MÁRCIA CAVALCANTE CONCEIÇÃO

OTIMIZAÇÃO DO PROCESSO DE EXTRAÇÃO E CARACTERIZAÇÃO DA MUCILAGEM DE ORA-PRO-NÓBIS (*Pereskia aculeata* Miller)

LAVRAS - MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, para a obtenção do título de Doutor.

Orientador

Dr. Jaime Vilela de Resende

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Ficha Catalográfica Elaborada pela Coordenadoria de Produtos e Serviços
da Biblioteca Universitária da UFLA

Conceição, Márcia Cavalcante.
Otimização do processo de extração e caracterização da mucilagem de ora-pro-nobis (*Pereskia aculeata* Miller) / Márcia Cavalcante Conceição. – Lavras : UFLA, 2013.
121 p. : il.

Tese (doutorado) – Universidade Federal de Lavras, 2013.
Orientador: Jaime Vilela de Resende.
Bibliografia.

1. Cactáceas. 2. Processamento de alimentos. 3. Hidrocolóides. 4. Ora-pro-nobis - Goma. 5. Alimentos - Aditivos. I. Universidade Federal de Lavras. II. Título.

CDD – 664.8054

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APROVADA em 23 de agosto de 2013.

Dr. Eduardo Valério de Barros Vilas Boas	UFLA
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Aos meus amados pais, Mário e Diná, pelo amor e apoio incondicional, pelo incentivo nos momentos difíceis, fornecendo-me tudo o que puderam para que eu pudesse alcançar meus objetivos. Por essa razão, gostaria de dedicar e reconhecer à vocês, minha imensa gratidão e amor.
Ao meu noivo, André, pelo amor, dedicação e compreensão durante todos esses anos de união.

DEDICO

AGRADECIMENTOS

Agradeço a Deus por ter tornado possível este momento, por me dar força durante todo este processo e não me deixar desistir perante as dificuldades.

À minha família, em especial meus pais, Mário e Diná, pelo amor incondicional, por acreditarem em mim e torcerem pelo meu sucesso. Às minhas irmãs, Aline e Adriana, mesmo distante, obrigada pela amizade e apoio.

Ao meu noivo, André Labegalini, meu companheiro de todas as horas, sempre me incentivando e acreditando em meus sonhos. Obrigada por todo amor, pela paciência e compreensão e principalmente, por me manter viva. Agradeço também a toda família Labegalini que me acolheu de forma tão carinhosa e especial. Obrigada por fazer parte dessa linda e grande família.

À Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo suporte financeiro para essa pesquisa.

À Universidade Federal de Lavras e ao Departamento de Ciência dos Alimentos, pela oportunidade concedida para realização do doutorado.

Aos professores da Universidade Federal de Lavras, em especial do Departamento de Ciência dos Alimentos e do Programa de Pós-Graduação em Ciência dos Alimentos, pelos ensinamentos, amizade e convívio.

Aos funcionários do Departamento de Ciência dos Alimentos, Tales, Creusa, Tina, Cidinha, Lucilene, Adriana e demais funcionários que direta ou indiretamente contribuíram para o bom andamento deste trabalho.

Ao meu orientador, Dr. Jaime Vilela de Resende, pelos ensinamentos, paciência, dedicação, amizade e pela compreensão, principalmente nos momentos difíceis na conclusão do doutorado. Obrigada por tudo!

Aos professores Dr. Eduardo Valério de Barros Vilas Boas, à Dra. Lanamar de Almeida Carlos, ao Dr. Luiz Ronaldo de Abreu e à Dra. Monica Elisabeth Torres Prado, pela participação da banca e pela colaboração para a melhoria e a qualidade deste trabalho.

Aos amigos de longe ou de perto, aos antigos e os mais recentes que me acompanharam nesta etapa, pela contribuição, ajuda e amizade, em especial aos colegas do laboratório de Refrigeração de Alimentos (LARA), Tânya, Cristina, Natália, Isis, Tales, Tatiane e às alunas de iniciação científica Luciana, Karen e Marina, vocês foram fundamentais para realização deste trabalho.

Ao professor Eduardo Alves e à aluna Olívia, do Departamento de Fitopatologia, pelo auxílio nas análises de microscopia eletrônica de varredura/espectroscopia de energia dispersiva de raio-x.

Ao Laboratório Multiusuário de Biomateriais do Departamento de Engenharia Florestal, na pessoa do aluno Thiago, que auxiliou na realização das análises de termogravimetria.

E a todos que direta ou indiretamente contribuíram para a conclusão deste trabalho. O meu eterno muito obrigada!

“A melhor de todas as coisas é aprender. O dinheiro pode ser perdido ou roubado, a saúde e a força podem falhar, mas o que você dedicou à sua mente é seu pra sempre.”

Louis L'amour

RESUMO GERAL

A mucilagem é um biopolímero de alto peso molecular que apresenta a capacidade de formar gel ou solução viscosa, e pode ser utilizada como modificadora de textura, agente gelificante, espessante, estabilizante e emulsionante na indústria de alimentos. Com o aumento da demanda por mucilagens, o mercado por novas fontes tornou-se promissor e as espécies de plantas nativas constituem uma alternativa para a produção de mucilagens específicas, por exemplo, podemos citar as folhas de *Pereskia aculeata* Miller, popularmente conhecida no Brasil como Ora-pró-nobis (OPN), que constitui material rico em mucilagem. Neste trabalho, a otimização do processo de extração de mucilagem das folhas do OPN foi desenvolvido. As variáveis independentes, avaliadas para determinar as condições ótimas de extração, foram a proporção de água: matéria prima e a temperatura de extração. Os resultados foram analisados utilizando o método de superfície de resposta. Usando-se a condição do processo otimizado, mucilagens foram preparadas e composição centesimal, conteúdo mineral, calorimetria diferencial de varredura (DSC), termogravimetria (TG), microestrutura eletrônica de varredura, espectroscopia de energia dispersiva de raios-x e capacidade de formação de emulsão por microscopia ótica foram analisados. A estabilidade dessas emulsões foi avaliada à temperatura ambiente e a 80 °C. As condições otimizadas foram uma proporção de água: matéria-prima de 2,46 e 3,70 $L.kg^{-1}$ e uma temperatura de extração entre 54,6 e 80 ºC. O produto otimizado obteve alto teor de proteína e minerais, baixo conteúdo de ácidos urônicos e carboidrato total. O espectro de infravermelho sugeriu que o produto obtido seja uma arabinogalactana-proteína (AGP). Os perfis de DSC apresentaram eventos endotérmicos e exotérmicos, altas temperaturas de transição vítrea (Tg) que sugerem estabilidade do produto. As curvas TG apresentaram alto teor de resíduo. As micrografias da mucilagem de OPN em pó apresentam uma alta porosidade, caracterizando um material higroscópico. A microscopia eletrônica de varredura/espectroscopia de energia dispersiva de raios-x confirmou que grandes quantidades de minerais estão presentes na amostra. As emulsões preparadas a 80 °C apresentaram maior estabilidade. Dessa forma, mucilagem das folhas do OPN, no processo otimizado, apresentou funcionalidades como aditivos alimentícios que podem ser utilizadas na indústria.

Palavras-chave: Cactus. Hidrocolóide. Goma. Aditivo. Processamento.

GENERAL ABSTRACT

Mucilage is a biopolymer of high molecular weight, which presents the capacity of forming a gel or viscous solution and which may be used as texture modifier, gelling agent, thickener, stabilizer and emulsifier in the food industry. With the increase in the demand for mucilage, the market has become promising for new sources and the native plant species constitute an alternative for the production of specific mucilage, for example, we may cite the *Pereskia acuteata* Miller leaves, commonly known in Brazil as Ora-pró-nobis (OPN), which is a material rich in mucilage. In this work, we developed an optimized process of mucilage extraction from the OPN leaves. The independent variables evaluated in order to determine the optimum extraction conditions were the proportion of water: raw materials and the extraction temperature. The results were analyzed using the response surface method. Using the optimized process condition, we prepared mucilage and analyzed the centesimal composition, mineral content, differential scanning calorimetry (DSC), thermogravimetry (TG), scanning electronic microstructure (SEM), spectroscopy of dispersive energy by x-rays and emulsion capacity by optic microscopy. The stability of these emulsions was evaluated at ambient temperature and at 80 ^{o}C. The optimized conditions were a proportion of 2.46 and 3.70 L.kg^{-1} water: raw material and an extraction temperature between 54.6 and 80 ^{o}C. The optimized product obtained a high protein and mineral content, low uronic acids and total carbohydrate content. The infrared spectrum suggested that the obtained product is an arabinogalactan protein (AGP). The DSC profiles presented endothermic and exothermic events, high glass transition (Tg) temperatures which suggests the stability of the product. The Tg curves presented high residue content. The micrographs of powder OPN mucilage presented high porosity, characterizing a hygroscopic material. The scanning electronic microstructure/ spectroscopy of dispersive energy by x-rays confirmed that large amounts of minerals are present in the sample. The capacity for emulsion formation of the product and high droplet coalescence was verified as being proportional to the reduction of powder gum concentration. The emulsions prepared at 80 ^{o}C presented higher stability. Thus, in an optimized process, OPN leaf mucilage presented functionality as food additives which may be used in the industry.

Keywords: Cactus. Hydrocolloid. Gum. Additive. Processing.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

As mucilagens são conhecidas como gomas, hidrocolóides ou polissacarídeos solúveis em água e, em alguns casos, são constituídas por proteínas. Biopolímeros de grande peso molecular podem ser encontradas em organismos de origem microbiana, animal ou vegetal, possuindo grande importância e destaque, pois apresentam uma imagem favorável diante dos consumidores, que buscam cada vez mais por produtos naturais, que proporcionem benefícios à sua saúde.

Nos vegetais, as mucilagens são obtidas de sementes, folhas, frutos ou exsudatos de plantas. Apresentam grande afinidade com a água, podendo formar géis ou soluções viscosas em sua presença, dessa forma, são utilizadas dentro da indústria de alimentos e em outros ramos, como modificadores de textura, estabilizantes, emulsificantes e espessantes.

O Brasil demanda uma grande quantidade de mucilagens em diferentes segmentos industriais, porém o país não produz o suficiente para atendê-los, portanto, é um grande importador de mucilagens. Como consequência, o consumidor paga por produtos mais caros, dificultando o acesso a certos produtos. Uma forma de contornar esse problema seria explorar a biodiversidade que o Brasil oferece, onde diversas plantas nativas podem constituir-se em novas fontes de mucilagens, com a vantagem de oferecer produtos naturais, de qualidade e baixo custo, além de atender às necessidades dos consumidores e empresas.

O uso de cactáceas vem se destacando por oferecer inúmeras vantagens e benefícios em sua aplicabilidade. Dentre elas, podemos destacar a *Pereskia aculeata* Miller, mais conhecida por ora-pro-nobis. Essa pode ser considerada

como fonte de fibras, vitaminas, destacando-se a vitamina C, minerais, como ferro e cálcio e também aminoácidos essenciais como a lisina, podendo suprir a ingestão diária recomendada. Além disso, apresenta alguns carotenoides e, principalmente, produz uma mucilagem constituída por arabinogalactanas, sendo obtida principalmente a partir das folhas.

As folhas dessa espécie são comestíveis e utilizadas na culinária regional no estado de Minas Gerais, sendo uma fonte de nutrientes para as populações de baixo poder aquisitivo. É considerada uma hortaliça não convencional por não possuir um cultivo difundido, sendo esquecida pela grande parte da população devido à falta de informações sobre sua rica composição e modo de preparo. Ainda são usadas em ornamentações de jardins ou como cercas vivas e também na medicina popular.

Em vista da grande importância da utilização dos aditivos na indústria de alimentos, relacionada aos aspectos econômicos do processo e aliada à necessidade de novas fontes de mucilagens e também à escassez de dados para produtos específicos. Objetivou-se, no presente trabalho, otimizar o processo de extração da mucilagem, a partir da folhas da *Pereskia aculeata* Miller através metodologia de superfície de resposta, analisar a composição química, propriedades térmicas e microestrutura das mucilagens no produto em pó, gel reconstituído e emulsões da *Pereskia aculeata* Miller (OPN) e avaliar o uso potencial do produto em pó, como agente emulsificante e estabilizante em aplicações alimentares.

2 REFERENCIAL TEÓRICO

2.1 Mucilagem

Na literatura são encontradas diversas designações para o termo mucilagem, como gomas, colóides hidrofílicos (ou hidrocolóides) ou ainda polissacarídeos solúveis em água (JAHANBIN et al., 2012).

As mucilagens alimentícias são biopolímeros hidrofílicos de alto peso molecular (principalmente polissacáridos e proteínas), usadas como ingredientes funcionais na indústria de alimentos para controle da microestrutura, textura, sabor e vida de prateleira. São extraídas de plantas, algas e fontes microbianas, assim como todas as gomas derivadas de exsudatos de plantas (FARAHNAKY et al., 2013; PRAJAPATI et al., 2013) e biopolímeros modificados pelos tratamentos químicos ou enzimáticos do amido e celulose (DICKINSON, 2003), e ainda de animais (tais como gelatina) (FARAHNAKY et al., 2013; PRAJAPATI et al., 2013). As mucilagens de vegetais têm a vantagem sobre aquelas de animais por causa de sua imagem favorável para os consumidores (VARDHANABHUTI; IKEDA, 2006), além de fornecerem maiores quantidades de mucilagem (PRAJAPATI et al., 2013).

Na indústria de alimentos, as mucilagens possuem grande aplicabilidade devido a sua capacidade, para formar gel ou soluções viscosas ou ainda estabilizar sistemas de emulsão (CEVOLI et al., 2013; MIRHOSSEINI; AMID, 2012). São utilizadas como fibra dietética, modificadores de textura, agentes gelificantes, espessantes, estabilizantes e emulsionantes, agentes de revestimento e de filmes de embalagem (CEVOLI et al., 2013; FARAHNAKY et al., 2013; LAI, LIANG, 2012; MIRHOSSEINI; AMID, 2012; MUÑOZ et al., 2012; PRAJAPATI et al., 2013; VARDHANABHUTI; IKEDA, 2006). Além disso,

são utilizados como controladores de sinérese (FARAHNAKY et al., 2013; MUÑOZ et al., 2012), e controladores da cristalização de gelo e açúcar (CEVOLI et al., 2013; FARAHNAKY et al., 2013).

As mucilagens aumentam a viscosidade do meio, mesmo em baixas concentrações, logo, essa propriedade permite que elas sejam o principal ingrediente em alimentos líquidos ou semissólidos. Geralmente, a viscosidade das soluções de mucilagem é influenciada por diversos parâmetros, tais como taxa de cisalhamento, concentração da mucilagem, temperatura, pH, força iônica e sais (FARAHNAKY et al., 2013). A seleção da mucilagem adequada para cada sistema alimentício depende das funções da mucilagem e das propriedades desejáveis nos alimentos. Além disso, seu preço e segurança são importantes (VARDHANABHUTI; IKEDA, 2006).

O comportamento das mucilagens influencia nas propriedades sensoriais dos alimentos, e, portanto, são utilizadas como aditivos alimentares importantes para realizar propósitos específicos. Esses ingredientes funcionais são amplamente utilizados em produtos lácteos e de panificação, alimentos enlatados, molhos para saladas, bebidas, sopas e outros alimentos processados para melhorar características de textura, sabor e vida de prateleira (CEVOLI et al., 2013).

A crescente demanda por mucilagens impulsiona a pesquisa por novas fontes que sejam econômicas e apresentem funcionalidades específicas (FARAHNAKY et al., 2013; NAJI; RAZAVI; KARAZHIYAN, 2012; RAZAVI; TAHERI; QUINCHIA, 2011), sendo necessário conhecer suas propriedades e características para melhor direcionar a aplicação desses aditivos naturais, podendo ser útil para projeto de processo e desenvolvimento de produto (MAURER; JUNGHANS; VILGIS, 2012).

2.2 Fontes de mucilagens

As mucilagens são amplamente encontradas na natureza podendo ser de origem microbiana, animal e vegetal. A composição de cada mucilagem é diferente, tendo em sua composição diferentes tipos de polissacarídeos que podem lhes conferir diversas funções (RENARD et al., 2012).

2.2.1 Mucilagens de origem microbiana

Os exopolissacarídeos (EPS) microbianos, também chamados de biopolímeros, são produzidos durante o crescimento de vários gêneros de bactérias. Apresentam grandes aplicações em produtos farmacêuticos, alimentícios, químicos e petroquímicos por causa de suas peculiares propriedades físicas e reológicas.

Ressalta-se que os polissacarídeos extraídos de plantas e algas ainda dominam o mercado de gomas devido ao baixo custo de produção, já os exopolissacarídeos ainda representam uma pequena fração do atual mercado de biopolímeros. Os principais fatores limitantes para a utilização de polissacarídeos estão associados ao seu custo de produção, porém possuem a vantagem de utilizar subprodutos ou resíduos agroindustriais como matéria-prima.

Dentre as mucilagens de origem microbiana podemos citar a goma xantana que é um polissacarídeo extracelular de elevado peso molecular produzido por fermentação pela bactéria *Xanthomonas campestres* (CHARCHOGHLYAN; PARK, 2013; FITZPATRICK et al., 2013; HEYMAN et al., 2013; XU et al., 2013). A goma dextrana produzida pela bactéria *Leuconostoc mesenteroides* (CHARCHOGHLYAN; PARK, 2013). A pupulana é polímero obtido a partir da fermentação por meio da levedura *Aureobasidium*

pullulans (PRAJAPATI; JANI; KHANDA, 2013). A goma gelana é um heteropolissacárido extracelular aniónico secretada pela bactéria *Sphingomonas elodea* (ROSAS-FLORES; RAMOS-RAMÍREZ; SALAZAR-MONTOYA, 2013).

2.2.2 Mucilagens de origem animal

A quitina é o polissacarídeo linear mais abundante (depois da celulose), encontrada naturalmente em exoesqueleto de crustáceos (caranguejo e cascas de camarão), insetos e fungos (*Rhizopus, Absidia, e Fusarium*) (KUMAR, 2000; NAIM et al., 2013; SATO et al., 2010). A partir da reação de desacetilação da quitina obtém-se a quitosana, que consiste num polissacarídeo catiônico de elevado peso molecular. Industrialmente é produzida por desacetilação química da quitina, utilizando uma base forte (GAO; ZHUB; ZHANG, 2013; SATO et al., 2010). Possui inúmeras aplicações nas áreas de agricultura e alimentos devido à sua excelente capacidade de formar filme, às suas atividades antimicrobianas e antifúngicas, biocompatibilidade, biodegradabilidade e não toxicidade para as pessoas (GAO; ZHUB; ZHANG, 2013).

A gelatina é uma proteína solúvel obtida da hidrólise parcial do colágeno, a principal proteína fibrosa constituinte em cartilagens, ossos, peles. Entretanto, a fonte, a idade do animal, e tipo de colágeno, são todos fatores intrínsecos influenciando as propriedades das gelatinas (GÓMEZ-GUILLÉN et al., 2011).

2.2.3 Mucilagens de origem vegetal

Várias partes da planta (por exemplo, frutas, sementes, folhas, tubérculos/raízes) assim como exsudatos de árvores, têm células superficiais

contendo gomas, mucilagens e compostos de fibras e proteínas (RANA et al., 2011). Do ponto de vista químico, eles são polissacarídeos (que constitui maior parte) ou proteínas (tal como gelatina).

Diversas espécies de plantas produzem exsudatos a partir do seu caule, em decorrência dos mecanismos de proteção contra danos mecânicos ou microbianos (MIRHOSSEINI; AMID, 2012). Há um grande número de espécies de plantas que estão a ser cultivadas e que são capazes de produzir gomas que podem ser implementadas na indústria alimentar como aditivos.

A maior parte das gomas de exsudatos de plantas pertence à família *Leguminosae* tais como *Acacia Senegal*, como uma fonte de goma arábica (NIE et al., 2013); *Astragalo spp*, como fonte de tragacanto; *Cyamopsis tetragonolobus*, como uma fonte de goma guar; *Ceratonia siliqua*, como uma fonte de goma de alfarroba (IBANEZ; FERRERO, 2003; MIRHOSSEINI; AMID, 2012); Sterculia urens, como fonte da goma karaya; *Anogeissus latifolia*, como fonte da goma ghatti (DESHMUKH et al., 2012).

Alguns frutos também são conhecidos por conterem quantidade notável de diversos compostos no que diz respeito ao nível de carboidratos, isso depende do fruto, da sua maturação e do período de tempo de armazenamento.

Atualmente, as pectinas comerciais vêm de casca de frutas cítricas e bagaço de maçã (MESBAHI; JAMALIAN; FARAHNAKY, 2005; YAPO, 2011). A crescente demanda industrial por pectinas, com diferentes capacidades de formar gel ou estabilizar produtos, intensificou a necessidade de diferentes tipos de pectinas ou derivados com propriedades predefinidas no mercado (VRIESMANN; TEÓFILO; PETKOWICZ, 2012).

Quimicamente, os polímeros de ácido D-galacturônico unidos por meio de ligações glicosídicas α-1,4 constituem o principal componente de materiais de pectina (CHAN; CHOO, 2013; JINDAL et al., 2013; NGOUÉMAZONG et al., 2012). Alguns dos grupos carboxílicos das moléculas do ácido galacturônico nas

cadeias de pectina são metil esterificados e a percentagem de grupos esterificados é expressa como DE (grau de esterificação). Dependendo do DE, as pectinas são divididas em dois grupos principais: pectina de alta metoxilação, com um DE superior a 50%, e pectina de baixo teor de metoxilação, com um DE inferior a 50% (CHAN; CHOO, 2013; JINDAL et al., 2013; MESBAHI; JAMALIAN; FARAHNAKY, 2005; NGOUÉMAZONG et al., 2012).

