
# UNIVERSIDADE ESTADUAL DE CAMPINAS

***FACULDADE DE ENGENHARIA DE ALIMENTOS***

JORGE MIGUEL MAGALHÃES VIEIRA


## “Rheology-functionality relationship in innovative flaxseed gum-based thickeners’ formulations for dysphagia patients”

---

## “Relação reologia-funcionalidade em novas formulações de espessantes para pacientes disfágicos baseadas em goma de linhaça”


**CAMPINAS**

**2019**

JORGE MIGUEL MAGALHÃES VIEIRA

# “Rheology-functionality relationship in innovative flaxseed gum-based thickeners’ formulations for dysphagia patients”

# “Relação reologia-funcionalidade em novas formulações de espessantes para pacientes disfágicos baseadas em goma de linhaça”

Thesis presented to the Faculty of Food Engineering of University of Campinas in partial fulfilment of the requirements for the degree of Doctor in Food Engineering.

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Doutor em Engenharia de Alimentos.

**Supervisor/Orientadora: Profa. Dra. Rosiane Lopes da Cunha**

**Co-supervisor/Co-orientador: Prof. Dr. António Augusto Martins de Oliveira Soares Vicente**

Este trabalho corresponde à versão final da Tese defendida pelo aluno Jorge Miguel Magalhães Vieira, e orientado pela Profa. Dra. Rosiane Lopes da Cunha

**CAMPINAS**

**2019**

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Márcia Regina Garbelini Sevillano - CRB 8/3647

V673r

Vieira, Jorge Miguel Magalhães, 1990-
Rheology-functionality relationship in innovative flaxseed gum-based thickeners' formulations for dysphagia patients / Jorge Miguel Magalhães Vieira. – Campinas, SP : [s.n.], 2019.

Orientador: Rosiane Lopes da Cunha.
Coorientador: António Augusto Martins Oliveira Soares Vicente.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Disfagia. 2. Espessantes. 3. Reologia. 4. Tribologia. I. Cunha, Rosiane Lopes da. II. Vicente, António Augusto Martins Oliveira Soares. III. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. IV. Título.

Informações para Biblioteca Digital

**Título em outro idioma:** Relação reologia-funcionalidade em novas formulações espessantes para pacientes disfágicos baseadas em goma de linhaça
**Palavras-chave em inglês:**
Dysphagia
Thickeners
Rheology
Tribology
**Área de concentração:** Engenharia de Alimentos
**Titulação:** Doutor em Engenharia de Alimentos
**Banca examinadora:**
Rosiane Lopes da Cunha [Orientador]
Flavia Maria Netto
Lorenzo Pastrana
Fabiana Perrechil Bonsanto
Caroline Joy Steel
**Data de defesa:** 29-08-2019
**Programa de Pós-Graduação:** Engenharia de Alimentos

**Identificação e informações acadêmicas do(a) aluno(a)**
- ORCID do autor: https://orcid.org/0000-0001-6239-8091
- Currículo Lattes do autor: http://lattes.cnpq.br/2359465353467288

## BANCA EXAMINADORA

**Profa. Dra. Rosiane Lopes da Cunha - Orientadora**
Faculdade de Engenharia de Alimentos (FEA) - DEA
Universidade Estadual de Campinas (UNICAMP), Campinas, SP

**Profa. Dra. Flávia Maria Netto – Membro Titular**
Faculdade de Engenharia de Alimentos (FEA) - DEPAN
Universidade Estadual de Campinas (UNICAMP), Campinas, SP

**Profa. Dra. Caroline Joy Steel – Membro Titular**
Faculdade de Engenharia de Alimentos (FEA) - DEPAN
Universidade Estadual de Campinas (UNICAMP), Campinas, SP

**Prof. Dr. Lorenzo Miguel Pastrana Castro - Membro Titular**
Department of Life Sciences
International Iberian Nanotechnology, Braga, Portugal

**Profa. Dra. Fabiana Perrechil Bonsanto – Membro Titular**
Universidade Federal de São Paulo (UNIFESP), São Paulo, SP

Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

Traço, sozinho, no meu cubículo de engenheiro, o plano,

Firmo o projeto, aqui isolado,

Remoto até de quem eu sou.

Ao lado, acompanhamento banalmente sinistro,

O tique-taque estalado das máquinas de escrever.

Que náusea da vida!

Que abjeção esta regularidade!

Que sono este ser assim!

Outrora, quando fui outro, eram castelos e cavaleiros

(Ilustrações, talvez, de qualquer livro de infância),

Outrora, quando fui verdadeiro ao meu sonho,

Eram grandes paisagens do Norte, explícitas de neve,

Eram grandes palmares do Sul, opulentos de verdes.

Outrora.

Ao lado, acompanhamento banalmente sinistro,

O tique-taque estalado das máquinas de escrever.

Temos todos duas vidas:

A verdadeira, que é a que sonhamos na infância,

E que continuamos sonhando, adultos, num substrato de névoa;

A falsa, que é a que vivemos em convivência com outros,

Que é a prática, a útil,

Aquela em que acabam por nos meter num caixão.

Na outra não há caixões, nem mortes,

Há só ilustrações de infância:

Grandes livros coloridos, para ver mas não ler;

Grandes páginas de cores para recordar mais tarde.

Na outra somos nós,

Na outra vivemos;

Nesta morremos, que é o que viver quer dizer;

Neste momento, pela náusea, vivo na outra ...

Mas ao lado, acompanhamento banalmente sinistro,

Ergue a voz o tique-taque estalado das máquinas de escrever.

***Álvaro de Campos in "Poemas"*** **(Heterónimo de Fernando Pessoa)**

## Agradecimentos

Apesar desta ser a parte da tese com menos peso científico, é uma parte com toneladas na minha vida, onde posso expressar a minha gratidão a quem tornou possível e me acompanhou nesta jornada. Sem me alongar mais, até porque o meu queixo ficou com comichão e meus olhos com uma alergia que se começa a propagar em forma de gotas, vou passar aos agradecimentos.

Primeiramente, aos dois obreiros e responsáveis pela maior aventura da minha vida, à professora Rosiane e ao professor Vicente o meu muito obrigado pela oportunidade de desenvolver um projeto que tanto prazer me deu (e dor de cabeça em alguns momentos) com uma orientação científica de excelência. Realmente fui/sou um privilegiado por ter sido supervisionado por dois exemplos de profissionalismo e seres humanos iluminados.

À banca examinadora pela disponibilidade e certamente correções, sugestões e conselhos pertinentes.

À Capes: o presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil. Código de Financiamento 001; E também à FAPESP pelo auxílio financeiro imprescindível no desenvolvimento da tese através do processo 2016/05448-8.

Ao Laboratório de Engenharia e Processos, nomeadamente a todos os amigos e colegas de trabalho com quem convivi e aprendi bastante ao longo destes 4 anos. Um agradecimento especial à Rapha, minha parceira desde o primeiro dia que cheguei, mais perdido que cego em tiroteio, sem dúvida fizeste a diferença nos meus dias. Ao Pierluigi, italiano com um fuso-horário próprio que o fazia chegar atrasado na maior parte das vezes, grande amigo, parceiro de viagens e que fez parte do meu melhor ano no Brasil. À Cris, Tati e Paulinha pelos sermões que eu precisava ouvir e pelos gestos de amizade contínuos que eu nunca vou esquecer (sermões da Paulinha nem pareciam sermões na verdade). Ao Antônio (nome profissional) ou Matias (nome social) pelas risadas constantes e por ser como um irmão mais velho (embora não parecesse). À Zil por ser minha confidente, uma pessoa e técnica incrível com um coração do tamanho do mundo. À Vanessinha por ser uma técnica incansável e com uma paciência digna de um Nobel. À Thais, Aureliano, Flávia, Nice, Karen, Camila, Matheus, Monalisa e Luiz por fazerem parecer fácil levantar cedo para ir trabalhar. Também à Andresa, Guilherme,

Paula, Ana Letícia, Aline, Karine, Tanara, Ana Carol, Aninha e Mariano que contribuíram para o bom ambiente dentro e fora do LEP.

Também aos muitos amigos e pessoas que conheci no Brasil e me marcaram, a todos vou levar no coração. Secret Truths, Constituição dos fatos, LEP Unofficial, Topersons, 100 Lanchitos, Citybanda, Saturday Night Fever, Bolinho de Chuva, La Casa de Papel, Three Little Birds, Sugar babies, Floripa Magic, Futebolada Mágica são alguns exemplos de grupos de whatsapp criados, criados para proporcionar momentos tão simples e com tantas memórias boas, desde noites de estudo até noites de risadas constantes e momentos de reflexão. Queria aqui destacar algumas das pessoas que mais me marcaram e ainda não foram citadas: Mari, Guta, Tulio, Eduardo, Fe, Flávia, João, Luizas, Mónia, Guilherme, Paulinha, Tales, Kezia e Pedro.

À Diana, por me acompanhar nas minhas aventuras e diferentes fases da vida e também por se manter sempre por perto, ainda que, por vezes, fisicamente distantes!

Por último à minha família, à minha madrinha e aos meus pais, a quem sou eternamente grato. À minha mãe e madrinha por serem uma figura de força, determinação e por demonstrar um amor incondicional. Se acham que as pessoas crescidas todas carregam maldade proveniente das marcas de que foram vítimas ao longo da vida, precisam conhecer o meu pai. Claramente foi o meu irmão que herdou o melhor dos dois, o que faz dele a pessoa mais importante e que mais admiro na minha vida. Peço a eles desculpa por ficar ausente tanto tempo, principalmente à minha mãe pelas caixas de esmolas que encheu no santuário da Senhora Aparecida para que ela me levasse de volta. Hoje, na reta final desta aventura, eles entendem e aperceberam-se que provavelmente foi um marco na minha vida que me ensinou muito e me fez crescer profissionalmente e acima de tudo pessoalmente, e que tudo valeu a pena.

Obrigado Brasil, serei eternamente grato pelo aprendizado e por todas as histórias felizes que em ti vivi.

**Resumo**

A deglutição em pacientes disfágicos é feita de forma desordenada, podendo resultar na aspiração de alimentos líquidos e conduzir à desidratação, desnutrição, asfixia e até mesmo à morte. Esta condição pode ser aliviada pela utilização de espessantes que modificam as propriedades reológicas dos alimentos líquidos para facilitar sua ingestão por pacientes com disfagia orofaríngea. A maioria dos produtos comerciais apresentam goma de xantana (XG) e amido modificado (MS) em suas formulações, sendo que a alternativa comercial para reduzir a rejeição sensorial por parte do disfágico é a incorporação de XG, sendo o MS o polissacarídeo mais utilizado para reduzir o custo. No entanto, a XG não pode ser ingerida por pacientes prematuros, pois pode provocar danos irreversíveis nos tecidos intestinais e o MS pode perder eficiência por ação da $\alpha$-amilase presente na saliva. Neste trabalho, a goma de linhaça (FG) demonstrou ser um potencial constituinte não convencional a ser incorporado em produtos espessantes direcionados para pacientes com disfagia. Dessa forma, o objetivo geral deste trabalho foi avaliar formulações com espessantes baseados em FG, XG e MS com foco no comportamento reológico e tribológico. Esses espessantes foram ainda avaliados quanto à sua digestibilidade *in vitro* e quantidade de glicose liberada. Individualmente, a FG, além do seu apelo nutricional, apresentou um comportamento não-newtoniano pseudoplástico. Ademais, as medidas oscilatórias mostraram um caráter predominantemente viscoso. Quando FG foi misturada com MS e/ou XG, variando a concentração dos biopolímeros em questão de acordo com um delineamento composto central rotacional em diferentes matrizes alimentares, todas as formulações apresentaram um comportamento altamente pseudoplástico. As medidas oscilatórias revelaram que o caráter elástico foi predominante nas formulações estudadas nas três matrizes consideradas, devido principalmente ao aumento da concentração em MS e XG. No entanto, o aumento da concentração de FG foi o fator mais significativo no aumento da viscosidade, independentemente da matriz alimentar e da taxa de deformação aplicada. A análise microscópica das estruturas de rede formadas de acordo com as diferentes interações de biopolímeros apoiou na discussão do comportamento reológico, fornecendo informações importantes para projetar novos espessantes para o gerenciamento da disfagia. Após a digestão *in vitro*, as formulações espessantes comerciais apresentaram uma liberação de glicose significativamente maior do que as formulações estudadas, sendo que um pequeno aumento na concentração de MS

representa um aumento significativo da quantidade de glicose liberada. Quanto aos resultados tribológicos, o aumento da concentração em FG (aumento da viscosidade) resultou num aumento da capacidade lubrificante, semelhante ao sucedido com os espessantes baseados em XG tanto na água quanto no leite, ao contrário do sucedido no suco de soja, demonstrando a significativa influência da complexidade estrutural das matrizes alimentares onde os espessantes serão incorporados. Foi observado um comportamento oposto para os produtos à base de MS, uma vez que o aumento da sua concentração, e consecutivamente da viscosidade, causou um aumento no coeficiente de atrito, independentemente da matriz estudada. Assim, avançámos a hipótese de que o valor do coeficiente de atrito observado para os diferentes biopolímeros poderia fornecer informações sobre semelhanças nas características sensoriais entre a XG e a FG.

Os resultados obtidos neste trabalho poderão contribuir não somente com o desenvolvimento de espessantes com maior aceitação e funcionalidade, abrindo a oportunidade de adaptar as características dos espessantes de acordo com as necessidades dos pacientes, mas também com a compreensão de que propriedades reológicas/tribológicas melhor correlacionam com a percepção sensorial dos pacientes disfágicos.

**Palavras-chave:** disfagia, deglutição, espessantes, reologia, tribologia.

**Abstract**

Patients with dysphagia present a disordered swallowing, resulting in aspiration of liquid foods and leading to dehydration, malnutrition, asphyxia and even death. This condition can be alleviated using thickeners that modify the liquid foods' rheological properties to facilitate their ingestion by patients with oropharyngeal dysphagia. Most commercial products are composed by xanthan gum (XG) and modified starch (MS), being that the alternative to reduce sensory rejection is the incorporation of XG, while MS is the most used polysaccharide to reduce the cost. However, XG cannot be ingested by preterm patients, since it may cause irreversible damage to the intestinal tissues, while MS may lose efficiency when mixed with the salivary α-amylase. In the present work flaxseed gum (FG) was proposed as a potential unconventional constituent to be incorporated into thickening products targeted to patients with dysphagia. Thus, the objective of this work was to evaluate biopolymeric (FG, XG and/or MS) thickener formulations focusing on their rheological and tribological behavior. These thickeners were further evaluated according to their *in vitro* digestibility and amount of glucose released. Individually, FG, besides its nutritional appeal, presented a non-Newtonian pseudoplastic behavior. In addition, the oscillatory measurements showed a predominantly viscous character. When FG was mixed with MS and/or XG, varying their concentration according to a central rotational compound design in different food matrices, all formulations showed a highly pseudoplastic behaviour. The oscillatory measurements revealed that the elastic character was predominant in the formulations studied, regardless of the matrix, mainly due to the increase in MS and XG concentration. However, the FG increased concentration was the most significant factor in the viscosity increase, independently of the food matrix and applied shear rate. The network structure formed by the biopolymers' interactions was analysed microscopically and supported the discussion on the rheological behavior, providing insights to design new thickeners for the management of dysphagia. After *in vitro* digestion, commercial thickener formulations showed significantly higher glucose release than the studied formulations. A small increase of concentration of MS caused a significant increase in the amount of glucose released. Regarding the tribological analysis, an increase of FG concentration (viscosity increase) increased the lubricating capacity of water and milk; a similar effect was observed with the XG-based thickeners in these matrices. On the other hand, when FG was added to soy juice, the coefficient of

friction increased, demonstrating the significant influence of the composition of the food matrices where the thickeners will be incorporated. An opposite behavior was observed for the MS-based products, since the increase of its concentration, and consequently the increased viscosity, caused an increase in the coefficient of friction, independently of the matrix studied. These observations led us to hypothesize that the coefficient of friction could provide information about similarities in the sensorial characteristics between XG and FG.

The results obtained in this work may contribute not only to the development of thickeners with greater acceptance and functionality, opening the opportunity to adapt the characteristics of the thickeners according to the patients' needs, but also to the understanding that the rheological/tribological properties provide a good correlation with sensory perception of dysphagic patients.

**Keywords:** dysphagia, swallowing, thickeners, rheology, tribology.

**Índice de Figuras**

**Capítulo II**



**Capítulo III**

**Capítulo V**

**Capítulo IX**

**Índice de Tabelas**

**Capítulo II**



**Capítulo III**



**Capítulo IV**

**Lista de Abreviaturas e Siglas**

***ABTS*** - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid

***AF*** - Acidic Fraction

***AIDS*** - Síndrome da Imunodeficiência Adquirida

***Ara*** - Arabinose

***B*** - Power law exponent

***BHA*** - Butylated Hydroxyanisole

***C*** - Concentration

***C**** - Chroma Parameter

***CCRD*** - Central Composite Rotational Design

***CoF*** - Coefficient of Friction

***CT*** - Starch-based Commercial Thickener

***DCCR*** - Delineamento Composto Central Rotacional

***DMSO*** - Dimethyl sulfoxide

***DNA*** - Deoxyribonucleic Acid

***DPPH*** - 2,2-diphenyl-1-picrylhydrazyl

***FDA*** - Food and Drugs Administration

***Ff*** - Friction Force

***FITC*** - Fluorescein-5-isothiocyanate

***FG*** - Flaxseed Gum

***Fuc*** - Fucose

***G**** - Complex modulus

***G'*** - Storage modulus

***G"*** - Loss modulus

***GAE*** - Gallic Acid

***Gal*** - Galactose

***GC/MS*** - Gas Chromatography with Mass Spectrometer

***Glc*** - Glucose

***H**** - Hue Angle

***HPLC*** - High Performance Liquid Chromatography

***HORAC*** - Hydroxyl Radical Antioxidant Capacity

***HTC*** - Hepatoma tissue culture

$IC_{50}$ - The half maximal inhibitory concentration

***IDDSI*** - International Dysphagia Diet Standardization Initiative

$\boldsymbol{k}$ - Consistency Index

***K*** - Fitting Parameter

***L**** - Lightness Parameter

***MS*** - Modified Starch

$\boldsymbol{n}$ - Flow Index

***NDD*** - National Dysphagia Diet

***NF*** - Neutral Fraction

***ORAC*** - Oxygen Radical Absorbance Capacity

***PDMS*** - Polydimethylsiloxane

***pI*** - Isoelectric Point

***Rha*** - Rhamnose

***RSA*** - Radical Scavenging Activity

***SFG*** - Soluble flaxseed gum

***SIF*** - Simulated Intestinal Fluid

***SGF*** - Simulated Gastric Fluid

***SSF*** - Simulated Salivary Fluid

***TE*** - MS-based Commercial Thickener

***TRAP*** - Total Radical-trapping Antioxidant Parameter

***TPC*** - Total Phenolic Content

***TU*** - XG-based Commercial Thickener

***UA*** - Uronic Acid

***UPLC*** - Ultra Performance Liquid Chromatography

***UV*** - Ultraviolet

***XG*** - Xanthan Gum

***Xyl*** - Xylose

$\boldsymbol{\delta}$ Phase angle between the applied strain and the stress response

$\Delta \boldsymbol{E}^*$ - Total Colour Difference

$\boldsymbol{\eta}$ - Viscosity

$\boldsymbol{\eta_{ap}}$ - Apparent Viscosity

$\boldsymbol{\eta^*}$ - Complex Viscosity at 50 $rad.s^{-1}$

$\boldsymbol{\sigma}$ - Shear Stress

$\boldsymbol{\sigma_0}$ - Dynamic yield stress

$\boldsymbol{\omega}$ - Angular Frequency

$\dot{\boldsymbol{\gamma}}$ - Shear Rate

# Índice

# *CAPÍTULO I*

*Introdução Geral*

*Objetivos*

*Estrutura da Tese*

## 1. Introdução Geral

A disfagia é um sintoma, definido como uma dificuldade ou incapacidade de deglutição de alimentos. Este distúrbio mecânico pode ser resultante não só de complicações neurológicas, tais como câncer no cérebro e pescoço, paralisia cerebral, doenças de Huntington, Parkinson ou Alzheimer, estenose benigna do esôfago, de AVC, como também do envelhecimento, uma vez que a pressão que a língua exerce sobre o bolo alimentar diminui dificultando a deglutição (MACKLEY et al., 2013; LEONARD et al., 2013). Como consequências, a disfagia pode causar a morte por asfixia (casos extremos), complicações associadas à desidratação e desnutrição (que podem provocar imunodeficiências graves) e/ou problemas pulmonares (O'LEARY *et al.*, 2010; LEONARD et al., 2013; GARIN et al., 2014).

Existem duas fases distintas relacionadas à disfagia, sendo a primeira, a orofaríngea, a de maior prevalência. A fase orofaríngea está associada à redução na sensibilidade faringolaríngea e consequentemente danos no córtex faríngeo, nos geradores de padrão central ou nos núcleos motores somáticos do cérebro. A segunda é a fase esofágica, que está associada a distúrbios que afetam o sistema nervoso entérico ou as camadas musculares esofágicas, com consequentes distúrbios neuromusculares ou processos inflamatórios que afetam o funcionamento do esfíncter esofágico inferior e/ou os movimentos do esôfago. O número de diagnósticos de disfagia tem aumentado, não só devido a uma maior vigilância e diagnóstico mais eficiente, mas também devido ao envelhecimento populacional (CHEN, 2009; CLAVÉ; SHAKER, 2015).

Estudos recentes revelaram números preocupantes, pois relatam que este sintoma afeta em torno de 8 % da população mundial, sendo que 30-40 % das pessoas apresentam idades superiores a 65 anos. Considerando apenas os idosos, em número representam cerca de 16 milhões de cidadãos com disfagia nos Estados Unidos, 30 milhões na Europa, 10 milhões no Japão e 6 milhões no Brasil, sendo que estes números têm tendência a crescer com o aumento da expectativa média de vida. Existe, portanto, a necessidade de intensificação do desenvolvimento de novas pesquisas com o intuito de melhorar estratégias de diagnóstico e de atuação clínica, visando a mais rápida recuperação e bem-estar dos pacientes através de uma deglutição segura (PAYNE et al., 2009; STUART; MOTZ, 2009; SEPTEMBER et al., 2014; GARIN et al., 2015).

A deglutição é uma função corporal complexa, essencial para a saúde humana, que ocorre como resultado de várias fases fisiológicas distintas, e resulta no transporte

seguro de um alimento líquido ou do bolo alimentar através de vários reflexos voluntários e involuntários que envolvem movimentos adequados e ordenados de músculos da cabeça e pescoço. A primeira intervenção é a da língua, que desempenha um papel importante aquando do ato de mastigar, exercendo pressão sobre o bolo alimentar (deficiências nos seus movimentos estão interligadas, muitas vezes, com a disfagia) e forçando-o para a faringe. Nesse percurso, o bolo alimentar passa pela epiglote (diafragma flexível que se encontra entre a faringe e a laringe e que dirige o ar para traqueia e os líquidos/alimentos para o esôfago) e desce até o estômago (O'LEARY et al., 2010; VALLONS et al., 2013; HORI *et al.*, 2015). Este processo, normalmente, pode demorar entre milisegundos até cerca de 20 s (MACKLEY et al., 2013) e a ele estão associadas forças de cisalhamento e extensionais que influenciam e, por sua vez, são influenciadas pelas propriedades reológicas do líquido/bolo alimentar. É importante salientar que, além das propriedades reológicas, outras características físicas como o volume do bolo alimentar e a temperatura são relevantes para o processo de deglutição (STUART; MOTZ, 2009). Contudo, as propriedades reológicas dos alimentos são geralmente influenciadas pela taxa de deformação aplicada, a qual depende da combinação da velocidade de deglutição e forças mecânicas aplicadas sobre eles. Líquidos pouco viscosos podem fluir rapidamente para a laringe antes das vias aéreas terem fechado, levando à aspiração para os pulmões e consequentes problemas pulmonares diretos como infecções ou até mesmo pneumonia aspirativa (O'LEARY et al., 2010; VALLONS et al., 2013).

Assim, é importante elevar a consistência/viscosidade dos líquidos de forma a permitir o adequado aporte hídrico, ao mesmo tempo que se reduz a incidência de aspiração e/ou asfixia (GARIN et al., 2014). Estas alterações permitem que, após a passagem pela boca/língua, o bolo alimentar se depare com um aumento de tempo de permanência no interior da garganta, obtendo-se assim um maior tempo de resposta reflexiva por parte dos músculos responsáveis pela deglutição quando este entra na faringe (DEWAR; JOYCE, 2006; MACKLEY et al., 2013; HORI et al., 2015). No entanto, podem ser desencadeados problemas resultantes desta ação se não for encontrada a viscosidade ideal, pois os alimentos podem se tornar menos aprazíveis em termos visuais e de textura (por serem excessivamente viscosos). Neste sentido, este trabalho visou suprir uma lacuna na estratégia de contornar os problemas associados à alimentação dos pacientes disfágicos, a partir do desenvolvimento de espessantes com foco na manipulação e melhora do comportamento reológico. A formulação e desenvolvimento

de misturas de biopolímeros para atuarem como espessantes alimentares visando facilitar a deglutição deve considerar que a viscosidade dos fluidos ingeridos deve estar dentro de um espectro relativamente estreito, e que o comportamento reológico e tribológico pode se alterar em distintas matrizes alimentares líquidas quando submetidas a variadas condições de consumo (VALLONS et al., 2013). Atualmente, de modo a suprir as necessidades básicas dos pacientes com disfagia no sentido de obter uma alimentação apropriada, são utilizados espessantes baseados principalmente em amido modificado e goma xantana. Embora estes dois biopolímeros apresentem pontos essenciais na modificação da textura dos alimentos líquidos de forma a permitir uma deglutição minimamente segura, ambos apresentam pontos com potencial impacto directo na saúde do consumidor. A goma xantana não é aconselhável para pacientes permaturos, havendo já casos de pacientes com inflamação intestinal, designada por enterocolite necrotizante (em alguns casos resultando na morte do paciente), causada pelo seu consumo (WOODS et al., 2012). Relativamente ao amido modificado, o problema é relacionado com o fato de este material correr o risco de perder a sua capacidade espessante ao entrar em contacto com a $\alpha$-amilase salivar (MARTINEZ et al., 2019). Assim, apesar do foco principal ter sido o estudo do comportamento reológico e tribológico dos diferentes polissacarídeos usados na formulação dos espessantes, também foram avaliados aspectos funcionais de um novo componente espessante proposto como possível alternativa ao mercado (goma de linhaça).

## 1.1.Objetivos

### 1.1.1. Geral

O objetivo geral deste projeto de pesquisa foi contribuir para o desenvolvimento de formulações de espessantes alimentares, usando como base amido gelatinizado, xantana e goma de linhaça, de forma a que tivessem propriedades adequadas para pacientes disfágicos.

### 1.1.2. Específicos

a) Avaliar o efeito das condições do processo de extração da goma de linhaça na sua composição (carboidratos e compostos fenólicos) e propriedades reológicas;

b) Preparar e caracterizar misturas de espessantes em água e matrizes alimentares a partir de propriedades reológicas em cisalhamento estacionário e ensaios oscilatórios. Analisar as variações de cor das misturas de espessantes adicionadas nas diferentes matrizes alimentares estudadas (água, leite e suco).
c) Analisar o comportamento tribológico dos diferentes polissacarídeos adicionados em matrizes alimentares para observar o possível desgaste do material, simulando o atrito epitelial com o palato duro;
d) Avaliar a digestibilidade *in vitro* dos produtos espessantes formulados para quantificar a glucose liberada pelas diferentes formulações.

### 1.2. Estrutura da Tese

A apresentação deste trabalho foi organizada em dez capítulos como descrito a seguir:

***Capítulo I***: Introdução geral

***Capítulo II***: Revisão bibliográfica

***Capítulo III****: Effect of extraction temperature on rheological behavior and antioxidant capacity of flaxseed gum*

Neste capítulo foi avaliado o efeito da temperatura de extração na composição e funcionalidade da goma de linhaça. Na caracterização da goma de linhaça extraída foram utilizadas técnicas de análise de compostos elementares fundamentais, potencial zeta e cromatografia líquida de alta performance (HPLC e uHPLC). Também foram avaliadas a capacidade antioxidade e propriedades reológicas da goma de linhaça extraída a diferentes temperaturas. Os efeitos da concentração da goma e a alteração do pH da matriz de incorporação do biopolímero também foram avaliados de forma a analisar a potencial aplicação deste polissacarídeo em formulações de espessantes.

***Capítulo IV****: Flaxseed gum-biopolymers interactions are driving the rheological behavior of oropharyngeal dysphagia-oriented products*

Neste capítulo foram analisados os efeitos das interações entre os diferentes biopolímeros estudados (amido gelatinizado, xantana e goma de linhaça) sobre as propriedades reológicas de matrizes aquosas contendo estes polissacarídeos, seguindo

um delineamento composto central rotacional (DCCR). A partir desta análise foi possível observar a importância da goma de linhaça na viscosidade e da xantana na elasticidade das formulações analisadas, sendo os efeitos preditos através de modelos matemáticos. No entanto, a interação do amido com os demais polissacarídeos foi fundamental para a compreensão do comportamento reológico observado. Além dos resultados das propriedades reológicas e de cor, também foi analisada a liberação de glicose de formulações otimizadas em diferentes níveis de consistência em comparação com espessantes comerciais.

***Capítulo V**: Rheology and soft tribology of thickened dispersions aiming at the development of oropharyngeal dysphagia-oriented products*

Neste capítulo foi realizado um estudo reológico e tribológico de soluções aquosas contendo os biopolímeros estudados no Capítulo 4, de modo a observar e relacionar as suas características viscosas, viscoelásticas e lubrificantes. Foi analisado o coeficiente de atrito, mantendo fixadas a velocidade e força aplicada nas soluções biopoliméricas e espessantes comerciais, buscando correlacionar esta propriedade física com propriedades sensoriais da fase final do processo de deglutição.

***Capítulo VI**: Discussão Geral*

Neste capítulo são discutidos e relacionados os resultados obtidos ao longo da tese.

***Capítulo VII**: Conclusões gerais*

Neste capítulo são relatadas as principais conclusões sobre os resultados obtidos.

***Capítulo VIII**: Referências Bibliográficas*

***Capítulo IX**: Apêndices*

Neste capítulo encontram-se resultados provenientes de ensaios efetuados na seleção dos biopolímeros usados nas formulações espessantes e dos estudos físicos efetuados (reologia e tribologia) em duas matrizes líquidas alimentares, leite e suco de soja com sabor a laranja.

### 1.3. Referências

CHEN, J. Food oral processing-A review. **Food Hydrocolloids**, v. 23, p. 1–25, 2009.

CLAVÉ, P.; SHAKER, R. Dysphagia: current reality and scope of the problem. Nature Reviews. **Gastroenterology and Hepatology**, v. 12, p. 259-270, 2015.

DEWAR, R.J.; JOYCE, M.J. Time-Dependent Rheology of Starch Thickeners and the Clinical Implications for Dysphagia Therapy. **Dysphagia**, v. 2006, p. 264–269, 2006.

GARIN, N. et al. Viscosity Differences between Thickened Beverages Suitable for Elderly Patients with Dysphagia. **Dysphagia**, v. 29, p. 483–488, 2014.

Feng, M.-C. et al. The Mortality and the Risk of Aspiration Pneumonia Related with Dysphagia in Stroke Patients. **Journal of Stroke and Cerebrovascular Diseases**, v. 28, n. 5, p. 1381-1387, 2019.

HORI, K. et al. Comparison of mechanical analyses and tongue pressure analyses during squeezing and swallowing of gels. **Food Hydrocolloids**, v. 44, p. 145-155, 2015.

LEONARD, R.J. et al. Effects of Bolus Rheology on Aspiration in Patients with Dysphagia. **Journal of the Academy of Nutrition and Dietetics**, p. 2212-2672, 2014.

MACKLEY, M.R. et al. The rheology and processing behavior of starch and gum-based dysphagia thickeners. **Journal of Rheology**, v. 57, n. 6, p. 1533-1553, 2013.

MARTÍNEZ, O. et al. Sensory perception and flow properties of dysphagia thickening formulas with different composition. **Food Hydrocolloids**, v. 90, p. 508-514, 2019.

O'LEARY, M. et al. Viscosity and Non-Newtonian Features of Thickened Fluids Used for Dysphagia Therapy. **Journal of Food Science**, v. 75, n. 6, p. 330-338, 2010.

PAYNE, C. et al. Consistently Inconsistent: Commercially Available Starch-Based Dysphagia Products. **Dysphagia**, v. 26, p. 27-33, 2011.

SEPTEMBER, C. et al. Implications of Changing the Amount of Thickener in Thickened Infant Formula for Infants with Dysphagia. **Dysphagia**, v. 29, p. 432–437, 2014.

STUART, S.; MOTZ, J.M. Viscosity in Infant Dysphagia Management: Comparison of Viscosity of Thickened Liquids Used in Assessment and Thickened Liquids Used in Treatment. **Dysphagia**, v. 24, p. 412–422, 2009.

VALLONS, K.J.R. et al. Effect of human saliva on the consistency of thickened drinks for individuals with dysphagia. **International Journal of Language & Communication Disorders**, v. 50, n. 2, p. 165–175, 2015.

WOODS, C.W. et al. Development of necrotizing enterocolitis in premature infants receiving thickened feeds using SimplyThick. **Journal of Perinatology**, v. 32, p. 150-152, 2012.

***CAPÍTULO II***

---

*Revisão Bibliográfica*

## 2. Revisão Bibliográfica

A alimentação é um dos fatores que mais interferem na qualidade e longevidade dos seres humanos, especialmente em pessoas que necessitam de cuidados especiais. Assim, nas últimas décadas, uma maior preocupação em estabelecer uma dieta cuidadosa e adequada para os diferentes tipos de pacientes tem sido observada, de forma a mitigar as consequências impostas pelos tratamentos hospitalares necessários.

### 2.1. Disfagia

Disfagia é uma dificuldade de deglutição de alimentos sólidos, semi-sólidos e líquidos. É um sintoma que pode resultar do envelhecimento, onde a pressão que a língua exerce sobre o alimento diminui, dificultando o processo normal de deglutição ou por desgaste epitelial, complicações neurológicas, câncer de cérebro e pescoço, paralisia cerebral, doença de Huntington, Parkinson ou Alzheimer, estenose benigna do esôfago, acidente vascular cerebral, entre outros distúrbios cerebrais (MACKLEY et al., 2013, LEONARD et al., 2013). É definida pela dificuldade de formar e/ou mover o bolo alimentar com segurança da boca para o estômago, o que pode levar à desnutrição, desidratação e outros efeitos negativos na qualidade de vida, como pneumonia por aspiração (O'LEARY et al., 2010, LEONARD et al., 2013, GARIN et al., 2014). Esse distúrbio pode aparecer em diferentes estágios da digestão, desde a perspectiva fisiopatológica, primeiramente na transição da boca para o esôfago, na disfagia orofaríngea e, posteriormente, do esôfago para o estômago na disfagia esofágica.

Os problemas causados pela disfagia orofaríngea e o aumento da sua relevância clínica provocam cada vez mais preocupação por parte dos profissionais de saúde nas mais diversas áreas, dado que esta disfunção na sensibilidade faríngea é a mais prevalente e grave. Este dano no córtex faríngeo dominante prevalece principalmente em pessoas idosas, pacientes com doenças neurológicas e neurodegenerativas e pacientes com doenças na cabeça e/ou pescoço. A disfagia orofaríngea causa complicações graves, o que pode levar à morbidez e, em caso extremos, à morte. Essas complicações incluem desnutrição e/ou desidratação, asfixia e aspiração traqueobrônquica que podem resultar em infecções respiratórias e pneumonia. Este sintoma tem um impacto enorme na qualidade de vida dos pacientes, pois por exemplo, mais de 41% das pessoas portadoras de disfagia orofaríngea sentem ansiedade ou pânico na hora de se hidratarem ou alimentarem e cerca de 36 % sentem necessidade de ajuda

nestes momentos. Estudos recentes demonstraram que a desnutrição e a sarcopenia estão altamente associadas com este distúrbio em idosos (CHEN, 2009; CLAVÉ; SHAKER, 2015). Clinicamente, a disfagia orofaríngea deve ser confirmada por videofluoroscopia ou por nasofibroscopia após a sua suspeita. A videofluoroscopia é atualmente o exame de referência para o estudo dos distúrbios da deglutição e permite orientar seu manejo, adaptando-se às melhores texturas alimentares e reabilitação (ABE et al., 2011). No entanto, é um exame que expõe os pacientes a radiações, é dispendioso e não muito acessível (PARIS et al., 2012).

Pacientes com disfagia esofágica apresentam sintomas de deglutição comprometida e lenta, distúrbios digestivos como regurgitação e perda de peso. Em comparação com a disfagia orofaríngea, os sintomas e complicações respiratórias são raros e geralmente são apenas observados em casos avançados e sem observação médica. A disfagia esofágica geralmente é diagnosticada e reconhecida pelos médicos através de técnicas de imagem, principalmente esofagoplastia, para avaliar as causas mecânicas intrínsecas (ex., síndrome dos anéis esofágicos, carcinomas, estenose péptica do refluxo gastroesofágico) ou extrínsecas (ex., compressão vascular, osteófitos da coluna cervical), neuromusculares (ex., acalasia esofágica, esclerodermia, espasmo esofágico difuso, esfíncter esofágico inferior hipertensivo), inflamatórias (ex., esofagite eosinofílica, lesão cáustica do esôfago e infeções esofágicas provocadas por candidíase, esofagite associada ao vírus da AIDS, entre outras) ou por anormalidades no corpo esofágico e no esfíncter inferior (SANTORO et al., 2011; LO et al., 2019).

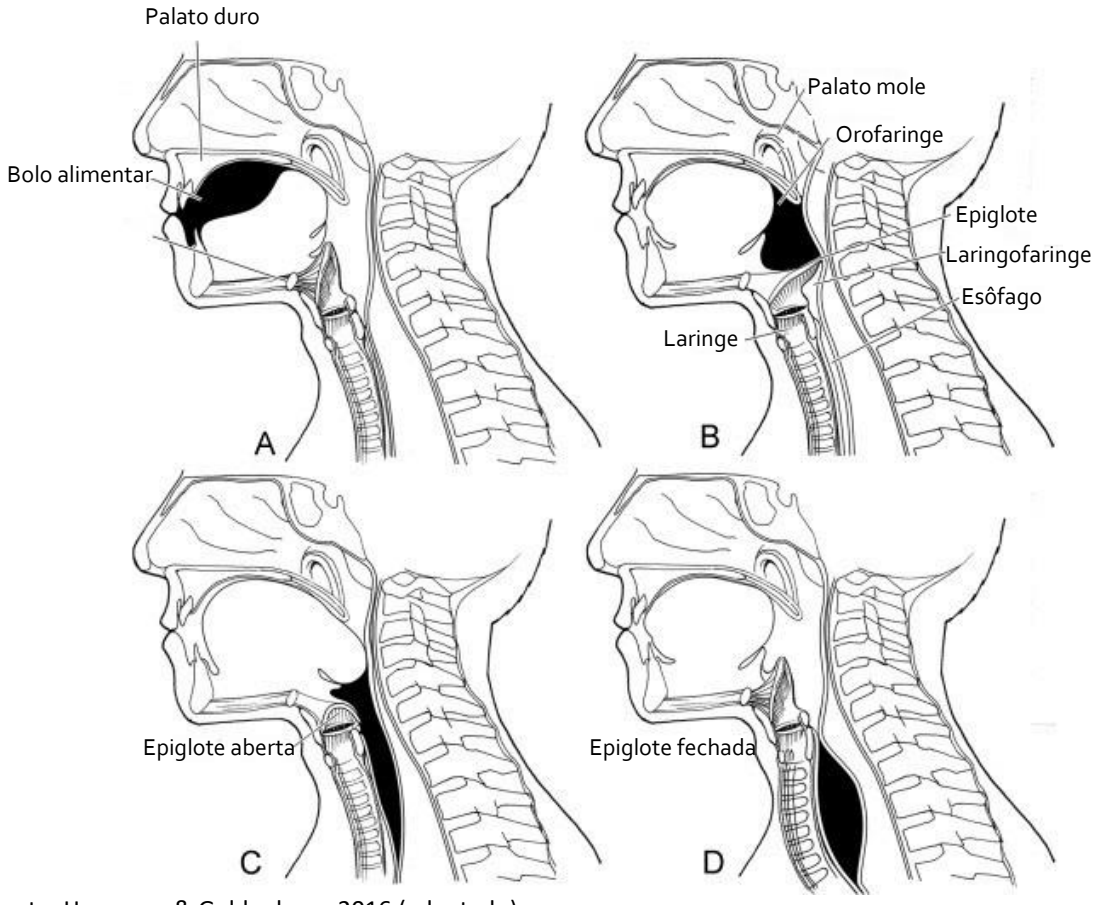
### 2.2. Deglutição

A deglutição efetuada de forma normal é um processo complexo e coordenado que envolve estruturas faciais, palatais, supra-hioides e faríngeas e requer a interação apropriada entre várias áreas do sistema nervoso central, nervos cranianos, tronco encefálico e cerebelar, componentes motores, psicológicos e sensoriais, e receptores periféricos de pressão, temperatura, estímulos químicos e água (FORSTER et al., 2011). Também requer a integridade anatômica da orofaringe e da laringe e a função neuromuscular dos músculos estriados cervicais preservada, incluindo o esfíncter esofágico superior, de forma a obter uma coordenação adequada com o sistema respiratório. Atualmente são consideradas como normais quatro fases de deglutição, preparatória oral, oral propulsiva, faríngea e esofágica (ERTEKIN; AYDOGDU, 2003;

CHEN et al., 2009). A primeira fase, preparatória oral, fase intermédia entre a mastigação e o início da deglutição, acontece de forma controlada e voluntária, sendo que esta depende de indivíduo para indivíduo e das caraterísticas do alimento, fluido ou sólido. No caso de um líquido, quando colocado na boca, dado o seu grau de coesão, a sua manutenção entre a língua e a região anterior do palato duro é muito importante no preparo para a deglutição (ERTEKIN; AYDOGDU, 2003). Este acontecimento, normalmente, é realizado através da pressão efetuada pela língua, que em forma de concha, empurra o bolo alimentar contra o palato duro (HENNESSY; GOLDENBERG, 2016). Se este processo falhar, o bolo alimentar passa diretamente para a faringe antes que a deglutição oro-faríngea seja desencadeada, isto é, antes que a via aérea feche, levando a que o fluido possa ser aspirado. Para os sólidos, o alimento é mastigado e o bolo alimentar é formado pela ação dos lábios, da língua, da saliva e das mandíbulas. A mastigação envolve movimentos do maxilar e sincroniza-se com o transporte dos alimentos da língua e bochechas para os molares. O alimento mastigado é misturado com saliva, transportado pela garganta e coletado na orofaringe onde o bolo alimentar é formado antes da deglutição (CAMPBELL et al., 2017). A segunda fase, oral propulsiva, envolve a transferência do bolo alimentar da boca através da faringe para o esôfago e é causada principalmente pela ação de aperto da língua contra o palato, proporcionando forças motrizes para impulsionar o material engolido em todo o esfíncter esofágico superior com resistência mínima (CHEN et al., 2009). A terceira fase, faríngea, é uma sequência automática e involuntária de movimentos neuromusculares que começa quando o bolo alimentar passa a garganta impulsionado pela pressão efetuada pela língua (CHEN; LOLIVRET, 2011). Nesta complexa fase é necessária uma boa coordenação temporal entre as funções respiratória e digestória (ERTEKIN; AYDOGDU, 2003). Ela inicia quando a resposta de deglutição foi desencadeada e o bolo alimentar passa pelo véu palatino, epiglote e pela região laringotraqueal protegida, atravessando o esfíncter esofágico superior, trajeto que dura entre 0,7 a 1,0 s. Pessoas com capacidade e coordenação motora têm uma resposta imediata à deglutição, com o fechamento rápido da epiglote e abertura rápida do esfíncter esofágico superior. Em contrapartida, pacientes com doenças neurológicas e/ou pessoas idosas podem sofrer com respostas reflexivas lentas, pois a epiglote pode não abrir oportunamente e ocorrer aspiração do bolo alimentar para a via respiratória (CHEN et al., 2009). Por fim, a quarta fase da deglutição, esofágica, é inconsciente, involuntária e mais lenta na deglutição, cerca de seis segundos, podendo ser mais rápida

em líquidos. O bolo alimentar entra no esôfago após a abertura do esfíncter esofágico superior, e é levado ao estômago através dos movimentos peristálticos de caráter descendente, que empurram o bolo alimentar do esôfago para o estômago (CLAVÉ; SHAKER, 2015). Na Figura 2.1 podem ser observadas as diferentes fases da deglutição.

**Figura 2.1.** Trajeto normal do bolo alimentar durante a deglutição. (A) Passagem do bolo alimentar para a orofaringe por ação predominante da língua. (B) A elevação do palato macio fecha a abertura da cavidade nasal a medida que o bolo alimentar passa para a faringe. (C) O bolo alimentar é propelido através da faringe após o fecho da epiglote e entra no esôfago quando da abertura do esfíncter esofágico superior. (D) O bolo alimentar passa para o esôfago, sendo transferido para o estômago através de ondas peristálticas. Após esta passagem o palato mole e a língua relaxam e a epiglote se abre para retomar a respiração.




Fonte: Hennessy & Goldenberg, 2016 (adaptado).

### 2.3. Mastigação e formação do bolo alimentar

A mastigação consiste em capacidades multifacetadas para cortar e esmagar alimentos, e misturá-los com saliva de modo a formar o bolo alimentar (ABE et al., 2011). Uma vez na boca, o alimento é manipulado pela língua, dentes, parte interna das bochechas e lábios com diferentes velocidades e pressões. Através dos vários estágios da mastigação, os alimentos são continuamente mordidos e misturados com a saliva até se converterem no bolo alimentar pronto para ser engolido (PRAKASH et al., 2013). O processo de mastigação pode ser afetado pela idade, gênero, estado dentário que obrigará a um maior número de ciclos de mastigação, produção de saliva, estado de saúde do indivíduo e, ainda, pelas propriedades dos alimentos (WODA et al., 2006).

