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Faculdade de Engenharia Agrícola

GISLAINE FERREIRA NOGUEIRA

INCORPORAÇÃO DE POLPA DE AMORA COMO ANTIOXIDANTE EM FILMES COMESTÍVEIS E BIODEGRADÁVEIS DE AMIDO DE ARARUTA

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Tese apresentada à Faculdade de Engenharia Agrícola da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Engenharia Agrícola, na Área de Tecnologia Pós-Colheita.

Orientador: Prof. Dr. Rafael Augustus de Oliveira

Co-orientadora: Profa. Dra. Farayde Matta Fakhouri

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“*Não é sobre chegar no topo do mundo e saber que venceu*
É sobre escalar e sentir que o caminho te fortaleceu”
(Ana Carolina Vilela Da Costa)

RESUMO GERAL

Embalagens de alimentos usualmente são provenientes de fontes não renováveis acarretando problemas ambientais. Uma das formas encontradas para minimizar esse impacto é o desenvolvimento de filmes biodegradáveis. O amido de araruta é uma matéria-prima potencial para elaboração desses filmes. Filmes podem servir como veículos de transporte de aditivos alimentares, como agentes antioxidantes extendendo a sua aplicabilidade como filmes antioxidantes e comestíveis. Amora preta é um fruto rico em compostos bioativos. A microencapsulação da sua polpa permite manter a estabilidade de seus compostos bioativos quando expostos a condições desfavoráveis, sendo uma tecnologia promissora para conservar sua funcionalidade, bem como aprimorar as propriedades funcionais dos filmes. Neste contexto, a primeira parte desse trabalho teve como objetivo a extração e caracterização do amido de araruta quanto às propriedades físico-quimicas, térmicas e estruturais. O amido apresentou alto teor de amilose, habilidade de gelificação e propriedades térmicas desejáveis para a sua utilização como agente encapsulante, bem como polímero na produção de filmes comestíveis e biodegradáveis. Posteriormente, avaliou-se sua capacidade como agente encapsulante em mistura com a goma arábica para a microencapsulação da polpa de amora por *spray drying e freeze drying*. Os polímeros apresentaram alta compatibilidade entre si e a polpa de amora, exibindo um efeito termoprotetor do ácido ascórbico e das antocianinas durante o processo de *spray drying*, confirmando sua adequação como agente encapsulante. Essa mistura foi capaz de tornar a polpa de amora mais estável, diminuindo a sua pegajosidade e aderência na parede da câmara de secagem e do ciclone via microencapsulação. O método de microencapsulação, a temperatura do ar de entrada e a concentração de agente encapsulante influenciaram significativamente as propriedades físico-químicas dos pós de amora. Na terceira parte desse trabalho foi avaliada a capacidade do amido de araruta como matriz formadora de filmes, bem como, veículo de transporte de polpa de amora para filmes comestíveis e antioxidantes. Os filmes preparados com amido de araruta e glicerol desprenderam das placas de suporte, eram manipuláveis, transparentes e inodoros, o que confirmam a sua aplicabilidade para esse fim. A concentração de amido e de glicerol utilizada na elaboração dos filmes afetaram significativamente a espessura e a solubilidade em água. A incorporação da amora na solução filmogênica transferiu ao filme compostos bioativos e atividade antioxidante. O tipo de incorporação, direta ou *sprinkling* de amora na solução filmogênica, influenciou na extração dos compostos bioativos, na capacidade antioxidante e solubilidade em água dos filmes. A espessura, a solubilidade em água, a permeabilidade ao vapor de água e a elongação aumentaram significativamente com o aumento da concentração de polpa de amora (20, 30 e 40%) nos filmes, enquanto a resistência à tração diminuiu. Houve um escurecimento dos filmes com polpa de amora após o processo de esterilização. Os filmes foram quase que totalmente desintegrados após 38 dias enterrados em compostagem orgânica, exibindo facilidade de biodesintegração. Considerando-se o conteúdo de compostos bioativos, atividade antioxidante e propriedades funcionais dos filmes de amido de araruta com polpa amora, sugerem-se a sua utilização como revestimentos coloridos para alimentos, como encapsulante e/ou embalagens modificadas como substitutos das embalagens de alimentos, intermediárias não biodegradáveis, ou ainda, para serem consumidos na forma de tiras de frutas.

Palavras-chave: antocianinas, secagem por pulverização, liofilização, pó, embalagem ativa.

ABSTRACT

Food packaging usually comes from non-renewable sources, causing environmental problems. One of the ways found to minimize this impact is the development of biodegradable films. Arrowroot starch is a potential raw material for production of these films. Films can serve as transport vehicles for food additive, as antioxidants, extending their applicability as antioxidant and edible films. Blackberry is a fruit rich in bioactive compounds. The microencapsulation of its pulp allows to maintain the stability of its bioactive compounds when exposed to unfavorable conditions, becoming a promising technology to conserve its functionality, as well as to improve the functional properties of films. In this context, the first part of this work had the objective of extracting and characterizing the arrowroot starch in relation to physicochemical, thermal and structural properties. The starch had high amylose content, gelling ability and desirable thermal properties for using as encapsulating agent, as well as polymer in edible and biodegradable films production. Subsequently, its capacity as encapsulating agent in mixture with gum arabic was evaluated for microencapsulation of blackberry pulp by spray drying and freeze drying. The polymers showed high compatibility between each other and of blackberry pulp, showing thermoprotective effect of ascorbic acid and anthocyanins during spray drying process, confirming its suitability as encapsulating agent. This mixture made blackberry pulp more stable, reducing its stickiness and adherence to the drying chamber and cyclone walls via microencapsulation. The microencapsulation method, the inlet air temperature and the encapsulating agent concentration significantly influenced physicochemical properties of blackberry powders. In the third part of this work, the capacity of arrowroot starch as film-forming matrix was evaluated, as well as carrier of blackberry pulp for edible and antioxidant films. Films prepared with arrowroot starch and glycerol detached from the support plates, were manipulable, transparent and odorless, confirming their applicability for this purpose. The concentration of starch and glycerol used in preparation of films significantly affected thickness and solubility in water. Incorporation of blackberry into the film-forming solution transferred bioactive compounds and antioxidant capacity to the films. The type of incorporation of blackberry, directly or by sprinkling, in the filmogenic solution influenced bioactive compounds extraction, antioxidant capacity and water solubility of the films. Thickness, water solubility, water vapor permeability and elongation increased significantly with increasing blackberry pulp concentration (20, 30 and 40%) in films, while tensile strength decreased. There was darkening of films with blackberry pulp after sterilization process. Films were almost completely disintegrated after 38 days buried in organic compost, exhibiting ease of biodesintegration. Considering the content of bioactive compounds, antioxidant capacity and functional properties of arrowroot starch films with blackberry pulp, it is suggested to use them as colorful food coatings, as encapsulant and/or modified packaging as substitutes for non-biodegradable intermediate food packaging, or to be consumed in the form of fruit stripes.

Keywords: anthocyanins, spray drying, freeze drying, powder, active packaging.

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ANEXO 2 330

Autorização da revista Drying Technology para uso do artigo intitulado “Effect of incorporation of blackberry particles on the physicochemical properties of edible films of arrowroot starch” 330

INTRODUÇÃO GERAL

INTRODUÇÃO GERAL

A maioria das embalagens, tradicionalmente utilizadas para alimentos, é produzida a partir de materiais sintéticos de fonte não renovável, que apesar de possuírem excelentes propriedades funcionais (baixa solubilidade em água e permeabilidade ao vapor de água, alta resitência à tração, flexibilidade) são consideradas não biodegradáveis e estão trazendo sérios problemas ambientais, devido à geração de elevadas quantidades de resíduos sólidos não degradáveis no ambiente (SOARES et al., 2005; AZEREDO, 2009; SOZER; KOKINI, 2009). No entanto, a utilização de embalagens é imprescindível, visto que estas possuem papel fundamental no controle das interações entre o alimento e o ambiente, protegendo e auxiliando na manutenção da qualidade do produto (TAWFIK; HUYGHEBAERT, 1999; SOARES et al., 2009), além da sua função básica de conter o alimento.

Este fator, juntamente com as preocupações ambientais, combinado com as exigências dos consumidores por produtos ecologicamente corretos, mais próximos ao natural com alta qualidade, tem chamado a atenção dos pesquisadores para o desenvolvimento de tecnologias que substituam o consumo de materiais provenientes de fósseis por processos sustentáveis e materiais de fontes renováveis (CORREA, 2008; SOARES et al., 2009). Nas últimas décadas, novas tecnologias de embalagens vêm sendo desenvolvidas em resposta a essa demanda, e uma das soluções encontradas foi o desenvolvimento de filmes biodegradáveis a partir de biopolímeros (FARIAS et al., 2012).

Os biopolímeros mais utilizados na elaboração de filmes biodegradáveis são as proteínas, os polissacarídeos e os lipídeos ou a combinação dos mesmos (CUQ et al., 1995; FAKHOURI et al., 2007). Dentre os polissacarídeos utilizados para produção de filmes, o amido é o biopolímero natural mais empregado, devido ao seu fácil processamento, baixo custo, abundância, biodegradabilidade, comestibilidade e fácil manipulação (MALI et al., 2002). É obtido em forma granular de fontes renováveis como ervilha, milho, mandioca, batata e araruta, entre outros (PETERSEN et al., 1999).

A araruta (*Maranta arundinaceae* L.) pertence à família *Marantaceae* e, seus rizomas apresentam um ao alto teor de fibras, o que dificulta seu consumo *in natura*. Além disso, a araruta apresenta um alto teor de amido em seus rizomas, por causa disso, tem sido utilizada especialmente para sua extração. O alto teor de amilose torna o amido de araruta um material polimérico promissor para ser utilizado na preparação de filmes biodegradáveis (LEONEL et al., 2002). Sua fécula forma uma película semelhante à celulose em resistência e transparência (AGOSTINI et al., 2009).

No entanto, filmes elaborados somente com amido são poucos flexíveis e quebradiços, apresentando propriedades físicas e mecânicas pobres, mesmo quando são incorporados de plastificantes, necessitando assim que suas propriedades sejam melhoradas para que possam ser utilizados como substitutos aos tradicionais plásticos (GONTARD et al., 1993). Neste sentido, estudos têm demonstrado que a adição de extratos, sucos e polpas de frutas, tais como extrato de *blueberry*, de sementes de uva e de bagaço de *cranberry*, suco de romã, purê de papaya, açaí, podem proporcionar modificações nas propriedades mecânicas e sensoriais, bem como na bioatividade, conferindo atividade antioxidante e antimicrobiana aos filmes em que foram incorporados (PARK; ZHAO, 2006; SIVAROOBAN et al., 2008; ZHANG et al., 2010; ESPITIA et al., 2014; OTONI et al., 2014; AZEREDO et al., 2016), o que poderá expandir suas aplicações como filmes bioativos e comestíveis.

Assim, antioxidantes naturais como os da amora-preta têm sido pesquisados para aplicações na indústria de alimentos. A amora-preta (*Rubus* spp) é uma fruta que apresenta alta qualidade nutricional, fonte de antioxidantes naturais como antocianinas e compostos fenólicos (WANG; LIN, 2000; MOYER et al., 2002). A presença de ácido elágico derivado do ácido gálico e ácido ascórbico (vitamina C) também foram constatados nos frutos (ANTUNES et al., 2006). Diversos estudos relataram a maior capacidade antioxidante de amoras com base em sua capacidade de absorção do radical oxigênio, em comparação com outros frutos, como morangos, framboesas vermelhas e uvas de vinho tinto (WANG; LIN, 2000; MOYER et al., 2002; CHO et al., 2004). Além disso, a microencapsulação da sua polpa como possibilidade de manter a estabilidade de seus compostos bioativos quando expostos a condições desfavoráveis (por exemplo, a temperatura elevada, luz, oxigênio) é uma alternativa viável e uma tecnologia promissora para conservar sua funcionalidade (SHAHIDI; HAN, 1993), bem como aprimorar as propriedades mecânicas e de barreira dos filmes à base de biopolímeros biodegradáveis como o amido.

Estudos apontaram que as propriedades dos filmes podem também ser melhoradas pela adição de compostos de reforço como as micropartículas e nanopartículas. Um exemplo disto é o estudo realizado por Chen et al. (2009). Nesta pesquisa, os autores desenvolveram nanobiocompósitos, utilizando nanocelulose obtida da casca de ervilha e uma matriz de amido. As propriedades de absorção ultravioleta, resistência à tração, transparência, elongação na ruptura e resistência a água dos filmes contendo nanocelulose apresentaram melhores resultados quando comparados com os filmes de amido puro.

Assim sendo, polpas de frutas na forma livre, bem como na forma microencapsulada, podem ser potencialmente utilizados como aditivos naturais para melhorar as propriedades físico-químicas e/ou bioativas de filmes comestíveis e biodegradáveis de amido para aplicações como embalagens ativas como substitutos de embalagens plásticas (WANG et al., 2012).

OBJETIVOS

Objetivo Geral

O objetivo do trabalho foi desenvolver e incorporar micropartículas contendo polpa de amora-preta na solução filmogênica de amido de araruta e glicerol para a produção de filmes antioxidantes e comestíveis.

Objetivos Específicos

I. Extrair e caracterizar amido de araruta quanto às propriedades físico-químicas, térmicas e microestruturais;

II. Avaliar a capacidade da mistura de amido de araruta e goma arábica como agente encapsulante da polpa de amora por *spray drying* e *freeze drying;*

III. Verificar a influência do uso de diferentes temperaturas do ar de entrada e diferentes concentrações de agente microencapsulante (mistura de amido de araruta/goma arábica) sobre as propriedades físico-químicas (rendimento, teor de água, atividade de água, tamanho médio das partículas, higroscopicidade, solubilidade, cor, conteúdo de antocianinas e ácido ascórbico e capacidade antioxidante) da polpa de amora em pó obtida por spray drying;

IV. Comparar as propriedades físico-químicas (rendimento, teor de água, atividade de água, tamanho médio das partículas, higroscopicidade, solubilidade, conteúdo de antocianinas e capacidade antioxidante) dos pós de polpa de amora microencapsulada por *spray drying* e *freeze drying*, assim como a polpa de amora seca por *freeze drying* sem agente encapsulante;

V. Verificar a influência da concentração do amido de araruta e do plastificante sobre a espessura, atividade de água, teor de água, permeabilidade ao vapor de água, solubilidade em água e resistência à tração dos filmes;

VI. Verificar a influência da concentração e da forma de incorporação (diretamente e por aspersão - *sprinkling*) da polpa de amora líquida, em pó e microencapsulada na solução filmogênica de amido de araruta e plastificante sobre a espessura, atividade de água, teor de água, permeabilidade ao vapor de água, solubilidade em água e resistência a tração, cor, conteúdo de antocianinas e capacidade antioxidante dos filmes resultantes;

VII. Avaliar a estabilidade dos filmes de amido de araruta e com polpa de amora em diferentes condições de pHs e frente ao processo de esterilização;

VIII. Verificar a biodesintegração dos filmes de amido de araruta e com polpa de amora em compostagem orgânica.

REFERÊNCIAS BIBLIOGRÁFICAS

AGOSTINI, J.S.; CÂNDIDO, A.C.S.; TEODÓSIO, T.K.C.; RODRIGUES, J.N.; GARCETE, G.J.; SCALON, S.P.Q. Atmosfera modificada e condições de armazenamento nas características físico-químicas de jabuticabas da cultivar ‘paulista’. *Ciência Rural*, Santa Maria, online, ISSN 0103-8478, 2009.

ANTUNES, L.E.C.; GONÇALVES, E.D.; TREVISAN, R. Alterações de compostos fenólicos e pectina em pós-colheita de frutos de amora-preta. *Revista Brasileira de Agrociência*, jan-mar; 12, 1, 57-61, 2006.

AZEREDO, H.M.C.; MORRUGARES-CARMONA, R.; WELLNER, N.; CROSS, K.; BAJKA, B.; WALDRON, K.W. Development of pectin films with pomegranate juice and citric acid. *Food Chemistry*, 198, 101–106, 2016.

BAJPAI, A.K.; GIRI, A. Swelling dynamics of a ternary interpenetrating polymer network (IPN) and controlled release of potassium nitrate as a model agrochemical. *Journal of Macromolecular Science*, 39, 75-102, 2002.

CHEN, Y.; LIU, C.; CHANG, P.R.; CAO, X.; ANDERSON, D.P. Bionanocomposite based on pea starch and cellulose nanowhiskers hydrolyzed from pea hull fibre: effect of hydrolysis time. *Carbohydrate polymers*, 76, 607-615, 2009.

CHO, M.J.; HOWARD, L.R.; PRIOR, R.L.; CLARK, J.R. Flavonoid glycosides and antioxidant capacity of various blackberry and red grape genotypes determined by high performance liquid chromatogrphy/ mass spectrometry. *Journal of the Science and Food Agriculture*, 84, 1771-1782, 2004.

CORREA, C.A. Bionanocompósitos e Novas Tendências em Engenharia de Biomassa. *Polímeros: Ciência e Tecnologia*, 18, 1, 2008.

CUQ, B.; GONTARD, N.; GUILBERT, S. Edible film and coating as active layers. In: ROONEY, M. L. (Ed.) *Active food packaging. London: Blackie Academic & Professional*, 1995. p. 111-142.

ESPITIA, P.J.P.; AVENA-BUSTILLOS, R.J.; DU, W.X.; TEÓFILO, R.F.; SOARES, N.F.; McHUGH, T.H. Optimal antimicrobial formulation and physical–mechanical properties of edible films based on açaí and pectin for food preservation. *Food Packaging and Shelf Life*, 2, 38-49, 2014.

FAKHOURI, F.M.; FONTES, L.C.B.; GONÇALVES, P.V.M.; MILANEZ, C.R.; STEEL, C.J.; COLLARES-QUEIROZ, F.P. Films and edible coatings based on native starches and gelatin in the conservation and sensory acceptance of Crimson grapes. *Ciências e Tecnologia de. Alimentos*, Campinas, 27, 2, 369-375, abr.-jun. 2007.

FARIAS, M.G.; FAKHOURI, F.M.; CARVALHO, C.W.P.; ASCHERI, J.L.P. Caracterização físico-química de filmes comestíveis de amido adicionado de acerola (*Malphigia emarginata* D.C.). *Quimica Nova*, 35, 2, 262-267, 2012.

GONTARD, N.; GUILBERT, S.; CUQ, J.L. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science*, 58, 1, 206-211, 1993.

LEONEL, M.; CEREDA, M.P.; SARMENTO, S.B.S. Arrowroot (*Maranta arundinacea*) as a Possible Raw Material for Starch Industries. *Brazilian Journal Food Technology*, 5, 151-155, 2002.

MALI, S.; GROSSMANN, M.; GARCÍA, M.; MARTINO, M.Y.; ZARITZKY, N. Microstructural characterization of yam starch films. *Carbohydrate Polymers*, 50, 379-386, 2002.

MOYER, R.A.; HUMMER, K.E.; FINN, C.E.; FREI, B.; WROLSTAD, R.E. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. *Journal of Agriculture and Food Chemistry*, 50, 519-525, 2002.

OTONI, C.G.; DE MOURA, M.R.; AOUADA, F.A.; CAMILLOTO, G.P.; CRUZ, R.S.; LOREVICE, M.V.; SOARES, N.F.F.; MATTOSO, L.H.C. Antimicrobial and physical mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids*, 41, 188–194, 2014.

PARK, S.; ZHAO, Y.Y. Development and characterization of edible films from cranberry pomace extracts. *Journal of Food Science*, 71, 2, E95–E101, 2006.

PETERSEN, K.; NIELSEN, P.; BERTELSEN, G.; LAWTHER, M.; OLSEN, M.; NILSSON, N.Y.; MORTENSEN, G. Potential of biobased materials for food packaging. *Food Science and Technology*, 10, 2, 52 - 68, 1999.

SHAHIDI, F.; HAN, X.Q. Encapsulation of food ingredients. *Food Science and Nutrition*, 33, 6, 501-547, 1993.

SIVAROOBAN, T.; HETTIARACHCHY, N.S.; JOHNSON, M.G. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. *Food Research International*, 41, 8, 781–785, 2008.

SOARES, R.M.D.; LIMA, A.M.F.; OLIVEIRA, R.V.B.; PIRES, A.T.N.; SOLDI, V. Thermal degration of biodegradable edible filme based on xanthan and starches from different sources. *Polymer Degradation and Stability*, 90, 449, 2005.

SOARES, N.F.F.; SILVA, W.A.; PIRES, A.C.S.; CAMILLOTO, G.P.; SILVA, S.S. Novos desenvolvimentos e aplicações em embalagens de alimentos. *Revista Ceres*, 56, 4, 370-378, 2009.

SOZER, N.; KOKINI, J. Review nanotechnology and its applications in the food sector. *Trends in Biotechnology*, 27, 82–89, 2009.

TAWFIK, M.S.; HUYGHEBAERT, A. Interaction of Packaging Materials and Vegetable Oils: Oil Stability. *Food Chemistry*, 64, 451-459, 1999.

WANG, S.Y.; LIN, H.S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental *stage. Journal of Agricultural and Food Chemistry*, 48, 140-146, 2000.

WANG, S.; MARCONE, M.F.; BARBUT, S.; LIM, L.T. The impact of anthocyaninrich red raspberry extract (ARRE) on the properties of edible soy protein isolates (SPI) films. *Journal of Food Science*, 77, 4, C497–C505, 2012.

ZHANG, C.; GUO, K.A.; MA, Y.; MA, D.; LI, X.H.; ZHAO, X.Y. Incorporations of blueberry extracts into soybean-protein-isolate film preserve qualities of packaged lard. *International Journal of Food Science and Technology*, 45, 9, 1801–1806, 2010.

APRESENTAÇÃO DA TESE

O trabalho está estruturado em capítulos, conforme descrito a seguir.

No Capítulo 1, "Revisão Bibliográfica", são apresentados os fundamentos teóricos dos tópicos abordados no presente trabalho.

O Capítulo 2, "Extraction and characterization of arrowroot starch and its application in films", reúne os ensaios de caracterização do amido de araruta. Nele também se avaliou a capacidade do amido de araruta em ser utilizado como matriz formadora de filmes biodegradáveis, bem como a influência da sua concentração e do plastificante nas propriedades funcionais do filme.

No Capítulo 3, "Bioactive films of arrowroot starch and blackberry pulp: physical, mechanical and barrier properties and stability to pH and sterilization, o foco principal foi a incorporação de polpa de amora na forma líquida na solução fimogênica de amido de araruta para a produção de filmes bioativos. Avaliou-se também a influência da sua presença e da variação da sua concentração nas propriedades funcionais do filme de araruta.

O Capítulo 4, "Microencapsulation of blackberry pulp with arrowroot starch and gum arabic mixture by spray drying", reúne os ensaios experimentais realizados para a avaliação da influência da temperatura do ar de entrada e da concentração de agente encapsulante sobre as propriedades físico-químicas (rendimento, teor de água, atividade de água, tamanho médio, higroscopicidade, solubilidade) da polpa de amora em pó.

No Capítulo 5, "Influence of spray drying in bioactives compounds of blackberry pulp", foi obtido pó de polpa de amora microencapsulada com a mistura de amido de araruta e goma arábica utilizada como agente encapsulante por *spray drying*. Neste estudo, o efeito das condições de secagem (temperatura do ar de entrada e da concentração de agente encapsulante) foi avaliado sobre a cor, conteúdo de antocianinas e ácido ascórbico e capacidade antioxidante dos pós produzidos.

O Capítulo 6, "Effect of incorporation of blackberry particles on the physico-chemical properties of edible films of arrowroot starch", avaliou as propriedades físico-quimicas de filmes de amido de araruta, plastificados com glicerol, incorporados diretamente e por *sprinkling* com 0, 20, 30 e 40% de pó de polpa de amora microencapsulada com a mistura de amido de araruta e goma arábica como agente encapsulante por *spray drying*.

No Capítulo 7, "Incorporation of blackberry pulp of particles obtained by freeze drying in edible films of arrowroot starch", foi investigada a incorporação direta e por *sprinkling* de polpa de amora e polpa de amora microencapsulada por *freeze drying*, obtidas na forma de pó, na solução fimogênica de amido de araruta para produção de filmes comestíveis e antioxidantes. A influência do tipo de incorporação e da variação da concentração de pó de amora nas propriedades funcionais do filme de amido de araruta foi investigada.

O Capítulo 8, “Antioxidant films of arrowroot starch and blackberry pulp and potential use as active food packaging”, teve como foco principal a incorporação direta e por *sprinkling*, de pó de polpa de amora e pó de polpa de amora microencapsulada por *freeze drying* e *spray drying* na solução de formadora de filme de amido de araruta. A influência do tipo de incorporação e a variação da concentração de pós de amora nas propriedades estruturais, propriedades antioxidantes, estabilidade ao pH e esterilização e biodesintegração de filmes de amido de araruta foram investigados para avaliar a sua aplicação como fimes ativos, comestíveis e biodegradáveis.

O Capítulo 9, “Discussão Geral”, reúne os principais resultados e discussão de uma forma global de todos os 8 capítulos deste trabalho.

CAPÍTULO 1. Revisão Bibliográfica

Revisão Bibliográfica

1. Embalagens Plásticas

Diversos processos químicos e físicos têm sido estudados com o intuito de serem aplicados para preservar a qualidade dos alimentos. Em geral, os processos são empregados de forma combinada, visto a importância da proteção física de uma embalagem para manter a qualidade dos produtos e prolongar a sua vida de prateleira (DEBEAUFORT et al., 1998).

A função primordial das embalagens, além de conter o alimento, é agir no controle das interações entre o alimento e o ambiente externo (TAWFIK; HUYGHEBAERT, 1999; SOARES et al., 2009). Particularmente, atuam protegendo e conservando o alimento acondicionado contra contaminações químicas e biológicas, facilitando o manuseio, armazenagem e o transporte (VERMEIREN et al., 1999).

A maioria das embalagens utilizadas em larga escala para embalar alimentos é produzida a partir de materiais não biodegradáveis como o petróleo, sendo os mais empregados o polietileno tereftalato (PET), policloreto de vinila (PVC), polietileno (PE), propileno (PP), poliéster (PS) e poliamida (PA). O uso destes materiais plásticos é grande, principalmente por sua disponibilidade, baixo custo e características funcionais úteis como maleabilidade, leveza, transparência, facilidade de impressão, boa selagem térmica, resistência à tração e barreira ao oxigênio (ALVES et al., 2006; SIRACUSA et al., 2008; SOUZA et al., 2010).

Apesar das excelentes propriedades físicas e mecânicas, o uso crescente destes materiais plásticos tem gerado preocupação devido a problemas de contaminação ambiental decorrentes do descarte. Isto porque, apesar de todos os tipos de plásticos poderem ser reciclados, somente cerca de 7% realmente tomam este destino (FIESP, 2009), e a sua reciclagem consome grandes quantidades de energia térmica (PARRA et al., 2004). O restante é abandonado em aterros (80%) ou incinerado (8%) (FIESP, 2009).

Contudo, a maioria dos plásticos é de difícil degradação, o que significa que eles levarão um longo tempo para decomporem-se naturalmente. Alguns exigem mais de 100 anos para degradarem-se totalmente, fato que gera um grande impacto ambiental (FRANCHETTI; MARCONATO, 2006).

Diante disso, a substituição de materiais oriundos exclusivamente de derivados de petróleo é uma nova tendência mundial. Vários países já reconheceram a necessidade de reduzir a enorme quantidade de materiais de difícil degradação, desempenhando esforços nas pesquisas no sentido de encontrar alternativas ecologicamente viáveis. Uma das soluções encontradas é o desenvolvimento de filmes e embalagens biodegradáveis a partir de polímeros provenientes de fontes renováveis (FARIAS et al., 2012).

2. Filmes biodegradáveis à base de amido

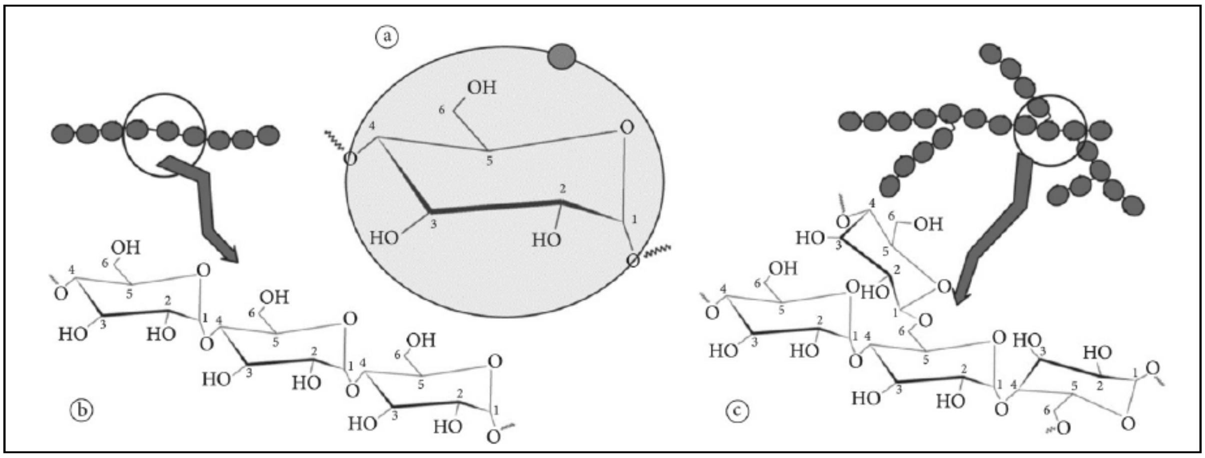
Os filmes são estruturas utilizadas para envolver alimentos. Podem ser definidos como uma fina película contínua formada separadamente do alimento e posteriormente aplicado sobre o mesmo (KROCHTA; MULDER-JOHNSTON, 1997). Os filmes possuem a função de inibir ou reduzir a migração da umidade, vapor de água, oxigênio, aromas dentre outros, pois agem como uma barreira semipermeável (GUILBERT et al., 1996). Além disso, os filmes podem conter e introduzir aditivos nos alimentos, como vitaminas, agentes antioxidantes e antimicrobianos, entre outros. Quando preparados a partir de materiais que podem ser completamente degradados por ação de microrganismos são considerados biodegradáveis, sendo chamados de filmes biodegradáveis (KROCHTA et al., 1997).

Em virtude disso, vários polímeros biodegradáveis produzidos a partir de fontes renováveis e naturais têm sido estudados para utilização como materiais formadores de filmes, tais como celulose, amido e proteínas (YOON et al., 2012). Dentre estes materiais, o amido é um dos biopolímeros mais promissores, em particular pela excelente biodegrabilidade, baixo custo de produção, obtenção a partir de recursos renováveis e por serem capazes de formar uma matriz contínua (PETERSEN et al., 1999).

2.1. Amido

O amido é um polissacarídeo de reserva de energia dos vegetais, formado de unidades de glicose ligadas entre si e representado pela fórmula geral $(C_6H_{10}O_5)_n + {}_xH_2O$ (FRANCO et al., 2002). Apresenta dois tipos de polímeros de glicose: amilose e amilopectina, com estruturas e funcionalidades diferentes.

A amilose é um polímero linear com unidades D-glicose ligadas por ligações α (1-4) que conferem à molécula uma estrutura helicoidal, enquanto que a amilopectina é um polímero altamente ramificado, menos hidrossolúvel que a amilose, com unidades D-glicose ligadas através de ligações α (1-4) e as ramificações em α (1-6) (Figura 1) (SHIMAZU et al., 2007).



Fonte: Alcázar-Alay e Meireles (2015).

Figura 1. Estrutura básica de (a) unidades de glicose, (b) amilose e (c) amilopectina, juntamente com a rotulagem dos átomos e ângulos de torção.

O amido apresenta-se na forma de pequenos grânulos semicristalinos. Em solução aquosa, quando visto através de microscópio de luz polarizada, o grânulo de amido é birrefringente. A refração pelas suas regiões cristalinas resulta na "Cruz de Malta", que é típica para cada tipo de amido e caracteriza a orientação radial das macromoléculas. O centro da Cruz, ou "hilum" é considerado o ponto original do crescimento do grânulo. Segundo Lajolo e Menezes (2006), essa propriedade de birrefringência é relacionada com o alto grau de orientação molecular interna, não tendo qualquer relação com a forma cristalina em particular.

O amido pode ser extraído comercialmente a partir de diversas fontes, tais como milho, batata, mandioca e arroz (CEREDA et al., 2002; THIRÉ et al., 2004). Nos últimos anos, o amido de milho tem sido a matéria prima predominante para a produção de polímeros biodegradáveis, talvez por ser a principal fonte de amido produzido no mundo (64%), seguido pelo de batata-doce (13%) e pelo de mandioca (11%) (SOUZA et al., 2010).

O amido é amplamente utilizado na indústria de alimentos, em diversos tipos de alimentos, e a necessidade da modificação de suas características para atender a crescente demanda do mercado, leva a uma crescente pesquisa sobre possíveis modificações do amido. Ao mesmo tempo, a busca por novas fontes naturais de amido não convencionais, que possam ser aproveitadas tanto pelo consumidor, quanto pela indústria alimentícia vêm sendo incentivada. Nesse sentido, os rizomas de araruta (*Maranta arundinacea* Linn) se destacam, uma vez que é uma fonte de amido não convencional, sem uma importância socioeconômica em muitos países e, por isso, não é considerada como uma matéria-prima de alta prioridade (GORDILLO et al., 2014).

2.1.2. Amido de araruta

A araruta (*Maranta arundinaceae* L.) pertence à família *Marantaceae* e é uma planta originária da América do Sul, ocorrendo em toda a região costeira, desde as Guianas até o Rio de Janeiro (NEVES et al., 2005). Além da América do Sul, a araruta também é cultivada nas Índias Ocidentais (Jamaica e São Vicente), Austrália, Sudeste Asiático e África do Sul e do Leste (CHARLES et al., 2016).

O termo “araruta” é atribuído a “arrow-root”, criado pelos colonizadores ingleses com base nos termos indígenas. “Aru-aru” é a designação dada pelos nativos sul-americanos e que significa farinha ou flecha, pois eles utilizavam a raiz da planta como antídoto contra o veneno de flechas, uma vez que tem efeito balsâmico aliviante sobre as feridas (ALBUQUERQUE; PINHEIRO, 1970). O macerado fresco dos rizomas contém substâncias ácidas e consta que os índios aplicavam como compressas contra feridas provocadas por flechas ou como antídoto nas picadas de insetos e outros animais peçonhentos, como as cobras (NEVES et al., 2005).

A araruta é uma planta herbácea perene rizomatosa (Figura 2). Os rizomas são caules prostrados que crescem horizontalmente sob o solo e que emite raízes, folhas e ramos a partir de seus nós (NEVES et al., 2005). Os rizomas da araruta apresentam tamanhos oscilando entre 10 e 25 cm, são fusiformes, alongados e apresentam pequenos segmentos, separados entre si por leves estrangulamentos providos de escamas (Figura 3) (DA SILVA, MONTEIRO, 1969; LEONEL; CEREDA, 2002). Crescem formando touceiras que podem chegar a 1,2 m de altura. As folhas são alternadas, têm a forma de lança, com longos pecíolos e apresentam um pulvino bastante proeminente na base que possibilita movimentos diuturnos às folhas que se colocam fechadas e eretas ao entardecer. Suas flores são brancas e seu fruto é muito pequeno e as sementes são avermelhadas (PIO CORRÊA, 1984; NEVES et al., 2005).

Arquivo pessoal.

Figura 2. Plantação de araruta do tipo comum.

Arquivo pessoal.

Figura 3. Rizomas de araruta do tipo comum.

No Brasil, a comum, a creoula e a banana, são os cultivares de importância de araruta, sendo as duas primeiras as mais predominantes. Devido ao alto teor de amido em seus rizomas, a araruta tem sido utilizada especialmente para sua extração. Comercialmente, as plantas da cultivar comum são as mais difundidas talvez por produzirem o amido de melhor qualidade (ZÁRATE et al., 2007).

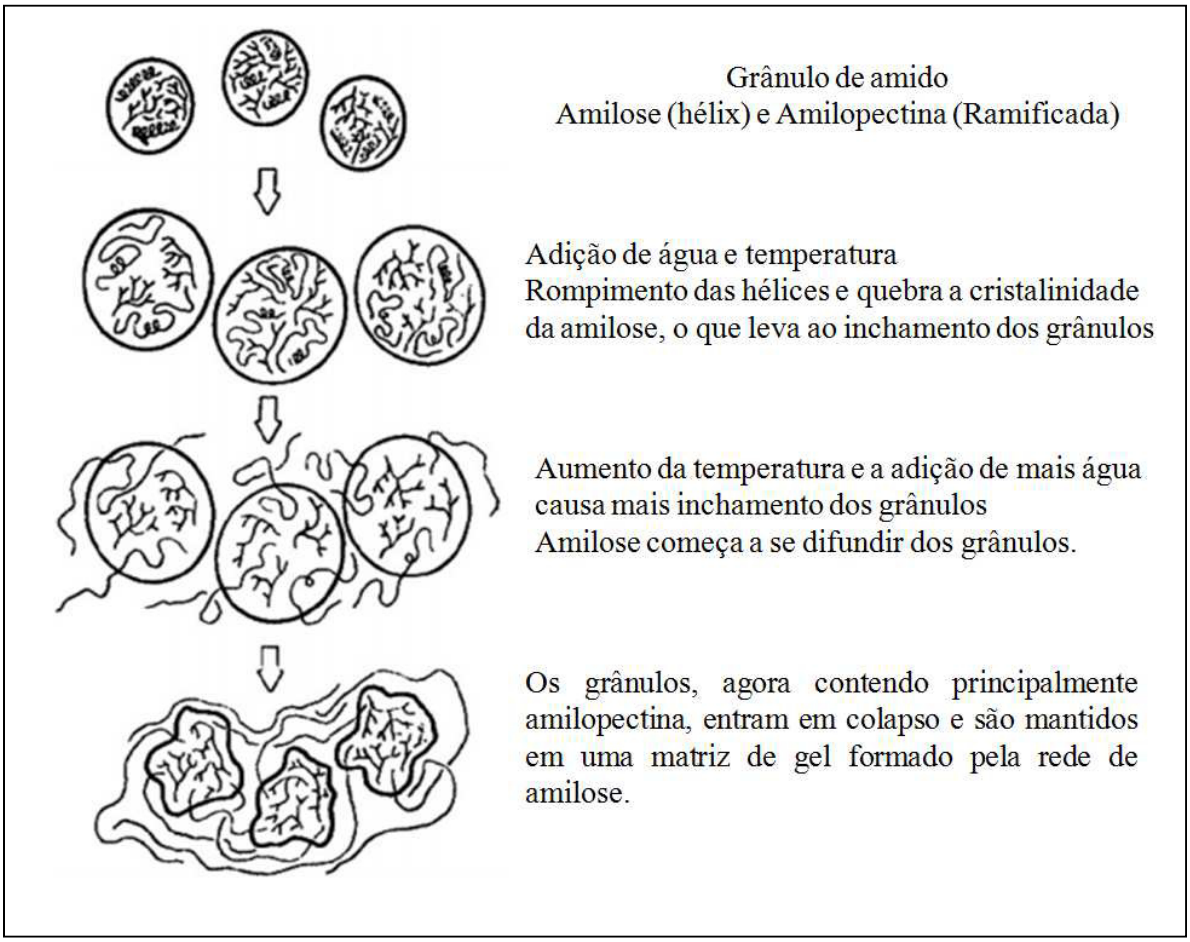
O amido de araruta é muito empregado na preparação de produtos de panificação (LEONEL et al., 2002), como nas confecções de biscoitos, bolos, cremes e doces, sendo recomendado, sobretudo, para convalescentes e crianças de 6 a 8 meses particularmente devido a sua ótima digestibilidade (ZÁRATE; VIEIRA, 2005). Outra característica importante dos alimentos feitos com o amido de araruta é a ausência de glúten, o que o torna recomendável para pessoas com doença celíaca (NEVES et al., 2005). Consequentemente, a sua importância atual está muito relacionada com as características culinárias peculiares de seu amido, o qual alcança preços elevados no mercado internacional (MONTEIRO; PERESSIN, 2002; LEONEL et al., 2002).

Além da sua importância na indústria alimentícia, devido às características de fácil digestibilidade e capacidade de gelatinização, o amido de araruta tem um grande potencial para ser empregado como agente encapsulante, bem como biopolímero para produção de filmes comestíveis e biodegradáveis. A estrutura da amilopectina de tipo A, com uma maior proporção de cadeias ramificadas mais curtas, presente no amido de araruta, pode formar uma matriz de parede com potencial para aprisionar ingredientes ativos (WINARTI et al., 2015). Além disso, o amido de araruta apresenta teor de amilose, variando de 16 a 27%, conforme reportado por Moorthy (2002). Esta quantidade elevada de amilose tem fundamental importância na sua escolha como material formador de filmes, pois interfere diretamente nas suas características finais. Isto porque, as propriedades tecnológicas de filmes de amilose são em geral melhores do que os de amilopectina, principalmente quando se trata de resistência mecânica à tração e propriedades de barreira (RINDLAV-WESTLING et al., 1998). Filmes de amilopectina tendem a serem mais frágeis e quebradiços. Em virtude disso, o uso do amido na produção de filmes baseia-se nas propriedades físicas, químicas e funcionais da amilose para formar géis e no seu desempenho para formar filmes.

2.1.3 Gelatinização do Amido

O amido é praticamente insolúvel em água fria e essa insolubilidade do grânulo deve-se as fortes ligações de hidrogênio que mantém as cadeias de amido unidas. Entretanto, quando amido é aquecido, em excesso de água (>60%), o mesmo sofre uma transição de fase ordenada para desordenada, chamada gelatinização, em uma faixa de temperatura característica de cada de fonte de amido (HOOVER, 2001).

Com o aquecimento do amido em excesso de água, as ligações de hidrogênio presentes na área amorfa são rompidas, permitindo o intumescimento do grânulo. A desestabilização e expansão da região amorfa durante o aquecimento de uma suspensão de amido facilita a posterior desestabilização da região cristalina por tirar moléculas das mesmas, conforme a temperatura se eleva. Nessa condição, em certa temperatura, a energia do sistema torna-se suficiente para vencer as ligações de hidrogênio entre as cadeias da amilose e amilopecina, o que leva perda da cristalinidade e o desaparecimento da ordem estrutural. Enquanto isso, as regiões amorfas são solvatadas e os grânulos incham rapidamente, acarretando no seu rompimento, o que permite que a amilose seja lixiviada para a fase aquosa entre os grânulos (BILIADERIS, 1991), conforme pode ser visualizado na Figura 4. Assim, em essência a gelatinização está associada à difusão de água no grânulo, devido à absorção de água pela região amorfa, hidratação e expansão radial dos grânulos de amido, perda de birrefringência óptica, absorção de calor, perda de ordem cristalina, desenrolamento e dissociação de hélices duplas (nas regiões cristalinas) e lixiviação de amilose (HOOVER, 2001).



Grânulo de amido
Amilose (hélix) e Amilopectina (Ramificada)

Adição de água e temperatura
Rompimento das hélices e quebra a cristalinidade da amilose, o que leva ao inchamento dos grânulos

Aumento da temperatura e a adição de mais água causa mais inchamento dos grânulos
Amilose começa a se difundir dos grânulos.

Os grânulos, agora contendo principalmente amilopectina, entram em colapso e são mantidos em uma matriz de gel formado pela rede de amilose.

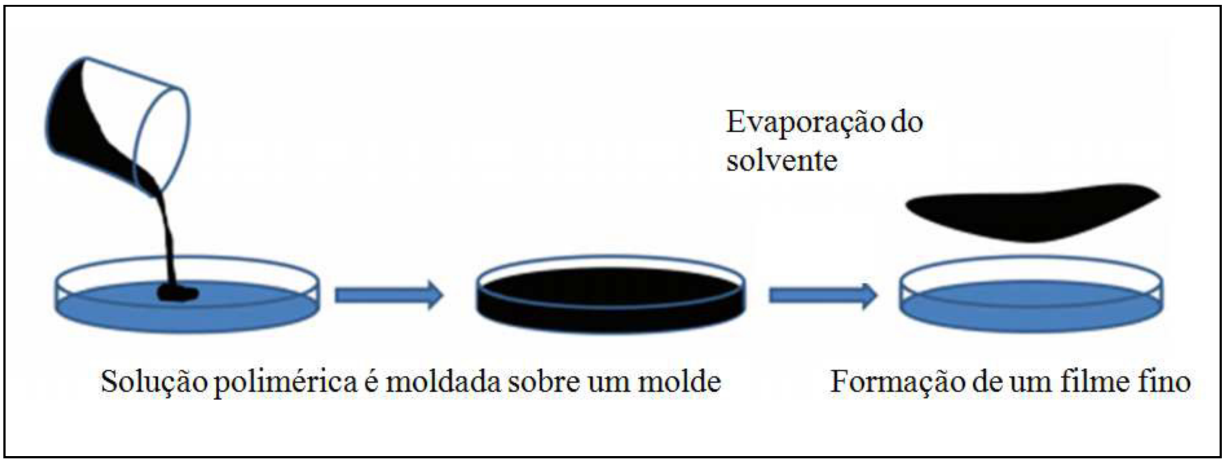
Fonte: adaptado de XIE et al. (2014).

Figura 4. Gelatinização do amido.

Após a gelatinização, as moléculas de amilose, em virtude de sua linearidade, orientam-se paralelamente, aproximando-se suficientemente para formar ligações de hidrogênio entre as hidroxilas de polímeros adjacentes (fenômeno de retrogradação). Ocorre redução do volume livre, diminuindo a afinidade do polímero pela água, podendo o amido gelatinizado formar filmes estáveis e flexíveis (WURZBURG, 1986).

2.1.4 Formação de filmes pelo método de *casting*

Geralmente em escala laboratorial, filmes biodegradáveis à base de amido são produzidos pelo método de *casting* (DONHOWE; FENNEMA, 1994). O princípio da formação dos filmes se baseia na dispersão de macromoléculas (amido) em um solvente ou mistura de solventes adequados, obtendo-se assim a suspensão formadora de filme que é submetida a gelatinização térmica e, em seguida, a mesma é depositada sobre um molde ou superfície para a secagem (WARD; NUSSINOVITCH, 1997), como pode ser visualizado na Figura 5. Pela moldagem tradicional, os filmes devem ser secos a baixas temperaturas para não quebrarem durante esta etapa (FISHMAN et al., 2000). É desejável também que o teor de água esteja entre 5-8% no filme seco para facilitar sua remoção do molde (THARANATHAN, 2003).



Fonte: Sayyar et al. (2017).

Figura 5. Formação de filme polimérico pela técnica de *casting*.

Nesta técnica, após a gelatinização térmica dos grânulos em presença de excesso de água, a amilose e amilopectina se dispersam na solução aquosa e, durante a secagem, se reorganizam, formando uma matriz contínua que dá origem aos filmes. Geralmente a formação do filme envolve associações inter e intramoleculares ou ligações cruzadas das cadeias poliméricas formando uma rede tridimensional semi-rígida que aprisiona e imobiliza o solvente. O grau de coesão depende da estrutura do polímero, solvente utilizado, temperatura e presença de outras moléculas tais como plastificantes (THARANATHAN, 2003).

Os plastificantes são empregados em filmes com o intuito de melhorar tanto suas propriedades físicas quanto mecânicas, unindo possíveis fissuras na matriz filmogênica. Devido ao fato dos filmes elaborados somente com amido serem pouco flexíveis e quebradiços, suas propriedades necessitam ser melhoradas para que possam ser utilizados como substitutos aos tradicionais plásticos e, para isso, os plastificantes devem ser compatíveis com o biopolímero (GONTARD et al., 1993).

Em filmes de amido, os plastificantes mais estudados são os polióis, como o glicerol e o sorbitol. Quando são adicionados à solução filmogênica modificam a organização molecular da rede aumentando o volume livre na molécula. Essa ação do plastificante normalmente causa redução das interações intermoleculares entre as cadeias adjacentes do amido, resultando no aumento da mobilidade dessas cadeias (GONTARD et al., 1993). Consequentemente, ocorrem alterações no material como o aumento da flexibilidade, extensibilidade e distensibilidade, seguido por diminuição da resistência mecânica, temperatura de transição vítrea e barreira a gases e vapor de água (GROSSMAN et al., 2007).

Além dos plastificantes, outros aditivos alimentares, incluindo agentes antimicrobianos e antioxidantes, vitaminas, aromas e corantes, podem ser incorporados ao filme durante sua formação, a fim de aprimorar sua propriedade de proteção, bem como gerar propridades personalizadas ao filme como cor, aroma e sabor inovadores e propriedades antioxidantes e antimicrobianas (HAN; KROCHTA, 2007; GÓMEZ ESTACA et al., 2009; SALGADO et al., 2010).

2.1.5 Filmes Antioxidantes

Atualmente, os estudos sobre a incorporação de agentes antioxidantes em filmes comestíveis têm sido crescentes (SALGADO et al., 2010; NORONHA et al., 2014). Em virtude da oxidação ser uma das reações de degradação mais importante que ocorrem nos alimentos, limitando a sua conservação.

A oxidação é geralmente considerada como o principal fator no desenvolvimento da rancidez de óleos e gorduras, constituindo um dos mecanismos mais frequentes de deterioração e redução da vida útil dos alimentos (VERMEIREN et al., 1999). Além de alterar o sabor (rancificação) e a qualidade nutritiva (perda de vitaminas e ácidos graxos essenciais) dos alimentos, a oxidação resulta em compostos reativos e tóxicos que representam um perigo e tornam-se inaceitáveis para os consumidores (LAGUERRE et al., 2007). No entanto, pode ser inibida por baixas temperaturas, exclusão do oxigênio e pela presença de antioxidantes. Os antioxidantes são amplamente utilizados como aditivos em alimentos para melhorar a estabilidade à oxidação lipídica e prolongar a vida útil do produto (VERMEIREN et al., 2002).

A incorporação de antioxidantes no filme pode contribuir para a manutenção da qualidade do alimento no qual ele será aplicado. Isso porque, em vez dos antioxidantes serem adicionados diretamente no alimento, será incorporado no filme. Com a interação intencional do filme e o alimento e /ou meio em que estiver inserido, a liberação dos antioxidantes ocorrerá de forma controlada sobre a superfície do alimento, onde passará a agir evitando a oxidação e a formação de sabores e texturas alimentares indesejáveis (DANIELLI et al., 2008; NORONHA et al., 2014).

Para isso, é obrigatório que os materiais do filme ou da embalagem mantenham as substâncias (os compostos bioativos como antocianinas, flavonoides, vitaminas entre outros) biodisponíveis e nas melhores condições até que sejam liberadas para o alimento e aplicado de forma controlável (SOZER; KOKINI; 2009; NORONHA et al., 2014). Além disso, a liberação do antioxidante deve ser suficientemente abundante para garantir a eficiente proteção antioxidativa (NORONHA et al., 2014).

Em decorrência desses aspectos, inúmeras pesquisas estão sendo conduzidas neste sentido, como por exemplo, a incorporação de extrato de chá verde em filmes de quitosana (SIRIPATRAWAN; HARTE, 2010); extrato de framboesa vermelha, rico em antocianinas em filmes de proteína isolada de soja (WANG et al., 2012a); extrato natural de beterraba e cenoura em filmes de hidroxipropilmetilcelulose (AKHTAR et al., 2012); óleo resina de alecrim, orégano, azeitona, pimentão, alho, cebola e *cranberry* em filmes de quitosana, carboximetilcelulose e caseína (PONCE et al., 2008), entre muitos estudos.

Diversas pesquisas têm relatado que a incorporação de extratos vegetais ou polpas de frutas as soluções formadoras de filmes transferem compostos bioativos com propriedades antioxidantes para os filmes resultantes e alterações das propriedades mecânicas, térmicas e de permeabilidade ao vapor de água dos filmes. Um exemplo disto foi observado quando o extrato de *blueberry* foi adicionado em filmes de proteína isolada de soja, que não apenas melhorou a resistência à tração e permeabilidade ao vapor de água, mas também simultaneamente transmitiu capacidade antioxidante ao filme, atrasando a oxidação e a hidrólise de gorduras de porco embaladas com o mesmo, durante o armazenamento (36 °C, 40% UR, durante 5 semanas) (ZHANG et al., 2010). Em outro estudo, os filmes incorporados com polpas de manga, acerola ou seriguela desempenharam efeito antioxidante sobre o azeite de dendê durante os 40 dias de armazenamento, podendo ser aplicados para o controle da oxidação deste produto (DANTAS et al., 2015). Já a adição de extrato de bagaço de *cranberry* forneceu a filmes de proteína de soja isolada uma cor vermelho brilhante e um excepcionalmente forte sabor de *cranberry* (PARK; ZHAO, 2006).

Filmes contendo polpa de amora mostraram atividade antiinflamatória e maior viabilidade celular, o que é encorajador no desenvolvimento de filmes comestíveis com propriedades nutracêuticas (GUITIÉRREZ, 2017). A aplicação de amora em filmes é promissora e merece ser mais explorada, uma vez que esta fruta se constitui em um excelente recurso agrícola por combinar propriedades nutricionais e atividades biológicas em um mesmo alimento, o que pode trazer benefícios tanto para o alimento em que será embalado devido aos antioxidantes, quanto para o consumidor em termos nutricionais. A amora é uma rica fonte de compostos bioativos com atividades antiproliferativa, antioxidante, antimicrobiana, anti-inflamatória e anticancerígena (LEUNG, 2008; MASISI et al., 2016).

3. Amora-preta

A amora-preta (*Rubus* spp.) pertence à família *Rosaceae*, gênero *Rubus*, formando um grupo diverso e bem difundido, para o qual se estima existir de 400 a 500 espécies, conhecidas como *berries*, cujo termo é utilizado para descrever qualquer fruta pequena, de sabor adocicado e formato arredondado (JEPSON; CRAIG, 2005), incluindo framboesas e amoras-pretas cultivadas na América, Europa, África e Ásia (LORENZI et al., 2006).

A amora-preta (*Rubus fruticosus*), cultivar Tupy, é a mais cultivada no Brasil e é resultado do cruzamento entre as cultivares Uruguai x Comanche, realizado na EMBRAPA Clima Temperado em 1982. A variedade Tupy apresenta plantas de porte ereto, com espinhos, produz frutas grandes (6 gramas), coloração preta e uniforme, sabor equilibrado em acidez e açúcar, consistente e firme, semente pequena, película resistente e aroma ativo, como pode ser visto na Figura 6 (SANTOS; RASEIRA, 1988; ANTUNES, 2002).

Fonte: Antunes et al. (2014).

Figura 6- Amoreira-preta, cultivar Tupy, floração e frutificação na região de Pelotas-RS.

As amoras- pretas são consumidas geralmente como frutas frescas ou processadas (congelados, desidratados, etc.) e são muito utilizadas usadas na elaboração industrial de uma ampla variedade de produtos alimentares, como cereais, produtos lácteos, sucos, geleias e licores (MORALES et al., 1996).

As amoras-pretas são compostas de cerca de 92 % de água (FERRARI et al., 2012), contém altos teores de fibras, vitaminas e minerais essenciais, além de ser uma fonte importante de compostos fenólicos que contribuem para sua alta capacidade antioxidante (ELISIA et al., 2007; HAGER et al., 2008; MACHADO et al., 2015). Os fenóis totais em amoras variam de 114 para 1056 mg / 100 g de peso fresco e este valor foi superior a outros tipos de bagas, como os cranberries (120-315 mg / 100 g de peso fresco) ou morangos (43-443 mg / 100 g de peso fresco) (HOWARD; HAGER, 2007; SZAJDEK; BOROWSKA, 2008). Os principais polifenóis que estão presentes nas amoras são as antocianinas e os elagitaninos (KAUME et al., 2012). A cianidina-3-glucosídeo e a cianidina-3-rutinosídeo são as principais antocianinas presentes na amora (FAN-CHIANG; WROLSTAD, 2005). As antocianinas são pigmentos naturais responsáveis pela cor azul, violeta e vermelha das frutas e têm sido amplamente estudadas devido o seu potencial para atuar como um antioxidante (SEERAM et al., 2001).

O efeito antioxidante dos compostos fenólicos tem sido relacionado com a sua capacidade de doar hidrogênios ou elétrons aos radicais livres (RICE-EVANS et al., 1996). Os fenólicos presentes nas *berries* têm mostrado ter propriedades eliminadoras de radicais livres contra radicais superóxido ($O_2^{\bullet -}$), peróxido de hidrogênio (H_2O_2), radicais hidroxilo ($\bullet OH$) e oxigênio singleto ($^1O_2$), e também a capacidade de poder inibir a peroxidação lipídica, bem como proteínas e lipídeos em lipossomas de oxidação (WANG; JIAO, 2000; SEERAM; HEBER, 2007).

No entanto, a utilização de seus efeitos benéficos é limitada, pois compostos bioativos apresentam instabilidade quando expostos a temperaturas elevadas, luz, oxigênio, entre outros, alterando suas propriedades funcionais e antioxidantes (SILVA et al., 2010). A aplicação de tecnologias de microencapsulação é uma alternativa viável para manter a estabilidade dos compostos bioativos durante o processamento dos alimentos (SHAHIDI; HAN, 1993). A microencapsulação consiste em recobrir um componente ativo com uma matriz polimérica, ambos assumindo dimensões microscópicas (AZEREDO, 2005), com o intuito de formar uma barreira física que o protegerá das condições adversas do meio, estabilizando e aumentando a vida útil do material encapsulado, além de controlar a sua liberação (SHAHIDI; HAN, 1993).

4 Microencapsulação

O conceito de microencapsulação surgiu da idealização do modelo celular, no qual o núcleo é envolvido por uma membrana semipermeável, que o protege do meio externo e, simultaneamente, controla a entrada e saída de substâncias na célula. De modo similar, a microcápsula consiste em uma camada de agente encapsulante, que atua como um filme protetor, isolando o material do núcleo e controlando sua liberação mediante os estímulos específicos, evitando efeitos de sua exposição inadequada (JIZOMOTO et al., 1993; ROCHA et al., 2011).

Define-se a microencapsulação como uma tecnologia de empacotamento que, com finas coberturas poliméricas aplicáveis em compostos sólidos ou líquidos formam partículas denominadas microcápsulas ou microesferas (SHAHIDI; HAN, 1993; GIBBS et al., 1999; JAFARI, et al., 2008). No caso de se obter uma microcápsula, há a formação de um núcleo que contém o material ativo, protegido por uma membrana do encapsulante, enquanto que na microesfera o material ativo encapsulado está disperso por toda a rede polimérica, formando geralmente um material homogêneo (DESAI; PARK, 2005). As partículas obtidas por esse processo geralmente apresentam formato esférico, podendo ter tamanhos variando de 1 a 1000 µm (SILVA et al., 2003) e vem sendo aplicadas com sucesso na indústria farmacêutica, alimentícia, agropecuária, dentre outras (SHAHIDI; HAN, 1993; RÉ, 2000; DUBEY et al., 2009).

Algumas vantagens da aplicação da microencapsulação na indústria têm sido salientadas como: facilitar a manipulação ao converter um líquido à forma sólida; diminuir a reatividade do material encapsulado em relação ao ambiente externo; promover a liberação controlada; mascarar sabor e/ou gosto indesejável; prolongar a vida de prateleira; aumentar a estabilidade; permitir a separação de materiais reativos (JACKSON; LEE, 1991; SHAHIDI; HAN, 1993; DUBEY et al., 2009; BURGAIN et al., 2011).

Em virtude disto, o uso da tecnologia de microencapsulação é extenso e, já tem sido utilizada pela indústria por mais de 60 anos. No setor alimentício os materiais que vem sendo encapsulados são os ácidos, bases, vitaminas, sais, gases, aminoácidos, óleos essenciais, corantes, enzimas e micro-organismos (DESAI; PARK, 2005). O seu foco principal é o desenvolvimento de partículas que tenham a capacidade de proteger a substância ativa do calor, umidade, oxidação, reações químicas ou de outras condições extremas, aumentando a estabilidade e mantendo a sua viabilidade, sendo capaz de liberá-la quando necessário (ROSENBERG et al., 1990; GOUIN, 2004; FÁVARO-TRINDADE et al., 2008).

Houve uma contribuição importante também na área farmacêutica, pois a encapsulação permitiu o desenvolvimento de fórmulas de liberação controlada, ou seja, aquelas com a capacidade de liberar os agentes ativos apenas nos órgãos onde devem agir ou onde serão absorvidos. Em tais produtos, o princípio ativo protegido é liberado gradativamente por meio de estímulos adequados, tais como mudança de pH, rompimento físico e dissolução, dentre outros (RÉ, 2000; FREIBERG; ZHU, 2004).

Na área agropecuária, a encapsulação de pesticidas tem tornado a aplicação do produto mais eficaz, minimizando a perda do agrotóxico por degradação, evaporação ou dissolução e escoamento para fontes de água, prevenindo a contaminação ambiental (KUMBAR; AMINABHAVI, 2002; BAJPAI; GIRI, 2002). Além disso, ocorre também um aumento da eficiência do agente ativo por unidade de área de aplicação e um período de proteção prolongado, quando se compara com a mesma quantidade de pesticida aplicada de forma convencional. Em questão de segurança, os pesticidas encapsulados reduzem o perigo de intoxicação, pois minimizam o tempo de exposição do usuário durante o seu manuseio (SCHER, 1999; RÉ, 2000; TSUJI, 2001).

A forma das partículas também é bastante variável em razão do material de parede e do método de microencapsulação utilizado para o seu preparo (JACKSON; LEE, 1991). Diferentes métodos são descritos para a preparação das micropartículas, que podem ser divididos em físico-químicos, físicos e químicos. Entre os métodos físico-químicos estão: coacervação simples ou complexa (separação de fase aquosa), evaporação emulsão-solvente (separação por fase orgânica), emulsão-solidificação, envolvimento lipossômico, e os métodos físicos compreendem o *spray drying*, *spray coating*, *spray chilling*, leito fluidizado, extrusão, centrifugação com múltiplos orifícios, co-cristalização, liofilização. Os métodos químicos envolvem a polimerização interfacial e inclusão molecular (SHAHIDI; HAN, 1993; GIBBS et al., 1999).

A escolha do método de microencapsulação é orientada pelo custo, propriedades do material de parede e do material a ser encapsulado, a aplicação e os mecanismos desejados de aplicação (SHAHIDI; HAN, 1993; AZEREDO, 2005).

4.1 *Spray drying*

Apesar de todas as técnicas desenvolvidas para a microencapsulação de ingredientes alimentares, a secagem por atomização (s*pray drying*) é a mais comum, devido ao seu custo relativamente baixo, equipamentos disponíveis, eficiência, por ser rápida e reprodutível permitindo fácil aumento de escala, quando comparada com outras técnicas de microencapsulação, justificando a preferência em termos industriais (ESTEVINHO et al., 2013).

A técnica de *spray drying* tem sido muito utilizada na indústria alimentícia para preparação de aromas e aditivos alimentares encapsulados na forma de pó (DESAI; PARK, 2005). Em virtude da redução da atividade da água nas amostras a técnica de s*pray drying* é geralmente utilizada para aumentar a estabilidade microbiológica do produto e evitar o risco de degradação de químicos e/ou biológicos durante o armazenamento (GHARSALLAOUI et al., 2007).

A secagem por *spray drying* é uma operação unitária através da qual um produto líquido é atomizado numa corrente de gás quente (geralmente ar), para obter-se instantaneamente um pó. O líquido de alimentação pode ser uma solução, uma emulsão ou uma suspensão. O processo contínuo de secagem envolve a combinação de algumas etapas, tais como a atomização, contato da mistura do *spray* com o ar de secagem, evaporação e produção do produto do ar de secagem (SHAHIDI; HAN, 1993; GHARSALLAOUI et al., 2007; ESTEVINHO et al., 2013).

Basicamente esta técnica consiste na preparação de uma dispersão do material encapsulante com posterior homogeneização do material ativo (GIBBS et al., 1999). A mistura resultante é levada ao *spray dryer* e pulverizada através de um atomizador sob pressão para a câmara a alta temperatura. À medida que as partículas são lançadas ao meio gasoso elas tomam formato esférico, com o material ativo empacotado no interior do agente encapsulante. Em contato com ar quente, a água se evapora rapidamente da partícula, devido à alta relação da área de superfície/volume das partículas. Com isso, o tempo de sua exposição ao calor é curto (geralmente não ultrapassa segundos), e a temperatura do núcleo não ultrapassa 100 ºC, o que reduz a ocorrência de alterações indesejáveis em compostos termossensíveis. O material seco é separado do ar de secagem e é coletado (Figura 7) (AZEREDO, 2005; DESAI; PARK, 2005; MADENE et al., 2006).

A otimização deste processo depende da avaliação conjunta entre vários parâmetros tais como: aquecimento, volume de ar, tipo de bico atomizador, características do produto, vazão do material a ser seco ou do sistema de atomização, temperatura do ar de secagem e os parâmetros de formulação (LANNES; MEDEIROS, 2003).

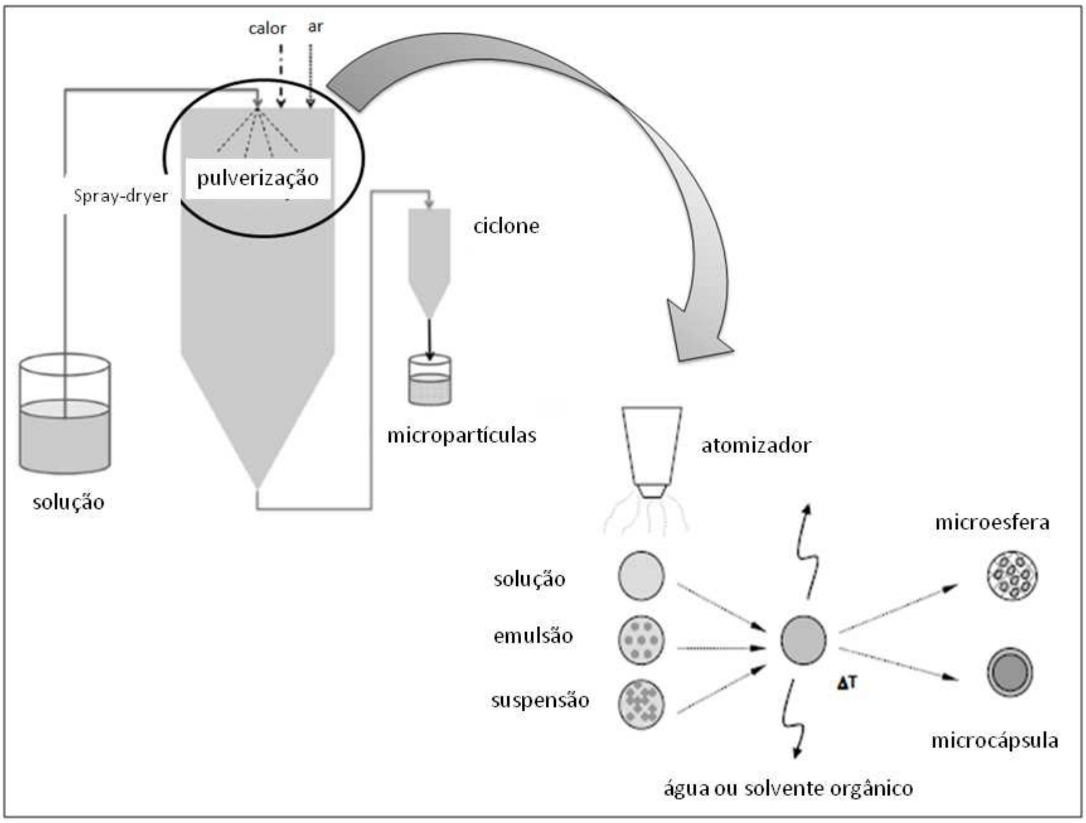
A atomização pode ser efetuada por diferentes tipos de atomizadores como o atomizador pneumático, bocal de pressão, disco giratório, bico de fluído e bocal sônico (GHARSALLAOUI et al., 2007). Esta etapa de atomização é decisiva na técnica de *spray drying*, pelo fato das condições da formulação inicial definir a distribuição inicial do tamanho das gotículas e poderem afetar as características do material seco final (RÉ, 2006). Como regra, o aumento da energia fornecida para o atomizador diminui o tamanho das gotas formadas. Para a mesma quantidade de energia, o tamanho das partículas formadas aumenta com o aumento da taxa de alimentação (solução com o agente de encapsulação e substância a encapsular). Por outro lado, o tamanho das partículas também aumenta com a viscosidade e tensão superficial do líquido de alimentação (GHARSALLAOUI et al., 2007).

Além disso, com o aumento da temperatura do ar de saída do equipamento, obtêm-se partículas com menor quantidade de água (RÉ, 2006). Tipicamente, a temperatura de entrada do ar quente é entre 150 a 220 ºC e a evaporação ocorre muito rapidamente. Em seguida, a temperatura diminui normalmente a temperaturas moderadas de 50 a 80 ºC (GHARSALLAOUI et al., 2007; de VOS et al., 2010; ESTEVINHO et al., 2013). O efeito da temperatura no tamanho da partícula parece altamente dependente do material que está sendo secado (RÉ, 2006).

As micropartículas produzidas por este processo são geralmente do tipo matricial, com o núcleo distribuído na forma de micropartículas na matriz seca do material encapsulante (AZEREDO, 2005). No entanto, dependendo da formulação inicial, podendo esta ser uma solução, emulsão ou suspensão, as partículas obtidas pode ser microesferas ou microcápsulas, conforme mostrado na Figura 7.

Além disso, dependendo do material utilizado na alimentação e das condições da operação, a produção do pó pode atingir dimensões desde muito finas (10-50 µm) a partículas de grandes dimensões (2-3 mm) (MARTÍNEZ et al., 2004). Quando se é produzido um pó muito fino de micropartículas é necessário de mais processamentos, tal como o processo de aglomeração, no qual as partículas encapsuladas são tratadas com vapor, que induz a coesão e a formação de partículas maiores, evitando assim a geração de problemas de separação em misturas secas (AZEREDO, 2005).

A principal limitação da técnica de secagem por s*pray drying* em microencapsulação é o número limitado de materiais de parede disponíveis, os quais devem ser solúveis em água a um nível aceitável, em virtude da maioria dos processos de secagem na indústria de alimentos serem realizadas a partir de formulações aquosas de alimentação (GOUIN, 2004; GHARSALLAOUI et al., 2007).



Fonte: Adaptado a partir de Ré (2006) e de Vos et al. (2010).

Figura 7. Representação esquemática do processo de secagem por s*pray drying* e os tipos de micropartículas obtidas dependendo da formulação inicial.

A escolha do material de parede para a microencapsulação por s*pray drying* é fundamentalmente determinante para a eficiência de encapsulação e estabilidade da micropartícula. Para isso, os critérios para a seleção dos materiais baseiam-se principalmente nas suas propriedades físico-químicas, como a capacidade de formação de filme e compatibilidade, sua viscosidade, higroscopicidade, habilidade de selar e segurar o material do núcleo e suas propriedades de liberação do material do núcleo conforme a aplicação a que se destinam (AZEREDO, 2005). Por não se encontrarem encapsulantes que sigam todos os critérios, em muitos casos, utilizam-se suas combinações (DESAI, PARK, 2005; RÉ, 2006).

Na indústria alimentícia a microencapsulação emprega formulações contendo compostos bioativos a serem preservados em misturas com agentes encapsulantes dos mais variados, como o amido ou seus derivados, proteínas, gomas e lipídios (ABURTO et al., 1998). Dentre as gomas, a goma arábica é a mais utilizada como agente encapsulante por *spray drying*, graças à sua solubilidade, baixa viscosidade, boas propriedades emulsificantes, sabor suave e alta estabilidade oxidativa conferida a óleos (REINECCIUS, 1988; THEVENET; 1995).

A goma arábica é constituída por um arranjo altamente ramificado de galactose, arabinose, ramnose e ácido glicurônico, contendo ainda cerca de 2% de um componente protéico ligado covalentemente a esse arranjo molecular, exercendo um papel crucial na determinação das propriedades emulsificantes da goma. No entanto, a goma arábica possui um alto custo e problemas de disponibilidade, uma vez que é produzida em regiões sujeitas a variações climáticas imprevisíveis e conflitos políticos, o que pode comprometer sua oferta (AZEREDO, 2005). Assim, a busca por substitutos totais ou parciais para a goma arábica tem sido incentivada. A utilização da mistura de amido de araruta e goma arábica como agente encapsulante por *spray drying* pode ser inovadora, uma vez que até o momento, ao que se sabe não existem trabalhos publicados nesse sentido.

4.2 *Freeze drying*

Outra técnica de microencapsulação e desidratação muito utilizada na área de alimentos é a liofilização ou *freeze drying*. Nessa técnica o material ativo é solubilizado com o material de parede, congelado e seco por sublimação, sob alto vácuo e baixa umidade (FODA et al., 1970; DO et al., 1975).

Basicamente, o processo de *freeze drying* compreende o congelamento, a secagem primária e a secagem secundária. A etapa de congelamento é importante, pois estabelece a morfologia do material congelado e isso pode afetar a duração das próximas etapas de secagem. Nessa fase, a água contida no alimento é resfriada rapidamente, ocorrendo a cristalização do gelo. Durante a secagem primária, o passo mais longo da liofilização, os cristais de gelo são removidos por sublimação que ocorre sob vácuo e adição de calor por meio das placas de aquecimento, que tem a temperatura inicial elevada, formando poros no interior do produto. Os tamanhos desses poros estão diretamente relacionados com os tamanhos dos cristais de gelo. Em seguida, o material parcialmente seco permanece no liofilizador por cerca de 2 a 6 horas, sob vácuo, para a evaporação de grande parte da água residual, sendo essa etapa chamada de secagem secundária ou de dessorção (ORDÓÑEZ, 2005). O resultado é um bolo seco estruturalmente intacto, com aparência de esponja no seu interior, que pode ser facilmente reduzido a pó.

A técnica de *freeze drying* destaca-se entre as demais, pela aplicação de baixas temperaturas, o que propicia a preservação dos materiais bioativos sensíveis a altas temperaturas (GOUIN, 2004; AZEREDO, 2005). Franceschinis et al. (2014), que relataram teor de antocianinas de 162 mg/100g para suco de amora-preta seco por *freeze drying* e de 70 mg /100 g para suco de amora-preta seco por *spray drying*. Ao passo que as perdas dos compostos bioativos no processo de secagem por *spray drying* estão ligadas à grande superfície exposta ao ar (OBEROI; SOGI, 2015) e altas temperaturas, as perdas dos compostos bioativos durante *freeze drying* está associada à moagem do material após a liofilização (KUCK; NOREÑA, 2016), por isso a investigação da microencapsulação por ambos os métodos é tão importante. Além do mais, *freeze drying* é de alto custo e de longo tempo de processo em comparação com *spray drying*, o que limita a sua aplicabilidade comercial (AZEREDO, 2005).

5. Incorporação de micropartículas em filmes

Atualmente vários estudos têm investigado a incorporação de compostos ativos em filmes à base de biopolímeros para aplicações como embalagens ativas para alimentos. Essa incorporação de compostos ativos nos filmes deve ser de acordo com as características do sistema de embalagem desejada, com o agente antioxidante utilizado, bem como, com o alimento em que os filmes serão aplicados (SADAKA et al., 2014). Em virtude disso, algumas formas de incorporação têm sido relatadas (CHANDRA; RUSTGI, 1998; SADAKA et al., 2014), tais como:

a) Produzir o filme utilizando polímeros inerentemente antioxidantes;

b) Misturar diretamente os compostos ativos aos polímeros no processo de fabricação do filme, através da adsorção ou de incorporação em massa no polímero;

c) Encapsular os compostos ativos, mantendo-os presos dentro de um material de parede, formando micro ou nanopartículas. Em seguida, realizar a incorporação através da mistura física direta das micro ou nanopartículas ao polímero no processo de elaboração do filme;

d) Enxerto, que é uma reação em que o agente ativo é ligado covalentemente à cadeia principal de uma macromolécula como cadeias laterais e, posteriormente, o mesmo é misturado a matriz formadora do filme.

Dentre estas estratégias, a microencapsulação tem se destacado, pois além de manter a estabilidade e a viabilidade dos compostos ativos quando expostos a condições desfavoráveis (por exemplo à temperatura elevada, luz, oxigênio) e permitir sua liberação controlada mediante estímulos específicos (SHAHIDI; HAN, 1993), tem se mostrado como uma alternativa viável para o aprimoramento das propriedades mecânicas e de barreira dos filmes à base de biopolímeros biodegradáveis.

Sanchez-Garcia et al. (2008) desenvolveram e caracterizaram um novo nanocompósito de policaprolactona com propriedades de barreira melhoradas e com liberação controlada de timol. Os filmes biodegradáveis antimicrobianos foram preparados pelo método de *casting*, enquanto que Chen et al. (2009), prepararam nanopartículas com ambas as propriedades antioxidantes e antibacterianas contendo eugenol e carvacrol para aplicações em embalagens de alimentos.

Guarda et al. (2011) demonstraram a possibilidade de utilização do agente antimicrobiano microencapsulado (timol ou carvacrol) como revestimento em filmes de polipropileno bi-orientado axialmente para conservação de alimentos frescos. Foi demonstrado que a capacidade antimicrobiana não foi alterada pela microencapsulação, mas a taxa de liberação dos agentes ativos foi inferior e pode ser controlada, em comparação com filmes com agentes antimicrobianos incorporados diretamente em polímeros.

Recentemente, a incorporação de nanopartículas na solução formadora de filme para produção de filmes de amido com estrutura e propriedade modificada, mostrou-se promissora. Shi et al. (2013) relataram pela primeira vez o método de preparação e as características de filmes de amido incorporados com nanopartículas de amido obtidas por *spray drying* e *freeze drying*. Estes autores estudaram a morfologia, a cristalinidade, as propriedades físicas e mecânicas dos filmes e relataram que a adição de ambas as nanopartículas de amido em filmes de amido aumentou a rugosidade da superfície, diminuiu o grau de cristalinidade em 23,5% e a permeabilidade ao vapor de água em 44%, respectivamente, comparados aos dos filmes de amido.

