UNIVERSIDADE FEDERAL DE LAVRAS

TATIANA NUNES AMARAL

COMPORTAMENTO REOLÓGICO E PROPRIEDADES TÉRMICAS DA GOMA DA *Pereskia aculeata* Miller ADICIONADA DE SOLUTOS E HIDROCOLOIDES COMERCIAIS E UMA APLICAÇÃO EM BEBIDA LÁCTEA FERMENTADA

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, área de concentração em Ciência dos Alimentos, para a obtenção de título de Doutor.

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2016

A Deus, aos meus pais Solange e Edson e ao meu marido Jayme
pelo amor e incentivo sempre.

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RESUMO GERAL

A mucilagem da *Pereskia aculeata* Miller apresenta propriedades de aplicação na indústria como espessante, gelificante e/ou emulsificante. O conhecimento desta mucilagem em sistemas alimentícios pode ser estudado em soluções aquosas, importante no estudo de componentes isolados. Produtos lácteos fermentados podem ser produzidos com hidrocoloides, como goma guar (GG) e goma arábica (GA), para manter a qualidade durante a vida de prateleira como substituto de gordura. Os objetivos, neste trabalho, foram caracterizar o comportamento reológico e as propriedades térmicas da mucilagem do ora-pro-nobis em diferentes soluções modelo, avaliando a aplicação dos hidrocoloides em bebida láctea fermentada. Mucilagem do ora-pro-nobis foi extraída e as gomas preparadas por agitação (30 minutos/75 °C). Parâmetros reológicos e termogravimétricos foram avaliados. O planejamento experimental foi dividido em fatorial fracionado 2^{5-1} para a seleção de fatores (mucilagem de ORA-PRO-NOBIS, sacarose, cloreto de sódio, cloreto de cálcio e pH) e fatorial completo 5x3x3 (mucilagem de ora-pro-nobis, sacarose e cloreto de sódio). Delineamento de mistura foi aplicado aos estudos em soluções modelo compostas de mucilagem de ora-pro-nobis, GA, GG, sacarose, cloreto de sódio e água deionizada. Propriedades reológicas foram avaliadas e um mix de hidrocoloides foi definido e aplicado a bebidas lácteas fermentadas. Propriedades reológicas e microestruturais foram estudadas. Soluções modelo estudadas para a seleção de fatores apresentaram comportamento pseudoplástico na presença de mucilagem de ora-pro-nobis, tendo efeito significante na viscosidade aparente. Análises termogravimétricas mostraram a presença de três fases distintas da perda de massa com o incremento de temperatura, o que pode ser relacionado à perda de água, a mudanças na conformação dos polissacarídeos e finalizando com material mineral remanescente. Mucilagem de ora-pro-nobis, sacarose e cloreto de sódio foram as variáveis com efeitos significantes nas respostas termogravimétricas. Nos estudos no fatorial completo, as amostras apresentaram comportamento newtoniano com 0% a 1,25% mucilagem de ora-pro-nobis e comportamento da Lei da Potência com 2,50% a 5,00% de mucilagem de ora-pro-nobis, com predominância de comportamento elástico (G' > G'' a 1Hz). A viscosidade aparente teve uma tendência de crescimento com o incremento da concentração de mucilagem de ora-pro-nobis. Os valores de G' foram maiores que G'', confirmando o caráter predominante elástico das amostras. O incremento nas concentrações de mucilagem de ora-pro-nobis aumentou o comportamento elástico, contribuindo para a formação de géis mais fortes. Maiores valores de viscosidade aparente das soluções modelo com diferentes hidrocoloides ocorreram na região referente ao mix de hidrocoloides, composta de 0,7 mucilagem de ora-pro-nobis, 0,0 GA e 0,3 GG, resultando em 0,5-0,7

Pa.s. Sacarose teve maior interferência que o cloreto de sódio na viscosidade aparente. O efeito da viscosidade aparente, G’ e G” foi discutido e as soluções modelo apresentaram comportamento antitixotrópico e pseudoplástico, com bom ajuste para o modelo reológico da lei da potência. As amostras de leite fermentado apresentaram bom ajuste para a lei da potência, comportamentos pseudoplásticos e tixotrópicos e tiveram comportamento elástico (G’>G”). A aplicação do mix de hidrocoloides aumentou a viscosidade aparente e o pH das amostras. A avaliação da microestrutura confirmou os resultados. A aplicação do mix de hidrocoloides com mucilagem de ora-pro-nobis em leites fermentados foi viável.

Palavras-chave: Aditivo de alimentos. Cloreto de sódio. Microestrutura. Sacarose. Sinergia. Viscosidade.

GENERAL ABSTRACT

Pereskia aculeata Miller mucilage presents properties that can be used in industry as thickener, gelling agent and/or emulsifier. The understanding of OPN mucilage in food systems can be studied in aqueous solutions, which are widely used for studying of isolated compounds. Fermented milk products can be produced with hydrocolloids, such as guar gum (GG) and gum Arabic (GA), to maintain their quality during the shelf-life period and to replace fat. The objectives of this study were to characterize the rheological behavior and thermal properties of OPN mucilage in different model solutions, and to evaluate the application of hydrocolloids in fermented milk beverages. OPN mucilage were extracted and the gums were prepared stirring for 30 min at 75°C. Rheological and thermogravimetric parameters were evaluated. The experimental design was divided into a 2^{5-1} fractional factorial for screening factors (OPN mucilage, sucrose sodium chloride, calcium chloride and pH) and a 5x3x3 factorial (OPN mucilage, sucrose and sodium chloride). A mixture design was applied to studies of model solutions composed by OPN mucilage, GA, GG, sucrose, sodium chloride and deionized water. Rheological properties were evaluated. A hydrocolloid mix was defined and applied to a fermented milk beverage. Rheological and microstructural properties were studied. Model solutions studied for the selection factors presented pseudoplastic behavior in the presence of OPN mucilage, having significant effect over the apparent viscosity. Thermogravimetric analysis showed the presence of three distinct phases of mass loss with increasing temperatures, and may be related to water loss, change in conformation of polysaccharides, ending with remaining mineral material. OPN mucilage, sucrose and sodium chloride had significant effect over thermogravimetry responses. In full factorial studies, the samples presented Newtonian behavior with 0-1.25% OPN mucilage, and Power Law behavior with 2.50-5.00% OPN mucilage with a predominance of elastic behavior (G' > G" at 1Hz). Apparent viscosity had an increasing tendency with the increased concentration OPN mucilage. G' values are higher than those of G", confirming elastic predominant characteristic of the samples. Increasing OPN mucilage concentration increases this elastic behavior, contributing to the formation of stronger gels.The highest values for apparent viscosity of the model solutions with different hydrocolloids occurred on the region with hydrocolloids mix composed of 0.7 OPN mucilage, 0.0 GA and 0.3 GG, resulting in 0.5-0.7 Pa.s. Sucrose presented greater interference than sodium chloride over apparent viscosity. The effect of apparent viscosity, G’ and G”, were discussed, and the model solutions presented anti-thixotropic and pseudoplastic behavior with good fit to the Power Law rheological model. Fermented milk samples presented good fit to Power law and pseudoplastic and thixotropic behavior, also presenting predominant elastic behavior (G’>G”). The application of hydrocolloids mix increased the apparent viscosity and pH of the samples. The microstructure evaluation confirmed the results. Application of hydrocolloids mix with OPN mucilage on fermented milk showed feasibility.

Keywords: Food additive. Microstructure. Sodium chloride. Sucrose. Synergy. Viscosity.

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LISTA DE SIGLAS

$CaCl_2$	Calcium chloride
CAPC	Chemistry Analysis and Prospection Center
FL	Fermented low fat milk
FLH	Fermented low fat milk with hydrocolloids
FW	Fermented whole milk
FWH	Fermented whole milk with hydrocolloids
GA	Gum arabic
GG	Guar gum
L	Low fat milk
LH	Low fat milk with hydrocolloids
NaCl	Sodium chloride
OPN	*Pereskia aculeata* Miller
SUC	Sucrose
TG	Thermogravimetry
T_{endset}	Temperature of final destruction (TG)
W	Whole milk
WH	Whole milk with hydrocollois

LISTA DE SÍMBOLOS

I	Components
J	Number of points
K	Consistency coefficient
N	Flow behavior index
q	Total number of components
$\hat{y}$	Predicted value
$\hat{\beta}$	Parameter estimative
$\hat{\beta}$	Parameter value
μ	Viscosity (Pa.s)
τ	Shear stress (Pa)
$\dot{\gamma}$	Shear rate (s^{-1})

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PRIMEIRA PARTE

1 INTRODUÇÃO

Os aditivos utilizados nas indústrias de alimentos têm funções importantes para a garantia da produção de alimentos de boa qualidade e possibilitam a aplicação de tecnologias de processamento. Um exemplo desses aditivos são os hidrocoloides, também conhecidos como gomas ou mucilagens, que têm funções estruturais para os produtos, como dar características de espessamento e gelificação. Novas fontes de extração de hidrocoloides são objetos de estudo para aumentar a oferta no mercado e abrir novas possibilidades de aplicações.

O ora-pro-nobis (*Pereskia aculeata* Miller) é uma hortaliça não convencional rica em nutrientes como proteínas, fibras, carboidratos, ferro e o cálcio. Estas características possibilitam o estudo de sua mucilagem como nova fonte de hidrocoloides, além da disponibilidade no Brasil e do relativo baixo custo de produção.

O conhecimento do comportamento dos hidrocoloides possibilita a correta aplicação nos meios alimentícios, sendo definido por meio de estudos das características reológicas, físico-químicas e microestruturais, entre outros. Estas informações auxiliam na previsão do comportamento nos meios nos quais os hidrocoloides vão ser aplicados.

Como os alimentos são sistemas complexos, os estudos, normalmente, são realizados em soluções modelo compostas de componentes comuns neles, como sacarose e cloreto de sódio. Parâmetros como pH, temperatura e concentração de solutos interferem na capacidade dos hidrocoloides de formar géis. Com a definição do comportamento em soluções modelo, a aplicação em sistemas alimentícios reais se torna mais assertiva.

Os objetivos, no presente trabalho, foram avaliar o comportamento dos hidrocoloides do ora-pro-nobis em presença de cátions e sacarose em soluções modelo, possíveis sinergias com hidrocoloides comerciais e aplicação em bebida láctea fermentada.

2 REFERENCIAL TEÓRICO

2.1 Aditivos de alimentos

Aditivos alimentares são ingredientes adicionados intencionalmente aos alimentos, com o objetivo de modificar suas características físicas, químicas, biológicas ou sensoriais, sem finalidade nutricional. Os aditivos são classificados de acordo com a função exercida e entre eles estão os espessantes, que aumentam a viscosidade de um alimento, e os geleificantes, que conferem textura por meio da formação de gel segundo a Agência Nacional de Vigilância Sanitária, ANVISA, (BRASIL, 1997). Os hidrocoloides são exemplos de aditivos espessantes e gelificantes. Com base em princípios da análise de risco, a ANVISA determina quais aditivos são permitidos para cada categoria de alimentos, com suas funções e limites máximos de uso, visando proporcionar o efeito tecnológico desejado sem oferecer risco à saúde humana (ANVISA, 2016).

Continuamente, universidades e centros de pesquisa estudam novos aditivos para aplicação em alimentos, com o objetivo de diminuir impactos de sazonalidade e variação de preços que ocorrem usualmente no mercado, além da utilização de recursos regionais como fonte dos aditivos. A pesquisa de novas fontes de hidrocoloides é um exemplo dessas pesquisas, os quais são aditivos de larga aplicação na indústria de alimentos com diversas funções, como espessante, gelificante e emulsificante (AMIN et al., 2007; KARAZHIYAN; RAZAVI; PHILLIPS, 2011; THANATCHA; PRANEE, 2011; ZINJARDE, 2014).

2.2 Hidrocoloides

2.2.1 Definições

O termo hidrocoloides refere-se a uma série de polímeros de cadeia longa, polissacarídeos e proteínas, os quais são totalmente ou parcialmente solúveis em água. Esses compostos são amplamente utilizados como aditivos alimentares em uma variedade de setores industriais, para realizar funções como espessar e/ou gelificar soluções aquosas, estabilizar espumas, modificar e/ou controlar as propriedades de fluxo e a textura dos alimentos líquidos e das bebidas, inibir a formação de cristais de gelo e de açúcar, controlar a liberação de sabores, modificar as propriedades de deformação de produtos semissólidos e substitutos de gordura, entre outros, no intuito de melhorar a qualidade do produto final (ADITIVOS E INGREDIENTES, 2012).

Esses aditivos têm grande quantidade de grupos hidroxila em sua cadeia, o que garante grande afinidade para a ligação com moléculas de água, tornando-os hidrofílicos. Além disso, quando em contato com a água, produzem uma dispersão, que é um sistema intermediário entre uma verdadeira solução e uma suspensão, exibindo as propriedades de um coloide. Consequentemente, eles são apropriadamente denominados "hidrocoloides" (SAHA; BHATTACHARYA, 2010). Assim, a principal razão para o extensivo uso de hidrocoloides em alimentos é por se ligarem à água e por modificarem, dessa maneira, as propriedades dos componentes dos alimentos (LI; NIE, 2014).

Os hidrocoloides também são conhecidos como gomas ou mucilagens. Segundo PRAJAPATI et al. (2013), as mucilagens são materiais viscosos, encontradas normalmente em células especiais de camadas externas de sementes.

Tanto as proteínas como os polissacarídeos contribuem para as propriedades estruturais e texturais dos alimentos, por meio da sua agregação e do comportamento de gelificação. Além disso, as proteínas são conhecidas por sua capacidade de emulsificação e de formar espumas. As proteínas atribuem aos hidrocoloides sua excelente capacidade de hidrofilicidade e polidispersidade, abaixam a tensão interfacial devido à sua adsorção na interface, podendo formar películas nessa interface, proporcionando a repulsão eletrostática e esférica entre as gotas, e os polissacarídeos por suas propriedades de retenção de água e espessamento (CORREDIG; SHARAFBAFI; KRISTO, 2011; DICKINSON, 2003; AKEN, 2002).

Os hidrocoloides de origem vegetal são encontrados nas plantas superiores, obtidos de exsudatos, sementes, frutos e tubérculos. Esses hidrocoloides encontrados em espécies vegetais formam um subgrupo denominado gomas. São exemplos de gomas utilizadas na indústria de alimentos a goma arábica, os alginatos e a agarose (CUNHA; PAULA; FEITOSA, 2009).

Os hidrocoloides podem ser classificados tradicionalmente de acordo com sua fonte de extração. Eles podem ser exsudados de planta, como as gomas acácia, arábica, karaya e ghatti; extraídos de algas, como ágar-ágar, alginato e carragena; extraídos de plantas, como a pectina, a celulose e o amido; extraídos de sementes, como as gomas guar, alfarroba e tara, e retirados de animais, como a gelatina e a quitina. Além das gomas naturais existem as sintéticas, os polímeros vinílicos, entre outros, e as semissintéticas, como a carboximetilcelulose, como exemplo (NUSSINOVITCH; HIRASHIMA, 2014).

Segundo PENNA (2002), a escolha e aplicação de um hidrocoloide deve considerar as características funcionais desses polímeros; a aparência do produto final; a compatibilidade com os constituintes do produto; a conservação (condições bacteriológicas, compatibilidade com conservantes e com bactérias); as considerações legais (toxicidade, padrões de identidade e níveis permitidos); a estabilidade (temperatura, tempo, umidade e embalagem); as propriedades e os tipos de aplicação (mistura seca, líquido concentrado ou diluído, gel ou pasta); o uso combinado (sinergismo ou antagonismo) e a reologia (consistência, tixotropia e dilatabilidade).

2.2.2 Mercado

O "Global Hydrocolloid Market Report" estimou em 2013, que para o ano de 2018 o mercado global de hidrocoloides está projetado para alcançar US$7 bilhões sendo a América do Norte o maior consumidor desses aditivos alimentares.

O relatório do “MicroMarket Monitor” indicou que o mercado de alimentos e bebidas nos quais são utilizados hidrocoloides na América do Norte terá um aumento de cerca de US$ 2049,3 milhões, em 2013, para US $ 2583,4 milhões, em 2018, o que resulta em crescimento de 4,7%, de 2013 a 2018. Já o “Hydrocolloids Market – Global Trend & Forecast to 2019” projetou, em 2014, que este mercado está estimado em US$ 7911,1 milhões, para 2019, mostrando que o aumento no consumo de hidrocoloides é uma tendência mundial.

Apesar da riqueza da flora brasileira, pouco se conhece e se investe nas gomas produzidas por ela e, dessa maneira, o Brasil destina um grande aporte financeiro para a importação de gomas (LIMA JUNIOR et al., 2013). Esse fato, aliado ao cenário mundial de consumo de hidrocoloides, deve impulsionar pesquisas na busca por novas fontes de hidrocoloides e estudos a respeito da aplicação desses compostos.

2.2.3 Sinergia

Os hidrocoloides têm sido amplamente estudados por pesquisadores, tanto do ponto de vista funcional quanto nutricional. A qualidade do produto depende de um equilíbrio de interações entre os diferentes tipos de ingredientes, incluindo os hidrocoloides (LI; NIE, 2014).

As misturas de hidrocoloides são comumente utilizadas para conferir características reológicas desejadas aos alimentos, além de, muitas vezes, serem utilizadas no intuito de reduzir custos no produto final. Com a associação de dois hidrocoloides pode ocorrer precipitação ou gelificação. Os hidrocoloides de carga oposta (por exemplo, uma proteína abaixo do seu ponto isoelétrico e um polissacarídeo aniônico) são suscetíveis de associação e formação de um precipitado, enquanto há evidências que mostram que, para algumas moléculas de polissacarídeos rígidos, a associação resulta em formação de gel (ADITIVOS E INGREDIENTES, 2012).

Quando os hidrocoloides são aplicados em baixas concentrações, grandes volumes de solvente podem ser ligados ao seu redor, como mostrado na Figura 1. As cadeias macromoleculares de cada polímero podem se enovelar, formando estruturas globulares (Figura 1b). Logo, um aumento da concentração pode causar separação de fases (Figura 1 c e d). Diferentes associações e interações polímero-polímero e polímero-solvente podem resultar num emaranhado com componentes misturados (Figura 1f) ou num emaranhado de fases (Figura 1e). O emaranhado misturado pode formar gel (Figura 1g). Em concentrações mais elevadas, a separação de fase pode acontecer de modo a facilitar a liberdade de movimento. Isto ocorre devido à similaridade nas formas moleculares dos hidrocoloides presentes na mistura (Figura 1h) (CHAPLIN, 2016; GIANNOULI; MORRIS, 2003).

