micropartículas de própolis foi até 41% sem manitol e 39% com manitol. A microencapsulação manteve a atividade da

própolis contra Staphylococcus aureus (BRUSCHI et al., 2003). Outro composto que também pode ser usado como agente encapsulante é a ciclodextrina (CD), que foi utilizadas para encapsular licopeno, após 180 dias de estocagem a 15 ºC, o licopeno se manteve constante no complexo licopeno-y-CD e reduziu cerca de 80% no complexo licopeno-ß-CD. A estabilidade a luz foi ótima, apresentando 100% de retenção em 40 dias de armazenamento (MATIOLI & RODRIGUEZ-AMAYA, 2003).

Tonon et al. (2009) avaliaram as propriedades físico-químicas e morfológicas de pó de açaí encapsulados com diferentes agentes, entre eles maltodextrina, goma arábica e amido de tapioca. Os resultados mostraram que as amostras produzidas com maltodextrina e goma arábica apresentaram o menor tamanho e maior higroscopicidade. O pó produzido com o amido de tapioca apresentou a menor higroscopicidade e solubilidade e o maior diâmetro médio. Em relação a morfologia, todas as partículas apresentaram superfícies esféricas, exceto as produzidas com o amido de tapioca, que exibia superfícies arredondadas e lisas.

Fenólicos da casca de romã foram micro encapsulados em *spray drying* com quatro tipos diferentes de maltodextrina, resultados evidenciaram que não foram observadas diferenças entre as maltodextrinas usadas para revestimentos. Também não houve diferença estatística significativa no conteúdo fenólico das microcápsulas para os períodos de armazenamento de 90 dias a 4 ° C ($p > 0,05$) (ÇAM et al., 2014).

Flores et al. (2014) avaliaram as propriedades de micropartículas de extrato de bagaço de mirtilo encapsulado em *spray-dried* com proteína isolada do soro de leite. O armazenamento revelou cinética de degradação de primeira ordem para antocianinas monoméricas, um aumento de fenólicos totais e ligeiro aumento na capacidade antioxidante das micropartículas.

Extratos fenólicos de amora-preta foram microencapsulados por liofilização utilizando ß-ciclodextrina, quitosana, xantana e hidrogel como agentes encapsulantes. Os autores concluíram que a eficiência de encapsulação dependeu do composto fenólico e do agente encapsulante utilizado, ácido gálico e epicatequina foram predominantes em microcápsulas revestidas com ß-ciclodextrina e xantana. A maior atividade antioxidante foi apresentada pelas microcápsulas revestidas com ß-ciclodextrina (84,43%) e xantana (90,75%) (ROSA et al., 2014).

Kuck & Noreña (2016) encapsularam fenólicos extraídos da casca da uva utilizando como agentes encapsulantes goma árabica, polidextrose e goma guar parcialmente hidrolisada. A retenção de fenólicos e antocianinas variou de 81,4% a 95,3%, e 80,8% a

99,6%, respectivamente, enquanto a retenção de atividade antioxidante variou de 45,4% a 83,7%.

Oléo de Palma e betacaroteno foram encapsulados com quitosana / tripolifosfato de sódio e quitosana / carboximetilcelulose. A eficiência de encapsulação foi superior a 95%, e o rendimento de micropartículas revestidas com quitosana / tripolifosfato de sódio foi de aproximadamente 55%, enquanto que a das micropartículas revestidas com quitosana / carboximetilcelulose foi de 87% (RUTZ et al., 2016).

Microcápsulas de antocianinas extraídas do bagaço de uva com diferentes materiais de parede foram aplicadas em filmes biodegradáveis ativos. Como materiais de parede foram utilizados a goma arábica e a maltodextrina, segundo os autores os filmes produzidos com antocianinas encapsulados com maltodextrina apresentaram propriedades melhores e maior proteção frente a oxidação de óleo (STOLL et al., 2016).

3.2.4. Resíduos de capsulas nutracêuticas

A indústria farmacêutica assim como a de alimentos também é uma grande geradora de resíduos, porém nem todos os resíduos produzidos por ela podem ser aproveitados, por conterem traços de medicamentos, já os resíduos da produção de cápsulas nutracuêticas de óleos de chia, coco, cartamos e linhaça podem ser perfeitamente utilizados. Esses resíduos são compostos apenas por gelatina, glicerol e pequenas quantidades residuais do óleo que foi embalado.

A quantidade de capsulas nutracêuticas produzidas pela indústria farmacêutica tem aumentado nos últimos anos, isso acontece porque esses produtos se referem a substâncias que são ou fazem parte de um alimento, e que fornecem efeitos benéficos para a saúde, auxiliando na prevenção e no tratamento de doenças. Segundo Penner et al. (2005) o termo "nutracêutico" também pode ser designado a suplementos alimentares que contém substância bioativa, na forma concentrada derivada originalmente de um alimento.

O aumento na produção das capsulas consequentemente provoca um aumento na quantidade de material residual. Esse material gera altos custos para empresa quando submetido a tratamento adequado e quando descartado no solo provoca danos negativos no meio ambiente.

O aproveitamento deste resíduo para a elaboração de filmes biodegradáveis é interessante, principalmente devido à composição desse material ser de glicerol, gelatina e água, ingredientes básicos para formulação de filmes a base de gelatina. Campo et al. (2016) desenvolveram filmes biodegradáveis com resíduos de cápsulas nutracêuticas de óleo de cártamo: amido de milho em diferentes proporções (20: 4, 30: 4, 40: 4 e 50: 4). Os autores observaram uma diminuição da resistência à tração e do módulo de Young e um aumento do alongamento à ruptura com o aumento no conteúdo dos resíduos (CAMPO et al., 2016). Resíduos de capsulas nutracêuticas de óleo de linhaça também foram utilizados para elaboração de filmes biodegradáveis (Iahnke et al. 2015; Iahnke et al. 2016)

3.3. Filmes comestíveis e embalagens ativas

A embalagem é um componente essencial na indústria de alimentos, sendo imprescindível para que o alimento mantenha suas características organolépticas, possua qualidade e promova segurança. Portanto, é necessário avaliar qual embalagem mais adequada para cada tipo de matéria-prima (THARANATHAN, 2003).

O Brasil é um grande produtor de alimentos e, consequentemente, usa grande quantidade de embalagens. Esta alta utilização pode ser vista como um problema, tanto econômico, uma vez que representa grande parte do custo dos alimentos, quanto ambiental, já que as embalagens sintéticas são mais utilizadas devido sua grande disponibilidade, baixo custo, boas características mecânicas e boa barreira aos gases e aos aromas, porém essas embalagens são de difícil deterioração.

As embalagens são utilizadas para prolongar a vida de prateleira de alimentos protegendo-os mecanicamente e evitando contaminações biológicas e químicas. Entretanto, na tentativa de satisfazer consumidores cada vez mais exigentes, surgem as chamadas embalagens ativas, que, além de proteger, interagem com o produto proporcionando benefícios extras em relação às embalagens convencionais (BRODY, 2001; BRAGA & PERES, 2010).

Os filmes e coberturas possuem a função de bloquear ou reduzir a migração de umidade, oxigênio, dióxido de carbono, lipídios, aromas, dentre outros, pois geram barreiras semipermeáveis. Existe diferença entre filme e cobertura, a cobertura é uma fina camada de material aplicado e formado diretamente na superfície do produto, enquanto que o filme é pré-formado separadamente e aplicado posteriormente sobre o produto (FAKHOURI et al., 2007). Esses podem ser classificados em comestíveis e/ou biodegradáveis, dependendo dos

constituintes utilizados para sua produção e da quantidade das substâncias empregadas (SHIH, 1996). Além disso, podem transportar ingredientes alimentícios como: antioxidantes naturais (terpenos, tocoferóis, carotenoides e vitaminas), antimicrobianos e flavorizantes, e/ou melhorar a integridade mecânica ou as características de manuseio do alimento (KROCHTA & MULDER-JOHNSTON, 1997; BROINIZI et al., 2007).

Tradicionalmente, os materiais de embalagens têm sido selecionados no sentido de ter a mínima interação com o alimento que acondicionam constituindo, assim, barreiras inertes, com a função de proteger o produto embalado. Entretanto, na última década, diversos estudos (GRISI et al., 2008; MACHADO et al., 2010; SOUZA et al., 2011; FERREIRA et al., 2014; IAHNKE et al., 2016) têm desenvolvido sistemas de embalagens com o objetivo de interagir com o alimento, pelo uso de matrizes biodegradáveis e aditivos naturais, planejados para corrigir deficiências das embalagens passivas, e sendo chamadas de embalagens biodegradáveis ativas.

Embalagens ativas podem ser definidas como embalagens que modificam as condições do ambiente que o alimento se encontra com o objetivo de prolongar a sua vida útil, sem alterar as características sensoriais e de segurança, e manter a qualidade do alimento (VERMEIREN et al., 1999). Essas embalagens podem ser classificadas em sistemas absorvedores e sistemas emissores. Os sistemas absorvedores se caracterizam por remover os compostos indesejáveis que aceleram a degradação do produto alimentício como: oxigênio, excesso de água, etileno, dióxido de carbono e outros compostos específicos. Os sistemas emissores adicionam ativamente compostos ao produto embalado ou ao espaço livre da embalagem como: dióxido de carbono, etanol, antioxidantes ou conservantes, entre outros (VERMEIREN et al., 1999; KRUIJF et al., 2002).

Existem ainda as embalagens ativas inteligentes que constituem um sistema que monitora as condições do alimento em tempo real, dando informações sobre sua qualidade durante o transporte e armazenamento (KRUIJF et al., 2002). Exemplos são os indicadores de tempo-temperatura, frescor, microrganismos patogênicos, oxigênio, além de sensores e biossensores (AHVENAINEN, 2003). A aplicação dessas embalagens em alimentos proporciona aumento significativo da quantidade de informações que o consumidor pode obter por meio da embalagem e também facilita a transmissão, já que a qualidade do produto pode ser informada apenas pela coloração da etiqueta presente na embalagem (BRAGA & PERES, 2010).

As embalagens ativas vêm sendo empregadas em diversos produtos alimentícios como cárneos, massas frescas e pães, nozes e similares, queijos, frutas, hortaliças frescas e sucos, etc. (SARANTÓPOULOS et al., 1996). Entretanto, o uso comercial de filmes comestíveis formulados a partir de matrizes biodegradáveis tem sido limitado por causa de problemas relacionados às propriedades mecânicas usualmente pobres e fraca barreira à umidade, quando comparados aos polímeros sintéticos (SOUZA et al., 2012).

Cada alimento tem seu mecanismo de degradação característico, que muda de acordo com a sua composição e tipo de processamento a que foi submetido. Sendo assim, muitos processos podem acontecer no interior das embalagens que vão depender das propriedades do alimento e da forma como ele interage com o ambiente externo. Com o objetivo de diminuir a degradação desses produtos, vários mecanismos podem ser empregados, sendo o controle da composição de gases e vapores uma das principais maneiras (BRAGA & PERES, 2010).

A embalagem ativa deve obedecer os seguintes requisitos: (a) ser segura em termos de saúde pública; (b) absorver/emitir o gás ou vapor de interesse em velocidade adequada; (c) oferecer elevada capacidade de absorção do gás ou vapor de interesse; (d) não causar reações paralelas adversas; (e) não causar modificações organolépticas no produto; (f) manter-se estável durante a estocagem; (g) ser compacta; e (h) apresentar custo compatível com a aplicação (SARANTÓPOULOS et al., 1996).

3.3.1. Biopolímeros utilizados para produção dos filmes

Os biopolímeros mais utilizados na elaboração de filmes e coberturas comestíveis são as proteínas (gelatina, caseína, ovoalbumina, glúten de trigo, zeína e proteínas miofibrilares), os polissacarídeos (amido e seus derivados, pectina, celulose e seus derivados, quitosana, alginato e carragena) e os lipídios (monoglicerídeos acetilados, ácido esteárico, ceras e ésteres de ácido graxo) ou a combinação dos mesmos (FAKHOURI et al., 2007).

Entre os biopolímeros de polissarídeos, destaca-se o amido que é considerado uma matéria promissora devido ao seu preço, disponibilidade e capacidade como um amido termoplástico para a produção de filmes biodegradáveis (ANDRADE-MOLINA et al., 2013). No entanto, os filmes à base de amido apresentam baixa resistência mecânica e baixa proteção ao vapor d’água se comparados aos plásticos provenientes do petróleo; entretanto, se adicionados de alguns aditivos, essas características podem ser melhoradas (VEIGA-SANTOS et al., 2008). O amido de mandioca proporciona boas características para formação

de filmes que, além de serem comestíveis, são de baixo custo quando comparadas às ceras comerciais. Esses, quando aplicados diretamente ao alimento, ainda proporcionam bom aspecto e brilho intenso (DAMASCENO et al., 2003).

O segundo polissacarídeo natural mais abundante após a celulose é a quitosana, um polissacárideo linear natural, derivado parcialmente da quitina. A quitosana é disponível comercialmente e obtida de fontes renováveis abundantes, principalmente de resíduos de crustáceos. Devido sua não toxicidade, biodegradabilidade, biocompatibilidade e propriedade antimicrobiana, o uso de quitosana para conservação de alimentos é bastante interessante (ZHANG et al., 2014). Os filmes ou revestimentos de quitosana são flexíveis, porém também são fortes e difíceis de rasgar, por suas propriedades mecânicas podem ser comparados aos polímeros de força média, porém esse biopolímero é sensível à umidade, o que limita sua aplicação em alimentos (ZHANG et al., 2014). Para reduzir a permeabilidade ao vapor da água de filmes de quitosana podem ser incorporados lipídios aos filmes.

Filmes à base de proteínas possuem melhores propriedades de barreira ao oxigênio e dióxido de carbono e propriedades mecânicas do que filmes de polissacarídeos. Vários tipos de proteína têm sido usadas em filmes biodegradáveis e comestíveis tais como proteína de soja, caseinato de sódio, glúten de trigo, proteínas de ervilha e gelatinas (HANANI et al., 2014).

Entre os filmes proteicos biodegradáveis, destacam-se os filmes de gelatina que possuem elevado potencial de aplicação comercial em embalagens de alimentos através de suas características. A gelatina é uma proteína de origem animal obtida do colágeno por hidrólise ácida ou básica, e amplamente utilizada na indústria alimentícia e farmacêutica. Além disso, a gelatina no Brasil é produzida em abundância, a baixo custo e com propriedades funcionais adequadas para a fabricação de filmes (HANANI et al., 2014) e pode ser extraída de pele, ossos e tecidos conjuntivos de animais (KARIM & BHAT, 2009). A gelatina é comumente utilizada para encapsular materiais de baixa umidade ou ingredientes alimentícios e farmacêuticos, na forma oleosa. A gelatina também pode agir como agente espessante, gelificante, estabilizante, emulsificante e possuem facilidade de manuseio e utilização (HANANI et al., 2014).

Como os biopolímeros tendem a interagir com o alimento torna-se interessante o uso de compostos antioxidantes que possam auxiliar no aumento da vida de prateleira do produto embalado.

3.3.2. *Filmes biodegradáveis adicionados de antioxidantes naturais*

A incorporação de antioxidantes naturais em substituição aos sintéticos em filmes biodegradáveis vem se mostrando uma ótima alternativa como é comprovado por inúmeros estudos. Esses antioxidantes podem ser extraídos de inúmeras fontes, como chá (SIRIPATRAWAN & HARTE, 2010; WAMBURA et al., 2012; GIMÉNEZ et al., 2013; YANG et al., 2016;), sementes e bagaço de uva (MORADI et al., 2012; FERREIRA et al., 2014; STOLL et al., 2016), óleos essenciais (BONILLA et al., 2012; MARTUCCI et al., 2015, PAGNO et al., 2016; HOSSEINI et al., 2016; ATARÉS & CHIRALT, 2016) mirtilo e cranberry (ZANG et al., 2010; WANG et al., 2012).

Antioxidantes naturais podem ser encontrados em grande quantidade em resíduos do processamento de frutas e vegetais, podendo ser facilmente extraídos ou simplesmente secos e na forma de farinhas incorporados em filmes comestíveis, tornando-os filmes de maior valor agregado com propriedades bioativas e funcionais (GALANAKIS, 2012).

Muitos trabalhos vem sendo desenvolvidos visando a adição dos antioxidantes obtidos de fontes naturais em polímeros biodegradáveis. GIMÉNEZ et al. (2013) adicionaram extrato de chá verde em filmes de ágar e gelatina e verificaram que a presença de gelatina na matriz do filme dificultou a liberação de compostos fenólicos totais. No entanto, a atividade antimicrobiana dos filmes não foi afetada pela presença de gelatina.

Extratos antioxidantes naturais de chá verde, de semente de uva e extrato de folhas de gingko foram aplicados em filmes de gelatina. No estudo desenvolvido por Li et al. (2014). Esse estudou concluiu que esses extratos são uma boa opção para o desenvolvimento de embalagens de alimentos ativas objetivando prolongar a vida útil de produtos alimentícios. Os autores verificaram também que o extrato de chá verde quando adicionado aos filmes reduziu a permeabilidade ao vapor da água.

Filmes à base de quitosana e metilcelulose adicionados de resveratrol, um antioxidante natural obtido de plantas, mostraram-se eficientes para o revestimento de produtos alimentares, podendo minimizar ou prevenir processos de oxidação, preservando a qualidade nutricional e prolongando a vida útil dos alimentos (PASTOR et al., 2013).

Extratos de frutas com elevado teor de compostos fenólicos e antocianinas como mirtilo e uva são um potencial ingrediente como antioxidante natural, mostrando capacidade in vitro e in vivo (SHIRLEY et al., 2007; OGAWA et al., 2008; GRUZ et al., 2013). Estudo realizado por Zang et al. (2010) avaliou o efeito do extrato de mirtilo incorporado em filme

comestível de proteína de soja isolada na qualidade de banha de porco embalada comparando com o efeito da vitamina E (10%) ou Butil Hidroxi Anisol (BHA) (10%). A capacidade antioxidante do filme incorporado com o extrato de mirtilo foi superior ao incorporado com a vitamina E e semelhante ao que incorporou BHA. Além disso, o filme com extrato de mirtilo mostrou maior capacidade de retardar a hidrólise da banha do que o filme com BHA. A incorporação de extrato de mirtilo, além de melhorar as propriedades mecânicas e de barreira, também retardou a oxidação e hidrólise da banha embalada.

Extrato aquoso de bagaço de uva, um extrato de cera de uva e óleo de semente de uva, foram adicionados em filmes de quitosana, os autores verificaram que todos os filmes contendo extrato e óleo de semente de uva tiveram atividade antioxidante maior que os filmes controle pelo método DPPH (Ferreira et al., 2014).

As sementes e as cascas das frutas também podem ser ótimas fontes de antioxidantes. Extrato de semente de toranja e óleo essencial de limão foram incorporados como agentes ativos em filmes de amido de milho e quitosana e diminuíram a rigidez e a resistência dos filmes porém aumentaram o alongamento e apresentaram boas propriedades funcionais e atividade antibacteriana (BOF et al., 2016).

Du et al. (2011) avaliaram as propriedades físicas e antibacterianas de filmes comestíveis adicionados de polifenóis extraídos da casca de maçã em filmes de purê de maçã. Os autores comprovaram que o filme comestível de maçã com polifenóis de casca de maçã mostrou-se eficaz contra *L. monocytogenes*. A presença dos polifenóis também reduziu a permeabilidade ao vapor de água dos filmes e aumentaram o alongamento.

O aproveitamento dos resíduos para obtenção de farinhas é uma maneira bastante fácil e barata de utilização do resíduo e quando incorporadas aos filmes podem melhor características tecnológicas como resistência a tração além de agregar poder antioxidante. Nascimento et al. (2012) desenvolveram filmes utilizando farinha de mesocarpo de maracujá e observaram que filmes feitos com 5 % de farinha apresentaram maior resistência a tração e maiores valores de modulo de Young quando comparados aos filmes de amido. Ferreira et al. (2016) verificaram que a incorporação de farinha de casca de batata melhorou o teor de sólidos totais, o teor de amido, a densidade e a resistência à tração dos filmes biodegradáveis.

Para que muitos desses antioxidantes extraídos de fontes naturais sejam preservados da degradação por agentes externos ou se solubilizem de maneira adequada no biopolímero, torna-se interessante a microencapsulação desses compostos antes da incorporação na matriz do filme. A incorporação de microcápsulas de antocianinas extraídas de bagaço de uva em

filmes ativos biodegradáveis de amido de mandioca, apresentaram potencial antioxidante pois protegeram o óleo de girassol da formação de peróxidos e em relação as propriedades mecânicas os filmes adicionados de antocianinas encapsuladas com maltodextrina apresentaram os melhores resultados de resistência a tração e alto valor de alongamento (STOLL et al., 2016).

Micropartículas de lipídicas de ácido ascórbico foram incorporadas em filmes de amido de banana verde, resultando em filmes com menor permeabilidade ao vapor de água e alongamento, bem como maior resistência à tração em comparação com filmes sem micropartículas. As micropartículas atuaram como agentes protetores da atividade antioxidante do ácido ascórbico durante o processo de produção do filme (SARTORI & MENEGALLI, 2016).

A aplicação dos antioxidantes naturais em matriz biopoliméricas é uma maneira conveniente de agregar valor a embalagem e evitar ou retardar a oxidação dos produtos, essa aplicação pode ser feita na forma de extratos, óleos, farinhas e micropartículas, como foi evidenciado pelos estudos apresentados acima. Isso se torna ainda mais interessante se esses antioxidantes naturais forem obtidos a partir resíduos da indústria de processamento de frutas.

CAPÍTULO 4

4. Metodologia

As análises dos artigos presentes nos capítulos 5 e 6 foram realizadas no Laboratório de Compostos Bioativos, Instituto de Ciência e Tecnologia de Alimentos (UFRGS) e as análises referentes aos artigos dos capítulos 7 e 8 foram realizadas no LEAF (Linking Landscape, Environment, Agriculture and Food), Instituto Superior de Agronomia, Universidade de Lisboa.

Para melhor entendimento e vizualição das etapas do trabalho e de como ele foi dividido foi desenhado um fluxograma, apresentado na Figura 1.

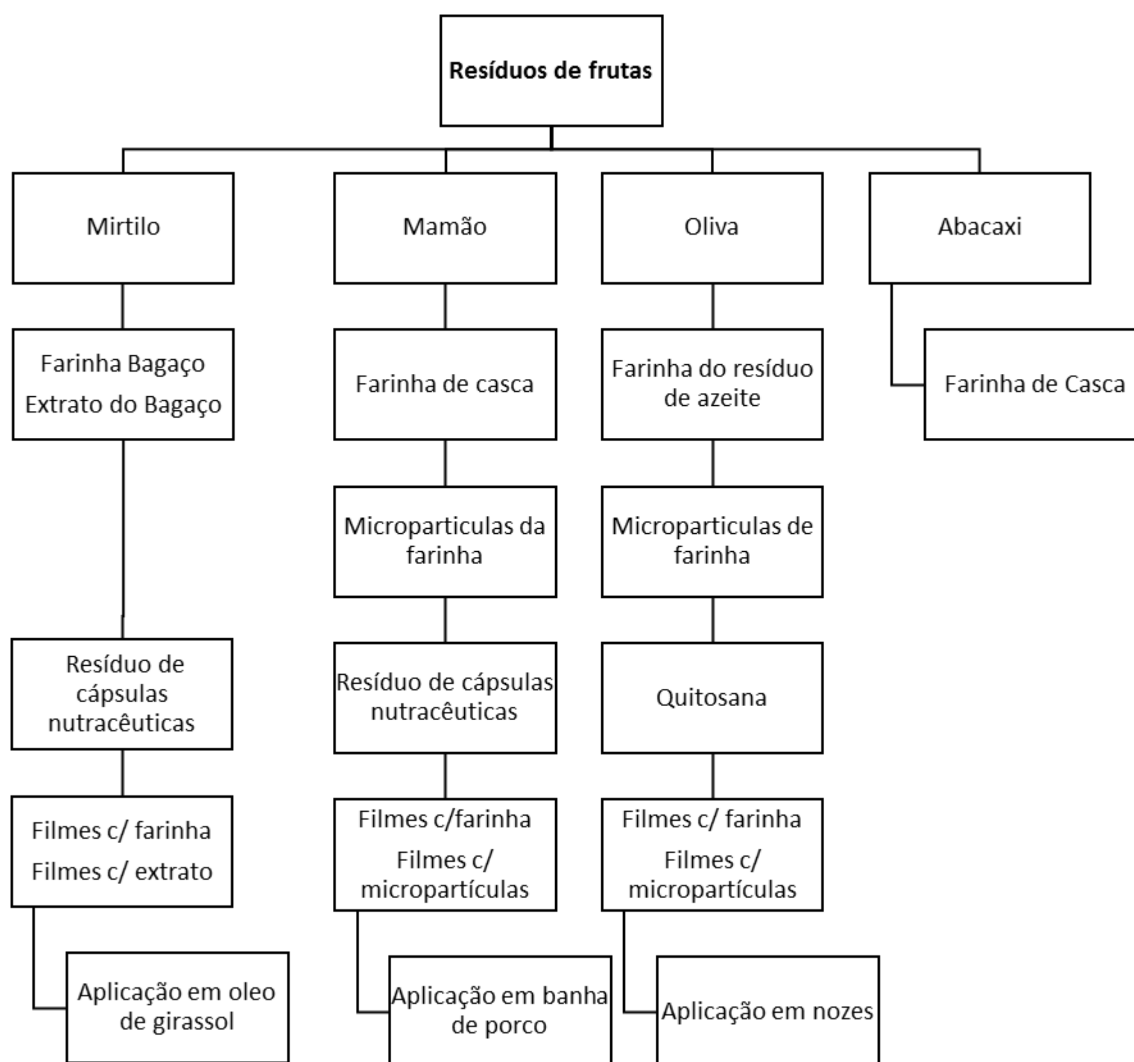

Figura 1. Fluxograma das etapas desenvolvidas durante esse trabalho.

4.1. Matéria-prima

Para o desenvolvimento desse trabalho foram utilizados resíduos do processamento de frutas como mirtilo (bagaço), mamão (cascas), abacaxi (cascas) e oliva (bagaço) devido à grande quantidade de resíduos gerados em seu processamento. A quantidade dos resíduos utilizados chegou a aproximadamente 45 % de bagaço de mirtilo, 25 % de casca de mamão, 40 % de casca de abacaxi e 85 % de resíduo de oliva, em relação ao peso inicial de cada fruta.

Resíduos do processamento de azeite de oliva foram fornecidos pela empresa Olivas do Sul, localizada na cidade de Cachoeira do Sul-RS, e as cascas do abacaxi resultantes de seu processamento mínimo foi fornecido pela Degasperi Atacadista, Estrela-RS. As cascas de abacaxi coletadas foram higienizadas em solução de hipoclorito de sódio (150 mg/L) por 15 min e armazenada a -18 °C para posterior utilização.

Resíduos de mamão e mirtilo foram obtidos a partir do processamento das frutas no Laboratório de Compostos Bioativos da UFRGS. Mamões da variedade Formosa (*Carica papaya*), foram adquiridos na "Central de Abastecimento (CEASA)", localizado em Porto Alegre (RS / Brasil) e os mirtilos orgânicos (*Vaccinium* spp.), fruto da cultivar Delite (grupo rabbiteye) da "Fazenda Viva o Verde ", em Camaquã (RS / Brasil). As frutas foram higienizadas em solução de hipoclorito de sódio (150 mg/L) por 15 min e os resíduos foram armazenados a -18 °C para posterior utilização.

O resíduo (bagaço) de mirtilo foi obtido utilizando um extrator doméstico tipo centrífuga (Walita-Philips®), em que o suco foi separado do resíduo (bagaço) e as cascas de mamão foram obtidas manualmente.

Os resíduo do processamento de capsulas nutracêuticas de óleo de chia que foram fornecidos pelo Laboratório Farmacêutico Químico Tiaraju, localizado na cidade de Santo Ângelo (RS/Brasil). Os resíduos são compostos basicamente por gelatina (48,2 %), água (30%) e glicerol (21,8%).

A quitosana utilizada na preparação dos filmes foi adquirida na Golden-Shell Biochemical Co., Ltd., localizado na China, tinha com um grau de desacetilação (DD) superior a 85%.

4.2. Obtenção da farinhas

Os resíduos de todas as frutas foram submetidos a secagem em estufa de circulação de ar forçado (Modelo B5AFD, Marca DeLeo) a 55 ºC, por 24 horas. Após resfriamento até

temperatura ambiente (25ºC) os mesmos foram triturados em moinho (Modelo MCF55, Marca Bertel, Brasil). O resíduo triturado foi separado através de peneiras sob agitação em partículas menores do que 125 mm (115 mesh). A fibra obtida foi embalada em seladora a vácuo (Modelo F200, Fastvac, Brasil) e armazenadas no escuro em temperatura (25 º C).

4.3. Caracterização das farinhas

As farinhas de mamão, abacaxi, mirtilo e oliva que foram obtidas nesse experimento foram analisadas quanto a suas propriedades físico-químicas, funcionais, e antioxidante.

4.3.1. Análises físico-químicas

4.3.1.1. Composição proximal

As análises físico-químicas de umidade, cinzas, proteína, lipídios foram determinadas segundo a AOAC (1990). O teor de umidade foi determinado a 105 ºC (DeLeo, modelo de 48 TLK, Brasil). O teor de lipídeos foi determinado utilizando um extrator de Soxhlet (Foss Soxtec, modelo 2055 TM, Dinamarca). O teor total de proteína (N x 6,25) foi determinado pelo método de Kjeldahl. O teor de cinzas foi realizado em mufla (Elektro Therm Linn, 312,6 SO LM 1729, Alemanha) a 550 ° C. O conteúdo de carboidratos foi determinado por diferença. Os resultados foram expressos em gramas por 100 g de matéria seca (bs). As fibras alimentares totais, solúvel e insolúvel, foram determinadas segundo o método enzimático-gravimétrico descrito pela AOAC (1990). Estas análises foram realizadas em triplicata.

4.3.1.2. Solubilidade

A solubilidade foi determinada de acordo com Cano-Chauca et al. (2005). Para cada 0,5 g de amostra de farinha foi adicionada a 50 mL de água destilada em um tubo de centrífuga e a mistura foi homogeneizada utilizando um vórtex (Quimis, modelo Q920-A2, Brasil) durante 1 min; os tubos foram centrifugados (3000 g, durante 5 min, Sigma, modelo 4K15, Inglaterra). Uma alíquota (25 mL) do sobrenadante foi removido para uma cápsula de porcelana, previamente tarada e transferida para estufa de secagem a 105 ° C durante 24 h e a solubilidade (%) foi então calculada pela diferença em peso.

4.3.1.3. Capacidade de retenção de água (CRA) e capacidade de retenção de óleo (CRO)

Para análise da CRA e da CRO foi empregada técnica utilizada por Fernández-López et al. (2009). Foram adicionados 30 mL de água destilada/óleo de girassol a 1 g da farinha. A suspensão foi homogeneizada por 1 min em vórtex e deixada em temperatura ambiente por 24 h. Após a centrifugação (3000 × *g* por 20 min), o sobrenadante foi removido e o resíduo pesado. A capacidade de retenção de água foi expressa em gramas de água/óleo por gramas de amostra seca.

4.3.2. Determinação de compostos bioativos das farinhas

4.3.2.1. Preparação do extrato

Para elaboração dos extratos utilizados nas análises de compostos fenólicos e DPPH, pesou-se 1 g de amostra de farinha, em tubos falcon de 50 mL, adicionou-se 20 mL de metanol 50% homogeneizou-se em ultra turrax (IKA, Ultra-Turrax_ T25 digital, Alemanha) por 1 minuto e deixou-se em repouso por 60 minutos à temperatura ambiente. Os tubos foram centrifugados a 25.000 g durante 15 minutos, em seguida o sobrenadante foi transferido para um balão volumétrico de 50 mL. A partir do resíduo da primeira extração, adicionou-se 20 mL de acetona 70 %, homogeneizou-se novamente por 1 min e deixou-se em repouso por 60 minutos à temperatura ambiente. Novamente as amostras foram centrifugadas a 25.000 g durante 15 minutos, o sobrenadante foi transferido para o balão volumétrico contendo o primeiro sobrenadante e o volume foi completado para 50 mL com água destilada. Os extratos foram realizados em triplicata.

4.3.2.2. Determinação dos compostos fenólicos totais

A determinação do teor de compostos fenólicos totais presentes nas farinhas foi realizada pelo método espectrofotométrico de Folin-Ciocalteau (SINGLETON & ROSSI, 1965). Para quantificação dos compostos fenólicos totais, uma alíquota de 20 µL do extrato combinada com 1580 uL de água e 100 µL do reagente Folin Ciocaltau’s 1N, reagiu por 3 min, e foi adicionado de 300 µL de Na_2CO_3 1N. Após 2h de incubação a absorbância foi medida a 725nm. Para quantificação foi realizada uma curva analítica (medidas em triplicata)

com ácido gálico, e os resultados foram expressos como miligramas de equivalentes de ácido gálico (GAE) por grama de amostra seca (mg / g).

4.3.2.3. DPPH

O método DPPH que foi utilizado se baseia na captura do radical DPPH (radical que pode ser obtido diretamente por dissolução do reagente em meio orgânico) por antioxidante produzindo um decréscimo na absorbância a 515nm (RUFINO et al., 2007). A partir do extrato elaborado no item 4.3.2.1, foram preparadas três diluições. Para determinação da atividade antioxidante uma alíquota de 100 µL de cada diluição do extrato foi combinada com 3900 µL da solução de DPPH e após a reação por 40 min às leituras foram realizadas a 515 nm em espectrofotômetro, sendo o metanol, utilizado como branco (BRAND-WILLIAMS et al., 1995). As análises do extrato foram feitas em triplicata e os resultados apresentados em g de amostra / g de DPPH. Para cálculo do percentual de atividade antioxidante as absorbâncias utilizadas foram do extrato com concentração de 15 %.

4.3.2.4. Análise de carotenoides

O extrato de carotenoides de farinhas dos resíduos de mamão, oliva e abacaxi foram preparados de acordo com Mercadante et al. (1998). A extração dos pigmentos foi realizada com acetona gelada até a completa descoloração da amostra e a saponificação com solução de KOH 10 % em metanol realizada durante a noite a temperatura ambiente. Posteriormente o extrato foi lavado para remoção dos álcalis e concentrado em rota evaporador (T < 35 ºC). A amostra foi transferida para um frasco âmbar, foi seca em corrente de nitrogênio e armazenada a –18 °C para posterior análise em cromatografia liquida de alta eficiência (CLAE).

A análise por CLAE foi realizada utilizando coluna polimérica de fase reversa C30 YMC (3 mm × 250 mm × 4,6 mm), usando um gradiente de eluição fase móvel de água / metanol / terc-metil-butiléter (MTBE) a partir de 5:90:5, atingindo 0:95:5 em 12 min, 0:89:11 em 25 min, 0:75:25 em 40 min, e 00:50:50 em 60 min, com um fluxo de 1,0 mL/min e temperatura de 33 ºC (ZANATTA & MERCADANTE, 2007).

Para quantificação dos compostos foi construída curva padrão de luteína (1 mg/L a 65 mg/L), zeaxantina (1 mg/L a 40 mg/L), criptoxantina (4 mg/L a 100 mg/L), α-caroteno (2 mg/L a 25 mg/L) e β-caroteno (5 mg/L a 50 mg/L). Os padrões utilizados de β-criptoxantina

(Pureza >97%), β-caroteno (Pureza >93%), α-caroteno, (Pureza >95%), zeaxantina (Pureza >95%) foram adquiridos da Sigma Chemical St. Louis, MO, EUA, O padrão de carotenoides luteína (Pureza >95%), adquirido da Indofine Chermical Company Inc. Hillsborough. Os resultados foram expressos em miligramas por 100 gramas de amostra seca.

4.3.2.5. Antocianinas

As antocianinas foram extraídas exaustivamente de uma amostra de farinha de resíduos de mirtilo (1 g), utilizando uma solução a 1% de HCl em metanol e Ultra Turrax® (IKA Ultra Turrax digitais, T25, Alemanha). A injeção das amostras foi realizada no HPLC no mesmo dia da extração, uma vez que os extratos não foram concentrados no rota evaporador. As antocianinas foram quantificadas por HPLC e identificadas em comparação com os padrões apropriados. A análise por HPLC foi realizada num sistema Agilent 1100 Series HPLC equipado com um sistema de bombeamento de solvente quaternário (G1311A - DE14917573 Agilent Série 1100) e detector UV-Visível (G1314B - DE71358944 Agilent Série 1100).

Os pigmentos foram separados em uma coluna de fase reversa C18 CLC-ODS de 5 µm, 250 x 4,6 mm (Shimadzu, Quioto, Japão), utilizando como fase móvel um gradiente linear de eluição com 4% de ácido fosfórico aquoso/acetonitrila de 85:15 (v / v) a 20:80 (v / v) em 25 minutos, em uma corrida cromatográfica de 10 minutos, de acordo com as condições estabelecidas experimentalmente por Vargas et al. (2016a). O fluxo da fase móvel foi de 1,0 mL/min, e o volume de injecção foi de 5 uL. A temperatura de coluna foi mantida a 29 ° C e os cromatogramas foram processados a 520 nm.

A extração das antocianinas e injeção no cromatógrafo foram realizadas em triplicata, a identificação das antocianinas foi realizada através da comparação dos tempos de retenção (tR) das amostras com o tR dos padrões comerciais obtidos a partir de Sigma-Aldrich (St. Louis, MO, EUA).