Diante disso, a incorporação de agentes antioxidantes naturais na forma de micropartículas para melhorar as propriedades físico-quimicas e ou bioativas de filmes biodegradáveis para aplicações como embalagens ativas como substituto de embalagens plásticas é potencialmente promissora.

5.1Propriedades dos filmes

O conhecimento das propriedades funcionais dos filmes é de fundamental importância e interesse científico e tecnológico, devido aos requisitos e exigências que os diversos filmes devem apresentar para serem utilizados como substitutos das embalagens convencionais, já que destas propriedades dependem muitas das aplicações industriais.

5.1.1 Propriedades mecânicas

As propriedades mecânicas dos filmes biodegradáveis estão diretamente relacionadas com a natureza do material filmogênico utilizado e com a coesão da estrutura da matriz polimérica, a qual está relacionada com a aptidão do polímero em formar fortes e/ou numerosas ligações em nível molecular entre duas cadeias poliméricas dificultando, assim, sua separação quando submetida a forças mecânicas (GONTARD, 1991). Assim as propriedades mecânicas são aquelas que determinam a resposta destes materiais às influências mecânicas externas (CANEVAROLO et al., 2004). As metodologias de análise das propriedades mecânicas se baseiam principalmente nas normas da ASTM (ASTM D-882-91, 1996), que se aplicam à determinação das propriedades de tração de filmes com espessura inferior a 1 mm e a 638-93 (ASTM D-638-93, 1993), para filmes com espessura igual ou superior a 1 mm.

Dentre as propriedades mecânicas de maior interesse em filmes estão as relacionadas com a resistência à tração, ou seja, a força necessária para estirar ou deformar o filme e a deformação alcançada pelo mesmo até a ruptura (BARRETO, 2003). Tais propriedades incluem:

a) Tensão máxima de ruptura (σ, dada em MPa): é a máxima tensão suportada pelo filme até o momento de sua ruptura;

b) Elongação máxima (ε, dada em %): é a medida de maleabilidade do filme e pode ser considerada como uma característica que define a habilidade do filme em deformar antes de ocorrer sua ruptura. Baixos valores de elongação implicam em filmes quebradiços (MACLEOD et al., 1997).

c) Módulo de elasticidade ou módulo de Young (E, dado em MPa): é a relação linear entre a tensão aplicada e a deformação sofrida, que é determinado pela inclinação da curva de tensão versus deformação na região elástica (VAN DE VELDE; KIEKENS, 2002). O módulo de elasticidade informa a respeito da dureza ou resistência que um material apresenta à deformação plástica. Quanto maior o módulo, mais resistente à deformação é o material (CALLISTER, 1997).

Estas propriedades são dependentes das condições ambientais, como temperatura e umidade relativa do ar. As características mecânicas de um filme polimérico são também influenciadas pela temperatura de transição vítrea do polímero (BARRETO, 2003).

As propriedades mecânicas servem como base de comparação do desempenho mecânico dos diferentes polímeros, uma vez que estão diretamente relacionadas à natureza do material filmogênico utilizado e com a coesão da matriz polimérica formada que está relacionada com a distribuição e a concentração de interações inter e intramoleculares na estrutura filmogênica (CUQ et al., 1998).

As características mecânicas devem ser suficientes para manter a integridade através das práticas de produção e manipulação porque qualquer dano ao filme, como perfurações e rasgos, danifica as propriedades de barreira e proteção. Assim a quantificação de dados para as características mecânicas dos filmes é essencial para o dimensionamento e projeto dos processos de embalagem e para que se alcancem características desejáveis de aplicação específica (BARRETO, 2003).

5.1.2 Propriedades de Barreira

Filmes preparados com polissacarídeos apresentam alta taxa de permeabilidade ao vapor de água, devido principalmente, à grande solubilidade apresentada por estes. Assim, este é um parâmetro muito importante que comumente é investigado.

A permeabilidade é definida como uma propriedade do par filme/permeado (gás, vapor de água, oxigênio, gás carbônico) em condições bem definidas de temperatura, umidade relativa, espessura e diferença de pressão parcial. Permeabilidade, entretanto, não deve ser confundida com transporte através de poros, uma vez que a mesma consiste em um processo de solução e difusão no qual o vapor se dissolve de um lado do filme e se difunde até o outro lado do mesmo (McHUGH; KROCHTA, 1994). Em um meio contínuo, homogêneo e isotrópico, desprezando-se os defeitos, a permeabilidade (Pva) é produto da difusividade (D), que representa a mobilidade das moléculas do permeado através do polímero e do coeficiente de solubilidade (S), representado pela concentração do permeante no interior do filme em equilíbrio com uma pressão externa (GONTARD, 1991). Na prática, a permeabilidade (Pva) é determinada a partir de um fluxo em regime estacionário pela Equação 1.

$$Pva = We/\ At\ (p_1-p_2) \qquad (1)$$

Onde:
W = aumento de peso do sistema (g);
e = espessura do filme (m);
A = área exposta do filme (m^2);
(p_1-p_2) = diferença de pressão entre os dois lados do filme (mmHg);
Pva = permeabilidade ao vapor de água (g.mm)/(m^2.dia.mmHg);
t = tempo (dia).

Essa característica física é importante para o controle da migração de vapor de água entre o produto e o meio ambiente. Quando a atividade de água do alimento é maior do que a umidade relativa do ambiente na qual este se encontra, ocorre a migração de água do alimento para o ambiente, provocando alterações das suas características físicas como textura, aparência e sensoriais. Em caso contrário, ocorre a troca de umidade do meio ambiente para o interior do alimento, podendo criar condições favoráveis para o desenvolvimento de micro-organismos ou ainda provocar a perda de textura e da aparência característica (LABUZA, 1980).

Além da permeabilidade ao vapor de água, difusidades adequadas do CO_2 e do O_2 no filme utilizado para a embalagem são essenciais para manter a atmosfera dentro do produto, sem causar desenvolvimento de odores desagradáveis ou danos fisiológicos nas condições ideiais de armazenamento (CHITARRA; CHITARRA, 2005). Por outro lado, o controle das trocas gasosas, particularmente do oxigênio, permite o melhor controle da maturação das frutas ou a redução da oxidação de alimentos sensíveis ao oxigênio (GENNADIOS; WELLER, 1990). A utilização de filmes que apresentam baixa taxa de permeabilidade a gases, como os sintetizados por polissacarídeos, que reduzem o acesso do oxigênio aos tecidos, são úteis para minimizar possíveis alterações no alimento (KESTER; FENNEMA, 1986).

A barreira aos gases de uma embalagem é avaliada pela quantidade de gás que passa através de uma unidade de área dela, por unidade de tempo, sob determinada temperatura, certo gradiente de pressão do gás-teste e sob determinada umidade relativa. A unidade utilizada para expressar a permeação é dada em cm^3 gás (CNTP)/m^2.dia^{-1}. 1 atm^{-1}. Sendo que, uma embalagem é considerada como de alta barreira ao O_2 quando apresenta taxa de permeabilidade ao mesmo inferior a 15 $cm^3/m^2.dia^{-1}$ (OLIVEIRA, 1990).

5.1.3 Solubilidade em água

A solubilidade em água de filmes biodegradáveis é de grande importância, uma vez que muitos dos filmes elaborados a partir de carboidratos e proteínas possuem grande afinidade com a água.

Para determinar a solubilidade de materiais biodegradáveis, uma das metodologias mais utilizadas é a proposta por Gontard et al. (1992). A solubilidade em água é avaliada pela porcentagem de peso seco não solubilizado, após a imersão da amostra em água por 24 horas. Pela diferença entre o peso seco inicial da amostra e peso seco após a etapa de solubilização, calcula-se a porção solúvel da amostra.

5.1.4 Propriedades Estruturais

A Microscopia Eletrônica de Varredura (MEV) permite alcançar aumentos muito superiores ao da microscopia óptica, possibilitando a visualização de possíveis imperfeições, porosidades, separação dos componentes dos filmes em camadas, estrutura da superfície e da seção transversal. Além disso, a MEV produz imagens a partir de um feixe de elétrons que varre a superfície de uma amostra, isto é, uma imagem é produzida por feixes de elétrons que são refletidos na superfície da amostra a ser analisada (CALLISTER, 1997).

Entretanto, se o filme é diretamente submetido ao feixe de luz, ele será degradado. Uma alternativa é utilizar um feixe de elétrons de intensidade bem baixa, 5 a 10 kV. Para melhor visualização da imagem, é necessário metalizar a amostra com uma fina camada de ouro, já que a maioria dos materiais biodegradáveis é à base de carbono.

5.1.5 Cristalinidade

A cristalinidade de filmes biodegradáveis pode ser facilmente determinada através de difração por raios-X, que pode revelar detalhes de estrutura interna do material da ordem de 10^{-8} cm de tamanho (CULLITY, 1978). A cristalinidade pode ser definida como a medida do grau de organização das moléculas de um polímero (MAIA; PORTE; SOUZA, 2000). Pela difração, pode-se determinar se um material é amorfo, semi-amorfo ou cristalino. Também permite avaliar a cristalização durante a estocagem do filme. Para os filmes semi-cristalinos ou cristalinos, a difração permite identificar qual ou quais elementos são responsáveis pela cristalinidade do material (CULLITY, 1978).

5.1.6 Propriedades Térmicas

Outra característica importante a ser analisada no filme de amido é sua propriedade térmica. Entende-se por análises térmicas o conjunto de técnicas que permitem medir as mudanças de um comportamento físico em função da temperatura.

A calorimetria diferencial de varredura (DSC- *Differential Scanning Calorimetry*) é a técnica na qual se mede a diferença de energia fornecida à amostra em relação a um material de referência, enquanto ambos são submetidos a uma programação controlada de temperatura. A medida de temperatura é feita por meio de termopares fixados à base do suporte da amostra e da referência. Alterações de temperatura da amostra são devido às variações de entalpia endotérmicas ou exotérmicas, decorrentes de transições físicas ou de reações químicas. A área contida sob o pico é representativa da variação de entalpia (ΔH) sofrida pela amostra. As variações de entalpia são chamadas transições de primeira ordem (fusão, cristalização, vaporização, solidificação e adsorção). As transições térmicas ditas de segunda ordem são acompanhadas de variação da capacidade calorífica da amostra, juntamente com variações dimensionais e viscoelásticas (como, por exemplo, a transição vítrea Tg), mas não apresentam variações de entalpia, não gerando picos nas curvas de DSC e sim, alterações na linha de base (COUPLAND et al., 2000).

Já a Termogravimetria é a técnica na qual a mudança de massa de uma substância é medida em função da temperatura enquanto esta é submetida a uma programação controlada. O termo Análise Termogravimétrica (TGA) é comumente empregado, particularmente em polímeros, no lugar de TG por ser seu procedente histórico e para minimizar a confusão verbal com Tg, a abreviação da temperatura de transição vítrea. Problemas adicionais podem ocorrer em pesquisas computadorizadas, já que ambas as abreviaturas são aceitas pela IUPAC (LUCAS et al., 2001). O equipamento utilizado na análise termogravimétrica é basicamente constituído por uma microbalança, um forno, termopares e um sistema de fluxo de gás.

6. Referências Bibliográficas

ABURTO, L.C.; TAVARES, D.Q.; MARTUCCI, E.T. Microencapsulação de óleo essencial de laranja. *Ciências Tecnologia Alimentos*, 18, 1, 45-48, 1998.

AKHTAR, M.J.; JACQUOT, M.; JASNIEWSKI, J.; JACQUOT, C.; IMRAN, M.; JAMSHIDIAN, M.; PARIS, C.; DESOBRY, S. Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract. *Carbohydrate Polymers*, 89, 1150 – 1158, 2012.

ALBUQUERQUE, M.; PINHEIRO, E. *Tuberosas feculentas*. Belém: IPEAN, 1970. 115 p. (Fitotecnia, 1).

ALCÁZAR-ALAY, S.C.; MEIRELES, M.A.A. Physicochemical properties, modifications and applications of starches from different botanical sources. *Food Science and Technology*, Campinas, 35, 2, 215-236, Abr.-Jun. 2015

ALVES, V.; COSTA, N.; HILLIOU, L.; LAROTONDA, F.; GONÇALVES, M.; SERANO, A.; COELHOSO, I. Design of biodegradable composite films for food packaging. *Desalination*, 199, 331-333, 2006.

ANTUNES, L.E.C. Blackberry: a new crop option to Brazil. *Ciência Rural*, Santa Maria, 32, 1, 151-158, 2002.

AZEREDO, H.M.C. Encapsulação: aplicação à tecnologia de alimentos. *Alimentos e Nutrição*, Araraquara, 16, 1, 89-97, 2005.

AZEREDO, H.M.C. Nanocomposites for food packaging applications. *Food Research International,* 42, 1240 – 1253, 2009.

BARRETO, P.L.M. *Propriedades Físico-Químicas de Soluções Formadoras e de Filmes de Caseinato de Sódio Plastificados com Sorbitol*; Tese Doutorado, 2003.

BILIADERIS, C. G. The strutucture and interactions of starch with food. *Canadian Journal of Physiology and Pharmacology*, 69, 60-78, 1991.

BURGAIN, J.; GAIANI, C.; LINDER, M.; SCHER, J. Encapsulation of probiotic living cells: from laboratory scale to industrial applications. *Journal of Food Engineering*, 104, 467-483, 2011.

CALLISTER, W.D. *Materials Science and Engineering: an introduction.* 4 ed., USA: John Wiley & Sons, Inc, 1997.

CEREDA, M.P.; FRANCO, C.M.L.; DAIUTO, È.R.; DEMIATE, I.M.; CARVALHO, L.J.C. B.; LEONEL, M.; VILPOUX, O.F.; SARMENTO, S.B.S. *Propriedades gerais do amido.* Série culturas de tuberosas amiláceas Latino Americanas, v.1. São Paulo: Fundação Cargill, p. 204, 2002.

CHANDRA, R.; RUSTGI, R. Biodegradable polymers. *Progress in Polymer Science*, 23, 1273-335, 1998.

CHARLES, A.L.; CATO, K.; HUANG, T-C.; CHANG, Y-H.; CIOU, J-Y.; CHANG, J-S; LIN, H-H. Functional properties of arrowroot starch in cassava and sweet potato composite starches. *Food Hydrocolloids*, 53, 187-191, 2016.

CHEN, F.; SHI, Z.; NEOH, K.G.; KANG, E.T. Antioxidant and antibacterial activities of eugenol and carvacrol-grafted chitosan nanoparticles. *Biotechnology and Bioengineering*, 104, 1, 30–39, 2009.

CHITARRA, M.I.F.; CHITARRA, A.B. *Pós-colheita de frutas e hortaliças: fisiologia e manuseio*. 2.ed.rev.e ampl. Lavras: UFLA, 2005. 785 p.

COUPLAND, J.N.; SHAW, N.B.; MONAHAN, F.J.; O'RIORDAN, D.; O'SULLIVAN, M. Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films. *Journal of Food Engineering, Oxford*, 43, 1, 25-30, 2000.

CULLITY, B. D. *Elements of X-ray diffraction.* Addison-Wesley Publishing Company, Inc. Massachusetts, 1978.

CUQ, B.; GONTARD, N.; GUILBERT, S. Proteins as agricultural polymers for packaging production. *Cereal Chemistry*, 75, 1, 1-9, 1998.

DAINELLI, D.; GONTARD, N.; SPYROPOULOS, D.; ZONDERVAN-VAN DEN BEUKEND, E.; TOBBACKE, P. Active and intelligent food packaging: Legal aspects and safety concerns. *Trends in Food Science and Technology*, 19,103–112, 2008.

DANTAS, E.A.; COSTA, S.S.; CRUZ, L.S.; BRAMONT, W.B.; COSTA, A.C.; PADILHA, F.F.; DRUZIAN, J.I.; MACHADO, B.A.S. Characterization and evaluation of the antioxidant properties of biodegradable films incorporated with tropical fruit pulps. *Ciência Rural*, Santa Maria, 45, 1, 142-148, jan, 2015.

DA SILVA, J.R; MONTEIRO D.A. *Cultura da araruta industrial: Instituto Agronômico de Campinas, seção de raízes e tuberculos*, Divisão de plantas industriais, Boletim n. 190, Julho 1969, 11p.

DEBEAUFORT, F.; QUEZADA-GALLO, J. A.; VOILLEY, A. Edible films and coatings: tomorrow packaging: a review. *Critical Reviews in Food Science and Nutrition*, 38, 4, 299-313, 1998.

DESAI, K.G.H.; PARK, H.J. Recent Developments in Microencapsulation of Food Ingredients. *Drying Technology*, 23, 1361–1394, 2005.

DO, J.Y.; SRISANGNAM, C.; SALUNKHE, D.K.; RAHMAN, A.R. Freeze-dehydrated and compressed sour cherries. *Journal Food Technology*, 10, 191-201, 1975.

DONHOWE, I.G.; FENNEMA, O.R. Edible films and coatings: characteristics, formation, definitions and testing methods. In: KROCHTA, J.M., BALDWIN, E.A., NISPEROS-CARRIEDO, M.O. (Ed.). *Edible coatings and films toilmprove food quality*. Lancaster, PA (USA): Technomic Publishing Co., p. 1-25, 1994.

DUBEY, R.; SHAMI, T.C.; BASHKER RAO, K.U. Microencapsulation technology and applications. *Defence Science Journal*, 59, 1, 82-95, 2009.

ELISIA, I.; HU, C.; POPOVICH, D.G.; KITTS, D.D. Antioxidant assessment of an anthocyanin-enriched blackberry extract. *Food Chemistry*, 101, 3, 1052–1058, 2007.

ESTEVINHO, B.N.; ROCHA, F.; SANTOS, L.; ALVES, A. Microencapsulation with chitosan by spray drying for industry applications - A review. *Trends in Food Science & Technology*, 31, 138-155, 2013.

FAN-CHIANG, H.-J.; WROLSTAD, R.E. Anthocyanin pigment composition of blackberries. *Journal of Food Science*, 70, C198–C202, 2005.

FARIAS, M.G.; FAKHOURI, F.M.; CARVALHO, C.W.P.; ASCHERI, J.L.P. Caracterização físico-química de filmes comestíveis de amido adicionado de acerola (*Malphigia emarginata* D.C.). *Química Nova*, 35, 2, 262-267, 2012.

FAVARO-TRINDADE, C.S.; PINHO, S.C.; ROCHA, G.A. Review: Microencapsulation of food ingredientes. *Brazilian Journal of Food Technology*, 11, 2, 103-112, abr.- jun., 2008.

FERRARI. C.C.; RIBEIRO. C.P.; AGUIRRE. J.M. Spray drying of blackberry pulp using maltodextrin as carrier agent. *Brazilian Journal of Food Technology*, 15, 157-165, 2012.

FIESP. *Análise Setorial de Mercado: Produtos transformados de plástico para o APL transformador de plásticos da região do Grande ABC Paulista*, 2009.

FISHMAN, M.L.; COFFIN, D.R.; KONSTANCE, R.P.; ONWULATA, C.I. Extrusion of pectin/starch blends plasticized with glycerol. *Carbohydrate Polymers*. Oxford, 41, 4, 317-325, 2000.

FODA, Y.H.; HAMED, M.G.E.; ABD-ALLAH, M.A. Preservation of orange and guava juices by freeze-drying. *Food Technology*, 24, 12, 74-80, 1970.

FRANCESCHINIS, L.; SALVATORI, D.M.; SOSA, N.; SCHEBOR, C. Physical and functional properties of blackberry freeze-and spray-dried powders. *Drying Technology*, 32, 154-207, 2014.

FRANCHETTI, S.M.M.; MARCONATO, J.C. Polímeros biodegradáveis – uma solução parcial para diminuir a quantidade dos resíduos plásticos. *Química Nova*, São Paulo, 29, 4, 811-816, 2006.

FRANCO, C.M.L.; DAIUTO, E.R.; DEMIATE, I.M.; CARVALHO, L.J.C.B.; LEONEL, M.; VILPOUX, O.F.; SARMENTO, S.B.S. Propriedades do amido, In: *Propriedades gerais do amido, série culturas de tuberosas amiláceas Latino Americanas*. Campinas: Fundação Cargil, 1, 141-184, 2002.

FREIBERG, S.; ZHU, X.X. Polymer microspheres for controlled drug release. *International Journal of Pharmaceutics*, 282, 1-18, 2004.

GENNADIOS, A.; WELLER, C. L. Edible films and coatings from wheat and corn proteins. *Food Technology*, 44-63, 1990.

GHARSALLAOUI, A.; ROUDAUT, G.; CHAMBIN, O.; VOILLEY, A.; SAUREL, R. Applications of spray drying in microencapsulation of food ingredients: An overview. *Food Research International,* 40, 1107–1121, 2007.

GIBBS, B.F.; KERMASHA, S.; ALLI, I.; MULLIGAN, C.N. Encapsulation in the food industry: a review. *International Journal of Food Sciences and Nutrition*, 50, 213-224, 1999.

GÓMEZ ESTACA, J.; GIMÉNEZ, B.; GÓMEZ-GUILLÉN, C.; MONTERO, P. Incorporation of antioxidant borage extract into edible films based on sole skin or a commercial fish skin gelatin. *Journal of Food Engineering*, 92, 78–85, 2009.

GONTARD, N.; GUILBERT, S.; CUQ, J.L. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science*, 58, 1, 206-211, 1993.

GONTARD, N.; GUILBERT, S.; CUQ, J.L. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*, 57, 1, 190-199, 1992.

GONTARD, N. *Films et enrobages comestibles: etude et amélioration des proprietés filmogène du gluten. Montpellier*: Université des Sciences et Techniques du Languedoc, 1991. 174p. These de Doctorat.

GORDILLO, C.A.S.; VALENCIA, G.A.; ZAPATA, R.A.V.; HENAO, A.C.A. Physicochemical Characterization of Arrowroot Starch (*Maranta arundinacea* Linn) and Glycerol/Arrowroot Starch Membranes. *International Journal of Food Engineering,*10, 4, 727–735, 2014.

GOUIN, S. Microencapsulation: industrial appraisal of existing technologies and trends. *Trends in Food Science and Technology*, 15, 330-347, 2004.

GROSSMAN, M.V.E.; MALI, S.; SHIMAZU, A.A. Efeitos plastificante e antiplastificante do glicerol e do sorbitol em filmes biodegradáveis de amido de mandioca. *Ciências Agrárias*, 28, 1, 79-88, 2007.

GUARDA, A.; RUBILAR, J.F.; MILTZ, J.; GALOTTO, M.J. The antimicrobial activity ofmicroencapsulated thymol and carvacrol. *International Journal of Food Microbiology*, 146, 144–150, 2011.

GUILBERT, S.; GONTARD, N.; GOSSIS, L.G.M. Prolongation of the shelf-life of perishable food products using biodegradable films amd coatings. *Lebesmittel Wissenschaft & Technologie*, 29, 1-2, 10-17, 1996.

GUTIÉRREZ, T.J. Surface and nutraceutical properties of edible films made from starchy sources with and without added blackberry pulp. *Carbohydrate Polymers*, 165, 169-179. 2017.

HAGER, T.J.; HOWARD, L.R.; LIYANAGE, R.; LAY, J.O.; PRIOR, R.L. Ellagitannin composition of blackberry as determined by HPLC-ESI-MS and MALDI-TOF-MS. *Journal of Agricultural and Food Chemistry*, 56, 3, 661–669, 2008.

HAN, J.H.; KROCHTA, J.M. Physical properties of whey protein coating solutions and films containing antioxidants. *Journal of Food Science*, 72, 5, E308–E314, 2007.

HOOVER, R. Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45, 3, 253-267, 2001.

HOWARD, L.; HAGER, T. *Berry fruit phytochemicals*. In: ZHAO, Y. (Ed.), Berry Fruit: Value-Added Products for Health Promotion. Taylor & Francis Group, pp. 73–104, 2007.

JACKSON, L.S.; LEE, K. Microencapsulation and the food-industry. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie*, 24, 4, 289-297, 1991.

JAFARI, S.M.; ASSADPOOR, E.; HE, Y.; BHANDARI, B. Encapsulation Efficiency of Food Flavours and Oils during Spray Drying. *Drying Technology*, 26, 7, 816-835, 2008.

JEPSON R.G.; CRAIG J.C. *The American Heritage Science Dictionary*. North America: Houghton Mifflin Company, 2005. p. 704.

JIZOMOTO, H.; KANAOKA, E.; SUGITA, K.; HIRANO, K. Gelatin-Acacia microcapsules for trapping micro oil droplets containing lipophilic dugs and ready disintegration in the gastrointestinal tract. *Pharmaceutical Research*, 10, 8, 1115- 1122, 1993.

KAUME, L.; HOWARD, L.R.; DEVAREDDY, L. The blackberry fruit: a review on its composition and chemistry, metabolism and bioavailability and health benefits. *Journal of Agricultural and Food Chemistry*, 60, 23, 5716–5727, 2012.

KESTER, J.J.; FENNEMA, O. Edible films and coatings: a review. *Food Technology*, 40, 12, 47-59, 1986.

KROCHTA, J.M.; MULDER-JOHNSTON, C. de.Edible and biodegradable polymer films: Challenges and opportunities. *Food Technology*, 51, 2, 61- 73, 1997.

KUCK, L.S.; NOREÑA, C.P.Z. Microencapsulation of grape (*Vitilabrusca* var. Bordo) skin phenol extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. *Food Chemistry*, 194, 569-576, 2016.

KUMBAR, S.G.; AMINABHAVI, T.M. Preparation and characterization of interpenetrating network beads of poly(vinyl alcohol)-grafted-poly(acrylamide) with sodium alginate and their controlled release characteristics for cypermethrin pesticide. *Journal of Applied Polymer Science*, 84, 552-560, 2002.

LABUZA, T.P. The effect of water activity on reaction Kinects of food deterioration. *Food Technology*, 34, 4, 36-41, 59, 1980.

LAGUERRE, M.; LECOMTE, J.; VILLENEUVE, P. Evaluation of the ability of antioxidants to counteract lipid oxidation: existing methods, new trends and challenges. *Progress in Lipid Research*, 46, 244-282, 2007.

LAJOLO, F.M.; MENEZES, E.W. *Carbohidratos en alimentos regionales Iberoamericanos*. São Paulo: Universidade de São Paulo, 2006. 648p.

LANNES, S.C.S.; MEDEIROS, M. L. Processamento de achocolatado de cupuaçu por spray-dryer. *Revista Brasileira de Ciências Farmacêuticas*, 39, 1, 2003.

LEONEL, M.; CEREDA, M.P.; SARMENTO, S.B.S. Arrowroot (*Maranta arundinacea*) as a Possible Raw Material for Starch Industries. *Brazilian Journal Food Technology*, 5, 151-155, 2002.

LEONEL, M.; CEREDA, M.P. Caracterização físico-química de algumas tuberosas amiláceas. *Ciências e Tecnologia de Alimentos*, 22, 1, 2002.

LEUNG, I.Y.F. Macular pigment: New clinical methods of detection and the role of carotenoids in age-related macular degeneration. *Optometry – Journal of the American Optometric Associati*on, 79, 5, 266–272, 2008.

LORENZI, H.; BACHER, L.; LACERDA, M.; SARTORI, S. *Frutas brasileiras e exóticas cultivadas: de consumo in natura*. Nova Odessa: Instituto Plantarum, 2006. p.627.

LUCAS, E.F.; SOARES, B.G.; MONTERIO, E.E.C. Caracterização de Polímeros: Determinação de Peso Molecular e Análises Térmicas. *E-papers*, Rio de Janeiro, 2001.

MACHADO, A.P.F.; PASQUEL-REÁTEGUI, J.L.; BARBERO, G.F.; MARTÍNEZ, J. Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International*, 77, 675–683, 2015.

MACLEOD, G.S.; FELL, J.T.; COLLET, J.H. Studies on the physical properties of mixed pectin/ ethylcellulose films intended for colonic drug delivery. *International Journal of Pharmaceutics*, 157, 53, 1997.

MADENE, A.; JACQUOT, M.; SCHER, J.; DESOBRY, S. Flavour encapsulation and controlled release- a review. *International Journal of food Science and Technology*, 41, 1-21, 2006.

MAIA, L.H.; PORTE, A.; SOUZA, V.F. *Filmes comestíveis: aspectos gerais, propriedades de barreira a umidade e oxigênio*. Boletim do CEPPA, Curitiba, 18, 1, 105-128, 2000.

MARTÍNEZ, H.F.; REVILLA, G.O.; VELÁZQUEZ, T.G. Optimal Spray-Drier Encapsulation Process of Orange Oil. Proceedings of the 14th *International Drying Symposium,* A, 621-627, 2004.

MASISI, K.; BETA, T.; MOGHADASIAN, M.H. Antioxidant properties of diverse cereal grains: A review on in vitro and in vivo studies. *Food Chemistry*, 196, 90–97, 2016.

McHUGH, T.H.; KROCHTA, J.M. Milk protein based edible films and coating. *Food Technology*, 48, 1, 97-103, 1994.

MONTEIRO, D.A.; PERESSIN, V.A. Cultura da araruta. In: CEREDA, M.P. *Agricultura: tuberosas amiláceas latino-americanas*. São Paulo: Fundação Cargill, 440-447, 2002.

MOORTHY, S.N. Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54, 559-592, 2002.

MORALES, A.L.; ALBARRACÍN, D.; RODRÍGUEZ, J.; DUQUE, C. Volatile constituents from Andes berry (*Rubus glaucus* Benth.). *Journal of High Resolution Chromatography*, 19, 585–587, 1996.

NEVES, M.C.P.; COELHO, I.S.; ALMEIDA, D.L. Araruta: resgate de um cultivo tradicional. *Seropédica: EMBRAPA*, 2005. 4 p. (Comunicado Técnico, 79).

PARK, S.; ZHAO, Y.Y. Development and characterization of edible films from cranberry pomace extracts. *Journal of Food Science*, 71, 2, E95–E101, 2006.

PARRA, D.F.; TADINI, C.C.; PONCE, P.; LUGÃO, A.B. Mechanical properties and water vapor transmission in some blends of cassava starch edible films. *Carbohydrate Polymers*, 58, 475-481, 2004.

PETERSEN, K.; NIELSEN, P.; BERTELSEN, G.; LAWTHER, M.; OLSEN, M.; NILSSON, N.Y.; MORTENSEN, G. Potential of biobased materials for food packaging. *Food Science and Technology*, 10, 2, 52 - 68, 1999.

PIO CORRÊA, M. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. Rio de Janeiro: Imprensa Nacional, 1, 150-151, 309p.. Editado pelo Serviço de Informação Agrícola, 1984.

PONCE, A.G.; ROURA, S.I.; VALLEA, C.E.D.; MOREIRA, M.R. Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: *In vitro* and *in vivo* studies. *Postharvest Biology and Technology*, 49, 294–300, 2008.

NORONHA, C.M.; CARVALHO, S.M.; LINO, R.C.; BARRETO, P.L.M. Characterization of antioxidant methylcellulose film incorporated with α-tocopherol nanocapsules. *Food Chemistry*, 159, 529–535, 2014.

OBEROI, D.P.S.; SOGI, D.S. Effect of drying methods and maltodextrin concentration on pigment content of watermelon juice powder. *Journal of Food Engineering*, 165, 172-78, 2015.

OLIVEIRA, L.M. As opções em alta barreira para embalagens de alimentos. *CETEA Informativo, Campinas*, 2, 4, 1990.

ORDÓÑEZ, J. A. *Tecnologia de alimentos: Componentes dos alimentos e processos*. Porto Alegre: Artmed, 1, p.294, 2005.

RÉ, M.I. Microencapsulação: em busca de produtos "inteligentes". *Ciência hoje*, 27, 162, 24-29, 2000.

RÉ, M.I. Formulation drug delivery system by spray drying. *Drying Technology*, 24, 433-446, 2006.

REINECCIUS, G.A. *Spray-drying of food flavors*. In: RISCH, S.J.; REINECCIUS, G.A. Flavor encapsulation. Washington, DC: ACS, 1988. p.55-66, (ACS Symposium Series, 370.).

RICE-EVANS, C.A.; MILLER, N.J.; PAGANGA, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology Medicine*, Amsterdam, 20, 7, 933-956, 1996.

RINDLAV-WESTLING, A.; STADING, M.; GATENHOLM, P. Crystallinity and morphology in films of starch, amylose and amylopectin blends. *Biomacromolecules*, 3, 1, 84–91, 2002.

ROCHA, G.A.; FÁVARO-TRINDADE, C.S.; GROSSO, C.R.F. Microencapsulation of lycopeno by spray drying: caracterization, stability and application of microcapsules. *Food Bioproducts Processing*, 90, 1, 37-42, 2012.

ROSENBERG, M.; KOPELMAN, I.J.; TALMON, Y. Factors affecting retention in spray-drying microencapsulation of volatile materials. *Journal of Agricultural and Food Chemistry*, 38, 1288-1294, 1990.

SADAKA, F.; NGUIMJEU, C.; BRACHAIS, C-H.; VROMAN, I.; TIGHZERT, L.; COUVERCELLE, J-P. Review on antimicrobial packaging containing essential oils and their active biomolecules. *Innovative Food Science and Emerging Technologies*, xxx, xxx–xxx, 2014.< http://dx.doi.org/10.1016/j.ifset.2014.03.002>.

SALGADO, P.R.; ORTIZ, S.E.M.; PETRUCCELLI, S.; MAURI, A.N. Biodegradable sunflower protein films naturally activated with antioxidant compounds. *Food Hydrocolloids*, 24, 525–533, 2010.

SANCHEZ-GARCIA, M.D.; OCIO, M.J.; GIMENEZ, E.; LAGARON, J.M. Novel polycaprolactone nanocomposites containing thymol of interest in antimicrobial film and coating applications. *Journal of Plastic Film and Sheeting*, 24, 3–4, p. 239–251, 2008.

SANTOS, A.M. dos; RASEIRA, M. do C.B. *Lançamento de cultivares de amoreira-preta.* Pelotas, RS: EMBRAPA – CNPFT, 1988. n.p. (EMBRAPA: Informativo 23).

SAYYAR, S.; OFFICER, D.L.; WALLACE, G.G. Fabrication of 3D structures from graphene-based biocomposites. *Journal of Materials Chemistry B*, 5, 3462--3482, 2017.

SCHER, B.H. *Controlled release delivery systems for pesticides*. Ed. Marcel Dekker, New York, 1999.

SEERAM, N.P.; MOMIN, R.A.; NAIR, M.G.; BOURQUIN, L.D. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine*, 8, 362–369, 2001.

SEERAM, N.P.; HEBER, D. Impact of berry phytochemicals on human health: Effects beyond antioxidant. *American Chemical Society Symposium Series*, 956, 326–336, 2007.

SHAHIDI, F.; HAN, X.Q. Encapsulation of food ingredients. *Food Science and Nutrition*, 33, 6, 501-547,1993.

SHI, A.-M.; WANG, L.-J.; LI, D., ADHIKARI, B. Characterization of starch filmscontaining starch nanoparticles: part 1: Physical and mechanical properties. *Carbohydrate Polymers*, 96, 593–601, 2013.

SHIMAZU, A.A.; MALI, S.; GROSSMANN, M.V.E. Efeito plastificante e antiplastificante do glycerol e do sorbitol em filmes biodegradáveis de amido de mandioca. *Ciências Agrárias*, 8, 1, 79-88, 2007.

SILVA, C.; RIBEIRO, A.; FERREIRA, D.; VEIGA, F. Administração oral de peptídeos e proteínas: II. Aplicação de métodos de microencapsulação. *Brazilian Journal of Pharmaceutical Sciences*, 39, 1, jan.- mar., 2003.

SILVA, G.J.F.; CONSTANT, P.B.L.; FIGUEIREDO, R.W.; MOURA, S.M. Formulação e estabilidade de corantes de antocianinas extraídas das cascas de jabuticaba (*Myrciaria ssp.). Alimentos e Nutrição, Araraquara*, 21, 3, 429-436, jul./set. 2010.

SIRACUSA, V.; ROCCULI, P.; ROMANI, S.; DALLA ROSA, M. Biodegradable polymers for food packaging: a review. *Trends in Food Science & Technology*, 19, 634-643, 2008.

SIRIPATRAWAN, U.; HARTE, B.R. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids*, 24, 770 – 775, 2010.

SOARES, N.F.F.; SILVA, W.A.; PIRES, A.C.S.; CAMILLOTO, G.P.; SILVA, S.S. Novos desenvolvimentos e aplicações em embalagens de alimentos. *Revista Ceres*, 56, 4, 370-378, 2009.

SOUZA, A.C.; DITCHFIELD, C.; TADINI, C. C. Biodegradable films based on biopolymers for food industries. In PASSOS, M.L., RIBEIRO, C.P. (Eds), *Innovation in Food Engineering: New techniques and products*, Boca Raton, FL: CRC Press, p. 511-537, 2010.

SOZER, N.; KOKINI, J. Review nanotechnology and its applications in the food sector. *Trends in Biotechnology*, 27, 82–89, 2009.

SZAJDEK, A.; BOROWSKA, E. Bioactive compounds and health-promoting properties of berry fruits: a review. *Plant Foods for Human Nutrition,* 63, 147–156, 2008.

TAWFIK, M.S.; HUYGHEBAERT, A. Interaction of Packaging Materials and Vegetable Oils: Oil Stability. *Food Chemistry*, 64, 451-459, 1999.

THARANATHAN, R.N. Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technoloy*, 14, 3, 71-78, 2003.

THEVENET, F. *Acacia gums: natural encapsulation agent for food ingredients*. In: RISCH, S.J.; REINECCIUS, G.A. Encapsulation and controlled release of food ingredients. Washington, DC: ACS, 1995. p.51-90. (ACS Symposium Series, 590).

THIRÉ, R.M.S.M.; SIMÃO, R.A.; ARAÚJO, P.J.G.; ACHETE, C.A.; ANDRADE, C.T. Redução da hidrofilicidade de filmes biodegradáveis à base de amido por polimerização por plasma. *Polímeros: Ciência e Tecnologia*, 14, 1, 57-62, 2004.

TSUJI, K. Microencapsulation of pesticides and their improved handing safety. *Journal of Microencapsulation*, 18, 137-147, 2001.

VAN DE VELDE, K.; KIEKENS, P. Development of a flax/polypropylene composite with optimal mechanical characteristics by fiber and matrix modification. *Journal of Thermoplastic Composite Materials*, 15, 4, 281-300, 2002.

VERMEIREN, L.; DEVLIEGHERE, F.; VAN BEEST, M.; KRUIJF, N.; DEBEVERE, J. Development in the active packaging of food. *Trends in Food Science & Tecnology*, 10, 3, 77-86, 1999.

VERMEIREN, L.; DEVLIEGHERE, F.; DEVEBERE, J. Effectiveness of some recent antimicrobial packaging concepts. *Food Additives and Contaminants*, 19, 163-171, 2002.

de VOS, P.; FAAS, M.M.; SPASOJEVIC, M.; SIKKEMA, J. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal*, 20, 4, 292- 302, 2010.

XIE, F.; POLLET, E.; HALLEY, P. J.; AVÉROUS, L. Advanced Nano-biocomposites Based on Starch. *Polysaccharides,* 2014. DOI 10.1007/978-3-319-16298-0_50.

WANG, S.Y.; JIAO, H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of Agricultural and Food Chemistry*, 48, 5677–5684, 2000.

WANG, S.; MARCONE, M.F.; BARBUT, S.; LIM, L.T. The impact of anthocyanin-rich red raspberry extract (ARRE) on the properties of edible soy protein isolates (SPI) films. *Journal of Food Science,* 77, 4, C497–C505, 2012a.

WARD, G.; NUSSINOVITCH, A. Characterizing the gloss properties of hydrocolloid films. *Food Hydrocolloids*, 11, 4, 357-365. 1997.

WINARTI, C.; RICHANA, N.; SUNARTI, T.C. Effect of acid hydrolysis on the characteristics of andrographolide-loaded arrowroot starch nanoparticles. *International Journal of Food Engineering,* 1, 1, 2015.

WURZBURG, O B. *Modified starches: properties and uses*: CRC Press Inc., Boca Raton, p. 277, 1986.

YOON, S.-D.; PARK, M.-H.; BYUN, H.-S. Mechanical and water barrier properties of starch/PVA composite films by adding nano-sized poly (methyl methacrylate-co-acrylamide) particles. *Carbohydrate Polymers*, 87, 676–686, 2012.

ZÁRATE, N.A.H.; VIEIRA, M.C. Produção da araruta ‘comum’ proveniente de três tipos de propágulos. *Ciência e agrotecnologia,* Lavras, 29, 5, 995-1000, set./out., 2005.

ZÁRATE, N.A.H.; VIEIRA, M.C.; GIULIANA, A.R.; STARCH KLAMT, M.F.; BASSI MORENO, L.; MARCONDES de SOUZA, C. Produção da araruta ‘comum’, solteira e consorciada com alface e cenoura. *BOTÁNICA*, Acta Científica Venezolana, 58, 1, 1-5, 2007.

ZHANG, C.; GUO, K.A.; MA, Y.; MA, D.; LI, X.H.; ZHAO, X.Y. Incorporations of blueberry extracts into soybean-protein-isolate film preserve qualities of packaged lard. *International Journal of Food Science and Technology*, 45, 9, 1801–1806, 2010.

CAPÍTULO 2- Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films

Este capítulo foi publicado pela revista *Carbohydrate Polymers* e está apresentado no formato de artigo científico (Anexo 1).

Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films

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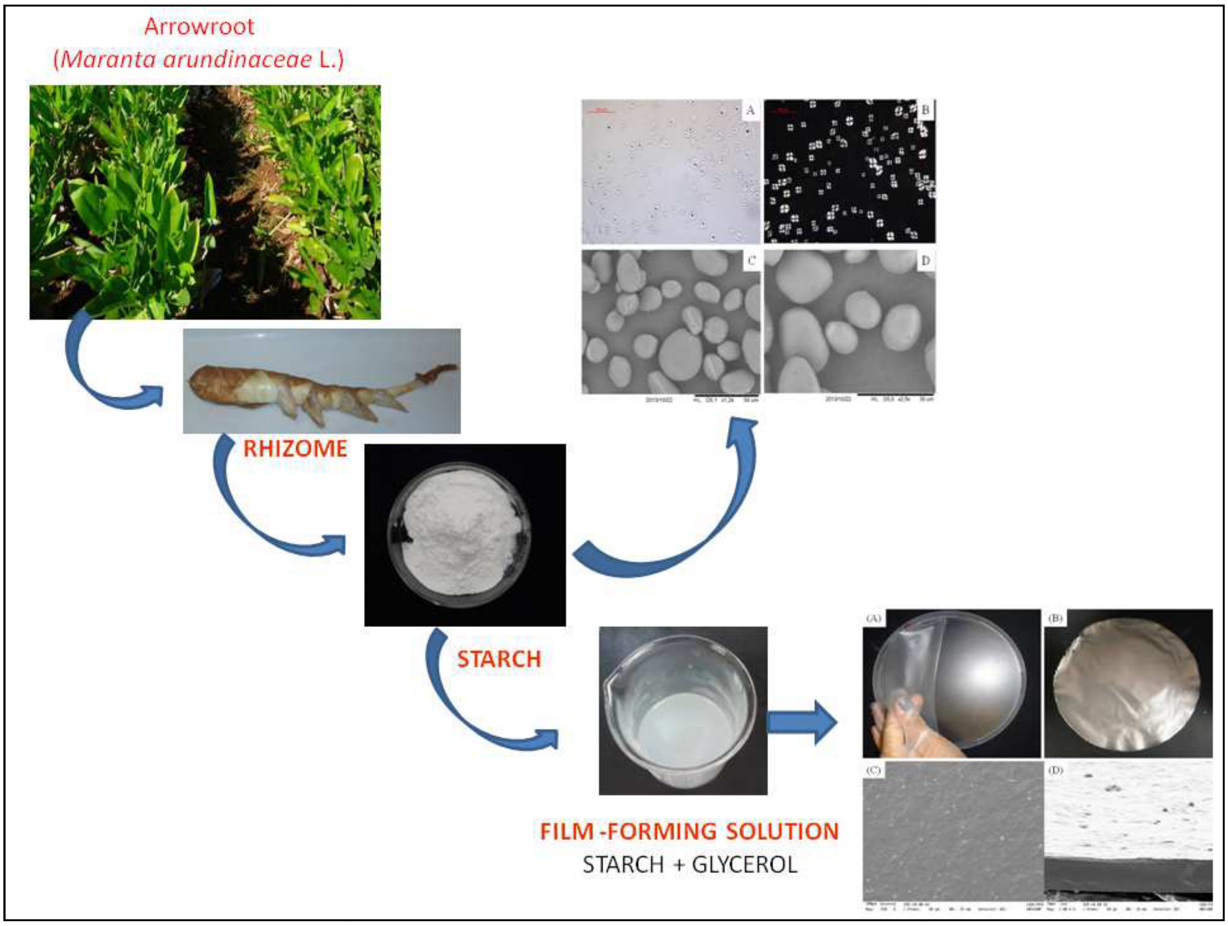
Highlight

- High starch content of *Maranta arundinaceae* L. rhizomes.
- *Maranta arundinaceae* L. starch is a C-type polymorph and has high amylose content.
- Edible films from arrowroot starch were prepared and characterized.
- Arrowroot is a very promising source of starch for application in films.
- Films were thicker and more soluble in high concentrations of starch and glycerol.

Abstract – This research work aimed extraction and characterization of arrowroot starch. Besides, the effects of different concentrations of starch (2.59 - 5.41%, mass / mass) and concentrations of glycerol (9.95 - 24.08%, mass versus starch mass) on films properties were evaluated by a rotational central composite 2^2 experimental design. Arrowroot starch showed high amylose content (35%). Low values were found for the swelling power and solubility index. The X-ray diffraction showed "C" type crystalline structures, while thermogram showed Tg around of 118 and 120 °C. The thermogravimetric analysis showed that 40% of mass loss of starch occurred between 330 and 410 °C. The films were homogeneous, transparent and manageable. Starch and glycerol concentrations played a significant role in thickness and solubility in water of films, but was not significant for water vapor permeability and tensile strength. Therefore, arrowroot is a very promising starch source for application in films.

Keywords: *Maranta arundinaceae* L; packing; X-ray diffraction; thermogravimetry; tensile strength; water solubility.

Graphical Abstract



1. Introduction

Currently, edible and biodegradable films have been used as a new strategy to reduce the severe environmental impact caused by using non-biodegradable petroleum packaging. Edible or biodegradable films are usually made from naturally compounds, such as proteins, lipids, polysaccharides or mixtures thereof (Genskowsky et al., 2015). Among polysaccharides, starch is the one with the greatest potentiality, due to its high capacity to form a continuous matrix, besides the advantage of being low cost, abundant and renewable, and exist in many ways depending on the origin of raw material (Sartori & Menegalli, 2016). The search for new natural sources of starch has been encouraged, as, with increasing population growth, there may be a shortage of common starches, such as corn, potatoes and wheat, for industrial applications in the future. In this sense, the arrowroot (*Maranta arundinacea* Linn) rhizomes stand out, since it is a source of unconventional starch without socioeconomic importance in many countries and therefore is not considered as a high priority raw material, which has not yet been studied (Gordillo, Valencia, Zapata, & Henao, 2014).

Arrowroot (*Maranta arundinaceae* L.) belongs to Marantaceae family and is a large perennial herb found in tropical forest. The plant is naturalized in Florida, but is grown mainly in the West Indies (Jamaica and St. Vincent), Australia, Southeast Asia and South and East Africa (Charles et al., 2016). In Brazil, there are three important cultivars: common, creole and banana (Leonel & Cereda, 2002). Economically, arrowroot has been used especially for starch extraction, due to the high starch content in its rhizomes. Arrowroot starch has the advantage of excellent digestibility (Villas-Boas & Franco, 2016), gelling ability (Charles et al., 2016; Hoover, 2001), and special physicochemical characteristics such as high amylose content (ranged from 16 to 27%, Moorthy, 2002), which is desirable for the production of films with good functional properties (Romero-Bastida, Bello-Pérez, Velazquez & Alvarez-Ramirez, 2015; Fakhoury et al., 2012; Li et al., 2011; Tharanathan, 2003).

The few studies that have been reported about arrowroot starch include the arrowroot starch behavior in composite starches (Charles et al., 2016), arrowroot starch carboxymethylation (Kooijman, Ganzeveld, Manurung, & Heeres, 2003), gelatinization profiles for the arrowroot starch (Hoover, 2001) and Erdman (1986) that compared some physical properties of commercial starch produced in West Indies with the starch of arrowroot cultivated in the United States.

Since the number of research papers about arrowroot starch is scarce, it is imperative to carry out new detailed studies regarding its physical-chemical, thermal and microstructural characterization, aiming to provide information that contributes to its applicability as a raw-starchy material. This characterization is particularly important as this starch has great potential for replacing conventional starch due to its functionality as a hydrocolloid, thickening and gelling agent, as well as encapsulating and coating agent and biodegradable food packaging and pharmaceutical products and food packaging.

Thus, the objective of this research work was to obtain and characterize arrowroot starch and to study the influence of its concentration as well as the plasticizer on thickness, water activity, water content, water vapor permeability, water solubility and tensile strength of the film.

2. Material and methods

2.1. Raw material

For starch extraction, samples of arrowroot rhizomes were obtained in experimental field of Faculty of Agronomy, Federal University of Grande Dourados, Mato Grosso do Sul, Brazil. For preparation of biodegradable films, the extracted starch was used as film-forming matrix, glycerol P.A. (Reagen, Quimibrás Indústrias Químicas S.A.- Rio de Janeiro, Brazil) was used as plasticizing agent and distilled water as solvent.

2.2 Extraction of arrowroot starch

Arrowroot starch was extracted according to methodology developed by Cruz and El Dash (1984) with adaptations. Arrowroot rhizomes were selected, peeled, sanitized with piped water, sliced and immersed in metabisulfite of potassium solution (0.03%, m/m) for 15 min. Then, its crushing was carried out with deionized water, in the ratio of 1: 2 (m / m) of arrowroot to water, high-speed stainless steel industrial blender (Spolu, Brazil), for 5 minutes, until obtaining a homogeneous mass. The obtained mass was filtered in a double cotton cloth. The mass washing process with deionized water was repeated three times for fiber separation and complete removal of starch. After approximately 12 h, with the starch sedimentation, its separation of the water was carried out by manual flow. The resulting starch was oven dried with air circulation at 60 °C for 4 h. The obtained starch was ground in a hammer mill (MR Manesco and Ranieri LTDA, model MR020, Piracicaba, Brazil), sieved (28 mesh) and packed in plastic bags, for further analyses.

2.3 Characterization of starch

2.3.1 Proximal composition and amylose content of starch

The yield of starch extraction on dry basis was calculated by taking the initial mass of arrowroot and the amount of starch obtained per 1 kg of sample. The moisture content was gravimetrically determined by drying the sample at 105 °C in a convective oven, during 24 h (AOAC, 2006). The fat contents of the starch was determined by gravimetric method after extraction using a Soxhlet apparatus and petroleum ether (A.O.A.C. Official Methods of Analysis, 2006), while the protein and ash contents were estimated by Kjeldhal and incineration methods (A.O.A.C. Official Methods of Analysis, 2006), respectively. Total carbohydrate was determined by the difference to 100%.

The amylose content was determined by colorimetric method, which is based on transmission of light through a colored complex which amylose forms upon reacting with iodine, according to methodology described by Martinez and Cuevas (1989), with adaptations (Zavareze et al., 2009). 100 mg sample of arrowroot starch, previously defatted in petroleum ether, was transferred to a 100 mL volumetric flask, with 1 mL of ethyl alcohol 96% GL and 9 mL of 1 N NaOH solution and placed in a 100 °C water bath for 10 min, being cooled for 30 min. Then, the volume was filled with distilled water. From each sample, a 5 mL aliquot was taken and transferred to a 100 mL volumetric flask, in which 1 mL of 1 N acetic acid and 2 mL of 2% (w / v) iodine solution were added. Then, volume of each volumetric flask was filled with distilled water. For fitting of standard curve, 40 mg of pure amylose was submitted to the same procedure used for arrowroot starch samples. Aliquots of 1, 2, 3, 4, and 5 mL of the volumetric flask were removed and so were added 0.2; 0.4; 0.6; 0.8 and 1 mL of acetic acid and 0.4; 0.8; 1.2; 1.6 and 2 mL of iodine, respectively. Then, the volume was completed at 100 mL with distilled water. The absorption reading was measured 30 min after addition of the iodine solution at 610 nm, using an ultraviolet spectrophotometer (model Q798U2 M, Quimis, Brazil). The results were expressed as percentage of amylose content, calculated by adjusting the potato amylose (Sigma-Aldrich, United States of America) calibration curve at concentrations ranging from 0.4 to 4 mg mL^{-1}.

2.3.2. Microstructure of starch

The microstructure of starch granules was examined using Optical Microscope (DMLM-Leica, Cambridge, England). Granules dispersed in glycerol were observed in transmitted light mode with magnification of 200 times with and without polarized light. Starch granules were also observed in Scanning Electron Microscope (SEM) (TM3000 - HITACHI - Tokyo, Japan). Powder sample was placed on double-sided carbon adhesive tape adhered to stub, submitted to application of a gold layer for 2 minutes and observed in scanning electron microscope operated at 20 kV.

2.3.3 Water absorption index (WAI) and water solubility index (WSI) of starch

The water absorption index (WAI) and water solubility index (WSI) of starch was determined in triplicate, according to Schoch (1964) with modifications. Suspensions of 0.2 g (d.s.) of starch in 18 g of distilled water were placed in centrifuge tubes and maintained at 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C and 90 °C for 30 minutes in a Dubnoff metabolic bath (model SL157, Solab) being lightly stirred (150 rpm) every 5 minutes. Then the weight of blend was completed to 20 g with addition of distilled water. The samples were homogenized and centrifuged at 4010 rpm for 15 minutes. The supernatant was oven dried at 105 °C until constant weight. The gel remaining in the tube was considered as wet and heavy sediment. The water absorption index (WAI) and water solubility index (WSI) were calculated according to equations 1 and 2, respectively.

$$\mathrm{WAI}\ (\mathrm{g.g^{-1}}) = \frac{\mathrm{Wg}}{\mathrm{W-Ws}} \tag{1}$$

$$\mathrm{WSI}\ (\%) = \frac{\mathrm{Ws}}{\mathrm{W}}\ \mathrm{x}\ 100 \tag{2}$$

Where: ‘Wg’ was weight of sediment (g), ‘W’ was weight of dry solids in original sample (g) and ‘Ws’ was weight of dissolved solids in supernatant (g).

2.3.4. X-ray diffractometry

The X-ray analysis was performed in sample of starch in powder form using a X-ray diffractometer (X'Pert model, Philips). The used X-ray source was a CoKα type radiation with a wavelength of λ=1.54056 Å (Almelo, Netherlands), under the following conditions: Voltage and current of 40kV and 40mA, respectively; Scanning range: 2Θ from 5 to 30°; pitch: 0.1°; speed: 1° / min, equipped with secondary graphite beam monochromator.

2.3.5. Differential Scanning Calorimetry (DSC)

Thermal properties of the starch were studied using a Differential Scanning Calorimeter (DSC1, Mettler Toledo, Schwerzenbach, Switzerland). 10 mg starch sample was weighed into a microanalytical scale (MX5-Mettler Toledo, Schwerzenbach, Switzerland), into an aluminum dish (40 µl). For reference, an empty aluminum cap was used. The sample was submitted to a heating program of 25 °C to and 100 °C at rate of 10 °C / min, in an inert atmosphere (50 mL / min of N_2). When temperature of 100 °C was reached, the sample was held for 10 minutes at this temperature. After this first scan, the measurement cells were cooled with liquid nitrogen to 25 °C, followed by a second heating sweep of 25 °C to 270 °C at a rate of 10 °C / min in an inert environment (50 ml / min of N_2). The glass transition temperature (Tg) was calculated as the baseline inflection point, caused by discontinuity of specific heat of the sample.

2.3.6. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of starch was performed on a thermogravimetric analyzer (TGA-50M, Shimadzu, Kyoto, Japan). A mass of approximately 8 mg, platinum crucible, nitrogen inert atmosphere of 30 mL min^{-1} were used, with heating rate of 10 °C min^{-1}, at temperature range of 25-600 °C, to measure the degradation of starch. The weight loss and weight derivative at different temperature ranges were determined from the TGA curves. The derivative weight percent was used to measure and compare the peak temperatures.

2.4 Preparation of film-forming solutions and Experimental planning

Arrowroot starch films were prepared following casting technique. Film-forming solutions were prepared by dispersing starch in distilled water (mass / mass) and heated at 85°C in a thermostatic bath, under constant stirring, for about 5 minutes. Then, glycerol was added to starch solution, proportionally to the mass of macromolecule and homogenized. Aliquots of 25 ml of the resulting solutions were dispensed into Plexiglas plates with 12 centimeters in diameter. The films were dried for 12 h at room temperature (25 ± 5 °C). After drying, the films were conditioned at 25 °C and 55 ± 3% relative humidity for 48 h before their characterization.

The effects of different concentrations of starch (2.59 - 5.41%, mass / mass) and different concentrations of glycerol (9.95 - 24.08%, mass versus starch mass) on the films properties were evaluated by a rotational central composite 2^2 experimental design, with 11 experimental runs, as described in **Table 1.** The response variables (dependent variables) were thickness, water vapor permeability, water solubility and tensile strength.

2.5 Characterization of arrowroot starch films

2.5.1 Visual aspect

Visual and tactile analyses were performed in order to select the most homogeneous films and were flexible for handling. Films that did not exhibit such characteristics were rejected.

2.5.2 Film Thickness

Films thicknesses were measured with accuracy of ± 0.001 mm, at ten different regions of each film, using a micrometer (model MDC 25M, Mitutoyo brand, Japan).

2.5.3 Solubility in water

Water solubility of films was determined according to the method proposed by Gontard, Guilbert and Cuq (1992). Films samples were cut into disks of 2 cm in diameter, in triplicate, dried at 105 °C for 24 h and weighed. The dehydrated samples were immersed individually in 50 mL beakers filled with distilled water, and maintained under slow agitation (75 rpm) for 24 h at 25 ± 2 °C. After this period, not solubilized samples were removed and dried (105 °C for 24 h) to determine the final dry mass. Solubility was expressed according to Equation (3).

$$\text{Solubilized material}(\%) = \frac{m_{si} - m_{sf}}{m_{si}} \text{ x } 100 \qquad (3)$$

In which: 'm_{si}' is the initial dry mass of films (g), 'm_{sf}' is the final dry mass of non-solubilized films (g).

2.5.4 Water vapor permeability

Water vapor permeability rate of films was determined gravimetrically based on ASTM E96-80 method (1989), using an acrylic cell, with a central opening, in which the film was fixed. The bottom of the cell was filled with dried calcium chloride, generating a dry environment inside (0% relative humidity at 25 °C). This cell was placed in desiccator containing saturated sodium chloride (75 ± 3% RH).

Water vapor transferred through films was determined by mass gain of calcium chloride. The cell weight was recorded daily for at least 7 days. The film thickness consisted on average of 5 random measurements made on different parts of film. The water vapor permeation rate (PVA) was performed in triplicate and calculated by Equation (4).

$$PVA = \frac{e}{A\ x\ \Delta p}\ x\dot{\mathrm{M}} \tag{4}$$

In which: 'PVA' is permeability to water vapor (g.mm / m^2.day.kPa), 'e' is mean film thickness (mm), 'A' is permeation area (m^2), 'Δp' is partial vapor pressure difference between two sides of films (kPa, at 25 ºC), '$\dot{\mathrm{M}}$' is absorbed moisture rate calculated by linear regression of weight gain and time, in steady state (g/day).

2.5.5 Mechanical properties

The tensile strength of the films were determined using a texturometer operated according to ASTM standard method D 882-83 (1980), with modifications (Tanada-Palmu, Hélen & Hyvonen, 2000). For each treatment, 6 films samples were cut into rectangles of 100 mm length and 25 mm wide. Their thickness values were randomly measured in 5 different parts of each sample before starting the analyses. In order to perform the tests, the films were fixed by two distal claws initially 50 mm apart, which moved at a speed of 1 mm / s. The tensile strength was calculated by Equation (5).

$$\mathrm{RT} = \frac{\mathrm{Fm}}{\mathrm{A}} \tag{5}$$

In which: 'RT' corresponds to tensile strength (MPa), 'Fm' is the maximum force at the moment of film rupture (N) and 'A' is cross-sectional area of films (m^2).

2.5.6 Microstructure of the film

The microstructure of the film was examined under a scanning electron microscope (Leo 440i, Electron Microscopy/Oxford, Cambridge, England). Films sample was placed on double-sided carbon adhesive tape adhered to stub, submitted to application of a gold layer (model K450, Sputter Coater EMITECH, Kent, United Kingdom) and observed in scanning electron microscope operated at 20 kV.

2.6 Statistical analysis

The results of responses of experimental design were evaluated using Statistica 9.0 software (StatSoft, South America). Significant differences were evaluated by analysis of variance (ANOVA) and Tukey test at 5% level of significance, using SAS software (Cary, NC, USA).

3. Results and discussion

3.1. Proximal composition and amylose content of starch

Extraction of arrowroot starch resulted in a white inodorous powder, with yield of 16 % on dry basis, similar to values found by Ferrari, Leonel & Sarmento (2005), corresponding to 18%. The arrowroot starch had the following proximal composition: 15.24 ± 0.19% of moisture content, 0.33 ± 0.01% of ashes, 0.40 ± 0.03% of proteins, 0.12 ± 0.01% of lipids and 83.91± 0.00% of carbohydrates, on dry basis. The values obtained are in agreement with those reported (Villas-Boas & Franco, 2016; Ferrari et al., 2005; Leonel, Cereda & Sarmento, 2002).

The low percentage of ashes, proteins and lipids shows the high quality and purity of the extracted starch. The determination of amount of proteins, lipids and mineral salts present in starch is essential, since these substances are considered as contaminants in the product and can interfere in physicochemical and technological properties of product (Leonel & Cereda, 2002). In addition to these components, amylose content has also an important effect on chemical properties of starch and, therefore, will determine its applications (Martinez & Prodolliet, 1996). The arrowroot starch had a total content of 35.20 ± 1.63% of amylose in its composition, higher than that found by Erdman (1986) of 19.9%. Variation of 16 to 27% for total amylose content in arrowroot starch was reported by Moorthy (2002).

The high amylose content of arrowroot of starch could allow its application in production of films with good technological properties, especially when it comes to mechanical resistance and barrier properties (Romero-Bastida et al., 2015; Fakhoury et al., 2012; Li et al., 2011; Tharanathan, 2003).

3.2. Microstructure of starch

Size distribution and microstructure of arrowroot starch was observed by optical microscopy (**Fig. 1 A** and **B**) and scanning electron microscopy (**Fig.** 1 C and D). When the granules were exposed to polarized light (**Fig. 1**B), it was possible to observe by optical microscopy the shape of the Maltese cross, evidencing birefringence and indicating presence of crystalline regions in starch (Riley, Wheatley & Asemota, 2006).

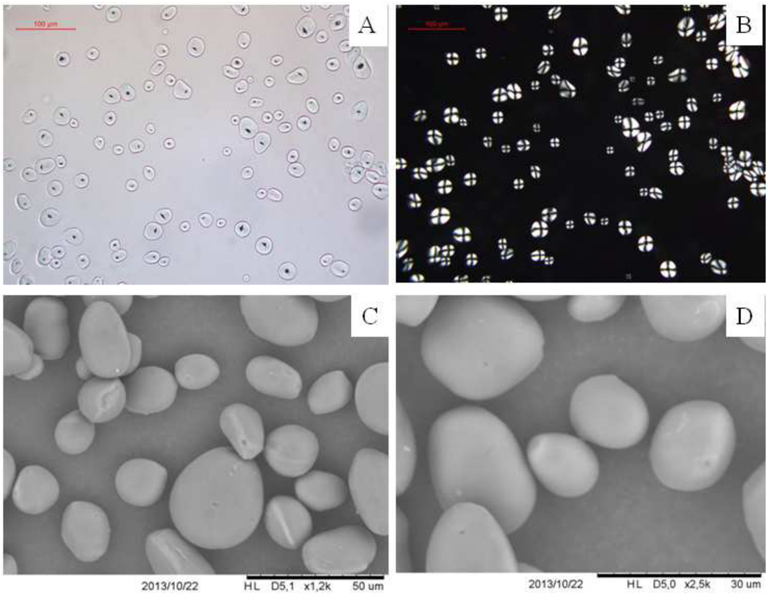


Figure 1. Optical microscopy (OM) and scanning electron microscopy (SEM) images of starch: (A) OM, 100 μm bar; (B) OM with polarized light, bar 100 μm; (C) SEM, 50 μm bar; (D) SEM, 30 μm bar.

As can be seen in **Figure 1**, starch granules presented heterogeneous size and circular shape, from ellipsoid to oval. The surface of starch arrowroot granules is smooth, with no evidence of fissures (**Fig. 1C** and **D**).

3.3 Water absorption index (WAI) and water solubility index (WSI) of arrowroot starch

The results obtained for water absorption index (WAI) and for solubility index (WSI) of arrowroot starch as function of temperature are shown in **Figure 2**.

Starch granules did not swell appreciably at temperatures below 60 °C, similar to that reported by Granados, Guzman, Acevedo, Díaz and Herrera (2014). This slow increase in swelling power with increasing temperature indicates that the internal associative forces that maintain the granule of bead structure were still strong and intense (Hoover, Hughes, Chung & Liu, 2010), thus resisting swelling. According to Hoover et al. (2010), for most starches extracted from seeds, such as black beans, lentils, peas, chickpeas, among others, no granule swelling or amylose leaching was measurable at temperatures below 60 °C.

At temperatures above 60 °C, however, the arrowroot starch granules swelled rapidly. The increase in swelling power of arrowroot starch was 2.17 ± 0.21 g / g (60 °C) and 11.32 ± 0.53 g / g (90 °C) (**Figure 2**). Continuous heating of water temperature causes a vigorous vibration of molecules of starch granules, causing rupture of intermolecular hydrogen bonds in amorphous areas. Thus, water molecules bind to exposed hydroxyl groups of amylose and amylopectin by hydrogen bonding, increasing granule size due to swelling and partial solubilization of polymers, especially amylose (Hoover, 2001).

Figure 2 shows that solubility of arrowroot starch also began to increase at 60 °C from 1.59 ± 0.60% to 17.22 ± 1.43% at 90 °C. The same behavior was reported by Pérez and Lares (2005) that found solubility of 2.09% at 60 °C and 13.22% at 90 °C for arrowroot starch.

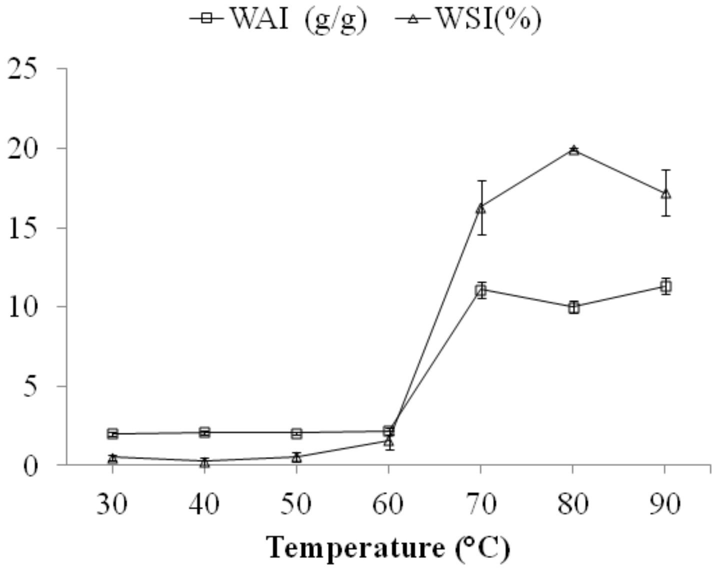


Figure 2. Water absorption index (WAI) and solubility index (WSI) of arrowroot starch as function of temperature.

3.4 X-ray diffractometry

The X-ray diffraction pattern of arrowroot starch shown in **Figure 3** indicated a mixture of polymorphs type A and B, a pattern that can be referred to as type C. Some of the peaks observed for arrowroot starch were similar to those found for cereal starches, such as the 2θ peak = 15.42°, typical of type A pattern. However, clear differences indicated presence of type B crystals, such as the peak observed at 2θ = 5.68°, the peak 2θ = 17.42°, which was the most prominent and the peak 2θ = 23.14°, the widest.

The B-type crystallinity pattern exhibited by arrowroot starch may be related in large part to the long branch chains of amylopectin (Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda, 2003), while A-type is particular to the short branch chains of amylopectin (Franklin et al., 2017).

Villas-Boas and Franco (2016) found for arrowroot native starch of A-type crystallinity, characterized by principal peaks at 15°, 17°, 18° and 23° in agreement with Moorthy (2002), who reported type A crystalline structures in arrowroot, cassava and other tuber starches as yams.

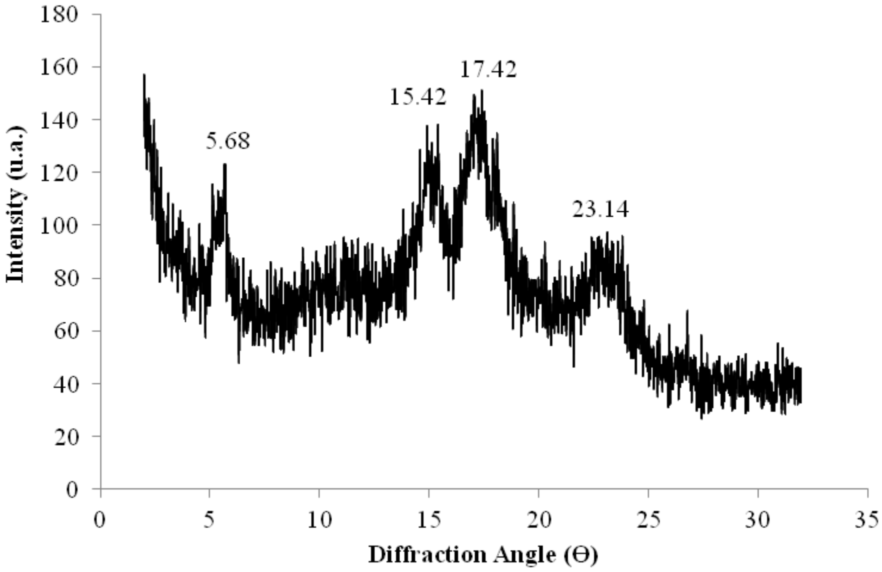


Figure 3. X-ray diffraction of arrowroot starch.

3.5. Differential Scanning Calorimetry (DSC)

An important data taken from the DSC curves is glass transition temperature (Tg). Tg is a value referring to a temperature range that, during heating of a polymeric material, allows the amorphous chains to acquire mobility (Schlemmer, Sales, & Resck, 2010). The thermogram of arrowroot starch shown in **Figure 4**A indicated its Tg at 120.30 °C, with the beginning of this transition around 118.37 °C and the end near of 120.34 °C.

For Chuang, Panyoyai, Katopo, Shanks and Kasapis (2016), potato starch thermogram with moisture content of 3.7% m / m (UR 11%) presented Tg of 161.72 °C and for the same sample with moisture content of 18.8% m / M (UR 75%), Tg of 141.91 °C. Chuang et al. (2017) found Tg of 150.10 °C and 137.50 °C for tapioca starch films with moisture content of 7.34% w / w (23% relative humidity) and 19.52% w / w (75% relative humidity), respectively. The increase in moisture content acts as a plasticizer that increases molecular mobility of amorphous regions in starch matrices, reducing Tg (Kasapis, 2005; Slade & Levine, 1994).

In addition to moisture, the Tg presented by starch also depends on its amylose and amylopectin content, the molecular interactions between starch and low molecular weight cosolutes and the nature of measurement protocol used (Perdomo et al., 2009).

The endothermic peak around 140.80 °C observed in thermogram (**Fig. 4** A) of arrowroot starch was attributed mainly to the evaporation of water and other volatiles that may be present in starch.

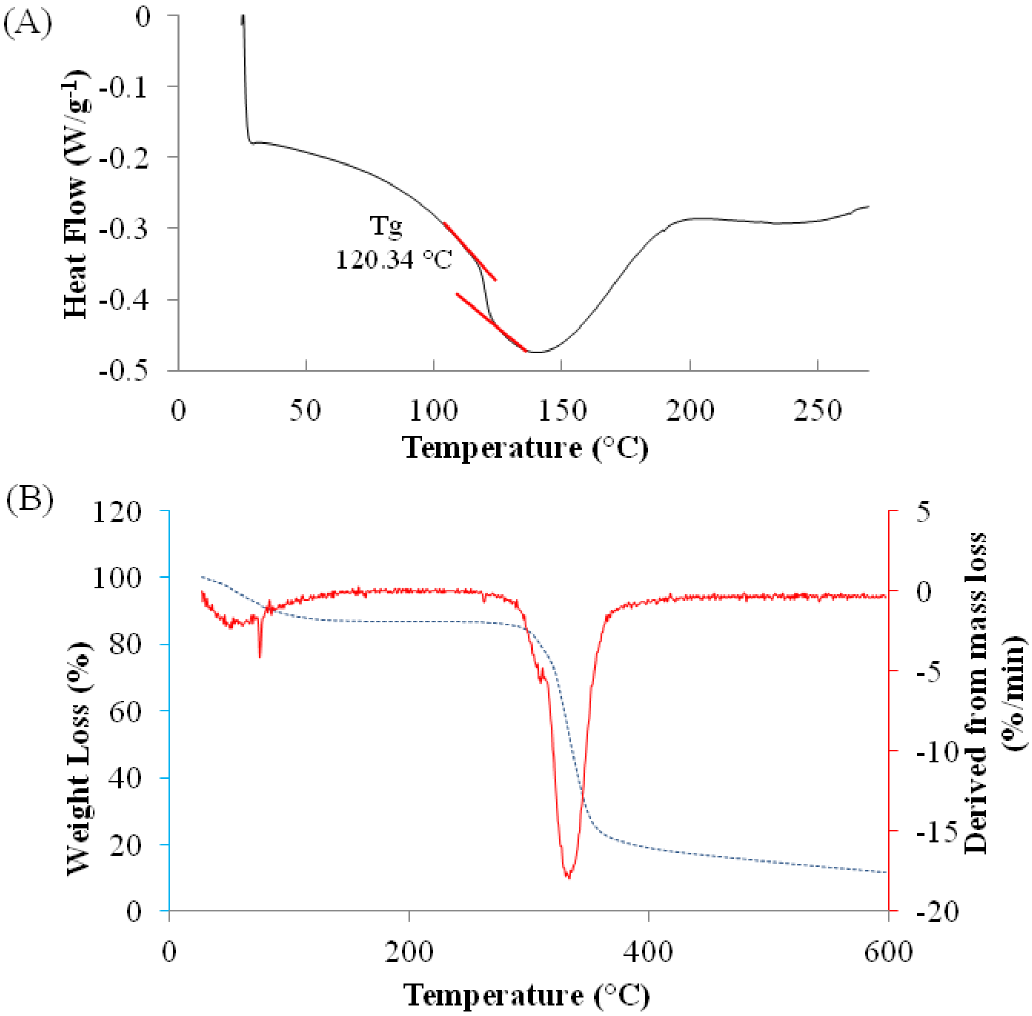


Figure 4. DSC curve (A) and thermogram (B) obtained for arrowroot starch containing 15.24 ± 0.19% moisture content (d.b.).

3.6. Thermogravimetric analysis (TGA)

Thermogravimetry analysis (TGA), assists in the study of thermal degradation of starch material. **Figure 4** B shows that starch had two stages of mass loss in temperature range of 25 to 600 °C. The first phase of mass loss corresponded to evaporation of water absorbed in starch material, which generally occurs in temperature range of approximately from 25 to 200 °C (Franklin et al., 2017). It was found that this mass loss was 13%, which was approximately the initial moisture content of arrowroot starch (15.24 ± 0.19%).

The second phase of mass loss (approximately 40%) occurred between 330 °C and 410 °C due to depolymerization of starch macromolecule (**Fig. 4**B). Carbonization and ash formation occurred at temperatures above 550 °C, while almost complete degradation of starch can be observed around 600 °C. For starch of *Curcuma angustifolia*, the reduction in mass was up to 4.35% in temperature range of 35 to 200 °C and 76% between 275 and 345 °C (Franklin et al., 2017).

Figure 4B also shows the curve derived from the main stage corresponding to degradation of the studied starch. The well-defined peak suggests possibility of a simple degradation mechanism involving amylose and amylopectin, as observed by Franklin et al. (2017) for starch of *Curcuma angustifolia.* In view of these results, it is possible to state that arrowroot starch is thermally stable and presents desirable characteristics for production of biodegradable and edible packages or films.

3.7 Characterization of arrowroot starch films

3.7.1 Visual aspects

All prepared films presented homogeneous surface, without bubbles and visible absence of insoluble particles. Regarding to handling characteristics, all films after drying could be removed from support plates without tearing, and could be manipulated (**Fig. 5** A and B).

Visual appearance of films was not affected by different concentrations of arrowroot starch and glycerol used in their formulation. All films were transparent and odourless, similar to oil packages, as shown in **Figure 5** A and B. One of the faces of films was brilliant (surface in contact with support plate during film drying) and another was matte (surface exposed to air during drying), as also observed by Basiak et al. (2017) for wheat, corn and potato starch films.

