SERGIO HENRIQUE SILVA

EXTRACTION AND APPLICATION OF ORA-PRO-NOBIS (*Pereskia aculeata* Miller) MUCILAGE IN FREEZE-DRIED PETIT SUISSE CHEESE

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 do título de Doutor.

Prof. Dr. Jaime Vilela de Resende
Orientador

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Dra. Isis Celena Amaral	IFNMG
Dra. Lizzy Ayra Alcântara Veríssimo	UFLA
Dra. Tatiana Nunes Amaral	UVJM
Dr. Fabiano Freire Costa	UFJF

Prof. Dr. Jaime Vilela de Resende
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“Porque Deus é o que opera em vós tanto o querer como o efetuar, segundo a sua boa vontade”. (Filipenses 2:13)

RESUMO

A mucilagens provenientes da folha (ML) e do fruto em estádio de maturação verde (MFR) do ora-pro-nobis (OPN) (*Pereskia aculeata* Miller) foram utilizadas para agir como agente espessante, estabilizante juntamente com um mix de carragenas (MIX) em diferentes concentrações para produção de 14 amostras de queijo Petit Suisse. As amostras frescas preparadas tiveram perfis de textura (firmeza, adesividade e mastigabilidade), sinérese (mL) e viscosidade ($Pa \cdot s^{-1}$) analisados e submetidas ao processo de liofilização e novamente avaliadas após o processo de reidratação. Além disso, foi determinada a capacidade de retenção de água. As propriedades texturais, firmeza, adesividade e a mastigabilidade aumentaram (em módulo) com o aumento das concentrações das ML, MFR e MIX em todos tratamentos e após a reidratação os valores dos mesmos parâmetros diminuíram. Anteriormente à liofilização e após a reidratação, as amostras exibiram comportamento pseudoplástico quando submetidas a medidas reológicas de curvas de escoamento. Maior concentração das mucilagens ML e MFR juntamente com o MIX resultou em um aumento do módulo da viscosidade aparente e do índice de consistência. As amostras recém preparadas apresentaram viscosidade aparente ($\eta_{1,76}$) e índice de consistência (K) ligeiramente superiores aos das formulações reidratadas. Dentre as amostras frescas (2, 3, 4, 5, 6, 11, e 12) não ocorreu sinérese, já para as amostras reidratadas, apenas as amostras (3, 5 e 6) não apresentaram sinérese. Os rendimentos de secagem para as emulsões liofilizadas variaram entre 64,89±4,97% até 72,91±2,26%. Os espectros de infra-vernelho exibiram diferenças entre as ML e MFR mesmo apresentando picos semelhantes devido às características da classe de hidrocoloides. Este estudo demonstrou que tanto a ML quanto a MFR mostraram boa interação com o Mix de carragenas na elaboração de queijo Petit Suisse liofilizado. Devido a interação da arabinogalactana presente na ML com a estrutura do queijo Petit Suisse a amostra com 1,5% ML + 1% MIX apresentou melhores resultados (fresca e reidratada), pois apresentou alta viscosidade e WAC e não apresentou sinérese.

Palavras-chave: Biopolímeros. Queijo. Liofilização.

ABSTRACT

The mucilages from the leaf (ML) and the fruit in the green maturation stage (MFR) of the ora-pro-nobis (OPN) were used to act as a thickening, stabilizing agent together with a carrageenan mixture (MIX) in different concentrations for production of 14 samples of Petit Suisse cheese. The fresh samples prepared had profiles of texture (firmness, adhesiveness and chewability), syneresis (mL) and viscosity (Pa • s^{-1}) analyzed and then were subjected to the freeze-dryingand evaluated after rehydration process. In addition, the water holding capacity was determined. Textural properties, firmness, adhesiveness and chewability increased (in module) with the ML, MFR and MIX concentrations increased in all treatments and after rehydration these parameters values decreased. Prior to freeze-dryingand after rehydration, the samples exhibited pseudoplastic behavior when subjected to rheological measurements of flow curves. Higher concentration of the ML and MFR mucilages together with the MIX resulted in an increase in the apparent viscosity module and the consistency index. The freshly prepared samples showed apparent viscosity ($\eta_{1,76}$) and consistency index (K) slightly higher than the rehydrated formulations. Among the fresh samples (2, 3, 4, 5, 6, 11, and 12) there was no syneresis, whereas for the rehydrated samples, only the samples (3, 5 and 6) did not show syneresis. Drying yields for lyophilized emulsions ranged from 64.89 ± 4.97% to 72.91 ± 2.26%. The infrared spectra showed differences between ML and MFR even with similar peaks due to the characteristics of the the hydrocolloids. This study demonstrated that both ML and MFR showed good interaction with the mix of carrageenans in the freeze-dried Petit Suisse cheese. Due to the interaction of arabinogalactan present in the ML withthe Petit Suisse structure, the sample with 1.5% ML + 1% MIX showed better results (fresh and rehydrated), as it presented high viscosity and WAC and did not show syneresis.

Keywords: Biopolymer, Cheese. Freeze-drying.

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A indústria de produtos lácteos está sempre atualizando e inovando na hora de colocar um produto à venda. Segundo a FAO (2017), nos próximos 10 anos é esperado um crescimento de 1,7% ao ano neste setor. Com esta expansão, a demanda por produtos diferenciados e por públicos específicos se faz necessária para acompanhar o ritmo de crescimento. Para a produção de derivados fermentados do leite a necessidade da cadeia do frio torna mais dispendiosa e difícil o armazenamento destes produtos.

A busca contínua dos pesquisadores no ramo de alimentos por novos biopolímeros que possam ser utilizados na fabricação de alimentos processados torna-se um desafio a fim de melhorar as características físico-química de um produto. A aplicação dessas macromoléculas visa melhorar os atributos textura, estabilidade, proteção de compostos bioativos sensíveis incorporados ao meio, substituição da gordura, entre outros.

Os parâmetros sensoriais e de textura dos produtos lácteos fermentados são de grande relevância por desempenharem um papel importante na aceitação do produto por parte dos consumidores. Para melhorar a estrutura do gel formado na produção dos derivados lácteos fermentados, estabilizantes e espessantes geralmente são adicionados para evitar sinérese especialmente em produtos com baixo teor de gordura. A reologia é o estudo do comportamento deformacional e fluxo de matéria quando uma amostra é submetida a tensões ao longo de um intervalo de tempo, e inclui a avaliação de propriedades como elasticidade, viscosidade e plasticidade. Logo, o estudo reológico dos lácteos fermentados auxilia no controle de suas características de textura ao longo da produção, armazenamento e consumo.

A planta *Pereskia aculeata* Miller, popularmente conhecida no Brasil como ora-pro-nobis (OPN), é um vegetal pertencente à família Cactaceae e que apresenta alto conteúdo de proteínas e mucilagem em suas folhas e frutos em estádios de maturação verde, o que tem despertado o interesse de indústrias farmacêuticas e de alimentos para sua utilização como matéria-prima em suas formulações.

Buscando avaliar a técnica de liofilização como método para prolongar a vida útil do queijo Petit Suisse elaborado com mucilagem de ora-pro-nobis e mix de carragenas, o objetivo deste trabalho foi avaliar as propriedades reológicas e texturais além da caracterização microscópica do queijo Petit Suisse recém preparado e após a liofilização.

2 REFERENCIAL TEÓRICO

2.1 Queijo Petit Suisse

O queijo Petit Suisse é um queijo de altíssima umidade, a ser consumido fresco, de acordo com a recomendação estabelecida no Regulamento Técnico de Identidade e Qualidade do Queijo Petit Suisse. Quando em sua elaboração tenham sido adicionados ingredientes opcionais não lácteos, até o máximo de 30 % m/m, classifica-se como queijo Petit Suisse com adições. No caso em que os ingredientes opcionais sejam exclusivamente açúcares e/ou se adicionam substâncias aromatizantes/saborizantes, classificam-se como queijo Petit Suisse com açucares e/ou aromatizantes/saborizantes. O queijo Petit Suisse deve ser envasado em material adequado às condições de armazenamento previstas, de forma a conferir ao produto uma proteção adequada, e deve ser conservado e comercializado à temperatura não superior a 10 ºC. O queijo Petit Suisse deve cumprir o estabelecido no Regulamento Técnico sobre Padrões Microbiológicos para Alimentos, para queijos de muita alta umidade com bactérias lácteas abundantes e viáveis (BRASIL, 2000).

Como ingredientes obrigatórios para a fabricação do Petit Suisse estão: leite e/ou leite reconstituído, bactérias lácteas específicas e/ou coalho e/ou outras enzimas coagulantes apropriadas. Como ingredientes opcionais, podem ser empregados leite concentrado, creme, manteiga, gordura anidra de leite, caseinatos alimentícios, proteínas lácteas, outros ingredientes sólidos de origem láctea, soros lácteos, concentrados de soros lácteos, frutas em forma de pedaços, polpa, suco e outro à base de frutas (BRASIL, 2000).

O queijo Petit Suisse é semelhante ao queijo *quark* em relação às características de elasticidade, viscosidade e viscoelasticidade que determinam as suas propriedades reológicas e influenciam na sua consistência e estabilidade final. O queijo Quark é um queijo coalho não maturado e macio que no Brasil é principalmente utilizado como base para elaboração do queijo Petit Suisse. As propriedades reológicas destes tipos de queijos apresentam alta relevância por afetarem diretamente as técnicas de manipulação, envase, textura de consumo, capacidade de manter a forma sobre determinada temperatura além da capacidade de reter gases e formar olhaduras. Aspectos como o arranjo estrutural e sua microestrutura, o estado físico de seus componentes e sua macroestrutura (a maior ou menor presença de pontos heterogêneos como junções e/ou rachaduras) refletem diretamente sobre essas propriedades (FOX et al, 2000).

Tradicionalmente na fabricação do queijo Petit Suisse é realizada a dessoragem que reduz o rendimento final do produto além do subproduto do soro do queijo. Sendo assim, uma

das formas de aumentar o rendimento do queijo Petit Suisse e reduzir a produção de soro de queijo é eliminar a etapa de dessoragem do processo de produção, produzindo o queijo Petit Suisse com retenção de soro. Para isso são utilizados aditivos como os hidrocoloides. Estes podem interagir com as proteínas do leite, gerando um produto final com estabilidade e consistência diferenciadas (GLICKSMAN, 1986)

No Brasil, o produto é consumido como sobremesa e as vendas são direcionadas principalmente para o público infantil, mostrando que pesquisas com o intuito de tornar esse queijo mais atrativo para a população adulta devem ser realizadas (PRUDENCIO et al., 2008). Bigliardi e Galati (2013) demonstram que esforços para criação e inovação de produtos são realizados para atender aos consumidores e aumentar a competitividade entre empresas que participam do mesmo ramo alimentício.

2.2 Sinerese

A sinérese é a expulsão gradativa do soro do leite durante o armazenamento de produtos lácteos fermentados, devido à instabilidade e a contração da rede de gel pelo rearranjo de ligações entre agregados de proteínas na estrutura do produto (AICHINGER et al., 2003). Através da quantificação deste soro expulso do produto, é determinado o índice de sinérese, que tem impacto no teor de umidade e na textura do produto (CASTILLO et al., 2006). A baixa acidez (pH<4,6) o baixo teor de sólidos, altas temperaturas de incubação bem como outros fatores podem gerar a sinérese.

Durante o armazenamento de um produto como um iogurte, um gel lácteo ou um quark a sinérese representa para os consumidores um defeito quando observado a presença de um liquido sobrenadante na superfície do produto. Sendo assim a vida útil desses produtos pode ser associada à sinérese em diferentes tipos de géis (LUCEY, 2001; AICHINGER et al., 2003; TIJSKENS; BAERDEMAEKER, 2004).

2.3 Hidrocoloides

Os hidrocoloides podem ser proteínas, polissacarídeos e polímeros de extensas cadeias, os quais apresentam total ou parcial solubilidade em água. Na indústria de alimentos esses compostos são utilizados como aditivos para funções do tipo: 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).

A grande presença do grupamento hidroxila nas cadeias dos hidrocoloides tornam estes hidrofílicos, e 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). Com isso, o principal propósito para o uso dos hidrocoloides em alimentos é por se ligarem à água, modificando as propriedades estruturais dos alimentos (LI; NIE, 2014).

As proteínas e os polissacarídeos atuam nas 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 estérica entre as gotas, e os polissacarídeos por suas propriedades de retenção de água e espessamento (CORREDIG; SHARAFBAFI; KRISTO, 2011; DICKINSON, 2003; AKEN, 2002).

Gomas e mucilagens são outras denominações que podem ser atribuídas aos hidrocoloides. As mucilagens são materiais viscosos, encontradas normalmente em células especiais de camadas externas de sementes (PRAJAPATI et al., 2013). 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).

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 estérica entre as gotas, e os polissacarídeos por suas propriedades de retenção de água e espessamento (CORREDIG; SHARAFBAFI; KRISTO, 2011; DICKINSON, 2003; AKEN, 2002).

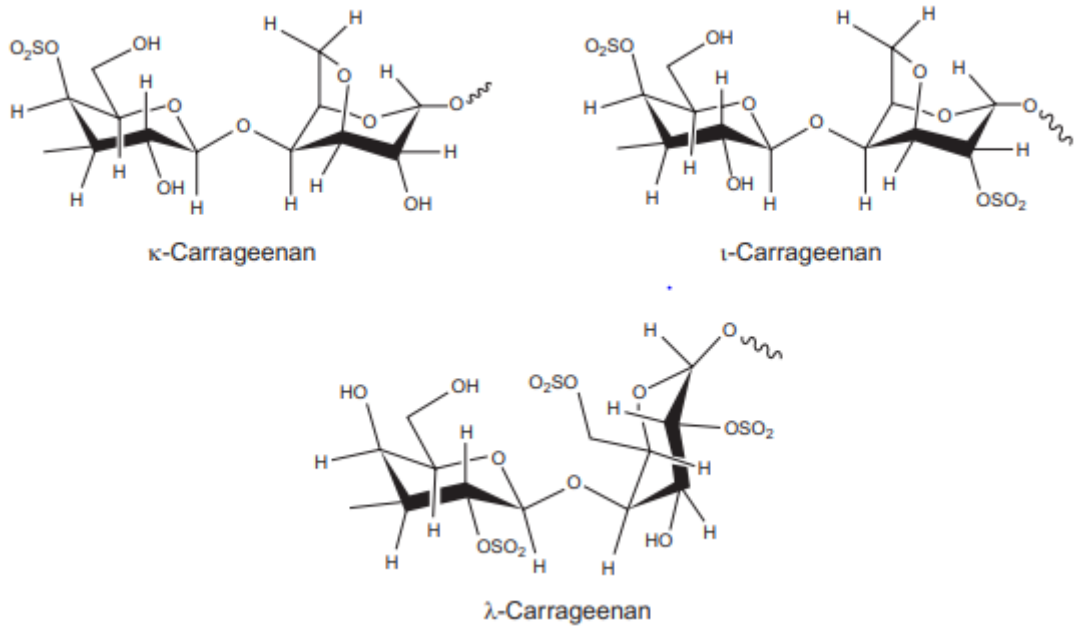
2.4 Carragena

Carragenas são hidrocoloides extraídos de algas vermelhas. São moléculas grandes e altamente flexíveis que formando estruturas helicoidais proporcionando uma capacidade para formar variedades de géis sobre diferentes temperaturas. Possuem a propriedade de fazer ligações de hidrogênio com a água, aumentando o volume do produto, trazendo vantagens em relação ao custo final. O poder de geleificação da carragena é muito maior em leite, devido à sua interação com a caseína. Utilizando-se concentrações de carragenas bem menores do que em sistemas aquosos obtêm-se géis de mesma textura (SANTOS; BRUNIERA; GARCIA, 2008).