As antocianinas identificadas na fibra de resíduos de mirtilo foram quantificadas através da construção de curva padrão com antocianinas glicosiladas, tais como cianidina-3-glicosídeo (Sigma-Aldrich, 95% de pureza), delfinidina-3-β -D-glicosídeo (Santa Cruz Biotechnology, 95% pureza), pelargonidina-3-glicosídeo (Sigma-Aldrich, 97% de pureza) e a malvidina-3-glicosídeo (Sigma-Aldrich, 90% de pureza), e as antocianinas agliconas tais

como delfinidina aglicona (Sigma-Aldrich, 95% de pureza), cianidina aglicona e malvidina aglicona.

4.4. Obtenção dos ingredientes ativos adicionados aos filmes

4.4.1. Farinha de bagaço de mirtilo

O bagaço de mirtilo foi submetido a secagem em estufa de circulação de ar forçado (Modelo B5AFD, Marca DeLeo) a 55 ºC, por 24 horas. Após resfriamento até temperatura ambiente (25ºC) os mesmos foram triturados em moinho (Modelo MCF55, Marca Bertel, Brasil). O resíduo triturado foi separado através de peneiras sob agitação em partículas menores do que 125 mm (115 mesh). A fibra obtida foi embalada em seladora a vácuo (Modelo F200, Fastvac, Brasil) e armazenadas no escuro em temperatura (25 º C).

4.4.2. Extrato de bagaço de mirtilo

O extrato foi elaborado a partir do bagaço obtido da extração de suco de mirtilo, utilizando etanol P.A como solvente na concentração de 1:1. O resíduo juntamente com o solvente foi homogeneizado com auxilio de ultra turrax (IKA, Ultra-Turrax_ T25 digital, Alemanha) por 1 minuto, sob temperatura ambiente (≈ 25 ºC) sendo posteriormente centrifugados a 3.000 g (Sigma, modelo 4K15, Inglaterra) durante 15 minutos para obtenção do extrato (sobrenadante).

4.4.3. Farinha de casca de mamão e bagaço de oliva

As cascas de mamão e o bagaço de oliva foram congelados em ultra-freezer a -40 ºC durante 48 horas e posteriormente liofilizadas (Freeze Dryer Liotop, L101, Brasil) por 5 dias. Os resíduos foram triturados em um moinho (Bertel Brand, Modelo MCF55, Brasil) e separados utilizando peneiras para análise de tamanho de partícula (Bertel, Brasil); As partículas separadas foram inferiores a 500 mm (malha 35). A farinha foi embalada em seladora a vácuo (Sealer ECOVAC, Modelo ECOVAC 40, Itália) e armazenada no escuro à temperatura ambiente (~ 25 ° C).

4.4.4. Micropartículas de farinha de casca de mamão

Primeiramente foi realizado um ensaio preliminar onde foram testadas quatro formulações diferentes de micropartículas, uma apenas com água e farinha de casca de mamão (5 g) e outras três com farinha e diferentes concentrações de resíduos de cápsulas de gelatina, em relação à quantidade de água adicionada (1, 1,5 e 2%). Para a preparação das micropartículas a farinha foi misturada com água (150 mL) e homogeneizada em agitador magnético (Velp Scientifica, Modelo Arex, Itália) durante 40 min. A gelatina foi dissolvida separadamente num banho de água a 60 ° C, com uma parte da água da formulação. A gelatina dissolvida foi adicionada a mistura de farinha e água sob agitação constante durante 10 min. Após estes passos adicionou-se tween 80 (0,1%) e a solução foi levada ao homogeneizador Ultra Turrax® (IKA Ultra Turrax digital, Modelo T25 básico, Alemanha) durante 30 segundos. Cada formulação foi bombeada a 6 ml/min (velocidade de bomba de 20%) através de uma bomba peristáltica para o secador por pulverização “spray dryer” (LabPlant, Model SD-05, Reino Unido) na temperatura de 160 ºC.

4.4.5. Micropartículas de farinha de bagaço de oliva

Para a preparação das micropartículas de oliva, misturou-se 5 g de resíduo de azeite de oliva com água (150 mL) e agitou-se em agitador magnético (Velp Scientifica, Modelo Arex, Itália) durante 40 min. Nesta mistura foi adicionado tween 80 (0,1 g) e subsequentemente a solução foi colocada sob agitação num homogeneizador Ultra Turrax® (IKA Ultra Turrax digital, Model T25 noções básicas, Alemanha) durante 60 segundos. A solução foi bombeada a 8 mL/min através de uma bomba peristáltica para o secador por pulverização (LabPlant, Modelo SD-05, Reino Unido) sob uma temperatura de ar de secagem de 160 ºC.

4.5. Filmes biodegradáveis

4.5.1. Filmes de resíduos de capsulas nutracêuticas de gelatina

A preparação das soluções filmogênicas foi realizada segundo a técnica *casting*, onde inicialmente, o resíduo das cápsulas nutracêuticas (gelatina) foi dissolvido em água, 50 g de gelatina para cada 100 mL de água. Posteriormente a solução foi aquecida até a temperatura

até 60 °C, sob agitação constante, por 30 minutos. As fibras, ou os extratos de compostos bioativos, foram incorporados após resfriamento da solução filmogênica (40 ºC) para não ocorrer perda de atividade antioxidante. As soluções filmogênicas foram então colocadas em um dessecador submetido a vácuo durante 2 minutos e após colocados em um banho ultrasom para remoção de bolhas de ar. As diferentes soluções filmogênicas foram pesadas em placas de Petri de poliestireno (0.13 g/cm2) e colocadas em estufa com circulação de ar (30 ± 2 °C) por 18 a 20 horas. Os filmes obtidos (com adição de fibras e com extratos de compostos bioativos) foram acondicionados (58 ± 2 % UR, 25 °C) em dessecadores contendo solução saturada de nitrato de magnésio por 2 dias até o momento das análises (VEIGA-SANTOS, et al., 2008).

4.5.2. Filmes de quitosana

Para o preparo da solução filmogênica, primeiro a quitosana (CH) (2% p / p) foi dissolvida em solução aquosa de ácido acético (1,0% v/v) sob agitação constante até a dissolução completa da quitosana. Após este período, adicionou-se glicerol (1,0% p / p) sob agitação. A farinha de resíduos de azeite de oliva e as micropartículas foram previamente dissolvidos em 50 ml da solução filmogênica sob agitação magnética antes de serem incorporados no resto da solução e agitados sob agitação durante 30 min. A solução filmogênica foi colocada em um banho ultra som durante 30 minutos para remoção de bolhas de ar. Pesou-se 0,50 g/cm^2 de solução em placas de Petri, que foram colocadas em estufa (Modelo D -78532, Mark Binder, Alemanha) a 40 ° C durante 48 h. As películas foram mantidas a 48% de umidade relativa e ≈ 25 ° C, durante 48 h antes das análises de caracterização.

4.6. Caracterização dos filmes

4.6.1. Microscopia eletrônica de varredura (MEV)

Imagens foram obtidas em um equipamento JEOL modelo JSM 5800 a 5KV com aumentos entre 100 a 1000 vezes.

4.6.2. Permeabilidade ao Vapor de Água (PVA)

Foi determinado o ganho de massa por cada célula de difusão através de pesagens em balança analítica (AY 220, Shimadzu) no tempo 0 e após 24h. A permeabilidade ao vapor de água das amostras foi determinada em triplicata pelo método gravimétrico da ASTM E96-00 descrito por Sarantópoulos et al. (2002) utilizando a Equação (3) descrita abaixo:

$$PVA = \frac{W \cdot L}{A \cdot t \cdot \Delta p} \quad (3)$$

Onde W é a taxa de ganho de massa (água) pela célula de permeação ($g.h^{-1}$), L a espessura (mm), A área (m^2), t é o tempo de permeação (h), diferença de pressão entre os dois lados da película (Pa).

4.6.3. Transmissão de luz

A transmissão de luz ultravioleta e visível através do filme foi determinada de acordo com Fang et al. (2002) utilizando um espectrofotómetro UV (Shimadzu Model UV-1800, Japão). As amostras de filmes foram cortadas em tiras retangulares (4,5 cm de comprimento e 1,2 cm de largura) e colocadas em cubetas de quartzo. As medições de transmitância foram feitas em comprimentos de onda entre 200 e 800 nm, e a opacidade foi calculada dividindo os valores de absorvância (nm) pela espessura da película (mm). Todas as determinações foram realizadas em triplicata.

4.6.4. Propriedades mecânicas

A resistência à tração, porcentagem de elongação na ruptura e o módulo de Young foram determinadas segundo ASTM D882-95 (2009), com um texturômetro (TA.XT plus, Stable Micro Systems, UK) com uma célula de carga de 5 kg, sendo a distância entre as garras de 55 mm e a velocidade do teste de 1mm/seg. Os filmes foram recortados formando corpos de prova de 80 mm de comprimento e 25 mm de largura. Os filmes foram condicionados por dois dias à temperatura ambiente e umidade relativa de 58 % antes da medição.

4.6.5. Solubilidade em água

A solubilidade em água dos filmes foi determinada em triplicata, sendo que amostras dos filmes foram recortadas em discos de 2 cm de diâmetro. A porcentagem inicial da matéria seca de cada amostra foi determinada em estufa a 105 °C por 24 horas. Após a primeira pesagem, as amostras foram imersas em recipientes contendo 30 mL de água destilada, e agitadas lenta e periodicamente por 24 horas e a 25 °C. Após este período, as amostras foram filtradas utilizando filtro de papel pré-pesados e posteriormente secos a 105 °C durante 24 h (DeLeo forno, modelo TLK 48, Brasil). O material resultante foi pesado para a determinação do peso seco final (Wf). A análise foi realizada em triplicata e determinado de acordo com a Eq. (4):

$$WS\ (\%) = W_i - W_f / W_i * 100 \quad (4)$$

onde Wi é o peso seco inicial da amostra (g), e Wf é o peso seco final da amostra (g).

4.6.6. Espessura

A espessura dos filmes foi determinada utilizando-se um micrometro digital (Modelo MDC-25, Mitutoyo Corp. Tóquio, Japão) com escala de 0-25mm e precisão de 0,001mm. Os valores apresentados representarão a média de cinco medidas feitas aleatoriamente ao longo de cada amostra avaliada. A espessura final corresponde a média aritmética de cinco pontos aleatórios de cada amostra (KECHICHIAN et al., 2010).

4.6.7. Conteúdo de umidade

O conteúdo de umidade foi determinado de acordo com a AOAC (1990) utilizando estufa com circulação e renovação de ar (DeLeo forno, modelo TLK 48, Brasil) a 110ºC até peso constante (base seca).

4.6.8. Isotermas de sorção de água

As isotermas de sorção de água foram determinadas pelo método gravimétrico, de acordo com Alves et al. (2011). As amostras de filme com dimensões de 10 mm x 10 mm foram previamente armazenadas em um dessecador com uma umidade relativa de 48% durante 48 h. As amostras foram pesadas e colocadas em dessecadores com diferentes soluções salinas saturadas: LiCl, $MgCl_2.6H_2O$, $Mg(NO_3)_2.6H_2O$, $NaNO_3$, NaCl, KBr e KCl, com atividade de água a 25 ºC de 0,112; 0,332; 0,534; 0,743; 0,753; 0,809 e 0,843, respectivamente. Após 3 semanas as amostras foram pesadas, assegurando que o equilíbrio foi atingido. Para cada valor de atividade de água foram realizadas três repetições.

4.6.9. Análise térmica

O método termogravimétrico foi realizado para análise das propriedades térmicas dos filmes em atmosfera de Argônio, em equipamento Shimadzu model TGA-50. As amostras foram aquecidas a partir da temperatura ambiente até 600 °C com a velocidade de aquecimento de 10 °C / min.

4.6.10. Análise de cor

A análise de cor realizada nos filmes de mirtilo foi avaliada durante um período de 35 dias, os filmes ficaram expostos a luz e temperatura ambiente de aproximadamente 25 ºC, os filmes foram avaliados nos dias 0, 7, 14, 21, 28 e 35. Já os demais filmes foram analisados apenas uma vez, no primeiro dia após sua produção. As leituras foram realizada em cinco pontos diferentes de cada filme utilizando um colorímetro (Minolta®, modelo CR400, Japão) e de acordo com o sistema de cor CIE-L**a*b** foram avaliados os valores L* (luminosidade) que variam do preto (0) ao branco (100), os valores do croma *a** que variam do verde (-60) ao vermelho (+60) e os valores do croma *b**.

4.6.11. Avaliação da Atividade antioxidante dos filmes pelo método DPPH

A atividade antioxidante dos filmes foi determinada de acordo com metodologia utilizada por Ferreira et al. (2014), com algumas modificações. Pedaços de 1 cm^2 de filme foram colocados em uma solução de 2,2-diphenyl-1- picrylhydrazyl (DPPH) em metanol (0,06 mM) e deixou-se reagir no escuro por um período de 120 min com agitação em vortex (Quimis, Modelo Q920-A2, Brasil) a cada 30 min. Após esse período a absorbância da solução a 515 nm foi de medida em espectrofotômetro (Shimadzu, modelo UV-1800), assim como, a absorbância da solução de DPPH sem filme. Todas as medições foram realizadas em triplicata.

4.6.12. Avaliação da Atividade antioxidante dos filmes pelo método FRAP

O método FRAP (Ferric Reducing Antioxidant Power) descrito por Pulido et al. (2000) foi utilizado para medir a potência antioxidante reduzida de ferro como alternativa à determinação da redução de fluidos biológicos de ferro e soluções aquosas de compostos puros. Os filmes foram cortados em pedaços de 1 cm^2 e pesados, colocados em tubos com 270 µL de água destilada misturada com 2,7 mL de reagente FRAP, os tubos foram agitados e mantidos em banho-maria a 37 °C. Os resultados foram analisados com um espectrofotômetro (595nm) (Unicam UV4 UV / Vis, Reino Unido) após 30 minutos de reação. O reagente FRAP foi utilizado como branco para calibrar o espectrofotómetro. Foi construída uma curva padrão de $FeSO_4.7H_2O$ (mM) para a absorbância a 595 nm. Os resultados foram expressos em µmol $FeSO_4.7H_2O$ equivalentes / g de matéria seca ou película seca. Todas as determinações foram realizadas em triplicata.

4.7.Aplicação dos filmes como embalagem de produtos alimentícios

4.7.1. Filmes com resíduo de mirtilo

Um teste acelerado foi utilizado para avaliar o efeito protetor do filme contra a oxidação do óleo de girassol. Para a análise foram selecionadas três formulações de filme:

duas películas com antioxidantes e a formulação de controle. Os filmes com antioxidantes foram escolhidos com base na determinação feita por análise de DPPH, e os filmes que tinham a maior atividade antioxidante foram selecionados para o teste. Assim, uma película com concentração de fibra de 0,15 g / mL (FF15) e uma película com 50 mL de extrato (FE50) foram cortadas em pedaços de 10 cm x 7,5 cm e seladas (Sealer Model F200, Fastvac, Brasil) para formar embalagens, com uma Contendo 7 g de óleo de girassol, e foram armazenados em câmara a 40 ° C na presença de luz durante 13 dias, com análises realizadas nos dias zero, 3, 8 e 13. Para o controle, o óleo de girassol foi acondicionado em embalagens plásticas fechadas foram colocadas Para a câmara. A progressão da oxidação do óleo foi monitorada pela análise do valor do índice de peróxido.

4.7.2. Filmes com resíduo de mamão

A banha de porco foi embalada com filmes incorporados de microcápsulas de casca de mamão que tinham a maior atividade antioxidante pelos métodos DPPH e FRAP. Para a análise foram selecionadas três formulações: duas com antioxidantes (uma com pó de casca de mamão e outra com micropartículas) e o controle. Os filmes foram cortados em pedaços de 7 cm x 5,5 cm e foram selados (Sealer ECOVAC, Modelo ECOVAC 40, Itália) para formar embalagens, com uma contendo 7 g de banha, e foram armazenados numa câmara climática (Aralab, modelo FitoClima 600 PDH, Portugal) a 40 ° C na presença de luz fluorescente durante 22 dias e 35% de umidade, com ensaios realizados nos dias zero, 3, 8, 13, 17 e 22. Um controle fechado foi usado durante o experimento onde a banha foi acondicionada em embalagens fechadas de polietileno. Foram analisadas três embalagens de banha de cada filme e três amostras de cada controle. As análises realizadas foram índice de peróxidos, compostos secundários (dienos e trienos) e perfil de ácidos graxos.

4.7.3. Filmes com resíduo de oliva

As amostras de nozes foram doadas pela Horta D'el Rey pomares e viveiros, localizadas em Estremoz, Portugal. As nozes foram colhidas 1 semana antes de serem utilizadas no teste. As nozes foram ligeiramente trituradas na Bimby TM31 (Vorwerk, Portugal) e imediatamente embaladas com películas que apresentaram a maior atividade antioxidante de cada formulação com pó e micropartículas de resíduos de azeitona em pó

pelos métodos DPPH e FRAP. No ensaio foram utilizados três filmes diferentes para embalar as nozes, um com resíduos de azeitona em pó, outro com micropartículas em pó e outro apenas quitosana. Dois controles foram usados durante o teste, um controle em embalagem plástica de polipropileno (a mesma usado pelo fornecedor das nozes para comercializá-lo), e outro controle aberto. Os filmes de quitosana e o plástico foram cortados em 14 cm x 10 cm, dobrados e selados para formar embalagens, onde foi colocado 25 g de nozes. Os pacotes e controles foram armazenados em câmara climática (Aralab, modelo FitoClima 600 PDH, Portugal) a 55 ºC na incidência de luz fluorescente (300 $\mu mol/m^2/s$) durante 31 dias e 35% de umidade. Para os ensaios de peróxidos, compostos secundários e ácidos graxos, o óleo foi extraído das nozes no dia da análise com o suporte de uma prensa mecânica. As análises no óleo de nozes foram realizadas nos dias 0, 5, 10, 19, 26 e 31. Foram analisadas três amostras de cada filme e dos controles para cada dia de análise. As análises realizadas foram índice de peróxidos, compostos secundários e perfil de ácidos graxos.

4.7.4. Análises realizadas nas amostras de produtos durante os experimentos

4.7.4.1. Índice de Peróxidos

A progressão da oxidação das amostras foi monitorizada pela análise do valor do índice de peróxido (IP) seguindo o método descrito por AOCS (2009). O IP foi expresso como mEq de oxigênio por quilograma de amostra.

4.7.4.2. Os compostos secundários (dienos e trienos)

Os produtos de oxidação foram quantificados (dienos e trienos) pelo método espectrométrico IUPAC (1979) II.D.23 que determina a absorbância da amostra em determinados comprimentos de onda do espectro ultravioleta e fornece uma indicação do seu grau de oxidação. Isto ocorre porque os produtos de oxidação (dienos conjugados (232 nm) e para trienos conjugados ou compostos secundários a (270 nm) são apresentados nos espectros característicos na região ultravioleta.

4.7.4.3. Perfil dos Ácidos graxos

A determinação do perfil dos ácidos graxos foi baseada no procedimento experimental previamente descrito por Bandarra et al. (1997). Amostras em triplicata (300 mg de massa seca por amostra) foram dissolvidas em 5 mL de cloreto de acetila/ metanol (1:19 v/v), agitada e aquecida a 80 °C durante 1 h. Após arrefecimento, adicionou-se 1 mL de água destilada Milli-Q e 2 mL de n-heptano, e as amostras foram agitadas e centrifugadas (2300g, 5 min) até a separação de fases. O teor de umidade da fase superior foi removido usando sulfato de sódio anidro. Foi injetada uma alíquota (2 ml) da fase superior num cromatógrafo de gás (Varian Star 3800 CP, Walnut Creek, CA, EUA) equipado com um detector de ionização à chama a 250 °C para ácidos graxos. A separação foi realizada com gás hélio como transportador a um caudal de 1 mL/min numa coluna capilar DB-WAX (30 m de comprimento x 0,32 mm de diâmetro interno, 0,25 mm de espessura, Hewlett-Packard, Albertville, MN) programada na temperatura de 180 ºC durante 5 min, aumentou para 220 ºC a 4 ºC/min e manteve-se a 220 ºC durante 5 min com o injetor a 250 ºC. A identificação dos ácidos graxos foi realizada através da comparação dos tempos de retenção com os padrões da Sigma. Os conteúdos de ácidos graxos foram quantificados (em peso) como percentagem (%) dos ácidos totais.

4.8. Análise Estatística

Os resultados das avaliações foram submetidos à ANOVA e teste de comparação de médias de Tukey ao nível de 5% de probabilidade, através do programa Statistica 12.

CAPÍTULO 5

Evaluation of bioactive compounds, chemical and technological properties of fruits byproducts powder

Tainara de Moraes Crizel, Vanessa Stahl Hermes, Alessandro de Oliveira Rios, Simone Hickmann Flôres

Abstract

Millions of tons of fruit byproducts are discarded globally every day by food processing industries, which is a considerable loss regarding the nutritional and antioxidant properties of the waste. The goal of this workwas to evaluate the physico-chemical, technological, antioxidant properties and characterization of carotenoids for papaya, pineapple, olive byproducts and anthocyanins for blueberry byproducts. The results indicated that these byproducts are good sources of total dietary fiber (TDF), especially olive byproducts (53.68%). The powder from papaya byproducts showed the highest values for the technological characteristics of water holding capacity (8.93 g water/g powder) and solubility (59.91%). All of the powders exhibited a good ability to reduce Folin Ciocalteu reagent and a high DPPH radical scavenging capacity, especially the powder from blueberry byproducts, which also exhibited a high level of anthocyanins (2063.4 mg/100 g). The carotenoid content was highest in the papaya powder sample, followed by the pineapple and olive powders. The results of this study indicate the high potential application of these powder byproducts as functional ingredients in food products because they can be considered a good source of antioxidant compounds.

Keywords: byproducts; fiber; powder; antioxidants.

1. Introduction

A large quantity of byproducts is generated daily by the food processing industry, primarily due to the production of juices, jellies, candies and fresh-cut fruits. It is estimated that whole processed fruits can generate byproducts comprising approximately 30% to 90% of these fruits, including peels, seeds and pulp. In the fresh-cut fruit industry, the byproducts are mainly peels and seeds of different shapes and sizes (Ayala-Zavala et al. 2011).

These byproducts can present fibers and bioactive components, such as phenolic compounds, carotenoids and anthocyanins. However, they are typically used to produce animal feed or they are discarded, which causes a significant environmental problem (O'shea et al. 2012; Tarazona-Díaz and Aguayo, 2013). Considering their functional, technological and nutritional properties, these byproducts can be applied as food ingredients, providing outstanding health benefits.

Byproducts from the fruit processing industry contains high total dietary fiber content. Dietary fiber has been underlined as a healthy component for humans because of its capacity to protect against coronary disease and to reduce cholesterol, diabetes and constipation (Figuerola et al. 2005). Furthermore, the byproducts present other properties that allow its use as food additives, such as antimicrobials, antioxidants, flavorings, pigments, and thickening agents (Ayala-Zavala et al. 2011).

Byproducts from different fruits and vegetables have been evaluated, such as those from apple, potato, cucumber, melon and watermelon (Tarazona-Díaz and Aguayo, 2013), mango (Ajila et al. 2008), and pineapple, guava and passion fruit (Martinez et al., 2012). However, the studies did not characterize completely the functional and antioxidant profiles of these byproducts, specially related to powder obtained from it. Most of the researches generally reported only one characterization profile or used part of the residue (Sangeeta & Mahanta, 2015; Morais Ribeiro da Silva et al. 2014; O'shea et al. 2012).

The application of these byproducts in food is a promising field for the food industry. The addition or replacement of wheat powder by byproducts powder with high content of fiber and bioactive compounds in products consumed every day by the general population, as bakery products (Bhol et al., 2015; Singh et al., 2015) , is essential and viable (Lima et al., 2014). This would be a way to supply the intake of these compounds so important to the health of people (Elleuch et al., 2011).

Therefore, this study aimed to determine the chemical, technological and in vitro antioxidant properties of the powder obtained from byproducts of processing fruits, including pineapple, papaya, blueberry and olive.

2. Materials and Methods

2.1. Byproducts

Fresh pineapple byproducts were provided by the Pure Juice Company located in Porto Alegre (RS/Brazil). Olive byproducts were provided by the Olivas do Sul Company in the city of Cachoeira do Sul (RS/Brazil). Papaya fruit (Formosa) was purchased in Central de Abastecimento (CEASA) located in Porto Alegre (RS/Brazil), and organic blueberries *(Vaccinium spp.)*, fruit of the cultivar Delite (Rabbiteye group), were obtained from the "*Fazenda Viva o Verde*" in Camaquã (RS/Brazil). The fruits were acquired at the same time and were processed in the Laboratory of Bioactive Compounds at UFRGS. The blueberry residue (bagasse) was obtained using a domestic centrifugal-type extractor (Walita-Philips®) in which the juice was separated from the residue (pomace). The papaya peels were obtained manually. The residues were subsequently stored at –18 °C until analysis. Pineapple and papaya byproducts were composed only of peels, and the olive and blueberry byproducts were composed of peels, pulp and seeds.

2.2. Powder Production

The powder of the byproducts was prepared according to the method by Crizel et al. (2013). The powder was produced by drying the residue in an oven with forced air circulation (DeLeo, Model B5AFD,Brazil) at 55 °C for 24 hours and subsequently grinding it in a mill (Bertel Brand, Model MCF55, Brazil). The milled powder was separated using sieves for particle size analysis (Bertel, Brazil); Particles smaller than 125 mm were separated. The powder was packed in a vacuum sealer (Fastvac, model F200, Brazil) and maintained in the dark at room temperature (25 ºC).

2.3. Analysis

2.3.1. Proximate Composition

The powders were analyzed according to the AOAC (1990). The carbohydrate content was assessed by the difference method. The results were presented in grams per 100 g of dry matter (DM). These analyses were executed in triplicate.

2.3.2. Powder composition

The total dietary fiber, soluble fiber and insoluble fiber measured by enzyme-gravimetric method according to AOAC (1990) (method 991.43).

2.3.3. Functional properties

The water holding capacity (WHC) was determined using a method similar to that of the OHC, substituting the sunflower oil with distilled water. The result was expressed as grams of water per gram of dry powder sample.

The oil holding capacity (OHC) analysis was performed according to Fernández-López et al. (2009) with minor adjustments. Thirty milliliters of sunflower oil was added to 1 g of fiber sample, and the suspension was homogenized in a vortex (Quimis, Model Q920-A2, Brazil) for 1 min, followed by storage for 24 h at room temperature. After centrifugation (3000 g for 20 min, Sigma, model 4K15, England), the supernatant was withdrawn, and the residue was weighed. The oil retention capacity was expressed in grams of oil per gram of dry powder sample.

The solubility analysis of the powders was performed according to Cano-Chauca et al. (2005).

2.4. Determination of bioactive compounds

2.4.1. Preparation of the extracts

For the phenolic compound and DPPH analyses, 1 g of powder sample was used, and the extraction was carried out with 50% methanol (20 mL x 1) and 70% acetone (20 mL x 1) in an Ultra Turrax® homogenizer (IKA Ultra Turrax digital, T25, Germany) for 1 min. Then, the sample was left for 60 min at room temperature (25 ºC). The extract was centrifuged (25000 *g* for 15 min, Hitachi, model CR216III, Japan), and the supernatant was stored in an amber flask.

2.4.2. Total phenolic content

The total phenolic content of different powders obtained from fruit byproducts were determined using the Folin-Ciocalteu spectrophotometric method. According to Singleton and Rossi (1965), gallic acid was used as a standard, and the absorbance was measured at 725 nm. For quantification, an analytical calibration curve was constructed (measurements in triplicate), and the results are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample (mg/g).

2.4.3. Antioxidant Activity-DPPH method

The method is based on the capture of the 2.2-diphenyl-1-picrylhydrazyl (DPPH•) radical by antioxidant compounds present in extracts, producing a decrease in the absorbance at 515 nm (Rodrigues et al. 2014). From the extract previously described, (2.3.4.1) solutions with different dilution factors were prepared. For the antioxidant activity analysis, 100 uL of each diluted extract was combined with 3900 uL of DPPH solution, and after 40 min of reaction time, the readings were taken with a spectrophotometer (set to 515 nm) using methanol as a control (Brand-Williams et al. 1995). The extract samples were analyzed in triplicate, and the results are expressed as the concentration of antioxidant necessary to reduce the initial amount of free radicals by 50% (EC_{50}). The IC_{50} value is the final concentration in mg mL^{-1} of the dry extract required to decrease or inhibit 50% of the initial DPPH concentration, and it was determined by linear regression. An test to detect interference was performed only the extract without the addition of DPPH.

2.4.4. Carotenoid extraction, identification and quantification

The carotenoid extracts of papaya, olive and pineapple byproduct powders were prepared according to Mercadante et al. (1998). The pigments were extracted with chilled acetone in an Ultra Turrax® homogenizer (IKA Ultra Turrax digital, T25, Germany) until the sample was completely discolored. Then, the extracts were saponified overnight with 10% KOH in a methanol solution in the dark and at room temperature. Then, the extracts were washed to eliminate the alkali content and were concentrated using a rotary evaporator

(Fisatom, model 801/802, Brazil). The concentrated extract was placed to an amber flask, subjected to a stream of nitrogen until it was completely dried and stored in freezer at -18 ºC until the moment of analysis in high performance liquid chromatography (HPLC).

HPLC analysis was performed on an Agilent 1100 Series HPLC system equipped with a quaternary solvent pumping system (G1311A–DE14917573 Agilent 1100 Series) and a UV/Vis detector (G1314B–DE71358944 Agilent 1100 Series). A 250 mm x 4.6 mm i.d., 3 μm, C30 reversed phase polymeric column was applied (YMC, Japan). The wavelength was set in 450 nm. The mobile phase was water:methanol:tert-methyl butyl ether (MTBE) (J.T.Baker and Mallinckrodt, EUA) with the gradient starting at 5:90:5, reaching 0:95:5 in 12 min, reaching 0:89:11 in 25 min, reaching 0:75:25 in 40 min, finally reaching finally reaching 0:50:50 at 60 min applying a flow rate of 1 mL/min at 33 ºC (Zanatta and Mercadante, 2007). The injection volume of the extract was 5 μL and the total run time was 50 min.

Standard curves of cryptoxanthin (4-100 mg/L), zeaxanthin (1-40 mg/L), lutein (1-65 mg/L), β-carotene (5-50 mg/L) and α-carotene (2-25 mg/L) were used to quantify the carotenoids. The standard of carotenoids cryptoxanthin (purity > 97%), zeaxanthin (purity > 95%), β-carotene (purity > 93%) and α-carotene (purity > 95%) and were acquired from Sigma Chemical (USA). Lutein (purity > 95%) was obtained from Indofine Chemical Company Inc. (Hillsborough, N.J, USA). The results are expressed in milligrams per 100 g of dry sample.

2.4.5. Anthocyanin extraction, identification and quantification

The anthocyanins were exhaustively extracted from the powder of blueberry byproducts. Approximately 1 g of the powder was mixed with 1% HCl in methanol in an Ultra Turrax® homogenizer. The injection into the HPLC to quantify the anthocyanins was carried out on the same day as the extraction because the extracts were not concentrated in the rotary evaporator. The anthocyanins were identified in comparison with the appropriate standards. HPLC analysis was performed using the same system as that used for the carotenoid analysis. However, to separate the anthocyanins, a C18 CLC-ODS reversed phase column (250 mm x 4.6 mm i.d., 5 μm, Shimadzu, Kyoto, Japan) was used, which was different from that used for the carotenoid analysis. A linear gradient elution of a mobile phase consisting of 4% aqueous phosphoric acid:acetonitrile from 85:15 (v/v) to 20:80 (v/v) after 12 min was used according to the conditions established experimentally by Zanatta et al.

(2005). The flow rate was 1.0 mL/min, and the injection volume was 5 μL. The temperature of the column was maintained at 29 °C, and Chromatograms were acquired in 520 nm. The extraction and injection into the chromatograph of the anthocyanin extracts were performed in triplicate, and the compounds were identified comparing retention times (tR) obtained for samples with the standards purchased from Sigma-Aldrich® (St. Louis, MO, USA).

Standard curves were determined with glycosylated anthocyanins, such as cyanidin-3-glucoside (Sigma–Aldrich, 95% purity), delphinidin-3-β–D-glucoside (Santa Cruz Biotechnology, 95% purity), pelargonidin-3-glucoside (Sigma–Aldrich, 97% purity) and malvidin-3-glucoside (Sigma–Aldrich, 90% purity), as well as the aglycones of the anthocyanins, such as aglycone delphinidin (Sigma–Aldrich, 95% purity), aglycone cianidin and aglycone malvidin.

2.5. Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and Tukey's test at a significance level of 0.05 using Statistica 11.0 software (STATSOFT Inc.).

3. Results and Discussion

3.1. Proximate Composition

In Table 1, the approximate compositions of the various types of powders are presented. The proximal compositions of the byproduct powders showed significant differences ($p < 0.05$) among the different sources related to the moisture, lipids, ash, proteins and carbohydrates.

The papaya byproduct powder presented the highest moisture content. This may be due to differences in the initial moisture contents of each byproduct because the drying time and temperature were the same for all of the powders. The powder from olive byproducts showed the lowest moisture content, which can be explained by the high lipid content present in this residue (23.08 g/100 g DM).

The oil contents in the residues varied with the extraction methods used in the fruit during processing in industry. The lowest lipid content was obtained in the powder from pineapple byproducts (1.02 g/100 g DM), followed by the papaya (2.48 g /100 g DM) and blueberry (4.13 g/100 g DM) byproduct powders. These values are in agreement with the

quantities present in the powder of other types of fruit byproducts, such as orange (1.83 g/100 g DM), apple (4.46 g/100 g) and mango (2.2 g/100 g DM) (Crizel et al. 2013; Ajila et al. 2008; Figuerola et al. 2005).

Table 1. Chemical compositions of the powders from pineapple, papaya, blueberry and olive byproducts

Byproduct powder	Moisture (g/100 g DM)*	Proteins (g/100 g DM)*	Lipids (g/100 g DM)*	Ash (g/100 g DM)*	Carbohydrates (g/100 g DM)*[1]
Pineapple	13.56 ± 0.43[b]	6.72 ± 0.34[b]	1.02 ± 0.01[d]	4.58 ± 0.04[b]	87.68 ± 0.39[a]
Papaya	15.85 ± 0.57[a]	17.72 ± 0.81[a]	2.48 ± 0.40[c]	9.09 ± 1.76[a]	70.71 ± 1.73[c]
Blueberry	7.91 ± 0.59[c]	7.55 ± 0.33[b]	4.13 ± 0.19[b]	4.14 ± 0.17[b]	84.18 ± 0.45[b]
Olive	1.41 ± 0.08[d]	5.27 ± 0.53[c]	23.08 ± 0.42[a]	3.87 ± 0.15b	67.78 ± 0.58[d]

DM: dry matter. * Results are the means of three determinations ± standard deviation. Different letters in the same line are significantly different as determined by Tukey’s test ($p \leq 0.05$).

[1] Determined by the difference.

The protein contents of the pineapple and blueberry byproduct powders (6.72 g/100 g and 7.55 g/100 g DM, respectively) were considered equal and were different from the other samples, while the papaya byproduct powder sample showed the highest protein content (17.72 g/100 g DM). The values obtained in this study were higher than the results reported by López-Marcos et al. (2015) for lemon albedo dietary fiber (5.25 g/100 g DM) and by Martinez et al. (2012) for guava (4.8 g/100 g DM), pineapple (4.0 g/100 g DM) and passion fruit (6.2 g/100 g) co-products.

Dietary fiber has been considered an important nutrient in the human diet. Many researchers have found fruit and vegetable byproducts to be a good source of dietary fiber (O'shea et al. 2012). The pineapple, papaya, blueberry and olive byproduct powders showed total dietary fiber (TDF) contents (Table 2) ranging from 32.23 to 53.68 g/100 g DM. These powder contents are greater than the TDF contents of other fruit fiber sources, such as mango (28.05 g/100 g DM) (Vergara, Valencia et al., 2007), residues of grapefruit juice extraction (44.2 g/100 g DM) (Figuerola et al. 2005), and peach and pear processing byproducts (35.8 g/100 g DM and 36.1 g/100 g DM, respectively) (Grigelmo-Miguel and Martin-Belloso, 1999). The TDF contents of pineapple, papaya, blueberry and olive byproducts are similar to

the fiber obtained from cereals, such as wheat bran (44%), and their contents are higher than oat bran fiber (23.8%) (Grigelmo-Miguel and Martin-Belloso, 1999).

Table 2. Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) of pineapple, papaya, blueberry and olive byproduct powders

Byproduct powder	TDF*	IDF*	SDF*
Pineapple	45.23 ± 0.35^{b}	37.58 ± 1.62^{b}	7.65 ± 1.97^{a}
Papaya	32.23 ± 0.49^{c}	30.34 ± 0.20^{c}	1.87 ± 0.29^{b}
Blueberry	47.51 ± 0.05^{b}	45.95 ± 0.85^{a}	1.55 ± 0.81^{b}
Olive	53.68 ± 0.97^{a}	49.34 ± 1.39^{a}	4.34 ± 0.42^{ab}

TDF (Total dietary fiber); IDF (Insoluble dietary fiber); SDF (Soluble dietary fiber); *Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey's test ($p \leq 0.05$).

In all of the byproduct powders, the insoluble dietary fiber (IDF) fraction was higher than the soluble dietary fiber (SDF) portion, whereas approximately 90% of the TDF content belongs to the insoluble fraction. These large quantities of IDF in the byproduct powders of these fruits indicate the presence of significant amounts of celluloses and hemicelluloses (Martínez et al. 2012). The advantages of IDF on human health include an increase in satiety and a contribution to the smooth functioning of the intestinal tract (Elleuch et al. 2011).