Diferentes pectinas podem ter diferentes cadeias laterais de arabinose, galactana, arabinogalactana, glicose, manose e xilose. Nos alimentos, a pectina é usada principalmente em doces e geléias como um agente de gelificação e espessante. Também é utilizado em bebidas, molhos, xaropes e outros alimentos para se obter uma textura desejável (JINDAL et al., 2013; MESBAHI; JAMALIAN; FARAHNAKY, 2005).

Grãos de cereais, sementes de leguminosas, tubérculos e certas frutas contêm de 30 a 85% de amido numa base de peso seco. Os amidos comerciais são obtidos principalmente a partir de milho amarelo, embora batata, trigo, arroz e sorgo também sejam fontes significativas. O amido é o principal polissacarídeo de reserva de muitas plantas e constitui um polímero de baixo custo, ocorrendo na forma de grânulos. Devido a sua espessura e propriedades de gelificação, é utilizado na indústria de alimentos (VRIESMANN; SILVEIRA; PETKOWICZ, 2009).

O amido consiste numa mistura de dois polissacarídeos: amilose e amilopectina (MIRHOSSEINI; AMID, 2012). A amilose é um polissacarídeo com cadeia linear de D-glucose, enquanto a amilopectina é um polímero ramificado, também, de D-glucose (VRIESMANN; SILVEIRA; PETKOWICZ, 2009).

Galactomanana é conhecido como um polissacarídeo linear que constitui a reserva de energia em endospermas de sementes de plantas leguminosas. Elas são mucilagens altamente solúveis proporcionando soluções aquosas viscosas e

estáveis. Elas apresentam diferentes propriedades físico-químicas e reológicas, dependendo da proporção de manose /galactose (M / G) (MIRHOSSEINI; AMID, 2012). As galactomananas são extraídas principalmente a partir do endosperma das sementes das Leguminosas para fins comerciais, por exemplo, a goma guar (*Cyamopsis tetragonolobus*), goma alfarroba (*Ceratonia siliqua*) e goma tara (*Caesalpinia spinosa*).

As algas comestíveis basicamente contêm elevadas proporções de polissacarídeos, juntamente com vários outros compostos potencialmente benéficos, tais como a proteína de boa qualidade, ácidos graxos insaturados essenciais, altas concentrações de vitaminas, compostos bioativos com conhecidas propriedades antioxidantes, e são excelente fonte de minerais e fibras alimentares (FERNÁNDEZ-MARTÍN et al., 2009; LÓPEZ-LÓPEZ; COFRADES; JIMÉNEZ-COLMENERO, 2009; LÓPEZ-LÓPEZ et al., 2009). São utilizadas como matéria-prima para a produção industrial de alguns ingredientes purificados (agar, carragena, alginatos) utilizados no processamento de alimentos (LÓPEZ-LÓPEZ et al., 2009).

Dentre as algas marinhas, as vermelhas e as marrons são aquelas a partir das quais são extraídos os polissacarídeos mais utilizados na indústria (VARELA; FISZMAN, 2011). Das algas vermelhas são obtidas as carragenanas- este é o nome genérico para uma família de polissacarídeos obtidos por extração a partir de certas espécies de algas vermelhas (*Rhodophyta*). São obtidos a partir de diferentes espécies de *Rhodophyta: Gigartina, Chondrus crispus, Euchema e Hypnea* (CAMPO et al., 2009).

Os alginatos, polissacarídeos aniônicos mais abundantes, são produzidos a partir de duas fontes, as algas marrons e bactérias (DRAGET; TAYLOR, 2011; FERNÁNDEZ-MARTÍN et al., 2009; GOH; HENG; CHAN, 2012). Eles são extraídos de espécies de algas marrons como: *Macrocystis pyrifera,*

Laminaria hyperborea, Laminaria digitata, Laminaria japonica e Ascophyllum nodosum (DRAGET; TAYLOR, 2011; GOH; HENG; CHAN, 2012).

Assim, as mucilagens apresentam ampla distribuição entre os vegetais, possuindo uma vantagem quando comparadas às de origem animal, já que o consumidor possui maior preferência e aceitabilidade por produtos naturais.

O Brasil, por ser um grande importador de mucilagens, o mercado brasileiro de novas fontes de mucilagens torna-se bastante interessante, pois plantas nativas pouco exploradas podem oferecer um produto natural, de qualidade e baixo custo, além de atender às necessidades das empresas. Um exemplo disso é a *Pereskia aculeata* Miller, mais conhecida como ora-pro-nóbis, que apresenta alto teor de mucilagem, sendo também uma fonte de nutrientes. Essa espécie é amplamente utilizada na culinária regional do estado de Minas Gerais, como planta ornamental ou na medicina popular. Diante disso, essa cactácea merece maiores estudos para difundir sua aplicação na indústria de alimentos e em outros ramos industriais.

2.3 *Pereskia Aculeata* Miller (Ora-pro-nóbis)

Entre as inúmeras famílias de plantas encontradas na flora brasileira, as cactáceas, chamam atenção pela sua rusticidade e beleza (DUARTE; HAYASHI, 2005). A família Cactaceae compreende 127 gêneros e 1.438 espécies, divididas em quatro subfamílias: Cactoideae, Maihuenioideae, Opuntioideae e Pereskiodeae (CALVENTE et al., 2011). Dessas, a última é considerada a menos evoluída (DUARTE; HAYASHI, 2005; FARAGO et al., 2004; TURRA et al., 2007).

O gênero Pereskia é considerado o menos avançado da família, com cerca de 25 espécies de cactos folheares, distribuídos em várias regiões do mundo (TURRA et al., 2007). 17 espécies, desse gênero, pertencem à subfamília

Pereskioideae (EDWARDS; NYFELER; DONOGHUE, 2005). Algumas espécies são utilizadas na medicina e culinária popular e apresentam alto valor nutricional (DUARTE; HAYASHI, 2005).

Entre as espécies podemos destacar a *Pereskia aculeata* Miller, também conhecida como ora-pro-nóbis, trepadeira-limão, groselha-de-barbados (DUARTE; HAYASHI, 2005; MARSARO-JÚNIOR et al., 2011), groselha-da-américa (AGOSTINE-COSTA et al., 2012; ROCHA et al., 2008; ROSA; SOUZA, 2003), lobrobô (ROCHA et al., 2008), carne-de-pobre, carne-de-negro (BRASIL, 2010; MARTINEVSKI et al., 2013).

A origem do seu nome surgiu por pessoas que colhiam a planta no quintal de um padre, enquanto ele rezava: ora pro nóbis. O nome científico é uma homenagem ao botânico francês do século 16, Nicolas Claude Fabri de Pereisc.

A *Pereskia aculeata* Miller (ora-pro-nóbis) é um cacto nativo que pode ser encontrado em trópicos americanos, como a região sul dos Estados Unidos (Florida) (BRASIL, 2010; MARTINEVSKI et al., 2013; TAKEITI et al., 2009) e no Brasil (BRASIL, 2010; MARTINEVSKI et al., 2013). Nesse, é amplamente distribuída entre os estados da Bahia e Rio Grande do Sul. (AGOSTINE-COSTA et al., 2012; DUARTE; HAYSASHI, 2005; MAZIA; SATOR, 2012; ROSA; SOUZA, 2003; TAKEITI et al., 2009; TOFANELLI; RESENDE, 2011).

Esta espécie é considerada uma erva daninha ambiental em alguns países, como África do Sul (AGOSTINE-COSTA et al., 2012; PATERSON; DOWNIE; HILL, 2009). De acordo com Duarte e Haysashi (2005), a *Pereskia aculeata* Miller ocorre em terras áridas ou levemente áridas. Almeida-Filho e Cambraia (1974) relatam que ela é nativa da América Tropical, além de ser largamente encontrada na Índia Oriental. Já Marsaro-Júnior et al. (2011) relatam que a cactácea em questão é nativa do Brasil e distribuída em todo o Nordeste, Centro-Centro-Oeste, Sudeste e Sul do país.

O ora-pro-nóbis, que no latim significa “rogai por nós”, é uma trepadeira arbustiva considerada detentora do maior número de caracteres primitivos da família Cactaceae (DUARTE; HAYASHI, 2005; ROSA; SOUZA, 2003; SATOR et al., 2010). Ela pode atingir 10 m de altura e apresenta caule fino, com ramos longos sublenhosos ou lenhosos, nos quais se inserem folhas lisas, largas, suculentas e de cor verde escuro com muitos espinhos. No final dos ramos, podem surgir flores terminais solitárias ou em cimeiras curtas (DUARTE; HAYSASHI, 2005; MARSARO-JÚNIOR et al., 2011), pequenas e de coloração branca (BRASIL, 2010; MARTINEVSKI et al., 2013), os frutos são esféricos do tipo baga de coloração amarela quando maduros (BRASIL, 2010; MARSARO-JÚNIOR et al., 2011; MARTINEVSKI et al., 2013), apresentam presença de mucilagem (“baba”) na planta (ALBUQUERQUE; SABAA-SRUR; FREIMAN, 1991; MERCÊ et al., 2001a, 2001b; TOFANELLI; RESENDE, 2011). Possui taxa de crescimento moderado (MARSARO-JÚNIOR et al., 2011) e caracteriza-se por um desenvolvimento vegetativo, durante o ano inteiro (ALMEIDA FILHO; CAMBRAIA, 1974). O maior índice de consumo está localizado nas antigas regiões mineradoras do estado de Minas Gerais (ALBUQUERQUE; SABAA-SRUR; FREIMAN, 1991; DIAS et al., 2005).

Esta cactácea tem grande importância ornamental, alimentícia e medicinal. A planta pode ser cultivada para fins de produção de mel pelos apicultores, pois apresenta floração rica em pólen e néctar. A floração ocorre nos meses de janeiro a abril (FARAGO et al., 2004).

Na medicina, a grande vantagem da planta é no abrandamento dos processos inflamatórios e na recuperação da pele, em casos de queimadura. As folhas são usadas popularmente como emolientes; os frutos, como expectorante e antissifilítico (DUARTE; HAYASHI, 2005; ROSA; SOUZA, 2003; SATOR et al., 2010).

As folhas, por apresentarem alto teor de proteínas e fibras (KAZAMA et al., 2012), juntamente com a ausência de toxicidade das mesmas (AGOSTINE-COSTA et al., 2012; MERCE et al., 2001a, 2001b; ROSA; SOUZA, 2003) e presença significativa de ferro e cálcio (KAZAMA et al., 2012; ROCHA et al., 2008), podem ser usadas como importante alimento. Adicionalmente, são consumidas na culinária regional brasileira, levando indústrias alimentícias a incluí-las em complementos alimentares, devido ao alto teor do biopolímero arabinogalactana (DUARTE; HAYASHI, 2005; FARAGO et al., 2004; MERCÊ et al., 2001a, 2001b). Em virtude da produção dessa mucilagem, possui excelente perspectiva como um aditivo não apenas para a indústria alimentar, mas também para outros usos industriais (KAZAMA et al., 2012; KIM et al., 2013).

Esta hortaliça possui folhas suculentas e comestíveis, podendo ser usada em várias preparações, como farinhas, saladas, refogados, tortas e massas alimentícias como o macarrão (ROCHA et al., 2008), além do preparo de pratos típicos do estado brasileiro de Minas Gerais (MARSARO-JÚNIOR et al., 2011). Embora tenha um alto potencial de utilização, ela ainda é cultivada e distribuída de forma limitada, restrita a determinadas localidades ou regiões, exercendo grande influência na alimentação e na cultura de populações tradicionais. Além disso, por não está inserida numa cadeia produtiva propriamente dita, diferentemente das hortaliças convencionais (batata, tomate, repolho, alface, etc.), não desperta o interesse comercial por parte de empresas de sementes, fertilizantes ou agroquímicos (BRASIL, 2010).

Frequentemente, hortaliças não convencionais como a taioba, o ora-pro-nóbis, o maxixe, a serralha, a mostarda dentre outros são “esquecidos” e deixados de lado, podendo ser uma são uma alternativa alimentar e uma opção de diversificação cultural, na atividade agropecuária, sobretudo na agricultura familiar, para populações rurais e urbanas de baixa renda (ALMEIDA; LISA;

CORREA, 2012; ROCHA et al., 2008). Cita-se o ora-pro-nóbis, presente na culinária de algumas localidades de Minas Gerais, como no município de Sabará onde essa planta faz parte dos hábitos alimentares da população e das manifestações culturais com a realização anual do festival do ora-pro-nóbis (BRASIL, 2010).

Segundo Kinupp e Barros (2008), as frutas e hortaliças não convencionais, geralmente apresentam teores de minerais e proteínas significativamente maiores do que as plantas domesticadas, além de serem mais ricas em fibras e compostos com funções antioxidantes. Devido aos elevados teores de proteínas apresentados pelas cactáceas do gênero Pereskia, essa planta é denominada “carne de pobre” (ROCHA et al., 2008).

Os teores de proteína em matéria seca observados em 100g de folhas da *Pereskia aculeata* foram de 25,5g; 25,4g; 27,4; 24,7g e 28,0 g de acordo com Almeida Filho e Cambraia (1974), Dayrell (1977), Mercê et al. (2001a), Silva e Pinto (2005) e Takeiti et al. (2009). De acordo com Rocha et al. (2008), a qualidade das proteínas de origem vegetal é considerada de baixo valor biológico, visto que são incompletas quanto à composição de aminoácidos, no entanto, ainda constituem uma boa fonte proteica para populações de baixo poder aquisitivo que têm acesso limitado a proteínas animais. Segundo Takeiti et al. (2009), a digestibilidade proteica das folhas de ora-pro-nóbis observada foi de 75,9%, já Cambraia (1980) reportou valores na ordem de 85%.

Nas folhas foram encontrados altos teores de lisina, um aminoácido essencial na nutrição humana, sendo superiores aos encontrados em couve, alface e espinafre (ALBUQUERQUE; SABAA-SRUR; FREIMAN, 1991; ALMEIDA FILHO; CAMBRAIA, 1974; CAMBRAIA, 1980; DAYRELL, 1977). Almeida-Filho e Cambraia (1974), Cambraia (1980) e Dayrell (1977) relataram que o alto teor de proteína encontrado nas folhas e os níveis de aminoácidos essenciais que o compõem, exceto para a metionina, foram

considerados maiores do que o mínimo recomendado pela FAO (Food and Agriculture Organization) como necessário para consumo humano. Takeiti et al. (2009) observaram que os aminoácidos mais abundantes foram o triptofano e o ácido glutâmico.

Observou-se o alto teor de mucilagem nas folhas de *Pereskia aculeata* (DUARTE; HAYASHI, 2005; MERCÊ et al., 2001a, 2001b; ROSA; SOUZA, 2003) além de heterossacarídeos (SIERAKOWSKI; GORIN; REICHER, 1987, 1990), arabinogalactanas (MERCÊ et al., 2001a) e galactomananas (MERCÊ et al., 2001b). Os arabinogalactanos e as galactomananas são biopolímeros com potencial aplicação na associação a íons de Fe (III), Co (II), Mn (II) e Ni (II) e também nas indústrias alimentícia e farmacêutica.

Takeiti et al. (2009) destacam que essa planta é uma boa fonte de minerais e vitaminas. Considerando a ingestão diária recomendada de minerais e vitaminas para adultos, as folhas de ora-pro-nóbis, na quantidade de 100 g dia^{-1}, suprem a necessidade dos minerais, para cálcio, magnésio, zinco, e ferro, assim como para a vitamina C. Nos frutos da *Pereskia aculeata* foram detectados 71,70±1,90 µg g-1 de carotenoides totais, apresentando substâncias bioativas com propriedade provitamina (AGOSTINI-COSTA et al., 2012).

REFERÊNCIAS

AGOSTINI-COSTA, T. S. et al. Carotenoids profile and total polyphenols in fruits of *Pereskia aculeata* Miller. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 34, n. 1, p. 234-238, 2012.

ALBUQUERQUE, M. G. P. T.; SABAA-SRUR, A. U. O.; FREIMAN, L. O. Composição centesimal e escore de amino-ácidos em três espécies de ora-pronobis (*Pereskia aculeata* Mill., *P. bleu* De Candolle e *P. pereskia* (L) Karsten). **Boletim SBCTA**, Campinas, v. 25, n. 1, p. 7-12, 1991.

ALMEIDA, M. E.; LISA, F.; CORRÊA, A. D. Utilização de cactáceas do gênero *Pereskia* na alimentação humana em um município de Minas Gerais. **Ciência Rural**, Santa Maria, v. 42, n. 4, p. 751-756, abr. 2012.

ALMEIDA FILHO, J.; CAMBRAIA, J. Estudo do valor nutritivo do ora-pro nóbis (*Pereskia aculeata* Mill.). **Revista Ceres**, Viçosa, MG, v. 21, n. 114, p. 105-111, 1974.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Manual de hortaliças não convencionais**. Belo Horizonte, 2010. 94 p.

CALVENTE, A. et al. Molecular phylogeny of tribe Rhipsalideae (Cactaceae) and taxonomic implications for *Schlumbergera* and *Hatiora*. **Molecular Phylogenetics and Evolution**, Orlando, v. 58, n. 3, p. 456-468, Mar. 2011.

CAMBRAIA, J. **Valor nutricional do ora-pro-nóbis**. Viçosa, MG: UFV, 1980. 3 p. (Informe Técnico).

CAMPO, V. L. et al. Carrageenans: biological properties, chemical modifications and structural analysis: a review. **Carbohydrate Polymers**, Barking, v. 77, n. 2, p. 167-180, June 2009.

CEVOLI, C. et al. Rheological characterisation of selected food hydrocolloids by traditional and simplified techniques. **Food Hydrocolloids**, Oxford, v. 33, n. 1, p. 142-150, Aug. 2013.

CHAN, S. Y.; CHOO, W. S. Effect of extraction conditions on the yield and chemical properties of Pectin from cocoa husks. **Food Chemistry**, London, v. 141, n. 4, p. 3752-3758, Dec. 2013.

CHARCHOGHLYAN, A. H.; PARK, H. D. Characteristics of a novel bacterial polysaccharide consisted of glucose and mannose as major components. **Food Hydrocolloids**, Oxford, v. 30, n. 2, p. 512-518, Mar. 2013.

DAYRELL, M. S. **Extração e estudo do valor nutritivo de proteínas de folhas de ora pro nobis (*Pereskia aculeata* Mill)**. 1977. 106 f. Tese (Doutorado em Ciências) - Universidade Federal de Minas Gerais, Belo Horizonte, 1977.

DESHMUKH, A. S. et al. Gum ghatti: a promising polysaccharide for pharmaceutical applications. **Carbohydrate Polymers**, Barking, v. 87, n. 2, p. 980-986, 2012.

DIAS, A. C. P. et al. Avaliação do consumo de hortaliças não convencionais pelos usuários das Unidades do Programa Saúde da Família (PSF) de Diamantina, MG. **Alimentos e Nutrição**, Araraquara, v. 16, n. 3, p. 279-284, 2005.

DICKINSON, E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. **Food Hydrocolloids**, Oxford, v. 17, n. 1, p. 25-39, Jan. 2003.

DRAGET, K.; TAYLOR, C. Chemical, physical and biological properties of alginates and their biomedical implications. **Food Hydrocolloids**, Oxford, v. 25, n. 2, p. 251-256, Mar. 2011.

DUARTE, M. R.; HAYASHI, S. S. Estudo anatômico de folha e caule de *Pereskia aculeata* Mill. (Cactaceae). **Revista Brasileira de Farmacologia**, Rio de Janeiro, v. 15, n. 2, p. 103-109, 2005.

EDWARDS, E. J.; NYFELER, R.; DONOGHUE, M. J. Basal cactus phylogeny: implications of *Pereskia* (Cactaceae) paraphyly for the transition to the cactus life form. **American Journal of Botany**, Columbus, v. 92, n. 7, p. 1177-1188, July 2005.

FARAGO, P. V. et al. Análise morfo-anatômica de folhas de *Pereskia grandifolia* Haw., Cactaceae. **Acta Farmaceutica Bonaerense**, Buenos Aires, v. 23, n. 3, p. 323-327, 2004.

FARAHNAKY, A. et al. Effect of various salts and pH condition on rheological properties of *Salvia macrosiphon* hydrocolloid solutions. **Journal of Food Engineering**, Essex, v. 116, n. 4, p. 782-788, June 2013.

FERNÁNDEZ-MARTÍN, F. et al. Influence of adding Sea Spaghetti seaweed and replacing the animal fat with olive oil or a konjac gel on pork meat batter gelation: potential protein/alginate association. **Meat Science**, Barking, v. 83, n. 2, p. 209-217, Oct. 2009.

FITZPATRICK, P. et al. Control of the properties of xanthan/glucomannan mixed gels by varying xanthan fine structure. **Carbohydrate Polymers**, Barking, v. 92, n. 2, p. 1018-1025, Feb. 2013.

GAO, P.; ZHUB, Z.; ZHANG, P. Effects of chitosan–glucose complex coating on postharvest quality and shelf life of table grapes. **Carbohydrate Polymers**, Barking, v. 95, n. 1, p. 371-378, June 2013.

GOH, C. H.; HENG, P. W. S.; CHAN, L. W. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. **Carbohydrate Polymers**, Barking, v. 88, n. 1, p. 1-12, Mar. 2012.

GÓMEZ-GUILLÉN, M. C. et al. Functional and bioactive properties of collagen and gelatin from alternative sources: a review. **Food Hydrocolloids**, Oxford, v. 25, n. 8, p. 1813-1827, Dec. 2011.

HEYMAN, B. et al. Influence of xanthan transition on the rheological properties of waxy starches. **Carbohydrate Polymers**, Barking, v. 96, n. 2, p. 568-577, July 2013.

IBANEZ, M. C.; FERRERO, C. Extraction and characterization of the hydrocolloid from *Prosopis flexuosa* DC seeds. **Food Research International**, Barking, v. 36, n. 5, p. 455-460, 2003.