As carragenas são polissacarídeos de alta massa molecular composto por unidades repetidas de galactose e 3,6-anidrogalactose, sulfatados e não sulfatados. As unidades são unidas por ligações alfa 1-3 e beta 1-4 glicosídicas alternadas. Em vista de suas propriedades gelificantes, espessantes e estabilizadoras, elas são amplamente utilizadas na indústria de alimentos. Apresenta grande aplicação na indústria de produtos lácteos e cárneos, devido à sua forte interação com proteínas, influenciando na textura final do alimento (KARIDURAGANAVAR et al., 2014).

Carragena é uma mistura complexa de, no mínimo, cinco polímeros distintos, designados kappa (K), lambda (l), mu (m), iota (i) e nu (n). O que as diferencia é a presença de grupamentos sulfatados na estrutura molecular. Dentre os diferentes tipos de carragena as mais utilizadas são: Kappa, a Iota e Lambda carragena. A Kappa, forma géis fortes e rígidos na presença de íons potássio; reage com proteínas lácteas. A Iota, forma géis macios na presença de íons cálcio. A Lambda não forma gel e é usado para aumentar a viscosidade derivados lácteos (VILLANUEVA; MONTAÑO, 2003). A estrutura dessas três formas de carragenas está apresentada na Figura 1.

Figura 1: Estrutura das carragenas tipo kappa, iota, e lambda.



Fonte: (KARIDURAGANAVAR et al., 2014).

A Tabela 1 apresenta os comprimentos de onda de absorbância característicos, dos grupos funcionais e se esses grupos estão presentes ou não em cada tipo de carragena, e em seguida, na Tabela 2 são apresentados os comportamentos de solubilidade dos três tipos de carragena em diferentes meios (THERKELSEN, 1993).

Tabela 1: Grupos funcionais característicos de cada carragenana e as respectivas absorbâncias no espectro do infravermelho

		Absorbância		
Comprimento de onda (cm^{-1})	**Grupo funcional**	**Kappa**	**Iota**	**Lambda**
1210 - 1260	Èster Sulfano	Muito elevada	Muito elevada	Muito elevada
1010 - 1080	Ligação Glicosídica	Muito elevada	Muito elevada	Muito elevada
928 - 933	3,6-anidro-D-galactose	Elevada	Elevada	Nula ou reduzida
840 - 850	D-galactose-4-sulfato	Média	Média	Nula
820 - 830	D-galactose-2-sulfato	Nula	Nula	Média
810 - 820	D-galactose-6-sulfato	Nula	Nula	Média
800 - 805	3,6-anidro-D-galactose-2-sulfato	Nula ou reduzida	Média	Nula

Fonte. (THERKELSEN, 1993)

Tabela 2: Características de solubilidade das carragenas tipo kappa, iota e lambda em diferentes meios.

Meio	**Kappa carragena**	**Iota Carragena**	**Lambda carragena**
Água quente	Solúvel acima de 60°C	Solúvel acima de 60 °C	Solúvel
Água fria	Sais de sódio solúveis. Sais de potássio e de cálcio (insolúveis)	Sais de sódio solúveis. Os sais de cálcio originam dispersões tixotrópicas.	Solúvel
Leite quente	Solúvel	Solúvel	Solúvel
Leite frio	Sais de sódio, cálcio e potássio, insolúvel, mais apresentam marcada dilatação	Insolúvel	Solúvel
Soluções concentradas de açúcar	Solúvel a quente	Dificilmente solúvel	Solúvel a quente
Soluções concentradas de sais	Insolúvel	Solúvel a quente	Solúvel a quente

Fonte. (THERKELSEN, 1993).

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

A *Pereskia aculeata* Miller é um cacto nativo brasileiro que pertencente à família Cactaceae sendo comumente conhecida como ora-pro-nobis (OPN). Suas folhas (Figura 2.1) possuem alto valor nutricional devido à presença de quantidades consideráveis de proteína, vitamina, fibra dietética total, carboidratos e minerais (cálcio, magnésio, manganês e zinco) (Pinto & Scio, 2014; TAKEITI et al., 2009). Por não apresentarem toxicidade, encontram aplicação na indústria de alimentos e farmacêutica (Almeida-Filho; Cambraia, 1974). Este cactáceo apresenta frutos (Figrua 2.2) que, quando jovens, possuem consistência carnosa e viscosa, alto teor de umidade, parede espessa com bractéolas verdes e espinhos em suas axilas (Rosa & Souza, 2003).

Figura 2: (a) Folha do OPN (b) Fruto verde OPN

(a) (b)

Fonte: (SILVA et al., 2017)

Pesquisas com este cacto apresentam 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) demonstraram que as folhas do OPN apresentam alto valor nutritivo com 25,5% de proteína em matéria seca, próximo ao encontrado por Dayrell; Vieira (1977), de 25,14% em matéria seca. Sierakowski et al. (1987), mostrou que 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.

Um trabalho sobre a extração do polissacarídeo da mucilagem do ora-pro-nobis realizado por Sierakowski et al. (1987) revelou a presença do biopolímero arabinogalactana. O estudo mostrou, ainda, que o polissacarídeo presente é constituído por uma heteroglicana formada, principalmente, por arabinose e galactose, contendo também raminose e ácido galacturônico.

Arabinogalactana 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).

A mucilagem extraída da folha do OPN tem sido amplamente aplicada em estudos das propriedades de formação de gel e produção de suspensões viscosas (Lima Junior et al.,

2013), na formação de nanoemulsão (Lago et al., 2019) e em misturas com outros hidrocoloides para produção de bebida fermentada de leite com alta viscosidade e teor de proteínas (Amaral et al., 2018). Entretanto, um único relato sobre a extração e aplicação da mucilagem obtida do fruto verde do ora-pro-nobis (MFV OPN) foi de Silva et al., (2017), demonstrando que a mucilagem liofilizada extraída do fruto verde OPN apresenta: 19% de proteínas, elevada estabilidade térmica, baixa atividade de água, coloração amarela clara e estrutura de alta porosidade com pouca aglomeração. O aumento da concentração de mucilagem extraída do fruto verde de OPN, contribuiu para o aumento da viscosidade aparente, capacidade de emulsificação, estabilidade e diminuição do tamanho médio das gotículas de óleo na emulsão.

2.6 Liofilização

A liofilização é um processo de secagem que possui como maior vantagem o não uso do calor como meio secante, tornando-se um processo a ser aplicado em alimentos termo-sensíveis, de forma que as qualidades nutricionais e sensoriais sejam preservadas. Além disso, possibilita a redução extrema do peso do produto, alta solubilidade, longa vida útil a temperatura moderada e a possibilidade de reidratação (CARPENTER et al., 1999). A figua 3, mostra a imagem de um liofilizador.

Figura 3: Liofilizador

Fonte:(Terroni, 2019).

A liofilização é um processo diferenciado de desidratação de produtos, pois ocorre em condições negativas de pressão e temperatura, possibilitando o processo de sublimação onde a

água previamente congelada (estado sólido) passe diretamente ao estado gasoso (sem passar pelo estado líquido) (KUMAR et al., 2011). O processo de liofilização começa com o congelamento dos alimentos abaixo de -40 °C. O tipo e a velocidade de congelamento atuam na estrutura final do alimento, devido à distribuição dos poros na sua estrutura e do tamanho e da localização dos cristais de gelo formados. Na liofilização, a formação de cristais de gelo grandes, com geração de uma rede cristalina, proporciona uma boa estrutura porosa, que facilitará o escape de vapor d'água durante a liofilização, bem como a entrada da água em sua posterior reidratação, porém, esse efeito pode causar sinérese em certos produtos e alterar as suas características estruturais (LIU et al., 2008).

O processo de desidratação o material liofilizado passa por dois tipos de secagem: uma é a secagem primaria onde a água é removida por sublimação que ocorre sob vácuo e com a adição de calor. Parte significativa do calor latente de sublimação é consumida quando as moléculas passam do estado sólido ao gasoso, devido a este fenômeno, a temperatura do alimento congelado decresce. Como tal, é necessário fornecer mais calor ao produto, que pode ser favorecido por condução, convecção ou radiação. O final da desidratação primária pode ser constatado pelo aumento da temperatura do produto num valor próximo ao do ambiente ou pela observação visual quando desaparece a interface entre camada seca e camada congelada (ORDÓÑEZ, 2005).

Após a sublimação do gelo o alimento ainda contém agua, e com a elevação da temperatura até aproximadamente 40° C essa agua é eliminada por dessorção. Para obtenção de um produto estável o conteúdo de umidade deve ser reduzido a cerca de 2% a 8 %, que corresponde a água fortemente ligada. Outra possibilidade é a finalização através de outro método de secagem, em decorrência dos elevados custos do processo de liofilização. Ao término da secagem antes da retirada do alimento da câmara, deve haver a introdução de um gás inerte, em geral, utiliza-se o nitrogênio, para rompimento do vácuo, pois se ocorrer à entrada de ar na câmara, os produtos imediatamente absorveriam umidade. O tempo desta etapa é cerca de 30 a 50 % do tempo gasto com a etapa anterior (BOSS, 2004).

3 CONCLUSÃO GERAL

Os hidrocoloides estão presentes na maioria das indústrias de alimentos, grande parte na forma de aditivos importados. A demanda por esses ingredientes tem-se elevado, sendo que a composição ou a fração deste aditivo quando adicionado em alimentos depende do suprimento assegurado, da qualidade e, sobretudo do preço. Devido aos custos de alguns destes hidrocoloides, ainda existe espaço no mercado para novas fontes que desempenhem

funcionalidades mais específicas, interações sinergéticas e melhoria das propriedades funcionais em alimentos. Fontes alternativas de hidrocoloides, como o ora-pro-nobis, são pesquisadas para suprir a necessidade das indústrias alimentícia e farmacêutica. Um fator relevante para impulsionar estas pesquisas é o grande interesse destas indústrias em matéria prima naturais, as quais podem ser produzidas de forma eficiente, a partir de fontes vegetais nativas de baixo custo e com qualidade.

A técnica de liofilização permite o armazenamento de um alimento sobre as corretas condições por vários anos se o produto for fabricado de forma segura e com qualidade, além de proporcionar o seu transporte sem condição de refrigeração. Sendo assim, pesquisas devem continuar aprimorando a técnica e aplicação em alimentos para ampliar cada vez mais a gama dos alimentos liofilizados.

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SEGUNDA PARTE–ARTIGO 1

EXTRACTION PROCESSES AND CHARACTERIZATION OF THE MUCILAGE OBTAINED FROM GREEN FRUITS OF *Pereskia aculeata* MILLER

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Sérgio Henrique Silva[a], Isabelle Cristina Oliveira Neves[a], Natália Leite Oliveria[a], Ana Cristina Freitas de Oliveira[a], Amanda Maria Teixeira Lago[a], Tales Márcio de Oliveira Giarolla[a], Jaime Vilela de Resende[a*]

[a] Federal University of Lavras, Department of Food Science, Laboratory of Food Refrigeration, P.O. Box 3037, 37200-000, Lavras, Minas Gerais, Brazil

*Corresponding author: Jaime Vilela de Resende; e-mail address: jvresende@dca.ufla.br

Abstract

Pereskia aculeata Miller is a cactus known for its non-toxic mucilage. This mucilage is usually extracted from its leaves and fruits, and is rich in the polysaccharide arabinogalactan and proteins, enabling its application as an emulsifier and a stabilizer in the food industry. In this work, the mucilage powder was obtained from green fruits of *Pereskia aculeata* Miller and characterized to determine its physicochemical, thermal, and microstructural properties. In addition, the technological characteristics of the reconstituted powder were evaluated. The freezing point of the solutions tested, regardless of the concentration of mucilage used, was on average −2 °C. Through the micrograph of the powder, it was possible to visualize an amorphous structure with high porosity and heterogeneity in relation to the average grain diameter. Infrared spectroscopy revealed the presence of bands and peaks characteristic of polysaccharides and protein chains. By thermogravimetric analysis, the thermal degradation of these biopolymers was observed in the range of 290 °C to 350 °C. The glass transition temperature and melting point (endothermic event) of the material were observed at −5 °C and at 68 °C, respectively. All the mucilage

solutions studied showed pseudoplastic behavior. Increasing the mucilage concentration of the medium contributed to the increase in the apparent viscosity, emulsification capacity, emulsion stability, and decrease in the mean size of the oil droplets. When compared to the mucilage powder extracted from the leaves, the mucilage powder extracted from the fruit showed approximately twice the protein content with low water activity and a clarified product with light yellow coloration.

Keywords: Fruits, Arabinogalactan; Proteins; Emulsion; Rheology.

1. Introduction

The search for new sources of food hydrocolloids that exhibit specific functionalities (structural, physicochemical, and functional properties) is an active area of research with emphasis on mucilages. Mucilage is defined as a complex carbohydrate polymer with high water absorption capacity, being frequently used as thickeners, emulsifiers, binders, gelling agents, and stabilizers in food and pharmaceutical products (Sáenz et al., 2004). Studies allow the industries to select the appropriate mucilages according to their specific needs without extensive trial and error. This mucilage can be obtained from locally available sources at a low cost (Geresh et al., 2000; Prajapati et al., 2013). Mucilage can be extracted from plants (Basella alba, Asplenium australasicum, Okra), fruits (Coccinia indica fruit), seeds (Chia, Cress seed) and other sources (Ameena et al., 2010; Behrouzian et al., 2014; Capitani et al., 2016; Hung and Lai, 2019; Motiwala et al., 2015; Zeng and Lai, 2014).

Pereskia aculeata Miller is a cactus native to Brazil and found from the southern region of the country to the northeast, being popularly known in the country as ora-pro-nobis (OPN) (Mercê et al., 2001). Being members of the Cactaceae family, OPN leaves have high nutritional value due to the presence of considerable amounts of proteins, vitamins, total dietary fibers, carbohydrates, and minerals (calcium, magnesium, manganese, and zinc) (Pinto and Scio, 2014; Takeiti et al., 2009). Furthermore, because they do not present toxicity and exhibit high mucilaginous content, the OPN leaves find application in the food and pharmaceutical industry (Almeida Filho and Cambraia, 1974). The OPN leaves are a viable alternative for obtaining mucilage.

In this light, efforts have been made to improve the extraction of hydrocolloids from the leaves of this cactus and to apply them as stabilizing and emulsifying agents (Conceição et al., 2014; Lima Junior et al., 2013; Martin et al., 2017). This is viable due to their adsorption characteristics in interfaces (Junqueira et al., 2018), gel formation and production (Lima

Junior et al., 2013), nanoemulsion formation (Lago et al., 2019), and when combined with other hydrocolloids for the production of fermented milk beverages with high viscosity and protein content (Amaral et al., 2018). In addition, the mucilage from OPN leaves is able to form cohesive and flexible biodegradable films with smooth surfaces without cracks, which can be used as the primary packaging material of light sensitive foods (Oliveira et al., 2019). New studies have emerged with the same purpose, in which the production of novel chia-mucilage nanocomposite films is proposed. As presented by the authors, this mucilage can be a suitable matrix for production of films when blended with other polymers such as cellulose nanofibers and starch nanocrystals. These films can present a variety of applications in food packaging and coating industry, due to the biodegradable, biocompatible, non-toxic, highly antioxidant and antimicrobial nature, as well as the mechanical and hydrophobic properties (Mujtaba et al., 2019a, 2019b).