A primeira mordida é normalmente vista como o início do processo mastigatório. Geralmente, a percepção sensorial recebida da primeira mordida abrange uma ampla gama de características de textura, como por exemplo, a dureza, elasticidade e coesão. Com base no padrão de movimento do maxilar, três fases distintas podem ser encontradas neste processo denominadas de abertura, fechamento rápido e fechamento lento. A força aplicada durante a primeira mordida é diretamente proporcional à natureza mecânica e geométrica dos alimentos (CHEN, 2009). Pesquisas efetuadas demonstraram que para um alimento elástico cada mordida demorou cerca de 0,8 s e para um alimento plástico, a força de mordida aumentou e levou cerca de 1,8 s. Já para materiais frágeis, o ciclo de mordida foi o mais curto, menos de 0,5 s (MIOCHE; PEYRON, 1995). Após a primeira mordida, as partículas são fragmentadas e devidamente lubrificadas pela saliva para formar um bolo alimentar que possa ser engolido com segurança e conforto (CHEN, 2015). O bolo alimentar pode ser definido como uma mistura de partículas reduzidas de alimentos mastigados envolvidos por saliva. Assim sendo, tanto as propriedades mecânicas dos alimentos quanto a taxa de fluxo de saliva são fatores importantes na formação do bolo alimentar, e consequentemente na deglutição. Assim como para a primeira mordida, o seguimento do processo de mastigação e o número de ciclos de mastigação também variam de indivíduo para indivíduo e de alimento para alimento, sendo que a reologia dos alimentos é o fator mais influente neste processo e em toda a atividade muscular envolvida no processamento oral (ENGELEN et al., 2005).

### 2.3.1. Deformação e fluxo do bolo alimentar durante o processamento oral

As pesquisas realizadas sobre a deformação e o fluxo do bolo alimentar são limitadas, embora tenham sido publicados inúmeros trabalhos sobre a fisiologia do fluxo do bolo alimentar (KU et al., 2007; PRAKASH, 2017; VAN ECK et al., 2019; WANG et al., 2019). Em um estudo sobre um melhor entendimento da deformação do bolo alimentar foi observado que uma maior viscosidade causou um atraso no trânsito oral e faríngeo do bolo alimentar e uma maior duração das ondas peristálticas da faringe com abertura prolongada do esfíncter esofágico superior (DANTAS et al., 1990). Outros estudos com essa temática demonstraram resultados similares, em que sugerem que a capacidade de fluxo e/ou a deformabilidade do bolo alimentar é o fator que mais afeta a facilidade de engolir (TAKAHASHI et al., 2003). No entanto, devido à natureza geométrica diferente da cavidade oral, faringe e esofaringe de indivíduo para indivíduo, seria de esperar um comportamento diferente do fluxo do bolo alimentar nas quatro fases de deglutição (ASSAD-BUSTILLOS et al., 2019; WANG et al., 2019).

Independentemente dos progressos obtidos através dos estudos efetuados até agora, o conhecimento sobre a taxa de deformação do bolo alimentar (ou a taxa de cisalhamento) é ainda muito limitado. Atualmente, nenhuma técnica é 100 % confiável para medir este parâmetro *in vivo* e a geometria oral irregular torna ainda mais difícil a caracterização da deformação. Para justificar essa afirmação serão mostrados alguns exemplos do quanto pode variar a taxa de deformação de estudo para estudo. Cutler *et al.* (1983) mostraram que a viscosidade do fluido obtida a uma taxa de cisalhamento de 10 $s^{-1}$ era mais relevante para a percepção sensorial, embora se acredite que a taxa deformação num bolo alimentar possa ser diferente dessa predição. Já Nicosia & Robbins (2001), para simular a deglutição da cavidade oral para a faringe, utilizaram uma taxa de cisalhamento de até 3000 $s^{-1}$ para um bolo alimentar de viscosidade de 1 Pa.s, partindo do pressuposto de que uma pressão de 13,3 kPa foi aplicada pela língua contra o palato. Mesmo que não haja evidência experimental direta, deve-se dizer que taxas de deformação tão elevadas são muito pouco prováveis. Meng *et al.* (2005), por outro lado, previu uma taxa de deformação de cerca de 400 $s^{-1}$.

A deformação do bolo alimentar é esperada não só em cisalhamento, mas também em escoamento extensional. Isso pode ser visto claramente a partir de videoendoscopia e imagens de MRI (Ressonância Magnética) em tempo real (BUETTNER et al., 2001), mostrando o bolo sendo extensivamente estirado durante a deglutição. Supondo que um

bolo de 15 ml seja engolido a uma velocidade de 0,5 $m.s^{-1}$ (HASEGAWA et al., 2005) em um tempo de trânsito de 1 s, pode-se estimar que este poderia ser elongado a uma taxa de cerca de 1 $s^{-1}$. Infelizmente, pouca atenção tem sido dada à reologia extensional do processamento e tração oral dos alimentos, embora seja relevante para entender o processo de deglutição (CHEN; LOLIVRET, 2011; MACKLEY et al., 2013). Uma razão importante para a falta de progresso na reologia extensional de alimentos é devido à limitação das técnicas experimentais. No entanto, com os recentes desenvolvimentos dos reômetros extensionais, mais resultados nesta área poderão ficar disponíveis no futuro (CHEN et al., 2009; JAISHANKAR et al., 2015; YUAN et al., 2018).

## 2.4. Reologia e o processamento oral de alimentos

O processamento oral, desde a primeira mordida até engolir, visa garantir que o alimento seja transformado a partir da sua forma e tamanho inicial em uma forma confortável para engolir (bolo alimentar), garantindo uma apreciação completa da textura e do sabor. Esse processo é intrinsecamente dependente das características reológicas do alimento ingerido.

### 2.4.1. Papel da reologia na deglutição

A chave para uma deglutição segura é a coordenação entre os atributos reológicos do bolo alimentar, as forças de propulsão aplicadas pela musculatura orofaríngea e os movimentos biomecânicos utilizados para proteger a via aérea. Desta forma, vários motivos podem interferir numa deglutição normal e segura de alimentos e bebidas espessadas por pacientes com disfagia, como defeitos no controle da cavidade oral, o comprometimento na preparação do bolo alimentar, excesso ou insuficiência de viscosidade (entre outros atributos reológicos) e/ou reações tardias da faringe (CHEN et al., 2009). A reologia é a ciência que estuda a deformação e o escoamento de corpos sólidos ou fluidos e, portanto, tem papel chave no processo de deglutição (GERMAIN et al., 2015). Nos últimos anos, vários métodos para a avaliação da consistência da dieta disfágica durante o processamento oral têm surgido e acompanhado o avanço do entendimento das diferentes necessidades deste tipo de pacientes e os processos de deglutição diferenciados de acordo com a gravidade do distúrbio associados, de forma a estabelecer possíveis correlações entre as propriedades reológicas necessárias tentando não abdicar de uma percepção sensorial agradável (CHEN; LOLIVRET, 2011; ROSS et al., 2019). Os ensaios reológicos em diferentes deformações podem proporcionar

informação fulcral sobre a consistência e resistência em fluir do alimento durante o consumo. Uma caracterização completa dos alimentos espessados em termos reológicos engloba ensaios em estado estacionário e em estado dinâmico, onde informações específicas são obtidas. A análise reológica em estado estacionário permite obter a viscosidade em função da taxa de cisalhamento através de curvas de escoamento, com a vantagem de prever a influência de fatores como a concentração biopolimérica, presença e tamanho de partículas em suspensão, temperatura, entre outros, sobre a viscosidade. De acordo com o comportamento reológico viscoso, os fluidos podem ser basicamente classificados em Newtonianos e não-Newtonianos. No caso dos fluidos Newtonianos, a tensão de cisalhamento ($\sigma$) é diretamente proporcional à taxa de cisalhamento ($\dot{\gamma}$), como é indicado na Equação (2.1):

$$\sigma = \eta \times \dot{\gamma} \qquad \text{(Eq. 2.1)}$$

Onde a viscosidade ($\eta$) do fluido independe da taxa de cisalhamento aplicada.

Por outro lado, nos fluidos não-Newtonianos a relação entre tensão de cisalhamento e taxa de cisalhamento não é linear, podendo apresentar um comportamento dependente (fluido tixotrópico ou reopético) ou independente do tempo (com ou sem tensão residual) (STEFFE, 1996). A maioria dos fluidos não-Newtonianos, assim como os alimentos líquidos espessados, em geral, apresentam comportamento não-Newtoniano independente do tempo (fluidos pseudoplásticos ou fluidos dilatantes), podendo o seu comportamento reológico ser descrito de acordo com a ausência de tensão residual (Lei de Ostwald-de-Waele ou Lei da Potência - Equação 2.2) ou com a presença de tensão residual (Herschel-Bulkley - Equação 2.3). Isto significa que a viscosidade aparente cai com o aumento da tensão de cisalhamento, independentemente do tempo de aplicação da força, sendo que ao findar a causa deformante, o fluido volta à viscosidade inicial.

$$\sigma = k \times \dot{\gamma}^n \qquad \text{(Eq. 2.2)}$$

$$\sigma = \sigma_0 + k \times \dot{\gamma}^n \qquad \text{(Eq. 2.3)}$$

Onde, $\sigma$ representa a tensão de cisalhamento, $\sigma_0$ é a tensão residual, $k$ o índice de consistência, $\dot{\gamma}$ a taxa de cisalhamento e *n* representa o índice de escoamento.

O índice de consistência é uma constante de proporcionalidade simples, sendo que um valor alto significa que o material é muito consistente, enquanto que o índice de escoamento ou de fluxo, *n*, demonstra o grau de pseudoplasticidade do fluido, sendo que valores de *n* mais próximos de 1 indicam maior similaridade ao comportamento newtoniano. No caso dos líquidos espessados direcionados para a dieta disfágica, frequentemente associam um menor *n* com uma maior facilidade e uma melhor sensação aquando da deglutição (CHO; YOO, 2014; ROSS et al., 2019; TORRES et al., 2019).

Em relação à medição reológica em estado dinâmico, a viscoelasticidade dos alimentos líquidos espessados é analisada através de ensaios onde são aplicadas tensões que oscilam com o tempo, sendo esta caracterização também de relativa importância. Como respostas destes ensaios, resultantes no espectro mecânico do material analisado, são mensuradas as propriedades viscosas e elásticas dos fluidos de acordo com tratamento matemático em função da frequência, através dos módulos de dissipação (*G''*) e de armazenamento (*G'*), respetivamente. De um modo geral, este tipo de ensaio permite classificar uma dispersão como: solução diluída, solução concentrada (sistema de redes entrelaçadas) e gel fraco ou gel forte, dependendo da relação *G'* e *G''*. Para essa interpretação, muitas vezes é calculada a tangente da mudança de fase ou ângulo de fase (*tan δ*), também ele observado em função da frequência. A *tan δ* é diretamente relacionada à perda de energia por ciclo dividida pela energia armazenada por ciclo, como pode ser observado na seguinte Equação (2.4):

$$\tan\delta = \frac{G''}{G'} \qquad \text{(Eq. 2.4)}$$

Dado que *G'* está relacionado à componente elástica do material analisado, quanto maior o valor de *G'* menor o valor de *tan δ*, e logicamente, quanto menor o valor de *tan δ*, maior a rigidez estrutural do material. Por outro lado, quanto maior o valor de *G''* maior o valor *de tan δ*, logo maior a flexibilidade do mesmo. Como os alimentos líquidos espessados se tratam de materiais viscoelásticos, o *δ* será sempre maior que 0 e menor que 90 °, pois *δ* = 0 ° representa uma resposta puramente elástica segundo a Lei de Hooke (sólido Hookeano) e *δ* = 90 ° representa uma resposta puramente viscosa por parte do material analisado segundo a Lei de Newton (Líquido Newtoniano). Assim, quanto mais próximo um alimento líquido espessado tiver de 0 °, mais estruturado será

o material e quanto mais próximo de 90 ° maior será a sua componente líquida, apresentando uma estrutura mais flexível.

Estes parâmetros, acoplados aos parâmetros obtidos no ensaio estacionário são relevantes para a previsão de estabilidade, interações entre hidrocolóides e atributos de textura final dos alimentos formulados (BARNES et al., 1989; CHO; YOO, 2014). Posto isto, atualmente é possível projetar alimentos de acordo com certos critérios reológicos de forma a atender a necessidades específicas. No caso dos espessantes direcionados para disfágicos, é possível obter as características desejadas apenas escolhendo a concentração do espessante (MACKLEY et al., 2013; ZARGARIAAN, 2013; PARK et al., 2016).

Existem produtos espessantes comercialmente disponíveis e destinados especificamente para uso em dietas disfágicas. Como esses produtos alteram as propriedades sensoriais dos alimentos ou bebidas, a sua aceitabilidade também pode ser modificada. Assim, a relação entre os métodos fundamentais e empíricos retirados das análises reológicas podem ter impacto no controle de qualidade, relação com análise sensorial ou ainda uso em padrões oficiais no que diz respeito à avaliação e formulação de espessantes para disfágicos (PAYNE et al., 2011; VALLONS et al., 2013; GARCIA et al., 2005; JOYNER, 2018; KROP et al., 2019). A coordenação entre práticas clínicas e estudos reológicos é um ponto importante no manejo dos alimentos, principalmente líquidos, a qual deve ser revista de modo a obter um contorno estratégico para os processos terapêuticos a serem aplicados em pacientes com disfagia (DEWAR; JOYCE, 2006). Por isso, existem alguns desafios para produzir alimentos mais viscosos/consistentes sem interferir muito nas características sensoriais dos alimentos convencionais (ZARGARAAN., 2013). Atualmente, o aumento da viscosidade de alimentos líquidos é o principal cuidado paliativo tanto hospitalar como domiciliar utilizado como estratégia para contornar os problemas associados ao processo de deglutição por parte de pacientes disfágicos. De forma a padronizar a linguagem na área da saúde, em 2002, a *American Dietetic Association* (agora designada por Academy of Nutrition and Dietetics) desenvolveu e publicou a *National Dysphagia Diet* (NDD) que traz nomenclaturas que categorizam os alimentos adequados aos pacientes disfágicos de acordo com a sua viscosidade como pode ser observado na Tabela 2.1. Apenas três consistências das quatro apresentadas na Tabela 2.1 se apresentam como adequadas na dieta disfágica de acordo com as necessidades apresentadas pelos pacientes

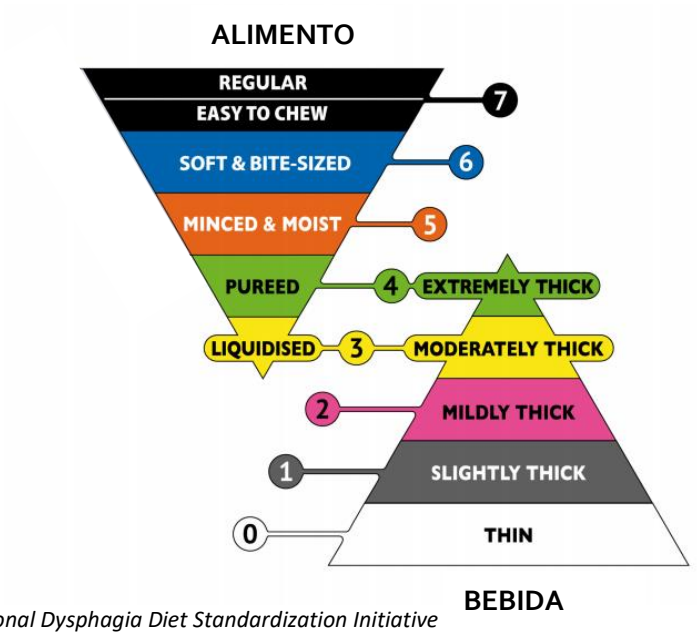
relativamente às viscosidades, são elas a *nectar-like*, *honey-like* e *spoon thick* ou *pudding thick*.

**Tabela 2.1.** Recomendações dos limites de viscosidade e correspondente nomenclatura, elaboradas pela *American Dietetic Association* em 2002.

| Classe de consistência | Viscosidade mínima (cP) | Viscosidade máxima (cP) |
|---|---|---|
| ***Thin*** | 1 | 50 |
| ***Nectar-like*** | 51 | 350 |
| ***Honey-like*** | 351 | 1750 |
| ***Spoon ou Pudding thick*** | 1750 | - |

Deve ser salientado que desde 2015, a *International Dysphagia Diet Standardization Initiative* (IDDSI) está remodelando essa categorização de forma a obter uma terminologia padrão mundial para as diferentes consistências tanto para alimentos sólidos como líquidos, a qual contempla todas as culturas e idades (Figura 2.2).

**Figura 2.2.** Pirâmides criadas e propostas pela *International Dysphagia Diet Standardization Initiative* para a padronização global da categorização dos alimentos líquidos e sólidos.




Fonte: *International Dysphagia Diet Standardization Initiative*

Esta categorização resulta da análise da consistência dos alimentos líquidos espessados através de métodos simples que visam, por exemplo, observar o movimento dos mesmos através de seringas ou em colheres (INTERNATIONAL DYSPHAGIA DIET STANDARDIZATION INITIATIVE, 2017).

A pirâmide relacionada com os líquidos de acordo com a IDDSI categoriza em: a) *Thin* que representa líquidos com capacidade de fluir como água colocando os pacientes disfágicos em risco, b) *Slightly Thick* é considerado ligeiramente mais espesso que a água e com características semelhantes à categorização *Thin* apresentada pela NDD, c) *Mildly Thick* apresenta semelhanças à categorização *Nectar-like* de acordo com a NDD, sendo adequada para casos em que o controle da língua na deglutição apresenta deficiências, c) *Moderately Thick* é a alternativa quando o paciente apresenta um controle insuficiente para ingerir o líquido *Mildly Thick*, a qual é comparável à categorização de *Honey-like* da NDD, d) por fim a *Extremely Thick*, deve ser ingerida através de uma colher, embora também não necessite de mastigação. No entanto, esta descrição de alimentos líquidos é meramente qualitativa, apresentando-se de forma subjetiva, o que dificulta não só a categorização de alimentos como também o seu manejo por parte das pessoas responsáveis por cuidar dos pacientes (HADDE; CHEN, 2019). Desta forma, garantir um produto com propriedades reológicas adequadas e quantificáveis é indispensável para obter uma deglutição segura por pacientes com disfagia.

Os espessantes comerciais têm sido extensamente avaliados por parte da comunidade científica, mas algumas fontes espessantes convencionais e não-convencionais, além dos novos métodos de análise, estão sendo equacionadas dada a necessidade em desenvolver alternativas às existentes no mercado (GERMAIN et al., 2006; GALLEGOS et al., 2012; ROFES et al., 2014; MORET-TATAY et al., 2015; PARK et al., 2016; TORRES et al., 2019).

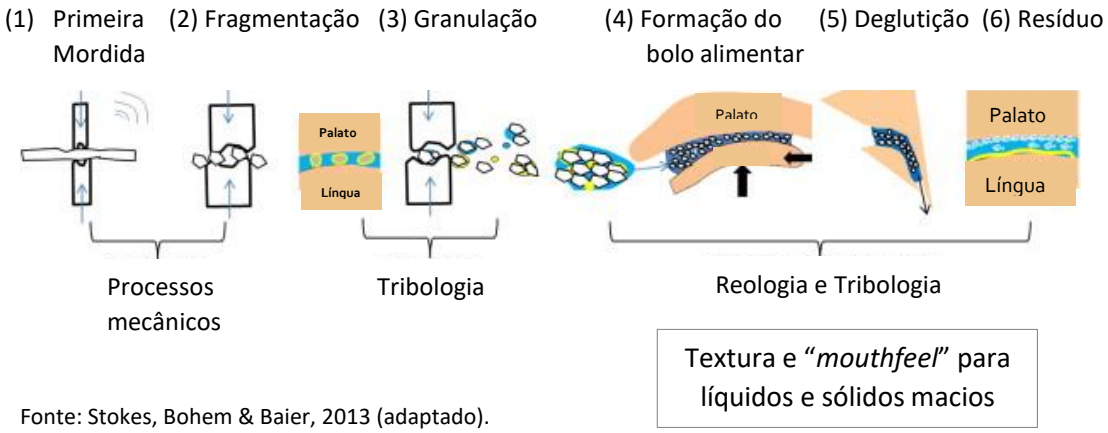
Atualmente, o foco da pesquisa tem se concentrado na análise do comportamento reológico de fluidos espessados em condições de estado estacionário com aplicação de forças de cisalhamento e extensionais, avaliando a viscosidade dos fluidos e também os possíveis efeitos das propriedades físico-químicas das matrizes alimentares líquidas onde os espessantes são incorporados (SOPADE et al., 2008; MACKLEY et al., 2013, HE et al., 2016; HADDE; CHEN, 2019). No entanto, ainda não se sabe se a viscosidade extensional e em cisalhamento são os únicos parâmetros relacionados ao fluxo do bolo alimentar. E é precisamente com esse pensamento que novas alternativas ao estudo dos

espessantes alimentares surgem como é o caso da tribologia, ferramenta complementar à reologia.

### 2.5. Tribologia

As propriedades mecânicas e reológicas têm sido amplamente usadas para compreender e descrever as propriedades de um alimento e a percepção sensorial associada. No entanto, a medida que o processamento oral continua e o tamanho das partículas de alimentos se reduz, a reologia por si só pode não ser tão eficaz na explicação das propriedades de textura e no comportamento oral dos alimentos (SCHOLTEN, 2017). Portanto, abordagens integradoras que combinam a reologia com a tribologia, uma análise da lubrificação através do cisalhamento de camadas hidrodinâmicas muito finas, apresentam um painel mais robusto para uma compreensão mais completa e assertiva sobre as prováveis contribuições da estrutura alimentar nos perfis sensoriais como está exemplificado na Figura 2.3.

**Figura 2.3.** Esquema representativo das diferentes fases durante o processamento oral de alimentos sólidos (1 - 6) e líquidos (4 - 6). A tribologia surge no ponto 3 devido às interações que ocorrem entre as partículas, bem como nas superfícies orais. Nas fases 4, 5 e 6 ocorrem as interações entre as superfícies orais relevantes para os líquidos.




Fonte: Stokes, Bohem & Baier, 2013 (adaptado).

Por esta razão, a tribologia está emergindo como uma importante ferramenta de pesquisa para quantificar os mecanismos físicos que ocorrem durante os processos fisiológicos orais, pois as propriedades lubrificantes dos alimentos são medidas usando o mesmo princípio empregado na engenharia mecânica para analisar as propriedades de atrito, desgaste e lubrificante de superfícies que se encontram em interação e

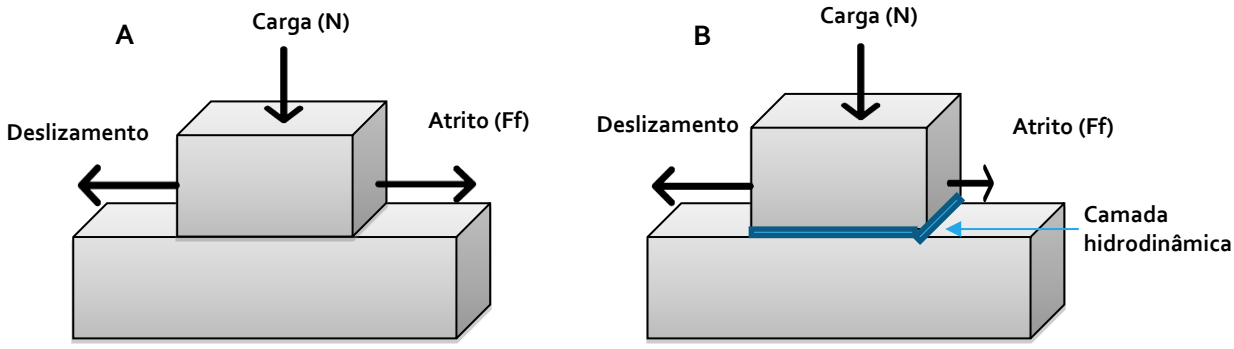
movimento relativo (SELWAY; STOKES, 2013). Nessas interações estão incluídas o ranger de dentes, interações língua-palato, língua-dentes, dentes-alimento, língua-alimento e lábios. Entre as superfícies interativas, língua-palato e língua-alimentos são provavelmente as mais relevantes a caracterizar (CHEN; STOKES, 2012). Assim, a escolha de um sistema tribológico fisiologicamente relevante, bem como as superfícies simuladoras das cavidades orais são primordiais para a aplicação bem sucedida da tribologia como um indicador para a percepção sensorial.

A principal propriedade obtida em um teste de tribologia é o coeficiente de atrito (Eq. 2.5), calculado como a razão entre a força de atrito medida e a carga normal aplicada (PRAKASH et al., 2013). Este coeficiente depende apenas das propriedades das superfícies em contato, podendo variar significativamente de acordo com a carga superficial e a viscosidade do fluido, quando uma fina camada de fluido está confinada entre duas superfícies. Quanto mais secas as superfícies, maior será a força de atrito (Figura 2.4A e 2.4B). No entanto, quando as superfícies em movimento relativo possuem uma camada hidrodinâmica entre elas, o atrito pode ser amenizado e até pode evitar praticamente que as superfícies entrem em contato, podendo a camada servir de lubrificante para as duas superfícies móveis. A lubrificação dessa camada pode evitar que as superfícies travem, resultando em desgaste severo da superfície e alto de atrito (CHEN; STOKES, 2012; SCHOLTEN, 2017).

$$CoF = \frac{Ff}{N} \qquad \text{(Eq. 2.5)}$$

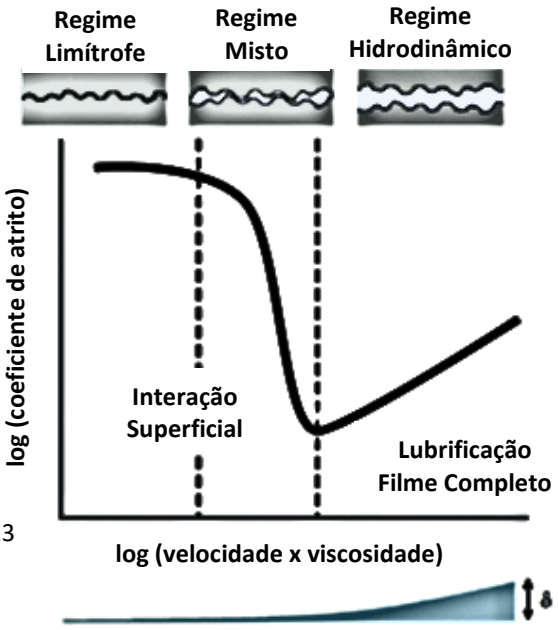
em que $Cof$ representa o coeficiente de atrito, $F_f$ a força de atrito e $N$ a carga aplicada.

**Figura 2.4.** Esquema representativo das forças envolvidas para analisar o coeficiente de atrito entre as superfícies em contato (A), podendo variar significativamente de acordo com a carga superficial e a viscosidade do fluido quando uma fina camada de fluido está presa entre as duas superfícies (B).



O atrito depende de vários parâmetros, como a viscosidade do lubrificante e as propriedades das superfícies. Com o aumento da velocidade de arraste, a lubrificação se torna mais eficaz e o coeficiente de atrito diminui. O coeficiente de atrito é geralmente representado através da curva de Stribeck, apresentada na Figura 2.5, na qual três regimes podem ser identificados dependendo da velocidade de deslizamento. O primeiro regime denomina-se de regime limítrofe (*Boundary*), no qual a velocidade de deslizamento é baixa e as duas superfícies mantêm-se em contato, observando-se um coeficiente de atrito praticamente constante. Seguidamente, aparece o regime misto (*Mixed regime)*, em que o deslizamento do lubrificante é suficiente para provocar uma separação entre as duas superfícies, pois a espessura da camada lubrificante começa a suportar parte da carga. O coeficiente de atrito, geralmente, atinge um mínimo nesta etapa. Por fim, ocorre o regime hidrodinâmico (*Hydrodynamic regime*), em que o aumento da velocidade de deslizamento/arraste do lubrificante resulta em um aumento adicional na pressão, capaz de separar completamente as superfícies. Este regime é referido como lubrificação hidrodinâmica, pois o atrito aumenta novamente com a velocidade, porém permanece menor do que no regime limítrofe (SCHOLTEN, 2017).

**Figura 2.5.** Curva de Stribeck, representando os diferentes regimes de lubrificação, com a respectiva espessura da camada hidrodinâmica, δ, relacionando o coeficiente de atrito com o aumento da velocidade.




Fonte: Selway e Stokes, 2013

Vários estudos têm sido realizados de forma a entender o comportamento e percepção oral ao longo da deglutição, sendo a maior parte dessas abordagens essencialmente baseadas em reologia estacionária usando forças de cisalhamento (PRAKASH et al., 2013). No entanto, está clara a limitação do uso de apenas abordagem reológica em alimentos fluidos e semi-fluidos, pois as características de textura são uma combinação do comportamento de fluxo do fluido e propriedades de superfície. Cremosidade, consistência e fluidez são exemplos típicos de características de textura que não podem ser explicadas satisfatoriamente por um simples teste de reologia (KOKINI; CUSSLER, 1984; KOKINI, 1987; CHEN; STOKES, 2012). O espessamento ou viscosidade, por exemplo, acreditava-se serem características de textura relativamente fáceis de prever através de medidas de fluxo em cisalhamento (curvas de escoamento). No entanto, tais ações estão associadas à formação combinada de forças normais e de cisalhamento na boca, gerando uma sensação de atrito/lubrificação entre palato e língua com o bolo alimentar que atua como lubrificante. Essas ações orais no estágio final da deglutição não estão mais associadas à deformação inicial do bolo alimentar, e sim, à tribologia. Duas das principais sensações que podem ser avaliadas através da tribologia são a suavidade (*smoothness*), untuosidade (*oily mouth coating*) e cremosidade, características de textura geralmente ligadas à presença de óleo/gordura (PRAKASH et al., 2013; SHARMA et al., 2017).

### 2.6. Produtos direcionados para pacientes disfágicos

As dietas com textura modificada prescritas para contornar os problemas associados aos alimentos, principalmente líquidos, para pacientes com disfagia, foram desenvolvidas utilizando espessantes focados em manipular e melhorar o comportamento reológico/viscoso dos alimentos. É importante aumentar a consistência/viscosidade do líquido através dos espessantes, permitindo uma passagem entre a boca/língua e a garganta com um tempo de residência maior em cada lugar percorrido, obtendo assim uma resposta reflexiva mais longa dos músculos responsáveis pela deglutição quando entra na faringe e, posteriormente, na laringe (DEWAR; JOYCE, 2006, MACKLEY et al., 2013, HORI et al., 2015).

Os espessantes são compostos à base de biopolímeros que, quando adicionados a um determinado fluido, aumentam sua viscosidade fazendo com que a deglutição se torne mais segura, quando adicionados em concentração adequada (STUART; MOTZ, 2009). Portanto, é necessário avaliar a concentração adequada para cada tipo de alimento e

situação de consumo (GARIN et al., 2014). Geralmente, os espessantes direcionados para pacientes com disfagia já existentes no mercado são compostos por amido modificado (pré-gelatinizado) ou goma xantana e, em alguns casos, há adição de outros constituintes, como a goma guar, maltodextrina e/ou agente indutor da gelificação como o cloreto de potássio. O uso de espessantes à base de goma pode oferecer vantagens em relação aos produtos à base de amido. Ao contrário do amido, as gomas não são naturalmente degradadas pela amilase presente na saliva além de atingir uma alta viscosidade a baixas concentrações, pondo em causa a sua função no problema em análise uma vez que são transformados em moléculas menores ainda na boca, resultando em perda das propriedades reológicas do fluido com ele espessado e, portanto, em um processo de deglutição menos seguro (PAYNE et al., 2011; LEONARD et al, 2013; VALLONS et al, 2015). Além disso, a grande variedade de produtos espessantes a base de amido modificado presentes no mercado apresentam grandes diferenças de viscosidade entre si e com relação aos padrões estabelecidos, demonstrando que os pacientes podem estar ingerindo alimentos com características muito diferentes das que são aconselháveis (PAYNE et al., 2011). Outro ponto relevante é que esses produtos resultam em aporte calórico para os pacientes e deve-se destacar o elevado índice reológicas interessantes, tem uma vantagem em relação à maioria das gomas e ao amido, que é a possibilidade de reduzir problemas associados com obstipação em pacientes disfágicos, problema associado à medicação que estão sujeitos como é o caso, por exemplo, de analgésicos (opióides) e antidepressivos (tricíclicos) (CUI et al, 1996; WANG et al, 2008). O rendimento da extração da goma de linhaça depende do método utilizado, oscilando entre 3,5 e 9,4 % (m/m) e, como consequência das condições de processo, as concentrações dos compostos descritos anteriormente podem se alterar, como é o caso dos fitoesteróis (OOMAH et al., 1995, CHEN et al., 2006). Estes esteróis vegetais, são conhecidos por apresentar propriedades potencialmente redutoras do colesterol de lipoproteínas de baixa densidade (LDL-C) no sangue, um fator de impacto no risco associado ao desenvolvimento de doenças cardiovasculares (HENDRICKS et al., 2003, RAS et al., 2015). Testes em ratos associam a ingestão de fitoesteróis a uma possível redução/alteração no metabolismo da testosterona (hormônio responsável por determinadas características masculinas) através de suas interações com a enzima 5-alfa-redutase (AWAD et al., 1998). No entanto, ensaios clínicos em humanos mostram que os níveis hormonais no sexo masculino (testosterona livre e total) e feminino (hormônio luteinizante, hormônio folículo

estimulante, beta-estradiol e progesterona), bem como todos os parâmetros hematológicos medidos não foram afetados a longo prazo pela ingestão diária de fitoesteróis (HENDRICKS et al., 2003). Vários autores, através de estudos realizados, demonstraram que o consumo de 50 g/dia de linhaça não demonstrou quaisquer efeitos adversos ao homem (MUIR; WESTCOTT, 2003, MARTINCHIK et al., 2012). A ingestão de outros subprodutos do óleo da linhaça é também, muitas vezes, recomendada devido aos possíveis benefícios, uma vez que estes podem funcionar como aliados na inibição da oxidação de colesterol, diminuindo o risco de outras doenças crônicas, tais como diabetes tipo 2, câncer ou ainda no combate à doença de Alzheimer (AWAD et al., 2000, ZANQUI et al., 2015).

Posto isto, pode-se concluir que há todo o interesse no desenvolvimento de novas alternativas de espessantes, seguras, para que os diferentes grupos de doentes disfágicos tenham disponíveis produtos com capacidade para corresponder às diferentes necessidades apresentadas, providenciando propriedades terapêuticas acopladas. Assim, o foco principal deste trabalho foi relacionar a área de engenharia de alimentos e saúde, buscando entender com mais profundidade os mecanismos de deglutição que poderá permitir encontrar novos padrões de propriedades reológicas adequadas dos líquidos a serem ingeridos por pacientes disfágicos.

## 2.7. Referências

ABE, R. et al. Videoendoscopic measurement of food bolus formation for quantitative evaluation of masticatory function. **Journal of Prosthodontic Research**, v. 55, n. 3, p. 171-178, 2011.

ASSAD-BUSTILLOS et al. Relationships of oral comfort perception and bolus properties in the elderly with salivary flow rate and oral health status for two soft cereal foods. **Food Research International**, v. 118, p. 13-21, 2019.

AWAD, A.B. et al. Phytosterol feeding induces alteration in testosterone metabolism in rat tissues. **Journal of Nutricional Biochemistry**, v. 9, n. 12, p. 712-717, 1998.

AWAD, A.B. et. al. Dietary phytosterol inhibits the growth and metastasis of MDA-MB-231 human breast cancer cells grown in SCID mice. **Anticancer Research**, v. 20, n. 2A, 821-824, 2000.

BARNES, H. A. Shear-Thickening (“Dilatancy”) in Suspensions of Nonaggregating Solid Particles Dispersed in Newtonian Liquids. **Journal of Rheology**, v. 33, p. 329-366, 1989.

BUETTNER, A. et al. Observation of the swallow process by application of videofluoroscopy and real time magnetic resonance imaging-Consequence for retronasal aroma stimulation. **Chemical senses**, v. 26, p. 1211-1219, 2001.

CAMPBELL, C.L. et al. Designing foods for satiety: The roles of food structure and oral processing in satiation and satiety. **Food Structure**, v. 13, p. 1-12, 2017.

CHAN, P. et al. Studies of the shear and extensional rheology of casein, waxy maize starch and their mixtures. **Food hydrocolloids**, v. 21, p. 716-725, 2007.

CHEN, J. et al. Flaxseed and its components reduce metastasis after surgical excision of solid human breast tumor in nude mice. **Cancer Letters**, v. 234, n. 2, p. 168–175, 2006.

CHEN, J. Food oral processing-A review. **Food Hydrocolloids**, n. 23, p. 1–25, 2009.

CHEN, J. Food oral processing: Mechanisms and implications of food oral destruction. **Trends in Food Science & Technology**, v. 45, n. 2, p. 222-228, 2015.

CHEN, J.; LOLIVRET, L. The determining role of bolus rheology in triggering a swallowing. Food Hydrocolloids, v. 25, n. 3, p. 325-332, 2011.

CHEN, J.; STOKES, J.R. Rheology and tribology: Two distinctive regimes of food texture sensation. **Trends in Food Science & Technology**, v. 25, n. 1, p. 4-12, 2012.

CHO, H.M.; YOO, B. Rheological Characteristics of Cold Thickened Beverages Containing Xanthan GumeBased Food Thickeners Used for Dysphagia Diets. **Journal of the Academy of Nutrition and** Dietetics, v. 115, n. 1, p. 106-111, 2014.

CLAVÉ, P.; SHAKER, R. Dysphagia: current reality and scope of the problem. Nature Reviews. **Gastroenterology and Hepatology**, v. 12, p. 259-270, 2015.

CUI, W. et al. Influence of genotype on chemical composition and rheological properties of flaxseed gums. **Food hydrocolloids**, v. 10, n. 2, p. 221-227, 1996.

CUTLER, A.N. et al. Oral Perception of Viscosity in fluid foods and model systems. **Food and Nutrition Press**, v. 14, p. 377-395, 1983.

DANTAS, R.O. et al. Effect of swallowed bolus variables on oral and pharyngeal phases of swallowing. **American journal of physiology**, v. 258, p. 675-681, 1990.

DEWAR, R.J.; JOYCE, M.J. Time-Dependent Rheology of Starch Thickeners and the Clinical Implications for Dysphagia Therapy. **Dysphagia**, v. 2006, p. 264–269, 2006.

ENGELEN, L. et al. The influence of product and oral characteristics on swallowing. **Archives of oral biology**, v. 50, p. 739-746, 2005.

ERTEKIN, C.; AYDOGDU, I. Neurophysiology of swallowing. **Clinical Neurophysiology**, v. 111, p. 2226–2244, 2003.

FORSTER, A. Et al. Oropharyngeal dysphagia in older adults: A review. **European Geriatric Medicine**, v. 2, p. 356–362, 2011.

GALLEGOS, C., et al. Rheology and Dysphagia: Na Overview. **Anual Transactions of the Nordic Rheology Society**, v. 20, p. 3-10, 2012.

GARCIA, J.M. et al. Viscosity Measurements of Nectar- and Honey-thick Liquids: Product, Liquid, and Time Comparisons. **Dysphagia**, v. 20, p. 325–335, 2005.

GARIN, N. Viscosity Differences between Thickened Beverages Suitable for Elderly Patients with Dysphagia. **Dysphagia**, v. 29, p. 483–488, 2014.

GERMAIN, I. et al. Rheological characterization of thickened beverages used in the treatment of dysphagia. **Journal of Food Engineering**, v. 73, p. 64-74, 2006.

HADDE, E.K.; CHEN, J. Shear and extensional rheological characterization of thickened fluid for dysphagia management. **Journal of Food Engineering**, v. 245, p. 18-23, 2019.

HASEGAWA, A. et al. Velocity of swallowed gel food in the pharynx by ultrasonic method. **Journal of the Japanese society for food science and technology**, v. 52, p. 441-447, 2005.

HE, Q. et al. Predicting sensory perceptions of thickened solutions based on rheological analysis. **Food Hydrocolloids**, v. 61, p. 221-232, 2016.

HENDRIKS, H.F. et al. Safety of long-term consumption of plant sterol esters-enriched spread. European Journal of Clinical Nutrition. **Nature**, v. 57, n. 5, p. 681-92, 2003.

HENNESSY, M.; GOLDENBERG, D. Surgical anatomy and physiology of swallowing. **Operative Techniques in Otolaryngology-Head and Neck Surgery**, v. 27, n. 2, p. 60-66, 2016.

HIIEMAE, K. et al. Natural bites, food consistency and feeding in man. **Archives of oral biology**, v. 41, p. 429-441, 1996.

HORI, K. et al. Comparison of mechanical analyses and tongue pressure analyses during squeezing and swallowing of gels. **Food Hydrocolloids**, v. 44, p. 145-155, 2015.

JAISHANKAR, J. et al. Probing hydrogen bond interactions in a shear thickening polysaccharide using nonlinear shear and extensional rheology. **Carbohydrate Polymers**, v. 123, p. 136–145, 2015.

JOYNER, H.S. Explaining food texture through rheology. **Current Opinion in Food Science**, v. 21, p. 7-14, 2018.

KOKINI, J.L. The physical basis of liquid food texture and texture–taste interactions. **Journal of Food Engineering**, v. 6, p. 51-81, 1987.

KOKINI, J.L.; CUSSLER, E.L. Predicting the texture of liquid and melting semi-solid foods. **Journal of Food Science,** v. 48, p. 1221-1225, 1984.

KROP, E.M. et al. On relating rheology and oral tribology to sensory properties in hydrogels. **Food Hydrocolloids**, v. 88, p. 101-113, 2019.

KU, P.K., et al. Early swallowing problems in a cohort of patients with nasopharyngeal carcinoma: Symptomatology and videofluoroscopic findings. **Laryngoscope**, v. 117, p. 142-146, 2007.

LEONARD, R.J. et al. Effects of Bolus Rheology on Aspiration in Patients with Dysphagia. **Journal of the Academy of Nutrition and Dietetics**, p. 2212-2672, 2014.

LLAVERIAS, G. et al. Phytosterols inhibit the tumor growth and lipoprotein oxidizability induced by a high-fat diet in mice with inherited breast cancer. **Journal of Nutricional Biochemistry**, v. 24, n. 1, p. 39-48, 2013.

LO, W.-L. et al. Dysphagia and risk of aspiration pneumonia: A nonrandomized, pair-matched cohort study. **Journal of Dental Sciences**, available online 22 March 2019, In Press, Corrected Proof.

MACKLEY, M.R. et al. The rheology and processing behavior of starch and gum-based dysphagia thickeners. **Journal of Rheology**, v. 57, n. 6, p. 1533-1553, 2013.

MARTINCHIK, A.N. et al. Nutritional value and functional properties of flaxseed. **Voprosy pitaniia**, v. 81, n. 3, p. 4-10, 2012.

MENG, Y. et al. Computer simulation of the pharyngeal bolus transport of Newtonian and Non-Newtonian fluids. **Food and Bioproducts Processing**, v. 83, n. 4, p. 297-305, 2005.

MIOCHE, L.; PEYRON, M.A. Bite force displayed during assessment of hardness in various texture contexts. **Archives of oral biology**, v. 40, p. 415-423, 1995.

MORET-TATAY, A. et al. Commercial thickeners used by patients with dysphagia: Rheological and structural behaviour in different food matrices. **Food Hydrocolloids**, v. 5, p. 318-326, 2014.

MUIR, A. D. AND WESTCOTT, N. D. Flaxseed constituents and human health. In Muir, A.D. and Westcott, N.D. (eds). Flax: the genus Linum, 243–51. London: **Taylor & Francis**, 2003.

NATIONAL DYSPHAGIA DIET TASK FORCE, 2002. National dysphagia diet: standardization for optimal care. Chicago, IL, **American Dietetic Association**.

NICOSIA, M.A.; ROBBINS, J. The fluid mechanism of bolus ejection from the oral cavity. **Journal of biomechanics**, v. 34, p. 1537-1544, 2001.

O'LEARY, M. et al. Viscosity and Non-Newtonian Features of Thickened Fluids Used for Dysphagia Therapy. **Journal of Food Science**, v. 75, n. 6, p. 330-338, 2010.

ONG, J.J.-X. et al. Sensory characteristics of liquids thickened with commercial thickeners to levels specified in the International Dysphagia Diet Standardization Initiative (IDDSI) framework. **Food Hydrocolloids**, v. 79, p. 208-217, 2018.

OOMAH, B.D. et al. Phenolic acids in flaxseed. **Journal of Agricultural and Food Chemistry**, v. 43, n. 8, 2016-2019, 1995.

PARIS, G. et al. Clinical screening of oropharyngeal dysphagia in patients with ALS. **Annals of Physical and Rehabilitation Medicine**, v. 55, n. 9-10, p. 601-608, 2012.

PARK, J.-W. et al. Fork test: A new simple and reliable consistency measurement for the dysphagia diet. **Geriatric Nursing**, v. 37, p. 292-295, 2016.

PAYNE, C. et al. Consistently Inconsistent: Commercially Available Starch-Based Dysphagia Products. **Dysphagia**, v. 26, p. 27–33, 2011.

PRAKASH, S. et al. Applications of tribology in studying food oral processing and texture perception. **Food Research International**, v. 54, n. 2, p. 1627-1635, 2013.

PRAKASH, S. Chapter 4 - From Rheology to Tribology: Applications of Tribology in Studying Food Oral Processing and Texture Perception. Advances in Food Rheology and Its Applications; **Woodhead Publishing Series in Food Science, Technology and Nutrition**, 65-86, 2017.

RAS, R.T. et al. Intake of phytosterols from natural sources and risk of cardiovascular disease in the European Prospective Investigation into Cancer and

Nutrition-the Netherlands (EPIC-NL) population. **European Journal of Preventive Cardiology**, v. 22, n. 8, p. 1067-1075, 2015.