JAHANBIN, K. et al. Isolation, purification and characterization of a new gum from Acanthophyllum bracteatum roots. **Food Hydrocolloids**, Oxford, v. 27, n. 1, p. 14-21, 2012.

JINDAL, M. et al. Aegle marmelos fruit pectin for food and pharmaceuticals: physico-chemical, rheological and functional performance. **Carbohydrate Polymers**, Barking, v. 93, n. 2, p. 386-394, 2013.

KAZAMA, C. C. et al. Involvement of arginine-vasopressin in the diuretic and hypotensive effects of *Pereskia grandifolia* Haw. (Cactaceae). **Journal of Ethnopharmacology**, Lausanne, v. 144, n. 1, p. 86-93, Oct. 2012.

KIM, J. H. et al. Mucilage removal from cactus cladodes (*Opuntia humifusa* Raf.) by enzymatic treatment to improve extraction efficiency and radical scavenging activity. **Food Science and Technology**, Trivandrum, v. 51, n. 1, p. 337-342, Apr. 2013.

KINUPP, V. F.; BARROS, I. B. I. Teores de proteína e minerais de espécies nativas, potenciais hortaliças e frutas Protein and mineral contents of native species, potential vegetables, and fruits. **Ciência e Tecnologia de Alimentos**, Campinas, v. 28, n. 4, p. 846-857, out./dez. 2008.

KUMAR, M. N. V. R. A review of chitin and chitosan applications. **Reactive & Functional Polymers**, New York, v. 46, n. 1, p. 1-27, Nov. 2000.

LAI, L. S.; LIANG, H. Y. Chemical compositions and some physical properties of the water and alkali-extracted mucilage from the young fronds of Asplenium australasicum (J. Sm.) Hook. **Food Hydrocolloids**, Oxford, v. 26, n. 2, p. 344-349, Mar. 2012.

LÓPEZ-LÓPEZ, I.; COFRADES, S.; JIMÉNEZ-COLMENERO, F. Low-fat frankfurters enriched with *n-3* PUFA and edible seaweed: effects of olive oil and chilled storage on physicochemical, sensory and microbial characteristics. **Meat Science**, Barking, v. 83, n. 1, p. 148-154, Sept. 2009.

LÓPEZ-LÓPEZ, I. et al. Design and nutritional properties of potential functional frankfurters based on lipid formulation, added seaweed and low salt content. **Meat Science**, Barking, v. 83, n. 2, p. 255-262, Oct. 2009.

MARSARO-JÚNIOR, A. L. et al. First report of natural infestation of *Pereskia aculeata* Mill. (cactaceae) by *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Brazil. **Revista de Agricultura**, Recife, v. 86, n. 2, p. 151-154, 2011.

MARTINEVSKI, C. S. et al. Utilização de bertalha (*Anredera Cordifolia* (TEN.) Steenis) e ora-pro-nobis (*Pereskia aculeata* Mill.) na elaboração de pães. **Brazilian Journal of Food and Nutrition**, Araraquara, v. 24, n. 3, jul./set. 2013. Disponível em: <http://serv-bib.fcfar.unesp.br/seer/index.php/alimentos/article/view/2251>. Acesso em: 10 abr. 2013.

MAURER, S.; JUNGHANS, A.; VILGIS, T. A. Impact of xanthan gum , sucrose and fructose on the viscoelastic properties of agarose hydrogels. **Food Hydrocolloids**, Oxford, v. 29, n. 2, p. 298-307, 2012.

MAZIA, R. S.; SARTOR, C. F. P. Influência do tipo de solo usado para o cultivo de *Pereskia aculeata* sobre propriedade protéica. **Revista Saúde e Pesquisa**, Maringá, v. 5, n. 1, p. 59-65, jan./abr. 2012.

MERCÊ, A. L. R. et al. Complexes of arabinogalactan of *Pereskia aculeata* and Co^{2}+, Cu^{2+}, Mn^{2+}, and Ni^{2+}. **Bioresource Techonology**, Essex, v. 76, n. 1, p. 29-37, 2001a.

MERCÊ, A. L. R. et al. Fe(III): galactomannan solid and aqueous complexes: potentiometric, EPR spectroscopy and thermal data. **Journal of Brazilian Chemical Society**, Campinas, v. 12, n. 6, p. 791-798, 2001b.

MESBAHI, G.; JAMALIAN, J.; FARAHNAKY, A. A comparative study on functional properties of beet and citrus pectins in food systems. **Food Hydrocolloids**, Oxford, v. 19, n. 4, p. 731-738, July 2005.

MIRHOSSEINI, H.; AMID, B. T. A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. **Food Research International**, Barking, v. 46, n. 1, p. 387-398, 2012.

MUÑOZ, L. A. et al. Chia seeds: microstructure, mucilage extraction and hydration. **Journal of Food Engineering**, Essex, v. 108, n. 1, p. 216-224, Jan. 2012.

NAIM, A. A. et al. Chemical modification of chitin by grafting with polystyrene using ammonium persulfate initiator. **Carbohydrate Polymers**, Barking, 2013. In press.

NAJI, S.; RAZAVI, S. M. A.; KARAZHIYAN, H. Effect of thermal treatments on functional properties of cress seed (*Lepidium sativum*) and xanthan gums: a comparative study. **Food Hydrocolloids**, Oxford, v. 28, n. 1, p. 75-81, July 2012.

NGOUÉMAZONG, D. E. et al. Effect of de-methylesterification on network development and nature of Ca^{2+} -pectin gels: towards understanding structureefunction relations of pectin. **Food Hydrocolloids**, Oxford, v. 26, n. 1, p. 89-98, Jan. 2012.

NIE, S. P. et al. A further amendment to the classical core structure of gum arabic (*Acacia senegal*). **Food Hydrocolloids**, Oxford, v. 31, n. 1, p. 42-48, 2013.

PATERSON, I. D.; DOWNIE, D. A.; HILL, M. P. Using molecular methods to determine the origin of weed populations or *Pereskia aculeate* in South Africa and its relevance to biological control. **Biological Control**, Orlando, v. 48, n. 1, p. 84-91, Feb. 2009.

PRAJAPATI, V. D. et al. Pharmaceutical applications of various natural gums, mucilages and their modified forms. **Carbohydrate Polymers**, Barking, v. 92, n. 2, p. 1685-1699, 2013.

PRAJAPATI, V. D.; JANI, G. K.; KHANDA, S. M. Pullulan: an exopolysaccharide and its various applications. **Carbohydrate Polymers**, Barking, v. 95, n. 1, p. 540-549, June 2013.

RANA, V. et al. Modified gums: approaches and applications in drug delivery. **Carbohydrate Polymers**, Barking, v. 83, n. 3, p. 1031-1047, Jan. 2011.

RAZAVI, S. M. A.; TAHERI, H.; QUINCHIA, L. A. Steady shear flow properties of wild sage (*Salvia macrosiphon*) seed gum as a function of

concentration and temperature. **Food Hydrocolloids**, Oxford, v. 25, n. 3, p. 451-458, May 2011.

RENARD, D. et al. Structure of arabinogalactan-protein from Acacia gum: from porous ellipsoids to supramolecular architectures. **Carbohydrate Polymers**, Barking, v. 90, n. 1, p. 322-332, 2012.

ROCHA, D. R. C. et al. Macarrão adicionado de ora-pro-nóbis (*Pereskia aculeata* Miller) desidratado. **Alimentos e Nutrição**, Araraquara, v. 19, n. 4, p. 459-465, 2008.

ROSA, S. M.; SOUZA, L. A. Morfo-anatomia do fruto (hipanto, pericarpo e semente) em desenvolvimento de *Pereskia aculeata* Miller (Cactaceae). **Acta Scientarium Biological Sciences**, Maringá, v. 25, n. 2, p. 415-428, 2003.

ROSAS-FLORES, W.; RAMOS-RAMÍREZ, E. G.; SALAZAR-MONTOYA, J. A. Microencapsulation of *Lactobacillus helveticus* and *Lactobacillus delbrueckii* using alginate and gellan gum. **Carbohydrate Polymers**, Barking, v. 98, n. 1, p. 1011-1017, Oct. 2013.

SARTOR, C. P. R. et al. Estudo da ação cicatrizante das folhas de *Pereskia aculeata*. **Revista Saúde e Pesquisa**, Maringá, v. 3, n. 2, p. 149-154, maio/ago. 2010.

SATO, K. et al. Analysis of a change in bacterial community in different environments with addition of chitin or chitosan. **Journal of Bioscience and Bioengineering**, Osaka, v. 109, n. 5, p. 472-478, 2010.

SIERAKOWSKI, M. R.; GORIN, P. A. J.; REICHER, F. Location of O-acetyl groups in the heteropolysaccharide of the cactus *Pereskia aculeata*. **Carbohydrate Research**, Amsterdam, v. 201, n. 2, p. 277-284, 1990.

SIERAKOWSKI, M. R.; GORIN, P. A. J.; REICHER, F. Some structural features of a heteropolysaccharide from the leaves of the cactus *Pereskia aculeata*. **Phytochemistry**, Saint Paul, v. 26, n. 6, p. 1709-1713, Dec. 1987.

SILVA, M. C.; PINTO, N. A. V. D. Teores de nutrientes nas folhas de taioba, ora-pro-nóbis, serralha e mostarda coletadas no município de Diamantina. In: FUNDAÇÃO EDUCACIONAL CIENTÍFICA E TECNOLOGIA DA UFVJA, 8., 2005, Diamantina. **Anais...** Diamantina: UFVJA, 2005. p. 86.

TAKEITI, C. Y. et al. Nutritive evaluation of non-conventional leafy vegetable (*Pereskia aculeata* Miller). **International Journal of Food Sciences and Nutrition**, Hants, v. 60, n. 1, p. 148-160, 2009.

TOFANELLI, M. B. D.; RESENDE, S. G. Sistemas de condução na produção de folhas de ora-pro-nobis. **Pesquisa Agropecuária Tropical**, Goiânia, v. 41, n. 3, p. 466-469, jul./set. 2011.

TURRA, A. F. et al. Avaliação das propriedades antioxidantes e susceptibilidade antimicrobiana de *Pereskia grandifolia* Haworth (Cactaceae). **Arquivos de Ciências da Saúde da UNIPAR**, Cascavel, v. 11, n. 1, p. 9-14, 2007.

VARDHANABHUTI, B.; IKEDA, S. Isolation and characterization of hydrocolloids from monoi (*Cissampelos pareira*) leaves. **Food Hydrocolloids**, Oxford, v. 20, n. 6, p. 885-891, Aug. 2006.

VARELA, P.; FISZMAN, S. M. Food hydrocolloids hydrocolloids in fried foods: a review. **Food Hydrocolloids**, Oxford, v. 25, n. 8, p. 1801-1812, 2011.

VRIESMANN, L. C.; SILVEIRA, J. L. M.; PETKOWICZ, C. L. O. Chemical and rheological properties of a starch-rich fraction from the pulp of the fruit cupuassu (*Theobroma grandiflorum*). **Materials Science and Engineering**, Lausanne, v. C29, n. 2, p. 651-656, Mar. 2009.

VRIESMANN, L. C.; TEÓFILO, R. F.; PETKOWICZ, C. L. O. Extraction and characterization of pectin from cacao pod husks (*Theobroma cacao L.*) with citric acid. **Food Science and Technology**, Trivandrum, v. 49, n. 1, p. 108-116, Nov. 2012.

XU, L. et al. The comparison of rheological properties of aqueous welan gum and xanthan gum solutions. **Carbohydrate Polymers**, Barking, v. 92, n. 1, p. 516-522, Jan. 2013.

YAPO, B. M. Pectic substances: from simple pectic polysaccharides to complex pectins: a new hypothetical model. **Carbohydrate Polymers**, Barking, v. 86, n. 2, p. 373-385, 2011.

SEGUNDA PARTE - ARTIGOS

ARTIGO 1 *Response surface methodology for optimization of the mucilage extraction process from Pereskia aculeata Miller*

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(Parte de artigo preparado de acordo com as normas da revista Food Hydrocolloid – publicado em Food Hydrocolloids 33 (2013) 38-47, 2013)

ABSTRACT

In this report, a process for hydrocolloid extraction from *Pereskia aculeata* Miller (Barbados gooseberry), popularly known in Brazil as Ora-pro-nóbis (OPN), was developed. In the process, several operations, such as extraction, pressing, filtration, precipitation, grinding and drying, were required. The independent variables evaluated to determine the optimum extraction conditions were the ratio of water: raw material and the extraction temperature. The significant results at each stage were analyzed using the response surface method. The conditions that presented the highest precipitate yield, highest pH value, highest hue value, highest filtrate viscosity and minimum flow rate value were a water:raw material ratio of 2.46-3.70 L/kg and an extraction temperature between 54.6-80 °C. The powdered product obtained was found to be close to yellow in color and with functionalities that can be used in the food industry.

Key words: gum, extraction, emulsion, thickening agent, *Pereskia aculeata* Miller.

1. INTRODUCTION

Hydrocolloids are widely used in food systems for several purposes, such as gelling agents, texture modifiers and stabilizers. Polysaccharides with large, linear, flexible structures increase viscosity even at low concentrations.

Due to these properties, hydrocolloids are often utilized as the main ingredients in certain types of solid and semi-solid foods (Vardhanabhuti & Ikeda; 2006). The hydrocolloids added in foods should present neutral flavor, be thermostable and easy to disperse, provide body, confer resistance to temperature variations, be absent of microorganism pathogens and have low costs.

Hydrocolloids extracted from plants have an advantage over those of animal origin due to their positive image in the eyes of consumers. Starch, pectin, galactomannans, carrageenans, alginates and cellulose and its derivatives are the principal hydrocolloids of plants origin. There is still a market for new hydrocolloid sources that meet the demand for ingredients with more specific functions, synergistic interactions and improvement of these functional properties in foods. Only a few plants species are currently cultivated to obtain gums to be used as additives in the food industry, and many of them are from the *Leguminosae* family. Some examples are as follows: *Acácia senegal*, the source of arabic gum; *Astragalus* spp., the source of tragacanth; *Cyamopsis tetragonolobus*, the source of guar gum; and *Ceratonia siliqua*, the source of locust gum (Ibañez & Ferrero, 2003).

In Brazil, the hydrocolloids used in food applications are from imported products, in spite of the fact that there are native plants that present high potential for hydrocolloid production, though their commercial and industrial uses have not been fully explored (Mercê , Landaluze, Mangrich, Szpoganicz &

Sierakowskui, 2001). In the state of Minas Gerais, Brazil, *Pereskia aculeata* Miller called as ora-pro-nobis (OPN) is consumed and appreciated to such an extent in the traditional dishes served in restaurants of historical cities that it has begun to be cultivated for commercial use. OPN belongs to the *Cactaceae* family and has scandent habits. The high protein and fiber content and the absence of leaf toxicity (Almeida-Filho & Cambraia, 1974) of this species make it a useful and important food source. The leaves are also an emollient, and the fruits have expectorant and antisyphilitic properties. In Brazil, this species is found from the northeast region to the south of the country. It preferentially grows on the borders of the forest and in the forests clearings (Rosa & Souza; 2003).

In addition to not possessing any toxic properties, OPN is extremely rich in high-quality proteins. Analyses conducted on OPN leaves show that they are composed of 25% protein and have high digestibility (85%). In addition to presenting a well-balanced composition, the leaves have an exceptionally high content of certain essential amino acids, particularly lysine, whose content in OPN is higher to that of the cabbage, lettuce and spinach. The protein and essential amino acid levels (except methionine) reported are substantially higher than the minimum amount recommended by the Food and Agriculture Organization of the United Nations (FAO) as necessary for human consumption (Sierakowski, Gorin, Reicher, & Corrêa, 1987). The nutritional benefits of the

OPN leaves were also revealed in a study that evaluated the nutritional components in terms of approximate composition, minerals, vitamins, proteins content and digestibility of the OPN leaf (Takeiti, Antônio, Motta, Collares-Queiroz & Park, 2009).

Polysaccharide extraction starting from plant sources can be performed with several solvents. Diluted acids such as 0.1 N HCl are usually used in the commercial extraction of pectin; however, some hydrolysis will occur, depending on the conditions. Sodium bicarbonate and sodium carbonate have been used to extract gums from the leaves of the hsian-tsao (Vardhanabhuti & Ikeda, 2006). However, the most frequently used method is the combination of cold water with ethanol and/or isopropanol and and/or acetone. The extraction of the mucilage from the pulp of the cactus *Opuntia fícus-indica* was performed by Medina-Torres, Brito-de la Fuente, Torrestiana-Sanchez & Katthain (2000) using acetone for the precipitation at a pulp: acetone ratio of 1:2. The precipitate was collected, washed with isopropyl alcohol and dried (Medina-Torres, Brito-de la Fuente, Torrestiana-Sanchez & Alonso, 2003). Ibañez & Ferrero (2003) used two different means of extraction of the hydrocolloid from *Prosopis flexuosa* DC seeds. The first method is based on extraction in alkaline medium where the seeds were macerated in a 0.5% NaOH (weight/weight) solution. The second method utilized extraction in neutral medium by immersion of the seeds in hot water. Sepúlveda, Sáenz, Aliaga, & Aceituno (2007) extracted mucilage

from the Opuntia spp. after obtaining the pulp by grinding and homogenization in water with 1:5 and 1:7 pulp:water ratios. To reduce the amount of alcohol used in the precipitation, the volume of the mucilage solution was reduced to one third of the initial volume by concentration in rotary evaporator.

Statistical methods have been satisfactorily applied to optimize system constituents and other critical variables for the extraction of biomolecules. These methods overcome the limitations of the optimization of simple parameters, in which one simple variable is changed while other variables are maintained at a constant level, that are time-consuming, demand many experiments and are not reliable (Arockiasamy & Banik, 2008). The response surfaces methodology has been successfully used to optimize the extraction process of new hydrocolloids by Wu, Cui, Tang, & Gu (2007), Arockiasamy & Banik (2008) and Koocheki, Taherian, Razavi & Bostan (2009).

Due to the presence of large amounts of gum, the presence of the biopolymer arabinogalactan, the high protein content, the economic importance that OPN cultivation is gaining in various areas of Brazil, the simplicity and high productivity of cultivation and mainly the enormous interest of the food and pharmaceutical industries in its processing, the objective of this work was to investigate the extraction process of the hydrocolloids/ mucilages of the *Pereskia aculeata* Miller (OPN) and to optimize the parameters involved in the various operations using response surface methodology.

2. MATERIAL AND METHODS

2.1 Experimental design

For the study of the optimum formulations and process operational parameters, a central composite rotational design was used (CCRD), using 11 assays with 4 axial points, 4 extreme points and 3 central points, to evaluate the reproducibility of the process with calculation of the experimental error (Rodrigues & Iemma, 2005). The values used are shown in Table 1.

Table 1 Experimental design.

Assays	Coded variables		Real variables	
	X_1	X_2	Temperature (°C)	Water quantity (L/kg)
1	-1	-1	46	1.5
2	-1	+1	46	3.6
3	+1	-1	75	1.5
4	+1	+1	75	3.6
5	-1.41	0	40	2.5
6	+1.41	0	80	2.5
7	0	-1.41	60	1.0
8	0	+1.41	60	4.0
9	0	0	60	2.5
10	0	0	60	2.5
11	0	0	60	2.5

X_1 is the temperature of the extraction water (°C), and X_2 is the volume of water per kg of the raw material.

2.2 Obtaining the hydrocolloid

The *Pereskia aculeata* Miller raw material was harvested in the municipal district of Itutinga, Minas Gerais, Brazil. All of the samples were

harvested at the same place to reduce interference due to the alterations in species composition that can be caused by the variability of available nutrients in the soil and climatic alterations. After harvest, the leaves, flowers, sprouts, thorns and stems were taken to the laboratory. They were washed in running water, manually preselected and placed in polyethylene bags that were sealed, identified and stored in a freezer. To obtain the final product in a powdered form, an extraction process was developed with the various operations shown in the flowchart in Figure 1.

2.2.1 Extraction 1: homogenization of the sample and hot extraction

Raw material (1 kg) containing leaves, stems, thorns and sprouts were homogenized at temperatures of 80 °C in different amounts of water using an industrial blender (Metvisa, model LG10, São Paulo, Brazil) for 10 min, until all the parts were triturated. The triturated material was transferred to glass receptacles and placed in a thermostatic bath (Quimis model q-215-2, São Paulo, Brazil) with controlled temperatures. The range of temperatures tested was from 40 to 80 °C in accordance with the experimental plan (Table 1). The extraction period was 6 h under constant agitation. The temperature of the bath was monitored with a temperature sensor (K-type thermocouple).

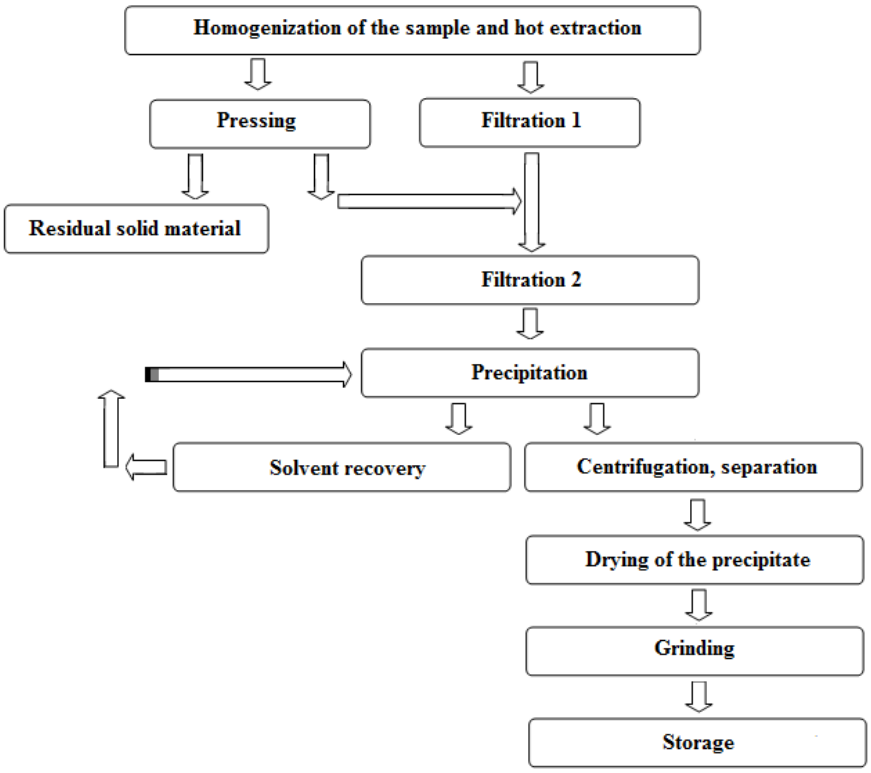


Figure 1 Flow chart of the operation for obtaining hydrocolloid from powdered OPN (leaves, stems, thorns and sprouts).