OPN plant also bears fruits which are not frequently used. However, when young, the green fruits have a fleshy pulp, viscous consistency, and high moisture content, thereby showing high potential to be used as an additive in the food and pharmaceutical industries. Currently, to our knowledge, there is no literature describing the extraction of mucilage from OPN green fruits and therefore, there is much to be explored in relation to the practical application of this method and the product obtained. Successful application of this mucilage may increase the use of native plants with high productivity and simple cultivation requirements, besides offering alternatives to the use of conventional emulsifiers. Only few studies have performed the morphological and anatomical characterization of the fruits of *Pereskia aculeata* Miller and described their profile of total carotenoids and phenolics (AgostiniCosta et al., 2012; Rosa and Souza, 2003). In this context, it is possible to infer that the use of the mucilage from OPN green fruits is a promising approach to improve the stability of colloidal systems.

Both the extraction and drying methods for obtaining powdered mucilage directly influence the functional properties of this material. Cold extraction is usually preferred for a new plant extract to obtain a high content of compounds, high yield of powder, and strong antioxidant activity. In contrast to hot extraction, this method avoids the loss of hydrolysable or thermolabile compounds (He et al., 2018). Among the mucilage drying processes, the freeze-drying method is highlighted because it does not involve the use of high temperatures, thus maintaining the physicochemical and rheological properties of the material (Junqueira et al., 2018).

To determine the potential use of mucilage extracted from a new source using a different method as a food ingredient, it is first necessary to study its composition along with its physicochemical properties as well as to evaluate the benefits that it can bring to the product (Prajapati et al., 2013). Such studies on the extraction and application of the mucilage from OPN green fruit have not yet been performed. Therefore, the aim of this study was to extract the mucilage from OPN green fruits by cold extraction and freeze-drying processes and to investigate its physicochemical, thermal, microstructural, and rheological properties. Furthermore, emulsions obtained from such solutions were evaluated in terms of emulsifying capacity, emulsion stability, and average droplet diameter.

2. Material and Methods

2.1 Obtaining fruits

The fruits of *Pereskia aculeata* Miller were collected in the state of green maturation in the cities of Nazareno, Minas Gerais, Brazil, during the fall of 2018.

2.2 Extraction and purification of mucilage

OPN green fruits were harvested, washed, sanitized, and the leaves and surface thorns were removed. After this step, the green fruits were pressed with a force of 2 tons in a hydraulic press (Tecnal TE 058, Brazil) and the extracted material was vacuum filtered by Buchner funnel coupled to a vacuum pump (Primar MC 1284, Brazil). The filter element was a layer of three organza cloths. Ethanol 99.5% v/v (ISOFAR, Brazil) was added to the filtrate at a ratio of 1:3 v/v (extract:ethanol) for precipitation of the mucilage. The precipitate was frozen at −75 °C in an ultra-freezer with a static air system (Coldlab CL 120- 86 V, Brazil) and freeze-dried at −40 °C with vacuum pressure of 0.998 mbar for 36 h (Edwards, L4KR, Brazil). The dried material was ground in a ball mill (SP Labor SP-38, Brazil) for 2 min to obtain powdered mucilage. The powdered products were hermetically packed and stored in vacuum desiccators.

2.3 Characterization of the mucilage powder of the OPN fruit

2.3.1 Proximal and mineral composition of OPN green fruit mucilage

Chemical analysis of the moisture content (method no. 967.08), protein content (method no. 988.05), fat content (method no. 2003.06), crude fiber content (method no. 958.06), and ash content (method no. 942.05) (AOAC, 2005) was performed. Moisture and ash contents were determined by gravimetric method in chamber at 105 °C and by incineration at 550 °C, respectively. Protein content was estimated using the Kjeldahl method and the protein concentration was estimated using a nitrogen conversion factor of 6.25. Fat content was measured by Soxhlet extraction with petroleum ether and crude fiber content was determined by acid hydrolysis followed by vacuum filtration. The carbohydrate levels were calculated using the following formula: 100 – (moisture + fat + protein + ash + crude fiber). Mineral analysis was performed on the sample extracts obtained by organic digestion with 2:1 (v/v) nitric-perchloric acid and followed the standards suggested by Malavolta et al. (1997).

2.3.2 Water activity (a_w)

The determination of the water activity of the mucilage was determined in triplicate with a Dew Point Hygrometer (Aqualab Decagon Services 3TE, USA) at 25 °C.

2.3.3 Colorimetric analysis

The color of the powder mucilage was measured on the Konica Minolta Spectrophotometer CM-5 colorimeter using the C *, C *, h * cylindrical coordinate color system. The parameters L *, chroma saturation index (C *) and Hue angle (h *) were measured with three replicates. L * indicates brightness and varies from 0 to 100, C * indicates saturation, starts with 0 in the center and increases as the distance from the center. The Hue hue angle starts at the + a * axis and is given in degrees; 0 °, + a *, (red); 90 °, + b * (yellow); 180 °, -a * (green); and 270 °, -b * (blue).

2.3.4 Infrared Spectroscopy Analysis (FTIR)

The mucilage sample was subjected to Fourier transform infrared spectroscopy using the attenuated total reflectance (ATR) technique in a Bruker FTIR spectrophotometer (Vertex 70,

USA). The analyzed infrared region was 4000 cm−1 to 500 cm−1 with 64 scans and a resolution of 4 cm^{-1}.

2.3.5 Thermal Analysis

2.3.5.1 Thermogravimetric analysis (TGA)

The TGA experiments were conducted in a nitrogen atmosphere, varying the temperature from 21 °C to 520 °C at a heating rate of 10 °C min^{-1} using a Shimadzu DTG-60H (Shimadzu Corporation, Japan).

2.3.5.2 Differential exploratory calorimetry (DSC)

A temperature-modulated differential scanning calorimeter (Shimadzu DSC-60A, Japan) was used to evaluate the thermal behavior of the powdered product. The temperature and heat flow of the instrument was calibrated using indium and zinc, and the temperature control system used liquid nitrogen as a refrigerant. Aluminum pots were used and the weight of each sample was approximately 5 mg. The temperature protocol used for the samples consisted of equilibrating the samples at -20 °C and then heating them to 220 °C at a rate of 3 °C.min^{-1}.

2.3.6 Scanning electron microscopy (SEM)

Micrographs of the lyophilized sample were examined on a scanning electron microscope (LEO EVO 40 XVP, Carl Zeiss, Germany) at an acceleration voltage of 20 kV. Prior to observation, the powder mucilage was fixed in stubs using double-sided carbon tape and sprinkled with gold at 200 A for 180 seconds to make the sample conductive.

2.4 Reconstitution of OPN green fruit mucilage

The mucilage was reconstituted in distilled water at 80 °C for 30 min under agitation and then kept in a thermostatic cabinet (Eletrolab, EL202, Brazil) at 4 °C for 12 h to allow complete hydration. Five solutions were prepared with the concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 g·100 mL^{-1} of aqueous phase.

2.5Emulsion preparation

To verify the functional properties of the reconstituted mucilage, five emulsions were prepared using 10 g of commercial sunflower oil (Sinhá, Caramuru Alimentos S.A., Brazil) and 40 g of reconstituted mucilage at each concentration.

2.5.1 *Rheological properties*

The rheological behavior of emulsions was tested in triplicates at 20 °C. The viscosities of the emulsions were determined using a concentric roller rotational viscometer (Brookfield DVIII Ultra, Brookfield Engineering Laboratories, USA). The SC4-18 spindle was used for 0.5% solution, subjected to an increasing ramp with shear rate ranging from 0.01 s^{-1} to 224.41 s^{-1} . This solution obtained 18 points for 3 min of testing. For the other concentrations, the SC4-31 spindle was used in an increasing ramp with a deformation rate of 0.034 s^{-1} to 57.83 s^{-1} . The power law model (Eq. 1) was fitted to the shear stress and shear rate data to determine the flow profile of the fluids.

$$\tau = K\dot{\gamma}^n \quad (1)$$

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

2.5.2 Emulsion formation (EC) and emulsion stability (ES)

The functional properties of the reconstituted mucilage were tested for their emulsion formation and emulsion stability using solutions at different mucilage concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 g, 100 mL^{-1}). Emulsion formation (EC) was determined by preparing emulsions containing 10 g of commercial sunflower oil (Sinhá, Caramuru Alimentos S.A., Brazil) and 40 g of reconstituted mucilage at each concentration. The samples were submitted to mechanical agitation in Turrax homogenizer (Tecnal TE102, Brazil) at 2500 rpm for 3 min. Emulsion capacity was determined using Equation 2 (Lima Junior et al., 2013).

$$EC\ (\%) = \frac{Emulsion\ volume\ (mL)}{(Total\ Volume\ of\ Fluid\ (mL)} x100 \quad (2)$$

To verify the stability of the emulsions (ES) prepared using the reconstituted mucilage at different concentrations, the emulsions were allowed to stand for 30 min in a room heated to 20 °C and another sample in a thermostatic bath (Solab, SL150, São Paulo, Brazil) at 80 °C. Then the samples were centrifuged (SPLABOR, sp-701, Brazil) at 1271 × g for 10 min and the distinct phases were separated and weighed. The stability of the emulsion was determined using Equation 3.

$$ES\ (\%) = \frac{Final\ emulsin\ (g) mass}{Fluid\ total\ mass\ (g)} x100 \quad (3)$$

2.5.3 Mean droplet diameter and size distribution (d_{32})

The polydispersity and mean droplet diameter of the emulsions were measured using a static light scattering instrument (Mastersizer 3000, Malvern Instruments Ltd, Malvern, UK) at 25øC. The refractive indexes of sunflower oil and aqueous phase used were 1,481 and 1,330, respectively. The droplet diameter of each sample was plotted as the weighted average diameter (d_{32}), and the polydispersity (Span) was calculated from Equation 4.

$$Span = \frac{d_{90} - d_{10}}{d_{50}} \quad (4)$$

where d_{10}, d_{50}, and d_{90} are the drop diameters (μm) at 10, 50 and 90% of the cumulative volume respectively (Bai, McClements, 2016; Bai et al., 2016). Five replicates were performed in each analysis.

2.5.4 Freezing point

In order to determine the freezing point of the reconstituted mucilage solutions (0.5, 1.0, 1.5, 2.0 and 2.5 g • 100 mL^{-1}), T-type thermocouples were used in a data acquisition system (National Instruments NI SCXI 1000, Hungary), monitoring the freezing curve of each solution when placed in an ultrathermostat bath set at -7 °C (New Ethics 521 / 3DE, Brazil). The analyses were performed in triplicate.

2.6 Statistical analyzes

For statistical analysis, polynomial models were fitted generically to the data (Equation 5) to explain the effect of the mucilage concentration on the response variables (viscosity, emulsifying capacity, stabilizer, d_{32}) through a regression equation.

$$\hat{Y} = a + bx + cx^2 \quad (5)$$

where, $\hat{Y}$ corresponds to the parameters analyzed, x is the concentration of mucilage and a, b, c, are the coefficients of the model.

Analysis of variance (ANOVA) was used to examine the statistical significance of the terms in the regression equation using Statistical Analysis System (SAS University Edition, Cary, USA, 2016). The graphs were plotted using SigmaPlot 11.0 software (Systat Software Inc., California, USA, 2008). The data obtained from the analyses were subjected to a self-scaling-type pretreatment followed by a principal component analysis (PCA) and hierarchical cluster analysis (HCA) with the aid of Chemoface software (version 1.61) (Nunes et al., 2012).

3. Results and Discussion

3.1 Proximal and mineral composition of OPN green fruit mucilage

The yield of mucilage in terms of the amount of powder obtained was 10.0 ± 0.5 g mucilage/kg green fruit (Fig. 1).

Figure 1: Fruit of the OPN

Source: Author (2019)

Table 1 shows the proximal composition of OPN green fruit mucilage and its comparison with composition of OPN leaves mucilage. The protein content of OPN green

fruit mucilage (19.89%) was higher than that found in leaves mucilage as observed in studies by Lima Junior et al. (2013) (10.47%), Martin et al. (2017) (19.00%) and Oliveira et al. (2019) (8.89%).

Table 1: Proximal composition (%) of the OPN green fruit mucilage compared to the OPN leaves mucilage.

	Moisture (%)*	Carbohydrates (%)	Proteins (%)	Ash (%)	Lipids (%)	Fibers (%)
MFR	2.97 ±0.69	67.20 ± 0.00	19.8± 0.19	8.30±0.05	0	4.57±0.87
ML	6.08±0.12	78.93± 0.29	8.89 ± 0.17	9.99±0.03	1.68± 0.16	0.49± 0.29

* All values were expressed on dry basis, except for moisture.

These variations may have occurred due to the differences in the process of extraction of OPN mucilage, different parts used to obtain the mucilage (leaves or fruits), and differences in plant location, climatic conditions and soil composition. A high protein content is desirable as it can improve the emulsifying ability of the mucilage by increasing the viscosity of the colloidal system. A high protein content can also improve the nutrition profile of food products of interest.

Table 2 shows the results of minerals contents for OPN green fruit mucilage and its comparison with composition of OPN leaves mucilage. The mineral content of OPN green fruit mucilage is close to the amount of minerals required to meet the need of one adult per day (Takeiti et al., 2009). Besides this, the amount of minerals found in OPN green fruit mucilage was higher than that in OPN leaves mucilage for P, K, Ca, Fe and lower for Mg, Mn, Zn, and Na. According to the results of Martin et al. (2017) the leaf mucilage did not present the minerals S, B and Cu.

Table 2: Mineral composition in (g . 100 g-1) of the mucilage of the OPN (MFV) green fruit compared to the mucilage of the OPN (MF).

Minerals	MFV.OPN (%)	MF.OPN (%) (Martin et al., 2017)
P	0.200 ± 0.030	0.094 ± 0.001
K	1.800 ± 0.520	0.300 ± 0.001
Ca	2.700 ± 0.800	0.150 ± 0.000
Mg	0.070 ± 0.100	1.600 ± 0.020
S	0.200 ± 0.260	-
B	0.001 ± 0.001	-
Cu	0.002 ± 0.001	-
Mn	0.004 ± 0.004	0.010 ± 0.001
Zn	0.005 ± 0.000	0.300 ± 0.001
Fe	0.040 ± 0.010	0.010 ± 0.002
Na	0.017 ± 0.001	2.000 ± 0.080

3.2 Water activity (aw)

The knowledge of the water activity of products is important to predict food stability, being an indicator of microbial growth rate and chemical reactions (Maneffa et al., 2017). The water activity found for mucilage powder was 0.199 ± 0.012, remaining in the range considered safe against microbial development (aw <0.60) (Barbosa-Canovas et al., 2007).

3.3 Colorimetric analysis

The color parameters observed for OPN green fruit mucilage were: L* 73.14 ± 0.33, C* 12.10 ± 0.27 and h* 81.56 ± 0.05. In the color space, L* indicates brightness. Therefore, values closer to 100 indicate lighter material. C* indicates the chroma (intensity of a particular color) and h* is hue angle. The Hue angle parameter demonstrates that values closer to 90° are related to colors closer to yellow. Thus, the mucilage shows light yellow coloration (Fig. 2). These results are satisfactory as the gums already available in the market have a clear coloring. According to Yang and McClements (2013), the knowledge of the optical properties of the additives is of great importance for their correct application in products, since dark coloration can limit its use. The heat extraction process reported by Conceição et

al. (2014) and Lima Junior et al. (2013) resulted in a mucilage of dark color because of clorophyll, necessitating a clarification step in a fixed-bed column. In the present study, it was observed that the pressing used in the cold extraction was less aggressive to the plant cells, which minimized the transfer of pigment to the extract, and consequently generated a clear mucilage.