ROFES, L. et al. The effects of a xanthan gum-based thickener on the swallowing function of patients with dysphagia. **Alimentary Pharmacology and Therapeutics**, v. 39, p. 1169-1179, 2014.

ROSS, A.I.V. et al. Relationships between shear rheology and sensory attributes of hydrocolloid-thickened fluids designed to compensate for impairments in oral manipulation and swallowing. **Journal of Food Engineering,** v. 263, p. 123-131, 2019.

SANTORO, P.P. et al. Otolaryngology and Speech Therapy evaluation in the assessment of oropharyngeal dysphagia: a combined protocol proposal. **Brazilian Journal of Otorhinolaryngology**, v. 77, n. 2, p. 201-213, 2011.

SCHOLTEN, E, 2017. Composite foods: from structure to sensory perception. **Food and Function**, v. 8, n. 2, 481-497.

SELWAY, N.; STOKES, J.R. Insights into the dynamics of oral lubrication and mouthfeel using soft tribology: Differentiating semi-fluid foods with similar rheology. **Food Research International,** v. 54, p. 423–431, 2013.

SEPTEMBER, C. et al. Implications of Changing the Amount of Thickener in Thickened Infant Formula for Infants with Dysphagia. **Dysphagia**, v. 29, p. 432–437, 2014.

SHARMA, M. et al. Effect of hydrocolloid type on texture of pureed carrots: Rheological and sensory measures. **Food Hydrocolloids**, v. 63, p. 478-487, 2017.

SILVA, F.G.D et al. Identification of peptides released from flaxseed (*Linum usitatissimum*) protein by Alcalase® hydrolysis: Antioxidant activity. **LWT - Food Science and Technology**, v. 76, Part A, p. 140-146, 2017.

SOPADE, P.A. et al. Rheological characterization of food thickeners marketed in Australia in various media for the management of dysphagia. III. Fruit juice as a dispersing medium. **Journal of Food Engineering**, v. 86, p. 604–615, 2008.

STEFFE, J.F. Rheological Methods in Food Process Engineering. 2nd Edition, Freeman Press, East Lansing, 1996.

STOKES, J. R. et al. Oral processing, texture and mouthfeel: From rheology to tribology and beyond. **Current Opinion in Colloid & Interface Science**, v. 18, p. 349–359, 2013.

TAKAHASHI, T. et al. Effects of physical properties and oral perception on transit speed and passing time of semiliquid foods from the mid-pharynx to the hypopharynx. **Journal of Texture Studies**, v. 33, p. 585-598, 2003.

TORRES, O. et al. Gellan gum: A new member in the dysphagia thickener family. **Biotribology**, v. 17, p. 8-18, 2019.

VALLONS, K.J.R. et al. Effect of human saliva on the consistency of thickened drinks for individuals with dysphagia. **International Journal of Language & Communication Disorders**, v. 50, n. 2, p. 165–175, 2015.

VAN ECK, A. et al. Oral processing behavior and dynamic sensory perception of composite foods: Toppings assist saliva in bolus formation. **Food Quality and Preference**, v. 71, p. 497-509, 2019.

WANG, X. et al. Topographic study of human tongue in relation to oral tribology. **Food Hydrocolloids**, v. 95, p. 116-121, 2010.

WODA, A. et al. Adaptation of healthy mastication to factors pertaining to the individual or to the food. **Physiology and Behavior**, v. 89, p. 28-35, 2006.

WOODS, C.W. et al. Development of necrotizing enterocolitis in premature infants receiving thickened feeds using SimplyThick. **Journal of Perinatology**, v. 32, p. 150-152, 2012.

YUAN, B. et al. Extensional and shear rheology of a food hydrocolloid. **Food Hydrocolloids**, v. 74, p. 296-306, 2018.

ZANQUI, A.B. et al. Subcritical extraction of flaxseed oil with n-propane: Composition and purity. **Food Chemistry**, v. 188, n. 1, p. 452–458, 2015.

ZARGARAAN, A. et al. Rheological aspects of dysphagia-oriented food products: A mini review. **Food Science and Human Wellness**, v. 2, p. 173–178, 2013.

***CAPÍTULO III***

# *Effect of extraction temperature on rheological behavior and antioxidant capacity of flaxseed gum*

*Published manuscript in Carbohydrate Polymers, v. 213, 217-227, 2019*

## 3. Effect of extraction temperature on rheological behavior and antioxidant capacity of flaxseed gum

J.M. Vieira [1], R.A. Mantovani [1], M.F.J. Raposo[2], M.A. Coimbra [2], A.A. Vicente [3], R.L. Cunha [1*]

[1] *Department of Food Engineering, Faculty of Food Engineering, University of Campinas (UNICAMP), 13083-862, Campinas, SP, Brazil*

[2] *Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

[3] *CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

*Corresponding author:
E-mail: **rosiane@unicamp.br**
Phone: **+55 19 3521 4047**

### Abstract

Soluble flaxseed gum (SFG) extracted at different temperatures (25, 40, and 60 °C) was analyzed in relation to the yield, polysaccharides and phenolics composition, surface charge, color, and rheological properties. The yield of SFG extract increased as the extraction temperature increased. The SFG xylan was the main component regardless the extraction temperature, but a reduction of substituents on the xylose chain was observed when increasing the extraction temperature. The phenolic compounds were also affected by the extraction temperature, influencing the antioxidant capacity of the gum. For all the extraction temperatures, SFG aqueous solutions showed a shear time-independent and shear-thinning behavior. Furthermore, oscillatory measurements showed a prevailing viscous character, but the decrease of the extraction temperature resulted in an increase of both *G'* and *G"*. Therefore, SFG extracted at low extraction temperatures showed higher viscous and elastic properties, while high extraction temperatures increased the antioxidant activity.

**Keywords:** Flaxseed polysaccharides; xylan; extraction temperature; antioxidant capacity; rheology.

### 3.1. Introduction

The soluble portion of flaxseed (*Linum usitatissimum* L.) gum (SFG) or mucilage is contained mainly in the hull mucous epidermis. This polysaccharide can be easily extracted by soaking the flaxseed in water, and the efficiency of the process depends mainly on the temperature (Cui, Mazza, Oomah & Biliaderis, 1994b). SFG shows great potential to be used in the food industry owing to its sustainable, biodegradable and functional properties, and bio-safe characteristics. It can be used as a thickener or a stabilizer/emulsifier in food systems, promoting interesting texture/rheological properties due to its high water-solubility and structural interaction with other hydrocolloids such as starch, guar gum or proteins (Wang *et al.*, 2008, Li, Li, Wang, Wu & Adhikari, 2012, Chen, Huang, Wang, Li & Adhikari, 2016), but its natural bioactive compounds can be equally useful for the enrichment of food products (Cui, Mazza & Biliaderis, 1994a; Cui & Mazza, 1996; Kennedy & Huang, 2003). The intake of SFG as dietary fiber can result in an improvement of the intestinal tract transit, reduced risk of diabetes and coronary heart diseases, decrease in the cholesterol and sugar absorption into the blood, decrease in the incidence of obesity, prevention of colorectal cancer, and other health benefits, such as help in treating the symptoms of depression, irritable bowel syndrome and osteoporosis (Morris & Vaisey-Genser, 2003; Mirhosseini & Amid, 2012; Liu, Shim, Poth & Reaney, 2016).

The SFG is composed by a neutral and an acidic fraction of polysaccharides and proteins (Cui *et al.*, 1994a; Qian, Cui, Nikiforuc & Goff, 2012a; Elboutachfaiti *et al.*, 2017). According to Qian, Cui, Wu & Goff (2012b), the main sugar of the neutral fraction (NF) of the polysaccharides is xylose (68.2 %), followed by arabinose (20.2 %), galactose (7.9 %) and glucose (3.7 %), whereas the acidic fraction (AF) is mainly composed by uronic acids (38.7 %), containing also rhamnose (38.3 %) and galactose (35.2 %) in the same proportion. Fucose (14.7 %) and xylose (8.9 %) are also present but in lower percentages, while arabinose (2.9 %) is the less abundant sugar. However, the polysaccharides composition and molecular structures can vary depending on the cultivars/genotype, the environment, the extraction conditions and dehydration process after extraction (Ziolkovska, 2012; Roulard, Petit, Mesnard & Rhazi, 2016). Thus, when these conditions vary, rheological and other functional properties may be significantly affected (Cui *et al.*, 1994a; Cui & Mazza, 1996).

Flaxseed is also a valuable natural source of phenolic compounds, including lignans, phenolic acids, flavonoids, phenylpropanoids, and tannins (Kasote, 2013). There is a vast number of studies reporting that these antioxidant components have pharmacological properties including antidiabetic, antihypertensive, immunomodulatory, anti-inflammatory and neuroprotective properties. The major compounds of flaxseed lignans are phytoestrogens (Herchi *et al.*, 2011; Hao & Beta, 2012; Alu'datt, Rababah, Ereifej & Alli, 2013). These compounds are usually associated to the SFG polysaccharides and can be co-extracted during the preparation of the gum, which, although providing antioxidant activity, may cause some setbacks and controversy in food applications due to presence of phytoestrogens as endocrine disruptors (Patisaul & Jefferson, 2010). Therefore, more studies on the phenolic compounds of flaxseed gum and their bioactivity are required.

Thus, in the present work, it is hypothesized that the definition of the extraction conditions of flaxseed gum can be used to tailor its properties according to the envisioned food and pharmaceutical applications. For this, we studied the influence of the extraction temperature on the composition and structural features of flaxseed gum relating such features with the antioxidant capacity and rheological behavior of SFG in aqueous solutions at different pH conditions.

## 3.2. Materials and Methods

### 3.2.1. Materials

Golden flaxseeds were produced in South of Brazil and kindly provided by CISBRA Ltda (Panambi, RS, Brazil). Ethanol was obtained from Dinamica (Brazil); Methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl) and BHA (butylated hydroxyanisole) were purchased from Sigma (USA).

### 3.2.2. Extraction of SFG

A physical procedure was used to obtain the polysaccharides with high molecular weight according to Cui *et al.* (1994b), with some modifications. Firstly, golden flaxseeds were washed with distilled water to remove dirt from the surface. Then, flaxseeds were soaked in distilled water at a flaxseed-to-water concentration of 10 % (w/w). This extraction process was made under stirring using an Ultra-Turrax system (IKA RW 20 digital, Brazil) for 5 h at 400 rpm and at three different temperatures (25,

40 and 60 °C). The soaked seeds were filtered (35 Mesh Tyler, Granutest, Brazil) and centrifuged at 11,200 *g* during 10 min. The water containing the dissolved SFG was treated with 99.5 % ethanol (1:1) to separate and remove the low molecular weight polysaccharides. Ethanol was then evaporated, and the dialyzed precipitates were freeze-dried (LS3000, Terroni, Brazil).

The SFG yield was determined using the following equation:

$$Yield(\%) = \frac{SFG}{Seed} \times 100 \qquad \text{(Eq. 3.1)}$$

where "SFG" represents the total mass of water-soluble portion of flaxseed gum in g (dry weight) after lyophilization and "Seed" represents the mass of flaxseeds used for the extraction in g (dry weight).

### 3.2.3. Preparation of SFG aqueous solutions

Aqueous SFG formulations with four concentrations of mucilage (0.75, 1.5, 2.25 and 3 % (w/w)) were prepared by dissolving the SFG in deionized water, under magnetic stirring during 3 h at 400 rpm and room temperature. The rheological properties of these formulations were evaluated at pH 3 (0.1 mol. $L^{-1}$ citric acid solution) and 6.5 (distilled water).

### 3.2.4. Fundamental elemental components and protein content

The fundamental elemental components (carbon, hydrogen, nitrogen and sulfur) were evaluated on a CHNS-O analyzer (Flash 2000, ThermoScientific, UK). Freeze-dried samples of SFG were crushed and homogenized, then weighed into a crucible, closed, and finally placed in the autosampler for instrumental analysis. Protein content was determined using the flaxseed specific factor N × 5.30 for the conversion of nitrogen to SFG protein. All the nitrogen content was considered as protein since the non-protein nitrogen in flaxseed gum is quite low (Qian *et al.*, 2012b).

### 3.2.5. Sugar and Glycosidic-linkage analyses

SFG samples were submitted to a dialysis (12-14 kDa cut-off) in order to obtain the polymeric material. Dialysis was carried out in a walk-in chamber against distilled water at 4 °C under constant stirring during four days, with two water renewals per day. The retentate was centrifuged at 4 °C and 15,000 rpm during 15 min; the supernatant

was concentrated, frozen and freeze-dried. Determination of sugars was performed before and after dialysis while linkage analysis was carried out only after dialysis of samples.

#### 3.2.5.1. Determination of sugars composition

After being submitted to a pre-hydrolysis with 72 % $H_2SO_4$, during 3 h, at room temperature, samples were hydrolyzed with 1 mol.$L^{-1}$ $H_2SO_4$ in a heating block, at 100 °C, during 2,5 h. After the first hour, a 500 µl-aliquot was collected from each tube for the analysis of uronic acids, which were determined colorimetrically according to the method referred by Nunes *et al.* (2012). Galacturonic acid was used as the standard. Total neutral sugars were determined according to the method of Nunes *et al.* (2012). Briefly, neutral monosaccharides were reduced with $NaBH_4$, acetylated with acetic anhydride in the presence of 1-methylimidazole, and the alditol acetates were extracted with dichloromethane. 2-deoxyglucose was used as the internal standard. After being dissolved in anhydrous acetone, the extracted alditol acetates were analyzed on a GC-FID (Perkin Elmer – Clarus 400, Massachusetts, USA) provided with a capillary column DB-225 (30 m length, 0.25 mm internal diameter, 0.15 mm film thickness). The injector temperature was 220 °C and the detector temperature 230 °C. The oven temperature was kept at 220 °C for 7 min; then the temperature increased at 5 °C/min up to 240 °C. Hydrogen was the carrier gas that was injected at 4 Bar. Retention times of standards were used to determine and quantify the sugar composition of each of the samples.

#### 3.2.5.2. Glycosidic-linkage analysis

In order to determine and characterize the glycosidic linkages, the various fractions of the polysaccharides were activated with NaOH pellets after being dispersed in DMSO, according to the method of Ciucanu & Kerek (1984) as indicated by Nunes *et al.* (2012). After being methylated with $CH_3I$, each sample was dissolved in $CHCl_3$:MeOH (1:1, v/v), and the solution was dialysed three times against 50 % EtOH in distilled water. Then the solution was vacuum dried. Methylated polysaccharides were hydrolysed with 2 M TFA during 1 h in a heating block at 121 °C, vacuum dried, reduced with $NaBD_4$ and acetylated with anhydride acetic in the presence of 1-methylimidazole. The partially methylated alditol acetates were analysed by GC/MS (Shimadzu GC-2010 Plus).

### 3.2.6. Zeta potential

Samples extracted at different temperatures were diluted in MilliQ water (Direct-Q3, Millipore, USA) to a concentration of 0.05 % (w/w) before being placed in the measuring chamber of microelectrophoresis (Zetasizer Nano-ZS, Malvern Instruments Ltd., UK). Zeta potential was determined as a function of pH, between 2 and 8. The Smoluchowsky model was used to convert the electrophoretic mobility measurement into zeta potential values. The samples were measured in triplicate at 25 °C.

### 3.2.7. Antioxidant activity

Radical scavenging activity of the SFG was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method according to Blois (1958), with some modifications. Briefly, 2.5 mL of DPPH (60 µM in methanol) were mixed with 0.2 mL of methanol and 0.3 mL of the sample dissolved in methanol (containing 10 mg $mL^{-1}$). After vortexing, each solution was stored in the dark for 30 min at room temperature. Then 0.2 mL of each sample was transferred into a Multiskan FC 96-well microplate to measure absorbance at 517 nm (Thermo Scientific, EUA) and the activity was expressed as the percentage of radical scavenging activity (% RSA) relative to the control. All experiments were conducted in triplicate, using the following equation:

$$RSA(\%) = \left( \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100 \qquad \text{(Eq. 3.2)}$$

where $Abs_{\text{sample}}$ and $Abs_{\text{control}}$ represent the absorbance of the sample solution and the absorbance of the control, respectively. Methanol was used as the control and butylated hydroxyanisole (BHA) was used as the reference antioxidant.

### 3.2.8. Total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu method as described by Wong-Paz *et al.* (2015). Firstly, the samples were dissolved in distilled water to the concentration of 10 mg/mL (w/v). In order to determine TPC, 800 µL of each sample were mixed with 800 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA), shaken and left for 5 min. Then 800 µL of $Na_2C0_3$ (0.01 M) were added, shaken and left for another 5 min. Finally, the solution was diluted with 5 mL of distilled water and the absorbance was read at 790 nm. A calibration curve was prepared using standard solutions of gallic acid (80, 160, 240, 320 and 400 mg/L, $R^2$ = 0.9938). All experiments were performed in

triplicate. The TPC was expressed as gallic acid equivalent per 100 grams (mg GAE/100 g).

### 3.2.9. Phenolic compounds

Freeze-dried SFG samples extracted at different temperatures were analyzed using a Shimatzu Nexpera X2 UHPLC chromatograph equipped with a Diode Array Detector (Shimadzu, SPD-M20A), according to the methodology used by Sluiter *et al.* (2008), with some modifications. A 300 mg of each sample were weighted into different pressure tubes and then 3 mL of 72 % sulfuric acid were added and mixed with a Teflon stir rod for 1 min, until the sample was thoroughly mixed. After that, sample tubes were incubated in water bath for 60 min at 30°C. Finally, the acid was diluted to a 4 % concentration by adding 84 mL of deionized water. Before being analysed, the samples were neutralized using calcium carbonate to pH 5-6. Separation was performed on a reversed-phase Aquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle size; from Waters) and a precolumn of the same material, at 40 °C. The flow rate was 0.4 mL $min^{-1}$ with an injected volume of 1 µl. The HPLC grade solvents used were formic acid 0.1 % (v/v) in water (up to 100 %) as solvent A and acetonitrile as solvent B. The elution gradient for solvent B was as follows: from 0.0 to 5.5 min eluent B at 5 %, from 5.5 to 17 min a linear increase to 60 %, from 17.0 to 18.5 min a linear increase to 100 %; then the column was equilibrated from 18.5 to 30.0 min at 5 %. Phenolic compounds were identified comparing their UV spectra and retention times with those of corresponding standards. The various compounds were quantified and identified at different wavelengths: caffeic acid at 320 nm, gallic acid at 280 nm, vanillic acid at 254 nm and ellagic acid at 250 nm.

### 3.2.10. Colorimetry analysis

The color of SFG solutions (indicated in the section 2.3) was measured in triplicate using an Ultra Scan Vis 1043 (Hunter Lab, model Color Quest II, USA) with reflectance mode, CIELab scale $L^*$ (lightness), $a^*$ and $b^*$ (chromaticity parameters), D65 as illuminant and a 10° observer angle as a reference system. Cylindrical coordinates $C^*$ (chroma, represents the intensity) (Eq. 3.3) and $H^*$ (hue angle) (Eq. 3.4) were calculated from parameters $a^*$ and $b^*$, according to:

$$C^* = \sqrt{\left(a^{*2} + b^{*2}\right)} \qquad \text{(Eq. 3.3)}$$

$$H* = \arctan\left(\frac{b*}{a*}\right) \quad \text{(Eq. 3.4)}$$

### 3.2.11. Rheological behavior

Flow curves of SFG solutions (from section 2.3) were obtained using a Physica MCR301 modular compact rheometer (Anton Paar, Graz, Austria) with a stainless-steel plate geometry (75 mm) and 100 µm gap. An up–down–up step program with various shear stresses range for each sample was used to provide shear rate between 0 to 1000 $s^{-1}$ at 25 ºC. This wide range includes a number of processes to mimic mastication (Mantovani, Cavallieri, Netto & Cunha, 2013), and also flowing and mixing. Newtonian (Eq. 3.5) and power-law equation (Eq. 3.6) were fitted to the data to obtain the rheological properties.

$$\sigma = \eta \times \dot{\gamma} \quad \text{(Eq. 3.5)}$$

$$\sigma = k \times \dot{\gamma}^{n} \quad \text{(Eq. 3.6)}$$

where $\sigma$ is the shear stress (Pa), $\eta$ is the viscosity (Pa.s), $k$ is the consistency index (Pa.s$^n$), $\dot{\gamma}$ is the shear rate ($s^{-1}$) and $n$ is the flow index. Eq. 3.7 was adjusted to the viscosity data according to a power law model in order to evaluate the effect of polysaccharide concentration on viscosity:

$$\eta_{ap}(50\ s^{-1}) = K \times C^{B} \quad \text{(Eq. 3.7)}$$

where, $\eta_{ap}$ $(50\ s^{-1})$ represents the apparent viscosity at a shear rate of 50 $s^{-1}$ (Pa.s), $K$ is a fitting parameter (Pa.s), $C$ is the concentration of SFG (%) and $B$ is the power law exponent (dimensionless) that represents the viscosity dependence with the concentration.

Oscillatory measurements of the SFG solutions (from section2.3) were performed using a stress-controlled AR1500ex rheometer (TA Instruments, USA) with a stainless-steel cone-plate geometry (6.0 cm, 2° angle, truncation 67 µm). The viscoelastic properties were evaluated using a frequency sweep between 0.1 and 10 Hz within the linear viscoelasticity domain. These measurements were done at 25 °C after one day of samples storage. The contributions of the elastic and viscous characteristics were evaluated from storage ($G'$) and loss ($G''$) moduli, respectively.

### 3.2.12. Statistical analysis

The data were analyzed using Sigma Plot 11 and Microsoft Excel (Office 365) software. Data were subjected to analysis of variance (ANOVA) ($p < 0.05$) and the means were compared using the Tukey's HSD test to examine if differences between treatments were significant ($\alpha = 0.05$).

## 3.3. Results and discussion

### 3.3.1. Physicochemical properties

The extraction temperature had a significant impact on the yield, composition and characteristics of SFG. The yield of SFG extraction increased with increasing temperature extraction, and the results were in accordance with the ones reported by Cui, Mazza, Oomah & Biliaderis (1994b) and Kaushik, Dowling, Adhikari, Barrow & Adhikari (2017), showing an almost two-fold increase when comparing the extraction at 25 °C (5.7 % w/w) with the extraction at 60 °C (10 % w/w) as shown in Table 3.3 ($p < 0.05$).

Variations in the concentrations of the various components in plant extracts might be due to the origin, growing conditions and diagenetic alteration of source materials (Fujine, 2014). This means that, although SFG characterization has already been performed (Cui *et al.*, 1994a, Cui & Mazza, 1996), such values may not adequately represent the samples used in the present work. Therefore, in order to compare the chemical composition of SFG extracted at different temperatures, samples were evaluated by fundamental elemental composition, zeta potential and total sugars and linkage analyses.

The elemental composition of SFG extracted at different temperatures is presented in Table 3.1. Nitrogen content increased significantly ($p > 0.05$) with increasing the extraction temperature, which means that the protein content varied between 4.3 ± 0.1 % and 13.8 ± 0.2 % (w/w), and this is within the range reported by Kaushik *et al.* (2017). This increase of SFG protein content with the extraction temperature was also observed by Cui *et al.* (1994a), leading to the conclusion that SFG extracted at 60 °C should have better interfacial and emulsifying properties, as demonstrated by Cui & Mazza (1996). Carbon was the major constituent for all the extraction temperatures, indicating the presence of a high content of carbohydrates and some protein in the extracted polysaccharide. Therefore, although an increase in the yield of SFG extraction

has been observed, the polysaccharide purity of the extracted decreased with increasing extraction temperature, since the protein yield was also greater at higher temperatures.

**Table 3.1.** Effect of extraction temperature on the yield, elemental composition and protein content of SFG.

| Extraction temperature (°C) | Yield (%, w/w) | N (%, w/w) | C (%, w/w) | H (%, w/w) | Protein content (%, w/w) |
|---|---|---|---|---|---|
| ***25*** | $5.7^{c}$ | $0.82 \pm 0.01^{c}$ | $33.93 \pm 0.27^{b}$ | $6.14 \pm 0.06^{a}$ | $4.33 \pm 0.07^{c}$ |
| ***40*** | $6.9^{b}$ | $1.18 \pm 0.02^{b}$ | $34.83 \pm 0.32^{a}$ | $6.26 \pm 0.09^{a}$ | $6.26 \pm 0.12^{b}$ |
| ***60*** | $10.0^{a}$ | $2.60 \pm 0.04^{a}$ | $35.11 \pm 0.54^{a}$ | $6.23 \pm 0.11^{a}$ | $13.80 \pm 0.21^{a}$ |

a-c Different letters in the same column correspond to statistically different samples for a 95% confidence level.

The zeta potential of SFG was always negative for pH values between 2 and 8, but a decrease of the absolute value was obtained (Figure 3.1) with decreasing pH values. The maximum values of zeta potential for each extraction temperature (25 °C, 40 °C and 60 °C) were −29.37, −34.57 and −35.1 mV, respectively. The isoelectric point (pI) of flaxseed protein isolate is pH 4.2 (Kaushik *et al.*, 2016), but it could not be observed because of the low protein content of the SFG extracted at different temperatures. The lower zeta potential of SFG extracted at higher temperature (or increased anionic character) could be associated to the higher protein content extracted at higher temperatures leading to a more pronounced negative charge at pH above the pI (Kaushik *et al.*, 2017). Indeed, the surface charge became very close to zero at pH near 2, this result agrees with those of Kaushik *et al.* (2017). At this pH, the protein is positively charged, and it is near to the polysaccharide pKa (Liu *et al.*, 2017). The maximum negative charge for all SFG samples extracted at different temperatures was observed from pH 6 to 8 and the highest absolute values of surface charge density were observed at higher extraction temperatures, with no difference between 40 °C and 60 °C.

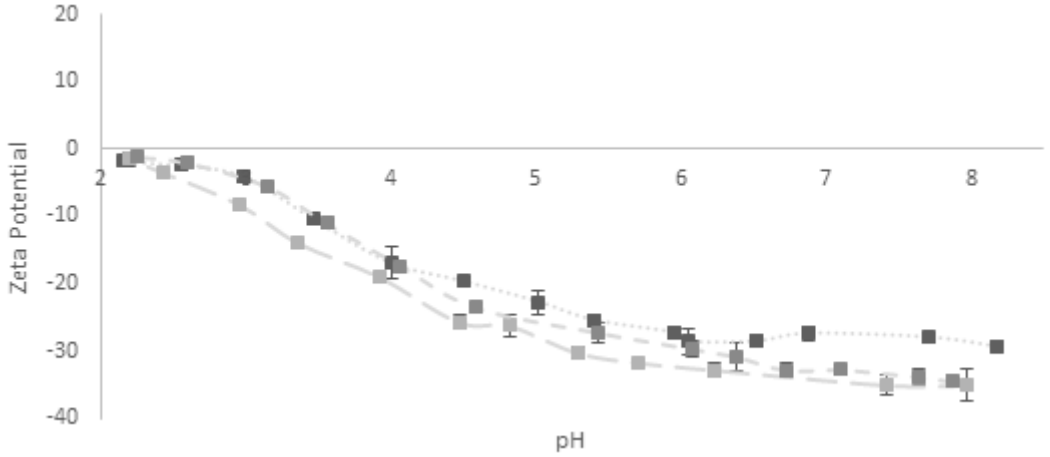



**Figure 3.1.** Zeta potential of SFG aqueous solutions obtained at different extraction temperatures (■ 25 °C, ■ 40 °C and ■ 60 °C). Error bars correspond to a significant difference at $p < 0.05$.

The proportion of polymeric material of the samples recovered after dialysis (12-14 kDa cutoff) correspond only to 53 %, 61 %, and 50 % for SFG extracted at 25 °C, 40 °C and 60 °C, respectively (Table 3.2). Nevertheless, after dialysis, the total sugars that composed each sample remained similar for the 25 °C samples (67 % and 63 % before and after dialysis, respectively), and slightly increased from 53 % to 63% for 40 °C samples and from 47 % to 62 % for 60 °C samples. The sugars composition of the samples determined before and after dialysis allowed also to observe that the main component in all samples was xylose, accounting for about one third in all samples. The dialyzed samples are also rich in uronic acids, accounting also for one third of the carbohydrate's composition, together with 13-14 mol% galactose, 8-9 mol% arabinose, and also rhamnose, fucose, and glucose in amounts ranging from 2 to 5 mol%. As the percentage of rhamnose, galactose and glucose are higher in the raw than in the dialyzed samples, it can be inferred that these carbohydrates are components of low molecular weight polysaccharides. Similar results were reported by Anderson and Lowe (1947) and Cui *et al.* (1994) for the composition of crude and dialyzed flaxseed gum, except for rhamnose that occurs in lower concentrations in the present study. This seems to be due to the incomplete hydrolysis of the aldobiouronic acid (GalA-Rha) component of the type-I rhamnogalacturonan of flaxseed gum, reported to require at least 6 h at 100 °C at 2 M $H_2SO_4$ to reach a maximum of release of the rhamnose residues (Emaga, Rabetafka, Blecker & Paquot, 2012).

**Table 3.2.** Sugar profile and yield of recovering after dialysis of the polysaccharides from flaxseed gum.

| SFG | Yield* | mol % | | | | | | | Total sugars |
|---|---|---|---|---|---|---|---|---|---|
| | (%, w/w) | Rha | Fuc | Ara | Xyl | Gal | Glc | UA | (%, w/w) |
| *Before dialysis* | | | | | | | | | |
| 25 ºC | | 8.0 | 5.1 | 9.1 | 34.5 | 18.5 | 12.6 | 12.3 | 66.5 |
| 40 ºC | | 8.0 | 4.8 | 9.6 | 36.2 | 18.9 | 6.9 | 15.6 | 53.3 |
| 60 ºC | | 5.8 | 2.6 | 8.2 | 27.7 | 16.6 | 25.3 | 13.8 | 47.4 |
| *After dialysis* | | | | | | | | | |
| 25 ºC | 52.9 | 5.0 | 4.2 | 7.5 | 29.8 | 13.7 | 2.5 | 37.3 | 63.0 |
| 40 ºC | 60.9 | 4.6 | 4.3 | 8.3 | 32.9 | 13.4 | 2.7 | 34.8 | 62.9 |
| 60 ºC | 49.5 | 4.2 | 3.9 | 9.0 | 30.5 | 13.4 | 3.1 | 35.8 | 61.5 |

* Yield after dialysis

The methylation analysis performed to the dialyzed samples allowed to observe that the xylose residues are mainly 1,4-linked (18-22 mol%), representing the unbranched main backbone of the xylan, where proportion of disubstituted 1,2,3,4-Xyl residues accounts for 9-11 mol% and the terminal residues account for 14-19 mol% (Table 3.2). The relative percentage of the linkages of the xylose residues are quite similar for the higher temperatures of extraction (40 °C and 60 °C), presenting a linear backbone of 1,4-linked xylose, with 22 % of unsubstituted residues and 3 % of *O*-2 monosubstituted residues and 9-10 % of disubstituted residues. At these temperatures of extraction, the relative percentage of disubstituted xylose residues are lower than the one observed for 25 °C, possibly by the higher extractability of the debranched polysaccharides at higher temperature and/or by debranching reactions due to the higher lability of the substituents, thereby increasing the non-substituted units along the 1,4-linked xylose main chain. In these samples it was also quantified arabinose residues, mainly as 1,5-linked, 1,2,3,5-linked, and terminally-linked, which are characteristic of flaxseed arabinoxylan, possibly as substituents at *O*-2 and/or *O*-3 positions of the xylan backbone, together with terminally-linked galactose and xylose residues (Naran, Chen & Carpita, 2008). The occurrence of 1,2,3-linked rhamnose together with the presence of galactose with a large diversity of linkages, including the terminally-linked, 1,4-Gal, 1,6-Gal, 1,4,6-Gal (Table 3.3), as well as uronic acids (Table 3.2), supports the presence of the characteristic homorhamnan domain of the rhamnogalacturonans of flaxseed mucilage (Qian *et al.*, 2012a).

These results show that the extracts, although rich in arabinoxylans (Cui *et al.*, 1994; Guilloux *et al.*, 2009; Ding, Qian, Goff, Wang & Cui, 2018), also have pectic polysaccharides. Nevertheless, the absence of 1,4 and 1,4,6-Glc shows that the xyloglucan reported by Ding, Cui., Goff, Chen, Wang & Han (2015), Ding, Cui, Goff, Guo & Wang (2016) and Ray, Paynel, Morvan, Lerouge, Driouich & Ray (2013) is not present in these extracts, as the xyloglucan requires alkali solutions to be extracted.

**Table 3.3.** Methylation analysis of the polymers extracted from flaxseed gum at three different temperatures, after being dialyzed

| Linkage type | 25 ºC | 40 ºC | 60 ºC |
|---|---|---|---|
| 2,3-Rha*p* | 5.0 | 4.5 | 8.4 |
| 2,4-Rha*p* | 0.4 | 0.7 | 0.5 |
| **Total Rha** | **5.5 (8)** | **5.2 (7)** | **8.9 (7)** |
| t-Fuc*p* | 1.2 | 3.6 | 1.9 |
| 2,3-Fuc*p* | 4.3 | 4.6 | 4.8 |
| **Total Fuc** | **5.5 (7)** | **8.2 (7)** | **6.7 (6)** |
| t-Ara*f* | 2.1 | 3.5 | 2.1 |
| 3-Ara*f* | 0.9 | 1.2 | 1.9 |
| 5-Ara*f* | 5.3 | 4.0 | 2.7 |
| 2,3,5-Ara*f* | 5.1 | 3.4 | 5.0 |
| **Total Ara** | **13.4 (12)** | **12.1 (13)** | **11.8 (14)** |
| t-Xyl*p* | 19.0 | 14.4 | 15.5 |
| 4-Xyl*p* | 17.5 | 22.4 | 21.6 |
| 2,4-Xyl*p* | 1.1 | 2.9 | 3.3 |
| 3,4-Xyl*p* | 0.5 | 0.9 | 1.1 |
| 2,3,4-Xyl*p* | 11.1 | 9.8 | 8.8 |
| **Total Xyl** | **49.2 (47)** | **50.4 (49)** | **50.2 (48)** |
| t-Gal | 8.7 | 7.1 | 8.4 |
| 4-Gal*p* | 6.5 | 4.2 | 2.6 |
| 6-Gal*p* | 2.5 | 3.3 | 3.1 |
| 3,4-Gal*p* | 0.2 | 0.2 | 0.2 |
| 3,6-Gal*p* | 0.3 | 0.2 | 0.2 |
| 4,6-Gal*p* | 1.0 | 1.6 | 1.2 |
| 2,3,4-Gal*p* | 0.4 | 0.8 | 0.6 |
| galactitol | 0.7 | 1.2 | 0.7 |
| **Total Gal** | **20.4 (22)** | **18.6 (21)** | **17.0 (21)** |
| t-Glc*p* | 5.7 | 5.0 | 5.2 |
| 3,4,6-Glc*p* | | 0.2 | 0.1 |
| **Total Glc** | **5.7 (4)** | **5.2 (4)** | **5.3 (5)** |

### 3.3.2. SFG phenolic compounds and antioxidant capacity

SFG samples extracted at 60 °C showed higher ($p < 0.05$) antioxidant activity than samples extracted at lower extraction temperatures (Table 3.4). Previous works have shown the relationship between antioxidant activity and the concentration of phenolic compounds in plants, correlating several analyses of antioxidant capacity, for example, ORAC (Oxygen Radical Absorbance Capacity), TRAP (Total Radical-trapping Antioxidant Parameter), DPPH (2,2-diphenyl-1-picrylhydrazyl method), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and HORAC (Hydroxyl Radical Antioxidant Capacity), with the phenolic content. After those studies, the researchers observed that an increase in phenolic content leads to an increase of the antioxidant activity (Číž, Čížová, Denev, Kratchanova, Slavov & Lojek, 2010, Rajurkar &. Hande, 2010, Seçzyk, Swieca, Dziki & Gawlik-Dziki, 2017). In addition, Hao and Beta (2012) observed that the antioxidant activity of flaxseed hull exhibited a large variation between different varieties, with $IC_{50}$ values ranging between 4.95-8.23 g $L^{-1}$ of phenolic compounds. Further, the antioxidant activity of free phenolic compounds extracted from the full fat flaxseed under heating was higher (62.3 %) when compared to the free phenolic compounds extracted without heat treatment (44.0 %) (Alu'datt *et al.*, 2016).

A number of studies using similar analyses have shown that the total phenolic content could be used as an indicator of antioxidant activity (Oliveira *et al.*, 2012; Pilluza & Bullita, 2009; Abozed, El-kalyoubi, Abdelrashid & Salama, 2014) although the total phenolic content does not incorporate all the antioxidants. Moreover, the structure of the antioxidants and the interactions between them should also be considered. Therefore, it is reasonable to consider that the antioxidant activity of the extracts can be related with the presence of some individual active phenolic compounds and their synergism in the mixture (Pilluza & Bullita, 2009). In the present work, it was verified that the increase in antioxidant activity was directly related to with the content of phenolic compounds.

In this study, the total phenolic content was estimated from the reaction between the Folin-Ciocalteu reagent and phenolic benzene rings. It was observed that the TPC of SFG was significantly influenced by the extraction temperature: the SFG extracted at 60 °C showed the highest ($p < 0.05$) TPC values, followed by those extracted at 40 and 25 °C (Table 3.4). Due to the affinity between the phenolic compounds and the protein

bonds, it is probable that some phenolic compounds have been extracted in greater quantity at higher temperatures by dragging since the increase of the extraction temperature also increased the protein content. These results compare well with those reported for guar gum (15.0 mg GAE. 100 $g^{-1}$) and are half of those reported for locust bean gum (33.0 mg GAE. 100 $g^{-1}$) (Hamdani & Wani, 2017).

In order to confirm the antioxidant activity and relate with the TPC profile, phenolic compounds present in the various SFG samples were determined by UHPLC (Table 3.4).

**Table 3.4.** Effect of the extraction temperature on phenolic compounds profile and antioxidant activity of SFG.

| **Extraction temperature** | **25 °C** | **40 °C** | **60 °C** |
|---|---|---|---|
| **Antioxidant activity (% RSA) *** | $4.39 \pm 1.52^{c}$ | $12.27 \pm 2.87^{b}$ | $29.64 \pm 2.39^{a}$ |
| **TPC (mg GAE. 100 $g^{-1}$) *** | $12.37 \pm 0.59^{b}$ | $13.01 \pm 0.20^{b}$ | $18.60 \pm 0.08^{a}$ |
| **Phenolic compound (mg.$L^{-1}$)** | | | |
| Caffeic acid | $6.58 \pm 0.06^{a}$ | $6.39 \pm 0.02^{b}$ | $6.06 \pm 0.11^{c}$ |
| p-cumaric-acid+epicatechin | $1.60 \pm 0.14^{a}$ | $1.43 \pm 0.00^{b}$ | $1.43 \pm 0.00^{b}$ |
| Ellagic acid | $1.18 \pm 0.54^{a}$ | $1.05 \pm 0.07^{a}$ | $3.14 \pm 0.46^{b}$ |
| Cinnamic acid | $2.28 \pm 0.02^{a}$ | $2.26 \pm 0.01^{a}$ | $2.27 \pm 0.01^{a}$ |
| Vanillic acid | - | - | $5.42 \pm 0.00$ |

a-c Different letters in the same line correspond to statistically different samples for a 95% confidence level.

*Sample at 10 mg/mL

The quantitative and qualitative composition of phenolic compounds in extracted SFG was dependent on the extraction temperature, although more obvious for 60 °C and mainly for ellagic and vanillic acids. Caffeic acid and p-cumaric-acid + epicatechin concentrations decreased and ellagic acid concentration increased with increasing extraction temperature. The amount of extracted phenolic compounds detectable by the methodology used was higher for extraction at 60 °C (18.32 mg.$L^{-1}$) followed by

extraction at 25 °C (11.64 $mg.L^{-1}$) and 40 °C (11.13 $mg.L^{-1}$). These results agree with the TPC values, however it was observed that the antioxidant activity measured in the SFG extracted at 40 °C was about three times greater than at 25 °C. This fact may be due to a) other extracted non-phenolic compounds which also exhibit antioxidant activity (extracted dry matter at 40 °C was higher when compared with extraction at 25 °C, as can be observed in Table 3.1) or b) a high number of interactions between phenolic compounds and proteins extracted in greater quantity at higher temperatures, making difficult to identify a given compound as a phenolic compound.

Almeida, Cavalcante & Vicentini (2016) studied the cytotoxicity, antiproliferative activity, and protection from DNA-induced damage in HTC cells, showing that vanillic acid was effective at protecting DNA from damage at any concentration between 1.684 $mg.L^{-1}$ and 16.84 $mg.L^{-1}$. In this study, the vanillic acid values detected were 5.42 $mg.L^{-1}$, which is in the range studied by the mentioned authors. The affinity of phenolic compounds to conjugate with major food components such as proteins, carbohydrates, lipids and minerals is due to the presence of an aromatic ring with hydroxyl groups and carboxylic acids, which is the case of vanillic acid; in this case, such affinity may have been the cause of the presence of this phenolic compound in SFG extracted at 60 °C (Sabally, 2006; Alu'datt *et al.*, 2016). Lutz, Lugli, Bitsh, Schlatter & Lutz (1997) studied the dose-response effect of different caffeic acid concentrations in rats, concluding that this compound can present anti-tumor properties in concentrations above 0.05 %. They also claim that, according to data collected, concentrations above 2 % of caffeic acid may have anticarcinogenic properties. Since for all extraction temperatures, the concentration of caffeic acid is approximately 0.0006%, it can be concluded that the dose of SFG to be consumed by rats should be high to observe some therapeutical effect. Regarding ellagic acid, previous studies have shown that even at very low concentrations this compound has a high antioxidant activity (Festa, Aglitti, Duranti, Ricordy, Perticone & Cozzi, 2001; Han, Lee & Kim, 2006; Kilic, Yeşiloğlu & Bayrak, 2014). Further, Priyadarsini, Khopde, Kumar & Mohan (2002) demonstrated that the ellagic acid concentration required to inhibit 50 % of lipid peroxidation was about 0.95 $mg.L^{-1}$, which is lower than the amount present in SFG (between 1.05 – 3.14 $m.L^{-1}$). Cinnamic acid was also detected and its concentration kept constant regardless the extraction temperature. Vanillic acid was only detected for SFG extracted at 60 °C; perhaps the extraction of this compound is also enhanced with temperature and the amount extracted at lower temperatures kept below the detection limits of the method.

Similar results were observed by Sytar, Hemmerich, Zivcak, Rauh & Bresti (2018), who studied the composition of 26 medicinal plants. All of them showed high antioxidant activity, and vanillic acid was present as the major phenolic compound in some of them (extraction temperatures above 60 °C were used for these analyzes).

These compounds have been widely studied, since they provide protection e.g. from the deleterious effects of oxidative stress (Cremonini, Bettaieb, Haj, Fraga & Oteiza, 2016). While antioxidant effects are the most studied in the literature, this being both a consequence and a motivation for the very extensive amount of work reported so far, it is also true that many other biological activities have been identified and demonstrated. For example, ellagic acid (and its dimeric derivative) also exhibits anti-mutagenic, anti-carcinogenic and anti-inflammatory activity (Feng *et al.,* 2009), and caffeic acid shows anti-dementia properties, contributing to reduce the progression of neuronal degenerations such as Alzheimer's disease (Mallik *et al.,* 2016; Akomolafea *et al.*, 2017). Further, cinnamic acid and its derivatives have attracted attention due to their anticarcinogenic, antimicrobial, antidiabetic, anticonvulsant, antidepressant, neuroprotective, analgesic, anti-inflammatory, muscle relaxant and sedative properties (Oishi, Yamamotoa, Oikea, Ohkurae & Taniguchif., 2017). Furthermore, vanillic acid has been associated with a variety of pharmacological activities, such as anti-carcinogenic, anti-apoptotic and anti-inflammatory but it has become most popular for its pleasant creamy odor that is widely used in fragrances, and licensed as a food additive, due to its distinct vanilla flavor (Gitzinger, Kemmer, Fluri, El-Baba, Weber & Fussenegger, 2012; Vinothiya & Ashokkumar, 2017). This acid has also shown to reduce the action of amylase, the primary human carbohydrase enzyme, thus reducing the efficiency of the digestive process in the mouth (Dupuis, Tsao, Yada & Liu, 2017).

The significant changes observed in the SFG composition upon extraction at different temperatures, particularly those regarding phenolic compounds; both their qualitative and quantitative compositions lead us to believe that it is possible to tailor to some extent the bioactive/functional properties of SFG extracts by controlling the extraction temperature.

### 3.3.3. Colorimetric analyses

Color is a crucial parameter with a significant role in the acceptability of foods. Reflectance spectrophotometry results indicated a change in the color of the samples mainly due to a significant ($p < 0.05$) increase of the lightness parameter ($L^*$) when

decreasing SFG extraction temperature (Table 3.5). This may be important when using SFG as food ingredient, depending on SFG concentration used in the final food formulation. Samples extracted at 60 °C also showed a higher chroma ($C^*$) value, which is associated with color saturation and tended to increase with SFG concentration. This is possibly due to the increase in phenolic compounds content in the SFG extract (as shown in section 3.3.2), to the higher concentration of proteins (as reported in section 3.3.1) or to the occurrence of Maillard reaction (especially relevant at 60 °C, considering the 5 h of extraction time). The Hue angle (all below 2°), $H^*$, a measure of color intensity, was located in the first quadrant, between yellow and red, and showed no significant differences between 40 and 60 °C extraction temperature. The obtained values for $H^*$ were higher for samples extracted at 25 °C; the same happened for the value of $L^*$, which means that the addition of SFG extracted at lower temperatures will exert less influence on the color of food products.