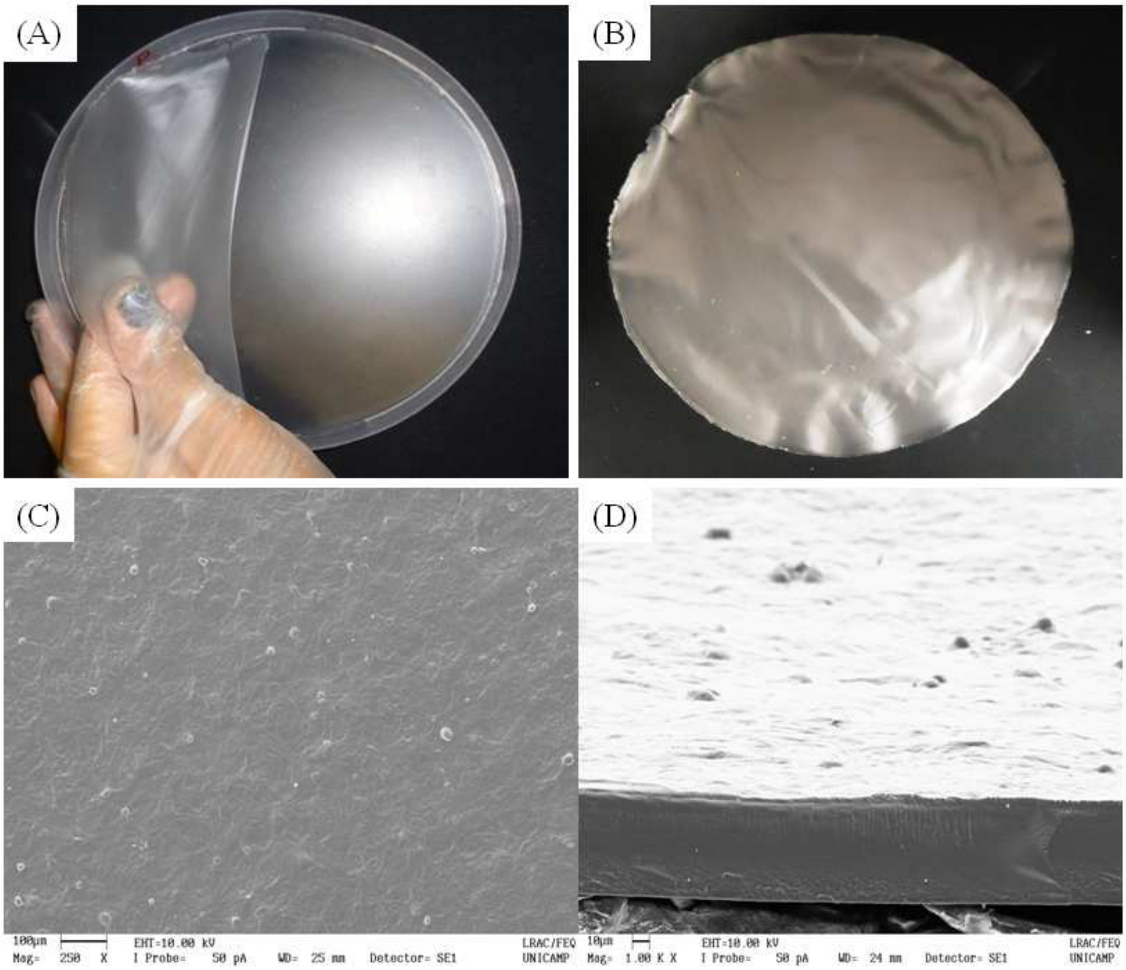


Figure 5. Photography (A and B) and scanning electron microscopy (C and D) of film produced with 4% (mass / mass) arrowroot starch and 17% glycerol (mass / mass of starch).

3.7.2 Statistical analysis

The experimental responses are presented in **Table 1**, while regression coefficients representing them as functions of independent variables are presented in **Table 2**. Only models for thickness and water solubility were significant ($p < 0.05$), and presented coefficients of determination of (R^2) of 0.98 and 0.67, indicating that models explain 98% and 67%, respectively, of observed data variation. The models graphical representation (contour plots) are shown in **Figure 6**, which can be analyzed along with **Table 2**.

Table 1. Experimental conditions and responses.

	Independent variables		Dependent Variables			
Runs	Arrowroot starch (%)	Glycerol (%)	Thickness (mm)	WS (%)	WVP g.mm/m^{2}daykPa	TS (MPa)
1	3 (-1)*	12 (-1)	0.035 ± 0.004 ^{ed}	6.46 ± 0.67 ^{g}	8.14 ± 0.23 ^{b}	14.31 ± 1.92 ^{dc}
2	3 (-1)	22 (1)	0.044 ± 0.003 ^{d}	10.55 ± 0.37 ^{de}	8.71 ± 0.09 ^{a}	12.57 ± 1.89 ^{dc}
3	5 (1)	12 (-1)	0.070 ± 0.012 ^{bac}	8.06 ± 0.44 ^{fg}	5.11 ± 0.19 ^{d}	14.21 ± 1.71 ^{dc}
4	5 (1)	22 (1)	0.077 ± 0.014 ^{ba}	16.71 ± 1.23 ^{a}	7.77 ± 0.14 ^{b}	11.28 ± 1.46 ^{d}
5	2.6 (-1.41)	17 (0)	0.026 ± 0.008 ^{e}	12.34 ± 0.62 ^{dc}	6.71 ± 0.04 ^{c}	16.87 ± 4.31 ^{bc}
6	5.4 (1.41)	17 (0)	0.082 ± 0.011 ^{a}	14.10 ± 0.07 ^{bc}	3.60 ± 0.03 ^{fg}	21.24 ± 2.47 ^{ba}
7	4 (0)	9.9 (-1.41)	0.063 ± 0.009 ^{c}	9.20 ± 0.62 ^{fe}	4.01 ± 0.15 ^{fe}	23.60 ± 3.98 ^{a}
8	4 (0)	24 (1.41)	0.068 ± 0.009 ^{bc}	16.32 ± 0.69 ^{a}	4.30 ± 0.23 ^{e}	24.11 ± 3.43 ^{a}
9	4 (0)	17 (0)	0.076 ± 0.009 ^{bac}	13.71 ± 0.50 ^{bc}	3.03 ± 0.39 ^{h}	25.79 ± 4.16 ^{a}
10	4 (0)	17 (0)	0.071 ± 0.007 ^{bac}	13.74 ± 0.33 ^{bc}	3.21 ± 0.05 ^{hg}	24.12 ± 3.06 ^{a}
11	4 (0)	17 (0)	0.071 ± 0.011 ^{bac}	15.02 ± 0.91^{ba}	2.90 ± 0.04 ^{h}	22.50 ± 1.82 ^{a}

*Numbers in parentheses correspond to coded variables. Coded values of variables are based on a central composite design; Uncoded values are the real experimental values of starch and glycerol concentration variables. WS is solubility in water; WVP is water vapor permeability; TS is tensile strength. Thickness values are mean ± standard deviation of 10 determinations. The values of WS and WVP are mean ± standard deviation of 3 determinations, and RT is mean ± standard deviation of 8 determinations. The means with different letters overlapped in column differ significantly ($p < 0.05$).

Table 2. Regression equations (for the coded variables) and statistical parameters of the models.

Regression	Fmodel/Ftabulated	R^2
Thickness = 0.073 + 0.018 S – 0.010 S^2 + 0.003 G – 0.004 G^2	78.43/4.53	98.12
WS = 12.38 +1.28S+ 2.85 G	8.49/4.46	67.97

WS is water solubility; S is starch concentration (coded values ranging from -1.41 to +1.41, according to Table 1); G is glycerol concentration (coded values ranging from -1.41 to +1.41, according to Table 1). Regression terms were significant ($p < 0.05$).

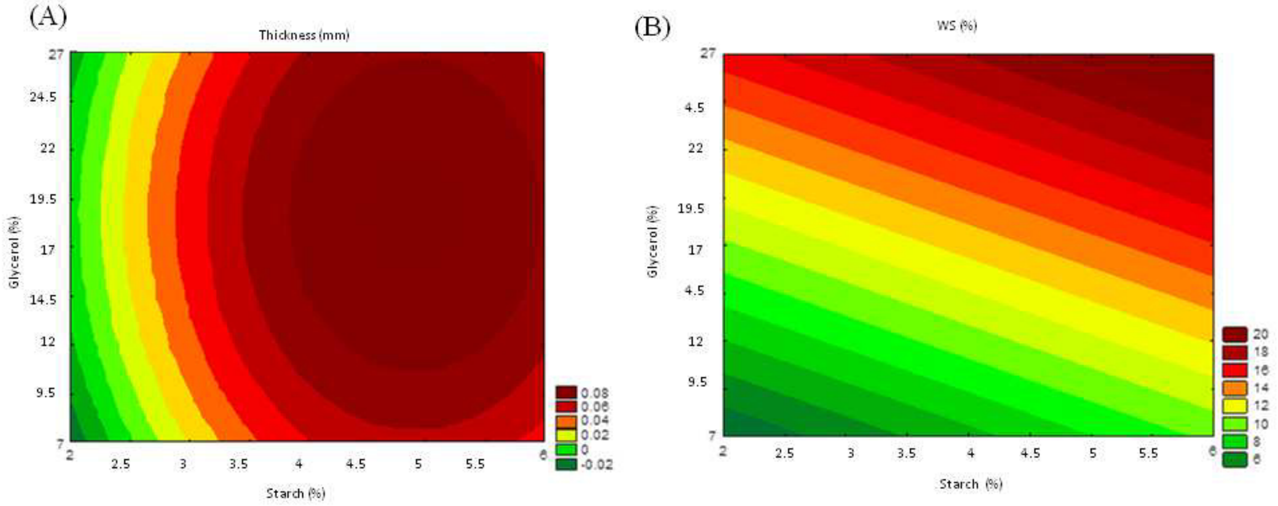


Figure 6 Contour plots representing thickness (A) and WS (B) of films with different concentrations of arrowroot starch and glycerol.

3.7.3 Film Thickness

Table 2 and **Figure 6**A show that concentrations of arrowroot starch and glycerol had positive linear effects ($p < 0.05$) on thickness of films, i.e., the higher their concentrations, higher film thickness. Concentration of arrowroot starch and glycerol also exerted a negative quadratic effect ($p < 0.05$) on thickness of films.

Thicknesses of arrowroot starch films ranged from 0.026 ± 0.008 to 0.082 ± 0.011 mm. Fakhoury et al. (2012) also observed that increasing the amount of starch or gelatin in films resulted in increase in their thickness when plasticized with glycerol and sorbitol. The author obtained thickness ranging from 0.034 to 0.075 mm. The increase in thickness is explained by increase in amount of dry matter in same volume of film-forming solution deposited per unit area in each support plate.

3.7.4 Solubility in water

Arrowroot starch films showed solubility in water ranging from 6.46 ± 0.67 to 16.71 ± 1.23%. These films were less soluble than cassava starch and gelatin plasticized with glycerol which presented solubility varying from 21.49% to 39.51% (Fakhoury et al, 2012).

Basiak et al. (2017) obtained water solubility about 14.52%, 30.16% and 44.76%, for potato, wheat and corn starch films, respectively. This characteristic may be related to its different amylose contents. According to the authors, higher amylose content creates higher solubility index for films.

In present study, **Table 2** and **Figure 6**B show that concentrations of starch and glycerol exerted positive linear effects ($p < 0.05$) on solubility, i.e., with increasing concentration, the water solubility of the films produced increased. Besides, concentration of glycerol had more significant effect on water solubility than starch concentration.

Probably, incorporation of plasticizer has caused changes in polymer grid of the film. The incorporation of plasticizer into biopolymers modifies the three-dimensional molecular organization of polymer grid, reducing intermolecular attraction forces and increasing free volume of system (Sothornvit & Krochta, 2000). Consequently, the grid becomes less dense, enabling permeation of water in its structure and its solubilization.

The increased concentration of starch and glycerol made film more soluble. This is an advantageous feature in case the package is ingested together with the food product. However, when the food is liquid or aqueous, films with high solubility are not indicated. In these cases, lower concentrations of starch and glycerol should be used for their production.

3.7.5 Water vapor permeability and Mechanical properties

The results of statistical analysis applied to experimental data of water vapor permeability and tensile strength did not show significant linear, quadratic and interaction effects of factors at confidence level of 95% ($p > 0.05$). However, arrowroot starch films showed tensile strength (range of 11.28 ± 1.46 to 25.79 ± 4.16 MPa) higher when compared to other starch films, such as rice, wheat, sago, potato, chickpeas, bananas, corn, in what the tensile strength ranged from 0.93 to 10 MPa (Basiak et al., 2017; Colussi et al., 2017; Al-Hassan & Norziah, 2012; Muscat, Adhikari, Adhikari & Chaudhary, 2012; Farahnaky, Saberi & Majzoobi, 2012; Torres, Troncoso, Torres, Díaz, & Amaya, 2011). These values strongly depend on the content of plasticizer and amylose, thickness, water content and additives (Basiak et al., 2017) used in their production.

Arrowroot starch films showed variation from 2.90 ± 0.04 to 8.71 ± 0.09 gmm / m^2daykPa (Table 1), for water vapor permeability, similar to values obtained for wheat (6.05 10^{-10} gm^{-1} s^{-1} Pa^{-1}), maize (8.72 10^{-10} gm^{-1} s^{-1} Pa^{-1}) and potato films (1.24 10^{-10} gm^{-1} s^{-1} Pa^{-1}) (Basiak et al., 2017). Native and acetylated rice starch films with medium and high amylose contents showed water vapor permeability varying from 5.33 to 10.33 gmm / m^2daykPa (Colussi et al., 2017).

Water vapor permeability is the measurement of the amount of moisture that passes through unit area of material per unit time (Basiak, Lenart & Debeaufort, 2016). According to Sartori and Menegalli (2016) and Hernandez (1994), the transfer of moisture usually occurs through the hydrophilic portion of a barrier and is directly related to hydrophilic-hydrophobic ratio of its components. Natural bipolymers used to make edible films are generally hydrophilic, as starch (Basiak et al., 2016).

The formulation of central point was the one presented the lowest water vapor permeability rate and the highest tensile strength, **Table 1**. This formulation (concentration of 4% starch (m / m) and 17% glycerol (m / m)) was able to produce films with structured, organized and compacted chains, which probably made it difficult the passage of water due to least mobility and generated mechanically resistant films. This can be confirmed in **Figure 5** C and D by SEM images of the surface and cross-section of films. The film surface was homogeneous and its structure was dense.

4. Conclusions

The physical-chemical, thermal and microstructural properties characterization of arrowroot starch were carried out. Arrowroot starch granules were circular, ellipsoid and oval, with different sizes. This starch also presented low protein, lipids, ashes and high amylose content, which are desirable attributes for many applications. There has been an increase in swelling power and water solubility of arrowroot starch granules at temperatures above 60 °C. The X-ray diffraction of arrowroot starch revealed a "C" type crystalline structure generally found for cereals and legumes. The starch had a Tg around 120.30 °C. The thermogravimetric analysis showed that 40% of the mass loss related to depolymerization of starch occurred between 330 and 410 °C. Arrowroot starch films were homogeneous, transparent and odourless. Films were thicker and more soluble in high concentrations of starch and glycerol. The increase in thickness occurred due to the increase in amount of dry matter, in same volume of filmogenic solution, deposited per unit area per support plate. Water solubility of films was strongly influenced by concentration of glycerol. Arrowroot is a very promising source of starch for applications in films.

Conflict of interest

The authors have declared no conflict of interest.

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References

Al-Hassan, A. A., & Norziah, M. H. (2012). Starch-gelatin edible films: Water vapour permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, 26, 108–117.

A.O.A.C. Official Methods of Analysis. (2006) (18th ed.). *Association of Official Analytical Chemists*, Gaithersburg, Maryland.

ASTM (1989). Standard test methods for water vapor transmission of materials. Method E96 e 80. In *Annual book of American standard testing methods*. Philadelphia: ASTM.

ASTM (1980). Standard test methods for tensile properties of thim plastic sheeting. Method D 882-83. In Annual *book of American standard testing methods*. Philadelphia: ASTM.

Basiak, E., Lenart, A., & Debeaufort, F. (2016). Effects of carbohydrate/protein ratio on the microstructure and the barrier and sorption properties of wheat starch–whey protein blend edible films. *Journal of the Science of Food and Agriculture* (DOI 10.1002/jsfa.7807).

Basiak, E., Lenart, A., & Debeaufort, F. (2017). Effect of starch type on the physico-chemical properties of edible films. *International Journal of Biological Macromolecules***,** 98, 348–356.

Charles, A. L., Cato, K., Huang, T. C., Chang, Y. H., Ciou, J. Y., Chang, J. S., & Lin, H. H. (2016). Functional properties of arrowroot starch in cassava and sweet potato composite starches. *Food Hydrocolloids*, 53, 187-191.

Chuang, L., Panyoyai, N., Katopo, L., Shanks, R., & Kasapis, S. (2016). Calcium chloride effects on the glass transition of condensed systems of potato starch. *Food Chemistry*, 199, 791–798.

Chuang, L.; PanyoyaI, N.; Shanks, R.A.; & Kasapis, S. (2017). Effect of salt on the glass transition of condensed tapioca starch systems. *Food Chemistry*, 229, 120–126.

Colussi, R., Pinto, V. Z., Halal, S. L. M. E., Biduski, B., Prietto, L., Castilhos, D. D., Zavareze, E. R., & Dias, A. R. G. (2017). Acetylated rice starches films with different levels of amylose: Mechanical, water vapor barrier, thermal, and biodegradability properties. *Food Chemistry*, 221, 1614–1620.

Cruz, R., & El Dash, A. A. M. (1984). Amido de chuchu (*Seichium edule*, Swartz). Efeito de fosfatação em sua viscosidade. Boletim SBCTA, 18 (4), 371-378.

Erdman, M. D. (1986). Starch from arrowroot (Maranta arundinacea) grown at Tifton, Georgia. *Cereal Chemistry*, 63, 277-279

Fakhoury, F. M., Martelli, S. M., Bertan, L. C., Yamashita, F., Innocentini Mei, L. H., & Queiroz, F. P. C. (2012). Edible films made from blends of manioc starch and gelatin – Influence of different types of plasticizer and different levels of macromolecules on their properties. *LWT - Food Science and Technology*, 49, 149-154.

Farahnaky, A., Saberi, B., & Majzoobi, M. (2012). Effect of glycerol on physical and mechanical properties of wheat starch edible films. *Journal of Texture Studies,* 44, 176–186.

Ferrari, T. B., Leonel, M., & Sarmento, S. B. S. (2005). Características dos rizomas e do amido de araruta (*Maranta arundinacea*) em diferentes estádios de desenvolvimento da planta. *Brazilian Journal of Food Technology*, 8 (2), 93-98.

Franklin, M. E. E., Pushpadass, H. A., Kumar, B., Kulkarni, S., Muthurayappa, M., Kandasamy, R., Venkatachalam, P., & Vellingiri, P. (2017). Physicochemical, thermal, pasting and microstructural characterization of commercial *Curcuma angustifolia* starch. *Food Hydrocolloids,* 67, 27-36.

Genskowsky, E., Puente, L. A., Pérez-Álvarez, J. A., Fernandez-Lopez, J., Muñoz, L. A., & Viuda-Martos, M. (2015). Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT - Food Science and Technology*, 64, 1057-1062.

Gontard, N., Guilbert, S., & Cuq, J. L. (1992). Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*., 57, 190-199.doi. 10.1111 / j.1365-2621.1992.tb05453.x

Gordillo, C. A. S., Valencia, G. A., Zapata, R. A. V, & Henao, A. C. A. (2014). Physicochemical Characterization of Arrowroot Starch (*Maranta arundinacea* Linn) and Glycerol/Arrowroot Starch Membranes. *International Journal of Food Engineering*, 10, 4, 727–735.

Granados, C. C., Guzman, C. E., Acevedo C. D., Díaz, M. M., & Herrera, A. A. (2014). Propiedades funcionales del almidon de sagu (*Maranta arundinacea*). *Colombia Biotecnología en el Sector Agropecuario y Agroindustrial***,** Cauca, 12 (2), 90-96.

Hernández, E. (1994). Edible coatings for lipids and resins. In: *Edible Coatings and Films to Improve Food Quality*, edited by Krochta, J.M., Baldwin, E.A., Nisperos-Carriedo, M.O. pp. 279-304. Tcchnomic Publishing Co.. Lancaster. PA.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers,* 45 (3), 253-267.

Hoover, R., Hughes, T., Chung, H. J., & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: a review. *Food Research International,* 43, 399–413.

Kasapis, S. (2005). Glass transition phenomena in dehydrated model systems and foods: A review. *Drying Technology*, 23, 731–757.

Kooijman, L. M., Ganzeveld, K. J., Manurung, R. M., & Heeres, H. J. (2003). Experimental studies on the carboxymethylation of arrowroot starch in isopropanol-water media. *Starch-Starke*, 55, 495-503.

Leonel, M., Cereda, M. P., & Sarmento, S. B. S. (2002). Arrowroot (*Maranta arundinacea*) as a Possible Raw Material for Starch Industries. *Brazilian Journal Food Technology*, 5, 151-155.

Leonel, M., & Cereda, M. P. (2002) Caracterização físico-química de algumas tuberosas amiláceas.*Ciências e Tecnologias de Alimentos*, 22 (1), 65-69.

Li, M., Liu, P., Zou, W., Yu, L., Xie, F., Pu, H., Liu, H., & Chen, L. (2011). Extrusion processing and characterization of edible starch films with different amylose contents, *Journal of Food Engineering*, 106 (1), 95-101.

Martinez, C., & Cuevas, F. (1989). *Evaluación de la calidad culinaria y molinera del arroz.* Guia de estudo, CIAT.

Martinez, C., & Prodolliet, J. (1996). Determination of amylose in cereal and non-cereal starches by a colorimetric assay: Collaborative study. *Starch*, 48, 81-85.

Moorthy, S. N. (2002). Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54, 559-592.

Muscat, D., Adhikari, B., Adhikari, R., & Chaudhary, D.S. (2012). Comparative study of film forming behaviour of low and high amylose starches using glycerol and xylitol as plasticizers, *Journal of Food Engineering*, 109, 189–201.

Perdomo, J., Cova, A., Sandoval, A. J., Garía, L., Laredo, E., & Müller, A. J. (2009). Glass transition temperatures and water sorption isotherms of cassava starch. *Carbohydrate Polymers,* 76, 305–3013.

Pérez, E., & Lares, M. (2005). Chemical Composition, Mineral Profile, and Functional Properties of Canna (*Canna edulis*) and Arrowroot (*Maranta spp.)* Starches. *Plant Foods for Human Nutrition*, 60,113–116.

Riley, C., Wheatley, A., & Asemota, H. (2006). Isolation and Characterization of starch from eight Dioscorea alata cultivars grown in Jamaica, *African Journal of Biotechnology*, 15 (17), 1528-1536.

Romero-Bastida, C. A., Bello-Pérez, L. A., Velazquez, G. J., & Alvarez-Ramirez, J. (2015). Effect of the addition order and amylose content on mechanical, barrier and structural properties of films made with starch and montmorillonite. *Carbohydrate Polymers*, 127, 195–201.

Sartori, T., & Menegalli, F. C. (2016). Development and characterization of unripe banana starch films incorporated with solid lipid microparticles containing ascorbic acid. *Food Hydrocolloids,* 55, 210-219.

Schlemmer, D., Sales, M. J. A., & Resck, I. S. (2010). Preparação, Caracterização e Degradação de Blendas PS/TPS Usando Glicerol e Óleo de Buriti como Plastificantes. *Polímeros: Ciência e Tecnologia*, 20 (1), 6-13.

Schoch, T. J. (1964). Swelling power and solubility of granular starches. In: Whistler, R. L. *Methods in Carbohydrate Chemistry; Starch.* New York: Academic Press, 106- 109.

Sothornvit, R., & Krochta, J. M. (2000). Plasticizer effect on oxygen permeability of β-lactoglobulina films. *Journal of Agricultural and Food Chemistry*, 48, 12, 6298-6302, 2000.

Slade, L., & Levine, H. (1994). Water and glass transition - dependence of the glass transition on composition and chemical structure: Special implications for flour functionality in cookie baking. *Journal of Food Engineering*, 22, 143–188.

Tanada-Palmu, P. S., Hélen, H., & Hyvonen, L. (2000). Preparation, properties and applications of wheat gluten edible biofilms. *Agricultural And Food Science in Finland,* 9, 23-25.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future**.** *Trends in Food Science & Technoloy*, 14 (3), 71-78.

Thitipraphunkul, K., Uttapap, D., Piyachomkwan, K., & Takeda, Y. A. (2003) Comparative study of edible canna (*Canna edulis*) starch from cultivars. part I. Chemical composition and physicochemical properties. *Carbohydrate Polymers*, 53, 317–324.

Torres, F. G., Troncoso, O. P., Torres, C., Díaz, D. A., & Amaya, E. (2011). Biodegradability and mechanical properties of starch films from *Andean crops*. *International Journal of Biological Macromolecules,* 48, 603–606.

Villas-Boas, F., & Franco, C. M. L. (2016). Effect of bacterial β-amylase and fungal α-amylase on the digestibility and structural characteristics of potato and arrowroot starches. *Food Hydrocolloids*, 52, 795- 803.

Zavareze, E. da R., El Halal, S. L. M., Pereira, J.M., Radünz, A. L., Elias, M. C., & Dias, A. R. G. (2009). Chemical characterization and extraction yield of rice starch with different amylose contents. *Brazilian Journal of Food Technology*, II SSA, janeiro.

CAPÍTULO 3- Bioactive films of arrowroot starch and blackberry pulp: physical, mechanical and barrier properties and stability to pH and sterilization

Este capítulo será submetido à revista *Food Hydrocolloyds* e está apresentado no formato de artigo científico

Bioactive films of arrowroot starch and blackberry pulp: Physical, mechanical and barrier properties and stability to pH and sterilization

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Highlight

- Bioactive films of arrowroot starch and blackberry pulp were developed.
- The effects of blackberry pulp on arrowroot starch films were studied.
- Pulp blackberry acted as plasticizer, improving elongation.
- The blackberry transferred bioactive compounds and antioxidant capacity to films.
- There was a darkening of the films with blackberry pulp after their sterilization.

Abstract: The influence of incorporation of blackberry pulp on properties of arrowroot starch films was studied. The blackberry pulp transferred bioactive compounds, antioxidant capacity and color for arrowroot starch films. Increasing the concentration of blackberry pulp (from 0 to 40%, mass / mass of dry starch) in the film resulted in increased thickness (from 0.065 to 0.133 mm), increased elongation (from 3.18 to 13.59%), decreased tensile strength (from 22.71 to 3.97 MPa), increased water vapor permeability (from 3.62 to 4.60 gmm/m^{2}daykPa) and solubility in water (from 14.18 to 25.46%). The films were stable to different media, maintaining the same initial diameter dimensions after immersion in acidic, neutral and alkaline solutions, but their color intensity was reduced, probably due to the release of anthocyanins to the solutions. There was darkening of the films with blackberry pulp after sterilization process.

Keywords: packaging, anthocyanins, color, sterilization, biopolymers.

1. Introduction

The development of edible films and coatings with fruit purees, pulp or juice tract has been extensively studied in the last few decades (Azeredo, Morrugares-Carmona, Wellner, Crooss, Bjaka, & Waldron, 2016; Espitia, Avena-Bustillos, Du, Teófilo, Soares, & McHugh, 2014; Otoni et al., 2014; Farias, Fakhouri, Carvalho, & Ascheri, 2012; Sothornvit & Rodsamran, 2008; Kaya & Maskan, 2003). The edible films and coatings are structures used to act as a barrier between food and surrounding environment, helping the external packaging in its protective role. The incorporation of fruits in polymeric matrix provide bioactive compounds, antimicrobial and antioxidant properties, desirable color and flavor to the resultant film, extending its applicability such as sushi wraps, fruit stripes, or colorful coatings for specific foods (Azeredo et al., 2016).

Blackberry (*Rubus fruticosus*), cv. Tupy, is a fruit rich phenolic compounds, such as phenolic acids, tannins and anthocyanins (Machado, Pasquel-Reátegui, Barbero, & Martínez, 2015). The fruits are much appreciated by consumers, by its sweet taste and its intense red color (D'Agostino et al., 2015). Your color is determined by anthocyanins content and composition, which are flavonoids widely distributed in nature, responsible for most of the blue, violet and all shades of red colors, present in flowers and fruits (Yamashita, Chung, Santos, Mayer, Moraes, & Branco, 2017; Yamamoto et al., 2015). The color displayed by anthocyanins and its stability depends on several factors (Azeredo et al., 2016; Fernandes, Faria, Calhau, Freitas, & Mateus, 2014). The anthocyanins are highly sensitive to degradation reactions when exposed to high temperature, light, oxygen, especially the pH and while the presence of copigments are the most determining factors of anthocyanin stability and color (Weber, Boch, & Schieber, 2017; Azeredo et al., 2016; Patras, Brunton, Donnell, & Tiwari, 2010).

Arrowroot starch may be an interesting matrix for improving the stability of anthocyanins and for producing edible films. Arrowroot starch has high amylose content, a desirable feature for the production of films with good technological properties, with stronger and more flexible mechanical characteristics (Tharanathan, 2003). According to Moorthy (2002), the total amylose content in arrowroot starch ranged from 16 to 27%. Additionally, the incorporation of fruits pulp could improve the plasticizing properties of biomaterials, generating innovative barrier and mechanical properties to the biopolymer (Azeredo et al., 2016; Otoni et al., 2014).

This study was developed to evaluate the influence of blackberry pulp on some physical, mechanical and barrier properties and stability to pH and sterilization of arrowroot starch films for applications in which a bright red color may be desirable, such as fruit stripes or food packaging.

2. Materials and methods

2.1. Materials

Frozen fruits of blackberry (*Rubus fruticosus*), cv Tupy, were acquired from "Agro Monte Verde Eirelli", company of Cambuí – MG, Brazil, and used in this research work. Pulp was obtained by grinding blackberry fruit in a blender, which was previously thawed in the refrigerator (8 °C) for 24 h. Then, pulp was sieved to remove the seeds, homogenized and packed in polypropylene bottles and coated with aluminium foil to protect against photodegradation. The samples were stored in freezer at -40 ± 3 °C. Blackberry soluble solids content was 9 °Brix, determined with a digital refractometer with a 0–90 °Brix range and 0.2 °Brix resolution (Reichert, Model AR200, USA). The pulp presented total solids content of 10.3 g/100g of pulp.

In elaboration of edible biodegradable films, arrowroot starch containing 15.24 ± 0.19% of water, 0.40 ± 0.03% of protein, 0.12 ± 0.01 % of fat, 0.33 ± 0.01% ash and 83.91 ± 0.00% of carbohydrates (AOAC, 2006) and amylose content of 35.20 ± 1.63% determined according to methodology described by Martinez and Cuevas (1989), with adaptations (Zavareze et al., 2009), was used as film forming matrix and glycerol P.A. (Reagen, Quimibrás Indústrias Químicas S.A.- Rio de Janeiro, Brazil) as plasticizing agent. All other reagents used for the analysis presented analytical grade.

2.2 Preparation of film-forming suspension

The film-forming suspension was obtained by dispersing arrowroot starch in distilled water (4%, mass / mass, as optimized by Nogueira, Fakhouri & Oliveira, 2018) and heating to 85 ± 2 °C in a thermostatic bath (TECNAL, Brazil), with constant agitation, for about 5 minutes. The pulp of blackberry (BP) was added directly in film-forming suspension in different concentrations, 0%, 20%, 30% and 40% (blackberry solids mass / mass of dry starch) and homogenized. Glycerol was added to the solution at concentration of 17% (mass/mass of total solids), as optimized by Nogueira, Fakhouri & Oliveira (2018). Aliquots of 25 mL of resulting suspensions were distributed on the support plate (12 cm diameter). Films were dried at room temperature (25 ± 5°C), until they could be easily removed from the support, approximately for 24 h. The films were conditioned at 25 °C and 55 ± 3% of relative humidity for 48 h, before their characterization.

2.3 Films characterization

2.3.1 Visual aspect

Visual and tactile analyses were performed in order to select the most homogeneous films, which had continuity and which were flexible for handling. Films that did not exhibit such characteristics were rejected.

2.3.2 Microstructure

Electronic Scanning Electron Microscope with X-ray Dispersive Energy Detector (SEM) bench (model of MEV: Leo 440i, model of EDS: 6070, Leo 440i - LEO Electron Microscopy/Oxford- Cambridge, Inglaterra) was used to observe the morphological characteristics of the surface and cross section developed for sample of the films formulations. Films sample was placed on double-sided carbon adhesive tape adhered to stub, submitted to application of a gold layer (model K450, Sputter Coater EMITECH, Kent, United Kingdom) and observed in scanning electron microscope operated at 20 kV.

2.3.3 Color determination

Color parameters were determined using a Hunter Lab colorimeter (Color Quest XE 2819, USA). The equipment was set with D65 illuminant and calibrated with a standard white reflector plate. Three films of each treatment were evaluated. A CIE-Lab (International Commission of L'Eclairage) color scale was used to measure the degree of lightness (L*), redness (+a*) or greenness (-a*), and yellowness (+b*) or blueness (-b*) of the films. The total color difference (*ΔE **) of the films were expressed according to Equation 1.

$$\Delta E^{*} = [(L^{*} - L^{*}{}_{p})^{2} + (a^{*} - a^{*}{}_{p})^{2} + (b^{*} - b^{*}{}_{p})^{2}]^{0,5} \qquad (1)$$

In which $L^{*}{}_{p}$, $a^{*}{}_{p}$ e $b^{*}{}_{p}$ are color parameters of the standard film (F0%) in standard white system CIELab.

2.3.4 Water activity and Moisture content

Water activity was measured in triplicate by direct reading using an AquaLab Lite water activity meter (Decagon Devices Inc., Pullman, USA), operating at 25 °C. The samples were circularly sized, 35 mm in diameter and placed in plastic capsules on the equipment. Moisture content of the films was determined in triplicate by air-forced oven drying at 105 ºC for 24 h (AOAC, 2006).

2.3.5 Total anthocyanins content

Total anthocyanins content of films and blackberry pulp was determined according to method described by Sims and Gamon (2002), with adaptations. Film samples were previously macerated in liquid nitrogen, weighed in triplicate, and homogenized with 3 mL of cold solution of acetone/Tris-HCl (80:20, volume/volume, pH 7.8, 0.2M) for 1 minute. The samples remained at repose for 1 hour, protected from light. Then, it was centrifuged for 15 minutes at 3500 rpm and the supernatants were immediately taken to reading in ultraviolet spectrophotometer (model Q798U2M, Quimis, Brazil) in visible region at 537 nanometers (anthocyanins). The acetone/Tris-HCl solution was used as blank sample. The absorbance values were converted to mg/100g of blackberry pulp solids.

2.3.6 Antioxidant property

The antioxidant capacity by FRAP method was performed using the methodology described by Rufino et al. (2006). In acid medium, ferric complex tripyridyltriazine (TPTZ) is reduced to its deep blue ferrous form in presence of antioxidants, causing an increase in absorbance at 595 nm. To prepare the FRAP reagent, 25 mL of 0.3 M acetate buffer solution (pH 3.6), 2.5 mL of 10 mM TPTZ solution (prepared with 40 mM HCl) and 2.5 mL of 20 mM aqueous ferric chloride solution.

Samples were extracted in methanol solution (50% methanol in distilled water, v/v) and then in acetone solution (70% acetone in distilled water, v/v) to determine the antioxidant property. Extraction was performed by placing ~ 1 g of sample (dry basis), 40 mL of 50% methanol and 40 mL of 70% acetone in a volumetric flask. The volume was filled with 100 mL with distilled water. Aliquot of 90 µL each diluted extract was transferred into test tubes along with 270 µL of distilled water and 2.7 mL of freshly prepared FRAP reagent. This mixture was homogenized and maintained in a water bath at 37 °C. After 30 minutes, reading was performed at 595 nm in an ultraviolet spectrophotometer (model Q798U2M, Quimis, Brazil). The FRAP reagent was used as blank sample to calibrate the spectrophotometer. For quantification of antioxidant capacity, a standard curve was constructed using aqueous solutions of Fe (II) in concentration range of 0 to 2000 µM. The determination of antioxidant capacity was expressed in µM ferrous sulfate / g solids. All the analyses were performed in duplicate.

2.3.7 Film Thickness

Films thicknesses were measured with accuracy of ± 0.001 mm, at ten different regions of the film, using a micrometer (Mitutoyo, model MDC 25M, MFG, Japan).

2.3.8 Solubility in water

Water solubility of the films was determined according to the method proposed by Gontard, Guilbert, and Cuq (1992). Samples of the films were cut into disks of 2 cm in diameter, in triplicate, dried at 105 °C for 24 h and weighed. The dehydrated samples were individually immersed in 50 mL beakers filled with distilled water, and maintained under slow mechanical agitation (75 rpm) for 24 h at 25 ± 2 °C. After this period, not solubilized samples were removed and dried (105 °C for 24 h) to determine the final dry mass. Solubility was expressed according to Equation (2).

$$\text{Solubilized material}(\%) = \frac{\mathrm{m_{si}} - \mathrm{m_{sf}}}{\mathrm{m_{si}}} \text{ x } 100 \tag{2}$$

In which 'm_{si}' is the initial dry mass of the films (g), 'm_{sf}' is the final dry mass of the non-solubilized films (g).

2.3.9 Water vapor permeability

Water vapor permeability rate of the films was determined gravimetrically based on ASTM E96-80 method (1989), using an acrylic cell, with a central opening (diameter of 4.3 cm), in which the film was fixed. The bottom of the cell was filled with dried calcium chloride, creating a dry environment inside (0% relative humidity at 25 °C). This cell was placed in desiccator containing saturated sodium chloride (75 ± 3% RH).

Water vapor transferred through the film was determined by mass gain of calcium chloride. The cell weight was recorded daily for at least 7 days. The film thickness consisted on the average of 5 random measurements made on different parts of the film. The water vapor permeation rate (PVA) was performed in triplicate and calculated by Equation (3).

$$PVA = \frac{e}{A\,x\,\Delta p}\,x\dot{\mathrm{M}} \tag{3}$$

In which '*PVA*' is permeability to water vapor (g.mm / m^2.day.kPa), '*e*' is mean film thickness (mm), '*A*' is permeation area (m^2), '*Δp*' is partial vapor pressure difference between two sides of films (kPa, at 25 ºC), '$\dot{\mathrm{M}}$' is absorbed moisture rate, calculated by linear regression of weight gain and time, in steady state (g/day).

2.3.10 Mechanical properties

The tensile strength and elongation at break of the films were determined using a texturometer operated according to ASTM standard method D 882-83 (1980), with modifications (Tanada-Palmu, Hélen, & Hyvonen, 2000). For each treatment, 6 films samples were cut into rectangles (100 mm x 25 mm). Its thickness was randomly measured in 5 different parts of sample before analysis. For testing, films were fixed by two distal claws initially 50 mm apart, which moved at speed of 1 mm / s. The tensile strength was calculated by Equation 4. The elongation at break was determined by Equation 5.

$$\mathrm{RT} = \frac{\mathrm{Fm}}{\mathrm{A}} \qquad (4)$$

In which RT corresponds to tensile strength (MPa), Fm is the maximum force at the moment of film rupture (N) and A is cross-sectional area of the film (m^2).

$$\mathrm{E} = \frac{\mathrm{dr}-\mathrm{di}}{\mathrm{dr}}\,\mathrm{x}100 \qquad (5)$$

In which E is elongation (%), dr is the distance at the moment of rupture and di is the initial separation distance (m).

2.3.11 Stability in acidic, neutral and alkaline solutions

The stability of the films in acid, neutral and alkaline solutions was evaluated following the methodology described by Jaramillo, Gutiérrez, Goyanes, Bernal and Famá (2016), with modifications. Samples of the films were cut into disks of 2 cm in diameter, in triplicate, and immersed in containers with 10 mL of standard solutions of hydrochloric acid (pH = 3), distilled water (pH= 7) and sodium hydroxide (pH = 10). The containers were sealed and kept at 5 °C for 12 days. After this period, not solubilized samples were removed and dried (105 °C for 24 h) to determine the final dry mass. Solubility was expressed according to Equation (2), presented in item 2.3.8. Color characteristics of acid, neutral and alkyl solutions after removal of films was determined using a ultraviolet spectrophotometer (model Q798U2M, Quimis, Brazil), with measurement of solutions absorbance at 420, 520 and 620 nm and using water as blank (OIV, 2006). Color intensity was calculated by total sum of absorbance (Abs 420 + Abs 520 + Abs 620). Changes in appearance of the samples were recorded with a camera (Kodak, model Easy Share Z712 IS, 7.1 mega pixels).

2.3.12 Stability of films on sterilization process

Stability of the films on sterilization temperature were assessed by visual changes in appearance. Samples of the films cut into disks of 2 cm in diameter were placed in sealed containers and submitted to 127 °C for 15 minutes in an autoclave. Changes in appearance of the samples were recorded with camera (Kodak, model Easy Share Z712 IS, 7.1 mega pixels).

2.4 Statistical analysis

Significant differences between average results were evaluated by analysis of variance (ANOVA) and Tukey test at 5% of significance level, using SAS software (Cary, NC, USA).

3. Results and discussion

3.1 Films characterization

3.1.1 Visual aspect

In general, the films 0%, 20% BP, 30% BP and 40% BP (liquid blackberry pulp) had good appearance and transparency; were homogeneous and very flexible to handling, and no fractures or grooves were evident. The incorporation of blackberry pulp into films did not make them sticky or fragile to manipulate, but by increasing concentration of blackberry pulp in film-forming solution caused an increase in intensity of reddish coloration in the resulting films, visible to the naked eye. This color of the films can be attractive for elaboration of certain food packages.

3.1.2 Microstructure

Figure 1 A and **B** showed that Film 0% presented a smooth and homogeneous surface. The arrowroot starch promoted formation of an organized, continuous and dense network. For 40% BL films, the **Figure 1 C-F** showed rougher surfaces, continuous regions and disorganized regions due to the presence of blackberry pulp structures within arrowroot starch matrix formed during drying of the films. This fact confirms that blackberry pulp was integrated in arrowroot starch matrix, showing good compatibility between the materials. The presence of structures in blackberry pulp is due to its non-filtration, since the intention was to use the maximum of the solid material of blackberry, rich in nutritional compounds.

Azeredo et al. (2016) also observed changes in microstructure of pectin films with the incorporation of pomegranate juice and citric acid. According to the authors, films containing pomegranate juice presented rougher surfaces than the one without this component, maybe because of the presence of monosaccharide (glucose and fructose) crystallites formed during the film drying.

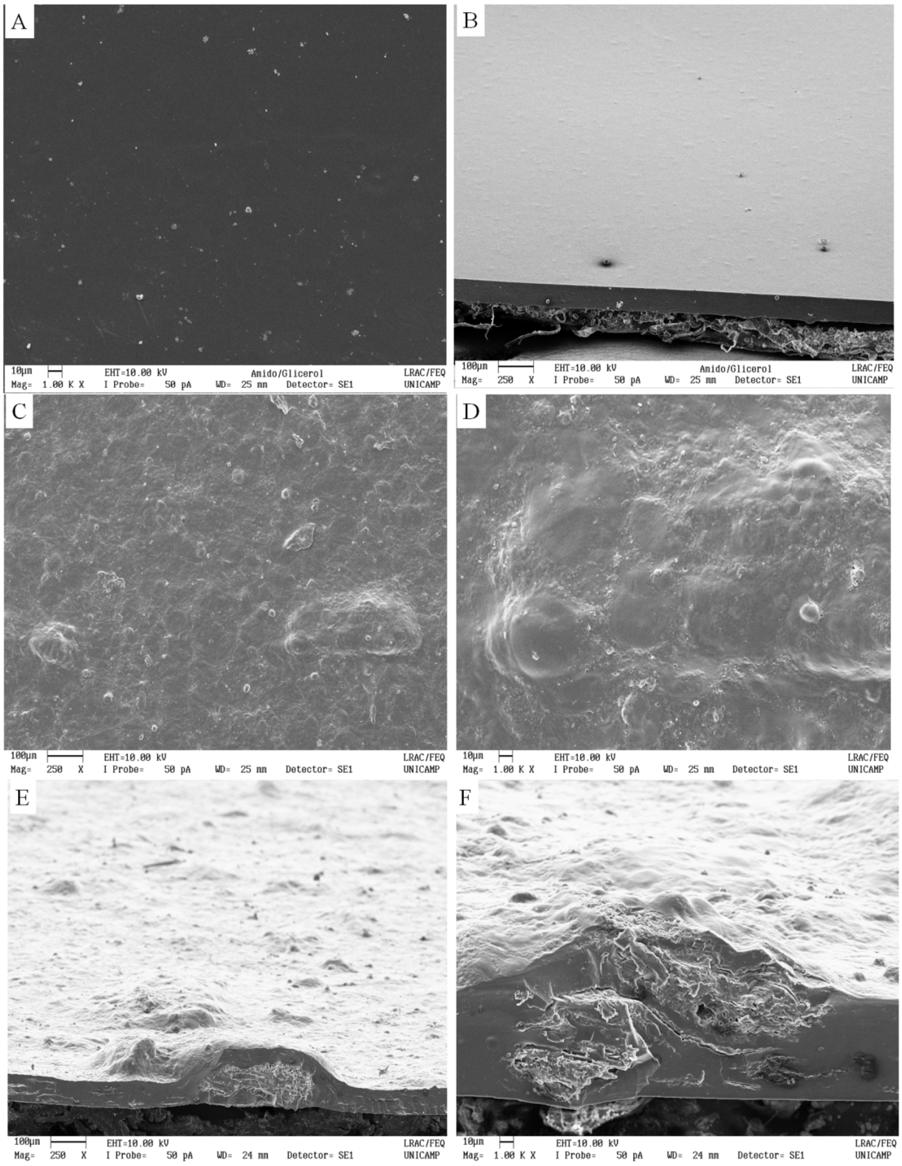


Figure 1. SEM images of the of 0% and 40% BP films: (A) 0% - surface of the control film, 1000x magnification; (B) 0% - cross section of the control film, 250 x magnification; (C and D) 40% BP- surface of the film, 250 and 1000x magnification, respectively; (E and F) 40% BP - cross section of the film, 250 and 1000x magnification, respectively.

3.1.3 Color determination

Differing from control film (0%) which was transparent and odourless, films incorporated with blackberry pulp exhibited notorious color and flavor typical and desirable of blackberry. The blackberry pulp presented colorimetric parameters of 22.64 ± 0.29 for *L**, 16.18 ± 2.24 for *a** and 3.20 ± 0.89 for *b**. Thus, as can be seen in **Table 1**, edible films with blackberry pulp developed colorimetric parameters directly related to that found for blackberry pulp. Based on the colorimetric data, it is possible to observe that the incorporation of blackberry in the arrowroot starch film makes it less transparent and more colorful, with tones ranging from violet to magenta.

Similar behavior was observed for açaí-pectin based edible films, which presented average colorimetric parameters (L^* = 26.96; a^* = 7.17; b^* = 2.66) related to those reported for açaí pulp, with color tones ranging from red to orange-yellow (Espitia et al., 2014).

Table 1. Color parameters of films with 0%, 20%, 30% and 40% of blackberry pulp (BP).

Response variables	FILMS			
	0%	20% BP	30% BP	40% BP
L	91.54 ± 1.16 ^{a}	73.32 ± 2.59 ^{b}	69.61 ± 3.62 ^{b}	54.02 ± 1.45 ^{c}
a^*	1.96 ± 0.09 ^{d}	17.48 ± 1.28 ^{c}	25.12 ± 2.18 ^{b}	36.28 ± 0.70 ^{a}
b^*	-8.64 ± 0.46 ^{b}	-5.56 ± 3.46 ^{ba}	-6.43 ± 0.86 ^{ba}	-2.26 ± 0.31 ^{a}
ΔE	-	24.33 ± 2.44 ^{b}	31.98 ± 4.10 ^{b}	51.24 ± 1.57 ^{a}

BP is liquid blackberry pulp. Same letters in the same column show no statistical difference ($p > 0.05$).

The increase in concentration of blackberry pulp (20% to 40% BP) incorporated in arrowroot starch film (0%) caused a statistically significant increase ($p<0.05$) in *a** and *b** values, leading to decrease of luminosity *L** and increase of total color difference ΔE^* (**Table 1**). Despite the increase in values of *b** with the increase of pulp added in the films, negative values were obtained, showing a tendency to blue color. For coordinate *a**, an expressive increase in their values were observed, evidencing a tendency towards red color and the presence of anthocyanins, as expected. The anthocyanins are pigments, responsible for the red to purple shades of plant foods, like blackberry. So, the coordinate *a** is directly related to the anthocyanins content of blackberry pulp (Yamashita et al., 2017; Laokuldilok & Kanha, 2015).

3.1.4 Water activity and moisture content

Edible films with fruit pulp should have low water activity to be relatively stable against microbial proliferation, and hydrolytic and enzymatic reactions, at room temperature and conditions of usual relative humidity (24 °C and 40% RH) (McHugh, Huxsoll, & Krochta, 1996).

The water activity and moisture content obtained for control films (0%) and films containing blackberry pulp ranged from 0.43 to 0.49 and ranged from 11.30 to 12.53%, respectively, after storage of filmes samples in desiccators at 25°C and 55±3% of relative humidity for 48h (**Table 2**). Similar results were obtained for films of wheat starch and grape juice, with water activity of 0.47 ± 0.03 and moisture content of 11.2 ± 0.1 g/100 g (Kaya & Maskan, 2003). Pure mango films presented moisture contents and water activity of 7.96 ± 1.22% and 0.52 ± 0.03, respectively (Sothornvit & Rodsamran, 2008). This result showed the potential of applying edible films with fruit pulp as a wrapper for food products such as frozen and dried foods, reducing many layers of laminated plastic packaging requirements and providing nutritional value for food products (Sothornvit & Rodsamran, 2008).

Table 2. Water activity, moisture content, anthocyanin content and antioxidant capacity of films with 0%, 20%, 30% and 40% of blackberry pulp (BP).

	FILMS			
Response variables	0%	20% BP	30% BP	40% BP
Aw at 25 °C	0.43 ± 0.05 ^{b}	0.49 ± 0.01 ^{a}	0.46 ± 0.01 ^{ba}	0.46 ± 0.01 ^{ba}
Moisture content (%)	11.30 ± 0.01 ^{ba}	11.38 ± 0.47 ^{b}	12.53 ± 0.44 ^{a}	12.15 ± 0.28 ^{ba}
Total Anthocyanin (mg /100 g of blackberry solids)	0.32 ± 0.12 ^{c*}	37.43 ± 4.31 ^{b}	54.20 ± 4.43 ^{a}	61.57 ± 1.56 ^{a}
FRAP (µmol ferrous sulfate / g of blackberry solids)	14.39 ± 2.15 ^{b*}	481.16 ± 12.44 ^{a}	479.60 ± 7.59 ^{a}	482.74± 0.05 ^{a}

*BP is liquid blackberry pulp. Film 0% anthocyanin content and antioxidant capacity is expressed by total solids, Total Anthocyanin (mg / 100 g of total solids) and FRAP (µmol ferrous sulfate / g of total solids). Same letters in the same column show no statistical difference ($p > 0.05$).

3.1.5 Anthocyanin content and antioxidant capacity

The **Table 2** presents anthocyanin content and antioxidant capacity of films, after drying process. Arrowroot film (0%) had an insignificant antioxidant capacity.

Anthocyanins content and antioxidant capacity of blackberry films are directly related to their content in blackberry pulp. However, there was a significant decrease in anthocyanin content and antioxidant capacity for films with blackberry pulp when compared to blackberry pulp. Blackberry pulp presented anthocyanin content of 109.26 ± 1.75 (mg/100g of blackberry solids) and antioxidant capacity of 782.36 ± 4.67 (μmol ferrous sulfate / g of blackberry solids). Anthocyanins are bioactive compounds sensitive to temperature, light, pH and oxygen (Patras et al., 2010). Consequently, anthocyanins have presented great susceptibility to degradation when exposed to environmental factors during film production, resulting in its decrease in antioxidant capacity.

Jaramillo, Seligra, Goyanes, Bernal and Famá (2015) reported a loss of 13 and 22% in antioxidant capacity during drying of cassava starch films with 5% and 22% of mate herb extract, respectively. Maniglia, Tessaro, Lucas and Tapia-Blácido (2017) also noted that the antioxidant capacity of babassu starch films decreased only slightly. According to the authors, the phenolic compounds may have been lost due to oxidation of phenolic groups by heating applied during preparation of filmogenic solution and during the film drying process.

However, it is observed that the content of anthocyanin and antioxidant capacity increased with incorporation of blackberry pulp in arrowroot starch film. The 20%, 30% e 40% BP films differed significantly ($p<0.05$) from 0% film in anthocyanin content and antioxidant capacity.

Although there was an increase in anthocyanins content of with the addition of concentration of blackberry pulp in the film, the statistical analysis showed no differences in antioxidant capacity between 20, 30 and 40% BP films.

Similar behavior was observed by Chang-Bravo, López-Córdoba and Martino (2014), for extracts of yerba mate and propolis. For both extracts, DPPH radical scavenging activity increased proportionally to their polyphenols content until reaching a plateau in which the antioxidant capacity became independent of the concentration.

In this work, further on anthocyanins, other bioactive compounds such phenolic acids, tannins and ascorbic acid (Machado et al., 2015), are probably present, contributing to the total antioxidant capacity of blackberry pulp, as well as the films.

3.1.6 Film thickness, solubility in water, water vapor permeability and mechanical properties

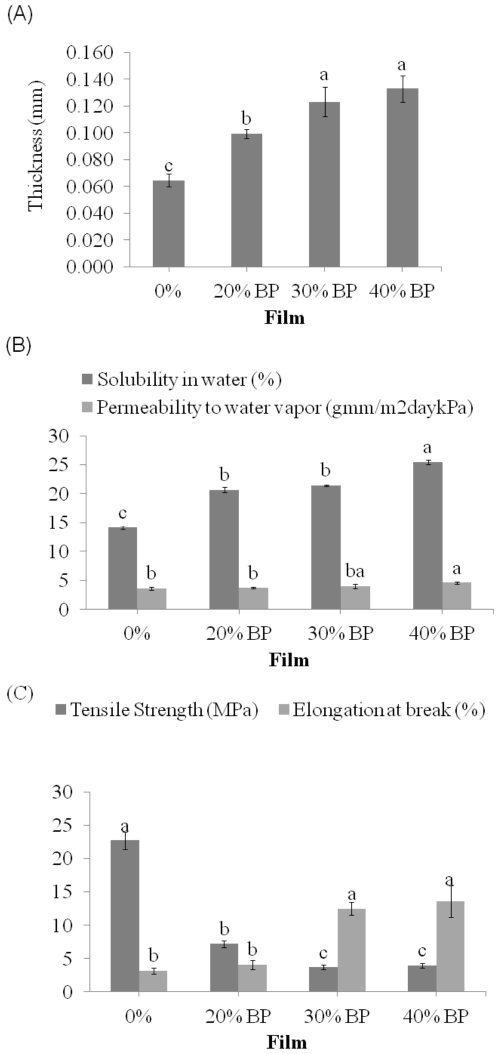
Figure 2 A shows the films thickness. Compared to control film (0%), the films thickness significantly increased ($p<0.05$) with increasing concentration of 20, 30 and 40% BP. This increase in thickness is due to the increase in amount of blackberry pulp solids incorporated in the same volume of film-forming solution deposited on the plate support. Sothornvit and Rodsamran (2008) produced films of mango purée with average thickness of 0.170 ± 0.02 mm, films thicker than the present study. Remembering that thickness presented by films is directly influenced by the amount of solids in the film-forming solution deposited on support plates.

The water solubility and water vapor permeability results and mechanical properties (elongation at break and tensile strength) obtained for films samples are shown in **Figures 2B** and **C**, respectively. Water solubility, permeability to water vapor and elongation at break increased significantly ($p<0.05$) with increasing concentration of blackberry pulp (20, 30 and 40%) in films, while the tensile strength decreases, when compared to control film (0%). Zhai et al. (2017) also found a falling behavior in tensile strength with addition of roselle (*Hibiseus sabdariffa* L.) anthocyanins, increasing from 30 to 120 mg / 100 g of starch, while the elongation at break increased.

The incorporation of blackberry pulp into the arrowroot starch film has made it more soluble and permeable in water, more flexible and less rigid. In fact, blackberry, besides being a source of bioactive compounds, vitamins, essential minerals and fibers, contains proteins, lipids and sugars (Machado et al., 2015). Thus, these structures were incorporated when blackberry pulp was added in arrowroot starch films, disrupting and promoting discontinuity of polymer matrix and making them less mechanically resistant and more soluble. On the other hand, the starch matrix of 0% film was much more organized than film 40% BL, probably due to the greater number of interactions of amylose-amylose, amylopectin-amylopectin and amylose-amylopectin (Mali, Grossmann, & Yamashita, 2010) which allowed the production of resistant films with good barrier properties. Thus, SEM observations allow explaining these results (**Figure 1**). Similar behavior was reported by Maniglia et al. (2017) for films made with babassu mesocarp flour and with starch isolated from babassu mesocarp.

In addition, some studies reported a plasticizer effect of sugars present in fruit pulps when added into polymeric films (Azeredo et al., 2016; Otoni et al., 2014). Azeredo et al. (2016) noted that increasing pomegranate juice/water ratio from 0/100 to 100/0 in pectin films resulted in enhanced elongation (from 2% to 20%), decreased strength (from 10 to <2 MPa) and elastic modulus (from 93 to <10 MPa), increased water vapor permeability (from 3 to 9 g.mm.kPa^{-1}.h^{-1}.m^{-2}), and decreased insoluble matter (from 35% to 24%).

When the plasticizer is incorporated in the film, it modifies the interactions between the macromolecules, increasing the free volume of the system, resulting in an increase in motility of the chains and, consequently, causing reduction in glass transition temperature (Tg) of the system. (Ivanič, Jochec-Mošková, Janigová, & Chodák, 2017; Bergo et al., 2008). The network becomes less dense, facilitating permeation of water in its structure and solubilization, reducing stiffness and increasing extensibility and flexibility of films. Thus, inclusion of plasticizers can affect all the physical properties of films (Bergo et al., 2008).



Same letters show no statistical difference ($p > 0.05$).

Figure 2. Thickness (mm) (A), solubility in water (%) and permeability to water vapor (g.mm/m^2.day.kPa) (B), tensile strength (MPa) and elongation at break (%) (C) of films with 0%, 20%, 30% and 40% of blackberry pulp (BP).

3.1.7 Stability in acidic, neutral and alkaline solutions

The study of stability of films submitted to different pH conditions is an important reference property, considering the potential use of these new polymer films in replacement of intermediate synthetic packaging for food coatings with a wide pH range (fruits, meat and cheese, among others) (Jaramillo et al., 2016). In addition, edible films can be consumed with food, being exposed to changes in pH as they pass through the human gastrointestinal tract. So, it is very important to evaluate the influence of pH on its stability, since it can interfere in the release of anthocyanins into the medium.

The **Figure 3** shows images of the films 0%, 20% BP, 30% BP and 40% BP, after immersion in acid, neutral or alkaline media for 12 days. It can be noted that all samples did not show large variations in diameter, maintaining the same dimensions at the start of the test (2 cm in diameter), showing significant stability in these media. These results differing from the behavior observed by Jaramillo et al. (2016), who noticed a swelling on cassava starch edible films with yerba mate extract when submitted to different pH conditions.

Although there were no changes in films size, it can be observed in **Figure 3** that the films with blackberry pulp had a decrease in intensity of their initial color after immersion in solutions with different pHs. Anthocyanins are natural water-soluble pigments that have wide response ranges to pH variation (Zhai et al., 2017). Probably the films were hydrated when they were immersed in the solutions, causing the solubilization of anthocyanins pigment to the media. This behavior suggests that films with blackberry pulp have potential application for using as controlled release of anthocyanins for food systems.

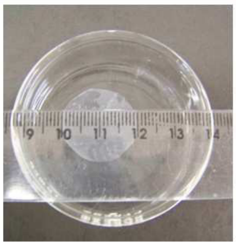
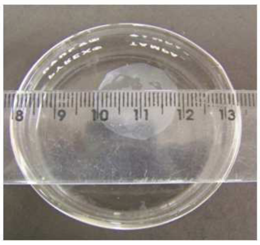
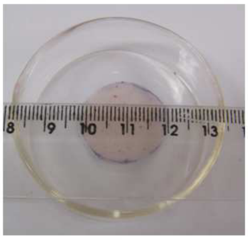
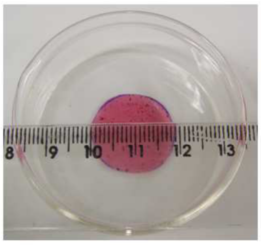
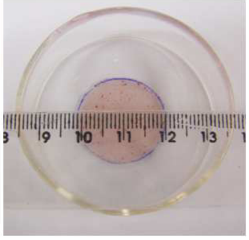
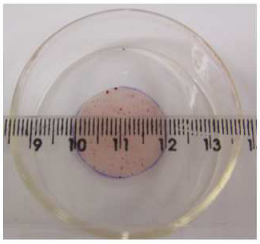
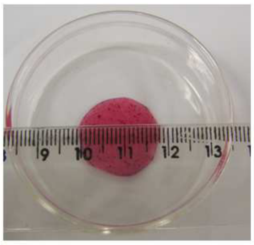
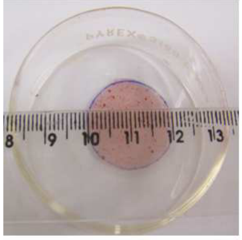
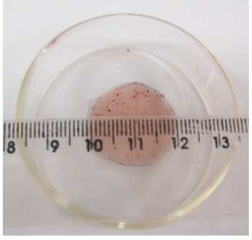
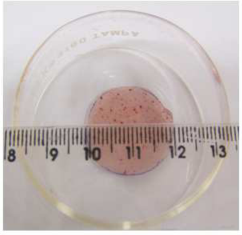
	Stability in acidic, neutral and alkaline solutions			
	Day 0		**After 12 days**	
Film	Film dry	pH3	pH7	pH 10
0%				
Solubility (%)		15.69 ± 0.67 Ca	16.97 ± 1.62 Ca	16.15 ± 0.78 Ca
20% BP				
Solubility (%)		19.82 ± 1.79 Bc	22.61 ± 1.69 Ba	21.57 ± 2.05 Bb
30% BP				
Solubility (%)		29.57 ± 1.51 Aa	26.04 ± 0.78 BAa	25.83 ± 2.24 BAa
40% BP				
Solubility (%)		26.92 ± 2.16 Aa	28.87 ± 1.01 Aa	26.93 ± 0.48 Aa

Figure 3. Digital photographs of arrowroot starch films with 0%, 20% BP, 30% BP and 40% BP, immersed in alkaline (pH 10), neutral (pH 7) and acid media (pH 3) after 12 days. Same uppercase letters in the same column and same lowercase letters in the line show no statistical difference ($p > 0.05$).

It was possible to visualize a slightly pinkish coloration in alkaline, neutral and acidic solutions, after removal of the films. A significant increase ($p<0.05$) in color intensity of solutions in which blackberry pulp films were immersed compared to solutions in which 0%

films were immersed was observed. In addition, the solutions in which the 0% films were immersed presented very low values at the absorbance 520 nm (**Table 3**). The value of the absorbancy at 520 nm can be directly related to the total anthocyanins content, but it is greatly affect by value of pH (Yamamoto et al., 2015).

Table 3. Color characteristics of acid, neutral and alkyl solutions after removal of films.

Response variables	pH 3	pH 7	pH 10
0%			
Abs 420	0.018 ± 0.005 Bba	0.011 ± 0.004 EDb	0.023 ± 0.004 Ea
Abs 520	0.008 ± 0.004 Bb	0.004 ± 0.002 Eb	0.022 ± 0.002 Ea
Abs 620	0.005 ± 0.001 Ba	0.005 ± 0.001 Ea	0.002 ± 0.001 Fb
Color intensity	0.031 ± 0.010 Bba	0.020 ± 0.005 Cb	0.047 ± 0.005 Ba
20% BP			
Abs 420	0.019 ± 0.003 Bb	0.026 ± 0.008 ECDba	0.039 ± 0.005 BCa
Abs 520	0.019 ± 0.003 Bb	0.025 ± 0.002 ECDb	0.050 ± 0.004 BAa
Abs 620	0.003 ± 0.001 Bb	0.063 ± 0.009 BCa	0.008 ± 0.003 Fb
Color intensity	0.040 ± 0.003 Bc	0.114 ± 0.012 Ba	0.097 ± 0.009 Ab
30% BP			
Abs 420	0.033 ± 0.004 BAb	0.037 ± 0.003 ECDba	0.047 ± 0.003 Ba
Abs 520	0.035 ± 0.003 BAb	0.037 ± 0.002 ECDb	0.060 ± 0.004 Aa
Abs 620	0.002 ± 0.001 Bb	0.119 ± 0.034 Aa	0.009 ± 0.005 Fb
Color intensity	0.071 ± 0.004 BAb	0.194 ± 0.031 Aa	0.115 ± 0.002 Ab
40% BP			
Abs 420	0.066 ± 0.028 Aa	0.054 ± 0.003 BECDa	0.028 ± 0.005 DEa
Abs 520	0.075 ± 0.041 Aa	0.056 ± 0.001 BCDa	0.034 ± 0.003 DCa
Abs 620	0.005 ± 0.002 Bb	0.101 ± 0.049 BAa	0.002 ± 0.002 Fb
Color intensity	0.146 ± 0.068 Aa	0.212 ± 0.048 Aa	0.064 ± 0.009 Ba

Same uppercase letters in the same column and same lowercase letters in the line show no statistical difference ($p > 0.05$).

Different anthocyanins generally express distinct color response to pH variation, originated by their structure transformation (Zhai et al., 2017; Garber, Odendaal, & Carlson, 2013; Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). An acidic environment is necessary to form the colored flavylium cation, which induces red color and has been shown to be the most stabile form of anthocyanins (Weber et al., 2017; Castañeda-Ovando et al., 2009). The color intensity of an aqueous solution containing anthocyanins also can be changed by phenomenon of copigmentation, which in turn is affected by pH value, the nature of the copigment or the concentration ratio between copigments and anthocyanins (Yamamoto et al., 2015; Boulton, 2001).

The solubilization of anthocyanins may have helped in solubilization of the films. As more blackberry pulp was incorporated into the film, higher was its solubilization compared to the 0% arrowroot starch film. This fact happened due to the plasticizing effect caused by sugars (Azeredo et al., 2016; Otoni et al., 2014) or interruption of polymer chain caused by structures present in blackberry pulp, which would have allowed a greater mobility of starch chains, favoring its interaction with media solutions. These data were consistent with the water solubility results of films, shown in section 3.1.6.

3.1.8 Stability of films on sterilization process

Depending on the purpose of the application of edible films, they can pack or coat various foods which, according to their characteristics, have to be submitted to sterilization processes, in order to obtain the desired quality and safety factors for their commercialization. The sterilization of food in pressurized autoclaves for inactivation of possible spores and microorganisms present in the product is one of the most used in food preservation (Teixeira & Tucker, 1997).

Figure 4 shows images of films before and after submitting to autoclaving at 127 °C for 15 minutes. It is evident that all films maintained their physical integrity, without presence of cracks or apparent holes, besides remaining handleable after process of sterilization. In view of this, it can be suggested that these films supported the sterilization temperature, and this characteristic can be attributed to the starch, which has shown stability to heat.

For the 0% film, no changes were observed in its color (**Figure 4**). However, in comparison with their visual appearance before sterilization, the films with blackberry pulp showed changes in their color from reddish to brown, and this may be due to the caramelization of sugars, which generates dark compounds. Blackberry is a fruit rich in sugars, as glucose, fructose and sucrose (Kafkas, Koşar, Türemiş, & Başer, 2006).

It is also very likely that this color change behavior may be related to the Maillard reaction (non-enzymatic darkening), which refers to a complex set of reactions, initiated by reaction between amines and reducing sugars, which together at high temperature decompose and condense into an insoluble brown product known as melanoidines (Damodaran, Parkin, & Fennema, 2010). The blackberry pulp may have supplied the amine components and the reducing sugars for that reaction (Machado et al., 2015).

It is also important to emphasize that anthocyanins are sensitive and unstable at high temperatures (Rigon & Noreña, 2016; Eldridge, Repko, & Mumper, 2014; Ferrari, Ribeiro, & Aguirre, 2012), and therefore, it is quite possible that they have degraded during sterilization process. At high temperatures anthocyanin degradation can occur by two mechanisms: the first one is hydrolysis of the 3-glucoside to form the unstable aglicone, and the second is hydrolytic opening of the heterocyclic ring, forming chalcone, which is a colorless anthocyanin structure that, at high temperatures, is degraded into an insoluble brown-colored phenolic (Machado et al., 2015; Simpson, 1985).

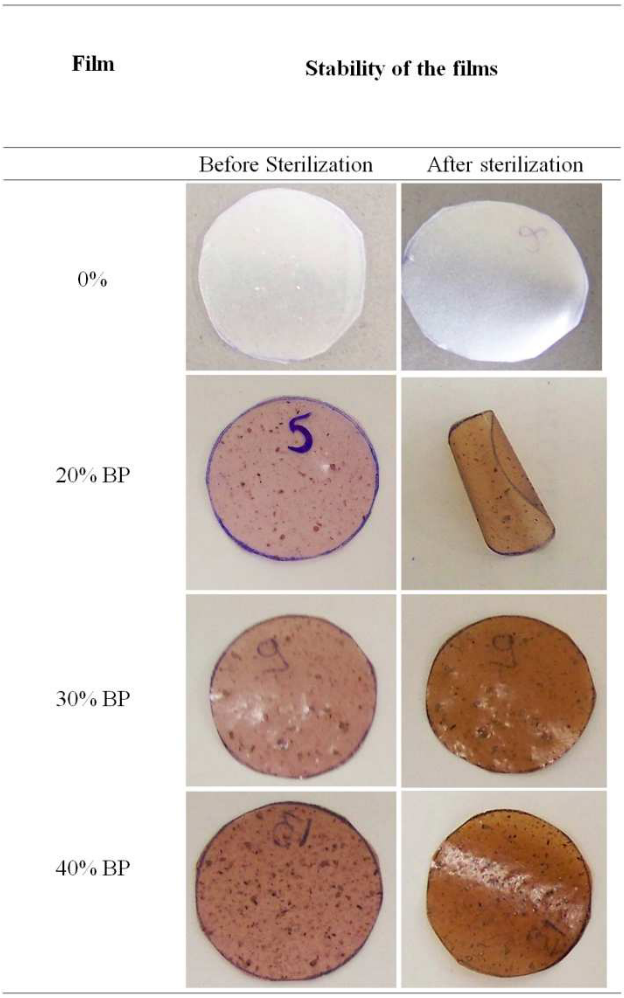


Figure 4. Digital photographs of the arrowroot starch films with 0%, 20% BP, 30% BP and 40% BP, before and after being submitted to sterilization by autoclaving at 127 °C for 15 minutes.

4. Conclusions

Films of arrowroot starch and blackberry pulp had good appearance, homogeneity and flexibility in handling, no fractures or grooves were evident. Increasing incorporation of blackberry pulp into the film-forming solution caused a growing reddish color in resulting films, visible to the naked eye, which may be attractive for the preparation of food packaging. Incorporation of blackberry pulp in film-forming suspension transferred bioactive compounds and antioxidant capacity to the resulting films, as well as typical flavor and color of blackberry pulp. The films with blackberry pulp was (20, 30, 40% BP) mechanically less resistant, thicker, more flexible, soluble and permeable to water vapor than the starch film (0%). These differences in the properties of the films are due to the structures derived from blackberry pulp, such as proteins, fibers, lipids, sugars and bioactive compounds, which generate discontinuity of polymer matrix. The film (0%) was stronger, more rigid, less soluble and less flexible, probably because its structure is more continuous, organized and dense, as confirmed by SEM images. The films had the same initial diameter dimensions after being immersed in acidic, neutral and alkaline solution, but their color intensity was reduced, probably due to the release of anthocyanins to the solutions. There was darkening of the films with blackberry pulp after sterilization process.

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References

A.O.A.C. Official *Methods of Analysis*. (2006) (18th ed.). Association of Official Analytical Chemists, Gaithersburg, Maryland.

ASTM (1980). Standard test methods for tensile properties of thim plastic sheeting. Method D 882-83. In *Annual book of American standard testing methods*. Philadelphia: ASTM.

ASTM (1989). Standard test methods for water vapor transmission of materials. Method E96 e 80. In *Annual book of American standard testing methods*. Philadelphia: ASTM.

Azeredo, H.M.C., Morrugares-Carmona, R., Wellner, N., Cross, K., Bajka, B., & Waldron, K.W. (2016). Development of pectin films with pomegranate juice and citric acid. *Food Chemistry*, 198, 101–106.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, 52, 67–87.

Bergo, P.V.A., Carvalho, R.A., Sobral, P.J.A., Dos Santos, R.M.C., Da Silva, F.B.R., Prison, J.M., Solorza-Feria, J., & Habitante, A.M.Q.B. (2008). Physical Properties of Edible Films Based on Cassava Starch as Affected by the Plasticizer Concentration. *Packaging Technology & Science*, 21, 85–89.

Castañeda-Ovando, A., Pacheco-Hernández, M.D.L., Páez-Hernández, M.E., Rodríguez, J.A., & Galán-Vidal, C.A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, 113, 4, 859-871.

Chang-Bravo, L., López-Córdoba, A., & Martino, M. (2014). Biopolymeric matrices made of carrageenan and corn starch for the antioxidant extracts delivery of Cuban red propolis and yerba mate. *Reactive & Functional Polymers*, 85, 11–19.

D'Agostino, M.F., Sanz, J., Sanz, M.L., Giuffrè, A.M., Sicari, V., & Soria, A.C. (2015). Optimization of a Solid-Phase Microextraction method for the Gas Chromatography–Mass Spectrometry analysis of blackberry (*Rubus ulmifolius Schott*) fruit volatiles. *Food Chemistry,* 178, 10–17.

Damodaran, S., Parkin, K.L., Fennema, O.R. (2010). *Química de alimentos de Fennema.* 4. ed. Porto Alegre : Artmed, p. 900.

Eldridge, J.A., Repko, D., & Mumper, R.J. (2014). Retention of Polyphenolic Species in Spray-Dried Blackberry Extract Using Mannitol as a Thermoprotectant. *Journal of Medicinal Food*, 17, 10, 1064–1069.

Espitia, P.J.P., Avena-Bustillos, R.J., Du, W.X., Teófilo, R.F., Soares, N.F., & McHugh, T.H. (2014). Optimal antimicrobial formulation and physical–mechanical properties of edible films based on açaí and pectin for food preservation. *Food Packaging and Shelf Life*, 2, 38-49.

Farias, M.G., Fakhouri, F.M., Carvalho, C.W.P. d., Ascheri, J.L.R. (2012). Caracterização físico-química de filmes comestíveis de amido adicionado de acerola (*Malphigia emarginata* D.C.). *Química Nova*, 35, 546-552. doi.org/10.1590/S0100-40422012000300020

Fernandes, I., Faria, A., Calhau, C., Freitas, V., & Mateus, N. (2014). Bioavailability of anthocyanins and derivatives. *Journal of Functional Foods*, 7, 54–66.

Ferrari, C.C., Ribeiro, C.P., & Aguirre, J.M. (2012). Spray drying of blackberry pulp using maltodextrin as carrier agent. *Brazilian Journal of Food Technology*, 15, 157-165.

Garber, K.C.A., Odendaal, A.Y., & Carlson, E.E. (2013). Plant pigment identification: A classroom and outreach activity. *Journal of Chemical Education*, 90, 6, 755-759.

Gontard, N., Guilbert, S., & Cuq, J.L. (1992). Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*, 57, 190-199.

Ivanič, F., Jochec-Mošková, D., Janigová, I., & Chodák, I. (2017). Physical properties of starch plasticized by a mixture of plasticizers. *European Polymer Journal*, 93, 843-849.

Jaramillo, C.M., Gutiérrez, T.J., Goyanes, S., Bernal, C., & Famá, L. (2016). Biodegradability and plasticizing effect of yerba mate extract oncassava starch edible films. *Carbohydrate Polymers*, 151, 150–159.

Jaramillo, C.M., Seligra, P.G., Goyanes, S., Bernal, C., & Famá, L. (2015). Biofilms based on cassava starch containing extract of yerba mate as antioxidant and plasticizer. *Starch/Stärke*, 67, 780–789.

Kafkas, E., Koşar, M., Türemiş, N., & Başer, K.H.C. (2006). Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. *Food Chemistry*. 2006, 97, 732-736.

Kaya, S., & Maskan, A. (2003). Watter vapor permeability of pestil (a fruit leather) made from boiled grape juice with starch. *Journal of Food Engineering*, 57, 295-299.

Laokuldilok, T., & Kanha, N. (2015). Effects of processing conditions on powder properties of black glutinous rice (*Oryza sativa* L.) bran anthocyanins produced by spray drying and freeze drying. *LWT-Food Science and Technology*, 64, 405-411.

Machado, A.P.da F., Pasquel-Reátegui, J.L., Barbero, G.F., & Martínez, J. (2015). Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International*, 77, 675–683.

Mali, S., Grossmann, M.V.E., & Yamashita, F. (2010). Filmes de amido: Produção, propriedades e potential de utilização. *Seminário: Ciências Agrárias*, 31, 1, 137-156.

Maniglia, B.C., Tessaro, L., Lucas, A.A., & Tapia-Blácido, D.R. (2017). Bioactive films based on babassu mesocarp flour and starch. *Food Hydrocolloids*, 70, 383-391.

Martinez, C., & Cuevas, F. (1989). *Evaluación de la calidad culinaria y molinera del arroz.* Guia de estudo, CIAT.

Mchugh, T.H., Huxsoll, C.C., & Krochta, J.M. (1996). Permeability properties of fruit puree edible films. *Journal Food Science*. 1996, 61, 88–91.

Moorthy, S.N. (2002). Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54, 559-592.