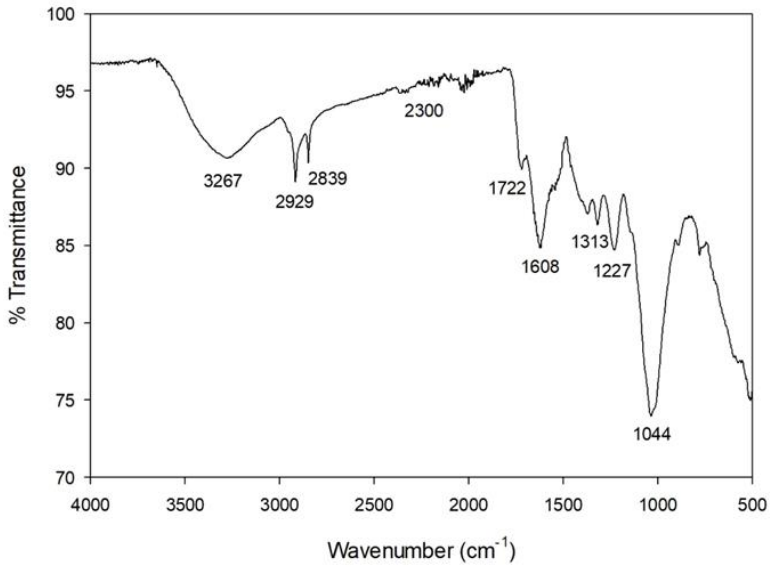
Figure 2: Mucilage powder of the OPN green fruit.

3.4 Infrared Analysis (IR)

In Figure 3 the IR spectra of mucilage of the OPN green fruit are shown and in Table 3 their assignments. A band with broad peak was observed at 3267 cm^{-1}, which is characterized by the presence of O-H bonds, demonstrating affinity of the polymers present in the mucilage by water molecules, making them hydrophilic compounds (Saha; Bhattacharya, 2010). Between 2928 cm^{-1} and 2839 cm^{-1}, characteristic peaks of organic compounds corresponding to the -CH_2 (sp^3) group were observed, and around 2300 cm^{-1} correlated with a C N. Bands binding at 1722 cm^{-1} and 1608 cm^{-1} were observed indicating the presence of a bond (─COOR) and also flexural vibration of the N-H plane, respectively. The absorptions at the wave number 1313 cm^{-1} demonstrate the presence of the -CH_2 and -CH_3 bonds and at 1044 cm^{-1} the bands may be related to the C-O stretch vibration. In Figure 3 the IR spectra of mucilage of the OPN green fruit are shown and in Table 3 their assignments. Analyzing the spectrum, a broad peak band at 3267 cm^{-1} is observed, which characterizes the presence of O-H bonds present in thecarbohydrates. Thus demonstrating that the polymers present in the mucilage are hydrophilic compounds (Saha; Bhattacharya, 2010). Between 2928 cm^{-1} and 2839 cm^{-1}, characteristic peaks of organic compounds corresponding to the -CH_2 (sp^3) group were observed, and around 2300 cm^{-1} correlated with a C-N. Bands binding at 1722 cm^{-1} and 1608 cm^{-1} were observed indicating the presence of a bond (─COOR) and flexural vibration

of the N-H plane, respectively. The transmittance at the wave number 1313 cm^{-1} shows the presence of the -CH_2 and -CH_3 bonds and at 1044 cm^{-1} the bands may be related to the C-O stretch vibration.

Figure 3: FTIR spectrum freeze-dried mucilage of OPN green fruit.



Conceição et al. (2014) presented the mucilage spectrum of the OPN sheet with the bands and peaks characteristic of polysaccharides with the presence of protein components. The mucilage of the OPN green fruit showed a similar spectrum, but the fruit showed an additional peak in the 1722 cm^{-1} band. The presence of the carboxylic group in the mucilage of the OPN sheet can serve as a site for ion bonds, which contribute to the ability to gel (Razavi et al., 2014). The emulsifying properties of OPN mucilage are related to the presence of protein components with a fraction of arabinogalactan (Lima Junior et al., 2013; Randall et al., 1988; Randall et al., 1989).

Table 3: FTIR peaks characteristic of the lyophilized mucilage of the OPN green fruit.

Frequência (cm^{-1})	Assignments
3267	─O─H association stretching vibration
2928 – 2839	─CH_2 (sp^3) antisymmetric stretching vibration
2300	–C ≡N, associação alongamento vibração
1722	(─COOR) ester bond stretching vibration
1608	─N─H plane bending vibration
1313	─CH_2, e CH_3 bending vibration
1044	C─O stretching vibration

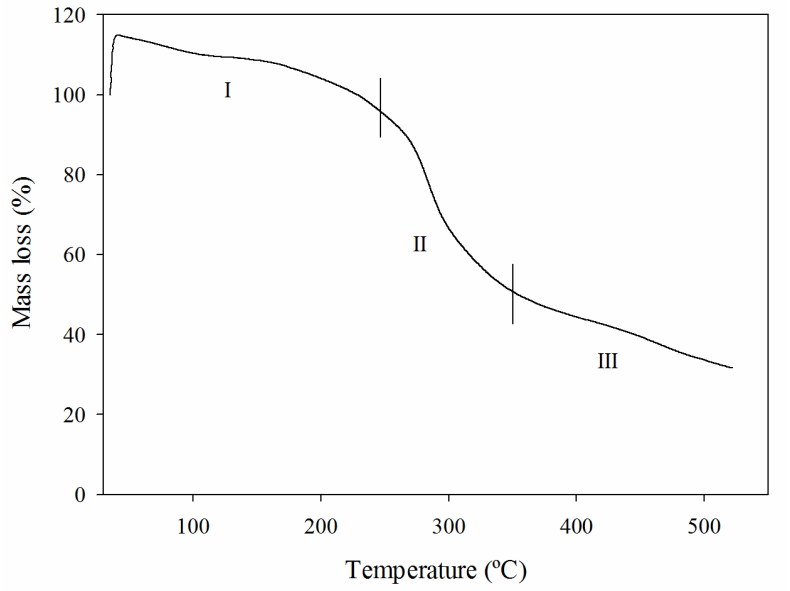
Source: Wang et al. (2014).

3.5 Thermal Analyzes

3.5.1 Thermogravimetric Analysis (TGA)

Thermal analysis methods are useful for understanding the thermal stability of the polymers under study and for predicting their thermal behavior during manufacturing and marketing. Thermogravimetric analysis is used to determine the initial decomposition temperature of various polymers (Mothé and Azevedo, 2009). The TGA curve of OPN green fruit mucilage is shown in Fig. 4.

Figure 4: TGA curve of the lyophilized mucilage of the OPN green fruit.



There well-defined events can be observed in the temperature range of 25 °C to 500 °C. The first one, stage I, corresponds to the volatilization of the moisture absorbed by the sample or volatiles (100 °C) with a loss of 5% of its initial mass. The second event, stage II, occurs between 180 °C and 400 °C, with maximum degradation rate at 285 °C (peaks in the DTG curve, data not shown). This stage can be attributed to degradation onset of the material. In this stage, a change in the conformation of the biopolymer is observed, followed by breakage of the branches, which results in the degradation of main components present, the polysaccharides and proteins. This stage is characterized by the largest mass loss of the sample (44%). The third event, stage III, from 350 °C to 500 °C can be attributed to the oxidative degradation of carbon and mineral residues present in the sample. Here, the mass loss observed was 12%. The complete degradation of the material did not occur, as the experiment was conducted in a nitrogen atmosphere, leaving approximately 39% residue at the end of the run.

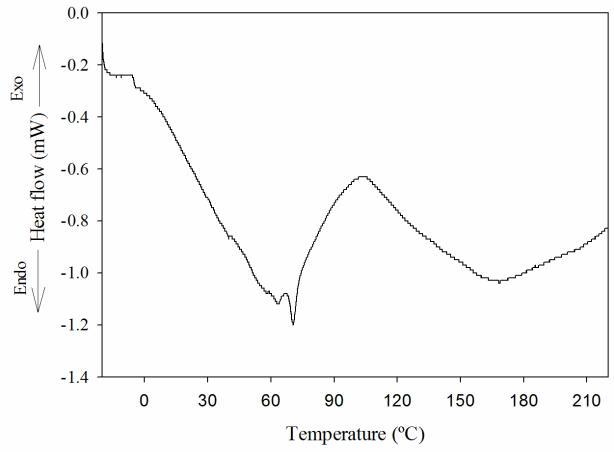
Similar bands representing stage II were observed in other mucilages such as tamarind seed mucilage (peak at 175 °C), cashew gum and gum arabic (252 °C), carboxymethyl tara gum (285 °C) and Opuntia dillenii haw mucilage (300 °C) (Alpizar-Reyes et al., 2017; Han et al., 2016; Mothé and Rao, 2000; Santos et al., 2019). Conceição et al. (2014); Martin et al. (2017); Mercê et al. (2001) and Oliveira et al. (2019) observed similar values for OPN leaves mucilage. According to Conceição et al. (2014) and Oliveira et al. (2019), the highest loss in mass was observed between 221 °C and 320 °C, where the material contained 83% polysaccharides and protein components. Martin et al. (2017) and Mercê et al. (2001) showed that the major loss in mass occurred near 250 °C. Thus, both OPN leaves mucilage and OPN green fruit mucilage showed similarities in the resistance to temperature up to 200 °C.

3.5.2 Differential scanning calorimetry (DSC)

DSC is a technique that determines the enthalpy and temperature variations in a sample and the reference during the phase and state transitions during the test (Sun, 2012), being relevant for studies of freezing, evaporation, dehydration and conservation processes (Mothé, Rao, 2000). Figure 5 shows the DSC curve obtained for the powder mucilage of the OPN green fruit, where a typical second order transition was first observed due to baseline extrapolation at -5 °C, characteristic of the glass transition of the material. At 68 °C, the thermogram detected an endothermic event that started at about 61 °C and ended at 73 °C, and can be attributed to the melting point of the sample. An exothermic event was also

identified in the thermogram at approximately 102 °C, which may be associated with boiling water (Pachuau et al., 2012).

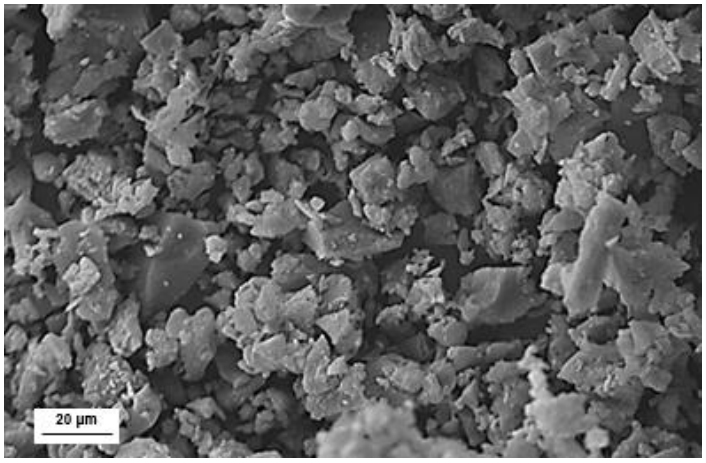
Figure 5: DSC curve of the lyophilized mucilage of the OPN green fruit.



3.6 Scanning Electron Microscopy (SEM)

The morphology of dehydrated mucilage can be affected by the extraction and purification method in the preparation of the product (Qian et al., 2009). The surface analysis of the mucilage powder extracted from the green fruit of the OPN was performed using SEM, as shown in Figure 6. Through the image, it is possible to visualize an amorphous structure with high porosity and little agglomeration, indicating low interaction between the particles, which gives stability to the powder. Wang, Ellis, and Ross-Murphy (2002, 2003 and 2006) reported that particle size and specific surface influences hydration and gum behavior, which in turn influence intrinsic viscosity and molecular mass.

Figure 6: Scanning eletron micrography of the lyophilized mucilage of the green OPN fruit. Magnification = 1250x.



3.7 Reconstitution of the mucilage of the powdered product for emulsion

3.7.1 Rheology properties

The Power Law model was adjusted to the shear stress and strain rate data obtained by the rheological study of the solutions prepared from the mucilage powder of the OPN green fruit, presenting high correlation coefficient (R^2> 0.996) and low values for the square root of the mean square error (RMSE <1,245). All the mucilage concentrations studied presented a pseudoplastic behavior (n <1, Table 4) which is characterized by the decay of the viscosity as the shear rate applied to the fluid is increased. This is due to the ordering of the chains of the molecules present in the suspension which, at rest, are disordered, and as tension is applied, begin to organize, reducing the viscosity of the medium (Steffe, 1996).

Table 4: Parameters of the Law of Power model and apparent viscosity at 50 s^{-1} (η_{50}).

Mucilage concentration (% m/v)	K (Pa·s^n)	n (-)	RMSE	R^2	η_{50} (mPa·s)
0.5	0.50±0.00	0.65±0.00	0.008	0.999	12.99 ± 0.11
1.0	2.77±0.00	0.53±0.00	0.361	0.997	44.69 ± 0.06
1.5	5.53±0.22	0.47±0.01	0.493	0.998	69.76 ± 0.55
2.0	9.78±0.15	0.42±0.00	0.802	0.997	101.02 ± 0.27
2.5	16.27±0.43	0.38±0.01	1.245	0.996	145.76 ± 0.07

The apparent viscosity at 50 s^{-1} presented a statistical difference ($p < 0.05$) between emulsions with different concentrations of hydrocolloid, in which Table 5 shows the adjusted regression for this parameter (R^2 = 98.79%). Figure 7a shows that increasing the mucilage concentration in solution contributed to the proportional increase of the apparent viscosity (η 50) and the consistency index (K), which may arouse the interest of industries for the use of this hydrocolloid as a thickening agent in formulations. Lima Junior et al. (2013) and Junqueira et al. (2018) found similar results for the rheological behavior of solutions prepared with mucilage extracted from the OPN sheet.The higher amount of protein and polysaccharides in the continuous emulsion phase may hinder the movement of the chains in solution, which is characterized by an increase in the viscosity of the medium (Maskan; Göğüş, 2000).

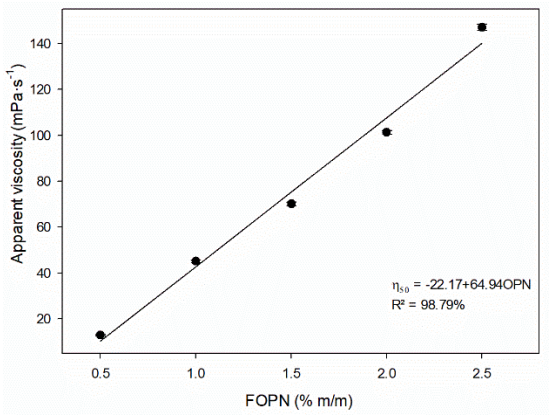
3.7.2 Emulsion formation (EC), emulsion stability (ES), d32

The emulsion formation capacity, stability at 20 and 80 °C, mean particle diameter showed a statistical difference between the treatments ($p < 0.05$), so adjusted the first and second order regression models, Figure 7, the parameters of these presented in Table 5.

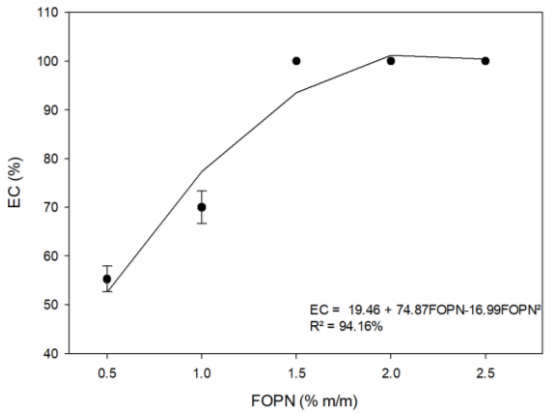
Table 5: Regression equations of the emulsifying capacity (EC) models, stabilizing capacity at 20 °C (ES20 °C), stabilizing capacity at 80 °C (ES80 °C), d_{32}.