Se os dois hidrocoloides não se associam, como geralmente é o caso, a baixas concentrações eles existirão como uma única fase homogênea, enquanto a concentrações mais elevadas poderão separar-se em duas fases líquidas. Se um ou ambos os hidrocoloides formarem géis de forma independente, a separação de fases e gelificação ocorrerão simultaneamente. As características do gel resultante dependerão das taxas relativas desses dois processos. A cuidadosa seleção do tipo e da concentração de hidrocoloides pode levar à formação de uma ampla gama de texturas de gel com propriedades mecânicas de dois ou mais compostos diferentes, sendo esta uma área que merece considerável atenção (ADITIVOS E INGREDIENTES, 2012).

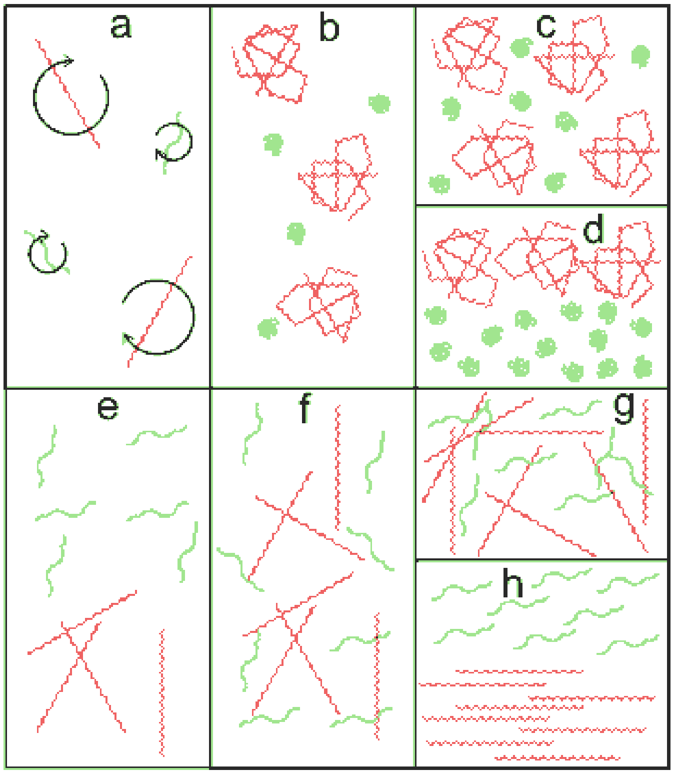

Figura 1 Conformação das misturas de hidrocoloides em solução

Fonte: Chaplin (2016).

2.3 Ora-pro-nobis (*Pereskia aculeata* Miller) e seus hidrocoloides

Pereskia aculeata Miller é uma espécie pertencente à subfamília Pereskioideae, considerada detentora do maior número de caracteres primitivos da família Cactaceae. Representantes do gênero ocorrem somente em regiões mésicas ou levemente áridas e consistem de plantas com caules finos, sublenhosos ou lenhosos, nos quais se inserem folhas largas com poucos espinhos na base e surgem flores terminais solitárias ou em cimeiras curtas. Denominada comumente de ora-pro-nobis, trepadeira-limão e groselha-de-barbados, *P. aculeata* é uma trepadeira semilenhosa, que pode atingir 10 m de altura, com ramos longos e espinhos na axila das folhas elípticas e carnosas (DUARTE; HAYASHI, 2005). Na Figura 2 está representado o ramo vegetativo do ora-pro-nobis, evidenciando a disposição das folhas e a presença de espinhos.

Figura 2 Ramo vegetativo do ora-pro-nobis (*Pereskia aculeata Miller*)
Fonte: Duarte e Hayashi (2005).

Segundo BRASIL (2010), o ora-pro-nobis é classificado como uma hortaliça não convencional, que são aquelas com distribuição limitada, restrita a determinadas localidades ou regiões, exercendo grande influência na alimentação e na cultura de populações tradicionais. Além disso, são espécies que não estão organizadas como cadeia produtiva propriamente dita, diferentemente das hortaliças convencionais (batata, tomate, repolho, alface, etc.), não despertando o interesse comercial por parte de empresas de sementes, fertilizantes ou agroquímicos. No entanto, a espécie aparece cada vez mais em estudos de centros de pesquisas que vêm explorando-a das mais diversas formas.

Em alguns dos estudos relevantes relativos ao ora-pro-nobis verificaram-se as características que podem viabilizar o uso das folhas em aplicações diversas como tecnológica ou nutricional, levando em consideração suas ricas propriedades nutricionais.

ALMEIDA FILHO; CAMBRAIA (1974) foram os primeiros pesquisadores a estudar a composição das folhas de ora-pro-nobis, concluindo que elas apresentam alto valor nutritivo. Os pesquisadores determinaram nível de proteínas de 25,5% em matéria seca, próximo ao encontrado por DAYRELL; VIEIRA (1977), de 25,14% em matéria seca. Devido ao valor proteico muito elevado, quando comparado com ao de outros vegetais, esta espécie é também chamada, popularmente, de carne dos pobres.

Segundo SIERAKOWSKI et al. (1987), além do alto nível proteico, as proteínas da espécie são altamente digeríveis (85%). Além disso, as folhas do ora-pro-nobis têm um teor elevado de aminoácidos essenciais, principalmente lisina, que é maior que em muitos alimentos utilizados para comparação e mais elevado que as quantidades mínimas recomentadas pela Organização de Alimentação e Agricultura das Nações Unidas para o consumo humano. No entanto, em estudos realizados por TAKEITI et al. (2009) foi demonstrado que o principal aminoácido encontrado nas suas folhas é o triptofano, representando cerca de 20% dos aminoácidos.

TAKEITI et al. (2009) também determinaram a composição nutricional geral das folhas de ora-pro-nobis. Os valores estão apresentados nas Tabelas 1 e 2, respectivamente. O teor de ferro presente nas folhas merece destaque, apresentando quantidade superior, em 100 g de folha, que a ingestão diária recomendada.

Estudos realizados por SIERAKOWSKI et al. (1987) sobre a extração do polissacarídeo da mucilagem do ora-pro-nobis revelam a presença do biopolímero arabinogalactana. Os estudos mostram, ainda, que o polissacarídeo presente é constituído por uma heteroglicana formada, principalmente, por arabinose e galactose, contendo também raminose e ácido galacturônico. Esses monômeros foram identificadas por técnicas de análise de polissacarídeos convencionais e as proporções molares dos monômeros arabinose, galactose, ramnose e ácido galacturônico encontradas foram de 5,1: 8,2: 1,8: 1,0, respectivamente.

Arabinogalactana (Figura 3) é um polissacarídeo solúvel em água, que pode ser extraído de uma grande variedade de plantas, apresentando propriedades peculiares e estando, na maioria das vezes, associado a proteínas (PAULSEN; BARSETT, 2005). São macromoléculas caracterizadas por uma elevada proporção de hidratos de carbono em que a galactose e a arabinose são os monossacarídeos predominantes (FINCHER; STONE, 1983).

Industrialmente, as arabinogalactanas são utilizadas por fazerem parte da composição da goma arábica extraída da acácia-senegal, proveniente de árvores de diferentes espécies de *Acacia*. Já com enfoque biológico, as principais arabinogalactanas utilizadas são provenientes do lariço (*Larix occidentalis* e *Larix dahurica*), uma árvore típica da região norte do globo, sendo descrita por apresentar propriedades imunomoduladoras.

Tabela 1 Composição nutricional geral das folhas de OPN (%)

Componentes	Matéria úmida	Matéria seca
Umidade	89,5	-
Proteínas totais	3,1	28,4
Lipídios	0,4	4,1
Cinzas	1,7	16,1
Fibra bruta	1,0	9,8
Fibra dietética solúvel	0,5	5,2
Fibra dietética insolúvel	3,3	33,9

Fonte: Takeiti et al. (2009).

Tabela 2 Conteúdo de minerais em folhas frescas de OPN (mg/100g)

Minerais	Folhas frescas (mg/100g)
Cálcio	3,4
Magnésio	1,9
Potássio	1,6
Fósforo	156,0
Manganês	46,4
Zinco	26,7
Ferro	14,2
Boro	5,5
Cobre	1,4

Fonte: Takeiti et al. (2009).

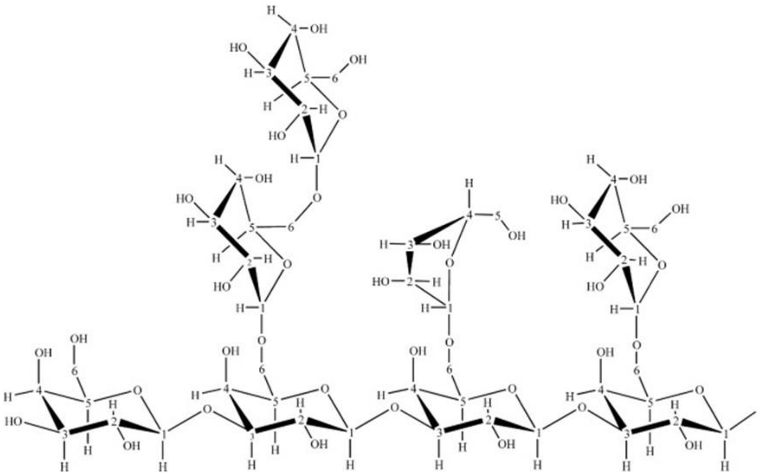

Figura 3 Estrutura molecular da arabinogalactana

Fonte: Paulsen e Barsett (2005)

A goma arábica é um aditivo amplamente utilizado na produção de alimentos, medicamentos e cosméticos. Devido ao pequeno volume hidrodinâmico das moléculas compactadas de arabinogalactana, fornecida pela estrutura altamente ramificada, a goma arábica resulta em soluções de baixa viscosidade. Além disso, outras frações presentes nas moléculas do polissacarídeo são associadas com proteínas, resultando em um componente de massa molecular elevada, denotado arabinogalactano-proteína, que tem propriedades emulsificantes (LEE; MCCLEMENTS, 2010; YADAV et al., 2007).

A presença de alto teor proteico e de arabinogalactana na espécie também despertou interesse para o estudo de uma potencial nova fonte de extração de aditivos. O processo de extração desse novo aditivo foi estudado e otimizado por LIMA JUNIOR et al. (2013). A estabilidade térmica e a microestrutura dos hidrocoloides extraídos do ora-pro-nobis e da goma formada por esses hidrocoloides foram estudadas por CONCEIÇÃO et al. (2014). O produto obtido em pó nestes estudos mostrou-se visualmente apresentável, com cor clara, semelhante à cor de hidrocoloides comerciais, como a pectina, e com propriedades que podem ser empregadas na indústria (espessante, gelificante, emulsificante).

2.4 Bebida láctea fermentada

Leites fermentados são produtos adicionados ou não de outras substâncias alimentícias, obtidos por coagulação e diminuição do pH do leite, ou leite reconstituído, adicionados ou não de outros produtos lácteos, por fermentação láctica mediante ação de cultivos de microrganismos específicos, os quais devem ser viáveis, ativos e abundantes no produto final, durante o prazo de validade. De acordo com a legislação, os cultivos ou microrganismos empregados na fermentação definem a denominação do produto que pode ser iogurte, leite fermentado, leite acidófilo, kefir, kumys e coalhada (BRASIL, 2007).

Na Tabela 3 estão representados os tipos de bebidas lácteas fermentadas e os respectivos microrganismos responsáveis pela fermentação (BRASIL, 2007). A composição química do produto lácteo é influenciada diretamente pela atividade metabólica da bactéria, que interage intensamente com o meio ao converter determinados componentes em produtos metabólitos durante o seu crescimento. Os carboidratos disponíveis e as proteínas do leite são os componentes mais utilizados pelo metabolismo bacteriano. Os leites fermentados podem ser classificados, de acordo com o teor de gordura, como com creme (>6,0 g/100 g), integral (mínimo 3,0 g/100 g), parcialmente desnatado (máximo 2,9 g/100 g) e desnatado (máximo 0,5 g/100 g).

Tabela 3 Tipos de leite fermentado e respectivos cultivos

Leite fermentado	Cultivos responsáveis pela fermentação
Iogurte	*Streptococcus salivarius subsp. thermophilus* e *Lactobacillus delbrueckii subsp. Bulgaricus* e/ou outras bactérias acido-lácticas
Leite fermentado	*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium sp.*, *Streptococus salivarius subsp. Thermophiles* e/ou outras bactérias acido-lácticas.
	Leite acidófilo: *Lactobacillus acidophilus* exclusivamente
Kefir	grãos de *Kefir*, *Lactobacillus kefir*, espécies dos gêneros *Leuconostoc*, *Lactococcus* e *Acetobacter* com produção de ácido láctico, etanol e dióxido de carbono. Os grãos de *Kefir* são constituídos por leveduras fermentadoras de lactose (*Kluyveromyces marxianus*) e leveduras não fermentadoras de lactose (*Saccharomyces omnisporus* e *Saccharomyces cerevisae* e *Saccharomyces exiguus*), *Lactobacillus casei*, *Bifidobaterium sp.* e *Streptococcus salivarius subsp. thermophilus*.
Kumys	*Lactobacillus delbrueckii subsp. bulgaricus* e *Kluyveromyces marxianus*
Coalhada	Cultivos individuais ou mistos de bactérias mesofílicas produtoras de ácido láctico

As propriedades físicas do leite fermentado são seus principais critérios de qualidade, além de suas propriedades sensoriais. Uma viscosidade inaceitável e separação do soro são os principais defeitos de qualidade física nesses produtos. Estes problemas são intensificados pela redução do teor de gordura. A faixa de pH atingida nos leites fermentados (3,4-4,6) gera instabilidade nas moléculas de caseína e é necessária a adição de estabilizantes para a prevenção da agregação de proteínas, para que a bebida tenha boa aceitação sensorial. Estabilizantes são importantes para o controle de propriedades como textura e viscosidade, permitindo a aplicação de processo adequado e produção de bebida de boa qualidade. Goma de alfarroba, carragenina, pectina e amido estão entre os estabilizadores comuns utilizados neste tipo de produtos. Caseínas no leite (pH neutro) estão presentes em forma de micelas estabilizadas por interações repulsivas. Durante a acidificação, as micelas se agregam (ponto isoelétrico pH=4,6) pelo colapso da camada de k-caseína na superfície da micela. Neste meio, a adição de polissacarídeos pode estabilizar o sistema, formando interações proteínas-polissacarídeos de natureza eletrostática (KOKSOY; KILIC, 2004; NAKAMURA et al., 2006; OZEN; KILIC, 2009).

2.5 Reologia

A reologia é uma ciência, citada pela primeira vez em 1928, por Eugene C. Bigham, que estuda o escoamento e a deformação de materiais. As determinações reológicas permitem, assim, obter informações sobre a forma como um determinado material reage, quando sujeito a uma solicitação de caráter mecânico, que é, no fundo, a expressão da sua estrutura que resulta, por sua vez, da sua composição química, das diferentes interações entre os seus vários componentes e do processamento a que foi sujeito (CASTRO, 2003).

Na área alimentar, o conhecimento do comportamento reológico tem desempenhado papel cada vez mais importante, permitindo melhor compreensão do comportamento mecânico dos alimentos, nem sempre fácil de prever e interpretar, como consequência da complexidade da sua composição e dos processos a que são submetidos. Além disso, o estudo reológico permite o melhoramento e o controle das propriedades funcionais de produtos convencionais e facilita o desenvolvimento de novos produtos. As propriedades mecânicas/sensoriais são determinantes na aceitabilidade de produtos alimentares, e a avaliação objetiva dessas propriedades é do domínio da reologia (CASTRO, 2003)

O conhecimento do comportamento reológico dos alimentos também é útil no dimensionamento de sistemas de tubulação, trocadores de calor, filtros, bombas, misturadores e homogeneizadores, entre outros. O comportamento reológico dos materiais é muito variado e complexo. Além da natureza do material, esse comportamento pode se diferenciar, dependendo das condições a que o material encontra-se submetido, sendo o nível de tensão, a velocidade de deformação, o tempo e a temperatura determinantes nesse comportamento (CASTRO, 2003; STEFFE, 2006).

2.5.1 Comportamentos reológicos dos materiais

A reologia clássica começa com a consideração de dois materiais ideais: o sólido elástico e o líquido viscoso. O sólido elástico é um material com forma definida que, quando deformado por uma força externa dentro de certos limites, irá retornar à sua forma e dimensões originais após a remoção dessa força. O líquido viscoso não tem forma definida e irá escoar, irreversivelmente, com a aplicação de uma força externa. Na reologia de sólidos a propriedade de maior interesse é a elasticidade e em líquidos, a viscosidade (TONELI; MURR; PARK, 2005).

A viscosidade é a propriedade associada à resistência interna que o fluido oferece à deformação por cisalhamento. De outra maneira, pode-se dizer que a viscosidade corresponde ao atrito interno nos fluidos devido, basicamente, às interações intermoleculares, sendo, em geral, função da temperatura (LIVI, 2004; RAO, 2007).

Os fluidos newtonianos têm relação linear entre a tensão de cisalhamento e a taxa de cisalhamento com ordenada zero. As propriedades reológicas desses fluidos independem de um histórico anterior de cisalhamento e dependem somente da composição e temperatura. Em um reograma típico de fluido newtoniano, o declive da linha aumenta com o aumento da viscosidade. Para fluidos newtonianos, a viscosidade é obtida por meio de uma única medida de tensão de cisalhamento e taxa de deformação. Porém, para identificar se um fluido é newtoniano ou não newtoniano, são necessários vários dados de tensão de cisalhamento nas correspondentes taxas de deformação, para então se avaliar o comportamento (STEFFE, 2006; VIDAL-BEZERRA, 2000). Esses fluidos seguem a Lei de Newton da viscosidade apresentada na Equação 1

$$\sigma = \mu \cdot \dot{\gamma} \tag{1}$$

em que σ: tensão de cisalhamento (Pa), μ: viscosidade newtoniana (Pa.s), $\dot{\gamma}$: taxa de deformação (s^{-1}).