**Table 3.5.** *L**, *C** and *H** values of SFG solutions obtained at different extraction temperatures (25 °C, 40 °C and 60 °C) and prepared with varied SFG concentrations (0.75 %, 1.5 %, 2.25 % and 3 % w/w).

| Extraction Temperature | SFG (%) | *L** | *C** | *H** |
|---|---|---|---|---|
| 25 °C | **0.75** | 87.0 ± 0.10$^{a}$ | 1.0 ± 0.10$^{h}$ | 1.1 ± 0.02$^{b}$ |
| | **1.5** | 85.2 ± 0.10$^{c}$ | 0.76 ± 0.10$^{j}$ | 1.3 ± 0.11$^{a}$ |
| | **2.25** | 85.5 ± 0.20$^{c}$ | 1.15 ± 0.12$^{g}$ | 1.4 ± 0.12$^{a}$ |
| | **3** | 85.4 ± 0.01$^{c}$ | 1.29 ± 0.03$^{f}$ | 1.4 ± 0.01$^{a}$ |
| 40 °C | **0.75** | 85.9 ± 0.11$^{b}$ | 0.91 ± 0.02$^{h}$ | 0.7 ± 0.12$^{c}$ |
| | **1.5** | 85.8 ± 0.01$^{b}$ | 2.14 ± 0.14$^{e}$ | 0.8 ± 0.01$^{c}$ |
| | **2.25** | 85.6 ± 0.10$^{b}$ | 2.57 ± 0.02$^{d}$ | 0.8 ± 0.02$^{c}$ |
| | **3** | 85.5 ± 0.10$^{b,c}$ | 3.18 ± 0.01$^{c}$ | 0.8 ± 0.02$^{c}$ |
| 60 °C | **0.75** | 80.1 ± 0.10$^{e}$ | 1.8 ± 0.21$^{e}$ | 0.7 ± 0.13$^{c}$ |
| | **1.5** | 80.9 ± 0.02$^{d}$ | 3.3 ± 0.10$^{b}$ | 0.7 ± 0.04$^{c}$ |
| | **2.25** | 81.2 ± 0.22$^{d}$ | 5.5 ± 0.03$^{a}$ | 0.8 ± 0.01$^{c}$ |
| | **3** | 81.1 ± 0.23$^{d}$ | 5.4 ± 0.14$^{a}$ | 0.8 ± 0.01$^{c}$ |

a-h Different letters in the same column correspond to statistically different samples for a 95% confidence level.

### 3.3.4. Rheological properties

Rheological properties of SFG solutions can be significantly affected by variables such as shear rate and time, pH and extraction temperature of the polysaccharide (Cui *et al.*, 1994b, Kaushik *et al.*, 2017), which will be influenced by the concentration of polysaccharide needed to achieve the desired viscosity or other rheological characteristic. Thus, the study of rheological properties was performed to evaluate thickening properties and viscoelastic behavior of SFG aqueous solution. The influence of pH and SFG concentration on rheological properties was assessed under isothermal conditions.

#### 3.3.4.1. Flow curves analysis

All samples presented a shear time-independent behavior. The arbitrarily positioned chains of polymer molecules when the fluid is at rest become aligned in the same direction of the flow as shear rate increases, decreasing the solution viscosity (Koocheki, Reza-Taherian & Bostan, 2013). A similar behavior was observed for dispersions of chia (*Salvia hispanica* L.) mucilage (Capitani, Ixtaina, Nolasco & Tomás, 2012), *Opuntia ficus indica* (Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez & Katthain, 2000), *Lepidium sativum* (Karazhiyan *et al.*, 2009), tragacanth (Chenlo, Moreira & Silva, 2010) and *Lepidium perfoliatum* (Koocheki *et al.*, 2013) gums. The effects of the extraction temperature, SFG concentration and pH on flow curves are indicated in Table 3.6. SFG solutions showed a shear-thinning behavior, as *n* values were lower than 1 regardless of the extraction temperature.

Apparent viscosity values at a fixed shear rate of 50 $s^{-1}$ ($\eta_{ap\ 50}$) for the different extraction conditions are shown in Figure 3.2 This shear rate value was chosen as representative of the food mastication (Wood, 1968). Increasing SFG concentration caused an increase in the apparent viscosity of the solutions (Table 3.6), possibly due to the higher content of total solids in the solution, hindering the intermolecular movement induced by hydrodynamic forces (Capitani *et al.*, 2015). The increase of the extraction temperature decreased the apparent viscosity and pseudoplasticity of the SFG solutions, which can be directly related to: a) an increase of protein content and b) interaction between polysaccharide chains and proteins (mixed and discontinuous network) (Fedeniuk & Biliaderis, 1994; Qian *et al.*, 2012b). Apparent viscosity values tended to decrease with decreasing pH values mainly at low SFG concentrations, which could be related to the lower magnitude of surface charge density (or solubility) when compared to SFG solutions at pH 6.5 (Figure 3.2). Thus, the decrease in viscosity could be attributed to a lower repulsion between SFG compounds (Hosseini, Reza, Mozafari, Hojjatoleslamy & Rousta, 2017).

**Table 3.6.** Steady state rheological properties of aqueous solutions of SFG obtained at different extraction temperatures (25 °C, 40 °C and 60 °C). SFG solutions were prepared at various concentrations and pH values. Rheological measurements were obtained in triplicate at 25 °C.

| *Extraction Temperature* | *SFG (%)* | *pH 6.5* | | *pH 3* | |
|---|---|---|---|---|---|
| | | ***n*** | ***k* (Pa.s$^n$)** | ***n*** | ***k* (Pa.s$^n$)** |
| **25 (°C)** | 0.75 | 0.83 ± 0.00$^{b}$ | 0.05 ± 0.00$^{g}$ | 0.86 ± 0.01$^{b}$ | 0.03 ± 0.00$^{h}$ |
| | 1.5 | 0.72 ± 0.01$^{c}$ | 0.28 ± 0.01$^{f}$ | 0.70 ± 0.01$^{c}$ | 0.26 ± 0.00$^{f}$ |
| | 2.25 | 0.60 ± 0.00$^{e}$ | 1.56 ± 0.03$^{c}$ | 0.61 ± 0.04$^{d,e}$ | 1.43 ± 0.00$^{c}$ |
| | 3 | 0.58 ± 0.01$^{f}$ | 3.12 ± 0.08$^{a}$ | 0.56 ± 0.01$^{e}$ | 3.06 ± 0.00$^{a}$ |
| **40 (°C)** | 0.75 | 0.83 ± 0.00$^{b}$ | 0.05 ± 0.00$^{g}$ | 0.87 ± 0.01$^{b}$ | 0.03 ± 0.00$^{h}$ |
| | 1.5 | 0.71 ± 0.00$^{c}$ | 0.28 ± 0.00$^{f}$ | 0.81 ± 0.09$^{b}$ | 0.31 ± 0.10$^{e,f}$ |
| | 2.25 | 0.67 ± 0.03$^{d}$ | 0.63 ± 0.50$^{d}$ | 0.64 ± 0.00$^{d}$ | 0.62 ± 0.01$^{d}$ |
| | 3 | 0.56 ± 0.02$^{f}$ | 2.66 ± 0.02$^{b}$ | 0.59 ± 0.03$^{e}$ | 2.09 ± 0.05$^{b}$ |
| **60 (°C)** | 0.75 | 0.93 ± 0.00$^{a}$ | 0.01 ± 0.00$^{h}$ | 0.99 ± 0.03$^{a}$ | 0.01 ± 0.00$^{i}$ |
| | 1.5 | 0.83 ± 0.01$^{b}$ | 0.06 ± 0.00$^{g}$ | 0.87 ± 0.01$^{b}$ | 0.04 ± 0.01$^{h}$ |
| | 2.25 | 0.73 ± 0.02$^{c}$ | 0.27 ± 0.01$^{f}$ | 0.84 ± 0.02$^{b}$ | 0.08 ± 0.01$^{g}$ |
| | 3 | 0.73 ± 0.03$^{c}$ | 0.55 ± 0.00$^{e}$ | 0.72 ± 0.01$^{c}$ | 0.36 ± 0.00$^{e}$ |

a-h Different letters in the same column correspond to statistically different samples for a 95% confidence level.

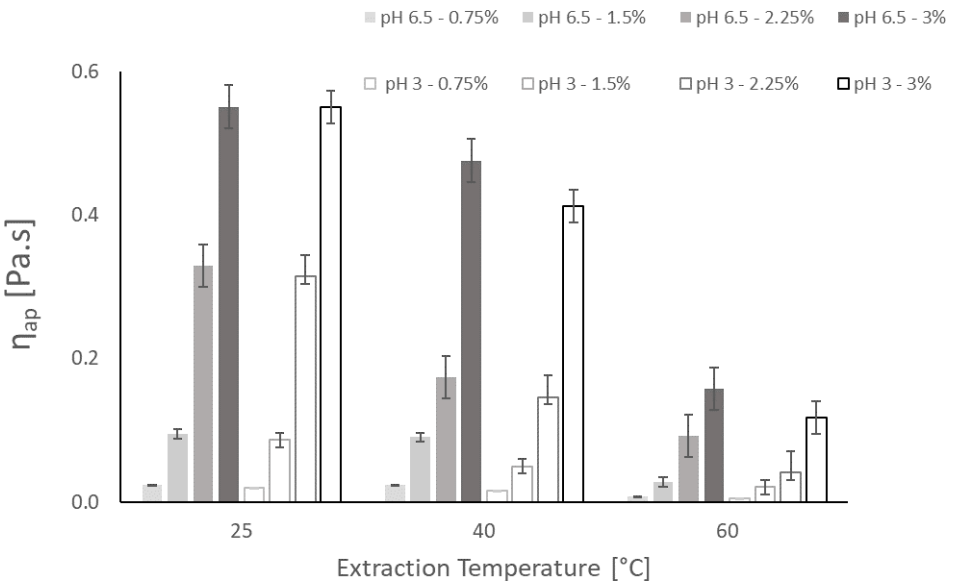



**Figure 3.2.** Apparent viscosity of SFG solutions obtained at different extraction temperatures (25 °C, 40 °C and 60 °C) and pH values, at a fixed shear rate of 50 $s^{-1}$. Full and empty symbols correspond to solutions at pH 6.5 and 3, respectively.

In addition, data of apparent viscosity (at 50 $s^{-1}$) for different concentrations of flaxseed gum and obtained at different extraction temperatures were adjusted by equation 3.7. Results presented in Table 3.7 confirm that the increase of the SFG extraction temperature caused a decrease in the viscous behavior of the aqueous solutions (decrease of *K* value). The effect of SFG concentration on the viscosity of aqueous FG solutions (*B* value) at different pH values was also affected by the extraction temperature ($p < 0.05$). At pH 3 a significant decrease of the power law exponent (*B* value) of the SFG extracted at the highest temperature means that in this condition the increase of SFG concentration exerted a minor effect on the viscosity. This behavior can be associated to a higher amount of some compounds extracted at 60 °C, such as phenolic compounds and proteins, and possibly to the reduction of substituents in the xylose chains (reported in Table 3.2), which may contribute to a lesser extent of interchain bonds and thus to a lower viscosity. This behavior was not observed at pH 6.5, since parameter *B* remained practically constant for all the extraction temperatures, indicating that the phenolic compounds and proteins are more sensitive to acid pH values.

**Table 3.7.** Fitting parameters obtained from the power law equation relating viscosity at 50 $s^{-1}$ and SFG concentration. SFG was extracted at different extraction temperatures (25 °C, 40 °C and 60 °C) and aqueous solutions were prepared at varied pH (3 and 6.5).

| | ***25 °C*** | | | ***40 °C*** | | | ***60 °C*** | | |
|---|---|---|---|---|---|---|---|---|---|
| **pH** | **K** | **B** | **$r^2$** | **K** | **B** | **$r^2$** | **K** | **B** | **$r^2$** |
| ***3*** | $0.0341^{a}$ | $2.550^{a}$ | 0.9922 | $0.0382^{a}$ | $2.124^{b}$ | 0.9386 | $0.0139^{b}$ | $1.712^{c}$ | 0.9454 |
| ***6.5*** | $0.0458^{a}$ | $2.250^{a}$ | 0.9886 | $0.0424^{a}$ | $2.013^{b}$ | 0.9770 | $0.0139^{b}$ | $2.334^{a}$ | 0.9949 |

a-c Different letters in the same line for each parameter correspond to statistically different samples for a 95% confidence level.

### 3.3.4.2 Oscillatory analysis

The effects of the extraction temperature, concentration and pH of SFG solutions on viscoelastic properties are shown in Figures 3.3 A (pH 6.5) and 3.3 B (pH 3). For all concentrations, regardless the pH of the SFG solution and the extraction conditions, the samples presented a predominance of viscous properties, the liquid-like properties predominate over that of solid-like, in the frequency range of 0.1-10 Hz, as the viscous modulus (*G''*) was always greater than the elastic modulus (*G'*). Although SFG solutions exhibit mainly a viscous behavior, previous works have shown that when this xylan is dissolved together with another hydrocolloid, both *G'* and *G''* increased indicating the formation of a stronger network. For example, Li *et al.* (2012) obtained higher values of *G'* and *G''* for solutions with 15 % of casein plus 0.5 % SFG when compared to solutions with 19% of casein, as well as showing that solutions with 15 % of casein had lesser *G'* and *G''* than solutions with 15 % of casein plus 0.5 % of SFG. This is possibly due to the establishment of interactions between the polymer chains of the different gums, pointing to the possibility of improved tailoring of SFG's textural properties through combinations with other hydrocolloids.

Higher *G'* and *G''* values were observed for SFG extracted at lower temperatures and these results agree with the trend observed by Cui, Mazza, Oomah & Biliaderis (1994). Similar tendency was observed for higher SFG concentrations. Therefore, the lowest values of *G'* and *G''* were observed for the sample with the lowest amount of SFG (0.75 % w/w) extracted at 60 °C. In addition, *G'* and *G''* were more frequency-dependent for an extraction temperature of 60 °C, which can be associated with the formation of a less complex structure (again, possibly due to the lower amount of

interactions, as a consequence of the reduction of substituents in the xylose chains – see Table 3.2) as also observed by Cui, Kenaschuk & Mazza (1996). Polysaccharides extracted from yellow flaxseeds presented higher *G'* and *G"* properties and apparent viscosity at higher levels of xylose followed by arabinose and galactose (Cui *et al.*, 1994). Therefore, one of the factors that may have influenced the increase of rheological properties is the high xylose and arabinose content observed before dialysis (Table 3.2). However, SFG extracted at 40 °C and 60 °C presented weaker rheological properties, despite of the xylose and arabinose content of SFG extracted at 40 °C was similar to 25 °C. These results could be associated to the decrease of water absorption capacity (WAC) of SFG with the increase of the extraction temperature, since polysaccharide can be not properly swollen at 40 °C. SFG extracted at lower temperatures showed higher pseudoplastic character, which can be associated to flaxseed structure significantly swollen, leading to a more visible deformation under shear forces. As observed for the apparent viscosity values (Figure 3.2), the decrease of pH exerted a negative effect on the rheological properties, being this effect more pronounced at lower concentrations (Figure 3.3 A and 3.3 B).

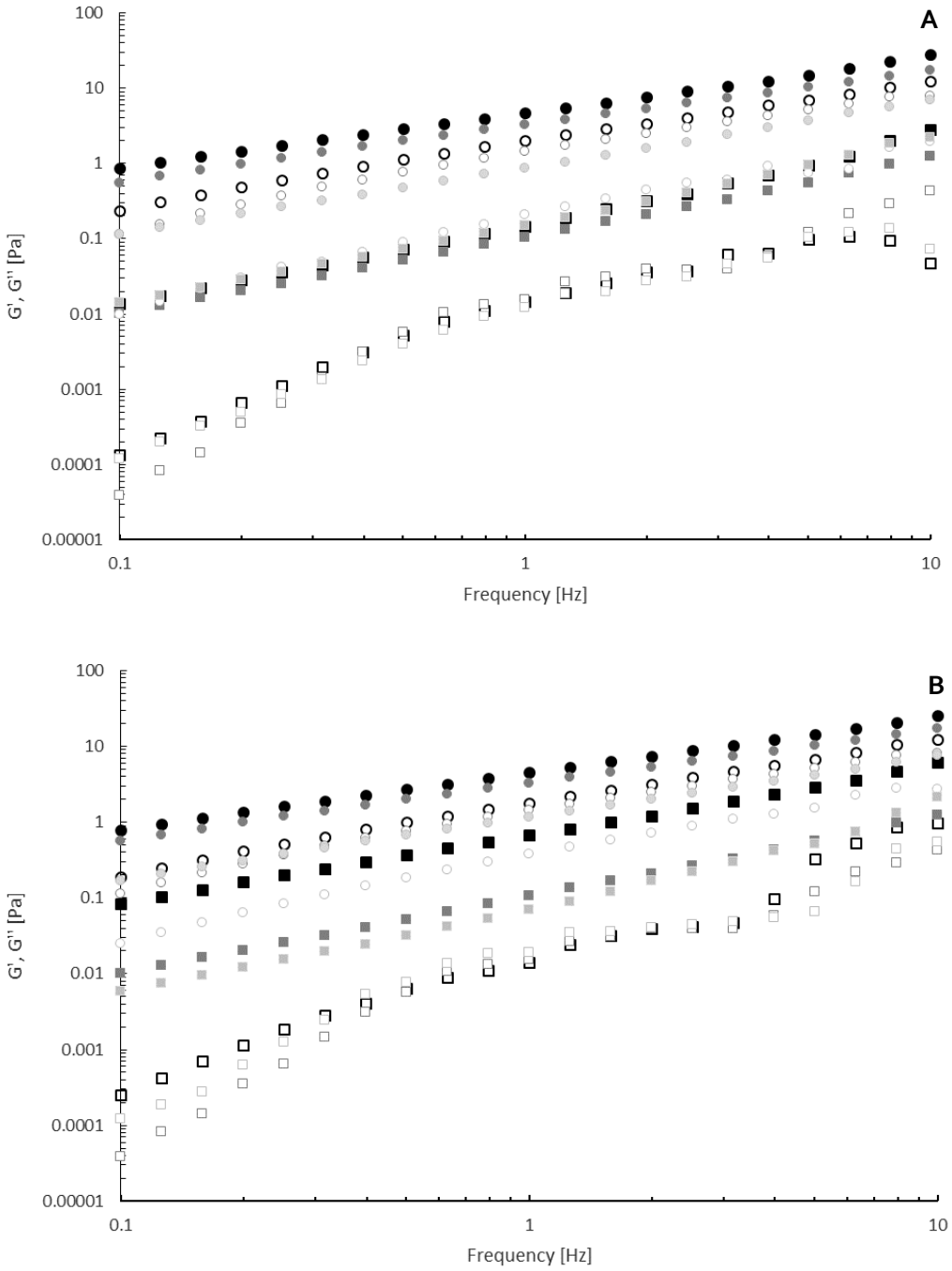



**Figure 3.3.** Elastic modulus (open symbols) and viscous modulus (closed symbols) as a function of frequency under isothermal (25 °C) conditions for solutions of SFG extracted at 25 °C (black symbols), 40 °C (dark grey symbols) and 60 °C (light grey symbols) in pH 6.5 (A) and pH 3 (B) for the extreme concentrations studied: (■) 0.75 % and (●) 3 % (w/w).

### 3.4. Conclusions

The extraction temperature affected SFG composition and physical properties (rheology and color). In particular, it was shown that the composition in phenolic compounds (caffeic acid, p-cumaric-acid + epicatechin, ellagic acid, cinnamic acid and vanillic acid were identified and quantified) was affected by the extraction temperature, which might have influenced the antioxidant capacity of the samples. Given that the concentrations of different phenolic compounds were affected differently for each of the extraction temperatures, it is hypothesized that the resulting SFG extracts may have diverse bioactive/functional properties.

The rheological properties at low and high deformations were negatively affected by the increase of the extraction temperature. Such a behavior can be related to the protein content increase, the reduction of substituents in the xylose chains, and/or the interaction between protein and polysaccharide molecules.

Overall, the extraction temperature affected both the biological/functional activities and the rheological properties of SFG: viscous properties decreased with increasing extraction temperature and phenolic composition changes leading to a higher antioxidant capacity.

### 3.5. Acknowledgements

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the PhD fellowship and Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP) for the financial support (Process numbers 2016/05448-8; 2011/51707-1; EMU 2009/54137-1; 2007/58017-5; 2006/03263-9; 2004/08517-3). We would also like to thank QOPNA (Quimica Orgânica, Produtos Naturais e Agroalimentares, University of Aveiro, Portugal) and Dr. Zlatina Genisheva (Centro de Engenharia Biológica, University of Minho, Portugal) for their valuable help in the sugar and phenolic compounds determinations.

### 3.6. References

Abozed, S.S., El-kalyoubi, A., Abdelrashid, A., Salama, M.F. (2014). Total phenolic contents and antioxidant activities of various solvent extracts from whole wheat and bran. *Annals of Agricultural Sciences*, 59(1), 63-67.

Akomolafea, S.F., Akinyemib, A.J., Ogunsuyi, O.B., Oyeleye, S.I., Oboh, G., Adeoyo, O.O., Allismitha, Y.R. (2017). Effect of caffeine, caffeic acid and their various combinations on enzymes of cholinergic, monoaminergic and purinergic systems critical to neurodegeneration in rat brain-In vitro. *NeuroToxicology*, 62, 6-13.

Almeida, I.V., Cavalcante, F.M.L., Vicentini, V.E.P. (2016). Different responses of vanillic acid, a phenolic compound, in HTC cells: cytotoxicity, antiproliferative activity, and protection from DNA-induced damage. *Genetic and Molecular Research*, 15(4), 1-12.

Alu'datt, M.H., Rababah, T., Ereifej, K., Alli, I. (2013). Distribution, antioxidant and characterisation of phenolic compounds in soybeans, flaxseed and olives. *Food Chemistry*, 139, 93–99.

Alu'datt, M.H., Rababah, T., Alhamad, M.N., Gammoh, S., Ereifej, K., Kubow, S., Alli, I. (2016). Characterization and antioxidant activities of phenolic interactions identified in byproducts of soybean and flaxseed protein isolation. *Food Hydrocolloids*, 61, 119-127.

Anderson, E., Lowe, H.J. (1947). The composition of flaxseed mucilage. *Journal of. Biology and Chemistry*, 168, 289-297.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199–1200.

Capitani, M.I., Ixtaina, V.Y., Nolasco, S.M., Tomás, M.C. (2012). Microstructure, chemical composition and mucilage exudation of chia (*Salvia hispanica* L.) nutlets from Argentina. *Journal of the Science of Food and Agriculture*, 93 (15), 3856-3862.

Capitani, M.I., Corzo-Rios, L.J., Chel-Guerrero, L.A., Betancur-Ancona, D.A., Nolasco, S.M., Tomás, M.C. (2015). Rheological properties of aqueous dispersions of chia (*Salvia hispanica* L.) mucilage. *Journal of Food Engineering*, 149, 70-77.

Chen, H., Xu, S., Wang, Z. (2006). Gelation properties of flaxseed gum. *Journal of Food Engineering*, 77, 295–303.

Chen, C., Huang, X., Wang, L.J. Li, D., Adhikari, B. (2016). Effect of flaxseed gum on the rheological properties of peanut protein isolate dispersions and gels. *LWT*, 74, 528-533.

Chenlo, F., Moreira, R., Silva, C. (2010). Rheological properties of aqueous dispersions of tragacanth and guar gums at different concentrations. *Journal of Texture Studies*, 41, 396–415.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, 131(2), 209-217.

Číž, M, Čížová, H., Denev, P., Kratchanova, M., Slavov, A., Lojek, A. (2010). Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control*, 21(4), 518-523.

Cremonini, E., Bettaieb, A., Haj, F.J., Fraga, C.G., Oteiza, P.I. (2016). (-)-Epicatechin improves insulin sensitivity in high fat diet-fed mice. *Archives of Biochemistry and Biophysics*, 13-21.

Cui, W., Kenaschuk, E., Mazza, G. (1996). Influence of genotype on chemical composition and rheological properties of flaxseed gums. *Food hydrocolloids*, 10(2), 221-227.

Cui, W., Mazza, G., Biliaderis, C.G (1994a). Chemical Structure, Molecular Size Distributions, and Rheological Properties of Flaxseed Gum. *Journal of Agricultural and Food Chemistry*, 42 (9), 1891–1895.

Cui, W., Mazza, G., Oomah, B.D., Biliaderis, C.G. (1994b). Optimization of an Aqueous Extraction Process for Flaxseed Gum by Response Surface Methodology. *Lebensmittel-Wissenschaft und –Technologie*, 27, 363-369.

Cui, W. and Mazza, G. (1996). Phvsicochemical characteristics of flaxseed gum. *Food Research International*, 29, 397-402.

Ding, H.H., Cui, S.W., Goff, H.D., Chen, J., Wang, Q., Han, N.F. (2015). Arabinan-rich rhamnogalacturonan-I from flaxseed kernel cell wall. *Food Hydrocolloids*, 47, 158-167.

Ding, H.H., Cui, S.W., Goff, H.D., Guo, Q., Wang, Q. (2016). Xyloglucans from flaxseed kernel cell wall: Structural and conformational characterization. *Carbohydrate Polymers*, 151, 538-545.

Ding, H.H., Qian, K., Goff, H.D., Wang, Q., Cui, S.W. (2018). Structural and conformational characterization of arabynoxylans from flaxseed mucilage. *Food Chemistry*, 254, 266-271.

Dupuis, J.H., Tsao, R., Yada, R.Y., Liu, Q. (2017). Physicochemical properties and in vitro digestibility of potato starch after inclusion with vanillic acid. *LWT - Food Science and Technology* 85, 218-224.

Emaga, T.H., Rabetafka, N., Blecker, C.S., Paquot, M. (2012). Kinetics of the hydrolysis of polysaccharide galacturonic acid and neutral sugars chains from flaxseed mucilage. *Biotechnology, Agronomy and Society and Environment*, 16(2), 139- 147.

Elboutachfaiti, R., Delattre, C., Anthony Quérro, A., Roulard, R., Julie Duchêne, J., Mesnard, F., Petit, E. (2017). Fractionation and structural characterization of six purified rhamnogalacturonans type I from flaxseed mucilage. *Food Hydrocolloids*, 62, 273-279.

Fedeniuk, R.W. and Biliaderis, C.G. (1994). Composition and Physicochemical Properties of Linseed (*Linum usitatissimum* L.) Mucilage. *Journal of Agricultural and Food Chemistry*, 42(2), 240–247.

Feng, Y., Yang, S.-g., Du, X.-t., Zhang, X., Sun, X.-x., Zhao, M., Sun, G.-y., Liu, R.-t. (2009). Ellagic acid promotes Aβ42 fibrillization and inhibits Aβ42-induced neurotoxicity. *Biochemical and Biophysical Research Communications*, 390, 1250–1254.

Festa, F., Aglitti, T., Duranti, G., Ricordy, R., Perticone, P., Cozzi, R. (2001). Strong antioxidant activity of ellagic acid in mammalian cells in vitro revealed by the comet assay. *Anticancer Research*, 21(6), 3903-8.

Fujine, K., 2008. FlashEA 1112 Elemental Analyzer (CHNS): User Guide. International Ocean Discovery Program, CHNS Analyzer User Guide, 1-12.

Guilloux, K., Gaillard, I., Courtois, J., Courtois, B., Petit, E. (2009). Production of arabinoxylan-oligosaccharides from flaxsedd (Linum usitatissimum). *Journal of Agricultural and Food Chemistry*, 57, 11308-11313.

Gitzinger, M., Kemmer, C., Fluri, D.A., El-Baba, M.D., Weber, W., Fussenegger, M. (2012). The food additive vanillic acid controls transgene expression in mammalian cells and mice. *Nucleic Acids Research*, 40, e37.

Hamdani, A.F and Wani, A. (2017). Guar and Locust bean gum: Composition, total phenolic content, antioxidant and antinutritional characterisation. *Bioactive Carbohydrates and Dietary Fibre*, 11, 53-59.

Han, D.H., Lee, M.J., Kim, J.H. (2006). Antioxidant and apoptosis-inducing activities of ellagic acid. *Anticancer Research*, 26(5), 3601-3606.

Hao, M. & Beta, T. (2012). Qualitative and quantitative analysis of the major phenolic compounds as antioxidants in barley and flaxseed hulls using HPLC/MS/MS. *Journal of the Science of Food and Agriculture*, 92, 2062–2068.

Herchi, W., Sakouhi, F., Arráez-Román, D., Segura-Carretero, A., Boukhchina, S., Kallel, H., Fernández-Gutierrez, A., 2011. Changes in the content of phenolic compounds in flaxseed oil during development. *Journal of the American Oil Chemists Society*, 88, 1135–1142.

Hosseini, E., Reza, H., Mozafari, M., Hojjatoleslamy, M., Rousta, E. (2017). Influence of temperature, pH and salts on rheological properties of bitter almond gum. *Food Science and Technology*, 37(3), 437-443.

Karazhiyan, H., Razavi, S.M.A., Phillips, G.O., Fang, Y., Al-Assaf, S., Nishinari, K., Farhoosh, R. (2009). Rheological properties of *Lepidium sativum* seed extract as a function of concentration, temperature and time. *Food Hydrocolloids*, 23(8), 2062–2068.

Kasote, D.M. (2013). Flaxseed phenolics as natural antioxidants. *International Food Research Journal*, 20(1), 27-34.

Kaushik P, Dowling K, Barrow CJ, Adhikari B. (2015). Complex coacervation between flaxseed protein isolate and flaxseed gum. *Food Research International*, 72, 91-97.

Kaushik, P., Dowling, K., McKnight, S., Barrow, C.J., Wang, B., Adhikari, B., 2016. Preparation, characterization and functional properties of flax seed protein isolate. *Food Chemistry*, 197, 212-220.

Kaushik, P., Dowling, K., Adhikari, R., Barrow, C.J., Adhikari, B. (2017). Effect of extraction temperature on composition, structure and functional properties of flaxseed gum. *Food Chemistry*, 215, 333–340.

Kennedy & Huang (2003). Polysaccharide Gums from Agricultural Products: Steve W. Cui (Book review). Carbohydrate Polymers, 52(2), 198.

Kilic, I., Yeşiloğlu, Y., Bayrak, Y. (2014). Spectroscopic studies on the antioxidant activity of ellagic acid. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 130, 447-52.

Koocheki, A., Reza-Taherian, A., Bostan, A. (2013). Studies on the steady shear flow behavior and functional properties of Lepidium perfoliatum seed gum. *Food Research International*, 50, 446–456.

Kuhnle, G.G.C, Dell'Aquila, C., Low, Y.-L., Kussmaul, M., Bingham, S.A. (2007). Extraction and Quantification of Phytoestrogens in Foods Using Automated Solid-Phase Extraction and LC/MS/MS. *Analytical Chemistry*, 79, 9234-9239.

Li, X.Y., Li, D., Wang, L.J., Wu, M., Adhikari, B. (2012). The effect of addition of flaxseed gum on the rheological behavior of mixed flaxseed gum–casein gels. *Carbohydrate Polymers*, 88 (4), 1214-1220.

Li, Y., Dai, F., Jin, X.L., Ma, M.M., Wang, Y.H., Ren, X.R., Zhou, B. (2014). An effective strategy to develop active cinnamic acid-directed antioxidants based on elongating the conjugated chains. *Food Chemistry*, 158, 41–47.

Liu, J., Shim, Y.Y., Poth, A.G., Reaney, M.J.T. (2016). Conlinin inflaxseed (*Linum usitatissimum L*.) gum and its contribution to emulsification properties. *Food Hydrocolloids*, 52, 963-971.

Liu, J., Shim, Y.Y., Shen, J., Wang, Y., Reaney, M.J.T. (2017). Whey protein isolate and flaxseed (Linum usitatissimum L.) gum electrostatic coacervates: Turbidity and rheology. *Food Hydrocolloids*, 64, 18-27.

Lutz, U., Lugli, S., Bitsch, A., Schlatter, J., Lutz, W.K. (1997). Dose Response for the Stimulation of Cell Division by Caffeic Acid in Forestomach and Kidney of the Male F344 Rat. *Fundamental and Applied Toxicology*, 39(2), 131-137.

Mallik, S.B., Mudgal, J., Nampoothiri, M., Hall, S., Anoopkumar–Dukie, S., Grant, G., Rao, C.M., Arora, D. (2016). Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. *Neuroscience Letters*, 632, 218–223.

Mantovani, R.A., Cavallieri, A.L.F, Netto, F.M., Cunha, R.L. (2013). Stability and *in vitro* digestibility of emulsions containing lecithin and whey protein. *Food and function*, 4, 1322-1331.

Mazza, G. & Biliaderis, C. (1989). Functional properties of flaxseed mucilage. *Journal of Food Science*, 54 (5), 1302–1305.

Medina-Torres, L., Brito-De La Fuente, E., Torrestiana-Sanchez, B., Katthain, R. (2000). Rheological properties of the mucilage gum (*Opuntia ficus indica*). *Food Hydrocolloids*, 14, 417–424.

Mirhosseini, H. & Amid, B.T. (2012). A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. *Food Research International*, 46, 387–398.

Morris, D.H. & Vaisey-Genser, M. (2003). FLAXSEED. *Encyclopedia of Food Sciences and Nutrition* (2nd Edition), 2525–2531.

Naran, R., Chen, G., Carpita, N.C. (2008). Novel rhamnogalacturonan I and arabinoxylan polysaccharides of flaxseed mucilage. *Plant Physiology*, 148(1), 132-141.

Nunes, C, Silva, L.M., Fernandes, A.P., Guiné, R., Domingues, M.R.M., Coimbra, M.A. (2012). Occurrence of cellobiose residues directly linked to galacturonic acid in pectic polysaccharides, *Carbohydrate Polymers*, 87, 620–626.

Oishia, K., Yamamotoa, S., Oikea, H., Ohkurae, N., Taniguchif, M. (2017). Cinnamic acid shortens the period of the circadian clock in mice. *Biochemistry and Biophysics Reports*, 9, 232-239.

Oliveira, A.M.F., Pinheiro, L.S., Pereira, C.K.S., Matias, W.N., Gomes, R.A., Chaves, O.S., Souza, M.F.V., Almeida, R.N., Assis, T.S. (2012). Total Phenolic Content and Antioxidant Activity of Some Malvaceae Family Species. *Antioxidants (Basel)*, 1(1), 33–43.

Patisaul, H.B. & Jefferson, W. (2010). The pros and cons of phytoestrogens. *Frontiers in Neuroendocrinology*, 31(4), 400–419.

Piluzza, G. & Bullitta, S. (2011). Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. *Pharmaceutical Biology*, 49(3), 240–247.

Priyadarsini, K.I., Khopde, S.M., Kumar, Mohan, H. (2002). Free Radical Studies of Ellagic Acid, a Natural Phenolic Antioxidant. *Journal of Agricultural and Food Chemistry*, 50, 2200−2206.

Qian, K.Y., Cui, S.W., Nikiforuk, J., Goff, H.D. (2012a). Structural elucidation of rhamnogalacturonans from flaxseed hulls. *Carbohydrate Research*, 362, 47-55.

Qian, K.Y., Cui, S.W., Wu, Y., Goff, H.D. (2012b). Flaxseed gum from flaxseed hulls: extraction, fractionation and characterization. *Food Hydrocolloids*, 28, 275-283.

Seçzyk, L., Swieca, M., Dziki, D., Anders, A., Gawlik-Dziki, U. (2017). Antioxidant, nutritional and functional characteristics of wheat bread enriched with ground flaxseed hulls. *Food Chemistry* 214, 32–38.

Sluiter, A., Hames, B.,Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker , D. (2008). Determination of Structural Carbohydrates and Lignin in Biomass. *National Renewable Energy Laboratory*.

Sytar, O., Hemmerich, I., Zivcak, M., Rauh, C., Brestic, M. (2018). Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. *Saudi Journal of Biological Sciences*, 25(4), 631-641.

Rajurkar & Hande, S. M. (2011). Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants. *Indian Journal of Pharmaceutical Sciences*, 73(2), 146-151.

Ray, S., Paynel, F, Morvan, C., Lerouge, P., Driouich, A., Ray, B. (2013). Characterization of mucilage polysaccharides, arabinogalactanproteins and cell-wall hemicellulosic polysaccharides isolated from flax seed meal: A wealth of structural moieties. *Carbohydrate Polymers*, 93, 651–660.

Roulard, R., Petit, E., Mesnard, F., Rhazi, L. (2016). Molecular investigations of flaxseed mucilage polysaccharides. *International Journal of Biological Macromolecules*, 86, 840-847.

Sabally, K. (2006). Lipase-catalyzed synthesis of selected phenolic lipid organic solvent media. (Ph.D Thesis) McGill University, Montreal, Canada.

Vinothiya, K. & Ashokkumar, N. (2017). Modulatory effect of vanillic acid on antioxidant status in high fat diet-induced changes in diabetic hypertensive rats. *Biomedicine & Pharmacotherapy*, 87, 640–652.

Wang, Y., Wang, L.J., Li, D., Özkan, N., Dong, X.D., Mao, Z.H. (2008). Effect of flaxseed gum addition on rheological properties of native maize starch. *Journal of Food Engineering*, 89(1), 87-92.

Wang, S.T., Chou, C.T., Su, N.W. (2017). A food-grade self-nanoemulsifying delivery system for enhancing oral bioavailability of ellagic acid. *Journal of Functional Foods*, 34, 207–215.

Wong-Paz, J.E., Contreras-Esquivel, J.C., Rodríguez-Herrera, R., Carrillo-Inungaray, M.L., López, L.I., Nevárez-Moorillón, G. V., Aguilar, C.N. (2015). Total phenolic content, in vitro antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. *Asian Pacific Journal of Tropical Medicine*, 8, 104–111.

Wood, G.N. (1968). Centric occlusion, centric relation, and the mandibular posture. *The Journal of Prosthetic Dentistry*, 20 (4), 292-306.

Wu, Z.M., Yu, Z.J., Cui, Z.Q., Peng, L.Y., Li, H.R., Zhang, C.L., Shen, H.Q., Yi, P.F., Fu, B.D. (2017). In vitro antiviral efficacy of caffeic acid against canine distemper virus. *Microbial Pathogenesis*, 110, 240-244.

Ziolkovska, A. (2012). Laws of flaxseed mucilage extraction. *Food Hydrocolloids*, 26, 197-204.

***CAPÍTULO IV***

# *Flaxseed gum-biopolymers interactions driving rheological behavior of oropharyngeal dysphagia-oriented products*

*To be submitted to Food Hydrocolloids*

## 4. Flaxseed gum-biopolymers interactions driving rheological behavior of oropharyngeal dysphagia-oriented products

J.M. Vieira [1], C.C.P. Andrade [2], T. P. Santos [1], P.K. Okuro [1], S.T. Garcia [1], M.I. Rodrigues [1], A.A. Vicente [2], R.L. Cunha [1*]

[1]*Department of Food Engineering, Faculty of Food Engineering, University of Campinas (UNICAMP), 13083-862, Campinas, SP, Brazil*

[2] *Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

*Corresponding author:

E-mail: **rosiane@unicamp.br**

Phone: **+55 19 3521 4047**

### Abstract

Viscosity-modified diet through thickeners is used as a strategy to circumvent swallowing problems by oropharyngeal dysphagia patients. Most commercial products present xanthan and starch in their formulations, but flaxseed gum (FG) is a potential thickener for liquid food that provides additional health benefits. FG was mixed either with modified starch (MS) and/or xanthan gum (XG), varying biopolymers' concentration according to a central composite rotational design (CCDR) in which rheological and colour properties in water were the evaluated responses. All formulations showed a shear time-independent and shear-thinning behaviour, mainly influenced by XG and MS concentrations. Oscillatory measurements presented a prevailing elastic character of the formulations, attributed to the MS and mainly to XG that in spite of the lower concentration presented similar influence on this rheological property. However, the increase of FG concentration was the most significant factor influencing viscosity, but also favoured an increase of both viscoelastic moduli, mainly *G'*. Analysis of the microstructure disclosed different network structures as a result of biopolymers interactions, which was related to rheological behaviour, giving insights to design new thickeners for dysphagia management. In addition, the amount of glucose released after *in vitro* digestion was evaluated and compared to a commercial MS-based

thickener. Interestingly, the commercial formulation showed a glucose release significantly higher than the proposed FG/MS/XG-based formulations. These results open the opportunity to tailor the rheological characteristics of food systems by adding and combining natural ingredients, improving technological and nutritional properties.

**Keywords:** dysphagia, thickeners, flaxseed gum, rheology, glucose load.

### 4.1. Introduction

Oropharyngeal dysphagia is the most prevalent and severe stage of dysphagia, since this pharyngolaryngeal sensitivity is a swallowing dysfunction, occurring during food transition from the mouth to the oesophagus. This mechanical disorder may be associated with aging, neurological complications, brain and neck cancer, cerebral palsy, Huntington's, Parkinson's or Alzheimer's diseases, benign oesophageal structure, cerebrovascular accident among others (Mackley *et al.*, 2013; Leonard *et al.*, 2013). Consequently, dysphagia can cause undernutrition, dehydration, aspiration pneumonia and even, in extreme cases, death (O'Leary, Hanson & Smith, 2010; Leonard *et al.*, 2013; Garin *et al.*, 2014). As a strategy to overcome the difficulty to swallow by the dysphagia patients, texture modification of liquid food using natural thickeners (biopolymers) has been investigated (September, Nicholson & Cichero, 2014; Clavé & Shaker, 2015). In this approach, viscosity must be risen in order to increase bolus passage time from the mouth to the throat and allowing thereby a longer reflective response by the muscles that are responsible for swallowing when it enters in the pharynx and, posteriorly, reaches the larynx (Dewar & Joyce, 2006a, Dewar & Joyce, 2006b; Hori *et al.*, 2015).

Generally, beverage thickeners available on the market are comprised of pre-gelatinized modified starch (soluble in water and able to behave as an instant thickener) and/or xanthan gum. In some cases, other polysaccharides, such as guar gum or maltodextrin, can be added as gelling agents (Moret-Tatay, Rodríguez-García, Martí-Bonmatí, Hernando & Hernandez, 2015). Starch is a low-cost ingredient that consists mainly of two α-1,4-D-glucose polymers: the almost linear amylose and the highly branched [5–6% α-1,6-linkages] amylopectin. Pre-gelatinization of the starch is necessary to convert starch into a soluble form in order to be completely dispersed and show fast viscosity development as necessary for an ingredient in dysphagia diet

(Tester, Karkalas & Qi, 2004; Tako, Tamaki, Teruya, & Takeda, 2014; Kanmani *et al.*, 2018). However, the high glucose load is a critical point of this polysaccharide, since its ingestion can result in either aggravation or development of chronic metabolic diseases. Moreover, starch is partly hydrolysed in the mouth by the amylase from saliva, decreasing the viscosity of the thickened foods. Unlike starch, gums do not need additional treatment and are not degraded by saliva amylase, keeping the viscosity more stable during the swallowing process (Leonard *et al.*, 2013, Vallons, Helmens, & Oudhuis 2015, Torres *et al.*, 2019). Xanthan gum (XG) is a high molecular weight heteropolysaccharide that presents 1,4-linked β-D-glucose residues as the primary structure and trisaccharide side chains with two molecules of mannose and one glucuronic acid linked to D-glucose at the backbone (Jo, Bak & Yoo, 2018). This hydrophilic colloid is an anionic polymer stable in a wide range of temperature and pH values. XG presents the advantage of being well accepted in terms of viscosity/texture by dysphagia patients when incorporated into beverages, due to its particular flow properties showing pronounced shear-thinning behaviour, owing to a rigid and rod-like conformation (Achayuthakan & Suphantharika, 2008; Jo *et al.*, 2018).

Flaxseed gum is a by-product of food oil industry that is found in the flaxseed hulls. The soluble part of the flaxseed gum (FG) is a promising biopolymer to be incorporated into a thickening product, capable of responding to the difficulties presented by dysphagia patients, since it exhibits thickening capacity coupled to nutritional properties (Vieira *et al.*, 2019). FG is mainly composed of neutral and acidic polysaccharides with a small fraction of proteins (5-10 % depending on extraction temperature) (Cui, Mazza & Biliaderis, 1994; Qian, Cui, Nikiforuc & Goff, 2012; Elboutachfaiti *et al.*, 2017). The main sugar of the neutral fraction of the polysaccharides is xylose, followed by arabinose, galactose and glucose, whereas the acidic fraction is mainly composed of uronic acids, and also rhamnose and galactose (Cui, Kenaschuk & Mazza, 1996; Vieira *et al.*, 2019). FG also presents phenolic compounds that could exhibit pharmacological properties including antidiabetic, antihypertensive, immunomodulatory, anti-inflammatory and neuroprotective properties (Vieira *et al.*, 2019). Moreover, FG fibres intake can improve intestinal tract transit, promoting an improvement in postprandial glycemia and weight control, reducing risk of diabetes and coronary heart diseases and decreasing cholesterol, besides preventing colorectal cancer and other health benefits (Foster-Powell, Holt & Brand-Miller, 2002; Ding *et al.*, 2014; Morris & Vaisey-Genser,

2003; Mirhosseini & Amid, 2012; Liu, Shim, Poth & Reaney, 2016; Moczkowska *et al.*, 2019).

Usually, modified liquid foods targeted for dysphagia patients are described according to recommendations from the guidelines of the National Dysphagia Diet (NDD), elaborated in 2002 by the American Dietetic Association. In the attempt to standardize the viscosity of liquid foods for dysphagia diets, four levels of consistency (expressed in centipoise) have been suggested: (*i*) Thin (1-50 cP), (*ii*) Nectar-like (51-350 cP), (*iii*) Honey-like (351-1750 cP) and (*iv*) Spoon or Pudding thick (>1750 cP). However, only the last three are recommended in the palliative care of dysphagia patients (Zargaraan, Rastmanesh, Fadavi, Zayeri & Mohammadifar, 2013). In general, the addition of thickeners to food formulations modifies their rheological properties, which become shear-thinning fluids and, in some cases, a yield stress can also be observed. A shear-thinning behaviour means that viscosity decreases with an increase of shear rate. The shear rate exerted on the food varies according to the physiological capacity of the oral cavity, the level of dysphagia of each patient and also the physicochemical properties of the ingested food. Thus, although Wood (1968) has stated that the shear rate during swallowing is around 50 $s^{-1}$ (used as a reference by the National Dysphagia Diet - NDD), there is limited scientific evidence for this standard, and oral process shear rates may range from 10 to 1000 $s^{-1}$ (Shama & Sherman, 1973). A shear rate of 10 $s^{-1}$ for liquids could be more reasonable according to Cutler, Morris & Taylor (1984), but Meng, Rao & Datta (2005) proposed 400 $s^{-1}$ as an indicator of oral shear rates. Therefore, several authors have reported that the shear rate definition given by NDD is inconclusive and subjective. In addition, other rheological parameters, such as the flow index ($n$), complex viscosity at 50 rad.$s^{-1}$ ($\eta^*$) and the relation between $G'$ (storage modulus, representing elastic behaviour) and $G''$ (loss modulus, associated to viscous behaviour), may give extra information about the sensory consistency of foods, allowing to draw some conclusions about the possibility to returning the pleasure of swallowing to these patients with restricted diet (Richardson *et al.*, 1989).