Mechanical properties	Regression model $\hat{Y} = a + bx + cx^2$			
	a	b	c	R^2
Apparent Viscosity (mPa·s)	-22.17	64.94	-	98.79
EC	19.47	74.87	-16.99	94.16
$ES_{20°C}$	27.50	13.47	-	97.90
$ES_{80°C}$	27.60	12.74	-	98.34
d_{32}	23.55	-7.60	1.40	90.49

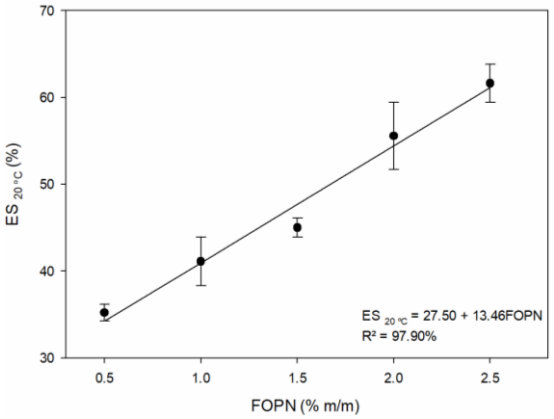
Figure 7: Regression curves of the adjusted models for emulsifying capacity (EC), stabilizing capacity at 20 ºC (ES20ºC), stabilizing capacity at 80 ºC (ES80ºC) and the average diameter (d_{32}).



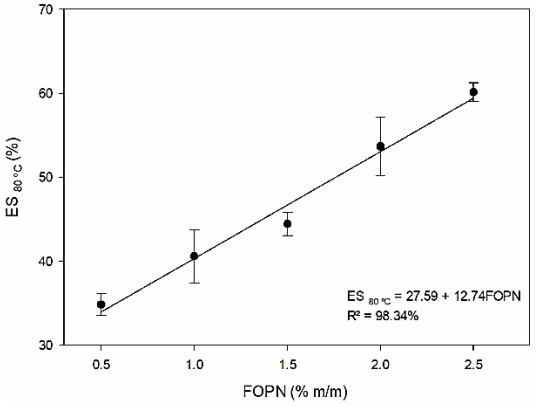
(a)



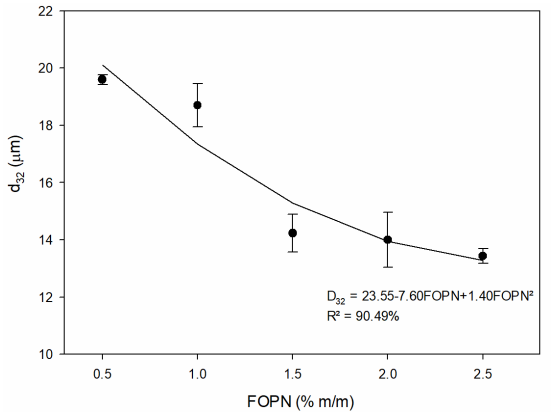
(b)



(c)



(d)



(e)

It is observed in Figure 7b that increasing the mucilage concentration in the medium contributed to the increase of the emulsifying capacity in the solutions. At concentrations of 0.5% (m/v) and 1% (m/v), the EC was 5.26% and 70.01%, respectively, being equal to 100% for the other concentrations studied. As shown by the analysis of centesimal composition, the

mucilage extracted from the OPN green fruit has high protein content (19.89%), molecules capable of diffusing in solution and adsorbing at the interface, modifying its three-dimensional structure in order to expose residues of hydrophilic amino acids for the aqueous portion and the hydrophobic amino acid residues for the oil phase. In this way, the interfacial tension of the system is reduced and the formation of a colloidal system, like the emulsions, is possible, remaining stable for a certain period of time (Lam; Nickerson, 2013). Thus, the increased mucilage concentration in solution was able to increase the emulsifying capacity due to the greater amount of proteins available to stabilize the interface. From 1.5% (m/v), a saturation of the interface is perceived, which means that the amount of surface agents present in the medium was sufficient to cover the dispersed phase oil molecules formed, for the amount of oil and agitation mode used. Lima Junior et al. (2013) found an emulsion formation capacity of 83% for emulsion prepared with 1.0% (w/v) mucilage extracted from the OPN sheet and canola oil, a result lower than that found in this work. The good results of the emulsifying capacity of this mucilage highlight the importance of the continuity of work with emulsified foods.

The stabilizing capacity of the emulsions was measured at 20 °C and 80 °C in order to study the effect of temperature on the stability of the colloidal system. An increase in ES as a function of the mucilage concentration in solution at both temperatures was observed, Figure 7c and 7d, reaching maximum stability when prepared with 2.5% (w/v) mucilage. As discussed, for the emulsion-forming ability, the larger amount of proteins present in solution and available to stabilize the interface contributes to greater stability of the emulsions. Moreover, increasing the mucilage concentration in the medium makes the continuous phase more viscous, making it difficult to move the oil droplets and delaying the effects of flocculation, waxing and consequent phase separation (Jafari et al., 2012; Rodriguez Patino, Pilosof, 2011). This result is in agreement with the one found by Junqueira et al. (2018) which observed an increase in the stability of emulsions (20 ° C), prepared with the gum extracted from OPN sheets and soybean oil, as the concentration of mucilage in the solution was increased. The elevation of temperature resulted in slightly greater destabilization in the emulsions. Emulsions at 20 °C obtained higher stability when compared to the same emulsion concentration at 80 °C. A similar result was found by Lima Junior et al. (2013) and Conceição et al. (2014). The mucilage of OPN presents high nitrogen content and is rich in proteins, being these components the ones responsible for the emulsifying capacity and emulsion stabilization (Lam; Nickerson, 2013; Rodriguez Patino, Pilosof, 2011; Junqueira et al., 2018; Sierakowski et al. al., 1987, Conceição et al., 2014).

The values of the mean diameter (d_{32}) of the droplets of the emulsions were in the range of 13.43 <d_{32}<19.06 μm. The increase in mucilage concentration influenced the formation of smaller oil droplet diameters in the emulsions (Figure 7e). The higher amount of protein (surface agent) is available in solution when more mucilage is used, favoring the stabilization of the system by reducing the surface tension and the formation of smaller diameter oil droplets during the formation of the emulsion (McClements, 2015). Conceição et al. (2014), through a microscopic technique, showed that for emulsion with 2% of the mucilage extracted from the OPN sheet, the diameter of the emulsions ranged from 2.0 to 10.0 μm. Lago et al. (2019) reported that the increase in mucilage concentration of the OPN sheet (%), together with lower concentrations of soybean oil (%), favored the formation of the nanoemulsion.

3.7.3 Freezing point of emulsions

The measurement of the start temperature for a freeze-drying process is an important analysis as it can be used to estimate thermophysical properties of food. During the freezing step, it is primordial to predict the required time for freezing the entire material in order to ensure product quality and equipment efficiency (Rahman, 1994). The amount of minerals present in the sample is one of the factors that can modify freezing temperature (Potter and Hotchkiss, 2012).

In this study, the difference in freezing points was not statistically significant among treatments ($p > 0.05$) with an average value of (−2.10 ± 0.05) °C. These results showed that the mucilage did not interfere with the freezing process within the mucilage concentration range tested (0.5%–2.5% w/w).

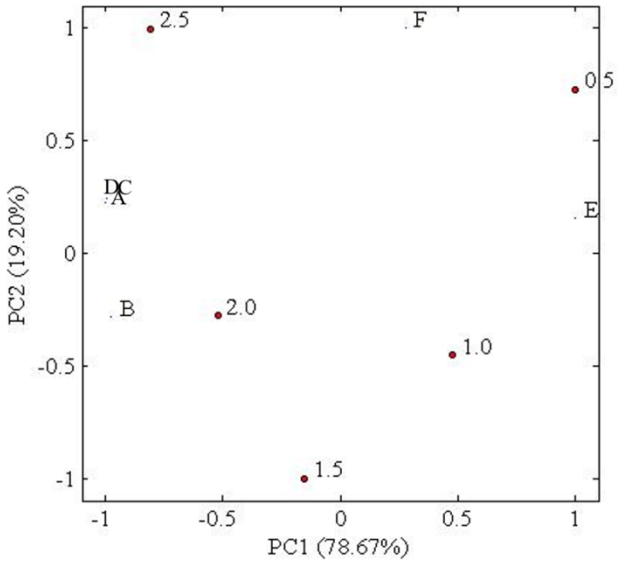
3.8. Principal component analysis (PCA)

Using an exploratory investigation, a graph containing the scores and observations of the analyzed emulsions was generated (Fig. 8a). It was found that PC1 and PC2 showed 97.87% of the total data variations. The sample containing 2.5% of OPN green fruit mucilage presented higher viscosity, emulsifying capacity and emulsion stability at 20 °C and 80 °C. It also showed lower average particle size. Whereas, the emulsion prepared with 0.5% OPN green fruit mucilage showed the highest average particle diameter. From the dendrogram (Fig.

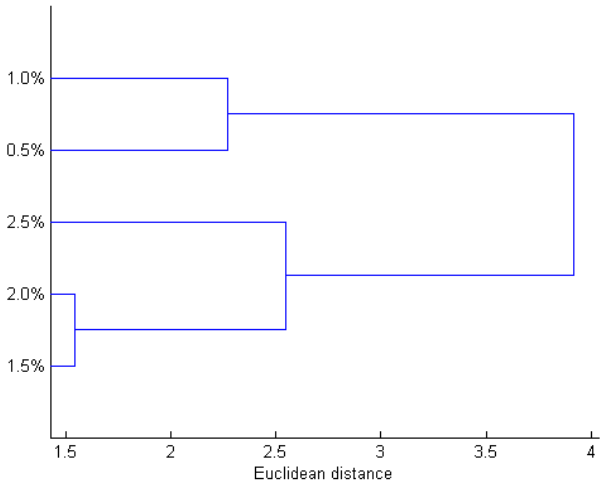
8b), it is evident that samples containing 2.0% and 1.5% of OPN green fruit mucilage are more similar to each other, as the Euclidian distance between them is smaller.

Figure 8: Pattern recognition of emulsions with different mucilage concentrations of OPN green fruit (a) PCA, (b) HCA.

(a)



(b)



4 Conclusion

The OPN green fruit mucilage presented high protein content and thermal stability, low water activity, light yellow coloration, and structure of high porosity with little agglomeration. These factors demonstrate the feasibility of using this mucilage as an emulsion. In addition, increasing the concentration of OPN green fruit mucilage in emulsion resulted in an increase in apparent viscosity, emulsifying capacity, emulsion stability, and a decrease in the mean droplet size. These features allow its incorporation in food matrices as an emulsifier, stabilizer, and thickener. The OPN green fruit mucilage concentrations are established from 1.5% (w/w) for application in colloidal systems, as these concentrations exhibited better emulsifying and functional properties, making this mucilage a promising additive to be used in the food industry. Therefore, the use of cold extraction and freeze-drying processes for the extraction of OPN green fruit mucilage is viable for generating a good quality additive with high application potential in food and pharmaceutical matrices.

Declaration of interest

The authors declare there is no conflict of interest in this research.

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TERCEIRA PARTE – ARTIGO 2

EXTRACTION AND APPLICATION OF ORA-PRO-NOBIS (*Pereskia aculeata* Miller) MUCILAGE IN FREEZE-DRIED PETIT SUISSE CHEESE

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Sérgio Henrique Silva[a], Isabelle Cristina Oliveira Neves[a], Ana Cristina Freitas de Oliveira Meira[a], Ana Cláudia Silveira Alexandre[a], Natália Leite Oliveira[a], Jaime Vilela de Resende[a]*.

[a]Department of Food Science, Federal University of Lavras, Av. Doutor Sylvio Menicucci, 1001 – Centro, Zip code: 37200-900, Lavras, Minas Gerais, Brazil.

***Corresponding author:** Jaime Vilela de Resende

Full postal address: Department of Food Science, Federal University of Lavras, Av. Doutor Sylvio Menicucci, 1001 – Centro, Zip code: 37200-900, Lavras, Minas Gerais, Brazil

e-mail adress: jvresende@ufla.br

Telephone: +55 35 3829-1050.

ABSTRACT

Mucilage extracts from the leaves (ML) and fruits (MFR) of ora-pro-nobis were used as stabilizing agent with a mixture of carrageenans (MIX) at different concentrations for Petit Suisse cheese production. The texture (firmness, adhesion and chewability), syneresis (mL), and viscosity ($Pa.s^{-1}$) profiles of the fresh samples were analyzed and subjected to freeze-drying process. Textural properties increased as concentrations increased, and after rehydration, the values decreased. Fresh and rehydrated samples exhibited pseudoplastic behavior. Greater concentration of ML, MFR and MIX increased the apparent viscosity (η) and consistency index (K). Fresh samples showed η at 1.76 s^{-1} and K slightly higher than rehydrated formulations. Syneresis occurred among some fresh samples(1, 7, 8, 9, 10, 13, and 14) and rehydrated samples (1, 2, 4, 7, 8, 9, 10, 11, 12, 13, and 14). Drying yields for freeze-dried emulsions ranged from 64.89–72.91%. This study demonstrates that both ML and MFR showed promising interaction with the MIX in creating freeze-dried Petit Suisse cheese.

Keywords: Biopolymer; Cheese; Freeze-drying; Powder; Stabilizing.

1. Introduction

Petit Suisse is a type of creamy and soft fresh cheese from France that has a delicate and sweet taste with a texture closer to a thick yogurt than cheese (Prudencio, Prude, Gris, Tomazi, & Marilde T Bordignon-luiz, 2008). This cheese is produced by coagulating milk using rennet and mesophilic bacteria with the possible addition of other food substances (Esmerino et al., 2015), besides being a popular dairy product worldwide that is generally consumed by children but is well-received by all age groups (Matias, Bedani, Castro, & Saad, 2014).

In fermented dairy products, gel formation is the most important functional property because it is directly linked to the structural product stability. Stabilizers and thickeners are used to improve the gel structure formed in these foods, and consequently, they improve the sensory aspects and reduce syneresis in storage (Amaral et al., 2018). Presently, there is a trend in the food industry to replace synthetic ingredients with natural products (McClements, 2015). Hydrocolloids are related to proteins, polysaccharides, and polymers consisting of extensive chains that can be applied to food. These biopolymers act as structuring, thickening, or glazing agents (Junqueira, 2018).

Pereskia aculeata Miller has been proposed as a new source of hydrocolloids due to the presence of large amounts of polysaccharides and proteins in its leaves and fruits in the green maturation stage (Lago et al., 2019). Sierakowski, A.J. Gorin, Reicher, & Corres (1987) described this biopolymer as an arabinogalactan, a water-soluble polysaccharide consisting of arabinose, galactose, rhamnose, and galacturonic acid, with protein branches in OPN mucilage. OPN-derived mucilage extracts have been studied for stabilization and emulsification (Conceição, Junqueira, Guedes Silva, Prado, & De Resende, 2014; Lima Junior et al., 2013), formation and stabilization of nano-emulsions (Lago et al., 2019), formation of biodegradable films (Oliveira et al., 2019), production of fermented milk beverages (Amaral et al., 2018), and microencapsulation of α-tocopherol (Neves et al., 2020).