Due to the insoluble and soluble properties of fiber present in powder, it has a variety of technological attributes, such as gelling, water binding, thickening and structure building, allowing its use as a replacement for fat. Therefore, fiber has been used as an ingredient with specific functions in food production. These properties are important for allowing utilization of the fiber or powder rich in fiber in the fabrication of different foods, such as bakery and meat products, snacks and diabetic beverages (Elleuch et al. 2011; O'shea et al. 2012).

3.2. Functional properties

The functional properties of powder, such as its water and oil holding capacities and solubility, are very important because they determine the functionality of the powder in the foods. The papaya powder showed the highest WHC value of 8.93 g water/g powder, followed by pineapple powder (6.06 g water/g powder), and both presented significant

differences ($p \leq 0.05$) compared to the other powders. Viuda-Martos et al. (2012) analyzed pomegranate whole fruit juice bagasse and obtained WHC values of 4.9 g water/g dry fiber. According López-Marcos et al. (2015) the water is associated with important in dietary fiber properties, water would affect metabolic activity of fiber in the gastrointestinal tract.

Powder with a high WHC can improve the technological characteristics of the foods to which it is added, such as decreasing the calories, avoiding syneresis, and changing the viscosity and texture of the final product (Grigelmo-Miguel and Martin-Belloso, 1999).

The OHC of the powder depends on the chemical and physical structures of the polysaccharides. This property is important to avoid fat loss during the cooking process; consequently, it has an auxiliary use in flavor preservation (Martínez et al. 2012). The values of OHC ranged from 2.7 to 3.81 g oil/g powder, and the highest values were obtained for the pineapple and papaya byproduct powders, followed by blueberry and olive powders. The OHC values of these powders were higher than the results reported by Figuerola et al. (2005) for apple fiber (0.60 to 1.45 g/g) and grapefruit (1.20 to 1.52 g/g), as well as the values obtained by Martínez et al. (2012) for mango (1.6 g/g), passion fruit (0.9 g/g) and guava (0.7 g/g) co-products. In addition, the particle size can also influence this capacity in which the smaller the particle size, the higher the oil holding capacity because the smaller particles have larger surface areas (Viuda-Martos et al. 2012).

The solubility of the powder is associated with the structure of its polysaccharides, and whereas these compounds are classified as regular (insoluble) or irregular (soluble) according to their structure (Elleuch et al. 2011). The solubilities of the powders (Table 3) ranged from 24.19% to 59.91%, and the highest value was presented by the papaya byproduct powder sample, followed by pineapple, blueberry and olive powders, with significant differences ($p \leq 0.05$) found among all of the powders. The byproduct powders analyzed in this study presented higher values compared to other powders, such as the fiber in orange byproducts, which demonstrated a solubility value of 28.95% (Crizel et al. 2013).

Table 3. Technological properties, total phenolic compounds and antioxidant activities of the pineapple, papaya, blueberry and olive byproduct powders.

Byproduct powder	WHC (g water/g powder)*	OHC (g oil/g powder)*	Solubility (%)*	Total phenolic content (mg/g GAE)*	Antioxidant Activity (IC 50 in mg powder)*
Pineapple	6.06 ± 0.29^{b}	3.45 ± 0.09^{a}	55.00 ± 0.13^{b}	12.28 ± 0.04^{c}	23.96 ± 1.90^{a}
Papaya	8.93 ± 1.05^{a}	3.81 ± 0.58^{a}	59.91 ± 0.23^{a}	15.35 ± 0.63^{b}	23.55 ± 0.68^{a}
Blueberry	4.01 ± 0.03^{c}	3.21 ± 0.16^{ab}	40.15 ± 0.55^{c}	23.59 ± 0.85^{a}	4.62 ± 0.18^{c}
Olive	3.57 ± 0.21^{c}	2.60 ± 0.08^{b}	24.19 ± 0.36^{d}	10.02 ± 0.98^{c}	19.17 ± 1.01^{b}

* Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey's test ($p \leq 0.05$).

3.3. Total phenolic content

The total phenolic content of the byproduct powders was evaluated and expressed as gallic acid equivalents. The samples evaluated presented values ranging from 10.02 to 23.59 mg GAE/g. The blueberry byproduct powder sample showed the greatest total phenolic content ($p \leq 0.05$), followed by the papaya powder sample and then the pineapple and olive powders, which were not significantly different from each other. These values are in agreement with data reported in the literature for byproducts of freezer-dried fruits, including guava (19.87 mg GAE/g), soursop (14.39 mg GAE/g), papaya (7.83 mg GAE/g), mango (3.76 mg GAE/g) and passion fruit (4.51 mg GAE/g) (Morais Ribeiro da Silva et al., 2014).

Su and Silva (2006) evaluated the phenolic content using the Folin-Ciocalteu method in blueberry pomace obtained by juice extraction, and the result (20.7 mg GAE/g) was similar to the powder byproducts analyzed in this study.

3.4. Antioxidant Activity-DPPH method

The antioxidant activity evaluates the capacity of substances to deactivate the DPPH radical by electron transfers. The results of the byproduct powders antioxidant capacities were expressed using the efficient concentration (EC_{50}), which represents the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (Magalhães et al. 2008).

The DPPH analysis revealed the best results for the blueberry byproduct powder sample, followed by the olive, papaya and pineapple powders. Studies reported that high blueberry antioxidant activity might be related to the high content of phenolic compounds because among many fruits and vegetables, blueberries can be considered a major source of antioxidants (Vrhovsek et al. 2012).

A study performed by Guimarães et al. (2010) obtained antioxidant activity EC_{50} values (mg/mL) lower than that of blueberry powder when assessing the polar fractions of orange and grapefruit peel (4.99 mg/mL and 5.15 mg/mL, respectively).

The contents and types of phenolic and other antioxidant compounds in fruit byproducts depend on numerous factors, which can include differences in the varieties, maturity and season; environmental aspects, such as the soil type and climate; genetic factors; and the processing method, including the extraction methods and solvent used (Martínez et al. 2012). Factors such as the temperature, time and type of drying also influence the amount of antioxidant compounds in the powder.

3.5. Carotenoid identification and quantification

Table 4 shows the carotenoid compositions of the pineapple, papaya and olive byproducts powders. The papaya powder sample showed the highest content of total carotenoids, including lutein, zeaxanthin, cryptoxanthin and β-carotene. Statistical analysis showed that the pineapple and olive byproduct powders did not differ in their carotenoid contents but showed a difference in their major carotenoid. Lutein was the main carotenoid in pineapple powder, and pineapple powder was the only one to present α-carotene.

Table 4. Carotenoid compositions of the pineapple, papaya and olive byproduct powders

Peak Nº	Carotenoids	Retention time (min)	Concentration (mg/100g (DM))*		
			Byproduct powder		
			Pineapple	Papaya	Olive
1	Lutein	17.790	1.12 ± 0.17^{b}	2.41 ± 0.05^{a}	0.26 ± 0.03^{c}
2	Zeaxanthin	20.746	0.10 ± 0.02^{b}	0.73 ± 0.08^{a}	0.10 ± 0.001^{b}
3	Cryptoxanthin	31.212	ND	9.21 ± 0.31^{a}	0.34 ± 0.01^{b}
4	α- carotene	37.746	0.058 ± 0.003	ND	ND
5	β-carotene	42.203	0.75 ± 0.04^{b}	3.21 ± 0.36^{a}	0.94 ± 0.07^{b}
Total Carotenoids			2.02 ± 0.13^{b}	15.56 ± 0.35^{a}	1.64 ± 0.06^{b}

* Results are the means of three determinations ± standard deviation. Different letters in the same line are significantly different as determined by Tukey's test ($p \leq 0.05$). ND (not detected)

The pineapple, papaya and olive byproducts powders showed total carotenoid concentrations higher than those obtained by Crizel et al. (2013) for orange byproduct fiber, including F1 (peel, bagasse and seed fiber) and F2 (peel fiber) with contents of 0.95 mg/100 g and 1.21 mg/100 g, respectively. The carotenoid contents obtained in this study were also higher than those for avocado peel (1.52 mg/100 g) and banana peel (0.4 mg/100 g) (Wang et al., 2010).

Sentanin and Amaya (2007) evaluated the carotenoid levels in formosa papaya determined by HPLC and obtained lower values than the cryptoxanthin and β-carotene contents (7.0 and 1.2 ug/g, respectively) found in the powder of papaya byproducts because carotenoids are usually more concentrated in the peels than in the pulp of the fruits. Geographical effects also influence the level of carotenoids relative to the soil and climate where the fruits were produced because exposure to sunlight and elevated temperatures increases carotenoid biosynthesis in fruits (Rodriguez-Amaya 2001).

Morais Ribeiro da Silva et al. (2014) quantified the β-carotene in freeze-dried byproducts of tropical fruits, including pineapple (peels and pulp leftovers) and papaya (peels, pulp leftovers, and seeds) and obtained contents of 0.16 mg/100 g and 0.49 mg/100 g, respectively. These results were lower than those demonstrated in this study. This difference can be explained by differences in the compositions of the byproducts as well as by the type,

temperature and time of drying, processing method and solvent utilized in the extraction and particle size.

Carotenoids have numerous important functions for human health, as protect humans from severe disorders, such as cardiovascular disease, cancer, age-related macular degeneration, and cataracts, because of the provitamin A activity that some of them possess (Fratianni et al. 2010). Research has been conducted towards elevating the levels of these compounds in foods thus, it becomes important to use ingredients that contain higher levels of these compounds and that exhibit low production costs, such as powder obtained from industrial residues.

3.6. Anthocyanins identification and quantification

Anthocyanins have significant antioxidant activities and are considered one of the best sources of antioxidants in the diet; thus, they play an important role in the prevention of neuronal diseases, cardiovascular diseases, cancer and diabetes (Patrasa et al. 2010). Due to these benefits, the addition of these compounds into the human diet is of great importance.

Table 5 shows the results of the anthocyanins obtained and quantitatively analyzed in the powder of blueberry byproducts (Figure 1D).

Table 5. Anthocyanins of blueberry byproducts powder and methanol:acetone extracts

Peak	**Anthocyanin**	**Retention Time (min)**	**Blueberry byproducts powder**
			Anthocyanins content (mg/100 g)*
1	Delphinidin 3-Glucoside	4.45	824.9 ± 11.9[a]
2	Cyanidin 3-Glucoside	4.89	303 ± 7.4[a]
3	Malvidin 3-Glucoside	5.68	513.2 ± 13[a]
4	Delphinidin Aglycone	5.76	47.8 ± 5[a]
5	Pelargonidin 3-Glucoside	5.92	222.7 ± 9.8[a]
6	Cyanidin Aglycone	6.38	112.8 ± 0.5
7	Malvidin Aglycone	7.09	38.9 ± 0.2
	Total Anthocyanins		2063.4 ± 17[a]

* Results are the means of three determinations ± standard deviation. Different letters in the same line are significantly different as determined by Tukey test ($p \leq 0.05$). ND (not detected)

The blueberry byproduct powder sample exhibited a high anthocyanin content of 2063.4 mg/100 g, with delphinidin 3-glucoside being the anthocyanin found in the greatest amount (824.9 mg/100 g), followed malvidin 3-glucoside, cyaniding 3-glucoside, pelargonidin 3-glucoside, cyaniding aglycone, malvidin aglicone and delphinidin aglycone. Reque et al. (2014) identified delphinidin 3-glucoside and malvidin 3-glucoside as the major anthocyanins in blueberry fruit.

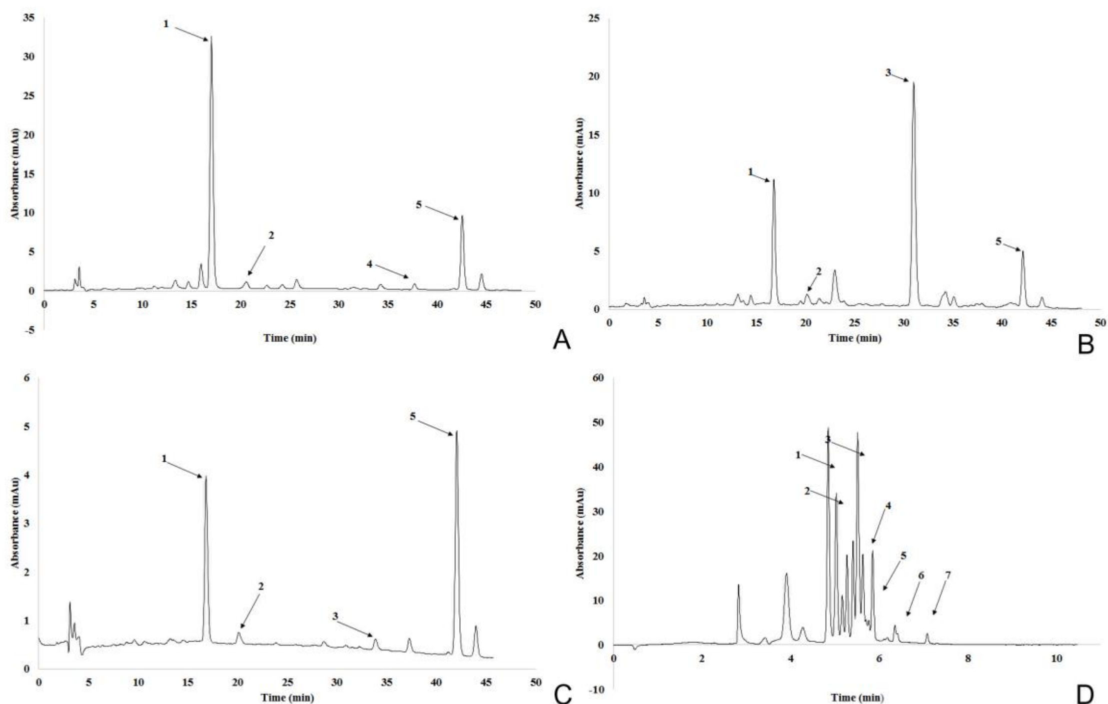

Figure 1. Chromatogram, obtained by HPLC, of carotenoids from byproduct powder pineapple (A), papaya (B) and olive (C) and of anthocyanins from byproduct powder blueberry (D).

The high anthocyanin content in blueberry powder justified its high Folin reduction capability and high ability to disable the DPPH radical, which were superior to the other powders analyzed in this study.

The determination of the total anthocyanins of a frozen rabbiteye blueberry pomace sample obtained by processing juice was performed by Su and Silva (2006), showing an 11.9 mg/g DM total anthocyanin content using a pH differential method. The result obtained in this study (20.63 mg/ g DM) was higher than the result exhibited by Su and Silva; many factors can be responsible for this difference. The anthocyanin content can vary according to cultivars and native species of the berries that exist, the season and growing location of different methods and the solvents used, all of which may contribute to the variance in the reported levels of anthocyanins, phenolics, and antioxidants (Moyer et al. 2002). According to Nicoué et al. (2007) who determined total anthocyanins by HPLC-DAD, this method showed lower values than analysis by the pH differential method. The powder drying process also influenced the total anthocyanins, particularly at high temperatures and for long periods because the process can destroy phenolic compounds. Studies show that the drying

temperature suitable for maintaining the antioxidant activities of products is 60 ºC, suggesting that antioxidant compounds have a higher resistance to heat degradation (Patras et al. 2010).

Due to the antioxidant profile of the powder from blueberry byproducts, an interesting use for it could be as an ingredient in the development of functional foods with the objective of improving health. The application of byproducts in the production of powder makes their use even more interesting because of their low economic value and because they are environmentally friendly.

4. Conclusions

The results confirm the high potential for the recovery of waste derived from the fruit processing industry to obtain dietary powder. The nutritional, functional and antioxidant properties of the powder enables its application as an ingredient in many products consumed by the human population.

The powders showed good functional properties, especially the papaya byproduct powder sample, which showed the highest WHC, OHC and solubility values, thus enabling its use to improve the texture and reduce the calories in food. All of the powders exhibited high TDF contents in their composition, high Folin reduction capacities and good abilities to disable the DPPH radical, with the blueberry byproduct powder sample obtaining the highest values for these properties and the highest concentration of anthocyanins. The carotenoid content was significant in papaya powder, followed by the pineapple and olive byproduct powders compared to other plant sources.

The use of these byproducts becomes interesting and viable in the food industry due to their low cost, environmental preservation and essential properties related to human health because powder rich in fiber is associated with bioactive compounds.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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CAPÍTULO 6

Artigo publicado na revista *Industrial Crops and Products*

Valorization of food-grade industrial waste in the obtaining active biodegradable films for packaging.

Tainara de Moraes Crizel, Tania Maria Haas Costa, Alessandro de Oliveira Rios, Simone Hickmann Flôres.

Abstract

The use of industrial waste as a material for the development of biodegradable and active packaging is economical and environmentally appealing. The aim of this study was to develop and characterize biodegradable and antioxidant packaging using wastes from the production of gelatin capsules and ingredients obtained from waste of juice blueberry processing. Fiber and ethanolic extract, obtained from the blueberry waste, were used at different weights in film formulation based on gelatin capsules waste: FF series with fiber concentration of 0.05, 0.10 and 0.15 g/mL; FE series with 30, 40 and 50 mL extract; and a control formulation. The morphological, mechanical, barrier, optical, thermal and antioxidant (AA) properties of the films were analyzed. The results suggest that the addition of fiber promoted a decrease in tensile strength from 2.51 (control formulation) to 1.51 MPa (0.15 g fiber/mL) and a increase in water vapor permeability from 59 (control formulation) to 99 (0.15 g fiber/mL) h g.mm/m2 kPa. However, the addition of fiber also provided a significant increase in the UV light barrier at 500 nm (+ 0.16%) in AA films (+67.36%) and was effective in reducing lipid oxidation of sunflower oil. Films with added extract did not exhibit altered mechanical or barrier properties compared with the control formulation and were effective as a barrier to UV light. Furthermore, these films exhibited very stable antioxidant capacity for 28 days. The use of gelatin capsules and blueberry juice processing waste are promising for the development of environmental friendly packaging to be used in food preservation.

Keywords: waste; gelatin films; blueberry; food packaging.

1. Introduction

In recent years, interest in biodegradable packaging as an alternative to synthetic packaging and non-biodegradable petroleum-based packages has increased in light of their promise to reduce the impact of packaging on the environment (Andrade-Molina et al., 2013). Adding antioxidants to these biodegradable packaging materials represents a promising alternative to reduce the oxidation of food products (Gómez-Estaca et al., 2014; Iahnke et al., 2015). These oxidative processes can cause the degradation of meat proteins, pigments and lipids, limiting the shelf life of food (Liu et al., 2010).

In this context, several studies have been conducted to evaluate the addition of antioxidants from natural sources, such as resveratrol (Pastor et al., 2013), plant extracts (Li et al., 2014), essential oils of herbs and spices (Wang et al., 2013), to biopolymers in packaging to replace synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as these synthetic antioxidants can constitute a potential danger to the health of consumers (Moure et al., 2001). Natural antioxidants can also be extracted from food industry waste, especially from the fruit processing industry due to the presence of compounds with high antioxidant value, including carotenoids and phenolic compounds (Gómez-Estaca et al, 2014; Mirabella et al., 2014; Su and Silva, 2006). According to Reque et al. (2014), blueberry pomace is rich in antioxidants, such as anthocyanins, because these compounds are preferably found in the blueberry fruit peel. These compounds play an important role in preventing cardiovascular disease, cancer and diabetes (Kończak and Zhang, 2004).

Different biopolymers can be used to produce films, including gelatin, which is obtained by thermal degradation of collagen. Gelatin represents an optimum material for biofilm formation due to its functional and biodegradable properties (Jridi et al., 2014). The waste produces of oil nutraceutical capsules (chia, linseed, coconut, and safflower) are comprised mainly of gelatin, which is generated in large quantities at a high waste treatment cost to the industry. This material can be 100% availed to obtain biodegradable and active biofilm, as it contains the gelatin, water and glycerol that are the important components for improving the properties of the films.

The aim of this study was to develop and characterize antioxidant biodegradable packaging using wastes from the production of nutraceutical capsules as biopolymer and blueberry juice processing waste.

2. Materials and Methods

2.1. Materials

The waste from the chia oil nutraceutical capsules (gelatin capsules) production were provided by the Laboratory Chemical Pharmaceutical Tiaraju, located in Santo Angelo (RS / Brazil). The waste is composed of gelatin (48.2%), water (30%) and glycerol (21.8%). Organic frozen blueberries (*Vaccinium* sp.) of the cultivar Delite (Rabbiteye) were purchased from "*Fazenda Viva o Verde*" in Camaquã (RS / Brazil).

2.2. Obtainment of fiber and extract from blueberry pomace

The blueberry pomace was obtained using a centrifugal extractor (Walita-Philips®) where the juice was separated from the bagasse. The blueberry pomace dietary fiber was prepared in accordance with the methodology developed by Crizel et al. (2013). The fresh blueberry pomace was dried in an oven with forced air circulation (DeLeo, Model B5AFD, Brazil) at 55°C for 24 h. After cooling to room temperature (~25ºC), the dried product was crushed in a mill (Model MCF55, Bertel Brand, Brazil) and was separated by screens on agitation. The separated particles were smaller than 125 mm (mesh 115). The fiber was packed in vacuum sealer (Model F200, Fastvac, Brazil) and stored in the dark at room temperature (~25°C).

To obtain the extract was used the fresh blueberry pomace and PA ethanol as a solvent at a concentration of 1:1 on Ultra Turrax® (Ultra Turrax® (IKA Ultra Turrax digital, T25, Germany) for 1 min and subsequently centrifuged at 3000 g (Sigma, 4K15 model, England) for 15 min. The supernatant was obtained as the ethanolic extract.

2.3. Film Formulation

The film formulations were based from preliminary tests, including seven formulations: three formulations with different concentrations of blueberry pomace dietary fiber (FF5- 0.05 g/mL, FF10- 0.10 g/mL and FF15- 0.15 g/mL), three different quantity of blueberry pomace extract added to replace the amount of water in the formulation (FE30- 30 mL, FE40- 40 mL and FE50- 50 mL) and a control formulation (FC- without extract and

without fiber). The percentage of fiber and extract added were calculated based on the volume of water in the formulation control. Table 1 presents the formulations of all films developed.

Table 1. Film formuls based on gelatin capsules waste and different additions of fiber (FF) and extract (FE) from blueberry pomace.

Formulation	FF5	FF10	FF15	FE30	FE40	FE50	FC
Capsules waste (g)	50	50	50	50	50	50	50
Water (mL)	100	100	100	70	60	50	100
Blueberry fiber (g/mL)*	0.05	0.10	0.15	-	-	-	-
Blueberry extract (mL)**	-	-	-	30	40	50	-

*Concentration fiber based on the amount of water in the formulation; ** Quantity of blueberry pomace extract added to replace the amount of water in the formulation.

2.4. Characterization of the waste processing nutraceutical capsules

The physicochemical analysis of moisture, ash, protein and lipids were determined in the waste according to AOAC (1990). The carbohydrate content was estimated by difference (the carbohydrate content was determined by weighing and subtracting weights of the other substances). All analyzes were performed in triplicate. The results were expressed in g / 100 g dry matter basis.

2.5. Film preparation

For the preparation of the filmogenic solution, 50 g of nutraceutical capsule residue was dissolved in water, according to the volumes presented in Table 1, in a water bath at 60°C (Model 752A, Mark Fisatom) under constant stirring for 30 min. The filmogenic solution was cooled to 40°C, when the extract or the fiber was added under constant stirring for 5 min. After this period, the filmogenic solution was placed in a vacuum desiccator for 2 min,

followed by ultrasound bath for 30 min to remove the air bubbles. The solution was placed in polystyrene petri dishes at 0.13 g/cm^2 on each plate. The plates were placed in an oven with forced air circulation (Model B5AFD, Mark DeLeo) at 30°C for 20 h. The films were stored (58% RH, 25°C) in desiccators containing a saturated solution of magnesium nitrate for 48 h before the characterization.

2.6. Film Characterization

2.6.1. Morphological analysis of the films

Scanning electron microscopy (SEM) was used to observe the morphology of the films, the material distribution in the matrix and the possible separation of the film layers. The samples were attached on an aluminum base with conductive adhesive double-sided tape, metallized with a thin layer of gold and observed in a scanning electron microscope (Model JSM 5800). All samples were examined with an accelerating voltage of 5 kV and a magnification of 100x for films with fiber and 500x for films with extract and the control film. Both the side in contact with air and the side in contact with plate were observed.

2.6.2. Film thickness measurement

The thickness of the films was measured at 5 random points on the film using a digital micrometer (Model MDC-25, Mitutoyo Corp. Tokyo, Japan) with a range from zero to 25 mm and 0.001-mm precision.

2.6.3. Mechanical properties

The tensile strength (TS), percentage elongation at break (% E) and elastic modulus (EM) or Young's Modulus (YM) were measured according to the standard method ASTM D882-09 (2009), with a Texture analyzer (TA.XT plus, Stable Micro Systems, UK) with a 5-kg load cell, a distance between the grips of 55 mm and a speed of 1 mm/sec test. For these analyses, the films were cut into rectangular strips (80 mm - 25 mm), and conditioned at 23ºC and 50% humidity for 48 h before testing. The thickness was measured at five different points in each strip. TS and YM were expressed in MPa and % E in percentage (%), and the average value of ten measurements was taken.

2.6.4. Water vapor permeability (WVP)

The WVP was measured according to the methodology proposed by Mei et al. (2013), with modifications. First permeation cell (internal diameter: 63 mm, height: 25 mm) were filled with granular anhydrous calcium chloride, where the films were sealed hermetically. Each cell was weighed on an analytical balance (AY 220, Shimadzu) and placed in a glass chamber with a saturated solution of sodium chloride, providing a gradient of 75% humidity (RH) at 25°C. Weight gain was determined by the difference between weight at time zero and 24 h. The analysis was performed in triplicate and the value was determined by the following equation (1):

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

where *w* is the weight gain rate (water) (g) by the permeation cell, *L* is the thickness of the film (mm), *A* is the permeation area (m^2), *t* is the time of permeation (h), and *Δp* is the water vapor pressure difference between the two sides of the film (Pa).

2.6.5. Moisture content

The humidity of the films was determined in triplicate using 2-cm diameter circles of each film sample, previously weighed and placed in a drying oven (Model TLK 48, Mark DeLeo, Brazil) for 24 h at a temperature of 105°C. The humidity was measured as the difference between the weight at zero and 24 h.

2.6.6. Water solubility (WS)

The solubility of the films was determined according to the method by Pelissari et al. (2013) with modifications. Briefly, the amount of dry matter of the initial and final samples was determined by drying the samples at 105℃ for 24 h. The films were cut into discs with a diameter of 2 cm, weighed and placed in capsules with 30 ml of distilled water and kept under stirring in a 25° C water bath for 24 h. Subsequently, the samples were filtered using pre-weighed filter paper and then dried at 105°C for 24 h (Model TLK 48, Mark DeLeo, Brazil).

The resulting material was weighed to determine the final dry weight (Wf). The analysis was performed in triplicate and determined using the following equation (2):

$$WS (\%) = W_i – W_f / W_i *100 \qquad (2)$$

where W_i is the initial dry weight of the sample (g), and W_f is the final dry weight of the sample (g).

2.6.7. Thermal properties

The thermogravimetric method was performed to analyze the thermal properties of the films in an atmosphere of argon using Shimadzu model TGA-50 equipment. Samples were heated from room temperature to 600ºC at a heating rate of 10ºC/min.

2.6.8. Light transmission

The transmission of ultraviolet and visible light through the film was determined according Fang et al. (2002) using a UV spectrophotometer (Shimadzu Model UV-1800, Japan). The film samples were cut into rectangular strips (4.5 cm long and 1.2 cm wide) and were placed in quartz cuvettes. Transmittance measurements were taken at wavelengths between 200 and 800 nm, and transparency was calculated by dividing transmittance values (nm) by film thickness (mm). All determinations were performed in triplicate.

2.6.9. Color Analysis

The color analysis was performed on the films added with fiber and extract blueberry waste exposed to light and ambient temperature (25°C) for 35 days, and the evaluation was performed on days zero, 7, 14, 21, 28 and 35, measuring different points of each film using a colorimeter (Minolta®, model CR400, Japan) and following the color system of the CIE-L* a* b*. The parameter L* (luminosity) ranged from black (0) to white (100), the values of the chroma a* range from green (-60) to red (+60), and chroma b* values range from blue (-60) to yellow (+60).

2.6.10. Determination of antioxidant activity

The antioxidant activity of the films was determined according to the methodology used by Ferreira et al. (2014), with modifications. In summary, 1-cm^2 pieces of film were placed in a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol (0.06 mM) to react in the dark for a period of 120 min with vortexing (Quimis, Model Q920-A2, Brazil) every 30 min. After this time, the absorbance of the solution was measured at 515 nm in a spectrophotometer (Shimadzu UV-1800 model, Japan), as was the absorbance of a DPPH solution without film. All measurements were performed in triplicate. The antioxidant activity was evaluated on days zero, 7, 14, 21, 28 and 35. The films were examined under light and ambient temperature (approximately 25°C), and the antioxidant activity of the films was determined by the percentage of inhibition of DPPH.

2.6.11. Oxidative rancidity test in sunflower oil

An accelerated test was used to evaluate the protective effect of the film against the sunflower oil oxidation. Three film formulations were selected for the analysis: two films with antioxidants and the control formulation. The films with antioxidants were chosen based on the determination made by DPPH analysis, and the films that had the highest antioxidant activity were selected for the test. Thus, a film with fiber concentration 0.15 g/mL (FF15) and a film with 50 mL extract (FE50) were cut in 10-cm x 7.5-cm pieces and were sealed (Sealer Model F200, Fastvac, Brazil) to form packages, with one containing 7 g of sunflower oil, and were stored in a chamber at 40°C in the presence of light for 13 days, with tests conducted on days zero, 3, 8 and 13. For the control, sunflower oil was conditioned in closed plastic packages were put into the chamber.

The progression of oil oxidation was monitored by the analysis of the peroxide index value (PI) following the method described by AOCS (1993). The PI was expressed as mEq of oxygen per kilogram of sample.

2.6.12. Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and Tukey's test with a 0.05 significance level using Statistica software 12.0 (STATSOFT Inc., São Paulo, Brazil).

3. Results and discussion

3.1. Composition of chia seed oil nutraceutical capsules waste

With respect to the chemical composition of chia oil capsules waste, as expected, protein was the major compound (49.73 ± 0.72%), as the primary material of the capsules is gelatin. According to Al-Hassan and Norziah (2012), the proteins exhibit good adhesion to hydrophilic surfaces and provide good barrier function against O_2 and CO_2. Films formed from gelatin are clear, flexible and resistant when obtained from the melting of the gelatin in aqueous solution in the presence of plasticizers (Gennadios et al., 1994).

The moisture content of this waste was 34.20 ± 0.22%, and the ash content was approximately 0.23 ± 0.03%. The waste lipid content was 1.75 ± 0.19%, which is considered high in light of the raw material used, but this value arises from the migration from the chia oil encapsulated by the gelatin capsules. According to Jiménez et al. (2010), hydrophobic materials such as lipids present in the waste may cause a significant decrease in TS, the percentage of E and YM of the films due to the discontinuity of the polymer film matrix, as lipids are unable to form a cohesive and continuous matrix.

3.2. Film characterization

3.2.1. Film morphology

The images of the biofilms with added fibers (FF5, FF10 and FF15) from SEM are presented in Figure 1. The face of the films that stayed in contact with air during the drying period exhibited a rough and uniform surface. The higher fiber concentration was correlated with greater roughness of the films, and cracks were not observed on this side of the film.

SEM analysis was also performed for raw blueberry byproduct fiber (Supplementary material, Figure 1) and revealed the presence of isolated particles equal or smaller than 100 micrometers. The fibers exhibited a strong tendency to agglomerate, which may have affected the formation of the microstructure of the films. Müller et al. (2009) studied starch films with added cellulose fiber (0.50 g fiber / g starch) and observed, by SEM, that the starch films also exhibited random distributions of cellulose fibers without pores or cracks.

Analyzing the films images from the side that remained in contact with the plate revealed the presence of some cracks in isolated spots of FF10 and FF15 and in higher

quantity in FF5. Cracks did not compromise the integrity or flexibility of the film macro-structure but interfered negatively with the mechanical properties of tensile strength.

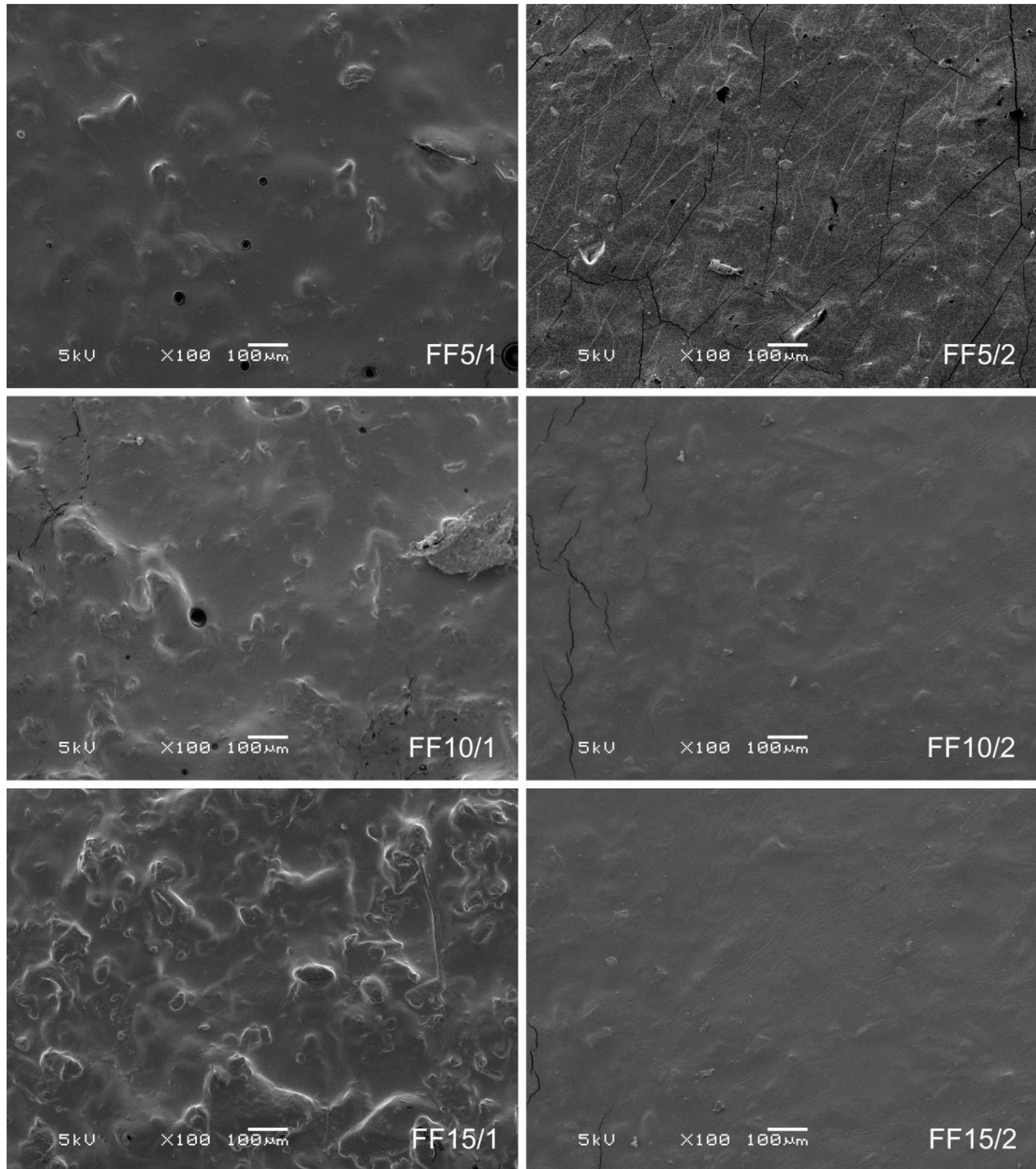

Figure 1 - Scanning Electron Microscopy (SEM) imgens (100 x magnification) of films surface added blueberry fiber (FF); 1 -air side of the film; 2- plate side of the film.

In Figure 2, micrographs of the films with the addition of blueberry bagasse extracts are presented. The ethanolic extract is composed mainly of anthocyanins originally present in fruit emphasizing the Delphinidin, Malvidin and Cyanidin-3-Glucoside (Reque et al. (2014).

SEM analysis was performed on both sides of the film, FE30, and no significant difference in the micrographs was observed. Thus, the remaining formulations were analyzed by SEM only on the side of the film that was in contact with air during the drying period. The films exhibited a homogeneous structure (500x magnification) without bubbles, but some cracks in isolated points of FE30 were noted. In Figure 2 (A2) and (B), some small insoluble particles, which can be derived from the gelatin that did not dissolve completely in the filmogenic solution or from oil particles originating from the capsules residue which were suspended, can be observed. The same particles were observed in FC (Figure 2D). Kavoosi et al. (2014) evaluated the effect of adding *Zataria multiflora* oil to gelatin films, and the micrographs revealed that the addition of oil increased the roughness and opacity of the film. Furthermore, the incorporation of oil caused homogeneously distributed bubbles in the film structure.

The films were evaluated at higher magnifications (1000x) (Supplementary material, Figures 2 and 3), and it was possible to observe homogeneity, without cracks and bubbles. In FF5 and FF10, some oil stains from the capsules waste were noted.