Due to the significant increase in the number of dysphagia patients, the need to intensify the development of new scientific researches in the area is a reality, aiming at a better and faster recovery and well-being of patients. For this, a detailed evaluation of the rheological behaviour of thickener solutions using different techniques and responses was performed, as well as a study of the glucose supply of these potential thickeners after their intake. These thickeners were composed by FG, MS and XG in

different concentration following a central composite rotational design allowing to understand the effect of each polysaccharide on rheological properties. Therefore, the aim of this work was to design new alternative thickeners, which should be safe and capable of responding to the needs of different groups of dysphagia patients.

## 4.2. Material and Methods

### 4.2.1. Material

Golden flaxseeds were kindly provided by CISBRA Ltda (Panambi, RS, Brazil). Xanthan gum (XG) and modified (pregelatinized) starch (MS) were donated by Danisco (Brazil) and Cargill (Brazil), respectively. The starch-based commercial thickener (CT) (Thick&Easy, Hormel) was purchased from a local pharmacy (São Paulo, Brazil). The following salts were used to prepare simulated digestion fluids: KCl, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$, HCl, $CaCl_2(H_2O)_2$, NaOH (Merck, Germany) and $KH_2PO_4$ (J.T. Baker, USA). The digestive enzymes porcine α-amylase (activity 9.65 $IU.mg^{-1}$ solid), porcine pepsin (activity 2512.16 $IU.mg^{-1}$ solid), porcine trypsin (activity 6.07 $IU.mg^{-1}$ solid) and porcine bile extract were purchased from Merck (Germany). Glucose Activity Assay Kit was bought from LaborLab (Brazil).

### 4.2.2. Extraction of FG and preparation of thickeners

FG was extracted at 25 °C according to Vieira *et al.* (2019) to obtain a composition approximately 67 % of carbohydrates, 5 % of protein and 13 % of phenolic compounds, responsible for the best rheological properties (Chapter III). Thickener formulations were prepared by dissolving FG, MS and XG in distilled water under mechanical stirring during 30 min at 800 rpm and room temperature (25 °C) to obtain a homogeneous solution. Then, samples were rested for an hour before being subjected to further analyses. Formulations were prepared following an experimental design described in section 2.3.

### 4.2.3. Experimental design

A Central Composite Rotational Design (CCRD), $2^3$, added of three replicates at the central point and six axial point essays, was performed for the three thickeners (FG, MS and XG) chosen based on a preliminary experimental design (Plackett-Burman Design, Appendix A) using five polysaccharides (FG, MS, XG, guar gum and pectin). Solutions

of each single biopolymer (FG, MS or XG) were prepared at intermediate and maximum studied concentrations and considered as control systems. Independent variables, *x1* = FG (0 - 3 %, w/v), *x2* = MS (0 - 3 %, w/v) and *x3* = XG (0 - 0.5 %, w/v), were studied (for details on formulations, please see Table 4.1) based on the importance of each compound for the following responses (dependent variables): viscosity ($\eta$) at different shear rates (10, 50 and 400 $s^{-1}$), complex viscosity at 50 rad/s ($\eta^*$), flow index ($n$), storage ($G'$) and loss ($G''$) moduli. Analysis of variance (ANOVA) of statistically significant regression coefficients ($p < 0.1$) was used to evaluate predictive models. Then, the response surfaces and contour curves were plotted in order to estimate the concentration range of each formulation's component according to the targeted rheological properties. One formulation representing each categorization of the NDD guidelines (using $\eta$ at 50 $s^{-1}$) was predicted and experimentally validated aiming at verifying the efficiency of the models. All experimental design analyses were performed by *Protimiza* software (www.http://experimental-design.protimiza.com.br) (Rodrigues & Iemma, 2015).

### 4.2.4. Rheological measurements

Rheological properties of thickeners' solutions were obtained using an AR1500ex rheometer (TA Instruments, USA) with a stainless-steel cone-plate geometry (6.0 cm, 2° angle, truncation 67 μm). All the measurements were performed at 25 °C. Flow curves were obtained by an up–down–up step program using different shear stresses range to provide shear rate ranging from 0 to 400 $s^{-1}$. Newtonian (Eq. 4.1) and power-law models (Eq. 4.1) were fitted to the data to obtain rheological properties.

$$\sigma = \eta . \dot{\gamma} \qquad \text{(Eq. 4.1)}$$

$$\sigma = k . \dot{\gamma}^n \qquad \text{(Eq. 4.2)}$$

where $\sigma$ is the shear stress (Pa), $\eta$ is the viscosity (Pa.s), $k$ is the consistency index ($Pa.s^n$), $\dot{\gamma}$ is the shear rate ($s^{-1}$) and $n$ is the flow index.

The viscoelastic properties were evaluated using a frequency sweep between 0.1 and 10 Hz within the linear viscoelasticity domain. The contributions of the elastic and viscous characteristics were analysed from storage ($G'$) and loss ($G''$) moduli, respectively. The loss tangent ($tan\,\delta$) was evaluated to define the prevailing behaviour

elastic or viscous. $Tan\ \delta < 1$ indicates a predominantly elastic behaviour, whereas $tan\ \delta > 1$ denotes a predominantly viscous behaviour, that is directly related to the energy lost per cycle divided by the energy stored per cycle (Eq. 4.3). The complex viscosity ($\eta^*$) (Eq. 4.4) was calculated since this property can be also correlated to the perception of thickness (He *et al.*, 2016), especially of viscoelastic materials.

$$tan\ \delta = \frac{G''}{G'} \quad \text{(Eq. 4.3)}$$

$$\eta^* = \frac{G^*}{\omega} \quad \text{(Eq. 4.4)}$$

where $\delta$ is the phase angle between the applied strain and the stress response, $G^*$ is the complex modulus and $\omega$ is the angular frequency.

The level of difficulty to chew and/or swallow food by dysphagia patients can vary considerably, which causes a clinical practice problem, and this may complicate medical follow-up and increase the medical errors (Germain, Dufresne & Ramaswamy, 2006). Thus, the effects of thickener formulations in water on varied rheological properties were evaluated.

Apparent viscosity values at a fixed shear rate of 10 $s^{-1}$ ($\eta_{ap\ 10}$) (indicator of oral shear rate according to Cutler *et al.*, 1984), 50 $s^{-1}$ ($\eta_{ap\ 50}$) (used as a reference by the National Dysphagia Diet - NDD) and 400 $s^{-1}$ ($\eta_{ap\ 400}$) (representative value to which a liquid food is subjected during the swallowing process according to Meng *et al.*, 2005); complex viscosity ($\eta^*$) at 50 $rad.s^{-1}$ (angular frequency used for quantitative correlation with panel scores), flow index *n* (as a shear-thinning indicator) and viscoelastic properties represented by elastic (*G'*) and viscous (*G''*) moduli at a fixed frequency of 1 Hz and *tan* $\delta$ were evaluated for the different formulations studied according to the CCRD.

### 4.2.5. Colorimetry analysis

The colour of thickened aqueous solutions was measured in triplicate using an Ultra Scan Vis 1043 (Hunter Lab, model Colour Quest II, USA) with reflectance mode, CIELab scale, D65 as illuminant and a 10° observer angle as a reference system. The cylindrical coordinate $C^*$ (chroma) was evaluated, representing the colour intensity (Eq. 4.5). Total colour differences ($\Delta E^*$) (Eq. 4.6) between the samples before and after

thickeners addition were evaluated and calculated from $L^*$ (lightness), $a^*$ and $b^*$ (chromaticity parameters):

$$C* = \sqrt{\left(a*^2 + b*^2\right)} \quad \text{(Eq. 4.5)}$$

$$\Delta E* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad \text{(Eq. 4.6)}$$

being $\Delta L = (L^* - L_0^*)$, $\Delta a = (a^* - a_0^*)$ and $\Delta b = (b^* - b_0^*)$, where, $L_0^*$, $a_0^*$ and $b_0^*$ are the initial colour values and $L^*$, $a^*$ and $b^*$ are the colour values of water matrix containing thickeners.

### 4.2.6. Fluorescence microscopy

The morphology of network formed by single biopolymers (FG, MS and XG) and their corresponding mixtures (MS-XG and MS-FG) was analyzed using a fluorescent microscope (Axio Scope.A1, Carl Zeiss, Germany) according to Tromp, van de Veld, van Riel & Paques (2001), with modifications. Images were taken using the "ZENLite" software package with 10x and 40x objective lens in order to observe interactions between the biopolymers.

XG and FG were stained with FITC (fluorescein-5-isothiocyanate) as follows. Both biopolymers (0.5 g of XG or 0.75 g of FG) were suspended separately in 50 mL dimethyl sulfoxide (DMSO) and FITC (70 mg). Pyridine (200 μL) and dibutyltin dilaurate (40 μL) were added to the mixture before heating for 4 h at 100 °C. Afterwards, both solutions were diluted into 200 mL isopropyl alcohol. Finally, the mixture was stored at 4 °C overnight and the yellow suspension was removed. To remove unbound dye, this suspension was re-dissolved in 45 mL DMSO for 2 h, precipitated in 150 mL isopropanol and dried under vacuum at 70 °C.

For single MS staining with Rhodamine B, MS was firstly dissolved (1.5 % w/v) in distilled water under magnetic stirring during 3 h at 600 rpm and room temperature. Then, 4 % v/v (regarding the total volume) of 0.2 % aqueous Rhodamine B solution was added. The stained MS was subjected to magnetic stirring at 200 rpm for 24 h at room temperature to obtain a homogenous dye distribution.

To analyse the network of the binary formulations (MS-FG and MS-XG), the stained gums (FG or XG) were mixed with MS (1.5 % w/w) in a ratio of MS:gum 11:1

on dry basis. The mixture was stirred during 4 h at room temperature, then 4 % v/v of the Rhodamine B solution was added, and the stirring was kept for more 20 h.

### 4.2.7. *In vitro* static digestion

Formulations were selected to be subjected to *in vitro* static digestion analysis (5 mL) considering the three different levels of consistency assigned by NDD. A formulation without MS was also evaluated in order to observe the impact of this biopolymer on the final amount of glucose in relation to the studied gums. To compare these formulations with a commercial product based on MS, nectar and spoon like consistency systems were prepared (extreme available cases) according to the label of the product. Gastrointestinal *in vitro* digestion was performed according to the standardized static model by Minekus *et al.* (2014), at 37 °C. Firstly, thickened samples were mixed with the simulated salivary fluid (SSF) for two minutes (oral phase). After that, simulated gastric fluid (SGF), porcine pepsin and calcium chloride were added to this mixture, and the pH was adjusted to 3 (gastric phase). The mixture was subjected to magnetic stirring at 100 rpm during 2 h. Finally, simulated intestinal fluid (SIF), pancreatin, calcium chloride and bile extract were added to the mixture with the same stirring velocity and temperature for 2 h more, at pH 7 (intestinal phase). Adjustments of the corresponding pH values were performed during the whole timeframe of the experiment. Samples were removed at different times to analyze the glucose released.

### 4.2.8. Glucose released

Glucose concentration was measured using the Glucose Activity Assay Kit (LaborLAb, Brazil) according to the manufacturer's instructions and converted to amount of glucose released (mg). Sampling was carried out after oral phase simulation (2 min), and during gastric phase (32, 62, 92 and 122 min) and intestinal phase (152, 182, 212 and 242 min) along the *in vitro* digestion. Each sample was analysed in duplicate.

### 4.2.9. Statistical analyses

The experimental design was evaluated using the "Protimiza Experimental Design" software ($\alpha$ = 0.1). Colour data were subjected to analysis of variance (ANOVA) ($p$ < 0.05) and the means were compared using Tukey's HSD test to examine if differences between formulations were significant ($\alpha$ = 0.1).

## 4.3. Results and discussion

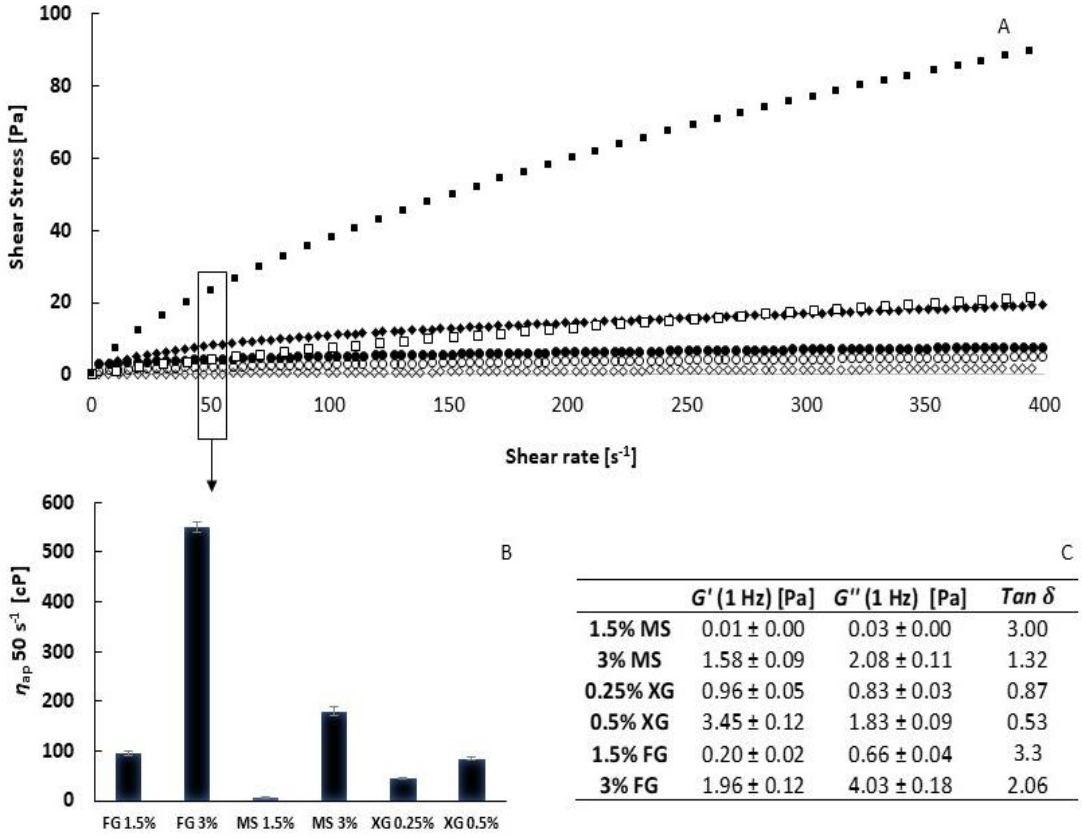
### 4.3.1. Rheological properties

All thickened liquid systems did not show phase separation or phases with reduced viscosity, aiming the reduction of their flow velocity from the mouth down to the oesophagus and thereby avoiding pulmonary aspiration caused by a dysfunctional airway protection.

#### 4.3.1.1 Biopolymer suspensions

The rheological behaviour of aqueous solutions of individual biopolymers (FG, MS and XG) was studied within the concentration range (intermediate and maximum levels) used to prepare the thickened formulations (see section 4.3.2). Figure 4.1 A shows that all flow curves presented a non-Newtonian shear-thinning behaviour (although at the lowest concentration of MS (0.75 %), the Newtonian fluid model was well-fitted). A slight thixotropy was observed for the MS solutions, which is in accordance with Anastadiases *et al.* (2002), and a shear time-independent behavior was verified for FG and XG aqueous solutions. The apparent viscosity values at 50 $s^{-1}$, used as a reference by the NDD to simulate the shear rate in the mouth, are shown in Figure 4.1 B. FG showed the highest viscosity (551 ± 10 cP) followed by MS (180 ± 9 cP) and XG (84 ± 3 cP) at the highest biopolymer concentration. On the other hand, at the intermediate concentration, MS (1.5 %) showed a very low apparent viscosity (6 ± 1 cP) even when compared to the 0.25 % of XG concentration (45 ± 2 cP).

Viscoelastic properties concerning $G'$ and $G''$ are presented in Figure 4.1 C. Regardless of biopolymers concentrations, the character of the solutions was not altered. FG and MS showed a prevailing viscous character ($tan\ \delta > 1$) whereas XG exhibited a predominated elastic behaviour (gel-like behaviour) ($tan\ \delta < 1$). At similar concentration of MS and FG, $G'$ and $G''$ values were higher for FG, but $G'$ was lower than XG at smaller concentration (0.5 %). The prevailing viscous behaviour ($G'' > G'$) of FG was also observed by Qian, Cui, Wu and Goff (2012), showing that this gum has a good thickening capacity.




| | G′ (1 Hz) [Pa] | G″ (1 Hz) [Pa] | Tan δ |
|---|---|---|---|
| 1.5% MS | 0.01 ± 0.00 | 0.03 ± 0.00 | 3.00 |
| 3% MS | 1.58 ± 0.09 | 2.08 ± 0.11 | 1.32 |
| 0.25% XG | 0.96 ± 0.05 | 0.83 ± 0.03 | 0.87 |
| 0.5% XG | 3.45 ± 0.12 | 1.83 ± 0.09 | 0.53 |
| 1.5% FG | 0.20 ± 0.02 | 0.66 ± 0.04 | 3.3 |
| 3% FG | 1.96 ± 0.12 | 4.03 ± 0.18 | 2.06 |

**Figure 4.1.** (A) Flow curves (FG (■), MS (◆) and XG (●)) of the maximum (full symbols) and intermediate (open symbols) concentrations of the used biopolymers, (B) apparent viscosity at 50 $s^{-1}$ (highlighted) and (C) loss ($G'$) and storage ($G''$) moduli and $tan\ \delta$ at 1 Hz.

#### 4.3.1.2. Thickeners' formulations

The flow curves of formulations that showed the highest viscosities (Formulations 4, 8 and 10, Table 4.1 in section 4.3.2) and the lowest viscosities (Formulations 1, 5 and 9, Table 4.1 in section 4.3.2) are represented in Figure 4.2 A. All thickeners solutions presented a shear time independent behaviour and a shear thinning non-Newtonian behaviour, as *n* values were lower than 1, which implies a decrease in the apparent viscosity at increased shear rates. Chains of polymer molecules are arbitrarily positioned when the thickened solutions are at quiescent conditions but after application of shear forces, these molecules become aligned in the same flow direction. The hydrodynamic forces deform aggregates that eventually disrupt resulting in a reduction of viscosity in the shear thinning fluids (Koocheki, Reza-Taherian & Bostan, 2013; Farahmandfar *et al.*, 2017).

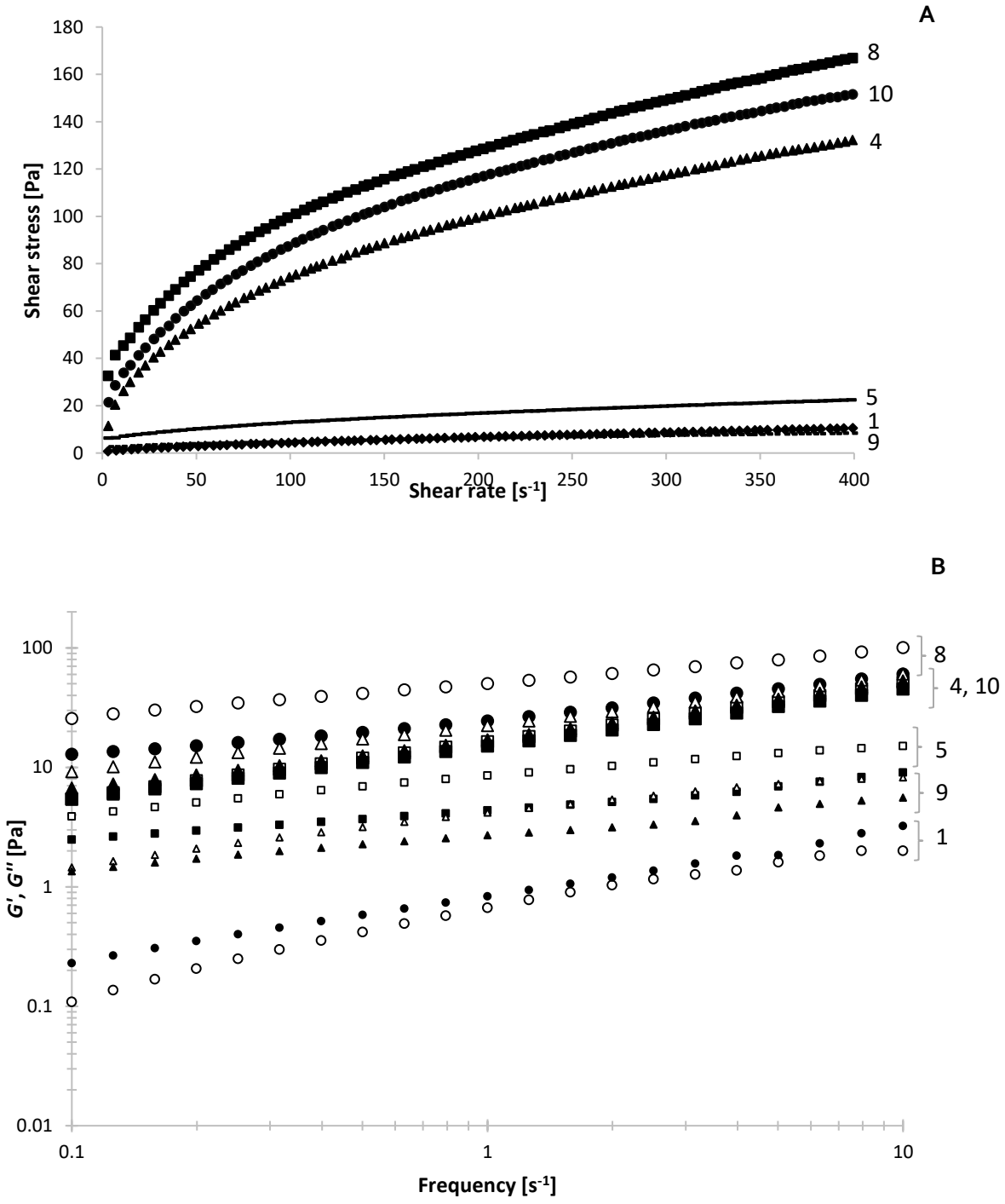



**Figure 4.2.** Representative flow curves of thickener formulations dissolved in water (A1). Highest viscosity: 4 (▲) (2.4 % FG, 2.4 % MS, 0.1 % XG), 8 (■) (2.4 % FG, 2.4 % MS, 0.4 % XG) and 10 (●) (3 % FG, 1.5 % MS, 0.25 % XG). Lowest viscosity: 1 (◆) (0.6 % FG, 0.6 % MS, 0.1 % XG), 5 (-) (0.6 % FG, 0.6 % MS, 0.4 % XG) and 9 (●) (1.5 % MS, 0.25 % XG). Elastic modulus (open symbols) and viscous modulus (closed symbols) (A2) as a function of frequency under isothermal (25 °C) conditions for the

extreme viscosity of thickener solutions (Lowest viscosity (small symbols): Formulation 1 (0.6 % FG, 0.6 % MS, 0.1 % XG (•); Formulation 5: 0.6 % FG, 0.6 % MS, 0.4 % XG (■); Formulation 9: 1.5 % MS, 0.25 % XG (▲)). Highest viscosity (large symbols): Formulation 4: 2.4 % FG, 2.4 % MS, 0.1 % XG (■); Formulation 8: 2.4 % FG, 2.4 % MS, 0.4 % XG (•); Formulation 10: 3 % FG, 1.5 % MS, 0.25 % XG (▲)) (A2).

Viscoelastic properties of these formulations were observed from oscillatory rheology. The magnitudes of *G'* and *G''* increased with the increase of frequency, indicating a slight frequency dependence. Higher *G'* and *G''* values were obtained for thickeners with higher concentration of FG and MS (represented by the extreme cases in Figure 4.2 B). It is noteworthy that most of the formulations presented a prevailing elastic behaviour (Figure 4.2 B) within the frequency range 0.1-10 Hz (*G' > G''*), which was not observed for single biopolymers except XG (Figure 4.1 C). Only Formulation 1, with the lowest concentration of each studied polymer, exhibited a prevailing viscous behaviour (liquid-like). Although individually FG can present a prevailing viscous behaviour at the studied concentrations (Figure 1.2), when it is dissolved together with another hydrocolloid a stronger network with elastic behaviour can be generated, increasing its energy storage capacity (Li *et al.*, 2012, Vieira *et al.*, 2019).

### 4.3.2. CCRD analysis of rheological properties

Apparent viscosity values at a fixed shear rate of 10 $s^{-1}$ ($\eta_{ap\ 10}$), 50 $s^{-1}$ ($\eta_{ap\ 50}$) and 400 $s^{-1}$ ($\eta_{ap\ 400}$), complex viscosity at 50 $rad.s^{-1}$ ($\eta^*$) flow index ($n$), loss tangent ($tan\ \delta$), elastic ($G'$) and viscous ($G''$) moduli at a fixed frequency of 1 Hz are shown in Table 4.1 for the different studied formulations according to CCRD.

**Table 4.1.** Rheological properties *G'* (1Hz), *G'* (1Hz)*'*, *tan δ* (1Hz), *n* (flow index), apparent viscosity at different shear rates ($\eta_{ap\ 10}$, $\eta_{ap\ 50}$ and $\eta_{ap\ 400}$) and complex viscosity at 50 rad.s-1 ($\eta^*$) of the thickener formulations (1-17) containing flaxseed gum (FG), modified starch (MS) and xanthan gum (XG) dispersed in water according to CCRD matrix.

| TF | FG (*x1*) | MS (*x2*) | XG (*x3*) | $\eta_{ap\ 10}$ | $\eta_{ap\ 50}$ | $\eta_{ap\ 400}$ | $\eta^*$ | *n* | *G'* (1 Hz) | *G''* (1 Hz) | *tan δ* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (%) | (%) | (%) | (cP) | (cP) | (cP) | (Pa.s) | | (Pa) | (Pa) | (1 Hz) |
| 1 | -1 (0.6)* | -1 (0.6) | -1 (0.1) | 105 | 57 | 26 | 0.15 | 0.62 | 0.66 | 0.83 | 1.26 |
| 2 | 1 (2.4) | -1 (0.6) | -1 (0.1) | 1265 | 529 | 172 | 1.15 | 0.46 | 11.25 | 7.54 | 0.67 |
| 3 | -1 (0.6) | 1 (2.4) | -1 (0.1) | 568 | 206 | 56 | 1.80 | 0.37 | 6.05 | 5.64 | 0.93 |
| 4 | 1 (2.4) | 1 (2.4) | -1 (0.1) | 2652 | 1064 | 327 | 3.10 | 0.43 | 16.76 | 14.83 | 0.89 |
| 5 | -1 (0.6) | -1 (0.6) | 1 (0.4) | 705 | 274 | 81 | 1.30 | 0.41 | 8.62 | 4.37 | 0.51 |
| 6 | 1 (2.4) | -1 (0.6) | 1 (0.4) | 908 | 477 | 287 | 0.55 | 0.52 | 4.17 | 6.17 | 1.48 |
| 7 | -1 (0.6) | 1 (2.4) | 1 (0.4) | 1275 | 440 | 112 | 4.50 | 0.34 | 21.79 | 10.54 | 0.48 |
| 8 | 1 (2.4) | 1 (2.4) | 1 (0.4) | 4199 | 1528 | 414 | 8.70 | 0.37 | 57.98 | 25.00 | 0.43 |
| 9 | -1.68 (0) | 0 (1.5) | 0 (0.25) | 229 | 80 | 21 | 0.70 | 0.35 | 4.31 | 2.70 | 0.63 |
| 10 | 1.68 (3) | 0 (1.5) | 0 (0.25) | 3356 | 1299 | 381 | 3.76 | 0.41 | 21.90 | 16.70 | 0.60 |
| 11 | 0 (1.5) | -1.68 (0) | 0 (0.25) | 387 | 190 | 76 | 0.67 | 0.56 | 3.71 | 2.99 | 0.81 |
| 12 | 0 (1.5) | 1.68 (3) | 0 (0.25) | 2553 | 931 | 253 | 5.80 | 0.37 | 37.69 | 19.69 | 0.52 |
| 13 | 0 (1.5) | 0 (1.5) | -1.68 (0) | 458 | 241 | 105 | 0.28 | 0.60 | 0.67 | 1.73 | 2.58 |
| 14 | 0 (1.5) | 0 (1.5) | 1.68 (0.5) | 2303 | 776 | 190 | 4.61 | 0.32 | 32.17 | 13.30 | 0.41 |
| 15 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 983 | 442 | 127 | 1.93 | 0.49 | 8.90 | 5.37 | 0.60 |
| 16 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 1023 | 451 | 142 | 1.91 | 0.50 | 9.50 | 6.19 | 0.65 |
| 17 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 996 | 422 | 139 | 1.94 | 0.47 | 8.27 | 5.99 | 0.72 |

*Coded and uncoded values (in parenthesis) representing biopolymers concentration.

In general, an increase of FG, MS and XG concentration caused an increase in the apparent viscosity and pseudoplasticity of the formulations. A higher content of total solids in solution can hinder the intermolecular movement induced by hydrodynamic forces, indicating a good interaction (or at least non-repulsive interactions) between the different components (Capitani *et al.*, 2015). Viscosity values at a shear rate of 10 $s^{-1}$, 50 $s^{-1}$ and 400 $s^{-1}$ ranged from 105 to 4199 cP, 57 to 1528 cP and 21 to 414 cP, respectively. Nectar-like (Formulations 1, 3, 5, 9, 11 and 13) and honey-like (Formulations 2, 4, 6, 7, 8, 10, 12, 14, 15, 16 and 17) consistency was achieved, although there were two formulations that approached the spoon-like consistency (Formulations 8 and 10), both containing high FG concentration.

Only Formulation 1 may compromise the swallowing safety for patients with dysphagia, regarding the viscosity of shear rate of 50 $s^{-1}$, since this measured viscosity was near the lowest recommended limit value (50 cP). This low viscosity can provide a quicker flow from the mouth to the laryngeal airway before it is closed, leading to aspiration into the lungs (O'Leary *et al.*, 2010). The combination of biopolymers (FG,

MS and XG) concentrations according to the experimental design could establish positive interactions in terms of the viscosity, since the quantity of total solids in some thickening formulations was low in comparison to individual biopolymers (Figure 4.1) and commercial thickeners. Regarding the studied formulations, it was observed that only 1.3 % w/v of total solids (Formulation 1) was necessary to achieve nectar-like consistency, while to reach values close to the spoon-thick category, 5.2 % w/v of total solids were required. Previous works have studied the rheological behaviour of commercial thickeners for dysphagia patients and observed that the XG-based thickener only required a thickener concentration of 0.88 % w/v in aqueous solutions (total solids) to obtain the nectar-like viscosity, although a thickener concentration above 11.5 % (w/v) was needed to reach a viscosity near the category of spoon-thick (1345 ± 87 cP). Similar content was necessary to observe high viscosity values in a commercial XG+MS-based thickener (11% w/v) but a higher amount of total solids was used (1.5 % w/v) to obtain the nectar-like category. On the other hand, the commercial MS-based thickener needed a concentration close to 7 % w/v to achieve spoon-like categorization (1691 ± 218 cP), but 4 % w/v of total solids had to be incorporated to reach the nectar-like behaviour (Hadde & Chen, 2019). In other words, the biopolymers' interactions in the present study demonstrate an efficiency which is similar to the best scenario with respect to commercial thickeners (XG- and XG+MS- based) in order to reach the nectar-like consistency, but using a significantly lower amount of total solids to achieve equally high consistency values.

Apparent viscosity is related to the flow velocity of a food bolus, but other rheological properties can complement information associated to sensory perception. Pseudoplasticity (represented by the flow index (*n*)) and complex viscosity at 50 rad.s$^{-1}$ ($\eta^*$) are associated to sliminess in the mouth and perceived ease of swallowing (Richardson *et al.*, 1989). The flow index of all samples, which indicates the extent of shear-thinning behaviour, ranged from 0.32–0.62 like in most high molecular weight polymers (Mandala, Savvas & Kostaropoulos, 2004). The lowest *n* value belongs to the thickened formulation with the highest concentration of XG (Formulation 14). A more pronounced shear-thinning behaviour allows easier swallowing of the thickened liquids by dysphagic patients while also reducing organoleptic sliminess, resulting in a pleasant mouthfeel (Jo *et al.*, 2018). On the other hand, the highest *n* value was obtained with Formulation 1, the polysaccharide mixture with the lowest biopolymer concentrations, and Formulation 13, a thickened solution without XG.

*Tan* $\delta$ smaller than the unity and high *G'* values have been suggested as a rheological criterion for safe-swallowing (Steffe, 1996; Jo *et al.*, 2018). As shown in Table 4.1, most of the samples exhibited a weak gel-like behaviour, including the formulations without FG (Formulation 9) and MS (Formulation 11), since *G'* values were higher than *G″* values (*tan* $\delta$ was lower than 1) regardless of the frequency (between 0.1-10 Hz). The formulation with the highest concentration of XG (Formulation 14) showed the lowest value of *tan* $\delta$ (0.41), while the formulation without XG (Formulation 13) showed the highest *tan* $\delta$ value (2.58) or prevailing viscous behaviour, indicating a potential difficulty in the dysphagic swallowing process for formulations without xanthan addition. Considering this assumption, it can be concluded that interactions between XG and MS/FG could contribute even more positively to the elastic properties and, consequently, to the swallowing process.

#### 4.3.2.1. Multivariate analysis: mathematical modelling

Once the rheological behaviour is a critical factor to be analysed in dysphagia patients' diet, mathematical models were fitted to the experimental data, to allow a more consistent description of the effect of thickener combinations in the formulations. Thus, concentration of FG (*x1*), MS (*x2*) and XG (*x3*) were analysed to evaluate the effect on the rheological properties using a CCRD (Table 4.1). The regression coefficients of the mathematical models used to predict the rheological properties of the different formulations were calculated and mathematical models were built for the responses of $\eta_{ap\ 10}$, $\eta_{ap\ 50}$, $\eta_{ap\ 400}$, $\eta*$, *n*, *G'* and *G''*. All properties shown in Table 4.2 presented a significant ($p < 0.1$) non-linear behaviour as a function of the variables. The correlation coefficients and the F-test ($F_{calc}/F_{tab} > 1$) showed that the models were reliable. Only statistically significant ($p < 0.1$) coefficients were maintained and the validity of each model was verified through Analysis of Variance (ANOVA). It should be noted that central points (Formulations 15, 16 and 17) showed repeatability for all parameters (Table 4.1) and, therefore, the study can be considered valid.

The same studied formulations incorporated in water according to the CCRD were analysed in milk and soy juice matrices, where the F-test were reliable and central points presented repeatability (data and discussion presented in appendix B).

**Table 4.2.** Percentage of variance explained ($R^2$), calculated F ($F_{calc}/F_{tab}$) for the responses: apparent viscosity at different shear rates ($\eta_{ap\ 10}$, $\eta_{ap\ 50}$, and $\eta_{ap\ 400}$), complex viscosity ($\eta*$) flow index ($n$) and viscoelastic properties ($G'$ and $G''$), by analysis of variance (ANOVA). Statistically significant models have $F_{calc}/F_{tab}$ values greater than 1.

| **Response equations** | $R^2$ | $F_{calc}/F_{tab}$ |
|---|---|---|
| $\eta_{ap\ 10}$ (cP) = 1251.45 + 851.54x1 + 684.91x2 + 410.15x3 + 455.75x1x2 + 251.50x2x3 + 196.80x1$^2$ | 0.96 | 14.63 |
| $\eta_{ap\ 50}$ (cP) = 496.72 + 341.94x1 + 230.50x2 + 129.01x3 + 158.86x1x2 + 66.58x2x3 + 70.45x1$^2$ | 0.98 | 30.16 |
| $\eta_{ap\ 400}$ (cP) = 143.82 + 112.11x1 + 46.97x2 + 33.32x3 + 27.81x1x2 + 11.29x1x3 + 23.36x1$^2$ + 10.44x2$^2$ | 0.99 | 49.56 |
| $n$ = 0.47 - 0.06x2 – 0.05x3 + 0.03x1x3 - 0.03x1$^2$ | 0.80 | 4.80 |
| $G'$ (1Hz) (Pa) = 12.50 + 6.05x1 + 9.89x2 + 8.11x3 + 5.10x1x2 + 7.01x2x3 + 3.07x2$^2$ | 0.92 | 7.48 |
| $G''$ (1Hz) (Pa) = 6.52 + 4.08x1 + 4.77x2 + 2.69x3 + 1.89x1x2 + 1.61x2x3 + 1.13x1$^2$ + 1.71x2$^2$ | 0.97 | 16.57 |
| $\eta*$ (Pa.s) = 2.20 + 0.80x1 + 1.73x2 + 1.18x3 + 0.66x1x2 + 0.97x2x3 + 0.39x2$^2$ | 0.96 | 13.11 |

x1 - FG
x2 - MS
x3 - XG

From the analysis of the mathematical models it was possible to observe that the viscosity was positively and significantly influenced by all biopolymers regardless of the applied shear rate in the following order: FG > MS > XG. Figure 4.3 shows the values of the apparent viscosity at a shear rate of 50 $s^{-1}$ obtained experimentally and predicted by the model as a function of the concentration of FG, MS and XG. By the explained variation ($R^2$), the results presented a good fit and this fact is representative for all the studied shear rates. Besides, MS presented a high positive interaction with FG as can be observed in Figure 4.3B. MS also showed a positive interaction with XG at lowest shear rates, while FG+XG interaction was evident only at 400 $s^{-1}$. Under tested conditions, XG presented a minor influence on viscosity compared to the other biopolymers. However, considering that the concentration range used for this gum (0-0.5 %) was quite smaller than those used for other biopolymers (0-3 %), XG exerted a strong influence in viscosity. Xanthan can form high viscosity solutions, especially at low shear rates, as a consequence of XG aggregates formed through hydrogen bonding and polymer entanglements (Russ *et al.*, 2014). Under the influence of high shear rates, the steady shear viscosity of XG solutions is decreased due to the disentanglement of

the polymer network and the partial alignment of the individual macromolecules in the direction of the shear flow (Song, Kim & Chang, 2006) in a more marked way than the other biopolymers, resulting in a strong influence of this gum on *n* values or pseudoplasticity (as shown in Table 4.2). Interactions between MS and/or XG with other polysaccharides have been studied with promising results in the most diverse areas (Heyman, De Vos, Meeren &, Dewettinck, 2014; Zhan, Ridout, Brownsey & Morris, 1993). When aqueous dispersions of MS and XG were compared with mixed gums systems (XG-MS or XG-other gums), an increase of the viscosity was also observed, which may be related to the larger shear forces exerted on the mixed structure than those encountered in individual systems (Mandala, Palogou & Kostaropoulos, 2002).

Despite of the low viscosity and pseudoplasticity (Figure 4.1) of MS aqueous solutions, this biopolymer showed a strong influence on both parameters when incorporated in solutions together with XG and FG (Table 4.2). A potential synergy between MS and XG was observed from the decrease of the flow index in previous rheological studies of this mixed system (Kim & Yoo, 2006; Hadde & Chen, 2019), which can have resulted in the commercialization of products containing both biopolymers for dysphagia patients. Szczesniak and Farkas (1963) reported that high weight polysaccharides with a high *n* value lead to a slimy feel in the mouth, showing that XG addition can favour sensory acceptance of thickeners. The *n* value of FG varied between 0.83 and 0.58 for the concentrations of 0.75 % and 3 % (w/w), respectively (Vieira *et al.*, 2019). Therefore, an equilibrated addition of FG, MS and XG can hinder an organoleptic sliminess feeling when consuming the thickened liquids.

Dynamic rheological analysis of thickened liquids has shown that viscoelastic properties (*G', G''*) were positively influenced by all biopolymers (Table 4.2). Elastic properties, represented by the *G'* value, are associated to the bolus formation helping in a safe and easy swallowing (Jo *et al.*, 2018). Figure 4.1 showed that FG exhibits a prevailing viscous rather than elastic behaviour up to a concentration of 3 % w/w. However, according to Table 4.2, this biopolymer showed a significant positive influence on the elastic properties (Vieira *et al.*, 2019) and synergistic interaction with MS in aqueous solution. A similar behaviour was observed for MS but in a more relevant way, since starch showed interaction with both FG and XG according to the established mathematical model represented in Table 4.2. This result displays the relevant synergistic interactions between modified starch and other biopolymers, since

the MS aqueous solution showed low elastic properties (Figure 4.1). Previous studies dealing with xanthan/starch mixtures showed that XG strengthened the interactions with modified starch, since the elastic modulus of the mixed system was higher than the pure additive contribution for both starch and xanthan (Abdulmola *et al.*, 1996; Kim & Yoo, 2006; Russ, Zielbauer, Ghebremedhin & Vilgis, 2016). Oppositely, XG alone induces an overall increase in elastic behaviour even at low concentrations (Figure 4.1) (Choi & Yoo, 2009; Choi *et al.*, 2014; Choppe, Puaud, Nicolai & Benyahia, 2014), since this gum is generally capable of establishing strong intermolecular associations (Heyman, De Vos, Van der Meerer & Dewettinck, 2014). XG could surround modified starch forming a binder thin film and thus forming a stronger network (Mandala & Palogou, 2003). These facts may explain why XG impact on elastic properties (*G'*) was higher than FG and close to MS although the studied XG concentration range was lower than FG and MS. Therefore, a small content of XG added in FG and MS solutions can increase the solid-like behaviour and even change rheological behaviour from viscous to elastic prevailing. However, it is remarkable that MS was the component with the greatest influence on both viscoelastic properties (*G'* and *G''*).

Complex viscosity $\eta^*$ can also give information about the sensory consistency, as perceived thickness, of the viscoelastic weak gels as the studied thickened formulations (Richardson *et al.*, 1989). The increase in MS concentration was the greatest contribution to complex viscosity, followed by that of XG. Indeed, the highest value of $\eta^*$ was usually observed for formulations with higher MS concentrations. This fact once again demonstrates that the mixture of starch with other hydrocolloids shows synergistic effects, improving the stability and the viscosity of the thickened solutions (Shalviri, Liu, Abdekhodaie & Wu, 2010). Thus, formulations with higher $\eta^*$ represent structured gels, promoting a safer and pleasant swallowing, whereas those with low $\eta^*$ presents a more fluid-like consistency that can result in aspiration more easily (Veiga, Cunha, Viotto & Petenate, 2000; He, Hort & Wolf, 2016).

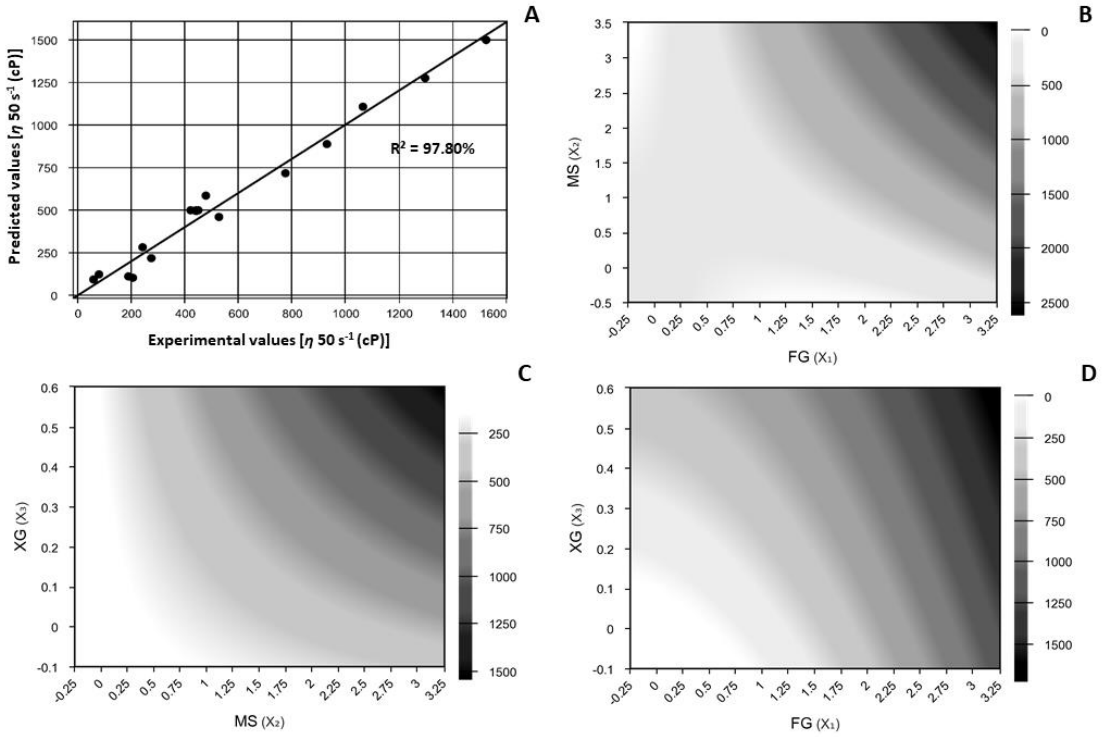



**Figure 4.3.** Experimental versus predicted values for response $\eta_{ap\ 50}$ (A) and the surface response of $\eta_{ap\ 50}$ (see grey scales on the right side of each graph) as a function of significant interactions of FG, MS and XG concentrations in thickened formulations for: XG concentration fixed at 0.25 % (B), FG concentration fixed at 1.5 % (C) and MS concentration fixed at 1.5 % (D).