Os fluidos que seguem a lei de Newton, ou seja, sua viscosidade é constante, independente da velocidade de deformação ou do tempo (podendo, contudo, variar com a temperatura ou pressão), recebem o nome de fluidos newtonianos. Não existem, naturalmente, fluidos ideais, mas tão somente fluidos com comportamento que se aproxima do ideal, como é o caso de líquidos puros, soluções verdadeiras diluídas e alguns sistemas coloidais (BOBBIO; BOBBIO, 2001).

Todos os fluidos que não apresentam relação linear entre a tensão e o cisalhamento e a taxa de cisalhamento podem ser chamados de fluido não newtoniano. Para fluidos não newtonianos o termo viscosidade é substituído por η_{ap}, que é a viscosidade aparente e é função do gradiente de velocidade (CASTRO, 2003; PATIL; MAGDUM, 2005; STEFFE, 2006; VIDAL-BEZERRA, 2000).

$$\eta_{ap} = \frac{\sigma}{\gamma} \tag{2}$$

em que: η_{ap} = Viscosidade aparente (Pa.s); σ = Tensão de cisalhamento ($N.m^{-2}$ = Pa); γ = Taxa de cisalhamento (s^{-1}).

Esses fluidos podem ser dependentes do tempo ou independentes do tempo. No primeiro caso, a viscosidade aparente dos fluidos varia tanto com a taxa de deformação quanto com a duração de sua aplicação, podendo ser classificados como tixotrópicos ou reopéticos. A diminuição da viscosidade aparente com o tempo de cisalhamento, a uma condição constante de temperatura e taxa de deformação, caracteriza um material tixotrópico, enquanto fluidos que têm a sua viscosidade aparente aumentada com o tempo são denominados reopéticos. Grande parte dos fluidos alimentícios, como pastas de frutas e vegetais, *ketchup*, mostarda e comida de bebê, apresentam comportamento tixotrópico (BARBOSA-CÁNOVAS; IBARZ; PELEG, 1993; CHOI; YOO, 2004). Essas definições levam à classificação reológica apresentada na Figura 4.

O comportamento não newtoniano resulta, geralmente, de suspensões coloidais de partículas com flexibilidade e formas diversas que podem formar entre si ligações temporárias ou estruturais internas mais ou menos complexas, chegando ao estabelecimento de redes mais ou menos organizadas em forma de emulsão ou gel (TONELI; MURR; PARK, 2005). Como objetos não simétricos passam uns pelos outros. O mesmo acontece durante o escoamento; seu tamanho, forma e coesividade determinam quanta força é requerida para movê-los. Em outra taxa de escoamento, o alinhamento dos objetos pode ser diferente e mais ou menos força será requerida para manter o movimento (PATIL; MAGDUM, 2005).

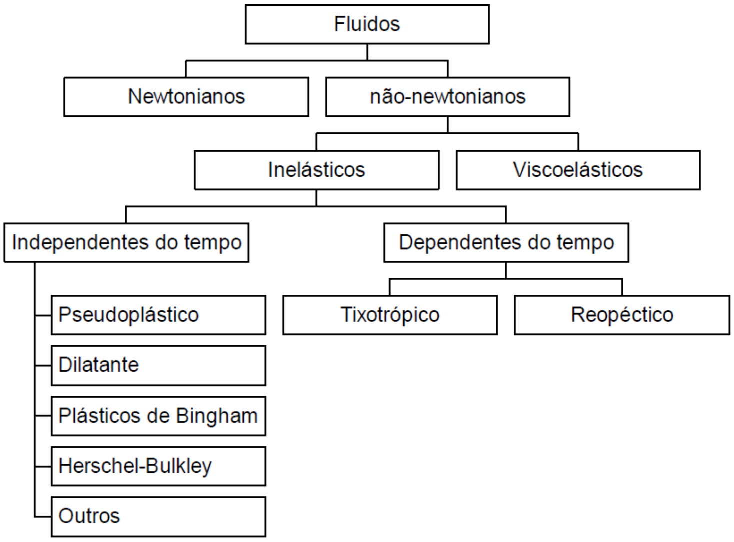

Figura 4 Classificação reológica dos fluidos

Fonte: Toneli, Murr e Park (2005)

O comportamento não newtoniano pode ser expresso por uma dependência da viscosidade em relação à velocidade de deformação ou por uma dependência da viscosidade em relação ao tempo. Nestes casos, a viscosidade deverá ser designada, para cada velocidade de deformação ou para cada tempo, por viscosidade aparente (η). Estes materiais são designados de viscoelásticos, uma vez que combinam as características de um fluido viscoso com as de um sólido elástico (PATIL; MAGDUM, 2005).

Fluidos não newtonianos inelásticos são todos aqueles que, de alguma maneira, não se comportam de acordo com a relação descrita pela Equação (1), quando são submetidos a uma deformação. Eles podem ainda ser classificados como dependentes ou independentes do tempo (TONELI; MURR; PARK, 2005).

Fluidos com comportamento reológico independente do tempo, sob condições de temperatura e composição constantes, apresentam viscosidade aparente dependente somente da taxa de deformação ou da tensão de cisalhamento. São exemplos deste comportamento os fluidos plásticos de Bingham, os pseudoplásticos e os dilatantes. Para o caso de fluidos com comportamento dependente do tempo, a viscosidade aparente também depende da duração dessa taxa de deformação, como ocorre nos fluidos tixotrópicos e reopéticos (TONELI; MURR; PARK, 2005).

2.5.2 Modelos matemáticos utilizados para descrição do comportamento reológico

A descrição do comportamento reológico dos materiais é feita por meio de modelos que relacionam como a tensão de cisalhamento varia com a taxa de deformação. Dentre os modelos matemáticos existentes, alguns dos mais aplicados para sistemas de alimentos são Ostwald-De-Waelle (lei da potência), plástico de Bingham, Herschel-Bulkley e Casson.

Devido às grandes variações nas características dos fluidos, pesquisadores observaram curvas de escoamento obtidas experimentalmente e, por meio dessas curvas, obtiveram modelos matemáticos que podem ser ajustados para cada fluido específico, descrevendo seu comportamento reológico. Esses modelos descrevem o comportamento reológico relacionando a tensão de cisalhamento com a taxa de deformação. Os modelos reológicos são úteis para relacionar propriedades reológicas de um fluido com grandezas práticas, como concentração, temperatura, pH e índice de maturação, entre outros. Esses modelos podem ser isotérmicos ou não isotérmicos. No primeiro caso, descrevem o comportamento de um fluido a uma temperatura fixa e, no último, incluem relações específicas, como funções da temperatura. Dependendo do modelo utilizado, têm ou não tensão inicial (TONELI; MURR; PARK, 2005).

Na Tabela 4 são apresentados as equações e os valores característicos dos parâmetros para as diferentes classificações dos fluidos, além dos exemplos mais encontrados dentro de cada classificação.

Tabela 4 Classificação dos modelos reológicos aplicados a curvas de escoamento

Classificação do fluido	Equação constitutiva	σ_0	k	n	Exemplos típicos
Newtoniano	$\sigma = k(\dot{\gamma})$	0	>0	1	Água, leite, mel, suco de fruta clarificado
Pseudoplástico*	$\sigma = k(\dot{\gamma})^n$	0	>0	$0<n<1$	Purê de banana, suco concentrado de laranja
Dilatante*	$\sigma = k(\dot{\gamma})^n$	0	>0	$1<n<\infty$	Suspensão 40% amido de milho
Plástico de Bingham	$\sigma = \sigma_0 + k(\dot{\gamma})$	>0	>0	1	Pasta de dente, purê de tomate
Hershel-Bulkley	$\sigma = \sigma_0 + k(\dot{\gamma})^n$	>0	>0	$1<n<\infty$	Pasta de peixe picado, uva passa
Casson	$(\sigma)^{0.5} = \sigma_0 + k(\dot{\gamma})^{0.5}$	>0	>0	0.5	Suspensões de partículas em meio newtoniano como o chocolate

* Classificações de fluido a partir da equação da lei da Potência.
Fonte: Steffe (2006).

As soluções de gomas, geralmente, são fluidos não newtonianos com comportamento pseudoplástico. Diversos modelos têm sido aplicados para descrever o comportamento reológico de soluções de hidrocoloides, como, por exemplo, modelos lineares (newtoniano ou Bingham), lei da potência (Ostwald-de-Waele), lei da potência com tensão residual (Herschel-Bulkley) e o modelo de Casson. Dentre estes, o da lei da potência é, talvez, o mais utilizado para fluidos não newtonianos e é extensivamente utilizado para descrever as propriedades de escoamento de líquidos, tanto em análises teóricas quanto em aplicações práticas da engenharia. As soluções de hidrocoloides também podem apresentar propriedades reológicas dependentes do tempo, principalmente a tixotropia (TONELI; MURR; PARK, 2005).

2.5.3 Tipos de ensaios para avaliação reológica dos materiais

A avaliação do comportamento reológico dos materiais pode ser realizada por testes estacionários, oscilatórios e estáticos (CASTRO, 2003; TONELI; MURR; PARK, 2005). Os testes em regime estacionário consistem na aplicação de um gradiente de tensão ou velocidade de corte, linear ou logarítmico, unidirecional relativamente à amostra em estudo. É usualmente utilizado para a determinação de viscosidade aparente, curvas de escoamento ou viscosidade. Mesmo sendo uma técnica importante, ocorre a destruição da estrutura dos sistemas, pelo que não descreve por completo o comportamento reológico dos materiais.

O princípio da análise oscilatória consiste em testar a amostra de uma forma não destrutiva. É aplicada à amostra uma tensão (deformação) que é uma função sinoidal do tempo e registra-se a deformação (tensão) resultante. É possível realizar vários tipos de testes oscilatórios em que o varrimento é realizado em tensão, frequência, tempo ou temperatura.

Enquanto, nos testes oscilatórios, a tensão aplicada varia com uma onda sinoidal, nos testes estáticos, ou ensaios de fluência-recuperação, a amostra é submetida a uma tensão constante durante um período prolongado, permitindo a análise dos elementos estruturais com tempos de resposta mais longos. Dessa forma, é aplicada instantaneamente à amostra em estudo uma tensão constante durante um período de tempo (S), período durante o qual a deformação sofrida pela amostra é registrada. Neste tempo, a tensão é instantaneamente removida, mas a variação da deformação continua a ser registrada. Os resultados são analisados em termos de susceptibilidade (Pa), definida como a razão entre a deformação resultante e a tensão constante aplicada.

3 CONCLUSÕES

As soluções modelo analisadas no Artigo 1 da Parte 2 apresentaram comportamento pseudoplástico em presença da mucilagem de ora-pro-nobis, sendo a mucilagem o fator significativo em relação à viscosidade aparente das amostras. As análises termigravimétricas revelaram um comportamento dividido em três etapas: perda de água, mudança na conformação dos biopolímeros e perda final de massa. A seleção de fatores foi realizada pelo planejamento fatorial fracionado e os fatores estatisticamente significativos para as respostas de reologia e termogravimetria foram mucilagem de ora-pro-nobis, sacarose e cloreto de sódio. A faixa de pH estudada e a concentração de $CaCl_2$ não afetaram significativamente a viscosidade aparente das amostras.

A partir do fatorial fracionado considerando a variação dos fatores selecionados foi verificado que as amostras com baixa concentração de mucilagem de ora-pro-nobis (0% a 1,25%) apresentaram comportamento newtoniano e as demais amostras (2,5%-5,0% mucilagem ora-pro-nobis) já tiveram um comportamento diferente, ajustando-se à lei da potência. Houve predominância do comportamento elástico das amostras (G’>G” a 1 Hz de frequência). O aumento da concentração da mucilagem do ora-pro-nobis contribuiu para a formação de géis mais fortes.

No Artigo 2 foram realizados testes de mistura em solução modelo para a definição de um mix de hidrocoloides e seguinte aplicação em bebida láctea fermentada. O mix de hidrocoloides nas regiões de maiores valores de viscosidade aparente foi 0,7% de mucilagem de ora-pro-nobis, 0,0 GA e 0,3 GG. A sacarose teve uma maior interferência na viscosidade aparente das soluções modelo que o cloreto de sódio. As soluções modelo apresentaram comportamento pseudoplástico ajustando-se ao modelo da lei da potência. Os leites fermentados tiveram bom ajuste à lei da potência, apresentaram comportamento tixotrópico, predominância de características elásticas (G’>G”) e sua microestrutura evidenciou a importância da ligação entre os hidrocoloides e os componentes do leite, como a caseína na formação da bebida.

A aplicação da mucilagem do ora-pro-nobis foi analisada como viável, tanto nos testes com soluções modelo quanto na bebida láctea fermentada, auxiliando na formação de gel, no incremento na viscosidade aparente e, consequentemente, contribuindo para a estabilidade das amostras.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 Behavior of *Pereskia aculeata* Miller mucilage in the presence of cations and sucrose

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ABSTRACT

Pereskia aculeata Miller mucilage presents properties that can be approached in industry as a thickener, gelling agent and/ or as an emulsifier. The understanding of the use of OPN mucilage in food systems can be studied in aqueous solutions, widely used for the study of isolated compounds. The objectives of this study were to characterize the rheological behavior and thermal properties of OPN mucilage in different model solutions. OPN mucilage were extracted and the gums were prepared by stirring for 30 min under 75°C. Rheological and thermogravimetric parameters were evaluated. The experimental design was divided in a fractional factorial 2^{5-1} to the screening of factors (OPN mucilage, sucrose sodium chloride, calcium chloride and pH) and then to a 5x3x3 full factorial (OPN mucilage, sucrose and sodium chloride). Model solutions studied for selection of factors presented pseudoplastic behavior in the presence of OPN mucilage, which was the factor with significant effect on the apparent viscosity. Thermogravimetric analysis showed the presence of three distinct phases of mass loss with increasing temperature, which may be related to water loss, change in conformation of polysaccharides and to the remaining mineral material. OPN mucilage, sucrose and sodium chloride were the variables with significant effect on thermogravimetric responses. In full factorial studies, the samples presented Newtonian behavior with 0-1.25% OPN mucilage and Power Law behavior with 2.50-5.00% OPN mucilage, with a predominance of elastic behavior (G' > G" at 1Hz). Apparent viscosity had an increasing tendency with increasing concentration of OPN mucilage. G' values were higher than those of G", confirming the elastic predominant character of the samples. Increasing OPN mucilage concentration increased this elastic behavior, contributing to the formation of stronger gels.

Keywords: Food additive. Rheology. Sodium chloride. Sucrose. Thermogravimetry.

1. INTRODUCTION

Hydrocolloids are additives used in the food industry as moisture-control, thickeners, gelling agents, stabilizers of different kinds of dispersions, dietary fibers, encapsulants, crystallization inhibitors and even as emulsifiers (Dickinson, 2003; Rincón, Muñoz, Ramírez, Galán, & Alfaro, 2014). Hydrocolloids are obtained from a wide range of natural raw materials including microorganisms, land and sea plants and animal connective tissues. Hydrocolloids obtained from plants have the advantage over those from animals because of their friendly image towards consumers. Starch, pectin, galactomannans, carrageenan, alginates, as well as cellulose and its derivatives are the main plant hydrocolloids (Vardhanabhuti & Ikeda, 2006).

A large number of plants can produce the complex polysaccharides commercially known as plant based gums. The plant gum exudates and seed gums are the complex polysaccharides/carbohydrate polymers commonly used as a dietary fiber, thickening agent, foaming agent, film, emulsifier, stabilizer and drug delivery agent. (Razavi, Cui, Guo, & Ding, 2014; Salehi, Kashaninejad, Tadayyon, & Arabameri, 2015). The development of new sources of vegetable hydrocolloids is widely studied (Gannasin, Adzahan, Hamzah, Mustafa, & Muhammad, 2015; Petera et al., 2015; Razavi, Cui, & Ding, 2016; Ritzoulis et al., 2014).

Lima Junior et al (2013) developed the extraction process of *Pereskia aculeata* Miller hydrocolloids, proving that the specie is an alternative source of the additive. This plant is popular known in Brazil as ora-pro-nobis (OPN) and is a cactaceae that presents a rich composition with the presence of gum, biopolymer arabinogalactan, high protein content (28,4g/100g), minerals and has a simple cultivation and high productivity. OPN mucilage presented properties that can be used in industry as a thickener, gelling agent and/ or emulsifier (Conceição, Junqueira, Guedes Silva, Prado, & de Resende, 2014; Takeiti, Antonio, Motta, Collares-Queiroz, & Park, 2009), is necessary for studies to understand the new hydrocolloid behavior on complex systems such as foods. Model solutions are widely used for the study of isolated compounds in aqueous solutions and allow a better understanding of the interactions.

The application of hydrocolloids requires relative low concentrations to get desired viscosity values and/or emulsifier capacity in the food industry. Some of the food application is in liquid systems where the interactions between the polymers and the dispersion medium play an important role. Other properties as dissolution and processing temperature, storage time, interactions between different gums or different food ingredients and other factors that promote aggregation or phase separation with increase or decrease in viscosity of the systems must be also considered (Chenlo, Moreira, Pereira, & Silva, 2009; Torres, Vázquez-Vila, & Pereira-Gonçalvez, 2015).

Knowledge of the rheological properties of food gum aqueous dispersions is essential to assess the consistency of commercial products at quiescent state, textural properties, design of unit operations (pumping, mixing, heat-exchange, bottling, packaging) and the product engineering (Rincón et al., 2014). Because of differences in gum structure and extrinsic conditions within the fluid food system, the rheological properties are quite different from one gum solution to another (Marcotte, Hoshahili, & Ramaswamy, 2001).

The objectives of this study were to characterize the rheological behavior and thermal properties of the mucilage extracted from OPN leaves in different model solutions and also to study the interactions of the additives and OPN mucilage to try to explain the differences between the rheological and thermal properties. This operation will give guidelines for future applications of OPN mucilage in foods, which are complex systems.

2. MATERIALS AND METHODS

2.1 Extraction of OPN mucilage

Extraction of mucilage from OPN leaves was conducted according to a process adapted from Lima Junior et al. (2013), as shown in Figure 1.