2.2.2 Extraction 2: pressing

The solid material resulting from Extraction 1 was submitted to pressing in a hydraulic press (Tecnal, model TE 058, Campinas, Brazil). During the pressing, the pressure exerted was controlled from 16.88 MPa to 19.95 MPa, and

the liquid product obtained at this stage (Extract 2) was mixed with Extract 1 before being filtered. The residual solid material was discarded.

2.2.3 Filtration 1: buchner funnel under high vacuum

The mixture was filtered in a buchner funnel using organza fabric as filtering element and a double stage pump for high vacuum production. The product obtained in this stage was named filtrate 1.

2.2.4 Filtration 2: fixed-bed column with activated carbon

Filtrate 1 was placed in a fixed-bed column to remove pigments and insoluble solids. The experimental assembly for the filtration process in the fixed-bed column is shown in Figure 2. The columns were built with cylindrical polyvinyl chloride tubes 1.00 m in height and 0.11 m in diameter. The bed in the column was composed of 0.80 m of activated carbon (Scientific Exodus, São Paulo, Brazil) with a 1-2 mm particle size. Filtration with activated carbon is a process that demands an extended period of time, which can result in the development of microorganisms. To avoid their growth, the filtration process in the fixed-bed column was conducted entirely in an inert atmosphere using compressed nitrogen gas at a pressure of 1.2 atm.

2.2.5 Precipitation, solvent recovery, drying, grinding and storage

Filtrate 2 was subjected to precipitation in ethyl alcohol (95%) at a 3:1 proportion of alcohol to each L of Filtrate 2. The wash procedure was conducted

three times, and the precipitation time was 90 min for each wash. After the third wash, the precipitate was separated by centrifugation (Fanem, model 206 BC, Brazil). After centrifugation and separation of the precipitate, the solvent in the supernatant solution was recovered using a rotary evaporator and reused in the process as shown in Figure 1. The drying of the precipitate was conducted under vacuum in an oven (Nova Ética, model 440/2D, Brazil), at 40 ºC for 18 h. The dry products were removed from the plates and ground in a ball mill; wrapped and stored in tightly closed containers containing silica gel; and protected from light and humidity.

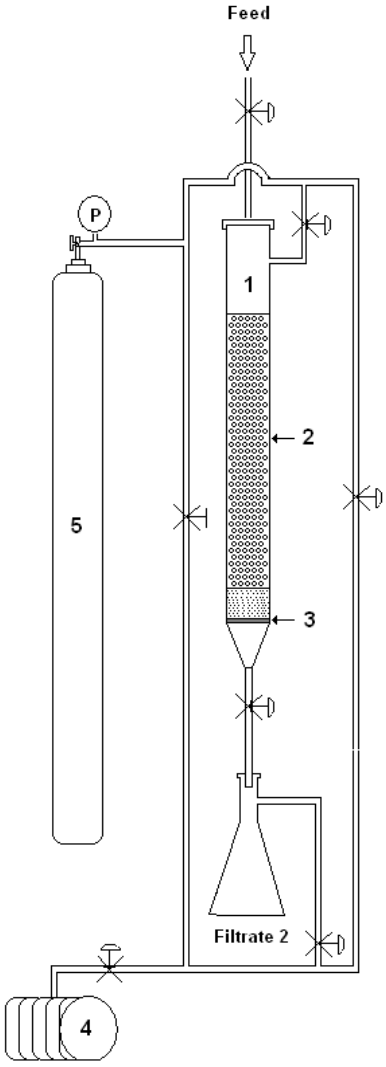


Figure 2 Fixed-bed column with activated carbon. (1) Fixed-bed columns, (2) activated carbon with a granulometry of 1-2 mm, (3) support with organza fabric, (4) vacuum pump and (5) nitrogen gas, (P) manometer.

2.3 Characterizations of filtrates 1 and 2

The pH of the filtrates was determined using a digital potentiometer (Micronal, model 320, Brazil) (Instituto Adolfo Lutz - IAL, 2008). The rheological measurements were obtained using a concentric cylinder rotational viscometer (Brookfield DVIII Ultra, Brookfield Engineering Laboratories, Stoughton, USA), a small sample adapter 13R/RP (19.05 mm diameter and 64.77 mm depth) and a SC4-18 coaxial shear sensor (17.48 mm diameter and 31.72 mm length). The samples were submitted to an increasing shear rate ramp that varied linearly from 0.10 s^{-1} to 100.0 s^{-1}, which is in the range of interest of food texture studies (Fernández, Alvarez & Canet, 2008). All of the rheological parameters were obtained using Reocalc software (Version V.3.1, Brookfield Engineering Laboratories, Stoughton, USA) for data capture. The rheological parameters were adjusted to the Herschel-Bulkley model (Equation 1) and the power law (Equation 2).

$$\sigma = \sigma_{0H} + k\dot{\gamma}^n \quad (1)$$

$$\sigma = k\dot{\gamma}^n \quad (2)$$

where σ = shear stress (Pa); k = consistency index (Pa.s); $\dot{\gamma}$ = shear rate (s^{-1}); n= flow behavior index and σ_{0H} = initial shear stress (Pa).

The instrumental analysis of color was conducted in a Minolta CR 200 colorimeter under the International Commission on Illumination system. The L* value expresses the brightness such that a value closer to 100 indicates a lighter product. The a* values indicate a tendency towards coloration from green (-) to red (+); the b* values indicate a tendency of coloration from blue (-) towards yellow (+). The hue angle, which indicates the chromatic shade (attribute where the color is perceived), was evaluated in each assay using Equation 3 (McGuire, 1992).

$$H^* = \tan^{-1}(b^*/a^*) \quad (3)$$

The yield was calculated after precipitation by amount (in weight) of the precipitate produced per unit of volume of the Filtrate 2, with the result expressed as a percentage.

2.4 Statistical analysis

The results of all of the analyses were evaluated by the response surface method using Statistica 8.0 software, with the polynomial used to adjust the model defined by Equation 4.

$$y = \beta_0 + \beta_1 X_1 + \beta_{11} X_1^2 + \beta_2 X_2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (4)$$

where β_0, β_1, β_{11}, β_2, β_{22}, β_{12}, are the regression coefficients; X_1 is the extraction temperature; X_2 is the proportion of water used per kg of raw material; and ε is the experimental error. The criteria used for the adaptation of the model were the determination coefficient values (R^2>80%) and variance analyses.

3. RESULTS AND DISCUSSION

3.1 Analysis of operating conditions

Table 2 presents the correlation coefficients, the calculated F value and the regression coefficients for each order with their respective p-values for the significant variables involved in the different stages of the process applied in the complete codified model shown in Equation 6.

The usual test of significance of the adjusted regression equation is the null hypothesis test, which involves the calculation of the F value and comparing this calculated value with the tabulated value, $F_{\alpha,p-1,N-p}$, where N is the number of observations, p is the number of adjusted parameters and α is the level of significance. If the calculated F value exceeds the tabulated $F_{\alpha,p-1,N-p}$ value, then it is inferred with an α level of significance that the variation accounted for by the model is significantly higher than the unexplained variation. In other words, higher calculated F value indicates a better adjustment. It was observed that practically all of the calculated F values for the curve adjustments presented in

Table 2 are above the tabulated F value, which for this experiment was 5.05, indicating that the parameters are significant (Khuri & Cornell, 1996).

Another parameter presented in Table 2 is the coefficient of determination (R^2). The R^2 value is a measure of the proportion of the variation of the values observed around the average explained by the adjusted model. In variance analysis shown in Table 2, the variation percentage explained by the regression is above 80%, but that value should not be compared to 100% because of the contribution due to the pure error, which is a measure of the random error that affects the responses (Barros neto, Scarminio & Bruns, 1996).

Table 2 Analysis of the regression coefficients for significant variables in the extraction process.

	pH (F1)		**Viscosity (F2)**		**Hue (F2)**		**Flow index, n (F2)**		**Yield (PPT)**	
	Coef. of regression	p-value	Coef. of regression	p-value	Coef. of regression	p-value	Coef. of regression	p-value	Coef. of regression	p-value
β_0	4.907	0.000	9.217	0.221	0.991	0.000	0.803	0.000	4.517	0.000
β_1	0.085	0.049*	33.615	0.009*	-0.034	0.721	-0.148	0.002*	0.169	0.606
β_{11}	-0.312	0.073	20.066	0.091	0.340	0.025*	-0.004	0.896	0.170	0.661
β_2	-0.200	0.144	-10.828	0.237	0.016	0.870	0.0558	0.080	0.963	0.026*
β_{22}	0.275	0.102	3.211	0.752	0.296	0.041*	-0.059	0.108	-0.785	0.085
β_{12}	-0.592	0.015*	-5.635	0.642	-0.179	0.222	-0.020	0.602	0.070	0.878
$F_{calculated}$	5.49		25.85		21.49		8.62		6.40	
R^2	85.49%		82.67%		85.75%		89.61%		86.23%	

* Significant at the 5% confidence level. F1 = Filtrate 1; F2 = Filtrate 2; PPT = precipitate.

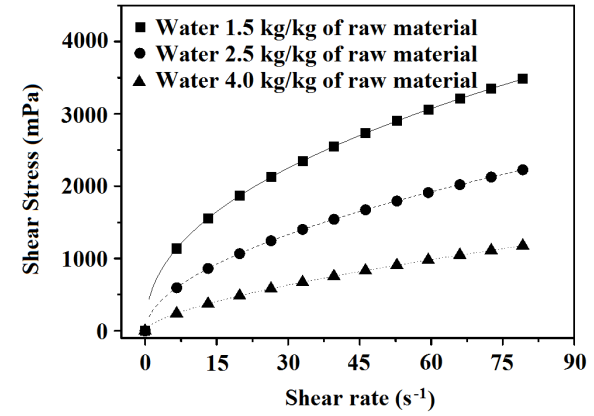
For the pH parameter of Filtrate 1 (F1), $F_{calculated}$ was higher than $F_{tabulated}$, and the coefficients of determination presented values superior to 80%, indicating a good adjustment of the complete model. Table 2 show that the temperature had influences of a linear order on the pH values, and the interaction of the temperature and water: raw material ratio variables were significant. In this case, the extraction temperature influenced the pH of Filtrate 1.

Koocheki et al. (2010) and Wu et al., (2007) performed studies on *Alyssum Homolocarpum* seeds and *Sterculia* seeds, respectively, in which pH control during the extraction is undertaken with the addition of acid and/or alkaline solutions, seeking a higher yield and increased ease in the final processing of the different species.

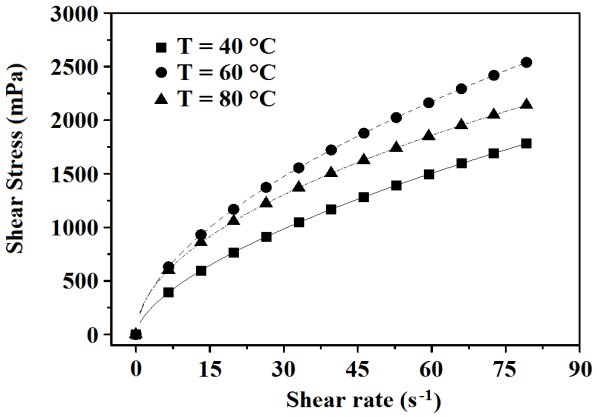
Koocheki et al. (2010) varied the experimental conditions of temperature, seed proportion and pH when conducting the mucilage extraction from seeds of *Alyssum homolocarpum*. The pH parameters were fixed and adjusted for the values of 4.0, 7.0 and 10.0. Such adjustments were made with NaOH and HCl solutions. The authors concluded that pH influenced parameters such as viscosity, protein content and the rheological parameters of the extracted mucilage. However, it did not have a significant effect on the final yield (Koocheki et al., 2010). Wu et al. (2007) concluded that the pH had a significant effect on the yield and viscosity results when obtaining polysaccharides extracted from fruits from Sterculia (*Semen Sterculiae Lychnophorae*) seeds,

where the optimum extraction condition was at a neutral pH. The other variables involved in the process were temperature, extraction time and water:seed ratio.

Figure 3 presents the rheograms obtained for Filtrate 1, where the shear stress is correlated with the shear rate and shows the effect of the variation of the proportion of the amount of water per kg of raw material and the extraction temperature on the rheological parameters. The figure shows that for all of the treatments, it is possible to verify the non-linearity between the shear stress and the shear rate that characterizes a shear-thinning fluid behavior with yield stress (Chabra & Richardson, 2008).



(a)



(b)

Figure 3 Relationship between the shear stress (mPa) and shear rate (s^{-1}) in a filtrate with (a) one extraction temperature (60 °C) and different proportions of water: raw materials, and (b) one water: raw material proportion (2.5 L/kg) and different extraction temperatures.

Table 3 shows the rheological parameters obtained for Filtrate 1 adjusted by the Herschel-Bulkley (HB) model, which presented a better correlation coefficient between all tested models. In Figure 3 and Table 3, it can be observed that the increase of the shear stress in the function of the shear rate is inversely proportional to the ratio of water used in the extraction process and is proportional to the temperature increase.

Table 3 Rheological parameters for Filtrate 1.

	Herschel-Bulkley model			
	k (mPa s)	**n**	**σ_{0H} (mPa)**	**R^2**
1	487.1	0.45	0.06	100
2	35.8	0.79	0.07	99.7
3	467.8	0.45	0.06	96.6
4	264.8	0.45	0.16	99.9
5	123.8	0.61	0.16	99.9
6	230.3	0.51	0.02	100
7	486	0.43	0.07	99.7
8	71.1	0.64	0.12	100
9	209.4	0.56	0.17	99.9
10	172.7	0.55	0.16	99.9
11	276.5	0.48	0.04	100

The consistency index (k) in the Herschel-Bulkley model of Filtrate 1 increases with the reduction of the proportion of water in relation to the amount of raw materials and with the increase of the temperature. The flow index (n) deviates from the behavior of a Newtonian fluid as the water: raw material ratio is reduced and the temperature is increased.

Figure 4 was obtained by Lima-Junior (2011) and shows the variation of the pH values of the samples for all treatments after passage through the fixed-bed column (Filtrate 2) compared with that of Filtrate 1. An increase can be observed in the pH values in Filtrate 2. This result can be explained by the retention of suspended particles within the material in the column increasing the values of the pH from a solution that was approximately neutral to more basic values (Lima Junior, 2011)(Figure 4). (Lima-Júnior, 2011).

This elevation in pH by the passage of Filtrate 1 through the column is due to the H^+ ion adsorption in the activated carbon bed through the interaction of charges present in these layers (Lima Junior, 2011). The opposite behavior was shown for the viscosity parameters. During the flow of the extract through the fixed-bed column, in addition to the removal of pigments, solid particles that were initially suspended and retained in the column were eliminated, thus reducing the viscosity of the samples (data not shown) by 22% on average. The viscosity was increased by the temperature in a linear manner such that the higher extraction temperature used, the higher the viscosity of Filtrate 2.

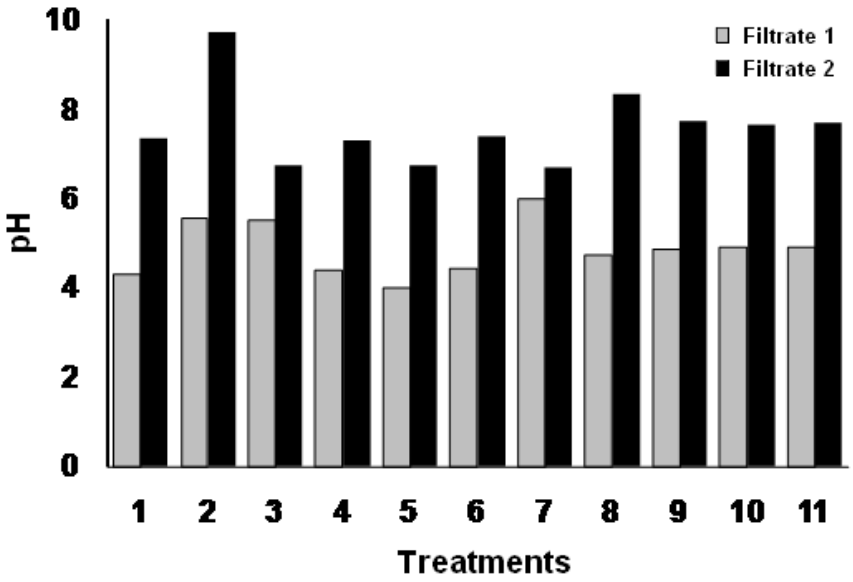


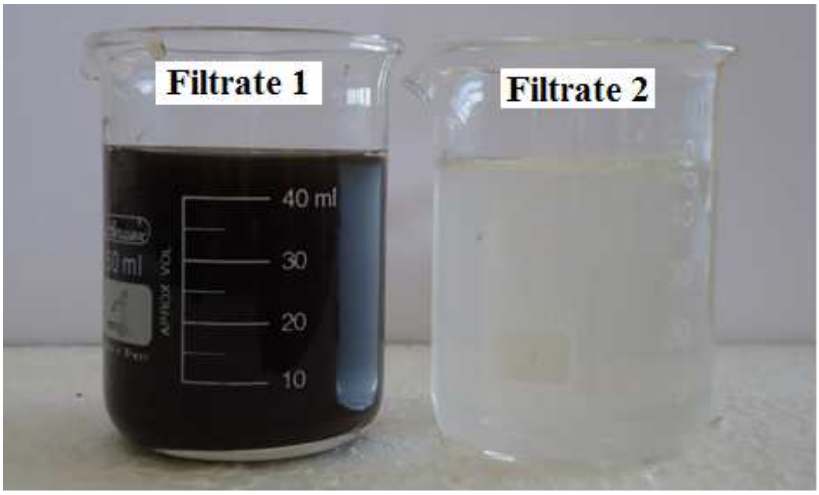
Figure 4 Comparison of the pH values after passing through the fixed-bed column (Lima Junior 2011).

The main application and objective of the filtration in the fixed-bed activated carbon column is the clarification of the product, which was significantly improved after Filtrate 1 was passed over the column. It is clear that there was an increase in the parameter relative to the hue value when compared to Filtrate 1, as shown in Figure 5. This parameter indicates how much closer to neutral colors (white, gray or black) the analyzed extract is (Figure 5A and 5 B). The increase in the hue angle parameter is analyzed considering that values close to zero are related to colors close to red that have

an angular value equal to 0°. For yellow, the angular value is equal to 90°. When passing the filtrate through the fixed-bed column, all of the assays presented values indicating a color closer to yellow. Therefore, the column was efficient in the pigment reduction of Filtrate 1.

The hue value was significantly influenced by the extraction temperature and water:raw material proportion, and the quadratic terms were significant (Table 2). A higher temperature resulted in the observation of a higher hue value.

In the study of the rheological behavior of Filtrate 2, the models that provided the best adjustment coefficients were those of Herschel-Buckley and the power law. Although we observed that the consistency index parameter (k) increases with the increase in temperature and decreases with the increase in the water raw: materials ratio (data not shown), there was not a good adjustment of the complete model. For the fluid behavior index parameter (n), the generation of the contour surfaces was practicable, and the coefficient of determination value was 89.60%. For the power law model, higher temperatures result in lower fluid behavior index (n) values and, consequently, higher k values, which indicates a more viscous filtrate.



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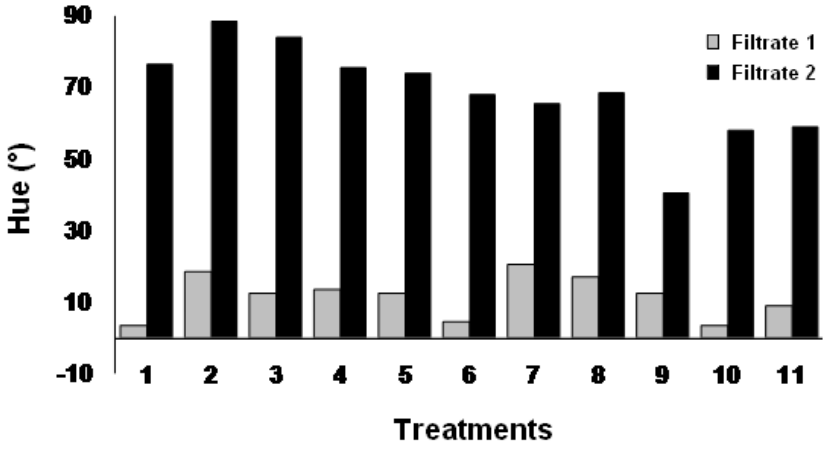


(

b)

Figure 5 Comparison of the hue angle values of Filtrates 1 and 2 (before and after passing through the column, respectively).

Table 2 also contains the results obtained for the precipitation yield. Based on the data in Table 2, the precipitate yield was significantly influenced by the water: raw material ratio used in the extraction in a linearly positive manner ($P < 0.05$). The yield found in the extraction process of hydrocolloids from OPN was inferior to 1%, obtaining an average of 2.37g of powdered hydrocolloid for each kilogram of plant.