Another possibility of thickener for application in fermented dairy products is carrageenan. This additive is are large, highly flexible molecules extracted from red algae that form helicoidal structures, which can be used to prepare various gel types at different temperatures (Necas & Bartosikova, 2013). Carrageenans possess gelling, thickening, and stabilizing properties, and they are widely used in the dairy and meat industries due to their strong interaction with proteins, which influences the final texture of food (Kariduraganavar, Kittur, & Kamble, 2014).

Consumer demand for the convenience of consuming food drives the food industry to seek different conservation techniques for fresh products. Thus, the search for conservation techniques that maintain the initial characteristics of a fresh product after processing it is necessary to gain preference in consumer purchasing and extend shelf life (Taylor, Oikonomopoulou, Krokida, Oikonomopoulou, & Krokida, 2012). Freeze-drying is a conservation technique that dehydrates a food product and mostly maintains the initial characteristics of the product when rehydrated (Krokida, Karathanos, & Maroulis, 1998). Additionally, freeze-dried products do not need refrigeration during storage due to low water activity, which retards microbial growth, and using low temperatures during dehydration helps in retaining color and aroma (Khairnar, Rajesh, Harwalkar, Chaudhari, & Kishor, 2013).

The textural parameters of fermented dairy products are of great relevance, as they play an important role in the acceptance of the product by consumers. The presence of arabinogalactan in ML and the high protein content in MFR provide a good interaction with the carrageenan mix in the structural arrangement of a food, due to the groups present in these biometrics. Considering the practicality of non-refrigerated consumption and the high shelf life of a powdered food, this study aimed to produce freeze-dried Petit Suisse cheese, using mucilages from the fruit, OPN leaf and carrageenan mixture and assessing the rheological properties, textural and microstructural of fresh and rehydrated Petit Suisse cheese after freeze drying.

2. Materials and methods

The following materials were used for preparing the cheese analyzed in the current study: whole milk (Itambé, Uberlândia, MG, Brazil), rennet (HA-LA®, Horsholm, Denmark), mesophilic culture of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* (Chr.

Hansen, Horsholm, Denmark), sucrose (PA) (Isofar, Duque de Caxias, RJ, Brazil), and carrageenan mixture (Gemacom Tech, Juíz de Fora, MG, Brazil).

2.1 Production of Petit Suisse Cheeses

Petit Suisse cheeses were prepared with different concentrations (Table 1) of mucilage extracted from the OPN leaf (ML), OPN green ripening fruit (MFR), and carrageenan mixture (MIX). The milk was mixed with 10% (w/w) sugar plus thickeners and subjected to heat treatment at 85 °C for 30 min. A Turrax homogenizer (Tecnal TE102, Brazil) was used for homogenization at 18000 rpm for 10 min, and this mixture was cooled to 35 °C. The mesophilic culture (0.5 mL/L) and rennet (0.08 mL/L) were added to the mixture, agitated, and packed for fermentation in a Nova Ética oven, model 440 (Ethik technology, São Paulo, Brazil) at 30 °C for 14–16 h, until pH 4.9 was attained.

Table 1: Concentrations of mucilage extracts and carrageenan mixture.

Petit Suisse Cheese Formulations	Mucilage (%)	MIX (%)
1	0.5 ML	0.5
2	1.0 ML	0.5
3	1.5 ML	0.5
4	0.5 ML	1.0
5	1.0 ML	1.0
6	1.5 ML	1.0
7	0.5 MFR	0.5
8	1.0 MFR	0.5
9	1.5 MFR	0.5
10	0.5 MFR	1.0
11	1.0MFR	1.0
12	1.5 MFR	1.0
13	-	0.5
14	-	1.0

Both OPN mucilages were extracted according to Lima Junior et al. (2013) and Silva et al. (2019). The Lactoscan ultrasonic milk analyzer G0041P (Bulgaria) was used for the

characterization of whole milk in terms of fat (%), protein (%), lactose (%), pH, density (g/ml), solids (%), and water (%). For the determination of titratable acidity (°D), the method described by the Adolfo Lutz Institute (2008) was used, and the protein content was estimated using the Kjeldahl method. The protein concentration was estimated according to the nitrogen conversion factor of 6.25.

At the end of fermentation, half of the samples was stored at a temperature of 8 °C to be analyzed (fresh), and the other half was frozen at -75 °C in an ultra-freezer with a static air system (Coldlab CL 120-86 V, Brazil) for 24 h. Frozen samples were dehydrated in a freeze-dryer (Edwards, L4KR, Brazil) at -40 °C with a vacuum pressure of 0.998 mbar for 72 h. After freeze-drying, the dried samples were ground and stored in vacuum desiccators. All samples were produced in triplicate.

2.2 Analysis of the texture profile

Texture analyses of the different treatments were performed on a TA-XT2i model texturometer (Micro Systems Stable, Godalming, England). The samples were analyzed at 25 °C using a 20 mm probe, and placed 3 cm high inside cylindrical jars (6 cm diameter *x* 4 cm high). The probe was programmed to penetrate 1.5 cm into the samples at a speed of 1 mm s^{-1}. All data were analyzed using Exponent Lite Express software (Micro Systems Stable, Godalming, UK). The parameters analyzed were hardness, adhesiveness, and chewability.

2.3 Rheological properties

The rheological parameters of the samples were determined using a concentric rotary viscometer (Brookfield DVIII Ultra, Brookfield Engineering Laboratories, USA) at 30 °C. The SC4-25 spindle was subjected to an increasing ramp with a shear rate ranging from 0.5 s^{-1}

and a speed of 2.5 rpm. The Power Law model (Eq. 1) was adjusted to the shear stress and shear rate data to determine the fluid flow profile.

$$\sigma = k\dot{\gamma}^n \quad (1)$$

where, σ is the shear stress (Pa), k is the consistency index (Pa s^n), $\dot{\gamma}$ is the strain rate (s^{-1}), and n is the flow behavior index (dimensionless). The apparent viscosity was presented with a deformation rate of 1.76 s^{-1} because this value represents the maximum deformation rate measured by the equipment for the most viscous sample.

2.4 Syneresis

Syneresis was measured according to the methodology of Riener, Noci, Cronin, Morgan, & Lyng (2010) using 30 g of cheese without stirring before freeze-drying and 30 g of the rehydrated cheese with manual stirring, spread evenly on a Quantity No. 1 filter paper (J. Prolab, São José dos Pinhais, Paraná, Brazil) in a funnel placed on top of a 50 mL graduated cylinder. The measuring cylinder was then held at 4 °C for 5 h. The volume of liquid collected was recorded, and the result was expressed as a percentage (%).

2.5 Colorimetric analysis

The colorimetric properties of fresh samples were examined using a Konica Minolta spectrophotometer CM-5 equipped with the cylindrical coordinate color system luminosity (L*), chroma saturation index (C*), hue angle (h°).

2.6 Freeze-drying and yield

The samples were weighed, frozen at -75 °C (Coldlab CL 120-86 V, Brazil) and freeze-dried at -40 °C (vacuum pressure of 0.998 mbar) for 36 h (Edwards, L4KR, Brazil).

The yield (%) of the drying process was considered equal to the dry mass (g) of the freeze-dried product over the initial mass (wet mass, g) of the sample.

$$Yield(\%) = \frac{drymass}{wet\ mass} * 100 \qquad (2)$$

2.7 Water activity (a_w)

Water activity of the freeze-dried samples was determined with a dew point hygrometer (Aqualab Decagon Services 3TE, USA) at 25 °C.

2.8 Scanning electron microscopy

The micrographs of freeze-dried samples were examined using a scanning electron microscope (LEO EVO 40 XVP, Carl Zeiss, Germany) at an acceleration voltage of 20 kV. Before observation, the mucilage was fixed in stubs using double-sided carbon tape and sprinkled with gold at 200 A for 180 s to make the sample conductive.

2.9 Rehydration and water absorption capacity (WAC)

The samples were rehydrated with the total volume of sublimated water in the freeze-drying process, calculated by the difference between the initial weight and the final weight. Water was incorporated into the powder by shaking it with a dessert spoon, simulating the practical application at the time of consumption. An adapted version of the centrifugation method described by (Riener et al., 2010) was used to measure WAC. Each sample (10 g) was weighed into centrifuge tubes and centrifuged at 3000 x g for 15 min at 20 °C. The supernatant was separated, and the samples were re-weighed. The WAC was expressed as the weight of drained whey per 100 g of cheese.

2.10 Statistical analysis

The results were subjected to an analysis of variance (ANOVA) and a Tukey test ($P<0.05$) for the comparison of means and determination of the statistical difference between treatments for fresh and rehydrated Petit Suisse cheeses. All analyses were performed in triplicate, and the results are expressed as the mean ± standard deviation. The statistical package SAS University Edition (SAS Institute Inc., Cary, NC, USA) was used in these analyses.

3 Results and discussion

3.1 Fresh Petit Suisse Cheeses

3.1.1 pH and syneresis

The whole milk used to prepare the Petit Suisse cheeses presented fat 3.142±0.006% (w/v); density 1,028±0.064 g/mL; lactose 3.949±0.006%; solids 8.051±0.017%; protein 2.761±0.006%; titratable acidity 13.774±0.38 °D; and pH 6.625±0.0181. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of the milk. During acidification of the milk, the pH reduces below 4.6 (isoelectric point of casein) forming gels that cause changes in protein-protein and mid-protein interactions (Helena et al., 2019). Protein gelification occurs at pH 5.2–5.4 for pasteurized milk (Lee & Lucey, 2010).

Syneresis is the gradual expulsion of whey from milk during the storage of fermented dairy products. It occurs due to the instability and contraction of the gel network through the rearrangement of bonds between protein aggregates in the product structure (Aichinger et al., 2003). By measuring the whey expelled from the product, the index of syneresis is determined, which has an impact on the moisture content and texture of the product (Castillo, Lucey, Wang, & Payne, 2006). Table 2 presents the initial and final pH and syneresis index of fresh Petit Suisse samples.

Table 2:PH values and syneresis obtained for Petit Suisse cheeses produced with ML, MFR or MIX.

Sample	% (w/w)	pH_i	pH_f	Syneresis (%)
1	0.5 ML + 0.5 MIX	6.71±0.02^{b}	4.55±0.06^{a}	6.00±0.04^{e}
2	1 ML + 0.5 MIX	6.70±0.08^{b}	4.76±0.15^{b}	0.00±0.00^{a}
3	1.5 ML + 0.5 MIX	6.72±0.03^{b}	4.80±0.22^{b}	0.00±0.00^{a}
4	0.5 ML + 1 MIX	6.64±0.06^{b}	4.83±0.18^{b}	0.00±0.00^{a}
5	1 ML + 1 MIX	6.61±0.01^{b}	4.93±0.18^{b}	0.00±0.00^{a}
6	1.5 ML + 1 MIX	6.66±0.04^{b}	4.92±0.19^{b}	0.00±0.00^{a}
7	0.5 MFR + 0.5 MIX	6.58±0.01^{b}	4.78±0.01^{b}	5.00±0.06^{d}
8	1 MFR + 0.5 MIX	6.34±0.01$^{a.\ b}$	4.57±0.15^{a}	4.50±0.02^{d}
9	1.5 MFR + 0.5 MIX	6.18±0.01^{a}	4.58±0.02^{a}	1.66±0.07^{c}
10	0.5 MFR + 1 MIX	6.42±0.00$^{a.\ b}$	4.64 ±0.03$^{a.\ b}$	0.72±0.01^{b}
11	1 MFR + 1 MIX	6.32±0.08$^{a.\ b}$	4.56±0.08^{a}	0.00±0.00^{a}
12	1.5 MFR + 1 MIX	6.19±0.04^{a}	4.61 ±0.01^{a}	0.00±0.00^{a}
13	0.5 MIX	6.63±0.10^{b}	4.85±0.13^{b}	13.9±0.02^{g}
14	1 MIX	6.56±0.08^{b}	4.98±0.48^{b}	10.0±0.02^{f}

* Different lower-case letters in the columns indicate a significant difference ($p<0.05$) by the Tukey test. ML = leaf mucilage, MFR = fruit mucilage, MIX = carrageenan mix. Number of replications = 3.

Treatments 13 and 14 were performed only with the carrageenan mixture for comparison with the samples formulated with ML and MFR plus MIX. Samples 1, 7, 8, 9, 10, 13, and 14 presented syneresis, and the highest values were obtained for samples 13 and 14. The interaction between MIX plus ML and MFR in treatments 2, 3, 4, 5, 6, 11, and 12 resulted in no syneresis. This phenomenon occurred because this interaction resulted in a greater thickening capacity in the system, trapping the water in the gel structure. The arabinogalactan chain present in OPN-derived mucilage may confer high viscosity to aqueous solutions due to its high molecular weight, conformation, and interactive properties (Conceição et al., 2014). Besides, carrageenan is a sulfated galactan with a strong negative charge that can interact with other electrically charged macromolecules, such as proteins, to obtain gels with various textures (Therkelsen, 1993).

3.1.2 Color

The samples of fresh Petit Suisse showed significant differences (P<0.05) in L* (Table 3) between the treatments.

Table 3: Colorimetric analysis of fresh Petit Suisse samples.

Sample	% (w/w)	L*	C*	h°
1	0.5 ML + 0.5 MIX	60.42 ± 0.01^{c}	9.40 ± 0.01^{a}	84.20 ± 0.00^{c}
2	1 ML + 0.5 MIX	54.46 ± 0.01^{b}	$10.65\pm0.04^{b,c}$	88.86 ± 0.06^{e}
3	1.5 ML + 0.5 MIX	49.76 ± 0.00^{a}	10.98 ± 0.01^{f}	82.26 ± 0.05^{b}
4	0.5 ML + 1 MIX	60.76 ± 0.01^{c}	$10.52\pm0.00^{b,c}$	86.59 ± 0.03^{d}
5	1 ML + 1 MIX	54.44 ± 0.00^{b}	$10.59\pm0.01^{b,c}$	84.25 ± 0.08^{c}
6	1.5 ML + 1 MIX	49.20 ± 0.01^{a}	10.86 ± 0.01^{c}	82.33 ± 0.01^{b}
7	0.5 MFR + 0.5 MIX	74.22 ± 0.01^{j}	11.17 ± 0.01^{d}	90.05 ± 0.01^{f}
8	1 MFR + 0.5 MIX	70.60 ± 0.00^{e}	13.24 ± 0.01^{f}	84.64 ± 0.00^{c}
9	1.5 MFR + 0.5 MIX	65.47 ± 0.04^{d}	14.05 ± 0.01^{k}	81.01 ± 0.03^{a}
10	0.5 MFR + 1 MIX	75.28 ± 0.01^{f}	12.76 ± 0.01^{e}	87.95 ± 0.05^{d}
11	1 MFR + 1 MIX	70.82 ± 0.01^{e}	13.54 ± 0.00^{f}	84.74 ± 0.04^{c}
12	1.5 MFR + 1 MIX	66.69 ± 0.20^{d}	14.32 ± 0.01^{g}	82.48 ± 0.02^{b}
13	0.5 MIX	86.67 ± 0.03^{g}	10.09 ± 0.01^{b}	100.88 ± 0.04^{g}
14	1 MIX	86.50 ± 0.04^{g}	10.14 ± 0.06^{b}	101.64 ± 0.06^{g}

* Different lower case letters in the columns indicate a significant difference (p<0.05) by the Tukey test. ML = leaf mucilage, MFR = fruit mucilage, MIX = carrageenan mix. Number of replications = 3.