Nogueira, G.F., Fakhouri, F.M., & de Oliveira, R.A. (2018). Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films. *Carbohydrate Polymers*.https://doi.org/10.1016/j.carbpol.2018.01.024

Otoni, C.G., De Moura, M.R., Aouada, F.A., Camilloto, G.P., Cruz, R.S., Lorevice, M.V., Soares, N.F.F., & Mattoso, L.H.C. (2014). Antimicrobial and physical mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids*, 41, 188–194.

O.I.V. *International Oenological Codex* (2006). Recueil des methodes internationales d'analyse des vins et des moûts, Volume 1 (edition 2006). Paris: Office International de la Vigne et du Vin.

Patras, A., Brunton, N.P., Donnell, C.O., & Tiwari, B.K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, 21, 3 -11.

Rigon, R.T., & Noreña, C.P.Z. (2016). Microencapsulation by spray-drying of bioactive compounds extracted from blackberry (*Rubus fruticosus*). *Journal of Food Science and Technology*, 53, 3, 1515–1524.

Rufino, M., Alves, R., De Brito, E.S., De Moraes, S.M, Sampaio, C.G., Pérez-Jiménez, J.E., & Saura-Calixto, F.D. (2006). Metodologia científica: determinação de atividade antioxidante total em frutas pelo método de redução de ferro (FRAP). *Comunicado Técnico on line. EMBRAPA*, Fortaleza.

Simpson, K. L. (1985). *Chemical changes in natural food pigments*. New York: Van Nostrand Reinhold.

Sims, D.A., & Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, 81, 337-354.

Sothornvit, R., Rodsamran, P. (2008). Effect of a mango film on quality of whole and minimally processed mangoes. *Postharvest Biology and Technology*, 47, 3, 407-415.

Tanada-Palmu, P.S., Hélen, H., & Hyvonen, L. (2000). Preparation, properties and applications of wheat gluten edible biofilms. *Agricultural And Food Science in Finland*, 9, 23-25.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technoloy*, 14, 3, 71-78.

Teixeira, A.A., & Tucker, G.S. (1997). On-line retort control in thermal sterilization of canned foods, *Food Control*, 8, 1, 13 – 20.

Yamashita, C., Chung, M.M.S., dos Santos, C., Mayer, C.R.M., Moraes, I.C.F., & Branco, I.G. (2017). Microencapsulation of an anthocyanin-rich blackberry (spp.) by-product extract by freeze-drying. *LWT - Food Science and Technology*, 84, 256-262.

Yamamoto, L.Y., de Assis, A.M., Roberto, S.R., Bovolenta, Y.R., Nixdorf, S.L., García-Romero, E., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2015). Application of abscisic acid (S-ABA) to cv. Isabel grapes (*Vitis vinifera* × *Vitis labrusca*) for color improvement: Effects on color, phenolic composition and antioxidant capacity of their grape juice. *Food Research International*, 77, 572–583.

Weber, F., Boch, K., & Schieber, A. (2017). Influence of copigmentation on the stability of spray dried anthocyanins from blackberry. LWT- Food Science and Technology, 75, 72-77.

Zavareze, E. da R., El Halal, S. L. M., Pereira, J.M., Radünz, A. L., Elias, M. C., & Dias, A. R. G. (2009). Chemical characterization and extraction yield of rice starch with different amylose contents. *Brazilian Journal of Food Technology*, II SSA, janeiro.

Zhai, X., Shi, J., Zou, X., Wang, S., Jiang, C., Zhang, J., Huang, X., Zhang, W., & Holmes, M. (2017). Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roselle anthocyanins for fish freshness monitoring. *Food Hydrocolloids,* 69, 308-317.

CAPÍTULO 4- Microencapsulation of blackberry pulp with arrowroot starch and gum arabic mixture by spray drying

Este capítulo foi submetido à revista *Journal of Microencapsulation* e está apresentado no formato de artigo científico.

Microencapsulation of blackberry pulp with arrowroot starch and gum arabic mixture by spray drying

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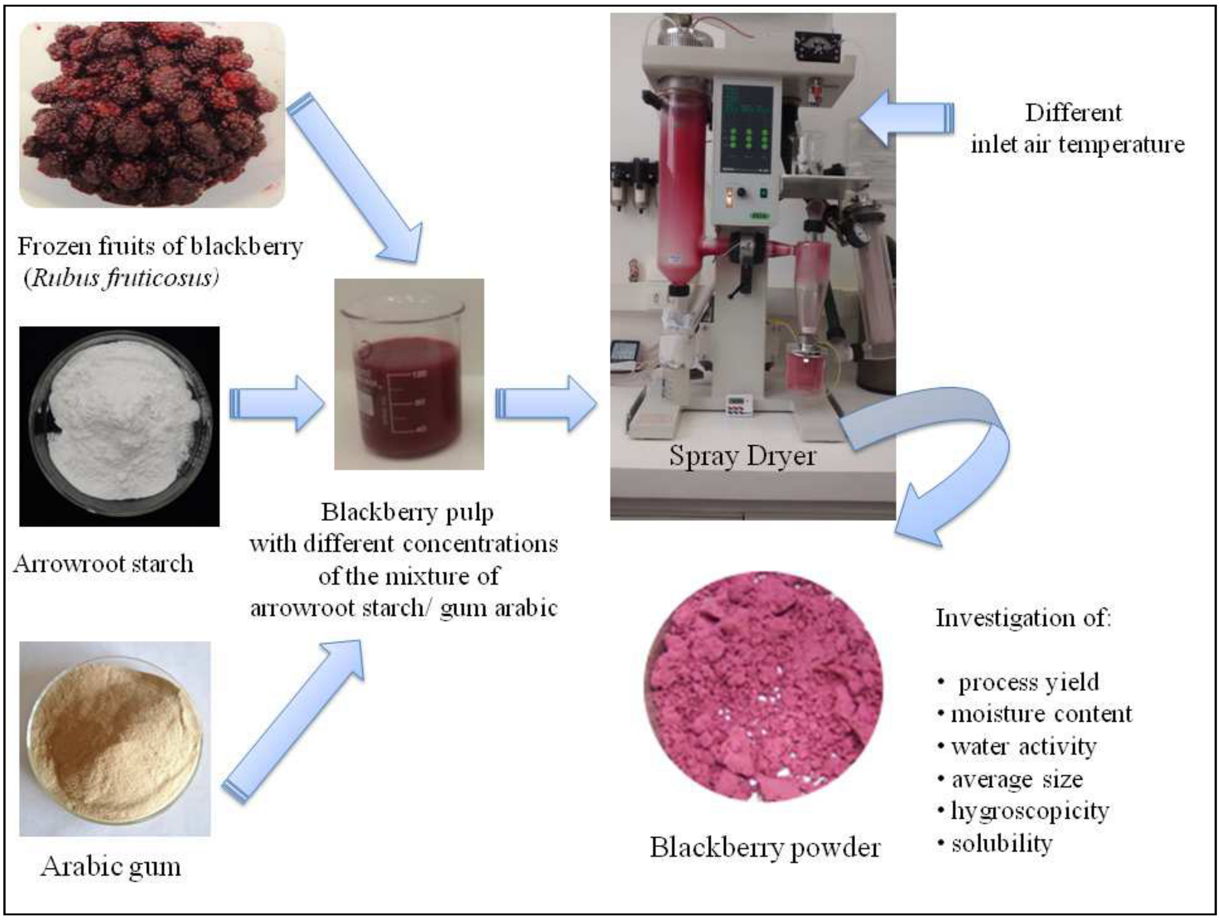
Highlight

- Arrowroot starch and gum Arabic showed Tg above 100°C.
- Arrowroot starch and gum Arabic mixture was used in blackberry pulp spray drying.
- The physicochemical properties of blackberry powder were evaluated.
- Encapsulating agent influenced yield, solubility and hygroscopicity of powders.
- Inlet air temperature influenced yield, solubility and hygroscopicity of powders.

Abstract: This research work aimed to obtain blackberry pulp powder by spray drying and, by an experimental design, evaluated the effect of inlet air temperature (100–150°C) and encapsulating agent concentration (1:0.5–1:2, blackberry pulp solids:arrowroot starch/gum Arabic) on the physicochemical properties of the powders. Arrowroot starch and gum Arabic present Tg values above 100°C; hence it was possible to employ them as carriers in blackberry pulp spray drying in order to increase Tg of the system. The powders produced showed a marked reddish color as well as characteristic flavor of blackberry pulp, being capable of being a sensorially attractive product. Arrowroot starch/gum Arabic mixture (1:1, w/w) was used as encapsulating agent. Powder yield and solubility increased with increasing encapsulating agent concentration, whereas hygroscopicity decreased. Yield, solubility and hygroscopicity of the powders increased and water activity decreased, with increasing inlet air temperature. The powders presented low moisture content and water activity. Temperature of 143°C and encapsulating agent concentration of 1:1.78 were the ideal conditions to obtain high yield and blackberry powders that are soluble in water with less hygroscopic.

Keywords: glass transition temperature, process yield, moisture content, average size, hygroscopicity, solubility.

Graphical Abstract



1. Introduction

Rubus fruticosus or blackberry, belonging to the Rosaceae family, grows abundantly in regions of cold weather, in the north of Europe, Australia, part of Asia, North America and South America [1, 2].

The fruits are highly appreciated by consumers, not only for the combination of their appealing color and desirable flavor and taste, but also for their high nutritional value [3]. In addition to high contents of fibers, vitamins, and essential minerals, blackberry is an important source of phenolic compounds, such as phenolic acids, tannins and anthocyanins, which have high antioxidant capacity [4, 5, 6]. Its consumption has been associated with benefits for human physical and mental health (antioxidant, anticancer, anti-inflammatory and anti-neurodegenerative activities) [7, 8, 9].

However, blackberry is one of the most perishable berries because of its thin and fragile skin and high respiration and transpiration rates, which limit its consumption in the fresh form [2, 10]. During postharvest period, there are fast changes in the blackberry's physicochemical and nutritional properties, such as mass loss, texture alteration and microbiological deterioration. Most blackberries destined for fresh markets became unmarketable after 2 or 3 days when held at 0°C due to decay [10, 11]. About 60% of its production is lost if processed improperly [12].

For this reason, spray drying is an alternative for the processing of blackberry, since the powder obtained is easier to handle, package and transport than the fruit itself, and also provides a high-quality product with prolonged shelf life [13].

More specifically, spray drying is a technological process in which a fluid product is transformed into a powder by atomizing it into a hot gas stream [14]. The feed flow rate, the inlet and outlet air temperatures, atomizer speed, feed concentration, feed temperature and inlet air flow rate are process parameters of spray drying that influence physicochemical properties of the powders produced [15, 16]. Different parameters can be controlled during spray drying process in order to obtain desired characteristics in the final product [17].

However, some intrinsic drawbacks, such as stickiness, hygroscopicity and low solubility may occur. In addition, the adhesion of droplets to the drying chamber walls decreases the process yield [13]. The sticky behavior of juices or fruit pulp powders is attributed to the presence of low molecular weight sugars and organic acids in their composition, which have low glass transition temperatures [18, 19, 20].

Alternatively, high molecular weight encapsulating agents are added to pulps or fruit juices prior to the drying process in order to increase the glass transition temperature of the system and to reduce such problems [18]. Gum arabic, maltodextrins, starches, gelatin, methyl cellulose, gum tragacanth, alginates, pectin, silicon dioxide, tricalciumphosphate, glycerol monostearate and mixtures of some of them are some examples of encapsulating and coating agents used in spray drying [21].

Among starches, arrowroot starch has not yet been used as an encapsulating agent. However it presents a great potential for application for being multifunctional, non-toxic, biodegradable, blood-compatible and bioactive [22, 23]. Due to its high digestibility, gelling and thickener ability, arrowroot starch [24, 25] has been widely used in preparation of bakery products, as in confection of biscuits, cakes, creams and sweets [26].

However, arrowroot starch cannot be easily processed hot, which limits its application in industries. Thus, to increase the functionality of arrowroot starch, it is blended with other polymers to make biocomposites, creating a biomaterial with more functional applications [22]. The mixture of arrowroot starch with gum Arabic has great potential for use as encapsulating agent, since gum Arabic presents many desirable characteristics of a good wall material for drying encapsulating techniques, such as increasing glass transition temperature, imparting high solubility, low viscosity and good emulsifying properties to feed dispersions [27].

This study aimed to produce powdered pulp from blackberry by spray drying using mixture of gum Arabic and arrowroot starch as encapsulating agent, in order to evaluate the impact of drying conditions on the physical properties of spray-dried pulp.

2. Materials and methods

2.1. Blackberry fruits

Frozen fruits of blackberry (*Rubus fruticosus*), cv Tupy, were acquired from "Agro Monte Verde Eirelli", company of Cambuí – MG, Brazil, and used in this research work. Pulp was obtained by grinding blackberry fruits in a blender, previously thawed in refrigerator (8°C) for 24 h. Then, the pulp was sieved to remove seeds, homogenized and packed in polypropylene bottles and coated with aluminium foil to protect against photodegradation. The samples were stored in freezer at -40 ± 3°C until drying. Pulp soluble solids content was 9°Brix, determined with a manual refractometer with a 0–90°Brix range and 0.2°Brix resolution (Reichert, Model AR200, USA). The pulp presented total solids content of 10.3 g/100g of pulp.

2.2. Carrier agent

The encapsulating agents used were gum Arabic Instantgum® (colloids Naturels, São Paulo, Brazil) containing 14.00±0.10% of moisture content, 1.38±0.16% of proteins, 0.37±0.02% of lipids, 3.70±0.10% of ash and 80.46±0.00% of carbohydrates [28], and arrowroot starch containing 15.24±0.19% of water, 0.40±0.03% of protein, 0.12±0.01% of fat, 0.33±0.01% of ash and 83.91±0.00% of carbohydrates [28]. The arrowroot starch was extracted according to the methodology developed by Cruz and El Dash [29]. Arrowroot rhizomes used in the extraction of the starch were obtained in the Faculty of Agronomy, Federal University of Grande Dourados, Mato Grosso do Sul, Brazil, in 2014, in a biome of cerrado and Mata Atlântica. All other reagents used for the analyses presented analytical grade.

2.2.1 Differential Scanning Calorimetry (DSC) of carrier agents

The thermal properties of arrowroot starch and gum Arabic (powder) were studied using a Differential Scanning Calorimeter (DSC1, Mettler Toledo, Schwerzenbach, Switzerland). Aliquots of 10 mg of the samples were weighed in a microanalytical scale (MX5-Mettler Toledo, Schwerzenbach, Switzerland), in an aluminum pan (40 μl). For reference, an empty aluminum pan was used. The samples were submitted to heating program between 25 and 100°C at a rate of 10°C/min, in an inert atmosphere (50 mL/min of N_2). When temperature reached 100 °C, samples were held for 10 minutes at this temperature. After this first scan, the measurement cells were cooled with liquid nitrogen to 25°C, followed by a second heating sweep between 25 and 270°C at rate of 10°C/min in an inert atmosphere (50 mL/min of N_2). The glass transition temperature (Tg) was calculated as the baseline inflection point, caused by the discontinuity of specific heat of the sample.

2.3. Experimental Design

Drying experimental runs were conducted following an experimental design using mixture of arrowroot starch/gum Arabic (1:1, weight/weight) as encapsulating agent. A central composite design – including 4 factorial runs, 4 experimental runs in axial conditions and 3 repetitions of central point, totalling 11 runs – was developed (**Table 1**) to study the effects of dryer inlet air temperature (100–150°C) and encapsulating agent concentration in relation to total solids present in blackberry pulp (1:0.5–1:2, blackberry pulp solids/encapsulating agent, weight/weight) on the powder properties. The response variables considered were drying process yield, moisture content, water activity, hygroscopicity, solubility and particle size.

2.3.1 Microcapsule production

The solution of encapsulating agents and blackberry pulp was performed in mixer type homogenizer at room temperature for 5 minutes. The solutions obtained were diluted in water in a ratio of 1:2 to facilitate spraying, due to its high viscosity.

The resulting homogenized solutions were immediately fed into laboratory spray dryer (Model B191, Büchi, Flawil, Switzerland) equipped with a drying chamber (diameter of 110 mm and height of 435 mm) and dual fluid atomizer nozzle with an orifice of 0.5 mm in diameter, using the following process conditions: airflow of 19 m^3/h; pressurized air flow of 0.6 m^3/h; feed mass flow of 0.2 kg/h. The resultant powder was stored in polyethylene packages in desiccators until its characterization.

2.4. Powder characterization

2.4.1 Drying process yield

Drying process yield was determined by the ratio between powder solids mass and feeding solids mass of blackberry pulp.

2.4.2. Moisture content and water activity

The powder’s moisture content was gravimetrically obtained by vacuum oven at 60°C until constant weight, in triplicate [28]. Water activity (Aw) was determined in triplicate by direct reading at 25°C, using a water activity meter (Aqualab, Decagon Devices, USA).

2.4.3. Hygroscopicity of blackberry powders

Hygroscopicity was determined according to methodology proposed by Cai and Corke [30], with some modifications. Approximately 1 g of each powder sample was placed in a hermetic container at 25°C, with a saturated NaCl solution, to create a 75.7% relative humidity atmosphere. The samples were weighed, in triplicate, after seven days and the hygroscopicity was determined and expressed in g of adsorbed water per 100 g of dry solid (100 g g^{-1}).

2.4.4. Solubility of the blackberry powders

Solubility was determined according to the method described by Eastman and Moore (1984), cited by Cano-Chauca et al. [31]. The method consists in adding 1 g of sample to a vessel containing 100 mL of distilled water, operating with high-speed magnetic stirring – level 4 of magnetic stirrer for 5 minutes, followed by centrifugation at 3000 g for 5 minutes. Aliquot of 25 mL of supernatant was removed and kept in the oven at 105°C until constant weight. The solubility was calculated by weight difference.

2.4.5. Particle size distribution

The particle size distribution was determined by a particle size analyzer based on laser diffraction (Mastersizer 2000, Malvern Instruments, UK). The mean diameter was determined based on the diameter of a sphere with equivalent volume (De Brouckere Mean Diameter, D[4.3]). Samples were analyzed in triplicate with dispersion in 99.5% ethanol.

2.5 Statistical analysis

The results of variable response of the experimental design were evaluated using Statistica 9.0 software. Significant differences between mean results were evaluated by analysis of variance (ANOVA) and Tukey test at 5% level of significance, using SAS software (Cary, NC, USA).

3. Results and discussion

3.1. Differential Scanning Calorimetry (DSC) of the carrier agents

Glass transition temperature (Tg) is an important information obtained from DSC curves. Tg is a value referring to a temperature range that, during the heating of a polymeric material, allows the amorphous chains to acquire mobility [32]. Phase transitions in foods are often a result of changes in composition or temperature during processing or storage.

Knowing the transition temperatures and thermodynamic quantities is important to understand processes such as dehydration, evaporation, freezing and conservation [33].

According to Araújo-Díaz et al. [18], for any substance or compound to be used as an encapsulating agent, it must present a Tg value above boiling temperature of water (approximately 100°C). This is due to the encapsulating agents being alternatively employed in spray drying process of pulps or fruit juices, with the main function of increasing Tg of the system, since these agents are characterized by high molecular weight, low viscosity and Tg in the range of 100–188 °C [34].

Considering that, carbohydrate polymers, arrowroot starch and gum Arabic present Tg values above 100°C and, hence, can be employed as carriers in spray drying blackberry pulp.

The thermogram of arrowroot starch, with moisture content of 15.24% (dry base), presented in Figure 1A, showed Tg of 118.19°C, with the beginning of this transition at about 116.52°C and the end at about 118.20°C. The endothermic peak around 140°C observed in thermogram (**Figure 1A**) of arrowroot starch was attributed mainly to evaporation of water present in starch.

The thermogram of potato starch with moisture content of 3.7% w/w (UR 11%) showed Tg of 161.72°C, while for the same sample with moisture content of 18.8% w/w (UR 75%) Tg of 141.91°C [35]. The Tg presented by starch depends heavily on moisture content of amylose and amylopectin, molecular interactions between starch and low molecular weight co-solutes and the inherent characteristics of the used measurement protocol [36].

For gum Arabic with moisture content of 14% (dry base), the thermogram showed Tg of 125.8°C, with the beginning of this transition at around 124.16°C and the end at around 125.97°C (**Figure 1B**). A similar endothermic peak for gum Arabic was reported by Mothé and Rao [33] at about 90°C. According to the authors, this peak appeared due to the melting of crystallites during heating of gum Arabic with lower moisture content (0–40%).

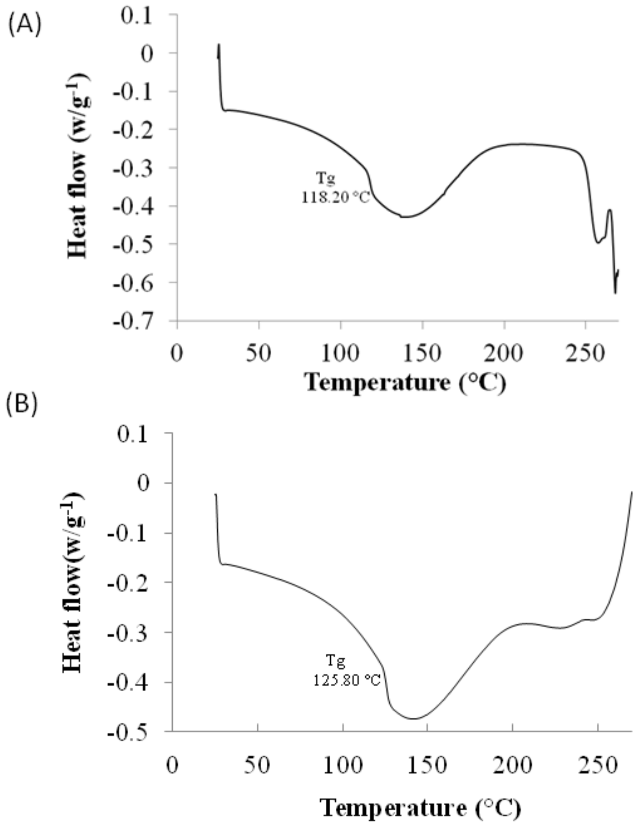


Figure 1. DSC curve obtained for arrowroot starch (A) and gum arabic (B).

3.2 Experimental design

The experimental data for process yield (Y), moisture content (M), water activity (Aw), average size (D[4.3]), hygroscopicity (H) and solubility (S) were determined using 11 combinations of the independent variables, as shown in **Table 1**.

The results were fitted using a second-order regression model. The codified regression coefficients, F-values, p-values and coefficients of determination (R^2) are presented in **Table 2**. Nonsignificant terms, at confidence level of 95%, were eliminated and the predictive models were tested for adequacy and accuracy of fit by ANOVA. When calculated F-value (Fc) was greater than the tabulated F-value (Ft), the variation was explained by the regression and not by residues. Thus, the regression was significant, and the model could be considered predictive. **Figure 2** shows the response surfaces generated by the proposed models.

Table 1. Experimental design for blackberry spray drying with arrowroot starch and gum Arabic mixture (1:1, m/m) as encapsulating agent.

T	XT	XP	Y	M	Aw	D[4.3])	H	S
1	107 (-1)	1 : 0.72 (-1)	49.57±1.21 ^{ba}	2.28±0.94 ^{cd}	0.26±0.06 ^{cb}	54.81±2.15 ^{g}	12.07±0.19 ^{de}	68.56±0.60 ^{bc}
2	107 (-1)	1 : 1.78 (1)	50.62±1.16 ^{ba}	3.69±1.01 ^{b}	0.29±0.04 ^{b}	71.50±0.57 ^{e}	10.93±0.17 ^{f}	58.39±0.99 ^{d}
3	143 (1)	1 : 0.72 (-1)	34.40±4.95 ^{dc}	1.67±0.67 ^{cde}	0.15±0.03 ^{e}	84.15±1.44 ^{d}	15.31±0.17 ^{a}	71.63±0.73 ^{b}
4	143 (1)	1 : 1.78 (1)	50.06±3.08 ^{ba}	0.97±0.57 ^{e}	0.18±0.03 ^{e}	63.11±0.84 ^{f}	12.99±0.04 ^{c}	74.47±0.56 ^{ba}
5	100 (-1.41)	1 : 1.25 (0)	28.77±1.74 ^{d}	5.87±1.47 ^{a}	0.36±0.04 ^{a}	74.31±0.26 ^{e}	12.61±0.19 ^{dc}	64.08±1.61 ^{dc}
6	150 (1.41)	1: 1.25 (0)	36.70±2.18 ^{c}	3.89±0.78 ^{b}	0.26±0.06 ^{cbd}	66.14±0.32 ^{f}	13.54±0.10 ^{b}	79.37±5.25 ^{a}
7	125 (0)	1 : 0.5 (-1.41)	44.61±3.32 ^{b}	3.91±1.01 ^{b}	0.26±0.02 ^{cb}	105.98±0.84 ^{b}	13.56±0.29 ^{b}	63.39±1.13 ^{dc}
8	125 (0)	1 : 2 (1.41)	56.54±1.38 ^{a}	1.43±0.26 ^{de}	0.17±0.06 ^{e}	50.94±0.61 ^{g}	11.66±0.24 ^{e}	60.22±1.31 ^{d}
9	125 (0)	1 : 1.25 (0)	56.95±3.62 ^{a}	1.58±0.56 ^{cde}	0.19±0.03 ^{ed}	94.10±2.48 ^{c}	12.79±0.17 ^{c}	60.15±5.62 ^{d}
10	125 (0)	1:1.25 (0)	53.32±1.41 ^{a}	1.99±0.66 ^{cde}	0.18±0.02 ^{e}	93.15±1.14 ^{c}	11.91±0.20 ^{e}	61.87±0.93 ^{d}
11	125 (0)	1 : 1.25 (0)	53.17±1.68 ^{a}	2.74±0.82 ^{cb}	0.20±0.04 ^{ced}	119.79±3.54 ^{a}	12.15±0.11 ^{de}	62.41±1.14 ^{d}

The independent variables correspond to the real values. Values in parentheses correspond to the coded values. XT, inlet air temperature (°C); XP, encapsulating agent concentration (1:0.5–1:2, blackberry pulp solids/encapsulating agent, weight/weight); Y, process yield (%); M, moisture content (%); Aw, water activity (decimal); D[4.3], average size (μm); H, hygroscopicity (g of adsorbed water/100 g of solids); S, solubility (%). The values of Y, D[4.3] and H represent means of three determinations. The values of M, S and Aw represent the means of eleven, four and nine determinations, respectively.

Table 2. Regression coefficients and adjusted R^2 for the final reduced models.

Regression coefficient	**Y**	**Aw**	**H**	**S**
Mean	53.76	0.19	12.68	61.98
Linear				
XT	Ns	-0.045	0.83	5.10
XP	4.32	Ns	-0.77	Ns
Quadratic				
XT^2	-9.46	0.049	Ns	5.34
XP^2	Ns	Ns	Ns	Ns
Interaction				
XT x XP	Ns	Ns	Ns	3.25
R^2	0.774	0.765	0.734	0.938
Fc	13.67	13.01	11.02	35.37

XT, inlet air temperature (°C); XP, encapsulating agent concentration (1:0.5–1:2, blackberry pulp solids/encapsulating agent, weight/weight). Y, process yield (%); Aw, water activity (decimal); H, hygroscopicity (g of adsorbed water/100 g of solids); S, solubility (%); R^2, regression coefficients; *Fc*, F calculated; ns, nonsignificant ($p>0.05$).

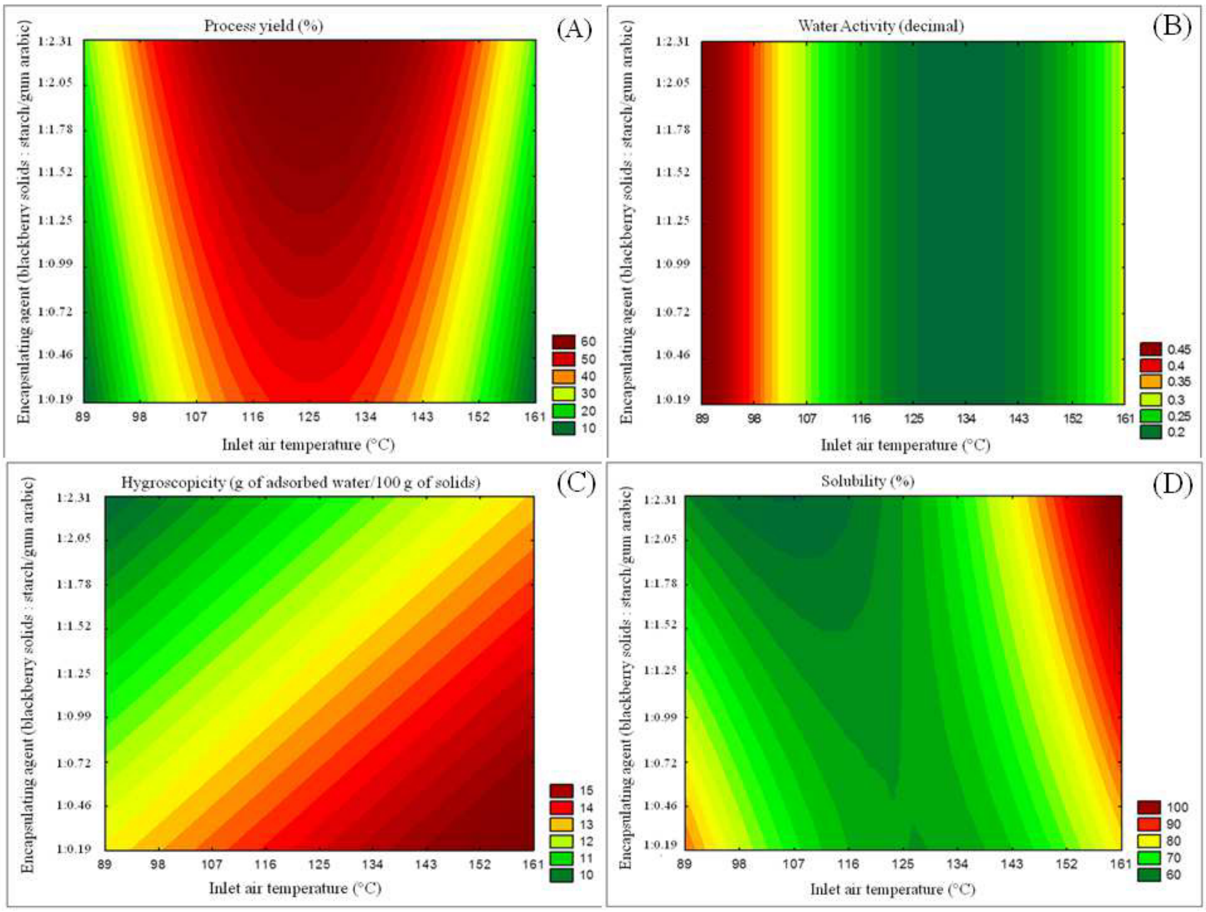


Figure 2. Model contour plots for process yield (%) (A); water activity (decimal) (B); hygroscopicity (g of adsorbed water/100 g of solids) (C); solubility (%) (D), as a function of inlet air temperature (°C) and encapsulating agent concentration (blackberry pulp solids/encapsulating agent, weight/weight).

3.2.1 Process yield

According to **Table 1**, the experimental process yield values for blackberry pulp spray drying using arrowroot starch and gum Arabic mixture as encapsulating agent ranged between 28.77 and 56.95%. These values were similar to those obtained for spray drying of açai of 34.3–55.7% [37], for jussara pulp of 33.88 to 76.55% [13], for beetroot juice ranged between 41.31% and 54.63% [38]. Eldridge, Repko and Mumper [39] demonstrated a recovery of 61.3% of blackberry powder contained at 6.3:1 (w/w) mannitol:blackberry extract.

Blackberry is a fruit rich in sugars of low molecular weight such as glucose, fructose and sucrose [40], which have low Tg value: Tg 32°C, Tg 14.5°C, and Tg 45°C, respectively [19]. Tg presented by its pulp suffers direct influence from the present sugars, which makes the spray drying process difficult. In this work, it was not possible to obtain powdered blackberry pulp without addition of encapsulating agent, because the pulp behaved as syrup, sticking to the walls of dryer and obtaining a caked product instead of a dry powder. Without carrier, pomegranate juice formed a hard glass film on spray dryer chamber walls in dehydration process [41].

With incorporation of encapsulating agent in blackberry pulp before drying process, it was possible to obtain powder, but at low concentrations of encapsulating agent in relation to the total blackberry pulp solids, a large powder deposit was still observed in the walls of drying chamber and cyclone. This same behavior was reported by Igual et al. [21] and Santana et al. [13] during drying of lulo pulp and jussara pulp, respectively. Retention of product on the chamber wall is undesirable [13]. Process yield corresponds to product recovery and is mainly determined by the powder collection efficiency [42].

The blackberry pulp drying process yield was significantly ($p<0.05$) influenced by inlet air temperature of the dryer and encapsulating agent concentration, which presented quadratic negative effect and positive linear effect, respectively (Table 2).

The highest yields were obtained when higher encapsulating agent concentrations and inlet air temperature were used, as shown in **Figure 2A**. The experimental run 8 and the central point runs presented the highest drying process yield values (53.17–56.95%), which were significantly different ($p<0.05$) from results of other experimental runs. This behavior indicates that addition of encapsulating agent to blackberry pulp before spray drying was sufficient for a possible increase of glass transition temperature of system. Increased encapsulating agent concentration favored blackberry pulp drying, reducing stickiness of powders encapsulation by arrowroot starch and gum Arabic mixture, consequently decreasing adherence of solids in drying chamber, which resulted in increased yield values. These data indicate that the arrowroot starch and gum Arabic mixture used as encapsulating agent presented compatibility between them and with blackberry pulp, confirming their adequacy as drying carrier.

As for temperature, the increase in dryer's inlet air temperature caused increase in process yield, which could be attributed to the greater efficiency of heat and mass transfer processes [38]. However, at temperatures above 143°C, there was decrease in yield, probably due to the caramelization of encapsulating agents, as well as of sugars present in blackberry pulp [43], which resulted in more adhesion to wall, so the amount of powder production and yield was reduced.

All the produced powders showed marked reddish color and flavor characteristics of blackberry fruit (**Figure 3**).

3.2.2 Moisture content

The results of statistical analysis applied to experimental data of the powders moisture content showed that the effects of linear, quadratic and interaction factors were not significant at 95% confidence interval (p>0.05). Due to these nonsignificant factors, it was not possible to construct a predictive mathematical model for behavior of moisture content of blackberry powders in function of independent variables.

However, **Table 1** shows that all powders produced presented moisture content values lower than 6%. Ferrari et al. [2] obtained approximately 3% moisture content for blackberry juice powder produced by spray drying using inlet air temperature of 160°C and 5% of maltodextrin as encapsulating agent (w/w). The moisture content of sumac extract powders ranged between 2.94 and 4.22% (wet basis) [44] and 0.9 to 7% for lulo powders [21].

For powder products, moisture content had great impact on flowability, stickiness and storage stability because of its plasticizing effects and crystallization behavior [45]. Since water acts as plasticizer, only a small amount is required to change glass transition temperature, which increases the food matrix mobility during storage and causes alterations in powder [46]. Thus, low water contents are desirable for powders. Any experimental run within the studied range for inlet air temperature and encapsulating agent concentration will result in formation of powder with mean moisture content below 6% that is desirable to keep it microbiologically stable.

3.2.3 Water activity

Water activity values of powders ranged from 0.18±0.03 to 0.36±0.04 (**Table 1**). Cagaita (*Eugenia dysenterica* DC.) fruit extract powders presented water activity values lower than 0.3 [17]. In the present study, except for run 5, that showed water activity of 0.36±0.04, results of all other runs were below the limit of 0.30, indicating that they are microbiologically stable, as there is no microbial growth below this value, as well as that there is delay in non-enzymatic browning, one of the main reactions of deterioration [47].

The powders water activity was not significantly influenced ($p>0.05$) by the encapsulating agent concentration. This means that powders produced with any encapsulating agent concentration within the range studied will present low values for water activity. On the other hand, the dryer's inlet air temperature significantly influenced ($p<0.05$) the water activity (**Table 2**).

Figure 2B shows that the lowest water activity values are found at inlet air temperatures above 100°C, regardless of the encapsulating agent concentration. This may be due to higher heat transfer to the particle, resulting in greater driving force for water evaporation, thus producing powders with lower moisture content and water activity [17].

3.2.4 Average Size

Powder particles of all the experimental runs presented varied and large sizes, with diameters ranging from 50.94±0.61 to 119.79±3.54 µm (**Table 2**). Mango powders produced with maltodextrin by spray drying ranged from 0.47 to 549 µm, while, without maltodextrin they ranged from 1.9 to 955 µm [48].

Drying temperatures and encapsulating agent concentration are parameters that may affect the particle size of powders obtained by spray drying. When drying is performed at higher temperatures, the process is faster than drying at lower temperatures, leading to immediate formation of particles and avoiding particle shrinkage. Similarly, encapsulating agent concentration increases feed viscosity and results in particles with larger diameters [49]. Higher liquid viscosities result in larger particles in atomizers and larger particles in spray drying chamber [50].

Besides the encapsulating agent, high content of sugars and acids in the fruit also increases viscosity of feed mixture during spraying in drying chamber. This introduces agglomeration and, consequently, produces larger particles [51]. Solutions of blackberry pulp and encapsulating agent used in feeding were visually very viscous, which may have led to particles with larger diameters.

Araujo-Díaz et al. [18] observed a general contraction of the volume of blueberry powder without encapsulating agent, indicating collapse of microstructure of the sample and suggesting crystallization of low melting sugars present in fruit juice. Islam et al. [52] observed with scanning electron microscopy that orange juice/maltodextrin 60:40 powders were fused and clumped together and that orange juice/maltodextrin 50:50 powders were agglomerated. Agglomerations were not found in the powders produced with higher maltodextrin ratio (orange juice/maltodextrin 40:60 and orange juice/maltodextrin 30:70).

In this study, inlet air temperature and encapsulating agent concentration did not significantly affect particle size. The results of statistical analysis applied to the experimental data of the powders average size showed that the effects of linear, quadratic and interaction factors were not significant at 95% confidence level ($p>0.05$). Due to these nonsignificant factors, it was not possible to construct a predictive mathematical model for the behavior of average size of blackberry powders in function of independent variables.

It is believed that, in this work, the presence of larger particles was due to a possible agglomeration process. Similar behavior was observed by Zotarelli et al. [48] for mango powder obtained by spray drying. This can be attributed to the formation of bridges between the smallest particles, resulting in agglomeration that formed larger particles [48].

3.2.5 Hygroscopicity

Hygroscopicity is a material's capacity to absorb moisture from the surrounding environment, and it is an important property to be considered, due to its influence on food stability [17]. The blackberry powders were stored for 7 days in desiccators with saturated NaCl solution and 75.7% relative humidity at 25°C. As shown in **Figure 3**, after 3 days in this condition, the powders of all tests absorbed water from environment, causing agglomeration of the particles and darkening of the powders. After 7 days, powders had a very sticky appearance, indicating that blackberry powders are unstable and hygroscopic, which may hinder their handling.

Figure 3. Photographs of the blackberry powders, of the 11 experimental runs, stored for 7 days in desiccator, at 25 °C and relative humidity of 75%.

Hygroscopicity values of powders obtained by blackberry pulp drying using an arrowroot starch and gum arabic mixture as encapsulating agent ranged from 10.93±0.17 to 15.31±0.17 g of adsorbed water/100 g of solids (**Table 1**). These results were lower than those found by Daza et al. [17] for cagaita powders obtained by using gum arabic (14.8–18.8 g of absorbed water per 100 g of sample) and inulin (13.8 and 19.4 g of absorbed water per 100 g of sample) as carrier. Dried beetroot juice particles presented hygroscopicity values of 14.46–20.68 g of water/100 g of dry matter [38].

Inlet air temperature and encapsulating agent concentration significantly influenced ($p<0.05$) the hygroscopicity of blackberry powders, which presented positive and negative linear effect, respectively (**Table 2**). Inlet air temperature was the variable that most influenced the final product's hygroscopicity.

According to **Figure 2C**, the lowest values of hygroscopicity were obtained when lower inlet air temperature and higher encapsulating agent concentration were used. This same behavior was observed by Bazaria and Kumar [38] for dried beetroot juice particles. According to the authors, this could be well explained by the increasing moisture content with lower inlet air temperature. Powders with very low moisture content tend to be more hygroscopic. The capacity to adsorb ambient moisture is related to the water concentration gradient between the product and the surrounding air [37].

As for the encapsulating agent concentration, both arrowroot starch and gum arabic are materials with low hygroscopicity; consequently, blackberry pulp microencapsulation tends to reduce the resulting powder's hygroscopicity. This fact demonstrated the capacity of arrowroot starch and gum arabic mixture as encapsulating agent to improve the hygroscopicity values of the blackberry powders obtained and make them more stable. These results are in agreement with those observed for orange juice, cactus pear juice, cagaita fruit extracts and beetroot juice powders obtained by spray drying, which showed decreased hygroscopicity due to increased carrier agent concentration [45, 17, 38, 53].

Hygroscopicity of orange juice powder decreased from 0.195±0.02 to 0.143±0.01 g H_2O/g with increasing maltodextrin concentration [45]. In this study, the blackberry powder of run 3 produced with inlet air temperature of 143 °C and ratio of blackberry pulp solids and encapsulating agent of 1:0.72 was the most hygroscopic (15.31 g of adsorbed water/100 g of solids), whereas the powder of run 2, produced with inlet air temperature of 107 °C and ratio of blackberry pulp solids and encapsulating agent of 1:1.78, was the least hygroscopic (10.93 g of adsorbed water/100 g of solids).

3.2.6 Solubility

Solubility is another important parameter that may be evaluated after powder production, because it can potentially affect some of their properties, such as the reconstitution of dry extract or the availability of encapsulated compounds in a food system [17]. Solubility of blackberry powder particles ranged from 58.39±0.99 to 79.37±5.25% (**Table 1**), lower than those reported by Daza et al. [17] and Santana et al. [13] in their studies on spray dried cagaita (*Eugenia dysenterica* DC.) fruit extracts obtained by using gum arabic (94.4–97.8%) and inulin (87.7–95.9%) and spray dried jussara pulp produced by using ternary mixture of gum arabic/maltodextrin/whey protein concentrate (78.81–93.46%) and gum arabic/modified starch/soy protein isolate (81.23–92.75%), respectively.

The differences in solubility can be explained by chemical structure of each carrier agent. The occurrence of water adsorption by a carbohydrate is attributed to the links between hydrogen present in water molecules and hydroxyl groups available in amorphous regions of substrate as well as in regions that have a crystalline surface [54].

The solubility of blackberry powders was significantly ($p<0.05$) influenced by variable inlet air temperature, presenting a positive linear and quadratic effect. The interaction between inlet air temperature and encapsulating agent concentration also showed a significant positive effect on the solubility of blackberry powders (**Table 2**).

Less soluble powders were obtained at lower inlet air temperatures and higher encapsulating agent concentrations (**Figure 2D)**, similar to the behavior observed for hygroscopicity. Although gum arabic is highly soluble [17], arrowroot starch in its natural form is highly insoluble in water at room temperature, which probably contributed to the decrease of blackberry powders solubility.

On the other hand, more soluble powders were produced when extreme inlet air temperatures were used, as shown in **Figure 2D**. Higher temperatures result in faster drying rates, avoiding the particle encountering during drying, maintaining the initial structure formed. Consequently, more porous or fragmented particles are produced at higher temperatures, increasing the surface of particles exposed to water, facilitating the access of water into its structure, hence favoring solubilization [43].

3.2.7 Optimization of process parameters and analysis of the model

The optimum spray drying conditions for blackberry pulp were determined by surface response methodology, in order to obtain higher process yield and soluble powders in water with low hygroscopic. Due to variation in particle size causing agglomeration it was not possible to generate a predictive mathematical model for the behavior of average size of blackberry powders as a function of independent variables, this response variable was not considered for choosing optimal drying conditions.

Moisture content and water activity were low for all runs (moisture content < 6% and water activity < 0.36). Therefore, powders produced with any inlet air temperature and encapsulating agent concentration within the studied range would present good microbiological stability, hence these response variables were not considered to choose optimal drying conditions.

Analyzing the model for process yield (**Figure 2A**), the highest values were observed using intermediate inlet air temperature and high encapsulating agent concentrations. According to **Figure 2C**, the lowest values for hygroscopicity were obtained when lower inlet air temperature and higher encapsulating agent concentration were used. On the other hand, more soluble powders were produced at extreme inlet air temperatures and high encapsulating agent concentration, as shown in **Figure 2D.**

Thus, the temperature of 143 °C was recommended as optimized condition, since at higher temperatures, powder solubility increased and process yield were reduced at temperatures above 143 °C. As process yield and powder solubility increased with increasing encapsulating agent concentration and hygroscopicity decreased, the ratio of blackberry pulp solids to encapsulating agent of 1:1.78 was used as optimum condition. Run 4, with inlet air temperature of 143 °C and encapsulating agent concentration of 1:1.78 (blackberry pulp solids:arrowroot starch/gum arabic) was capable of producing powders with low hygroscopicity (12.99 g of adsorbed water/100 g of solids), good solubility in water (74.47%), and satisfactory process yield (50.06%), as shown in **Table 1**.

The optimized conditions for blackberry pulp spray drying were experimentally reproduced and process yield, hygroscopicity and solubility were determined in triplicate, as shown in **Table 3**, with predicted mean and experimental values according to the quadratic model.

Table 3. Predicted, experimental and relative deviation values for the responses at optimum conditions (143°C inlet air temperature and 1:1.78 ratio of blackberry pulp solids to encapsulating agent) for spray dried blackberry pulp powder.

Responses	**Experimental value**	**Predicted Value**	**% Relative Deviation ***
Drying Process Yield (%)	57.69±8.97	48.62	15.72
Hygroscopicity (g of adsorbed water/100 g of solids)	17.22±0.45	12.68	26.36
Solubility in water (%)	59.64±1.15	75.67	26.88

* Relative deviation = ABS[(experimental value - predicted value) / experimental value] * 100.

As expected, the powder produced in optimized condition had low moisture content and water activity, 1.45±0.51% and 0.13±0.04, respectively, indicating microbiological stability. The values observed for process yield, hygroscopicity, and solubility were relatively close to those observed previously for run 4, as shown in **Table 1**. Based on the values for relative deviation (% DR) obtained for each response variable, the optimization methodology used was considered satisfactory.

4. Conclusions

Arrowroot starch and gum arabic present Tg values above 100 °C; hence it was possible to employ them as carriers in blackberry pulp spray drying in order to increase the Tg of the material. The produced powders showed a marked reddish color as well as characteristic flavor of blackberry pulp, being capable of being a sensorially attractive product. The powders presented moisture content values lower than 6% and water activity below 0.36, indicating microbiological stability. The particles presented varied and large size, suggesting possible agglomeration. Increasing encapsulating agent concentration increased the yield of blackberry pulp drying process and solubility of the powders in water and decreased their hygroscopicity. The arrowroot starch and gum arabic mixture used as encapsulating agent was capable of making blackberry pulp more stable by encapsulation. Particles produced with higher drying inlet air temperatures showed higher values for solubility in water. High temperatures increased process yield; however, at temperatures above 143 °C there was reduction possibly due to caramelization of the encapsulating agent and sugars present in blackberry pulp. Temperature of 143 °C and encapsulating agent concentration of 1:1.78 were the ideal conditions to obtain high yield and blackberry powders that are soluble in water and less hygroscopic.

Conflict of interest

The authors have declared no conflict of interest.

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References

[1] A. Gomar, A. Hosseini, N. Mirazi, Preventive effect of *Rubus fruticosus* on learning and memory impairment in an experimental model of diabetic neuropathy in male rats, PharmaNutrition 2 (2014) 155–160.

[2] C.C. Ferrari, C.P. Ribeiro, J.M. Aguirre, Spray drying of blackberry pulp using maltodextrin as carrier agent, Brazilian Journal of Food Technology 15(2012) 157-165.

[3] M.F. D'Agostino, J. Sanz, M.L. Sanz, A.M. Giuffrè, V. Sicari, A.C. Soria, Optimization of a Solid-Phase Microextraction method for the Gas Chromatography–Mass Spectrometry analysis of blackberry (*Rubus ulmifolius* Schott) fruit volatiles, Food Chemistry 178 (2015) 10–17.

[4] A.P.F. Machado, J.L. Pasquel-Reátegui, G.F. Barbero, J. Martínez, Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods, Food Research International 77 (2015) 675–683.

[5] T.J. Hager, L.R. Howard, R. Liyanage, J.O. Lay, R.L. Prior, Ellagitannin composition of blackberry as determined by HPLC-ESI-MS and MALDI-TOF-MS, Journal of Agricultural and Food Chemistry 56 (2008) 661–669.

[6] I. Elisia, C. Hu, D.G. Popovich, D.D. Kitts, Antioxidant assessment of an anthocyanin-enriched blackberry extract, Food Chemistry 101 (2007) 1052–1058.

[7] M. Zia-Ul-Haq, M. Riaz, V. De Feo, H.Z. Jaafar, M. Moga, *Rubus fruticosus* L.: constituents, biological activities and health related uses, Molecules 19 (2014) 10998–11029.

[8] L. Tavares, I. Figueira, D. Macedo, G.J. McDougall, M.C. Leitão, H.L.A. Vieira, D. Stewart, P.M. Alves, R.B. Ferreira, C.N. Santos, Neuroprotective effect of blackberry

(*Rubus* sp.) polyphenols is potentiated after simulated gastrointestinal digestion, Food Chemistry 131 (2012) 1443–1452.

[9] J. Dai, J.D. Patel, R.J. Mumper, Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties, Journal of Medicinal Food 10 (2007) 258–65.

[10] M. Joo, N. Lewandowski, R. Auras, J. Harte, E. Almenar, Comparative shelf life study of blackberry fruit in bio-based and petroleum-based containers under retail storage conditions, Food Chemistry 126 (2011) 1734–1740.

[11] R.E. Hardenburg, A.E. Watada, C.Y. Wang, The commercial storage of fruits, vegetables and commercial nursery stocks. US Department of agriculture, Agriculture Handbook (1986) (p. 66).

[12] C.A.J. Romero, B.D.V. Yépez, Ultrasound as pretreatment to convective drying of Andean blackberry (*Rubus glaucus* Benth), Ultrasonics Sonochemistry 22 (2015) 205–210.

[13] A. Santana, D.M. Cano-Higuita, R.A. de Oliveira, V.R.N. Telis, Influence of different combinations of wall materials on the microencapsulation of jussara pulp (*Euterpe edulis*) by spray drying, Food Chemistry 212 (2016) 1–9.

[14] A. Gharsallaoui, G. Roudaut, O. Chambin, A. Voilley, R. Saurel, Applications of spray-drying in microencapsulation of food ingredients: An overview, Food Research International 40 (2007) 1107–1121.

[15] G.R. Chegini, B. Ghobadian, Effect of spray-drying conditions on physical properties of orange juice powder, Drying Technology 23 (2005) 657–668.

[16] M.I. Ré, Microencapsulation by spray drying, Drying Technology 16 (1998) 1195-1236.

[17] L.D. Daza, A. Fujita, C.S. Fávaro-Trindade, J.N. Rodrigues-Ract, D. Granato, M.I. Genovese, Effect of spray drying conditions on the physical properties of Cagaita (*Eugenia dysenterica* DC.) fruit extracts, Food and Bioproducts Processing 97(2016) 20-29.

[18] S.B. Araujo-Díaz, C. Leyva-Porras, P. Aguirre-Bañuelos, C. Álvarez-Salas, Z. Saavedra-Leos, Evaluation of the physical properties and conservation of the antioxidants content, employing inulin and maltodextrin in the spraydrying of blueberry juice, Carbohydrate Polymers 167 (2017) 317–325.

[19] M.Z. Saavedra-Leos, C. Alvarez-Salas, M. Esneider-Alcalá, A. Toxqui-Terán, S.A. Pérez-García, M. Ruiz-Cabrera, Towards an improvedcalorimetric methodology for glass transition temperature determination inamorphous sugars, CyTA − Journal of Food 10 (2012) 258–267.

[20] B.R. Bhandari, N. Datta, T. Howes, Problems associated with spray drying of sugar-rich foods, Drying Technology 15 (1997) 671–684.

[21] M. Igual, S. Ramires, L.H. Mosquera, N. Martínez-Navarrete, Optimization of spray drying conditions for lulo (*Solanum quitoense* L.) pulp, Powder Technology 256 (2014) 233–238.

[22] C.S. Wu, H.T. Liao, Interface design and reinforced features of arrowroot (*Maranta arundinacea*) starch/polyester-based membranes: Preparation, antioxidant activity, and cytocompatibility, Materials Science and Engineering C 70 (2017) 54–61.

[23] C. Winarti, N. Richana, T.C. Sunarti, Effect of acid hydrolysis on the characteristics of andrographolide-loaded arrowroot starch nanoparticles, International Journal of Food Engineering 1 (2015) 29–33.

[24] F. Villas-Boas, C.M.L. Franco, Effect of bacterial β-amylase and fungal α-amylase on the digestibility and structural characteristics of potato and arrowroot starches, Food Hydrocolloids 52 (2016) 795- 803.

[25] R. Hoover, Composition, molecular structure, and physicochemical properties of tuber and root starches: a review, Carbohydrate Polymers, 45 (2001) 253 -267.

[26] M. Leonel, M.P. Cereda, S.B.S. Sarmento, Arrowroot (*Maranta arundinacea*) as a Possible Raw Material for Starch Industries. Brazilian Journal Food Technology 5 (2002) 151-155.

[27] Ramírez, M.J., Giraldo, G.I., & Orrego, C.E. (2015). Modeling and stability of polyphenol in spray-dried and freeze-dried fruit encapsulates. Powder Technology, 277, 89–96.

[28] A.O.A.C. Official Methods of Analysis, (18th ed.). Association of Official Analytical Chemists, Gaithersburg, Maryland (2006).

[29] R. Cruz, A.A.M. El Dash, Amido de chuchu (*Seichium edule,* Swartz). Efeito de fosfatação em sua viscosidade. Boletim SBCTA, 18, 4, 371-378 (1984).

[30] Y.Z. Cai, H. Corke, Production and properties of spray-dried *Amaranthus* betacyanin pigments. Journal of Food Science 65 (2000) 1248-1252.

[31] M. Cano-Chauca, P.C. Stringheta, A.M. Ramos, J. Cal-Vidal, Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization, Innovative Food Science and Emerging Technologies 5(2005) 420–428.

[32] D. Schlemmer, M.J.A. Sales, I.S. Resck, Preparação, Caracterização e Degradação de Blendas PS/TPS Usando Glicerol e Óleo de Buriti como Plastificantes, Polímeros: Ciência e Tecnologia 20 (2010) 6-13.

[33] C.G. Mothé, M.A. Rao, Thermal behavior of gum Arabic in comparison with cashew gum. Thermochimica Acta 357-358 (2000) 9-13.

[34] Z. Saavedra -Leos, C. Leyva-Porras, S.B. Araujo-Díaz, A. Toxqui-Terán, A.J. Borrás-Enríquez, Technological application of maltodextrins according to the degree of polymerization, Molecules 20 (2015) 21067–21081.

[35] L. Chuang, N. Panyoyai, L. Katopo, R. Shanks, S. Kasapis, Calcium chloride effects on the glass transition of condensed systems of potato starch, Food Chemistry 199 (2016) 791–798.

[36] J. Perdomo, A. Cova, A.J. Sandoval, L. Garía, E. Laredo, A.J. Müller, Glass transition temperatures and water sorption isotherms of cassava starch, Carbohydrate Polymers 76 (2009) 305–3013.

[37] R.V. Tonon, C. Brabet, M.D. Hubinger, Influence of process conditions on the physicochemical properties of açai (*Euterpe oleraceae* Mart.) powder produced by spray drying, Journal of Food Engineering 88 (2008) 411–418.

[38] B. Bazaria, P. Kumar, Optimization of spray drying parameters for beetroot juice powder using response surface methodology (RSM), Journal of the Saudi Society of Agricultural Sciences xxx (2016) xxx–xxx.

[39] J.A. Eldridge, D. Repko, R.J. Mumper, Retention of Polyphenolic Species in Spray-Dried Blackberry Extract Using Mannitol as a Thermoprotectant, Journal of Medicinal Food 17 (2014) 1064–1069.

[40] E. Kafkas, M. Kosar, N. Türemis, K.H.C. Baser, Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey, Food Chemistry, 97 (2006) 732-736.

[41] S. Yousefi, Z. Emam-Djomeh, S.M. Mousavi, Effect of carrier type and spray drying on the physicochemical properties of powdered and reconstituted pomegranate juice (*Punica granatum* L.), Journal of Food Science and Technology 48 (2011) 677–684.

[42] S. Wang, T. Langrish, A review of process simulations and the use of additives in spray drying. Food Research International 42 (2009) 13–25.

[43] V.B. Souza, M. Thomazini, J.C.C. Balieiro, C.S. Fávaro-Trindade, Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained

from vinification by products of the Bordo grape (*Vitis labrusca*), Food and Bioproducts Processing 93 (2015) 39–50.

[44] G. Caliskan, S.N. Dirim, The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders, Powder Technology 287 (2016) 308–314.

[45] M.Z. Islam, Y. Kitamura, Y. Yamano, M. Kitamura, Effect of vacuum spray drying on the physicochemical properties, water sorption and glass transition phenomenon of orange juice powder, Journal of Food Engineering 169 (2016) 131–140.

[46] A.M. Goula, K.G. Adamopoulos, A new technique for spray drying orange juice concentrate, Innovative Food Science and Emerging Technologies 11 (2010) 342-351.

[47] D.S. Reid, O.R. Fennema, Água e gelo. In: S. Damodaran, K.L. Parkin, O.R. Fennema, (Eds.), Química de Alimentos de FENNEMA. Artmed, Porto Alegre, 25–74 (2010).

[48] M.F. Zotarelli, V.M. da Silva, A. Durigon, M.D. Hubinger, J.B. Laurindo, Production of mango powder by spray drying and cast-tape drying, Powder Technology 305 (2017) 447–454.

[49] A.V. Calomeni, V.B. de Souza, F.L. Tulini, M. Thomazini, L.C. Ostroschi, S.M. de Alencar, A.P. Massarioli, J.C.C. Balieiro, R.A. de Carvalho, C.S Favaro-Trindade, Characterization of antioxidant and antimicrobial properties of spray-dried extracts from peanut skins, Food and Bioproducts Processing 105(2017) 215-223.

[50] S.M. Jafari, M.G. Ghalenoei, D. Dehnad, Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder, Powder Technology 311(2017) 59-65.

[51] A.K. Shrestha, T. Ua-arak, B.P. Adhikari, T. Howes, B.R. Bhandari, Glass transition behavior of spray dried orange juice powder measured by differential scanning calorimetry (DSC) and thermal mechanical compression test (TMCT), International Journal of Food Properties 10 (2007) 661–673.

[52] M.Z. Islam, Y. Kitamura, M. Kokawa, K. Monalisa, F.H. Tsai, S. Miyamura, Effects of micro wet milling and vacuum spray drying on the physicochemical and antioxidant properties of orange (*Citrus unshiu*) juice with pulp powder. Food and Bioproducts Processing 101 (2017) 132–144.

[53] G.R. Rodriguez-Hernandez, R. Gonzalez-Garcia, A. Grajales-Lagunes, M.A. Ruiz-Cabrera, M. Abud-Archila, Spray-drying of cactus pear juice (*Opuntia streptacantha*): effect on the physicochemical properties of powder and reconstituted product, Drying Technology 23 (2005) 955–973.

[54] A.N. Negrão-Murakami, G.L. Nunes, S.S. Pinto, F.S. Murakami, E.R. Amante, J.C.C. Petrus, E.S. Prudêncio, R.D.M.C. Amboni, Influence of DE-value of maltodextrin on the physicochemical properties, antioxidant activity, and storage stability of spray dried concentrated mate (*Ilex paraguariensis* A. St. Hil.), LWT - Food Science and Technology 79 (2017) 561-567.

CAPÍTULO 5- Influence of spray drying in bioactives compounds of blackberry pulp

Este capítulo foi submetido à revista *Food Chemistry* e está apresentado no formato de artigo científico.

Influence of spray drying in bioactive compounds of blackberry pulp

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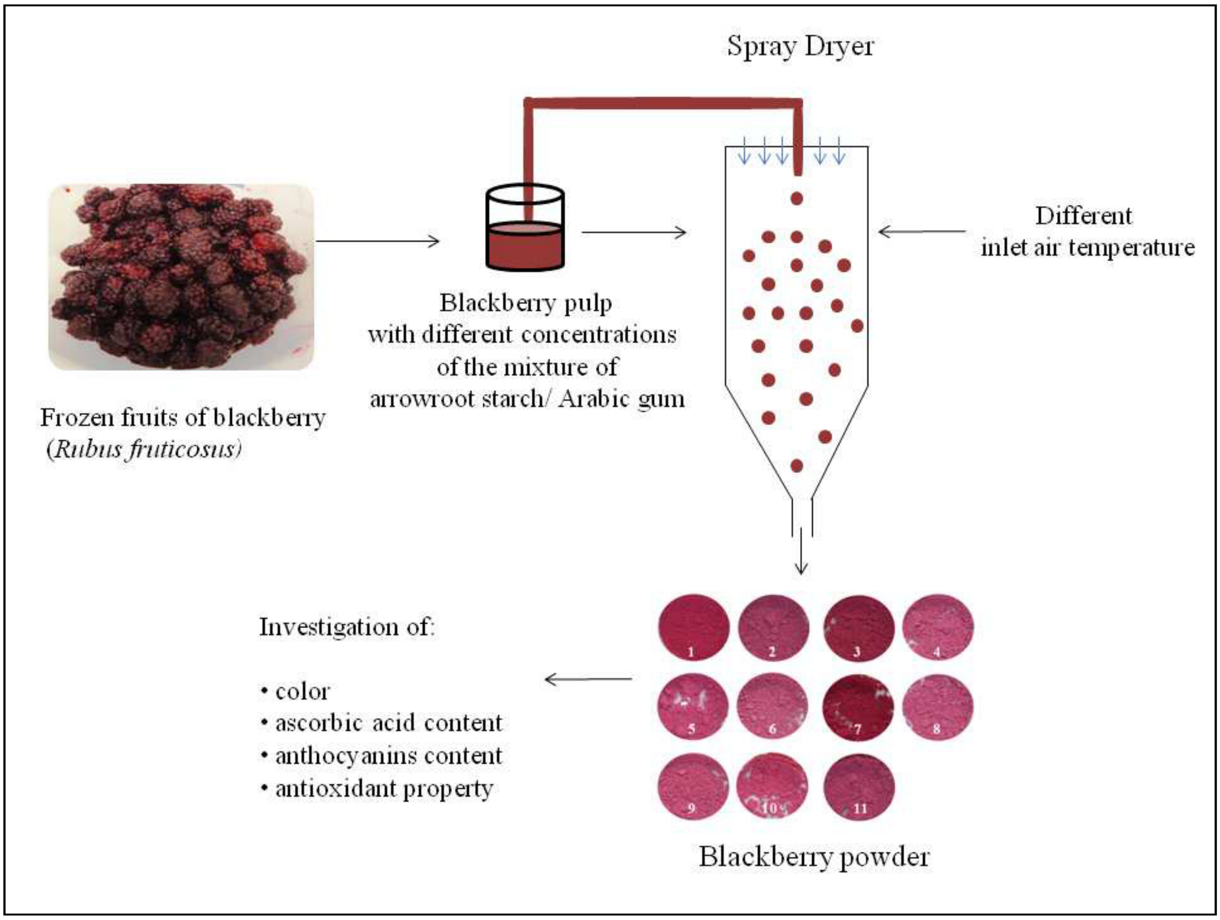
Highlight

- Arrowroot starch and gum arabic were employed in drying of blackberry pulp.
- Arrowroot starch / gum arabic exhibited thermoprotective effect during spray drying.
- Encapsulating agent influences the ascorbic acid content of blackberry powder.
- Blackberry powder with high anthocyanin content was obtained at high temperatures.
- Blackberry powder shows antioxidant property.

Abstract: The objective of this research work was to obtain blackberry pulp in microencapsulated powder with arrowroot starch/ gum arabic mixture by spray drying. Experimental design, with 11 runs, was performed to evaluate the effects of inlet air temperature (100-150°C) and encapsulating agent concentration (1:0.5 - 1:2, blackberry pulp solids: arrowroot starch/gum arabic) on functional properties of powders. The ascorbic acid content and luminosity of powder increased with increasing encapsulating agent concentrations, whereas *b** values decreased. Increasing inlet air temperature and decreasing encapsulating agent concentration, the content of anthocyanins also increased. The powders were able to reduce Fe^{+3} and to trap free radicals, showing antioxidant property. The temperature of 143°C and concentration of encapsulating agent 1:1.78 were the ideal conditions to have high content of ascorbic acid, content of anthocyanins and antioxidant properties.

Keywords: arrowroot starch, gum arabic, inlet air temperature, anthocyanins, ascorbic acid, antioxidant property.

Graphical Abstract



1. Introduction

Blackberry, originally from Asia, is currently produced in countries of Europe, North America and South America, growing in regions of cold weather (Ferrari, Ribeiro, & Aguirre, 2012). In addition to its attractive color and flavor characteristics, blackberry (*Rubus fruticosus*), cv. Tupy, is a fruit rich in phenolic compounds, such as phenolic acids, tannins and anthocyanins, which have high antioxidant capacity (Machado, Pasquel-Reátegui, Barbero & Martínez, 2015). Studies with fruits rich in phenolic compounds, specifically anthocyanins, show that these bioactive compounds provide functional benefits for humans, reducing free radicals that carry chain reactions able to damage cells, acting in the prevention of certain cancers and cardiovascular diseases, and also acting on the immune system, due to their antioxidant action (Masisi, Beta & Moghadasian, 2016; Leung, 2008).

However, blackberry presents short shelf life, due its fragile structure, high respiratory rate and high moisture content (92% in wet basis) (Ferrari et al., 2012). Besides, it is a seasonal fruit, restricting its use as a source of antioxidants and limiting consumption and market availability.

Spray drying of blackberry pulp is an alternative for processing this fruit, since the powder obtained is easier to handle, packing and transport than the fruit itself, and also provides a high-quality product with prolonged shelf life (Rigon & Noreña, 2016; Eldridge, Repko & Mumper, 2014; Ferrari et al., 2012).

Spray drying is the most widely used process for drying liquids and extracts due to its short time of contact with drying medium and high rate of evaporation, generating a high quality product compared to conventional drying methods with relatively low costs. Although spray drying is widely used to obtain food powders, due to its high operation temperature, some quality losses can be observed (Caliskan & Dirim, 2016). This is because the bioactive compounds are unstable due to their sensitivity to high temperature, light, pH, oxygen, among other processing conditions, changing their functional and antioxidant properties (Patras, Brunton, Donnell & Tiwari, 2010).

In this context, microencapsulation can be a good alternative to maintain and stabilize these bioactive compounds and promote controlled release of active ingredient (Desai, Liu & Park, 2006). Although most often considered a dehydration process, spray drying can also be used as an encapsulation method when it incorporates “active” material within a protective matrix, which is essentially inert to the encapsulated material (Ré, 1998). Different encapsulating agents were used as carriers and coating agents during spray drying of blackberry pulp, among them: gum arabic and polydextrose (Rigon & Noreña, 2016), mannitol (Eldridge et al., 2014), maltodextrin 20 DE (Ferrari et al., 2012) and maltodextrin 19 DE (Weber & Schieber, 2017).

Gum arabic is widely used in food industry. It is nontoxic, odorless and tasteless. It interacts with water and has a wide range of applications, such as emulsification, texture control and flavor encapsulation (Mothé & Rao, 2000). Until now, no study used arrowroot starch as encapsulating agent during spray drying. The arrowroot starch and gum arabic mixture has great application potential as encapsulating agent, since arrowroot starch has great functionality for being a hydrocolloid, thickening and gelling agent (Hoover, 2001). It also presents good digestibility (Villas-Boas & Franco, 2016) and special physicochemical characteristics, such as high amylose content (Moorthy, 2002).

Physicochemical properties of powders produced by spray drying depend on the encapsulating agent and also the operational parameters like feed flow rate, inlet and outlet air temperatures, atomizer speed, feed concentration, feed temperature and inlet air flow rate (Chegini & Ghobadian, 2005, Ré, 1998). Among them, the inlet and/or outlet air temperatures are the most effective factors for spray drying to be optimized (Igual, Ramires, Mosquera & Martínez-Navarrete, 2014). The aim of this research work, therefore, was to optimize the spray drying conditions of blackberry (*Rubus fruticosus*) pulp to produce a stable powder with high nutritional and functional values. The inlet air temperature and the concentration of mixture of arrowroot starch/gum arabic used as encapsulating agent were considered as the process variables.

2. Materials and methods

2.1. Blackberry fruits

In this study, frozen fruits of blackberry (*Rubus fruticosus*), cv Tupy, were acquired from “Agro Monte Verde Eirelli”, a company from city of Cambuí, MG, Brazil. Pulp was obtained by grinding blackberry fruits in a blender, previously thawed in the refrigerator (8°C) for 24 h. Then, pulp was sieved to remove the seeds, homogenized, packed in polypropylene bottles and coated with aluminum foil to protect against photodegradation. The samples were stored in a freezer at -40 ± 3°C until drying. Pulp soluble solids content was 9°Brix (Reichert, Model AR200, USA). The pulp presented total solids content of 10.3 g/100g of pulp.

2.2. Encapsulating agent

Arrowroot rhizomes were obtained in the Faculty of Agronomy, Federal University of Grande Dourados, Mato Grosso do Sul, Brazil, in a biome of *cerrado* and Atlantic Forest. The arrowroot starch was extracted according to the methodology developed by Cruz and El Dash (1984). The following encapsulating agents were used: arrowroot starch containing 15.24 ± 0.19% of water, 0.40 ± 0.03% of protein, 0.12 ± 0.01 % of fat, 0.33 ± 0.01% of ash and 83.91 ± 0.00% of carbohydrates (AOAC, 2006); and gum arabic (Instantgum®, colloids Naturels, São Paulo, Brazil) containing 14.00 ± 0.10% of water content, 1.38 ± 0.16% of proteins, 0.37 ± 0.02% of lipids, 3.70 ± 0.10% of ash and 80.46 ± 0.00% of carbohydrates (AOAC, 2006). All other reagents used for the analyses presented analytical grade.

2.3. Microcapsule production, experimental design and analysis

Drying experimental runs were conducted following an experimental design using an arrowroot starch/gum arabic mixture (1:1, w/w) as encapsulating agent. A central composite design, including 4 factorial runs, 4 experimental runs in axial conditions and 3 repetitions of central point, totaling 11 runs, was developed (**Table 1**) to study the effects of dryer inlet air temperature (100 to 150°C) and encapsulating agent concentration in relation to the total solids present in blackberry pulp (1:0.5 to 1:2, solids of blackberry pulp/encapsulating agent, weight/weight) on the functional properties of the powders. The solution of encapsulating agents and blackberry pulp was performed in a mixer type homogenizer at room temperature for 5 minutes. The solutions obtained were diluted in water in a 1:2 ratio to facilitate spraying, due to its high viscosity.

Spray drying of the samples was performed in a laboratory spray dryer (Model B191, Büchi, Flawil, Switzerland) equipped with a drying chamber (diameter of 110 mm and height of 435 mm) and dual fluid atomizer nozzle with an orifice of 0.5 mm of diameter, employing the following process conditions: airflow 19 m^3/h; pressurized air flow of 0.6 m^3/h and feed mass flow of 0.2 kg/h. The outlet air temperature was monitored to observe its variation as a function of pulp feeding. The resultant powder was stored in polyethylene packages in desiccators. The response variables considered were color, ascorbic acid content, anthocyanins content and antioxidant capacity.

2.4. Powder characterization

2.4.1. Samples color

The color parameters of powder samples were determined by reflectance, using a colorimeter (Ultra Scan Vis 1043, Hunter Lab, Reston, Virginia, USA) with CIELab scale (L^*, a^*, b^*). The measurements were performed at 25°C and using a D-65 illuminant (RSEX mode). Color measurements were expressed in terms of luminosity L^* (L^*=0 for black and

L^*=100 for white) and chromaticity, defined by a* ($+a^*$ for red and $-a^*$ for green) and b^* ($+b^*$ for yellow and $-b^*$ for blue).

2.4.2 Ascorbic acid content

Ascorbic acid content was determined according to the protocol described by Jagota and Dani (1982) with adaptations. The technique involves reaction of Folin Ciocalteu reagent with strong reducing agents, predominantly ascorbic acid, producing a product of blue color measurable by colorimetric method. Aliquot of 0.2 g of sample was weighed and 2.8 mL of 10% trichloroacetic acid was added. For estimation of ascorbic acid, 0.5 mL of the respective extract was diluted with 1.5 mL of double-distilled water. Then, 0.2 mL of dilution, Folin reagent was added to the extract and the samples were vigorously shaken. After 10 min, absorbance was measured at 760 nm using a spectrophotometer. The results were expressed as mg of ascorbic acid /100 g of blackberry pulp solids, calculated by adjusting the calibration curve of ascorbic acid at concentrations ranging from 0 to 75 μg mL^{-1}. All analyses were performed in triplicate.

2.4.3 Anthocyanins content

Anthocyanins in powders were determined according to the method employed by Sims and Gamon (2002) with adaptations. Samples were weighed in triplicate and homogenized with 3 mL of cold solution of acetone/Tris-HCl (80:20, volume/volume, pH 7.8 0.2M) for 1 minute. The samples remained at repose for 1 hour, protected from light. Then, they were centrifuged for 15 minutes at 3500 rpm and the supernatants were immediately taken to be read in the spectrophotometer (B422 model, Micronal) in a visible region at 537 nanometers (anthocyanins). The acetone/tris-HCl solution was used as blank sample. The absorbance values were converted to mg/100g of blackberry pulp solids.

2.4.4 Antioxidant property

2.4.4.1 Extraction samples

Samples were extracted in methanol solution (50% methanol in distilled water, v/v) and then in acetone solution (70% acetone in distilled water, v/v) to determine their antioxidant capacity. Extraction was performed by placing ~ 0.1 g of sample (dry basis), 40 mL of 50% methanol and 40 mL of 70% acetone in a volumetric flask. The volume was completed until 100 mL of distilled water.

2.4.4.2 FRAP

The determination of antioxidant capacity by FRAP method was performed using methodology described by Rufino et al. (2006). In acid medium, ferric complex tripyridyltriazine (TPTZ) was reduced to its deep blue ferrous form in presence of antioxidants, causing an increase in absorbance at 595 nm. To prepare the FRAP reagent, 25 mL of 0.3 M acetate buffer solution (pH 3.6), 2.5 mL of 10 mM TPTZ solution (prepared with 40 mM HCl), and 2.5 mL of 20 mM aqueous ferric chloride solution were used. Aliquot of 90 μL of each diluted extract was transferred into test tubes along with 270 μL of distilled water and 2.7 mL of freshly prepared FRAP reagent. This mixture was homogenized and maintained in water bath at 37°C. After 30 minutes, absorbance was read at 595 nm in a spectrophotometer (model B422, Micronal). FRAP reagent was used as blank sample to calibrate the spectrophotometer. For quantification of antioxidant capacity, a standard curve was created using aqueous solutions of Fe (II) in concentration range of 0 to 2000 μM. Antioxidant capacity was expressed in μM ferrous sulfate/g solids. All analyses were performed in triplicate.

2.4.4.3 DPPH

The antioxidant capacity of extracts, measured by its DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical sequestering ability, was determined according to the methodology described by Rufino et al. (2007) using DPPH method. DPPH is a stable free radical that accepts an electron or hydrogen radical and can be reduced in presence of an antioxidant. Aliquots of 0.1 mL of these extracts were placed in different test tubes and added with 3.9 mL of DPPH (2,2-diphenyl-1-picryl-hydrazyl) solution in methyl alcohol. After shaking, tubes were kept away from light and after 45 minutes, the absorbance was measured at 515 nm in a spectrophotometer (model B422, Micronal). Methyl alcohol was used as blank sample to calibrate the spectrophotometer. Results were expressed in μg g^{-1} solids, in DPPH equivalent, calculated by adjusting the calibration curve for DPPH at concentrations ranging from 0 to 60 μM. By standard curve equation for each sample of blackberry powder extract, the concentrations necessary to inhibit 50% of the DPPH radical were calculated.

2.5 Statistical analysis

The results related to responses of the experimental design were evaluated using Statistica 9.0 software.

3. Results and discussion

3.1. Experimental design

The experimental data for color parameters, luminosity (*L**), coordinates *a** and *b**, ascorbic acid content (C) and anthocyanins (A), antioxidant capacity by DPPH and FRAP, were determined using 11 combinations of independent variables, as shown in **Table 1**.

A second-order regression model fitted the results. The codified regression coefficients, F-values and coefficients of determination (R^2) are presented in **Table 2**. Non-significant terms, considering a confidence level of 95%, were eliminated and the predictive models were tested for fitting adequacy and accuracy by ANOVA. When calculated F-value (Fc) was greater than tabulated F-value (Ft), the variation was explained by the regression and not by the residues. Thus, regression was significant and the model could be considered predictive. **Figures 1** and **2** show the model contour plots generated by the proposed models.

Table 1. Experimental design for spray drying of blackberry with arrowroot starch and gum arabic mixture (1:1, m/m) as encapsulating agent.