The methodology developed to obtain hydrocolloid from powdered OPN was natural and did not employ any type of chemical reagent throughout the process to facilitate the extraction. In gums obtained from fruits of the Malva nut (*Scaphium scaphigerum*), the results obtained by Somboonpanyakul, Wang, Cui, Barbut & Jantawat (2006) show that the yield for extraction in hot water was approximately 1%; in acid extractions, the yield was 6%; and in alkaline extractions, the yield was 20%. These findings clearly demonstrate that the presence of acid or alkaline agents favor the extraction, culminating in a higher yield. Wu, Cui, Eskin & Goff (2009) showed that in the fractionation of non-pectic polysaccharides of yellow mustard mucilage, precipitation with 75% ethanol was more efficient in increasing the precipitation yield when compared with the precipitation conducted in an ammonium sulfate $(NH_4)_2SO_4$ solution.

The temperature and seed: water ratio had similar linear effects on the yield of mucilage obtained from Qodume Shirazi seeds (*Alyssum homolocarpum*). The interaction among the pH and water:seed ratio terms had a

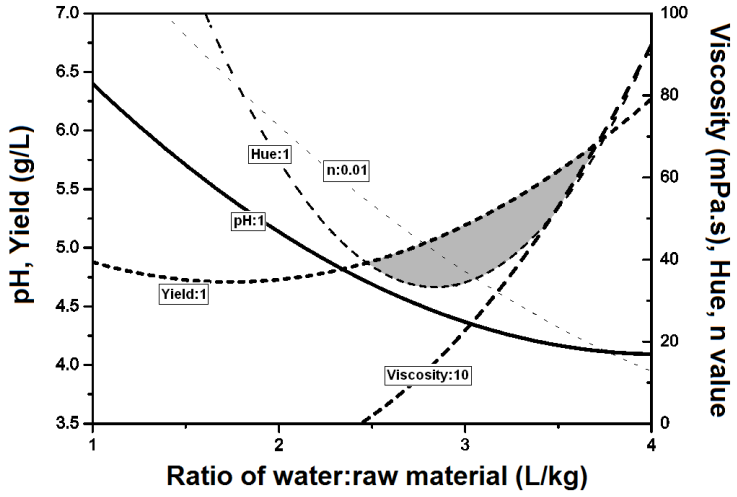
significant ($P < 0.05$) effect on the yield, and the water:seed ratio had highly significant quadratic effect coefficients ($P < 0.01$) (Koocheki et al., 2010).

3.2. Process optimization

Optimum conditions for the extraction of the OPN gum were determined to obtain the maximum precipitate yield, the pH value of Filtrate 1, the hue value of Filtrate 2, the viscosity of Filtrate 2 and the minimum flow value index of Filtrate 2. The optimum condition range for the extraction was determined by superimposing the contour surfaces of all the analyzed results. Figure 6A presents the superposition of the graphs obtained for the five responses that were evaluated as a function of the water: raw material ratio while maintaining a constant temperature at 75 °C. Figure 6B presents the graphs for the five responses as a function of the extraction temperature while maintaining a constant ratio of water at 2.5 L/kg raw materials.

These graphs show the best combination of factors for the extraction of OPN gum. Figure 6 A demonstrates that the water: raw material ratio of 2.46-3.70 L/kg is the range with the best combinations of factors. The shaded area in the graph with the six factors is the optimum area of extraction conditions that results in a higher pH and soluble solids value for Filtrate 1, a larger hue angle value of Filtrate 2, higher viscosity of Filtrate 2 and, most importantly, a higher yield value of the precipitate. Figure 6 B shows that the shaded area

corresponding to the optimum extraction temperature conditions is in the range from 54.6-80 °C.



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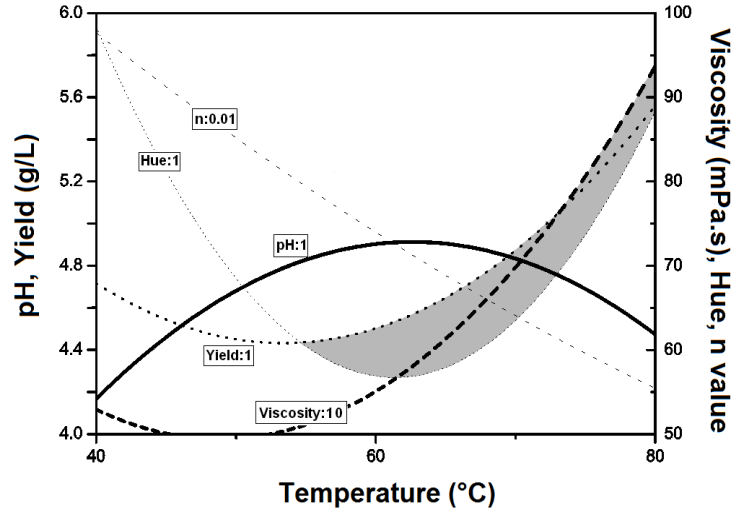

(b)

Figure 6 Optimal superposition region of the contour graphs of six responses evaluated as (a) a function of the water:raw material ratio at a constant temperature of 75 °C, and (b) as a function of temperature at a constant water: raw material 2.5 L/kg ratio. The shaded area in the graph is the optimum area of extraction conditions.

4. CONCLUSIONS

The process developed herein, involving multiple steps and only using ethanol as chemical agent, presented satisfactory results for obtaining the hydrocolloid in a natural way. The *Pereskia aculeata* Miller species proved to be an alternative source of hydrocolloids; thus, an industrial process is viable.

The conditions that presented a higher precipitate yield, a higher pH value of Filtrate 1, a higher hue value of Filtrate 2 (lighter product), a higher viscosity of Filtrate 2 and a minimum flow index value of Filtrate 2 were a water:raw material ratio of 2.46-3.70 L/kg and an extraction temperature in the range of 54.6-80 °C. The powdered product obtained presented a light color and had properties that can be used in industry as a thickener, gelling agent and/or emulsifier.

5. Acknowledgments

The authors wish to thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG- Brazil, CVZ APQ-01209/08), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Brazil) for financial support for this research.

6. REFERENCES

Arockiasamy, S. & Banik, R. M. (2008). Optimization of gellan gum production by *Sphingomonas paucimobilis* ATCC 31461 with noionic surfactants using central composite design. *Journal of Bioscience and Bioengineering*, 105, 204-210.

Barros Neto, B., Scarminio, I. S. & Bruns, R. E. (1996). Planejamento e otimização de experimentos. (2th ed.), Campinas:Editora da UNICAMP.

Chabra, R. P. & Richardson, J. F. (2008). *Non-Newtonian flow and applied rheology***:** engineering applications. 2nd ed. Oxford: Butterworth-Heinemann, 518 p.

Fernández, C., Alvarez, M. D. & Canet, W. (2008). Steady shear and yield stress data of fresh and frozen/thawed mashed potatoes: Effect of biopolymers addition. *Food Hydrocolloids*, 22,1381-1395.

Ibañez, M. C. & Ferrero, C. (2003). Extraction and characterization of the hydrocolloid from *Prosopis Flexuosa* DC seeds. *Food Research International*, 36, 455-460.

Khuri, A. I. & Cornell, J. A. (1996). *Response Surfaces - Designs and Analyses*. 2nd Edition, Marcel Dekker,Inc.

Koocheki, A., Mortazavi, S. A., Shahidi, F., Razavi, S. M. A., Kadkhodaee, R. & Milani, J. M. (2010). Optimizaton of mucilage extraction from Qodume Shirazi seed (Alyssum Homocarpum) using response surface methodology. *Journal of Food Process Engineering*, 33, 861-882.

Koocheki, A., Taherian, A. R., Razavi, S. M. A. & Bostan, A. (2009). Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from Lepidium perfoliatum seeds. *Food Hydrocolloids*, 23, 2369-2379.

Lima-Júnior, F. A. (2011). Desenvolvimento de processo de extração de hidrocoloides do ora-pro-nóbis (*Pereskia Aculeata* Miller). 129 p. Dissertação (Mestrado em Ciência dos Alimentos) – Universidade Federal de Lavras.

McGuire, R. G. (1992). Reporting of objective color measurements. *Horticultural science*, 27, 1254-1255.

Medina-Torres, L., Brito-de la Fuente, E., Torrestiana-Sanchez, B. & Katthain, R. (2000). Rheological properties of the mucilage gum (*Opuntia fícus indica*). *Food Hydrocolloids*, 14, 417-424.

Medina-Torres, L., Brito-de la Fuente, E., Torrestiana-Sanchez, B. & Alonso, S. (2003). Mechanical properties of gels formed by mixtures of mucilage gum (Opuntia fícus indica) and carrageenans. *Carbohydrate Polymers*, 52, 143-150.

Mercê, A. L. R., Landaluze, J. S., Mangrich, A. S., Szpoganicz, B. & Sierakowski, M. R. (2001). Complexes of arabinogalactan of *Pereskia aculeata* and Co^{2+}, Cu^{2+}, Mn^{2+}, and Ni^{2+}. *Bioresource Technology*, 76, 29-37.

Rodrigues, M. I. & Iemma, A. F. *Planejamento de experimentos e otimização de processos***.** Campinas: Casa de Pão, 2005. 326 p.

Rosa, S. M. & Souza, L. A. (2003). Morfo-anatomia do fruto (hipanto, pericarpo e semente) em desenvolvimento de *Pereskia aculeata* Miller (Cactaceae). *Acta Scientiarum, Biological Science*, 25, 415-428.

Sepúlveda, E., Sáenz, C., Aliaga, E. & Aceituno, C. (2007). Extraction and characterization of mucilage in *Opuntia* spp. *Journal of Arids Environments*, 68, 534-545.

Sierakowski, M., Gorin, P. A. J., Reicher, F. & Corrêa, J. B. C. (1987) Some structural features of a heteropolysaccharide from the leaves of the cactus *Pereskia aculeate. Phytochemistry*, 26, 1709-1713.

Somboonpanyakul, P., Wang, Q., Cui, W., Barbut, S. & Jantawat, P. (2006). Malva nut gum. (Part I): extraction and physicochemical characterization. *Carbohydrate Polymers*, 64, 247–253.

Takeiti, C. Y., Antônio, G. C., Motta, E. M. P., Collares-Queiroz, F. P. & Park, K. J. (2009). Nutritive evaluation of a non-conventional leafy vegetable (*Pereskia aculeata* Miller). *International Journal of Food Sciences and Nutrition*. 60, 148-160.

Vardhanabhuti, B. & Ikeda, S. (2006). Isolation and characterization of hydrocolloids from monoi (*Cissampelos pareira*) leaves. *Food Hydrocolloids*, 20, 885-891.

Wu, Y., Cui, W., Eskin, N.A.M. & Goff, H. D. (2009). Fractionation and partial characterization of non-pectic polysaccharides from wellow mustard mucilage. *Food Hydrocolloids*, 23, 1535-1541.

Wu, Y., Cui, S. W., Tang, J. & Gu, X. (2007). Optimization of extraction process of crude polysaccharides from boat-fruited *sterculia* seeds by response surface methodology. *Food Chemistry*, 105, 1599-1605.

ARTIGO 2 ***Thermal and microstructural stability of powdered gum extracted from Pereskia aculeata Miller leaves***

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(Artigo preparado de acordo com as normas da revista Food Hydrocolloids)

ABSTRACT

In this report, the thermal and microstructural stability of a powdered product extracted from *Pereskia aculeata* Miller, popularly known in Brazil as ora-pró-nóbis (OPN), was characterized. Using an optimized process condition, gums were prepared and the proximate composition, mineral content, thermal stability as differential scanning calorimetry (DSC) and thermogravimetry (TG), scanning electronic microstructure (SEM), spectroscopy of dispersive energy by x-rays and emulsion formation capacities by optical microscopy were analyzed. The obtained powdered product presented high protein and mineral content and low total carbohydrate and uronic acid values. The FT-IR spectrum suggests a arabinogalactan-protein. The stability of the emulsions prepared from powdered product was evaluated at room temperature and at 80°C. DSC thermal profiles of OPN powdered product showed endothermic and exothermic events that allows identify systems organization and samples destructions. TG curves for OPN gums show high residue value which is attributes to carbonaceous and minerals contents. The SEM micrographs of powdered OPN gum show a high porosity, differences in the particle sizes and smaller particles adhered in larger particles. The spongy aspect was characteristic suggest that the material is hygroscopic. Scanning electronic microscopy/Spectroscopy of Dispersive Energy by X-rays confirmed that large quantities of minerals are present in the samples. The emulsion formation capacity of the product was verified and strong droplets

coalescence as being proportional to the reduced powdered gum concentration. *Pereskia aculeata* Miller may be considered an alternative source for mucilage and its powdered product presents the potential use as an emulsifying and stabilizing agent for food applications.

Key words: powdered gum, microstructure, thermal analysis, emulsion stability, *Pereskia aculeata* Miller.

1 INTRODUCTION

The use of hydrocolloids from plants begin with the extraction operation with water, acid or alkaline solutions. Several studies (Lin & Lai, 2009; Lin et al, 2009; Lai & Liang 2012;Yapo, 2009a, 2009b, 2009c; Yapo & Koffi, 2008; Yapo et al, 2007a; Yapo et al, 2007b) have shown that plants parts and extraction conditions influenced significantly the productive and physicochemical characteristics of the gums. The characteristics such as the chemical compositions (including neutral sugars, ash, protein, degree of esterification methoxylation and acetylation), and molecular weight distribution affect the rheological characteristics and the function of these gums as gelling and thickening agents, as well as emulsifying agents influencing the emulsification ability and stability.

In accordance with the form of extraction and the source of origin, the hydrocolloids chemical structure varies and it can have one or more physical properties commercially useful. The use of these materials as additives in industrial processes is extensive in paint industries, paper, pharmaceutical and food (Mercê et al 2001).

The *Pereskia aculeata* Miller is a native Cactus found in the tropics of America, such as the southern region of the United States (Florida), and in Brazil. In Brazil, this cactacea is known as ora-pro-nobis (OPN) and this species is found from the northeast region to the south of the country. It preferentially grows on the borders of the forest and in the forests clearings (Rosa & Souza; 2003). OPN belongs to the *Cactaceae* family and has scandent habits. The high protein and fiber content and the absence of leaf toxicity (Almeida-Filho & Cambraia, 1974, Dayrell & Vieira, 1977; Butterworth and Wallace, 2005) of this species make it a useful and important food source. The leaves are also an emollient, and the fruits have expectorant and antisyphilitic properties.

In addition, the OPN do not possess any toxic properties and is extremely rich in proteins. Analyses conducted on OPN leaves show that they are composed of 25% protein and have high digestibility (85%). In addition to presenting a well-balanced composition, the leaves have an exceptionally high content of certain essential amino acids, particularly lysine, whose content in OPN is higher to that of the cabbage, lettuce and spinach. The protein and

essential amino acid levels (except for methionine) reported are substantially higher than the minimum amount recommended by the Food and Agriculture Organization of the United Nations (FAO) as necessary for human consumption (Sierakowski, Gorin, Reicher, & Corrêa, 1987). The nutritional benefits of the OPN leaves were also revealed in a study that evaluated the nutritional components in terms of approximate composition, minerals, vitamins, proteins content and digestibility of the OPN leaf (Takeiti, Antônio, Motta, Collares-Queiroz & Park, 2009).

Some aspects of the chemical structure of a heteropolysaccharide obtained from the OPN leaves were studied by Sierakowski et al., (1987). A mucilaginous water-soluble heteropolysaccharide containing 3.5% protein was isolated from the leaves and hydrolyzed, and the monomers were identified by conventional polysaccharide analysis techniques. The results showed that the leaves contained arabinose, galactose, rhamnose and galacturonic acid in a molar ratio of 5.1: 8.2: 1.8: 1.0. According to Sierakowski, Gorin, Reicher & Corrêa (1990), the polysaccharide complexes of the *Pereskia aculeata* Miller leaves are highly ramified, containing arabinofuranose, arabinopyranose, galactopyranose, galactopyranosyl, uronic acid and rhamnopyranose units.

The arabinogalactans (AGs) are structural polysaccharides with a complex molecular structure that is difficult to characterize (Aspinall, 1969; Aspinall, 1982; Whistler, 1970). They are present in all higher plants (Fincher,

Stone & Clarke, 1983). Several reports in the literature describe the structural elucidation of these polymers, which are found in leaves, stems, roots, flowers, and seeds as well as in high amounts in gums and vegetable exudates (Delgobo, Gorin, Jones & Iacomini, 1998; Fincher et al., 1983; Menestrina, Iacomini, Jones & Gorin, 1998). Studies of the complex nature of biopolymers (AG) extracted specifically from OPN leaves and their interactions with Co^{2+}, Cu^{2+}, Mn^{2+} and Ni^{2+} in terms of the thermal stability of the metallic compounds were conducted by Sierakowski et al., (1990) and Mercê et al., (2001), whose results suggested their potential use in the food and pharmaceutical industries.

Due to the presence of large amounts of gum, the presence of the biopolymer arabinogalactan, the high protein content, the economic importance that OPN cultivation is gaining in various areas of Brazil, the simplicity and high productivity of cultivation and mainly the enormous interest of the food and pharmaceutical industries in its processing, the objective of this work was to investigate the chemical composition, thermal properties and microstructure of the hydrocolloids/mucilages in the powdered product, reconstituted gel and emulsions of the *Pereskia aculeata* Miller (OPN). We also sought to evaluate the potential use of the powdered product as an emulsifying and stabilizing agent in food applications.

2 MATERIALS AND METHODS

2.1 Material

The P*ereskia aculeata* Miller raw material was harvested in the municipal district of Itutinga, Minas Gerais, Brazil. All of the samples were harvested at the same place to reduce interference due to the alterations in species composition that can be caused by the variability of available nutrients in the soil and climatic alterations. After harvest, the leaves were taken to the laboratory. They were washed in running water, manually preselected and placed in polyethylene bags that were sealed, identified and stored in a freezer until the experiments were begun. To obtain the final product in a powdered form, an extraction process was developed with the various operations shown in the flowchart in Figure 1.

2.2. Extraction process of leaf hydrocolloid of *Pereskia aculeata* Miller

The process to obtain powdered product was optimized in the various steps as shown with details in Lima Junior et al., 2013. The conditions that presented a higher precipitate yield, a higher pH value of Filtrate 1, a higher hue value of Filtrate 2 (lighter product), a higher viscosity of Filtrate 2 and a minimum flow index value of Filtrate 2 were a water:raw material ratio of 2.46-3.70 L/kg and an extraction temperature in the range of 54.6-80 °C.

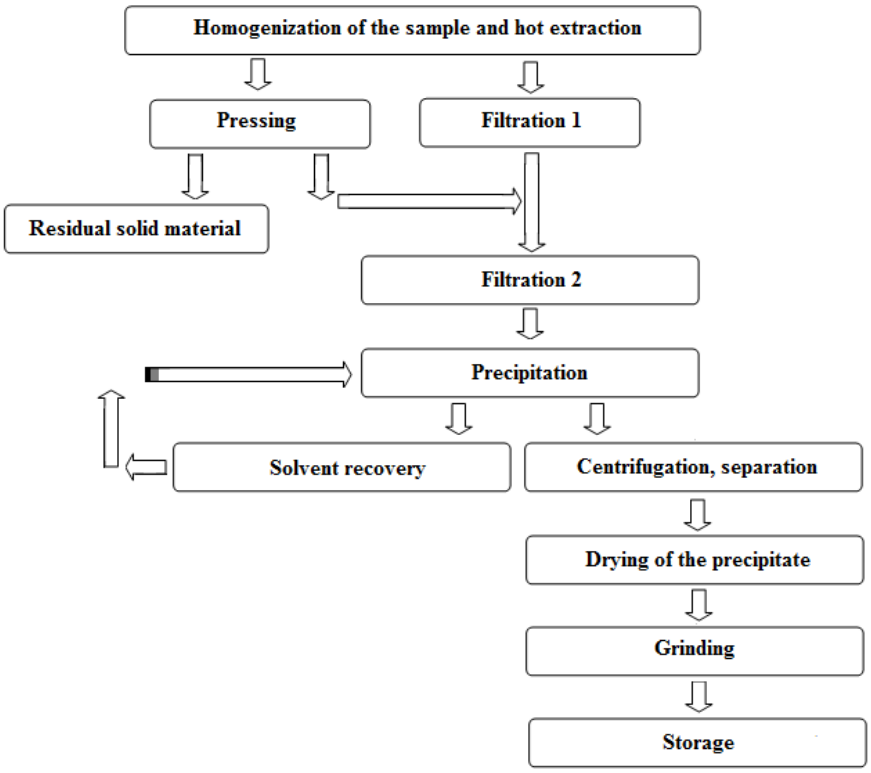


Figure 1 Flow chart of the operation for obtaining hydrocolloid from powdered OPN leaves.

2.3 Reconstitution of the powdered product

To analyze the behavior of the obtained product, assays conducted at 80 °C and a solution prepared from powdered product with a concentration 5g/100mL of water was chosen for the reconstitution tests of the product in the gel form based on the optimization results. The gel was maintained in a

thermostatic cabinet (Eletrolab, EL202, São Paulo, Brazil) at 4 °C for 12 hours until their complete hydration. One portion of the gel was freeze-dried at -40 °C during 18 hours and grinded in ball mill. Powdered product and dried gel were submitted to microstructural analysis. Powdered product and reconstituted gel were submitted to thermal analysis.

2.4 Chemical composition

The reconstitution analyses were carried out with samples produced with 2.5 L of water/kg raw material processed at a temperature of 75 °C, selected after the results of the optimization had been determined (Lima Junior et. al., 2013).

2.4.1. Proximate composition

The chemical analysis of moisture content, protein (determined by the Kjeldhal method, N x 6.25) content, lipid fraction (Soxlet method), fiber and ashes were carried out following the methodology indicated by the AOAC (2006).