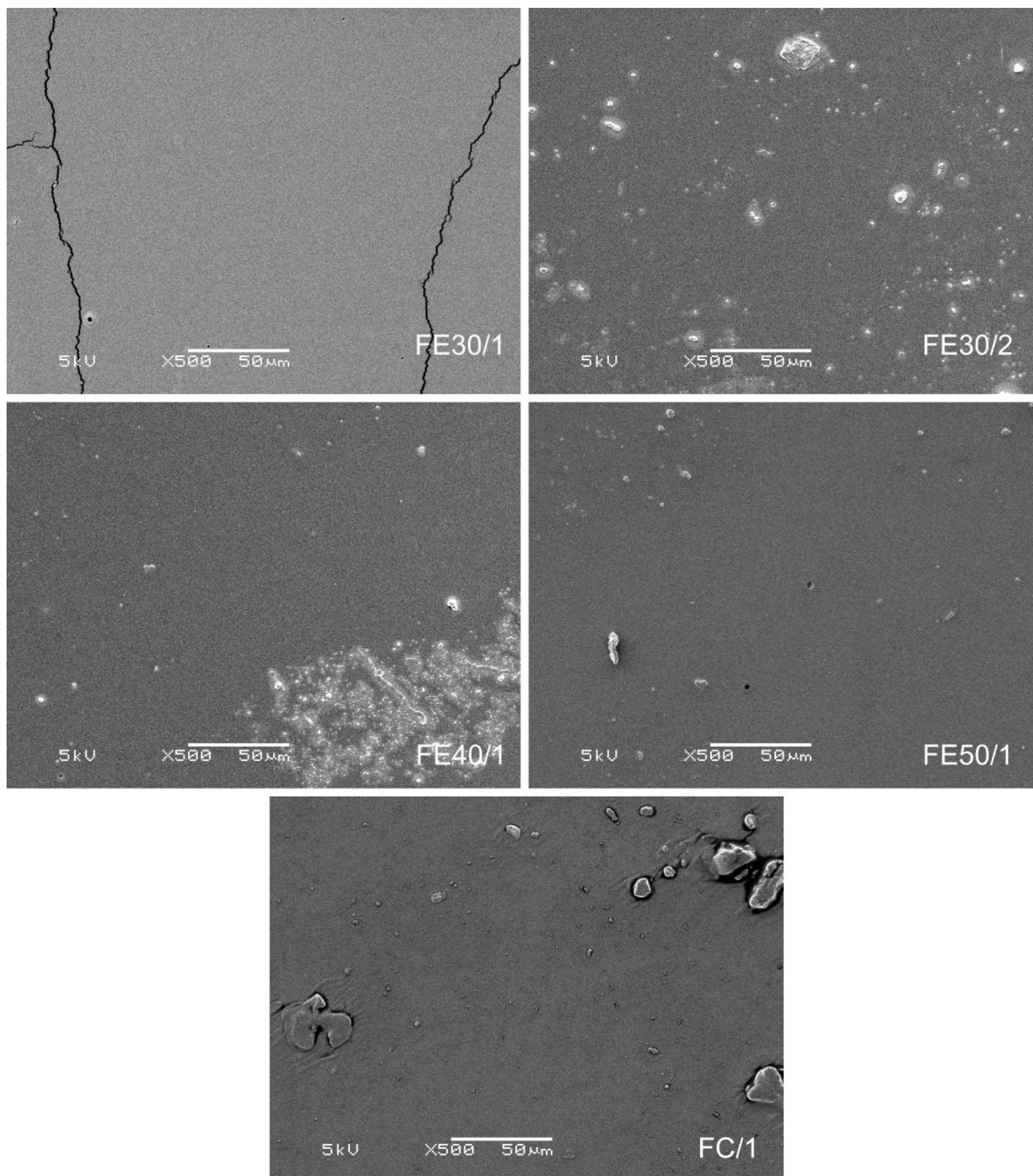

Figure 2 - Scanning Electron Microscopy (SEM) imgens (500 x magnification) of films surface added blueberry byproduct extract (FE); 1 -air side of the film; 2- plate side of the film.

3.2.2. Mechanical properties of films

Table 2 presents the results of the thickness, tensile strength (TS), percentage of elongation at break (% E) and Young's modulus (YM) analyses of all film formulations.

Significant differences (p <0.05) in thickness among the different film formulations analyzed were noted. The film formulations with the addition of blueberry bagasse fiber (FF5, FF10, FF15) exhibited the highest thickness values, and higher fiber concentrations in the film were correlated with greater thickness (0.385 mm). This result is consistent with the SEM analysis that revealed the presence of roughness caused by the fibers. This homogeneous distribution of fibers in the film caused an increase in the roughness and in the average thickness of the films. The addition of blueberry extract did not modify the thickness relative to FC.

Table 2. Thickness, tensile strength (TS), percentage elongation (E) and Young's modulus (YM) of the control films and films added of fiber (FF) and extract (FE) from blueberry pomace.

Film	Thickness (mm)*	TS (MPa)*	E[d] (%)*	YM (MPa)*
FF5	0.312 ± 0.001^{c}	1.35 ± 0.07^{b}	252 ± 20.95^{b}	433.91 ± 35.00^{c}
FF10	0.351 ± 0.005^{b}	1.31 ± 0.12^{b}	138.67 ± 7.77^{c}	535.02 ± 19.13^{b}
FF15	0.385 ± 0.007^{a}	1.51 ± 0.06^{b}	86.33 ± 5.13^{d}	735.83 ± 3.43^{a}
FE30	0.238 ± 0.007^{d}	2.56 ± 0.20^{a}	291.62 ± 15.62^{a}	175.51 ± 11.37^{d}
FE40	0.239 ± 0.009^{d}	2.54 ± 0.04^{a}	292.67 ± 2.89^{a}	222.38 ± 2.81^{d}
FE50	0.246 ± 0.008^{d}	2.87 ± 0.13^{a}	300.67 ± 5.51^{a}	196.92 ± 19.66^{d}
FC	0.236 ± 0.003^{d}	2.57 ± 0.17^{a}	257 ± 7^{b}	175.73 ± 6.52^{d}

Film with blueberry pomace dietary fiber (FF5 – 0.05 g/mL, FF10-0.10 g/mL, FF 15 -0.15 g/mL); Film with blueberry pomace extract (FE30- 30 mL, FE40- 40 mL and FE50-50 mL); *Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test (p < 0.05).

The mechanical properties of biopolymer films, including tensile strength, elongation and modulus of elasticity, are extremely important because the packaging material must possess adequate mechanical strength to maintain its integrity during handling and storage (Wihodo and Moraru, 2013).

The blueberry fiber added to the films caused a decrease in tensile strength and elongation values compared with the control film (FC). These results reflect the grade of chemical and structural incompatibility of gelatin and fiber. The cohesion of the film material is an important parameter that influences the mechanical strength, particularly if the materials

contain heterogeneous ingredients that are not compatible with the main biopolymers. The addition of fibers may have interfered in the gelatin structure, causing the breaking of bonds, which can also be observed in Figure 1 (SEM of FF5, FF10 and FF15), where the presence of cracks in some places was observed. The blueberry bagasse fiber possess a tendency to agglomerate (Supplementary material, Figure 1), and according to Gilfillan et al. (2012), the agglomeration of the fibers can reduce the connection that they have with the film matrix. Ideally, the fibers would exhibit good dispersion throughout the matrix, resulting in good tension transfer. Other factors, such as the high grade of impurity of the fiber or the addition of high quantities of fibers, can increase the difficulty of incorporating of the fibers in the matrix film (Wollerdorfer and Bader, 1998).

Although the addition of fiber reduced the TS, the values obtained were similar to those in starch films with fish skin gelatin studied by Al-Hassan and Norziah (2012), in the TS increased from 1.28 MPa to 1.67 MPa when the starch concentration increased.

The results of the mechanical properties of the films developed in this study differ from those obtained on films made with other biopolymers, such as starch. Several authors have reported that the addition of fibers is an alternative to improve the mechanical characteristics of films due to the strong bond between the fiber and the starch matrix (Curvelo at al., 2001; Gilfillan et al, 2012; Ma et al., 2005; Müller et al, 2009; Vallejos et al, 2011)

Films with added blueberry bagasse fiber exhibited high values of YM. Chiumarelli and Hubinger (2012) found that fibrous material has a high modulus of elasticity, whereas elastomers have low values, and plastics have an intermediate value. Dias et al. (2011) reported that the addition of cellulose fibers in rice flour film increased the tensile strength and the YM of these films.

The presence of the fibers increased the stiffness of the films but created failures due to the size of its agglomerates. These defects can be visualized cracks or internal pores that propagate under tensile stress, leading to rupture and thus reducing the TS and, in general, creating a more plastic than elastic behavior. This conduct is evidenced by the low values of elongation at break of the films with fibers.

Films with added extract did not exhibit significant differences in TS when compared with FC. Studies have demonstrated that the incorporation of a dispersed phase may cause a decrease in the modulus and tensile strength, which may be attributed to the presence of structural discontinuities that reduce the fracture resistance of the film (Bodini et al., 2013;

Monedero et al., 2009). This effect was not observed with the addition of blueberry bagasse extract, as there were no impairments of the film matrix or any consequent reduction of TS.

The film stretching percentage increased with the addition of the extract relative to FC. Similar results were obtained by Bittencourt et al. (2014) with the addition of turmeric ethanoic extract in gelatin films and concentrations above 100 g/100 g of gelatin resulted in an increase in the percentage of rupture elongation of the film. According to the authors, interactions between phenolic compounds (present in the extract) and gelatin peptides form covalent cross-links that can lead to a matrix that is more cohesive and flexible. Rattaya et al. (2009) added seaweed extract to fish skin films and observed a significant increase in the percentage of elongation.

Films with added extract did not exhibit alterations in YM; therefore, no significant difference in the FE30, FE40 and FE50 was observed relative to FC. The mechanical properties of the films with the addition of extract and even the control were higher than the results obtained by Hosseini et al. (2013), who studied films prepared with gelatin from cold water fish skin (TS 2.17± 0.97 MPa, rupture elongation of films 82.61 ± 20.11% and YM of 92.2 MPa).

3.2.3. Water-related properties

Table 3 presents the films' water vapor permeability (WVP). The WVP values of films with fiber were significantly higher ($p< 0.05$) than the FC and other films with added extract. As reported, the fibers interfered with the film structure, resulting in a fragile structure and with some cracks that may have changed the WVP of the films.

The addition of extract did not affect the WVP, and there was no significant difference between the biofilms with extract (FE) and FC. Giménez et al. (2013) also reported that the addition of green tea extract to gelatin and agar-agar films did not alter the WVP. The incorporation of raspberry ethanoic extract in soy isolated protein edible film also resulted in WVP values similar to the control film (Wang et al., 2012).

According to Park and Chinnan (1995), the permeability of the film can vary with the thickness due to the structural changes caused by swelling of the filmogenic matrix, which affects the film's structure and cause internal stresses that can influence the permeation. Thus, the WVP increases proportionally with film thickness.

It is essential to know the values of the WVP before to applying the film to some foods because these values determine the product type that can be packed in the film (e.g., fresh or dehydrated foods).

The moisture content obtained from the control formulation was not changed by the addition of fibers or extract from blueberry. Thus, the phenolic compounds present in the fiber and extract did not alter the hygroscopicity of the material at the tested concentrations.

Table 3. Water vapor permeability (WVP). water-solubility and moisture content of the control films and films added of fiber (FF) and extract (FE) from blueberry pomace.

Film	WVP ($g.mm/m^2$ h kPa)*	Water-solubility (%)*	Moisture content (%)*
FF5	0.99 ± 0.05^{a}	30.61 ± 0.31^{bc}	23.23 ± 0.88^{a}
FF10	0.93 ± 0.01^{a}	27.14 ± 0.18^{d}	20.63 ± 0.67^{a}
FF15	0.88 ± 0.04^{a}	26.02 ± 0.66^{d}	21.82 ± 1.19^{a}
FE30	0.57 ± 0.03^{b}	29.71 ± 0.06^{c}	22.77 ± 0.16^{a}
FE40	0.55 ± 0.00^{b}	32.42 ± 0.82^{b}	22.10 ± 0.52^{a}
FE50	0.63 ± 0.03^{b}	30.04 ± 2.13^{bc}	23.03 ± 0.75^{a}
FC	0.59 ± 0.01^{b}	42.13 ± 1.03^{a}	20.84 ± 0.31^{a}

Film with blueberry pomace dietary fiber (FF5 – 0.05 g/mL, FF10-0.10 g/mL, FF 15 -0.15 g/mL); Film with blueberry pomace extract (FE30- 30 mL, FE40- 40 mL and FE50-50 mL); *Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

The water solubility of the films decreased with the addition of the blueberry bagasse fiber and extract. FF10 and FF15 exhibited a lower percent solubility, and the fiber concentration of 0.15 g/mL fiber reduced the film solubility by 21%.

Similar results were obtained by Bittencourt et al. (2014) with gelatin film with added turmeric ethanolic extract, where an extract concentration of 100 to 200 g/100 g of gelatin significantly decreased the films' solubility. According to these authors, the interactions between the gelatin and the extract phenolic compounds justify these results. Values of low

water solubility in films embedded with green tea extract may be caused by the stronger structure of the film due to greater protein-polyphenol interaction (Wu et al., 2013).

3.2.4. Thermal stability

All films exhibited similar thermal degradation behaviors (Figure 3) and three primary stages of weight loss were identified. In the first stage between T_{room} (25°C) and 150°C, 13% weight loss that can be attributed to the loss of free water was noted. The second stage of weight loss was noted at temperatures between 150 and 210°C and may be associated with the loss of glycerol compounds, low weight molecular peptides and structurally bound water; loss reached approximately 23%. In the third stage, verified higher losses at temperature ranging from 210 to 400°C were observed. The weight loss in this phase was attributed to the degradation of gelatin protein. Similar behavior was observed in study by Wu et al., (2013). The results indicate that the film gelatin degradation began at approximately 210°C, after the complete loss of the films' moisture and glycerol content. This temperature was higher than in the study by Nuthong et al. (2009), in which the initial degradation temperature of porcine plasma protein film was observed at 170°C.

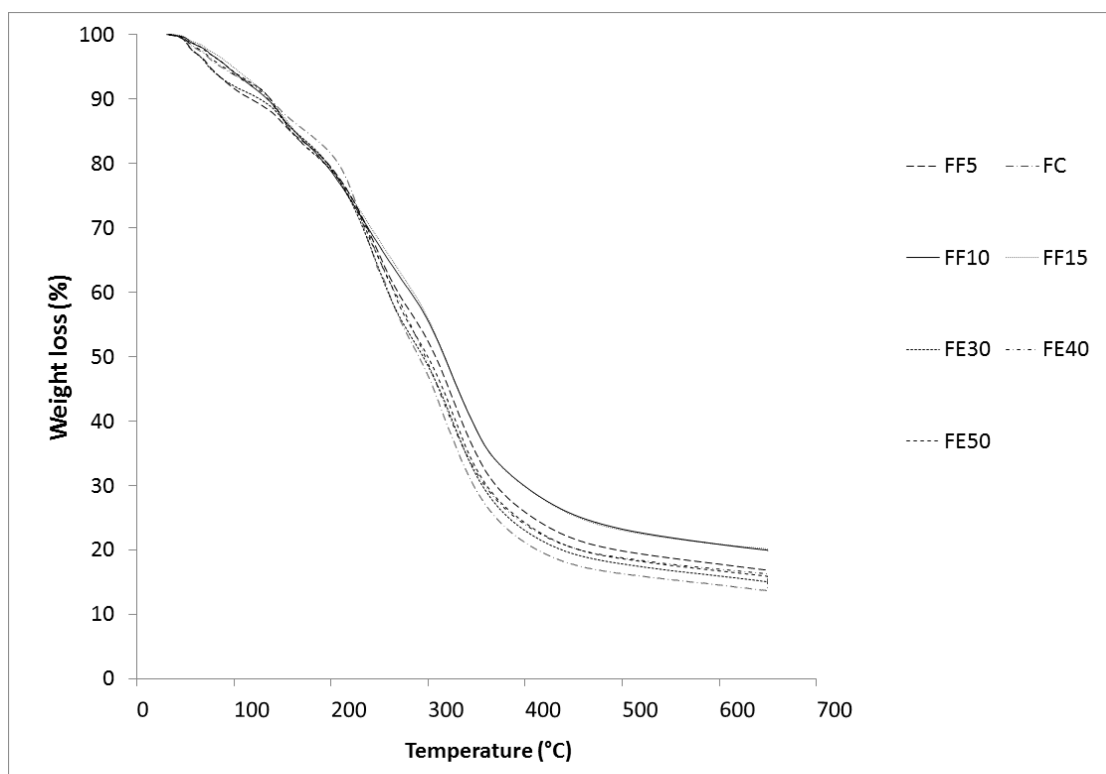

Figure 3 - Thermogravimetric Analysis (TGA) curves of films with fiber (FF) and extract (FE), comparatively with control film (FC).

3.2.5. Light transmission and opacity

Optical properties are important features for film applications, particularly if the film is used as a food coating surface or to improve the appearance of the product (Jridi et al., 2014).

The UV light transmission was very low (approximately 200 nm and 280 nm) for all films (Table 4), indicating that gelatin prevented the transmission of UV light in these wavelengths, irrespective of extract or fiber incorporation. According to Ahmad et al. (2012), protein-based films are a strong barrier to light due to the high content of aromatic amino acids that absorb UV light.

The light transmission in the visible range (350 to 800 nm) of the film with the addition of extract was lower than FC, and the addition of fiber decreased the film's transmittance values, indicating that it acted as a good barrier to radiation in the UV/visible light spectrum. This result can be explained by the large amount of anthocyanins in the film that absorb light in these wavelengths. These results are favorable, as food packaging with a high barrier in the UV/visible can prevent the lipid oxidation of food products (Bittencourt et al., 2014).

The percentage of transmittance (% T) in the films with fibers (FF5, FF10 and FF15) and extract (FE30, FE40 and FE50) were higher (transmitted lower light amount) in the UV-visible region (500 nm) compared with gelatin films with added natural antioxidant extracts, such as green tea extract (77.06%), grape seed extract (68.70%) and ginger extract (80.67%) (Li et al., 2014). The addition of blueberry byproduct extract also reduced the percentage of light transmittance of the film relative to FC. Gelatin films containing oregano and rosemary extracts developed by Gómez-Estaca et al. (2009) acted as a good barrier to UV and visible light in the range 200 to 400 nm.

With respect to the transparency values, the FC exhibited the lowest transparency value (the most transparent film), which were significantly difference from the films with antioxidants. The films with added fiber were less transparent, and transparency decreased

with the addition of fiber. The addition of extracts, even in high concentrations, caused no change in the film transparency.

3.2.6. Color Analysis

The luminosity parameter exhibited no significant difference ($p > 0.05$) among the fiber or extract film formulations over 35 days, but the films with added extract exhibited higher luminosity relative to the films with added fibers (Figure 4).

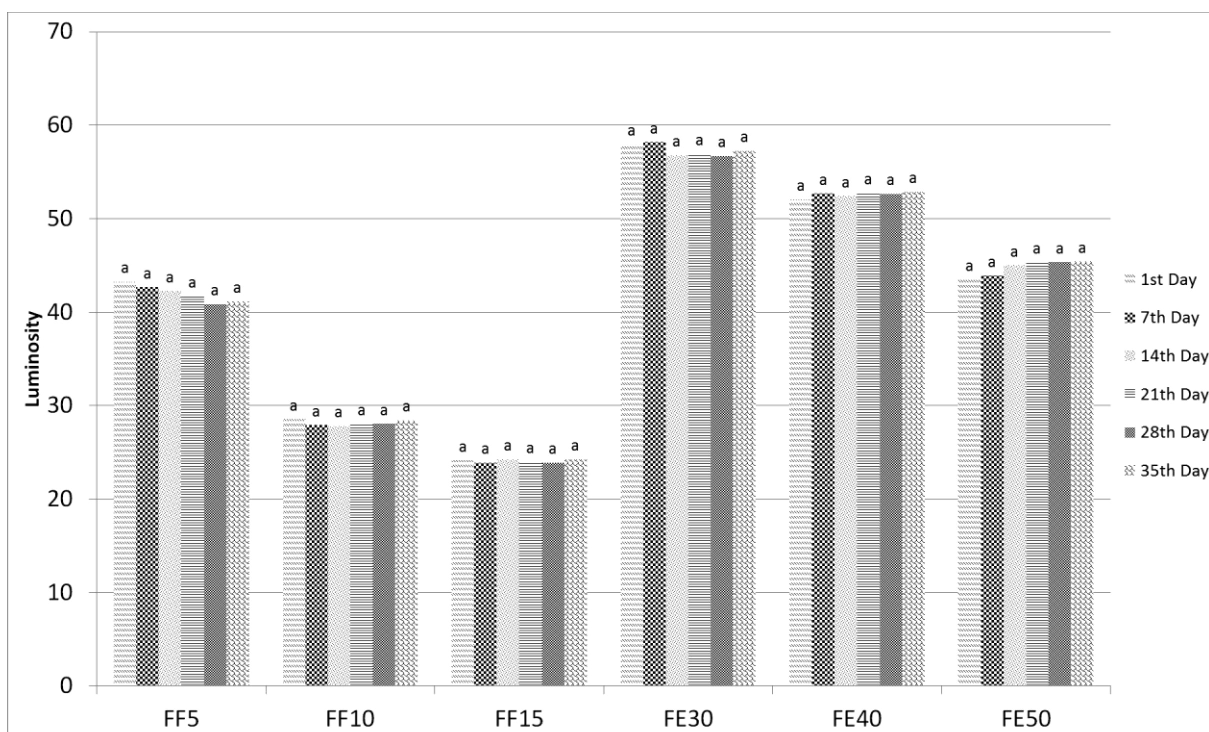

Figure 4 - Luminosity stability along 35 days of the films with fiber (FF5 – 0.05 g/mL, FF10- 0.10 g/mL, FF 15 -0.15 g/mL) and extract (FE30- 30 mL, FE40- 40 mL and FE50-50 mL).* Same letters mean no statistical difference in different storage periods of each formulation.

Over the analysis period, we observed an increase in the parameter a* (Figure 5), indicating that the films color changed, possibly due to degradation of anthocyanins. The FF15 was the most stable film to the color parameter, and a significant change in color was observed only after 21 days.

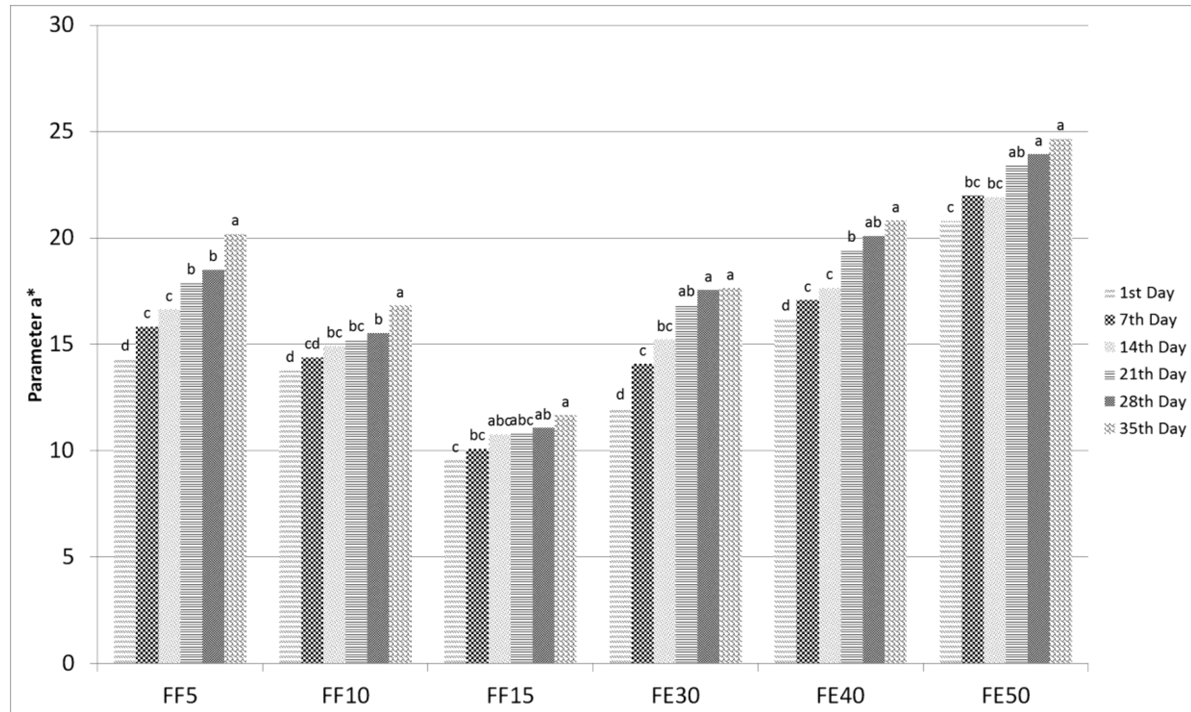

Figure 5 - Stability of the a * color parameter in films over 35 days of the films with fiber (FF5 – 0.05 g/mL, FF10-0.10 g/mL, FF 15 -0.15 g/mL) and extract (FE30- 30 mL, FE40- 40 mL and FE50-50 mL). * Same letters mean no statistical difference in different storage periods of each formulation.

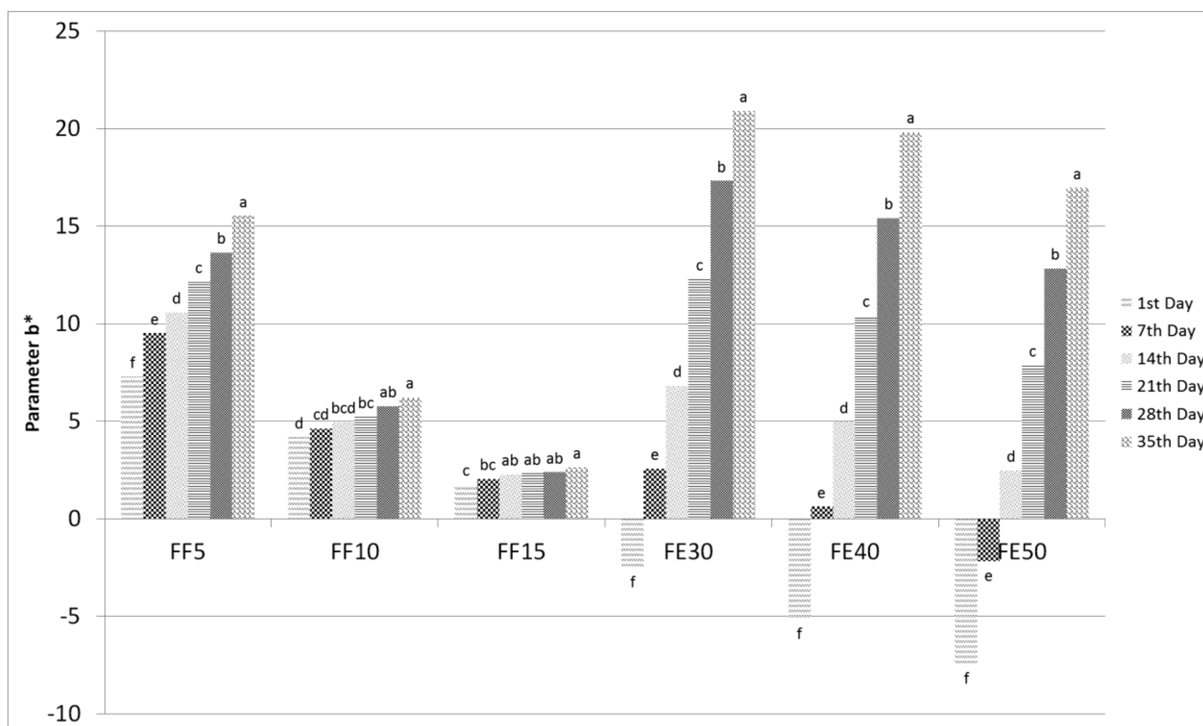

Figure 6 - Stability of the color parameter b * in films over 35 days of the films with fiber (FF5 – 0.05 g/mL, FF10-0.10 g/mL, FF 15 -0.15 g/mL) and extract (FE30- 30 mL, FE40- 40 mL and FE50-50 mL).* Same letters mean no statistical difference in different storage periods of each formulation.

All film formulations exhibited an increasing trend in parameter b* (yellow) over the 35-day period. This result reflects the degradation of anthocyanins present in the films. The films with added fiber suffered less degradation, and higher fiber content was correlated with less degradation, probably because the compounds are retained within the fiber and are thus less exposed to temperature and light. FF5 exhibited a significant increase in parameter b* in the first 7 days, and FF10 increased significantly only from the 21st day.

Over the first days, the films with the addition of the extract exhibited negative values of b* (blue) and tended to positive values (yellow), indicating pigment degradation, that was significant over time.

3.2.7. Antioxidant activity

The antioxidant properties of packaging materials were determined for 35 days in order to evaluate their stability under light incidence and room temperature.

Films with higher fiber concentrations and extract (FF15 and FE50) exhibited the highest antioxidant activity (AA) values (day 1), and there was no significant difference between them (Table 5). Thus, the addition of fiber and extract proportionally increased the AA of the films, as FF5 and FE30 were the films with lower AA values.

The stability of AA was greater in films with fiber concentration of 0.05 and 0.10 g/mL, as these films did not exhibit a significant decrease over 35 days. The films FF15, FE30 and FE40 behaved similarly, but only until the 28th day.

Ferreira et al. (2014) evaluated the influence of grape pomace extract (winemaking byproduct) on the properties of chitosan films. With respect to the antioxidant activity by DPPH method, the authors found that the aqueous extract obtained from bagasse (0.15% (w/v)) and the oil obtained from the grape seeds (0.75%) increased the antioxidant activity of the films (approximately 17% and 32% inhibition, respectively) relative to the control chitosan film (approximately 8%). Importantly, the reaction time of films with DPPH radical was 68 h. The results obtained by these authors were lower than those obtained for the films with blueberry fiber and bagasse extract developed in the present work, as the percentage of AA was significantly higher with a lower reaction time (2 h). However, the fiber and extract concentrations used in blueberry films were higher than those used by Ferreira et al. (2014).

Siripatrawan and Harte (2010) evaluated the antioxidant activity (by DPPH) in the filmogenic solution (FS) of chitosan with added green tea extract (3 mL FS plus 1 mL DPPH radical in methanol / 30 min), and formulations with 20% added extract resulted in 50% AA. The antioxidant activity was evaluated in gelatin films with turmeric ethanolic extract (0.5 ml FS plus 2.5 ml DPPH radical in ethanol / 3 h), and the films with higher extract concentrations (200 g / 100 g gelatin) exhibited higher AA values, approximately 80% (Bittencourt et al., 2014). The films with 100 g of turmeric extract reached the same concentration of extract / gelatin of the FE50 formulation of blueberry films. Thus, we were able to compare the percentage of AA, and the results were similar, approximately 60% for turmeric film and 62.7% for the activity in the films with blueberry extract.

Table 4. Films antioxidant activity (%) during a period of 35 days of the films with fiber (FF) and extract (FE) from blueberry pomace.

	1º Day	7º Day	14º Day	21º Day	28º Day	35º Day
FF5	35 ± 3^{aC}	33 ± 0^{a}	33 ± 0^{a}	33 ± 2^{a}	33 ± 0^{a}	31 ± 0^{a}
FF10	49 ± 1^{aB}	49 ± 5^{a}	49 ± 2^{a}	46 ± 2^{a}	44 ± 7^{a}	44 ± 1^{a}
FF15	67 ± 4^{aA}	67 ± 1^{a}	67 ± 2^{a}	63 ± 1^{ab}	63 ± 1^{ab}	58 ± 2^{b}
FE30	38 ± 0^{aC}	33 ± 1^{ab}	32 ± 0^{ab}	32 ± 4^{ab}	33 ± 0^{ab}	32 ± 1^{b}
FE40	50 ± 1^{aB}	46 ± 1^{a}	46 ± 2^{a}	44 ± 3^{ab}	43 ± 4^{ab}	37 ± 4^{b}
FE50	62 ± 1^{aA}	59 ± 3^{ab}	59 ± 1^{ab}	57 ± 1^{b}	50 ± 1^{c}	48 ± 1^{c}
FC	3 ± 0^{D}	-	-	-		

Film with blueberry pomace dietary fiber (FF5 – 0.05 g/mL, FF10-0.10 g/mL, FF 15 -0.15 g/mL); Film with blueberry pomace extract (FE30- 30 mL, FE40- 40 mL and FE50-50 mL); *Results are the means of three determinations ± standard deviation. Small letters in the same line and same capital letters in the same column do not differ statistically by the Tukey test at the 5 % level of significance.

The antioxidant properties of the films as protecting against sunflower oil oxidation were evaluated during 13 days and the results are shown in Figure 7.

The sunflower oil lipid oxidation protected with gelatin film (FC), gelatin film with blueberry fiber concentration of 0.15 g/mL (FF15), gelatin film with 50 mL blueberry extract and control in a transparent and closed plastic bag (CC). The oxidation was monitored by the formation of peroxide value (PV) over 13 days.

All gelatin films retarded the lipid oxidation of the oil during the period assessed. However, the unprotected oil samples (CC) were quickly oxidized.

After eight days, the gelatin films with added fiber and extract were significantly more effective in the control of oil oxidation compared with gelatin film without antioxidants. However, on the 13th day, only FF15 exhibited an effect against oil oxidation that differed significantly from the other samples. The oil protected by FF15 exhibited a constant PV and was stable under extreme conditions of temperature and light during this period.

The film with added extract (FE50) exhibited no significant difference in BC in the amount of peroxide formed in the oil at the end of 13 days. This may be associated with the low color stability of films with added extract (Figure 6). With respect to the antioxidant

activity, the films exhibited stability for 21 days at room temperature, but the extreme conditions applied in the oxidation analysis accelerated the film oxidization, decreasing their AA. Soy isolate protein films with added blueberry extract (0.5 g / 100 mL) were applied in lard to prevent oxidation, and in the films with extract, the peroxide value was lower (16.42%) that in the films without extract (5.36 meq kg^{-1}), demonstrating the antioxidant activity of blueberry extract (Zhang et al., 2010).

Atarés at al. (2010) evaluated the PV of sunflower oil protected with sodium caseinate films with the addition of cinnamon and ginger essential oils and found that the films exhibited the same protective effect as the control packed with aluminum.

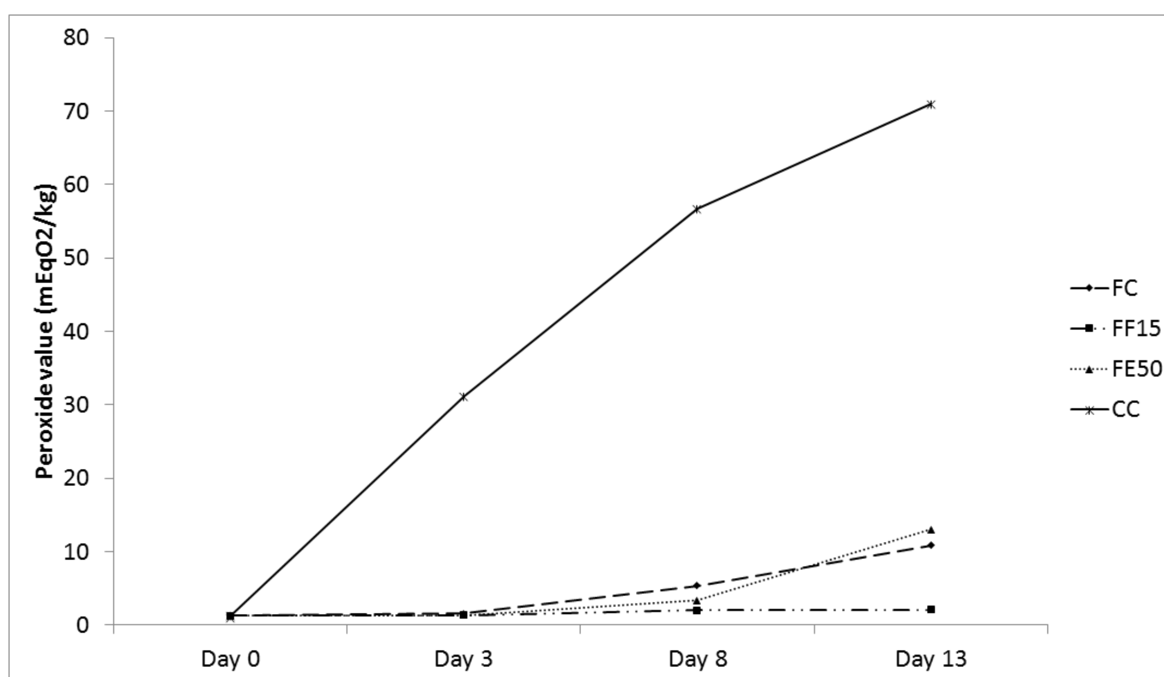

Figure 7 - Development of sunflower oil peroxide value protected by film control (FC), film with blueberry bagasse fiber of concentration 0.15 g/mL (FF15), film with 50 mL of blueberry bagasse extract (FE50), control in closed transparent plastic bottle (CC).

4. Conclusions

The results presented in this work illustrate the importance of the application of industry waste in the development of environmental friendly packaging. Films developed from gelatin capsule waste with ingredients obtained from blueberry juice processing waste are interesting with respect to the economic and environmental factors involved, as these materials represented materials discarded by industry, are biodegradable and exhibit low cost.