The samples from 7–12 prepared with MFR showed higher values of L*, indicating a lighter shade. Mucilage derived from the OPN fruit was stained a light-yellow color (Silva et al., 2019), unlike the mucilage extracted from the leaf, which shows dark green coloring (Conceição et al., 2014). Samples 13 and 14 obtained higher values ofL* because the carrageenan mixture shows no pigmentation. According to Yang & McClements, 2013, knowledge of the optical properties of additives is of great importance for their correct application in products since dark staining may limit their use. Chroma, is the saturation value C* (intensity of a specific color), represents the distance from the luminosity axis (L *) and starts at zero in the center. Hue angle (h°), indicates the color saturation of the object. Table 3

shows the color parameters of fresh samples and Figure 1 shows the images of fresh Petit Suisse samples.

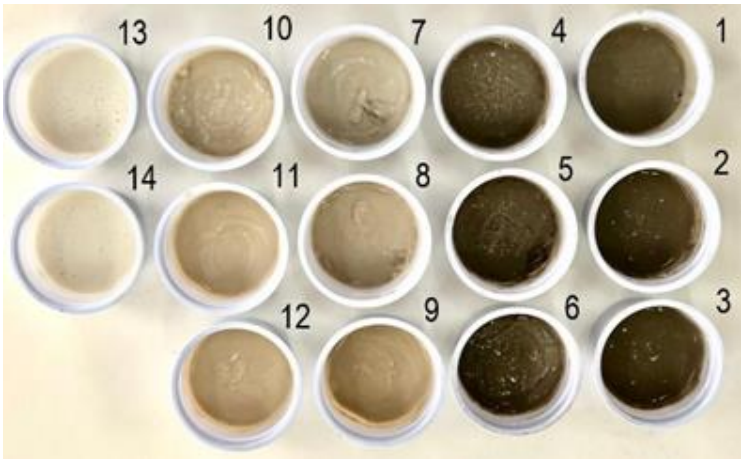


Figure 1: Images of fresh petit Suisse samples

3.1.3 Texture

Knowledge of the system's textural properties is fundamental to understanding the physical attributes of foods, which are crucial aspects of quality and general sensory acceptance by consumers (Helena et al., 2019). The instrumental texture profile is presented in Table 4 and significant differences ($P<0.05$) were found in the texture properties between all samples.

Table 4: Texture analysis of fresh Petit Suisse samples.

Sample	% (w/w)	HF	AF	CHF
1	0.5 ML + 0.5 MIX	92.70±1.92^{d}	-289.15±13.56^{g}	48.06±1.40$^{c. d}$
2	1 ML + 0.5 MIX	101.71±4.05$^{d. e}$	-337.65±18.43$^{d. e}$	54.66±3.01^{d}
3	1.5 ML + 0.5 MIX	108.32±4.97^{e}	-346.65±13.71^{d}	58.40±2.12^{d}
4	0.5 ML + 1 MIX	123.05±5.23^{g}	-374.16±13.37^{c}	63.64±3.81^{e}
5	1 ML + 1 MIX	142.36±7.11$^{i. j}$	-393.49±12.41^{b}	82.58±3.63^{g}
6	1.5 ML + 1 MIX	145.91±7.63^{j}	-483.16±23.85^{a}	82.51±3.96^{g}
7	0.5 MFR + 0.5 MIX	74.81±2.47^{b}	-189.11±5.66^{h}	33.95±1.01^{b}
8	1 MFR + 0.5 MIX	82.93±2.82^{c}	-287.43±12.28^{g}	40.88±1.93^{c}
9	1.5 MFR + 0.5 MIX	87.35±3.68$^{c. d}$	-296.67±13.39$^{f. g}$	42.67±2.68^{c}
10	0.5 MFR + 1 MIX	115.00±5.79^{f}	-103.50±4.76^{f}	65.57±3.45^{e}
11	1 MFR + 1 MIX	135.42±6.83^{h}	-333.78±14.35^{e}	71.30±2.47^{f}
12	1.5 MFR + 1 MIX	138.35±6.97^{h}	-351.29±4.23^{d}	80.69±3.49^{g}
13	0.5 MIX	25.16±1.45^{a}	-59.73±1.81^{i}	20.82±1.07^{a}
14	1 MIX	79.80±3.94^{b}	-196.87±5.51^{h}	32.19±1.55^{b}

* Different lower-case letters in the columns indicate a significant difference ($p<0.05$). (HF) = Firmness, (AF) = adhesion and (CHF) chewability, ML = mucilage of the leaf, MFR = mucilage of the fruit, MIX = mix of carrageenan. Number of replications = 3.

Hardness, adhesiveness, and chewability increased (in modulus) as the concentrations of mucilage increased in all treatments. The increase in the values of these parameters in dairy products is generally proportional to an increase in protein and fat concentrations. For samples prepared with ML, the values of all the analyzed parameters were higher than those for the formulations prepared with MFR.

3.1.4 Rheology

ML and MFR from OPN demonstrated properties that indicate their potential application in the industry as thickeners, gelling agents, and/or emulsifiers. The high capacity of emulsion formation and thermal stability of this emulsion was previously shown (Conceição et al., 2014; Silva et al., 2019). The rheological behavior and interactions between hydrocolloids and other major components in food formulations have been researched to explore their microstructure. According to Amaral et al. (2018), the use of two or more gums

in the formulation of a product may result in an improvement of its final quality due to the synergistic effects of the combination.

Emulsions, such as Petit Suisse cheese formulations, are systems generally characterized as non-Newtonian fluids (McClements, 2015). The Power Law model fit showed a high correlation coefficient for fresh samples ($R^2 \geq 88.40$). For all mucilage concentrations, Petit Suisse cheeses presented pseudoplastic behavior ($n<1$, Table 5), characterized by a decrease in apparent viscosity with increase in the shear rate applied to the fluid. This occurs due to the order of the chains of molecules present in the suspension, which are disordered at rest, and as stress is applied, begin to organize, reducing the viscosity of the medium (Steffe, 1996).

The apparent viscosity at 1.76 s^{-1} showed a statistical difference ($P<0.05$) between formulations with different concentrations of hydrocolloids by Tukey's Test. Table 5 shows that the increase in both mucilage concentration in the samples contributed to a proportional increase in apparent viscosity ($\eta_{1.76}$) and consistency index (K), and a decrease in the behavior index (n).

Table 5: Rheological data of fresh Petit Suisse cheeses. (K) = consistency index, (n) = behavior index and apparent viscosity at 1.76 s^{-1}.

Sample	% (w/w)	K ($Pa \cdot s^n$)	n(-)	R^2	$\eta_{1.76}$ ($Pa \cdot s^{-1}$)
1	0.5 ML + 0.5 MIX	36.88 ± 1.34^{c}	$0.29 \pm 0.03^{b.c}$	94.37	23.02 ± 1.13^{c}
2	1 ML + 0.5 MIX	$69.49 \pm 1.24^{e.f}$	0.25 ± 0.04^{b}	95.70	41.85 ± 1.10^{e}
3	1.5 ML + 0.5 MIX	78.52 ± 2.09^{h}	$0.23 \pm 0.02^{a.b}$	88.40	45.93 ± 2.21^{f}
4	0.5 ML + 1 MIX	$69.81 \pm 1.45^{e.f}$	0.26 ± 0.03^{b}	97.73	37.23 ± 1.61^{d}
5	1 ML + 1 MIX	$75.09 \pm 3.07^{g.h}$	$0.21 \pm 0.01^{a.b}$	96.60	49.67 ± 1.36^{g}
6	1.5 ML + 1 MIX	78.42 ± 2.67^{h}	0.17 ± 0.02^{a}	92.35	53.17 ± 2.64^{h}
7	0.5 MFR + 0.5 MIX	35.47 ± 1.63^{c}	0.30 ± 0.03^{c}	95.37	21.29 ± 0.98^{c}
8	1 MFR + 0.5 MIX	62.88 ± 3.55^{d}	0.28 ± 0.02^{b}	96.70	$39.61 \pm 1.45^{d.e}$
9	1.5 MFR + 0.5 MIX	76.41 ± 2.16^{g}	0.27 ± 0.01^{b}	94.00	44.35 ± 2.10^{f}
10	0.5 MFR + 1 MIX	68.27 ± 2.97^{e}	$0.22 \pm 0.03^{a.b}$	96.83	36.76 ± 1.53^{d}
11	1 MFR + 1 MIX	72.71 ± 2.67^{g}	$0.20 \pm 0.03^{a.b}$	96.47	48.15 ± 2.41^{g}
12	1.5 MFR + 1 MIX	79.41 ± 3.30^{h}	0.19 ± 0.02^{a}	98.03	51.29 ± 1.53^{h}
13	0.5 MIX	6.97 ± 1.06^{a}	0.41 ± 0.05^{e}	90.53	5.14 ± 0.53^{a}
14	1 MIX	12.56 ± 3.81^{b}	0.37 ± 0.02^{d}	92.30	9.57 ± 0.64^{b}

* Different tiny superscripts in the columns indicate significant difference ($p<0.05$) by Tukey's test, ML = leaf mucilage, MFR = fruit mucilage, MIX = carrageenan mix. Number of replications = 3.

Similar behavior was described by Junqueira et al. (2018) and Lago et al. (2019) with ML, and by Silva et al. (2019) with MFR. The higher amount of proteins and polysaccharides in these formulations resulted in the formation of a three-dimensional network through the interactions between the molecules. The formation of this network then limits the movement of chains in a solution capable of trapping water molecules, thereby increasing the medium viscosity and pseudoelasticity.

The treatments prepared using only the carrageenan mixture showed apparent viscosity ($\eta_{1.76}$) and consistency index (K) values lower than those of the formulations containing the OPN mucilages. This result indicates the potential industrial use of ML and MFR as thickening agents.

3.2 Freeze-dried Petit Suisse Cheese

After freeze-drying, the yield of the dried samples varied from 64.89–72.91% (Table 6).

Table 6: Physical-chemical results for Petit Suisse freeze-dried and rehydrated samples.

Sample	pH_r	Yield (%)	Syneresis (%)	WAC (%)	a_w	Protein (%)
1	4.52 ± 0.19^{a}	20.79 ± 2.64^{a}	1.66 ± 0.01^{b}	281 ± 8.49^{g}	0.29 ± 0.0^{c}	10.66 ± 0.24^{b}
2	4.57 ± 0.01^{a}	30.64 ± 2.50^{c}	0.56 ± 0.01^{b}	300 ± 12.83^{i}	0.29 ± 0.0^{c}	11.03 ± 0.13^{b}
3	4.56 ± 0.01^{a}	30.95 ± 3.33^{c}	0.00 ± 0.00^{a}	304 ± 9.90^{i}	0.29 ± 0.00^{c}	11.00 ± 0.18^{b}
4	4.57 ± 0.08^{a}	34.38 ± 4.93^{d}	1.10 ± 0.01^{b}	268 ± 7.78^{f}	0.29 ± 0.00^{c}	10.78 ± 0.27^{b}
5	4.41±0.13a	27.30 ± 2.26^{b}	0.00 ± 0.00^{a}	289 ± 9.69^{h}	0.28 ± 0.01^{b}	11.43 ± 0.23^{b}
6	4.51 ± 0.18^{a}	28.30 ± 3.75^{b}	0.00 ± 0.00^{a}	309 ± 10.61^{i}	0.28 ± 0.02^{b}	11.62 ± 0.19^{b}
7	$4.66\pm0.18^{a.b}$	31.81 ± 3.97^{c}	7.66 ± 0.03^{e}	219.50 ± 3.54^{b}	0.26 ± 0.00^{b}	12.42 ± 0.31^{c}
8	4.58 ± 0.12^{a}	$33.11\pm4.80^{c.d}$	4.43 ± 0.02^{d}	235.00 ± 8.49^{d}	0.26 ± 0.02^{b}	12.50 ± 0.32^{c}
9	4.54 ± 0.03^{a}	$32.65\pm2.4^{c.d}$	2.50 ± 0.01^{c}	248.50 ± 6.36^{e}	0.26 ± 0.01^{b}	12.74 ± 0.24^{c}
10	$4.63\pm0.15^{a.b}$	$33.03\pm3.80^{c.d}$	5.23 ± 0.01^{d}	224.00 ± 8.49^{c}	0.26 ± 0.00^{b}	12.22 ± 0.19^{c}
11	4.51 ± 0.01^{a}	$29.43\pm1.30^{b.c}$	3.33 ± 0.05^{c}	234.50 ± 7.78^{d}	0.26 ± 0.01^{b}	12.24 ± 0.16^{c}
12	4.50 ± 0.06^{a}	$29.06\pm3.04^{b.c}$	0.56 ± 0.01^{b}	249 ± 4.95^{e}	0.27 ± 0.00^{b}	12.40 ± 0.35^{c}
13	$4.73\pm0.20^{a.b}$	31.07 ± 3.86^{c}	22.3 ± 0.03^{g}	185.50 ± 6.36^{a}	0.23 ± 0.01^{a}	9.38 ± 0.21^{a}
14	4.76 ± 0.21^{b}	31.09 ± 4.75^{c}	19.1 ± 0.02^{f}	212.50 ± 9.19^{b}	0.26 ± 0.02^{b}	9.68 ± 0.24^{a}

* Different tiny superscripts in the columns indicate significant difference ($p<0.05$) by the Tukey test, pHr = pH rehydrated., ML = leaf mucilage, MFR = fruit mucilage, MIX = carrageenan mix. 1 = 0.5% ML + 0.5% MIX, 2 = 1% ML + 0.5% MIX, 3 = 1.5% ML + 0.5% MIX, 4 = 1% ML + 1% MIX, 5 = 1% ML + 1% MIX, 6 = 1.5% ML + 1% MIX, 7 = 0.5% MFR + 0.5% MIX, 7 = 1% MFR + 0.5% MIX, 8 = 1% MFR + 0.5% MIX, 9 = 1.5% MFR + 0.5% MIX, 10 = 0.5% MFR + 1% MIX, 11 = 1% MFR + 1% MIX, 12 = 1.5% MFR + 1% MIX, 13 = 0.5% MIX, 14 = 1% MIX. Number of replications = 3.

Knowledge of the products water activity is important to predict food stability, which is an indicator of microbial growth rate and chemical reactions (Silva et al., 2018). The mean a_w value determined for all Petit Suisse freeze-dried samples (Table 6) was approximately 0.26±0.03, which is within the range of a_w considered safe against microbial development ($a_w<0.60$) (Gustavo V. Barbosa-Cánovas, Anthony J. Fontana Jr., Shelly J. Schmidt, 2007).

The protein contents showed a statistical difference ($P<0.05$) between treatments (Table 6). The variation in protein contents ranged from 9.38–12.74%. The lowest protein concentrations were observed in samples 13 and 14 prepared only with carrageenan mixture.

Samples 7-12 presented higher protein contend because the MFR has the highest protein concentration among all the hydrocolloids used (Silva et al., 2019).

3.2.1 Syneresis

The syneresis results of the rehydrated Petit Suisse samples are presented in Table 6. Whey is one by-product in the food industry; therefore, the absence or reduction of syneresis represents an important technological improvement in the processing of Petit Suisse (Singh, Imam, Road, Pradesh, & Pradesh, 2014). Syneresis occurred in rehydrated samples 1, 2, 4, 7, 8, 9, 10, 11, 12, 13, and 14, and higher values were found in samples 13 and 14, consecutively. Samples 3,5 and 6 made with ML may not have shown syneresis due to the presence of arabinogalactan. In the production of the OPN ML, the leaves undergo an ultra-processing compared to the extraction of the MFR, releasing this macromolecule present in the cell walls of plants. Comparing the data of syneresis presented in Table 2, the fresh samples showed lower syneresis values compared to the rehydrated samples (Table 6) for all treatments. This effect is associated with loss in the structural properties of the samples due to freezing before lyophilization, changing the water retention, viscosity, gelation and emulsification capacities (Xiong, 1997).