2.4.2. Total carbohydrates

Total carbohydrates were determined by phenol-sulfuric method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956).

2.4.3. Mineral analysis

The minerals present in the extract and powdered product were determined by the method of Malavolta, Vitti & de Oliveira (1989).

2.4.5 Uronic acids contents

The uronic acid contentes were determined by the method of m-hydroxydiphenyl (MHDP) (Blumenkrantz & Asboe-Hansen, 1973).

2.5 Spectroscopy in the infrared region

The infrared (IR) spectra of powdered product were recorded in a (FTIR) double-beam spectrometer (Digilab Excalibur, serie FTS 3000), in KBr pellets, spectral range between 400 and 4000 cm^{-1} and resolution of $4cm^{-1}$.

2.6 Thermal analysis

2.6.1. Thermogravimetry (TG)

The analysis were carried out on a DTG-60H Shimadzu, Tokyo, Japan) at a heating rate of 2 °C/min in nitrogen atmosphere, from 21 to 520 °C.

2.6.2. Differential scanning calorimetry (DSC)

A modulate temperature differential calorimeter (DSC-60A, Shimadzu, Tokyo, Japan) was used to evaluate the thermal behavior of the powdered product and reconstituted gel. The instrument was calibrated for temperature and heat flow with indium and zinc, and the temperature control system used liquid

nitrogen as the cooling agent. Hermetically sealed stainless steel pans were used, and the sample size of each sample was approximately 6 mg. The temperature protocol used for samples consisted of equilibrating the samples at -100 ºC and then heating the samples a temperature rate of 3 ºC min^{-1} to 250 ºC.

2.7 Microstructural analyses

2.7.1. Scanning Electron Microscopy

The powdered product and dried reconstituted gel were fixed with double-sided carbon tape onto an aluminum support (stubs) that was sputter-coated under vacuum with a thin film of metallic gold using a Bal-Tec model SCD 050 evaporator (Balzers, Liechtenstein). A Nano Technology Systems (Carl Zeiss, Oberkochen, Germany) model Evo® 40 VP scanning electron microscope was used with an accelerating voltage of 20 kV and a working distance of 9 mm to obtain the digital images using the Leo User Interface software at varying magnifications. The images were processed using Corel Draw 14 Photo paint Software.

2.7.2 Scanning eletronic microscopy (SEM) / Spectroscopy of Dispersive Energy by X-rays

The powdered product and dried reconstituted gel were fixed with double-sided carbon tape onto an aluminum support (stubs) that was sputter-coated under vacuum with carbon using a Union CED 020 evaporator (Balzers,

Liechtenstein). A Nano Technology Systems (Carl Zeiss, Oberkochen, Germany) model Evo® 40 VP scanning electron microscope was used to obtain the digital images. The chemical compositions were qualified and quantified by Spectroscopy of Dispersive Energy by X-rays in the Quantax XFlash 5010 Bruker apparatus.

2.8 Reconstitution of the gum from the powdered product for emulsion preparation and analysis of the microstructure and emulsion stabilities.

The reconstitution were carried out with samples produced with 2.5 L of water/kg raw material processed at a temperature of 75 °C, selected after the results of the optimization had been determined.

The emulsion microstructures were determined by preparing an emulsion containing 10 g of commercial corn oil (Mazola, Cargill, São Paulo, Brazil) and 40 g of reconstituted gum with concentrations of 1.0, 2.0 and 3.0 g/100mL of water. The sample was submitted to mechanical agitation (Ika labortechnik, RW.20, Germany) for 3 minutes and homogenized in a blender (Tecnal, TE102, Brazil) at 20,500 rpm. The emulsion microstructure images were acquired using a light microscope (Meiji ML 5000, Meiji Techno America, Santa Clara, CA, USA) with an attached video camera (Cole-Palmer 49901-35, Cole-Palmer, Vernon Hills, IL, USA).

To verify the stability of the emulsion formed from the OPN gum, the emulsions were left at rest for 30 min at room temperature or in a thermostatic bath (Solab, mod. SL150, São Paulo, Brazil) at 80 °C. The samples were then centrifuged (Fanem, 206 BC, Brazil) at 2700 rpm (1,271 xg) for 10 min, and the final volume was measured. The emulsion stability was viewed using a light microscope as previously described.

3 RESULTS AND DISCUSSION

3.1. Centesimal composition and mineral analysis of the extract and powdered product

Table 1 shows the chemical composition and mineral concentrations of the Filtrate 1 and powdered product obtained using ratio of water:raw material and extraction temperature of 2.5 L/kg and 75° C, respectively, selected after the optimization.

The drying process reduced the moisture content 97.05 to 13.45%. Total protein contents were reduced after passage through the fixed-bed column (Filtrate 2) compared with that of Filtrate 1. The reduction was related to the residence time of the extract into the column (data not shown). This was also related to the high ash content found in the powdered product, suggesting that an interaction occurred between the extract and the activated carbon, causing the transference of particles from these to the filtered product.

The contents of total protein, lipid fraction, ash and total fiber in the extract were close to those reported by Almeida-Filho & Cambraia (1974), Albuquerque et al. (1991) and Takeite et al. (2009) for fresh OPN leaves: 25, 28 and 28.4% (dry basis) for total protein, 6.3, 6.8 and 4.1% (dry basis) for lipid fraction, 14.2, 20.1 and 16.1% (dry basis) for ash, and 7.7, 9.1 and 9.8% (dry basis) for total fiber, respectively. The protein content of 30% found in this work presented higher value when compared to those reported in literature. The results found for the lipid fraction, ash and total fiber contents presented lower values of 4.04, 14.09 and 6.46%, respectively.

These differences are due to external factors such as climate and soil in which the plant was cultivated, harvesting season and pre-processing. In this work, the leaves were frozen and stored refrigerated until the processing moment. The influence of external factors on the characteristics of the raw materials was proven by Almeida Filho & Cambraia (1974). These authors worked with OPN from two different regions of the state of Minas Gerais, Brazil. The results differed in the lipid fraction, fibers, ash and protein content analyses when compared to samples from different regions.

Table 1 Proximate and Mineral compositions of Filtrate 1 and product powdered.

Analysis*	Composition	
	Filtrate 1	**Powdered Product**
Moisture content (g/100g)*	97.05*	13.45*
Protein content (g/100g)	30.10	10.47
Carbohydrates (g/100g)	43.57	46.88
Ashes (g/100g)	14.09	42.54
Fibers	6.46	7.35
Lipid fraction (g/100g)	4.04	2.46
Uronic acids	0.44	1.39
P (mg/100g)	110	1,130
K (mg/100g)	1,470	2,420
Ca (mg/100g)	2,410	3,350
Mg (mg/100g)	400	450
B (mg/100g)	18.6	54.6
Cu (mg/100g)	8.00	31.80
Mn (mg/100g)	39.30	175.20
Zn (mg/100g)	45.50	93.30
Fe (mg/100g)	137.5	189.7

* All values were expressed in dry base, except moisture content.

Total carbohydrate content is often measured by the Dubois carbohydrate method (Dubois et al., 1956), and it is useful for sugars and polysaccharides. Crude gum contents, i.e., gum content based on all components

that contribute to the gum viscosity (protein, polysaccharides and cross-linking cations) were estimated with this method by the use of appropriate control samples (Abbott et al., 1995).

The extract (Filtrate 1) and the powdered mucilage obtained from *Pereskia aculeata* leaves presented 43.57% and 46.88% of total carbohydrate, respectively. These values are low when compared to those obtained by Ibanez & Ferrero (2003), for *Prosopis flexuosa* seeds and for the mucilage extracted from seeds by different procedures (in alkaline and neutral mediums) with 54% and 66.1 – 72.5% of total sugar content, respectively; Lin & Lai (2009), for hydrocolloids extracted from mulberry (*Morus alba* L.) leaves using different solvents (water andsodium bicarbonate) with 62.1 to 64.4%; Singthong et al. (2009), for Yanang (*Tiliacora triandra*) leaves, with 59.5%; and Xie et al. (2013), for polysaccharide extracted from *Cyclocarya paliurus* leaves, with 64.8% of total sugar content.

Similar results were found by Karazhiyan et al. (2011) for *Lepidium sativum* seeds, with 43.51% of total sugar content, and by Lin & Lai (2009), with 39.8% in mulberry leaves. The result variation found in literature for obtaining the mucilage is related to the use of different parts of the plants (leaves, seeds, fruits), species, geographic locations (climate and soil) and, especially, the extraction method and variations in parameters such as temperature, pH, solvent, etc.

Mucilages are complex polymeric substances of carbohydrate nature with a highly branched structure (Sepúlveda et al., 2007), and which contain varying proportions of neutral sugars, such as arabinose, galactose, rhamnose, xylose, glucose, manose and fucose, as well as acid sugars (uronic acids) in different proportions (Chitarra et al., 1998; Sepúlveda et al., 2007). The uronic acids present a carboxyl group and are mainly constituted of galacturonic acid. This last is the main pectin forming monomer and constituent of other gums (Chitarra et al., 1998).

The results presented in Table 1 for the uronic acid content analysis (0.44g/100g) show that these results are low when compared to those obtained by Singthong et al. (2009) for gum extracted from Yanang (*Tiliacora triandra*) leaves, and by Yamazaki et al. (2008) for hydrocolloids extracted from *Corchorus olitorius* leaves, which values were of 10g/100g of uronic acid. However, Xie et al. (2013) obtained 23.5% of uronic acid in the extraction of polysaccharides from *Cyclocarya paliurus* leaves.

Sierakowski et al. (1987) isolated water-soluble mucilaginous hetero-polysaccharide containing 3.5% of protein from *Pereskia aculeata* leaves. These hetero-polysaccharides contained arabinose, galactose, rhamnose and galacturonic acid in a molar ratio of 5.1:8.2:1.8:1.0. The physiochemical properties of the gums depend on the amount of the groups charged by carboxylic acids. The most common source for such groups is carbohydrates

with a carboxylic acid group (uronic acids) (Batsoulis et al., 2004). Furuta & Maeda (1999) found a 23.3% content of uronic acid in water-soluble soybean polysaccharides and suggest that they contain arabinogalactans, including galacturonic acid. We concluded that the increase in viscosity was caused by the uronic acids repelling each other in the polysaccharide molecule, the last being extended by this repellence.

Lai & Liang (2012) studied the effects of extraction conditions, including types of solvents (water and sodium bicarbonate) and extraction temperatures (25, 50, 70 and 90 ^{o}C), over the physicochemical properties of water and alkali-extracted mucilage from young fronds of *Asplenium australasicum* (J. Sm.) Hook. Sugar composition analysis revealed that the mucilage contained a significant amount of uronic acid (14.3 and 56.5%, based on total sugars). Lin & Lai (2009) also observed the influence of mucilage extraction conditions over uronic acid content for hydrocolloids extracted from mulberry (*Morus alba* L.) leaves with water or sodium bicarbonate, resulting in uronic acid contents of 33.3 and 28.4%, respectively.

The mineral analysis of the powdered product indicates a high concentration of calcium (3,350mg/100g), followed by potassium (2,420mg/100g), phosphorus (1,130mg/100g) magnesium (450mg/100g). For the Filtrate 1, phosphorus, potassium, calcium, magnesium and manganese contents were smaller when compared to those obtained by Takeite et al. (2009).

The same was observed by Almeida Filho & Cambraia (1974). The remaining minerals such as boron, copper and zinc presented results superior when compared with the same literature.

Lai & Liang (2009) observed differences in the mineral compositions of the hydrocolloids extracted from mulberry leaves using different solvents (deionized water and 0.14M sodium bicarbonate). The mucilage extracted with deionized water presented higher calcium (48mg/100g), magnesium (5mg/100g), iron (0.16mg/100g) and zinc (0.05mg/100g) content, while the mucilage extracted with the 0.14M sodium bicarbonate solution presented higher sodium (105mg/100g) and potassium (40mg/100g) content. Both presented values inferior to those found in mucilages obtained from OPN leaves. This probably occurs due to the different species, factors regarding harvest and also to the mucilage extraction procedures.

3.2 Infrared (IR) spectra

Polysaccharides, depending on their chemical structure, may possess one or more commercially useful physical properties (viscosity and gelation being two examples). The use of these materials as additives in industrial processes is extensive and in some form they have been used in paper, paint and pharmaceutical, and food industries. In the last few years, there has been an

increase in the use of polysaccharides as food and in industrial processes worldwide (Mercê et al., 2001).

The IR spectrum the powdered mucilage extracted from *Pereskia aculeata* Miller leaves is shown in Figure 2. Although IR sometimes does not prove to be useful with polymers because the spectra can appear simpler than expected due to accidental degeneracy of chemical by similar groups, similar infrared spectrum bands were found in the work of Mercê et al. (2001). The difference is only in intensity of the bands in the spectrum. Mercê et al. (2001) carried studies out of the complex nature of biopolymers (AG) extracted specifically from OPN leaves and their interactions with Co^{2+}, Cu^{2+}, Mn^{2+} and Ni^{2+} in terms of the thermal stability of the metallic compounds. In this work, Mercê et al. (2001) reported that a twist in the main chain of a biopolymer having rhamnose units linked (1 → 2) exists in its structure.

The FT-IR spectra of carbohydrates are used for determination of their structural features (Singthong et al., 2009). Carbohydrates show absorbance in the region 1200–800 cm−1 due to ring vibrations overlapped with stretching vibration of the hydroxyl groups and the glycosidic bond vibration (Kacuráková et al., 2000). This region is often called the fingerprint of molecules because it allows the identification of major chemical groups in polysaccharides: the position and intensity of the bands that are specific for each polysaccharide (Posé et al., 2012; Singthong et al., 2009). The region at 1200–800 cm-1, which

is dominated by stretching vibrations of C–O, C–C, ring structures and deformation vibrations of CH2 groups (Hori & Sugiyama 2003), was found to be useful for the identification of polysaccharides and is (Kacuráková et al., 2000).

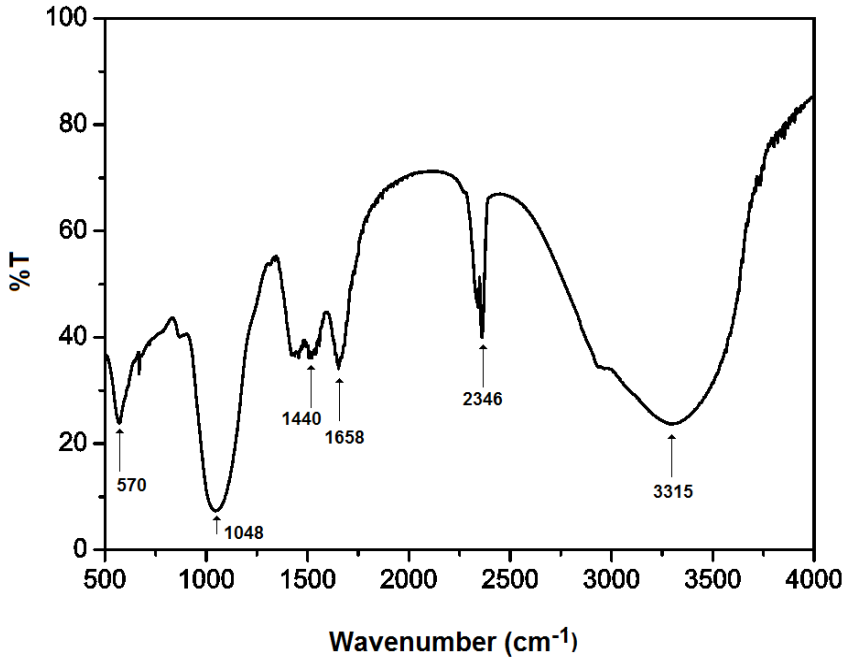


Figure 2. IR spectra of the powdered mucilage extracted from *Pereskia aculeata* Miller leaves.

The FT-IR data analysis showed a characteristic band in 1048cm^{-1}, which was attributed to polysaccharides with mannose, arabinose and rhamnose. The β-arabinogalactans presented one band around 1048cm^{-1} which may belong

to their particular components as arabinofuranose units in side branches (Kačuráková et al., 2000). According to Sierakowski et al. (1990), the polysaccharide complex of *Pereskia aculeata* leaves is highly ramified, containing arabinofuranose, arabinopyranose, galactopyranose, galactopyranosyl, uronic acid and rhamnopyranose units.

The absorption of around 1048cm^{-1} was attributed to the C-O (Capek, et al., 2013; Tajmir-Riahi, 1984), C-C stretching (Peng et al., 2012; Tajmir-Riahi, 1984) or C-OH bending (Singthong et al., 2009). The region at 1200-1000 cm-1 is dominated by ring vibrations overlapped with stretching vibrations of (C-OH) side groups and the (C-O-C) glycosidic bond vibration (Kačuráková et al., 2000).

From 1200 to 1800cm^{-1}, the distinctly smaller absorbance of “oses” means that the spectral signature of minor components of the polysaccharides - proteins and uronic acids - may be sought (Boulet et al., 2007). The proteins present specific absorption bands in the 1700-1500cm^{-1} region (Singthong et al., 2009). The wavenumbers in this region are usually associated with functional protein groups. The 1700-1600cm^{-1} band is associated with stretching vibrations of peptide bonds C=O and, therefore, directly related to the backbone confirmation, while 1600-1500cm^{-1} is associated with bending N-H vibrations (Capek et al., 2013).

Uronic acids are characterized by the carboxyl function which may lead to two absorbance peaks, a weak band in 1440cm^{-1} and a strong band in 1658 cm^{-1}, which may demonstrate the presence of the –COO- group, characteristic of vegetable gums (Boulet et al., 2007; Posé et al., 2012; Singh & Singh, 2011; Vinod et al., 2008). The absorption band in 1440cm^{-1} in the spectrum is assignable, especially, to the C-OH and C-CH bending vibrations (Tajmir-Riahi, 1984), and the band close to 1658cm^{-1} is due to the C=C (Singha et al., 2007) and C=O (Ehrenfreund-Kleinman et al., 2002) stretching.

The peak at about 2346cm^{-1} may be due to the C-H stretching of the CH_2 (Capek et al., 2013; Hu et al., 2011; Peng et al., 2012; Shah et al., 2013; Shing & Shing, 2011). The broad stretching peak around 3400cm^{-1} was ascribed to the hydroxyl groups (OH) of the monosaccharide units of arabinogalactans (Capek et al., 2013; Ehrenfreund-Kleinman et al., 2002; Hu et al., 2011; Peng et al., 2012; Shan, et al., 2013; Shing & Shing., 2011; Singha et al., 2007; Singthong et al., 2009; Tajmir-Riahi, 1984; Vinod et al., 2008).

The results suggested a hetero-polysaccharide with complex, branched structure, in addition to the association with proteins, constituting a special class of molecules, the arabinogalactan-proteins (AGPs).

The arabinogalactans (AGs) are structural polysaccharides with a complex molecular structure that is difficult to characterize (Aspinall, 1969; Aspinall, 1982; Whistler, 1970). Alone or associated with proteins mainly

present in plant cellular walls of both inferior and superior species, arabinogalactan has been the target of many structural studies (Mercê et al., 2001). Several reports in the literature describe the structural elucidation of these polymers, which are found in leaves, stems, roots, flowers, and seeds, as well as in high amounts in gums and vegetable exudates (Delgobo, Gorin, Jones & Iacomini, 1998; Fincher et al., 1983; Menestrina, Iacomini, Jones & Gorin, 1998).

Pereskia aculeata leaves are a mucilaginous material with 50% m/m composed of arabinogalactan polysaccharide (Sierakowski et al., 1987, 1990). The main interest in this biopolymer is its edibility (Mercê et al., 2001). Sierakowski et al. (1987) determined that the main chemical structures of the mucilaginous heteropolysaccharide of *P. aculeate* leaves were arabinose, galactose, rhamnose and galacturonic acid. For the *Pereskia aculeata* arabinogalactan, the arabinose to galactose ratio was 1:1.4.

3.3 Thermal analysis

3.3.1 Differential scanning calorimetry

Phase transitions in foods are often a result of changes in composition or temperature during processing or storage. Knowledge of transition temperatures and thermodynamic quantities are important to understand the processes such as: dehydration, evaporation, freezing and conservation.

Fig. 3 shows a comparison of DSC thermal profiles for gum OPN gel (5.0 g/100mL) and OPN powdered product. The curve of OPN powdered product showed an endothermic event, crystallite melting during heating, at about 81.6 °C (T_{onset}) and an exothermic event at about 218, 8 °C (T_{onset}), probably due to sample destruction. However with increasing water content (concentration of 5 g of OPN gum/100 mL of water) multiple melting endotherms were observed, which reflect the water and heat induced disorganization of crystallites. The samples with high water content showed single endotherms, which may be attributed to organization systems. Similar results were also found by Mothé and Rao (2000) that evaluated the thermal behavior of Arabic gum and cashew gum with various concentrations. The transition temperatures and estimation of associated enthalpies of the powdered OPN gum and gel with 5.0 g of OPN gum/100 mL of water are given in Table 2.

The OPN powdered gum presents elevated glass transition temperatures (Tg), which characterized thermal stability. This may be related to a high molecular weight constituent present in the material, as well as to the low humidity rate. The reconstituted gel of the OPN gum presents a higher amount of water in its constitution which leads to the water's plasticization and lower glass transition temperatures.

According to Roos (1995), the physical state of the foods is, generally, ruled by the transition phase of its main components. Since water is the main

component and diluent of the majority of the foods, it must significantly affect the physical state and the properties of the other compounds (Mothé and Rao, 2001). The water content of the materials has a strong influence over glass transition temperature. The water causes a drastic reduction in the Tg of food polymers (Slade and Levine, 1991).

The Tg varies with the composition of the foods, especially with the concentration of water. The knowledge of the glass transition temperature in regard to the water concentration of the foods is of extreme importance in the formulation and determination of the ideal food processing and storing conditions, maintaining the quality of the product for the longest possible time.