From the response surfaces and contour curves for viscosity at 50 $s^{-1}$ (Figure 4.3), three formulations were produced to confirm and validate the mathematical model, aiming at representing each categorization published by NDD (nectar-like, honey-like and spoon thick). Thus, to validate the predictions obtained with the mathematical models for the viscosity at 50 $s^{-1}$, new formulations within the studied concentration range were prepared, in triplicate, as shown in Table 4.3. Validation of the viscosity response at 50 $s^{-1}$ was selected for presentation to observe if the categorization was respected, since it currently represents the most relevant data for patient's safety. Concentrations of the three biopolymers were selected to obtain more bioactive formulations with representative viscosities of the three categorizations described by NDD.

**Table 4.3.** Predicted and experimental viscosity at a shear rate of 50 $s^{-1}$ obtained from new formulations for validation of the previous results.

| | | Formulation (%) | | | Experimental (cP) | Predicted (cP) | Relative Error (%) |
|---|---|---|---|---|---|---|---|
| **Matrix** | **Categorization** | **FG** | **MS** | **XG** | $\eta_{ap\ 50}$ | $\eta_{ap\ 50}$ | |
| | Nectar[1] | 1.5 | 0.15 | 0.1 | 121 ± 8 | 124 ± 62 | 2.4 |
| **Water** | Honey[2] | 1.5 | 1.5 | 0.25 | 455 ± 18 | 498 ± 26 | 8.6 |
| | Spoon[3] | 2.5 | 3 | 0.25 | 2023 ± 40 | 1860 ± 95 | 8.8 |

[1] 51-350 cP*
[2] 351-1750 cP*
[3] >1750 cP*

Experimental values of viscosity agreed with the model's prediction within the representative range of the categorization established by NDD. At lower viscosity, the experimental values were within the standard deviations of predicted values. However, for the categorization of spoon thick, although the viscosity presented experimental values above the expected by the model, they were still acceptable (relative error lower than 9 %). The experimental results obtained in this matrix are within the range recommended in the guidelines generated by the NND.

### 4.3.3. Fluorescence microscopy imaging

The improvement of the viscoelastic and pseudoplastic properties of the thickened formulations comprised of FG, XG and MS can be explained by the kind of structures formed (Figure 4.4) between the biopolymers. It was observed that the mixture between MS and FG forms a well-mixed structure with both biopolymers. MS presented a structure of embedded particles (Figure 4.4 B) promoting an intermolecular physical interaction (Figure 4.4 D) with the FG flexible polymer chains (Figure 4.4 A). We suggested from the interpretation of all available images (representative illustration) that the FG molecules are not only adsorbed on the MS particles but also entrapped and intertwined with them. Thus, the presence of a random coil polymer as FG presumably increases the number of interactions forming a more homogeneous three-dimensional physical structure of thickened formulations (Russ *et al.*, 2016). XG is also capable of association with the MS surface (Chaisawang & Suphantharika, 2006, Heyman *et al.*, 2014), but a micro-phase separation was previously observed between XG and tapioca starch (Russ *et al.*, 2016). According to Figure 4.4 E, the two effects were observed, since although a well-mixed structure was not observed between both polymers, an interaction between the two polymers was observed on the external surface of the

structure. Similar interaction was observed between a pregelatinized starch and ι-carrageenan (Russ *et al.*, 2014). These results suggest that XG (present in rod-like conformation) can associate with the swollen MS particules possibly through non-electrostatic interactions (Heyman *et al.*, 2014) forming a more heterogeneous structure.

Therefore, the mobility and diffusion of XG and FG molecules lead to the formation of a stronger physical structure when mixed with MS, influencing the rheological behaviour that results in a predominance of elastic over viscous behaviour (weak gel) (Chaisawang & Suphantharika, 2006; Heyman *et al.*, 2014).

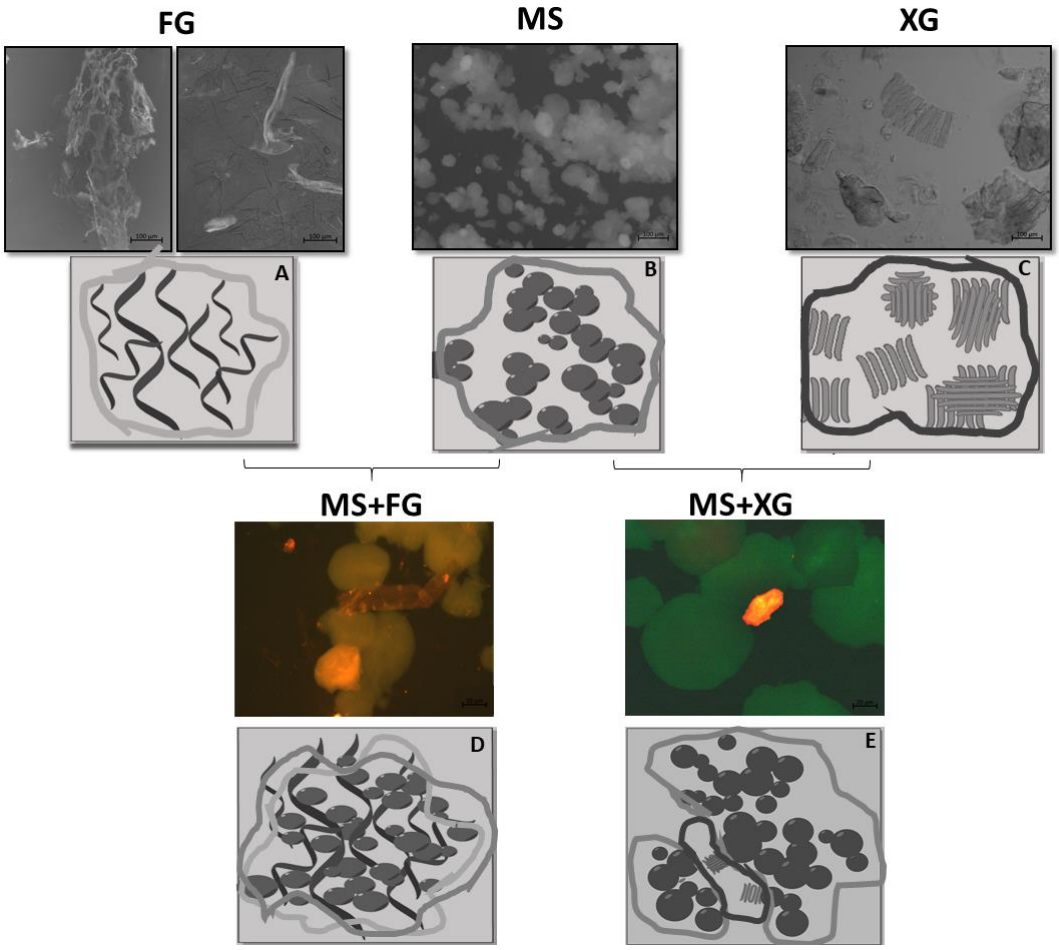



**Figure 4.4.** Fluorescence microscopy of 1.5 % w/w MS (A), 1.5 % w/w FG (B) and 0.5 % XG (C) (objective: 10×). 1.5 % w/w MS produced by the mixtures of MS (green)+FG (orange) (D) and MS (green)+XG (orange) (E) (objective: 40×) in 11–1 ratio). All pictures are followed by the respective schematic illustration of the suggested network structure.

### 4.3.3 Colorimetric analyses

The colour of the product plays a significant role on its acceptability for consumers. Colour parameters ($L^*$, $C^*$ and $\Delta E^*$) of thickened solutions were analysed and results are presented in Table 4.4. In general, thickeners addition modified the colour parameters observed from a colorimeter, although the visual appearance only showed greater opacity and a slight colour change.

**Table 4.4.** $L^*$, $C^*$ and $\Delta E^*$ values of control (C - water) and thickened formulations (TF).

| TF | FG (x1) (%) | MS (x2) (%) | XG (x3) (%) | L* | C* | ΔE* |
|---|---|---|---|---|---|---|
| **C** | - | - | - | 27.9 ± 0.2$^{j}$ | 0.6 ± 0.0$^{g}$ | - |
| **1** | -1 (0.6)* | -1 (0.6) | -1 (0.1) | 30.0 ± 0.1$^{h}$ | 0.5 ± 0.0$^{g}$ | 2.5 ± 0.1 |
| **2** | 1 (2.4) | -1 (0.6) | -1 (0.1) | 30.6 ± 0.1$^{g}$ | 0.6 ± 0.1$^{g}$ | 2.8 ± 0.3 |
| **3** | -1 (0.6) | 1 (2.4) | -1 (0.1) | 31.8 ± 0.1$^{f}$ | 1.3 ± 0.0$^{e}$ | 4.4 ± 0.2 |
| **4** | 1 (2.4) | 1 (2.4) | -1 (0.1) | 37.2 ± 0.8$^{d}$ | 2.0 ± 0.0$^{c}$ | 9.4 ± 0.8 |
| **5** | -1 (0.6) | -1 (0.6) | 1 (0.4) | 31.4 ± 0.2$^{f}$ | 1.4 ± 0.1$^{e}$ | 4.1 ± 0.1 |
| **6** | 1 (2.4) | -1 (0.6) | 1 (0.4) | 39.9 ± 0.3$^{b}$ | 2.8 ± 0.1$^{b}$ | 12.2 ± 0.7 |
| **7** | -1 (0.6) | 1 (2.4) | 1 (0.4) | 35.1 ± 0.3$^{e}$ | 0.8 ± 0.1$^{f}$ | 7.3 ± 0.2 |
| **8** | 1 (2.4) | 1 (2.4) | 1 (0.4) | 42.1 ± 0.2$^{a}$ | 2.7 ± 0.1$^{b}$ | 14.3 ± 0.9 |
| **9** | -1.68 (0) | 0 (1.5) | 0 (0.25) | 29.0 ± 0.1$^{i}$ | 1.7 ± 0.1$^{d}$ | 2.6 ± 0.1 |
| **10** | 1.68 (3) | 0 (1.5) | 0 (0.25) | 41.2 ± 0.2$^{b}$ | 3.4 ± 0.0$^{a}$ | 13.6 ± 0.3 |
| **11** | 0 (1.5) | -1.68 (0) | 0 (0.25) | 31.7 ± 0.2$^{f}$ | 0.4 ± 0.0$^{h}$ | 3.9 ± 0.3 |
| **12** | 0 (1.5) | 1.68 (3) | 0 (0.25) | 38.7 ± 0.2$^{c}$ | 1.4 ± 0.1$^{e}$ | 10.8 ± 0.4 |
| **13** | 0 (1.5) | 0 (1.5) | -1.68 (0) | 28.0 ± 0.2$^{j}$ | 1.1 ± 0.2$^{e}$ | 1.8 ± 0.1 |
| **14** | 0 (1.5) | 0 (1.5) | 1.68 (0.5) | 36.9 ± 0.2$^{d}$ | 0.6 ± 0.0$^{g}$ | 9.1 ± 0.1 |
| **15** | 0 (1.5) | 0 (1.5) | 0 (0.25) | 35.6 ± 0.5$^{e}$ | 0.6 ± 0.0$^{g}$ | 7.7 ± 0.4 |

a-k Different letters in the same column correspond to statistically different samples for a 95% confidence level.

*Coded and uncoded values (in parenthesis) representing biopolymers concentration.

Reflectance spectrophotometry indicated a change in the colour of the samples mainly due to a significant variation ($p < 0.05$) of the lightness ($L^*$) values for the different formulations. For example, $L^*$ value was 27.9 ± 0.2 for the control (water) and in formulation 8 this value increased to 42.1 ± 0.2 (extreme case). As relevant changes were observed in colour parameters, mathematical models were generated (Table 4.5) to analyse the impact of the biopolymers' concentration on this matrix. The colour parameters were mainly influenced by the FG concentration followed by MS and XG. FG exerted a significant ($p < 0.05$) effect on $L^*$ and chroma ($C^*$) (Tables 4.4 and 4.5);

the latter is associated to colour saturation that is indicating a higher colour intensity as can be seen in Formulations 4, 6, 8 and 10 (higher FG concentration). This fact was confirmed by $\Delta E^*$ values, since they were quite high (always $\Delta E^* > 8$) for these formulations, concluding that these thickeners formulations can present color alteration compared to the control matrix (Olivas and Barbosa-Cánovas, 2005). Formulations 1, 2, 3, 5, 7, 9, 11, 13 and 15 presented a $\Delta E^*$ designated as "very good" according to the American Society for Testing and Materials, since $\Delta E^*$ were always below 8, confirming the small colour differences observed directly by the eye. XG presented reduced influence on colour properties, showing only interaction effect with FG when evaluating colour difference with the control.

**Table 4.5.** Regression coefficients of the mathematical models generated (coded model) for the colour parameters ($L^*$, $C^*$ and $\Delta E^*$) of biopolymers dispersed in water, with the respective coefficient of determination ($R^2$). Statistically significant models have $F_{calc}/F_{tab}$ values greater than 1.

| **Response equations** | $R^2$ | $F_{calc}/F_{tab}$ |
|---|---|---|
| $L^* = 35.49 + 3.08x1 + 1.91x2 + 2.48x3 + 1.19x1x3 - 0.96x3^2$ | 0.96 | 19.00 |
| $C^* = 0.8 + 0.51x1 + 0.23x2 + 0.31x1x3 - 0.36x2x3 + 0.65x1^2$ | 0.86 | 5.21 |
| $\Delta E^* = 7.78 + 2.85x1 + 1.86x2 + 1.23x1x3 - 0.77x1^2$ | 0.96 | 20.58 |

x1 - FG
x2 - MS
x3 - XG

In sum, FG was the component that showed the greatest influence on $L^*$ and $C^*$ parameters, and this was mirrored in the impact that its concentration provokes on the colour modification, since the $\Delta E^*$ was more influenced by this biopolymer than by MS and XG. Thus, this biopolymer is fundamental to rheological properties, but it should be added in moderate concentration to avoid colour changes.

### 4.3.4. Glucose response

Static *in vitro* digestion was performed to quantify the amount of glucose released from the different thickener formulations, which is shown in Figure 4.5. At the end of the *in vitro* digestion process, higher glucose levels were observed for both systems represented by the commercial formulations, CTS (123.25 ± 6.18 mg) with a spoon-

thick consistency and CTN (73.35 ± 4.88 mg) with a nectar-like consistency, since these were the formulations containing the highest starch content (465 and 233 mg of starch to 5 ml of sample, respectively). Formulations 3 (46.89 ± 2.30 mg) and 8 (53.26 ± 3.25 mg), both with 2.4 % of MS, presented similar values of released glucose at the end of the *in vitro* digestion, despite the FG concentration was considerably higher in Formulation 8. Moreover, viscosity of Formulation 3 (206 cP) was quite lower than the Formulation 8 (1528 cP), which allows concluding that a spoon-thick consistency could be achieved with a reduced release/absorption of glucose. Or a nectar-like consistency could be produced with even lower released glucose content if the combination of biopolymers content considered a reduction of starch content.

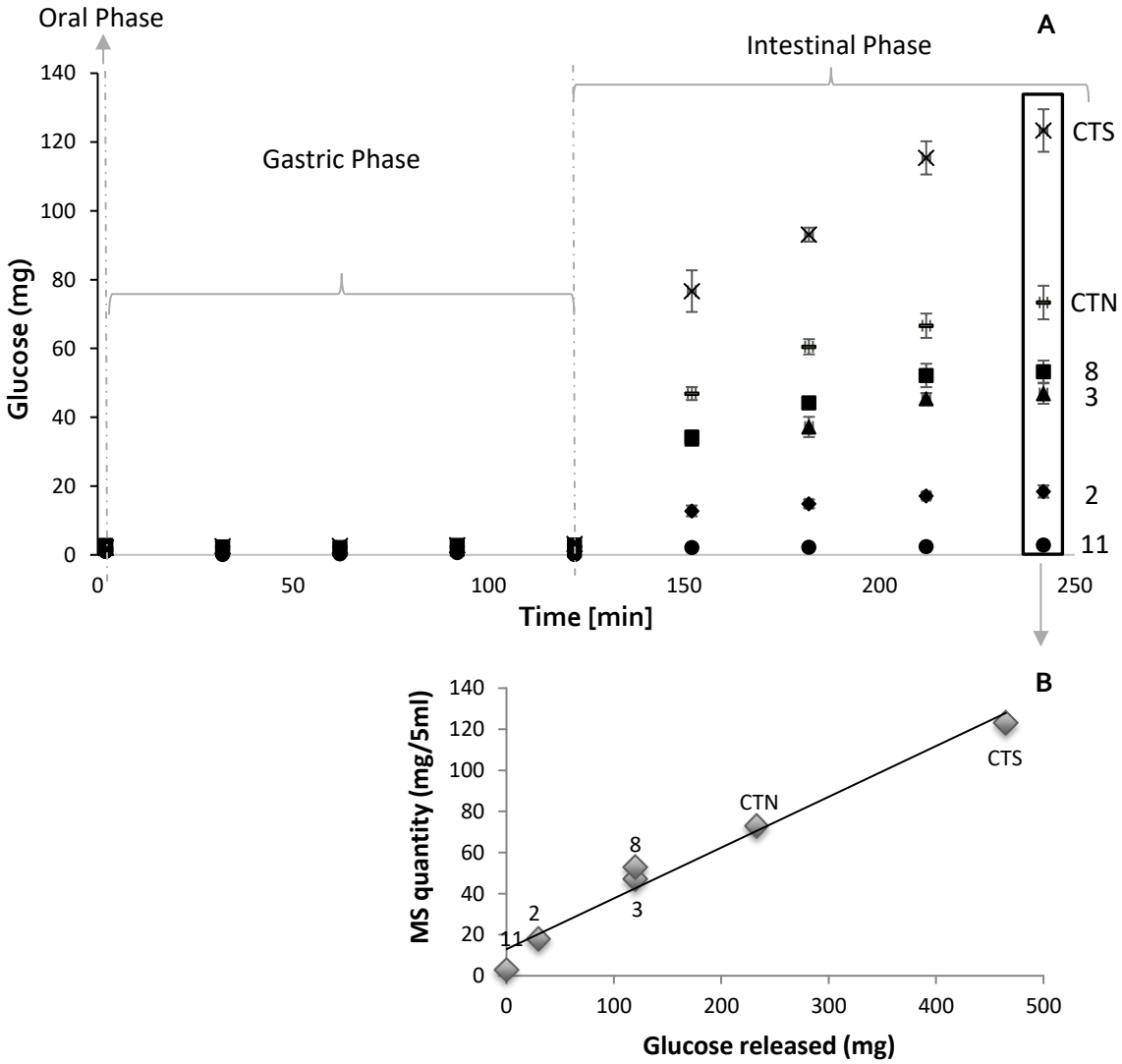



**Figure 4.5.** Amount of glucose released during the *in vitro* digestibility (A) mount of MS quantity to 5 mL of thickened solutions *versus* glucose released during the *in vitro*

digestibility (B) of the different thickened formulations: Commercial thickener - Spoon-thick consistency (CTS) (×); Commercial thickener - nectar consistency (CTN) (-); Formulations 2 (2.4 % FG, 0.6 % MS, 0.1 % XG) (◆); 3 (0.6 % FG, 2.4 % MS, 0.1 % XG) (■); 8 (2.4 % FG, 2.4 % MS, 0.4 % XG) (▲); 11 (1.5 % FG, 0.25 % XG) (●). It was considered the different phases: oral (0 to 2 min), gastric (2 to 122 min) and intestinal (122 to 242 min).

These results may be related to the following facts: a) the low amount of glucose present in the FG chain was not sufficient to increase the glucose released or b) FG incorporated in Formulation 8 results in a slower glucose release, since the FG dietary fibres may encapsulate starch resulting in a slowly available glucose (Englyst, Englyst, Hudson, Cole & Cummings, 1999). The former consideration (option a) is quite reasonable since Formulation 11 (without addition of MS) showed a very low glucose content (2.90 ± 0.34 mg), confirming that, glucose released from FG and XG was significantly lower than the MS. In addition, the formulation with honey-like consistency (Formulation 2) and intermediate MS concentration (0.6% MS) showed the lowest amount of glucose released (18.43 ± 1.80 mg) regarding to the other formulations with MS (with 2.4% MS). This result also emphasizes that the glucose content released is mostly from MS. These facts demonstrates, again, the importance of the interactions between the biopolymers and their influence on physical and nutritional properties, since the increase of FG concentration (and MS concentration decrease, 2.4 % to 0.6 %) increased the consistency/viscosity from nectar (Formulation 3 – 206 cP) to honey-like (Formulation 2 – 529 cP) with a significant decrease of glucose released because FG is richer in fibres, as can be observed in Figure 4.5B (Moczkowska, Karp, Niu & Kurek, 2019). This result suggests that FG is a healthier option for thickeners formulations, and may partially replace starch, depending on the desired viscosity levels, without compromising disphagic patient's safety.

### 4.4. Conclusions

A relation between functionality/rheological properties of thickeners and their benefits in terms of health, increasing fibers and phenolic content and decreasing glucose absorption, was considered regarding the incorporation of flaxseed gum (FG) in thickening formulations for dysphagia patients. FG showed the greatest positive

influence on viscosity followed by modified starch (MS). However, xanthan (XG) demonstrated a more significant contribution to the pseudoplasticity and elasticity of the formulations, even at lower concentrations than FG and MS. The influence of MS was mainly relevant in relation to the synergistic interactions with the other two biopolymers, showing the key role of this biopolymer on rheological properties of mixed systems. Regarding the colour, FG exerted a more significant influence on colour ($L^*$ and $C^*$) parameters, when compared with MS and XG, with a significant impact on $\Delta E^*$ at high FG concentration. In addition, it was observed that the glucose released from the thickened beverages based on high MS concentrations upon digestion was considerable, which can delay the recovery of dysphagia patients with problems of obesity and/or diabetes. Thus, thickened beverages based on FG may have advantages in this regard over those based on MS, improving the quality and longevity of dysphagia patients' life and diminishing the consequences on the nutritional profile of the diets imposed by the treatments to which they are subjected.

### 4.5. Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (Finance code 001); Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP) (Process numbers 2016/05448-8; 2011/51707-1; EMU 2009/54137-1; 2007/58017-5; 2006/03263-9; 2004/08517-3) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq- Process 307168/2016-6)).

### 4.6. References

Abdulmola, N.A., Hember, M.W.N., Richardson, R.K., Morris E.R., 1996. Effect of xanthan on the small-deformation rheology of crosslinked and uncrosslinked waxy maize starch. Carhohydrate Polymers, 31, 65-78.

Achayuthakan, P. and Suphantharika, M., 2008. Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. Carbohydrate Polymers, 71(1), 9-17.

Anastasiades, A., Thanou, S., Loulis, D., Stapatoris, A., Karapantsios, T.D., 2002. Rheological and physical characterization of pregelatinized maize starches. Journal of Food Engineering, 52, 57–66.

Capitani, M.I., Ixtaina, V.Y., Nolasco, S.M., Tomás, M.C., 2012. Microstructure, chemical composition and mucilage exudation of chia (*Salvia hispanica* L.) nutlets from Argentina. Journal of the Science of Food and Agriculture, 93 (15), 3856-3862.

Chaisawang, M. and Suphantharika, M., 2006. Pasting and rheological properties of native and anionic tapioca starches as modified by guar gum and xanthan gum. Food Hydrocolloids, 20(5), 641-649.

Choi, H.M. and Yoo, B., 2009. Steady and dynamic shear rheology of sweet potato starch–xanthan gum mixtures. Food Chemistry, 116(3), 638-643.

Choi, H., Mitchell, J.R., Gaddipatia, S.R.,Hill, S.E., Wolf, B., 2014. Shear rheology and filament stretching behaviour of xanthan gum and carboxymethyl cellulose solution in presence of saliva. Food Hydrocolloids, 40, 71-75.

Choppe, E., Puaud, F., Nicolai, T., Benyahia, L., 2010. Rheology of xanthan solutions as a function of temperature, concentration and ionic strength. Carbohydrate Polymers, 82(4), 1228-1235.

Clavé, P. and Shaker, R., 2015. Dysphagia: current reality and scope of the problem. Nature Reviews. Gastroenterology and Hepatology, 12, 259-270.

Cui, W., Mazza, G., Biliaderis, C.G, 1994. Chemical Structure, Molecular Size Distributions, and Rheological Properties of Flaxseed Gum. Journal of Agrucultural and Food Chemisry, 42 (9), 1891–1895.

Cui, W. and Mazza, G., 1996. Phvsicochemical characteristics of flaxseed gum. Food Research International, 29, 397-402.

Cutler, A.N., Morris, E.R., Taylor, L.J., 1983. Oral Perception of Viscosity in fluid foods and model systems. Food and Nutrition Press, 14, 377-395.

Dewar, R.J and Joyce, M.J., 2006a. The thixotropic and rheopectic behaviour of maize starch and maltodextrin thickeners used in dysphagia therapy. Carbohydrate Polymers 65, 296–305.

Dewar, R.J. and Joyce, M.J., 2006b. Time-Dependent Rheology of Starch Thickeners and the Clinical Implications for Dysphagia Therapy. Dysphagia, 21(4), 264–269.

Ding, H.H., Cui, S.W., Goff, H.D., Wang, Q., Chen, J., Han, N.F., 2014. Soluble polysaccharides from flaxseed kernel as a new source of dietary fibres: Extraction and physicochemical characterization. Food Research International, 56, 166–173.

Elboutachfaiti, R., Delattre, C., Anthony Quérro, A., Roulard, R., Julie Duchêne, J., Mesnard, F., Petit, E., 2017. Fractionation and structural characterization of six purified rhamnogalacturonans type I from flaxseed mucilage. *Food Hydrocolloids*, 62, 273-279.

Englyst, K.N., Englyst, H.N., Hudson, G.J., Cole, T.J., Cummings, J.H., 1999. Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. The American Journal of Clinical Nutrition, 69(3), 448–454.

Farahmandfar, R., Asnaashari, M., Salahi, M.R., Rad, T.K., 2017. Effects of basil seed gum, Cress seed gum and Quince seed gum on the physical, textural and rheological properties of whipped cream. International Journal of Biological Macromolecules, 98, 820–828.

Foster-Powell, K., Holt, S.H., Brand-Miller, J.C., 2002. International table of glycemic index and glycemic load values: 2002. The American Journal of Clinical Nutrition, 76(1), 5-56.

Garin, N., De Pourcq, J.T., Martín-Venegas, R., Cardona, D., Gich, I., Mangues, M.A., 2014. Viscosity Differences between Thickened Beverages Suitable for Elderly Patients with Dysphagia. Dysphagia, 29: 483–488.

Germain, I., Dufresne, T. & Ramaswamy, H.S., 2006. Rheological characterization of thickened beverages used in the treatment of dysphagia. Journal of Food Engineering, 73, 64-74.

Hadde, E.K. and Chen, J., 2019. Shear and extensional rheological characterization of thickened fluid for dysphagia management. Journal of Food Engineering, 245, 18-23.

He, Q., Hort, J., Wolf, B., 2016. Predicting sensory perceptions of thickened solutions based on rheological analysis. Food Hydrocolloids, 61, 221-232.

Heyman, B., De Vos, W. H., Depypere, F., Van der Meeren, P., Dewettinck, K., 2014. Guar and xanthan gum differentially affect shear induced breakdown of native waxy maize starch. Food Hydrocolloids, 35, 546–556.

Hori, K., Hayashi, H., Yokoyama, S., Ono, T., Ishihara, S., Magara, J., Taniguchi, H., Funami, T., Maeda, Y. and Inoue, M., 2015. Comparison of mechanical analyses and tongue pressure analyses during squeezing and swallowing of gels. Food Hydrocolloids 44:145-155.

Jo, W., Bak, J. H., Yoo, B., 2018. Rheological characterizations of concentrated binary gum mixtures with xanthan gum and galactomannans. International Journal of Biological Macromolecules, 114, 263–269.

Kanmani, N., Romano, N., Ebrahimi, M., Amin, S.M.N., Kamarudin, M.S., Karami, A., Kumard, V., 2018. Improvement of feed pellet characteristics by dietary pre-gelatinized starch and their subsequent effects on growth and physiology in tilapia. Food Chemistry, 239, 1037-1046.

Kim, C. and Yoo, B., 2006. Rheological properties of rice starch–xanthan gum mixtures. Journal of Food Engineering, 75(1), 120-128.

Koocheki, A., Reza-Taherian, A., Bostan, A., 2013. Studies on the steady shear flow behavior and functional properties of Lepidium perfoliatum seed gum. Food Research International, 50, 446–456.

Leonard, R.J., White, C., McKenzie, S., Belafsky, P.C., 2013. Effects of Bolus Rheology on Aspiration in Patients with Dysphagia. Journal of the Academy of Nutrition and Dietetics, 2212-2672.

Li, X.Y., Li, D., Wang, L.J., Wu, M., Adhikari, B., 2012. The effect of addition of flaxseed gum on the rheological behavior of mixed flaxseed gum–casein gels. Carbohydrate Polymers, 88 (4), 1214-1220.

Liu, J., Shim, Y.Y., Poth, A.G., Reaney, M.J.T., 2016. Conlinin inflaxseed (Linum usitatissimumL.) gum and its contribution to emulsification properties. Food Hydrocolloids, 52, 963-971.

Mackley, M.R., Tock, C., Anthony, R., Butler, S.A., Chapman, G. and Vadillo, D.C., 2013. The rheology and processing behavior of starch and gum-based dysphagia thickeners. Journal of Rheology, 57(6), 1533-1553.

Mandala, I.G., Palogou, E.D., Kostaropoulus, A. E., 2002. Influence of preparation and storage conditions on texture of xanthan-starch mixtures. Journal of Food Engineering, 53, 27-38.

Mandala I. and Palogou E., 2003. Effect of Preparation Conditions and Starch/Xanthan Concentration on Gelation Process of Potato Starch Systems. International Journal of Food Properties, 6, 311-328.

Mandala, I.G., Savvas, T.P, Kostaropoulos, A.E, 2004. Xanthan and locust bean gum influence on the rheology and structure of a white model-sauce. Journal of Food Engineering, 64(3), 335-342.

Meng, Y., Rao, M.A., Datta, K., 2005. Computer simulation of the pharyngeal bolus transport of Newtonian and Non-Newtonian fluids Food and Bioproducts Processing, 83(4), 297-305.

Minekus, M., Alminger, M., Alvito, P., Bohn, T., Ballance, S., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, Menard, O., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S.J., Weitschies, W., Brodkorb, A., 2014. A standardised static in vitro digestion method suitable for food – an international consensus. Food & Function, 5, 1113–1124.

Mirhosseini, H. and Amid, B.T., 2012. A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. Food Research International, 46, 387–398.

Moczkowska, M., Karpa, S., Niu, Y., Kureka, M.A., 2019. Enzymatic, enzymatic-ultrasonic and alkaline extraction of soluble dietary fibre from flaxseed – A physicochemical approach. Food Hydrocolloids, 90, 105-112.

Moret-Tatay, A., Rodríguez-García, J., Martí-Bonmat, E., Hernando, I., Hernández, M.J., 2015. Commercial thickeners used by patients with dysphagia: Rheological and structural behaviour in different food matrices. Food Hydrocolloids, 51, 318-326.

Morris, D.H. and Vaisey-Genser, M., 2003. FLAXSEED. Encyclopedia of Food Sciences and Nutrition (2nd Edition), 2525–2531.

National Dysphagia Diet Task Force (2002). National dysphagia diet: standardization for optimal care. Chicago, IL, American Dietetic Association.

O'Leary, M., Hanson, B. and Smith, C., 2010. Viscosity and Non-Newtonian Features of Thickened Fluids Used for Dysphagia Therap. Journal of Food Science, 75(6), 330-338.

Olivas, G.I. and Barbosa-Cánovas, G.V., 2005. Edible Coatings for Fresh-Cut Fruits. Critical Reviews in Food Science and Nutrition, 45, 657-670.

Qian, K.Y., Cui, S.W., Wu, Y., Goff, H.D., 2012. Flaxseed gum from flaxseed hulls: Extraction, fractionation, and characterization. Food Hydrocolloids, 28(2), 275-283.

Richardson, R. K., Morris, E.R., Ross-Murphy, S.B., Taylor, L.J., Dea, I.C.M., 1989. Characterization of the perceived texture of thickened systems by dynamic viscosity measurements. Food Hidrocolloids, Volume 3, 175-191.

Rodrigues, M.I. and Iemma, A.F., 2015. Experimental Design and Process Optimization. Taylor & Francis group (1st Edition).

Russ, N., Zielbauer, B.I., Ghebremedhin, M., Vilgis, T.A., 2016. Pre-gelatinized tapioca starch and its mixtures with xanthan gum and ι-carrageenan. Food Hydrocolloids, 56, 180-188.

September, C., Nicholson, T.M., Cichero, J.A.Y., 2014. Implications of Changing the Amount of Thickener in Thickened Infant Formula for Infants with Dysphagia. Dysphagia, 29, 432–437.

Shalviri, Liu, Q., Abdekhodaie, M.J., Wu, X.Y., 2010. Novel modified starch–xanthan gum hydrogels for controlled drug delivery: Synthesis and characterization. Carbohydrate Polymers, 79, 898–907.

Shama, F. and Sherman, P., 1973. Identification of stimuli controlling the sensory evaluation of viscosity II. Oral methods. Journal of Texture Studies, 4 (1), 111-118.

Song, K., Kim, Y., Chang, G., 2006. Rheology of concentrated xanthan gum solutions: Steady shear flow behaviour. Fibers and Polymers, 7(2), 129-138.

Sopade, P.A., Halley, P.J., Cichero, J.A.Y., Ward, L.C., Liu, J., Varliveli, S., 2008. Rheological characterization of food thickeners marketed in Australia in various media for the management of dysphagia. III. Fruit juice as a dispersing medium. Journal of Food Engineering, 86, 604–615.

Steffe, J.F. (1996). Rheological Methods in Food Process Engineering. 2nd Edition, Freeman Press, East Lansing.

Szczesniak, A.S. and Farkas, E., 1963. Objective characterization of the mouth feel of gum solutions. Journal of Food Science, 27, 381-385.

Tako, M., Tamaki, Y., Teruya, T., Takeda Y., 2014. The principles of starch gelatinization and retrogradation. Food and Nutritional Sciences, 5, 280-291.

Tester, R.F., Karkalas, J., Qi, X., 2004. Starch-composition, fine structure and architecture. Journal of Cereal Science, 39(2), 151-165.

Torres, O., Yamada, A., Rigby, N.M., Hanawa, T., Kawano, Y., Sarkar, A., 2019. Gellan gum: A new member in the dysphagia thickener family. Biotribology, 17, 8-18.

Tromp, R.H., van de Velde, F., van Riel, J., Paques, M., 2001. Confocal scanning light microscopy (CSLM) on mixtures of gelatine and polysaccharides. Food Research International, 34(10), 931-938.

Veiga, P.G., Cunha, R.L., Viotto, W.H., Penetate, A.J., 2000. Cacracterização química, reológica e aceitação sensorial do queijo petit suisse brasileiro. Ciência e Tecnologia de Alimentos, 20(3), 349-357.

Vieira, J.M., Mantovani, R.A., Raposo, M.F.J., Coimbra, M.A., Vicente, A.A., Cunha, R.L., 2019. Effect of extraction temperature on rheological behavior and antioxidant capacity of flaxseed gum. Carbohydrate Polymers, 213, 217-227.

Vallons, K.J.R., Helmens, H.J. and Oudhuis, A.A.C.M., 2015. Effect of human saliva on the consistency of thickened drinks for individuals with dysphagia. International Journal of Language & Communication Disorders, 50(2), 165–175.

Wood, G.N., 1968. Centric occlusion, centric relation, and the mandibular posture. The Journal of Prosthetic Dentistry, 20(4), 292-306.

Zhan, D.F., Ridout, M.J., Brownsey, G.J., Morris, V.J., 2003. Xanthan-locust bean gum interactions and gelation. Carbohydrate Polymers, 21(1), 53-58.

Zargaraan, A., Rastmanesh, R., Fadavi, G., Zayeri, F., Mohammadifar, M.A., 2013. Rheological aspects of dysphagia-oriented food products: A mini review. Food Science and Human Wellness, 2, 173–178.

## *CAPÍTULO V*

# *Rheology and soft tribology of thickened dispersions aiming the development of oropharyngeal dysphagia-oriented products*

*To be submitted to Current Research in Food Science*

## 5. Rheology and soft tribology of thickened dispersions aiming the development of oropharyngeal dysphagia-oriented products

J.M. Vieira [1], D.B., Salvaro [2], G.P. Maffezzolli [2], J.D.B., de Mello [2], A.A. Vicente [3], R.L. Cunha[1*]

[1]*Department of Food Engineering, Faculty of Food Engineering, University of Campinas (UNICAMP), 13083-862, Campinas, SP, Brazil*

[2] *Laboratory of Materials (LabMat), Department of Mechanical Engineering, Federal University of Santa Catarina, Florianópolis, SC, 88040-900, Brazil*

[3] *Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

*Corresponding author:

E-mail: **rosiane@unicamp.br**

Phone: **+55 19 3521 4047**

### Abstract

Tribology is a promising analytical tool to complement rheological analyses, with the potential of becoming a new quantification strategy to be used for a deeper understanding of swallowing mechanisms. Flaxseed gum (FG) (0.75-3 % w/v) was studied as a potential thickener in comparison to thickeners for dysphagia patients and polysaccharides usually available in these commercial formulations (xanthan gum (XG) and modified starch (MS)) from rheology and oral tribology measurements. Thus, the objective of this work was to carry out a comprehensive study to correlate the rheological and tribological responses of different biopolymer-based thickened solutions. For all the biopolymer solutions a constant friction coefficient value over the analysis time was observed. The increase in the concentration, and consequently in the viscosity, of the gums caused a decrease in the coefficient of friction, and consequently an increase in the lubricating capacity. An opposite behavior was observed for starch-based products, since the increase in viscosity caused an increase in the coefficient of friction or decrease in the lubricating capacity. The solutions based on MS presented a high pseudoplasticity at the highest concentration (3 % w/v) despite the low viscosity

and viscoelastic moduli compared with solutions based on gums. Thus, we hypothesized that the coefficient of friction of different biopolymers could provide information about sensory characteristics (as adhesion) and also on the capacity of the biopolymer to remain intact after friction contact occurring against the material present in the oral cavity.

**Keywords:** dysphagia, swallow, thickeners, flaxseed gum, rheology, tribology

### 5.1. Introduction

Dysphagia is a swallowing disorder caused by aging or neurological complications. In recent years the number of patients diagnosed with oropharyngeal dysphagia (the most prevalent and severe stage) has increased worldwide (affecting 8 % of the population). This disorder occurs in nearly 100 % of people with idiopathic Parkinson's disease and Huntington's disease and 30-65 % of people with stroke (Wang, 2010; Feng *et al.*, 2019; Torres *et al.*, 2019). This disturbance occurs in the food transition (mainly with liquid foods) from the oral cavity to the esophagus. Food aspiration is the major risk in this process, since the food *bolus* can accidentally enter the respiratory pipe blocking the airways (Chen, 2009; Clavé & Shaker, 2015). Oropharyngeal dysphagia is also secondarily associated with many death cases during hospitalization mainly caused by nutritional and hydration deficits and pneumonia (Mackley *et al.*, 2013; Leonard *et al.*, 2013, Feng *et al.*, 2019).

As a strategy to circumvent the problems associated with dysphagia patients, thickened liquid foods are usually prescribed in order to increase the bolus passage time from the mouth to the esophagus, allowing time for the longer response of the muscles responsible for swallowing typical of these patients (Dewar & Joyce, 2006, Mackley *et al.*, 2013, Hori *et al.*, 2015). Pregelatinized starch has been widely studied and used in commercially available products for dysphagia patients, being the most abundant and cheap alternative. Starch particles composed by amylose and amylopectin show the capacity to swell when incorporated in water, increasing the viscosity of the suspension. However, the action of amylase in the mouth may cause a thinning effect, which is considered as potentially unsafe (Martinez *et al.*, 2019). Therefore, in recent years, gum-based thickeners, with emphasis on xanthan gum, have emerged as an alternative, promoting a greater increase of viscosity and shear-thinning properties in aqueous systems. The rigid xanthan structure makes the systems containing this gum stable to

pH, ionic strength and temperature, favoring its use in commercial thickeners (Martinez *et al.*, 2019; Torres *et al.*, 2019).

Flaxseed gum (FG), extracted from the *Linum usitatissimum*, is a potential and promising thickener. FG is mainly composed by a neutral and an acidic polysaccharide fractions, proteins and phenolic compounds which confer pharmacological properties, such as antidiabetic, antihypertensive, immunomodulatory, anti-inflammatory and neuroprotective properties, and also may improve intestinal tract transit along with other health benefits (Cui, Mazza & Biliaderis, 1994; Qian, Cui, Nikiforuc & Goff, 2012; Elboutachfaiti *et al.*, 2017; Vieira *et al.*, 2019; Foster-Powell, Holt & Brand-Miller, 2002; Ding *et al.*, 2014; Liu, Shim, Poth & Reaney, 2016). In addition, this gum exhibits interesting rheological characteristics, and equally to xanthan gum, these characteristics are very stable at neutral and acidic pH (Vieira *et al.*, 2019).

Viscosity is a fundamental property obtained from rheological measurements, which is used as the most important criterion in the development of thickeners for dysphagia patients. An agreement published in the *National Dysphagia Diet* (2002) by *American Dietetic Association* was reached to establish a categorization according to the viscosity range values (Nectar-like (51-350 cP); Honey-like (351-1750 cP); Spoon thick (>1750 cP)) aiming at ensuring a safe swallowing and to facilitate palliative care procedures for different types of patients' needs, although it does not consider very relevant sensory aspects. These viscosity values are obtained at 50 $s^{-1}$, shear rate considered as of the swallowing process, although there is not a consensus in the scientific community about this value. A broad range of oral shear rates involving the bolus has been considered such as 10 $s^{-1}$ (Cutler, Morris & Taylor, 1983) or 400 $s^{-1}$ to correlate with oral shear rates (Meng, Rao & Datta, 2005). On the other hand, other rheological properties such as pseudoplasticity, viscoelastic properties and complex viscosity at 50 $rad.s^{-1}$ of thickened fluids may also give a good empirical correlation with sensorial characteristics or a probable mouthfeel, which may help in the choice of biopolymer to be used as a thickener to return the pleasure of eating to the dysphagia patients (Richardson *et al.*, 1989).

Tribology, the study of lubrication, friction and wear of compliant surfaces in relative motion, and has been used, among others, as a tool to understand the lubrication ability of polymer solutions, fluid gels and gelled particles and emulsions (Douaire, Stephenson & Norton, 2014). Recent research indicates that tribological analyses coupled with rheological measurements are an emerging field contributing to the

understanding of oral processing of food, sensory perception and surface properties of the interacting substrates, i.e., food and the epithelium in the mouth/throat (Chen & Stokes, 2012). A thin film of food is formed and squeezed between the tongue and hard palate in the last stage of mastication, and this is the process that must be simulated in the instrument used for soft tribological analysis (Upadhyay & Chen, 2019). The selection of a physiologically relevant tribological configuration is essential for the successful application of tribology: the choice of instrument, surfaces and the model food system should be considered. Several surfaces have been tested: polydimethylsiloxane (PDMS), rough plastic and rubber pads were used to simulate the tongue, while stainless steel, glass and PDMS were used to simulate the hard palate (Prakash, Tan & Chen, 2013). The coefficient of friction is the parameter commonly measured in tribological analyses; it is defined as the ratio of the friction force to the surface load. It can vary significantly with food properties, such as viscosity, and its magnitude depends on the chosen surface nature and characteristics (Stokes, Boehm, & Baier, 2013; Upadhyay & Chen, 2019). Thus, the coefficient of friction is an effective indication of the lubricating properties between the two surfaces at a given moving speed and surface load applied resulting in sensory/textural indicia. The tribological analysis may be useful due to frequent changes in perceived thickness observed in commercial thickened foods available for management of dysphagia. However, the correlation between a coefficient of friction and texture characteristics is still a challenge, given the complexity in establishing a bridge between oral tribology and sensory perception, since both are affected by multiple physical and chemical properties (Upadhyay & Chen, 2019).

The relationship between rheological and tribological properties may result in crucial data about the ability to avoid loss of thickening capacity during swallowing process associated with a lowering of the viscosity due to friction in the oral cavity. It should be noted that properties of thickened fluids under the same concentrations were analysed in order to compare the potential behaviour of a new component as flaxseed gum with the commonly used thickeners and commercial formulations. Therefore, the interest of this work was the use of tribological analysis associated to rheological properties to evaluate FG as a potential thickener to dysphagia patients establishing a comparison with commercial products.

## 5.2. Materials and Methods

### 5.2.1. Materials

Golden flaxseeds were kindly provided by CISBRA Ltda (Panambi, RS, Brazil). Xanthan gum was donated by Danisco (Brazil) and modified starch by Cargill (Brazil). Two commercial thickeners were used: one based on xanthan gum (with addition of maltodextrin and gelling potassium chloride) (ThickenUP, Nestlé) and another on modified starch (with addition of maltodextrin) (Thick&Easy, Hormel). The commercial thickeners were acquired in local market. The material used to simulate the tongue's surface was polydimethylsiloxane (PDMS) produced from a Sylgard 184 silicone elastomer kit (Dow Corning Corporation, U.S.A.). Base and curing agents were used in a mass ratio of 9:1 to obtain disks with 5 mm thickness sheet and 50 mm diameter. In order to eliminate air bubbles, the PDMS disks were cured for 2 h in the oven at 55 °C.

### 5.2.2. Thickened beverages preparation

Aqueous dispersions were prepared using the following thickeners: MS = modified starch; XG = xanthan gum; FG = flaxseed gum; TU = XG-based commercial thickener; TE = MS-based commercial thickener at concentrations of 0.75, 1.5, 2.25 and 3 (% w/w). The concentrations were chosen based on preliminary tests (data not shown), which showed the maximum lubrication limits under the conditions of the tribological analyses. All the samples were prepared by dissolution of thickeners at room temperature in distilled water using mechanical stirring for 30 min at 800 rpm.