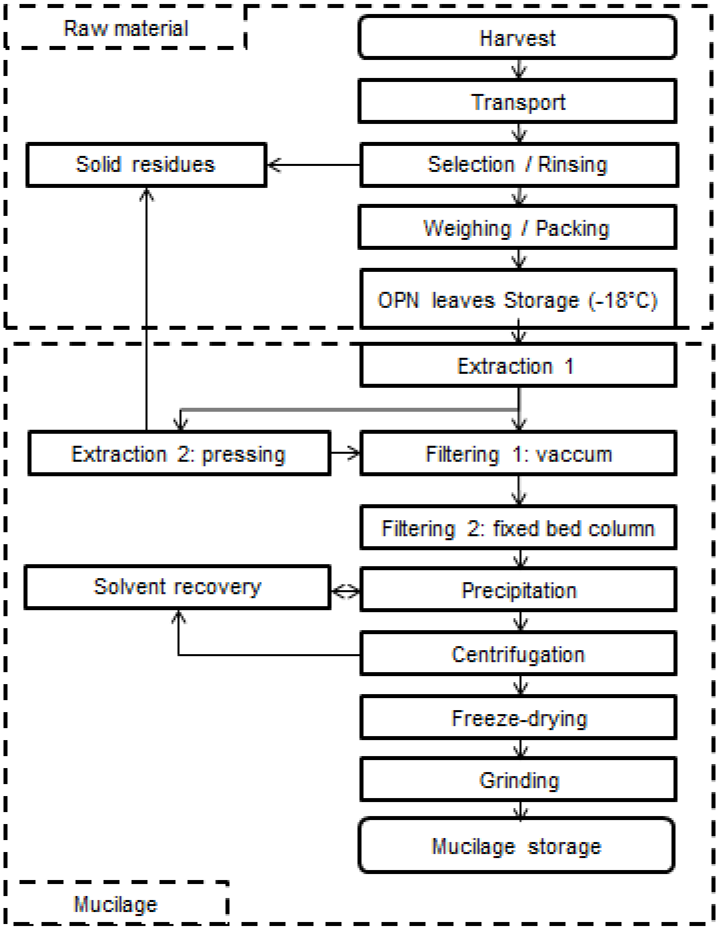


Figure 1: Extraction process of mucilage from OPN leaves

2.2.1 Raw material

Fresh OPN leaves were collected at the Federal University of Lavras (Lavras, Brazil) in the period of May-June 2015. They were harvested and transported to the Food Refrigeration Laboratory of the Department of Food Science. In the laboratory, the leaves were selected, rinsed, weighed and packed into identified polyethylene bags. The packaged material was stored in freezer (-18°C) until the mucilage extraction.

2.2.2 OPN mucilage extraction steps

Extraction 1 is the homogenization of OPN leaves in industrial blender (Metvisa LG10, São Paulo, Brazil) with boiling water and subsequent extraction in the thermostatic water bath (Solab SL 150, Piracicaba, Brazil) under 75±1°C for 6 hours.

Material resulted from Extraction 1 was submitted to the Extraction 2, pressing in hydraulic press (Tecnal TE 058, Campinas, Brazil). The liquid fractions derived from Extractions 1 and 2 passed through the Filtering 1. This step was carried out by vacuum (dual-stage pump) filtering the material in a Buchner funnel and organza fabric as a filter element, generating the Filtrate 1.

Filtrate 1 was placed in a fixed bed column containing activated carbon to remove insoluble solids and pigments (Filtering 2 phase). This column consists of a PVC tube with 1.00m and 0.11m long diameter, containing granulated activated carbon (Dinâmica Química 1-2mm). This process generated the Filtrate 2.

Filtrate 2 was precipitated with ethanol 95% (Sigma-Aldrich) at a ratio of 3:1 of alcohol per liter of Filtrated 2. The precipitate was centrifuged (centrifugal SP Labor SP701, Presidente Prudente, Brazil, 12 min, 628 xg) for maximum removal of alcohol and then dried in a freeze dryer (Edwards, São Paulo, Brazil). The dry material was ground in a ball mill (Marconi MA350, Brazil) for 1 minute and stored away from light and moisture. The ethyl alcohol used in these steps was recovered by distillation and reused for Precipitation.

2.2 Preparations of gums

The gums were prepared by homogenizing in a magnetic stirrer (30 min, 75°C) the components of each solution: buffer solution, sucrose (Isofar), sodium chloride (Sigma Aldrich), OPN mucilage, calcium chloride (Sigma Aldrich) and guar gum (Sigma-Aldrich), according to the experimental design (Item 2.5). Homogenized solutions were kept in a thermostatic cabinet (Eletrolab, EL202, São Paulo, Brazil) at 4°C during 18 hours for complete hydration before analysis.

2.3 Rheological studies

Rheological studies of gums were conducted using the rheometer HAAKE RheoStress 6000 (Thermo Scientific – Karlsruhe / Germany) coupled to a temperature controller HAAKE UTM Controller (Thermo Scientific) in FRL. The analysis were performed with parallel plate geometry (34.997 mm diameter) and GAP 1 mm.

Characterization of flow rheological behavior was performed by operation of the flow curve, subjecting the samples to a continuous shear rate ($\dot{\gamma}$) ramp between 0 and 300 s $^{-1}$ for 2 minutes, resulting in 100 readings of shear stress (τ). To the generation of each flow curve the samples were first stabilized during 3 minutes at 20°C and subjected to thixotropy breaking, by applying a continuous shear rate ($\dot{\gamma}$) ramp between 0 and 300 s^{-1} for 2 minutes and the same time to decrease gradient between 300 and 0 s^{-1}.

Data generated in the flow curve were fitted to the rheological models presented in Equation 1 (Newton) and Equation 2 (Power Law), given information as fluid classification and the parameters of adjustment. Thixotropy values were calculated by the difference of areas of the rise and fall curves prior to the generation of flow curve. The apparent viscosity values were calculated from the shear stress point of 100 s^{-1}, related to the value experienced by the fluid in industrial pipes (Steffe, 2006).

$$\tau = \mu \cdot \dot{\gamma} \qquad (1)$$

$$\tau = K \cdot (\dot{\gamma})^n \qquad (2)$$

where τ is shear stress (Pa), $\dot{\gamma}$ is shear rate (s^{-1}), μ is viscosity (Pa.s), K is the consistency coefficient and *n* is the flow behavior index.

Oscillatory tests were performed to study the viscoelastic properties of the samples. The definition of linear viscoelastic region was carried out by subjecting the samples to shear stress of 0.001Pa and 100Pa with a fixed frequency of 1Hz at 20°C. The frequency sweep curves were generated assuming a value of shear stress within the linear viscoelastic range, varying frequency ranged from 0.01Hz to 10Hz at 20°C. From this scan, it was observed the behavior of G ' and G ".

2.4 Thermogravimetry (TG)

Thermogravimetry experiments (TG) were conducted under a nitrogen atmosphere in room temperature (25 ± 1°C) of 520°C at a heating rate of 3°C min^{-1} (adapted from Conceição et al. (2014)) using DTA-TG-60H (Shimadzu Corporation, Kyoto, Japan) in CAPC. The responses evaluated were the temperature (°C) and mass loss (%).

2.5 Experimental design

The first part of the experimental design consisted of a fractional factorial design to identify medium ingredients that had a significant effect on the response. The major benefit of applying a factorial design is the reduced number of experiments that need to be carried out to obtain maximum information. The second part was a factorial design that allows the effect of a given factor to be determined at several levels of the other factors, so, the conclusions are valid over a range of experimental conditions (Gheshlaghi, Scharer, Moo-Young, & Douglas, 2005; Rodrigues & Iemma, 2005).

2.5.1 Screening factors

A fractional factorial design (2^{5-1}) was prepared in order to reduce the number of variables, evaluating rheological and thermal characteristics of the samples. Apparent viscosity response was selected for screening variables related to rheology (values regarding deformation rate of $100s^{-1}$) and related to thermogravimetry, the parameters were temperatures and weight loss (%). Fractional factorial design was applied and five medium ingredients were assessed, as shown in Table 1 (Rodrigues & Iemma, 2005). The factors evaluated in the study are shown in Table 2 with their coded and real values. In addition to the screening of the variables, it was possible to infer rheological and thermal characteristics of the samples that enrich the knowledge to continue the study.

Data were subjected to analysis in STATISTICA version 7 (Statsoft Inc). After completion of the trials, data obtained were subjected to a linear effect analysis to evaluate the pure error at 10% level of significance. In factor selection designs, working at this level of significance minimizes the risk of deleting factors that are important to the process.

Table 1: Fractional factorial design 2^{5-1}

Test	OPN mucilage	SUC	NaCl	$CaCl_2$	Buffer (pH)
1	−1	−1	−1	−1	+1
2	+1	−1	−1	−1	−1
3	−1	+1	−1	−1	−1
4	+1	+1	−1	−1	+1
5	−1	−1	+1	−1	−1
6	+1	−1	+1	−1	+1
7	−1	+1	+1	−1	+1
8	+1	+1	+1	−1	−1
9	−1	−1	−1	+1	−1
10	+1	−1	−1	+1	+1
11	−1	+1	−1	+1	+1
12	+1	+1	−1	+1	−1
13	−1	−1	+1	+1	+1
14	+1	−1	+1	+1	−1
15	−1	+1	+1	+1	−1
16	+1	+1	+1	+1	+1
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0

Table 2: Coded and real values of the fractional factorial design 2^{5-1}.

Factor	Code	-1	0	1
OPN mucilage (%)	X_1	0	1,5	3
SUC (%)	X_2	0	10	20
NaCl (%)	X_3	0	2	4
$CaCl_2$ (%)	X_4	0	0,5	1
Buffer (pH)	X_5	3	7	11

2.5.2 Factorial design

After the selection of factors, a new experimental design was executed, the completely randomized design applied to factorial (5x3x3). The factors studied and their levels are shown in Table 3. Outcome variables were the rheological data. The responses studied in experimental design were evaluated by analysis of variance (5% probability) and the significant factors were submitted to regression analysis, with the assistance of SAS Statsoft (licenced by Federal University of Lavras). The 45 tests were executed in 2 repetitions.

Table 3: Factors and levels considered to the factorial design 5x3x3.

OPN mucilage (%)	SUC (%)	NaCl (%)
0	0	0
1,25	10	2
2,50	20	4
3,75		
5,00		

3. RESULTS AND DISCUSSIONS

3.1. Screening factors

3.1.1. Rheological behavior and thermogravimetry

Figure 2 shows the variation of the apparent viscosity versus shear rate for some of the treatments specified in Table 1. According to the curves behavior, where the viscosity values decrease with the force until stabilization, the samples are classified as pseudoplastic (Steffe, 2006). From the structure of mucilage as polymer with long molecule chains connected and trapped water inside, little force can cause polymer rearrangement in the same direction with the input force and also flowing following the high force, so the increase of flow rate is related to the decrease of viscosity (Thanatcha & Pranee, 2011).

From Figure 2 it can be inferred that there are differences between treatments with OPN gum in the presence of sucrose, sodium chloride and calcium chloride ($CaCl_2$). The highest values for apparent viscosity are observed for systems with maximum concentration of OPN gum added and the maximum concentration of sucrose (treatment 4). The minimum values were obtained in treatment with minimal OPN mucilage concentrations added to minimum sucrose (SUC) and sodium chloride (NaCl) concentration (treatment 8). The pH values showed no notable differences considering all treatments. According to Thanatcha & Pranee (2011) guar gum and xanthan gum viscosities at low pH were not different from the basic condition.

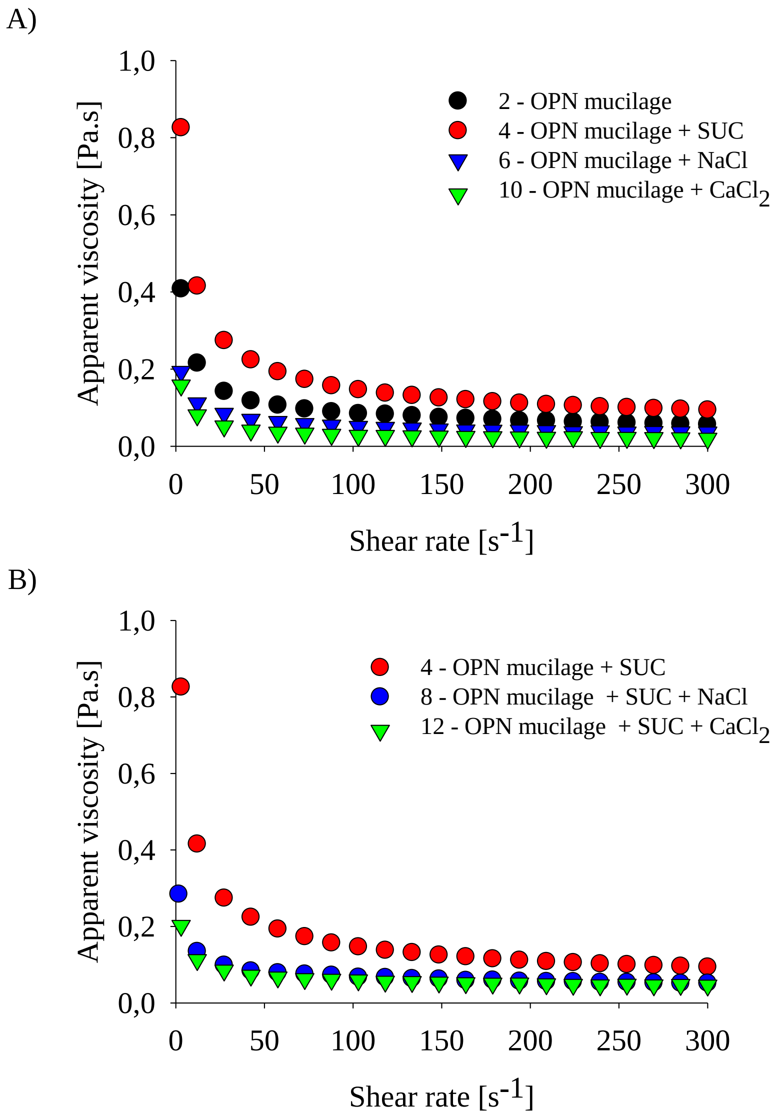


Figure 2: Variation of apparent viscosity versus shear rate of the treatments 2, 4, 6 and 10 (A) and 4, 8 and 12 (B).

The behavior of thermogravimetric curves (TG) are shown in Figure 3. Figure 3 (A) shows that the samples composed of OPN mucilage, OPN mucilage

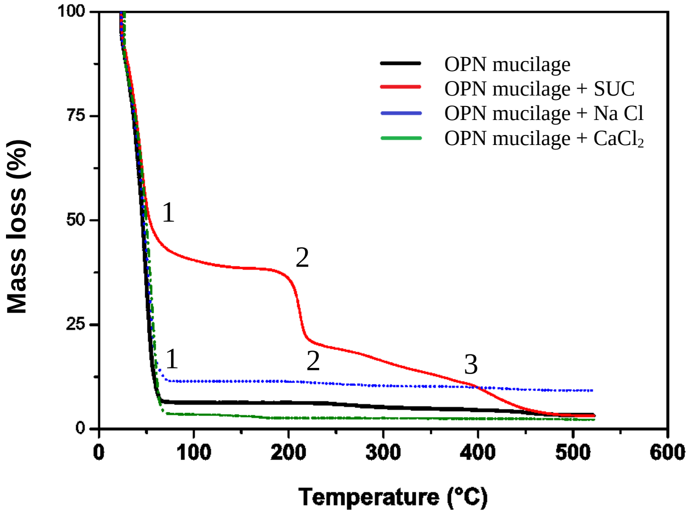
+ NaCl + $CaCl_2$ and OPN mucilage had similar weight loss behaviors with the temperature elevation, and a lower percentage of the loss was observed to OPN mucilage + NaCl sample. The abrupt decline on the beginning of the process, between room temperature and 65°C approximately, refers to the water loss of the systems. On temperatures near 240°C samples had a small decline in the thermogravimetry curves, suggesting the loss of organic compounds. The sample with presence of SUC presented a behavior divided into 3 phases as noted by Conceição et al, (2014). In the first phase, the sample mass loss refered to water loss occurred between room temperature and 55.7°C, representing a loss of 42.9% of the initial mass of the sample. Between the temperatures of 204.5°C and 219.5°C occurred event 2, generating a weight loss from 37.6% to 20.9%. There is a transition that can be attributed to a change in the conformation of the biopolymer, followed by breakage of the branches, because the TG associated curves show a significant loss of mass. This transition occurs due to the oxidative degradation of the samples (Mercê, Fernandes, Mangrich, Szpoganicz, & Sierakowski, 2001). The mass loss can be attributed to polysaccharides and proteinaceous components. The event 3 corresponds to the end of the sample destruction (between 270°C and 480°C) and refers to the remaining minerals and carbonaceous mass.

The samples presented on Figure 3 (B) are composed of OPN + SUC. The addition of NaCl shifted down the curve (higher weight loss) and the presence of $CaCl_2$ preserved the mass. The presented curves showed the 3 events described in Figure 3 (A).

Mothé & Rao (2000) studied the thermal behavior of arabic gum and cashew gum. Both consist of high molecular mass polysaccharides and interact with water, having applications such as emulsification and texture control. These particular characteristics and TG properties are similar to OPN mucilage. TG profiles showed that Arabic gum and cashew gum presented two phases: the first stage occurred at around 100.8°C, relative to the water loss and the second event occurred at 252.8°C (Tonset), attributed to polysaccharides. The residue could be carbonacious.

The values obtained for viscosity and TG, considering all treatments, were used in a fractional factorial design to reduce the number of factors as described in the next section.

A)



B)

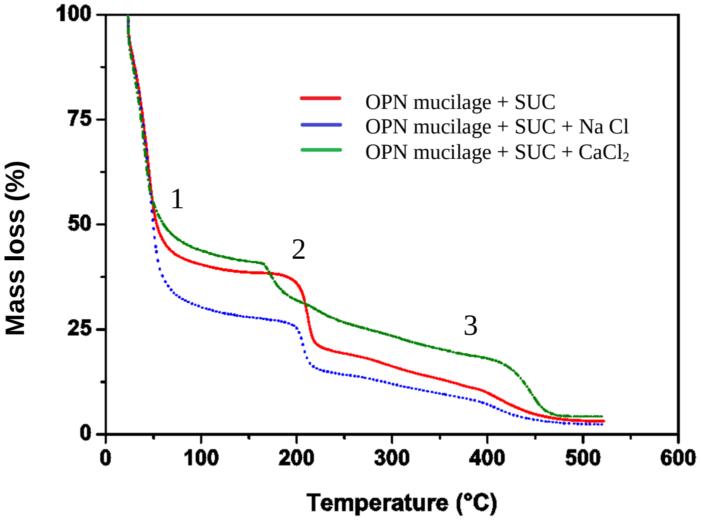


Figure 3: Thermogravimetric analyzes of the samples (A): OPN mucilage, OPN mucilage + SUC, OPN mucilage + NaCl and OPN mucilage + $CaCl_2$, (B): OPN mucilage + SUC, OPN mucilage + SUC + NaCl and OPN mucilage + SUC + $CaCl_2$.