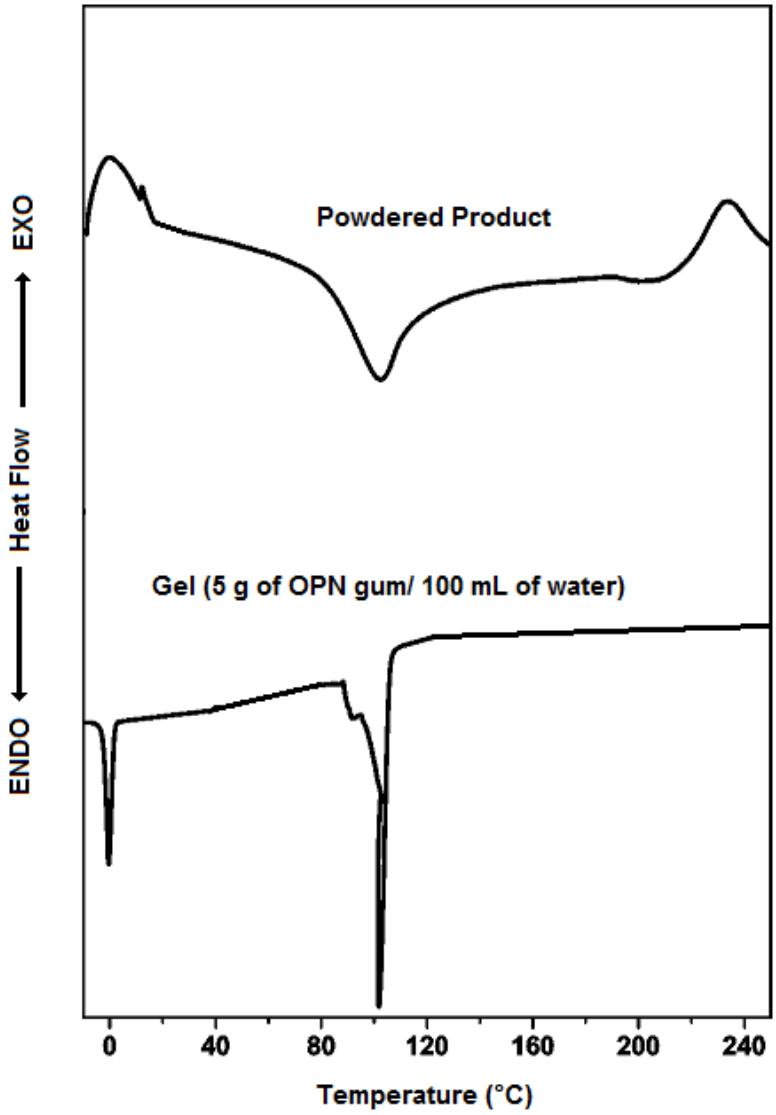


Figure 3 – Comparison of DSC thermograms of OPN gums in the powdered form and gel with 5.0 g of OPN gum/100 mL of water.

Table 2. DSC characteristics, transition temperatures and enthalpies in the powdered OPN gum and gel with 5.0 g of OPN gum/100 mL of water.

Endotherms peaks				
	T_{onset} (°C)	**T_{peak} (°C)**	**T_{end}(°C)**	**ΔH (J/g)**
OPN powdered gum	81.6	102.1	146.7	150.23
OPN gel (5.0 g/mL) 1° peak	-3.3	0.1	2.3	272.95
OPN gel (5.0 g/mL) 2° peak	92.8	102.5	115.5	1472.9
Exotherms peaks				
	T_{onset} (°C)	**T_{peak} (°C)**	**T_{end}(°C)**	**ΔH (J/g)**
OPN powdered gum	218.8	233.4	261.3	417.1

3.3.2. Thermogravimetry (TG)

Figure 4 shows TG curves for OPN powdered gum and OPN gel with concentration of 5.0 g/100 mL of water. The main observed thermal effects in Figure 4 can be described as follows. In two tested cases, after the buoyancy effects on the TG balance, at the very beginning of the run, there is an endothermic loss of adsorbed water in the biopolymer and its complexes (Mercê et al. 2001). The first stage occurred at around 64 °C, relative the water loss for the OPN in powder form and 45 °C (event 1) for the reconstituted gel with concentration of 5.0 g/100mL.

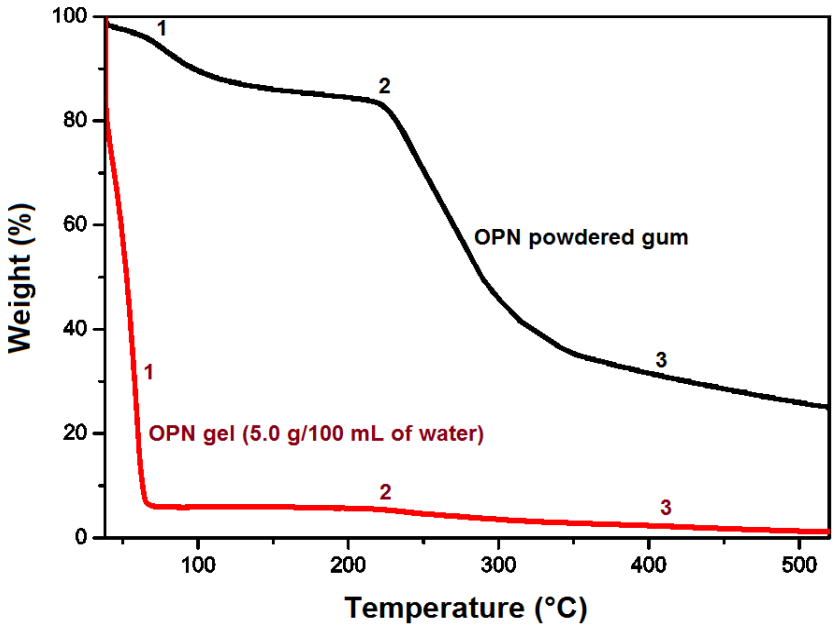


Figure 4 Thermogravimetric curves for OPN gums in the powdered form and gel with 5.0 g of OPN gum/100 mL of water.

Between 221 °C and 320 °C (event 2), there is a transition that could be assigned to a change in the conformation of the biopolymer followed by a break of branches, as the TG associated curves show a significant mass loss. This transition occurs due to oxidative degradation of the sample (Mercê et al., 2001). This mass loss can be attributed to polysaccharides and proteinaceous, with a composition 83 % and a residue of 44% in OPN powdered. The similar behavior were found by Mothé and Rao (2000) that resulted polysaccharides composition

of 73% and a residue of 15% in cashew gum and 65% of polysaccharides and a residue 20% for gum arabic in samples with low water content (0 % w/w). Final destruction (event 3) occurs in the temperature range of 390 to 430 °C. In this work we attributed that the high residue value is constituted of carbonaceous and minerals.

According to the thermogravimetric curves presented in Figure 4, the OPN powdered gum presents a relatively larger stability than the reconstituted OPN gum gel, possibly due to the higher water content in the gel.

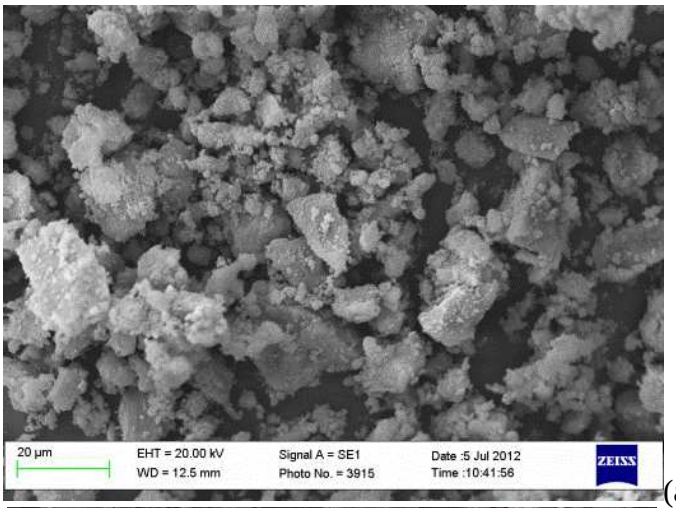
3.4 Microstructural analyses

3.4.1 Scanning eletronic microscopy (SEM)

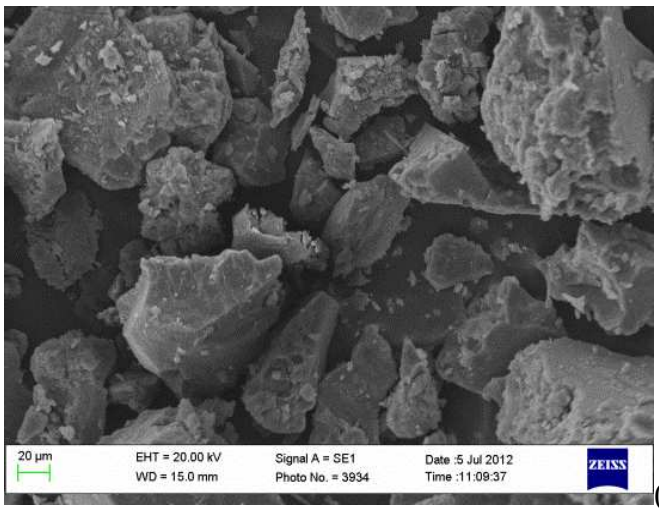
The analysis of the particle surfaces from OPN powdered gum and freeze-dried OPN gel with concentration of 5.0 g/100 mL of water was carried out at a three-dimensional level through electronic microscopy and the electromicrographs are presented in Figure 5a and 5b. Figure 5A refers to the OPN powdered gum, and it demonstrates the amorphous structures, high bulk porosity and strong attraction and adherence of the smaller particles to the surface of the larger particles.

One example of a system that involved the freeze-dried OPN gel with concentration of 5.0 g/100 mL of water is presented in Figure 5B. It was verified

that the particles were larger and the particles were uniform and did not strongly adhere to each other, verifying that the set contained scattered particles.



(a)



(b)

Figure 5 – Micrographs from scanning electronic microscopy of (a) OPN powdered gum and (b) freeze-dried OPN gel with concentration of 5.0 g/100 mL of water.

Comparing the Figures 5a and 5b, it is clear that the freeze-dried OPN gel structures are characterized by lower bulk porosity without a strong interaction among the particles. These features indicate that the structures were organized during the gelation and freeze-dried processes. Larger agglomerate with strong interactions and inter-particle adherence was observed in the powdered form. The non-interacting particles formed during the drying process could reduce the stickiness phenomenon. The electron micrographs presented for the gel at a concentration of 5g/100mL shows that with hydration of the molecules results an organized structure, having a uniform distribution and size of particles when compared to the hydrocolloid only. In Figure 5A should be noted that there is a higher porosity, differences in the particle sizes and smaller particles adhered in larger particles. The spongy aspect is also characteristic of a hygroscopic material.

3.4.2 Scanning eletronic microscopy (SEM)/Spectroscopy of Dispersive Energy by X-rays

The digital images of the powdered product and dried reconstituted gel were used to determine the mineral chemical compositions using spectroscopy of dispersive energy by X-rays as shown in Figure 6. The results of microanalyses for OPN powdered gum and freeze-dried OPN gels with

concentration of 5.0 g/100 mL of water are presented in Figures 7a and 7b, and Table 3, respectively.

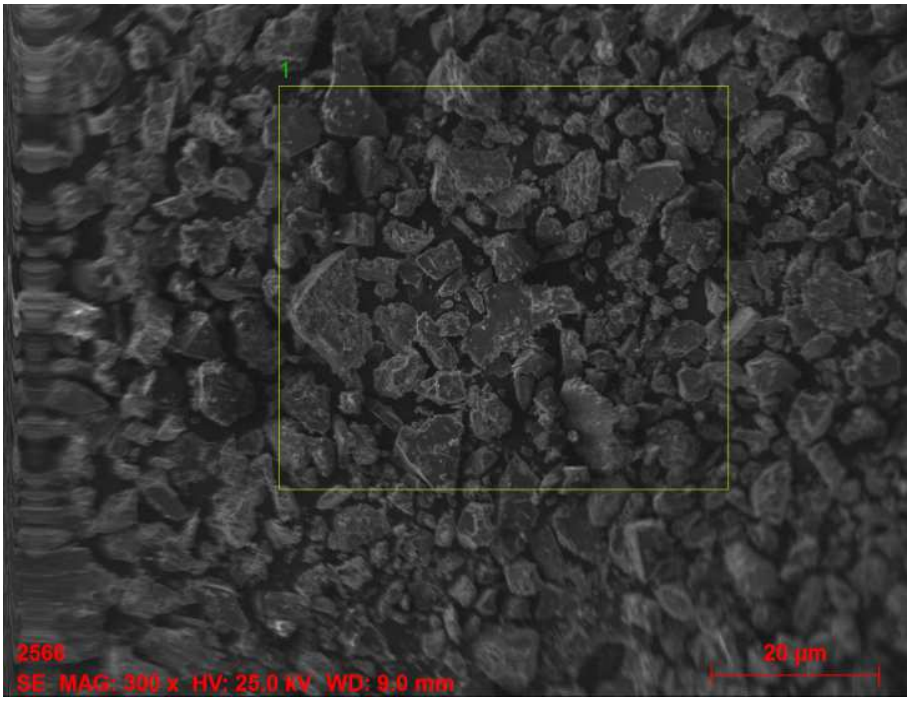
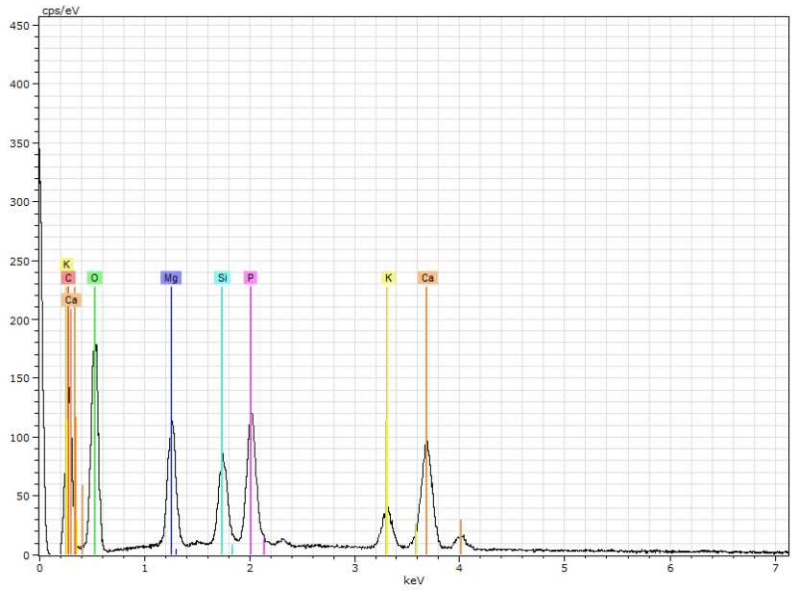
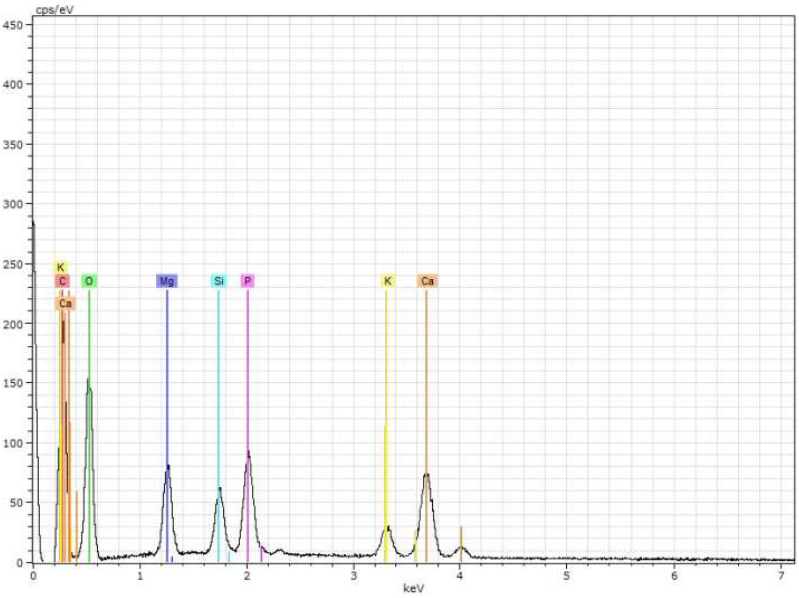


Figure 6 – Region identified in the digital image of freeze-dried OPN gel used in the mineral microanalyses.



(a)



(b)

Figure 7: Microanalysis of X-ray of hydrocolloid in (a) powdered form, and (b) gel with 5g/100mL of OPN gum.

Table3: Mass percentages of minerals present in the tested systems.

Mineral composition	**OPN powdered % (w/w)**	**Freeze-dried OPN gel % (w/w)**
Phosphorus	13,66	13,85
Potassium	5,10	5,350
Calcium	17,52	19,10
Magnesium	14,49	13,43
Silicon	6,46	6,00

The results shown in Table 3 confirm that large quantities of minerals are present and also that there were no significant differences in these parameters when the two systems are compared.

3.5 Emulsion microstructures

Tests were performed with various concentrations (1.0; 2.0 and 3.0 g/100 mL of OPN gum) to verify the emulsion microstructure and its stability at room temperature and at 80° C. One of the uses of hydrocolloids in the food industry is as an emulsion stabilizer. The microstructural analyses show the emulsion capacity in the product, and its performance increased with the increase of the powder concentration used in the preparation of the gum, as shown in Figures 8 and 9.

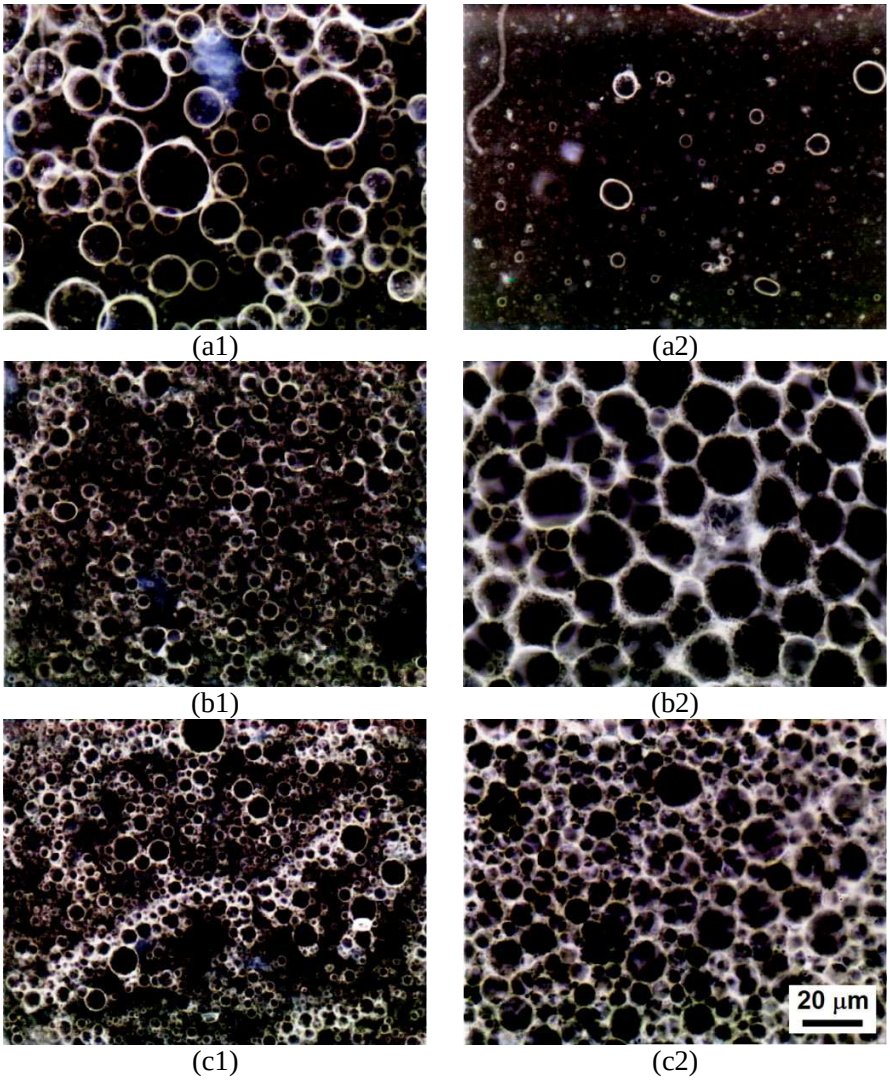


Figure 8 Micrographs of emulsions prepared at room temperature with different concentrations of OPN gum. (a) 1.0; (b) 2.0 and (c) 3.0 g of OPN/100 mL water; numbers (1) refer to fresh emulsion and (2) to emulsion after centrifugation.