### 5.2.3. Rheological properties

Rheological properties of the thickened food matrices were obtained using an AR1500ex rheometer (TA Instruments, USA) with a stainless-steel cone-plate geometry (6.0 cm, 2° angle, truncation 67 μm) or double concentric cylinders (rotating bob: external diameter 21.96 mm and internal diameter 20.38 mm, stationary cylinder: internal diameter of 20 mm and external diameter 22.38 mm). The choice of geometry depended on the viscosity of the thickened solution.

Flow curves were obtained by an up–down–up step program using different shear stress ranges to provide shear rates between 0 to 400 $s^{-1}$ at 25 ºC. The shear rate range was chosen in order to understand the rheological behaviour during the swallowing

process (Wood *et al.*, 1968, Cutler *et al.*, 1983). Newtonian (Eq. 5.1), power-law (Eq. 5.2) and Herschel-Bulckley (Eq. 5.3) equations were fitted to the data to obtain rheological properties:

$$\sigma = \eta . \dot{\gamma} \quad \text{(Eq. 5.1)}$$

$$\sigma = k . \dot{\gamma}^n \quad \text{(Eq. 5.2)}$$

$$\sigma = \sigma_0 + k . \dot{\gamma}^n \quad \text{(Eq. 5.3)}$$

where $\sigma$ is the shear stress (Pa), $\eta$ is the viscosity (Pa.s), $k$ is the consistency index (Pa.s$^n$), $\dot{\gamma}$ is the shear rate (s$^{-1}$) and $n$ is the flow index.

Oscillatory measurements were performed at 25 °C using a frequency sweep between 0.1 and 10 Hz within the linear viscoelastic domain. The contributions of the elastic and viscous properties were analysed from storage ($G'$) and loss ($G''$) moduli, respectively. The complex viscosity at 50 rad.s$^{-1}$ ($\eta^*$) (Eq. 5.4) was also evaluated, since this property showed a good correlation with the sensorial consistency of viscoelastic materials (He et al, 2016).

$$\eta^* = \frac{G^*}{\omega} \quad \text{Eq. (5.4)}$$

where $G^*$ is the vector sum of the elastic (G') and viscous (G") moduli and $\omega$ is the angular frequency (rad.s$^{-1}$).

### 5.2.4. Tribological measurements

Tribological tests were carried out at 25 °C using a reciprocating ball-on-plane configuration in an UMT-2 (CETR) equipment (USA) (Figure 5.1). The AISI 52100 steel ball (ø = 10 mm) slided without rolling on the PDMS plane surface (disc with 40 mm diameter and 5 mm thickness) during measurements, forming the rubbing contact (ball-on-disc). The ball was used to represent the palate and the PDMS disc was meant to mimic the human tongue surface. PDMS is currently a feasible choice for food tribology studies and it was selected due to its well-defined mechanical properties and surface chemistry. PDMS shows a low elastic modulus that could mimic the low-pressure contact typical in bio-lubrication processes (Prakash *et al.*, 2013; Zhang *et al.*, 2017).

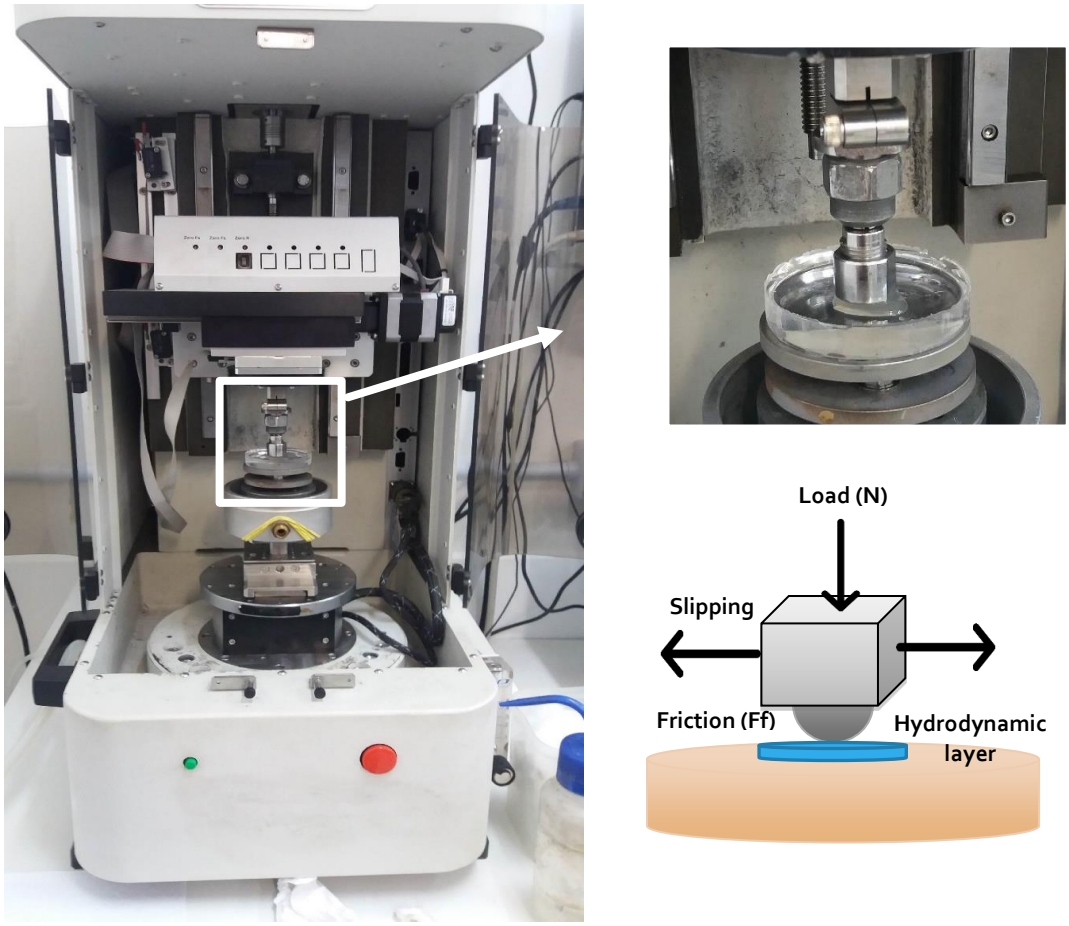



**Figure 5.1.** Tribometer (UMT-2 (CETR)) configuration and the schematic representation, which was used for all coefficient of friction measurements. A normal load (N) is applied and the friction force (*Ff*) measured while the steel surface slipped on the hydrodynamic layer at a given speed.

The wear track was 10 mm long with 2 Hz of reciprocating frequency, speed of 40 $mm.s^{-1}$, with a normal load of 5 N during 20 s (swallowing process occurs between milliseconds up to 20 s). The human tongue speeds have been estimated between 10 and 200 $mm.s^{-1}$ and the in-mouth force has been reported between 0.01 N and 10 N (Laiho, Williams, Poelman, Appelqvist, & Logan, 2017). The tribological measurements in this study are within this range (40 $mm.s^{-1}$ and 5 N). Besides that, this velocity value was chosen based on an earlier study have demonstrated that the maximum coefficient of friction was measured at 40 $mm.s^{-1}$ for a wide speed range (until 1000 $mm.s^{-1}$) evaluating different aqueous biopolymeric suspensions, including starch and xanthan gum (Zhang *et al.*, 2017; Torres *et al.*, 2019). It was placed 1.5 mL of each thickened sample on the PDMS surface and the tribological behaviour of the thickened matrices was tested. The coefficient of friction (*CoF*) was measured according to the Equation

5.5 and reported for each sample as a function of time (s). Reported results were taken at steady state conditions.

$$CoF = \frac{Ff}{N} \quad \text{(Eq. 5.5)}$$

where $Ff$ is the friction force (calculated as a function of the applied entrainment speed - 40 mm.s$^{-1}$ of the PDMS disc) and $N$ is the applied load (5 N). Results are presented as the average of five replicates with the corresponding standard deviation.

#### 5.2.5. Statistical analyses

The data were analyzed using Statistica 7.0 software and Microsoft Windows Excel 2017 software. Data were subjected to analysis of variance (ANOVA) ($p < 0.05$) and the means were compared using the Tukey's HSD test to examine if differences between formulations were significant ($\alpha = 0.05$).

## 5.3. Results and discussion

### 5.3.1. Rheological behaviour

Thickened liquids are used to reduce the flow velocity from the mouth to the oro-pharynx region in order to avoid aspiration of fluid into the pulmonary airways. In addition, no phase separation or syneresis should occur throughout the swallowing process (Germain, Dufresne & Ramaswamy, 2006), which means that the liquids should be homogeneous and stable. The flow curves of shear stress as a function of shear rate of each thickened solution are shown in Figure 5.2, while the rheological properties obtained from these curves ($n$ (flow index), $\sigma_0$ (yield stress), $k$ (consistency index) and the apparent viscosity at shear rates of 10 s$^{-1}$ ($\eta_{ap\ 10}$), 50 s$^{-1}$ ($\eta_{ap\ 50}$) and 400 s$^{-1}$ ($\eta_{ap\ 400}$)) are presented in Table 5.1. The shear rate range between 10 and 400 s$^{-1}$ encompasses the swallowing process and is commonly used by several authors to allow comparison of the studies (Shama & Sherman, 1973; Morris & Taylor, 1984; Meng *et al.*, 2005).

Regarding to the flow curves (Figure 5.2) obtained for the solutions with modified starch, a significant increase in the shear stress with the shear rate was observed for the MS concentration of 3 % (w/w) and for the TE concentration of 2.25 % w/w, leading to a significant increase in viscosity (Table 5.1). This fact agrees with previous studies that observed a more pronounced viscosity increase when the concentration of hydrocolloids allows to reach a viscosity range of 10 – 20 cP (Ferry *et al.*, 2006). In relation to the

other studied biopolymers, this abrupt change was observed for all the concentrations, since the viscosity values were higher than 10- 20 cP from 0.75 % w/w (Table 5.1). Three types of consistency according to NDD were reached for the different biopolymer solutions regarding the viscosity obtained at 50 $s^{-1}$. The two modified starch-based solutions presented the lowest viscosity values, being that 3% w/w MS was necessary for adequacy to the dysphagic diet. All xanthan gum-based solutions presented appropriate consistency for dysphagic consumption. Solutions thickened with TU presented nectar-like categorization, whereas two types of categorization were observed for solutions thickened with XG: nectar-like and honey-like. Finally, the FG thickened solutions showed nectar-like consistency between the concentrations of 1.5 and 2.25 % w/w and honey-like for the highest concentration. Thus, the individual thickening capacity of the modified starch was less efficient compared to both gums.

The steady shear rheological values (Table 1) are associated to the flow velocity of the food bolus (or viscosity), being that the flow index (*n*) and the consistency index (*k*) values are related to sliminess perceived in the mouth (Funami, 2011). All thickened solutions presented a shear time independent behaviour and a non-Newtonian shear-thinning behaviour, as *n* values were lower than 1, except the solution with 0.75 % w/w of MS. A shear thinning behaviour implies a decrease in the apparent viscosity at increased shear rates, which is commonly observed in polysaccharide solutions (Li, Qi, Sun & Wang, 2016; Jo *et al.*, 2018; Vieira *et al.*, 2019). The flow index of all samples varied between 0.10 ± 0.01 and 1.00 ± 0.03. The lowest *n* values of XG-based thickened solutions indicate that increasing the XG concentration in the formulations probably facilitates swallowing and reduces organoleptic viscosity (slimy feel), due to its greater pseudoplasticity, offering a pleasant and light mouthfeel (Szczesniak & Farkas, 1963; Cho & Yoo, 2014).

All xanthan gum-based and concentrated TU-based solutions showed yield stress, which was not observed for other biopolymer solutions. Apparent viscosity of gum-based solutions, regardless of the shear rate and concentration, were pointedly higher than those based in MS. The high apparent viscosity of XG-based solutions at 10 $s^{-1}$ could be a result of intermolecular association among polymer chains which resulted in the formation of a complex network of tangled rod-like molecules (Chapter IV), but the reduction of viscosity observed in higher shear rates pointed to a fast organization of these molecules with the flow field. However, the apparent viscosity of FG solutions at higher shear rates (400 $s^{-1}$) were higher than those of XG and XG-based commercial

thickener (TU), since FG presents a less pronounced shear thinning behaviour. The order of magnitude in apparent viscosity values at lower polysaccharide concentrations (0.75 and 1.5 % w/w) and lower shear rates (10 $s^{-1}$ and 50 $s^{-1}$) of thickened liquids was as follows: XG > TU > FG > TE > MS, while for the highest concentration (3 % w/w) and shear rate (400 $s^{-1}$) it was as follows: FG > XG > MS > TU > TE. These results indicate that the mixture of FG and XG may lead to higher shear stability at higher shear rates (higher viscosity) when compared with an XG-based product (Chapter IV). However previous studies have shown that fluids with very high viscosity could result in high cohesiveness, causing difficulty in the swallowing process due to the high resistance to stretching deformation, in particular in patients with weak tongue muscle and low oral pressure (Hadde & Chen, 2019). The occurrence of a $\sigma_o$ (yield stress) was only observed in XG-based solutions, as well as more pronounced elastic properties when compared to the other biopolymers (as shown in Figure 5.3). Values of the consistency index ($k$) and yield stress ($\sigma_o$) of XG solutions increased with the concentration (Table 5.1), indicating the formation of a stronger network, although the pseudoplasticity remains constant from a concentration of 1.5% w/w, according to the $n$ value (Cho & Yoo, 2015). Therefore, thickened products with high concentration of XG will have much higher viscosity than liquids thickened with FG and MS at similar concentration under lower shear rates (including at 50 $s^{-1}$, as can be seen in Table 5.1). Once again, the thickener and its concentration should be considered in detail for the final formulation used in dysphagic diet, since different properties will exhibit different roles during swallowing, e.g., elastic properties may result in better sensory properties, but the amounts of components that promote these properties must be regulated to avoid a solid-like behaviour at low swallowing velocity. This because an excess of yield stress requires higher tongue pressures and coordination to drive the bolus, which is often not plausible for patients with dysphagia (Miller & Watkin, 1996).

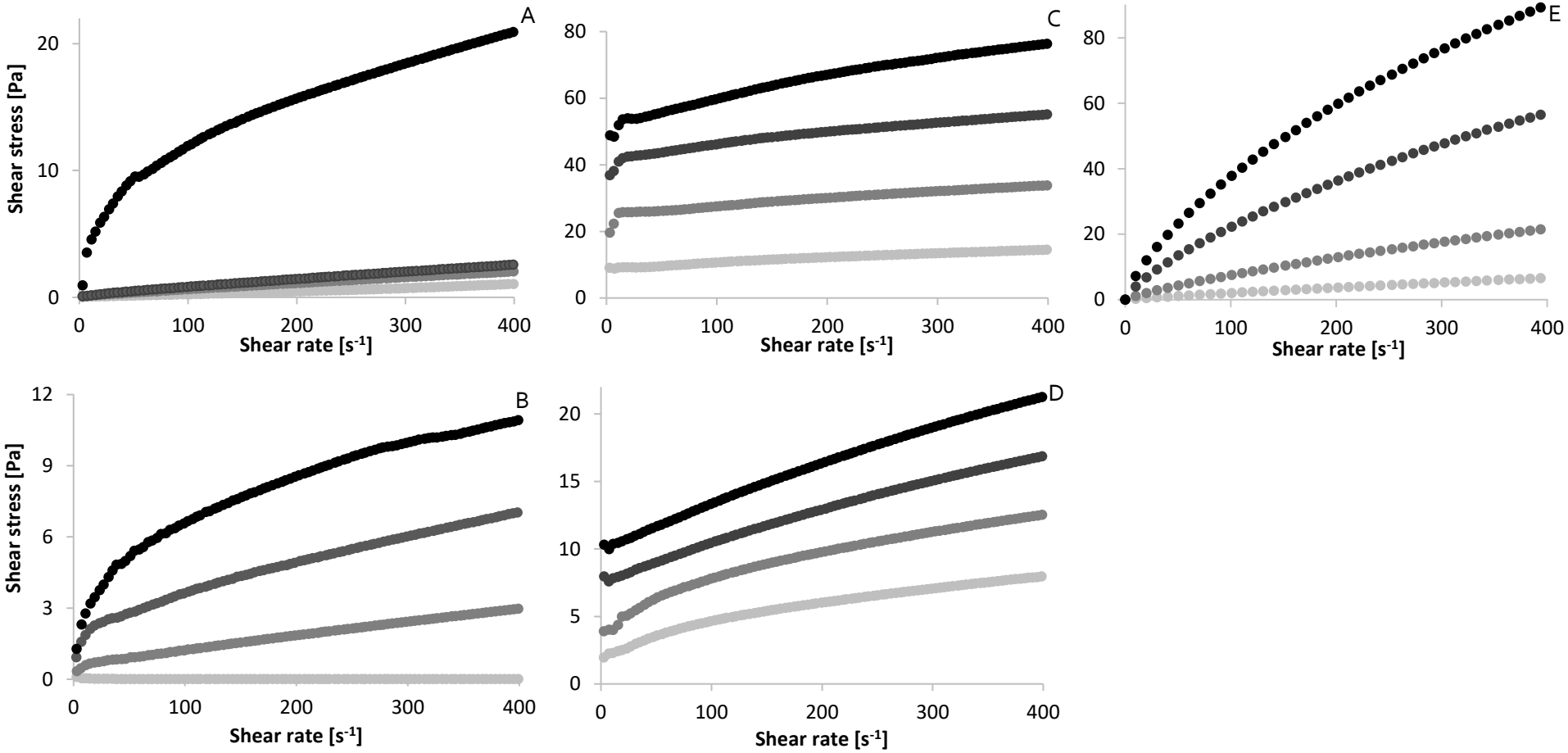



**Figure 5.2.** Flow curves of the studied biopolymer solutions based on modified starch (MS (A) and TE (B)), xanthan gum (XG (C), TU (D)) and flaxseed gum (FG (adapted from Vieira *et al.*, 2019) (E)), at different concentrations (0.75 % ●, 1.5 % ●, 2.25 % ● and 3 % ● w/w).

**Table 5.1.** Rheological properties ($n$, $k$, $\eta_{ap\ 10}$, $\eta_{ap\ 50}$, $\eta_{ap\ 400}$, $\sigma_0$) of the MS, FG, XG, TU and TE thickened solutions prepared at different concentrations.

| | (% w/w) | $n$ | $k$ [Pa.s$^n$] | $\eta_{ap\ 10}$ [cP] | $\eta_{ap\ 50}$ [cP] | $\eta_{ap\ 400}$ [cP] | $\sigma_0$ [Pa] |
|---|---|---|---|---|---|---|---|
| **MS** | **0.75** | 1.00 ± 0.03 | 0.002 ± 0.001 | 5 ± 1 | 3 ± 1 | 3 ± 1 | 0 |
| | **1.5** | 0.87 ± 0.03 | 0.011 ± 0.002 | 11 ± 2 | 7 ± 1 | 5 ± 1 | 0 |
| | **2.25** | 0.80 ± 0.05 | 0.032 ± 0.006 | 20 ± 2 | 14 ± 2 | 9 ± 2 | 0 |
| | **3** | 0.41 ± 0.05 | 1.824 ± 0.032 | 438 ± 7 | 191 ± 5 | 53 ± 3 | 0 |
| **FG** | **0.75** | 0.83 ± 0.02 | 0.047 ± 0.001 | 32 ± 2 | 24 ± 2 | 17 ± 2 | 0 |
| | **1.5** | 0.72 ± 0.01 | 0.287 ± 0.011 | 151 ± 5 | 96 ± 4 | 54 ± 3 | 0 |
| | **2.25** | 0.60 ± 0.03 | 1.561 ± 0.034 | 622 ± 11 | 327 ± 8 | 142 ± 5 | 0 |
| | **3** | 0.58 ± 0.01 | 3.178 ± 0.082 | 1208 ± 32 | 615 ± 24 | 257 ± 13 | 0 |
| **XG** | **0.75** | 0.17 ± 0.02 | 5.223 ± 0.253 | 1003 ± 12 | 195 ± 10 | 35 ± 5 | 8.87 ± 0.18 |
| | **1.5** | 0.11 ± 0.02 | 17.340 ± 0.352 | 2501 ± 34 | 521 ± 17 | 85 ± 5 | 21.45 ± 1.90 |
| | **2.25** | 0.10 ± 0.01 | 30.272 ± 0.730 | 4202 ± 94 | 906 ± 21 | 140 ± 5 | 37.43 ± 0.60 |
| | **3** | 0.11 ± 0.02 | 34.758 ± 1.572 | 5533 ± 101 | 1154 ± 26 | 192 ± 3 | 51.79 ± 2.96 |
| **TE** | **0.75** | 0.91 ± 0.03 | 0.009 ± 0.004 | 8 ± 2 | 7 ± 2 | 6 ± 2 | 0 |
| | **1.5** | 0.84 ± 0.03 | 0.022 ± 0.003 | 11 ± 2 | 7 ± 1 | 4 ± 1 | 0 |
| | **2.25** | 0.71 ± 0.02 | 0.331 ± 0.061 | 132 ± 9 | 69 ± 7 | 30 ± 4 | 0 |
| | **3** | 0.61 ± 0.03 | 1.511 ± 0.124 | 371 ± 13 | 139 ± 10 | 39 ± 5 | 0 |
| **TU** | **0.75** | 0.38 ± 0.03 | 0.718 ± 0.219 | 217 ± 11 | 74 ± 5 | 22 ± 2 | 0 |
| | **1.5** | 0.29 ± 0.02 | 2.085 ± 0.131 | 454 ± 13 | 125 ± 4 | 31 ± 3 | 0 |
| | **2.25** | 0.27 ± 0.02 | 3.035 ± 0.105 | 877 ± 22 | 185 ± 16 | 40 ± 4 | 7.39 ± 0.56 |
| | **3** | 0.26 ± 0.01 | 4.362 ± 0.139 | 1177 ± 38 | 236 ± 19 | 51 ± 2 | 9.94 ± 0.36 |

The viscoelastic rheological behaviour of the thickened solutions at different concentrations was analysed by oscillatory analysis. Figure 5.3 presents the dynamic frequency sweep tests that were carried out within the linear viscoelastic region to determine the frequency (or time of observation) dependence of the elastic (*G'*) and viscous (*G''*) moduli. Higher frequency dependence was observed for the viscoelastic moduli of thickening solutions based on MS and FG, when compared to those thickened with XG, mainly with the decrease in concentration. The dynamic rheological behaviour of the biopolymer solutions suggests that food liquids should be carefully prepared according to the thickener to support safe swallowing. The values of dynamic moduli

were also compared at 50 rad.s$^{-1}$ for all thickened solutions (Table 5.2). The viscoelastic moduli (*G'* and *G''*) of thickened solutions followed the order XG > TU > FG > MS > TE, indicating that MS-based solutions showed lower viscoelastic properties, as well as lower pseudoplasticity and apparent viscosity. As shown in the dynamic spectra, a predominance of elastic over viscous behaviour was observed for the XG-based samples. A typical gel behaviour was observed, since *G'* was always greater than *G''* and both exhibited a small frequency-dependence within the studied frequency range (Figure 5.3). This result indicates that xanthan-based thickeners have more promising properties for pleasant swallowing since elastic properties have been considered important parameters for palatability and smooth texture when compared with MS-based thickeners (Cho & Yoo, 2014). On the other hand, FG behaved as a predominant viscous material: *G"* was always greater than *G'*, and the frequency dependence of the moduli decreased with increasing FG concentration. However, the magnitude of both moduli, although smaller than that of XG, was greater than that of MS-based thickeners. Thus, in addition to the higher viscosity achieved for solutions with different concentrations of FG in relation to MS, FG solutions presented a significantly higher elastic parameter (*G'*).

The complex viscosity ($\eta^*$) is calculated from the quotient of *G** (vector sum of *G'* and *G"*) and the frequency. The complex viscosity at 50 rad.s$^{-1}$ ($\eta^*$) can be used to provide information on the stability and sensory consistency of viscoelastic foods (Richardson *et al.*, 1989; Farahmandfar, Asnaashari, Salahi & Rad, 2017) and this property is shown in Table 5.2. The value of $\eta^*$ for all samples increased with increasing concentration, and under the same biopolymer concentration, XG samples had a higher complex viscosity, followed by TU and FG samples. The lowest XG concentration showed no significant differences in $\eta^*$ when compared to the highest FG concentration, displaying the strong thickening capacity of xanthan. At the same concentration of MS and commercial MS-based thickened solutions, no significant differences were observed in this viscoelastic property. Thus, it can be concluded that higher values of $\eta^*$ are associated with a stable/structured gel (greater elastic property), which is potentially relevant for the swallowing process (Cho & Yoo, 2014; He *et al.*, 2016; Anvari & Joyner, 2018).

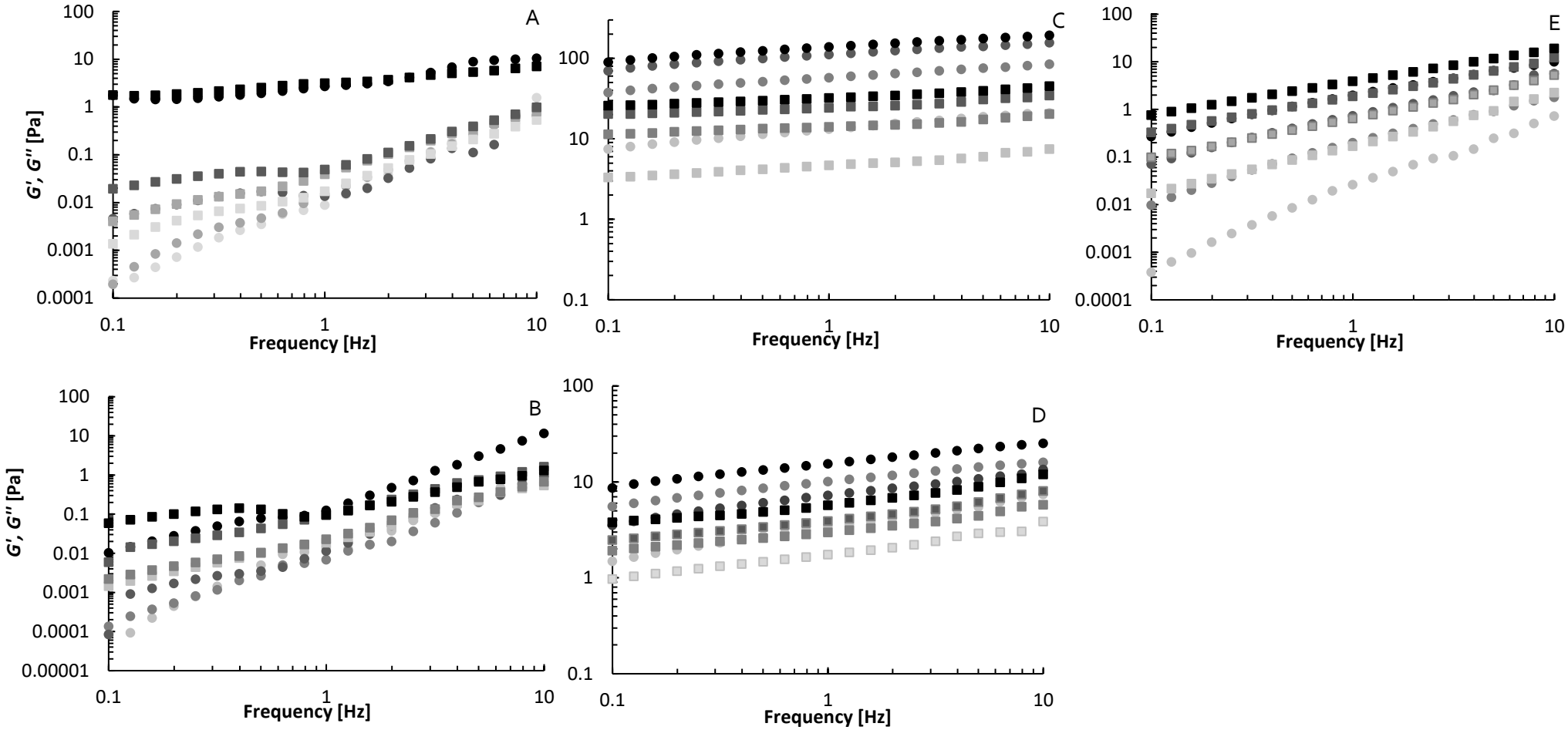



**Figure 5.3.** Elastic modulus (circular symbols) and viscous modulus (quadrangular symbols) as a function of frequency under isothermal (25 °C) conditions of the studied biopolymer solutions based on modified starch (MS (A) and TE (B)), xanthan gum (XG (C) and TU (D)) and flaxseed gum (FG (adapted from Vieira *et al.*, 2019) (E)), at different concentrations (0.75 % ●, 1.5 % ●, 2.25 % ● and 3 % ●).

**Table 5.2.** Storage and loss moduli (*G'* and *G''*) and complex viscosity at 50 rad/s ($\eta$*****) of the biopolymers solutions.

| | **Concentration (% w/w)** | ***G'* [Pa]** | ***G''* [Pa]** | **$\eta$ * [cP]** |
|---|---|---|---|---|
| **MS** | **0.75** | 0.46 ± 0.05 | 0.44 ± 0.03 | 14 ± 2 |
| | **1.5** | 0.64 ± 0.09 | 0.62 ± 0.07 | 17 ± 2 |
| | **2.25** | 0.65 ± 0.07 | 0.69 ± 0.06 | 18 ± 2 |
| | **3** | 1.00 ± 0.11 | 0.67 ± 0.04 | 176 ± 37 |
| **FG** | **0.75** | 0.05 ± 0.03 | 1.83 ± 0.15 | 37 ± 4 |
| | **1.5** | 1.04 ± 0.25 | 4.49 ± 0.13 | 86 ± 5 |
| | **2.25** | 4.95 ± 0.52 | 12.45 ± 0.30 | 269 ± 9 |
| | **3** | 10.04 ± 0.83 | 21.47 ± 1.94 | 451 ± 13 |
| **XG** | **0.75** | 21.17 ± 0.46 | 6.88 ± 0.08 | 445 ± 19 |
| | **1.5** | 81.12 ± 0.69 | 19.14 ± 0.10 | 1667 ± 4 |
| | **2.25** | 116.25 ± 7.00 | 25.74 ± 2.05 | 3200 ± 108 |
| | **3** | 186.75 ± 8.14 | 42.74 ± 3.65 | 3831 ± 137 |
| **TE** | **0.75** | 0.59 ± 0.09 | 0.39 ± 0.05 | 16 ± 2 |
| | **1.5** | 0.60 ± 0.04 | 0.49 ± 0.02 | 15 ± 1 |
| | **2.25** | 0.65 ± 0.08 | 0.73 ± 0.09 | 21 ± 6 |
| | **3** | 7.30 ± 0.15 | 1.00 ± 0.09 | 148 ± 4 |
| **TU** | **0.75** | 4.90 ± 0.66 | 2.78 ± 0.22 | 113 ± 11 |
| | **1.5** | 12.06 ± 2.12 | 5.88 ± 0.90 | 269 ± 37 |
| | **2.25** | 15.46 ± 3.02 | 6.58 ± 1.08 | 337 ± 20 |
| | **3** | 25.63 ± 1.32 | 11.52 ± 0.85 | 557 ± 33 |

### 5.3.2. Tribological properties

The emerging new methods to understand the swallowing process can be crucial in the development of thickeners for dysphagia patients. In this sense, soft tribology is an emerging tool in Food Science, correlating the coefficient of friction with some sensory properties, especially at the end of chewing (Hayashi *et al.*, 2016; Marconati *et al.*, 2019; Torres *et al.*, 2019).

The coefficient of friction as a function of time was evaluated for all the aqueous biopolymers dispersions and the responses for the lowest and highest concentrations of

thickeners are shown in Figures 5.4 and 5.5. Entrainment velocity and normal force were kept fixed while the coefficient of friction was measured over time in order to be correlated with the sensorial characteristics in the final stage of swallowing. Some authors consider that this perceived sensation results from the dynamic response of food products retained/held between the surfaces of the oral cavity, such as the tongue and palate (Selway & Stokes, 2013). The friction curves suggest that the friction coefficient of most formulations remains almost constant at the fixed speed (40 $mm.s^{-1}$) with a thin film of lubricant solution and dominant surface characteristics.

Figure 5.5 shows that the lubricating properties of MS and commercial suspensions were dependent on the concentration of thickener. No significant differences were observed between the coefficients of friction of water (control) and MS solution at 0.75 % w/w, being that their viscosity values were also similar. The coefficient of friction of the MS solution decreased from 0.75 to 2.25 % w/w to values lower than those of the coefficient of friction of water and this lubricant ability could be explained by the formation of a boundary layer of starch particles that prevented direct contact between the opposing steel-PDMS surfaces (lower friction). However, a strong increase of the coefficient of friction was observed for the highest MS concentration (minimum lubricant capacity). As shown in Table 5.1, a marked increase in the viscosity of the MS solution from 2.25 to 3 % w/w was also observed for the different shear rates, relating this behaviour with the sudden change in the coefficient of friction (practically doubled), that exceeded the coefficient of friction of the control (Figure 5.4 and 5.5). This behaviour modification by the thickeners surface in contact may interfere on the perceived texture and on the underpinning mechanisms applied for texture perception (Chen & Stokes, 2012; Liu *et al.*, 2015). A similar behaviour was observed for the commercial thickener based on MS, although it was less pronounced, and the coefficient of friction did not exceed the coefficient of friction of the control (water). This fact indicates that other compounds of the thickener, such as maltodextrin, could exhibit a lubricant effect in combination (or individually) with starch.

On the other hand, the dispersions produced with FG, XG and the XG-based commercial product (TU) showed a low friction coefficient in all studied concentrations (much smaller than water), thus presenting a good lubrication capacity. In addition, a reduction in the coefficient of friction was observed with increasing concentration of these thickeners, similar to that noticed for starch solutions up to 2.25 % w/w. The coefficient of friction of dispersions of FG did not change with the increase of polysaccharide concentration. Once a threshold concentration is achieved to form a particles layer that provides a complete barrier to surface

contact, a further increase in particles number does not have a marked influence on the coefficient of friction (Yakubov *et al.*, 2015, Zhang *et al.*, 2017). Figure 5.5 shows that XG and FG presented similar coefficient of friction for the studied concentration range, representing a similar surface lubrication capacity. However, unlike starch and FG dispersions, XG-containing systems increased their lubricating capacity with increased concentration and viscosity. At the maximum concentration used (3 % w/w) and consequently the highest viscosity, the commercial xanthan-based thickener, XG and FG solutions presented the lowest coefficient of friction with no significant differences between them. These results show evidence of sensorial divergences between gums and modified starch. Indeed, a sensory analysis with a trained panel showed that modified starch and commercial thickener based on modified starch were classified as "Denseness", while xanthan obtained higher values to "Oily mouth coating" (Sharma, Kristo, Corredig, & Duizer, 2017). Our results also suggest potential similarities in sensorial characteristics of FG and XG, according to their similar lubricating characteristics.

Our results indicate that the coefficient of friction would be constant during the chewing time if the force and velocity in the oral cavity were fixed. However, these parameters change during the swallowing process and therefore it is relevant to consider that these results are only qualitative allowing to compare the lubricant effect of the different thickeners. Therefore, lubrication properties varied depending on the biopolymer composition and concentration in solution, and the gum-based formulations have undergone minor changes for different concentrations and reached steady state in less time (Figure 5.4) when compared to those based in modified starch. Each biopolymer behaved differently when incorporated in water, thus obtaining varied rheological and tribological properties probably associated to the strong interactions between water and gums (xanthan and flaxseed) and their higher ability to a fast hydration than other types of hydrocolloids due to their numerous side chains, which could explain their lubricating properties capable of reducing the friction process (Qian *et al.*, 2012; Hamilton & Norton, 2016; Liu *et al.*, 2017, Sun *et al.*, 2018; Martinez *et al.*, 2019). Nguyena, Kravchuk, Bhandari and Prakash (2017) also observed that the increase, decrease or maintenance of the lubricant capacity of the original matrix (yoghurt, in their case) depended on the concentration/viscosity and nature of the incorporated material, and this had an impact on the sensory characteristics.

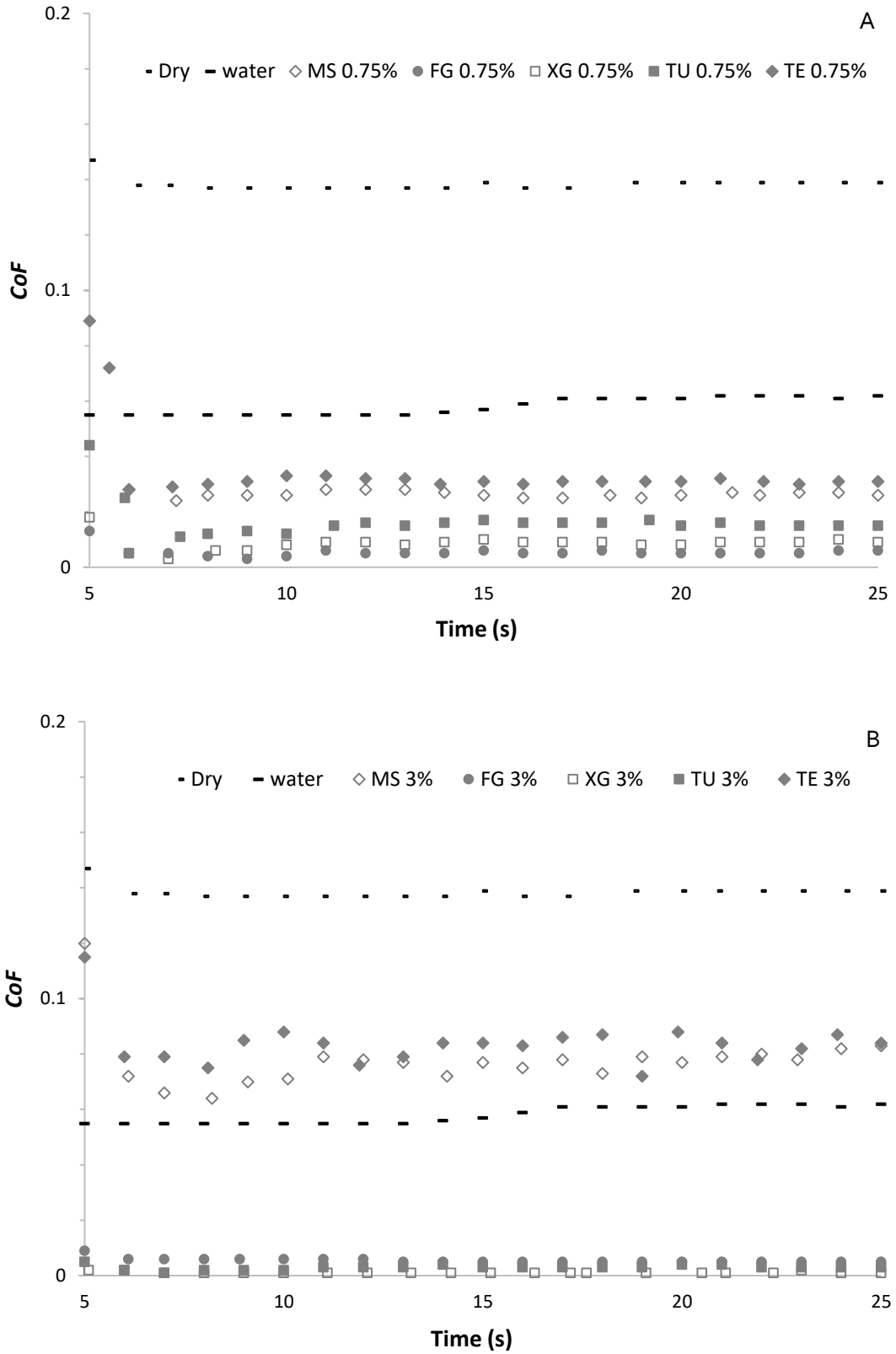



**Figure 5.4**. Coefficient of friction (*CoF*) *versus* time obtained at a fixed entrainment speed (40 $mm.s^{-1}$) for the different formulations produced at the lowest and highest concentrations (0.75 % (A) and 3 % w/w (B)).

It is noteworthy that despite the presence of residual stress and high viscosity at low shear rates observed for the different XG concentrations, the coefficient of friction was significantly lower with increasing velocity until to reach steady state (up to 5 s), followed by TU and FG (data not shown). In this way, the lubrication capacity of the thickened solutions cannot be directly related to the viscosity, since the characteristics and nature of the material are more relevant.

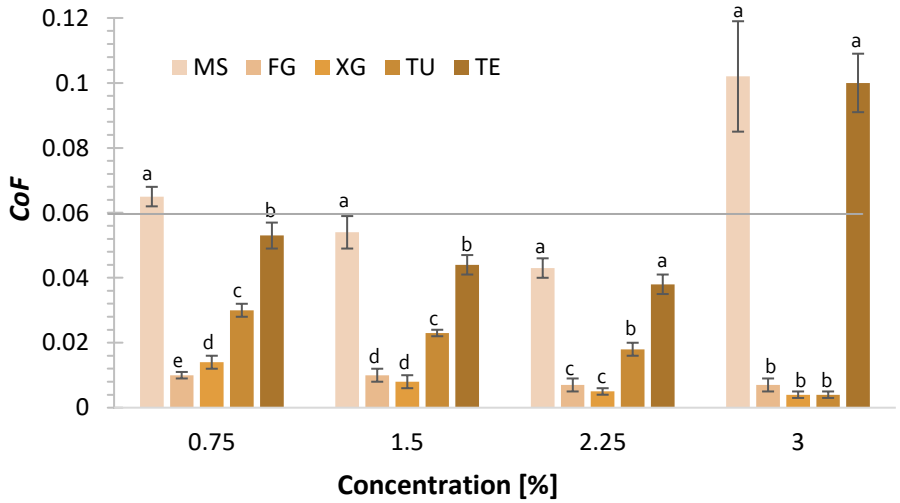



**Figure 5.4.** Coefficient of friction (*CoF*) of the thickeners measured from the tribological analysis at steady state regime. Crossed line represents the coefficient of friction of the control (water). Different letters (a-e) at the same concentration correspond to statistically different samples for a 95 % confidence level.

It should be evidenced that the order of magnitude of the coefficient of friction obtained for starch and xanthan agrees with the literature (Torres *et al.*, 2019). It is also to be noted that commercial XG-based products presented more components than MS products. This fact made all the rheological and tribological properties similar between starch-based solutions, unlike xanthan gum-based solutions, since the viscosity of the commercial product TU was different from xanthan, despite having similar elastic character and tribological properties.

It should be also noted that, the same studied concentrations of the thickeners incorporated in water according were also tribologically tested in milk and soy juice matrices using the same methodology (data and discussion presented in Appendix C).

## 5.4. Conclusions

The present study provided additional evidences of FG as a potential thickener to dysphagic patients. The rheological properties and lubricating characteristics were evaluated to cover forces and velocity during swallowing and the results were promising as compared to the commercially available thickeners. The increase in the biopolymer concentration significantly affected rheological properties, but XG showed the highest viscosity, pseudoplasticity and viscoelasticity, followed by FG. MS and the MS-based thickener only exerted a relevant impact on the viscosity, pseudoplasticity and viscoelastic properties (*G'*, *G''*) at the highest concentration studied (3 % w/w). Similar to rheological studies, the biopolymer nature and concentration had a significant impact on the tribological behavior. In general, similar lubricating capacity was observed between the gum-based formulations, since the coefficient of friction was lower to than that of water. On the other hand, MS based solutions tended to maintain the coefficient of friction similar to water (in low concentration) but increased at the highest studied concentration. Thus, the greater lubrication capacity of the gums can occur in the swallowing process and avoid the unpleasant stickiness feeling. Therefore, our results suggest that although FG shows less pseudoplasticity (such as MS) than XG-based products, FG exhibited a low coefficient of friction as XG-based products, which could be correlated with a pleasant sensation in the last step of swallowing. Thus, these rheological and tribological results coupled showed that the choice of the biopolymer base to be incorporated into a potential food thickener has a significant impact on the mechanical properties, thus leading to differences in the sensory texture. These findings, coupled with the high nutritional value of flaxseed gum may help in a faster recovery of dysphagia patients, and contribute to the mitigation of the consequences imposed by the treatments.

## 5.5. Acknowledgements

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (Finance code 001); Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP) (Process numbers 2016/05448-8; 2011/51707-1; EMU 2009/54137-1; 2007/58017-5; 2006/03263-9; 2004/08517-3) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

### 5.6. References

Anvari, M. and Joyner, H.S., 2018. Effect of fish gelatin and gum arabic interactions on concentrated emulsion large amplitude oscillatory shear behavior and tribological properties. Food Hydrocolloids, 79, 518-525.

Chen, J., 2009. Food oral processing – A review. Food Hydrocolloids, 23, 1-25.

Chen, J. and Stokes, J.R., 2012. Rheology and tribology: Two distinctive regimes of food texture sensation. Trends in Food Science & Technology, 25(1), 4-12.

Cho, H.M. and Yoo, B., 2014. Rheological Characteristics of Cold Thickened Beverages Containing Xanthan Gum–Based Food Thickeners Used for Dysphagia Diets. Journal of the Academy of Nutrition and Dietetics, 115(1), 106-111.

Cui, W., Mazza, G., Biliaderis, C.G, 1994. Chemical Structure, Molecular Size Distributions, and Rheological Properties of Flaxseed Gum. Journal of Agrucultural and Food Chemisry, 42 (9), 1891–1895.

Cutler, A.N., Morris, E.R., Taylor, L.J., 1983. Oral Perception of Viscosity in fluid foods and model systems. Food and Nutrition Press, 14, 377-395.

Clavé, P. and Shaker, R., 2015. Dysphagia: current reality and scope of the problem. Nature Reviews. Gastroenterology and Hepatology. 12: 259-270.

Cui, W., Mazza, G., Biliaderis, C.G, 1994. Chemical Structure, Molecular Size Distributions, and Rheological Properties of Flaxseed Gum. Journal of Agricultural and Food Chemistry, 42(9), 1891–1895.