3.1.2. Fractional factorial

Analysis of effects related to the apparent viscosity response (Pa.s, $100s^{-1}$) and TG parameters on the fractional factorial design 2^{5-1} are shown in Table 4. TG responses were defined by event, as showed in Figure 3 (section 3.1.1).

The indication of interference in apparent viscosity by adding OPN mucilage was confirmed in this study by the significance value and its positive effect on the viscosity of the samples (Table 4), causing an increase on the studied response. Lima Junior et al. (2013) defined that OPN mucilage is composed of 13.45g/100g moisture, protein (10.47g/100g), carbohydrates (46.88g/100g) and ashes (42.54g/100g) with significant levels of calcium, potassium, phosphorus, magnesium, sulfur and iron, the characteristic mineral of OPN. In the same study the authors tested different concentrations of OPN mucilage in aqueous solution (0.25 – 1.00%) and verified that the increase in the viscosity of the reconstituted system is directly proportional to the increase of OPN mucilage concentration in a linear manner, relating to OPN mucilage macromolecular structure.

Table 4: Estimation of effects and p-values of apparent viscosity ($100s^{-1}$), T_{endset} (1), T_{onset} (2), T_{onset} (3), M_{quant} (2) and M_{endset} (3).

Source Response	OPN mucilage	SUC	NaCl	$CaCl_2$	pH
Apparent Viscosity (Pa.s)	0.066 (0.006)*	0.011 $(0.323)^{ns}$	-0.011 $(0.331)^{ns}$	-0.020 $(0.124)^{ns}$	-0.001 $(0.947)^{ns}$
T_{endset} (1) (°C)	0.275 $(0.909)^{ns}$	-1.275 $(0.605)^{ns}$	1.225 $(0.619)^{ns}$	-0.225 $(0.925)^{ns}$	-4.075 $(0.163)^{ns}$
T_{onset} (2) (°C)	59.448 (0.008)*	-10.088 $(0.361)^{ns}$	7.238 $(0.497)^{ns}$	-9.688 $(0.378)^{ns}$	-9.963 $(0.366)^{ns}$
T_{onset} (3) (°C)	92.625 (0.033)*	77.325 (0.052)*	2.525 $(0.925)^{ns}$	55.950 $(0.109)^{ns}$	-56.050 $(0.109)^{ns}$
M_{quant} (2) (%)	3.370 $(0.033)^{ns}$	5.58 (0.008)*	4.980 $(0.011)^{ns}$	2.020 $(0.111)^{ns}$	-1.193 $(0.278)^{ns}$
M_{endset} (3) (%)	1.508 $(0.293)^{ns}$	-0.343 $(0.791)^{ns}$	5.493 (0.019)*	0.983 $(0.467)^{ns}$	-0,768 $(0.563)^{ns}$

Values in parentheses are p values.* p-value<0.10. ns: not significant. (1): TG event 1, (2): TG event 2 and (3): TG event 3. T_{endset}: final temperature of the event, T_{onset}: initial temperature of the event. M_{quant}: weight corresponding to the difference between the initial and final weights of the event. M_{endset}: final weight (%) of the event.

The TG event 1 corresponded to water loss of the samples and according to Table 4, the factors studied did not cause effect in the final temperature of the event (T_{endset} (1)). OPN mucilage had a positive effect on the initial temperature of event 2 (T_{onset} (2)), event that was corresponded to oxidative degradation of the samples. This result demonstrates that the presence of OPN mucilage allows sample integrity until temperatures around 200°C. On this same event was possible to analyse the mass loss data. As noted in Table 4, the difference between the initial mass and the final mass event (M_{quant} (2)) had a positive effect of sucrose. OPN mucilage and SUC had a positive effect on the onset degradation temperature of the samples (T_{onset} (3)) on the event 3. NaCl had a positive effect on the mass loss of event 3 (M_{endset} (3)). It is possible to infer that the behaviors of OPN mucilage, SUC and NaCl assisted in the thermal resistance of the samples in the various events.

3.2 Complete factorial evaluation

The factors selected for evaluation of rheological parameters in complete factorial format were OPN mucilage, SUC and NaCl. Factors and levels studied in this section are shown in Table 3 (item 2.5.2). The non-selected factors were fixed in pH 7 and absence of $CaCl_2$.

3.2.1. Rheological behavior

Rheological parameters of the gums prepared in accordance with the experimental design were evaluated (Table 3). It can be verified that the samples prepared with OPN mucilage between 0% and 1.25% fitted to the Newton model (R^2> 80%). The Newtonian samples had a tendency to increase the viscosity (μ) with the addition of solutes. The samples composed with OPN mucilage concentrations between 2.5% and 5.0% fitted to Power Law model. These samples showed good fit (R^2> 85%) and pseudoplastic behavior. This behavior was evidenced by the values of consistency coefficient higher than zero (k> 0) and index flow behavior values between zero and one (0 <n <1) (Steffe, 2006). Mucilages usually present a non-Newtonian behavior, such as *Ziziphus mauritiana* Lam. mucilage that fitted to the Power Law model in 5% solutions (Thanatcha & Pranee, 2011). The samples presented thyxotropic behavior and a tendency to higher values of thixotropy with the presence of OPN mucilage.

Figure 4 confirmed the Newtonian behavior of the samples with 0% and 1.25% of OPN mucilage, showing the linear tendency of the experimental points. The curves of the samples with 2.50%, 3.75% and 5.00% of OPN mucilage presented a non-Newtonian behavior, fitting the Power Law rheological model.

The vast majority of samples exhibited values of G' greater than G'' (1Hz) in all experiments as shown in Table 5. This reflects the predominance of elastic behavior of the samples, representing the resistance structure to contribute to a three-dimension network. Figure 5 exemplifies this behavior to the sample composed of 5% OPN mucilage + 20% SUC + 4% NaCl, but explores the whole range of frequency.

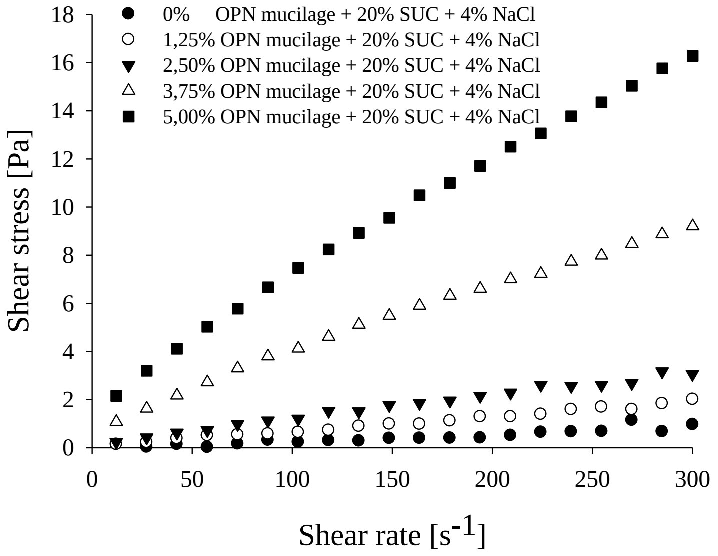


Figure 4: Flow curves of the samples composed of 20% SUC + 4% NaCl and varying OPN mucilage concentration.

Table 5: Mean values G’ (Pa) and G” (Pa) of the complete factorial tests at frequency of 1Hz

Test	OPN mucilage (%)	SUC (%)	NaCl(%)	G’ (Pa)	G” (Pa)
1	0	0	0	2.197	0.949
2	0	0	2	1,652	0.989
3	0	0	4	2.675	1.135
4	0	10	0	1.662	0.920
5	0	10	2	1.866	0.201
6	0	10	4	1.848	1.239
7	0	20	0	2.501	1.229
8	0	20	2	1.475	0.889
9	0	20	4	2.154	1.311
10	1.25	0	0	2.325	1,448
11	1.25	0	2	1.012	1.857

Table 5, conclusion

Test	OPN mucilage (%)	SUC (%)	NaCl(%)	G’ (Pa)	G” (Pa)
12	1.25	0	4	2.695	1.306
13	1.25	10	0	1.842	1.254
14	1.25	10	2	0.821	0.466
15	1.25	10	4	1.255	0.527
16	1.25	20	0	0.969	0.710
17	1.25	20	2	0.282	1.006
18	1.25	20	4	1.994	0.480
19	2.50	0	0	1.371	0.192
20	2.50	0	2	1.234	0.935
21	2.50	0	4	0.928	0.568
22	2.50	10	0	0.925	0.255
23	2.50	10	2	1.019	0.295
24	2.50	10	4	0.413	1.734
25	2.50	20	0	0.790	0.220
26	2.50	20	2	0.877	0.569
27	2.50	20	4	1.305	0.677
28	3.75	0	0	1.004	0.475
29	3.75	0	2	1.764	0.960
30	3.75	0	4	0.892	0.184
31	3.75	10	0	1.833	1.904
32	3.75	10	2	1.637	0.939
33	3.75	10	4	1.624	0.817
34	3.75	20	0	2.130	1.209
35	3.75	20	2	1.418	0.986
36	3.75	20	4	1.957	0.950
37	5.00	0	0	8.629	3.544
38	5.00	0	2	15.280	4.812
39	5.00	0	4	2.859	1.669
40	5.00	10	0	2.059	0.943
41	5.00	10	2	1.919	1.055
42	5.00	10	4	4.758	2.382
43	5.00	20	0	2.585	1.811
44	5.00	20	2	2.716	1.533
45	5.00	20	4	2.731	1.544

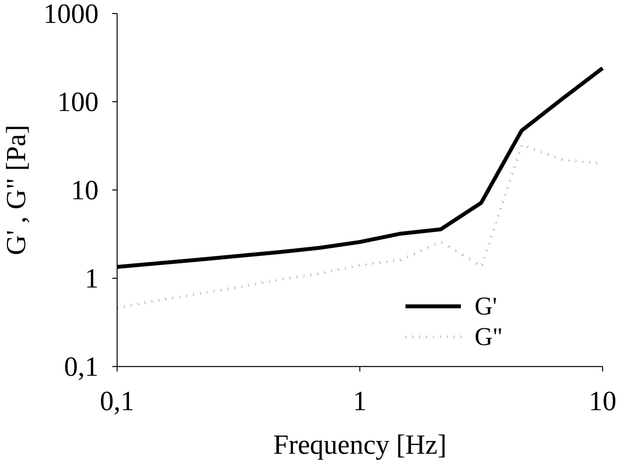


Figure 5: Viscoelastic properties of the sample composed of 5% OPN mucilage + 20% SUC + 4% NaCl.

3.2.2. Statistical evaluation of the rheological data

Responses obtained from the full factorial design (5x3x3) were subjected to analysis of variance, than the significant factors interactions were unfolded and the resultant regressions were analysed. The following rheological responses were studied: apparent viscosity ($100s^{-1}$), G', G'' and thixotropy.

The regressions of apparent viscosity in function of OPN mucilage are shown in Figure 6. Apparent viscosity had an increasing tendency with increasing concentration of OPN mucilage according to Figures 6 A, B, C and D. In the absence of NaCl and SUC the samples had linear behavior of viscosity with OPN mucilage increase. The presence of NaCl changed the behavior of the samples by adjusting the curves of quadratic equations. These behaviors can be observed in Figure 6 A. Figure 6 (B) shows that the behavior of the samples was similar to the 20% SUC and NaCl studied range, increasing the apparent viscosity linearly with the increasing of OPN mucilage concentration. Sucrose was maintained among linear increase in viscosity with OPN mucilage, as shown in Figure 6 C. With the maximum concentration of NaCl, sucrose changed the behavior of the curves passing from quadratic to linear, with greater values to the curve with maximum concentration of SUC (Figure 6D).

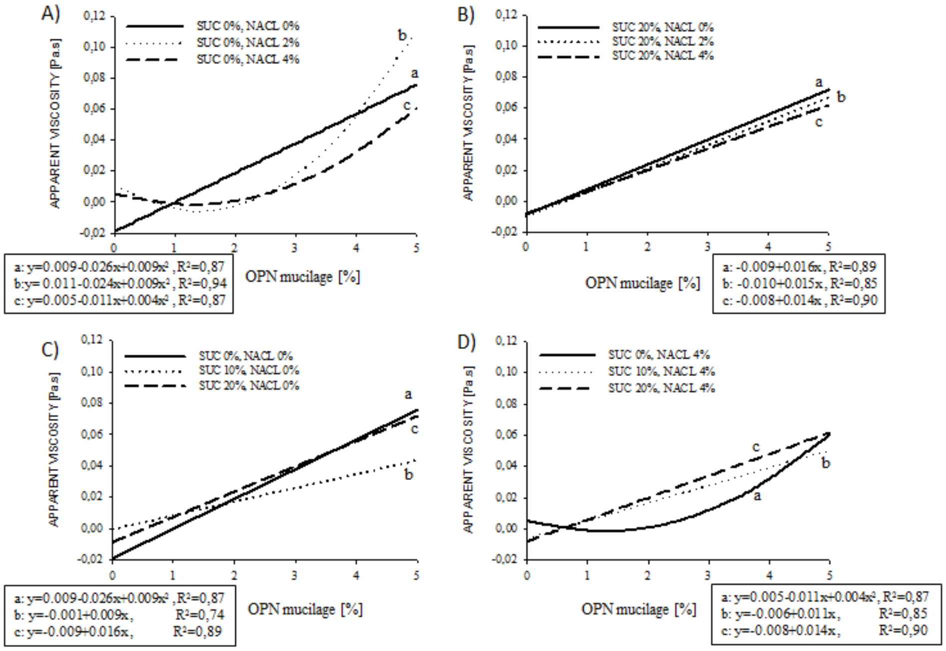


Figure 6: Unfolding of regressions from the triple interaction for the apparent viscosity response ($100 s^{-1}$) varying OPN mucilage, SUC and NACL levels. "y" = apparent viscosity (Pa.s) and "x" = OPN mucilage (%).

Higiro, Herald, Alavi, & Bean (2007) studied the effects of salt in xanthan and locust bean gum (LBG) interaction. They verified that the addition of salt did not affect the intrinsic viscosity of LBG in aqueous solution within 5 to 50 mM of NaCl, but the increase in ionic strength of the salt caused a significant decrease in intrinsic viscosity for gum blends.

The regressions performed for the significant factors of G' and G" are shown in Figures 7 A and B respectively. On Figure 7 is possible to verify that the G' values are higher than those of G", confirming the elastic predominant character of the samples. Increasing OPN mucilage concentration increases this elastic behavior, contributing to the formation of stronger gels.

Figure 8 (A, B, C and D) shows that the thixotropy values tended to increase with increasing concentration of OPN mucilage. In the absence of sucrose (Fig. 8 A), samples showed a quadratic fit. From 3% OPN, sample with 2% NaCl presented higher thixotropy values. With the maximum sucrose concentration (Fig. 8 B), the samples with 0% and 4% NaCl were similar, and 2% NaCl showed a quadratic fit and greater value than the others in a concentration of 5%. In the absence of NaCl (Fig. 8 C) the samples with presence of sucrose had similar behavior. Samples with a maximum NaCl concentration (Fig. 8 D) showed similar behavior, while in the presence of 20% SUC fit was linear.

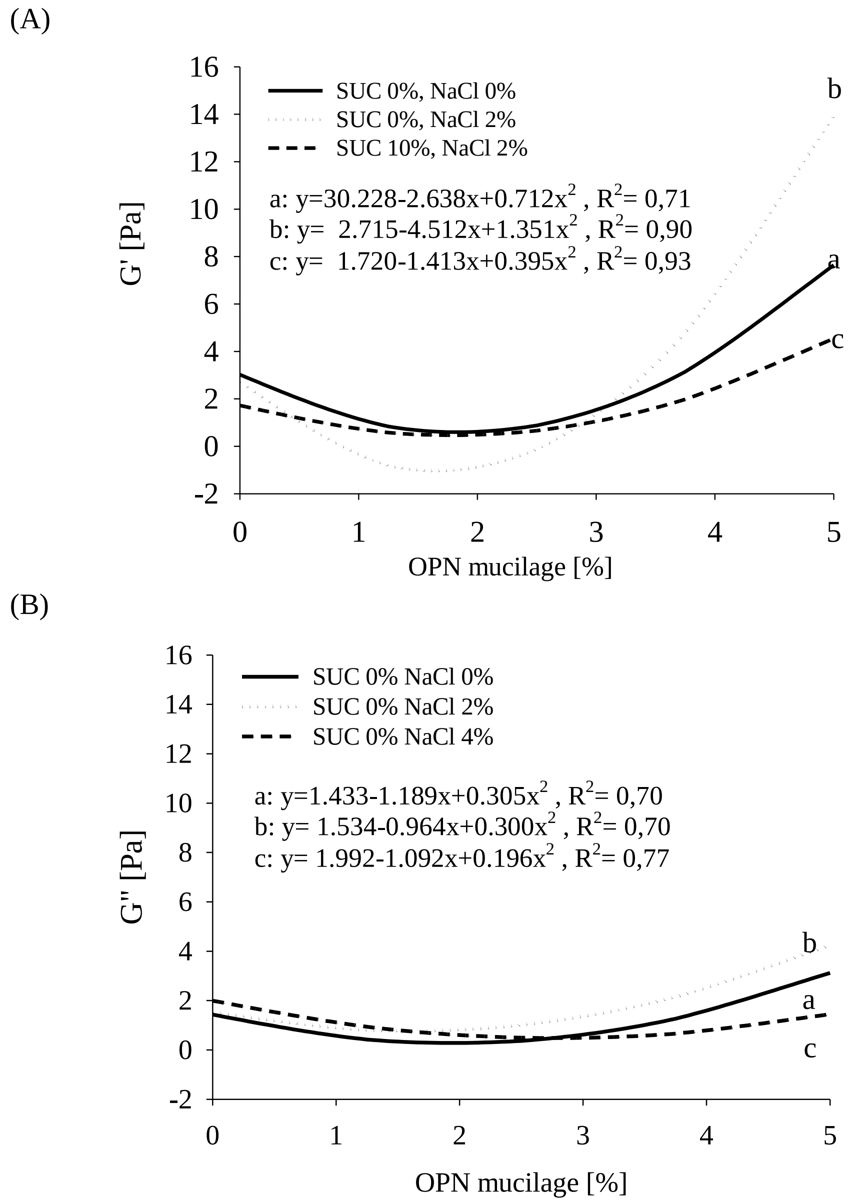
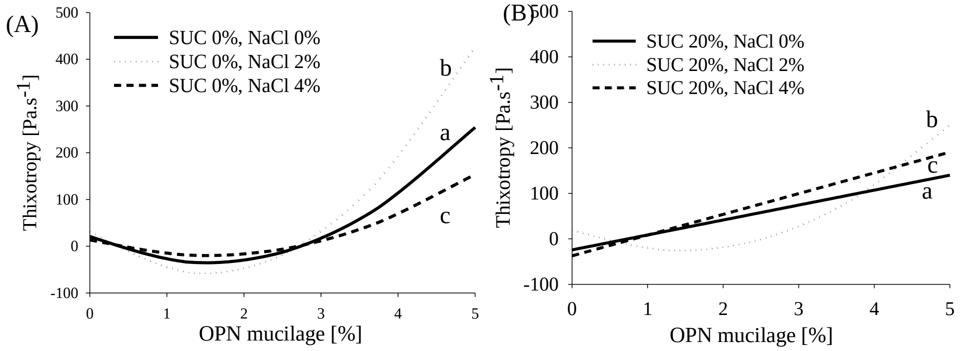
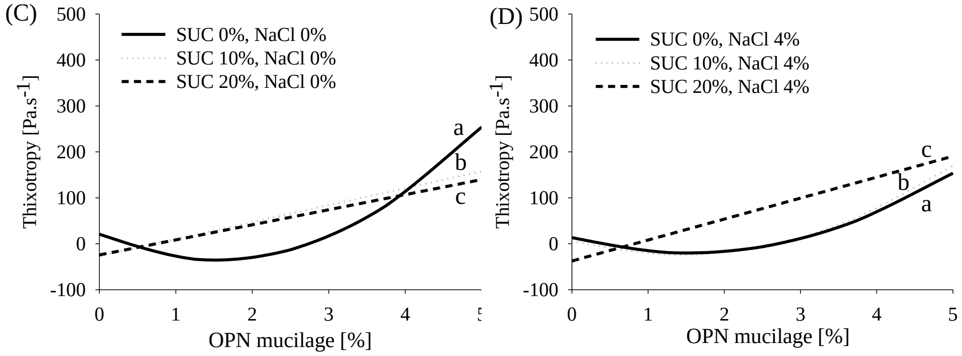