T	**XT**	**XP**	***L****	***a****	***b****	**C**	**A**	FRAP	DPPH
1	107 (-1)*	1:0.72 (-1)	49.50±0.27	13.32±0.49	2.92±0.11	145.68 ± 2.91	88.75 ± 5.55	890.64 ± 164.89	170.13 ± 15.91
2	107 (-1)	1:1.78 (1)	52.8±0.19	14.60±0.27	1.98±0.19	191.00 ± 4.45	83.07 ± 4.11	1130.08 ± 14.17	139.79 ± 21.13
3	143 (1)	1:0.72 (-1)	48.93±0.25	11.4±0.15	2.69±0.07	138.53 ± 3.16	120.37 ± 2.05	1013.72 ± 19.15	195.91 ± 1.46
4	143 (1)	1:1.78 (1)	52.38±0.53	12.38±0.35	1.88±0.10	194.70 ± 7.40	93.52 ± 2.37	1257.34 ± 268.86	161.77 ± 1.10
5	100 (-1.41)	1:1.25 (0)	46.41±0.84	7.96±1.25	2.47±0.13	184.12 ± 10.83	79.02 ± 1.42	1484.75 ± 213.47	135.40 ± 13.92
6	150 (1.41)	1:1.25 (0)	51.63±0.32	12.72±0.90	2.29±0.05	175.08 ± 2.21	96.56 ± 1.26	1951.67 ± 33.33	157.10 ± 29.31
7	125 (0)	1:0.5 (-1.41)	46.58±0.34	10.46±0.51	2.89±0.08	134.65 ± 7.78	103.91 ± 5.62	1151.28 ± 9.57	180.18 ± 3.19
8	125 (0)	1:2 (1.41)	53.31±0.60	13.88±0.66	1.76±0.11	193.04 ± 6.26	81.98 ± 0.47	620.35 ± 8.28	89.00 ± 2.75
9	125 (0)	1:1.25 (0)	51.85±0.71	13.77±0.95	2.37±0.04	173.33 ± 8.17	86.05 ± 0.73	900.19 ± 11.61	234.10 ± 23.20
10	125 (0)	1:1.25 (0)	50.35±0.49	13.91±0.88	2.38±0.04	169.96 ± 15.12	81.75 ± 3.33	1041.73 ± 246.27	189.18 ± 25.27
11	125 (0)	1:1.25 (0)	51.52±0.54	13.76±0.57	2.31±0.05	179.21 ± 10.16	83.43 ± 6.70	927.78 ± 7.98	206.46 ± 80.06

The independent variables correspond to the real values. Values enclosed in parentheses correspond to the coded values. XT: inlet air temperature (°C); XP: concentration of encapsulating agent (solids of blackberry pulp/encapsulating agent, weight/weight). *L**, *a** and *b**: color parameters; C: ascorbic acid (mg/100g of blackberry solids); A: anthocyanins (mg/100g of blackberry solids); FRAP (µmol ferrous sulfate/g of blackberry solids); DPPH (g of blackberry solids/g DPPH). The values of C, A, DPPH and FRAP represent the means of three determinations. The values of color parameters represent the means of nine determinations.

Table 2. Regression coefficients and adjusted R^2 for final reduced models.

Regression coefficient	**L***	**b***	**C**	**A**	**FRAP**	**DPPH**
Mean	50.48	2.36	175.72	90.76	869.38	209.91
Linear						
XT	Ns	-0.07	Ns	8.36	Ns	Ns
XP	2.03	-0.42	46.02	-7.94	Ns	-24.18
Quadratic						
XT^2	Ns	Ns	Ns	Ns	350.80	-25.21
XP^2	Ns	Ns	-13.42	Ns	Ns	-31.04
Interaction						
XT x XP	Ns	Ns	Ns	Ns	Ns	Ns
R^2	0.586	0.990	0.955	0.697	0.609	0.757
F	12.76	405.00	84.62	9.20	14.01	7.28

XT: inlet air temperature (°C); XP: concentration of encapsulating agent (solids of blackberry pulp/encapsulating agent, weight/weight); *L**, *a** and *b**: color parameters; C: ascorbic acid; A: anthocyanins; FRAP and DPPH: antioxidant capacity; R^2: regression coefficients; *F*: calculated F-values; ns: not significant ($p>0.05$).

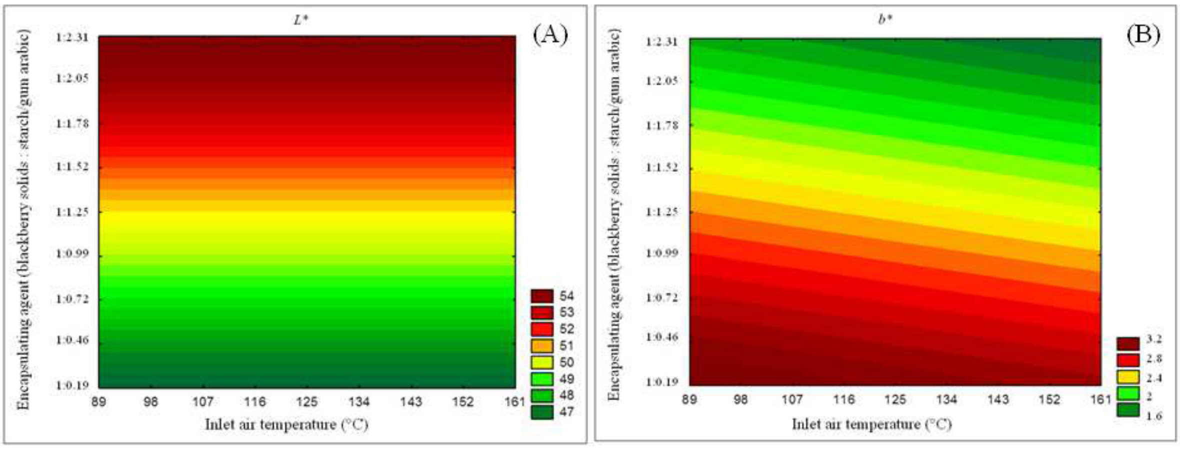


Figure 1. Model contour plots for *L** (A) and *b** (B), color parameters as a function of the air inlet temperature (°C) and concentration of encapsulating agent (solids of blackberry pulp/encapsulating agent, weight/weight).

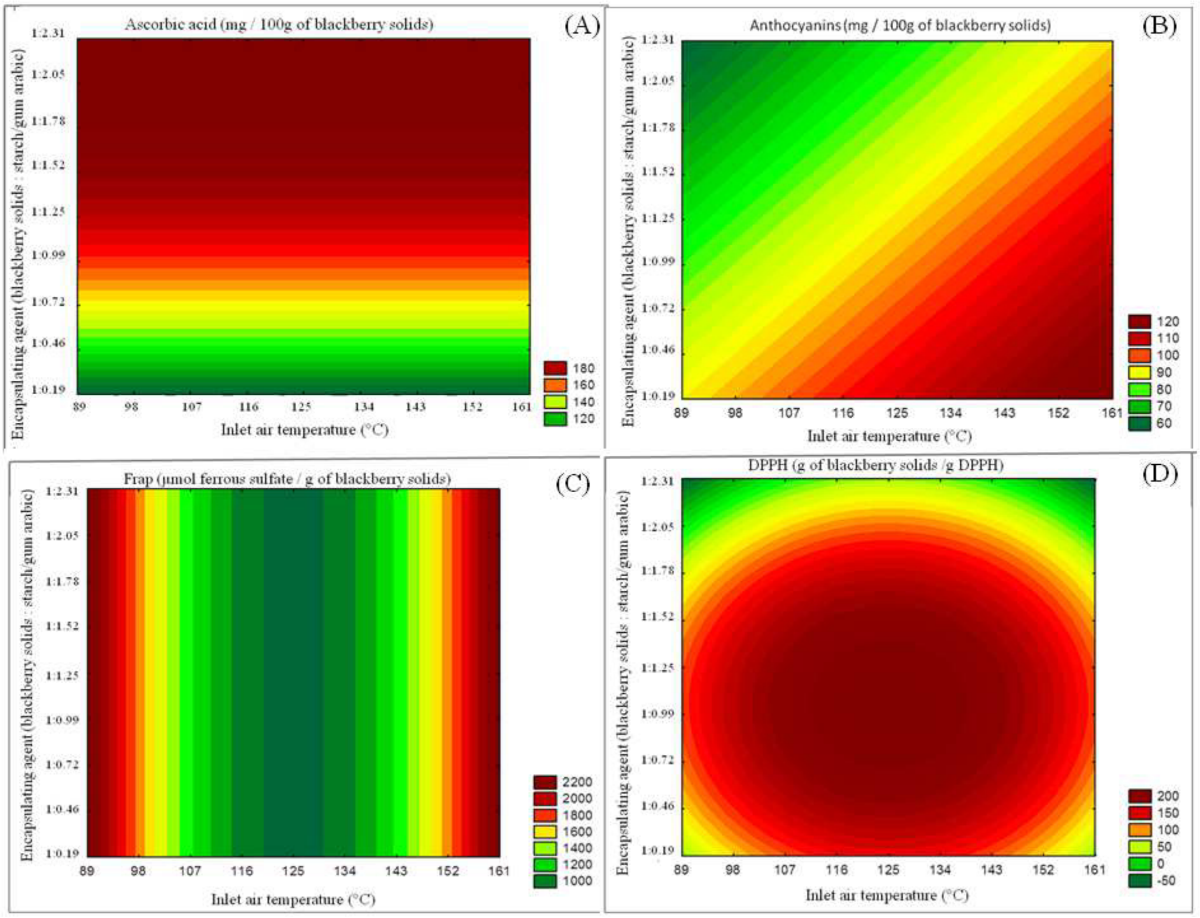


Figure 2. Model contour plots for ascorbic acid (mg/100g of blackberry solids) (A); anthocyanins (mg/100g of blackberry solids) (B); FRAP (µmol ferrous sulfate/g of blackberry solids) (C); DPPH (g of blackberry solids/g DPPH) as function of inlet air temperature (°C)

and of encapsulating agent concentration (solids of blackberry pulp/encapsulating agent, weight/weight) (D).

3.2 Color

An important quality factor for dried foods is color, since it reflects the sensory attractiveness, particularly for products like blackberry, which is also used as colorant (Yamashita, Chung, Santos, Mayer, Moraes & Branco, 2017). The color coordinates determined for blackberry powders (**Table 1**) revealed that the formulated microcapsules tended to present a reddish color, not having significant ($p>0.05$) influence in the values of a^* with the increase of inlet air temperature and encapsulating agent concentration, confirming their adequacy as drying carrier.

The a^* coordinate is directly related to the content of anthocyanins in the fruit, which is the pigment responsible for redness in blackberry powder (Yamashita et al., 2017; Jiménez-Aguilar, Ortega-Regules, Lozada-Ramírez, Pérez-Pérez, Vernon-Carter & Welti-Chanes, 2011). Spray drying conditions at high temperatures resulted in considerable loss of red color due to thermal degradation of pigments. During thermal processing, anthocyanins readily degrade and colorless form or undesirable brown-colored polymeric pigments (Jafari, Ghalenoei & Dehnad, 2017). Therefore, redness in blackberry powder is the main characteristic that indicates the best possible quality of results after drying.

Lower b^* values for encapsulated blackberry were obtained with increasing inlet air temperature and encapsulating agent concentration ($p<0.05$). However, they showed positive values, indicating a tendency toward color yellow (**Figure 1**). Islam, Kitamura, Kokawa, Monalisa, Tsai and Miyamura (2017) demonstrated that, with increasing maltodextrin solids content, the color of orange (*Citrus unshiu*) juice powders obtained by vacuum spray drying became lighter (higher L^* values), but the redness (a^*) and yellowness (b^*) values decreased.

The lightness (L^*) of blackberry powder was significantly ($p<0.05$) influenced by increasing encapsulating agent concentration used. Blackberry powders with lighter color were obtained when prepared with high encapsulating agent concentration, as can be seen in **Figure 3**. Similar results were observed by Daza, Fujita, Fávaro-Trindade, Rodrigues-Ract, Granato and Genovese (2016) for dried extracts from *Eugenia dysenterica* fruit and by Caliskan and Dirim (2016) for sumac extract powders obtained by spray drying. This happened because arrowroot starch and gum arabic mixture used as encapsulating agent showed clear coloration and with the increase of its concentration regarding the content of blackberry pulp solids, there is dilution of anthocyanin pigments in the resultant powder, as previously reported by Ferrari et al. (2012).

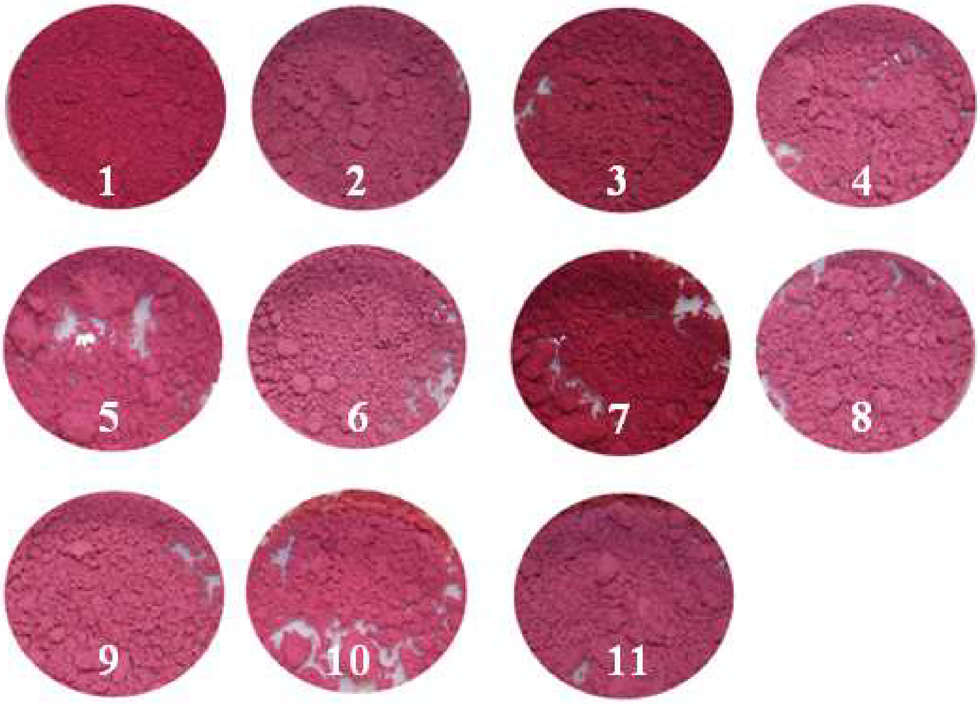


Figure 3. Microencapsulated powder of blackberry with arrowroot starch and gum arabic mixture from the 11 experimental design runs.

3.3 Ascorbic acid content

Ascorbic acid content in blackberry powders varied from 134.65 ± 7.78 to 194.70 ± 7.40 mg/100g of blackberry pulp solids (**Table 1**). Islam et al. (2017) reported ascorbic acid content varying from 7.04 ± 1.0 to 13.24 ± 1.03 mg/g of dry solid for orange juice produced by micro-wet milling and vacuum spray drying process.

Run 4 (**Table 1**), with inlet air temperature of 143°C and encapsulating agent concentration of 1:1.78 (blackberry pulp solids: arrowroot starch/ gum arabic), resulted in higher concentration of ascorbic acid after blackberry pulp drying.

The ascorbic acid content in blackberry powders was not significantly ($p>0.05$) influenced by inlet air temperature. On the other hand, encapsulating agent concentration significantly ($p<0.05$) influenced the ascorbic acid content in blackberry powders, which presented a positive linear effect and a quadratic negative effect (**Table 2**).

Higher encapsulating agent concentrations produced powders with higher ascorbic acid content (**Figure 2A**). This fact indicates that arrowroot starch and gum arabic mixture used as encapsulating agent presented compatibility between them and thermoprotective effect during drying process, which made the formation of blackberry particles more efficient, capable of protecting inner ascorbic acid against thermal degradation and oxidation.

The ascorbic acid is highly sensitive to heat and oxidation (Islam, Kitamura, Yamano & Kitamura, 2016). Studies have shown ascorbic acid losses in orange juice (Islam et al., 2016), tomato pulp (Goula & Adamopoulos, 2006) and lulo (*Solanum quitoense* L.) pulp during spray drying process (Igual et al., 2014). According to Igual et al. (2014), the incorporation of high molecular weight solutes reduced the powder's stickiness and helped the retention of nutritive and functional properties.

Gum arabic is the gum most commonly used as flavor encapsulating material, mainly due to its solubility, low viscosity, emulsification characteristics and good retention of volatile compounds (Igual et al., 2014). Unlike gum arabic, arrowroot starch has not yet been used as an encapsulating agent. Modified starches have also been used in encapsulation of bioactive compounds and volatile substances, conferring protection and stability (Charve & Reineccius, 2009; Baranauskiené, Bylaité, Zukauskaité & Venskutonis, 2007).

3.4 Anthocyanins content

Anthocyanins content of powders ranged from 79.02 ± 1.42 to 120.37 ± 2.05 mg/100g of blackberry pulp solids (**Table 1**). Ferrari et al. (2012) found anthocyanin content ranging from 129.33 ± 0.28 to 433.32 ± 9.03 mg/100g dry base, for spray drying blackberry pulp using maltodextrin (5, 15 or 25%) as encapsulating agent and drying air temperature of 160 or 180°C. For Rigon and Noreña (2016), the anthocyanins content ranged from 878.32 to 1300.83 mg/100 g on dry basis, for blackberry aqueous extract acidified with 2% citric acid, spray dried using gum arabic and polydextrose as encapsulating agents at concentrations of 10 and 15 % and temperatures from 140 to 160°C.

Anthocyanins content in powders was significantly ($p<0.05$) influenced by inlet air temperature and encapsulating agent concentration, which presented a positive linear effect and negative linear effect on anthocyanins content, respectively (**Table 2**). Souza, Thomazini, Balieiro and Fávaro-Trindade (2015) observed similar behavior for powdered pigment made by spray drying, obtained from vinification byproducts of Bordo red grape (*Vitis labrusca*). According to these authors, with the increased maltodextrin concentrations, anthocyanin retention was constant at the lowest temperature. However, there was an increase in retention when higher temperatures were employed.

Figure 2B shows that powders with higher anthocyanin contents were obtained with higher inlet air temperatures and lower encapsulating agent concentrations. Inlet air temperature was the most influential variable into anthocyanins content of blackberry pulp powders. Higher inlet air temperatures of blackberry pulp drying produced higher anthocyanin contents in resulting powders. This fact was not expected, as the increase in temperature tends to cause degradation of anthocyanins, since it is sensitive and unstable at high temperatures (Rigon & Noreña, 2016; Eldridge et al., 2014; Ferrari et al., 2012; Tonon & Hubinger, 2008). Exposure of phenolic compounds to high temperatures adversely affect their structure, causing structural breakage and resulting in the formation of different compounds, thereby reducing antioxidant capacity (Mishra, Sharma, Kumar, Saxena & Pandey, 2013).

However, it is supposed that, at high drying air temperatures, particle formation with blackberry pulp and arrowroot starch/gum arabic was faster and more efficient, consequently reducing thermal degradation and oxidation of anthocyanins. It is believed that the arrowroot starch and gum arabic mixture exhibited a thermoprotective effect during spray drying. Dangles and Brouillard (1992) stated that gum arabic is an excellent encapsulating agent that adequately involves the anthocyanin molecule, so that the flavylium cation is less vulnerable to the degradation factors, increasing anthocyanins stability.

Another kind of reaction that may explain this result is caramelization of encapsulating agent, as well as sugars present in the blackberry pulp. Caramelization yields dark compounds quantified in total anthocyanin content (Souza et al., 2015).

3.5 Antioxidant property

Fruits and their extracts have a very large variety of bioactive compounds, with antioxidant capacity. Consequently, measuring the antioxidant capacity of each compound individually becomes very difficult. The combination of one or more methods has been necessary for determination of total antioxidant capacity of fruits and their extracts (Genskowsky, Puente, Pérez-Álvarez, Fernandez-Lopez, Muñoz & Viuda-Martos, 2015). The antioxidant properties of blackberry powders were investigated using two different methods, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, and FRAP (ferric reducing antioxidant power) assay.

FRAP assay is usually used to determine antioxidant potential of plant materials (Genskowsky et al., 2015). FRAP method detects compounds with redox potential less than 0.7 V (redox potential Fe^{+3}-TPTZ), which acts by a simple electron transfer mechanism, but present reduced capacity to detect compounds that act by transfer of hydrogen (Prior, Wu & Schaich, 2005).

Antioxidant capacity of blackberry powder was analyzed by the ability of bioactive compounds present in these extracts to reduce ferrous iron. **Table 1** shows the results of antioxidant property of powders. All powder samples were able to reduce Fe^{+3}. The antioxidant property of powders was significantly ($p<0.05$) influenced by inlet air temperature, which presented a positive quadratic effect, in the capacity of reducing ferrous iron (**Table 2**). As shown in **Figure 2C**, blackberry powders exhibit high ferric reducing antioxidant capacity when were produced with the minimum and maximum inlet air temperature. The results of antioxidant capacity followed the same trend of anthocyanin content, indicating that these compounds are mainly responsible for antioxidant capacity observed in blackberry powder (**Figure 2B**).

The antioxidant capacity of blackberry powders also was evaluated by the DPPH elimination capacity in 50%. DPPH test is based on the capability of stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with H-donors, including phenolics (Roginsky & Lissi, 2005). DPPH is a commercial oxidant radical that can be reduced by antioxidants (Islam et al., 2017).

The median effective concentration (EC50) is a widely-used parameter to estimate antioxidant capacity. EC50 is the concentration that inhibits 50% of DPPH* radicals in solution. The lower the EC50, the higher are the antioxidant activities found (Medina-Torres et al., 2016). As can be seen in **Table 1**, all samples were able to trap free radicals. Antioxidant capacity in powders was significantly ($p<0.05$) influenced by inlet air temperature, which presented negative linear effect. Encapsulating agent concentration, which presented negative linear and quadratic effect on high antioxidant capacity determined by DPPH assay (**Table 2**). Therefore, blackberry powders with high antioxidant capacity were produced with inlet air temperatures values and encapsulating agent concentration minimum and maximum tested (**Figure 2D**), following the same behavior as FRAP assays.

These antioxidant properties presented by blackberry powders are related not only to anthocyanins, but also to other bioactive compounds such as phenolic acids, flavonoids, anthocyanins and ascorbic acid present in blackberry pulp, contributing to the total antioxidant capacity (Yamashita et al., 2017; Rigon & Noreña, 2016).

3.6 Optimization of process parameters and validation of the model

The optimum spray drying conditions for blackberry pulp were determined by response surface methodology to obtain higher values of content of ascorbic acid, anthocyanins and antioxidant property (FRAP and DPPH assays). Analyzing ascorbic acid model contour (**Figure 2A**), the highest response value was observed at inlet air temperature of 143°C and encapsulating agent concentration of 1:1.78 (blackberry pulp solids: arrowroot starch/gum arabic). For the content of anthocyanins in powders, the model contour shows (**Figure 2B**) that high values were obtained with higher inlet air temperatures and lower encapsulating agent concentrations. As shown in **Figure 2C and 2D**, blackberry powders exhibit high antioxidant capacity when were produced with extreme inlet air temperatures. Thus, the temperature of 143°C was recommended as the optimized condition, since the encapsulating agent concentration did not significantly ($p>0.05$) influence the antioxidant property and the highest value of vitamin C, while a considerable anthocyanin content was obtained at the encapsulating agent concentration of 1:1.78 (blackberry pulp solids: arrowroot starch/gum arabic). This concentration was used with optimum condition.

The optimum conditions were experimentally reproduced and content of ascorbic acid, anthocyanins and antioxidant property (FRAP and DPPH assays) were determined, as shown in **Table 3**, with means and experimental values predicted according to the quadratic model. The values found for content of ascorbic acid, anthocyanins and antioxidant property were relatively close to those found previously for run 4, as shown in **Table 1**. Based on the values of relative deviation (%RD) obtained for each response variable, the optimization methodology used was considered adequate (combination of variables chosen with their respective response range).

Table 3. Predicted, experimental and relative deviation values of the responses at optimum conditions (143°C inlet air temperature and 1:1.78 ratio of blackberry pulp solids to encapsulating agent) for spray dried blackberry pulp powder.

Responses	**Experimental value**	**Predicted Value**	**% Relative Deviation***
Anthocyanins (mg / 100g of blackberry solids)	88.05 ± 4.36	91.18	3.55
Ascorbic acid (mg / 100g of blackberry solids)	285.67 ± 1.84	208.32	27.08
FRAP (µmol ferrous sulfate / g of blackberry solids)	937.49 ± 3.94	1220.18	30.15
DPPH (g of blackberry solids/g DPPH)	197.73 ± 11.15	290.34	46.84

* Relative deviation = [(experimental value - predicted value)/experimental value] * 100.

4. Conclusions

The formulated microcapsules tended to present a reddish color. The increased encapsulating agent concentration resulted in powders with higher ascorbic acid content and lighter in color. Particles produced with higher inlet air temperatures and lower encapsulating agent concentration presented higher anthocyanins content values. The arrowroot starch and gum arabic mixture exhibited a thermoprotective effect during spray drying. All samples were able to reduce Fe^{+3} and trap free radicals, showing antioxidant property. The independent variables showed significant effects on the following responses: *L** and *b**, ascorbic acid, anthocyanins content and antioxidant capacity of the powders. Temperature of 143 °C and encapsulating agent concentration of 1:1.78 were the ideal conditions to obtain blackberry powder with high ascorbic acid content, anthocyanins and antioxidant property.

Conflict of interest

The authors have declared no conflict of interest.

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References

A.O.A.C. (2006). Official Methods of Analysis. 18th ed. *Association of Official Analytical Chemists*, Gaithersburg, Maryland.

Baranauskiené, R., Bylaité, E., Zukauskaité, J., & Venskutonis, R. P. (2007). Flavor Retention of peppermint (*Mentha piperita* L.) essential oil spray dryed in modified starches during encapsulation and storage. *Journal of Agricultural and Food Chemistry*, Chicago, 55, 8, 3027-3036.

Caliskan, G., & Dirim, S.N. (2016). The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders. *Powder Technology*, 287, 308–314.

Charve, J., & Reineccius, G. A. (2009). Encapsulation Performance of Proteins and Traditional Materials for Spray Dried Flavors. *Journal of Agricultural and Food Chemistry*, Chicago, 57, 6, 2486-2492.

Chegini, G.R., & Ghobadian, B. (2005). Effect of spray-drying conditions on physical properties of orange juice powder. *Drying Technology*, 23, 3, 657–668.

Cruz, R., & El Dash, A. A. M. (1984). Amido de chuchu (*Seichium edule*, Swartz). Efeito de fosfatação em sua viscosidade. *Boletim SBCTA*, 18, 4, 371-378.

Dangles, O., & Brouillard, R. (1992). A spectroscopic method based on the anthocyanin copigmentation interaction and applied to the quantitative study of molecular complexes. *Journal of the Chemical Society, Perkin Transactions*, 2, 247– 257.

Daza, L.D., Fujita, A., Fávaro-Trindade, C.S., Rodrigues-Ract, J. N., Granato, D., & Genovese, M. I. (2016). Effect of spray drying conditions on the physical properties of Cagaita (*Eugenia dysenterica* DC.) fruit extracts. *Food and Bioproducts Processing*, 97, 20-29.

Desai, K.G., Liu, C., & Park, H.J. (2006). Characteristics of vitamin C encapsulated tripolyphosphate-chitosan microspheres as affected by chitosan molecular weight. *Journal of Microencapsulation*, 23, 79-90.

Eldridge, J.A., Repko, D., & Mumper, R.J. (2014). Retention of Polyphenolic Species in Spray-Dried Blackberry Extract Using Mannitol as a Thermoprotectant. *Journal of Medicinal Food*, 17, 10, 1064–1069.

Ferrari, C.C., Ribeiro, C.P., & Aguirre, J.M. (2012). Spray drying of blackberry pulp using maltodextrin as carrier agent. *Brazilian Journal of Food Technology*, 15, 157-165.

Genskowsky, E., Puente, L. A., Pérez-Álvarez, J. A., Fernandez-Lopez, J., Muñoz, L. A., & Viuda-Martos, M. (2015). Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT - Food Science and Technology*, 64, 1057-1062.

Goula, A.M., & Adamopoulos, K.G. (2006). Retention of ascorbic acid during drying of tomato halves and tomato pulp. *Drying Technology*, 24, 57-64.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45, 3, 253 -267.

Igual, M., Ramires, S., Mosquera, L.H., & Martínez-Navarrete, N. (2014). Optimization of spray drying conditions for lulo (*Solanum quitoense* L.) pulp. *Powder Technology*, 256, 233–238.

Islam, M.Z., Kitamura, Y., Yamano, Y., & Kitamura, M., (2016). Effect of vacuum spray drying on the physicochemical properties, water sorption and glass transition phenomenon of orangejuice powder. *Journal of Food Engineering*,169, 131–140.

Islam, M.Z., Kitamura, Y., Kokawa, M., Monalisa, K., Tsai, F.H., & Miyamura, S. (2017). Effects of micro wet milling and vacuum spraydrying on the physicochemical and antioxidantproperties of orange (*Citrus unshiu*) juice with pulp powder. *Food and Bioproducts Processing*, 101, 132–144.

Jafari, S.M., Ghalenoei, M. G., & Dehnad, D. (2017). Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder. *Powder Technology*, 311, 59-65.

Jagota, S. K., & Dani, H. M. (1982). A New Calorimetric Technique for the Estimation of Vitamin C Using Folin Phenol Reagent. *Analytical Biochemistry*, 127, 178- 182.

Jiménez-Aguilar, D.M., Ortega-Regules, A.E., Lozada-Ramírez, J.D., Pérez-Pérez, M.C.I., Vernon-Carter, E.J., & Welti-Chanes, J. (2011) Color and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. *Journal of Food Composition and Analysis*, 24, 889-894.

Leung, I.Y.F. (2008). Macular pigment: New clinical methods of detection and the role of carotenoids in age-related macular degeneration. *Optometry – Journal of the American Optometric Association,* 79, 5, 266–272.

Machado, A.P.F., Pasquel-Reátegui, J.L., Barbero, G.F., & Martínez, J. (2015). Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International*, 77, 675–683.

Masisi, K., Beta, T., & Moghadasian, M. H. (2016). Antioxidant properties of diverse cereal grains: A review on in vitro and in vivo studies. *Food Chemistry*, 196, 90–97.

Medina-Torres, L., Calderas, F., Minjares, R., Femenia, A., Sánchez-Olivares, G., Gónzalez-Laredo, F.R., Santiago-Adame, R., Ramirez-Nuñez, D.M., Rodríguez-Ramírez, J., & Manero, O. (2016). Structure preservation of Aloe vera (*barbadensis Miller*) mucilage in a spray drying process. *LWT - Food Science and Technology*, 66, 93-100.

Mishra, A., Sharma, A.M., Kumar, S., Saxena, A.K., & Pandey, A.K. (2013). *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant, and anticancer activities. *BioMed Research International*, Article ID 915436, 1-10.

Moorthy, S. N. (2002). Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54, 559-592.

Mothé, C.G., & Rao, M.A. (2000). Thermal behavior of gum Arabic in comparison with cashew gum. *Thermochimica Acta*, 357-358, 9-13.

Patras, A., Brunton, N.P., Donnell, C.O., & Tiwari, B.K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, 21, 3 -11.

Prior, R.L., Wu, X., & Schaich, K. (2005). Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry*, 53, 4290-4302.

Ré, M. I. (1998). Microencapsulation by spray drying. *Drying Technology*, 16, 1195-1236.

Rigon, R. T., & Noreña, C. P. Z. (2016). Microencapsulation by spray-drying of bioactive compounds extracted from blackberry (*rubus fruticosus*). *Journal of Food Science and Technology*, 53, 3, 1515–1524.

Roginsky, V., & Lissi, E. A. (2005). Review of methods to determine chain-breaking antioxidant capacity in food. *Food Chemistry*, 92, 2, 235-254.

Rufino, M., Alves, R., De Brito, E. S., De Moraes, S. M, Sampaio, C. G., Pérez-Jiménez, J. E., & Saura-Calixto, F. D. (2006). Metodologia científica: determinação de atividade antioxidante total em frutas pelo método de redução de ferro (FRAP). *Comunicado Técnico* on line. EMBRAPA, Fortaleza.

Rufino, M.S.M., Alves, R.E., Brito, E.S., Morais, S.M., Sampaio, C.G., Jimenez, J.P., & Calixto, F.D.S. (2007). Determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH.*Comunicado Técnico* EMBRAPA, 127: 1-4.

Sims, D.A., & Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of environment*, 81, 337-354.

Souza, V.B., Thomazini, M., Balieiro, J.C.C., & Fávaro-Trindade, C.S. (2015). Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification by products of the Bordo grape (*Vitis labrusca*). *Food and Bioproducts Processing*, 93, 39–50.

Tonon, R.V., Brabet. C., & Hubinger, M.D. (2008). Influence of process conditions on the physicochemical properties of acai (*Euterpe oleraceae* Mart.) powder produced by spray drying. *Journal of Food Engineering*, 88, 411–418.

Villas-Boas, F., & Franco, C.M.L. (2016). Effect of bacterial β-amylase and fungal α-amylase on the digestibility and structural characteristics of potato and arrowroot starches. *Food Hydrocolloids*, 52, 795- 803.

Weber, F., Boch, K., & Schieber, A. (2017). Influence of copigmentation on the stability of spray dried anthocyanins from blackberry. *LWT- Food Science and Technology*, 75, 72-77.

Yamashita, C., Chung, M.M.S., dos Santos, C., Mayer, C.R.M., Moraes, I.C.F., & Branco, I.G. (2017). Microencapsulation of an anthocyanin-rich blackberry (spp.) by-product extract by freeze-drying. *LWT - Food Science and Technology*, 84, 256-262.

CAPÍTULO 6- Effect of incorporation of blackberry particles on the physicochemical properties of edible films of arrowroot starch

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Effect of incorporation of blackberry particles on the physicochemical properties of edible films of arrowroot starch

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Abstract: This work evaluated the physicochemical properties of arrowroot starch films plasticized with glycerol and incorporated directly and by sprinkling with 0, 20, 30 and 40% (blackberry solids mass / biopolymer mass) of blackberry particles obtained by spray drying. The films were characterized by thickness, color, water capacity, moisture content, water solubility, water vapor permeability, mechanical properties, anthocyanins content and antioxidant properties. Films incorporated directly and by sprinkling with blackberry particles showed an increase in thickness, water solubility, water vapor permeability and elongation at break as well as decrease in tensile strength compared to the control film. The incorporation of blackberry particles to films transferred bioactive compounds and antioxidant capacity, as well as color to resulting films.

Keywords: packaging, anthocyanins, powder, spray drying, casting.

1. Introduction

Currently, there is a significant interest in edible and biodegradable films made from renewable sources polymers to reduce the severe environmental impact caused by packaging resultant from non-biodegradable conventional sources. Edible or biodegradable films can be formed by compounds present in nature such as proteins, lipids and polysaccharides [1]. Among polysaccharides, starch has received attention due to its ability to form a continuous matrix, for being abundant and renewable, and it exists in various forms depending on the origin of the raw material [2].

Arrowroot (*Maranta arundinaceae* L.) belongs to the family *Marantaceae* and is a large perennial herb found in rainforest habitats [3]. Due to the high content of starch in its rhizomes, arrowroot has been used especially for its extraction. The arrowroot starch presents good digestibility [4], gelling ability [3], [5] and special physicochemical characteristics such as high amylose content (ranging from 16 to 27% for total amylose content [6]). This high amount of amylose has fundamental importance in its choice as film-forming material, as it interferes directly on its final characteristics. Starchy sources with high amylose contents produce films with good characteristics, especially when it comes to mechanical strength and barrier properties [7]. In addition to presenting diffusion barrier properties (humidity, gas and volatiles), films and coatings may be used as carriers for food additives [2], [8].

Recently, the elaboration of edible films with fruits pulp has been studied by several authors [1], [8], [9], [10], [11], [12]. The main advantages of using fruits pulp as components of film-forming suspension are natural antioxidants and antimicrobials transfer and combined biological activities, innovative colors and flavours addition, as well as suitable mechanical and barrier properties increment to the biopolymer [1], [8-12].

Blackberry (*Rubus fruticosus*), cv. Tupy, is a fruit rich in phenolic compounds, such as phenolic acids, tannins and anthocyanins, which has high antioxidant capacity [13]. Also, this fruit has great potential to be incorporated into arrowroot starch to form a biopolymer matrix to create edible films. However, using its beneficial effects is limited because its bioactive compounds exhibit instability when exposed to high temperature, light, pH, oxygen, among other processing conditions, changing their functional and antioxidant properties [14]. Food industry commonly uses microencapsulation to stabilize sensitive compounds and to promote controlled release of active ingredients [15]. Among the several microencapsulation techniques, spray drying is a method characterized by transformation of a liquid into a dry product, in powder form, by means of a relatively short drying time [16]. Although most often considered a dehydration process, spray drying can also be used as an encapsulation method when it incorporates ‘active’ material within a protective matrix, which is essentially inert to the encapsulated material [16]. It is frequently used for reducing moisture content, to maintain or increase the stability anthocyanins from blackberry [17], [18]. At the same time, the incorporation of this blackberry powder in the film-forming solution can promote controlled release of active ingredients from film for the food during storage. Sartori and Menegalli [2] show the microparticles lipid acted as protective agents of the antioxidant capacity of ascorbic acid during film production, remained up to 84% antioxidant capacity after drying process. In this context, this study aimed to produce and characterize arrowroot starch films incorporated directly by blackberry particles sprinkling.

2. Material and Methods

2.1 Materials

In this research work, frozen fruits of blackberry (*Rubus fruticosus)* cultivar Tupy (purchased from "Agro Monte Verde Eirelli", company from Cambuí – MG, Brazil) were used as raw material. The fruits were stored in ultrafreezer at - 43 ± 1 °C until pulp processing. For drying process of blackberry pulp, arrowroot starch and Instantgum® gum (Colloides Naturels, São Paulo, Brazil) were used as encapsulating agents. In elaboration of edible biodegradable films, arrowroot starch containing 15.24 ± 0.19% of water, 0.40 ± 0.03% of protein, 0.12 ± 0.01 % of fat, 0.33 ± 0.01% ash and 83.91 ± 0.00% of carbohydrates [19] and amylose content of 35.20 ± 1.63% [20] was used as film forming matrix and glycerol P.A. (Reagen, Quimibrás Indústrias Químicas S.A.- Rio de Janeiro, Brazil) as plasticizing agent. All other reagents used for the analysis presented analytical grade.

2.2 Spray drying of blackberry pulp

Pulp was obtained by grinding of blackberry fruits in a blender, previously thawed in refrigerator (8 °C) for 24 h. Then, pulp was sieved to remove the seeds, homogenized and packed in polypropylene bottles and coated with aluminum foil to protect against photodegradation. The samples were stored in freezer at -10 ± 1 °C until drying.

The previously thawed blackberry pulp sample (8 °C for 18 h) was added to the encapsulating agent, arrowroot starch and gum arabic mixture (1: 1 mass / mass) in a ratio of 1:1.78 (mass / mass, blackberry pulp solids for encapsulating agent). The content of total solids present in blackberry pulp was 10.3 g / 100 g of pulp. The solution of encapsulating agent and blackberry pulp was performed in a mixer type homogenizer at room temperature for 5 minutes. The solution obtained was diluted in water in proportion of 1:2 (w / w) to enable its spraying.

Spray drying of samples was carried out in a mini spray dryer (Model B191, Büchi, Flawil, Switzerland), with a double-flow atomizer nozzle with a 0.5 mm diameter orifice, with an airflow of 0.6 m^3 / h and air pressure of 8 bar. The feed mass flow rate was 0.2 kg / h and inlet and outlet air temperatures were 143 °C and 105.43 ± 3.13 °C, respectively.

The obtained powder was characterized for drying yield, moisture content, water capacity, hygroscopicity, water solubility and wettability, in triplicate. Drying process yield was determined by ratio between powder solids mass and feeding solids mass of blackberry pulp. The powder moisture content was gravimetrically obtained by vacuum oven at 60 °C until constant weight [19]. Water capacity was determined by direct reading at 25 °C, using AquaLab Lite apparatus (Decagon Devices Inc., Pullman, USA).

Hygroscopicity was determined according to methodology proposed by Cai and Corke [21], with some modifications. About 1 g of each powder sample was placed in desiccators containing saturated sodium chloride (NaCl) solution, equivalent to 75.7% relative humidity at 25 °C. After one week, the samples were weighed in triplicate and hygroscopicity was expressed in grams of water absorbed per 100 g of solids dry mass of the sample (g / 100g).

Solubility was determined according to the method of Eastman and Moore (1984), cited by Cano-Chauca et al. [22]. The method consisted of adding 1 g of sample to a vessel containing 100 mL of distilled water, operating with high-speed magnetic stirring - level 4 of magnetic stirrer for 5 minutes, followed by centrifugation at 3000 G for 5 minutes. Aliquot of 25 mL of supernatant was removed and brought to the oven at 105 °C until constant weight. The solubility was calculated by weight difference.

Wettability time was evaluated according to the method described by Hla and Hogekamp [23]. The method consisted in dropping of 1 g of powder over 400 g of distilled water at 25 °C in 600 mL beaker and visual measurement using a stopwatch to determine the time required for presence of powdered material on the water surface.

2.3 Incorporation of blackberry powder into film-forming suspension

2.3.1 Preparation of film-forming suspension

The film-forming suspension was obtained by dispersing arrowroot starch in distilled water (4%, mass / mass, as optimized by Nogueira et al. [38]). This suspension was heated to 85 ± 2 °C in a thermostatic bath (TECNAL, Brazil), with constant agitation, for about 5 minutes [38]. The blackberry powder was added to film-forming suspension in different concentrations, 0%, 20%, 30% and 40% (mass / mass of dry starch) and two different ways. The first incorporation method was directly in film-forming suspension and the second was by sprinkling into film-forming suspension. Glycerol was added to the solution at concentration of 17% (mass/mass of total solids).

2.3.2 Direct incorporation of blackberry powder into film-forming suspension (ID)

Blackberry powder was added directly into film-forming suspension and homogenized in previously mentioned proportions. Aliquots of 25 mL of resulting suspensions were distributed on the support plate (12 cm diameter). Films were dried at room temperature (25 ± 5°C), until they could be easily removed from the support, approximately for 24 h. The films were conditioned at 25°C and 55 ± 3% of relative humidity for 48 h, before their characterization.

2.3.3 Incorporation of blackberry powder by sprinkling into film-forming suspension (IS)

Aliquots of 25 mL of resulting film-forming suspension were distributed on the support plate (12 cm diameter). Blackberry powder was dispersed through a stainless steel sieve (53 mesh), homogeneously in all the surface area of film-forming suspension already disposed on the support. Then, films were dried for 24 h at room temperature (25 ± 5 °C). After drying, films were removed from the support and conditioned at 25 °C and 55 ± 3% relative humidity for 48 h, before their characterization.

2.4 Films characterization

2.4.1 Visual aspect

Visual and tactile analyses were performed in order to select the most homogeneous films (evaluation by presence of insoluble particles and uniform color), the ones which present continuity (no cracks or prone to cracking areas) and were flexible for handling in removing the films from plates). Films that did not exhibit such characteristics were rejected.

2.4.2 Color determination

Color parameters were determined using a Hunter Lab colorimeter (Color Quest XE 2819, USA). The equipment was set with D65 illuminant 108° observer angle and calibrated with a standard white reflector plate. Three films of each treatment were evaluated. The following color parameters were quantified, in the CIE (International Commission of L'Eclairage) scale $L*$, $a*$ and $b*$. The color measurements were expressed in terms of luminosity $L*$ ($L* = 0$ black and $L* = 100$ white) and chromaticity defined by $a*$ ($+a* =$ red and $-a* =$ green) and $b*$ ($+b* =$ yellow and $-b* =$ blue). The total color difference ($\Delta E*$) of the films were expressed according to Equation 1.

$$\Delta E^* = [(L^* - L^*_p)^2 + (a^* - a^*_p)^2 + (b^* - b^*_p)^2]^{0,5} \qquad (1)$$

Where L^*_p, a^*_p e b^*_p are color parameters of the standard film (F0%) in standard white system CIELab.

2.4.3 Film Thickness

Films thicknesses were measured with accuracy of ± 0.001 mm, at ten different regions of each film, using a micrometer (Mitutoyo, model MDC 25M, MFG / Japan).

2.4.4 Water activity and Moisture content

Water activity was measured in triplicate by direct reading using an AquaLab Lite water activity meter (Decagon Devices Inc., Pullman, USA), operating at 25 °C. The samples were circularly sized, 35 mm in diameter and placed in plastic capsules of equipment. Moisture content of the films was determined in triplicate by air-forced oven drying at 105 ºC for 24 h [19].

2.4.5 Solubility in water

Water solubility of the films was determined according to the method proposed by Gontard et al. [24]. Films samples were cut into disks of 2 cm in diameter, in triplicate, dried at 105 °C for 24 h and weight. The dehydrated samples were individually immersed in 50 mL beakers filled with distilled water, and maintained under slow mechanical agitation (75 rpm) for 24 h at 25 ± 2 °C. After this period, not solubilized samples were removed and dried (105 °C for 24 h) to determine the final dry mass. Solubility was expressed according to Equation 2.

$$\text{Solubilized material}(\%) = \frac{\mathrm{m_{si}} - \mathrm{m_{sf}}}{\mathrm{m_{si}}} \text{ x } 100 \tag{2}$$

Where *msi* is the initial dry mass of the films (g), *msf* is the final dry mass of the non-solubilized films (g).

2.4.6 Water vapor permeability

Water vapor permeability rate of the films was determined gravimetrically based on ASTM E96-80 method [25], using an acrylic cell, with a central opening (diameter of 4.3 cm), in which each film was fixed. The bottom of the cell was filled with dried calcium chloride, creating a dry environment inside (0% relative humidity at 25 °C). This cell was placed in desiccator containing saturated sodium chloride (75 ± 3% RH, measured in hygrometer), equalized for 48 h before analysis.

Water vapor transferred through the film was determined by mass gain of calcium chloride. The cell weight was daily recorded for, at least, 7 days. The film thickness consisted on the average of 5 random measurements made on different parts of films. The water vapor permeation rate (PVA) was performed in triplicate and calculated by Equation 3.

$$PVA = \frac{e}{A\, x\, \Delta p}\, x\dot{\mathrm{M}} \tag{3}$$

Where *PVA* is permeability to water vapor (g.mm / m^2.day.kPa), *e* is mean film thickness, *A* is permeation area (m^2), *Δp* is partial vapor pressure difference between two sides of films (kPa, at 25 ºC), $\dot{M}$ is absorbed moisture rate, calculated by linear regression of weight gain and time, in steady state (g/day).

2.4.7 Mechanical properties

The tensile strength and elongation at break of the films were determined using a texturometer operated according to ASTM standard method D 882-83 [26], with modifications [27]. For each treatment, 6 films samples were cut into rectangles (100 mm x 25 mm). Its thickness was randomly measured in 5 different parts of sample before analysis. For testing, films were fixed by two distal claws initially 50 mm apart, which moved at speed of 1 mm / s. The tensile strength was calculated by Equation 4. The elongation at break was determined by Equation 5.

$$\mathrm{RT} = \frac{\mathrm{Fm}}{\mathrm{A}} \tag{4}$$

Where RT corresponds to tensile strength (MPa), Fm is the maximum force at the moment of film rupture (N) and A is cross-sectional area of the film (m^2).

$$\mathrm{E} = \frac{\mathrm{dr}-\mathrm{di}}{\mathrm{dr}}\,\mathrm{x}100 \tag{5}$$

Where E is elongation (%), dr is the distance at the moment of rupture and di is the initial separation distance (cm).

2.4.8 Anthocyanins content

Determination of anthocyanins of films and powder was performed according to method employed by Sims and Gamon [28], with adaptations. Film samples were previously macerated in liquid nitrogen, weighed in triplicate, and homogenized with 3 mL of cold solution of acetone/Tris-HCl (80:20, volume/volume, pH 7.8 0.2M) for 1 minute. The samples remained at repose for 1 hour, protected from light. Then, it was centrifuged for 15 minutes at 3500 rpm and the supernatants were immediately taken to reading in spectrophotometer (B422 model, Micronal) in visible region at 537 nanometers (anthocyanins). The acetone / Tris-HCl solution was used as blank sample. The absorbance values were converted to mg/100g of blackberry pulp solids.

2.4.9 Antioxidant capacity

Determination of antioxidant capacity by ABTS$^{\bullet+}$ method estimates the capacity of the sample in sequester ABTS radicals [2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]. These ABTS radicals were formed by reaction of 140 mM potassium persulfate with standard 7 mM ABTS solution, for 16 h in dark ambient at room temperature. Then, ABTS (P.A.) was diluted with ethanol to obtain the absorbance value of 0.70 nm ± 0.05 nm to 734 nm. Samples were extracted in methanol solution (50% methanol in distilled water, v / v) and then in acetone solution (70% acetone in distilled water, v / v) to determine the antioxidant capacity. Extraction was performed by placing ~ 1 g of sample (dry basis), 40 mL of 50% methanol and 40 mL of 70% acetone in a volumetric flask. The volume was completed until 100 mL with distilled water. Extract aliquots of 30 µL were added to 3 mL of ABTS radical and stayed in dark ambient for 6 minutes. Standard curve was fitted with Trolox [6-hydroxy-2.5.7.8-tetrametilchroman-2-carboxylic acid] at concentrations ranging from 100 to 2000 µM. Results were calculated according to equation fitted by standard curve and expressed by $\mu g.g^{-1}$ solid of Trolox equivalent (TE). All the analyses were performed in triplicate.

2.5 Statistical analysis

Significant differences between average results were evaluated by analysis of variance (ANOVA) and Tukey test at 5% level of significance, using SAS software (Cary, NC, USA).

3. Results and Discussion

3.1 Characterization of blackberry powder

Table 1 presents the characterization of blackberry powder obtained by spray drying using mixture of arrowroot starch and gum arabic as encapsulating agents.

Drying parameters were standardized according to preliminary tests that resulted in the best retention of anthocyanins (77%) and drying process yield of approximately 57%.

Blackberry powder moisture content was 1.45% and water activity was less than 0.3. Thus, it can be considered biochemically or microbiologically quite stable [29]. In general, blackberry powder presented values of hygroscopicity, solubility and wettability similar to powders obtained by spray drying method [18], [29],[30]. Visually, the powder preserved the typical color of blackberry pulp (**Figure 1 A**).

Table 1. Characterization of blackberry powder obtained by spray drying.

Analysis	**Blackberry powder**
Process yield (%)	57.69 ± 8.97
Moisture content (%)	1.45 ± 0.51
Aw (decimal)	0.13 ± 0.04
Hygroscopicity (g of adsorbed water / 100g solids)	17.22 ± 0.45
Solubility in water (%)	59.64 ± 1.15
Wettability (min)	9.77 ± 0.22
Total Anthocyanins (mg / 100 g of blackberry solids)	88.05 ± 4.36
ABTS (µmol of Trolox / g of blackberry solids)	493.56 ± 3.36
Color	
*L**	48.89 ± 0.44
*a**	6.70 ± 0.65
*b**	2.59 ± 0.07

ABTS, 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid).

3.2 Films characterization

3.2.1Visual aspect

The resulting films were homogeneous, continuous, and flexible in handling, as shown in **Fig. 1**. The incorporation of blackberry powder into the films did not make it sticky, being easily manipulated.

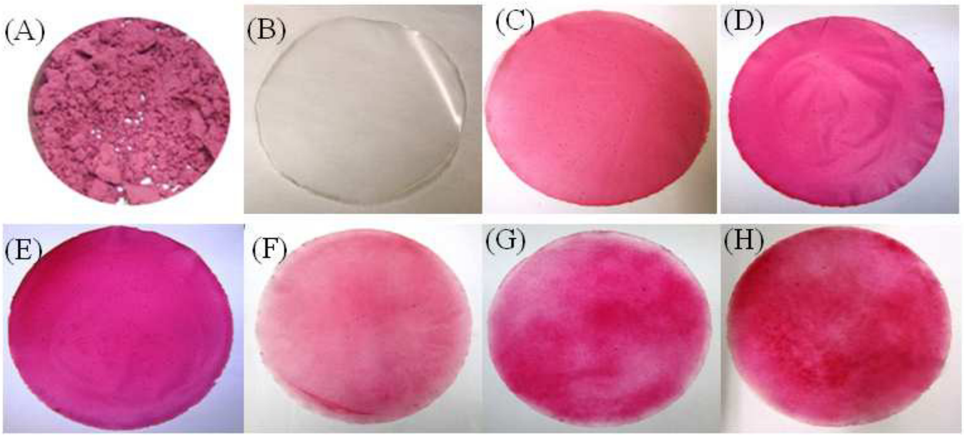

Figure 1. Appearance of blackberry powder (a) films: 0%, (b) control film without blackberry powder; 20% ID, (c) film incorporated directly with 20% blackberry powder; 30% ID, (d) film incorporated directly with 30% blackberry powder; 40% ID, (e) film incorporated directly with 40% blackberry powder; 20% IS, (f) film incorporated by sprinkling with 20% blackberry powder; 30% IS, (g) film incorporated by sprinkling with 30% blackberry powder; 40% IS, (h) film incorporated by sprinkling with 40% blackberry powder.

Using sieve to sprinkle blackberry powder over the film-forming suspension allowed powder to fall homogenously over the whole surface area of films (**Figure 1**). The films presented a rougher face to manual contact. It can be explained by the incorporated particles presence, while the face that came into contact with support plate during drying was smooth and bright. The films without blackberry incorporation (0%) did not present visible to naked eye particles in their matrix and presented two smooth faces to manual contact (**Figure 1 B**), deferring of films with blackberry incorporation (**Figure 1 C-H**).

It is believed that most of blackberry particles remained intact after their direct incorporation and by sprinkling to the film-forming suspension, since it was possible to visualize particles in resulting dry film by naked eye. This behavior was not expected for films with blackberry powder incorporated directly into the film-forming suspension. Due to the hydrophilic characteristic of blackberry particles which was confirmed by its water solubility of approximately 60% (Table 1), the particles were expected to dissolve. However, it is likely that this did not occurred because, during the starch gelatinization process, water molecules bind to the exposed hydroxyl groups of amylose and amylopectin by hydrogen bonds,[5] leaving little water available for interaction with the particle, which consequently hampered its solubilization. No data were found in the literature for starch films dried incorporated particles obtained by spray drying.

3.2.2 Colorimetric analysis

The color of food packaging is an important factor in terms of general appearance and consumer acceptance.[31] Films incorporated with blackberry exhibited color and flavor typical of blackberry powder differing from films without blackberry incorporation which were transparent and odourless, as shown in **Figure 1** and confirmed by results of color parameters, shown in **Table 2**.

Table 2. Color parameters of films samples with 0%, 20%, 30% and 40% of blackberry powder incorporated directly (ID) and by sprinkling (IS).

Films	$L*$	$a*$	$b*$	$\Delta E*$
0%	91.54 ± 1.16^{a}	1.96 ± 0.09^{d}	-8.11 ± 0.46^{d}	-
20% ID	67.43 ± 0.99^{b}	19.80 ± 0.32^{c}	-2.45 ± 0.87^{c}	30.64 ± 0.91^{c}
30% ID	56.49 ± 1.85^{ced}	26.05 ± 1.56^{b}	1.33 ± 0.84^{ba}	43.70 ± 2.03^{b}
40% ID	50.32 ± 1.87^{ed}	28.09 ± 0.59^{b}	4.43 ± 0.75^{a}	50.53 ± 1.91^{ba}
20% IS	60.43 ± 1.59^{cb}	26.26 ± 0.88^{b}	-2.92 ± 0.51^{c}	39.89 ± 1.78^{bc}
30% IS	58.25 ± 4.97^{cbd}	27.91 ± 3.59^{b}	-0.95 ± 0.75^{bc}	42.90 ± 6.15^{b}
40% IS	47.48 ± 5.57^{e}	33.56 ± 2.26^{a}	2.62 ± 2.61^{ba}	55.39 ± 6.20^{a}

Same letters in the same column show no statistical difference ($p > 0.05$).

Compared with control film (0%), film luminosity $L*$ decreased significantly ($p<0.05$) upon addition of blackberry powder. Sartori and Menegalli [2] also observed decreased luminosity and increased opacity for banana starch films incorporated with encapsulated ascorbic acid.

The increase in concentration of blackberry powder incorporated in arrowroot starch film caused a statistically significant increase ($p<0.05$) in $a*$ and $b*$ values, leading to decrease of luminosity $L*$ and increase of total color difference $\Delta E*$ (**Table 2**). This result means that the incorporation of blackberry powder made arrowroot starch film darker and less translucent.

The increase in $a*$ and $b*$ values based on blackberry powder concentration increase shows that films exhibited color shades ranging from red to yellow-orange. It was reported that $a*$ coordinate is attributed to anthocyanin content of the fruit [32], which is responsible for redness color of blackberry microcapsules. A lower $b*$ value for encapsulated blackberry represents greater yellowness [33].

3.2.3 Film Thickness

Figure 2 shows thickness of the films made from arrowroot starch incorporated with blackberry powder, which ranged from 0.065 to 0.153 mm. Thickness of the films significantly increased ($p<0.05$) with increasing concentration (0, 20, 30, 40 %) of blackberry powder incorporated directly (ID) and by sprinkling (IS) as compared to control film (0%). This difference occurred because of increasing concentration of blackberry powder incorporated solids in the same mass of film-forming suspension per unit area in carrier plate.

Farias et al. [8] also verified increasing in thickness as function of increasing of plasticizer and acerola pulp concentrations in cassava starch films. Rojas-Grau et al. [12] found a similar thickness band (0.135 to 0.155 mm) for films made with apple puree and essential oils. Films addition of encapsulated or non-encapsulated ascorbic acid presented thickness varying from 0.088 to 0.099 mm [2].

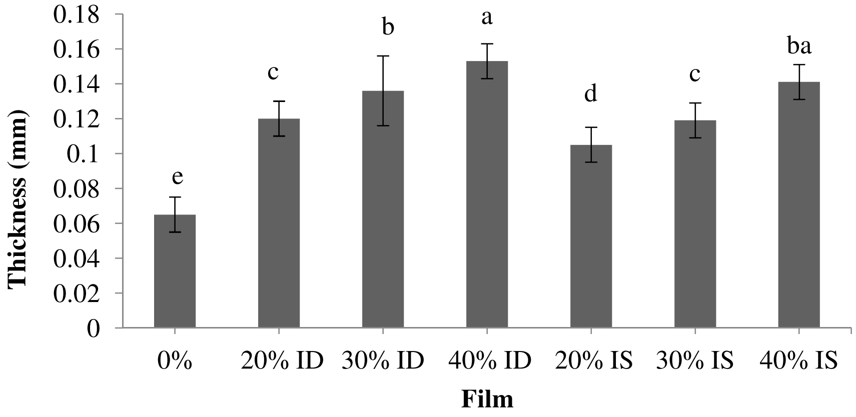

Figure 2. Film thickness (mm) with 0%, 20%, 30% and 40% of blackberry powder incorporated directly (ID) and by sprinkling (IS). Same letters show no statistical difference ($p > 0.05$).

3.2.4 Water activity and moisture content

Table 3 shows films water activity and moisture content values evaluated after storage of films samples in desiccator at 25 °C and 55 ± 3% of relative humidity for 48 h. The water activity values obtained for control films (0%) and films containing blackberry powder did not differ significantly and were averaging 0.42. Edible peach puree films presented water activity of 0.43 and moisture content of 14.4% [9].

The average moisture content of films varied from 9.66 ± 0.44 % to 11.84 ± 0.14 % (**Table 3**). The incorporation of 20% ID, 20% IS and 30% IS of blackberry powder in films has led to a significant decrease ($p \leq 0.05$) in moisture content of films as compared to control film (0%). Sartori and Menegalli [2] found opposite results, which moisture content of films increased with addition of encapsulated or non-encapsulated ascorbic acid as compared to control film ($p < 0.05$). Probably, the particles incorporation triggered a discontinuity of polymer matrix, favoring the release of water from the starch structure [2], [34].

Table 3. Water activity and moisture content of films with 0%, 20%, 30% and 40% of blackberry powder incorporated directly (ID) and by sprinkling (IS).

Films	Aw at 25 °C	Moisture content (%)
0%	0.43 ± 0.05^{a}	11.30 ± 0.10^{ba}
20% ID	0.42 ± 0.05^{a}	9.96 ± 0.12^{c}
30% ID	0.42 ± 0.07^{a}	10.95 ± 0.61^{b}
40% ID	0.44 ± 0.01^{a}	11.81 ± 0.17^{a}
20% IS	0.36 ± 0.05^{a}	9.66 ± 0.44^{c}
30% IS	0.41 ± 0.04^{a}	10.14 ± 0.12^{c}
40% IS	0.42 ± 0.02^{a}	11.84 ± 0.14^{a}

Same letters in the same column show no statistical difference ($p > 0.05$).

3.2.5 Solubility in water and permeability to water vapor

Figure 3 depicts water solubility and water vapor permeability results obtained for films samples. Water solubility and permeability to water vapor of films increased significantly ($p<0.05$) with increasing concentration of blackberry powder concentration (20, 30 and 40%) in films when compared to control film (0%). This behaviour can be attributed to blackberry powder, which is highly hygroscopic and hydrophilic (**Table 1**) giving the film greater tendency to bond with water molecules and therefore greater solubility and permeability.

Hernandez [35], Sartori and Menegalli [2], Rojas-Graü [12] had already postulated that moisture transfer usually occurs through the hydrophilic portion of a barrier and is directly related to the hydrophilic-hydrophobic ratio of its components. Thus, adding hydrophilic substances (e.g., blackberry powder) decreases moisture barrier properties of a film. In control films, without blackberry powder (0% films), the greater number of interactions of amylose-amylose, amylopectin-amylopectin and amylose-amylopectin formed during film drying favoured a smaller amount of hydrophilic groups available to interact with water, whereas in films with blackberry powder, greater number of hydrophilic groups would be present in resulting film matrix, allowing greater diffusion of water vapor through the film.

Our results are in agreement with those found by Azeredo et al [10] and Otoni et al. [11]. Regarding Azeredo et al [10], water vapor permeability was also significantly increased by incorporation of pomegranate juice, suggesting again its plasticizing effects, as well as the pectin dilution effect. Increasing pomegranate juice/water ratio from 0/100 to 100/0 resulted in increased water vapor permeability from 3 to 9 $g.mm.kPa^{-1}.h^{-1}.m^{-2}$ [10]. Otoni et al. [11] also found a significant increase ($p < 0.05$) in water vapor permeability in high and low degree of esterification pectin films with incorporation of papaya puree.

Water vapor permeability of cassava starch films added with acerola ranged from 0.208 to 0.273 ($g\ mm\ h^{-1}\ m^{-2}\ kPa^{-1}$) [8]. The authors observed that increasing pulp concentration up to 60% causes the increase of water vapor permeability. According to them, this increase can also be explained, because increasing pulp concentration causes an increase in films thickness, which influences the water vapor permeability. This fact indicates that, in this research work, increasing in films thickness with blackberry powder incorporation may also have influenced the increase of water vapor permeability.

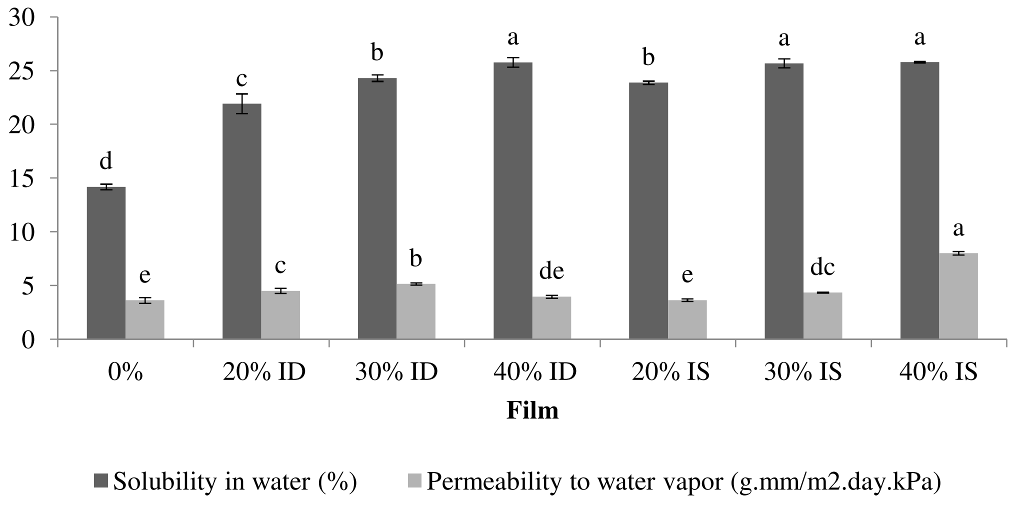

Figure 3. Effect of addition of blackberry powder on water vapor permeability and water solubility of films with 0%, 20%, 30% and 40% of blackberry powder incorporated directly (ID) and by sprinkling (IS). Same letters show no statistical difference ($p > 0.05$).

3.2.6 Mechanical properties

Figure 4 shows the mechanical properties (elongation at break and tensile strength) of films samples with 0%, 20%, 30% and 40% of blackberry powder incorporated directly (ID) and by sprinkling (IS). The blackberry powder incorporation in film significantly ($p<0.05$) affected all mechanical properties. The films with blackberry powder were less rigid and more flexible than the control film. It is observed that films with blackberry powder had lower and higher tensile strength and elongation at break values, respectively, than those obtained for control film (0%).

These results indicate a plasticizing effect of blackberry powder, which can be attributable to its sugar content. Blackberry is a fruit rich in sugars such as fructose, glucose and sucrose [36]. Studies have reported the plasticizing effect of sugars present in fruits when incorporated into polymer films [10], [11], [31].

Incorporation of plasticizer into biopolymers modifies the three-dimensional molecular organization of polymer matrix, reducing the intermolecular attraction force and raising free volume of the system [37], reducing stiffness and increasing extensibility and flexibility of films. Moreover, regarding Azeredo et al. [10], when added in large quantities, those sugars also decrease concentration of polymer matrix, which further weakens the films.

Films with blackberry powder incorporated by sprinkling 30% IS and 40% IS had the highest elongation results, differing significantly ($p<0.05$) from the films directly incorporated (ID). It is possible that the impact of sprinkling of particles on film-forming suspension has disrupted and promoted discontinuity of polymer matrix, increasing elongation of the film.

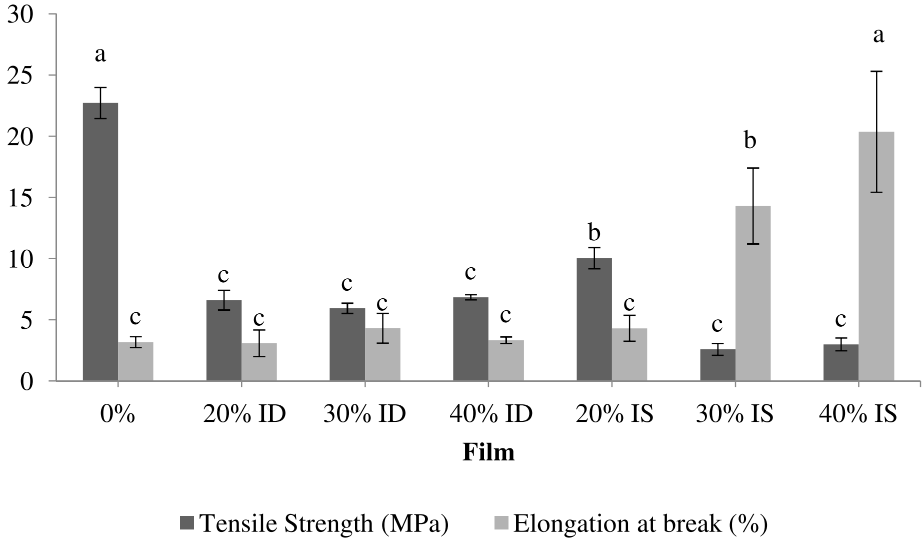

Figure 4. Mechanical properties of the films with 0%, 20%, 30% and 40% of blackberry powder incorporated directly (ID) and by sprinkling (IS). Same letters show no statistical difference ($p > 0.05$).

3.2.7 Anthocyanin content and antioxidant capacity

Table 4 shows anthocyanin content (mg / 100g of blackberry solids) and antioxidant capacity (µmol of Trolox / g of blackberry solids) of films after drying process. Arrowroot film without blackberry powder (0% film) showed an insignificant amount of anthocyanins and antioxidant capacity.

Incorporation of blackberry powder also resulted in significant increase ($p<0.05$) in anthocyanins content and antioxidant capacity of the films when compared to 0% film (without blackberry powder). Genskowsky et al. [1] also observed a significant increase in phenolic compounds content and antioxidant capacity in chitosan films after incorporation of maqui berry.

The antioxidant properties of arrowroot films with blackberry powder are related to the presence of anthocyanins from blackberry powder (**Table 1**) in edible films. The high content of anthocyanins in encapsulated blackberry pulp has been demonstrated [17], [18], [33]. The antioxidant capacity of blackberry is not the result of anthocyanins alone. Other constituents, such as phenolic acids, tannins, vitamin C as and pigments might contribute to total antioxidant capacity [13].

Sartori and Menegalli [2] observed that encapsulated ascorbic acid protected the antioxidant capacity of this compound during film processing.

Table 4. Anthocyanin content and antioxidant capacity of the films with 0%, 20%, 30% and 40% of blackberry powder incorporated directly (ID) and by sprinkling (IS).

Films	Total Anthocyanins (mg / 100 g of blackberry solids)	ABTS (µmol of Trolox / g of blackberry solids)
0%	0.32 ± 0.12^{d}	9.15 ± 6.51^{c}
20% ID	43.15 ± 2.51^{cb}	475.42 ± 8.38^{ba}
30% ID	37.96 ± 2.71^{c}	384.22 ± 4.13^{b}
40% ID	57.52 ± 2.67^{a}	416.25 ± 44.56^{b}
20% IS	60.63 ± 6.08^{a}	455.60 ± 40.90^{ba}
30% IS	47.81 ± 2.79^{b}	541.11 ± 46.22^{a}
40% IS	45.43 ± 0.63^{cb}	449.51 ± 67.76^{ba}

Same letters in the same column show no statistical difference ($p > 0.05$).

ABTS, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid).

For Film 0%, anthocyanin content is expressed in (mg/100 g of total solids) and antioxidant capacity is expressed in (µmol of Trolox/g of total solids).

4. Conclusions

The results of this study clearly demonstrated that arrowroot starch films added with blackberry powder had anthocyanins and antioxidant properties. Using encapsulated blackberry pulp protected the antioxidant capacity of this compound during film processing. Incorporation of blackberry powder in film-forming suspension transferred bioactive compounds to the resulting films, as well as typical flavor and color of blackberry powder. Furthermore, films incorporated with blackberry powder showed lower luminosity and higher values of a^* and b^* than the control films. Increasing incorporation of blackberry powder in arrowroot starch films caused increased thickness, water solubility and water vapor permeability of films. As for the mechanical properties of the films containing blackberry powder, their tensile strength and elongation at break were lower and higher, respectively. This effect originated from incorporation of particles triggered by a discontinuity of polymer matrix.

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References

[1] Genskowsky, E.; Puente, L. A.; Pérez-Álvarez, J. A.; Fernandez-Lopez, J.; Muñoz, L. A.; Viuda-Martos, M. Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT - Food Science and Technology*. **2015**, 64, 1057-1062. doi.org/10.1016/j.lwt.2015.07.026.

[2] Sartori, T.; Menegalli, F. C. Development and characterization of unripe banana starch films incorporated with solid lipid microparticles containing ascorbic acid. *Food Hydrocolloids*. **2016**, 55, 210-219. doi.org/10.1016/j.foodhyd.2015.11.018

[3] Charles, A.L.; Cato, K.; Huang, T-C., Chang, Y-H.; Ciou, J-Y.; Chang, J-S; Lin, H-H. Functional properties of arrowroot starch in cassava and sweet potato composite starches. *Food Hydrocolloids*. **2016**, 53, 187-191. doi.org/10.1016/j.foodhyd.2015.01.024.

[4] Villas-Boas, F.; Franco, C.M.L. Effect of bacterial β-amylase and fungal α-amylase on the digestibility and structural characteristics of potato and arrowroot starches. *Food Hydrocolloids*. **2016**, 52, 795- 803.doi.org/10.1016/j.foodhyd.2015.08.024

[5] Hoover, R. Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*. **2001**, 45, 3, 253 -267. doi.org/10.1016/S0144-8617(00)00260-5

[6] Moorthy, S. N. Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*. **2002**, 54, 559-592. doi 10.1002/1521-379X(200212)54:12<559::AID-STAR2222559>3.0.CO;2-F

[7] Tharanathan, R. N. Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technoloy*. **2003**, 14, 3, 71-78. doi 10.1016/S0924-2244(02)00280-7.

[8] Farias, M.G.; Fakhouri, F.M.; Carvalho, C.W.P. d.; Ascheri, J.L.R. Caracterização físico-química de filmes comestíveis de amido adicionado de acerola (*Malphigia emarginata* D.C.). *Química Nova*. **2012**, 35, 546-552. doi.org/10.1590/S0100-40422012000300020

[9] Mchugh, T.H.; Huxsoll, C.C.; Krochta, J.M. Permeability properties of fruit puree edible films. *Journal Food Science*. **1996**, 61, 88–91. doi.10.1111/j.1365-2621.1996.tb14732.x

[10] Azeredo, H.M.C.; Morrugares-Carmona, R.; Wellner, N.; Cross, K.; Bajka, B.; Waldron, K.W. Development of pectin films with pomegranate juice and citric acid. *Food Chemistry*. **2016**, 198, 101–106. doi.org/10.1016/j.foodchem.2015.10.117.

[11] Otoni, C.G.; De Moura, M.R.; Aouada, F.A.; Camilloto, G.P.; Cruz, R.S.; Lorevice, M.V.; Soares, N.F.F.; Mattoso, L. H. C. Antimicrobial and physical mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids,* **2014**. 41, 188–194.doi.org/10.1016/j.foodhyd.2014.04.013.

[12] Rojas-Graü, M.A.; Avena-Bustillos, R.J.; Friedman, M.; Henika, P.R.; Martín-Belloso,O.; McHugh, T. H. Mechanical, barrier, and antimicrobial properties of apple puree edible films containing essential oils. *Journal of Agricultural and Food Chemistry*. **2006**, 54, 9262–9267. doi. 10.1021 / jf061717u.

[13] Machado, A.P.da F.; Pasquel-Reátegui, J.L.; Barbero, G.F.; Martínez, J. Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.)

residues: a comparison with conventional methods. *Food Research International.* **2015**, 77, 675–683. doi.org/10.1016/j.foodres.2014.12.042.

[14] Patras, A.; Brunton, N.P.; Donnell, C.O.; Tiwari, B.K. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology.* **2010**, 21, 3 -11. doi:10.1016/j.tifs.2009.07.004

[15] Desai, K.G.; Liu, C.; Park, H.J. Characteristics of vitamin C encapsulated tripolyphosphate-chitosan microspheres as affected by chitosan molecular weight. *Journal of Microencapsulation.* **2006**, 23, 79-90.doi.org/10.1080/02652040500435360

[16] Ré, M. I. Microencapsulation by spray drying. *Drying Technology.* **1998**, 16, 1195-1236. doi.10.1080/07373939808917460

[17] Weber, F.; Boch, K.; Schieber, A. Influence of copigmentation on the stability of spray dried anthocyanins from blackberry. *LWT- Food Science and Technology.* **2017**, 75, 72-77. doi.org/10.10.16/ j.lwt.2016.08.042

[18] Ferrari, C.C.; Ribeiro, C.P.; Aguirre, J.M. Spray drying of blackberry pulp using maltodextrin as carrier agent. *Brazilian Journal of Food Technology.* **2012**. 15, 157-165. doi.org/10.1590/S1981-67232012005000009.

[19] A.O.A.C. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Gaithersburg, Maryland, **2006**.

[20] Martinez, C.; Cuevas, F. Evaluación de la calidad culinaria y molinera del arroz. *Guia de estudo*, CIAT, **1989**.

[21] Cai, Y. Z.; Corke, H. Production and properties of spray-dried Amaranthus betacyanin pigments. *Journal of Food Science.* **2000**, 65, 1248-1252. doi. 10.1111 / j.1365-2621.2000.tb10273.x

[22] Cano-Chauca, M.; Stringheta, P.C.; Ramos, A.M.; Cal-Vidal, J. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional

characterization. *Innovative Food Science and Emerging Technologies*. **2005**, 5, 420–428. doi.10.1016/j.ifset.2005.05.003

[23] Hla, P. K.; Hogekamp, S. Wetting behaviour of instanized cocoa beverage powders. *International Journal of Food Science and Technology*. **1999**, 34, 4, 335-342. doi.10.1046 / j.1365-2621.1999.00275.x

[24] Gontard, N; Guilbert, S.; Cuq, J.L. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*. **1992**, 57, 190-199.doi. 10.1111 / j.1365-2621.1992.tb05453.x

[25] ASTM. Standard test methods for water vapor transmission of materials. Method E96e80. In Annual book of American standard testing methods. Philadelphia: ASTM. **1989**.

[26] ASTM. Standard test methods for tensile properties of thim plastic sheeting. Method D 882-83. In Annual book of American standard testing methods. Philadelphia: ASTM. **1980**.

[27] Tanada-Palmu, P.S.; Hélen, H.; Hyvonen, L. Preparation, properties and applications of wheat gluten edible biofilms. *Agricultural And Food Science in Finland*. **2000**, 9, 23-25.

[28] Sims, D. A.; Gamon, J. A. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of environment*. **2002**, 81, 337-354. doi.org/10.1016/S0034-4257(02)00010-X

[29] Santhalakshmy, S.; Don Bosco, S.J.; Francis, S.; Sabeena, M. Effect of inlet temperature on physicochemical properties of spray-dried jamun fruit juice powder. *Powder Technology*. **2015**, 274, 37–43. doi.org/10.1016/j.powtec.2015.01.016.