The utilization of these wastes represents a great option for the production of biodegradable and active packaging, mainly because the added the fiber and extract blueberry waste did not affect the macro-structure of film formation and improved UV barrier properties, significantly decreasing the light transmission of the films and increasing the antioxidant activity associated with the ability to deactivate the DPPH radical, thus becoming stable to light and temperature environment. Furthermore films exhibited antioxidant effects against sunflower oil oxidation, which enables its use for the purpose of prolonging the shelf life of food products.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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Supplementary material

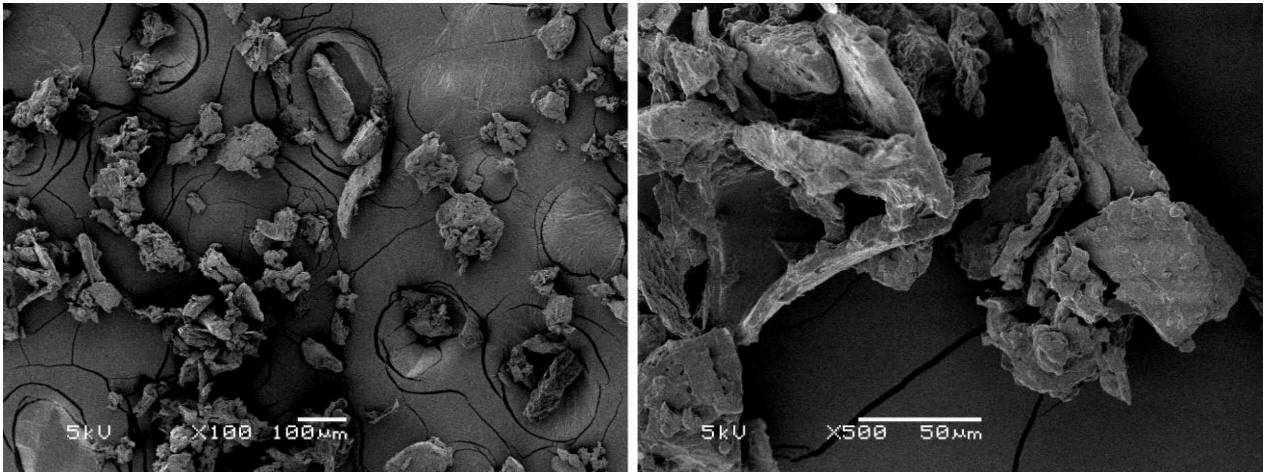

Figure 1 (Supplementary material) - Scanning electron microscopy (SEM) of the surface of the blueberry bagasse fiber (100 x and 500x magnification)

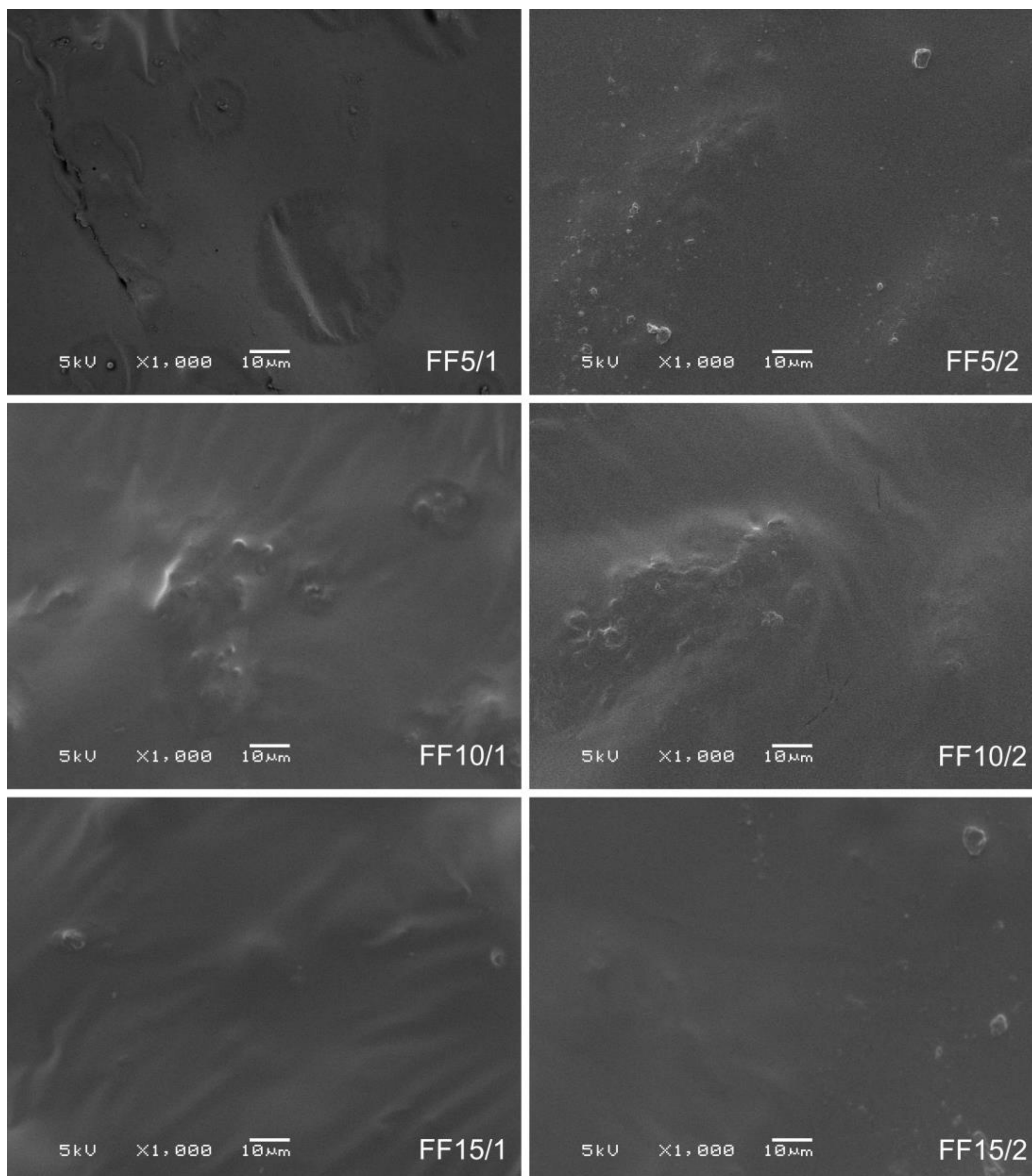

Figure 2 (Supplementary material) - Scanning Electron Microscopy (SEM) imgens (1000 x magnification) of films surface added blueberry pomace dietary fiber (FF5 – 0.05 g/mL, FF10-0.10 g/mL, FF 15 -0.15 g/mL); 1 -air side of the film; 2- plate side of the film.

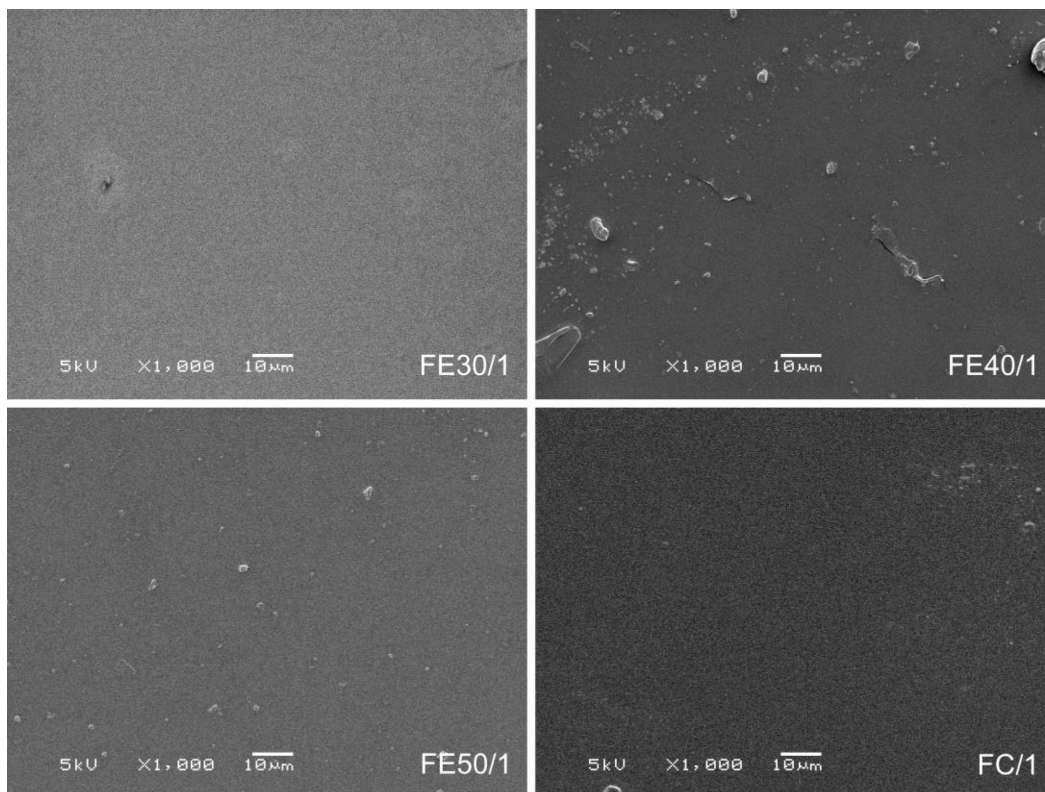

Figure 3 (Supplementary material) - Scanning Electron Microscopy (SEM) imgens (1000 x magnification) of films surface added blueberry pomace extract (FE30- 30 mL, FE40- 40 mL and FE50-50 mL); 1 -air side of the film; 2- plate side of the film.

CAPÍTULO 7

Biodegradable films based on gelatin and papaya peel microparticles with antioxidant properties

Tainara de Moraes Crizel[a], Alessandro de Oliveira Rios[a], Margarida Moldão-Martins[b], Vítor D. Alves[b], Simone Hickmann Flôres[a]*

[a] *Laboratório de Compostos Bioativos, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, RS, Brasil.*

[b]*LEAF—Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*

Abstract

Biodegradable and bioactive films were prepared using gelatin from nutraceutical capsules wastes, and natural antioxidants present in papaya peel. These films are intended to be an alternative to synthetic polyethylene packages in food preservation. Papaya peel was incorporated in the gelatin matrix as macroparticulate powder and in the form of microparticles, in different concentrations (2.5%, 5%, and 7.5%). The papaya peel powder microparticles were produced by spray drying with gelatin as wall material. The results indicated that microparticles of papaya peel powder originated a more continuous film matrix increasing the tensile strength and Young's modulus. Films with 5% and 7.5% papaya peel macroparticulate powder showed the highest antioxidant activity with values of 0.94 and 1.44 µmol Trolox equivalents (TE) / g dried film, respectively, when compared to films with microparticles (0.63 and 0.84 µmol Trolox equivalents (TE) / g dried film). When applied as packaging material for lard, the films with microparticles (7.5%) were the most efficient as active barriers (higher antioxidant activity), as a lower content of peroxides (3.47 mEq/kg) was quantified after 22 days. The addition of natural antioxidants through papaya peel microparticles is a promising strategy for the development of environmentally friendly packaging of food products with high fat content susceptible to oxidation.

Keywords: Gelatin films; papaya peel waste; microparticles; lard packaging.

1. Introduction

Along the last years, the research in food packaging has had a strong emphasis, particularly in what regards biodegradable packaging made from natural polymers for replacing petroleum-based plastic materials that are not eco-friendly (Shi et al., 2016). There is a broad range of natural polymers that can be used. Among them, gelatin stands out for its versatility in different industries such as food and pharmaceutical (Cozmuta et al., 2015). In the pharmaceutical industry, the gelatin is the base for production of oil nutraceutical capsules (chia, linseed, coconut, and safflower). However, in this production are generated large quantities of gelatin wastes that result in high recycling costs (Crizel, Costa, Rios & Flôres, 2016a).

The use of gelatin in edible films shows many advantages, namely the favorable mechanical behavior, the high oxygen-barrier property and excellent film forming ability (Pereda, Ponce, Marcovich, Ruseckaite & Martucci, 2011; Shi et al., 2016). The principal difficulty of its application relies on the low moisture-barrier due to its hydrophilic nature (Gómez-Guillén, Giménez, López-Caballero & Montero, 2011). Besides the ability to protect the food from drying, light, and oxygen, gelatin based biodegradable packaging may also protect against the action of microorganisms and oxidant agents with the incorporation of antimicrobials and antioxidants, respectively (Gómez-Guillén et al., 2009; Gómez-Estaca et al., 2014).

Natural antioxidants such as tocopherol (Noronha, Carvalho, Lino & Barreto, 2014), plant or tea extracts (Yang, Lee, Won & Song, 2016) and essential oils (Martucci, Gende, Neira & Ruseckaite, 2015) have been studied in biodegradable packaging for food preservation as an alternative to synthetic antioxidants. Also, waste originated from the processing of fruits and vegetables, such as blueberry, pineapple, papaya peel and carrot are rich in fibers and are a great natural source of bioactive compounds like phenolics and carotenoids (Porto Dalla Costa et al., 2016; Crizel, Hermes, Rios & Flôres, 2016b).

Vegetable fibers are essentially constituted by the cell wall polysaccharides, associated natural antioxidants, are low cost and renewable. However these fibers present poor mechanical properties, and some have low solubility, which makes difficult their incorporation in film matrices (Encalada, Basanta, Fissore, De'Nobili & Rojas, 2016; Versino, López & Garcia, 2015). Crizel et al. (2016a) and Iahnke, Costa, Rios & Flôres (2015) studied the incorporation of the waste fiber of blueberry, and carrot in gelatin films. Both studies presented a significant increase in the antioxidant activity of packaging films

produced, however, a decrease in their tensile strength was observed. Microencapsulation of the fibers is a promising pre-processing technique that may enable the preservation of the antioxidant compounds, being also useful to mask possible unpleasant tastes and odors. Furthermore, it may improve the technological functionality of the fibers by disposing of them in the form of microparticles that after incorporated in the gelatin film matrix, may enhance the mechanical properties, the thermal stability, and decrease the water adsorption (Strauss & Gibson, 2004; Gómez-Guillén et al., 2011).

Considering the potential use of food and nutraceutical industry by-products, the aim of this study was to produce and characterize bioactive antioxidant films based on gelatin and papaya peel, being later incorporated into the gelatin matrix as a powder or in the form of microparticles. Furthermore, the performance of selected film formulations in the preservation of food products with high fat content was evaluated using lard as the case study.

2. Materials and Methods

2.1. Materials

The waste from the chia oil nutraceutical capsules production (food grade gelatin capsules) was provided by the Laboratory Chemical Pharmaceutical Tiaraju, located in Santo Angelo (RS / Brazil). According to the company, the waste is composed of gelatin (48.2%), water (30%) and glycerol (21.8%). The papaya peels were obtained manually from papaya fruit (Formosa) purchased in Central de Abastecimento (CEASA) located in Porto Alegre (RS/Brazil).

2.2. Production of powder from papaya peels

The papaya peels were frozen in an ultra-freezer at -40 ºC for 48 hours and subsequently lyophilized (Freeze Dryer Liotop, L101, Brazil). The waste was ground in a mill (Bertel Brand, Model MCF55, Brazil) and the powder was separated using sieves for particle size analysis (Bertel, Brazil). The separated particles were smaller than 500 mm (mesh 35). The powder was packed in vacuum sealer (Sealer ECOVAC, Model ECOVAC 40, Italy) and stored in the dark at room temperature (~25°C).

2.3. Preparation of microparticles by spray dring

Firstly were produced four different microparticles formulations, one only with papaya peel powder and other three with powder and various concentrations of gelatin capsule waste, the gelatin was calculated in relation the quantity of added water (1, 1.5 and 2%). For the preparation of microparticles with gelatin, the powder (5 g) was mixed with water (150 mL) and stirred with a magnetic stirrer (Velp Scientifica, Model Arex, Italy) for 40 min. Gelatin was dissolved separately in a water bath at 60 ° C, with part of the water of the formulation. The gelatin solution was added to a mixture of powder and water under constant stirring for 10 min. After these steps tween 80 (0.1 %) was added and the solution was stirred with an Ultra Turrax® homogenizer (IKA Ultra Turrax digital, Model T25 basics, Germany) for 30 seconds at 5000 rpm. Each formulation was pumped at 6 mL/min with a peristaltic pump to the spray dryer (LabPlant, Model SD-05, United Kingdom), which operated with an air temperature of 160 ºC.

2.4. Film Formulation

Seven film formulations were obtained from preliminary tests: three formulations with different concentrations of papaya peel powder (2.5%, 5%, and 7.5%), three different concentrations of papaya peel powder microparticles (2.5%, 5% and 7.5%) and a control formulation (FC-without powder and microparticles). The percentage of powder and microparticles added were calculated based on the weight of gelatin capsule residue.

2.5. Film preparation

The films were prepared according to the casting method. For the filmogenic solution, 50 g of gelatin capsule residue was dissolved in 80 mL of water, in a water bath at 60 °C under constant stirring for 30 min. In the filmogenic solution after reaching the temperature to 40 °C, it was add the papaya peel powder and microparticles, previously dissolved in 20 ml of water, under constant stirring for 5 min. After this period, the filmogenic solution was placed in an ultrasound bath for 30 min to remove the air bubbles, 0.13 g/cm2 of the solution was weighed in polystyrene Petri dishes, that were placed in an oven (Model D -78532, Mark Binder, Germany) at 30°C for 24 h. The films were equilibrated at 48% relative humidity and ≈ 25 °C, for 48 h before the characterization.

2.6. Scanning Electron Microscopy of the microparticles and films

The microparticles and films cross sections morphology were observed used scanning electron microscopy (SEM) by field emission scanning electron microscopy (JEOL, Model JSM7001F, Japan). The samples were placed on mutual conductive adhesive tape on aluminum stubs and covered with a film of Au / Pd, about 30 nm thick in a sputter coater (Quorum Technologies, Model Q150T ES, United Kingdom). The microscopy was utilized for defined the best microparticles formulation.

2.7. Film Characterization

2.7.1. Mechanical properties

Mechanical properties were determined by tensile tests (tensile strength (TS), elongation at break (% E) and elastic modulus or Young's Modulus (YM) using a texture analyzer (TA-Xtplus, Stable Micro Systems, England) with a load cell of 5 kg. Measurements were made following the standard method ASTM D882-09 (2009), with rectangular film strips (80 mm - 25 mm) conditioned at 25 ºC and 48% humidity for 48 h before testing. The samples were attached between the grips with an initial separation of 6.0 cm, and the crosshead speed was set at 1.0 mm/s. Were evaluated ten strips of each film and the average value for TS and YM were expressed in MPa and % E in percentage (%).

2.7.2. Moisture content

The moisture content of the films was determined with 2-cm diameter circles of each film sample, previously weighed and positioned in a drying oven (Model D -78532, Mark Binder, Germany)) for 24 h at a temperature of 105°C. The humidity was measured in triplicate, and calculated as the difference between the initial weight and the final after drying for 24 h.

2.7.3. Water vapor permeability (WVP)

The WVP was measured gravimetrically according to the methodology recommended by Mei et al. (2013), with some modifications. The films samples were placed on the top of a glass permeation cell (diameter of 5 cm) containing granular anhydrous calcium chloride and

sealed with silicone. The cells were weighed and placed in a desiccator with a saturated solution of sodium chloride, providing a gradient of 75% humidity (RH) at 25°C. The mass gain was determined by the difference between weight at time zero and 24 h. The WVP was performed in using equation (1):

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

In which w is the weight gain rate (water) (g) by the permeation cell, l is the thickness of the film (mm), A is the permeation area (m2), t is the time of permeation (h), and Δp is the water vapor pressure difference between the two sides of the film (Pa).

2.7.4. Water solubility (WS)

The solubility in water was calculated as the percentage of dry matter of the film solubilized after immersion for 24 h in water at 25 °C. The films were cut into squares with a diameter of 2 cm, were stored in a desiccator with silica gel for 72 hours, after this period, the films were weighed and placed in capsules with 30 ml of distilled water and slowly and periodically agitated in a 25° C water bath for 24 h. Next day, the samples were filtered using pre-weighed filter paper and then dried at 105°C for 24 h (Model D -78532, Mark Binder, Germany). The capsules were weighed to determine the final dry weight (Wf). The analysis was performed in triplicate and determined using the following equation (2) (Pelissari et al.,2013).

$$WS\ (\%) = Wi - Wf / Wi * 100 \quad (2)$$

where Wi is the initial dry weight of the sample (g), and Wf is the final dry weight of the sample (g).

2.7.5. Water sorption isotherms

Water sorption isotherms were determined by the gravimetric method, according to Alves et al. (2011). Film samples with dimensions of 10 mm x 10 mm were previously stored in a desiccator with a relative humidity of 48% during 48 h. The samples were weighed and

placed in desiccators with different saturated salt solutions: LiCl, $MgCl_2.6H_2O$, $Mg(NO_3)_2.6H_2O$, $NaNO_3$, NaCl, KBr, and KCl, with water activity at 25 ºC of 0.112, 0.332, 0.534, 0.743, 0.753, 0.809 and 0.843, respectively. After 3 weeks the samples were weighed, ensuring that the equilibrium has been reached. For each water activity value were performed three replicates.

2.7.6. Light transmission and opacity

The barrier properties of films against ultraviolet (UV) and visible light were measured at selected wavelength between 200 and 800 nm using the method described by Fang, Tung, Britt, Yada, & Dalgleish (2002), using UV spectrophotometer (Unicam UV4 UV/Vis, United Kingdom). The film samples were cut into rectangular strips (4.5 cm long and 1.2 cm wide) and were placed in quartz cuvettes; the measurement was done in six different pieces of each sample. The opacity of the films was calculated by the following equation:

$$Opacity\ value = \frac{-logT_{600}}{x} \quad (3)$$

where T600 is the fractional transmittance at 600 nm and x is the equivalent film thickness (mm). The lower opacity value indicates the higher transparency of films.

2.7.7. Colour measurements

The color analysis was performed on all samples films, using colorimeter (Minolta CR-300, USA) and the CIELAB color space.

2.8. Antioxidant properties of powder, microparticles and film

2.8.1. Preparation of the powder and microparticles extracts

For DPPH and FRAP analyses were prepared extracts with powder and papaya peel microparticles, It was used 1 g of powder/microparticles sample with 40 mL of 50% methanol in an Ultra Turrax® homogenizer (IKA Ultra Turrax digital, Model T25 basics, Germany) for

1 min and the sample was left for 60 min at room temperature (25 ºC). The extract was centrifuged (25000 g for 1o min, Hermle Labortechnik, Model Z383k, Germany), and the supernatant was stored in an amber flask. In the precipitate was added 70% acetone and the same procedure as above was carried out. The supernatant was placed together with previous in the same amber flask. The extracts were made in triplicate.

2.8.2. DPPH radical scavenging capacity

For determination of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method in films was used methodology proposed by Ferreira, Nunes, Castro, Ferreira & Coimbra (2014), with modifications. The samples films were cut in 1-cm^2 pieces and weighed, after were placed in 3.9 mL methanolic DPPH solution (0.06 mM), to react in light absence, for 120 min with mixing tubes in vortex every 30 min, the absorbance of DPPH solution without film was measure. From the extracts (powder/microparticles) previously described, 100 uL of each extract was combined with 3900 uL of DPPH solution (0.06 mM), the reaction time was 40 min and methanol was used as a control. The absorbance of the solutions was measured at 515 nm in a spectrophotometer (Unicam UV4 UV/Vis, United Kingdom) (Brand-Williams, Cuvelier, & Berset, 1995). All measurements were performed in triplicate. A standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid from Aldrich) was conducted simultaneously. Antioxidant capacity was expressed as µmol Trolox equivalents (TE)/g dry matter or dry film.

2.8.3. FRAP

The FRAP method (Ferric Reducing Antioxidant Power) described by Pulido, Bravo & Saura-Calixto (2000) was used to measure the antioxidant power reduced iron as an alternative to determining the reduction of iron biological fluids and aqueous solutions of compounds pure. In the dark, an extract aliquot of 90 µL was transferred to test tubes, added up 270 uL of distilled water mixed with 2.7 mL FRAP reagent, the tubes were agitated and maintained in a water bath at 37 ° C. From The samples films were cut in 1-cm^2 pieces and weighed, placed in tubes with 270 µL of distilled water mixed with 2.7 mL FRAP reagent, the tubes were agitated and maintained in a water bath at 37 ° C. The results were analysed with a

spectrophotometer (595nm) (Unicam UV4 UV/Vis, United Kingdom) after 30 minutes of reaction. The FRAP reagent was used as white to calibrate the spectrophotometer. It was constructed a standard curve of FeSO4.7H2O (mM) to the absorbance at 595 nm. Results were expressed as μmol FeSO4.7H2O equivalents/g dry matter or dry film. All determinations were performed in triplicate.

2.9. Effect of antioxidant gelatin film on lard storage under accelerated conditions

The lard was packed with films that had the highest antioxidant activity by DPPH and FRAP methods. Three film formulations were selected for the analysis: two films with antioxidants (one with papaya peel powder and other with microparticles) and the control formulation. The films were cut into 7-cm x 5.5-cm pieces and were sealed (Sealer ECOVAC, Model ECOVAC 40, Italy) to form packages, with one containing 7 g of lard, and were stored in a chamber climatic (Aralab, model FitoClima 600 PDH, Portugal) at 40°C in the presence of fluorescent light for 22 days and 35 % humidity, with tests conducted on days zero, 3, 8, 13, 17 and 22. One control was used during the experiment into the chamber; lard was conditioned in closed packages polyethylene. Three packs of lard each film and three samples of each control were analyzed.

2.9.1. Peroxide index value

The progression of lard oxidation was monitored by the analysis of the peroxide index value (PI) following the method described by AOCS (2009). The PI was expressed as mEq of oxygen per kilogram of sample.

2.9.2. Secondary compounds (dienes and trienes)

Oxidation products were quantitated (dienes and trienes) by spectrophotometric method IUPAC (1979) II.D.23 number that determines the lard absorbance at certain wavelengths of the ultraviolet spectrum and provides an indication of its degree of oxidation. This is because oxidation products (conjugated dienes (232 nm) and for conjugated trienes or

secondary compounds at (270 nm) are presented in the characteristic spectra in the ultraviolet region.

2.9.3. Fatty Acid Profile

The determination of the FA profile was based on the experimental procedure previously described by Bandarra et al. (1997). Triplicate samples (300 mg of dry mass per sample) were dissolved in 5 mL of acetyl chloride/methanol (1:19 v/v), shaken, and heated at 80 C for 1 h. After cooling, 1 mL of Milli-Q distilled water, and 2 mL of n-heptane pro-analysis were added, and samples were shaken and centrifuged (2300g, 5 min) until phase separation. The moisture content of the upper phase was removed using anhydrous sodium sulfate. An aliquot (2 ml) of the upper phase was injected onto a gas chromatograph (Varian Star 3800 CP, Walnut Creek, CA, USA) equipped with an autosampler and fitted with a flame ionization detector at 250 C for fatty acid methyl ester (FAME) analysis. The separation was carried out with helium as carrier gas at a flow rate of 1 mL/min in a capillary column DB-WAX (30 m length x 0.32 mm internal diameter; 0.25 mm film thickness; Hewlett-Packard, Albertville, MN) programmed at 180 C for 5 min, raised to 220 at 4 C/min, and maintained at 220 ºC for 5 min with the injector at 250 C. FAME identification (% total FA) was accomplished through comparison of retention times with those of Sigma, Nu Check Preap and Larodan Fine Chemicals standards. The FAME contents were quantified (by weight) as a percentage (%) of the total FAMEs.

2.10. Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and Tukey's test with a 0.05 significance level using Statistica software 12.0 (STATSOFT Inc., São Paulo, Brazil).

3. Results and discussion

3.1. Morphology of the microparticles

Preliminary tests were made with different formulations of papaya peel powder microparticles, which was varied the gelatin content in the formulation and kept constant the other components. The morphology evaluated by SEM was used to select the best formulation.

Observing the SEM pictures (Figure 1), it is visible the difference between the various microparticle formulations, being noticeable that the formulation with 2% gelatin was the one that provided the most homogeneous microparticles regarding shape and size.

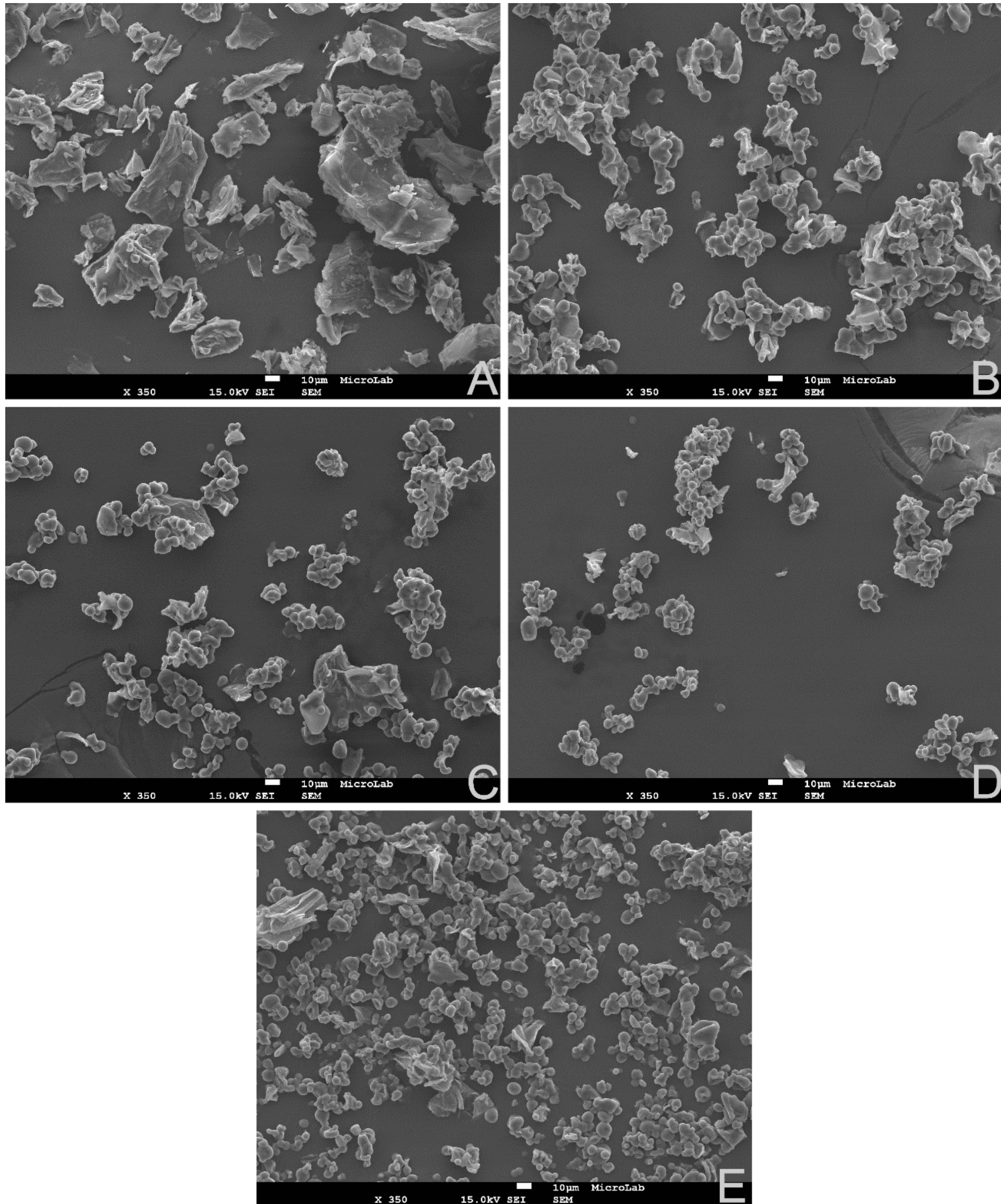

Figure 1. Scanning Electron Microscopy (SEM) imagens (350 x magnification): (A) papaya peel powder; (B) Microparticles without gelatin; (C) microparticles with 1% of gelatin; (D) microparticles with 1.5% of gelatin; (E) microparticles with 2.0% of gelatin.

In Figure 1 E the particles are more dispersed than in other formulations. This fact is is probably due to the higher amount of gelatin in the formulation, since according to Zhang, Ping & Xiao (2000) the presence of the plasticizer, in case the glycerol (present in the gelatin residue) is important since it promotes the formation of spherical and smooth surface microparticles, especially for microencapsulation by spray drying.

3.2. Film characterization

3.2.1. Morphology of the films

In Figure 2 are presented the Scanning Electron Microscopy (SEM) images of the upper surface and cross section of the films. It can be observed that the morphology of the films changed as more papaya peel powder was added to the biopolymer matrix. The surface became more heterogenous than control film, and it is possible to identify powder clusters, which resulted in greater roughness films, especially for 7.5% powder (Fig. 1 - C). As in the study of Crizel et al. (2016a), the bottom of the film, which was in contact with the plate, had a smooth surface without the presence of particles.

The effect of the addition of papaya powder microparticles in the films was easily noticed, as microparticles provided a smoother surface, without major imperfections in the matrix of the film. This is probably due to a better dispersion of the microparticles in the filmogenic solution, which are smaller than the powder, producing a more homogeneous films cross section eventually. A higher concentration of microparticles (Fig. 2 - F) resulted in improvements in the surface, leaving it smooth and thus similar to the control film (Fig. 2 - G). It is quite interesting considering that the good appearance of the packaging is of great importance to attract the consumer when buying a food product.

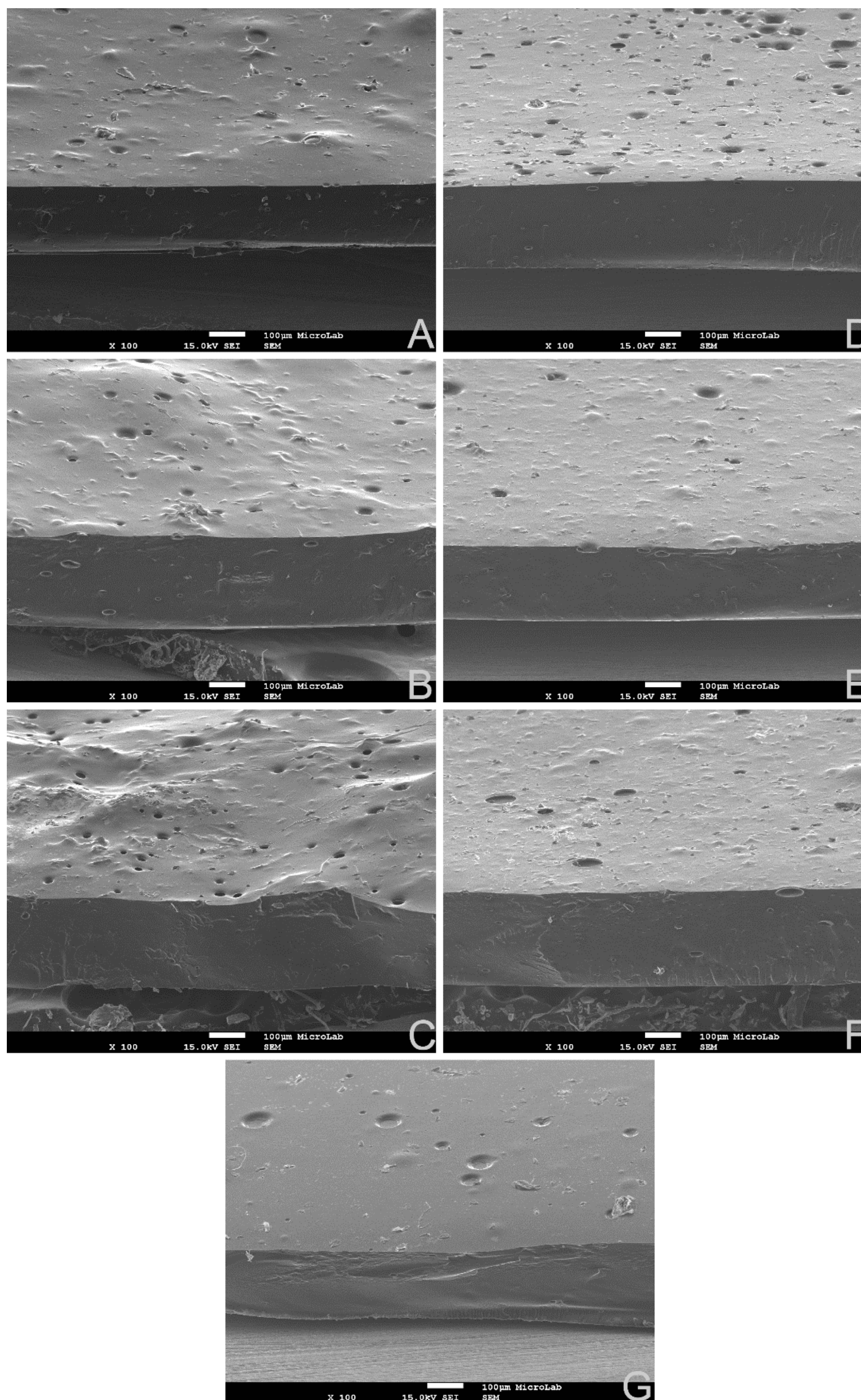

Figure 2. Scanning Electron Microscopy (SEM) images (100 x magnification) of films surface and cross section added papaya peel powder ((A) 2.5% powder, (B) 5.0% powder, (C) 7.5% powder), microparticles papaya peel powder ((D) 2.5% microparticles, (E) 5.0% microparticles, (F) 7.5% micro) and control film ((G) without powder or microparticles).

Biodegradable films made with microencapsulated powder particles of anthocyanins (wine grape pomace) using maltodextrin as wall material, resulted in films with a smoother surface, being particles partially dissolved in the films (Stoll, Costa, Jablonski, Flôres & Rios, 2016).

In all images of SEM including in the control formulation (Figure 2 - G) it is possible to visualize the presence of insoluble particles of gelatine, which can be explained by the renaturation of collagen during the film formation and heterogeneous distribution of solutes during evaporation in drying process, causing a higher concentration of solute on the surface of the film (Ghoshal, Mattea, Denner & Stapf, 2010). Also it is visible the presence of oil droplets that came from the residue of nutraceutical capsules used, according to Ma et al. (2012) gelatin films containing olive oil had a heterogeneous surface.

With expansion of cross section (300x magnification) (Supplementary material, Figure 1, (A, B and C)) of the film were perceptible some changes in the film structure, such as the lack of uniformity in the presence of some particles, apparently a less compact structure, and more brittle, differing images of films added to microparticles and control.