Figure 7: Unfolding of regressions from the triple interaction for the G’ (A) and G” (B) responses varying OPN mucilage, SUC and NACL levels. “y” = G’ or G” (Pa) and “x” = OPN mucilage (%).



$a: y=20.845-73.687x+24.066x^2$, $R^2= 0,84$
$b: y= 31.581-118.878x+39.585x^2$, $R^2= 0,90$
$c: y= 13.460-43.971x+14.400^2$, $R^2= 0,86$

$a: y=-24.351+32.833x$, $R^2= 0,82$
$b: y= 19.120-62.341x+21.741x^2$, $R^2= 0,96$
$c: y= -37.419+45.586x$, $R^2= 0,77$



$a: y=20.845-73.687x+24.066x^2$, $R^2= 0,84$
$b: y= -25.788+36.805$, $R^2= 0,87$
$c: y=-24.351+32.833x$, $R^2= 0,82$

$a: y= 13.460-43.971x+14.400^2$, $R^2= 0,86$
$b: y= 6.195-42.441x+15.047x^2$, $R^2= 0,90$
$c: y= -37.419+45.586x$, $R^2= 0,77$

Figure 8: Unfolding of regressions from the triple interaction for the thixotropy response varying OPN mucilage, SUC and NACL levels: (A) – samples 0% SUC, (B) – samples 20% SUC, (C) – samples 0% NaCl and (D) – samples 4% NaCl. “y” = Thixotropy (Pa.s^{-1}) and “x” = OPN mucilage (%).

4. CONCLUSIONS

Model solutions studied for selection of factors presented pseudoplastic behavior in the presence of OPN mucilage. OPN mucilage was the factor with significant effect on the apparent viscosity of the samples. Thermogravimetric analysis showed the presence of three distinct phases of mass loss with increasing temperature, which may be related to water loss, change in conformation of polysaccharides and remaining mineral material. OPN mucilage, SUC and NaCl were the variables with significant effect on thermogravimetry responses. In full factorial studies the rheological characteristics of the samples were identified, where the samples with Newtonian behavior were the ones that ranged from 0% to 1.25% OPN mucilage and Power Law samples presented 2.50% - 5.00%, with a predominance of elastic behavior (G' > G" at 1Hz). Apparent viscosity had an increasing tendency with increasing concentration of OPN mucilage. G' values were higher than those of G", confirming the elastic predominant character of the samples. Increasing OPN mucilage concentration increases this elastic behavior, contributing to the formation of stronger gels.

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(VERSÃO PRELIMINAR).

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ARTIGO 2 Rheological study of fermented milk beverage produced with *Pereskia aculeata* Miller mucilage and commercial hydrocolloids

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ABSTRACT

Fermented milk products can be produced with hydrocolloids, such as guar gum (GG) and gum Arabic (GA), to keep their quality during the shelf-life period and to replace fat. With the increasing demand for new hydrocolloids, *Pereskia aculeata* Miller mucilage extraction resulted in a potential additive as gelling agent and emulsifier. The study of rheology and the understanding of synergic interactions between hydrocolloids (OPN mucilage, GA, GG) and the components of model solutions and a fermented milk beverage are the aims of this study. OPN mucilage were extracted by the authors. Mixture design was applied to the model solutions studies, composed by OPN mucilage, GA, GG, sucrose, sodium chloride and deionized water. Rheological properties were evaluated. From these results, a hydrocolloid mix was defined and applied to a fermented milk beverage. Rheological and microstructural properties were studied. The highest values for apparent viscosity of the model solutions occurred on the region with hydrocolloids mix composed of 0,7 OPN mucilage, 0,0 GA and 0,3 GG, resulting in 0,5-0,7 Pa.s. Sucrose had greater interference than sodium chloride on apparent viscosity. The effect of apparent viscosity, G' and G", were discussed and the model solutions presented anti-thixotropic and pseudoplastic behavior with good fit to the Power Law rheological model. Fermented milk samples presented good fit to Power law, pseudoplastic and thixotropic behavior, and had predominant elastic behavior (G'>G"). The application of hydrocolloids mix increased the apparent viscosity and pH of the samples. The microstructure evaluation confirmed the results. Application of hydrocolloids mix with OPN mucilage on fermented milk presented to be viable.

***Keywords*:** Fermented milk beverage, Microstructure, Mixture design, Sodium chloride, Sucrose, Synergy

1. INTRODUCTION

Fermented milk products are obtained by pH decreasing and coagulation of milk added or not of other foodstuff and dairy products. The lactic fermentation takes place by action of specific microorganisms crops, which should be viable, active and abundant in the final product during its shelf-life period (MAPA, 2007). Gel formation is the most important functional property of fermented dairy products, and the rheological characteristics of their gel are affected by the selected starter culture (Casarotti, Monteiro, Moretti, & Penna, 2014). Stabilizers as hydrocolloids are commonly used in cultured products to control texture and reduce whey separation. Lower-fat versions of cultured products usually need careful selection of stabilizers to restore the creaminess and other attributes present in the full-fat product (Lucey, 2004).

Guar gum is a hydrocolloid processed from *Cyamopsis tetragonoloba* seeds. Its composition essentially consists of the galactose and mannose complex carbohydrate polymer. Industrial application of guar gum is possible because of its ability to form hydrogen bonding with water molecules, chiefly used as thickener and stabilizer (Mudgil, Barak, & Khatkar, 2014). Another hydrocolloid widely used in industry is gum arabic, an edible, dried, gummy exudate from the stems and branches of *Acacia senegal* and *A. seyal*. It is rich in non-viscous soluble fiber and it is used as a stabilizer, thickening agent and emulsifier (Ali, Ziada, & Blunden, 2009).

According to the increasing demand for hydrocolloids with specific functionality, finding new hydrocolloids sources with appropriate properties is an active area of study (Salehi, Kashaninejad, Tadayyon, & Arabameri, 2015). Lima Junior et al. (2013) developed a process that allows the extraction of hydrocolloids from *Pereskia aculeata* Miller leaves. This plant belongs to the Cactaceae family and is popularly known in Brazil as ora-pro-nobis (OPN). The protein content of the leaves was reported as high when compared with other vegetables and presents a high level of total dietary fiber, as well as considerable amounts of carbohydrates and minerals (calcium, magnesium, manganese and zinc) (Pinto & Scio, 2014; Takeiti, Antonio, Motta, Collares-Queiroz, & Park, 2009). The polysaccharide complexes of *P. aculeata* Miller leaves are highly ramified and contain arabinofuranose, arabinopyranose, galactopyranose, galactopyranosyl, uronic acid and rhamnopyranose units (Sierakowski, Gorin, Reicher, & Corrêa, 1990).

OPN proved to be an alternative source of mucilage, presenting properties that can be used in industry as a thickener, gelling agent and/ or emulsifier. OPN mucilage is rich in protein, carbohydrates and minerals, such as calcium, potassium and phosphorus. These findings were verified by the linear increase of the viscosity as a function of the concentration of the gum produced by the reconstitution of the powder as well as by the high emulsion formation capacity and thermal stability of this emulsion under different temperature conditions (Conceição, Junqueira, Guedes Silva, Prado, & de Resende, 2014; Lima Junior et al., 2013).

The study of rheological properties is important for the design of industrial processes such as fluid flow and pumps, it is also a parameter for the sensory characteristics and stability of food products. The thickening properties and viscoelastic behavior of hydrocolloids in solution can be significantly affected by variables such as shear rate and time, concentration of the compound, temperature, pressure, ionic strength and pH, among others. The analysis of individual or combined effects of these factors is important, especially when they will be used to modify food texture, and also in the design, evaluation and modeling of processes (Amid & Mirhosseini, 2012; Capitani et al., 2015; Karazhiyan et al., 2009).

Rheological behavior and the study of interactions between hydrocolloids and other main components in food formulations are researched in order to explore the microstructure and to characterize thickening systems. The use of two or more gums in the formulation of a product is extensive in the food industry due to the synergic effect of combined use. It may result in an improvement of the product quality and provide economic benefits by decreasing concentration of the gum in the formulation. Considering that apparent viscosity and physical stability of a food formulation could be modified by the presence of sugars and salts, at higher concentrations than used for hydrocolloids, the study of these mixtures is justified (Chenlo, Moreira, & Silva, 2011).

The aims of the present work were to study the rheology and to understand synergic interactions between hydrocolloids (OPN mucilage, gum Arabic and guar gum), sucrose and sodium chloride in model solutions by mixture design. Application of the optimized hydrocolloids mix in a fermented milk beverage was also explored.

2. MATERIALS AND METHODS

2.1 Extraction of OPN mucilage

The extraction process of OPN leaves mucilage was conducted according to an adaptation of Lima Junior et al. (2013) process, as shown in Figure 1.

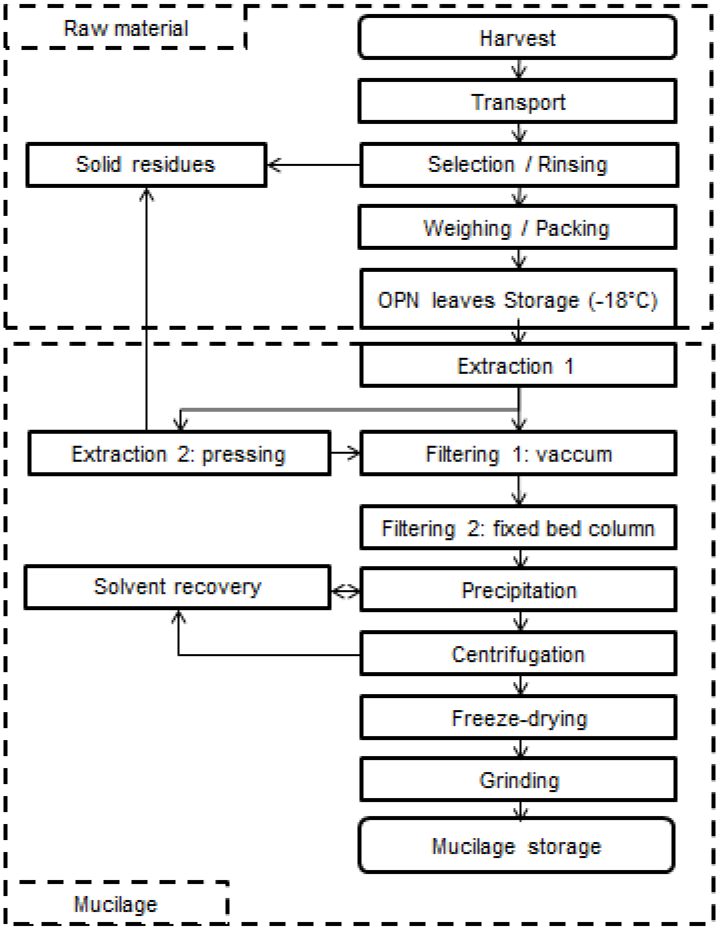

Figure 1: Extraction process of OPN leaves mucilage

2.1.1 Raw material

Fresh OPN leaves were obtained in the city of Lavras, Brazil, from May to June 2015. They were harvested and transported to the Food Refrigeration Laboratory of the Department of Food Science. In the laboratory, the leaves were selected, rinsed, weighed and packed in identified polyethylene bags. The packaged material was stored in freezer (-18°C) until the mucilage extraction.

2.1.2 OPN mucilage extraction

Extraction 1 is the homogenization of the leaves in industrial blender (Metvisa LG10, São Paulo, Brazil) with boiling water and subsequent extraction in the thermostatic water bath (Solab SL 150, Piracicaba, Brazil) to 75 ± 1°C during 6 hours. The resulting material of *Extraction 1* was submitted to the *Extraction 2*, pressing in hydraulic press (Tecnal TE 058, Campinas, Brazil). The liquid fractions derived from *Extractions 1 and 2* were passed through the *Filtering 1*. This step was carried out by filtering the material with vacuum (dual-stage pump) in Buchner funnel and organza fabric as a filter element, generating Filtrate 1.

The Filtrate 1 was placed in a fixed bed column containing activated carbon to remove insoluble solids and pigments (*Filtering 2* phase). This column consists of a PVC tube with 1.00m and 0.11m long diameter, containing granulated activated carbon (Dinâmica Química 1-2mm). This process generated

Filtrate 2. This filtrate was precipitated with ethanol 95% (Sigma-Aldrich) at a ratio of 3: 1 of alcohol per liter of Filtrated 2. The precipitate was centrifuged (centrifugal SP Labor SP701, Presidente Prudente, Brazil, 12 min, 628 G) for maximum removal of alcohol and dried in freeze-dryer (Edwards, São Paulo, Brazil). The dry material was ground in ball mill (Marconi MA350, Brazil) for 1 minute and stored away from light and moisture. The ethyl alcohol used in these steps was recovered by distillation and reused for Precipitation.

2.2 Preparation of model solutions

The model solutions were prepared by homogenizing in a magnetic stirrer (30 min, 75°C) the solutions components: deionized water, sucrose (Isofar), sodium chloride (Sigma Aldrich), OPN mucilage, gum arabic (Synth) and guar gum (Sigma-Aldrich) according to the experimental design (item 2.7). The homogenized solutions were kept in a thermostatic cabinet (Eletrolab, EL202, São Paulo, Brazil) at 4°C during 24 hours for complete hydration. Sodium chloride (NaCl) and sucrose (SUC) were chosen to compose the solutions because they are the most abundant low molecular food taste component (Antipova & Semenova, 1995).

2.3 Fermented milk beverage production

Fermented milk products were prepared with whole and low fat milk using the starter culture, comprised of *L. acidophilus*, *Bifidobacterium* and *S. thermophiles* (Bio Rich, Chr. Hansen), with sucrose (Isofar) and with or without inclusion of the hydrocolloids mix. The formulas of the samples were prepared according to the experimental design (item 2.7) and the composition of hydrocolloids mix was defined on the previous model solutions tests.

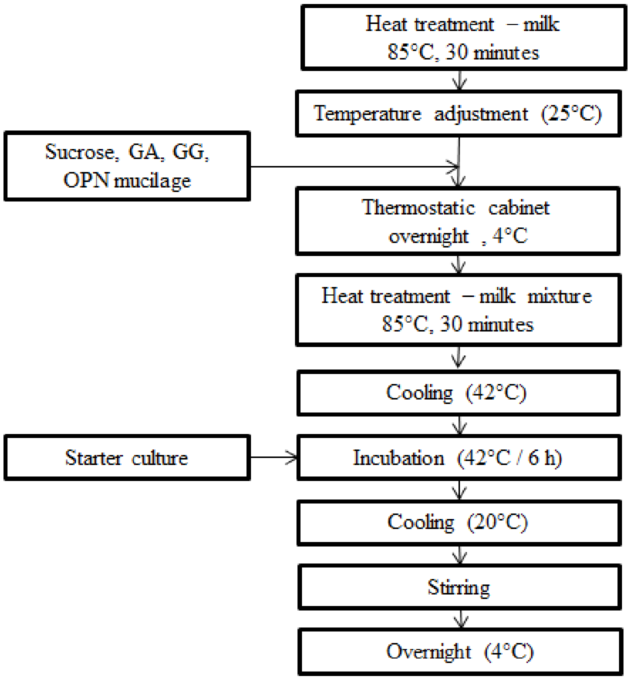

Figure 2: Fermented milk production flowchart

The fermented milk production flowchart is shown in Figure 2. The two types of milk were heat treated and cooled firstly. The correspondent SUC and hydrocolloids mix were added and stayed overnight on a thermostatic cabinet at 4°C (Eletrolab, EL202, São Paulo, Brazil) to complete hydrocolloids hydration. The milk solutions were heat treated to avoid the presence of undesirable microorganisms from the ingredients added and cooled up to 42°C. At that temperature the starter culture was added and the samples were incubated in oven at 42°C during 6 hours. After ripening the samples had the gel structure broken with slight agitation and stayed overnight at 4°C until the analysis.