[30] Tonon, R. V.; Brabet, C.; Hubinger, M.D. Influence of process conditions on the physicochemical properties of acai (*Euterpe oleraceae* Mart.) powder produced by

spray drying. *Journal of Food Engineering*, **2008**, 88, 411-418. doi.org/10.1016/j.jfooden.2008.02.029

[31] Espitia, P. J. P.; Avena-Bustillos, R. J.; Du, W. X.; Teófilo, R. F.; Soares, N. F.; McHugh, T. H. Optimal antimicrobial formulation and physical–mechanical properties of edible films based on açaí and pectin for food preservation. *Food Packaging and Shelf Life*. **2014**, 2, 38-49. doi.org/10.1016/j.fpsl.2014.06.002

[32] Jiménez-Aguilar, D.M.; Ortega-Regules, A.E.; Lozada-Ramírez, J.D.; Pérez-Pérez, M.C.I.; Vernon-Carter, E.J.; Welti-Chanes, J. Color and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. *Journal of Food Composition and Analysis*. **2011**, 24, 889-894. doi:10.1016/j.jfca.2011.04.012.

[33] Yamashita, C.; Chung, M.M.S.; dos Santos, C.; Mayer, C.R.M.; Moraes, I.C.F.; Branco, I.G. Microencapsulation of an anthocyanin-rich blackberry (spp.) by-product extract by freeze-drying. *LWT - Food Science and Technology*. **2017**, 84, 256-262. doi.org/10.1016/j.lwt.2017.05.063.

[34] Fabra, M. J.; Hambleton, A.; Talens, P.; Debeaufort, F.; Chiralt, A. Effect of ferulic acid and a-tocopherol antioxidants on properties of sodium caseinate edible films. *Food Hydrocolloids*. **2011**, 25, 1441-1447. doi:10.1016/j.foodhyd.2011.01.012

[35] Hernandez, E. Edible coatings from lipids and resins. In J. M. Krochta, E. A. Baldwin, & M. G. Nispero-Carriedo (Eds.), Edible coatings and films to improve food quality (pp. 279-303). Lancaster, PA, USA: Technomic Publ. Co. **1994**

[36] Kafkas, E.; Koşar, M.; Türemiş, N.; Başer, K.H.C. Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. *Food Chemistry*. **2006**, 97, 732-736.doi.org/10.1016/j.foodchem.2005.09.023

[37] Sothornvit, R.; Krochta, J. M. Plasticizer effect on oxygen permeability of β-lactoglobulina films. *Journal of Agricultural and Food Chemistry*. **2000**, 48, 6298-6302. DOI: 10.1021 / jf0008361

CAPÍTULO 7- Incorporation of blackberry pulp of particles obtained by freeze drying in edible films of arrowroot starch

Este capítulo será submetido à revista *Food Hydrocolloyds* e está apresentado no formato de artigo científico.

Incorporation of blackberry pulp of particles obtained by freeze drying in edible films of arrowroot starch

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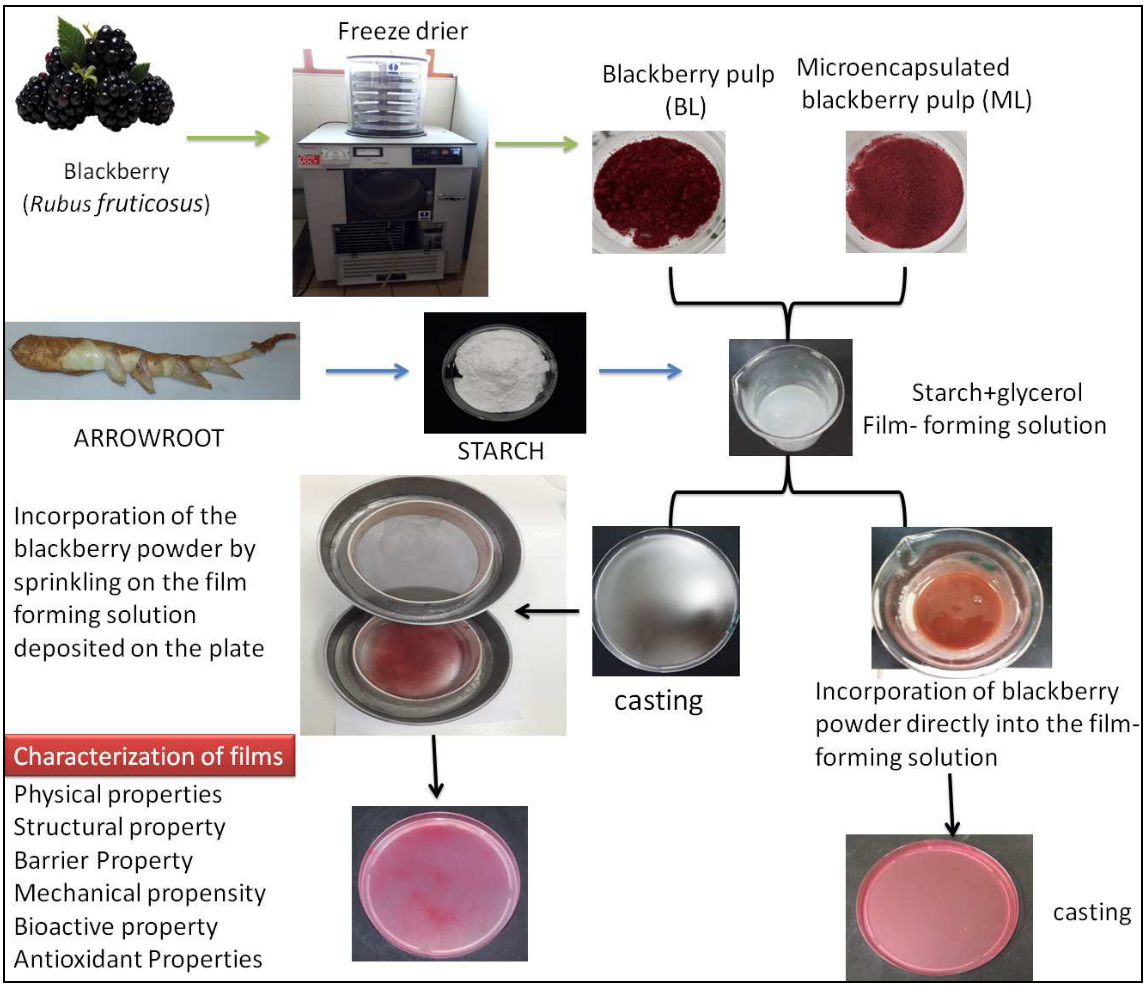
Highlight

- Biopolymeric films of starch and blackberry pulp microparticles were developed.
- Arrowroot starch film present structure organized, confirmed by SEM.
- The effect of the incorporation of blackberry powder in the starch film was studied.
- Films with blackberry powder were thicker, soluble in water and less resistant than the control film.
- Blackberry powder transferred color, anthocyanins and antioxidant capacity to films.
- The surface of films with blackberry powder was rough compared to the control film.
- Films incorporated with sprinkling blackberry powder had higher antioxidant capacity.

Abstract: Blackberry pulp (BL, without encapsulating agent) powder and microencapsulated blackberry pulp (ML) using mixture of gum arabic and arrowroot starch (1:1, mass / mass) by freeze drying were incorporated in the arrowroot starch film-forming solution directly (D) and by sprinkling (S), in concentrations of 0, 20, 30 and 40% (blackberry solids mass / biopolymer mass). The influence of type of incorporation and variation of the concentration of BL and ML powders in physical, mechanical and structural properties, thickness, water activity, moisture content, color, anthocyanins content and antioxidant properties of starch films were investigated. The addition of BL and ML powders increased thickness, water solubility, as well as decrease tensile strength compared to the control film (0%). Compared to the arrowroot starch film (0%), the surface of films with BL and ML powder became irregular and heterogeneous. When their blackberry powders content was 20%, the powder dispersed homogeneously in starch film-forming solution, resulting in compact film, which reduced the permeability to water vapor. However, when blackberry powder content was more than 20%, the powder particles began to aggregate, which reduced its interaction with arrowroot starch matrix, increasing permeability to water vapor of films. The incorporation of blackberry transferred anthocyanins, antioxidant capacity, as well as color to arrowroot starch films. Films incorporated with sprinkling blackberry powder had higher antioxidant capacity and were more soluble in water in relation to films directly incorporated, showing great potential to be used as vehicle for releasing of bioactive compounds into the surroundings.

Keywords: freeze drying; microstructure; water solubility; water vapor permeability; anthocyanins; antioxidant capacity.

Graphical Abstract



1. Introduction

Currently, edible and biodegradable films have been used as new strategy to reduce serious environmental problems caused by using of packaging made from petroleum-based materials, not biodegradable. Edible or biodegradable films are usually made from naturally compounds, such as proteins, lipids, polysaccharides or mixtures thereof (Genskowsky et al., 2015). Among polysaccharides, starch is probably the most promising material, due to its high capacity to form a continuous matrix, and exist in many ways depending on the origin of raw material (Sartori & Menegalli, 2016).

For these reasons, many studies have focused on development of starch-based films. Unfortunately, starch presents some drawbacks, such as their strong hydrophilic characteristic and poorer mechanical properties than conventional synthetic polymers used in food packaging industries, even with the addition of plasticizers such as glycerol (Dai, Qiu, Xiong, & Sun, 2015; Shi, Wang, Li, & Adhikari, 2013; Avella et al., 2005).

Thus, generally modifications in properties of starch-based films, have been necessary. Recently, the incorporation of micro and nano particles in film-forming solution to produce starch films with modified structure and properties have shown promise (Shi et al., 2013). Shi et al. (2013) reported for the first time, the preparation method and characteristics of starch films incorporating spray dried and vacuum freeze dried starch nanoparticles. These authors studied the morphology, crystallinity, physical and mechanical properties of films and reported that the addition of both starch nanoparticles in starch films increased roughness of surface, lowered degree of crystallinity by 23.5%, water vapor permeability by 44% and glass transition temperature by 4.3 °C, respectively compared to those of starch-only films.

Freeze drying is a technology that has been used for encapsulation of fruit pulps, as well as bioactive compounds sensitive to temperature, pH, light and oxygen, in the form of micro or nanoparticles (Yamashita et al., 2017). *Rubus fruticosus* or blackberry is a fruit highly appreciated by consumers, not only by their appealing color and desirable flavor and taste, but also for its high nutritional value (D'Agostino et al., 2015).

Blackberry is an important source of phenolic compounds, such as phenolic acids, tannins and anthocyanins, which has high antioxidant capacity (Machado, Pasquel-Reátegui, Barbero, & Martínez, 2015). Its encapsulation by freeze drying allows to preserve its color and its bioactive and antioxidants properties. Freeze-drying is a method based on dehydration by sublimation of a frozen product. During this procedure, blackberry pulp is homogenized with the encapsulating agent and then co-lyophilized, resulting in a dry material (Yamashita et al., 2017).

It is believed that the use of the same starch for production of particles and as the film matrix tends to facilitate further mixing during preparation of the film-forming solution than other compounds of different molecules (Shi et al., 2013). The incorporation of blackberry pulp into starch film can provide color, flavor, bioactive compounds and antioxidant capacity to it, and can be used successfully to produce starch films with some custom properties (Azeredo et al., 2016).

Arrowroot (*Maranta arundinaceae* L.) starch presented high amylose content. According to Moorthy (2002), the total amylose content in arrowroot starch ranged from 16 to 27%. For the films production with good technological properties, with stronger and more flexible mechanical characteristics, using starch with high amylose content is desirable

(Tharanathan, 2003). Thus, it is expected that arrowroot starch to be a promising matrix for producing edible and biodegradable films.

Until now, the formation and characteristics of arrowroot starch-based films containing blackberry pulp microparticles are not reported so far. Therefore, the objective of this work was to incorporate directly and by sprinkling, blackberry pulp powder and microencapsulated blackberry pulp in arrowroot starch film-forming solution.The influence of type of incorporation and variation of concentration of blackberry powders in physical, mechanical and structural properties, thickness, water activity, moisture content, color, anthocyanins content and antioxidant properties of starch films were investigated.

2. Materials and methods

2.1. Raw materials

In this work, frozen fruits of blackberry (*Rubus fruticosus*), cv Tupy, were acquired from "Agro Monte Verde Eirelli", company of Cambuí – MG, Brazil. Blackberry soluble solids content was 9 °Brix, determined with digital refractometer with 0–90 °Brix range and 0.2 °Brix resolution (Reichert, Model AR200, USA). Blackberry pulp presented total solids content of 10.3 g/100g of pulp.

For microencapsulation of blackberry pulp, arrowroot starch and Instantgum® gum arabic (Colloides Naturels, São Paulo, Brazil) were used as encapsulating agents. In elaboration of edible biodegradable films, arrowroot starch containing 15.24 ± 0.19% of water, 0.40 ± 0.03% of protein, 0.12 ± 0.01 % of fat, 0.33 ± 0.01% of ash and 83.91 ± 0.00% of carbohydrates (AOAC, 2006) and amylose content of 35.20 ± 1.63%, determined according to the methodology described by Martinez and Cuevas (1989), with adaptations (Zavareze et al., 2009), was used as film-forming matrix and glycerol P.A. (Reagen, Quimibrás Indústrias Químicas S.A.- Rio de Janeiro, Brazil) as plasticizing agent. All other reagents used for the analysis presented analytical grade.

2.2 Microencapsulation of blackberry pulp by freeze drying

Blackberry pulp was obtained by grinding the fruits in a blender, previously thawed in a refrigerator (8 °C) for 24 h, filtered through a sieve to remove the seeds and homogenized.

Blackberry pulp was added to the encapsulating agent, arrowroot starch and gum arabic mixture (1: 1 mass / mass) in a ratio of 1:1.78 (mass / mass, blackberry pulp solids for encapsulating agent). Solution of encapsulating agent and blackberry pulp was performed in a mixer type homogenizer at room temperature for 5 minutes.

Portions of frozen blackberry pulp with and without encapsulating agent was lyophilized (Mod. 501, Edwards Pirani, Crawley, West Sussex, UK), with an initial temperature of -40 °C, pressure of 0.1 mmHg, and final temperature of 25 °C per 2 h, with total cycle time of 48 h. The resulting product was ground in a hammer mill (MR Manesco and Ranieri LTDA, model MR020, Piracicaba- Brazil), sieved (28 mesh) and stored in polyethylene packages in desiccators, for further analysis.

2.2.1 Characterization of blackberry powder

Freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) were characterized for drying yield, moisture content, water activity, hygroscopicity, water solubility and wettability, in triplicate. Drying process yield was determined by ratio between powder solids mass and feeding solids mass of blackberry pulp. The powder moisture content was gravimetrically obtained by vacuum oven at 60 °C until constant weight (AOAC, 2006). Water activity was determined by direct reading at 25 °C, using AquaLab Lite apparatus (Decagon Devices Inc., Pullman, USA).

Hygroscopicity was determined according to methodology proposed by Cai and Corke (2000), with some modifications. About 1 g of each powder sample was placed in desiccators containing saturated sodium chloride (NaCl) solution, equivalent to 75.7% relative humidity at 25 °C. After one week, the samples were weighed in triplicate and hygroscopicity was expressed in grams of water absorbed per 100 g of solids dry mass of the sample (g / 100g).

Solubility was determined according to the method of Eastman and Moore (1984), cited by Cano-Chauca, Stringheta, Ramos and Cal-Vidal (2005). The method consisted of adding 1 g of sample to a vessel containing 100 mL of distilled water, operating with high-speed magnetic stirring - level 4 of magnetic stirrer for 5 minutes, followed by centrifugation at 3000 G for 5 minutes. Aliquot of 25 mL of supernatant was removed and brought to the oven at 105 °C until constant weight. Solubility was calculated by weight difference.

Wettability time was evaluated according to the method described by Hla and Hogekamp (1999). The method consisted in dropping 1 g of powder over 400 g of distilled water at 25 °C in 600 mL beaker and visual measurement using a stopwatch to determine the time required for presence of powdered material on the water surface.

2.3 Incorporation of blackberry into film-forming suspension

2.3.1 Preparation of film-forming suspension

Film-forming suspension was obtained by dispersing arrowroot starch in distilled water (4%, mass / mass, as optimized by Nogueira, Fakhouri & Oliveira, 2018). This suspension was heated to 85 ± 2 °C in a thermostatic bath (TECNAL, Brazil), with constant agitation, for about 5 minutes. The freeze dried blackberry pulp (BL) and the freeze dried microencapsulated blackberry pulp (ML) were added to film-forming suspension in different concentrations, 0%, 20%, 30% and 40% (mass / mass of dry starch) in two different ways. The first incorporation method was directly in film-forming suspension and the second one was by sprinkling into film-forming suspension. Glycerol was added to the solution at concentration of 17% (mass/mass of total solids, as optimized by Nogueira, Fakhouri & Oliveira, 2018).

2.3.2 Direct incorporation of blackberry powder into film-forming suspension (D)

Freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) were added directly into film-forming suspension and homogenized in previously mentioned proportions. Aliquots of 25 mL of resulting suspensions were distributed on support plates (12 cm diameter). Films were dried at room temperature (25 ± 5 °C), until they could be easily removed from the plates, approximately for 24 h. Films were conditioned at 25 °C and 55 ± 3% of relative humidity for 48 h, before their characterization.

2.3.3. Incorporation of blackberry powder by sprinkling into film-forming suspension (S)

Aliquots of 25 mL of resulting film-forming suspension (item 2.3.1) were distributed on support plates (12 cm diameter). Freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) were dispersed through a stainless steel sieve (53 mesh), homogeneously in all the surface area of film-forming suspension already disposed on the plates. Then, films were dried for 24 h at room temperature (25 ± 5 °C). After drying, films were removed from the support plates and conditioned at 25 °C and 55 ± 3% relative humidity for 48 h, before their characterization.

2.4 Films characterization

2.4.1 Visual aspect

Visual and tactile analyses were performed in order to select the most homogeneous films (evaluation by presence of insoluble particles and uniform coloration), which had continuity (no cracks or prone to cracking areas) and were flexible for handling in removing the films from plates). Films that did not exhibit such characteristics were rejected.

2.4.2 Microstructure

Electronic Scanning Electron Microscope with X-ray Dispersive Energy Detector (SEM) bench (model of MEV: Leo 440i, model of EDS: 6070, Leo 440i - LEO Electron Microscopy/Oxford- Cambridge, Inglaterra) was used to observe the morphological characteristics of the surface and cross section developed for sample of the films formulations. Films sample was placed on double-sided carbon adhesive tape adhered to stub, submitted to application of a gold layer (model K450, Sputter Coater EMITECH, Kent, United Kingdom) and observed in scanning electron microscope operated at 20 kV.

2.4.3 Color determination

Color parameters were determined using a Hunter Lab colorimeter (Color Quest XE 2819, USA). The equipment was set with D65 illuminant and calibrated with a standard white reflector plate. Three films of each treatment were evaluated. The following color parameters were quantified in the CIE (International Commission of L'Eclairage) scale (L^*, a^* and b^*). The color measurements were expressed in terms of luminosity L^* ($L^* = 0$ black and $L^* = 100$ white) and chromaticity defined by a^* ($+a^*$ = red and $-a^*$ = green) and b^* ($+b^*$ = yellow and $-b^*$ = blue). The total color difference (ΔE^*) of the films were expressed according to Equation 1.

$$\Delta E^{*} = [(L^{*} - L^{*}_{p})^{2} + (a^{*} - a^{*}_{p})^{2} + (b^{*} - b^{*}_{p})^{2}]^{0,5} \quad (1)$$

Where L^{*}_{p}, a^{*}_{p} e b^{*}_{p} are color parameters of the standard film (F0%) in standard white CIELab system.

2.4.4 Anthocyanins content

Determination of anthocyanins of films and powder was performed according to method employed by Sims and Gamon (2002), with adaptations. Film samples were previously macerated in liquid nitrogen, weighed in triplicate and homogenized with 3 mL of cold solution of acetone/Tris-HCl (80:20, volume/volume, pH 7.8 0.2M) for 1 minute. The samples remained at repose for 1 hour, protected from light. Then, it was centrifuged for 15 minutes at 3500 rpm and the supernatants were immediately taken to reading in spectrophotometer (B422 model, Micronal) in visible region at 537 nanometers (anthocyanins). The acetone / Tris-HCl solution was used as blank sample. The absorbance values were converted to mg/100g of blackberry pulp solids.

2.4.5 Antioxidant capacity

Determination of antioxidant capacity by ABTS•+ method estimates the capacity of the sample in sequester ABTS radicals [2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]. These ABTS radicals were formed by reaction of 140 mM potassium persulfate with standard 7 mM ABTS solution, for 16 h in dark ambient at room temperature. Then, ABTS (P.A.) was diluted with ethanol to obtain the absorbance value of 0.70 nm ± 0.05 nm to 734 nm. Samples were extracted in methanol solution (50% methanol in distilled water, v / v) and then in acetone solution (70% acetone in distilled water, v / v) to determine the antioxidant capacity. Extraction was performed by placing ~ 1 g of sample (dry basis), 40 mL of 50% methanol and 40 mL of 70% acetone in a volumetric flask. The volume was completed until 100 mL with distilled water. Extract aliquots of 30 µL were added to 3 mL of ABTS radical and stayed in dark ambient for 6 minutes. Standard curve was fitted with Trolox [6-hydroxy-2.5.7.8-tetrametilchroman-2-carboxylic acid] at concentrations ranging from 100 to 2000 µM. Results were calculated according to equation fitted by standard curve and expressed by $\mu g.g^{-1}$ solid of Trolox equivalent (TE). All the analyses were performed in triplicate.

2.4.6 Film Thickness, Water activity and Moisture content

Films thicknesses were measured with accuracy of ± 0.001 mm, at ten different regions of the film, using a micrometer (Mitutoyo brand, model MDC 25M, MFG / Japan). Water activity was measured in triplicate by direct reading using a water activity meter (AquaLab Lite, Decagon Devices Inc., Pullman, USA), operating at 25 °C. The samples were circularly sized, 35 mm in diameter and placed in plastic capsules of equipment. Moisture content of the films was determined in triplicate by air-forced oven drying at 105 ºC for 24 h (AOAC, 2006).

2.4.7 Solubility in water

Water solubility of films was determined according to the method proposed by Gontard, Guilbert, and Cuq (1992). Films samples were cut into disks of 2 cm in diameter, in triplicate, dried at 105 °C for 24 h and weighed. Dehydrated samples were immersed and individually placed in 50 mL beakers filled with distilled water, and maintained under slow mechanical agitation (75 rpm) for 24 h at 25 ± 2 °C. After this period, not solubilized samples were removed and dried (105 °C for 24 h) to determine the final dry mass. Solubility was expressed according to Equation (2).

$$\text{Solubilized material}(\%) = \frac{m_{si} - m_{sf}}{m_{si}} \text{ x } 100 \qquad (2)$$

In which 'm_{si}' is the initial dry mass of the films (g), 'm_{sf}' is the final dry mass of the non-solubilized films (g).

2.4.8 Water vapor permeability

Water vapor permeability rate of the films was determined gravimetrically based on ASTM E96-80 method (1989), using an acrylic cell, with a central opening (diameter of 4.3 cm), in which the film was fixed. The bottom of the cell was filled with dried calcium chloride, creating a dry environment inside (0% relative humidity at 25 ° C). This cell was placed in desiccator containing saturated sodium chloride (75 ± 3% RH, measured in Hygrometer), equalized for 48 h previously to analysis.

Water vapor transferred through the film was determined by mass gain of calcium chloride. The cell weight was recorded daily for, at least, 7 days. The film thickness consisted on the average of 5 random measurements made on different parts of the samples. The water vapor permeation rate (PVA) was performed in triplicate and calculated by Equation (3).

$$PVA = \frac{e}{A\,x\,\Delta p}\, x\dot{M} \qquad (3)$$

In which 'PVA' is permeability to water vapor (g.mm / m^2.day.kPa), 'e' is mean film thickness, 'A' is permeation area (m^2), 'Δp' is partial vapor pressure difference between two sides of films (kPa, at 25 ºC), '$\dot{M}$' is absorbed moisture rate, calculated by linear regression of weight gain and time, in steady state (g/day).

2.4.9 Mechanical properties

Tensile strength and elongation at break of films were determined using a texturometer operated according to ASTM standard method D 882-83 (1980), with modifications (Tanada-Palmu, Hélen, & Hyvonen, 2000). For each treatment, 6 films samples were cut into rectangles (100 mm x 25 mm). Thickness was randomly measured in 5 different parts of sample before analysis. For testing, films were fixed by two distal claws initially 50 mm apart, which moved at speed of 1 mm / s. Tensile strength was calculated by Equation 4. Elongation at break was determined by Equation 5.

$$\mathrm{RT} = \frac{\mathrm{Fm}}{\mathrm{A}} \qquad (4)$$

Where RT corresponds to tensile strength (MPa), Fm is maximum force at the moment of film rupture (N) and A is cross-sectional area of the film (m^2).

$$\mathrm{E} = \frac{\mathrm{dr} - \mathrm{di}}{\mathrm{dr}}\, \mathrm{x}100 \qquad (5)$$

Where E is elongation (%), dr is distance at the moment of rupture and di is initial separation distance (cm).

2.4.10 Statistical analysis

Significant differences between average results were evaluated by analysis of variance (ANOVA) and Tukey test at 5% level of significance, using SAS software (Cary, NC, USA).

3. Results and discussion

3.1 Characterization of blackberry powder

Figure 1 shows microstructure of BL and ML powders observed in SEM images. External morphology of freeze-dried BL and ML resembled a broken glass structure of variable sizes, with typical folds, slight cracks and porosity in surface due to the loss of water content during freeze-drying process. Similar characteristics were observed by Yamashita et al. (2017) and Franceschinis, Salvatori, Sosa and Schebor (2014) for freeze dried blackberry (*Rubus spp.*) powder.

Furthermore, for ML powder, it was possible to observe spherical particles distributed in their vitreous structures. It is believed that these particles are result of microencapsulation of blackberry pulp by mixture of arrowroot starch and gum arabic used as encapsulating agent. Shi et al. (2013) produced nanoparticles of starch by spray drying and freeze drying methods. Nanoparticles of starch produced by both methods were spherical and showed very similar morphology.

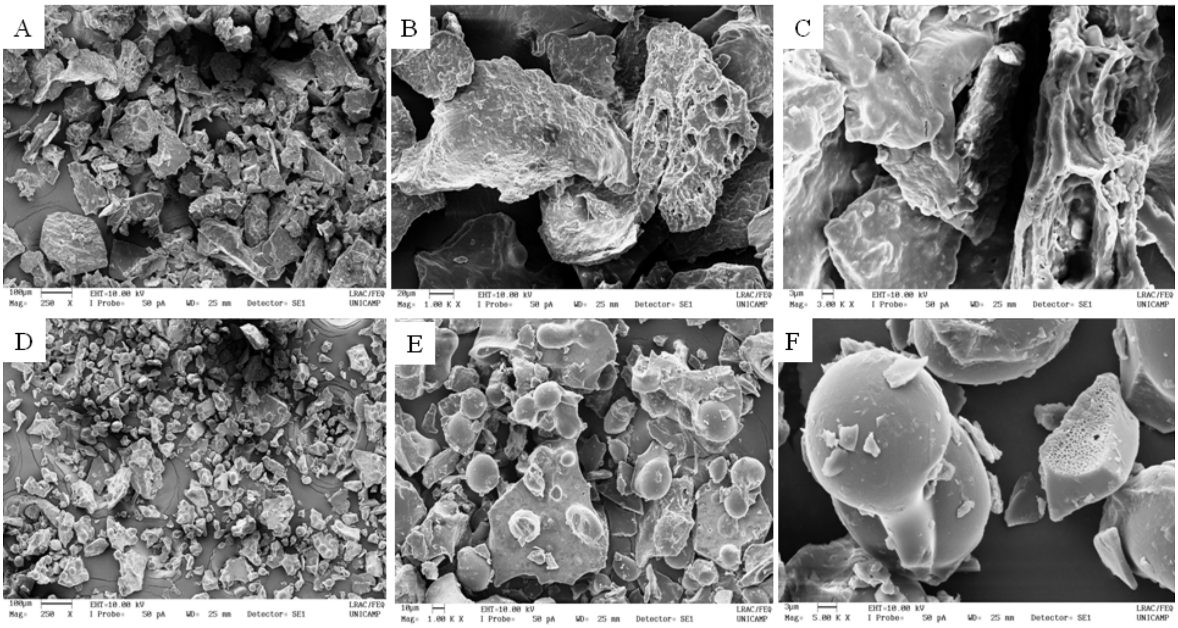


Figure 1. Scanning electron micrographs of freeze-dried blackberry pulp- BL (images A, B and C) and freeze-dried microencapsulated blackberry pul- ML (images D, E and F) powders: images A and D - 250x magnification, B and E - 1000x magnification, C and F - 3000x magnification.

Table 1 shows the results of characterization of blackberry pulp (BL) and microencapsulated blackberry pulp (ML) powder obtained by freeze-drying. Yield of blackberry pulp drying process with or without encapsulating agent was high. BL and ML powders presented low water content and water activity was less than 0.3, indicating that they are biochemically and microbiologically stable, since below that value (Aw <0.3), there are interruptions in non-enzymatic reactions and there is no growth of microorganisms, one of the main reactions of deterioration (Reid & Fennema, 2010).

ML powder was significantly ($p<0.05$) less hygroscopic and soluble than BL powder. This fact happened because arrowroot starch and gum arabic are materials with low hygroscopicity, consequently the microencapsulation of blackberry pulp tends to reduce the hygroscopicity of resulting powders. Besides that, although gum arabic is highly soluble (Daza et al., 2017), arrowroot starch in its native form presents low solubility in water at room temperature. This probably contributed to the decrease in solubility of blackberry powder. However, microencapsulation of blackberry pulp did not significantly ($p>0.05$) alter wettability of powder, which is desirable.

Table 1. Characterization of blackberry powder obtained by freeze drying.

Analysis	**Blackberry pulp (BL)**	**Microencapsulated blackberry pulp (ML)**
Process yield (%)	89.24 ± 2.81 ^{a}	95.86 ± 0.89 ^{a}
Moisture content (%)	10.72 ± 2.81 ^{a}	4.50 ± 0.31 ^{a}
Aw (decimal)	0.13 ± 0.01 ^{a}	0.11 ± 0.01 ^{b}
Hygroscopicity (g of adsorbed water / 100g solids)	21.28 ± 0.45 ^{a}	12.86 ± 0.1^{b}
Solubility in water (%)	61.26 ± 0.49 ^{a}	53.84 ± 0.76 ^{b}
Wettability (min)	0.99 ± 0.53 ^{a}	1.66 ± 0.01 ^{a}
Total Anthocyanins (mg / 100 g of blackberry solids)	125.27 ± 9.77 ^{a}	125.99 ± 5.25 ^{a}
ABTS (µmol of Trolox / g of blackberry solids)	288.43 ± 30.70 ^{a}	309.18 ± 34.09 ^{a}
Color		
L^*	47.29 ± 2.35 ^{b}	57.23 ± 1.57 ^{a}
a^*	14.18 ± 2.97 ^{a}	20.13 ± 1.17 ^{a}
b^*	4.95 ± 0.56 ^{a}	3.59 ± 0.08 ^{a}

Same letters in the same column show no statistical difference (p > 0.05).

Although microencapsulation is a common method to protect bioactive compounds against adverse environment conditions, such as pH, light and oxygen (Shahidi & Han, 1993), no significant differences (p>0.05) were observed between the values of anthocyanins and antioxidant properties of BL and ML powders. As freeze drying is a method that does not use high temperatures during drying process and is based on dehydration by sublimation of a frozen product, these factors probably contributed to maintenance of bioactive compounds and antioxidant capacity (Yamashita et al., 2017).

The resulting powders had a typical reddish color, typical of blackberry pulp. ML powder showed significantly (p<0.05) lighter coloration than BL powder, due to presence of encapsulating agent which has a lighter color.

3.2 Films characterization

3.2.1 Visual aspect

Incorporation of blackberry in film-forming solution transferred reddish color to arrowroot starch films. In larger concentrations more visually remarkable was the color. All films could be removed from the plates and, in general, had good appearance and transparency. Films with 0%, 20%, 30% and 40% freeze dried blackberry pulp directly incorporated (BLD) and freeze dried microencapsulated blackberry pulp directly incorporated (MLD) were visually homogeneous, continuous and very flexible to handling. On the other hand, films with blackberry pulp (BL) and microencapsulated blackberry pulp (ML) incorporated by sprinkling (S) were brittle and sensitive to handling.

3.2.2 Microstructure

Differences in microstructure of the films can be visualized in **Figure 2**, by scanning electron microscopy (SEM). Control film (0%) presented organized and continuous polymer matrix and regular surface (**Figure 2** A-C). Differing from the 0% film, the films developed with 40% of blackberry presented rougher and irregular surfaces and exhibited structural characteristics directly related to those observed for BL and ML powders.

Shi et al. (2013) also observed that incorporation of starch nanoparticles obtained by spray drying and freeze drying made the surface of starch film rough. According to the authors, protuberances found on film surface resulted of the presence of starch nanoparticles. It is believed that most of BL and ML structures remained intact after their incorporation to the film-forming suspension, since it was possible to visualize particles in the SEM films images. This behavior was not expected, as BL and ML powders presented hydrophilic characteristic with water solubility of approximately 61% and 54% at 25°C, respectively (**Table 1**), the particles were expected to dissolve. It is possible that, during the starch gelatinization process, water molecules bind to the exposed hydroxyl groups of amylose and amylopectin by hydrogen bonds (Hoover, 2001), leaving little water available for interaction with the BL and ML, which consequently hampered its solubilization.

The type of incorporation of the blackberry into filmogenic solution (directly or by sprinkling) influenced the structure of resulting films. The cross-section images of 40% BLD and MLD films (**Figure 2** D-F and J-L, respectively), showed continuous regions and disorganized regions due to the presence of BL and ML particle within arrowroot starch matrix formed during the films drying. BL and ML were integrated in arrowroot starch matrix, showing good compatibility between materials.

Some regions with BL and ML agglomerates could also be observed in films for both types of incorporation. This may be due to poor dispersion of powders in high concentrations in the film-forming solution, given its high viscosity. Similar characteristics were observed by Sartori and Menegalli (2016) for film containing solid lipid microparticles. Castillo et al. (2013) observed randomly dispersed nano-agglomerates and individual platelets of talc in nanocomposite films by TEM. Mukurumbira, Mellem and Amonsou (2017) observed that the incorporation of nanocrystals into starch films made the surface of films irregular and rough. According to the authors, these observed changes on film surfaces could be attributed to the presence of aggregated nanocrystals and, possibly, the interactions between nanocrystals and amylose in the starch. Ortega, Giannuzzi, Arce and García (2017) incorporated silver nanoparticles into starch films and also observed the presence of agglomerates of nanoparticles in the gelatinized starch suspension.

Using sieve to sprinkle BL and ML powder over the film-forming suspension allowed falling homogenously over the entire surface area of films. It can be seen in **Figure 2** G-I, in which, when 40% ML were sprayed under the film-forming suspension, a penetration of particles occurred in the starch matrix, while 40% BL remained on the surface. Probably, the ML are denser than BL (**Figure 2** M-O).

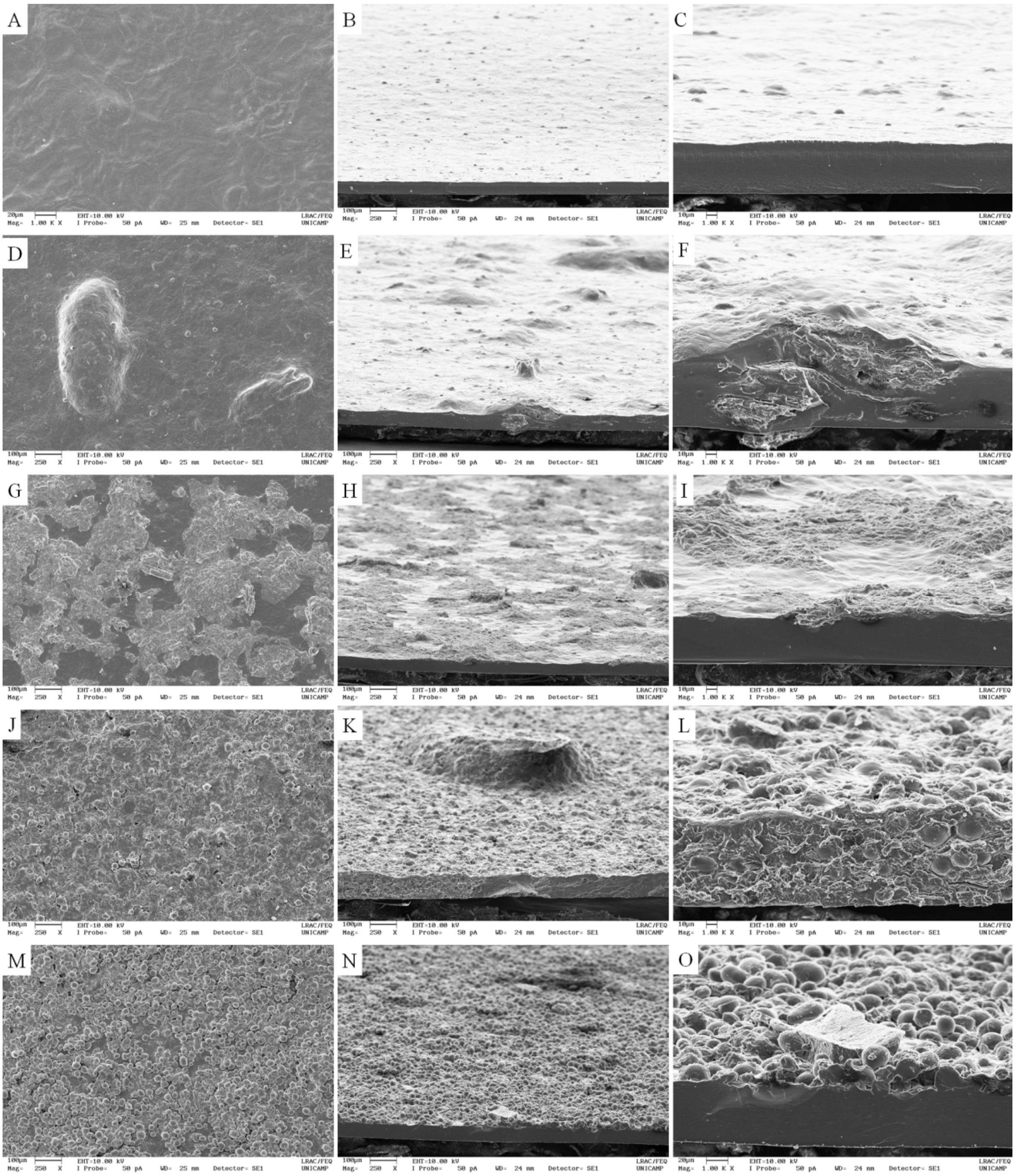


Figure 2. SEM images of the films with 0% and 40% of freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) incorporated directly (D) and by sprinkling (S): (A) surface of control film; (B and C) cross section of control film; (D) surface of 40% BLD film; (E and F) cross section of 40% BLD film; (G) surface of 40% BLS film; (H and I) cross section of 40% BLS film; (J) surface of 40% MLD film; (K and L) cross section of 40% MLD film; (J) surface of 40% MLS film; (K and L) cross section of 40% MLS film. Images A, C, F, I, L and O with 250x magnification and images B, D, E, G, H, J, K, M and N with 1000x magnification.

3.2.3 Color determination

Table 2 presents color parameters of films samples with 0%, 20%, 30% and 40% of blackberry pulp (BL) and microencapsulated blackberry pulp (ML) incorporated directly (D) and by sprinkling (S). Films incorporated with blackberry exhibited colorimetric parameters correlated with the parameters found for BL and ML powders (**Table 1**), differing significantly of the 0% film that was colorless.

The results indicate that incorporation and concentration of BL and ML significantly ($p < 0.05$) affected chromaticity parameters (a^* and b^*) of films. With incorporation of BL and ML in 0% film, values of b^* increased, going from negative to positive, showing a yellow color tendency. Similarly, Li et al. (2015) observed that introduction of starch nanocrystals resulted in yellowing of pea starch films.

A relevant increase in values of a^* also were observed, evidencing tendency towards red color and presence of anthocyanins pigments. It was reported that a^* coordinate is attributed to anthocyanin content of blackberry, which is responsible for redness color of BL and ML powders (Yamashita et al., 2017; Jiménez-Aguilar et al. 2011). Once again, low values indicate that developed films with BL and ML powder exhibited color shades ranging from red to yellow-orange, correlating with film visual observations.

Ortega et al. (2017) also observed that incorporation and concentration of silver nanoparticles significantly affected chromaticity parameters (a^* and b^*), although in both cases, the corresponding values were very low and, visually, nanocomposite films still remained colorless.

Regarding to luminosity (L^*), a significant effect ($p < 0.05$) was also found with addition of BL and ML powders, observing a decrease in this parameter with increasing concentration. As expected, color difference (*DE*) was significantly ($p < 0.05$) affected by both incorporation of BL and ML powders and their concentration. The increase in concentration from 20% to 40% of BL and ML incorporated directly and by sprinkling in arrowroot starch film (0%) caused statistically significant increase ($p<0.05$) in a^* and b^* values, leading to decrease of luminosity L^* and increase of total color difference ΔE^* (**Table 2**).

Table 2. Color parameters of films samples with 0%, 20%, 30% and 40% of freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) incorporated directly (D) and by sprinkling (S).

Films	*L**	*a**	*b**	*ΔE**
0%	91.54 ± 1.16 ^{a}	1.96 ± 0.09 ^{g}	-8.64 ± 0.46 ^{i}	-
20% BLD	63.16 ± 0.40 ^{b}	23.50 ± 0.18 ^{e}	1.35 ± 0.09 ^{hg}	37.00 ± 0.43 ^{de}
30% BLD	62.16 ± 0.48 ^{b}	24.60 ± 0.30 ^{dce}	1.69 ± 0.06 ^{g}	38.50 ± 0.56 ^{de}
40% BLD	47.28 ± 0.66 ^{edf}	35.05 ± 0.36 ^{a}	4.30 ± 0.16 ^{ba}	56.76 ± 0.76 ^{a}
20% BLS	57.89 ± 3.68 ^{cb}	27.44 ± 2.88 ^{c}	0.44 ± 0.29 ^{h}	43.17 ± 4.62 ^{dc}
30% BLS	46.67 ± 1.51 ^{edf}	34.31 ± 0.85 a	1.86 ± 0.36 ^{fg}	56.30 ± 1.75 ^{ba}
40% BLS	40.72 ± 3.87 ^{gf}	35.92 ± 0.61 ^{a}	3.52 ± 1.15 ^{bdc}	62.33 ± 3.70 ^{a}
20% MLD	62.78 ± 1.54 ^{b}	19.28 ± 0.68 ^{f}	3.11 ± 0.31 ^{dec}	35.57 ± 1.68 ^{e}
30% MLD	53.84 ± 0.37 ^{cd}	24.27 ± 0.24 ^{de}	4.09 ± 0.07 ^{bac}	45.61 ± 0.43 ^{c}
40% MLD	42.70 ± 0.70 ^{gf}	30.44 ± 0.11 ^{b}	4.72 ± 0.08 ^{a}	58.09 ± 0.66 ^{a}
20% MLS	49.89 ± 1.03 ^{ed}	25.98 ± 0.34 ^{dce}	2.21 ± 0.39 ^{feg}	49.29 ± 0.95 ^{bc}
30% MLS	42.96 ± 2.19 ^{egf}	30.62 ± 0.28 ^{b}	2.90 ± 0.35 ^{fde}	57.58 ± 1.93 ^{a}
40% MLS	36.27 ± 0.13 ^{g}	26.82 ± 1.50 ^{dc}	3.74 ± 0.29 ^{bdac}	61.94 ± 4.85 ^{a}

Same letters in the same column show no statistical difference ($p > 0.05$).

3.2.4 Anthocyanin content and antioxidant capacity

Table 3 shows anthocyanin content (mg/100g of blackberry solids) and antioxidant capacity (µmol of Trolox/g of blackberry solids) of films after drying process. In absence of blackberry powder (BL and ML), arrowroot starch film showed insignificant amount of anthocyanins and antioxidant capacity. Thus, it is clear that content of anthocyanins and antioxidant capacity presented by arrowroot starch films incorporated with BL and ML is directly related to their content in blackberry powders (**Table 1**).

It is important to note that, there was a decrease in anthocyanin content of films with BL and ML powders when compared to the initial amount in the respective powders. This happened because, anthocyanins present great susceptibility to degradation when exposed to environmental factors as temperature, light, pH and oxygen (Patras et al., 2010) during film production, resulting in its decrease on dried films. Maniglia, Tessaro, Lucas and Tapia-Blácido (2017) also reported losses of phenolic compounds due to a possible oxidation degradation of phenolic groups by heating applied during preparation of film-forming solution and during film drying process. Even so, in this work, the increase in concentration of blackberry incorporated in film led to a slight increase in content of anthocyanins in it.

Table 3. Anthocyanin content and antioxidant capacity of films with 0%, 20%, 30% and 40% of freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) incorporated directly (D) and by sprinkling (S).

Films	Total Anthocyanins (mg / 100 g of blackberry solids)	ABTS (µmol of Trolox / g of blackberry solids)
0%*	0.32 ± 0.12 ^{e}	9.15 ± 6.51 ^{f}
20% BLD	47.53 ± 6.06 ^{cd}	161.99 ± 10.54 ^{e}
30% BLD	40.23 ± 1.29 ^{cd}	180.68 ± 22.48 ^{ed}
40% BLD	76.47 ± 0.98 ^{a}	174.24 ± 51.73 ^{ed}
20% BLS	70.01 ± 9.65 ^{ba}	253.57 ± 24.68 ^{ed}
30% BLS	71.63 ± 6.96 ^{ba}	368.32 ± 37.02 ^{bac}
40% BLS	81.95 ± 12.83 ^{a}	408.24 ± 32.04 ^{a}
20% MLD	38.13 ± 0.55 ^{d}	272.64 ± 73.00 ^{dc}
30% MLD	41.79 ± 0.10 ^{cd}	274.55 ± 46.66 ^{dc}
40% MLD	39.39 ± 9.41 ^{cd}	278.93 ± 8.32 ^{bdc}
20% MLS	45.47 ± 2.13 ^{cd}	385.62 ± 18.54 ^{ba}
30% MLS	56.09 ± 1.22 ^{bc}	436.78 ± 24.48 ^{a}
40% MLS	55.68 ± 1.63 ^{bcd}	446.82 ± 39.66 ^{a}

Same letters in the same column show no statistical difference ($p > 0.05$).

*For 0% film, anthocyanin content and antioxidant capacity is expressed by total solids, Total Anthocyanin (mg / 100 g of total solids) and ABTS (µmol of Trolox / g of total solids).

In scientific literature, there are several works reporting a high correlation between content of phenolic compounds and antioxidant capacity (Genskowsky et al., 2015; Turumtay et al., 2014). For antioxidant capacity, the type of incorporation (direct or by sprinkling BL and ML) blackberry powders had more influence on antioxidant capacity than variation of its concentration in films. The films incorporated with 30 and 40% BL and ML by sprinkling were the ones with the highest antioxidant capacity. Probably the fact that the particles of BL and ML are on the surface of film has facilitated the extraction of bioactive compounds, due to a greater amount of particles in direct contact with the extraction solvent, consequently generating greater antioxidant activities. However, in direct incorporation, the BL and ML powders were integrated into the polymer matrix, which probably reduced their surface area in direct contact with extraction solvents. This fact may have hindered the extraction of bioactive compounds and consequently generated a lower antioxidant capacity.

In addition, it is important to emphasize that, there are huge varieties of bioactive compounds, with antioxidant capacity, contained in fruits extracts (Genskowsky et al., 2015). In addition to anthocyanins, other bioactive phenolic acids, tannins and ascorbic acid (Machado et al., 2015) may be present, contributing to the total antioxidant capacity of BL and ML powders, as well as in films with blackberry.

3.2.5 Water activity and moisture content

Table 4 shows films water activity and moisture content values evaluated after storage of films samples in desiccator at 25 °C and 55 ± 3% of relative humidity for 48 h. Water activity obtained for control film (0%) and films containing BL and ML ranged from 0.37 to 0.55. Films can be considered stable against microbial proliferation. According to Quek, Chock and Swedlund (2007), generally, food with $aw < 0.6$ is considered microbiologically stable and if any spoilage occurs, it is induced by chemical reactions rather than by microorganism.

Moisture content in control film (0%) and films containing BL and ML ranged from 7.88 to 13.65%. Similar results were found for films of amadumbe and potato starch containing amadumbe starch nanocrystals (0, 2.5, 5 and 10%), that presented moisture content ranged from 9.3 to 13.4 % and 14.8 to 16.7%, respectively (Mukurumbira et al., 2017).

Incorporation of BL and ML powders by sprinkling (S) in films has led to a significant decrease ($p < 0.05$) in moisture content of films as compared to films incorporated directly and control film (0%). Li et al. (2015) observed a decrease in moisture content with the incorporation of starch nanocrystals in pea starch films. In this study, probably the impact of falling particles on the film-forming solution caused by sprinkling may have triggered a discontinuity of polymer matrix on its surface, favoring the release of water from the starch structure. Low water content in BLS and MLS films may be one of the possible causes of these films have been brittle and fragile to handling.

Table 4. Water activity and moisture content of films with 0%, 20%, 30% and 40% of freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) incorporated directly (D) and by sprinkling (S).

Films	Aw at 25 °C	Moisture content (%)
0%	0.43 ± 0.05 ^{bc}	11.30 ± 0.10 ^{bdc}
20% BLD	0.37 ± 0.01 ^{c}	9.94 ± 1.02 ^{fedg}
30% BLD	0.37 ± 0.01 ^{c}	10.89 ± 0.62 ^{bedc}
40% BLD	0.40 ± 0.01 ^{bc}	13.65 ± 1.00 ^{a}
20% BLS	0.55 ± 0.09 ^{a}	8.72 ± 0.79 ^{fhg}
30% BLS	0.45 ± 0.03 ^{bc}	8.50 ± 0.84 ^{hg}
40% BLS	0.41 ± 0.02 ^{bc}	9.97 ± 1.17 ^{fedg}
20% MLD	0.40 ± 0.01 ^{bc}	10.42 ± 0.13 ^{fedc}
30% MLD	0.38 ± 0.01 ^{c}	12.14 ± 0.38 ^{bac}
40% MLD	0.39 ± 0.01 ^{c}	12.30 ± 0.83 ^{ba}
20% MLS	0.42 ± 0.01 ^{bc}	8.18 ± 0.28 ^{hg}
30% MLS	0.47 ± 0.03 ^{ba}	7.88 ± 0.72 ^{h}
40% MLS	0.45 ± 0.02 ^{bc}	9.22 ± 0.50 ^{fehg}

Same letters in the same column show no statistical difference ($p > 0.05$).

3.2.6 Film thickness, Solubility in water, Water vapor permeability and Mechanical properties

Table 5 shows thickness (mm), solubility in water (%), permeability to water vapor (g.mm/m^2.day.kPa), tensile strength (MPa) and elongation at break (%) of films with 0%, 20%, 30% and 40% of freeze dried blackberry pulp (BL) and freeze dried microencapsulated blackberry pulp (ML) incorporated directly (D) and by sprinkling (S).

Water solubility, permeability to water vapor and mechanical properties are directly influenced by thickness of film, among other factors (Ortega et al, 2017). Thickness of films (0%) made from arrowroot starch and of films incorporated with BL and ML powder ranged from 0.065 mm (0%) to 0.173 mm (40%MLS). Thickness of films significantly increased ($p<0.05$) with increasing concentration of BL and ML powder incorporated directly (D) and by sprinkling (S) as compared to control film (0%), although, in all cases, the film- forming suspension volume by plate area remained constant. The increased solid content and possible agglomeration of BL and ML in polymer matrix, evidenced by prominences on the surface of films by SEM (**Figure 2**), could explain this result. Ortega et al. (2017) also observed that the content of silver nanoparticles caused a slight increase in film thickness. The authors also attributed these results to increased solid content and possible agglomeration of silver nanoparticles.

Water solubility, permeability to water vapor and mechanical property are important parameters in choosing the applications of biopolymer films. Some applications, considering the potential use of these new polymer films in replacement of synthetic packaging, may require low water solubility, strength and flexibility to tolerate the typical effort encountered by packaging material during handling and transport of food, maintaining the integrity of the product (Mukurumbira et al., 2017; Slavutsky & Bertuzzi, 2014; Sadegh-Hassani & Mohammadi Nafchi, 2014). Some other applications such as in encapsulation, may require significantly higher solubility (Mukurumbira et al., 2017; Slavutsky & Bertuzzi, 2014), in order to allow release of encapsulated material into surroundings.

Incorporation and variation of BL and ML concentrations had significant effect ($p<0.05$) on solubility, tensile strength and elongation at break of starch films. In general, water solubility of films increased significantly ($p<0.05$), while tensile strength decreases, with increasing the BL and ML powder concentrations (20, 30 and 40%) in films, when compared to control film (0%), for direct incorporation. This behavior could be attributed to a possible reduction of intermolecular attraction forces caused by agglomeration of BL and ML, which afforded the disrupting and discontinuity of starch matrix (**Figure 2**). Consequently, polymer network was less dense, facilitating permeation of water in its structure and its solubilization, as well as reduceded its resistance and increasing flexibility. Films prepared with babassu mesocarp flour and with starch isolated from babassu mesocarp by casting exhibited similar behavior (Maniglia et al., 2017).

Incorporated films with BL and ML by sprinkling were more water soluble than films with blackberry powder incorporated directly. Probably, the location of blackberry particles have influenced this behavior. Contrary to direct incorporation, the incorporation by sprinkling allowed particles of powder stayed only in the surface of the films. As blackberry powders were porous and showed a high hydrophilicity, it is believed that, when the films were immersed in water, the particles rehydrated rapidly leading to their solubilization. Solubilization of the particles probably created holes in the surface of the films, which allowed the acess of water molecules into the starch matrix facilitating their solubilization.

As observed for water solubility, permeability to water vapor presented by films was also significantly affected ($p<0.05$) by incorporation of blackberry powder in the film, as well as by variation of its concentration. According Ludueña, Vázquez, and Alvarez (2012), the passage of water molecules through a polymeric material is the balance of three principal mechanisms: film crystallinity, tortuous pathways through the polymeric matrix and presence of structural defects on the surface.

The films with only 20% of powder presented decreasing permeability to water vapor in relation to the 0% film, as previously reported by Shi et al. (2013), for starch films containing spray dried and vacuum freeze dried starch nanoparticles. At low concentrations, powders of BL and ML were easily dispersed in film-forming solution, increasing compactness of the films, which may have hindered the passage of water molecules (Dai et al., 2015; Li et al., 2015; Shi et al., 2013). It is also possible that presence of blackberry powder particles within the starch matrix, as well as on the surface, has introduced a tortuous path for the passage of water molecules, which may have led to a decreasing behavior in water vapor permeability. In a single polymer film, the diffusible molecules migrate of a straight (middle) path that is perpendicular to the film surface. Whereas, in films with nanocomposites, the diffusion molecules must navigate through particles or platelets, as well as through interfacial zones of different permeability characteristics than those of the pure polymer (Ducan, 2011). In theory, the longer the diffusive pathway of the penetrant, in this case, the water molecules, the lower the permeability (Mukurumbira et al., 2017; Dai et al., 2015).

However, when more than 20% BL and ML of powder was incorporated directly, the particles tended to form aggregates, as observed in the microstructure of 40% BLD and MLD films (**Figure 2**). Aggregation of BL and ML particles reduces their active surface area to interact with the polymer matrix. This fact tends to weaken their adhesion at the starch matrix interface, destroying the orderly structure of the 0% film; increasing permeability to water vapor (Mukurumbira et al., 2017; Dai et al., 2015) and following the same trend observed for water solubility.

Table 5. Thickness (mm), solubility in water (%), permeability to water vapor (g.mm/m^2.day.kPa), tensile strength (MPa) and Elongation at break (%) of films with 0%, 20%, 30% and 40% of freeze dried blackberry pulp (BL) and the freeze dried microencapsulated blackberry pulp (ML) directly incorporated (D) and by sprinkling (S).

Films	Thickness (mm)	Solubility in water (%)	Permeability to water vapor (gmm/m^{2}daykPa)	Tensile Strength (MPa)	Elongation at break (%)
0%	0.065 ± 0.005^{d}	14.18 ± 0.26^{g}	3.62 ± 0.27^{hdfge}	22.71 ± 1.27^{a}	3.18 ± 0.44^{d}
20% BLD	0.092 ± 0.005^{dc}	21.64 ± 0.93^{fe}	3.03 ± 0.10^{hfge}	3.60 ± 0.33^{ih}	23.53 ± 3.60^{a}
30% BLD	0.121 ± 0.014^{bdaac}	22.76 ± 1.13^{dfe}	6.63 ± 0.39^{bc}	3.55 ± 0.12^{ih}	23.33 ± 0.72^{a}
40% BLD	0.154 ± 0.054^{bac}	26.14 ± 1.16^{dce}	5.40 ± 0.47^{dce}	2.73 ± 0.33^{i}	26.42 ± 1.40^{a}
20% BLS	0.082 ± 0.006^{d}	19.26 ± 1.68^{fe}	1.67 ± 0.12^{h}	10.84 ± 1.69^{b}	7.46 ± 2.55^{c}
30% BLS	0.098 ± 0.013^{bdc}	24.65 ± 1.95^{dce}	2.38 ± 0.45^{hg}	8.16 ± 0.64^{dc}	5.26 ± 1.55^{dc}
40% BLS	0.113 ± 0.016^{bdac}	27.98 ± 2.69^{bc}	3.47 ± 0.14^{hfge}	6.32 ± 0.85^{fe}	18.32 ± 4.69^{b}
20% MLD	0.150 ± 0.024^{bac}	21.74 ± 1.70^{fe}	2.43 ± 0.36^{hg}	7.02 ± 0.99^{de}	3.99 ± 0.76^{dc}
30% MLD	0.146 ± 0.022^{bac}	22.18 ± 0.36^{dfe}	7.80 ± 0.07^{ba}	5.62 ± 0.40^{feg}	3.28 ± 0.42^{d}
40% MLD	0.154 ± 0.010^{ba}	23.69 ± 0.77^{dfce}	9.23 ± 0.47^{a}	4.51 ± 0.29^{hg}	7.72 ± 0.50^{c}
20% MLS	0.147 ± 0.017^{bac}	27.14 ± 2.45^{dc}	4.42 ± 0.17^{dfge}	8.87 ± 0.86^{c}	4.32 ± 0.40^{dc}
30% MLS	0.153 ± 0.005^{bac}	33.89 ± 2.50^{a}	5.08 ± 1.87^{dfce}	6.98 ± 0.60^{de}	3.96 ± 0.71^{dc}
40% MLS	0.173 ± 0.011^{a}	32.33 ± 1.39^{ba}	5.57 ± 1.05^{dc}	4.82 ± 0.67^{fhg}	3.25 ± 0.96^{d}

Same letters in the same column show no statistical difference ($p > 0.05$).

4. Conclusions

The lyophilization method was successfully to obtain powdered blackberry pulp (BL), as well as in microencapsulated form (ML). Freeze dried BL and ML resembled a broken glass structure of variable sizes. For ML powder, it was possible to observe spherical particles distributed in their vitreous structures. The powders had a reddish color typical of the blackberry pulp.

Incorporated films with BL and ML powder exhibited structural characteristics directly related with those observed for BL and ML powders. Incorporation of blackberry powder has rendered the surface of the rough and irregular film, besides transferring color, anthocyanins and antioxidant capacity for arrowroot starch film (0%). The type of incorporation of blackberry into the filmogenic solution (direct - D or by sprinkling - S) influenced structure, antioxidant capacity, moisture content and water solubility of the resulting films.

Films with blackberry powder were less mechanically resistant, thicker, more flexible and more soluble than the film (0%). These differences in films properties are due to the structures derived from BL and ML, which generate the discontinuity of polymer matrix. Films with only 20% BL and ML presented lower water vapor permeability rates than the 0% film. This behavior was attributed to a better dispersion of blackberry powder at low concentrations in the film-forming solution, as well as the introduction of tortuous paths in starch matrix. At concentrations above 30%, there was an increase in water vapor permeability due to the presence of agglomerated blackberry dust particles. The film (0%) was stronger, rigider, less soluble and less flexible, probably because its structure is more continuous, organized and dense, as confirmed by SEM images. Films incorporated with sprinkling blackberry powder had higher antioxidant capacity and were more soluble in water, showing great potential to be used as a vehicle for releasing bioactive compounds into the surroundings.

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Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

References

A.O.A.C. Official Methods of Analysis. (2006) (18th ed.). *Association of Official Analytical Chemists*, Gaithersburg, Maryland.

ASTM (1980). Standard test methods for tensile properties of thim plastic sheeting. Method D 882-83. In *Annual book of American standard testing methods*. Philadelphia: ASTM.

ASTM (1989). Standard test methods for water vapor transmission of materials. Method E96 e 80. *In Annual book of American standard testing methods*. Philadelphia: ASTM.

Avella, M., De Vlieger, J.J., Errico, M.E., Fischer, S., Vacca, P., & Volpe, M.G. (2005). Biodegradable starch/clay nanocomposite films for food packaging applications. *Food Chemistry*, 93, 3, 467–474.

Azeredo, H.M.C., Morrugares-Carmona, R., Wellner, N., Cross, K., Bajka, B., & Waldron, K.W. (2016). Development of pectin films with pomegranate juice and citric acid. *Food Chemistry*, 198, 101–106.

Cai, Y.Z., & Corke, H. (2000). Production and properties of spray-dried Amaranthus betacyanin pigments. *Journal of Food Science*, 65, 1248-1252.

Cano-Chauca, M., Stringheta, P.C., Ramos, A.M., & Cal-Vidal, J. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science and Emerging Technologies*, 5, 420–428.

Castillo, L., López, O., López, C., Zaritzky, N., García, M. A., Barbosa, S., & Villar, M. (2013). Thermoplastic starch films reinforced with talc nanoparticles. *Carbohydrate Polymers*, 95, 664–674.

D'Agostino, M.F., Sanz, J., Sanz, M.L., Giuffrè, A.M., Sicari, V., & Soria, A.C. (2015). Optimization of a Solid-Phase Microextraction method for the Gas Chromatography–Mass Spectrometry analysis of blackberry (*Rubus ulmifolius* Schott) fruit volatiles. *Food Chemistry*, 178, 10–17.

Dai, L., Qiu, C., Xiong, L., & Sun, Q.J. (2015). Characterisation of corn starch-basedfilms reinforced with taro starch nanoparticles. *Food Chemistry*, 174, 82–88.

Daza, L.D., Fujita, A., Fávaro-Trindade, C.S., Rodrigues-Ract, J. N., Granato, D., & Genovese, M.I. (2016). Effect of spray drying conditions on the physical properties of Cagaita (*Eugenia dysenterica* DC.) fruit extracts. *Food and Bioproducts Processing*, 97, 20-29.

Duncan, T. V. (2011). Applications of nanotechnology in food packaging and foodsafety: barrier materials: Antimicrobials and sensors. *Journal of Colloid and Interface Science*, 363, 1–24.

Franceschinis, L., Salvatori, D.M., Sosa, N., & Schebor, C. (2014). Physical and functional properties of blackberry freeze-and spray-dried powders. *Drying Technology*, 32, 154-207.

Genskowsky, E., Puente, L. A., Pérez-Álvarez, J. A., Fernandez-Lopez, J., Muñoz, L. A., & Viuda-Martos, M. (2015). Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT - Food Science and Technology*, 64, 1057-1062.

Gontard, N., Guilbert, S., & Cuq, J.L. (1992). Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*, 57, 190-199.

Hla, P.K., & Hogekamp, S. (1999). Wetting behaviour of instanized cocoa beverage powders. *International Journal of Food Science and Technology*, 34, 4, 335-342.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45, 3, 253 -267.

Jiménez-Aguilar, D.M., Ortega-Regules, A.E., Lozada-Ramírez, J.D., Pérez-Pérez, M.C.I., Vernon-Carter, E.J., & Welti-Chanes, J. (2011) Color and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. *Journal of Food Composition and Analysis*, 24, 889-894.

Li, X., Qiu, C., Ji, N., Sun, C., Xiong, L., & Sun, Q. (2015). Mechanical: Barrier andmorphological properties of starch nanocrystals-reinforced pea starch films. *Carbohydrate Polymers*, 121, 155–162.

Ludueña, L., Vázquez, A., & Alvarez, V. (2012). Effect of lignocellulosic filler type and content on the behavior of polycaprolactone based eco-composites for packaging applications. *Carbohydrate Polymers*, 87, 411-421.

Machado, A.P. da F., Pasquel-Reátegui, J.L., Barbero, G.F., & Martínez, J. (2015). Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International*, 77, 675–683.

Maniglia, B.C., Tessaro, L., Lucas, A.A., & Tapia-Blácido, D.R. (2017). Bioactive films based on babassu mesocarp flour and starch. *Food Hydrocolloids*, 70, 383-391.

Martinez, C., & Cuevas, F. (1989). *Evaluación de la calidad culinaria y molinera del arroz.* Guia de estudo, CIAT.

Moorthy, S. N. (2002). Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54, 559-592.

Mukurumbira, A.R., Mellem, J.J., & Amonsou, E.O. (2017). Effects of amadumbe starch nanocrystals on the physicochemical properties of starch biocomposite films. *Carbohydrate Polymers*, 165, 142–148.

Nogueira, G.F., Fakhouri, F.M., de Oliveira, R.A. (2018). Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films. *Carbohydrate Polymers*, https://doi.org/10.1016/j.carbpol.2018.01.024

Ortega, F., Giannuzzi, L., Arce, V.B., & García, M.A. (2017). Active composite starch films containing green synthetized silver nanoparticles. *Food Hydrocolloids*, 70, 152-162.

Patras, A., Brunton, N.P., Donnell, C.O., & Tiwari, B.K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends in *Food Science & Technology*, 21, 3 -11.

Quek, S.Y., Chok, N.K., & Swedlund, P. (2007). The physicochemical properties of spary-dried watermelon powders. *Chemical Engineering and Processing: Process Intensification*, 46, 386-392.

Reid, D.S., & Fennema, O.R. (2010). Água e gelo. In: Damodaran, S.,Parkin, K.L., Fennema, O.R. (Eds.), *Química de Alimentos de FENNEMA*. Artmed, Porto Alegre, pp. 25–74.

Sadegh-Hassani, F., & Mohammadi Nafchi, A. (2014). Preparation andcharacterization of bionanocomposite films based on potato starch/halloysitenanoclay. *International Journal of Biological Macromolecules*, 67, 458–462.

Sartori, T., & Menegalli, F.C. (2016).Development and characterization of unripe banana starch films incorporated with solid lipid microparticles containing ascorbic acid. *Food Hydrocolloids*, 55, 210-219.

Shahidi, F., Han, X.Q. (1993). Encapsulation of food Ingredients. *Critical Reviews in Food Science and Nutrition*, 33, 501–47.

Shi, A.-M., Wang, L.-J., Li, D., & Adhikari, B. (2013). Characterization of starch filmscontaining starch nanoparticles: part 1: Physical and mechanical properties. *Carbohydrate Polymers*, 96, 593–601.

Sims, D.A., & Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of environment*, 81, 337-354.

Slavutsky, A. M., & Bertuzzi, M. A. (2014). Water barrier properties of starch filmsreinforced with cellulose nanocrystals obtained from sugarcane bagasse. *Carbohydrate Polymers*, 110, 53–61.

Tanada-Palmu, P.S., Hélen, H., & Hyvonen, L. (2000). Preparation, properties and applications of wheat gluten edible biofilms. *Agricultural And Food Science in Finland*, 9, 23-25.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technoloy*, 14, 3, 71-78

Turumtay, E. A., Islamoglu, F., Çavus, D., Sahin, H., Turumtay, H., & Vanholme, B. (2014). Correlation between phenolic compounds and antioxidant capacity of Anzer *tea (Thymus praecox* Opiz subsp. *caucasicus* var. *caucasicus*). *Industrial Crops and Products*, 52, 687- 694.

Yamashita, C., Chung, M.M.S., dos Santos, C., Mayer, C.R.M., Moraes, I.C.F., & Branco, I.G. (2017). Microencapsulation of an anthocyanin-rich blackberry (spp.) by-product extract by freeze-drying. *LWT - Food Science and Technology*, 84, 256-262.

Zavareze, E. da R., El Halal, S. L. M., Pereira, J.M., Radünz, A. L., Elias, M. C., & Dias, A. R. G. (2009). Chemical characterization and extraction yield of rice starch with different amylose contents. *Brazilian Journal of Food Technology*, II SSA, janeiro.

CAPÍTULO 8- Antioxidant films of arrowroot starch and blackberry pulp and potential use as active food packaging

Este capítulo será submetido à revista *Food Packaging and Shelf Life* e está apresentado no formato de artigo científico

Antioxidant films of arrowroot starch and blackberry pulp and potential use as active food packaging

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Highlight

- Arrowroot starch films and blackberry pulp microparticles were developed.
- Active films showed significant antioxidant capacity.
- Incorporation of blackberry powder caused heterogeneity in the starch matrix.
- Arrowroot starch films showed stability to pH and sterilization process.
- There was darkening of the films with blackberry pulp after their sterilization.
- Films showed almost entirely disintegration after 38-days of composting.

Abstract: Blackberry pulp powder (BL, without encapsulating agent) and microencapsulated blackberry pulp (ML, with encapsulating agent) obtained by freeze drying and spray drying were incorporated in arrowroot starch film-forming solution, in concentrations of 0, 20, 30 and 40% (blackberry solids mass / biopolymer mass), to produce active food packaging with antioxidant properties. Films with blackberry powder showed high antioxidant capacity ranging from 335.05 to 794.87 µmol ferrous sulfate/ g of blackberry solids, while control film had an insignificant antioxidant capacity. SEM images showed that incorporation of blackberry powder in the film-forming solution caused heterogeneity in the starch matrix and made its surface rough and irregular. As blackberry powder content increased, films showed an increase in their solubility in acidic, neutral and alkaline solutions. There was darkening of films with blackberry pulp after sterilization process. Films showed almost entirely disintegration after 38-days of composting.

Keywords: freeze drying; spray drying; casting; microstructure; sterilization; biodisintegration;

1. Introduction

Food packaging has a fundamental role in controlling the interactions between food and environment, protecting and helping to maintain product quality (Jaramillo, Gutiérrez, Goyanes, Bernal, & Famá, 2016). However, the polymers mainly used in this field are from non-renewable sources, which despite having excellent functional properties, are associated with environmental pollution issues (Piñeros-Hernandez, Medina-Jaramillo, López-Córdoba, & Goyanes, 2017).

Recently, reduction of environmental impact by using natural polymers in packages has been constituted as real alternative to the traditional polymers derived from petroleum (Piñeros-Hernandez et al., 2017; Jaramillo et al., 2016; Chang-Bravo, López-Córdoba, & Martino, 2014; Fakhouri et al., 2013; Cerruti et al., 2011). When these polymers are disposed in bioactive environments and are degraded by enzymatic action of living organisms, such as bacteria, yeasts, fungi, being converted at the end of degradation process into CO_2, H_2O and biomass under aerobic conditions and hydrocarbons, methane and biomass under anaerobic conditions, are called biodegradable (Mohee, Unmar, Mudhoo, & Khadoo, 2008).

In this sense, starch is one of the natural polymers that have been used to develop eco-friendly matrices for film applications and edible coatings in food industry. In addition to be able to form a continuous matrix, odorless, tasteless, colorless and non-toxic films, starch has the advantage of being abundant and renewable, and existes in various forms depending on its origin (Piñeros-Hernandez et al., 2017; Sartori & Menegalli, 2016).

Arrowroot (*Maranta arundinaceae* L.) starch is characterized as a promising material for production of edible and biodegradable films, because it presents good digestibility, gelling ability and high amylose content (ranging from 16 to 27% for total amylose content) (Villas-Boas & Franco, 2016; Moorthy, 2002; Hoover, 2001), which are desirable features for production of films with good technological properties (Tharanathan, 2003).

In addition, incorporation of natural additives, such as processing benefits, plasticizers, stabilizers, antibacterials and antioxidants into the film-forming solution, enables development of edible and biodegradable films with some customized properties (Piñeros-Hernandez et al., 2017; Jaramillo et al., 2016; Chang-Bravo et al., 2014; Cerruti et al., 2011). Studies have shown that incorporation of fruits pulp in polymeric matrix confer to the resultant film, bioactive compounds, antimicrobial and antioxidant properties, desirable color and flavor, extending its applicability such as fruit stripes or colorful coatings for specific foods (Azeredo et al., 2016; Otoni et al., 2014; Farias, Fakhouri, de Carvalho, & Ascheri, 2012).

Blackberry (*Rubus fruticosus*), cv. Tupy, is a fruit with appealing color and desirable sweet flavor, rich phenolic compounds, such as phenolic acids, tannins and anthocyanins, which has high antioxidant capacity (Machado, Pasquel-Reátegui, Barbero, & Martínez, 2015). However, blackberry's short post-harvest life is due to its high respiratory rate and its fragile structure, which limits its consumption in the fresh form (Ferrari, Ribeiro, & Aguirre, 2012). For this reason, drying of blackberry pulp by spray drying or freeze drying are alternative processes for preservation of its nutritional and organoleptic properties, extending shelf life, and preventing loss of volatile components during processing and storage (Araujo-Díaz, Leyva-Porras, Aguirre-Bañuelos, Álvarez-Salas, & Saavedra-Leos, 2017).

More specifically, spray drying is a technological process in which a fluid product is transformed into powder by atomizing it into a hot gas stream (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Freeze drying is based on dehydration by sublimation of a frozen product resulting in a dry material (Yamashita et al., 2017). Both processes can be used as encapsulation method when it incorporates 'active' material within a protective matrix, which is essentially inert to the material being encapsulated (Ré, 1998). The resulting products of this process are microparticles with diameters comprised in the range of μm-mm (Avellone et al., 2018). The incorporation of these blackberry pulp microparticles into starch matrix is believed to result in biodegradable, edible and active films with improved functional properties. Until now, to our knowledge there are no published studies of arrowroot starch films ingested with blackberry pulp microparticles.

Therefore, the objective of this work was to incorporate directly and by sprinkling, blackberry pulp powder and microencapsulated blackberry pulp by freeze drying and spray drying in arrowroot starch film-forming solution. The influence of type of incorporation and variation of blackberry powders concentration in structural properties, antioxidant properties, stability to pH and sterilization and biodesintegration of starch films were investigated to evaluate their application in the form of biodegradable, edible and active packaging.

2. Materials and methods

2.1. Materials

Frozen fruits of blackberry (*Rubus fruticosus*), cv Tupy, were acquired from "Agro Monte Verde Eirelli", company of Cambuí – MG, Brazil, and used in this research work. Pulp was obtained by grinding of blackberry fruit in a blender, previously thawed in the refrigerator (8 °C) for 24 h. Then, pulp was sieved to remove the seeds, homogenized and packed in polypropylene bottles and coated with aluminium foil to protect against photodegradation. The samples were stored in freezer at -40 ± 3 °C until drying. Blackberry soluble solids content was 9 °Brix, determined with a manual refractometer with a 0–90 °Brix range and 0.2 °Brix resolution (Reichert, Model AR200, USA). The pulp presented total solids content of 10.3 g/100g of pulp.

For microencapsulation of blackberry pulp, arrowroot starch and Instantgum® gum arabic (Colloides Naturels, São Paulo, Brazil) were used as encapsulating agents. In elaboration of edible biodegradable films, arrowroot starch containing 15.24 ± 0.19% of water, 0.40 ± 0.03% of protein, 0.12 ± 0.01 % of fat, 0.33 ± 0.01% of ashes and 83.91 ± 0.00% of carbohydrates (AOAC, 2006) and amylose content of 35.20 ± 1.63%, determined according to methodology described by Martinez and Cuevas (1989), with adaptations (Zavareze et al., 2009), were used as film forming matrix and glycerol P.A. (Reagen, Quimibrás Indústrias Químicas S.A.- Rio de Janeiro, Brazil) as plasticizing agent. All other reagents used for the analyses presented analytical grade.

2.2 Preparation of blackberry pulp microparticles

Blackberry microparticles were produced from homogenization of blackberry pulp with encapsulating agent, consisting of arrowroot starch and gum arabic mixture (1: 1 mass / mass) in ratio of 1:1.78 (mass / mass, blackberry pulp solids for encapsulating agent). Solution of encapsulating agent and blackberry pulp was performed in a mixer type homogenizer at room temperature for 5 minutes. Spray drying and freeze drying techniques were used to prepare blackberry pulp microparticles.

2.2.1 Spray drying

The freshly prepared solution of blackberry pulp and encapsulating agent was diluted in water in proportion of 1:2 (w / w) to enable its spraying. A bench-top spray dryer (Model B191, Büchi, Flawil, Switzerland) with feed mass flow rate of 0.2 kg / h was used in the process. Atomization was accomplished using a 0.5 mm double-flow atomizer nozzle. Compressed air was used as atomizing media and the inlet outlet air temperatures were 143°C and 105.43 ± 3.13 °C, respectively. Compressed air flow rate was 0.6 m^3 / h and its pressure was maintained at 8 bar. Powders were collected at cyclone and finally transferred for polyethylene packages. These spray dried blackberry microparticles were stored in desiccator containing dried allochroic silica gel at 25 °C.

2.2.2 Vacuum freeze drying

A portion of frozen blackberry pulp with and without encapsulating agent (encapsulating agent, consisting of arrowroot starch and gum arabic mixture (1: 1 mass / mass) in ratio of 1:1.78 (mass / mass, blackberry pulp solids for encapsulating agent) was freeze dried (Mod. 501, Edwards Pirani, Crawley, West Sussex, UK), with initial temperature of -40 °C, pressure of 0.1 mmHg and final temperature of 25 °C per 2 h, with total cycle time of 48 h. The product obtained was ground in hammer mill (MR Manesco and Ranieri LTDA, model MR020, Piracicaba- Brazil) and sieved (28 mesh). Freeze dried blackberry microparticles were stored in the same conditions ofof spray dried samples.