3.2.2. Effect on Mechanical Properties of films

Mechanical strength and extensibility are important features for a packaging film to preserve its integrity under external stress (Yang & Paulson, 2000). Table 1 shows the tensile strength (TS), percentage elongation (%E) and Young's modulus (YM) of all film formulations.

The TS and % E decreased with the addition of papaya peel powder in the films in relation to the control film. The cohesion of the constituents of the polymer matrix is paramount for the films mechanical strength, being necessary a good interaction between the fiber and polymer matrix (Sethi & Ray, 2015). The insoluble papaya peel powder and its impurity (Wollerdorfer and Bader, 1998) hinder interaction with the gelatin matrix causing a modification or disruption of the original structure of the polymeric matrix. Crizel et al. (2016a) observed similar results with the addition of blueberry bagasse fiber in gelatin films. The added of beet root and carrot residues in gelatin-based films decreased of films %E values (Iahnke, Costa, Rios & Flôres, 2016; Iahnke et al., 2015), due to lack of cohesion of residues with gelatin.

The TS and %E of the films with microparticles increased with the growth of microparticles content when compared to the formulations with powder. This increase was possibly due to the protein-protein interactions in films with microparticles resulting in the biopolymer matrix reinforcement. The microencapsulation turns powder more soluble, as the wall material used was the same as the one of the film, and provided a greater dispersion of the particles throughout the matrix, resulting in good tension transfer, consequently increasing the cohesion (Gilfillan, Nguyen, Sopade & Doherty, 2012).

The results for tensile strength of films with 7.5 % of microparticles were close to those of plastic films such as Low Density Polyethylene (LDPE) analyzed by Gennadios, McHugh, Weller & Krochta (1994) that obtained values between 8.6–17.3 MPa for LDPE films.

Table 1. Tensile strength (TS), percentage elongation (E) and Young's modulus (YM) of films with powder and microparticles papaya peel power and control film.

Film	TS (MPa)*	E^d (%)*	YM (MPa)*
Powder			
2.5%	1.86 ± 0.00^e	132.34 ± 1.09^d	856.84 ± 35.76^d
5.0%	1.62 ± 0.04^e	74.10 ± 2.41^e	758.57 ± 16.20^{de}
7.5%	1.09 ± 0.01^f	59.06 ± 0.65^f	664.37 ± 21.71^e
Microparticles			
2.5%	4.34 ± 0.02^c	154.52 ± 2.14^{bc}	2148.17 ± 149^b
5.0%	6.24 ± 0.33^b	142.15 ± 1.90^{cd}	2309.69 ± 23.24^{ab}
7.5%	7.67 ± 0.11^a	166.67 ± 0.19^{ab}	2457.70 ± 55.99^a
Control	3.41 ± 0.11^d	338.30 ± 10.11^a	1088.21 ± 73.55^c

*Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

The YM indicates how much the sample stretches without deforming, is quantified as the ratio between the stress and deformation of the sample (Chiumarelli & Hubinger, 2012). The YM values for control film were similar results found for films from tilapia skin gelatin, 1148.30 MPa (Nagarajan, Benjakul, Prodpran & Songtipya, 2014). There was a decrease in the YM values with the addition of powder; however, this reduction was only significant with the addition of 7.5%, in relation the control formulation. Iahnke et al. (2016) obtained YM

value of 674.72 MPa for gelatine films with 8% of beet root residue; similar results showed in this study for 7.5% of papaya peel powder (664.37 MPa).

Different results were obtained with the addition of microparticles, which increased the values of Young's modulus. These results reflect the chemical and structural compatibility between the gelatin matrix and microparticles. The performance was very high compared to other gelatin films made of different materials such as chitosan nanoparticles (467.2 MPa) (Hosseini, Rezaei, Zandi & Farahmandghavi, 2015) and nanoclays (1715.83 MPa) (Nagarajan et al., 2014).

3.2.3. Moisture content, water solubility, and water vapor permeability

The knowledge of moisture content (MC), water solubility and water vapor permeability of the film is crucial for food packaging, as the packaging should keep the moisture levels of product for better preservation (Ma et al., 2012).

The control film showed MC of 19.93% and the addition of powder not changed the MC of films, the same did not occur with the addition of powder microparticles, which decreased moisture of films.

Water vapor permeability (WVP) is the barrier property that usually determines the capacity of the film to protect the food from the environment (McHugh & Krochta, 1994). The results of the WVP of the films are shown in Table 2, which ranged between 1.26 and 1.75 g mm/ m2 h kPa. The WVP values of films with powder were significantly higher ($p < 0.05$) than the FC and other films with added of microparticles. As reported, the particles interfered with the film structure, resulting in a fragile structure and less cohesive. The lack of cohesion between the film matrix and powder can cause larger voids within the matrix which facilitates the water diffusion.

The addition of microparticles did not affect significantly ($p < 0.05$) the films WVP. The WVP values found in this study were high when compared to other biopolymers such as chitosan (Aguirre-Loredo, Rodríguez-Hernández, Morales-Sánchez, Gómez-Aldapa & Velazquez 2016) and whey protein (Galus & Kadzińska 2016), but the WVP values were lower than the results presented by Li, Miao, Wu, Chen & Zhang (2014) to fish skin gelatin films (2.63 g.m/m2.d.KPa). Li et al. (2014) incorporated natural antioxidants in the films formulations as grape seed extract, ginger extract and gingko leaf extract that did not change the WVP of the films.

Table 2. Moisture Content (MC), Water Solubility (WS) and Water Vapor Permeability (WVP) of the control films and films added of powder and microparticles from peel papaya.

Film	Moisture content (%)*	WVP (g.mm/m^2 h kPa)*	Water-solubility (%)*
Powder			
2.5%	20.82 ± 1.03^{a}	1.64 ± 0.02^{a}	58.11 ± 0.76^{b}
5.0%	20.91 ± 0.82^{a}	1.74 ± 0.05^{a}	65.89 ± 0.84^{a}
7.5%	20.60 ± 0.66^{a}	1.75 ± 0.03^{a}	67.75 ± 0.14^{a}
Microparticles			
2,5%	15.42 ± 0.88^{b}	1.45 ± 0.07^{b}	46.50 ± 1.11^{c}
5.0%	16.42 ± 0.42^{b}	1.32 ± 0.01^{b}	41.63 ± 1.38^{d}
7.5%	17.12 ± 0.57^{b}	1.31 ± 0.08^{b}	37.88 ± 0.55^{e}
Control	19.93 ± 0.20^{a}	1.26 ± 0.01^{b}	66.67 ± 0.04^{a}

*Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

The water-solubility of films was significantly ($p< 0.05$) reduced with the addition of 2.5% of papaya peel powder. However, this was not observed with the addition of higher concentrations of powder, which caused an increase in these but no different to the control film. An increase in the fiber addition can result in small cracks that make the water inlet and thereby enhance the solubility of the films.

The addition of microparticles results in a decrease in the films solubility values, where a higher concentration of the microparticles in the formulation promoted lower solubility values. This is because of microparticles reinforced structure of the film, as can already be evidenced by the results of mechanical properties, thereby reducing the gaps in the polymer-particle interface, consequently enters a minor amount of water and film solute diffusion will be reduced.

For the use of the films as packaging it is interesting that the film has a lower solubility for better food preservation, but higher solubility is beneficial during cooking of food products coated with edible films (Maizura, Fazilah, Norziah and Karim, 2007).

3.2.4. Water sorption isotherms

The sorption isotherm represents the variation of moisture content as a function of water activity. Figure 3 show the water sorption isotherms of film control, films with powder

and films with microparticles. The sorption isotherm curves of gelatin films showed a specific behavior of water vapor sensitive hydrophilic biopolymers when the relative humidity (% RH) increased.

The films water vapor sorption capacity showed a slight increase in water activity values inferior to 0.5 after there was an increase quickly, this behavior has been reported in some studies for hydrophilic films (Aguirre-Loredo et al., 2016; Al-Hassan & Norziah, 2012).

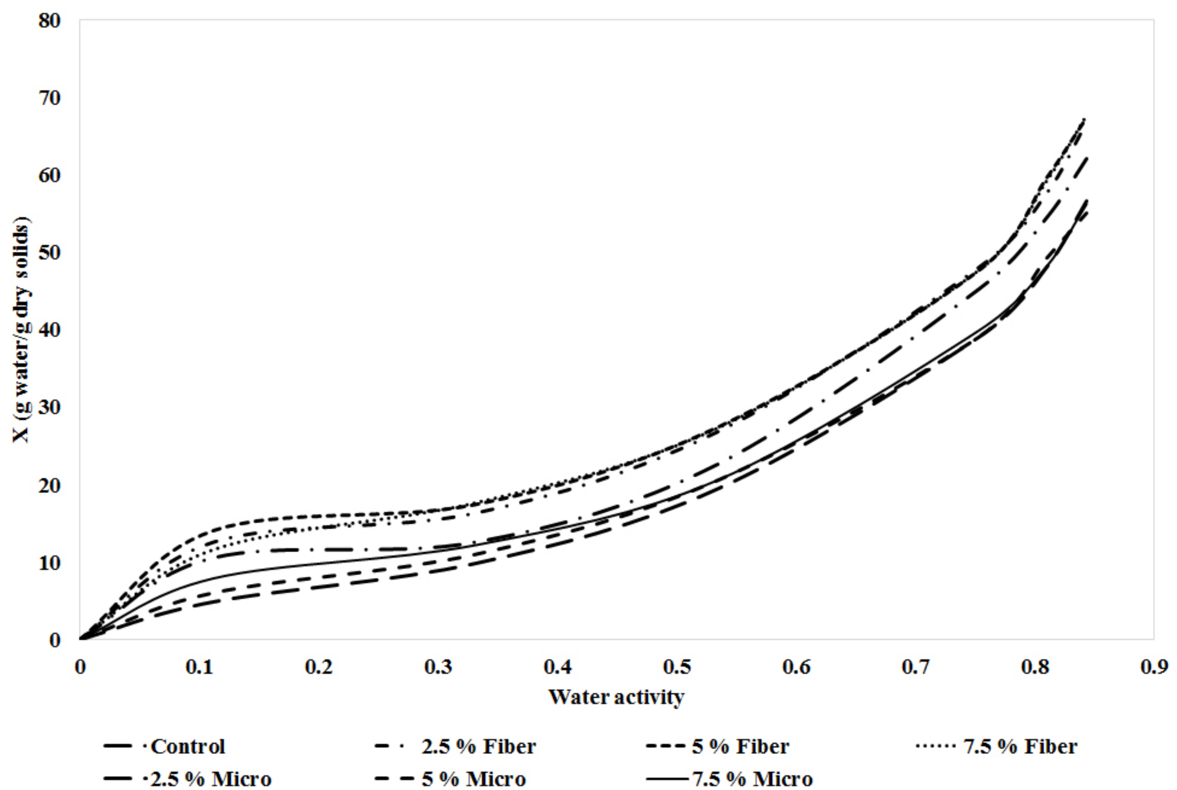

Figure 3. Water sorption isotherms of gelatin films.

By isotherms was observed that the equilibrium moisture content was higher in gelatin films with added papaya peel powder than those films with microparticles, due to the fact that the structure of films with microparticles is more compact than the films with powder, as evidenced by the results of mechanical properties, it causes the moisture has more difficulty in penetrating through the sample. As the powder does not solubilize the gelatin base, this results in changes in the morphology of the polymer (Figure 2) that may facilitate the entry of moisture in the film. Similar results were obtained from analysis of water solubility of the film, discussed above.

3.2.5. Light transmission and opacity

The presence of a film in food packaging is necessary to protect food product from the effects of light, especially UV radiation (Li et al., 2014). Transmission of UV and visible light at selected wavelengths in the range of 200–800 nm of films from gelatin incorporated with papaya peel powder and papaya peel powder microparticles is shown in Table 3.

Table 3. Light transmission (% T) and the opacity values of the control films and films added of papaya powder and papaya powder microparticles.

Film	**Light transmission at different wavelengths (%)**								**Opacity values***
	200	280	350	400	500	600	700	800	
Powder									
2.5%	0.00	0.01	16.78	33.58	48.71	58.49	61.23	62.47	0.73 ± 0.02^{d}
5.0%	0.00	0.01	8.12	19.01	30.96	40.79	43.34	44.33	1.14 ± 0.01^{b}
7.5%	0.00	0.00	1.99	7.63	17.64	29.18	32.37	33.46	1.21 ± 0.01^{a}
Micro									
2.5%	0.00	0.01	12.06	29.17	49.99	62.82	67.12	69.55	0.63 ± 0.02^{e}
5.0%	0.01	0.00	9.63	25.11	46.95	62.27	66.52	68.56	0.88 ± 0.02^{c}
7.5%	0.00	0.00	5.47	17.89	38.96	56.21	60.78	63.06	1.10 ± 0.05^{b}
Control	0.02	0.02	38.08	61.85	77.27	81.63	83.06	84.09	0.22 ± 0.01^{f}

*Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

The transmission of UV light was very low at 200 and 280 nm for all films, indicating that have excellent barrier properties against UV, mainly due high content of aromatic amino acids-tyrosine and tryptophan and in a less extent, phenylalanine and disulfide bonds which absorb UV light (Aitken & Learmonth, 1996). Higher UV light barrier ability was reported for gelatin films added of residues of minimally processed carrot (Iahnke et al., 2015). The results suggested that all films have a protective effect on the retardation of product oxidation promoted by UV light (Ahmad et al., 2015).

Transmission of visible light range (350–800 nm) of gelatin films with papaya peel powder decreased as the increased concentration powder. The addition of 7.5% of powder reached 33.46% of light transmittance at 800 nm, while control formulation transmitted 84.09%. The addition of microparticles also reduced the transmission light of the films in the visible region, but this decrease was smaller than the film with powder, possibly because the

phenolic compounds and carotenoids present in the powder were encapsulated, according to Li et al. (2014) the decreasing in light transmission may be attributed to the presence of these compounds.

About the opacity values, lower values indicate more transparent films. The addition of the powders and the microparticles increase the opacity of films, and films with higher concentrations of powder and microparticles (7.5%) are less transparent, thus improving light barrier properties. A study by Li et al. (2014) obtained lower opacity results for gelatin films added natural antioxidants extracts, such as green tea extract (0.570%), grape seed extract (0.070%) and ginger extract (0.570%). The difference in opacity values of film samples could be caused by the difference in color, thickness, the concentration of extracts or antioxidant added and their interaction with gelatin film matrix (Li et al., 2014).

3.2.6. Color measurements

Colour parameters of films prepared at different concentrations of powder, papaya peel powder microparticles, and control film are shown in Table 4. The luminosity values decreased with incorporation of powder and microparticles, the values were lower with the microparticles, as expected since the particles are encapsulated with gelatin.

The addition of powder and microparticles increased the parameter b* significantly (yellow) of films due to the presence of compounds yellow/orange characteristic of papaya peel powder. In the films with microparticles the compounds were more solubilized in the matrix of the film, so the values were higher than the films with powder at the same concentration.

The alterations in color of films were attributed to the coloring and concentration components of papaya peel powder or microparticles.

Table 4. Color parameters of films prepared with of powder and papaya peel powder microparticles and control film.

Film	L*	a*	b*
Powder			
2.5%	86.18 ±0.14 [b]	-1.74 ±0.03 [f]	26.91 ± 0.54 [e]
5.0%	84.73 ±0.07 [c]	-1.22 ±0.04 [d]	29.75 ± 0.96 [d]
7.5%	81.91 ±0.11 [e]	0.46 ± 0.00 [a]	37.27 ± 0.79 [c]
Micro			
2.5%	83.83 ±0.23 [d]	-1.89 ±0.04 [g]	35.64 ± 0.53 [c]
5.0%	82.39 ±0.48 [e]	-1.41 ±0.02 [e]	39.45 ± 0.96 [b]
7.5%	80.69 ±0.32 [f]	-0.32 ±0.01 [b]	45.82 ± 0.07 [a]
Control	92.56 ±0.27 [a]	-0.99 ±0.06 [c]	7.39 ± 0.36 [f]

Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

3.3. Antioxidant properties of powder, microparticles and film

3.3.1. Antioxidant by DPPH and FRAP methods

The antioxidant activity of powder and papaya peel microparticles determined by two different methods: DPPH that assay the capacity of scavenging free radicals and FRAP that evaluate the iron reducing power. The results obtained in each method were different. Regarding DPPH method, the microparticles showed the lowest antioxidant activity (45.05 ± 2.47 µmol Trolox equivalents (TE)/g dry matter) and for FRAP method the highest value (173.78 ± 1.96 µmol FeSO4.7H2O equivalents/g dry matter), significantly differing from papaya peel powder sample that showed antioxidant activity of 61.68 ±1.92 µmol Trolox equivalents (TE)/g dry matter and 148.50 ± 4.18 µmol FeSO4.7H2O equivalents/g dry matter.

By DPPH, microparticles had lower antioxidant activity than papaya peel powder, which can be justified by the loss of bioactive compounds during the process of formation of microparticles in spray dring, since the temperature employed in the process was 160 ° C.

It was possible to verify that the value of antioxidant activity varied according to the method used. According to Ferreira et al. (2014) if the method uses an organic solvent, as in DPPH, the most hydrophobic materials indication higher antioxidant activities, on the other land in an aqueous medium, as in FRAP method, the most hydrophilic materials shown the highest antioxidant activity. As the microparticles contain gelatin in their formulation, they

are more hydrophilic than powder, so would present higher values of antioxidant activity by Frap method, which was confirmed by the results obtained.

The antioxidant activity (AA) of films with powder and papaya peel powder microparticles and control film by DPPH and FRAP method were showed in Table 5. Film incorporated with powder and papaya peel microparticles showed higher antioxidant activity than the control film ($p < 0.05$). By method DPPH the films added powder showed higher antioxidant activity than the films added to microparticles in the same concentration, this is because the microparticles are made of powder and gelatin. Hence the added mass has less antioxidant compounds derived from the powder used in microparticles. The films analyzed in this study showed a higher antioxidant activity to films from fish skin gelatin incorporated with ginger and turmeric essential oils at different levels, considering the oil concentration added in the films (25% ginger oil – 0.26 μmol Trolox equivalents (TE) / g dried film and 25% turmeric root oil – 0.72 μmol Trolox equivalents (TE) / g dried film) (Tongnuanchan, Benjakul & Prodpran, 2013)

Considering the added residue concentration and reaction time of AA by DPPH method, you can see that both the papaya powder and the papaya microparticles have proved as optimal natural antioxidants when added to gelatin films, overcoming AA other films added waste such as beet and carrot, which at the concentration of 30% and 12.5% (relative to the amount of gelatin), respectively and reaction time of 8 hours ,showed a percentage inhibition of 42% and 32% (Iahnke et al., 2015; Iahnke et al., 2016).

Table 5. Antioxidant activity of films with powder and papaya peel powder microparticles and control film.

Film	DPPH (µmol Trolox equivalents (TE)/g dried film)*	DPPH - % RSA*	FRAP (µmol $FeSO_4.7H_2O$ equivalents/g dried film)*
Powder			
2.5 %	0.66 ± 0.00^c	23.09 ± 0.15^d	9.52 ± 0.52^c
5.0%	0.94 ± 0.01^b	41.14 ± 0.97^b	13.91 ± 0.06^b
7.5%	1.44 ± 0.07^a	53.65 ± 1.43^a	17.54 ± 0.65^a
Micro			
2.5%	0.39 ± 0.01^d	14.23 ± 0.47^e	8.53 ± 0.46^c
5.0%	0.63 ± 0.04^c	21.45 ± 0.34^d	13.34 ± 0.36^b
7.5%	0.84 ± 0.06^b	31.51 ± 2.01^c	17.37 ± 0.09^a
Control	0.23 ± 0.03^e	6.71 ± 0.54^f	1.36 ± 0.09^d

*Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

FRAP method is based on the capacity of antioxidants to reduce ferric tripyridyltriazine complex (Fe(III)-TPTZ) to the blue ferrous complex Fe(II)-TPTZ (Huang, Ou & Prior, 2005). The AA determined by FRAP test (Table 5) also showed a significant increase in AA when the powder and the microparticles were added to the film, compared to a control sample. However, there was no significant difference between AA films prepared with powder or papaya peel microparticles at the same concentration (2.5%, 5% and 7.5%).

Goméz-estaca et al. (2009) analyzed the antioxidant properties of films for FRAP method made from bovine-hide gelatin, with added oregano and rosemary extracts. For 1.25 g/100 ml (oregano) and 20 g/100 ml (rosemary) the films obtained 0.09 µmol FeSO4.7H2O equivalents/g film and 0.0598 µmol FeSO4.7H2O equivalents/g film, respectively. These results were much lower than those obtained in this study for films embedded with papaya waste.

3.4. Effect of antioxidant gelatin film on lard storage under accelerated conditions

3.4.1. Peroxide index value

Films with powder and microparticles that showed the highest values in DPPH and FRAP methods were used as packing lard in a test under accelerated conditions for 22 days

and had their antioxidant power analyzed by peroxides index of lard over the days; the results are presented in Table 6.

The gelatin films slowed significantly ($p < 0.05$) the oxidation of lard during the 22 days when compared to plastic packaging. However, the films with microparticles showed the best results, since it significantly delayed the formation of peroxides in the sample, even containing less antioxidant activity to the film with 7.5% powder by DPPH, this can be explained by the greater homogeneity and less water vapor permeability of microparticle film and also because in the microparticle the antioxidants compounds are preserved longer.

The control films and films with papaya peel powder retard the oxidation of lard when compared to plastic packaging, but at the end of 22 days, difference in peroxide value of the lard samples packaged with film control and the film samples with powder was higher than that in the earlier days, the peroxide values was 26% higher in the packaged sample with the film control, and that at the end of the 17 days the difference was 11%, this indicates that over the days the film control was losing its antioxidant forward to the papaya peel powder film. Li et al. (2014) evaluated the effect of gelatin films added antioxidants in retardation of pink oil oxidation, subjected to a temperature of 60 °C for seven days. All films have antioxidant power, mainly the films added green tea extract, grape seed extract, and gingko leaf extract. As in the present study the packed samples with control gelatin film obtained peroxide values like the film sample with added antioxidant extract (ginger), but higher than to other films with antioxidants.

Table 6. Analysis of peroxides (mEq/ kg), dienes, and trienes of lard packed in control film, the film added of 7.5% Powder and 7.5% microparticles and closed control in polyethylene packaging.

Peroxide Values *					
	Day 3	**Day 8**	**Day 13**	**Day 17**	**Day 22**
Film Control	3.12 ± 0.07^{bD}	3.27 ± 0.00^{bD}	3.97 ± 0.00^{bC}	4.51 ± 0.07^{bB}	5.65 ± 0.28^{bA}
Film 7.5 % Powder	2.78 ± 0.07^{cE}	3.22 ± 0.07^{bD}	3.66 ± 0.07^{cC}	4.05 ± 0.07^{bcB}	4.49 ± 0.07^{cA}
Film 7.5% Micro	1.88 ± 0.00^{dB}	1.98 ± 0.00^{cB}	2.18 ± 0.00^{dB}	2.78 ± 0.56^{cAB}	3.47 ± 0.00^{dA}
Closed control	22.41 ± 1.41^{aE}	51.41 ± 0.20^{aD}	116.62 ± 0.27^{aC}	234.68 ± 0.41^{aB}	413.93 ± 0.96^{aA}
Dienes*					
	Day 3	**Day 8**	**Day 13**	**Day 17**	**Day 22**
Film Control	2.56 ± 0.04^{bD}	2.65 ± 0.04^{bcCD}	2.89 ± 0.02^{bBC}	2.98 ± 0.11^{bB}	5.63 ± 0.13^{bA}
Film 7.5% Powder	2.54 ± 0.01^{bD}	2.70 ± 0.06^{bC}	2.84 ± 0.02^{bB}	2.83 ± 0.03^{bB}	2.99 ± 0.02^{cA}
Film 7.5% Micro	2.46 ± 0.02^{bC}	2.52 ± 0.00^{cBC}	2.77 ± 0.06^{bAB}	2.81 ± 0.12^{bAB}	2.92 ± 0.10^{cA}
Closed control	3.95 ± 0.02^{aD}	5.06 ± 0.00^{aD}	10.49 ± 0.01^{aC}	24.02 ± 0.04^{aB}	31.08 ± 1.17^{aA}
Trienes*					
	Day 3	**Day 8**	**Day 13**	**Day 17**	**Day 22**
Film Control	0.144 ± 0.02^{aB}	0.141 ± 0.02^{aB}	0.144 ± 0.01^{bB}	0.167 ± 0.01^{bB}	0.287 ± 0.02^{bA}
Film 7.5 % Powder	0.151 ± 0^{aC}	0.160 ± 0^{aC}	0.158 ± 0^{bC}	0.171 ± 0^{bB}	0.181 ± 0^{cA}
Film 7.5% Micro	0.125 ± 0^{abC}	0.132 ± 0^{aBC}	0.144 ± 0^{bAB}	0.155 ± 0^{bA}	0.157 ± 0.01^{cA}
Closed control	0.103 ± 0^{bC}	0.134 ± 0^{aC}	0.315 ± 0^{aC}	0.878 ± 0.10^{aB}	3.188 ± 0.11^{aA}

*Results are the means of three determinations ± standard deviation. Different superscript lower case letters in the same column indicate statistically significant differences as determined by Tukey test ($p < 0.05$).Different superscript capital letters in the same line indicate statistically significant differences ($p < 0.05$). Conjugated dienes ($^{1\%}$ ε_{1cm} [λ 232]). Conjugated trienes ($^{1\%}$ ε_{1cm} [λ 268]).

3.4.2. Secondary compounds (dienes and trienes)

Polyunsaturated fatty acids when oxidized, the resulting hydroperoxide formation and displacement of the double bonds, with consequent formation of conjugated diene. The conjugated dienes absorb at 232 nm (Table 6). The products of the secondary oxidation or trienes, in particular α-dicetones or unsaturated cetones, absorb 268 -272 nm (Table 6) (Jadhav, et al., 1996).

Dienes values in samples of lard packed with films and subjected to oxidation accelerated conditions were a similar profile to that presented to the peroxide values, where all films gelatin showed antioxidant power since fewer dienes were formed in the samples stored in these films compared the control sample kept in polyethylene packaging. In the 13th and 17th day, there was no significant difference ($p > 0.05$) between the samples packed in

gelatin control films, with powder and microparticles, and dienes formed in equal amounts in all packages. Already in the day 22, it can be seen that the formation of these compounds was significantly lower in packages of powder and microparticles, probably due to the presence of bioactive compounds.

The concentration of conjugated trienes (Table 6) progressively increased in all the lard packaging as a function of storage time, but the concentration of oxidation products was less in the samples packed with gelatin films especially in films with the addition of powder and the papaya peel microparticles on the 22nd day of experiment, in agreement with the result shown to dienes.

3.4.3. Fatty Acid Profile

Fatty acid profile of lard samples packed in gelatin and plastic films submitted to the test under accelerated conditions has been mentioned in Table 7. The analyze the profile of saturated fatty acids, monounsaturated and polyunsaturated lard over the days of the experiment under accelerated conditions can be seen that the films with gelatin protected more the product of changes in the fatty profile than plastic packaging.

In general, the fatty acid profiles of the samples of lard packed with gelatine control film, powder and microparticles were not changed during the experiment, because the packaged samples with polyethylene plastic were no difference of saturated fatty acid and polyunsaturated. The acids saturated fatty increased significantly over the days, and polyunsaturated fatty acids decreased significantly during the same period, this was due to the oxidation process caused by exposure to high temperature and direct incidence of light during storage.

In the days analyzed the film control and films added papaya residues showed the same effect antioxidant protection, the optical properties of gelatin films may have contributed to that result and also by a low permeability to oxygen, characteristic of these films and already reported in other studies (Wihodo & Moraru, 2013).

Table 7. Fatty Acid Profile of lard packed in control film, the film added of 7.5% Powder and 7.5% microparticles and closed control in polyethylene packaging.

Samples	Fatty Acid (%)*	Storage (days)				
		3	8	13	17	22
Film Control	SFA	39.67±0.23[ab]	40.93±0.37[a]	39.23±0.24[b]	38.61±0.33[b]	38.75±0.71[b]
	MUFA	43.78±0.13[a]	42.79±0.18[a]	43.24±1.16[a]	44.74±0.42[a]	44.41±0.17[a]
	PUFA	15.69±0.05[a]	15.30±0.11[a]	15.54±0.48[a]	15.90±0.07[a]	16.16±0.29[a]
Film 7.5 % Powder	SFA	40.60±0.18[ab]	39.79±0.36[ab]	42.82±1.49[a]	40.13±0.62[ab]	38.85±0.77[b]
	MUFA	44.18±0.14[a]	43.80±0.33[a]	42.59±1.16[a]	44.45±0.47[a]	44.33±0.59[a]
	PUFA	14.42±0.03[b]	15.61±0.11[a]	13.75±0.26[c]	14.61±0.11[b]	16.14±0.21[a]
Film 7.5% Micro	SFA	39.54±0.01[a]	38.45±0.29[a]	39.04±0.47[a]	39.06±0.81[a]	38.95±0.74[a]
	MUFA	43.87±0.12[a]	44.68±0.21[a]	44.28±0.42[a]	44.20±0.66[a]	44.37±0.36[a]
	PUFA	15.69±0.05[a]	16.04±0.06[a]	15.86±0.16[a]	15.92±0.18[a]	16.06±0.31[a]
Closed control	SFA	39.34±0.03[d]	40.04±0.43[cd]	41.13±0.15[bc]	42.02±0.03[ab]	43.25±0.54[a]
	MUFA	43.66± 0.18[a]	44.91±0.40[a]	44.15±0.94[a]	44.18±0.11[a]	45.06±0.06[a]
	PUFA	15.47±0.22[a]	14.82±0.43[a]	13.59±1.24[ab]	12.43±0.04[b]	9.691±0.07[c]

*Results are the means of three determinations ± standard deviation. Different letters in the same line are significantly different as determined by Tukey test ($p < 0.05$). Saturated Fatty Acids (SFA); Monounsaturated Fatty Acids (MUFA); Polyunsaturated Fatty Acids (PUFA).

4. Conclusions

Based on the results obtained in this study it was possible to demonstrate the use of waste from the food processing industry (papaya peel) and nutraceutical industry of chia oil nutraceutical capsules (gelatin) since from these wastes were possible to produce biodegradable packaging films with antioxidant activity.

The films presented a good physical integrity and appearance, but the inclusion of the papaya peel in the form of encapsulated microparticles with gelatin is essential to improve their mechanical properties and decrease their solubility. The films with microparticles presented a higher antioxidant effect. When tested as packaging material for lard, under high temperature (around 40ºC) and light incidence, the samples packaged in these films presented a significantly lower amount of oxidation compounds after 22 days. Also, gelatin films did not affect the profile of fatty acids of the lard samples during the experiment differing from control sample packed into polyethylene.

The packages obtained in this study are envisaged to fairly viable economically considering the material used in its production, which is derived from waste and totally

biodegradable, its antioxidant power, which assists in the shelf life of products, and its mechanical properties, which were similar to low density polyethylene.

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CAPÍTULO 8

Biodegradable active food packaging with chitosan and olive pomace

Tainara de Moraes Crizel[a], Alessandro de Oliveira Rios[a], Vítor D. Alves[b], Narcisa Bandarra[c], Margarida Moldão-Martins[b], Simone Hickmann Flôres[a]*

[a] *Laboratório de Compostos Bioativos, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, RS, Brasil.*

[b]*LEAF—Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*

[c] *IPMA - Instituto Português do Mar e da Atmosfera, Av. Brasilia 1449-006, Lisboa, Portugal.*

ABSTRACT

There is currently a strong trend towards the use of biodegradable packaging to replace synthetic. Therefore the objective of this work was to evaluate the effect of the addition of different concentrations of powder and microparticles of olive pomace powder in chitosan based films. The films were evaluated for their barrier, mechanical, optical and antioxidant properties. The protective effect of films against nut oxidation was also evaluated. The incorporation of olive residue powder into the chitosan matrix caused changes in the morphology, making the film more heterogeneous and rough. The addition of 10% of olive microparticles significantly improved the tensile strength (22.40 ± 0.22 MPA) of films without altering their original properties. The powder and the microparticles of olive increased the antioxidant capacity of the films that was proportional to the concentration of powder or microparticles added to the film. The films with 30% of powder or microparticles were effective as protective packaging against the oxidation of nuts during 31 days. The packages developed in this study are viable considering the material used in its production, totally biodegradable and added antioxidants naturally obtained from waste.

Keywords: Biodegradable packaging; chitosan; microparticles; nut oxidation; antioxidant capacity.

1. Introduction

The growing requirement for sustainability has increased the interest of researchers in the development of biodegradables packages from biopolymers incorporating bioactive compounds from wastes. The advantages of biopolymers in relation about polyethylenes are numerous such as rapid biodegradability, non-toxicity, biocompatibility with other biopolymers, easy interaction with food, the natural presence of antioxidant and antimicrobial compounds (Dutta, Tripathi, Mehrotra & Dutta, 2009; Hosseini, Rezaei, Zandi & Ghavi, 2013). Among the natural polymers used to biodegradable packaging development, chitosan stands out due to its excellent film forming ability, high mechanical strength and good barrier capacity (Martins, Cerqueira, & Vicente, 2012). Chitosan is obtaining by the deacetylation of chitin, a compound found mainly in crustacean shells (No & Meyers, 1995).

Aiming to prolong shelf life, maintain food quality and reduce the use of synthetic food additives many studies have evaluated the incorporation of natural antioxidants into chitosan films such as green tea extract (Siripatrawan & Noipha, 2012) and propolis (Siripatrawan& Vitchayakitti 2016). Compounds extracted from plants or obtained from food residues such as grape pomace extract (Ferreira, Nunes, Castro, Ferreira & Coimbra, 2014) and grapefruit seed extract (Bof, Jiménez, Locaso1, García, Chiralt, 2016) were used in active films. The integral use of residues from the food industry, mainly fruits and vegetables, is attractive due to its high content of bioactive compounds. The antioxidant potential of these residues has been proven in gelatine films added with blueberry bagasse (Crizel, Costa, Rios & Flôres, 2016a) and minimally processed beet residues (Iahnke, Costa, Rios & Flôres, 2016). Therefore the use of these flours can modify the structure and consequently the mechanical properties of films.

The olive oil industry generates tons of olive pomace worldwide. This residue is rich in dietary fiber, phenolic compounds and other antioxidants such as carotenoids (Crizel, Hermes, Rios & Flôres, 2016b) and usually being burned by industries (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, Fernández-Bolaños, 2015a). Furthermore, from this residue can be extracted pectin and according to Cardoso, Coimbra, & Lopes-da-Silva (2003), the gelling ability of this extract is similar to the citrus pectin sold commercially.

Considering the high antioxidant capacity of olive oil pomace, the objective of this work was to evaluate the effect of the incorporation of different concentrations of powder and microparticles on biodegradable chitosan films for food packaging.

2. Materials and Methods

2.1. Materials

The chitosan used in the preparation of films was acquired from Golden-Shell Biochemical Co., Ltd., located in China, and had a degree of deacetylation (DD) greater than 85%.

Regarding the olive pomace used, it was composed of fruit bagasse and lumps collected after the first pressing for oil extraction, and was provided by Olivas do Sul Company (Cachoeira do Sul city- RS/Brazil).

Fresh walnut (*Juglans regia L.,* cultivar Chandler*)* kernels were supplied by H. Reynolds de Souza, Estremoz, Portugal, packed in commercial film (PA/PE 90). Walnuts had been harvested during the period September–October 2015 and experiments were carried out during the period October-November.

The commercial film (polyamide cast flexible–polietylene – PA/PE 90) was purchased from AlemPack, Portugal, and presented an oxygen permeability value below 4.6×10^{-17} mol m/m^2sPa.

2.2. Production of olive pomace powder

Olive pomace was frozen in an ultra-freezer at -40 ºC for 48 hours and subsequently lyophilized (Freeze Dryer Liotop, L101, Brazil). The lyophilized material was ground in a mill (Bertel Brand, Model MCF55, Brazil). The lumps present in the waste and particles were smaller than 500 mm were separated using sieves for particle size analysis (Bertel, Brazil); The powder was packed in a vacuum sealer (Sealer ECOVAC, Model ECOVAC 40, Italy) and stored in the dark at room temperature (~25°C).

2.3. Preparation of microparticles by spray dring

For the preparation of microparticles, 5 g of olive pomace power were mixed with water (150 mL) and stirred with a magnetic stirrer (Velp Scientifica, Model Arex, Italy) for 40 min. Tween 80 (0.1 g) was added to this mixture and, subsequently, the solution was stirred using an Ultra Turrax® homogenizer (IKA Ultra Turrax digital, Model T25 basics, Germany) for 60 seconds at a stirring rate of 13500 rpm. The resulting suspension was pumped at 8

mL/min with a peristaltic pump to the spray dryer (LabPlant, Model SD-05, United Kingdom), operating with an inlet drying air temperature of 160 ºC.

2.4. Films preparation

The chitosan (CH) solutions with a concentration of 2% (w/w) were prepared by dissolving the chitosan powder in a 1% (w/w) aqueous acetic acid under constant stirring until complete dissolution, after which glycerol (1.0% w/w) was added.

Depending on the type of film to be prepared, olive pomace powder or microparticles were added to the filmogenic solution under magnetic stirring for 30 min. The resulting filmogenic solutions were treated in an ultrasound bath for 30 min to remove the dissolved air bubbles before being poured in polystyrene Petri dishes (0.50 g/cm^2) and dried in an oven (Binder, Model D -78532, Germany) at 40°C for 48 h. After drying the films were peeled off from the casting surface and maintained at 48% relative humidity and T ≈ 25 °C, for 48 h before their characterization.