2.4 Physical-chemical analysis - milk

Milk samples were characterized according to the density ($g.L^{-1}$) and contents (%) of fat, lactose, solids and proteins. To these determinations were used Lactoscan ultrasonic milk analyzer G0041P (Bulgaria). The pH value of the samples were determined in potentiometer Schott Handylab.

2.5 Rheological analysis

Rheological studies were conducted using the rheometer HAAKE RheoStress 6000 (Thermo Scientific – Karlsruhe / Germany) coupled to a temperature controller HAAKE UTM (Thermo Scientific).

2.5.1 Rheological tests of the model solutions

The model solutions were analysed with parallel plate geometry (34.997 mm diameter), GAP 1 mm at 20°C. Characterization of the flow rheological behavior was performed by the operation of the flow curve, subjecting the samples to a continuous ramp shear rate ($\dot{\gamma}$) between 0 and 300 s^{-1} for 2 minutes with 100 shear stress (τ) readings. For the generation of the flow curves, samples were first stabilized for 3 minutes at 20 °C and subjected to thixotropy breaking, by applying a continuous ramp shear rate ($\dot{\gamma}$) between 0 and 300 s^{-1} for 2 minutes for ramping and the same time to decrease gradient between 300 and $0s^{-1}$.

Oscillatory tests were performed to study the viscoelastic properties of the samples. The definition of the linear viscoelastic region was carried out by subjecting the samples to shear stress between 0.001 Pa and 100 Pa with a fixed frequency of 1 Hz and 20°C. The frequency sweep curves were generated assuming a value of shear stress within the linear viscoelastic range, varing frequency range from 0.01Hz to 10 Hz at 20°C.

2.5.2 Rheological tests of the fermented milk

Fermented milk samples were performed with cone plate geometry (34.998 mm diameter, 2°), GAP 0,105mm at 10°C (Braga & Cunha, 2004). Flow curves were obtained by submitting the samples to a continuous shear rate ($\dot{\gamma}$) ramp (0 to 300 s^{-1} / 2 minutes / 100 readings). For the generation of the flow curves, samples were first stabilized for 3 minutes at 10°C and subjected to thixotropy breaking (0 - 300 s^{-1} and 300 - 0 s^{-1} ramps).

The oscillatory tests were: definition of the linear viscoelastic region (shear stress between 0.001 Pa and 100 Pa / 1 Hz / 10°C) and frequency sweep curves assuming a value of shear stress within the linear viscoelastic range and frequency range from 0.01Hz to 10 Hz at 10°C.

2.5.3 Rheological responses

Data obtained on the flow curves were fitted to the Newton (Equation 1) and Power Law (Equation 2) rheological models, generating responses to the fluid classification and the adjusted model parameters. Thixotropy values were calculated by the difference between the curves areas of rise and fall prior to the generation of flow curve. The apparent viscosity values were calculated from the shear stress point of 100 s^{-1}, related to the value experienced by the fluid in industrial pipes (Steffe, 2006). From the frequency sweep curves were observed the behavior of G' and G" and the effect plots of G’ and G” at 1Hz of frequency.

$$\tau = \mu \cdot \dot{\gamma} \qquad (1)$$

$$\tau = K \cdot (\dot{\gamma})^n \qquad (2)$$

where τ is shear stress (Pa), $\dot{\gamma}$ is shear rate (s^{-1}), μ is viscosity (Pa.s), K is the consistency coefficient and *n* is the flow behavior index.

2.6 Microstructural analysis

2.6.1 Scanning electron microscopy (SEM)

The fermented milk samples were freeze-dried (Edwards, São Paulo, Brazil) (Espírito-Santo et al., 2013), fixed with double-sided carbon tape to aluminum supports (stubs) and sputter-coated under vacuum with a thin film of metallic gold using a Bal-Tec model MED 020 evaporator (Balzers, Liechtenstein). A scanning electron microscope (JEOL JSM 6360 LV, Akishima, Japan) was used at an accelerating voltage of 10 kV to obtain digital images at various magnifications. The images were processed using Corel Draw 14 Photo Paint software.

2.6.2 Fluorescence microscopy

Fluorescence microscopy was conducted in an Axio Observer Z1 microscope (Carl Zeiss Microimaging GmbH, Göttingen, Germany), and the images were taken using the Zeiss AxioVision 4.6 Image Program. The fluorochromes used were Rodamine BP.A (Dinâmica CAS [81-88-9]) and Red Nile (Sigma-Aldrich N3013-100mg). Rodamine was prepared according to Schmitt et al. (2001) and Red Nile according to technical information (ATTBioquest, 2012).

2.7 Experimental design and statistical analysis

2.7.1 Model solutions - Mixture design

The general purpose in a mixture design is to make possible to estimate the properties of a multicomponent system from a limited number of observations. The theory of experimentation and modeling for mixtures design encompasses designs and suitable polynomial regression to the specific tests involving proportions of components (Nepomucena, Silva, & Cirillo, 2013). Methods of experiments with mixtures represent an area of applied statistics, being important in food science and industry since all foods are mixtures of a number of different ingredients (Bjerke, Næs, & Ellekjær, 2000).

Mixing tests were developed taking as variables the hydrocolloids OPN mucilage, guar gum (GG) and gum arabic (GA). Three fixed systems were tested to apply the hydrocolloids mixtures (Table 1). The blends of OPN mucilage, GG and GA were prepared according to the mixture design with 9 points (Figure 3). The experimental domain of this research consisted of different proportions of each component: A (OPN mucilage) and B (GA) between 0 and 1, and C (GG) between 0 and 0.33. GG had its ratio restricted to 0.33 for better evaluation of the other studied gums. The component proportion of the blends is shown in Table 2. All samples with respective blends were prepared in 3 independent replications in order to allow error estimation. The quadratic model defined in Equation 3 was adjusted according to the proposed design (Statistica StatSoft 8).

Graphs were obtained based on this model in order to study the relationship between the effects of the components in the influence of the responses.

$$E(\hat{y}) = \sum_{i=1}^{q} \hat{\beta}_i^* x_i + \sum\sum_{i<j} \hat{\beta}_{ij}^* x_i x_j \qquad (3)$$

Where $\hat{y}$ is the value predicted by the quadratic model and $\hat{\beta}$ the estimate of the parameters relating to the principal effects and interactions, i= 1 component, q = 3 (total number of components) and j is the number of points.

Table 1: Fixed systems to the mixture tests

Fixed systems	Hydrocolloids mix (%)	Sucrose (%)	Sodium Cloride (%)	Deionized water (%)
System 1	3	10	2	85
System 2	3	10	0	87
System 3	3	0	2	95

Table 2: Mixture design with nine ratios of hydrocolloids blends.

Model solution	Component proportion (sum = 1)		
	OPN mucilage	GA	GG
1	0.2088	0.7088	0.0825
2	0.0000	0.6700	0.3300
3	0.7088	0.2088	0.0825
4	0.0000	1.0000	0.0000
5	1.0000	0.0000	0.0000
6	0.4175	0.4175	0.1650
7	0.6700	0.0000	0.3300
8	0.5438	0.2088	0.2475
9	0.2088	0.5438	0.2475
10	0.4175	0.4175	0.1650
11	0,4175	0.4175	0.1650

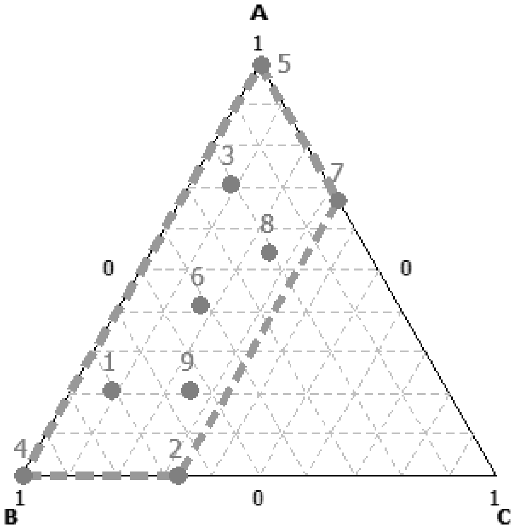

Figure 3: Mixture design with 9 coded points. A: OPN mucilage; B: GA and C: GG.

2.7.2 Fermented milk beverage application

A completely randomized factorial design was used for statistical analysis and performed with the SAS statistical software (licensed by UFLA). All experiments were conducted at least in duplicate and the data was presented as a mean of each experiment. T-test and Scott-Knott test were used to compare means, and results were considered significant for p-value < 0.05. Curves, rheological parameters of fitted models, goodness of fit (coefficients of determination (R^2) and root mean square error (RMSE)) were obtained by using SAS statistical software. The formulas of the fermented milk samples are shown in Table 3.

Table 3: Fermented milk formulas with hydrocolloids mix

Sample	Milk type	Milk (g)	Sucrose (g)	Starter culture (g)	Hydrocolloids mix (g)	
					OPN	GG
FW	W	89.964	10.000	0,036	0.000	0.000
FL	L	89.964	10.000	0,036	0.000	0.000
FWH	W	89.964	10.000	0,036	2.190	0.810
FLH	L	89.964	10.000	0,036	2.190	0.810
WH	W	89.964	10.000	0,036	2.190	0.810
LH	L	89.964	10.000	0,036	2.190	0.810

FW: fermented whole milk; FL: fermented low fat milk; FWH: fermented whole milk with hydrocolloids; FLH: fermented low fat milk with hydrocolloids; WH: whole milk with hydrocollois; LH: low fat milk with hydrocolloids, W: whole milk, L: low fat milk.

3. RESULTS AND DISCUSSIONS

3.1 Model solutions

3.1.1 Mixture study – rheological responses

The experimental values obtained for apparent viscosity (shear rate of $100s^{-1}$), storage modulus (G') and loss modulus (G") were adjusted to the Equation 3 and the respective parameters are shown in Table 4. All systems presented R^2>0.95 for the three responses. Significant components presented an expressive contribution to the prediction of the respective model.

The application of Table 4 equations resulted in the contour lines graphs showed on Figure 4. The curves show the apparent viscosity behavior of each system. System 1 comprises SUC, NaCl and the hydrocolloid mix, with maximum values of viscosity similar to the system 2, which is constituted of NaCl. System 3 (without SUC) even with similar behavior to other systems had a smaller increase in apparent viscosity of the samples with maximum value around 0.5 Pa.s. The highest values for apparent viscosity of the model solutions occurred on the region with hydrocolloids mix composed of 0,7 OPN mucilage, 0,0 GA and 0,3 GG, resulting in 0,5-0,7 Pa.s.

It can be inferred that SUC had greater interference than NaCl in apparent viscosity of the samples. Sucrose addition increases apparent viscosity of the systems and have been associated with synergistic effects with hydrocolloids. Among the possible mechanisms, Chenlo et al. (2011) proposed the sugar effect on the polymer–polymer molecular interactions, the specific interactions through hydrogen bonds between the polymer and the sugar–OH groups, more junction zones and more stable between the polymer chains and the effect of the spatial structure and the interaction forces between sugar–water on interactions with the polysaccharide.

Chenlo et al. (2011) verified that salt presence determined a slight decrease on apparent viscosity at low shear rates and showed significant variations of shear-thinning character in guar gum systems, considering that GG is a non-ionic polymer. On the other hand, Khouryieh, Herald, Aramouni, & Alavi (2007) observed no significant effect of the addition of sodium chloride on intrinsic viscosity of guar gum systems. The addition of salts to sugar solutions modifies the carbohydrate–water interactions (Seuvre & Mathlouthi, 2010).

Effect plot of each component in apparent viscosity is shown in Figure 4 (B, D, F). GG presented a rapid increase in the effect of apparent viscosity at the midpoint, GA had a rapid decrease to the midpoint then stabilized, and OPN mucilage had a slight decrease with linear tendency. The three systems showed similar behavior.

Table 4: Models and goodness of fit for apparent viscosity ($100s^{-1}$), G' and G" of the 3 systems of model solutions.

Apparent Viscosity (Pa.s) ($100s^{-1}$)

Parameter	System 1		System 2		System 3	
(β)	($\hat{\beta}$)	p-value	($\hat{\beta}$)	p-value	($\hat{\beta}$)	p-value
OPN	-0.002	0.95	0.002	0.95	0.004	0.81
AG	0.010	0.72	0.018	0.65	0.011	0.54
GG	-0.901	0.83	-6.741	0.27	-3.253	0.23
OPN * AG	-1.089	0.30	-2.363	0.12	-1.535*	0.04
OPN * GG	4.633	0.48	13.541	0.15	7.488*	0.09
AG * GG	2.835	0.66	11.199	0.22	6.100	0.14
R^2	0.996		0.993		0.998	

Storage modulus - G' [Pa]

Parameter	System 1		System 2		System 3	
(β)	($\hat{\beta}$)	p-value	($\hat{\beta}$)	p-value	($\hat{\beta}$)	p-value
OPN	1.571	0.15	1.304	0.42	1.773	0.27
AG	2.466*	0.06	1.783	0.29	3.032*	0.08
GG	-975.4*	0.01	71.92	0.76	-379.7	0.25
OPN * AG	-204.5*	0.01	-58.32	0.30	-111.2	0.13
OPN * GG	1441.9*	0.01	34.40	0.92	621.1	0.20
AG * GG	1456.1*	0,01	-16.90	0.96	576.4	0.24
R^2	0.985		0.994		0.948	

Loss modulus - G" [Pa]

Parameter	System 1		System 2		System 3	
(β)	($\hat{\beta}$)	p-value	($\hat{\beta}$)	p-value	($\hat{\beta}$)	p-value
OPN	0.806*	0.05	1.944*	0.10	0.483	0.25
AG	0.592*	0.10	1.009	0.33	0.724	0.12
GG	-653.5*	0.00	-167.7	0.28	384.7*	0.09
OPN * AG	-135.3*	0.00	-83.84*	0.05	43.83	0.23
OPN * GG	975.1*	0.00	342.1	0.16	-448.6	0.12
AG * GG	986.8*	0.00	313.2	0.19	-517.4*	0.10
R^2	0.998		0.995		0.997	

*p-value<0.10. ($\hat{\beta}$) = Estimation of parameters relating to the principal effects and interactions. OPN refers to OPN mucilage.

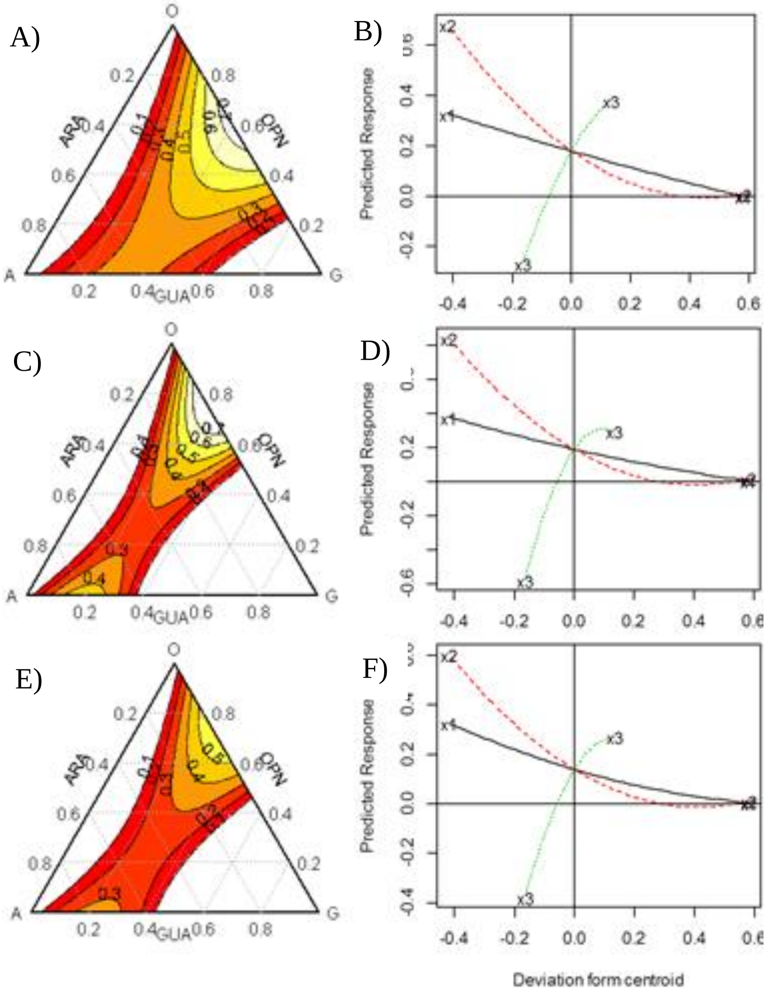

Figure 4: Contour lines (A: system 1, C: system 2, E: system 3) and Effect plot (B: system 1, D: system 2, F: system 3) of components for apparent viscosity (at 100 s^{-1}). x1 = OPN mucilage, x2 = GA and x3 = GG

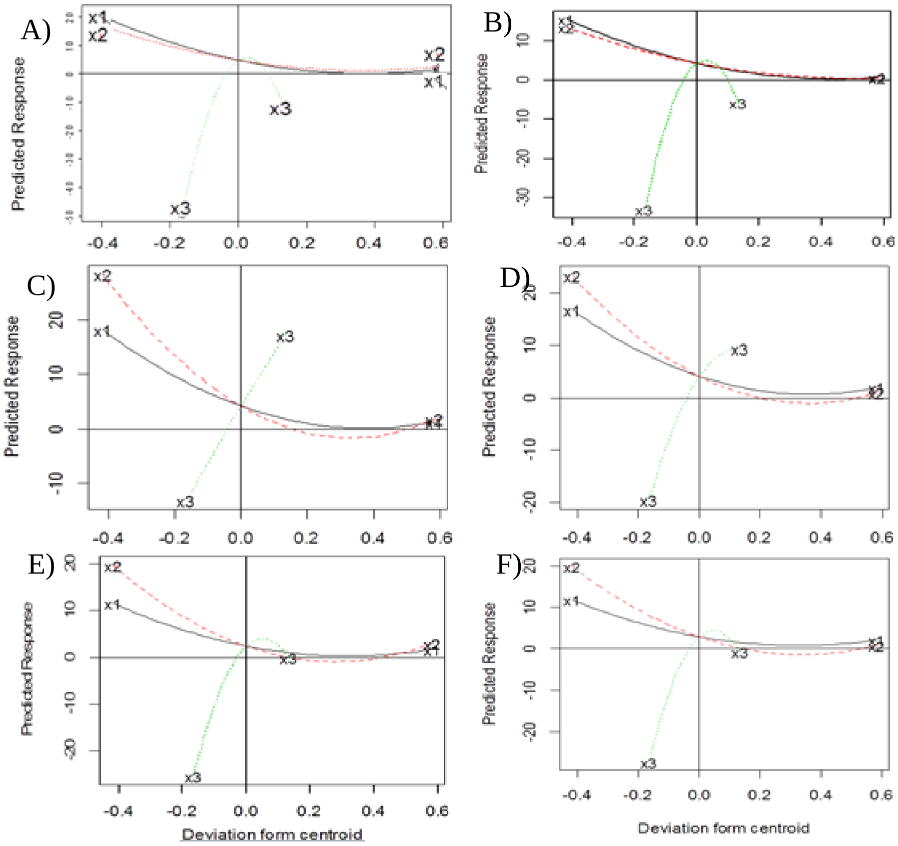

Figure 5: Effect plot of components for G’ at 1Hz (A: system 1, C: system 2 and E: system 3) and G” at 1Hz (B: system 1, D: system 2 and F: system 3). x1 = OPN mucilage, x2 = GA and x3 = GG.