2.2.3 Characterization of powders

Particle size distribution of powders was determined by particle analyzer based on laser diffraction (Mastersizer, Mastersizer 3000, Malvern Instruments, Worcestershire, U.K). Average diameter was determined by the diameter of a sphere with equivalent volume (diameter of the Brouckere, D[4.3]). Samples were analyzed in triplicate with dispersion in 99.5% ethanol and 1500 rpm shaking. Dried powder was dispersed in 99.5% ethanol in order to morphology be examined by Optical Microscope (DMLM-Leica, Cambridge, England).

The powders particles were also observed in bench Scanning Electron Microscope (SEM) (Leo 440i - MEV/EDS: LEO Electron Microscopy/Oxford, Cambridge, England).

Powder sample was placed on double-sided carbon adhesive tape adhered to stub, submitted to application of gold layer for 2 minutes (K450 -Sputter Coater EMITECH, Kent, United Kingdom) and observed in scanning electron microscope operated at 20 kV.

2.3 Incorporation of blackberry microparticles into film-forming suspension

2.3.1 Preparation of film-forming suspension

Film-forming suspension was obtained by dispersing arrowroot starch in distilled water (4%, mass / mass, as optimized by Nogueira, Fakhouri & Oliveira, 2018). This suspension was heated to 85 ± 2 °C in a thermostatic bath (TECNAL, Brazil), with constant agitation, for about 5 minutes. After starch gelatinization, blackberry powders were added to film-forming suspension in different concentrations, 0%, 20%, 30% and 40% (mass / mass of dry starch) and two different ways The first incorporation method was directly in film-forming suspension and the second one was by sprinkling into film-forming suspension. Glycerol was added to the solution at concentration of 17% (mass/mass of total solids, as optimized by Nogueira, Fakhouri & Oliveira, 2018).

2.3.2 Direct incorporation of blackberry powder into film-forming suspension (D)

The freeze dried blackberry pulp (BL), the freeze dried microencapsulated blackberry pulp (ML) and the spray dried microencapsulated blackberry pulp (MS) was added directly into film-forming suspension and homogenized in previously mentioned proportions. Aliquots of 25 mL of resulting film-forming suspension were distributed on 12 cm diameter support plates. Films were dried at room temperature (25 ± 5 °C), until they could be easily removed from the support plate, approximately for 24 h. Films were conditioned at 25 °C and 55 ± 3% of relative humidity for 48 h, before their characterization.

2.3.3 Incorporation of blackberry powder by sprinkling into film-forming suspension (S)

Aliquots of 25 mL of resulting film-forming suspension (item 2.3.1) were distributed on 12 cm diameter support plates. The freeze dried blackberry pulp (BL), the freeze dried microencapsulated blackberry pulp (ML) and the spray dried microencapsulated blackberry pulp (MS) was dispersed through a stainless steel sieve (53 mesh), homogeneously in all the surface area of film-forming suspension already disposed on the support plates. Then, films were dried for 24 h at room temperature (25 ± 5 °C). After drying, films were removed from the support plates and conditioned at 25 °C and 55 ± 3% relative humidity for 48 h, before their characterization. The nomenclature of films is presented in **Table 2**.

2.4 Films characterization

2.4.1 Morphology

Electronic Scanning Electron Microscope with X-ray Dispersive Energy Detector (SEM) bench (model of MEV: Leo 440i, model of EDS: 6070, Leo 440i - LEO Electron Microscopy/Oxford- Cambridge, Inglaterra) was used to observe the morphological characteristics of the surface and cross section developed for sample of the films formulations. Films sample was placed on double-sided carbon adhesive tape adhered to stub, submitted to application of a gold layer (model K450, Sputter Coater EMITECH, Kent, United Kingdom) and observed in scanning electron microscope operated at 20 kV.

2.4.2 Antioxidant property

The antioxidant capacity by FRAP method was performed using methodology described by Rufino et al. (2006). In acid media, ferric complex tripyridyltriazine (TPTZ) is reduced to its deep blue ferrous form in presence of antioxidants, causing an increase in absorbance at 595 nm. FRAP reagent was prepared with 25 mL of 0.3 M acetate buffer solution (pH 3.6), 2.5 mL of 10 mM TPTZ solution (prepared with 40 mM HCl) and 2.5 mL of 20 mM aqueous ferric chloride solution.

Film and powder samples were extracted in methanol solution (50% methanol in distilled water, v / v) and then in acetone solution (70% acetone in distilled water, v / v) to determine antioxidant property. Extraction was performed by placing ~ 1 g of sample (dry basis), 40 mL of 50% methanol and 40 mL of 70% acetone in a volumetric flask. The volume was completed until 100 mL of distilled water. Aliquots of 90 µL of each diluted extract was transferred into test tubes along with 270 µL of distilled water and 2.7 mL of freshly prepared FRAP reagent. This mixture was homogenized and maintained in water bath at 37 °C. After 30 minutes, reading was performed at 595 nm in ultraviolet spectrophotometer (model

Q798U2M, Quimis, Brazil). FRAP reagent was used as blank sample to calibrate the spectrophotometer. For quantification of antioxidant capacity, a standard curve was constructed using aqueous solutions of Fe (II) in concentration range of 0 to 2000 µM. The antioxidant capacity was expressed in µM ferrous sulfate / g solids. All the analyses were performed in duplicate.

2.4.3 Stability in acidic, neutral and alkaline solutions

Stability of films in acid, neutral and alkaline solutions was evaluated following methodology described by Jaramillo et al. (2016), with adaptations. Samples of films were cut into disks of 2 cm in diameter, in triplicate, and immersed in containers with 10 mL of standard solutions of hydrochloric acid (pH = 3), distilled water (pH = 7) and sodium hydroxide (pH = 10). The containers were sealed and kept at 5 °C for 12 days. After this period, not solubilized samples were removed and dried (105 °C for 24 h) to determine final dry mass. Solubility was expressed according to Equation (1). Changes in the appearance of the samples were recorded with a camera (model Easy Share Z712 IS, Kodak 7.1 mega pixels).

$$\text{Solubilized material}(\%) = \frac{m_{si} - m_{sf}}{m_{si}} \text{ x } 100 \qquad (1)$$

In which 'm_{si}' is initial dry mass of the films (g), 'm_{sf}' is final dry mass of non-solubilized films (g).

2.4.4 Stability of films on sterilization process

Stable films on sterilization temperature were assessed by visual changes in appearance and in thickness. Samples of films, cut into disks of 2 cm in diameter, were placed in sealed containers and submitted to 127 °C for 15 minutes in an autoclave. Thickness of each film sample was randomly measured in 5 different parts of sample before and after sterilization process. Changes in samples appearance were recorded with a camera (model Easy Share Z712 IS, Kodak, 7.1 mega pixels).

2.4.5 Biodesintegration test

Biodesintegration test of films was performed as reported by Jaramillo et al. (2016). Samples of films, cut out into disks of 2 cm in diameter, were buried at 1 e 2 cm depth in plastic trays containing organic composting (Class B1 organic fertilizer, Soilfert, composition: peat, ground pine bark, micro and macronutrient, enriched ash, 30% moisture, as reported by manufacturer). Plastic trays were incubated at room temperature (relative humidity of 85%) under aerobic conditions. Then, film samples were recovered at 0, 7, 14, 21, 28 and 38 days. The biodesingradation process was monitored by visual inspection of buried films: samples were collected and photographed periodically (Cerruti et al., 2011).

2.5 Statistical analysis

Significant differences between average results were evaluated by analysis of variance (ANOVA) and Tukey test at 5% of level of significance, using SAS software (Cary, NC, USA).

3. Results and discussion

3.1 Characterization of powders

Blackberry pulp microparticles were successfully obtained from spray drying and freeze drying techniques, using a mixture of arrowroot starch and gum arabic (1: 1 mass / mass) in a ratio of 1:1.78 (mass / mass, blackberry pulp solids for encapsulating agent) as encapsulating agent. However, drying of blackberry pulp without encapsulating agent was only possible by freeze drying.

There is great difficulty in drying fruit pulps by spray drying method without addition of encapsulating agent, since fruits are rich in low molecular weight sugars, which have low glass transition temperatures (Saavedra-Leos et al., 2012), which influences in transition temperature of dried particles, hindering the drying process by this method. Usually without addition of encapsulating agent, during the spray drying process, the fruit pulp behaves like a syrup, with a high stickiness, which leads to its adherence to drying chamber wall, obtaining a caked product or a hard film, instead of a dry powder (Yousefi, Emam-Djomeh, & Mousavi, 2011). With addition of encapsulating agent, the end glass transition temperature of the mixture of pulp and agents tends to increase, reducing the stickiness of pulp via microencapsulation and, consequently, decreasing its adhesion in drying chamber, making possible to collect the powder (Santana, Cano-Higuita, de Oliveira, & Telis, 2016; Igual, Ramires, Mosquera, & Martínez-Navarrete, 2014).

Visually, blackberry powders presented a typical reddish coloration of blackberry pulp (**Figure 1**). The ML and MS powders were lighter than BL, because the incorporation of encapsulating agent which exhibits light color and dilutes the anthocyanin pigment, responsible for the red color of blackberry (Ferrari at al., 2012).

Figure 1 shows the morphological characteristics of BL, ML and MS powders, observed by optical microscopy (OM) and scanning electron microscopy (SEM). Powders (BL and ML) obtained by freeze drying method, observed by optical microscopy, resembled crystals of varied sizes, whereas powder (MS), obtained by spray drying, seemed to be agglomerated with fine particles.

These observations were confirmed by SEM images. The external morphology of BL and ML powders is similar of broken glass structure, as observed by Yamashita et al. (2017). Curiously for ML powder, spherical particles could be visualized in the vitreous structures. Probably, these particles are due to the microencapsulation of blackberry pulp by encapsulating agent. For powder obtained by spray dryer (MS), besides spherical particles, oval and withered particles were also observed. From image of **Figure 1L**, it is evident that particles of MS powder coalesce into large irregularly shaped particles.

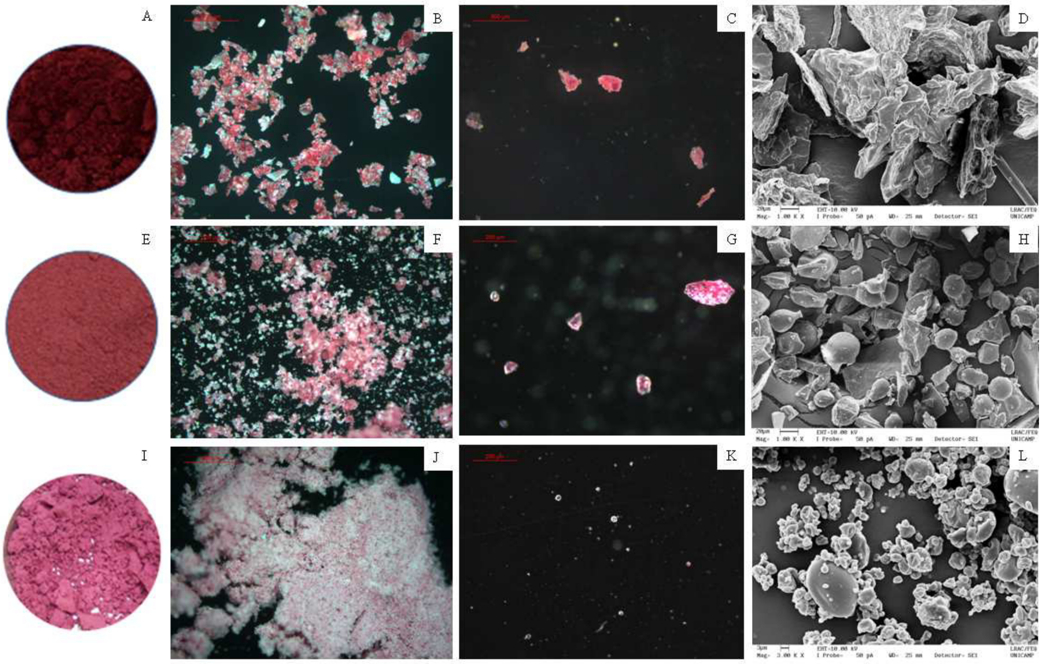

Figure 1. Photographic (column 1), optical microscopy (column 2 - dry samples and column 3 - sample dispersed in 99.5% ethanol) and scanning electron microscopy (column 4) images of blackberry powders: (A, B, C, D) freeze dried blackberry pulp (BL), (E, F, G, H) freeze dried microencapsulated blackberry pulp (ML); (I, J, K, L) spray dried microencapsulated blackberry pulp (MS). Scale = 500 µm (B, C, F and J); Scale = 200 µm (G and K); Scale = 20 µm (D and H); Scale = 3 µm (L).

Table 1 shows values of average diameter D[4,3] and size distribution of particles powders resulted from the different processes. The particles of BL and ML powders were significantly ($p<0.05$) larger than those of MS powder (**Table 1**). Particles of BL, ML and MS presented average diameter D[4,3] of 149.50 ± 2.92, 95.96 ± 5.87 and 9.82 ± 0.12 µm, respectively. Yamashita et al. (2017) prepared blackberry powder containing maltodextrin 10 and 20 DE by freeze drying and obtained particles which presented average diameter D[4,3] of 100.77± 1.47 and 98.99 ± 1.33 µm. On the other hand, Ferrari et al. (2012) obtained average diameter ranging from 13.33 ± 0.18 to 34.18 ± 1.98 µm for samples of blackberry juice powder, produced under different drying temperatures and concentrations of maltodextrin 20 DE, by spray drying.

These differences in size are due to the different production methods of powders. The size of dried particles obtained by spray drying depends on the size of spray droplets and the size of these droplets is dependent on atomizer type and operational parameter (e.g., nozzle rotation, nozzle pressure) and solids concentration of feed solution (Goula & Adamopoulos, 2004), while the size and format of particles produced by freeze drying depends on the type of milling and its operating conditions. In addition, milling time can also change the particle size distribution.

Although BL and ML powders were produced by the same method, significant differences ($p<0.05$) between the average particle sizes were found. It is possible that the incorporation of encapsulating agent to blackberry pulp has caused more friability of the solids, producing particles smaller than particles containing only blackberry pulp (Yamashita et al., 2017).

Table 1. Size distribution of the powder of freeze dried blackberry pulp (BL), freeze dried microencapsulated blackberry pulp (ML) and spray dried microencapsulated blackberry pulp (MS).

Powder	D [3.2] (μm)	D [4.3] (μm)	d 0.1 (μm)	d 0.5 (μm)	d 0.9 (μm)
BL	85.84 ± 2.86^{A}	149.50 ± 2.92^{A}	52.90 ± 1.82^{A}	140.60 ± 3.27^{A}	260.70 ± 4.50^{A}
ML	28.49 ± 0.61^{B}	95.96 ± 5.87^{B}	16.86 ± 0.31^{B}	70.70 ± 2.71^{B}	216.10 ± 17.59^{B}
MS	3.58 ± 0.05^{C}	9.82 ± 0.12^{C}	1.44 ± 0.03^{C}	5.70 ± 0.07^{C}	25.63 ± 0.35^{C}

Same letters in the same column show no statistical difference ($p > 0.05$).

3.2 Films characterization

3.2.1 Morphology

Figure 2 illustrates SEM cross-section images of films with 0% and 40% BL, ML and MS powders, incorporated directly (D) and by sprinkling (S). The surface of starch film in absence of microparticles is smooth and its polymer matrix is organized and dense (**Figure 2A**). With incorporation of BL (**Figure 2B and C**), ML (**Figure 2D and E**) and MS (**Figure 2F and G**) powders, the surface of arrowroot starch film became rough and irregular and this characteristic was more pronounced in films with ML and MS powders. Similar characteristics were observed by Ortega, Giannuzzi, Arce and García (2017), Sartori and Menegalli (2016), and Shi et al. (2013), for films added with silver nanoparticles, lipid microparticles and starch nanoparticles, respectively.

Microstructural characteristics presented by films with blackberry powder were directly influenced by morphological characteristics presented for each type of blackberry powder (BL, ML and MS), as well as by the method used for its incorporation in films (directly - D and by sprinkling - S). For films with blackberry powder directly incorporated into the film-forming solution, the irregularity shown on their surfaces is due to the protuberances caused by suspended particles as well as agglomerated within starch matrix. For films produced with sprinkling blackberry powder, the irregularities are due to the presence of particles on its surface. Furthermore, the presence of microparticles (ML and MS) produced by the spray drying and freeze drying methods had identical effect on surface morphology of starch films, as previously observed by Shi et al. (2013). The cracks of MSD and MSS films showed in **Figure 2F and G** were caused by sample preparation procedure, which is necessary for the analysis of films cross-section by SEM**.**

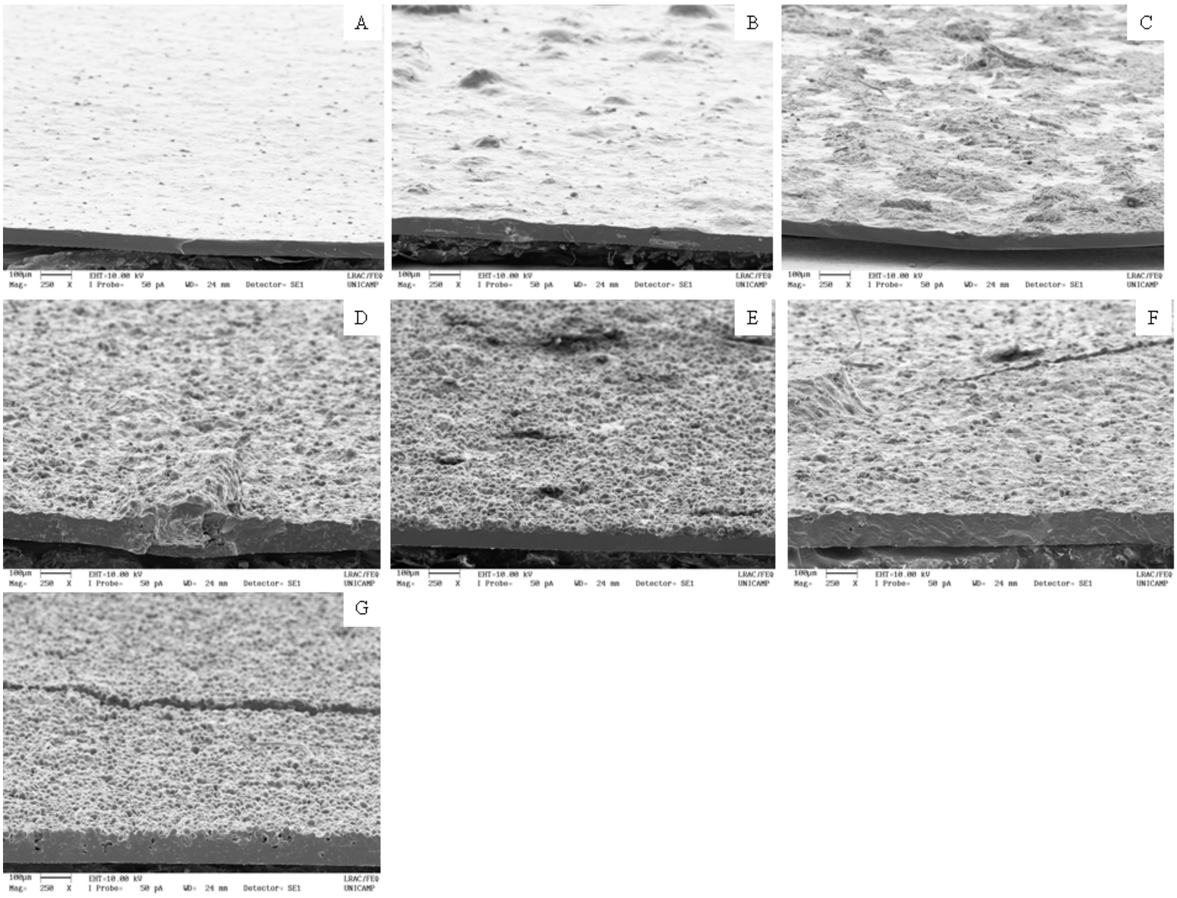

Figure 2. SEM images of cross section of films with 0% and 40% of freeze dried blackberry pulp (BL), freeze dried microencapsulated blackberry pulp (ML) and spray dried microencapsulated blackberry pulp (MS), incorporated directly (D) and by sprinkling (S): (A) 0%; (B) 40% BLD; (C) 40% BLS; (D) 40% MLD; (E) 40% MLS; (F) 40% MSD; (G) 40% MSS. All images at 250x magnification.

3.2.2 Antioxidant properties

Table 2 shows antioxidant capacity of films with 0%, 20%, 30% and 40% of BL, ML and MS powders, incorporated directly (D) and by sprinkling (S), after drying process, investigated using FRAP (ferric reducing antioxidant power) method assay. Basically, FRAP method actually measures ability of the bioactive compounds in these extracts to reduce ferric iron (Prior, Wu, & Schaich, 2005).

All films samples were able to reduce Fe $^{+3}$, but the arrowroot starch film (0%) without blackberry exhibit insignificant ferric reducing antioxidant capacity when compared to films with blackberry powder. Based on these results, it is evident that the blackberry powders (BL, ML and MS) incorporated in film-forming solution transfer antioxidant capacity to resulting arrowroot starch films.

BL, ML and MS powders presented antioxidant capacity of 845.29 ± 12.52, 837.87 ± 9.36 and 1036.94 ± 81.94 μmol ferrous sulfate/g of blackberry solids, respectively. It is observed that there was a significant decrease in antioxidant capacity for films with BL, ML and MS powders when the capacity found for each powder were compared. The bioactive compounds, as anthocyanins, are sensitive and unstable at temperature, light, pH and oxygen (Patras et al., 2010). According to Maniglia, Tessaro, Lucas and Tapia-Blácido (2017), phenolic compounds can be lost due to oxidation of phenolic groups by heating applied during preparation of filmogenic solution and during film drying process.

The variation in concentration of blackberry powder incorporated into the films from 20% to 30 and 40% significantly increased ($p<0.05$) the antioxidant capacity of MLD films, while decreasing antioxidant capacity of BLD and MSS films, and did not significantly ($p>0.05$) influence the antioxidant capacity of BLS, MLS and MSD films.

The type of incorporation (direct or by sprinkling) of BL, ML and MS blackberry powders had more influence on antioxidant capacity than the variation of its concentration in the films. In general, films incorporated with BL, ML and MS powders by sprinkling tended to present a higher antioxidant capacity than directly embedded films. Probably, the location of blackberry particles influenced this behavior. Unlikely direct incorporation, incorporation by sprinkling allowed that particles of powder would concentrate only on the film surface, thereby a greater amount of particles came into direct contact with extraction solvent, facilitating bioactive compounds extraction and, consequently, generating greater antioxidant activities.

Many works have reported that bioactive compounds content in the extract has a high correlation with antioxidant capacity (Genskowsky et al., 2015; Turumtay et al., 2014). Blackberry is an important source of phenolic compounds, such as phenolic acids, tannins and anthocyanins, which has high antioxidant capacity (Machado et al., 2015). Thereby, huge varieties of bioactive compounds may be contained in the extract, contributing in total antioxidant capacity of the BL, ML and MS powders, as well as in films with blackberry.

Table 2. Antioxidant capacity of films with 0%, 20%, 30% and 40% of freeze dried blackberry pulp (BL), freeze dried microencapsulated blackberry pulp (ML) and spray dried microencapsulated blackberry pulp (MS), incorporated directly (D) and by sprinkling (S).

Films	FRAP (μmol ferrous sulfate / g of blackberry solids)
0%*	10.69 ± 6.58^{I}
20% BLD	479.51 ± 11.29^{FG}
30% BLD	339.84 ± 9.22^{H}
40% BLD	335.05 ± 4.56^{H}
20% BLS	656.25 ± 2.81^{BC}
30% BLS	664.98 ± 3.93^{BC}
40% BLS	620.34 ± 52.79^{EDC}
20% MLD	363.89 ± 19.69^{HG}
30% MLD	498.35 ± 6.87^{EF}
40% MLD	511.15 ± 8.83^{EFD}
20% MLS	510.35 ± 13.51^{EFD}
30% MLS	504.63 ± 1.57^{EFD}
40% MLS	584.97 ± 90.91^{EFDC}
20% MSD	628.95 ± 7.25^{BDC}
30% MSD	562.23 ± 80.51^{EFDC}
40% MSD	675.94 ± 1.08^{BAC}
20% MSS	793.99 ± 12.30^{A}
30% MSS	794.87 ± 79.43^{BA}
40% MSS	552.05 ± 87.04^{EFDC}

Same letters in the same column show no statistical difference ($p > 0.05$). * For 0% film, antioxidant capacity is expressed by total solids; FRAP (μmol ferrous sulfate /g of total solids).

3.2.3 Stability in acidic, neutral and alkaline solutions

Stability of films submitted to different pH conditions is an important parameter to define the applications of biopolymer films. For applications whose purpose is to use such biopolymer films as substitute of synthetic intermediate packaging, low solubility at different pH conditions may be required for maintenance of its integrity, since foods to be packaged may have a wide pH range, for example, fruits, meats and cheeses, among others (Jaramillo et al., 2016). However, high solubility may be an advantageous feature in case the biopolymer film is used as an encapsulant or ingested with packaged product (Mukurumbira, Mellem, & Amonsou, 2017). In such cases, the film should be able to release encapsulated compounds to food system or during passage into the gastrointestinal tract after consumption.

Incorporation and variation of BL, ML and MS concentration had significant effect ($p<0.05$) on solubility of films. In general, solubility of films in acidic, neutral and alkaline solutions increased significantly ($p<0.05$), with increasing concentration of BL, ML and MS powder (20, 30 and 40%) when compared to control film (0%). The solubility of the films varied from 15.69 ± 0.67% (0% film, immersed in pH 3) to 52.70 ± 2.09% (40% MSS film, immersed in pH 10). Incorporation of particles of BL, ML and MS powders caused disrupting and discontinuity of starch matrix, as shown in SEM images (**Figure 2**). Consequently, the polymer network was less dense, facilitating permeation of water in its structure and solubilization.

In addition, it is believed that solubilization of anthocyanins also may have helped in solubilization of films, because it was possible to visualize changes in films color after their immersion for 12 days in acidic, neutral and alkaline solutions, as shown in **Figure 3**. As anthocyanins are natural water-soluble pigments (Zhai et al., 2017), probably, when films were immersed in the solutions, they hydrated causing release of anthocyanins pigment to the media. It is possible that anthocyanins solubilization have caused micro pores in films, which allowed the entry of water molecules into the starch polymer chains, increasing their solubilization. This behavior suggests that films with blackberry pulp have potential application for use as controlled release of anthocyanins for food systems.

As it is well known, several factors may influence the release rates of active compounds from biopolymeric matrices including chemical composition of active compounds, polymer–active compound interactions, matrix structure and physical state and surrounding medium conditions (Piñeros-Hernandez et al., 2017; Chang-Bravo et al., 2014). The pH variation of solutions did not significantly ($p<0.05$) affect solubility of films, with the exception of films 20% MLD and 30% MSS. Despite solubilization of films, no significant changes were observed related to its initial diameter (2 cm diameter) of films after its immersion for 12 days, in acidic, neutral or alkaline solutions, differently from that observed for cassava starch films with herb mate extract, which expanded after being submitted under different pH conditions (Jaramillo et al., 2016).

Table 3. Solubility (%) in acidic, neutral and alkaline solutions of films with 0%, 20%, 30% and 40% of freeze dried blackberry pulp (BL), freeze dried microencapsulated blackberry pulp (ML) and spray dried microencapsulated blackberry pulp (MS), incorporated directly (D) and by sprinkling (S).

	Stability in acidic, neutral and alkaline solutions			**Stability of films on sterilization process**	
Films	Solubility (%)			Thickness (mm)	
	pH 3	pH 7	pH 10	Before Sterilization	After Sterilization
0%	15.69 ± 0.67$^{\mathrm{Ha}}$	16.97 ± 1.62$^{\mathrm{Ga}}$	16.15 ± 0.78$^{\mathrm{Ha}}$	0.071± 0.009$^{\mathrm{Ha}}$	0.071 ±0.005$^{\mathrm{Ha}}$
20% BLD	26.34 ± 1.60$^{\mathrm{Ga}}$	24.35 ± 0.17$^{\mathrm{GFa}}$	25.38 ± 1.72$^{\mathrm{GHa}}$	0.105 ± 0.023$^{\mathrm{EHGFa}}$	0.088±0.011$^{\mathrm{GHa}}$
30% BLD	29.48 ± 0.52$^{\mathrm{FGa}}$	28.23 ± 1.89$^{\mathrm{EGDFa}}$	27.76 ± 0.62$^{\mathrm{GFa}}$	0.116± 0.015$^{\mathrm{EHDGCFa}}$	0.100± 0.007$^{\mathrm{FGHa}}$
40% BLD	29.61 ± 1.01$^{\mathrm{FGa}}$	29.78 ± 1.28$^{\mathrm{EGDFa}}$	31.33 ± 1.57$^{\mathrm{GFEa}}$	0.157 ±0.016$^{\mathrm{BDACa}}$	0.140±0.015$^{\mathrm{BDCa}}$
20% BLS	29.66 ± 1.39$^{\mathrm{FGa}}$	27.51 ± 4.26$^{\mathrm{EGDFa}}$	29.44 ± 3.93$^{\mathrm{GFEa}}$	0.090 ± 0.011$^{\mathrm{HGa}}$	0.091 ± 0.015$^{\mathrm{GHa}}$
30% BLS	29.92 ± 1.36$^{\mathrm{FGa}}$	32.57 ± 2.11$^{\mathrm{EDFCa}}$	30.43 ± 0.69$^{\mathrm{GFEa}}$	0.107 ± 0.013$^{\mathrm{EHDGFa}}$	0.109 ± 0.009$^{\mathrm{FGEDa}}$
40% BLS	41.90 ± 9.15$^{\mathrm{BDACa}}$	36.66 ± 3.76$^{\mathrm{EBDFCa}}$	37.64 ± 2.69$^{\mathrm{FEDCa}}$	0.101 ± 0.005$^{\mathrm{HGFa}}$	0.109 ±0.001$^{\mathrm{FGEDa}}$
20% MLD	28.66 ± 1.91$^{\mathrm{Ga}}$	24.79 ± 0.99$^{\mathrm{EGFb}}$	28.00 ± 0.91$^{\mathrm{GFba}}$	0.140 ± 0.06$^{\mathrm{EBDGCFa}}$	0.126 ± 0.006$^{\mathrm{FEDCa}}$
30% MLD	33.06 ± 0.38$^{\mathrm{FGDEa}}$	33.09 ± 0.65$^{\mathrm{EDFCa}}$	33.53 ± 0.41$^{\mathrm{GFEDCa}}$	0.176 ±0.031$^{\mathrm{BAa}}$	0.163±0.002$^{\mathrm{BAa}}$
40% MLD	38.53 ± 0.81$^{\mathrm{FBDECa}}$	37.73 ± 0.68$^{\mathrm{EBDACa}}$	38.00 ± 0.54$^{\mathrm{FEDCa}}$	0.165 ± 0.016$^{\mathrm{BACa}}$	0.153±0.013$^{\mathrm{BACa}}$
20% MLS	34.18 ± 4.96$^{\mathrm{FGDEa}}$	40.35 ± 8.71$^{\mathrm{BDACa}}$	35.86 ± 3.08$^{\mathrm{FEDCa}}$	0.130 ± 0.006$^{\mathrm{EBDGCFa}}$	0.136 ±0.004$^{\mathrm{BEDCa}}$
30% MLS	39.26 ± 4.81$^{\mathrm{BDECa}}$	37.00 ± 9.69$^{\mathrm{EBDFCa}}$	41.91 ± 8.91$^{\mathrm{BDCa}}$	0.155 ±0.020$^{\mathrm{BDACa}}$	0.155±0.010$^{\mathrm{BACa}}$
40% MLS	43.96 ± 3.39$^{\mathrm{BACa}}$	49.30 ± 4.35$^{\mathrm{BAa}}$	48.71 ± 2.50$^{\mathrm{BAa}}$	0.200 ±0.026$^{\mathrm{Aa}}$	0.165 ±0.009$^{\mathrm{BAa}}$
20% MSD	35.08 ± 0.73$^{\mathrm{FGDECa}}$	33.22 ± 0.76$^{\mathrm{EDFCa}}$	34.38 ± 1.28$^{\mathrm{GFEDCa}}$	0.135±0.012$^{\mathrm{EBDGCFa}}$	0.102 ± 0.007$^{\mathrm{FGEHa}}$
30% MSD	39.86 ± 1.19$^{\mathrm{BDECa}}$	39.28 ± 0.49$^{\mathrm{BDACa}}$	38.84 ± 0.77$^{\mathrm{BEDCa}}$	0.150±0.011$^{\mathrm{EBDACFa}}$	0.129 ± 0.018$^{\mathrm{FEDCa}}$
40% MSD	44.55 ± 0.78$^{\mathrm{BAa}}$	44.34 ± 0.63$^{\mathrm{BACa}}$	43.38 ± 0.42$^{\mathrm{BACa}}$	0.196±0.016$^{\mathrm{Aa}}$	0.176 ±0.002$^{\mathrm{Aa}}$
20% MSS	31.94 ± 1.45$^{\mathrm{FGEa}}$	38.42 ± 8.05$^{\mathrm{BDACa}}$	32.72 ± 6.03$^{\mathrm{GFEDa}}$	0.129±0.004$^{\mathrm{EBDGCFa}}$	0.105 ± 0.001$^{\mathrm{FGEb}}$
30% MSS	50.13 ± 2.75$^{\mathrm{Aa}}$	43.78 ± 2.61$^{\mathrm{BACba}}$	37.40 ± 6.67$^{\mathrm{FEDCb}}$	0.153± 0.009$^{\mathrm{EBDACa}}$	0.132 ±0.012$^{\mathrm{FBEDCa}}$
40% MSS	47.47 ± 4.56$^{\mathrm{BAa}}$	50.35 ± 5.91$^{\mathrm{Aa}}$	52.70 ± 2.09$^{\mathrm{Aa}}$	0.169±0.021$^{\mathrm{BAa}}$	0.146 ±0.023$^{\mathrm{BACb}}$

Same uppercase letters in the same column and same lowercase letters in the line show no statistical difference ($p > 0.05$).

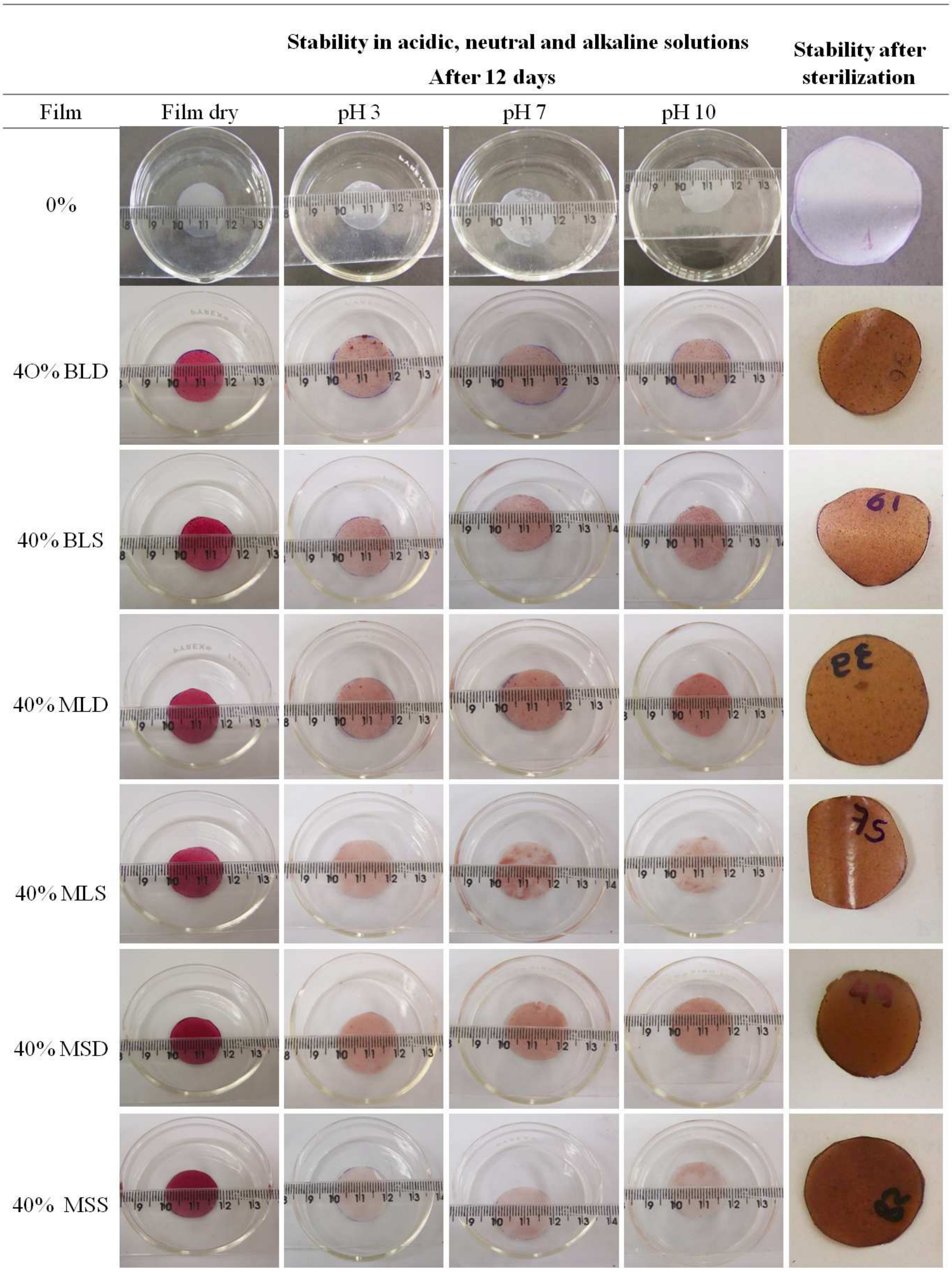

Figure 3. Digital photographs of arrowroot starch films with 0% and 40% of freeze dried blackberry pulp (BL), freeze dried microencapsulated blackberry pulp (ML) and spray dried microencapsulated blackberry pulp (MS), incorporated directly (D) and by sprinkling (S), immersed in alkaline (pH 10), neutral (pH 7) and acidic (pH 3) solutions, before and after 12 days and after being submitted to sterilization by autoclaving at 127 °C for 15 minutes.

3.2.4 Stability of films on sterilization process

Sterilization is a process that destroys all living organisms, spores and viruses in a pressurized vessel at high temperature. Often, sterilization of food is carried out in an autoclave at temperatures between 121 and 134 °C (Najafpour, 2015). Depending on the application of biopolymer film, it can be used for packing a variety of foods, which according to their nature need to be submitted to sterilization processes to obtain the desired quality and food safety factors required for commercialization. So, it is important to identify the damage caused to biopolymeric film by heating.

Table 3 shows films thickness before and after being autoclaved at 127 °C for 15 minutes. Except for 20 and 40% MSS films, sterilization process did not significantly ($p>0.05$) alter films thickness. The films maintained their physical integrity, without any cracks or holes visible to naked eye, after their sterilization.

All films with blackberry powders have suffered a noticeable change from their reddish color to brown after sterilization process, while film 0% remained colorless, as can be seen in **Figure 3**. Blackberry is a fruit rich in sugars, as glucose, fructose and sucrose (Kafkas, Koşar, Türemiş, & Başer, 2006). It is possible that heating has caused caramelization of these sugars generating dark compounds.

Another possible reason for darkening of films with blackberry powder is the formation of insoluble brown product from Maillard's reaction. Heated amino acids and sugars mixtures can lead to formation of melanoidins, similar to the extracts obtained at 100 °C from blackberry bagasse (Machado et al., 2015). In addition, it has been reported that anthocyanins at high temperature can be degraded into an insoluble brown-colored phenolic compound. Machado et al. (2015) reported that extracts, obtained from residues of blackberries by pressurized liquid extraction at 80 and 100 °C, had color varying from red to brown, indicating some anthocyanin degradation.

3.2.5 Biodesintegration in organic composting

Biodesintegration of films was evaluated by a qualitative study as function of time required for degradation of polymeric materials buried in organic vegetables composting. Unfortunately, it was not possible to evaluate degradation of films by their weight change, due to difficulty of removing soil residues adhered to the material, which could alter their precise weight. Thus, films degradation was only investigated by alteration of their visual appearance (Jaramillo et al., 2016).

Figure 4 shows the evolution of biodesintegration of films with 0% and 40% BL, ML and MS powder, incorporated directly (D) and by sprinkling (S). After 7 days of testing, all film samples showed strong changes in hue and integrity, suggesting the beginning of degradation of polymeric material, as well as of the anthocyanins, pigment responsible for the redness in blackberry powder (Yamashita et al., 2017; Jiménez-Aguilar et al., 2011).

The polymer degradation in a bioactive environment occurs by material fragmentation and subsequent mineralization. The action of heat and moisture as well as enzymatic activity of microorganisms abbreviate and fade the polymer chains, resulting in fragmentation residues of the polymer. Only if these polymer fragments are consumed by microorganisms as food and an energy source, they can be considered biodegradable (Mohee et al., 2008).

Biodesintegration of films occurred almost entirely after 38 days of testing (Figure 4), before 90 days that is the period that plastic materials are considered disintegrable, according to ISO 20200:2004. For cassava starch films, decomposition occurred almost entirely after 14 days of testing. After this time, integrity of cassava starch films containing polyphenols-rich rosemary extracts were better preserved, indicating that their biodegradation was delayed by presence of rosemary extract (Piñeros-Hernandez et al., 2017). Jaramillo et al. (2016) reported that addition of yerba mate extract decreased the biodegradation time of cassava starch films, observing complete disintegration of films before two weeks.

Xiong et al. (2008) observed that biodegradation rates of starch-based films with and without nano silicon dioxide (nano-SiO_2) content occurred in 100 days. The authors found that presence of nano-SiO_2 had no influence on biodegradation rate of films.

In this study, apparently the presence of blackberry powder particles did not alter biodesintegration of arrowroot starch films, showing that biodegradability is characteristic of chemical composition of both arrowroot starch and blackberry powder.

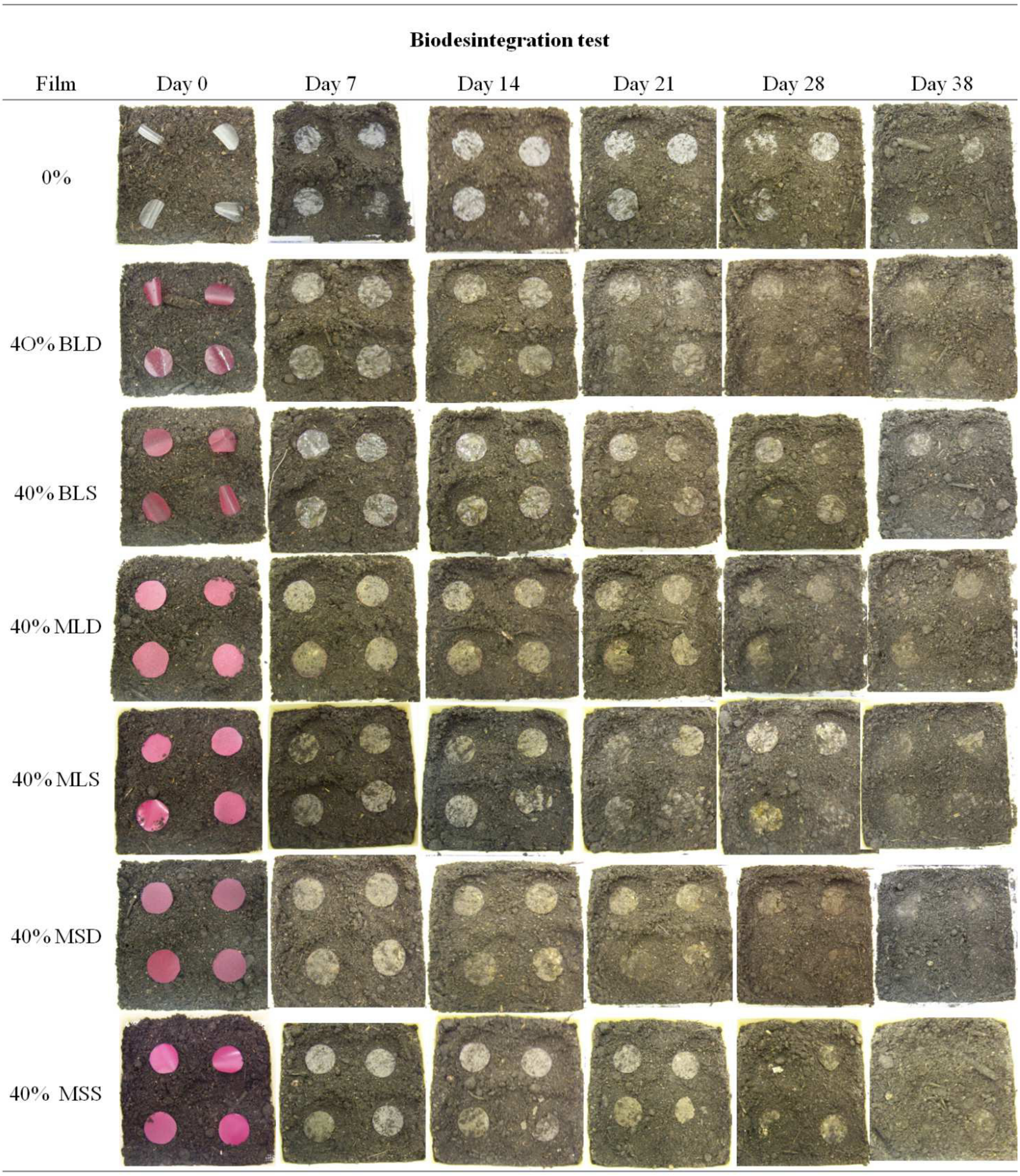

Figure 4. Evolution of biodesintegration of arrowroot starch films with 0% and 40% of freeze dried blackberry pulp (BL), freeze dried microencapsulated blackberry pulp (ML) and spray dried microencapsulated blackberry pulp (MS), incorporated directly (D) and by sprinkling (S), buried under solid composting material.

4. Conclusions

Freeze drying and spray drying methods were successfully used for powder microencapsulation of blackberry pulp (ML and MS) and only freeze drying method was able to dry blackberry pulp (BL) without encapsulant. Size and morphology of blackberry powder particles were influenced by presence of encapsulating agent and method of production. Blackberry microparticles obtained by spray drying were spherical, oval and withered and were significantly lower than obtained by freeze drying. External morphology of particles of BL and ML powder is similar to broken glass structure and spherical particles could be visualized in vitreous structures of particles of ML powder. All powders present a reddish color, typical of blackberry pulp, and antioxidant capacity.

Incorporated films with BL, ML and MS powder, exhibited structural characteristics directly related with those observed for each powder, as well as by method used for its incorporation in film (directly - D and by sprinkling - S). The incorporation of blackberry powder has made the surface of arrowroot film rough and irregular and caused discontinuity in its starch polymer matrix. Incorporation of blackberry powder in film-forming suspension transferred antioxidant capacity and color to the resulting films. As the blackberry powder content increased, films showed increased solubility in acid, neutral and alkaline solutions. Films containing blackberry powder have changed from their notorious reddish color to brown, after sterilization process. This darkening may be due to caramelization of sugars, Maillard reaction or degradation of anthocyanins. Its antioxidant characteristics and high solubility at different pH conditions make arrowroot starch films incorporated with blackberry powder very promising for using as active and edible packing and coating food, which allow a controlled release of bioactive compounds with high antioxidant capacity for food system.

The film of arrowroot starch without blackberry powder presented an insignificant antioxidant capacity. However, this film, when immersed on acidic, neutral and alkaline solutions, was the most stable, presenting low solubility when compared to others films. In addition, arrowroot starch film became colorless after sterilization process. The results suggest that the films of arrowroot starch have great potential for using as biopolymer films, as substitute for synthetic intermediate packaging. Biodesintegration of all films occurred almost entirely after 38 days, following the current trends to environmental care.

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Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

References

A.O.A.C. Official Methods of Analysis. (2006) (18th ed.). Association of Official Analytical Chemists, Gaithersburg, Maryland.

Araujo-Díaz, S.B., Leyva-Porras, C., Aguirre-Bañuelos, P., Álvarez-Salas, C., & Saavedra-Leos, Z. (2017). Evaluation of the physical properties and conservation of theantioxidants content, employing inulin and maltodextrin in the spraydrying of blueberry juice. *Carbohydrate Polymers*, 167, 317–325.

Avellone, G., Salvo, A., Costa, R., Saija, E., Bongiorno, D., Stefano, V.D., Calabrese, G., & Dugo, G. (2018). Investigation on the influence of spray-drying technology on the quality of Sicilian Nero d'Avola wines. *Food Chemistry*, 240, 222-230.

Azeredo, H.M.C., Morrugares-Carmona, R., Wellner, N., Cross, K., Bajka, B., & Waldron, K.W. (2016). Development of pectin films with pomegranate juice and citric acid. *Food Chemistry*, 198, 101–106.

Cerruti, P., Santagata, G., Gomez d'Ayala, G., Ambrogi, V., Carfagna, C., Malinconico, M., & Persico, P. (2011). Effect of a natural polyphenolic extract on the properties of abiodegradable starch-based polymer. *Polymer Degradation and Stability*, 96, 5,839–846.

Chang-Bravo, L., López-Córdoba, A., & Martino, M. (2014). Biopolymeric matrices made of carrageenan and corn starch for the antioxidant extracts delivery of Cuban red propolis and yerba mate. *Reactive & Functional Polymers*, 85, 11–19.

ČSN EN ISO 20200 "Plastics - Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test" (ISO 20200:2004).

Fakhouri, F.M., Costa, D., Yamashita, F., Martelli, S.M., Jesus, R.C., Alganer, K., Collares-Queiroz, F.P., Innocentini-Mei, L.H., (2013). Comparative study of processing methods for starch/gelatin films. *Carbohydrate Polymers*, 95, 681-689, ISSN 0144-8617, https://doi.org/10.1016/j.carbpol.2013.03.027.

Farias, M.G., Fakhouri, F.M., Carvalho, C.W.P. d., Ascheri, J.L.R. (2012). Caracterização físico-química de filmes comestíveis de amido adicionado de acerola (*Malphigia emarginata* D.C.). *Química Nova*, 35, 546-552. doi.org/10.1590/S0100-40422012000300020

Ferrari, C.C., Ribeiro, C.P., & Aguirre, J.M. (2012). Spray drying of blackberry pulp using maltodextrin as carrier agent. *Brazilian Journal of Food Technology*, 15, 157-165.

Genskowsky, E., Puente, L. A., Pérez-Álvarez, J. A., Fernandez-Lopez, J., Muñoz, L. A., & Viuda-Martos, M. (2015). Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT - Food Science and Technology*, 64, 1057-1062.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, 40, 1107–1121.

Goula, A.M., & Adamopoulos, K.G. (2006). Retention of ascorbic acid during drying of tomato halves and tomato pulp. *Drying Technology*, 24, 57-64.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45, 3, 253 -267.

Igual, M., Ramires, S., Mosquera, L.H., & Martínez-Navarrete, N. (2014). Optimization of spray drying conditions for lulo (*Solanum quitoense* L.) pulp. *Powder Technology*, 256, 233–238.

Jaramillo, C.M., Gutiérrez, T.J., Goyanes, S., Bernal, C., & Famá, L. (2016). Biodegradability and plasticizing effect of yerba mate extract oncassava starch edible films. *Carbohydrate Polymers*, 151, 150–159.

Jiménez-Aguilar, D.M., Ortega-Regules, A.E., Lozada-Ramírez, J.D., Pérez-Pérez, M.C.I., Vernon-Carter, E.J., & Welti-Chanes, J. (2011) Color and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. *Journal of Food Composition and Analysis*, 24, 889-894.

Kafkas, E., Koşar, M., Türemiş, N., & Başer, K.H.C. (2006). Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. *Food Chemistry*. 2006, 97, 732-736.

Machado, A.P.da F., Pasquel-Reátegui, J.L., Barbero, G.F., & Martínez, J. (2015). Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International*, 77, 675–683.

Maniglia, B.C., Tessaro, L., Lucas, A.A., & Tapia-Blácido, D.R. (2017). Bioactive films based on babassu mesocarp flour and starch. *Food Hydrocolloids*, 70, 383-391.

Martinez, C., & Cuevas, F. (1989). Evaluación de la calidad culinaria y molinera del arroz. Guia de estudo, CIAT.

Mohee, R., Unmar, G.D., Mudhoo, A., Khadoo, P. (2008). Biodegradability of biodegradable/ degradable plastic materials under aerobic and anaerobic conditions. *Waste Management*, 28, 1624- 1629.

Moorthy, S. N. (2002). Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54, 559-592.

Mukurumbira, A.R., Mellem, J.J., & Amonsou, E.O. (2017). Effects of amadumbe starch nanocrystals on the physicochemical properties of starch biocomposite films. *Carbohydrate Polymers*, 165, 142–148.

Najafpour, G. D. Chapter 15 - Sterilization, In Biochemical Engineering and Biotechnology (Second Edition), Elsevier, Amsterdam, 2015, Pages 435-452.

Nogueira, G.F., Fakhouri, F.M., de Oliveira, R.A. (2018). Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films. *Carbohydrate Polymers*, https://doi.org/10.1016/j.carbpol.2018.01.024

Ortega, F., Giannuzzi, L., Arce, V.B., & García, M.A. (2017). Active composite starch films containing green synthetized silver nanoparticles. *Food Hydrocolloids*, 70, 152-162.

Otoni, C.G., De Moura, M.R., Aouada, F.A., Camilloto, G.P., Cruz, R.S., Lorevice, M.V., Soares, N.F.F., & Mattoso, L.H.C. (2014). Antimicrobial and physical mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids*, 41, 188–194.

Patras, A., Brunton, N.P., Donnell, C.O., & Tiwari, B.K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, 21, 3 -11.

Piñeros-Hernandez, D., Medina-Jaramillo, C., López-Córdoba, A., & Goyanes, S. (2017). Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. *Food Hydrocolloids*, 63, 488-495.

Prior, R.L., Wu, X., & Schaich, K. (2005). Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry*, 53, 4290-4302.

Ré, M. I. (1998). Microencapsulation by spray drying. *Drying Technology*, 16, 1195-1236.

Rufino, M., Alves, R., De Brito, E.S., De Moraes, S.M, Sampaio, C.G., Pérez-Jiménez, J.E., & Saura-Calixto, F.D. (2006). Metodologia científica: determinação de atividade antioxidante total em frutas pelo método de redução de ferro (FRAP). Comunicado Técnico on line. EMBRAPA, Fortaleza.

Saavedra-Leos, M.Z., Alvarez-Salas, C., Esneider-Alcalá, M., Toxqui-Terán, A.,Pérez-García, S.A., & Ruiz-Cabrera, M. (2012). Towards an improvedcalorimetric methodology for glass transition temperature determination inamorphous sugars. *CyTA − Journal of Food*, 10, 4, 258–267.

Santana, A., Cano-Higuita, D.M., de Oliveira, R.A., & Telis, V.R.N. (2016). Influence of different combinations of wall materials on the microencapsulation of jussara pulp (*Euterpe edulis*) by spray drying. *Food Chemistry*, 212, 1–9.

Sartori, T., & Menegalli, F.C. (2016).Development and characterization of unripe banana starch films incorporated with solid lipid microparticles containing ascorbic acid. *Food Hydrocolloids*, 55, 210-219.

Shi, A.-M., Wang, L.-J., Li, D., & Adhikari, B. (2013). Characterization of starch filmscontaining starch nanoparticles: part 1: Physical and mechanical properties. *Carbohydrate Polymers*, 96, 593–601.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technoloy*, 14, 3, 71-78.

Turumtay, E. A., Islamoglu, F., Çavus, D., Sahin, H., Turumtay, H., & Vanholme, B. (2014). Correlation between phenolic compounds and antioxidant capacity of Anzer tea (*Thymus praecox* Opiz subsp. caucasicus var. caucasicus). *Industrial Crops and Products*, 52, 687- 694.

Villas-Boas, F., & Franco, C.M.L. (2016). Effect of bacterial β-amylase and fungal α-amylase on the digestibility and structural characteristics of potato and arrowroot starches. *Food Hydrocolloids*, 52, 795- 803.

Xiong, H., Tang, S., Tang, H., & Zou, P. (2008). The structure and properties of starch-based biodegradable film. *Carbohydrate Polymers*, 71, 2, 263–268.

Yamashita, C., Chung, M.M.S., dos Santos, C., Mayer, C.R.M., Moraes, I.C.F., & Branco, I.G. (2017). Microencapsulation of an anthocyanin-rich blackberry (spp.) by-product extract by freeze-drying. *LWT - Food Science and Technology*, 84, 256-262.

Yousefi, S., Emam-Djomeh, Z., & Mousavi, S.M. (2011). Effect of carrier type and spray drying on the physicochemical properties of powdered and reconstituted pomegranate juice (*Punica granatum* L.). *Journal of Food Science and Technology*, 48, 6, 677–684.

Zavareze, E. da R., El Halal, S. L. M., Pereira, J.M., Radünz, A. L., Elias, M. C., & Dias, A. R. G. (2009). Chemical characterization and extraction yield of rice starch with different amylose contents. *Brazilian Journal of Food Technology*, II SSA, janeiro.

Zhai, X., Shi, J., Zou, X., Wang, S., Jiang, C., Zhang, J., Huang, X., Zhang, W., & Holmes, M. (2017). Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roselle anthocyanins for fish freshness monitoring. *Food Hydrocolloids*, 69, 308-317.

CAPÍTULO 9- DISCUSSÃO GERAL

Extração e caracterização do amido de araruta

A primeira parte deste estudo se concentrou na extração e caracterização do amido de araruta. Durante o processamento dos rizomas de araruta para a extração do seu amido, observou-se a facilidade no seu descascamento, por suas cascas serem finas, como mostrado na Figura 1.

Figura 1. Foto do rizoma de araruta sendo descascado.

Algumas dificuldades foram encontradas durante a moagem dos rizomas devido ao seu alto teor de fibras longas. Ajustes na pré-moagem, como fatiamento dos rizomas e proporção de água foram extremamente importantes para a sua moagem e consequente extração do amido, pois é nessa etapa em que ocorre o rompimento das células para a liberação dos grânulos de amido (Figura 2).

Figura 2. Foto do processamento dos rizomas de araruta.

Embora tenham existido algumas dificuldades na moagem, os processos de extração, lavagem, sedimentação e drenagem foram eficientes garantindo a obtenção de um amido de boa qualidade e assegurando um bom rendimento (Figura 3).

Figura 3. Fotos dos processos de extração, lavagem, sedimentação para a obtenção do amido de araruta.

O rendimento do amido obtido foi de 16% em base seca, inferior ao valor encontrado por Ferrari et al. (2005), de 18 % (amido extraído a partir de plantas cultivadas por 12 meses) e por Leonel et al. (2002a), de 21%, mas superior aos valores encontrados para outras fontes amiláceas como mandioquinha salsa com 10,3% (MASTSUGUMA, 2006), biri com 11,44% (LEONEL et al., 2002b) e banana com 5 a 8% (FREITAS; TAVARES, 2005).

Observa-se na Figura 4 que o amido obtido apresentou uma coloração branca e sem impurezas aparentes, o que é considerado um fator positivo, pois a cor tem um forte impacto na sua qualidade. Qualquer pigmentação no amido poderá ser transferida para o produto final, influenciando na sua aplicação. Um valor elevado de luminosidade (valor *L**) e um baixo valor de croma são desejados para o amido (FRANKLIN et al., 2017). A cor, em termos dos valores de *L*, a** e *b** (sistema CIELab), foram 98,26 ± 1,75, -0,12 ± 0,038 e 2,81 ± 0,34, respectivamente, similares aos valores de 95,74, 0,01 e 3,30, obtidos por Franklin et al. (2017) para amido de *Curcuma angustifolia*. A partir do elevado valor de luminosidade e baixo valor dos componentes de croma (valores *a** e *b**), pode-se dizer que o amido de araruta obtido consistiu num pó branco.

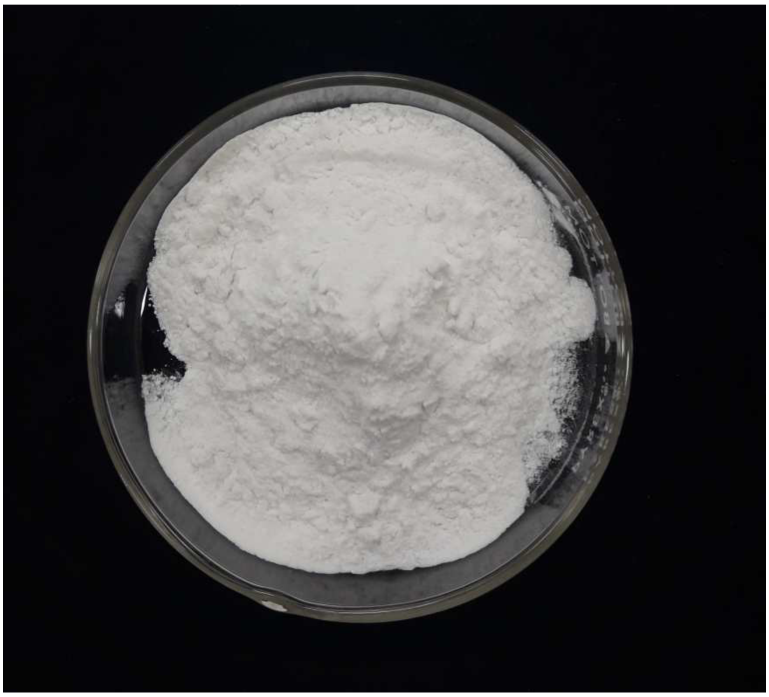

Figura 4. Aparência do amido de araruta.

A observação dos grânulos de amido por microscopia ótica e eletrônica de varredura revelou variações no seu tamanho e na sua morfologia, destacando-se as formas circular, cilíndrica, elipsoide e oval como já relatado anteriormente por Erdman (1986). Aparentemente os grânulos de amido apresentaram superfície lisa, sem presença de irregularidades, porosidade superficial ou evidência de fissuras. Além disso, não foram observadas impurezas ou fibras, o que demonstra grânulos íntegros e não danificados, confirmando a boa eficiência do processo da extração do amido.

Quanto a composição proximal encontrada para o amido de araruta, o valor do teor de água foi próximo a 15 % em base seca, superior aos valores reportados por Rodrigues (2014), de 10,9 %, por Ferrari et al. (2005), de 10,49 g/100 g^{-1} e por Leonel et al. (2002a), de 12,8 g/100 g^{-1}, mas dentro dos limites estabelecidos pela legislação brasileira. Segundo a legislação (BRASIL, 2005), farinhas, amido de cereais e farelos devem ter umidade máxima de 15,0 % (g/100 g^{-1}); para amido ou fécula de batata, de 21,0 % (g/100 g^{-1}) e para amido ou fécula de mandioca a umidade máxima deve ser de 18,0 % (g/100 g^{-1}). Valores acima deste teor de água são favoráveis para o desenvolvimento de microrganismos como bactérias, fungos e leveduras.

O amido apresentou baixo conteúdo de lipídeos (0,12 %), que concorda com os resultados encontrados por Villas-Boas e Franco (2016), de 0,13%, por Ferrari et al. (2005), de 0,10 %, para o amido extraído de planta com 12 meses e por Leonel et al. (2002a), de 0,11 g/100 g^{-1}. Quanto ao teor de proteínas, o valor encontrado foi de 0,40 %, e é um valor considerado relativamente baixo comparando-se com ao obtido por Cunha (2016), de 0,85 % para amido de araruta do tipo seta e redonda, por Clemente Granados et al. (2014), de 0,65 % e por Hernandez-Medina (2008), de 0,64 %, mais alto se comparado com o valor de 0,10 % encontrado por Villas-Boas e Franco (2016).

A pureza obtida pelo procedimento de extração do amido pôde ser confirmada, principalmente devido ao baixo teor de lipídeos, cinzas e proteínas. O somatório dos resultados obtidos de cinzas, lipídeos e proteínas foram inferior a 1%, o que é desejável, pois indica que o amido apresentou alto grau de pureza e que o processo de extração foi eficiente, visto que esses contaminantes são carreados durante esse processo e podem interferir nas propriedades físico-químicas e tecnológicas do amido (LEONEL; CEREDA, 2002).

Como esperado, o amido de araruta apresentou um conteúdo elevado de amilose, 35,20 ± 1,63%, superior ao encontrado por Erdman (1986) de 19,9% e, superior à variação de 16 a 27% relatada por Moorthy (2002), o que o torna vantajoso para a produção de filmes comestíveis e biodegradáveis com boas propriedades funcionais.

O amido também foi caracterizado quanto ao seu poder de inchamento (PI) e para o índice de solubilidade (IS) em função da temperatura. O poder de inchamento está relacionado com a capacidade do grânulo de amido em absorver água, enquanto que o índice de solubilidade é sua capacidade de reagir com a água e dissolver-se, indicando a extensão da associação existente entre a amilose e amilopectina. A extensão desta interação é influenciada pela razão amilose / amilopectina e pelas suas características em termos de peso molecular, distribuição, grau e comprimento de ramificação e conformação (HOOVER, 2001).

O poder de inchamento do amido de araruta foi de 2,17 ± 0,21 g/g a 60 °C para 11,32 ± 0,53 g/g a 90 °C e da solubilidade em água foi de 1,59 ± 0,60 % a 60°C para 17,22 ± 1,43 % a 90 °C. O aumento da temperatura acima de 60°C causou um aumento significativo no poder de inchamento e da solubilidade em água dos grânulos de amido de araruta. Comportamento semelhante foi reportado por Cunha (2016), que encontrou poder de inchamento de 4,64 ± 0,16 g/g (60 °C) a 10,26 ± 0,15 g/g (90 °C) e de 6,47 ± 0,11 g/g (60 °C) a 10,31 ± 0,03 g/g (90 °C) para amido de araruta do tipo redonda e seta, respectivamente. Para Pérez e Lares (2005), o amido de araruta mostrou poder de inchamento de 2,94 g/g (60 °C) a 16,19 g/g (90 °C). Hernández-Medina et al. (2008) encontraram poder de inchamento de 16,98 g/g (90 °C) para amido de araruta com 22,69% de amilose.

O aumento da temperatura enfraquece as forças associativas internas que mantêm a estrutura do grânulo, levando à ruptura das ligações de hidrogênio intermoleculares em áreas amorfas, o que permite que as moléculas de água se liguem aos grupos hidroxilos expostos de amilose e amilopectina por ligações de hidrogênio. Isso leva ao aumento do tamanho do grânulo devido ao inchamento e solubilização parcial dos polímeros, amilose e amilopectina (HOOVER, 2001).

Na temperatura de 80 °C houve um pico na solubilização (19,93 ± 0,07 %) do amido de araruta concomitante com uma queda sutil do poder de inchamento (10,00 ± 0,40 g/g). Esse comportamento provavelmente está relacionado com a quebra da estrutura do amido após alcançar inchamento máximo na temperatura de gelatinização, o que faz com que a amilose e amilopectina seja lixiviada a partir dos grânulos, aumentando consequentemente a solubilização e diminuindo a capacidade de absorção de água (CUNHA, 2016; MENDES, 2011).

De acordo com Ikegwu et al. (2010), o amido pode ser descrito como tendo um alto poder de inchamento, se sua solubilidade for maior ou igual 30%. A absorção máxima de água para os amidos é de cerca de 40 %. Sendo assim, pode-se afirmar que o amido de araruta do presente trabalho não está dentro da categoria de alto poder de inchamento.

A difratometria de raios-X é outra caracterização importante do amido, pois revela as características da estrutura cristalina dos grânulos de amido. A região cristalina do grânulo é formada pelas cadeias laterais de amilopectina, enquanto que a região amorfa é composta por amilose e ramificações de amilopectina. Dessa forma, a cristalinidade do grânulo está, particularmente, relacionada com as duplas hélices formadas pelas ramificações de amilopectina (HOOVER, 2001). O difratograma de raios-X do amido de araruta mostrou estrutura cristalina do tipo "C", uma mistura dos polimorfos do tipo A e B, geralmente encontrado para cereais e leguminosas (SINGH et al., 2003; ZHANG et al., 2005).

Outra informação relevante é a temperatura de transição vítrea (Tg), que é retirada da curva de calorimetria exploratória diferencial (DSC). Tradicionalmente, este tipo de termograma é utilizado para identificar o início e o fim da região de transição vítrea, sendo o ponto médio considerado como a temperatura de transição vítrea empírica (CHUANG et al., 2016; DU et al., 2016; CHUANG et al., 2017). O amido de araruta apresentou uma faixa de Tg em torno de 118 °C e 120 °C. Isso significa que em temperaturas abaixo desse valor, o amido de araruta se encontra no estado vítreo e com o seu aquecimento em tem temperaturas superiores a 120 °C, o polímero vítreo converte-se para um estado gomoso, passando a ter um comportamento elástico e flexível.

Na análise de termogravimetria (TG), a variação da massa da amostra é determinada em função da temperatura e/ou tempo, enquanto a amostra é submetida a uma programação controlada de temperatura. Essa análise auxilia no estudo de degradação térmica do material amiláceo. Quando o amido é submetido a temperaturas altas, ocorre uma série de modificações irreversíveis, com alteração estrutural do polímero e formação de produtos solúveis em água, como pirodextrinas. Em temperaturas mais elevadas, a despolimerização das macromoléculas conduz à formação de levoglucosanas, furfural e produtos voláteis e gasosos de baixo peso molecular (AGGARWAL; DOLLIMORE, 1998). Quando a temperatura ultrapassa os 300 °C ocorre a decomposição do amido, e um resíduo carbonáceo permanece depois que todos os produtos voláteis forem removidos.

Para o amido de araruta, a análise termogravimétrica mostrou uma primeira perda de massa de 13% entre as temperaturas de 25 e 200 °C, relacionada à evaporação da água absorvida no material amiláceo e uma segunda perda de massa de 40% entre as temperaturas 330 °C e 410 °C, devido à despolimerização da macromolécula de amido e decomposição dos seus constituintes minoritários, como proteínas e lipídios. Para Cunha (2016), as curvas de termogravimetria (TG) para amidos de araruta do tipo redonda e seta apresentaram a primeira perda de massa de 14 % e 13 % entre 24°C e 112 °C e entre 21 °C e 107 °C e uma segunda perda de massa de 49 % e 52 % entre 299 °C e 326 °C e entre 293 °C e 326 °C, respectivamente.

Diante de todos os resultados apresentados, foi possível concluir que o amido de araruta possui um alto teor de amilose, habilidade de gelificação e é termicamente estável, apresentando qualidades desejáveis para a sua utilização como agente encapsulante, polímero na produção de embalagens ou filmes biodegradáveis e comestíveis, além de características adequadas para aplicação na indústria alimentícia como agente espessante e gelificante.

Avaliação da capacidade do amido de araruta em ser utilizado como agente encapsulante.

Na segunda parte desse trabalho, foi investigada a capacidade do amido de araruta em ser utilizado como agente encapsulante. Para isso a polpa amora foi escolhida como material a ser encapsulado, uma vez que essa fruta é rica em compostos nutricionais e ao mesmo tempo é altamente perecível, devido à alta taxa de respiração, alto teor de água e estrutura frágil (ELISIA et al., 2007; HAGER et al., 2008; MACHADO et al., 2015).

Spray drying foi a tecnologia de conservação escolhida por ser, além de uma técnica de desidratação, uma técnica de microencapsulação. Entre as técnicas de processamento utilizadas para a preparação de alimentos na forma de pó (por exemplo, leito fluidizado com ar quente, liofilização, secagem por pulverização, etc.), *spray drying* é considerada econômica, flexível, de fácil operação e controle, com altas taxas de secagem e, as vezes ,de curto período de residência (ARAUJO-DÍAZ et al., 2017).

Durante os testes preliminares observou-se que a secagem da polpa de amora sem encapsulante ou utilizando apenas o amido de araruta como agente encapsulante seria muito difícil, pois uma grande quantidade de material aderiu-se a parede da câmara de secagem, gerando um rendimento insatisfatório.

A fim de melhorar esse comportamento e aumentar a funcionalidade do amido de araruta, adicionou-se goma arábica ao amido, na proporção de 1:1 (m/m). A goma arábica apresenta muitas características desejáveis para ser utilizada como encapsulante em técnicas de secagem, pois ela contribui para o aumento da temperatura de transição vítrea do sistema, confere alta solubilidade, baixa viscosidade e boas propriedades emulsionantes a dispersões alimentares (RAMÍREZ et al., 2015).

Além da adição da goma arábica ao amido de araruta, a solução resultante da mistura de agente encapsulante e polpa de amora teve que ser diluída em água na proporção 1:2 (m/m) para diminuir a viscosidade da mistura e facilitar sua pulverização na câmara de secagem. A partir desses ajustes foi possível avaliar a secagem, bem como a microencapsulação da polpa de amora por *spray drying* por meio de um planejamento experimental, variando-se a temperatura do ar de entrada (100 - 150 ° C) e a concentração do agente encapsulante (1: 0,5 - 1: 2, sólidos de polpa de amora: amido de araruta / goma arábica), totalizando11 ensaios de secagem.

Os limites de variação dos parâmetros (variáveis independentes) utilizados neste planejamento experimental foram determinados através de testes preliminares. O limite mínimo da concentração de agente encapsulante e da temperatura do ar de entrada foi determinado pela menor concentração de encapsulante e temperatura que permitisse a formação de pó de polpa de amora. Enquanto que o limite máximo para temperatura de ar de entrada foi determinado pela máxima temperatura de ar de entrada que permitisse a formação de pó de polpa de amora sem escurecimento excessivo (queimá-lo). Verificou-se nesses testes preliminares que não era possível a formação de pó de amora em temperaturas abaixo de 100 °C, pois estava abaixo da temperatura de ebulição da água (pressão atmosférica) e em temperaturas acima de 150 °C ocorria a gelatinização do amido ou sua caramelização, bem como dos açúcares presentes na amora, o que em muitas vezes levou ao entupimento do atomizador.

A temperatura ambiente, umidade relativa ambiente, temperatura da amostra e temperatura utilizada na alimentação do secador e do ar de saída foram monitorados em intervalos de 15 minutos durante os ensaios de secagem com o intuito de verificar a variação destas variáveis sobre o produto em pó.

Os resultados das variáveis monitoradas durante a realização de cada ensaio estão apresentados na Tabela 1. As soluções de polpa de amora acrescida de agente encapsulante apresentaram teor de sólidos solúveis variando de 4,71 a 7,32 °Brix e teor de água de 90,70 a 94,67 %. A adição de agente encapsulante antes da secagem em *spray dryer* aumentou o conteúdo de sólidos totais da mistura a ser alimentada no *spray dryer*, reduzindo a quantidade de água a ser evaporada durante o processo. Foi observado também que, quanto maior a proporção de agente encapsulante utilizada em relação ao de sólidos de polpa de amora, maior o teor de sólidos solúveis na mistura e, menor teor de água na solução. A temperatura da solução variou entre 26,38 e 31,56 °C.

A temperatura e umidade relativa ambiente oscilaram entre 29,49 e 32,92 °C, e 47,27 e 63,77 %, respectivamente. Como esperado para a temperatura do ar de saída do secador, observou-se que, quanto menor a temperatura de secagem utilizada, menor a temperatura de saída do ar do secador, variando entre 73,00 a 107,20 °C.

Em todos os ensaios, foi possível obter amora em pó, com cor avermelhada, característica da polpa de amora. A temperatura do ar de secagem e a concentração de agente encapsulante não influenciaram significativamente ($p>0,05$) o teor de água, tamanho, e os valores de a^* dos pós. De uma maneira geral, o aumento de temperatura do ar de entrada levou ao aumento da higroscopicidade, solubilidade, conteúdo de antocianinas e capacidade antioxidante (ensaio FRAP) dos pós, enquanto que o aumento da concentração do agente encapsulante elevou o rendimento do processo e o conteúdo de vitamina C.

As condições ótimas de secagem por pulverização para a polpa de amora foram determinadas pela metodologia da superfície de resposta, para obter maior rendimento do processo e pós com elevado conteúdo de ácido ascórbico, antocianinas e propriedades antioxidantes (ensaio FRAP). Os resultados da ANOVA, incluindo os coeficientes de regressão para as equações polinomiais codificadas de segunda ordem, os coeficientes de determinação (R^2), e os valores de F (F calculado e F tabulado), estão apresentados no Apêndice A.

Tabela 1. Variáveis monitoradas durante os 11 ensaios de secagem de polpa de amora com mistura de amido de araruta e goma arábica como agente encapsulante por spray drying.