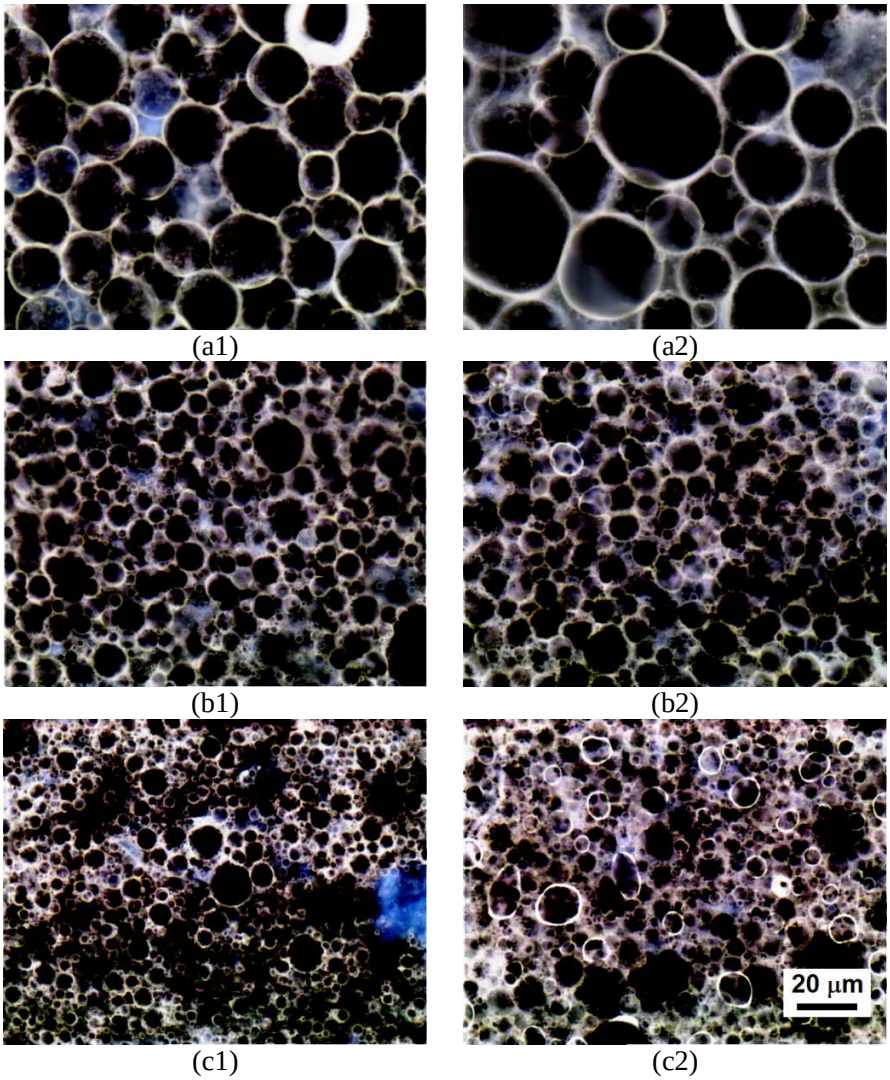


Figure 9 Micrographs of emulsions prepared at 80 °C with different concentrations of OPN gum. (a) 1.0; (b) 2.0 and (c) 3.0 g of OPN/100 mL water; numbers (1) refer to fresh emulsion and (2) to emulsion after centrifugation.

Figure 8(a1) shows that emulsions prepared with OPN gum with concentration of 1% at room temperature are unstable and disintegrate with the centrifugation. The droplets coalescence is observed in emulsions with 2.0 g of OPN gum/100 mL of water that had diameters of 2-10 μm (fig. 8b1) in the fresh emulsion and diameters of 5-22 μm in the emulsion after centrifugation. Figs. 8c1 and 8c2 also show the occurrence of droplets coalescence in the emulsions prepared with concentration of 3.0 g of OPN gum /100 mL of water, at room temperature. In this case the emulsions were more stable and the diameters were of 1-8 μm for fresh and 1-12 μm for centrifuged emulsion.

Fig. 9 shows the increased stability of emulsions that were prepared with OPN gum at 80° C when compared with those prepared at room temperature. Strong droplets coalescence was observed by the increase of diameter for the concentration of 1.0 g of OPN gum/100 mL of water (figs. 9a1 and a2). For concentrations of 2.0 and 3.0 g of OPN gum/100 mL of water (figs. 9b1, b2, c1 and c2) the emulsion droplets were numerous and with small diameters that remained unchanged after centrifugation.

Most hydrocolloids can act as stabilizers (stabilizing agents) of oil-in-water emulsions, but only a few can act as emulsifiers (emulsifying agents). The latter functionality requires substantial surface activity at the oil–water interface, and hence the ability to facilitate the formation and stabilization of fine droplets during and after emulsification (Dickinson, 2009).

To form a fine emulsion, large deformable drops must be broken down by the vigorous application of mechanical energy (Dickinson, 2009). Emulsification involves the sudden creation of a large amount of new liquid interface. The main role of the emulsifier is to adsorb at the surface of the freshly formed fine droplets and so prevent them from coalescing with their neighbors to form larger droplets again. When the emulsifier adsorbs too slowly, or is present at too low a concentration, most of the individual droplets formed during the intense energy dissipation of emulsification are not retained in the final emulsion. This may be due to breakage of the thin film between colliding droplets (coalescence) or sharing of the adsorbed layer between two droplets (bridging flocculation). The latter phenomenon is prevalent in concentrated emulsions (e.g., homogenized cream) which have a relatively low emulsifier/oil ratio, and in less concentrated systems containing mixed polymeric emulsifiers of different surface activity (Dickinson, 2009).

It is generally important that emulsion droplets are made as small as possible in order to minimize gravity creaming effects (Dickinson, 2009). The nature of the environmental conditions to which the system will be subjected is important to determine the bulk emulsifier concentration required to produce the minimum mean droplet size (maximum surface area per unit volume of oil). These conditions include factors such as temperature, pH, ionic strength, calcium ion content, and so on.

The most widely used polysaccharide emulsifiers in food applications are gum arabic (*Acacia senegal*), modified starches, modified celluloses, some kinds of pectin, and some galactomannans. The surface activity of these hydrocolloids has its molecular origin in either (i) the non-polar character of chemical groups attached to the hydrophilic polysaccharide backbone (in hydrophobically modified starch/cellulose) or (ii) the presence of a protein component linked covalently or physically to the polysaccharide (some gums, pectins, etc.).

The emulsifying properties of gum Arabic are associated with a high-molecular-weight fraction representing less than 30% of the total hydrocolloid (Randall, Phillips, & Williams, 1988). The protein is covalently bound to the carbohydrate in the form of a mixture of arabinogalactan–protein complexes, each containing several highly branched polysaccharide units linked to a common protein core. The protein chain firmly anchors the complex to the oil–water interface, and the charged polysaccharide units attached to the protein chain provide a steric barrier against droplet flocculation. Gum arabic is an extremely effective emulsifier at low pH, at high ionic strength, and in the presence of beverage colorings agents.

In a previous work (Lima Junior et al., 2013), tests were performed to verify the emulsion formation capacity of the reconstituted product and its stability at room temperature and at 80° C. The emulsion capacity in the product

was verified, and its performance increased with the increase of the powder concentration used in the preparation of the gum. The gums of *Pereskia aculeata* Miller obtained with a solution concentration of 1 g/100 mL presented an emulsion formation capacity of 83%.

A pure polysaccharide provides emulsion stability through solution viscosity, since it does not have surface active properties (Lima Junior et al., 2013). Most polysaccharides have some proteins in the extracts, and these may give some surface activity. A very few gums have a conjugated protein, like gum arabic, for example, which gives rise to its emulsifying properties. The data shown in Table 1 suggests that OPN is heteropolymolecular. Therefore, the OPN gum consists of molecules that differ in their sugar composition and their mode of linkage as well as in molecular mass (Randall, Phillips & Williams, 1989).

According to Sierakowski et al., (1990), the polysaccharide complexes of the OPN are highly ramified, containing arabinofuranose, arabinopyranose, galactopyranose, galactopyranosyl, uronic acid and rhamnopyranose units. In addition, OPN gum present high nitrogen content and is extremely rich in protein and the significance of these proteinaceous components can be responsible for formation capacity and emulsion stabilization (Randall, Phillips & Williams, 1988, Randall et al, 1989). There is also a reasonably good correlation between the limiting interfacial tension and the

nitrogen content of the Acacia gum (Dickinson, Murray, Stainsby & Douglas, 1988).

Dickinson et al., (1988) considered that the nitrogen content of the Acacia gum is a measure of the amount of bound protein (or polypeptide). OPN gum presented higher nitrogen contents (1.39 g/100g) than *Acacia* gum which importance of the proteinaceous components to the emulsification properties has been demonstrated (Randall et al., 1988). The surface and emulsifying properties of OPN gum were related to its macromolecular structure (Lima Junior, et al., 2013).

4 CONCLUSIONS

The extraction process of obtaining powdered OPN mucilage presented the ratio of water: raw material and extraction temperature of 2.5 L/kg and 75 °C, respectively, verified after the optimization. The mucilage shows high contents of protein and minerals such as calcium, potassium, phosphorus, magnesium and sulfur, and low contents of uronic acids and total carbohydrate. These results are influenced by many factors, such as the different parts of the plant used in obtaining the mucilage (leaves, seeds, fruits), plant species, geographic locations (climate and soil) and, especially, the extraction process conditions (temperature, pH, solvent, time, etc.).

The FT-IR spectrum suggested a hetero-polysaccharide with a branched complex structure, associated with proteins, which constituted arabinogalactan-proteins. Differential scanning calorimetry thermal profiles of OPN powdered product showed endothermic and exothermic events that allows identify systems organization and samples destructions. The OPN powdered product presented higher thermal stability when compared to the reconstituted gel from the OPN gum for presenting smaller water content and high glass transition temperatures. Thermogravimetry curves for OPN gums show high residue value which is attributed to its carbonaceous and mineral contents. The scanning electronic microstructure micrographs of OPN powdered gum show a high porosity, differences in particle sizes and smaller particles adhered to larger particles and a spongy aspect which suggest that the material is hygroscopic, while the freeze-dried OPN gel presented a more organized structure due to the hydration and reorganization of its molecules.

Scanning electronic microscopy/Spectroscopy of Dispersive Energy by X-rays confirmed that large quantities of minerals are present in the samples. The emulsion formation capacity of the product was proportionate to the increase of powdered gum concentration used for the preparation. Strong droplets coalescence as being proportional to the reduced powdered gum concentration. The emulsions prepared with OPN gum at 80 ^{o}C presented a higher stability when compared to those prepared at room temperature.

In front of this, *Pereskia aculeata* Miller (OPN) constitutes an alternative source for mucilage, with properties which may be used in the industry as a thickening, gelling and/or emulsifying agent for food applications.

5. ACKNOWLEDGMENTS

The authors wish to thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG- Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Brazil) for financial support for this research.

6 REFERENCES

Abbott, T. F., Nelsen, T. C., Kleiman, R. (1995). Seed surface gums content using the Dubois carbohydrate analysis. Industrial Crops and Products, 4, 185-192.

Almeida-Filho J, Cambraia J. 1974. Estudo do valor nutritivo do 'ora-pro-nobis' (*Pereskia aculeata* Mill.). Revista Ceres. 21:105111.

AOAC - Association of Official Analytical Chemists. Official methods of Analysis. 18 ed. Washington DC USA, 2006.

Aspinall, G. O. (1969). Gums and mucilages. *Advances in Carbohydrate Chemistry & Biochemistry*, 24, 333- 379.

Aspinall, G. O. (1982). *The polysaccharides*. New York: Academic. 1: 503.

Batsoulis, A. N., Nacos, M.K., Pappas, C. S., Tarantilis, P. A., Mavromoustakos, T., Polissiou, M. G. Determination of Uronic Acids in Isolated Hemicelluloses from Kenaf Using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) and the Curve-Fitting Deconvolution Method. Applied Spectroscopy, 58, 199-202. 2004.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. Analytical Biochemistry, 54, 484–489.

Boulet, J.C.; Williams, P.; Doco, T. (2007). A Fourier transform infrared spectroscopy study of wine polysaccharides. Carbohydrate Polymers, 69, 79-85.

Butterworth CA, Wallace RS. (2005). Molecular phylogenetics of the leafy cactus genus Pereskia (Cactaceae). System Bot. 30:800-808.

Capek, P.; Matulová, M.; Navarini, L.; Suggi-Liverani, F. (2013). Molecular heterogeneity of arabinogalactan-protein from *Coffea arabica* instant coffee. International Journal of Biological Macromolecules, 59, 402-407.

Chitarra, L. G., Chitarra, A. B., Machado, J. C., Menezes, J. B. (1998). Neutral sugars, uronic acids and electric conductivity of cottonseeds (*Gossypium hirsutum* L.) exudate in function of the chemical delinting. Brazilian Archives of Biology and Technology, 41 (1), 125-139.

Dayrell MS, Vieira EC. (1977). Leaf protein concentrate of the cactacea *Pereskia aculeata* Mill. I. Extraction and composition. Nutr Rep Int 15:529 - 537.

Delgobo, C. L., Gorin, P. A. J., Jones, C. & Iacomini, M. (1998) Gum heteropolysaccharides and free reducing mono- and oligosaccharides of *Anadenanthera colubrina. Phytochemistry*, 47, 1207-1214.

Dickinson, E., Murray, B. S., Stainsby, G. & Anderson, D. M. W. (1988). Surface activity and emulsifying behavior of some *Acacia* gums. *Food Hydrocolloids*, 2, 477-490.

Dickinson, E. (2009) Hydrocolloids as emulsifiers and emulsion stabilizers. Food Hydrocolloids, 1473–1482.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28, 350–356.

Ehrenfreund-Kleinman, T.; Azzam, T.; Falk, R.; Polacheck, I.; Golenser, J.; Dom, A. J. (2002). Synthesis and characterization of novel water soluble amphotericin B–arabinogalactan conjugates. Biomaterials, 23 1327-1335.

Fincher, C. B., Stone, B. A. & Clarke, A. E. (1983). Arabinogalactan-proteins: structure, biosynthesis and function. Annual *Review of* Plant Physiology, 34, 47-70.

Furuta, H.; Maeda, H. (1999). Rheological properties of water-soluble soybean polysaccharides extracted under weak acidic condition. Food Hydrocolloids. 13, 267–274.

Hori, R.; Sugiyama, J. (2003). A combined FT-IR microscopy and principal component analysis on softwood cell walls. Carbohydrate Polymers, 52, 449-453.

Hu, C.; Kong, Q.; Yang, D.; Pan, Y. (2011). Isolation and structural characterization of a novel galactomannan from *Eremurus anisopterus* (Ker. et Kir) Regel roots. Carbohydrate Polymers, 84, 402-406.

Ibañez, M. C. & Ferrero, C. (2003). Extraction and characterization of the hydrocolloid from *Prosopis Flexuosa* DC seeds. *Food Research International*, 36, 455-460.

Kačuráková, M.; Capeka, Sasinková, P.; V.; Wellner, N.; Ebringerova, A. (2000). FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohydrate Polymers. 43, 195-203.

Karazhiyan, H.; Razavi, S. M. A.; Phillips, G. O. (2011).Extraction optimization of a hydrocolloid extract from cress seed (*Lepidium sativum*) using response surface methodology. Food Hydrocolloids. 25, 915-920.

Lai, L.S. & Liang H.Y. (2012). Chemical compositions and some physical properties of the water and alkali-extracted mucilage from the young fronds of Asplenium australasicum (J. Sm.) Hook. Food Hydrocolloids. 26. 344-349.

Lima Junior, F. A., Conceição, M. C., Vilela de Resende, J., Junqueira, L. A., Pereira, C. G. ; Prado, M. E. T. (2013) Response surface methodology for optimization of the mucilage extraction process from *Pereskia aculeata* Miller. Food Hydrocolloids, v. 33, p. 38-47, 2013.

Lin, H. Y., & Lai, L. S. (2009). Isolation and viscometric characterization of hydrocolloids from mulberry (Morus alba L.) leaves. Food Hydrocolloids, 23(3), 840-848.

Lin, H. Y., Tsai, J. C., & Lai, L. S. (2009). Effect of salts on the rheology of hydrocolloids from mulberry (Morus alba L.) leaves in concentrated domain. Food Hydrocolloids, 23(8), 2331-2338.

Malavolta, E.; Vitti, G.C.; Oliveira, A.S. (1989). Avaliação do estado nutricional das plantas: princípios e aplicações. Piracicaba: Associação Brasileira para Pesquisa da Potássio e do Fosfato. 201p.

Menestrina, J. M., Iacomini, M., Jones, C., & Gorin, P. A. J. (1998). Similarity of monosaccharide, oligosaccharide and polysaccharide structures in gum exudate of *Anacardium occidentale*. Phytochemistry, New York, v. 47, p. 715-721.

Mercê, A.L.R., J.S. Landaluze, A., Mangrich, B. Szpoganicz & M.R. Sierakowski (2001). Complexes of arabinogalactan of *Pereskia aculeata* and Co^{2+}, Cu^{2+}, Mn^{2+}, and Ni^{2+}. Bioresource Technology. 76: 29-37.

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

Peng, H.; Wang, N.; Hu, Z.; Yu, Z.; Liu, Y.; Zhang, J.; Ruan, R. (2012) Physicochemical characterization of hemicelluloses from bamboo (*Phyllostachys pubescens Mazel*) stem. Industrial Crops and Products, 37, 41-50.

Posé, S.; Kirbyc, A. R.; Mercado, J. A.; Morris, V. J.; Quesada, M. A. (2012). Structural characterization of cell wall pectin fractions in ripe strawberry fruits using AFM. Carbohydrate Polymers, 88, 882– 890.

Randall, R. C., Phillips, G. O. & Williams, P. A. (1988). The role of the proteinaceous component on the emulsifying properties of gum arabic. *Food Hydrocolloids*, 2, 131-140.

Randall, R. C., Phillips, G. O. & Williams, P. A. (1989). Fractionation and characterization of gum from *Acacia Senegal*. *Food Hydrocolloids*, 3, 65-75.

Roos, Y. H. Phase transitions in foods. San Diego, California: Academic Press. 1995.

Rosa, S. M. & Souza, L. A. (2003). Morfo-anatomia do fruto (hipanto, pericarpo e semente) em desenvolvimento de *Pereskia aculeata* Miller (Cactaceae). *Acta Scientiarum, Biological Science*, 25, 415-428.

Sepúlveda, E., Sáenz, C., Aliaga E., Aceituno, C. (2007). Extraction and characterization of mucilage in *Opuntia* spp. Journal of Arid Environments, 68 534-545.

Shah, S. M.; Pathak, P. O.; Jain, A. S.; Barhate, C. R.; Nagarsenker, M. S. (2013). Synthesis, characterization, and in vitro evaluation of palmitoylated arabinogalactan with potential for liver targeting. Carbohydrate Research, 367 41-47.

Sierakowski, M.-R., Gorin, P.A.J., Reicher, F., Corrêa, J.B.C. (1987). Some structural features of a heteropolysaccharide from the leaves of the cactus Pereskia aculeata. Phytochem. 26, 1709-1713.

Sierakowski, M.R.; Gorin, P.A.J.; Reicher, F.; Corrêa, J.B.C. Location of *O*-acetyl groups in the heteropolysaccharide of the cactus *Pereskia aculeata*. (1990).Carbohydr Res., 201: 277-284.

Singh, V.; Singh, S. K. (2011). Synthesis and characterization of gum acacia inspired silica hybrid xerogels for mercury(II) adsorption. International Journal of Biological Macromolecules, 48, 445-451.

Singha, P. K.; Roy, S.; Dey, S. (2007). Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. against ethanol-induced toxicity in mice. Journal of Ethnopharmacology, 111, 13-21.

Singthong, J., W., Ningsanond, S., Cui, S. W. (2009). Extraction and physicochemical characterisation of polysaccharide gum from Yanang (Tiliacora triandra) leaves. Food Chemistry. 114, 1301-1307.

Slade, L., Levine, H. (1991). Beyond water activity: recente advances based on an alternative approach to the assessmentof food quality and safety. Critical Reviews in Food Science and Nutrition. 30, 115-360.

Takeiti, C. Y., Antonio, G. C., Motta, E. M. P., Collares-Queiroz, F. P., Park, K. J. Nutritive evaluation of a non-conventional leafy vegetable (*Pereskia aculeata* Miller). International Journal of Food Sciences and Nutrition, August 2009; 60(S1): 148-.160.

Tajmir-Riahi, H.-A. (1984). Infrared spectra of crystalline l-arabinose and two of its calcium complexes. Carbohydrate Research, 127, l-8.

Vinod, V. T. P.; Sashidhar, R. B.; Suresh, K. I.; Ramo Rao, B.; Vijaya Saradhic, U. V. R.; Prabhakar Rao, T. (2008). Morphological, physico-chemical and structural characterization of gum kondagogu (*Cochlospermum gossypium*): A tree gum from India. Food Hydrocolloids, 22, 899-915.

Xie, J. H.; Liu, X.; Shen, M. Y.; Nie, S. P.; Zhang, H.; Li, C.; Gong, D. M; Xie, M. Y. (2013). Purification, physicochemical characterisation and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves. Food Chemistry. 136, 1453–1460.

Whistler, R. L. *The carbohydrates: chemistry and biochemistry*. London: Academic, 1970. p. 447-458.

Yamazaki, E., Kurita, O., Matsumura, Y. Hydrocolloid from leaves of Corchorus olitorius and its synergistic effect on k-carrageenan gel strength. Food Hydrocolloids. 22, 819-825. 2008.

Yapo, B. M. (2009a). Biochemical characteristics and gelling capacity of pectin from yellow passion fruit rind as affected by acid extractant nature. Journal of Agricultural and Food Chemistry, 57, 1572-1578.

Yapo, B. M. (2009b). Pectin quantity, composition and physicochemical behaviour as influenced by the purification process. Food Research International, 42, 1197-1202.

Yapo, B. M. (2009c). Lemon juice improves the extractability and quality characteristics of pectin from yellow passion fruit by-product as compared with commercial citric acid extractant. Bioresource Technology, 100, 3147-3151.

Yapo, B. M., & Koffi, K. L. (2008). The polysaccharide composition of yellow passion fruit rind cell wall: chemical and macromolecular features of extracted pectins and hemicellulosic polysaccharides. Journal of the Science of Food and Agriculture, 88, 2125-2133.

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007a). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. Food Chemistry, 100, 1356-1364.

Yapo, B. M., Wathelet, B., & Paquot, M. (2007b). Comparison of alcohol precipitation and membrane filtration effects on sugar beet pulp pectin chemical features and surface properties. Food Hydrocolloids, 21, 245-255.

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