Effect plots of components in G’ and G” are shown in Figure 5. The effect behavior of G’ and G” in the same system are similar, varying between the 3 systems. OPN mucilage and GA presented similar effect on system 1, decreasing until the midpoint then stabilizing, while GG presented a rapid increase until the midpoint. System 3 had a similar behavior, but GA presented a greater reduction gradient than OPN mucilage. System 2, without NaCl, behaved differently from other systems. GG presented rapid linear increase related to centroid point to G’ and a similar behavior to G”. OPN mucilage presented a tendency to linear behavior with a smaller decrease than GA.

3.1.2 Rheological behavior

The rheological behavior of sucrose solutions is Newtonian in the whole range of concentration (Saggin & Coupland, 2004), but with the addition of solutes the system can change the behavior. In all 3 systems studied the samples with presence of GG had 99% adjustment to the rheological model of Power Law and presented pseudoplastic behavior. According to Galmarini, Baeza, Sanchez, Zamora, & Chirife (2011) the addition of 0.1 g/100g of GG to the sugar solutions resulted in a pseudoplastic behavior as observed for GG solutions in this study.

The samples composed only of GA as hydrocolloid had Newtonian behavior with adjustments above 75% in all 3 systems. The presence of solutes (SUC and/or NaCl) changed the GA behavior on aqueous solution, that is non-Newtonian according to Gómez-Díaz, Navaza, & Quintáns-Riveiro (2008). Arabic gum (AG) is a biopolymer that contains amphiphilic compounds (proteins, polysaccharide-protein complexes) and its main function in food industry is to act as an emulsifier or encapsulant of flavors.

The samples composed only of OPN mucilage as hydrocolloid had pseudoplastic behavior (R^2 > 87% Power Law) in the systems 1 and 3. System 2, without NaCl, presented Newtonian behavior (R^2 > 97%).

The tests composed only of OPN mucilage, considering the 3 systems, presented a thixotropic behavior. All the other samples presented anti-thixotropic behavior. The occurrence of anti-thixotropic behavior can be explained as a result of increased frequency of collisions of the dispersed particles or polymer molecules in suspension with increasing applied shear rate, resulting in an increase of the bonds between the particles over time (Martin, 1993).

3.2 Fermented milk beverage application

From the results obtained in model solutions tests (Item 3.1), it was defined the hydrocolloids mixture composed of 2.19% OPN mucilage and 0.81% GG to be applied on fermented milk beverage (Table 3), composing 3% of the whole formula. These values are within the optimum ranges of apparent viscosity of the model systems.

3.2.1 Milk physical-chemical characterization

Whole milk and low fat milk used in the production of fermented milk products were analyzed and the results are shown in Table 5. The two types of milk did not differ in pH values, proving that this parameter did not affect the results treatments of the fermented milk. The lactose, not fat solids, protein, fat content and therefore the density were significantly different. The main difference of the samples was fat content.

Table 5: Properties of the milk used to produce the fermented milk samples

Property	Whole milk	Low fat milk
pH	6.74 ± 0,03 ^{a}	6.77 ± 0,01 ^{a}
Density (g L^{-1})	30.80 ± 0,20 ^{a}	32.58 ± 0,07 ^{b}
Fat (%)	4.00 ± 0,16 ^{a}	0.58 ± 0,01 ^{b}
Lactose	4.78 ± 0,05 ^{a}	4.66 ± 0,03 ^{b}
Solids not fat (SNF) (%)	8.72 ± 0,12 ^{a}	8.45 ± 0,02 ^{b}
Total protein (%)	3.19 ± 0,03 ^{a}	3.12 ± 0,02 ^{b}

Data subjected to analysis of variance and the T-test at 5% significance level. Means in the lines followed by the same letter are statistically equal to each other.

3.2.2 Fermented milk rheology

Flow and viscosity curves of fermented milk samples were obtained (Figure 6) and their rheological data were fit to the Power Law model (Table 6). The samples showed good fit ($R^2 > 94\%$) and pseudoplastic behavior. This behavior was evidenced by the values of consistency coefficient higher than zero ($K > 0$) and index flow behavior values between zero and one ($0 < n < 1$) (Steffe, 2006). The *n* values were statistically higher for FL and FW samples, showing that they had higher pseudoplasticity, followed by WH and LH, and smaller values of *n* were found to FWH and FLH samples.

In Figure 6 (B) is verified that the samples are time dependent fluid, that is, by applying a constant shear rate the apparent viscosity of solutions decreases. The samples without hidrocolloids (FW and FL) presented the lower apparent viscosity profile.

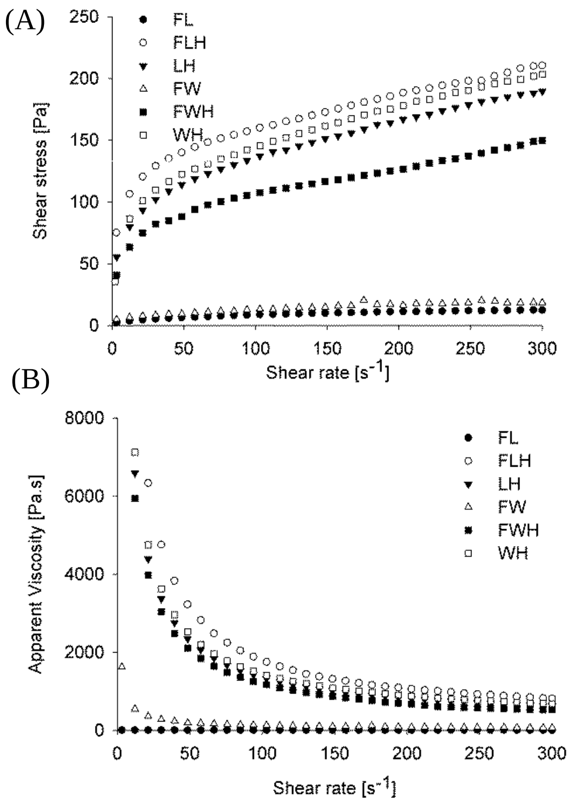

Figure 6: Flow curve (A) and apparent viscosity curve (B) of fermented milk samples.

Table 6: Power Law parameters of the fermented milk

Sample	R^2	Error	*K* (Pa.s^n)	*n*
FW	0.9729	0.6242	3.199 ± 0.641 [a]	0.315 ± 0.039 [c]
FL	0.9673	0.5977	1.663 ± 0.240 [a]	0.381 ± 0.004 [d]
FWH	0.9416	5.6372	40.515 ± 7.962 [b]	0.222 ± 0.038 [a]
FLH	0.9581	7.0534	64.987 ± 6.401 [c]	0.216 ± 0.017 [a]
WH	0.9729	0.6242	40.207 ± 2.527 [b]	0.280 ± 0.006 [b]
LH	0.9884	3.9877	41.457 ± 2.926 [b]	0.269 ± 0.011 [b]

Data subjected to analysis of variance and the T- test at 5% significance level. Means in the columns followed by the same letter are statistically equal to each other.

The studied samples showed thixotropic behavior and did not differ significantly, with an average of 2800 ± 1161 at 5% significance by Scott-Knott test. The pH values of the samples are shown in Table 7. From these values it is possible to confirm the correct fermentation on the inoculated samples by increasing acidity of the media. The addition of hydrocolloids reduced acidity of the samples.

The apparent viscosity data (100 s^{-1}) are presented in Table 7. Fermented milk without the addition of hydrocolloid had lower apparent viscosity and did not differ, followed by FWH and by the two samples without fermentation (LH and WH). The sample with higher apparent viscosity value was FLH. Exo-polysaccharides are some of fermentative metabolites derived from lactic acid bacteria and are useful in matrix-structure formation and stabilization of fermented dairy products (Beermann & Hartung, 2013).

Table 7: Fermented milk pH and rheology data

Sample	pH	η_{100}	G’	G”
FW	4,09 ± 0,02 ^{a}	0.151 ± 0.035 ^{a}	2.708 ± 72 ^{b}	1502 ± 72 ^{b}
FL	4,03 ± 0,01 ^{a}	0.096 ± 0.013 ^{a}	299 ± 96 ^{a}	84 ± 29 ^{a}
FWH	4,80 ± 0,10 ^{b}	1.098 ± 0,084 ^{b}	299 ± 63 ^{a}	140 ± 23 ^{a}
FLH	5,22 ± 0,59 ^{b}	1.731 ± 0.123 ^{d}	569 ± 68 ^{a}	221 ± 41 ^{a}
WH	8,49 ± 0,06 ^{c}	1.427 ± 0.050 ^{c}	242 ± 26 ^{a}	87 ± 07 ^{a}
LH	8,64 ± 0,04 ^{c}	1.405 ± 0.044 ^{c}	220 ± 28 ^{a}	80 ± 11 ^{a}

Data subjected to analysis of variance and the Scott-Knott mean test at 5% significance level. Means in the columns followed by the same letter are statistically equal to each other.

A synergistic effect on the apparent viscosity was observed in mixtures of polysaccharides with proteins, e.g. between guar gum and soy protein, so Galmarini et al. (2011) proposed the formation of weak electrostatic interactions and hydrogen bonds, forming a network that allows for greater water retention and results in a bigger increase in viscosity than that developed by the individual components in the solution.

The samples exhibited values of G' greater than G" in all experiments as stated in Table 7. This reflects the predominance of elastic behavior of the samples representing the resistance structure to contribute to a three dimension network. The interactions among hydrocolloids allow the formation of an elastic gel, related to an existing and low density interaction between chains, otherwise the gel would become rigid. The elasticity of the gel is related to the configuration of these chains (conformational entropy) (Groseberg & Khokhlov, 1994; Neves, 2015).

3.2.3 Fermented milk microstructure

Scanning electron microscope micrographs are presented on Figure 7. This technique evaluates the microstructure by scanning the surface of the specimen. The samples showed different microstructures in each treatment, but the behavior of whole and low fat milk showed no major differences. Fermented samples in the presence of hydrocolloids (Fig. 7 C, D) had higher globular structures than fermented samples without hydrocolloids (Fig. 7 A, B), representing aggregates of milk protein and hydrocolloids (Pang, Deeth, Prakash, & Bansal, 2016). A normal structure of fermented milk with casein network surrounded by a high number of pores of whey could be seen, as described by Espírito-Santo et al. (2013). Samples without fermentation (Fig. 7 E, F) had a smoother surface with little presence of globular structures.

Fluorescence photomicrographs of the samples are shown on Figure 8. The application of Red Nile showed the lipid structures, more evident in samples with whole milk (Fig. 8 B, F and J) (Valeur, 2002). Rodamine is a fluorochrome that evidences the biopolymer (protein + carbohydrate) structures. These structures are present in all samples as milk protein (casein micelles) and as hydrocolloids (Fig. 8 E, G, I, K).

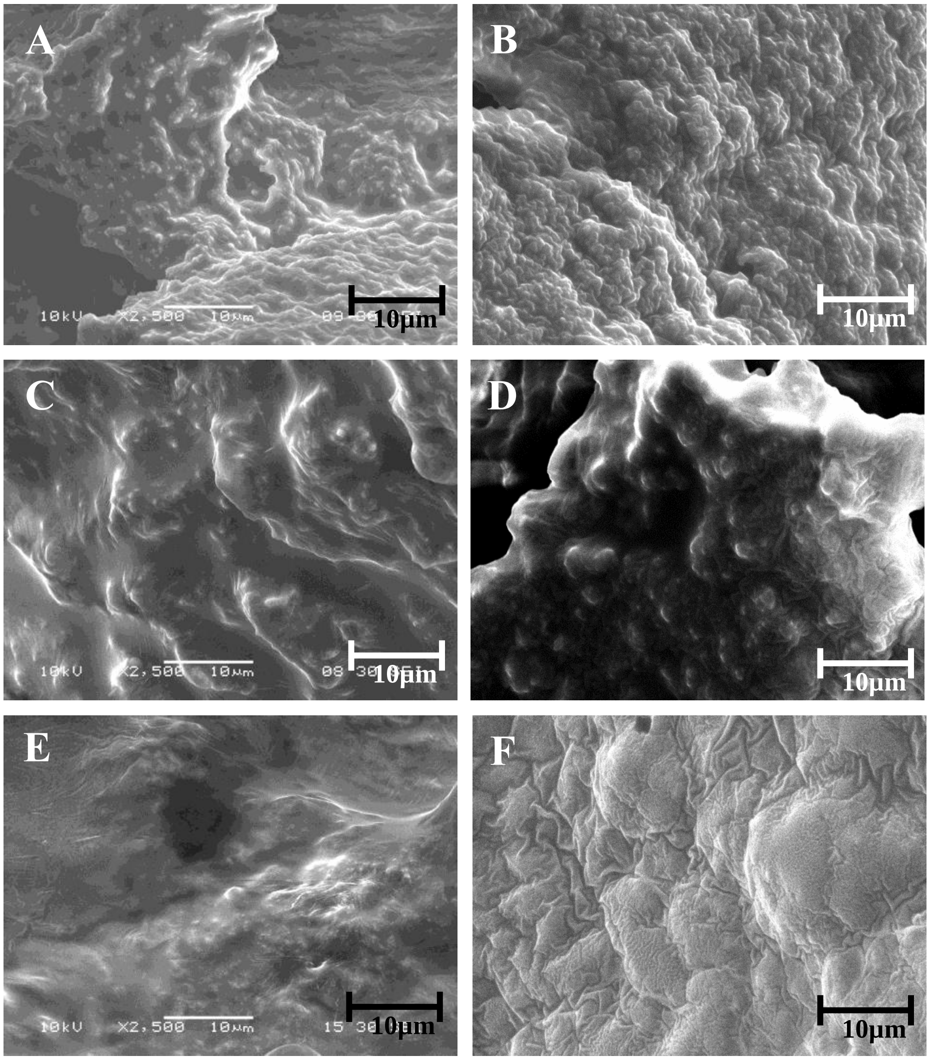

Figure 7: SEM micrographs of the samples: FW (A), FL (B), FWH (C), FLH (D), WH (E) and LH (F). Scale bars in the images are 10µm. 2.500x magnification.

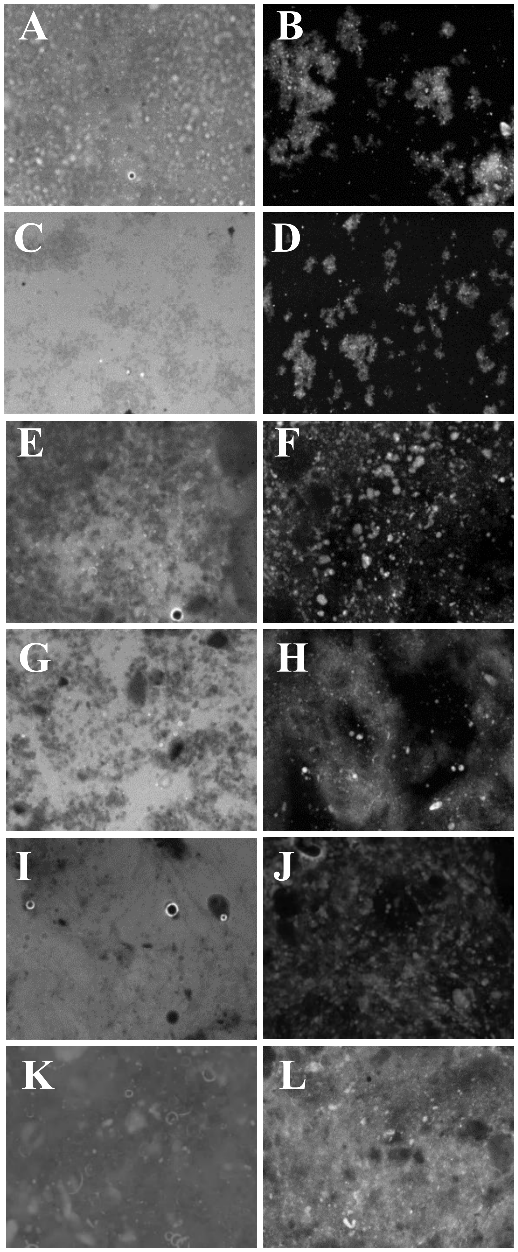

Figure 8: Fluorescence photomicrograph of the samples: Rodamine ((A): FW, (C): FL, (E): FWH, (G): FLH, (I): WH, (K): LH) and Red Nile ((B): FW, (D): FL, (F): FWH, (H): FLH, (J): WH, (L): LH). 100x magnification.

4. CONCLUSIONS

The highest values for apparent viscosity of the model solutions occurred on the region with hydrocolloids mix composed of 0.7 OPN mucilage, 0.0 GA and 0.3 GG, resulting in 0.5-0.7 Pa.s. Sucrose had greater interference than sodium chloride on apparent viscosity. The effect of apparent viscosity, G' and G", were discussed and the model solutions presented anti-thixotropic and pseudoplastic behavior with good fit to the Power Law rheological model.

Fermented milk samples presented good fit to Power law, pseudoplastic and thixotropic behavior, and had predominant elastic behavior (G'>G"). The application of hydrocolloids mix increased the apparent viscosity and pH of the samples. The microstructure evaluation confirmed the results. Application of hydrocolloids mix with OPN mucilage on fermented milk presented to be viable.

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