	Variáveis Independentes		**Variáveis Monitoradas**						
Ensaios	**Temperatura do ar de entrada (°C)**	**Proporção (amora: amido/GA)**	**SS da solução de entrada no spray dryer (°Brrix)**	**Teor de água da solução de entrada no spray dryer (%, bu)**	**Temperatura da amostra (°C)**	**Temperatura ambiente (°C)**	**Umidade relativa (%)**	**Temperatura do ar de entrada do secador (°C)**	**Temperatura do ar de saída do secador (°C)**
1	107 (-1)*	1 : 0,72 (-1)	5,27 ± 0,06	94,19 ± 0,03	26,87± 1,51	30,85 ± 1,45	52,87 ± 7,03	107	76,67 ± 3,11
2	107 (-1)	1 : 1,78 (1)	6,23 ± 0,06	91,17 ± 0,01	26,38 ± 1,26	29,49 ± 1,34	63,77 ± 3,48	107	73,00 ± 2,79
3	143 (1)	1 : 0,72 (-1)	4,71 ± 0,49	94,15 ± 0,01	29,57 ± 0,10	32,92 ± 1,24	43,79 ± 8,40	143	102,43 ± 6,61
4	143 (1)	1 : 1,78 (1)	7,32 ± 0,98	91,12 ± 0,01	29,86 ± 1,17	32,88 ± 1,08	49,29 ± 7,43	143	105,43 ± 3,13
5	99,54 (-1,41)	1 : 1,25 (0)	5,40 ± 0,20	92,99 ± 0,01	31,56 ± 2,06	31,05 ± 1,47	49,75 ± 4,30	100	73,13 ± 3,38
6	150,46 (1,41)	1: 1,25 (0)	5,43 ± 0,06	92,84 ± 0,01	31,2 ± 0,94	31,23 ± 1,16	48,93 ± 6,67	150	107,20 ± 4,28
7	125 (0)	1 : 0,5 (-1,41)	4,83 ± 0,06	94,67 ± 0,01	27,42 ± 0,99	31,50 ± 0,69	51,17 ± 6,58	125	91,58 ± 2,71
8	125 (0)	1 : 2 (1,41)	7,31 ± 1,13	90,70 ± 0,01	29,17 ± 1,22	32,17 ± 1,25	47,47 ± 3,98	125	92,60 ± 2,61
9	125 (0)	1 : 1,25 (0)	5,30 ± 0,08	92,55 ± 0,01	30,10 ± 1,06	32,21 ± 1,03	47,27 ± 6,13	125	90,53 ± 2,11
10	125 (0)	1:1,25 (0)	5,90 ± 0,10	92,96 ± 0,01	28,00 ± 1,35	31,23 ± 1,34	53,00 ± 5,20	125	89,83 ± 3,56
11	125 (0)	1 : 1,25 (0)	5,73 ± 0,06	92,51 ± 0,01	26,77 ± 1,24	30,08 ± 0,76	62,31 ± 2,10	125	90,38 ± 2,29

*Números entre parênteses correspondem às variáveis codificadas.

Com base nos modelos matemáticos, a temperatura de 143 °C e a proporção de 1: 1,78 (sólidos de polpa de amora : agente encapsulante) foram consideradas como condições otimizadas, uma vez que em alta temperatura e alta concentração de agente encapsulante os conteúdos de antocianinas e ácido ascórbico e a capacidade antioxidante aumentaram e o rendimento do processo foi reduzido a temperaturas superiores a 143 °C.

Acredita-se que essa concentração de agente encapsulante (1:1,78, sólidos de polpa de amora: amido de araruta / goma arábica) foi suficiente para um possível aumento da temperatura de transição vítrea do sistema. Dessa forma, essa concentração de agente encapsulante favoreceu a secagem da polpa de amora, reduzindo a adesividade por meio da sua encapsulação pela mistura de amido de araruta e goma arábica, aumentando o rendimento do processo. Na temperatura do ar de entrada de 143 °C ocorreu maior eficiência dos processos de transferência de calor e massa (BAZARIA; KUMAR, 2016) e isso pode ter levado a uma formação mais rápida e eficiente de partículas com polpa de amora encapsulada, reduzindo consequentemente a degradação por oxidação térmica das antocianinas e ácido ascórbico, o que refletiu na sua capacidade antioxidante. Isso indica que a mistura de amido de araruta e goma arábica utilizada como agente encapsulante apresentou uma alta compatibilidade entre eles e a polpa de amora, exibindo um efeito termoprotetor durante o processo de secagem por pulverização, confirmando sua adequação como agente encapsulante.

Como não foi possível obter a polpa de amora na forma de pó sem encapsulante pelo método de *spray drying*, o método de *freeze drying* foi utilizado para a secagem da polpa de amora sem encapsulante e com encapsulante na proporção de 1:1,78 de sólidos de polpa de amora para amido de araruta / goma arábica, concentração de agente encapsulante ótima selecionada durante os ensaios de *spray drying*. O intuito dessa parte do trabalho foi investigar a influência da presença de agente encapsulante, bem como do amido de araruta, nas propriedades físico-químicas dos pós obtidos pelos diferentes métodos, comparando-os ao pó de amora sem encapsulante. Embora o método de *spray drying* tenha a vantagem de ter um baixo custo quando comparado ao método *freeze drying*, ele utiliza altas temperaturas enquanto que o *freeze drying* utiliza baixas temperaturas. Isso pode ter um efeito direto nos compostos bioativos e capacidade antioxidante dos pós de amora e, dessa forma, essa comparação de métodos é extremamente importante.

Os resultados da caracterização do pó de amora obtido por *freeze drying* (BL), do pó de polpa de amora microencapsulada por *freeze drying* (ML) e microencapsulada por *spray drying* (MS) estão mostrados na Tabela 2.

Os três pós de amora foram obtidos com êxito pelos métodos de *freeze drying* e *spray drying*. Os pós apresentaram aroma adocicado característico da amora e cor avermelhada. No entanto, os pós ML e MS tiveram uma diminuição da intensidade da sua cor avermelhada com a adição do encapsulante. Isso porque o agente encapsulante apresenta coloração clara, o que provoca uma diluição do pigmento antocianina responsável pela cor avermelhada da amora.

Quanto às características morfológicas dos pós, os pós obtidos por *freeze drying* tinham aparência de vidro quebrado em vários tamanhos. Curiosamente para o pó ML, que continha o agente encapsulante, foi possível visualizar partículas esféricas nas estruturas vítreas. Acredita-se que essas partículas esféricas sejam resultado do processo de microencapsulação da polpa de amora pela mistura de amido de araruta e goma arábica, utilizada como agente encapsulante. Partículas esféricas a ovaladas, cheias e murchas, grandes e pequenas também foram encontradas no pó de amora obtido por *spray drying*.

Tabela 2**.** Caracterização da amora em pó obtida por *freeze drying* (BL), pó de amora microencapsulada por *freeze drying* (ML) e *spray drying* (MS).

Análises	**Amora em pó obtida por *freeze drying* (BL)**	**Amora em pó microencapsulada por *freeze drying* (ML)**	**Amora em pó microencapsulada por *spray drying* (MS)**
Rendimento de Secagem (%)	89,24 ± 2,81A	95,86 ± 0,89A	57,69 ± 8,97B
Teor de água (%)	10,72 ± 2,81A	4,50 ± 0,31B	1,30 ± 0,10B
Aw (decimal)	0,13 ± 0,01B	0,11 ± 0,01B	0,16 ± 0,02A
Diâmetro médio D [4.3] (µm)	149.50 ± 2.92A	95.96 ± 5.87B	9.82 ± 0.12C
Higroscopicidade (g de água adsorvida/ 100g de sólidos)	21,28 ± 0,45A	12,86 ± 0,14C	17,04 ± 0,30B
Solubilidade em água (%)	61,26 ± 0,49A	53.84± 0,76B	60,16± 0,60A
Molhabilidade (min)	0,99 ± 0,53B	1,66 ± 0,01B	9,77 ± 0,22A
Antocianinas Total (mg/100 g de sólidos de amora)	125,27 ± 9,77A	125,99 ± 5,25A	86,35 ± 3,37B
FRAP (µmol sulfato ferroso / g de sólidos de amora)	845,29±12,52B	837, 87 ± 9,36B	1036,94 ± 81,94A

As mesmas letras, na mesma linha, não apresentam diferença estatística (p> 0,05).

O rendimento do processo de secagem dos pós de amora obtidos por *freeze drying* foi significativamente ($p<0,05$) superior do que o valor encontrado para o pó obtido por *spray drying*. Isso porque, a perda de produto no método de *freeze drying* geralmente está relacionada com o resíduo de material que pode ter ficado na vidraria em que a solução foi preparada, ou na bandeja em que o material foi seco, enquanto que no método de *spray drying* as perdas se devem ao material aderido à câmara de secagem e ciclone, o qual geralmente não apresenta condições de ser aproveitado.

Os pós de amora com agente encapsulante (ML e MS) apresentaram teor de água significativamente ($p <0,05$) menor do que o pó de amora sem encapsulante (BL). A adição de agente encapsulante antes da secagem aumenta o conteúdo de sólidos totais da mistura, reduzindo a quantidade de água a ser evaporada, o que acarreta a diminuição da umidade dos pós produzidos (CALISKAN; DIRIM, 2016). A atividade de água encontrada para os pós estava abaixo de 0,3, indicando a sua estabilidade microbiológica, uma vez que abaixo desse valor ($Aw <0,3$) não há crescimento de microrganismos, uma das principais reações de deterioração do produto (REID; FENNEMA, 2010).

As partículas dos pós BL e ML foram significativamente ($p <0,05$) maiores do que as partículas do pó MS e, apesar dos pós BL e ML serem produzidos pelo mesmo método, as partículas do pó ML foram significativamente ($p <0,05$) menores do que as partículas do pó BL, sem encapsulante. A presença de agente encapsulante e o tipo de método utilizado para a produção dos pós influenciou o diâmetro médio das partículas dos pós. O tamanho das partículas obtidas por *spray drying* é diretamente influenciado pelo tamanho das gotículas de pulverização, sendo que o tamanho dessas gotículas é dependente do tipo de atomizador e propriedades físicas como, por exemplo, rotação do atomizador, pressão do atomizador e concentração de sólidos da solução de alimentação (GOULA; ADAMOPOULOS, 2004). Já o tamanho das partículas produzidas por *freeze drying* é consequência do tipo de moagem e das suas condições de operação.

O pó BL se mostrou mais higroscópico e mais solúvel em água do que os pós produzidos com agente encapsulante. O amido de araruta e a goma arábica utilizados como encapsulantes apresentam baixa higroscopicidade. Consequentemente, a microencapsulação da polpa de amora tende a reduzir a higroscopicidade do pó resultante. Além disso, embora a goma arábica seja altamente solúvel em água (DAZA et al., 2017), o amido de araruta na sua forma nativa apresenta baixa solubilidade na água à temperatura ambiente, o que provavelmente contribuiu para a diminuição da solubilidade do pó de amora. Esse comportamento indica que a polpa de amora realmente foi microencapsulada pela mistura de amido de araruta e a goma arábica e por ambos os métodos.

Os tempos de molhabilidade dos pós (BL e ML) obtidos por *freeze drying* foi significativamente (p<0,05) inferior ao valor encontrado para o pó obtido por *spray drying*. Isto ocorreu porque os pós obtidos por *freeze drying* apresentaram maior tamanho de partícula e eram porosos, conforme visualizado por MEV. Partículas de tamanhos maiores apresentam mais espaços entre elas, sendo mais facilmente penetradas pela água (FERRARI et al., 2012). Por outro lado, as partículas de pó obtidas por *spray drying* eram muito menores e menos porosas. Isso levou a uma maior formação de grumos quando eles entraram em contato com a água. A baixa molhabilidade dos pós muito finos se deve à sua elevada tensão superficial e a camada viscosa formada na superfície do líquido que impede o fluxo por capilaridade entre os poros intergranulares (SCHUBERT, 1993). Um tempo de molhabilidade muito alto pode representar um aspecto negativo em relação à reconstituição do produto, que deve ocorrer em questões de poucos segundos.

Quanto ao teor de antocianinas, os pós (BL e ML) de amora obtidos por *freeze drying*, apresentaram valores significativamente (p<0,05) superiores do que o pó obtido por *spray drying* (MS). Esses resultados estão de acordo com o comportamento observado por Franceschinis et al. (2014), que relataram teor de antocianinas de 162 mg/100g para suco de amora preta seco por *freeze drying* e de 70 mg /100 g para suco de amora preta seco por *spray drying*. As perdas dos compostos bioativos no processo de secagem por *spray drying* estão ligadas à grande superfície exposta ao ar (OBEROI; SOGI, 2015) e altas temperaturas, enquanto que a perda durante *freeze drying* está associada à moagem do material após a liofilização (KUCK; NOREÑA, 2016).

Assim como observado para as antocianinas, não foram encontradas diferenças significativas (p>0,05) entre os pós BL e ML, obtidos por *freeze drying* quanto à capacidade antioxidante. No entanto, o pó MS obtido por *spray drying* mostrou capacidade antioxidante superior os pós BL e MS obtidos por *freeze drying*. Essa diferença na capacidade antioxidante pode ser resultado da grande variedade de compostos bioativos, que além das antocianinas podem estar presentes nos pós de amora como vitamina C, taninos, ácidos fenólicos, contribuindo na capacidade antioxidante total.

Avaliação da capacidade do amido de araruta em ser utilizado como biopolímero na produção de filmes biativos, comestíveis e biodegradáveis.

A terceira parte desse trabalho teve como foco principal avaliar a capacidade do amido de araruta em formar filmes comestíveis. As propriedades tecnológicas apresentadas pelo filme são diretamente influenciadas pelo tipo de processo de produção, pela concentração do biopolímero, bem como do plastificante. A influência da concentração de amido de araruta (2,6 - 5,4%, massa/massa) e do plastificante (10 - 24%, massa em relação à massa de amido) sobre as propriedades funcionais (espessura, atividade de água, teor de água, permeabilidade ao vapor de água, solubilidade em água e resistência a tração) dos filmes produzidos pela técnica de *casting* foi avaliada por meio de um planejamento composto central rotacional, com 11 ensaios experimentais.

Inicialmente, por meio de testes preliminares, foram determinados os limites utilizados no planejamento experimental (Apêndice B). O limite mínimo da concentração de amido foi determinado pela menor concentração de biopolímero que permitisse a formação de filmes capazes de serem facilmente desprendidos das placas de suporte, e o limite máximo foi determinado pela concentração máxima de amido que permitisse o escoamento homogêneo da solução pela placa, devido a sua alta viscosidade. Para o glicerol, as concentrações limites foram estabelecidas a partir de estudos relatados na literatura (FAKHOURY et al., 2012).

Todos os filmes preparados puderam ser retirados das placas de suporte sem rasgar e eram manipuláveis, o que mostra a capacidade do amido em ser utilizado como matriz formadora de filme. Os filmes eram transparentes, incolores e de bom aspecto visual, sem bolhas e partículas insolúveis visíveis ao olho nu, como pode ser visualizado na Figura 5. Uma das faces do filme era brilhante (superfície em contato com a placa durante a secagem do filme) e outra fosca (superfície exposta ao ar durante a secagem do filme), como também observado por Basiak et al. (2017) para filmes de amido de trigo, milho e batata.

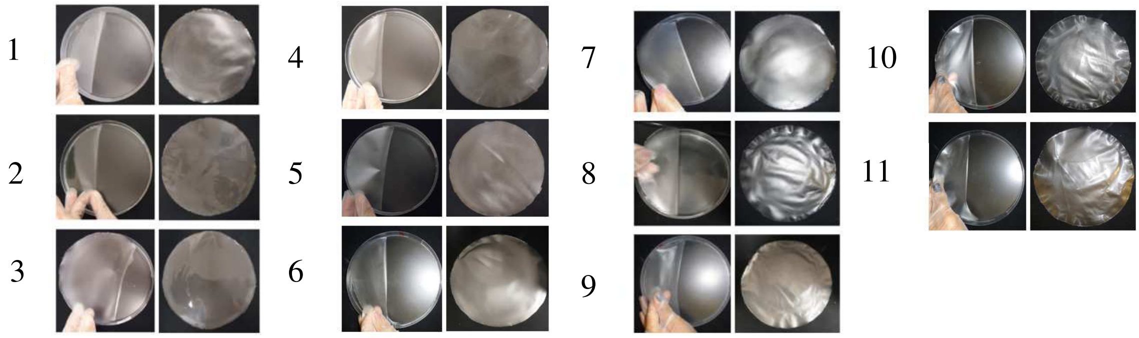

Figura 5. Imagens dos filmes obtidos nos ensaios do planejamento experimental.

Quanto as propriedades funcionais, a concentração do amido e do glicerol influenciaram significativamente ($p<0,05$) a espessura e a solubilidade em água apresentada pelos filmes. Os resultados da ANOVA, incluindo os coeficientes de regressão para as equações polinomiais codificadas de segunda ordem, os coeficientes de determinação (R^2), e os valores de F (F calculado e F tabulado), estão apresentados no Apêndice C.

Filmes mais espessos e mais solúveis em água foram obtidos quando altas concentrações de amido e glicerol foram utilizadas. O aumento da espessura ocorreu pelo aumento da quantidade de matéria seca num mesmo volume de solução filmogênica, depositado na placa de suporte, enquanto que a solubilidade em água dos filmes foi fortemente influenciada pela concentração de glicerol. A incorporação de plastificante no biopolímero reduz as forças de atração intermoleculares e eleva o volume livre do sistema, facilitando a permeação da água na sua estrutura e sua solubilização. Para aplicações como filmes comestíveis, a solubilização do filme de amido em água pode ser vantajosa em situações em que o filme será consumido com o produto, provocando poucas alterações nas propriedades sensoriais do alimento (FARIAS et al., 2012).

A variação da concentração do amido de araruta e do glicerol não afetou significativamente ($p >0,05$) os valores de permeabilidade ao vapor de água e a resistência à tração mostrada pelos filmes. Como um dos propósitos dos filmes comestíveis é também controlar a transferência de umidade entre o alimento e a atmosfera circundante, a permeabilidade ao vapor de água deve ser tão baixa quanto possível (FLORES et al., 2007; MARTINS et al., 2012; BASIAK et al., 2017).

Os filmes do ponto central foram os que apresentaram a menor permeabilidade ao vapor de água e a maior resistência à tração em comparação aos demais filmes e foram solúveis em água. Diante dos resultados e do exposto acima, o planejamento experimental permitiu selecionar a formulação do ponto central como promissora para aplicações como filme comestível. Para a validação dos modelos obtidos no planejamento experimental, foram realizados ensaios nas condições ótimas escolhidas, concentrações de 4% amido (massa/massa) e 17% de glicerol (massa/massa, em relação à massa de amido). Os resultados da validação experimental para filmes de amido de araruta, bem como os valores preditos pelos modelos e a diferença entre eles, estão apresentados na Tabela 3.

O filme apresentou-se manuseável e visualmente homogêneo e incolor. A permeabilidade ao vapor de água foi de 3,41 ± 0,13 g.mm/m^2.dia.kPa, enquanto que a resistência à tração foi de 17,87 ± 1,84 MPa. Os valores foram relativamente próximos aos encontrados anteriormente por esse estudo. Baseando-se nos valores de desvio relativo (% DR) obtido para cada variável resposta, considerou-se satisfatória a metodologia de otimização empregada (combinação de variáveis escolhidas com sua respectiva faixa de resposta).

Tabela 3. Valores preditos e experimentais das respostas em condições ótimas para filmes de amido de araruta e glicerol.

Análise	**Valor experimental**	**Valor predito** [a]	**% DR** [b]
Espessura (mm)	0,079 ± 0,11	0,073	7,59
Solubilidade em água (%)	14,75 ± 0,59	12,38	16,07

[a] Valores obtidos nas condições ótimas: concentrações de 4% amido (massa/massa) e 17% de glicerol (massa/massa, em relação à massa de amido).

[b] Desvio relativo = [(valor experimental - valor predito)/ valor experimental BF]*100.

A investigação sobre a utilização de filmes comestíveis como materiais de embalagem é contínua, devido ao grande potencial desses filmes em melhorar e manter a qualidade do alimento, possibilitar o alimento seguro e aumentar a vida de prateleira. Além de agir como uma barreira contra a difusão (umidade, gases e voláteis), eles podem ser utilizados como veículos de transporte para muitos tipos de aditivos alimentares, incluindo agentes antioxidantes, vitaminas, corantes, agentes antimicrobianos e aromas, melhorando assim a integridade do alimento (FARIAS et al., 2012).

A amora-preta é um fruto rico em compostos biologicamente ativos que apresentam atividades antioxidantes (MACHADO et al., 2015). Assim, a quarta parte desse trabalho teve como objetivo principal investigar a incorporação de polpa de amora na solução filmogênica de amido de araruta para a produção de filmes comestíveis com propriedades antioxidantes. As concentrações de 4% amido de araruta (m/m) e 17% glicerol (massa/massa, em relação à massa de amido) selecionadas por meio do planejamento experimental como condição ótima para a produção de filmes comestíveis foi utilizada como matriz biopolimérica a ser incorporada a amora.

A polpa de amora foi incorporada de três formas distintas: i) na forma líquida; ii) na forma de pó seco por freeze *drying;* iii) na forma de pó microencapsulado pelos métodos de *spray drying* e *freeze drying;* e em diferentes concentrações: 0%, 20%, 30% e 40% (m/m) em relação à massa do amido. Para a produção da polpa de amora microencapsulada por *spray drying* foi utilizada a temperatura do ar de entrada de 143 °C e a proporção de 1: 1,78 de sólidos de polpa de amora para agente encapsulante (mistura de amido de araruta e goma arábica, 1:1, m/m), conforme as condições otimizadas na segunda parte desse trabalho. Essa mesma concentração de agente encapsulante foi utilizada na obtenção da polpa de amora microencapsulada por *freeze drying*. Esses pós mostraram alta capacidade antioxidante e por isso, se apresentam como aditivos alimentares potenciais.

Além disso, a polpa de amora foi incorporada de 2 maneiras diferentes sendo a primeira a incorporação direta na solução filmogênica e a segunda por aspersão (*sprinkling)* sob a solução filmogênica. Ao todo foram elaborados 22 filmes. As formulações e as respectivas nomenclaturas estão resumidas na Tabela 4.

Tabela 4. Formulações de filmes de amido de araruta e polpa de amora produzidos.

Tratamentos	**Amora líquida** (B)	**Amora em pó obtida por *freeze drying*** (BL)	**Amora em pó microencapsulada por *freeze drying*** (ML)	**Amora em pó microencapsulada por *spray drying*** (MS)
Filme 0% amora (0%)	-	-	-	-
Incorporação da amora diretamente à solução filmogênica (D)				
Filme com 20% de amora líquida (20% BP)	20%	-	-	-
Filme com 30% de amora líquida (30% BP)	30%	-	-	-
Filme com 40% de amora líquida (40% BP)	40%	-	-	-
Filme com 20% de amora em pó obtida por *freeze drying* (20% BLD)	-	20%	-	-
Filme com 30% de amora em pó obtida por *freeze drying* (30% BLD)	-	30%	-	-
Filme com 40% de amora em pó obtida por *freeze drying* (40% BLD)	-	40%	-	-
Filme com 20% de amora microencapsulada por *freeze drying* (20% MLD)	-	-	20%	-
Filme com 30% de amora microencapsulada por *freeze drying* (30% MLD)	-	-	30%	-
Filme com 40% de amora microencapsulada por *freeze drying* (40% MLD)	-	-	40%	-
Filme com 20% de amora microencapsulada por *spray drying* (20% MSD)	-	-	-	20%
Filme com 30% de amora microencapsulada por *spray drying* (30% MSD)	-	-	-	30%
Filme com 40% de amora microencapsulada por *spray drying* (40% MSD)	-	-	-	40%
Incorporação por sprinkling do pó de amora sob a solução Filmogênica (S)				
Filme com 20% de amora em pó obtida por *freeze drying* (20% BLS)	-	20%	-	-
Filme com 30% de amora em pó obtida por *freeze drying* (30% BLS)	-	30%	-	-
Filme com 40% de amora em pó obtida por *freeze drying* (40% BLS)	-	40%	-	-
Filme com 20% de amora microencapsulada por *freeze drying* (20% MLS)	-	-	20%	-
Filme com 30% de amora microencapsulada por *freeze drying* (30% MLS)	-	-	30%	-
Filme com 40% de amora microencapsulada por *freeze drying* (40% MLS)	-	-	40%	-
Filme com 20% de amora microencapsulada por *spray drying* (20% MSS)	-	-	-	20%
Filme com 30% de amora microencapsulada por *spray drying* (30% MSS)	-	-	-	30%
Filme com 40% de amora microencapsulada por *spray drying* (40%MSS)	-	-	-	40%

De maneira geral, todos os filmes foram obtidos com êxito, pois puderam ser desprendidos das placas de suporte. Com exceção do filme 0% que era incolor e inodoro, todos os demais filmes mostraram aroma característico e coloração com tons róseos a avermelhados, típica da polpa de amora bem como do pó produzido a partir da mesma. A intensidade de cada cor variou em função da sua concentração no filme, mesmo a olho nu.

Os filmes feitos com a polpa de amora líquida foram os que apresentaram aroma, cor e sabor mais pronunciados da amora *in natura*. Os filmes produzidos pela incorporação direta de amora na solução filmogênica exibiram aspecto visual semelhante ao papel tipo celofane, ou seja, era evidente o brilho em ambas as faces do filme, enquanto que os filmes produzidos com incorporação por *sprinkling* tinham aparência fosca e aveludada no lado da face em que a amora ou as micropartículas foram adicionadas, se assemelhando ao papel camurça. Os filmes elaborados com 20 % amora ou micropartícula pulverizada eram quebradiços e sensíveis ao manuseio, mas todos os demais filmes produzidos foram maleáveis e manipuláveis.

Quanto às características microestruturais observadas por MEV, o filme 0% mostrou matriz polimérica organizada, contínua e densa. A incorporação direta da amora na solução filmogênica, produziu a heterogeneidade da matriz de amido, gerando algumas regiões desorganizadas dentro da matriz, devido à presença de partículas suspensas na rede polimérica ou aglomeradas.

A incorporação da amora por *sprinkling* foi realizada de forma homogênea, pois as partículas foram distribuídas por toda a área superficial do filme. Esses filmes mostraram uma matriz desorganizada na sua superfície e organizada logo abaixo da superfície. O pó de amora obtido por *spray drying* penetrou um pouco mais na superfície da solução filmogênica do que os pós produzidos por *freeze drying*. Os filmes elaborados com pó de amora exibiram características estruturais diretamente relacionadas com as observadas para cada pó. Curiosamente, a presença de micropartículas (ML e MS) produzidas por *freeze drying* e *spray drying* tiveram o mesmo efeito na morfologia do filme de amido de araruta quando foram incorporadas na solução filmogênica.

Como observado por MEV, a maioria das micropartículas da amora permaneceram intactas após serem incorporadas à solução filmogênica. Acredita-se que isso ocorreu porque durante o processo de gelatinização do amido, as moléculas de água se ligam aos grupos hidroxilos expostos de amilose e amilopectina por ligações de hidrogênio (HOOVER, 2001), deixando pouca água disponível para interação com as micropartículas, o que pode ter ajudado na manutenção da sua estrutura.

Todos os filmes que foram incorporados com amora, independentemente se na forma líquida ou na forma de pó, microencapsulada ou não, diretamente ou por *sprinkling*, apresentaram conteúdo de antocianinas e capacidade antioxidante significativos, diferindo estatisticamente do filme (0%) sem amora, que mostrou traço de antocianinas e capacidade antioxidante insignificante. A incorporação da amora na solução filmogênica transferiu compostos bioativos e atividade antioxidante ao filme resultante. Com isso fica evidente que a estratégia de incorporação da amora-preta ao filme de amido de araruta para produção de filmes antioxidantes é viável e promissora.

Na maioria dos casos, o tipo de incorporação direta ou por *sprinkling* de amora na solução filmogênica, teve mais influência no conteúdo de antocianinas e capacidade antioxidante do que a variação de sua concentração no filme. Os filmes incorporados por *sprinkling* quase sempre apresentaram valores de capacidade antioxidante maiores que os filmes com incorporação direta. Possivelmente, o fato de a amora estar na superfície do filme tenha facilitado a extração dos compostos bioativos, devido ao contato direto da amora com os solventes de extração. No caso da incorporação direta, a amora foi integrada dentro da matriz polimérica. Isso provavelmente dificultou a extração dos compostos bioativos pelos solventes de extração, uma vez que amido de araruta pode ter funcionado como uma barreira protetora.

Todos os filmes foram considerados microbiologicamente estáveis, pois apresentaram baixo conteúdo de água e atividade de água menor que 0.6 (QUEK et al., 2007). O aumento da concentração da amora incorporada ao filme levou a um aumento significativo ($p<0,05$) da espessura do filme de araruta. Esse aumento de espessura é decorrente do aumento do conteúdo de sólidos e protuberâncias encontradas na superfície dos filmes causado pela presença das estruturas ou micropartículas de amora no filme. Como visualizado por MEV, a incorporação da amora tornou a superfície do filme de amido de araruta áspera e irregular.

De maneira geral, a solubilidade em água, a permeabilidade ao vapor de água e o alongamento à ruptura aumentaram significativamente ($p <0,05$) com o aumento da concentração de polpa de amora (20, 30 e 40%) no filme, enquanto a resistência à tração diminuiu quando comparada ao do filme de 0%. Essa tendência foi observada para todos os filmes incorporados com a amora, independentemente se na forma líquida ou na forma de pó, microencapsulada ou não, diretamente ou por *sprinkling*.

A transferência de umidade geralmente ocorre através da porção hidrofílica de uma barreira e está diretamente relacionada com a relação hidrofílica - hidrofóbica de seus componentes (HERNANDEZ, 1994; SARTORI; MENEGALLI, 2016; ROJAS-GRAÜ et al., 2006). Assim, como os pós de amora são altamente higroscópicos e solúveis em água, a sua incorporação ao filme, provavelmente aumentou os números de grupos hidrofílicos presentes na matriz polimérica. Isso deu ao filme uma maior tendência à ligação com as moléculas de água, favorecendo a permeabilidade ao vapor de água e a sua solubilização. Além disso, as zonas de ruptura observadas na matriz polimérica promovida pela adição da amora na solução filmogênica também pode ter ajudado nesse comportamento. A presença de defeitos estruturais na matriz polimérica, bem como na superfície do filme, o torna menos resistente quanto à passagem de moléculas de água.

Outra possível causa, é o efeito plastificante propiciado pelos açúcares presentes na polpa de amora quando adicionada à matriz polimérica. Estudos têm demonstrado que o aumento do plastificante produz filmes menos rígidos e mais extensíveis, isto é, causa uma redução na resistência à tração e um aumento do alongamento à ruptura dos filmes, respectivamente. Essas tendências provavelmente são devido à redução das interações entre as cadeias de biopolímeros, que leva a uma maior mobilidade das cadeias do amido, com diminuição da temperatura da transição vítrea (BERGO et al., 2008; IVANIČ et al., 2017).

O mesmo comportamento encontrado na análise de solubilidade em água foi observado no estudo de estabilidade dos filmes submetidos em diferentes condições de pH, o que sugere que as prováveis causas para esse comportamento sejam as mesmas ou estão relacionadas. Em geral, a solubilidade dos filmes em soluções ácida (pH 3), neutra (pH 7) e alcalina (pH 10), aumentou significativamente ($p < 0{,}05$) com o aumento da concentração de amora (20, 30 e 40%) no filme quando comparado ao filme controle (0%). Além do mais, os filmes incorporados com amora por *sprinkling* foram mais solúveis em água que os filmes incorporados com amora diretamente, seguindo a mesma tendência observada para a capacidade antioxidante.

Esses resultados reforçam que a localização da polpa de amora na matriz polimérica influencia diretamente a solubilidade do filme, bem como a extração dos compostos bioativos. Como observado por MEV, a incorporação da amora por *sprinkling* permitiu que as partículas do pó de amora ficassem apenas na superfície dos filmes. Como os pós de amora apresentaram caráter hidrofílico, acredita-se que quando os filmes foram imersos em água, as partículas se hidrataram levando à sua solubilização. A solubilização das partículas possivelmente criou espaços vazios na superfície dos filmes, o que permitiu a entrada de moléculas de água na matriz do amido facilitando a sua solubilização. Já para os filmes incorporados diretamente, a matriz polimérica do amido serviu como uma barreira contra a solubilização das partículas de amora e consequentemente do filme.

A diminuição da intensidade da cor dos filmes de amora após imersão em diferentes condições de pH e a coloração rosa claro das soluções após remoção dos filmes contribuem para esses resultados. Como as antocianinas são pigmentos solúveis em água, é razoável que elas tenham sido liberadas para o meio circundante. Essa liberação das antocianinas também está relacionada com a solubilização do filme.

Embora tenha havido a solubilização dos filmes, não foram observadas grandes alterações na sua estrutura perceptíveis ao olho nu, com exceção pela alteração da cor. Aparentemente os filmes mantiveram o mesmo diâmetro do início da análise após 12 dias, imersos em diferentes condições de pHs.

Quanto à estabilidade frente ao processo de esterilização em autoclave a 127 °C por 15 minutos, para a maioria dos filmes não foram obervadas alterações significativas ($p>0,05$) na espessura. Todos os filmes continuaram íntegros, sem furos ou rachaduras após serem autoclavados. Porém, a alteração na coloração dos filmes com amora foi notória.

O filme 0% continuou incolor, enquanto que os filmes com amora se tornaram marrons. Isso sugere que o escurecimento está diretamente relacionado com a presença da amora no filme. Três possíveis mecanismos podem estar envolvidos nesse escurecimento do filme de amora, sendo o primeiro a degradação das antocianinas por oxidação térmica (MACHADO et al., 2015), o segundo é a reação de Maillard (escurecimento não enzimático) que ocorre quando componentes amina e açúcares estão presentes na mesma mistura e são aquecidos a altas temperaturas (DAMODARAN et al., 2010) e o terceiro é a caramelização dos açúcares presentes na amora.

Com relação à biodesintegração dos filmes em compostagem orgânica, observou-se que o todos os filmes exibiram fortes mudanças na tonalidade e integridade após 7 dias enterrados, sugerindo o início da degradação das antocianinas e do material polimérico. Após 38 dias de teste, todos os 22 filmes estavam quase que totalmente desintegrados, bem antes do prazo de 90 dias, período final de desintegração, de acordo com ISO 20200: 2004, para que os materiais plásticos sejam considerados desintegráveis. Considerando o curto período de degradação dos materiais observados neste trabalho, os resíduos desses materiais poderiam ser eliminados em jardins urbanos sem intervenção industrial, uma vez que o volume dos resíduos iria diminuir rapidamente devido a sua facilidade de desintegração. Isso também poderia ajudar a reduzir os gastos do governo com o processamento de materiais poliméricos (JARAMILLO et al., 2016).

Diante de todos os resultados, podem-se sugerir aplicações potenciais para os filmes elaborados nesse trabalho. O filme de amido de araruta e glicerol tem um grande potencial em ser utilizado como embalagens de alimentos em substituição às embalagens não biodegradáveis, pois dentre todos os filmes produzidos neste trabalho, ele foi o que apresentou a menor solubilidade em soluções aquosas, foi estável fisicamente frente ao processo de esterilização, foi resistente e flexível, e desintegrou-se em compostagem orgânica em menos de 90 dias.

Os filmes produzidos com a polpa de amora líquida ou seca por *freeze drying* mostraram características favoráveis para serem aplicados como revestimentos coloridos para alimentos específicos ou para serem consumidos na forma de tiras de frutas, pois foram os filmes que mais mantiveram o aroma, a cor e o sabor próximo ao da amora *in natura*. Além de serem solúveis em água, característica desejável caso o filme seja ingerido junto com o produto a ser embalado, esses filmes foram considerados fonte de antocianinas e antioxidantes.

Os filmes produzidos com polpa de amora microencapsulada, tanto por *spray drying* quanto por *freeze drying*, apresentaram características funcionais similares quando incorporados diretamente e quando incorporados por *sprinkling* e ambos se mostraram bioativos e antioxidantes. Baseando-se nisso, acredita-se que tanto os filmes incorporados diretamente quanto os filmes incorporados por *sprinkling,* tenham um grande potencial para serem utilizados como encapsulante e/ou embalagens ativas com capacidade de substituírem as embalagens de alimentos intermediárias não biodegradáveis, uma vez que foram solúveis em água, menos resistentes e mais flexíveis e apresentaram relativa permeabilidade ao vapor de água e se biodesintegraram em ambiente ativo.

Os filmes incorporados com polpa de amora microencapsulada diretamente na solução filmogênica foram solúveis em soluções aquosas de diferentes pHs, mas foram menos solúveis e apresentaram menos facilidade de liberação dos compostos antioxidantes quando comparados aos filmes incorporados por *sprinkling*. Diante disso, sugere-se que esses filmes incorporados diretamente possam ser aplicados como embalagens ativas em alimentos nos quais sejam necessários, liberação controlada de compostos antioxidantes em sistemas alimentares dentro de um espaço de tempo maior que 12 dias (período em que foi avaliada a solubilização dos filmes em diferentes condições de pHs). Talvez esse maior tempo em que o filme ficará em contato com o alimento poderá estimular a liberação dos compostos antioxidantes restantes. No entanto, os filmes incorporados com polpa de amora microencapsulada por *sprinkling* podem ser aplicados em alimentos, nos quais essa liberação de compostos antioxidantes deva ocorrer em um curto período de tempo.

No entanto, vale ressaltar que testes de migração de compostos bioativos em simulantes alimentares devem ser realizados para se obter informações mais conclusivas e específicas quanto às características dos potenciais alimentos, em que os filmes poderão ser aplicados, assim como entender os mecanismos envolvidos nessa liberação. Sabe-se que a quantidade de qualquer componente que migra para o alimento depende da sua concentração inicial no polímero e sua solubilidade, bem como a temperatura, tensões mecânicas e tempo de contato. É extremamente difícil medir essa migração direta de um filme para um alimento, uma vez que a maioria dos gêneros alimentícios tem composições complexas (Avella et al., 2005). Geralmente se avalia esse comportamento de migração do filme biopolimérico em simuladores de alimentos, tais como etanol 95 % e água, utilizados como simulantes de alimentos gordurosos e aquosos, respectivamente (PIREÑOS-HERANADEZ et al., 2017;

Busolo et al., 2015), em condições definidas de temperatura, tempo e modo estático / dinâmico. Ao final do teste, é aplicada uma técnica analítica apropriada para determinar a quantidade de composto presente no simulante e a partir disso, o grau de migração pode ser calculado (AVELLA et al., 2005).

Referências Bibliográficas

AGGARWAL, P.; DOLLIMORE, D. A thermal analysis investigation of partially hydrolyzed starch. *Thermochimica Acta*, Amsterdam, 447, 17-25, 1998.

ARAUJO-DÍAZ, S.B.; LEYVA-PORRAS, C.; AGUIRRE-BAÑUELOS, P.; ÁLVAREZ-SALAS, C.; SAAVEDRA-LEOS, Z. Evaluation of the physical properties and conservation of theantioxidants content, employing inulin and maltodextrin in the spraydrying of blueberry juice. *Carbohydrate Polymers*, 167, 317–325, 2017.

AVELLA, M.; DE VLIEGER, J.J.; ERRICO, M.E.; FISCHER, S.; VACCA, P.; VOLPE, M.G. Biodegradable starch/clay nanocomposite films for food packaging applications. *Food Chemistry*, 93, 3, 467–474, 2005.

BASIAK, E.; LENART, A.; DEBEAUFORT, F. Effect of starch type on the physico-chemical properties of edible films. *International Journal of Biological Macromolecules*, 98, 348–356, 2017.

BAZARIA, B.; KUMAR, P. Optimization of spray drying parameters for beetroot juice powder using response surface methodology (RSM). *Journal of the Saudi Society of Agricultural Sciences*, xxx, xxx–xxx., 2016.

BERGO, P.V.A.; CARVALHO, R.A.; SOBRAL, P.J.A.; DOS SANTOS, R.M.C.; DA SILVA, F.B.R.; PRISON, J.M.; SOLORZA-FERIA, J.; HABITANTE, A.M.Q.B. Physical

Properties of Edible Films Based on Cassava Starch as Affected by the Plasticizer Concentration. *Packaging Technology & Science*, 21, 85–89, 2008.

BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução – RDC nº 263, de 22 de setembro de 2005. *Aprova o regulamento técnico para produtos de cereais, amidos, farinhas e farelos*. Diário Oficial da República Federativa do Brasil, Brasília, 23 de setembro de 2005.

BUSOLO, M.A.; LAGARON, J.M. Antioxidant polyethylene films based on a resveratrol containing Clay of Interest in Food Packaging Applications. *Food Packaging and Shelf Life*, 6, 30-41, 2015.

CALISKAN, G.; DIRIM, S.N. The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders. *Powder Technology*, 287, 308–314, 2016.

CHUANG, L.; PANYOYAI, N.; SHANKS, R.A.; KASAPIS, S. Effect of salt on the glass transition of condensed tapioca starch systems. *Food Chemistry*, 229, 120–126, 2017.

CHUANG, L.; PANYOYAI, N.; KATOPO, L.; SHANKS, R.; KASAPIS, S. Calcium chloride effects on the glass transition of condensed systems of potato starch. *Food Chemistry*, 199, 791–798, 2016.

CLEMENTE GRANADOS, C.; ENRIQUE GUZMAN, C.; DIOFANOR ACEVEDO C.; MARÍA DÍAZ, M.; ANA HERRERA, A. Propiedades funcionales del almidon de sagu (Maranta arundinacea). *Colombia Biotecnología en el Sector Agropecuario y Agroindustrial*, Cauca, 12, 2, 90-96, jul./dic. 2014.

ČSN EN ISO 20200 "*Plastics - Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test*" (ISO 20200:2004).

CUNHA, A.L. *Caracterização do amido da araruta tipos seta e redonda, visando utilização na indústria de alimentos*. Lavras –MG, 2016. 113p. Dissertação (Mestrado em Ciências dos Alimentos). Universidade Federal de Lavras, 2016.

DAMODARAN, S.; PARKIN, K.L.; FENNEMA, O.R. Química de alimentos de Fennema, 4. ed. Porto Alegre: Artmed, 2010. *Química de alimentos de Fennema*. 4. ed. Porto Alegre : Artmed, p. 900, 2010.

DAZA, L.D.; FUJITA, A.; FÁVARO-TRINDADE, C.S.; RODRIGUES-RACT, J.N.; GRANATO, D.; GENOVESE, M.I. Effect of spray drying conditions on the physical properties of Cagaita (*Eugenia dysenterica* DC.) fruit extracts. *Food and Bioproducts Processing*, 97, 20-29, 2016.

DU, L.; BRENNER, T.; XIE, J.; MATSUKAWA, S.A study on phase separation behavior in kappa/iota carrageenan mixtures by micro DSC, rheological measurements and simulating water and cations migration between phases. *Food Hydrocolloids*, 55, 81–88, 2016.

ELISIA, I.; HU, C.; POPOVICH, D.G.; KITTS, D.D. Antioxidant assessment of an anthocyanin-enriched blackberry extract. *Food Chemistry*, 101, 3, 1052–1058, 2007.

ERDMAN, M.D. Starch from arrowroot (*Maranta arundinacea*) grown at Tifton, Georgia. *Cereal Chemistry*, 63, 277-279, 1986.

FAKHOURY, F.M.; MARTELLI, S.M.; BERTAN, L.C.; YAMASHITA, F.; INNOCENTINI MEI, L.H.; QUEIROZ, F.P.C. Edible films made from blends of manioc starch and gelatin –

Influence of different types of plasticizer and different levels of macromolecules on their properties. *LWT - Food Science and Technology*, 49, 149-154, 2012.

FARIAS, M.G.; FAKHOURI, F.M.; DE CARVALHO, C.W.P.; ASCHERI, J.L.R. Physicochemical characterization of edible starch films with barbados cherry (*Malphigia emarginata* DC). *Quimica Nova*, 35, 546-552, 2012.

FERRARI, T.B.; LEONEL, M.; SARMENTO, S.B.S. Características dos rizomas e do amido de araruta (*Maranta arundinacea*) em diferentes estádios de desenvolvimento da planta. *Brazilian Journal of Food Technology*, Campinas, 8, 2, 93-98, abr./jun. 2005.

FERRARI, C.C.; RIBEIRO, C.P.; AGUIRRE, J.M. Spray drying of blackberry pulp using maltodextrin as carrier agent. *Brazilian Journal of Food Technology*, 15, 157-165, 2012.

FLORES, S.; FAMÁ, L.; ROJAS, A.M.; GOYANES, S.; GERSCHENSON, L. Physical propertiesof tapioca-starch edible films: influence of filmmaking and potassiumsorbate, *Food Research International*, 40, 257–265, 2007.

FRANKLIN, M.E.E.; PUSHPADASS, H.A.; KUMAR, B.; KULKARNI, S.; MUTHURAYAPPA, M.; KANDASAMY, R.; VENKATACHALAM, P.; VELLINGIRI, P. Physicochemical, thermal, pasting and microstructural characterization of commercial *Curcuma angustifolia* starch. *Food Hydrocolloids*, 67, 27-36, 2017.

FREITAS, M.C.J.; TAVARES, D.Q. Caracterização do grânulo de amido de bananas (Musa AAA-Nanicão e Musa AAB-Terra). *Ciência e Tecnologia de Alimentos*, Campinas, 25, 2, 217-222, 2005.

GOULA, A.M.; ADAMOPOULOS, K.G. Retention of ascorbic acid during drying of tomato halves and tomato pulp. *Drying Technology*, 24, 57-64, 2006.

HAGER, T. J.; HOWARD, L. R.; LIYANAGE, R.; LAY, J.O.; PRIOR, R.L. Ellagitannin composition of blackberry as determined by HPLC-ESI-MS and MALDI-TOF-MS. *Journal of Agricultural and Food Chemistry*, 56, 3, 661–669, 2008.

HERNANDEZ, E. *Edible coatings from lipids and resins*. In KROCHTA, J.M.; BALDWIN, E.A.; NISPERO-CARRIEDO, M.G. (Eds.), Edible coatings and films to improve food quality (pp. 279-303). Lancaster, PA, USA: Technomic Publ. Co. 1994.

HERNANDEZ-MEDINA, M.; TORRUCO-UCO, J.G.; CHEL-GUERRERO, L.; BETANCUR-ANCONA, D. Caracterización fisicoquímica de almidones de tubérculos cultivados en Yucatán, México. *Ciência e Tecnologia de Alimentos*, Campinas, 28, 3, 718-726, 2008.

HOOVER, R. Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45, 3, 253-267, 2001.

IKEGWU, O.J.; OKECHUKWU, P.E.; EKUMANKANA, E.O. Physico-chemical and pasting characteristics of flour and starch from Achi Brachystegia eurycoma seed. *Journal of Food Technology*, 8, 58- 66, 2010.

IVANIČ, F.; JOCHEC-MOŠKOVÁ, D.; JANIGOVÁ, I.; CHODÁK, I. Physical properties of starch plasticized by a mixture of plasticizers. *European Polymer Journal*, 93, 843-849, 2017.

JARAMILLO, C.M.; GUTIÉRREZ, T.J.; GOYANES, S.; BERNAL, C.; FAMÁ, L. Biodegradability and plasticizing effect of yerba mate extract oncassava starch edible films. *Carbohydrate Polymers*, 151, 150–159, 2016.

KUCK, L.S.; NOREÑA, C.P.Z. Microencapsulation of grape (*Vitilabrusca* var. Bordo) skin phenol extract using Arabic gum, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. *Food Chemistry*, 194, 569-576, 2016.

LEONEL, M.; CEREDA, M.P.; SARMENTO, S.B.S. Arrowroot (*Maranta arundinacea*) as a Possible Raw Material for Starch Industries. *Brazilian Journal Food Technology*, 5:151-155, 2002a.

LEONEL, M.; SARMENTO, S.B.S.; CEREDA, M.P.; GUERREIRO, L.M.R. Extraction and Characterization of Biri Starch (*Canna edulis*). *Brazilian Journal Food Technology*, 5:27-32, 2002b.

LEONEL, M.; CEREDA, M.P. Caracterização físico-química de algumas tuberosas amiláceas. *Food Science Technology*, Campinas, 22, 1, 65-69, 2002.

MACHADO, A.P.F.; PASQUEL-REÁTEGUI, J.L.; BARBERO, G.F.; MARTÍNEZ, J. Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International*, 77, 675–683, 2015.

MARTINS, J.T.; CERQUEIRA, M.A.; BOURBON, A.I.; PINHEIRO, A.C.; SOUZA, B.W.S.; VICENTE, A.A. Synergistic effects between k-carrageen and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocolloids*, 29, 280–289, 2 012.

MASTSUGMA, L.S. *Caracterização do amido de mandioquinha salsa (Arracaia xanthorrhiza) nativo e modificado por oxidação*. Ponta Grossa, 2006. 112p. Dissertação (Mestrado em Ciências e Tecnologia de Alimentos). Universidade Estadual de Ponta Grossa, 2006.

MENDES, M.L.M. *Caracterização para fins industriais dos amidos nativo e modificados extraídos de amêndoas de sementes de manga, variedade "Tommy Atkins"*. 2011. 131 p. Tese

(Doutorado em Ciência e Tecnologia de Alimentos) - Universidade Federal da Paraíba, João Pessoa, 2011.

MOORTHY, S.N. Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54, 559-592, 2002.

OBEROI, D.P.S.; SOGI, D.S. Effect of drying methods and maltodextrin concentration on pigment content of watermelon juice powder. *Journal of Food Engineering*, 165, 172-78, 2015.

PÉREZ, E.; LARES, M.; GONZÁLEZ, Z. Some characteristics of sagu (*Canna edulis*) and zulu (*Maranta* sp) rhizomes. *Journal of Agricultural and Food Chemistry*, 45:2546-2549, 1997.

PIÑEROS-HERNANDEZ, D.; MEDINA-JARAMILLO, C.; LÓPEZ-CÓRDOBA, A.; GOYANES, S. Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. *Food Hydrocolloids*, 63, 488-495, 2017.

QUEK, S.Y.; CHOK, N.K.; SWEDLUND, P. The physicochemical properties of spary-dried watermelon powders. *Chemical Engineering and Processing: Process Intensification*, 46, 386-392, 2007.

RAMÍREZ, M.J.; GIRALDO, G.I.; ORREGO, C.E. Modeling and stability of polyphenol in spray-dried and freeze-dried fruit encapsulates. *Powder Technology*, 277, 89–96, 2015.

REID, D.S.; & FENNEMA, O.R. Água e gelo. In: DAMODARAN, S.; PARKIN, K.L.; FENNEMA, O.R. (Eds.), *Química de Alimentos de FENNEMA*. Artmed, Porto Alegre, pp. 25–74, 2010.

RODRIGUES, L.B.O. *Estudos reológicos e de textura dos géis de amido de araruta (Maranta arundinaceae L.) e dos géis adicionados de sacarose e concentrado protéico de soro*. Itapetinga – BA, 2014. 69p. Dissertação (Mestrado em Engenharia e Ciências de Alimentos). Universidade Estadual do Sudoeste da Bahia-UESB, 2014.

ROJAS-GRAÜ, M.A.; AVENA-BUSTILLOS, R.J.; FRIEDMAN, M.; HENIKA, P.R.; MARTÍN BELLOSO, O.; McHUGH, T. H. Mechanical, barrier, and antimicrobial properties of apple puree edible films containing essential oils. *Journal of Agricultural and Food Chemistry*, 54, 9262–9267, 2006.

SARTORI, T.; MENEGALLI, F.C. Development and characterization of unripe banana starch films incorporated with solid lipid microparticles containing ascorbic acid. *Food Hydrocolloids*, 55, 210-219, 2016.

SCHUBERT, H. Instantization of powdered food products. *International Chemical Engineering*, 33, 1, 28-45, 1993.

SINGH, N.; SINGH, J.; KAUR, L.; SODHI, N.S.; GILL, B.S. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry,* 81, 219–231, 2003.

VILLAS-BOAS, F.; FRANCO, C.M.L. Effect of bacterial β-amylase and fungal α-amylase on the digestibility and structural characteristics of potato and arrowroot starches. *Food Hydrocolloids*, 52, 795- 803, 2016.

ZHANG, P.; WHISTLER, R.L.; BEMILLER, J.N.; HAMAKE, B.R. Banana starch: production, physicochemical properties, and digestibility—a review. *Carbohydrate Polymers*, 59, 4, 443–458, 2005.

Conclusões Gerais

A extração do amido de araruta foi realizada de forma satisfatória. O amido apresentou grânulos circulares, elipsoides e ovais, de diferentes tamanhos e íntegros, sem fissuras ou rachaduras. O amido apresentou alto grau de pureza, contendo baixos teores de proteínas, lipídeos e cinzas e conteúdo elevado de amilose, 35,20 ± 1,63%. O difratograma de raios-X do amido de araruta revelou uma estrutura cristalina do tipo C, comumente encontrado para cereais e leguminosas. Os grânulos de amido de araruta incharam e se tornaram solúveis em água em temperaturas acima de 60 ºC, o que está relacionado com a sua habilidade de gelificação. O amido de araruta apresentou Tg em torno de 118 °C e 120 °C. A análise termogravimétrica mostrou que entre 330 °C e 410 °C ocorreu 40 % de perda de massa do amido, relacionada com a sua despolimerização. O alto teor de amilose, a habilidade de gelificação e as boas propriedades térmicas apresentadas pelo amido de araruta são qualidades desejáveis para a sua utilização como agente encapsulante, bem como polímero na produção de embalagens ou filmes comestíveis e biodegradáveis, além das características adequadas para sua aplicação na indústria alimentícia como agente espessante e gelificante.

Não foi possível a utilização do amido de araruta sozinho, como agente encapsulante da polpa de amora por *spray drying,* devido à grande quantidade de material que ficou aderida à parede da câmara de secagem e do ciclone, o que acarretou um rendimento insatisfatório. A incorporação da goma arábica ao amido melhorou esse comportamento e permitiu a sua aplicação como agente encapsulante pelo método de *spray drying*. Essa mistura utilizada como agente encapsulante foi capaz de tornar a polpa de amora mais estável, diminuindo a sua pegajosidade e aderência na parede da câmara de secagem e do ciclone via microencapsulação e, consequentemente, aumentando o rendimento do processo. O amido de araruta e goma arábica apresentou alta compatibilidade entre si e a polpa de amora, exibindo um efeito termoprotetor do ácido ascórbico e das antocianinas durante o processo de *spray drying*, confirmando sua adequação como agente encapsulante.

Em todos os ensaios experimentais, foi possível a formação da polpa de amora em pó microbiologicamente estável. Os pós exibiram notável cor rósea a avermelhada, bem como *flavour* típico da amora. A temperatura do ar de entrada e a concentração de agente encapsulante influenciaram significativamente as propriedades físico-químicas dos pós. O aumento da concentração do agente encapsulante resultou em pós com cores mais claras, com maiores teores de ácido ascórbico e capacidade antioxidante e menos higroscópicos. Pós de amora produzidos com altas temperaturas de ar de entrada se mostraram mais solúveis em água que os demais pós. A temperatura de 143 °C e a proporção de 1: 1,78 (sólidos de polpa de amora para agente encapsulante) foram consideradas como condições otimizadas do processo de secagem da polpa de amora por *spray drying*, uma vez que nesta temperatura e nesta concentração de agente encapsulante, os conteúdos de antocianinas e ácido ascórbico e a capacidade antioxidante e o rendimento do processo foram maiores quando comparados aos dos demais ensaios.

A mistura de amido de araruta e goma arábica também mostrou eficiência como agente encapsulante da polpa de amora pelo método de *freeze drying*. As imagens de MEV revelaram partículas esféricas nas estruturas vítreas presentes no pó da polpa de amora microencapsulada por *freeze drying*. Comparando-se o pó de amora microencapsulada por *spray drying* (MS) e por *freeze drying* (ML) com a polpa de amora sem encapsulante seca por *freeze drying* (BL), observou-se que os pós com agente encapsulante eram menos higroscópicos, apresentaram teores menores de água e tinham partículas com diâmetro médio menor. Quanto aos métodos de microencapsulação, *spray drying* e *freeze drying*, verificaram maiores rendimentos de processo quando se utilizou o método *freeze drying* para a secagem da polpa de amora. Além disso, partículas maiores foram obtidas pelo método *freeze drying* o que refletiu diretamente no tempo de molhabilidade dos pós. Os pós BL e ML obtidos por *freeze drying* apresentaram teores de antocianinas significativamente superiores aos do pó MS obtido por *spray drying*, e isso provavelmente se deve à utilização de baixas temperaturas no processo de secagem.

Em relação ao estudo em que o amido de araruta foi avaliado como matriz polimérica para produção de filmes comestíveis, a sua aplicabilidade para esse fim foi confirmada, pois todos os filmes preparados puderam ser retirados das placas de suporte sem rasgar e eram manipuláveis, transparentes, incolores e inodoros. A concentração de amido e de glicerol utilizada na elaboração do filme afetou significativamente a sua espessura e sua solubilidade em água. Quanto maior a concentração de amido e glicerol utilizada, mais espesso e solúvel o filme se mostrou. Já a solubilidade em água dos filmes foi fortemente influenciada pela concentração de glicerol. A concentração de amido de araruta e glicerol não influenciou significativamente a permeabilidade ao vapor de água e resistência a tração dos filmes. A menor permeabilidade ao vapor de água e a maior resistência à tração foram observadas para a formulação do ponto central, 4% (massa/massa) de amido e 17% de glicerol (massa/massa, em relação à massa de amido). Sendo assim, essa formulação foi selecionada para os ensaios posteriores, nos quais se estudou a incoporação da polpa de amora no filme.

Na investigação da incorporação de polpa de amora na solução filmogênica de amido de araruta para a produção de filmes comestíveis com propriedades antioxidantes, observou-se que essa estratégia é viável e promissora. A incorporação da amora na solução filmogênica transferiu ao filme de amido de araruta resultante, compostos bioativos e atividade antioxidante. O tipo de incorporação, direta ou por *sprinkling* de amora na solução filmogênica, influenciou na extração dos compostos bioativos, na capacidade antioxidante e solubilidade em água dos filmes. Em geral, filmes incorporados com amora por *sprinkling,* quase sempre apresentaram maiores valores de capacidade antioxidante e solubilidade em soluções aquosas, de diferentes condições de pHs em comparação com os filmes incorporados diretamente.

De maneira geral, a espessura, a solubilidade em água, a permeabilidade ao vapor de água e o alongamento à ruptura aumentaram significativamente ($p < 0,05$) com o aumento da concentração de polpa de amora (20, 30 e 40%) no filme, enquanto a resistência à tração diminuiu, quando comparado aos do filme de 0%. Essa tendência foi observada para todos os filmes incorporados com a amora, independentemente se na forma líquida ou na forma de pó, microencapsulada ou não, diretamente ou por *sprinkling*.

Houve um escurecimento dos filmes com polpa de amora após o processo de esterilização, possivelmente devido à caramelização dos açúcares, à reação de Maillard ou à degradação de antocianinas. Todos os filmes foram quase que totalmente desintegrados após 38 dias enterrados em compostagem orgânica, exibindo facilidade de biodesintegração.

O alto conteúdo de compostos bioativos, atividade antioxidante e propriedades funcionais dos filmes com amora sugerem sua utilização como revestimentos coloridos para alimentos específicos, como encapsulante e/ou embalagens ativas com capacidade de substituírem as embalagens de alimentos intermediárias não biodegradáveis, ou ainda, para serem consumidos na forma de tiras de frutas.

APÊNDICES

Apêndice A: Tabelas de efeitos e ANOVA do planejamento experimental da secagem de polpa de amora por *spray drying*

Nas Tabelas A.1 e A.2, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta rendimento de secagem, obtido no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 1 - Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta rendimento de secagem (%).

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Mean/Interc.	54,48	1,235759	44,0863	0,000514
(1)XT (L)	-1,3788	1,513489	-0,911	0,45845
XT (Q)	-19,3663	1,801413	-10,7506	0,008542
(2)XP (L)	8,6454	1,513489	5,7122	0,029307
XP (Q)	-1,5263	1,801413	-0,8473	0,486074
1L by 2L	6,805	2,140397	3,1793	0,086315

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 2-Análise de variância do modelo ajustado para a resposta rendimento de secagem.

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	702,55	2,00	351,28	13,67	4,46
Resíduo	205,53	8,00	25,69		
Falta ajuste	196,37	6,00	32,73	7,14	19,33
erro puro	9,16	2,00	4,58		
Total	908,08	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.3 e A.4, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta teor de água, obtido no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 3- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta teor de água dos pós (%).

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Mean/Interc.	2,10333	0,339624	6,19312	0,025095
(1)XT (L)	-1,53254	0,415953	-3,6844	0,066412
XT (Q)	1,96542	0,495083	3,96987	0,057988
(2)XP (L)	-0,69931	0,415953	-1,68123	0,234739
XP (Q)	-0,24458	0,495083	-0,49403	0,670214
1L by 2L	-1,055	0,588246	-1,79347	0,214759

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 4 - Análise de variância do modelo ajustado para a resposta teor de água dos pós (%).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	6,41	1,00	6,41	3,83	5,12
Resíduo	15,09	9,00	1,68		
Falta ajuste	14,40	7,00	2,06	5,94	19,35
erro puro	0,69	2,00	0,35		
Total	21,50	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.5 e A.6, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta atividade de água dos pós, obtida no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 5 - Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta atividade de água dos pós.

	Efeito Estimado	Erro Padrão	t(2)	P - valor
Mean/Interc.	0,190000	0,005774	32,9090	0,000922
(1)xT (L)	-0,090355	0,007071	-12,7782	0,006069
xT (Q)	0,098750	0,008416	11,7332	0,007186
(2)XP (L)	-0,016820	0,007071	-2,3787	0,140442
XP (Q)	0,003750	0,008416	0,4456	0,699499
1L by 2L	0,000000	0,010000	0,0000	1,000000

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 6- Análise de variância do modelo ajustado para a resposta atividade de água dos pós.

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	0,03	2,00	0,02	13,01	4,46
Resíduo	0,01	8,00	0,00		
Falta ajuste	0,01	6,00	0,00	15,59	19,33
erro puro	0,00	2,00	0,00		
Total	0,04	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.7 e A.8, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta higroscopicidade, obtida no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 7- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta higroscopicidade.

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Mean/Interc.	12,28333	0,262636	46,76938	0,000457
(1)xT (L)	1,65380	0,321662	5,14143	0,035810
xT (Q)	0,78292	0,382855	2,04494	0,177522
(2)XP (L)	-1,53675	0,321662	-4,77753	0,041128
XP (Q)	0,31792	0,382855	0,83038	0,493662
1L by 2L	-0,59000	0,454899	-1,29699	0,324098

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 8 - Análise de variância do modelo ajustado para a resposta higroscopicidade.

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	10,19	2,00	5,10	11,02	4,46
Resíduo	3,70	8,00	0,46		
Falta ajuste	3,29	6,00	0,55	2,65	19,33
erro puro	0,41	2,00	0,21		
Total	13,89	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.9 e A.10, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta solubilidade, obtida no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 9 - Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta solubilidade (%).

	Efeito Estimado	Erro Padrão	t(2)	P - valor
Mean/Interc.	61,47667	0,681404	90,22061	0,000123
(1)XT (L)	10,19333	0,834546	12,21423	0,006636
XT (Q)	10,99708	0,993308	11,07117	0,00806
(2)XP (L)	-2,95326	0,834546	-3,53877	0,071406
XP (Q)	1,07708	0,993308	1,08434	0,39153
1L by 2L	6,505	1,180226	5,51166	0,031377

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 10 - Análise de variância do modelo ajustado para a solubilidade (%).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	426,41	3,00	142,14	35,37	4,35
Resíduo	28,13	7,00	4,02		
Falta ajuste	25,34	5,00	5,07	3,64	19,3
erro puro	2,79	2,00	1,39		
Total	454,54	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.11 e A.12, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta *L**, obtido no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 11 - Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta *L**.

	Efeito Estimado	Erro Padrão	t(2)	P - valor
Mean/Interc.	51,24000	0,455082	112,5950	0,000079
(1)xT (L)	1,59555	0,557360	2,8627	0,103437
xT (Q)	-1,50875	0,663391	-2,2743	0,150792
(2)XP (L)	4,06941	0,557360	7,3012	0,018247
XP (Q)	-0,58375	0,663391	-0,8799	0,471701
1L by 2L	0,07000	0,788226	0,0888	0,937327

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 12 - Análise de variância do modelo ajustado para a resposta *L**.

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	33,12	1,00	33,12	12,76	5,12
Resíduo	23,36	9,00	2,60		
Falta ajuste	22,12	7,00	3,16	5,09	19,35
erro puro	1,24	2,00	0,62		
Total	56,48	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.13 e A.14, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta *b**, obtidas no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 13- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta *b**.

	Efeito Estimado	Erro Padrão	t(2)	P - valor
Mean/Interc.	2,353333	0,021858	107,6640	0,000086
(1)xT (L)	-0,146140	0,026771	-5,4590	0,031957
xT (Q)	0,034167	0,031863	1,0723	0,395816
(2)XP (L)	-0,837015	0,026771	-31,2662	0,001021
XP (Q)	-0,020833	0,031863	-0,6538	0,580350
1L by 2L	0,065000	0,037859	1,7169	0,228139

Valores em vermelho indicam parâmetro significativo a p < 0,05.

Tabela A 14 - Análise de variância do modelo ajustado para a resposta *b**.

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	1,44	2,00	0,72	405,00	4,46
Resíduo	0,01	8,00	0,00		
Falta ajuste	0,01	6,00	0,00	1,32	19,33
erro puro	0,00	2,00	0,00		
Total	1,46	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.15 e A.16, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta ácido ascórbico, obtidas no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 15- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para o ácido ascórbico.

	Efeito Estimado	Erro Padrão	t(2)	P - valor
Mean/Interc.	174,1667	3,005336	57,95248	0
(1)XT (L)	-4,0586	3,68077	-1,10266	0,3204
XT (Q)	3,3108	4,380992	0,75573	0,483874
(2)XP (L)	46,0165	3,68077	12,50186	0,000058
XP (Q)	-12,4442	4,380992	-2,84049	0,036226
1L by 2L	5,425	5,205395	1,04219	0,345071

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 16 - Análise de variância do modelo ajustado para a resposta ácido ascórbico.

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	4513,28	2,00	2256,64	84,62	4,46
Resíduo	213,33	8,00	26,67		
Falta ajuste	169,50	6,00	28,25	1,29	19,33
erro puro	43,83	2,00	21,92		
Total	4726,61	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.17 e A.18, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta antocininas, obtidas no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 17- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta teor de antocianinas.

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Mean/Interc.	83,7433	2,832967	29,56029	0,000001
(1)XT (L)	16,7188	3,469662	4,81857	0,004804
XT (Q)	7,0767	4,129724	1,71359	0,147273
(2)XP (L)	-15,8859	3,469662	-4,57852	0,005955
XP (Q)	12,2317	4,129724	2,96186	0,031451
1L by 2L	-10,585	4,906844	-2,15719	0,083483

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 18 - Análise de variância do modelo ajustado para a resposta teor de antocianinas.

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	1063,76	2,00	531,88	9,20	4,46
Resíduo	462,35	8,00	57,79		
Falta ajuste	452,96	6,00	75,49	16,08	19,33
erro puro	9,39	2,00	4,70		
Total	1526,12	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.19 e A.20, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta FRAP, obtido no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 19- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta capacidade antioxidante (FRAP).

	Efeito Estimado	Erro Padrão	t(2)	P - valor
Mean/Interc.	956,568	43,32038	22,08125	0,002045
(1)XT(L)	227,664	53,05642	4,29099	0,050252
XT(Q)	647,106	63,14977	10,24717	0,009389
(2)XP(L)	-66,947	53,05642	-1,2618	0,334246
XP(Q)	-185,283	63,14977	-2,93402	0,099183
1L by 2L	2,087	75,0331	0,02781	0,98034

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela A 20- Análise de variância do modelo ajustado para a resposta capacidade antioxidante (FRAP).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	760741	1	760741	14,01	5,12
Resíduo	488628	9	54292		
Falta ajuste	477368	7	68195,43	12,11	19,35
erro puro	11260	2	5630		
Total	1249369	10			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas A.21 e A.22, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta capacidade antioxidante (DPPH), obtido no planejamento experimental para a polpa de amora por *spray drying* utilizando mistura de amido de araruta e goma arábica como agente encapsulante.

Tabela A 21 - Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta capacidade antioxidante (DPPH).

	Efeito Estimado	Erro Padrão	t(2)	P - valor
Mean/Interc.	209,9123	14,10568	14,88141	0,000025
(1)XT(L)	19,6137	17,27586	1,13532	0,307714
XT(Q)	-50,4205	20,56238	-2,45207	0,057789
(2)XP(L)	-48,3538	17,27586	-2,79893	0,038042
XP(Q)	-62,0838	20,56238	-3,01929	0,02944
1L by 2L	-1,9012	24,43175	-0,07782	0,940992

Valores em vermelho indicam parâmetro significativo a p < 0,05.

Tabela A 22 - Análise de variância do modelo ajustado para a resposta capacidade antioxidante (DPPH).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	11716,17	3	3905,39	7,28	4,74
Resíduo	3757,56	7	536,7943		
Falta ajuste	2730,83	5	546,166	1,06	19,3
erro puro	1026,73	2	513,365		
Total	15473,73	10			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Apêndice B: Testes preliminares de produção filmes de amido de araruta

As imagens dos filmes produzidos nos testes preliminares para se determinar os limites mínimo e máximo da concentração de amido de araruta em solução a ser utilizada no planejamento experimental são apresentadas na Figura B.1.

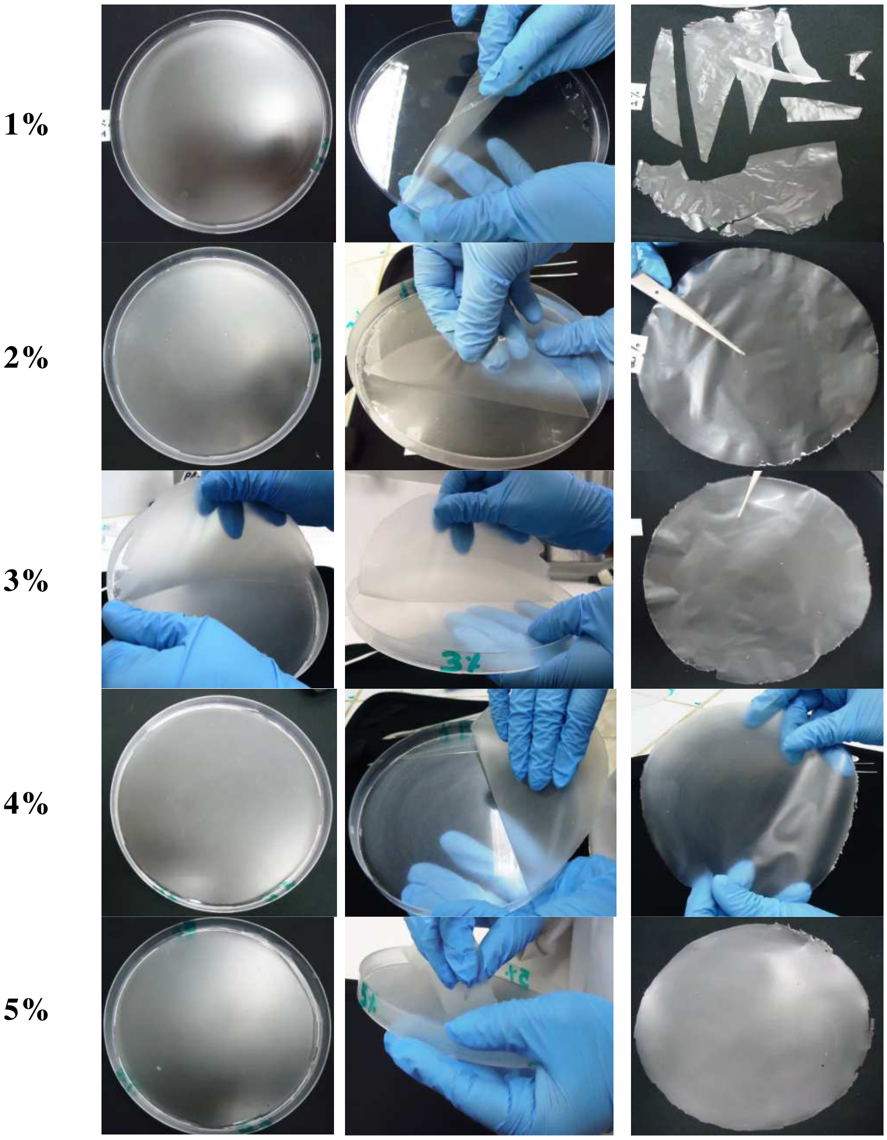

Figura B 1 Imagens dos filmes produzidos com as concentrações de 1, 2, 3, 4 e 5% amido de araruta em solução (m/m) e 10 % (massa/massa) de glicerol em relação à massa de amido nos testes preliminares.

O objetivo desse teste foi encontrar a concentração mínima de amido em solução que permitisse a formação do filme e seu desprendimento da placa de suporte. Além de determinar a concentração máxima que permitisse o escoamento da solução de amido na placa de suporte, visto que altas concentrações de amido tendem a ser muito viscosas.

Os filmes foram produzidos com 1, 2, 3, 4 e 5% (massa/massa) de amido de araruta em solução e 10% (massa/massa) de glicerol em relação à massa de amido. Como pode ser observado na Figura B.1, os filmes produzidos com 2, 3, 4 e 5% de amido desprenderam-se facilmente das placas de suporte, apresentando boa flexibilidade quanto ao manuseio, sem presença de partículas insolúveis, rupturas ou zonas quebradiças. Por outro lado, os filmes com 1% de amido não apresentaram facilidade de desprendimento da placa de suporte e acabaram-se rasgando na sua retirada.

Diante disso, o limite mínimo da concentração de amido escolhido foi de 2%, que permitiu a formação de filmes capazes de serem desprendidos do suporte sem rasgar. O limite máximo que permitiu o escoamento da solução no suporte, apesar da alta viscosidade foi de 5% de amido em solução.

Apêndice C: Tabelas de efeitos e ANOVA do planejamento experimental da produção de filmes de amido de araruta e glicerol

Nas Tabelas C.1 e C.2, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta, espessura obtidas no planejamento experimental para os filmes de amido de araruta (A) e glicerol (G).

Tabela C 1 - Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta espessura (µm).

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Média	0,072667	0,002056	35,33840	0,000000
(1)A (L)	0,036799	0,002518	14,61174	0,000027
A (Q)	-0,020292	0,002998	-6,76940	0,001069
(2)G (L)	0,005768	0,002518	2,29020	0,070632
G (Q)	-0,008792	0,002998	-2,93294	0,032522
Interação	-0,001000	0,003562	-0,28077	0,790134

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela C 2 - Análise de variância do modelo ajustado para a resposta espessura (µm).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	0,003368	4	0,000842	78,43	4,53
Resíduo	0,000064	6	0,000011		
Falta ajuste	0,000048	4	0,000012	1,43	19,25
erro puro	0,000017	2	0,000008		
Total	0,003433	10			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas C.3 e C.4, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta, solubilidade em água, obtidas no planejamento experimental para os filmes de amido de araruta e glicerol.

Tabela C 3- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta Solubilidade em água (%).

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Média	14,15667	0,431754	32,78877	0,000929
(1)A (L)	2,56225	0,528788	4,84552	0,040050
A (Q)	-2,20917	0,629384	-3,51005	0,072455
(2)G (L)	5,70230	0,528788	10,78372	0,008490
G (Q)	-2,66917	0,629384	-4,24092	0,051355
Interação	2,28000	0,747819	3,04887	0,092840

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela C 4- Análise de variância do modelo ajustado para a resposta solubilidade em água (%).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	78,16	2,00	39,08	8,49	4,46
Resíduo	36,83	8,00	4,60		
Falta ajuste	35,71	6,00	5,95	10,64	19,33
erro puro	1,12	2,00	0,56		
Total	114,99	10,00			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas C.5 e C.6, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta, Permeabilidade ao vapor de água, obtida no planejamento experimental para os filmes de amido de araruta e glicerol.

Tabela C 5- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta Permeabilidade ao vapor de água (g.mm/m^2.dia.kPa).

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Média	3,04667	0,089876	33,8984	0,000869
(1)A (L)	-2,09205	0,110076	-19,0056	0,002757
A (Q)	3,49708	0,131016	26,6920	0,001401
(2)G (L)	0,91003	0,110076	8,2673	0,014317
G (Q)	2,49708	0,131016	19,0593	0,002742
Interação	1,04500	0,155671	6,7129	0,021479

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela C 6- Análise de variância do modelo ajustado para a resposta Permeabilidade ao vapor de água (g.mm/m^2.dia.kPa).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	32,10	5	6,42	1,95	5,05
Resíduo	16,49	5	3,30		
Falta ajuste	16,45	3	5,48	226,22	19,16
erro puro	0,05	2	0,02		
Total	48,59	10			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

Nas Tabelas C.7 e C.8, estão apresentadas as estimativas dos efeitos lineares, quadráticos e das interações e a Análise de variância (ANOVA), respectivamente, para a resposta, Resistência à tração, obtida no planejamento experimental para os filmes de amido de araruta e glicerol.

Tabela C 7- Efeito estimado, erro puro, coeficiente t e grau de significância estatística, para a resposta Resistência a tração (MPa).

	Efeito Estimado	Erro Padrão	t(2)	P – valor
Média	24,13667	0,949778	25,41296	0,001545
(1)A (L)	1,01003	1,163235	0,86829	0,476774
A (Q)	-9,35667	1,384527	-6,75802	0,021202
(2)G (L)	-0,79969	1,163235	-0,68747	0,562805
G (Q)	-4,55667	1,384527	-3,29114	0,081232
Interação	-0,22000	1,645063	-0,13373	0,905856

Valores em vermelho indicam parâmetro significativo a $p < 0,05$.

Tabela C 8- Análise de variância do modelo ajustado para a resposta Resistência a tração (MPa).

Fonte de Variação	SQ	GL	MQ	Fcalculado	Ftabelado
Regressão	99,32	1	99,32	4,57	5,12
Resíduo	195,64	9	21,74		
Falta ajuste	190,23	7	27,18	10,04	19,35
erro puro	5,41	2	2,71		
Total	294,96	10			

SQ: Soma quadrática; GL: Grau de liberdade; QM: Quadrado médio.

ANEXOS

ANEXO 1

Autorização da revista Carbohydrate Polymers para uso do artigo intitulado “Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films”

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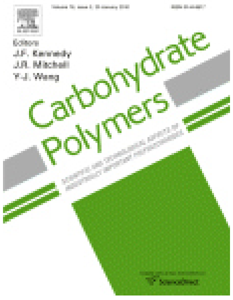

Title: Extraction and characterization of arrowroot (Maranta arundinaceae L.) starch and its application in edible films

Author: Gislaine Ferreira Nogueira,Farayde Matta Fakhouri,Rafael Augustus de Oliveira

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