3.2.2 WAC

The results of water retention capacity are shown in Table 6. The freeze-dried samples were rehydrated with the respective percentages of water noted at the primary and secondary drying stages of the freeze-drying process. Sample 13 presented lower WAC (185±6.36%) due to the lower concentration of the thickening agent in the medium, and thus, a lower capacity of gel network formation. Two types of interactions in the structural network of the samples can influence WAC, including the interaction between the hydrocolloids and water

by hydrogen bonding, and interaction between the hydrocolloids (ML, MFR and carrageenan macromolecules) by electrostatic, hydrophobic, or even hydrogen bonding interactions (Helena et al., 2019). For other samples, values above 210% were obtained for WAC due to higher concentrations of thickening agents in the medium, with sample 6 presenting the highest WAC (309±10.61%).

Higher WAC was obtained for treatments formulated with ML compared to those prepared with MFR. This result may be related to the greater number of groups capable of forming hydrogen bonds with water present in the mucilage chain, such as the hydroxyl group found in the arabinogalactan fraction and the $–NH_2$ and –COOH protein groups. In addition, the mucilage chain may have interacted with the carrageenan molecules via hydrogen bonds or ionic interactions due to the presence of the hydroxyl of the galactose monomers and $-SO_4$ group in their structure, forming a three-dimensional network that trapped water and contributed to increase the WAC in the product.

3.2.3 Color

Samples of freeze-dried Petit Suisse showed a significant difference ($P<0.05$) between the treatments. The values of L*, C*, and h° of the freeze-dried samples were similar to the values of the fresh samples. Table 7 shows the color parameters analyzed for freeze-dried samples.

Table 7: Colorimetric analysis of rehydrated Petit Suisse samples.

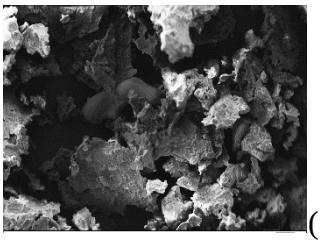
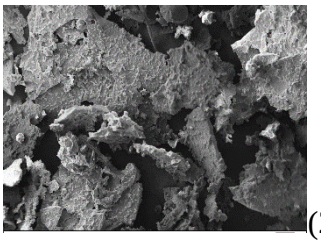
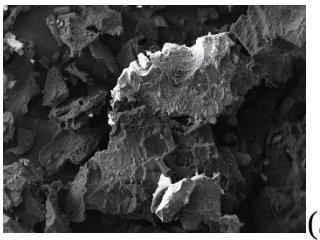
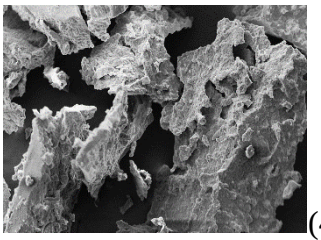
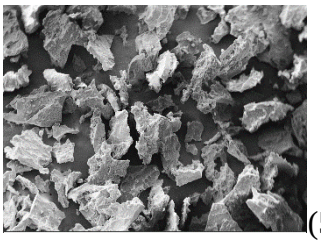
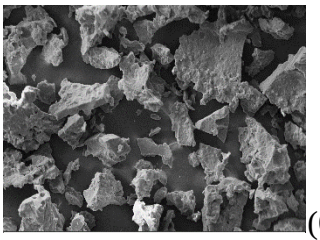
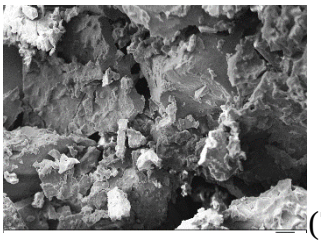
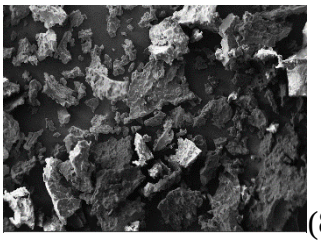
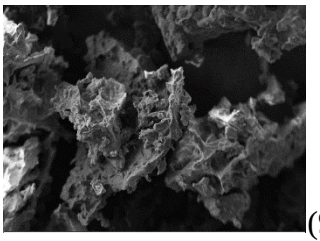
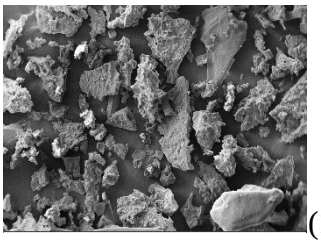
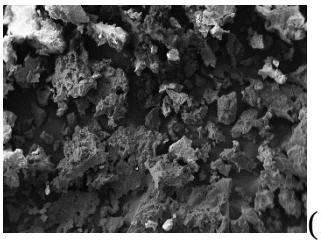
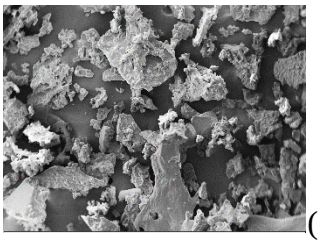
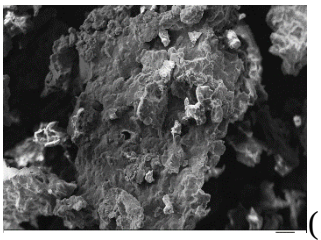
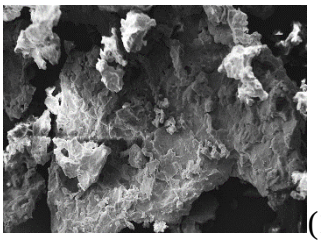
Sample	% (w/w)	L*	C*	h°
1	0.5 ML + 0.5 MIX	59.14±0.04^{c}	9.66±0.01^{a}	88.02±0.04^{e}
2	1 ML + 0.5 MIX	52.57±0.01^{b}	10.41±0.00^{b}	84.25±0.07^{c}
3	1.5 ML + 0.5 MIX	48.79±0.01^{a}	10.88±0.00^{b}	82.52±0.02^{b}
4	0.5 ML + 1 MIX	57.95±0.06^{c}	10.46±0.01^{b}	86.31±0.01^{d}
5	1 ML + 1 MIX	52.60±0.01^{b}	10.53±0.01^{b}	83.90±0.00^{c}
6	1.5 ML + 1 MIX	48.07±0.00^{a}	10.59±0.07^{b}	82.56±0.08^{b}
7	0.5 MFR + 0.5 MIX	71.32±0.01^{f}	11.27±0.00^{c}	88.32±0.00^{e}
8	1 MFR + 0.5 MIX	68.40±0.00^{e}	13.15±0.01^{d}	83.95±0.06^{c}
9	1.5 MFR + 0.5 MIX	64.54±0.02^{d}	14.26±0.00^{e}	81.11±0.01^{a}
10	0.5 MFR + 1 MIX	72.28±0.00^{f}	13.03±0.00^{m}	85.71±0.01^{d}
11	1 MFR + 1 MIX	69.37±0.01^{e}	13.52±0.00^{d}	83.41±0.01^{c}
12	1.5 MFR + 1 MIX	64.94±0.70^{d}	14.29±0.01^{e}	81.49±0.02^{a}
13	0.5 MIX	84.31±0.00^{g}	11.44±0.00^{c}	98.77±0.04^{f}
14	1 MIX	84.46±0.01^{g}	11.13±0.01^{c}	99.09±0.02^{f}

* Different tiny superscripts in the columns indicate significant difference ($p<0.05$) by Tukey's test, ML = leaf mucilage, MFR = fruit mucilage, MIX = carrageenan mix. Number of replications = 3.

3.2.4 Scanning electron microscopy

Scanning electron microscopy have proven to be useful for examining small-scale structural elements in cheese, such as the micropores formed in the sublimation of water during freeze-drying (Everett & Auty, 2008). The Figure 2 shows the photomicrographs of the freeze-dried samples.

Figure 2: Scanning eletron micrographs of the freeze-dried samples (1 – 14)

 (1)
 (2)
 (3)
 (4)
 (5)
 (6)
 (7)
 (8)
 (9)
 (10)
 (11)
 (12)
 (13)
 (14)

All figures show structures with higher porosities and little agglomeration, indicating low interaction between the particles. The freeze-drying process wherein structures with larger cavities were observed after sublimation of larger ice crystals. The process of sublimation of ice during lyophilization causes the formation of micavities in the product structure. The ice crystals that filled these microcavities were formed by the available water

and were formed in the pores of the protein matrix of the product (Amaral et al. 2018). Table 6 shows that the rehydrated samples 6 and 12 had higher protein content (11.62% and 12.40%, respectively) and pH (4.51 and 4.50, respectively). According to Harwalkar & Kalab (1986), the formation of large pores in a protein network can be caused by an increase in the positive electrical charge of the casein micelles at pH<4.6. The increase in the positive electric charge reduces intermicellar interactions, which result in the formation of an open (porous) structure (Amaral, 2018). Wang, Ellis, & Ross-Murphy (2002) and Wang, Ellis, & Ross-Murphy (2006) reported that in powders, particle size and specific surfaces influence hydration and material behavior.

3.2.5 Texture of rehydrated Petit Suisse Cheeses

The instrumental texture profile is shown in Table 8 demonstrating significant differences ($P<0.05$) in texture properties between all samples.

Table 8: Result of the texture analysis of the rehydrated samples of Petit Suisse cheeses. (HR) = Firmness, (AR) = adhesiveness, and (CHR) chewability.

Sample	% (w/w)	HR	AR	CHR
1	0.5 ML + 0.5 MIX	32.18±1.88^{c}	-131.71±5.88^{f}	21.65±1.05^{c}
2	1 ML + 0.5 MIX	36.83±1.46$^{c. d}$	-187.3±8.465$^{d. e}$	24.14±1.30$^{c. d}$
3	1.5 ML + 0.5 MIX	40.87±1.03$^{d. e}$	-356.43±14.29^{b}	27.29±1.43$^{d. e}$
4	0.5 ML + 1 MIX	44.34±2.70^{e}	-254.95±8.46^{b}	34.80±1.74^{g}
5	1 ML + 1 MIX	59.49±2.91^{f}	-452.93±18.30^{a}	42.88±2.08^{h}
6	1.5 ML + 1 MIX	64.71±2.12^{g}	-493.98±22.42^{a}	49.04±2.47^{i}
7	0.5 MFR + 0.5 MIX	38.35±1.42^{d}	-132.10±5.49^{f}	23.82±0.91$^{c. d}$
8	1 MFR + 0.5 MIX	45.48±3.16^{e}	-157.75±7.28$^{e. f}$	27.51±1.12$^{d. e}$
9	1.5 MFR + 0.5 MIX	48.81±2.39^{e}	-181.01±4.53$^{d. e}$	33.20±1.47$^{f. g}$
10	0.5 MFR + 1 MIX	38.46±1.87^{d}	-209.08±6.70$^{c. d}$	26.73±1.37$^{d. e}$
11	1 MFR + 1 MIX	44.90±2.08^{e}	-243.73±7.95$^{b. c}$	30.31±1.72$^{e. f}$
12	1.5 MFR + 1 MIX	47.86±1.38^{e}	-251.12±7.11$^{b. c}$	34.75±1.26^{g}
13	0.5 MIX	10.61±1.28^{a}	-18.29±1.78^{g}	11.84±0.80^{a}
14	1 MIX	17.20±1.82^{b}	-40.80±2.53^{g}	16.60±1.08^{b}

* Different tiny superscripts in the columns indicate significant difference ($p<0.05$) by Tukey's test. ML = leaf mucilage, MFR = fruit mucilage, MIX = carrageenan mix. Number of replications = 3.

Hardness, adhesiveness, and chewability of rehydrated samples increased (in modulus) as concentrations in all treatments increased. Amaral et al. (2018) demonstrated that the hardness and adhesiveness of fermented beverage samples are not indicative of susceptibility to syneresis but rather the pore sizes in the protein network.

3.2.6 Rheology

The Power Law model presented a high correlation coefficient to the flow curve data obtained for the rehydrated samples ($R^2 \geq 88.25$). As in fresh samples, for all mucilage concentrations studied, Petit Suisse cheeses showed pseudoplastic behavior ($n<1$, Table 9), characterized by a decrease in apparent viscosity with increments in shear rate.

Table 9: Rheological data of rehydrated Petit Suisse cheeses. (K) = consistency index, (n) = behavior index and ($\eta_{1.76}$) = apparent viscosity.

Sample	k (Pa·s^n)	n (-)	R^2	$\eta_{1.76}$ (Pa•s^{-1})
1	34.62±1.88^{c}	0.31±0.07$^{c.d}$	94.77	21.18±1.87^{c}
2	67.34±1.04^{e}	0.27±0.08^{c}	95.68	40.19±1.36^{e}
3	74.39±2.99$^{f.\ g}$	0.24±0.09^{b}	94.88	42.93±2.33^{e}
4	61.36±2.34^{d}	0.27±0.08^{c}	97.15	34.29±1.57^{d}
5	73.89±3.10^{f}	0.22±0.06^{a}	90.30	48.23±2.35 ^{g}
6	75.01±3.41^{g}	0.19±0.01^{a}	95.45	51.16±2.37^{g}
7	30.97±1.02^{c}	0.34±0.06^{d}	95.55	19.84±0.99^{c}
8	60.46±3.12^{d}	0.31±0.01$^{c.d}$	94.68	36.90±1.16^{d}
9	75.86±1.46^{g}	0.29±0.04^{c}	97.13	52.42±1.53^{h}
10	66.13±2.28^{e}	0.24±0.03^{b}	96.38	34.25±1.06^{d}
11	71.20±3.41^{f}	0.23±0.02$^{a.b}$	95.80	45.48±1.35^{f}
12	75.95±2.73^{g}	0.21±0.01^{a}	97.17	49.43±1.78^{g}
13	2.54±0.13^{a}	0.48±0.02^{f}	88.25	0.57±0.04^{a}
14	5.64±0.30^{b}	0.31±0.03^{e}	90.30	2.01±0.10^{b}

* Different tiny superscripts in the columns indicate significant difference ($p<0.05$) by Tukey's test. Number of replications = 3.

Moreover, it was possible to observe a statistical difference ($P<0.05$) between the apparent viscosity of the formulations. The increase in mucilage concentration in samples contributed to a proportional increase in apparent viscosity ($\eta_{1.76}$) and the consistency index (K), and a decrease in the behavior index (n).

Rehydrated samples showed lower apparent viscosity ($\eta_{1.76}$) and consistency index (K) than fresh formulations (Table 5), which was more pronounced for samples containing only the carrageenan mix. This can be explained by the breakdown of the three-dimensional structure formed by the fermentation of Petit Suisse cheese due to freezing, which is one of the operations in the freeze-drying process (Meza, Verdini, & Rubiolo, 2011).

4. Conclusion

For fresh samples, increments in ML and MFR concentrations resulted in higher viscosity and texture values and lower syneresis compared to samples prepared with MIX

only. The rehydrated samples differed from fresh samples in texture and syneresis, indicating that freezing influences the structure of Petit Suisse cheese. However, the rehydrated samples showed only a slight decrease in viscosity, indicating that the process can be used without significant effect on this parameter. The sample with 1.5% ML + 1% MIX showed better results (fresh and rehydrated) because it had high viscosity and WAC and did not show syneresis. Both ML and MFR showed good interactions with MIX, indicating that this hydrocolloid can be used in the elaboration of freeze-dried Petit Suisse cheese.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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