Seven different formulations of chitosan films with olive pomace were prepared: three formulations using the powder, described in item 2.2, (concentrations of 10%, 20% 30% in relation to the mass of chitosan used in the preparation of the film); three formulations with the microparticles obtained by spray drying, (added at concentrations of 10%, 20% and 30%), and a control formulation only with chitosan.

2.5. Scanning Electron Microscopy of the powder, microparticles and films

For scanning electron microscopy the samples were placed on mutual conductive adhesive tape on aluminum stubs and covered with a film of Au / Pd, about 30 nm thick in a sputter coater (Quorum Technologies, Model Q150T ES, United Kingdom). Olive pomace powder, microparticles, and all the film samples were analysed by field emission scanning electron microscopy (JEOL, Model JSM7001F, Japan).

2.6. Films Characterization

2.6.1. Water vapor permeability (WVP)

The films samples were placed in the superior part of a glass permeation cell (diameter of 5 cm) filled with granular anhydrous calcium chloride. The cells were sealed with silicone, weighed and placed in a desiccator that contained a saturated solution of sodium chloride, resulting in a gradient of 75% humidity (RH) at 25°C. The mass gain was determined by the difference between mass at time zero and 24 h. The WVP was performed in using equation (1):

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

In which w is the mass gain rate (water) (g) by the permeation cell, L is the thickness of the film (mm), A is the permeation area (m^2), t is the time of permeation (h), and Δp is the water vapor pressure difference between the two sides of the film (Pa).

2.6.2. Moisture content and Water solubility (WS)

For moisture analysis, squares of each film sample (2 x 2 cm) were weighed and placed in a drying oven (Model D -78532, Mark Binder, Germany) for 24 h at a temperature of 105 °C. The moisture analysis was carried out in triplicate, and calculated by the mass difference divided by the samples mass before drying.

The water solubility of films was performed according to the method used by Mei et al. (2013). Films squares (2x2 cm) were oven dried 105 ºC for 24 hours (Binder Model D - 78532, Germany). The films were weighed (Wi) and were immersed in 30 mL of distilled water and maintained under agitation in a 25 ºC water bath for 24 h. Afterwards, the samples were taken out and dried at 105 ºC for 24 h (Wf) (Binder Model D -78532, Germany). The WS analysis was performed in triplicate and determined using the following equation (2).

$$WS\,(\%) = W_i - W_f / W_i * 100 \qquad (2)$$

2.6.3. Water vapour sorption isotherms

The water vapor adsorption isotherms were determined according to Alves et al. (2011). Saturated solutions of different salts, such as LiCl, MgCl2.6H2O, Mg(NO3)2.6H2O,

NaNO3, NaCl, KBr and KCl, which result in different relative humidity environments at 25 ºC of 11%, 33%, 53%, 74%, 75%, 81% and 84%, respectively, were used. Triplicates of each film, with dimensions of 10 mm x 10 mm, were previously conditioned in a desiccator (48% humidity and during 48h) and weighted. Then, they were placed in the desiccators with the different relative humidity environments during three weeks, after which they were weighed again assuring that the equilibrium had been reached.

2.6.4. Color, light transmission, and opacity

Color of film samples was determined using a colorimeter (Minolta CR-300, USA). The color parameters analyzed were L* (lightness/brightness), a*(redness/greenness) and b* (yellowness/blueness) values.

Rectangular films strips (4.5 cm long and 1.2 cm wide) were placed in quartz cuvettes and their absorption spectra of films were measured at wavelengths ranging from 200 to 800 nm using an UV spectrophotometer (Unicam UV4 UV/Vis, United Kingdom). The measurement was done in six different strips of each sample. The opacity value of the films was calculated by the following equation:

$$Opacity\ value = \frac{-\log T_{600}}{x} \quad (3)$$

where T_{600} is the transmittance at 600 nm and x is the corresponding film thickness (mm). The lower opacity value, the higher transparency of the films.

2.6.5. Film thickness

The thickness of films was measured using a micrometer (Model MDC-25, MitutoyoCorp. Tokyo, Japan). Each film was measured in 10 different random locations.

2.6.6. Mechanical properties

Tensile test were carried out according to specifications of the standard method ASTM D882-09 (2009), using a texture analyzer (TA-Xtplus, Stable Micro Systems, England) with a

load cell of 1 kg. The films were cut into rectangular strips (80 mm x 25 mm) and stored under controlled conditions of moisture (48% humidity) and temperature (25 ºC) before analysis. Ten strips of each film formulation were analyzed. Tensile strength at break (TS), elongation at break (% E) and Young's Modulus (YM) were calculated.

2.7. Antioxidant properties of pomace powder, microparticles and films

2.7.1. Extracts of pomace powder and microparticles

Olive pomace powder and microparticles extracts were obtained by mixing 1 g of powder (or microparticles) with 40 mL of a methanol-water solution (50% each) and stirred with an Ultra Turrax® homogenizer (IKA Ultra Turrax digital, Model T25 basics, Germany) for 1 min at 13500 rpm. The overall mixture was stored in the dark at 25 ºC for 60 min and centrifuged (25000 xg for 10 min, Hermle Labortechnik, Model Z383k, Germany). The supernatant was separed and the pellet was extracted again with an acetone-water solution (70% acetone) using the same methodology described above. Finally, the recovered supernatants was mixed and stored in the same flask in the dark.

2.7.2. Ferric reducing antioxidant power

The ferric reducing antioxidant power (*FRAP)* method was performed according to Pulido, Bravo & Saura-Calixto (2000). A 90 µL aliquot of the extracts, obtained as described in the previous section, was transferred to glass tubes, added up with 270 uL of distilled water and mixed with 2.7 mL FRAP reagent, stirred in a vortex and maintained in a water bath at 37 °C.

In order to measure the antioxidant capacity of films, samples were cut in 1-cm^2 pieces and weighed, placed in tubes with 270 mL of distilled water mixed with 2.7 mL FRAP reagent, followed by stirring in vortex and maintained in a water bath at 37 °C.

For all cases, after 30 minutes of reaction, the absorbance at 595nm was measured using a spectrophotometer (Unicam UV4 UV/Vis, United Kingdom). The spectrophotometer was calibrated with FRAP reagent. The FRAP results were expressed as µmol $FeSO_4.7H_2O$ equivalents/g dry matter or dry film, using a standard curve of $FeSO_4.7H_2O$ (mM) as reference. Determinations of ferric reducing antioxidant activity were performed in triplicate.

2.7.3. DPPH radical scavenging capacity

Methodology recommended by Ferreira, Nunes, Castro, Ferreira & Coimbra (2014) was used for determination of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. One film sample (1 cm^2) was weighed and immersed in 3.9 mL methanolic DPPH solution (0.06 mM). The reaction occurred in light absence, for 120 min, stirring in vortex for 30 s every 30 min. Afterwards, the absorbance of the supernatant was measured at 515 nm using a spectrophotometer (Unicam UV4 UV/Vis, United Kingdom).

To evaluate the antioxidant activity of the powder and microparticles, the respective extracts obtained as described in section 2.7.1, 100 uL of each extract was mixed with 3900 µL of DPPH solution (0.06 mM), and the reaction lasted 40 min at room temperature in the dark. The reduction of DPPH radical in the solutions was evaluated by measuring the absorbance at 515 nm using a spectrophotometer (Unicam UV4 UV/Vis, United Kingdom) (Brand-Williams, Cuvelier, & Berset, 1995).

The results were expressed as µmol Trolox equivalents (TE)/g dry mater, based on a standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid from Aldrich). The tests were performed in triplicate.

2.8. Evaluation of antioxidant effect of chitosan film on walnuts storage under accelerated conditions

Fresh walnut (*Juglans regia L.,* cultivar Chandler*)* kernels were lightly crushed and immediately packed in bags (25 g / 140 x 100mm) from the selected films and the commercial one. In the test were used the films with olive pomace powder, with powder microparticles and two controls: film with only chitosan and a commercial film (PA/PE 90). The packages and controls were stored in a chamber climatic (Aralab, model FitoClima 600 PDH, Portugal) at 55 ºC, with incidence of UV fluorescent light (300 µmol/m^2/s) for 31 days and 35 % humidity.

To evaluate of protective effect of each film type over time, nuts from each bag were mechanically pressed and the extracted oil was analyzed in terms of peroxide index, secondary oxidation compounds and fatty acids. The analysis were performed on days 0, 5, 10, 19, 26 and 31. Three samples of each film and controls were analyzed in each day.

2.8.1. Peroxide index value

The peroxide index value (PI) of walnuts oil was evaluated by the method described by AOCS (2009). The PI was expressed as mEq of oxygen per kg of sample.

2.8.2. Secondary oxidation compounds (dienes and trienes)

Secondary compounds were quantitated in the oil samples by IUPAC method (1979) II.D.23 number, based on spectrophotometry (GBC Scientific Equipment, UV/VIS 916, Australia). In some wavelengths of the ultraviolet spectrum indicate the presence of oxidation products, that can be conjugated dienes (232 nm) and trienes (270 nm).

2.8.3. Fatty Acid Profile

The fatty acid profile (FA) analysis of walnuts oil was performed according to the methodology described Bandarra et al. (1997). For analysis, 300 mg of sample were dissolved in 5 mL of acetyl chloride/methanol (1:19 v/v), mixed and heated at 80 ºC for 60 min. To the cooled solution were added 1 mL of Milli-Q distilled water and 2 mL of n-heptane. The total mixture was centrifuged (2300xg) for 5 min. Anhydrous sodium sulfate was added to remove moisture from the supernatant. An 2 mL aliquot of supernatant was injected into a gas chromatograph (Varian Star 3800 CP, Walnut Creek, CA, USA) equipped with an autosampler and with a flame ionization detector at 250 ºC for fatty acid methyl ester (FAME) analysis. Helium gas was used as transporter for separation in a capillary column DB-WAX (30 m length x 0.32 mm internal diameter; 0.25 mm film thickness; Hewlett-Packard, Albertville, MN) at a flow rate of 1 mL/min. The temperature at 180 ºC for a time of 5 min, it was programmed to increase at 4 ºC/min to 220 ºC, being maintained at this temperature (220 ºC) for 5 min with the injector at 250 ºC. The qualitative analysis of fatty acids was performed through the comparison of retention times with Sigma, Nu Check Preap and Larodan Fine Chemicals standards. The results were expressed as a percentage (%) of the total FAMEs in the samples.

2.9. Statistical analysis

The Statistica software 12.0 (STATSOFT Inc., São Paulo, Brazil) was used to analyze the results obtained by analysis of variance (ANOVA) and Tukey's test with a $p<0.05$ significance level.

3. Results and discussion

3.1. Scanning Electron Microscopy of the powder, microparticles, and films

The characteristic morphology of olive powder particles and powder microparticles are shown in Figure 1. The results showed a noticeable difference between the particle size of olive residue powder and that of the microparticles obtained by spray drying. In addition, the morphology is also different, with the olive pomace powder showing a more irregular shape.

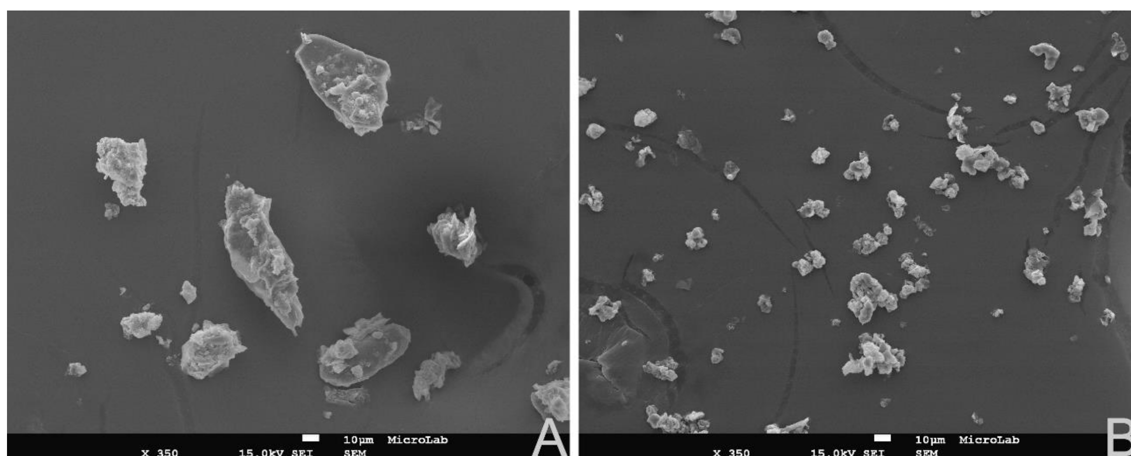

Figure 1- Scanning Electron Microscopy (SEM) images (350 x magnification): (A) olive pomace powder; (B) olive pomace powder microparticles.

The scanning electron microscopy (SEM) was used to visualize the effect of the addition of powder and olive pomace microparticles to the polymeric matrix. Figure 2 shows the microstructures of surface and cross section of each film. It is possible to observe that the surface of control chitosan film was smooth (Fig 2 G and Fig 3 G) and did not show cracks. However, there was a great difference in the superficial properties when compared to films with powder and microparticles. From these, the films with powder presented a more irregular surface, with many waves due to powder particles that were not solubilized in the chitosan matrix. Similar images were shown in a study developed by Crizel et al. (2016a) in gelatin films added with blueberry bagasse flour.

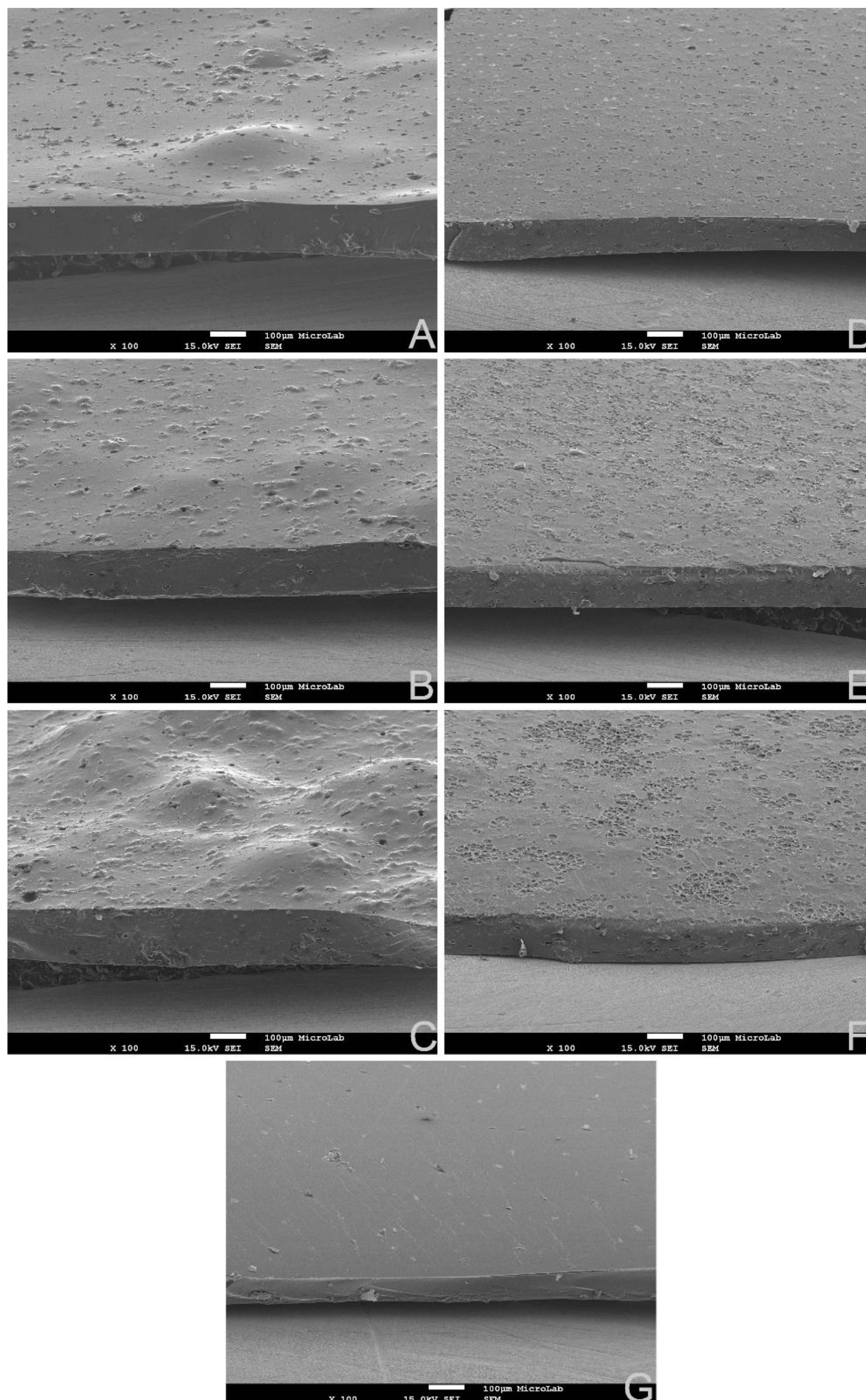

Figure 2 - Scanning Electron Microscopy (SEM) images (100 x magnification) of films surface and cross section added olive pomacepowder ((A) 10% powder, (B) 20% powder, (C) 30% powder), microparticles olive oil pomace powder ((D) 10% micro, (E) 20% micro, (F) 30% micro) and control film ((G) without powder or microparticles).

In the cross section of the film (Figure 2) was possible to verify the presence of a very irregular structure with the presence of air bubbles and oil droplets mainly in the films with 30% of olive powder and 20% and 30% of microparticles of olive powder. This fact may be associated with the presence of oil in olive pomace that is released to the solution during the homogeneization process (Crizel et al., 2016b). This effect is more clear when observing the films surface in more detail (Figures 3 D-F). According to Ma, Zhang, Critzer, Davidson, Zivanovic & Zhong (2016), the mixture of oil, water and surfactants may also result in coalescence and consequently increase in droplet size, which can be seen in Figure 3 F.

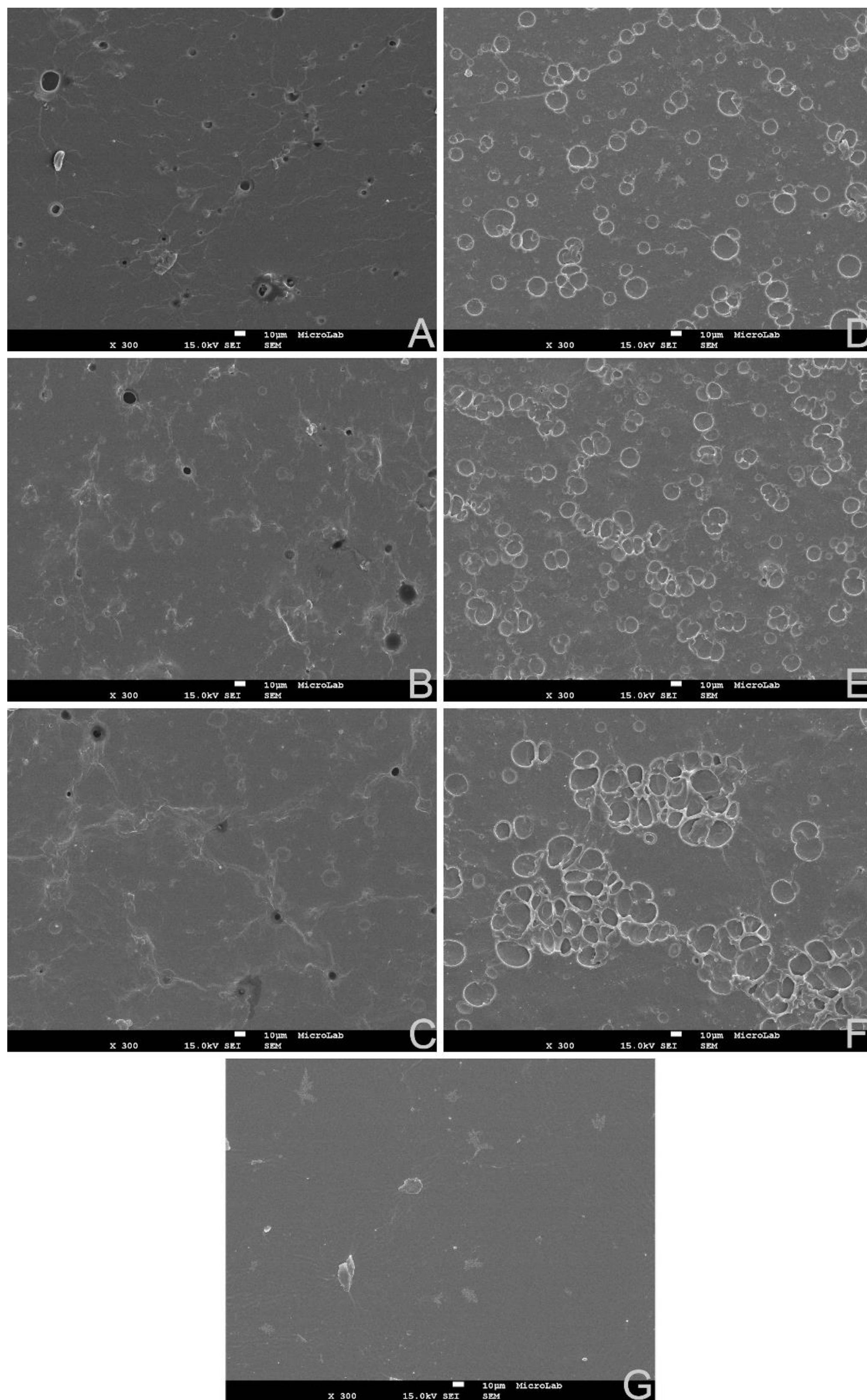

Figure 3- Scanning Electron Microscopy (SEM) images (300 x magnification) of films surface with olive pomace powder ((A) 10% powder, (B) 20% powder, (C) 30% powder), and olive pomace microparticles ((D) 10% micro, (E) 20% micro, (F) 30% micro) and control film ((G) without powder or microparticles).

Chitosan films added with essential oil of thyme shown images of electron microscopy similar to this study. These authors associated the presence of these pores with essential oil components (Hosseini et al., 2009).

3.2. *Film Characterization*

3.2.1. *Water vapor permeability (WVP)*

The shelf life of food products is directly related to the transfer of water between the product and the external environment in which they are inserted. Biodegradable films should reduce this transfer of water (Hosseini, Rezaei, Zandi & Farahmandghavi, 2016). The water vapor permeability of chitosan films (Table 1) was significantly increased ($p < 0.05$) by the addition of olive pomace powder. The addition of 30% of powder increased by 2.6 times the WVP of films when compared to control film. This fact is due to irregular structure (Figure 1 A, B and C) of powder and its insolubility in the matrix of the film, which can result in small cracks that facilitate the transfer of water through the film.

Unlike the olive pomace powder, the addition of the microparticles of olive pomace did not alter the WVP of the chitosan films, since the microparticles were smaller and solubilized better in the chitosan, forming a more cohesive intermolecular network and consequently hindering the passage of water by the film membrane. According to Martins et al. (2012) and Siripatrawan & Harte (2010) factors as film thickness, water sensitivity, chemical structure, and morphology may also affect the WVP of chitosan films.

Gelatin films added blueberry pomace dietary fiber showed higher WVP value than the control film (made with only gelatin). According to the authors, the fibers interfered in the structural matrix of the film and resulted in small cracks that allowed a greater permeability of water by the film (Crizel et al., 2016a).

Table 1. Water Vapor Permeability (WVP), Moisture Content (MC) and Water Solubility (WS) of the control films and films added of powder and microparticles from olive pomace.

Film	WVP (g.mm/m^2 h kPa)*	Moisture content (%)*	Water-solubility (%)*
Powder			
10%	1.32 ± 0.03^{bc}	18.87 ± 1.06^{a}	31.93 ± 4.22^{b}
20%	1.52 ± 0.05^{b}	17.76 ± 0.07^{a}	31.40 ± 4.35^{b}
30%	2.23 ± 0.14^{a}	16.47 ± 0.87^{ab}	44.55 ± 1.28^{a}
Microparticles			
10%	0.96 ± 0.04^{d}	18.97 ± 0.19^{a}	31.31 ± 3.59^{b}
20%	1.06 ± 0.06^{d}	17.38 ± 0.58^{ab}	28.64 ± 2.55^{b}
30%	1.09 ± 0.02^{cd}	15.04 ± 0.28^{b}	26.16 ± 2.13^{b}
Control	0.85 ± 0.00^{d}	18.28 ± 0.92^{a}	32.61 ± 0.73^{b}

*Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

3.2.2. Moisture content and Water Solubility

The addition of olive pomace powder did not affect significantly ($p > 0.05$) the moisture content of film compared to control film. Regarding the microparticles, the addition of 30% concentration reduced the moisture of films. Probably this reduction is directly associated with the moisture content of the microparticles. Studies indicate that chitosan films exhibit low solubility in water when compared to other biopolymers such as gelatine, for example (Jridi et al., 2014).

The water solubility of chitosan films (Table 1) was significantly higher by the incorporation of the 30% of olive pomace power. An increase in the concentration of the powder became the polymeric matrix of the film more irregular with larger spaces between the fiber and the polymer, thus resulting in the absorption of a greater amount of water and consequently higher solubility of the film.

The addition of microparticles did not alter significantly ($p > 0.05$) the solubility of films compared with the control sample, and this is due to the real cohesion between the microparticles and the chitosan matrix of the film. According to Crizel et al. (2016b) the residue of olive oil extraction is rich in lipids (23%), which may have contributed to these results. According to some studies with essential oils of thyme and cinnamon, in the concentration of 1 and 1.5%, the oils increase the cross-linking and the hydrophobicity of film

consequently decreasing the solubility (Hosseini, Razavi, & Mousavi, 2009; Ojagh, Rezaei, Razavi, & Hosseini, 2010). However, it should be considered that in this work the amount of residue and consequently added oil was lower than in the studies cited, but already it is observed a tendency that higher concentrations of olive pomace powder increase the solubility of films.

3.2.3. Water sorption isotherms

According Aguirre-loredo, Rodríguez-Hernández, Morales-Sánchez, Gómez-Aldapa & Velazquez (2016) in absorption isotherms it is possible to view three zones, in water activity (a_w): values less than 0.2 the water is adsorbed in the monolayer, between a_w of 0.2-0.65 the water is adsorbed in the multiple layers and in the final region of isotherm in a_w greater than 0.65 the condensation of water in the pores of film.

Figure 4 shows water sorption isotherms of chitosan films, where it was possible to evaluate the behavior of films in front of different a_w. It was verified that at low levels of a_w the films with 30% of micro and powder were very similar to the control film. In a_w above 0.5, all the films followed the same trend, and the films added to 20%, and 30% of microparticles absorbed less water than the others.

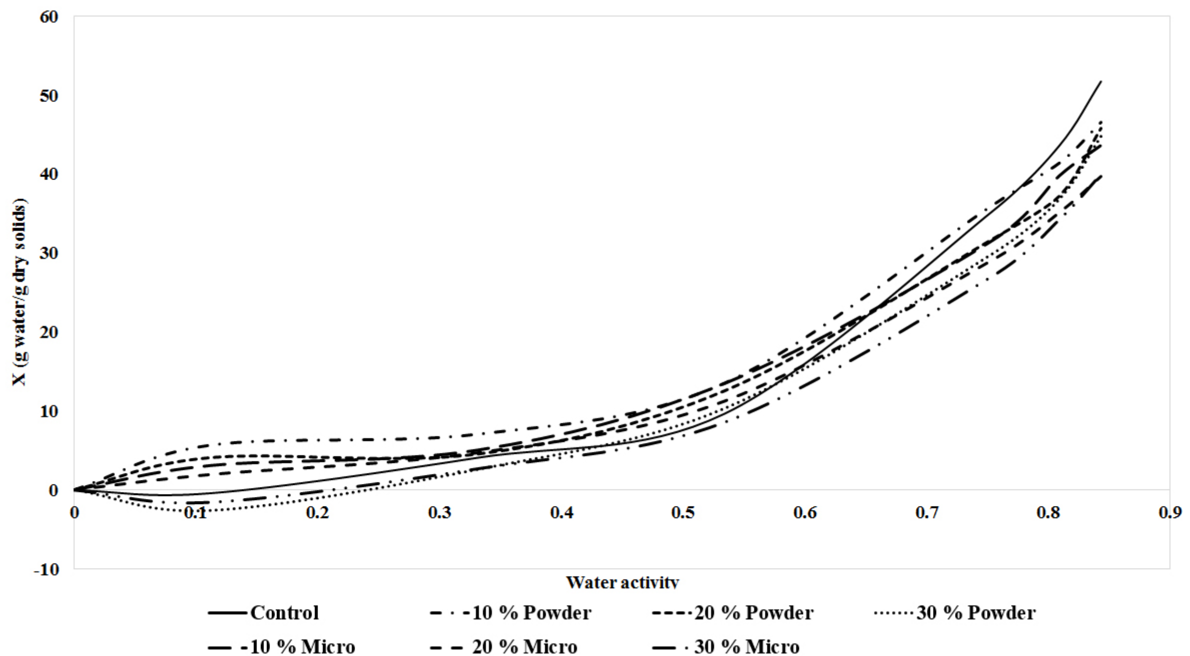

Figure 4. Water sorption isotherms of chitosan films.

In the upper, a_w of 0.809 and 0.843 the control film presented the highest values 43.63 and 51.72 g water / g dry solids, respectively. Similar behavior was obtained by Aguirre-loredo et al (2016) for chitosan films, where there was a small increase in moisture content at a_w 0.6 after an exponential increase.

3.2.4. Color, light transmission, and opacity

The optical properties are important to verify the effectiveness of packaging as a protective agent of food in the face of deterioration, loss of nutrients and flavors when exposed to visible and ultraviolet light (Martins et al., 2012).

The transmission of UV light and visible by the films at a wavelength of 200-800 nm is shown in Table 2. In the UV range of 200–280 nm the films incorporated of powder and microparticles of olive waste powder demonstrated excellent UV barrier properties, while the control film exhibited light transmission of 23.63% at wavelength 280 nm.

Table 2. Light transmission (% T) and the opacity values of the control films and films added of olive pomace powder and olive pomace powder microparticles.

Film	**Light transmission at different wavelengths (%)**								**Opacity values**
	200	280	350	400	500	600	700	800	
Powder									
10%	0.00	0.11	1.28	10.59	34.29	44.39	50.70	56.31	1.77 ± 0.13^{e}
20%	0.00	0.17	0.97	5.73	14.22	23.65	28.70	36.30	2.86 ± 0.02^{d}
30%	0.00	0.00	0.02	0.62	8.60	16.18	22.73	25.78	3.03 ± 0.06^{d}
Micro									
10%	0.00	0.24	1.21	12.35	35.94	47.79	54.51	59.45	3.51 ± 0.08^{c}
20%	0.00	0.00	0.00	0.66	11.25	23.44	32.48	38.32	4.30 ± 0.14^{b}
30%	0.00	0.00	0.10	1.66	11.84	21.10	28.50	32.45	6.03 ± 0.20^{a}
Control	0.08	23.63	42.50	69.74	86.99	89.94	90.46	91.17	0.68 ± 0.00^{f}

*Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

In the range of 350-800 visible light transmission absorbance values were higher in the films with the lowest concentrations of olive powder and microparticles. The light transmission was greater than 50% in the films with 10% of powder and 10% of microparticles. Already in the films with 30% of powder and of microparticles the values were of 25.78% and 32.45%, respectively.

The opacity values for the films added with powder and microparticles of olive residue powder were significantly higher when compared to the control film, indicating a lower transparency of the films. The opacity of films increased proportionally with the increase in powder and microparticles concentrations of formulations. All films added with microparticles presented higher opacity values than the films with powder, being less transparent and consequently more efficient in the light barrier. It occurs because the microparticles solubilized better in the matrix of the film (Figure supplementary material) resulting in a matrix and more homogeneous color, which did not happen in the films with the powder.

The addition of extracts may also increase opacity in films as reported by Siripatrawan & Harte (2010) with the incorporation of green tea extract in chitosan films also resulted in an increase in opacity where 20% extract led to an opacity of 3.019.

The effects of powder and olive pomace microparticles on chitosan film are shown in Table 3 and can be observed in Figure 5. Adding to powder and microparticles in the films significantly decreased the films lightness, the higher the concentration of powder and microparticles the lower the luminosity value of films.

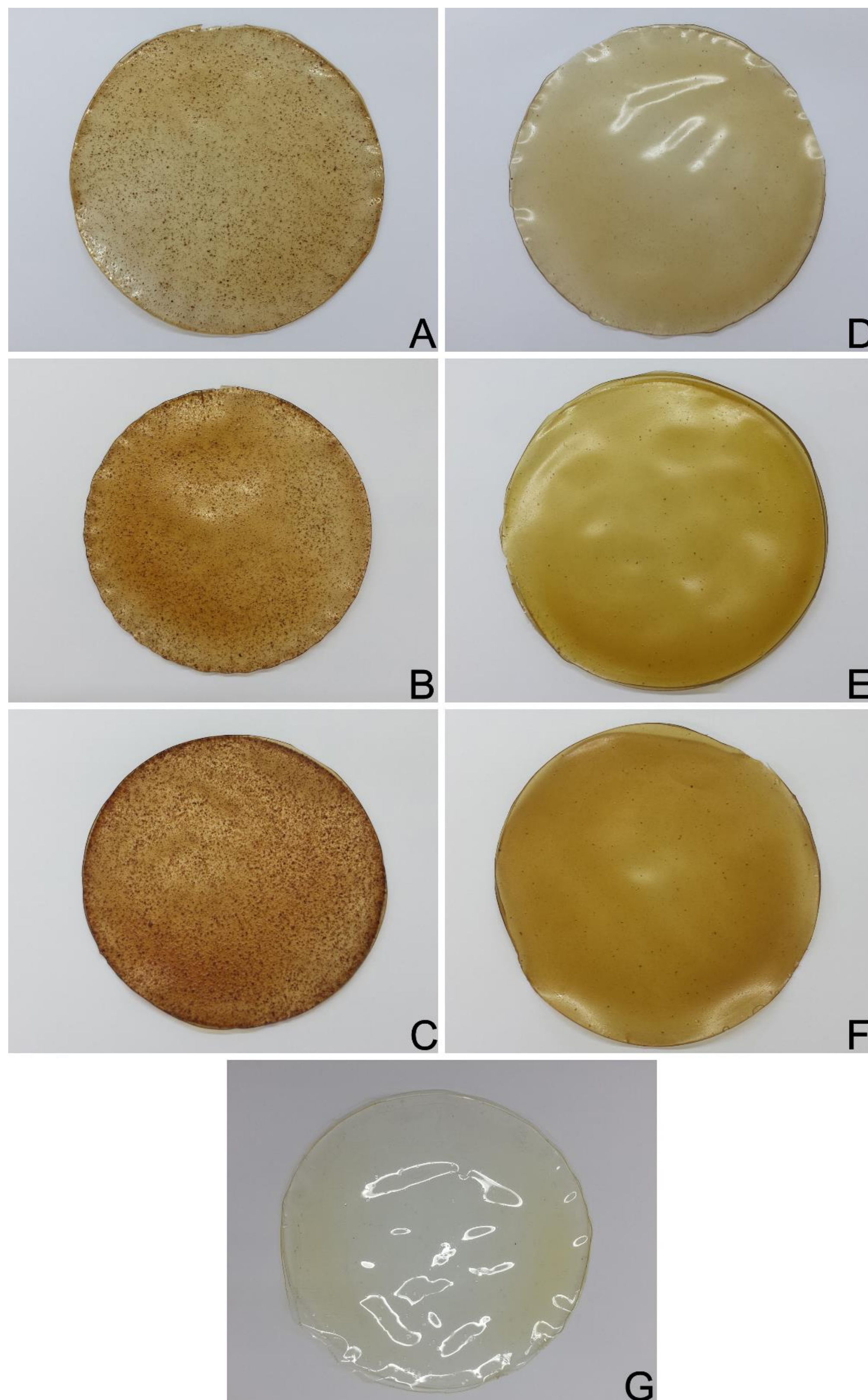

Figure 5. Visual appearance of films incorporated with 10%, 20% and 30% of olive pomace powder (A, B, and C) and olive pomace powder microparticles (D, E and F) and control film (G).

The addition of the wastes significantly increased the values of the parameters of color $a*$ (redness) and $b*$ (yellowness) about the control film, being that the powder left the films more redrawn than the microparticles. In relation to the parameter $b*$ both had practically the same behavior, with no significant difference between the formulations with the same concentration of powder and micro, except for the formulation with 30% of microparticles that presented the highest value differing from the others.

Table 3. Color parameters of films prepared with of powder and olive byproducts powder microparticles and control film.

	$L*$	$a*$	$b*$
Powder			
10%	71.12 ±0.11 [c]	3.83 ±0.14 [d]	31.29 ± 0.25 [c]
20%	61.80 ±0.74 [f]	9.60 ±0.42 [b]	38.94 ± 0.79 [b]
30%	56.32 ±0.46 [g]	15.26 ± 0.33 [a]	39.06 ± 0.73 [b]
Micro			
10%	74.16 ±0.10 [b]	2.59 ±0.09 [e]	30.74 ± 0.08 [c]
20%	67.37 ±0.41 [d]	6.31 ±0.24 [c]	38.83 ± 0.47 [b]
30%	64.73 ±0.54 [e]	6.40 ±0.09 [c]	42.35 ± 0.60 [a]
Control	88.63 ±1.45 [a]	-1.03 ±0.13 [f]	6.00 ± 0.31 [d]

Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

3.2.5. Film thickness

The thickness of the film was significantly increased when olive waste powder content increased (Table 4) because the powder becomes insoluble in the chitosan matrix and deposits on the surface of the film and increases its thickness. This fact did not occur with the addition of microparticles since these are smaller in size, not altering the thickness of the films that was equal to that of the control film.

3.2.6. Mechanical properties

In order to be used as packaging, it is necessary that the films are resistant to external stresses and maintain their integrity. Thus, the characterization of the mechanical properties of the films as tensile strength at break, elongation at break, and Young's modulus or elasticity

modulus becomes essential (Rao, Kanatt, Chawla, & Sharma, 2010). The tensile strength at break (TS), elongation at break (E) and Young's modulus (YM) of chitosan films are presented in Table 4.

From the obtained results it was observed that the incorporation of olive residue powder in the chitosan films decreased the tensile strength, the elongation at break and Young's modulus of the films. It is directly associated with the non-homogeneous structure of the film, caused by the presence of insoluble particles from the olive powder, as was previously observed in the SEM (Figure 2), causing the addition of powder to interfere in the chitosan matrix and consequently make the film more fragile. Studies that added flour from fruit and vegetable residues such as blueberries and beets in films that had biopolymer gelatin obtained similar results and attributed this to the lack of cohesion of waste flour with the film matrix (Crizel et al., 2016; Iahnke et al., 2016).

Table 4. Tensile strength (TS), elongation at break (E) and Young's modulus (YM) of films.

Film	Thickness (mm)*	TS (MPa)*	E^d (%)*	YM (MPa)*
Powder				
10%	0.218 ± 0.01^b	7.58 ± 0.23^c	17.57 ± 0.28^d	40.42 ± 0.29^b
20%	0.237 ± 0.01^b	5.49 ± 0.21^{cd}	$15.39 \pm 0.44d^e$	25.79 ± 2.55^c
30%	0.305 ± 0.00^a	2.89 ± 0.06^d	13.31 ± 0.77^e	12.39 ± 0.39^d
Microparticles				
10%	0.140 ± 0.01^c	22.40 ± 0.22^a	33.01 ± 0.66^a	35.90 ± 2.48^b
20%	0.140 ± 0.01^c	14.55 ± 0.43^b	23.58 ± 0.73^b	23.43 ± 1.63^c
30%	0.145 ± 0.01^c	6.51 ± 0.02^{cd}	20.93 ± 0.69^c	16.28 ± 0.83^d
Control	0.123 ± 0.00^c	16.76 ± 3.51^b	23.05 ± 1.36^{bc}	69.11 ± 2.10^a

*Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

The incorporation of 10% microparticles of olive residue powder significantly ($p < 0.05$) increased the tensile strength and elongation at break of films compared to the control film. The increase was approximately 34% for TS and 43% for E. However, a further increase in the concentration (up to 30% microparticles) generated a reduction in of values of TS comparing to the control formulation, and for E values the difference was not significant. The improvement of the mechanical properties of films incorporated with 10% microparticles can be attributed to the good interpenetration of these particles in the chitosan matrix, resulting in a more cohesive and resistant film.

It can be observed in SEM of films (Figure 3) that the increase in the addition of microparticles resulted in a greater amount of discontinuities in the chitosan matrix causing a loss of cohesion and consequently made its structure less resistant. Studies of Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009 and Martins et al., 2012 indicate that the presence of some essential oils in films can cause structural discontinuities that reduce the chain mobility and consequently less flexibility and the film's strength. However, there are also works indicating that the addition of small amounts of lipid in chitosan films makes its structure more compact (Pereda, Amica, & Marcovich, 2012). It would justify the results found in the present work, where the addition of 10% microparticles of olive powder increased the tensile strength of films and the addition of 30% reduced those values.

The results for the tensile strength of the films, especially the control film and the films with 10% and 20% of microparticles were quite high considering the TS values quoted by Gennadios, McHugh, Weller & Krochta (1994) for synthetic films that presented values between 8.6-17.3 MPa. The films with 10% of powder and with 30% of microparticles exhibited values of TS higher than to films elaborated with biopolymers of gelatin and added of fiber and extract of blueberry bagasse that presented values between 1.31 and 2.87 MPA (Crizel et al., 2016a).

Bonilla et al (2012) reported that the addition of essential oils of thyme and basil reduced the tensile strength and modulus of elasticity in chitosan films, but concluded that the increase in the elomgation at break of films depends on oil content added. Chitosan films combined with olive oil had their mechanical properties evaluated by Pereda, Amica, & Marcovich (2012). These authors concluded that the incorporation of oil in the films significantly increased the tensile strength, elasticity, and Young's Modulus and according to the authors there are no bibliographical references that prove increased elasticity as observed. It could be attributed to the difference in the degree of chitosan deacetylation used and interactions developed with components of olive oil that may involve several other factors such as humidity, the amount of added glycerol and drying temperature of film.

In relation to YM, a property that measures how much the film stretches without deforming, it was possible to observe that both the addition of olive powder and the addition of microparticles led to decrease in these values, which was inversely proportional to the amount of added residue. Ferreira et al (2014) reported that the amount of incorporated grape seed oil in the chitosan films is directly related to the YM since the addition of 0.3% oil

slightly increased the YM. However, there was a 30% decrease when the added oil content was doubled.

3.2.7. Antioxidant properties of olive pomace, microparticles, and film

The antioxidant activity of the olive pomace and microparticles of olive pomace were analyzed by the DPPH and FRAP methods. The DPPH method is based on the capture of free radicals, and the FRAP method evaluates the iron reduction ability. By the DPPH method, the antioxidant activity of powder was 143.51 ± 13.56 μmol Trolox equivalents (TE)/g dry matter, and the microparticles were 109.16 ± 2.01 μmol Trolox equivalents (TE)/g dry matter. Thus the powder value is statistically higher than the value of microparticles. The same trend was observed in FRAP method, the powder presented a higher value (136.51 ± 13.71 μmol FeSO4.7H2O equivalents/g dry matter) in relation to the microparticles (115.02 ± 7.57 μmol FeSO4.7H2O equivalents/g dry matter), but this difference was not significant ($p > 0.05$).

Table 5 shows the values of the antioxidant activity of chitosan films added with olive pomace. It can be observed that the incorporation of both (powder and microparticles) increased the antioxidant activity of films by both methods. Antioxidant activity increase by DPPH method was directly related to the amount of residue added in the formulation, and the films with 30% of powder and micro had the highest values of inhibition 39.45% and 42.56%, respectively, with no significant difference between them. According to Xie, Xu, & Liu (2001), this control can be explained by the ability of chitosan amino groups to interact with free radicals and to form stable macromolecular radicals and ammonium groups.

The antioxidant activity of chitosan films added to residues was evaluated in a study developed by Ferreira et al (2014), which incorporated 0.3% and 0.75% of grape seed oil to the films, obtaining approximately 25% and 35% of increase in antioxidant activity values but the reaction time of the analysis was 68 hours for 1 cm^2 of film. Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, & Fernández-López (2013) used the DPPH method and reported that the addition of 2% essential oil of thyme in chitosan films resulted in an antioxidant activity of 1.07 mg TE / g with a reaction time of 1 hour. Considering that in the present work powder residues were used and that the reaction time was 2 hours, its possible affirmed that the antioxidant power of films was quite significant.

Table 5. Antioxidant activity of films with powder and olive byproducts powder microparticles and control film.

Film	DPPH (μmol Trolox equivalents (TE)/g dried film)	DPPH - % RSA	FRAP (μmol $FeSO_4.7H_2O$ equivalents/g dried film)
Powder			
10%	0.88 ± 0.02^{d}	14.08 ± 1.36^{d}	4.32 ± 0.12^{e}
20%	1.32 ± 0.01^{c}	21.39 ± 1.25^{b}	7.79 ± 0.02^{c}
30%	2.48 ± 0.20^{a}	39.45 ± 1.59^{a}	7.84 ± 0.26^{c}
Microparticles			
10%	1.18 ± 0.13^{cd}	15.20 ± 2.50^{cd}	5.45 ± 0.26^{d}
20%	1.87 ± 0.02^{b}	20.98 ± 2.04^{bc}	9.09 ± 0.45^{b}
30%	2.73 ± 0.18^{a}	42.56 ± 2.02^{a}	11.28 ± 0.23^{a}
Control	0.43 ± 0.08^{e}	6.69 ± 0.68^{e}	0.27 ± 0.04^{f}

Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different as determined by Tukey test ($p < 0.05$).

3.2.8. Evaluation of antioxidant capacity of chitosan film on walnuts storage

The films with powder and microparticles that presented the highest antioxidant were selected for application as a nut packing and storage under high temperatures and light incidence. Nuts were pressed in a manual mechanical press for extraction of oil. Table 6 shows results of peroxides, dienes and trienes analysis of walnuts oil in film added of 30% microparticles, 30 % powder and control film and in two negative controls (polyethylene packaging and no packaging).

Chitosan films combined with olive residues presented significant protective effect against peroxide formation in the nuts during the 31 days of storage, differing ($p < 0.05$) from the samples packed with chitosan control film without olive residues and the other negative controls used (open plate and closed in Polyethylene packages). There was no difference ($p > 0.05$) in the oil peroxide formation of nuts packed with films containing powder and films with microparticles of olive residue powder. The control samples that were kept in open plates were those that obtained the highest index of peroxides in all the days of analysis, as expected.

The effects of chitosan coatings with green tea extract added on lipidic oxidation of nuts was evaluated by Sabaghi, Maghsoudlou, Khomeiri, & Ziaiifar (2015),. The coated nuts samples were stored for 18 weeks in polyethylene packages at room temperature. At the end of 18 weeks, the authors observed that the most effective coating in the protection against oxidation was that presented in its formulation 10 g / L of chitosan and 10 g / L of green tea extract, concluding that the determining factor was the increase in chitosan concentration and not in tea extract.

Table 6. Analysis of peroxides (PVs; mEq/ kg), dienes and trienes of walnuts oil in film added of 30% microparticles, 30 % powder and control film and in two controls closed in polyethylene packaging and open.

	Peroxide Values				
	Day 5	**Day 10**	**Day 19**	**Day 26**	**Day 31**
Film 30% Microparticles	0.25 ± 0.07^{abC}	0.75 ± 0.06^{bBC}	1.17 ± 0.14^{bAB}	1.56 ± 0.14^{cdAB}	1.78 ± 0.34^{dA}
Film 30% powder	0.25 ± 0.07^{abD}	0.71 ± 0.02^{bC}	0.86 ± 0.05^{bC}	1.20 ± 0.07^{dB}	1.65 ± 0.14^{dA}
Film control	0.20 ± 0.00^{bD}	0.88 ± 0.12^{bC}	2.06 ± 0.01^{aB}	2.13 ± 0.16^{bcB}	3.10 ± 0.03^{cA}
Plastic pack	0.45 ± 0.07^{aD}	1.08 ± 0.01^{bC}	1.09 ± 0.14^{bC}	2.88 ± 0.07^{abB}	5.09 ± 0.14^{bA}
Open control	0.20 ± 0.00^{bC}	2.27 ± 0.17^{aB}	2.62 ± 0.35^{aB}	3.36 ± 0.48^{aB}	6.40 ± 0.58^{aA}
	Dienes				
	Day 5	**Day 10**	**Day 19**	**Day 26**	**Day 31**
Film 30% Microparticles	0.08 ± 0.01^{dC}	0.10 ± 0.00^{cCB}	0.11 ± 0.00^{dB}	0.13 ± 0.00^{bA}	0.15 ± 0.01^{cA}
Film 30% powder	0.08 ± 0.00^{cdC}	0.11 ± 0.00^{bBC}	0.13 ± 0.00^{cAB}	0.16 ± 0.02^{abA}	0.16 ± 0.00^{cA}
Film control	0.10 ± 0.00^{bcD}	0.11 ± 0.00^{bD}	0.13 ± 0.00^{cC}	0.16 ± 0.00^{abB}	0.18 ± 0.00^{bcA}
Plastic pack	0.13 ± 0.01^{aC}	0.11 ± 0.00^{bBC}	0.16 ± 0.00^{bB}	0.16 ± 0.00^{abB}	0.20 ± 1.17^{abA}
Open control	0.11 ± 0.01^{abC}	0.13 ± 0.00^{aC}	0.19 ± 0.00^{aB}	0.20 ± 0.00^{aAB}	0.22 ± 0.00^{aA}
	Trienes				
	Day 5	**Day 10**	**Day 19**	**Day 26**	**Day 31**
Film 30% Microparticles	1.12 ± 0.01^{aC}	1.76 ± 0.00^{cB}	2.01 ± 0.02^{cAB}	2.50 ± 0.35^{bA}	2.23 ± 0.04^{dAB}
Film 30% powder	1.05 ± 0.02^{aD}	1.58 ± 0.03^{dC}	2.17 ± 0.01^{cB}	2.25 ± 0.00^{bA}	2.31 ± 0.02^{dA}
Film control	1.08 ± 0.03^{aE}	1.90 ± 0.02^{bcD}	2.50 ± 0.02^{bcC}	2.81 ± 0.04^{abB}	3.28 ± 0.00^{cA}
Plastic pack	1.11 ± 0.01^{aD}	2.06 ± 0.09^{abC}	2.83 ± 0.22^{abB}	2.77 ± 0.00^{abB}	3.81 ± 0.04^{bA}
Open control	1.16 ± 0.05^{aD}	2.20 ± 0.00^{aC}	3.29 ± 0.21^{aB}	3.39 ± 0.03^{aB}	4.01 ± 0.02^{aA}

*Results are the means of three determinations ± standard deviation. Different superscript lower case letters in the same column indicate statistically significant differences as determined by Tukey test ($p < 0.05$).Different superscript capital letters in the same line indicate statistically significant differences ($p < 0.05$). Conjugated dienes (1% Ɛ1cm [λ 232]). Conjugated trienes (1% Ɛ1cm [λ 268]).

Oxidation of fatty acids can lead to the formation of secondary compounds such as the dienes and trienes, identifiable on a spectrophotometer at 232 nm and 268-272 nm, respectively (Jadhav, et al., 1996).

At the end of the experiment, the diene formation was higher in the open and closed controls, and the value of dienes in the open control was significantly higher than the samples packed with chitosan film. There was no significant difference between the formation of secondary compounds in the samples of nuts packed with control chitosan film and the nuts packed with the chitosan films with olive residues.

The formation of trienes (Table 6) followed the same tendency of peroxide formation mentioned above, where the films with powder and microparticles of olive pomace industry significantly prevented the formation of these compounds in relation to the control samples evaluated, this is due the amount of antioxidant compounds such as phenolic compounds from the olive residues that are present in these packages (Crizel et al 2016b).

Analyzing the values of trienes formed in the nuts on the other days of storage, it was observed that the chitosan films presented no significant difference between them, on the 19th and the 26th day of analysis and that only on the 31st day there was a statistical difference between them, proving that the material of the biopolymer, chitosan, helped preserve the films during these days. Therefore at 31st day the presence of antioxidants was essential to maintain the protective effect of the packaging, being it in the form of powder or microparticles.

Table 7. Fatty Acid Profile of walnuts oil packed in control film, film added of 30% powder and 30% microparticles and open and closed control in polyethylene packaging.

Samples	Fatty Acid (%)*	Storage (days)				
		5	10	19	26	31
Film 30 % Micro	ω3	14.29±0.25[a]	14.17±0.06[a]	14.06±0.19[a]	14.12±0.16[a]	13.93±0.04[a]
	ω6	59.16±0.09[bc]	59.20±0.07[bc]	59.57±0.11[a]	59.44±0.18[ab]	59.10±0.03[c]
	ω3/ω6	0.24±0.00[a]	0.24±0.00[a]	0.24±0.00[a]	0.24±0.00[a]	0.24±0.00[a]
Film 30 % Flour	ω3	14.17±0.06[a]	13.95±0.10[a]	14.12±0.13[a]	13.88±0.19[a]	14.12±0.07[a]
	ω6	59.25±0.11[a]	59.35±0.12[a]	59.37±0.08[a]	59.42±0.10[a]	59.19±0.05[a]
	ω3/ω6	0.24±0.00[a]	0.24±0.09[a]	0.24±0.00[a]	0.23±0.00[a]	0.24±0.00[a]
Film control	ω3	14.20±0.11[a]	13.87±0.29[a]	14.07±0.07[a]	13.91±0.20[a]	13.94±0.24[a]
	ω6	59.08±0.23[a]	59.16±0.17[a]	59.14±0.28[a]	59.05±0.12[a]	59.02±0.05[a]
	ω3/ω6	0.24±0.00[a]	0.23±0.00[a]	0.24±0.00[a]	0.24±0.00[a]	0.24±0.00[a]
Closed control	ω3	14.25±0.36[a]	14.21±0.13[ab]	13.97±0.13[ab]	13.71±0.15[ab]	13.59±0.17[b]
	ω6	58.97± 0.27[b]	59.31±0.08[ab]	59.62±0.23[a]	59.42±0.23[ab]	59.55±0.17[ab]
	ω3/ω6	0.24±0.00[a]	0.24±0.01[a]	0.23±0.00[ab]	0.23±0.00[ab]	0.23±0.00[b]
Open control	ω3	14.31±0.25[a]	14.01±0.17[ab]	13.68±0.12[b]	13.80±0.13[b]	13.62±0.15[b]
	ω6	59.18±0.20[a]	59.15±0.24[a]	59.34±0.37[a]	59.34±0.29[a]	59.17±0.14[a]
	ω3/ω6	0.24±0.00[a]	0.24±0.00[ab]	0.23±0.00[b]	0.23±0.00[b]	0.23±0.00[b]

*Results are the means of three determinations ± standard deviation. Different letters in the same line are significantly different as determined by Tukey test ($p < 0.05$).

Omega-3 fatty acids are vital for health because of their beneficial effects on the heart and diseases such as diabetes, cancer, and neurological illness. These compounds have anti-inflammatory effects that can aid in the treatment of diseases (Kaur, Chugh & Gupta, 2012).The nuts contain a considerable amount of ω-3 fat, with a ω3 / ω 6 ratio of about 0.25. Taking into account the importance of these fatty acids, they were evaluated in the samples of oil extracted from the nuts that were packed in chitosan films, polyethylene plastics and kept open (Table 7). The oil of the nuts packed in the control chitosan films and in the films with olive residues, during the 31 days showed no difference in relation to their content of ω 3 and ω 6, although the value of w6 of the samples with chitosan film and microparticles had presented an elevation on the 19th day.

It was possible to identify small changes in the fatty acid profile of the control samples. In the nuts packed in polyethylene bags and in the samples kept open there was a significant reduction in the contents of ω 3 and consequently in the relation ω3 / ω 6. According to Kaur, Chugh & Gupta (2012) alpha-linolenic acid is very sensitive to degradation by light, heat and oxygen, which would justify this reduction in the values of the controls.

4. Conclusions

The results of the present work demonstrate that it was possible to use olive pomace in the development of biodegradable chitosan films, but the incorporation of these residues resulted in changes in the original properties of the films. The incorporated films of olive pomace presented a more heterogeneous and rough structure, with several imperfections that consequently reflected in the mechanical properties causing a reduction in tensile strength and the Young's Modulus. The lack of solubility of the powder in the chitosan matrix increased the permeability and water solubility of films, however, allowed the significant improvement of the antioxidant properties.

The chitosan based films with the addition of 10% microparticles of olive pomace revealed films with a more homogeneous and compact structure, besides a higher mechanical resistance without showing changes in water vapor permeability and solubility. Furthemore, the addition of microparticles improved the optical properties and significantly increased the antioxidant activity. The films added of olive residues showed protective effect against the oxidation of nuts during the 31 days in which they were stored under high temperature and incident conditions.

The packaging developed with microparticles of olive pomace proved to be a promising alternative to replace the plastic packaging currently found on supermarket shelves, mainly because they are made from residues, totally biodegradable, with high antioxidant capacity and their good mechanical properties.

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Supplementary material

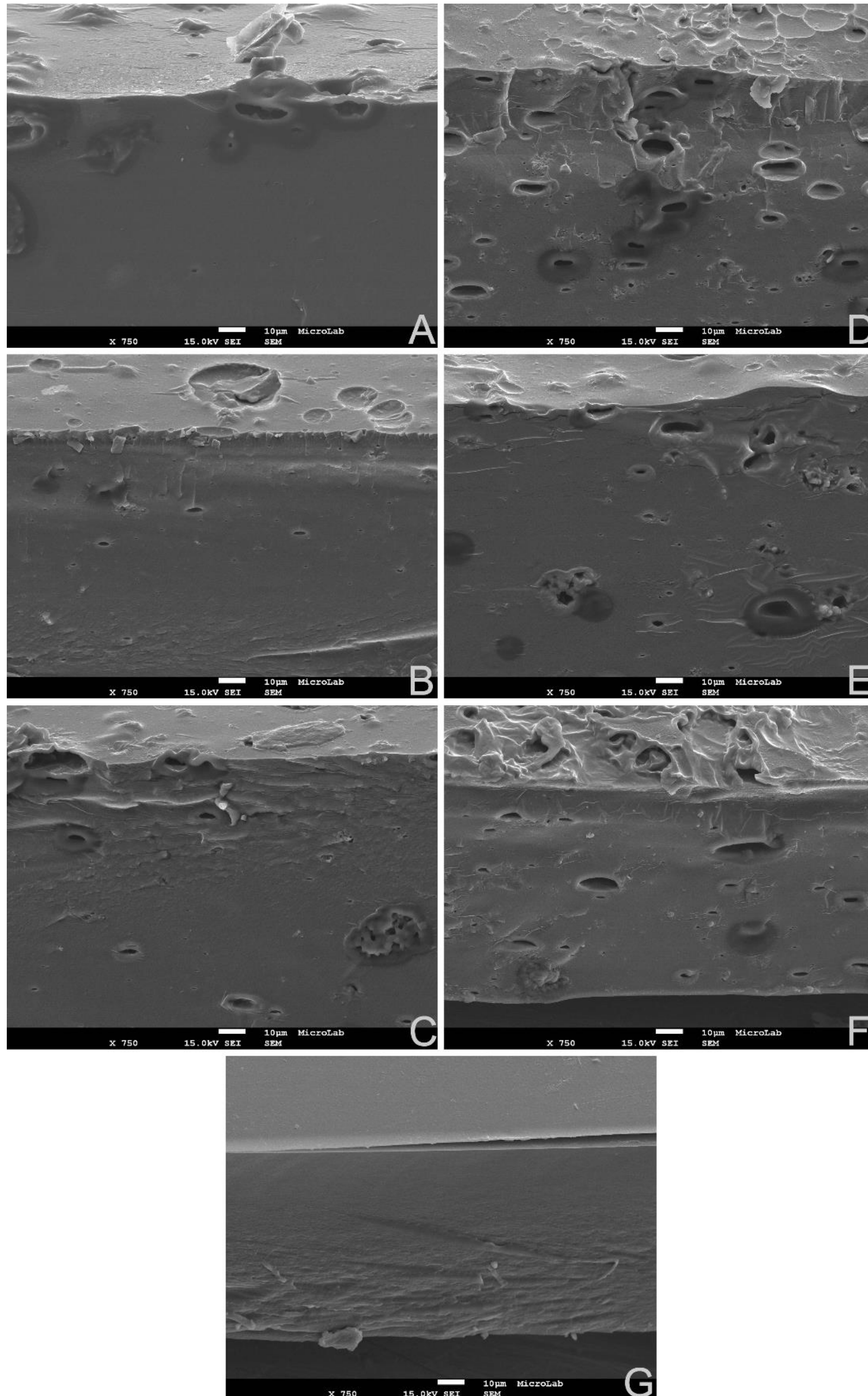

Figure 1 (Suplementary material) - Scanning Electron Microscopy (SEM) images (750 x magnification) of films cross section added olive waste powder ((A) 10% powder, (B) 20% powder, (C) 30% powder), microparticles olive waste powder ((D) 10% micro, (E) 20% micro, (F) 30% micro) and control film ((G) without powder or micro).

CAPÍTULO 9

9. Discussão Geral

O aproveitamento dos resíduos da indústria de processamento de alimentos é importante e viável, tanto pelo fator econômico como pela riqueza nutricional desses resíduos, fatores esses que foram comprovados ao longo deste trabalho.

A partir dos resíduos do processamento de frutas como abacaxi, mamão, mirtilo e oliva puderam ser obtidas farinhas, que apresentaram elevado teor de fibras, boas características tecnológicas e alto potencial antioxidante. As diferentes farinhas obtidas apresentaram propriedades diferentes frente as análises realizadas. (Tabela 1). A farinha de mamão, por exemplo, apresentou os maiores valores de capacidade de retenção de óleo e de água e solubilidade o que permitem sua aplicação em produtos a fim de melhorar a textura e reduzir valor calórico, além de ser rica em carotenoides, como a criptoxantina. A farinha de mirtilo foi a que apresentou o maior teor de compostos fenólicos e atividade antioxidante pelo método DPPH, além de altos teores de antocianinas, o que indica essa farinha como um excelente ingrediente funcional. Os resíduos de oliva, por sua vez, foram os que apresentaram os maiores valores de fibra dietética total (53,68 b.s) quando comparado as demais farinhas.

Juntamente com o interesse pelo aproveitamento dos resíduos de frutas está o interesse pelo desenvolvimento de embalagens biodegradáveis que possam substituir as tradicionais embalagens plásticas, que há anos se acumulam no solo e provocam danos ambientais. As embalagens biodegradáveis são obtidas a partir de biopolímeros como a gelatina e a quitosana, sendo que a gelatina utilizada no desenvolvimento dos filmes também pode ser orirunda de resíduos produzidos a partir de cápsulas nutracêuticas de óleo de chia oriundas da indústria farmacêutica. Os biopolímeros possuem inúmeras vantagens em relação aos polietilenos, como a rápida biodegradabilidade, a não toxicidade, a biocompatibilidade com outros biopolímeros e principalmente a fácil interação com os alimentos, que propicia o desenvolvimento de embalagens biodegradáveis e ativas, que podem ter papel antimicrobiano e antioxidante, o que ajuda na preservação do produto embalado e consequentemente aumenta sua vida de prateleira.

Neste trabalho foram desenvolvidas embalagens biodegradáveis e ativas utilizando como biopolímero gelatina oriunda dos resíduos de cápsulas nutracêuticas de óleo de chia e resíduos do processamento de mirtilo (bagaço) e casca de mamão, como agentes

antioxidantes. Também foram desenvolvidas embalagens utilizando a quitosana como biopolímero e resíduos da indústria do processamento de óleo de oliva (bagaço) como compostos antioxidantes.

A partir dos resultados obtidos ficou evidente o poder antioxidante que cada um dos ingredientes adicionados agregaram às embalagens. Entretanto, alguns desses ingredientes como as farinhas, quando adicionadas aos filmes modificaram às propriedades originais dos mesmos, como as propriedades mecânicas e de permeabilidade. Já ingredientes como o extrato de mirtilo e as micropartículas da farinha de mamão e oliva melhoraram algumas dessas propriedades e também agregaram poder antioxidante.

As farinhas de bagaço de mirtilo e de casca de mamão quando adicionadas na matriz de gelatina reduziram a tensão de ruptura e aumentaram a permeabilidade ao vapor da água, resultados semelhantes foram também obtidos quando a farinha de bagaço de oliva foi adicionada à matriz de quitosana. Isto está diretamente associado à estrutura não homogênea da película, causada pela presença de partículas insolúveis, como pode ser observado nas imagens de microscopia eletrônica de varredura apresentada nos artigos. Esses resultados refletem o grau de incompatibilidade química e estrutural dos biopolímeros com as farinhas. A coesão do material do filme é um parâmetro importante que influencia na resistência mecânica e na permeabilidade. Quando os materiais contêm ingredientes heterogêneos que não são compatíveis com os biopolímeros principais ocorre uma modificação ou interrupção da estrutura original da matriz polimérica.

Diante dos efeitos que as farinhas provocaram nas embalagens foram estudados outros ingredientes obtidos das mesmas fontes de resíduos que apresentassem potencial antioxidante semelhante sem modificar propriedades importantes das embalagens. Neste sentido, têm-se o extrato de bagaço de mirtilo nos filmes de gelatina que apresentou potencial antioxidante semelhante ao da farinha, sem alterar as propriedades mecânicas e de permeabilidade.

A fim de melhorar a solubilidade da farinha de casca de mamão na matriz do biopolímero foram desenvolvidas micropartículas das próprias farinhas, por atomização utilizando como material de parede a mesma gelatina utilizada na matriz filmogênica. Tal processo foi determinante para os bons resultados obtidos já que as micropartículas se solubilizaram perfeitamente na matriz de gelatina, e além de manter os resultados de permeabilidade do filme de gelatina controle (somente com gelatina) também reforçaram a estrutura, uma vez que quanto maior a concentração de micropartículas adicionadas maiores foram os valores de tensão das embalagens, ou seja, estas ficaram mais resistentes. Porém, as

embalagens com micropartículas de farinha de casca de mamão apresentaram menor atividade antioxidante pelo método DPPH do que as embalagens com farinha bruta, quando foram utilizadas como embalagem para banha e submetidas a condições elevadas de temperatura e incidência de luz, contudo apresentaram os melhores resultados já que no final de 22 dias as amostras de banha embaladas com filme de micropartículas continham menor valor de peróxidos do que as demais. Tal resultado pode estar relacionado a maior capacidade de proteção do material de parede e consequentemente maior estabilidade dos antioxidantes contidos na micropartícula.

As micropartículas da farinha de bagaço de oliva também melhoraram as propriedades mecânicas dos filmes de quitosana, porém diferentemente das micropartículas de mamão nos filmes de gelatina, que quanto maior a concentração adicionada maiores foram os valores de resistência dos filmes. Para os filmes de quitosana os melhores resultados foram obtidos com a menor concentração de micropartículas (10%), porém quando adicionados 20% de micropartículas não houve diferença significativa em relação à amostra controle (apenas com quitosana). Segundo alguns estudos encontrados na literatura a adição de pequenas quantidades de lipídios em filmes de quitosana torna a sua estrutura mais compacta, mas a presença de alguns óleos essenciais pode causar descontinuidades estruturais que reduzem a mobilidade da corrente e consequentemente menor é a flexibilidade e a força do filme.

A embalagem com 30% de micropartículas de farinha de oliva foi a que apresentou a maior atividade antioxidante, porém os menores valores de resistência à tração quando comparados aos demais filmes de quitosana desenvolvidos. Entretanto, quando comparamos os valores de resistência a tração com filmes de gelatina com extrato de mirtilo e micropartículas de farinha de mamão também desenvolvidos neste estudo, foi verificado que os valores foram bastante elevados. Ao comparar com filmes plásticos de polietileno os valores de tensão foram similares.

Para os valores de opacidade e atividade antioxidante os filmes que continham micropartículas apresentaram maiores resultados do que os filmes com farinha. Já no teste acelerado como embalagens de nozes, durante 31 dias, não houve diferença significativa na formação de peróxidos ou compostos secundários nas amostras de nozes, ou seja, ambas as embalagens protegeram as nozes da oxidação quando comparadas com as amostras controles (mantidas abertas e em embalagens plásticas de polietileno).

CAPÍTULO 10

10. Conclusão Geral

Os resultados apresentados ao longo desse trabalho confirmam o elevado potencial de aplicação dos resíduos oriundos do processamento de frutas, pelo alto teor de compostos antioxidantes. Esse potencial foi comprovado quando os resíduos na forma de farinhas, extrato ou micropartículas foram incorporados em biopolímeros de gelatina e quitosana e resultaram em embalagens ativas eficazes contra a oxidação de produtos como nozes, banha e óleo de girassol.

Os compostos bioativos na forma de farinha, extrato e microcápsula, de maneira geral, reforçaram as propriedades mecânicas e de barreira, proporcionando embalagens biodegradáveis mais resistentes e algumas delas com características de tração semelhantes as embalagens oriundas do petróleo.

Os filmes desenvolvidos neste estudo mostraram-se promissores como embalagens biodegradáveis e ativas para alguns alimentos, destacando-se por serem economicamente viáveis considerando o material utilizado na sua produção (derivado de resíduos e biodegradáveis), além do seu poder antioxidante, que auxilia na vida útil dos produtos, e de suas propriedades mecânicas, que são semelhantes aos plásticos de polietileno de baixa densidade.

Tabela 1. Propriedades mecânicas, de permeabilidade, solubilidade, umidade, atividade antioxidante e opacidade dos filmes desenvolvidos.

	Resistência a Tração (Mpa)	**Elongação (%)**	**Módulo de Young (Mpa)**	**PVA (g.mm/m^2 h kPa)**	**Solubilidade (%)**	**Umidade (%)**	**Atividade antioxidante (Método DPPH %)**	**Valores de opacidade**
Resíduo Mirtilo								
Fibra								
5%	1,35 ± 0,07	252 ± 20,95	433,91 ± 35,00	0,99 ± 0,05	30,61 ± 0,31	23,23 ± 0,88	35 ± 3	2,30 ± 0,01
10%	1,31 ± 0,12	138,67 ± 7,77	535,02 ± 19,13	0,93 ± 0,01	27,14 ± 0,18	20,63 ± 0,67	49 ± 1	5,87 ± 0,07
15%	1,51 ± 0,06	86,33 ± 5,13	735,83 ± 3,43	0,88 ± 0,04	26,02 ± 0,66	21,82 ± 1,19	67 ± 4	6,25 ± 0,10
Extrato								
30%	2,56 ± 0,20	291,62 ± 15,62	175,51 ± 11,37	0,57 ± 0,03	29,71 ± 0,06	22,77 ± 0,16	38 ± 0	1,38 ± 0,13
40%	2,54 ± 0,04	292,67 ± 2,89	222,38 ± 2,81	0,55 ± 0,00	32,42 ± 0,82	22,10 ± 0,52	50 ± 1	1,49 ± 0,09
50%	2,87 ± 0,13	300,67 ± 5,51	196,92 ± 19,66	0,63 ± 0,03	30,04 ± 2,13	23,03 ± 0,75	62 ± 1	1,59 ± 0,07
Controle gelatina	2,57 ± 0,17	257 ± 7	175,73 ± 6,52	0,59 ± 0,01	42,13 ± 1,03	20,84 ± 0,31	3 ± 0	0,20 ± 0,03
Resíduo mamão								
Pó								
2,5%	1,86 ± 0,00	132,34 ± 1,09	856,84 ± 35,76	1,64 ± 0,02	58,11 ± 0,76	20,82 ± 1,03	23,09 ± 0,15	0,73 ± 0,02
5,0%	1,62 ± 0,04	74,10 ± 2,41	758,57 ± 16,20	1,74 ± 0,05	65,89 ± 0,84	20,91 ± 0,82	41,14 ± 0,97	1,14 ± 0,01
7,5%	1,09 ± 0,01	59,06 ± 0,65	664,37 ± 21,71	1,75 ± 0,03	67,75 ± 0,14	20,60 ± 0,66	53,65 ± 1,43	1,21 ± 0,01
Micropar-ticulas								
2.5%	4,34 ± 0,02	154,52 ± 2,14	2148,17 ± 149	1,45 ± 0,07	46,50 ± 1,11	15,42 ± 0,88	14,23 ± 0,47	0,63 ± 0,02
5,0%	6,24 ± 0,33	142,15 ± 1,90	2309,69 ± 23,24	1,32 ± 0,01	41,63 ± 1,38	16,42 ± 0,42	21,45 ± 0,34	0,88 ± 0,02

7,5%	7,67 ± 0,11	166,67 ± 0,19	2457,70 ± 55,99	1,31 ± 0,08	37,88 ± 0,55	17,12 ± 0,57	31,51 ± 2,01	1,10 ± 0,05
Controle gelatina	3,41 ± 0,11	338,30 ± 10,11	1088,21 ± 73,55	1,26 ± 0,01	66,67 ± 0,04	19,93 ± 0,20	6,71 ± 0,54	0,22 ± 0,01
Resíduos Oliva								
Pó								
10%	7,58 ± 0,23	17,57 ± 0,28	40,42 ± 0,29	1,32 ± 0,03	31,93 ± 4,22	18,87 ± 1,06	14,08 ± 1,36	1,77 ± 0,13
20%	5,49 ± 0,21	15,39 ± 0,44	25,79 ± 2,55	1,52 ± 0,05	31,40 ± 4,35	17,76 ± 0,07	21,39 ± 1,25	2,86 ± 0,02
30%	2,89 ± 0,06	13,31 ± 0,77	12,39 ± 0,39	2,23 ± 0,14	44,55 ± 1,28	16,47 ± 0,87	39,45 ± 1,59	3,03 ± 0,06
Micropar-ticulas								
10%	22,40 ± 0,22	33,01 ± 0,66	35,90 ± 2,48	0,96 ± 0,04	31,31 ± 3,59	18,97 ± 0,19	15,20 ± 2,50	3,51 ± 0,08
20%	14,55 ± 0,43	23,58 ± 0,73	23,43 ± 1,63	1,06 ± 0,06	28,64 ± 2,55	17,38 ± 0,58	20,98 ± 2,04	4,30 ± 0,14
30%	6,51 ± 0,02	20,93 ± 0,69	16,28 ± 0,83	1,09 ± 0,02	26,16 ± 2,13	15,04 ± 0,28	42,56 ± 2,02	6,03 ± 0,20
Controle quitosana	16,76 ± 3,51	23,05 ± 1,36	69,11 ± 2,10	0,85 ± 0,00	32,61 ± 0,73	18,28 ± 0,92	6,69 ± 0,68	0,68 ± 0,00

PERSPECTIVAS FUTURAS

Como perspectivas futuras podemos considerar:

- a aplicação dos filmes em outros tipos de alimentos e avaliar o efeito do filme sobre eles;
- estudo de outros resíduos para produção de farinhas e ingredientes de filmes;
- obtenção de extratos dos compostos bioativos dos diferentes resíduos estudados e obtenção de micro e nanocápsulas destes extratos;
- adição de extratos micro e nanoencapsulados em filmes biodegradáveis de diferentes matrizes poliméricas;
- adaptação para produção do filme em escala industrial atrás de processos como a extrusão.

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