Dewar and Joyce, 2006. The thixotropic and rheopectic behaviour of maize starch and maltodextrin thickeners used in dysphagia therapy. Carbohydrate Polymers 65, 296–305.

Ding, H.H., Cui, S.W., Goff, H.D., Wang, Q., Chen, J., Han, N.F., 2014. Soluble polysaccharides from flaxseed kernel as a new source of dietary fibres: Extraction and physicochemical characterization. Food Research International, 56, 166–173.

Douaire, D., Stephenson, T., Norton, T., 2014. Soft tribology of oil-continuous emulsions. Journal of Food Engineering, 139, 24-30.

Elboutachfaiti, R., Delattre, C., Anthony Quérro, A., Roulard, R., Julie Duchêne, J., Mesnard, F., Petit, E., 2017. Fractionation and structural characterization of six purified rhamnogalacturonans type I from flaxseed mucilage. Food Hydrocolloids, 62, 273-279.

Farahmandfar, R., Asnaashari, M., Salahi, M.R., Rad, T.K., 2017. Effects of basil seed gum, Cress seed gum and Quince seed gum on the physical, textural and rheological

properties of whipped cream. International Journal of Biological Macromolecules, 98, 820–828.

Feng, M.-C., Lin, Y.-C., Chang, Y.-H., Chen, C.-H., Chiang, H.-C., Huang, L.-C., Yang, Y.-H., Hung, C.-H., 2019. The Mortality and the Risk of Aspiration Pneumonia Related with Dysphagia in Stroke Patients. Journal of Stroke and Cerebrovascular Diseases, 28(5), 1381-1387.

Ferry, A.-L., Hort, J., Mitchell, J.R., Cook, D.J., Lagarrigue, S., Pamies, B.V., 2006. Viscosity and flavour perception: Why is starch different from hydrocolloids? Food Hydrocolloids, 20, 855–862.

Foster-Powell, K., Holt, S.H., Brand-Miller, J.C., 2002. International table of glycemic index and glycemic load values: 2002. The American Journal of Clinical Nutrition, 76(1), 5-56.

Funami, T., 2011. Next target for food hydrocolloid studies: Texture design of foods using hydrocolloid technology. Food Hydrocolloids, 25(8), 1904-1914.

Germain, I., Dufresne, T., Ramaswamy, H.S., 2006. Rheological characterization of thickened beverages used in the treatment of dysphagia. Journal of Food Engineering, 73, 64-74.

Hadde, E.K. and Chen, J., 2019. Shear and extensional rheological characterization of thickened fluid for dysphagia management. Journal of Food Engineering, 245, 18-23.

He, Q., Hort, J., Wolf, B., 2016. Predicting sensory perceptions of thickened solutions based on rheological analysis. Food Hydrocolloids, 61, 221-232.

Hamilton, E. and Norton, I.T., 2016. Modification to the lubrication properties of xanthan gum fluid gels as a result of sunflower oil and triglyceride stabilised water in oil emulsion addition. Food Hydrocolloids, 55, 220-227.

Hayashi, M., Takei, R., Umene, S., Narita, K., Kato, K., Kobayashi, Y., Honma, Y., 2016. Tribological analysis of the surface of food gels. Food Hydrocolloids, 58, 343-346.

Hori, K., Hayashi, H., Yokoyama, S., Ono, T., Ishihara, S., Magara, J., Taniguchi, H., Funami, T., Maeda, Y. and Inoue, M., 2015. Comparison of mechanical analyses and tongue pressure analyses during squeezing and swallowing of gels. Food Hydrocolloids 44, 145-155.

Jo, W., Bak, J.H., Yoo, B., 2018. Rheological characterizations of concentrated binary gum mixtures with xanthan gum and galactomannans. International Journal of Biological Macromolecules, 114, 263-269.

Krop, E.M., Hetherington, M.M., Holmes, M., Miquel, S., Sarkar, A., 2019. On relating rheology and oral tribology to sensory properties in hydrogels. Food Hydrocolloids, 88, 101-113.

Laiho, S., Williams, R.P.W., Poelman, A., Appelqvist, I., Logan, A., 2017. Effect of whey protein phase volume on the tribology, rheology and sensory properties of fat-free stirred yoghurts. Food Hydrocolloids, 67, 166-177.

Leonard, R.J., White, C., McKenzie, S., Belafsky, P.C., 2014. Effects of Bolus Rheology on Aspiration in Patients with Dysphagia. Journal of the Academy of Nutrition and Dietetics, 2212-2672.

Li, N., Qi, G., Sun, X.S., Wang, D., 2016. Characterization of gum isolated from Camelina seed. Industrial Crops and Products, 83, 268-274.

Liu, K., Stieger, M., van der Linden, van de Velde, E, 2015. Fat droplet characteristics affect rheological, tribological and sensory properties of food gels. Food Hydrocolloids, 44: 244-259.

Liu, J., Shim, Y.Y., Poth, A.G., Reaney, M.J.T., 2016. Conlinin in flaxseed (*Linum usitatissimum* L.) gum and its contribution to emulsification properties. Food Hydrocolloids, 52, 963-971.

Liu, J., Shim, Y.Y., Shen, J., Wang, Y., Reaney, M.J.T. (2017). Whey protein isolate and flaxseed (*Linum usitatissimum* L.) gum electrostatic coacervates: Turbidity and rheology. Food Hydrocolloids, 64, 18-27.

Mackley, M.R., Tock, C., Anthony, R., Butler, S.A., Chapman, G. and Vadillo, D.C., 2013. The rheology and processing behavior of starch and gum-based dysphagia thickeners. Journal of Rheology, 57(6), 1533-1553.

Marconati, M., Engmann, J., Burbidge, A.S., Mathieu, V., Souchon, I., Ramaioli, M., 2019. A review of the approaches to predict the ease of swallowing and post-swallow residues. Trends in Food Science & Technology, 86, 281-297.

Martínez, O., Vicente, M.S., De Vega, M.C., Salmerón, J., 2019. Sensory perception and flow properties of dysphagia thickening formulas with different composition. Food Hydrocolloids, 90, 508–514.

Meng, Y., Rao, M.A., Datta, K., 2005. Computer simulation of the pharyngeal bolus transport of Newtonian and Non-Newtonian fluids Food and Bioproducts Processing, 83 (4), 297-305.

Miller, J.L and Watkin, K.L., 1996. The influence of bolus volume and viscosity on anterior lingual force during the oral stage of swallowing. Dysphagia, 11(2), 117-124.

Morris, D.H. and Vaisey-Genser, M., 2003. FLAXSEED. Encyclopedia of Food Sciences and Nutrition (2nd Edition), 2525–2531.

National Dysphagia Diet Task Force, 2002. National dysphagia diet: standardization for optimal care. Chicago, IL, American Dietetic Association.

Nguyena, P.T.M., Kravchuk, O., Bhandari, B., Prakash, S., 2017. Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. Food Hydrocolloids, 72, 90-104.

Prakash, S., Tan, Y.Y.D., Chen, J., 2013. Applications of tribology in studying food oral processing and texture perception. Food Research International, 54(2), 1627-1635.

Qian, K.Y., Cui, S.W., Nikiforuk, J., Goff, H.D., 2012. Structural elucidation of rhamnogalacturonans from flaxseed hulls. Carbohydrate Research, 362, 47-55.

Richardson, R.K., Morris, E.R., Ross-Murphy, S.B., Taylor, L.J., Dea, I.C.M., 1989. Characterization of the perceived texture of thickened systems by dynamic viscosity measurements. Food hydrocolloids, 3(3), 175-191.

Shama, F. and Sherman, P., 1973. Identification of stimuli controlling the sensory evaluation of viscosity II. Oral methods. Journal of Texture Studies, 4(1), 111-118.

Sharma, M., Kristo, E., Corredig, M., Duizer, L., 2017. Effect of hydrocolloid type on texture of pureed carrots: Rheological and sensory measures. Food Hydrocolloids, 63, 478-487.

Selway, N. and Stokes, J.R., 2013. Insights into the dynamics of oral lubrication and mouthfeel using soft tribology: Differentiating semi-fluid foods with similar rheology. Food Research International, 54, 423–431.

Stokes, J. R., Boehm, M. W., Baier, S. K., 2013. Oral processing, texture and mouthfeel: From rheology to tribology and beyond. Current Opinion in Colloid & Interface Science, 18, 349–359.

Sun, C., Liang, B., Sheng, H., Wang, R., Zhao, J., Zhang, Z., Zhang, M., 2018. Influence of initial protein structures and xanthan gum on the oxidative stability of O/W emulsions stabilized by whey protein. International Journal of Biological Macromolecules, 120, 34-44.

Szczesniak, A.S. and Farkas, E., 1963. Objective characterization of the mouthfeel of gum solutions. Journal of Food Science, 27, 381-385.

Upadhyay, R. and Chen, J., 2019. Smoothness as a tactile percept: Correlating ‘oral’ tribology with sensory measurements. Food Hydrocolloids, 87, 38-47.

Torres, O., Yamada, A., Rigby, N.M., Hanawa, T., Kawano, Y., Sarkar, A., 2019. Gellan gum: A new member in the dysphagia thickener family. Biotribology, 17, 8-18.

Vieira, J.M., Mantovani, R.A., Raposo, M.F.J., Coimbra, M.A., Vicente, A.A., Cunha, R.L., 2019. Effect of extraction temperature on rheological behavior and antioxidant capacity of flaxseed gum. Carbohydrate Polymers, 213, 217-227.

Wang, E.Q., 2010. Dysphagia. Encyclopedia of Movement Disorders, 365-367.

Yakubov, G.E., Branfield, T.E., Bongaerts, J.H.H., Stokes, J.R., 2015. Tribology of particle suspensions in rolling-slidding soft contacts. Biotribology, 3, 1-10.

Wood, G.N., 1968. Centric occlusion, centric relation, and the mandibular posture. The Journal of Prosthetic Dentistry, 20(4), 292-306.

Zhang, B., Selwayc, N., Shelat, K.J., Dhital, S., Stokes, J.R., Gidley, M.J., 2017. Tribology of swollen starch granule suspensions from maize and potato. Carbohydrate Polymers 155, 128–135.

***CAPÍTULO VI***

---

*Discussão Geral*

## 6. Discussão Geral

O aumento do número de pacientes diagnosticados com disfagia orofaríngea e a consequente intensificação na busca de novos tratamentos promovem a pesquisa de novos potenciais espessantes capazes de suprir as necessidades dos diversos tipos de doentes com este distúrbio. Esta tese apresenta a proposta de incorporação da goma de linhaça na composição de novos espessantes que sejam direcionados para pacientes disfágicos.

A extração e caracterização da goma da linhaça (FG) (Capítulo III) foi o primeiro e fulcral passo para o entendimento do potencial deste biopolímero como ingrediente de uma formulação de espessante. Primeiramente, o método de extração foi analisado de forma a se obter as características/propriedades necessárias para aplicação em uma possível formulação espessante destinada a pacientes com disfagia. A goma obtida por diferentes temperaturas de extração foi pormenorizadamente caraterizada em termos de composição, estrutura e funcionalidade, uma vez que se trata de um produto desenvolvido para ser ingerido diariamente. O rendimento da extração aumentou quase duas vezes com o incremento de temperatura para 60 °C (10 % m/m) quando comparado com a extração à 25 °C (5,7 % m/m), porém também foi observada alteração na composição e funcionalidade das gomas. O conteúdo proteico e o teor total de fenólicos (TPC) da FG também aumentaram com a temperatura de extração, sendo que a FG extraída a 60 °C apresentou os maiores valores de TPC. Devido à afinidade entre os compostos fenólicos e alguns grupamentos das proteínas, sugeriu-se que alguns compostos fenólicos foram extraídos em maior quantidade por arraste a altas temperaturas. A composição quantitativa e qualitativa dos compostos fenólicos da FG também foi dependente da temperatura de extração. As concentrações de ácido cafeico e ácido p-cumarico + epicatequina diminuíram, enquanto que o conteúdo de ácido elágico aumentou com a temperatura de extração. Por outro lado, ácido cinâmico também foi detectado e sua concentração manteve-se constante, independentemente da temperatura de extração. Já a presença do ácido vanílico foi detetada apenas na FG extraída à 60 °C.

Todas as soluções aquosas de FG extraída a diferentes temperaturas apresentaram comportamento pseudoplástico e viscoelástico, porém o aumento da temperatura de extração da goma diminuiu sua capacidade de aumentar a viscosidade, pseudoplasticidade e a viscoelasticidade das soluções. Estes resultados foram associados com o aumento do teor de proteína que promoveu a formação de uma rede mista e descontínua entre as cadeias de polissacarídeo e proteínas (FEDENIUK; BILIADERIS, 1994; QIAN et al., 2012). Assim, embora um aumento no rendimento de goma e uma maior capacidade antioxidante tenham

sido observados com o aumento da temperatura de extração, também foi notado o efeito deletério nas características almejadas para um agente espessante. Assim, a goma extraída a 25 °C (cuja composição se encontra na Tabela 6.1) foi selecionada para a continuidade do estudo, considerando que a alteração na composição com o aumento da temperatura de extração, menor quantidade de açúcares totais, responsáveis pela capacidade espessante, e maior teor proteico e fenólico, foi negativa em termos reológicos. Outro ponto importante na FG extraída a 25 °C foi que mostrou maior estabilidade reológica frente ao pH, ao contrário da FG extraída a temperaturas mais altas que mostrou menor estabilidade principalmente em baixas concentrações de FG.

**Tabela 6.1.** Características da goma de linhaça extraída a 25 °C.

| **Teor proteico (%)** | **Açúcares Totais (%)** | **Fenólicos Totais (mg GAE*. 100 $g^{-1}$)** | **Atividade Antioxidante (% RSA**)** | **Potencial Zeta** |
|---|---|---|---|---|
| 4,33 | 66,5 | 12,37 | 4,39 | −29,37 |

* *Galic Acid Equivalents*

** *Radical Scavenging Activity*

Após os ensaios reológicos da goma de linhaça em soluções aquosas em diferentes condições de pH, foi observado o comportamento da FG em diferentes concentrações e sua potencial sinergia com diferentes biopolímeros (amido pré-gelatinizado, xantana, guar e pectina) que pudessem ser incorporados nas formulações espessantes. A avaliação da interação entre os biopolímeros foi feita com o auxílio de planejamento experimental do tipo *Placket Burmann*, tendo como variáveis a concentração dos polissacarídeos e como resposta, além da viscosidade à taxa de deformação de 50 $s^{-1}$ conforme recomenda a NDD, também nas taxas de deformação de 10 e 400 $s^{-1}$, bem como parâmetros obtidos a partir de ensaios reológicos oscilatórios como *G'* e *G''* (Apêndice A). Após a análise desses resultados foi possível o delineamento de um planejamento experimental fatorial completo (Delineamento Composto Central Rotacional- DCCR) com as três variáveis de concentração biopolimérica que apresentaram impacto significativo nos parâmetros reológicos estudados (amido- MS, xantana- XG e goma de linhaça- FG), dentro do intervalo de concentrações analisado. Este planejamento experimental foi utilizado em três matrizes diferentes: água (Capítulo IV), leite e suco de soja com sabor de laranja (Apêndice B). Os resultados deste estudo contribuem para a compreensão do comportamento reológico dos diferentes tipos de espessantes em vários meios de dispersão, o que pode auxiliar nos cuidados paliativos de indivíduos com disfagia.

Para cada matriz foi gerado um modelo matemático com o intuito de predizer o comportamento reológico das misturas de polissacarídeos (viscosidade em diferentes taxas de cisalhamento, índice de comportamento do fluido, viscosidade complexa, módulos elástico- G' e viscoso- G") de acordo com as concentrações de cada um dos componentes das formulações. De acordo com os resultados obtidos neste estudo, observou-se que, independentemente da matriz analisada, a FG apresentou uma elevada capacidade de interação com os biopolímeros e foi o componente com maior influência no aumento da viscosidade. O amido também apresentou influência significativa no aumento da viscosidade das diferentes matrizes espessadas, apesar da sua baixa capacidade espessante nas concentrações estudadas quando avaliado sem a presença dos outros biopolímeros, vincando a sinergia obtida nas formulações estudadas. Quanto à goma xantana, apesar de uma menor influência na viscosidade, principalmente no suco de soja com sabor de laranja, foi possível observar que apresentou impacto significativo no aumento da pseudoplasticidade em todas as matrizes estudadas. De forma a confirmar a capacidade de predição das equações obtidas para predizer a viscosidade a 50 $s^{-1}$, foi efetuada uma validação para as categorizações de viscosidade dos produtos recomendados para pacientes com disfagia (*Nectar-like* (51-350 cP), *Honey-like* (351-1750 cP) e *Spoon thick* (>1750 cP)). Este estudo, além de poder funcionar como base de um novo produto espessante, pode auxiliar profissionais de saúde no cuidado paliativo de pacientes hospitalizados, dado que fornece indicações para formular e obter as propriedades reológicas de acordo com a necessidade do paciente, a nível de segurança na deglutição. Através desta validação foi confirmada a capacidade dos modelos matemáticos gerados em alcançar as viscosidades pretendidas de acordo com a concentração de cada componente. De forma a se ter um maior conhecimento sobre as formulações desenvolvidas, a quantidade de glicose liberada por alguns espessantes foi avaliada por meio de ensaios de digestibilidade *in vitro* e estes resultados comparados com um espessante comercial baseado em amido modificado. De acordo com os resultados obtidos, foi notório que os espessantes comerciais baseados em amido modificado apresentaram uma quantidade de glicose liberada significativamente superior às formulações desenvolvidas, apesar da viscosidade similar. Dentre os espessantes formulados neste trabalho, observou-se que o amido modificado foi o componente com maior impacto na liberação de glicose, sendo que os demais polissacarídeos não tiveram efeito sobre esta resposta. Assim sendo, os resultados apresentados podem gerar diferentes formulações de espessantes com variadas ou combinadas finalidades. Por exemplo, um maior aporte de fibras pode ser feito com a seleção de um espessante com maior quantidade de FG, enquanto que a obtenção de um espessante mais barato deveria ter maior

quantidade de MS na composição. Um *mouthfeel* mais agradável (associado à maior pseudoplasticidade) poderia ser alcançado com o aumento da XG e, por fim, se o produto pretendido for de baixo aporte de glicose, a concentração de MS deve ser baixa ou até nula.

Em paralelo com o estudo intensivo das características reológicas discutido anteriormente, foram analisadas as características tribológicas dos componentes individualmente incorporados nas diferentes matrizes propostas (água, leite e suco de soja com sabor de laranja (Capítulo V e Apêndice C). Com esta análise, através do coeficiente de atrito (propriedade tribológica), foi possível observar as propriedades lubrificantes dos polissacarídeos em contato com superfícies simuladoras da cavidade oral (língua e palato duro) em diferentes concentrações. As propriedades tribológicas de dois espessantes comerciais, um a base de amido e outro a base de xantana, foram comparadas com os biopolímeros avaliados neste estudo. Estas propriedades foram avaliadas com o intuito de ampliar o conhecimento da relação entre a capacidade de escoamento entre superfícies sólidas e o processo de deglutição, o qual está amplamente associado à habilidade de manter a viscosidade durante a passagem do bolo alimentar pela cavidade oral. Verificou-se que o perfil de lubrificação de todos os espessantes incorporados na água foi influenciado pela viscosidade das soluções biopoliméricas, uma vez que em baixas concentrações, as soluções contendo MS apresentaram praticamente a mesma viscosidade da água e não resultaram em modificação da capacidade de lubrificação na presença deste componente. No entanto, o aumento da concentração de amido reduziu a capacidade de lubrificação das matrizes embora tenha levado a um aumento da viscosidade. Por outro lado, a adição de gomas levou a aumento da lubrificação com a redução do coeficiente de atrito. Foram observados valores de coeficientes de atrito da mesma ordem de grandeza para as duas gomas estudadas (FG e XG) e o espessante baseado em XG, ou seja, apresentam lubrificação similar, o que sugere *mouthfeel* semelhante. Desta forma, foram obtidos indícios de que apesar de a FG não apresentar uma pseudoplasticidade tão alta como a XG, ela pode apresentar algumas semelhanças de facilidade/*mouthfeel* no processo de deglutição, uma vez que as características tribológicas de alimentos estão relacionadas com as percepções sensoriais (HAYASHI et al., 2016).

Nossos dados mostram evidências claras do potencial da FG como espessante direcionado para a população disfágica em geral, podendo mesmo, em casos específicos, funcionar como aporte nutricional ao mesmo tempo que supre as necessidades básicas dos pacientes devido às suas propriedades reológicas. Além disso, apresentam indícios de poderem aliar à segurança na deglutição, uma possível melhoria na sensação ao ingerir

aquando da sua interação com outros biopolímeros. O desenvolvimento de um novo produto baseado no design experimental estudado pode provocar uma melhoria na qualidade de vida dos pacientes com disfagia devolvendo-lhes o prazer e afastando o medo de comer e, quem sabe, desta forma evitar o mal-estar, dificuldades em mastigar e engolir que resultam na perda de peso, tosse, engasgo, desidratação e desnutrição e até o isolamento social causado por esta condição.

### 6.1. Referências Bibliográficas

FEDENIUK, R.W.; BILIADERIS, C.G. Composition and Physicochemical Properties of Linseed (*Linum usitatissimum* L.) Mucilage. **Journal of Agricultural and Food Chemistry**, v. 42, n. 2, p. 240–247, 1994.

QIAN, K.Y. et al. Structural elucidation of rhamnogalacturonans from flaxseed hulls. **Carbohydrate Research**, v. 362, p. 47-55, 2012.

HAYASHI, M. et al. Tribological analysis of the surface of food gels. **Food Hydrocolloids**, v. 58, p. 343-346, 2016.

***CAPÍTULO VII***

---

*Conclusão Geral*

*Sugestões para trabalhos futuros*

## 7. Conclusões Gerais

Com base nos resultados obtidos no decorrer deste trabalho foi possível concluir que a goma de linhaça (FG) apresenta um grande potencial para ser incorporado em uma vasta gama de produtos produzidos pela indústria alimentar devido às suas propriedades funcionais e nutricionais. A temperatura de extração influenciou significativamente o rendimento, composição e comportamento reológico da goma de linhaça. Temperaturas mais elevadas favoreceram a extração de mais compostos fenólicos, resultando em uma maior capacidade antioxidante da goma. No entanto, a goma de linhaça extraída em temperaturas mais baixas, quando adicionada nas matrizes estudadas, apresentou maior viscosidade, pseudoplasticidade e viscoelasticidade, as quais são propriedades importantes na formulação de espessantes para disfágicos. Além disso, a FG extraída na menor temperatura estudada (25 °C) apresentou propriedades reológicas mais estáveis frente ao pH, sendo, por isso, selecionada com maior potencial para incorporar em uma possível formulação espessante. Este potencial foi confirmado através da avaliação da interação dessa goma com outros polissacarídeos usando a técnica de planejamento experimental fatorial completo (DCCR), obtendo-se como resposta o comportamento reológico e cor. Foi observada uma elevada sinergia da FG com os outros biopolímeros (amido pré-gelatinizado- MS e goma xantana- XG), porém a maior influência no aumento da viscosidade foi dada pela goma de linhaça embora MS e XG exerceram importantes papéis no comportamento avaliado. O MS misturado com a XG e FG mostrou elevada influência no aumento da pseudoplasticidade e nas propriedades elásticas dos espessantes formulados, embora individualmente não tenha exercido efeito relevante nas propriedades reológicas. No entanto, a adição desse componente deve ser feita com moderação, pois estudos da digestibilidade *in vitro* mostraram que este ingrediente pode elevar a carga glicêmica absorvida, o que está associado a doenças crônicas como diabetes. Quanto à XG, terceiro e último componente das formulações estudadas, apesar de estar presente com um valor muito menor de concentração, teve uma influência relevante no aumento do comportamento elástico e pseudoplástico das formulações, com um possível papel na melhora das características sensoriais de consumo. Dessa forma, observou-se que os espessantes baseados em FG e XG apresentaram vantagens quanto ao comportamento reológico e à quantidade de glicose liberada.

Com o intuito de se obter mais informações sobre a deglutição dos constituintes escolhidos para as formulações espessantes, foram analisados seu comportamento reológico e tribológico, além de dois espessantes comerciais a fim de compará-los nas mesmas condições

de concentração e consumo. Observaram-se comportamentos de lubrificação similares para o FG, XG e o espessante comercial a base de XG. Apesar das propriedades reológicas apontarem diferenças entre FG e XG, as propriedades tribológicas mostraram semelhanças entre estas gomas. Posto isto, apesar de a deglutição ser um processo complexo e, embora a reologia e a tribologia de fluidos espessados não clarifiquem todos os fenômenos envolvidos durante esse processo, eles contribuem com propriedades mecânicas importantes dos mesmos, informações essas valiosas para a compreensão da deglutição e para delinear uma estratégia de manejo adequada. Além disso, os modelos matemáticos dos parâmetros reológicos obtidos neste estudo ajudarão em potenciais futuras formulações e/ou na comparação dos dados de viscosidade obtidos por outros métodos, como por exemplo por videofluoroscopia.

## 7.1. Sugestões para trabalhos futuros

- Medição da viscosidade extensional da goma de linhaça e formulação de espessantes, ampliando a análise reológica de forma a simular um potencial movimento do bolo alimentar na transição entre a fase final da cavidade oral/faringe até a laringe e posteriormente o esôfago;
- Ensaios tribológicos variando a velocidade e a força normal aplicada entre as superfícies de forma a observar o coeficiente de atrito em diferentes regimes;
- Incorporação da saliva nas análises tribológicas, de modo a observar se as características lubrificantes são alteradas;
- Confirmar a relação de dados instrumentais obtidas no reômetro e tribômetro com medidas sensoriais (por exemplo, coesividade e adesividade) de forma a confirmar as relações/sugestões feitas neste trabalho;
- Determinar o índice glicêmico e consequente carga glicêmica dos espessantes.

***CAPÍTULO XIII***

# *Referências Bibliográficas*

## 8. Referências Bibliográficas

ABDULMOLA, N.A. et al. Effect of xanthan on the small-deformation rheology of crosslinked and uncrosslinked waxy maize starch. **Carhohydrate Polymers**, v. 31, p. 65-78, 1996.

ABE, R. et al. Videoendoscopic measurement of food bolus formation for quantitative evaluation of masticatory function. **Journal of Prosthodontic Research**, v. 55, n. 3, p. 171-178, 2011.

ABOZED, S.S. et al. Total phenolic contents and antioxidant activities of various solvent extracts from whole wheat and bran. **Annals of Agricultural Sciences**, v. 59, n. 1, p. 63-67, 2014.

ACHAYUTHAKAN, P.; SUPHANTHARIKA, M. Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. **Carbohydrate Polymers**, v. 71, n. 1, p. 9-17, 2008.

AKOMOLAFEA, S.F. et al. Effect of caffeine, caffeic acid and their various combinations on enzymes of cholinergic, monoaminergic and purinergic systems critical to neurodegeneration in rat brain-In vitro. **NeuroToxicology**, v. 62, p. 6-13, 2017.

ALMEIDA, I.V. et al. Different responses of vanillic acid, a phenolic compound, in HTC cells: cytotoxicity, antiproliferative activity, and protection from DNA-induced damage. **Genetic and Molecular Research**, v. 15, n. 4, p. 1-12, 2016.

ALU'DATT, M.H. et al. Distribution, antioxidant and characterisation of phenolic compounds in soybeans, flaxseed and olives. **Food Chemistry**, v. 139, p. 93–99, 2013.

ALU'DATT, M.H. et al. Characterization and antioxidant activities of phenolic interactions identified in byproducts of soybean and flaxseed protein isolation. **Food Hydrocolloids**, v. 61, p. 119-127, 2016.

ANASTASIADES, A. et al. Rheological and physical characterization of pregelatinized maize starches. **Journal of Food Engineering**, v. 52, p. 57–66, 2002.

ANDERSON, E.; LOWE, H.J. The composition of flaxseed mucilage. **Journal of. Biology and Chemistry**, v. 168, p. 289-297, 1947.

ANVARI, M.; JOYNER, H.S. Effect of fish gelatin and gum arabic interactions on concentrated emulsion large amplitude oscillatory shear behavior and tribological properties. **Food Hydrocolloids**, v. 79, p. 518-525, 2018.

ASSAD-BUSTILLOS et al. Relationships of oral comfort perception and bolus properties in the elderly with salivary flow rate and oral health status for two soft cereal foods. **Food Research International**, v. 118, p. 13-21, 2019.

AWAD, A.B. et al. Phytosterol feeding induces alteration in testosterone metabolism in rat tissues. **Journal of Nutricional Biochemistry**, v. 9, n. 12, p. 712-717, 1998.

AWAD, A.B. et. al. Dietary phytosterol inhibits the growth and metastasis of MDA-MB-231 human breast cancer cells grown in SCID mice. **Anticancer Research**, v. 20, n. 2A, 821-824, 2000.

BARNES, H. A. Shear-Thickening ("Dilatancy") in Suspensions of Nonaggregating Solid Particles Dispersed in Newtonian Liquids. **Journal of Rheology**, v. 33, p. 329-366, 1989.

BLOIS, M.S. Antioxidant determinations by the use of a stable free radical. **Nature**, v. 181, n. 4617, p. 1199–1200, 1958.

BUETTNER, A. et al. Observation of the swallow process by application of videofluoroscopy and real time magnetic resonance imaging-Consequence for retronasal aroma stimulation. **Chemical senses**, v. 26, p. 1211-1219, 2001.

CAMPBELL, C.L. et al. Designing foods for satiety: The roles of food structure and oral processing in satiation and satiety. **Food Structure**, v. 13, p. 1-12, 2017.

CAPITANI, M.I. et al. Microstructure, chemical composition and mucilage exudation of chia (*Salvia hispanica* L.) nutlets from Argentina. **Journal of the Science of Food and Agriculture**, v. 93, n. 15, p. 3856-3862, 2012.

CAPITANI, M.I. et al. Rheological properties of aqueous dispersions of chia (*Salvia hispanica* L.) mucilage. **Journal of Food Engineering**, v. 149, p. 70-77, 2015.

CHAISAWANG, M.; SUPHANTHARIKA, M. Pasting and rheological properties of native and anionic tapioca starches as modified by guar gum and xanthan gum. **Food Hydrocolloid**s, v. 20, n. 5, p. 641-649, 2006.

CHAN, P. et al. Studies of the shear and extensional rheology of casein, waxy maize starch and their mixtures. **Food hydrocolloids**, v. 21, p. 716-725, 2007.

CHEN, J. et al. Flaxseed and its components reduce metastasis after surgical excision of solid human breast tumor in nude mice. **Cancer Letters**, v. 234, n. 2, p. 168–175, 2006.

CHEN, H. et al. Gelation properties of flaxseed gum. **Journal of Food Engineering**, v. 77, p. 295–303, 2006.

CHEN, J. Food oral processing-A review. **Food Hydrocolloids**, n. 23, p. 1–25, 2009.

CHEN, J.; LOLIVRET, L. The determining role of bolus rheology in triggering a swallowing. Food Hydrocolloids, v. 25, n. 3, p. 325-332, 2011.

CHEN, J.; STOKES, J.R. Rheology and tribology: Two distinctive regimes of food texture sensation. **Trends in Food Science & Technology**, v. 25, n. 1, p. 4-12, 2012.

CHEN, J. Food oral processing: Mechanisms and implications of food oral destruction. **Trends in Food Science & Technology**, v. 45, n. 2, p. 222-228, 2015.

CHEN, C. et al. Effect of flaxseed gum on the rheological properties of peanut protein isolate dispersions and gels. **LWT**, v. 74, p. 528-533, 2016.

CHENLO, F. et al. Rheological properties of aqueous dispersions of tragacanth and guar gums at different concentrations. **Journal of Texture Studies,** v. 41, p. 396–415, 2010.

CHO, H.M.; YOO, B. Steady and dynamic shear rheology of sweet potato starch–xanthan gum mixtures. **Food Chemistry**, v. 116, n. 3, p. 638-643, 2009.

CHO, H.M.; YOO, B. Rheological Characteristics of Cold Thickened Beverages Containing Xanthan Gum-Based Food Thickeners Used for Dysphagia Diets. **Journal of the Academy of Nutrition and Dietetics**, v. 115, n. 1, p. 106-111, 2014.

CHOI, H. et al. Shear rheology and filament stretching behaviour of xanthan gum and carboxymethyl cellulose solution in presence of saliva. **Food Hydrocolloids**, v. 40, p. 71-75, 2014.

CHOPPE, E. et al. Rheology of xanthan solutions as a function of temperature, concentration and ionic strength. **Carbohydrate Polymers**, v. 82, n. 4, p. 1228-1235, 2010.

CIUCANU, I.; KEREK, F. A simple and rapid method for the permethylation of carbohydrates. **Carbohydrate Research**, v. 131, n. 2, p. 209-217, 1984.

ČÍŽ, M. et al. Different methods for control and comparison of the antioxidant properties of vegetables. **Food Control**, v. 21, n. 4, p. 518-523, 2010.

CLAVÉ, P.; SHAKER, R. Dysphagia: current reality and scope of the problem. Nature Reviews. **Gastroenterology and Hepatology**, v. 12, p. 259-270, 2015.

CREMONINI, E. et al. (-)-Epicatechin improves insulin sensitivity in high fat diet-fed mice. **Archives of Biochemistry and Biophysics**, p. 13-21, 2016.

CUI, W. et al. Chemical Structure, Molecular Size Distributions, and Rheological Properties of Flaxseed Gum**. Journal of Agricultural and Food Chemistry**, v. 42, n. 9, p. 1891–1895, 1994a.

CUI, W. et al. Optimization of an Aqueous Extraction Process for Flaxseed Gum by Response Surface Methodology. **Lebensmittel-Wissenschaft und –Technologie**, v. 27, p. 363-369, 1994b.

CUI, W. et al. Influence of genotype on chemical composition and rheological properties of flaxseed gums. **Food hydrocolloids**, v. 10, n. 2, p. 221-227, 1996.

CUI, W.; MAZZA, G. Phvsicochemical characteristics of flaxseed gum. **Food Research International**, v. 29, p. 397-402, 1996.

CUTLER, A.N. et al. Oral Perception of Viscosity in fluid foods and model systems. **Food and Nutrition Press**, v. 14, p. 377-395, 1983.

DANTAS, R.O. et al. Effect of swallowed bolus variables on oral and pharyngeal phases of swallowing. **American journal of physiology**, v. 258, p. 675-681, 1990.

DEWAR, R.J AND JOYCE, M.J. The thixotropic and rheopectic behaviour of maize starch and maltodextrin thickeners used in dysphagia therapy. **Carbohydrate Polymers**, v. 65, p. 296–305, 2006a.

DEWAR, R.J. AND JOYCE, M.J. Time-Dependent Rheology of Starch Thickeners and the Clinical Implications for Dysphagia Therapy. **Dysphagia**, v. 21, n. 4, p. 264–269, 2006b.

DING, H.H. et al. Arabinan-rich rhamnogalacturonan-I from flaxseed kernel cell wall. **Food Hydrocolloids**, v. 47, p. 158-167, 2015.

DING, H.H. et al. Xyloglucans from flaxseed kernel cell wall: Structural and conformational characterization. **Carbohydrate Polymers**, v. 151, p. 538-545, 2016.

DING, H.H. et al. Structural and conformational characterization of arabynoxylans from flaxseed mucilage. **Food Chemistry**, v. 254, p. 266-271, 2018.

DING, H.H. et al. Soluble polysaccharides from flaxseed kernel as a new source of dietary fibres: Extraction and physicochemical characterization. **Food Research International**, v. 56, p. 166–173, 2014.

DOUAIRE, D. et al. Soft tribology of oil-continuous emulsions. **Journal of Food Engineering**, v. 139, p. 24-30, 2014.

DUPUIS, J.H. et al. Physicochemical properties and in vitro digestibility of potato starch after inclusion with vanillic acid. **LWT - Food Science and Technology**, v. 85, p. 218-224, 2017.

ELBOUTACHFAITI, R. et al. Fractionation and structural characterization of six purified rhamnogalacturonans type I from flaxseed mucilage. **Food Hydrocolloids**, v. 62, p. 273-279, 2017.

EMAGA, T.H. et al. Kinetics of the hydrolysis of polysaccharide galacturonic acid and neutral sugars chains from flaxseed mucilage**. Biotechnology, Agronomy and Society and Environment**, v. 16, n. 2, p. 139- 147, 2012.

ENGELEN, L. et al. The influence of product and oral characteristics on swallowing. **Archives of oral biology**, v. 50, p. 739-746, 2005.

ENGLYST, K.N. et al. Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. **The American Journal of Clinical Nutrition**, v. 69, n. 3, 448–454, 1999.

ERTEKIN, C.; AYDOGDU, I. Neurophysiology of swallowing. **Clinical Neurophysiology**, v. 111, p. 2226–2244, 2003.

FARAHMANDFAR, R. et al. Effects of basil seed gum, Cress seed gum and Quince seed gum on the physical, textural and rheological properties of whipped cream. International **Journal of Biological Macromolecules**, v. 98, p. 820–828, 2017.

FEDENIUK, R.W.; BILIADERIS, C.G. Composition and Physicochemical Properties of Linseed (*Linum usitatissimum* L.) Mucilage. **Journal of Agricultural and Food Chemistry**, v. 42, n. 2, p. 240–247, 1994.

FENG, Y.et al. Ellagic acid promotes Aβ42 fibrillization and inhibits Aβ42-induced neurotoxicity. **Biochemical and Biophysical Research Communications**, v. 390, p. 1250–1254, 2009.

FERRY, A.-L. et al. Viscosity and flavour perception: Why is starch different from hydrocolloids? **Food Hydrocolloids**, v. 20, p. 855–862, 2006.

FESTA, F. et al. Strong antioxidant activity of ellagic acid in mammalian cells in vitro revealed by the comet assay. **Anticancer Research**, v. 21, n. 6, p. 3903-3908, 2001.

FORSTER, A. Et al. Oropharyngeal dysphagia in older adults: A review. **European Geriatric Medicine**, v. 2, p. 356–362, 2011.

FOSTER-POWELL, K. et al. International table of glycemic index and glycemic load values: 2002. **The American Journal of Clinical Nutrition**, v. 76, n. 1, p. 5-56, 2002.

FUJINE, K. FlashEA 1112 Elemental Analyzer (CHNS): User Guide. International Ocean Discovery Program, CHNS Analyzer User Guide, 1-12, 2008.

FUNAMI, T. Next target for food hydrocolloid studies: Texture design of foods using hydrocolloid technology. **Food Hydrocolloids**, v. 25, n. 8, p. 1904-1914, 2011.

GALLEGOS, C., et al. Rheology and Dysphagia: Na Overview. **Anual Transactions of the Nordic Rheology Society**, v. 20, p. 3-10, 2012.

Gallegos, C., Quinchia, L., Ascanio, G., Salinas-Vásquez, M., Brito-de la Fuente, E., 2012. Rheology and Dysphagia: Na Overview. Anual Transactions of the Nordic Rheology Society, 20.

GARCIA, J.M. et al. Viscosity Measurements of Nectar- and Honey-thick Liquids: Product, Liquid, and Time Comparisons. **Dysphagia**, v. 20, p. 325–335, 2005.

GARIN, N. Viscosity Differences between Thickened Beverages Suitable for Elderly Patients with Dysphagia. **Dysphagia**, v. 29, p. 483–488, 2014.

GERMAIN, I. et al. Rheological characterization of thickened beverages used in the treatment of dysphagia. **Journal of Food Engineering**, v. 73, p. 64-74, 2006.

GUILLOUX, K. et al. Production of arabinoxylan-oligosaccharides from flaxsedd (*Linum usitatissimum*). **Journal of Agricultural and Food Chemistry**, v. 57, p. 11308-11313, 2009.

GITZINGER, M. et al. The food additive vanillic acid controls transgene expression in mammalian cells and mice. **Nucleic Acids Research**, v. 40, e37, 2012.

HAMDANI, A.F; WANI, A. Guar and Locust bean gum: Composition, total phenolic content, antioxidant and antinutritional characterisation. **Bioactive Carbohydrates and Dietary Fibre**, v. 11, p. 53-59, 2017.

HAMILTON, E.; NORTON, I.T. Modification to the lubrication properties of xanthan gum fluid gels as a result of sunflower oil and triglyceride stabilised water in oil emulsion addition. **Food Hydrocolloids**, v. 55, p. 220-227, 2016.

HAN, D.H. et al. Antioxidant and apoptosis-inducing activities of ellagic acid. **Anticancer Research**, v. 26, n. 5, p. 3601-3606, 2006.

HADDE, E.K.; CHEN, J. Shear and extensional rheological characterization of thickened fluid for dysphagia management. **Journal of Food Engineering**, v. 245, p. 18-23, 2019.

HAO, M.; BETA, T. Qualitative and quantitative analysis of the major phenolic compounds as antioxidants in barley and flaxseed hulls using HPLC/MS/MS. **Journal of the Science of Food and Agriculture**, v. 92, p. 2062–2068, 2012.

HASEGAWA, A. et al. Velocity of swallowed gel food in the pharynx by ultrasonic method. **Journal of the Japanese society for food science and technology**, v. 52, p. 441-447, 2005.

HAYASHI, M. et al. Tribological analysis of the surface of food gels. **Food Hydrocolloids**, v. 58, p. 343-346, 2016.

HE, Q. et al. Predicting sensory perceptions of thickened solutions based on rheological analysis. **Food Hydrocolloids**, v. 61, p. 221-232, 2016.

HENDRIKS, H.F. et al. Safety of long-term consumption of plant sterol esters-enriched spread. European Journal of Clinical Nutrition. **Nature**, v. 57, n. 5, p. 681-92, 2003.

HENNESSY, M.; GOLDENBERG, D. Surgical anatomy and physiology of swallowing. **Operative Techniques in Otolaryngology-Head and Neck Surgery**, v. 27, n. 2, p. 60-66, 2016.

HERCHI, W. et al. Changes in the content of phenolic compounds in flaxseed oil during development. **Journal of the American Oil Chemists Society**, v. 88, p. 1135–1142, 2011.

HEYMAN, B. et al. Guar and xanthan gum differentially affect shear induced breakdown of native waxy maize starch. **Food Hydrocolloids**, v. 35, p. 546–556, 2014.

HIIEMAE, K. et al. Natural bites, food consistency and feeding in man. **Archives of oral biology**, v. 41, p. 429-441, 1996.

HORI, K. et al. Comparison of mechanical analyses and tongue pressure analyses during squeezing and swallowing of gels. **Food Hydrocolloids**, v. 44, p. 145-155, 2015.

HOSSEINI, E. et al. Influence of temperature, pH and salts on rheological properties of bitter almond gum. **Food Science and Technology**, v. 37, n. 3, p. 437-443, 2017.

JAISHANKAR, J. et al. Probing hydrogen bond interactions in a shear thickening polysaccharide using nonlinear shear and extensional rheology. **Carbohydrate Polymers**, v. 123, p. 136–145, 2015.

JO, W. et al. Rheological characterizations of concentrated binary gum mixtures with xanthan gum and galactomannans. **International Journal of Biological Macromolecules**, v. 114, p. 263–269, 2018.

JOYNER, H.S. Explaining food texture through rheology. **Current Opinion in Food Science**, v. 21, p. 7-14, 2018.

KANMANI, N. et al. Improvement of feed pellet characteristics by dietary pre-gelatinized starch and their subsequent effects on growth and physiology in tilapia. **Food Chemistry**, v. 239, p. 1037-1046, 2018.

KARAZHIYAN, H. et al. Rheological properties of Lepidium sativum seed extract as a function of concentration, temperature and time. **Food Hydrocolloids**, v. 23, n. 8, p. 2062–2068, 2009.

KASOTE, D.M. Flaxseed phenolics as natural antioxidants. **International Food Research Journal**, v. 20, n. 1, p. 27-34, 2013.

KAUSHIK P. et al. Complex coacervation between flaxseed protein isolate and flaxseed gum. **Food Research International**, v. 72, p. 91-97, 2015.

KAUSHIK, P. et al. Preparation, characterization and functional properties of flax seed protein isolate. **Food Chemistry**, v. 197, p. 212-220, 2016.

KAUSHIK, P. et al. Effect of extraction temperature on composition, structure and functional properties of flaxseed gum. **Food Chemistry**, v. 215, p. 333–340, 2017.

KENNEDY; HUANG Polysaccharide Gums from Agricultural Products: Steve W. Cui (Book review). **Carbohydrate Polymers**, v. 52, n. 2, p. 198, 2003.

KILIC, I. et al. Spectroscopic studies on the antioxidant activity of ellagic acid. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy**, v. 130, p. 447-452, 2014.

KIM, C.; YOO, B. Rheological properties of rice starch–xanthan gum mixtures. **Journal of Food Engineering**, v. 75, n. 1, p. 120-128, 2006.

KOKINI, J.L. The physical basis of liquid food texture and texture–taste interactions. **Journal of Food Engineering**, v. 6, p. 51-81, 1987.

KOKINI, J.L.; CUSSLER, E.L. Predicting the texture of liquid and melting semi-solid foods. **Journal of Food Science,** v. 48, p. 1221-1225, 1984.

KOOCHEKI, A. et al. Studies on the steady shear flow behavior and functional properties of Lepidium perfoliatum seed gum. **Food Research International**, v. 50, p. 446–456, 2013.

KROP, E.M. et al. On relating rheology and oral tribology to sensory properties in hydrogels. **Food Hydrocolloids**, v. 88, p. 101-113, 2019.

KU, P.K., et al. Early swallowing problems in a cohort of patients with nasopharyngeal carcinoma: Symptomatology and videofluoroscopic findings. **Laryngoscope**, v. 117, p. 142-146, 2007.

KUHNLE, G.G.C. et al. Extraction and Quantification of Phytoestrogens in Foods Using Automated Solid-Phase Extraction and LC/MS/MS. **Analytical Chemistry**, v. 79, p. 9234-9239, 2007.

LAIHO, S. et al. Effect of whey protein phase volume on the tribology, rheology and sensory properties of fat-free stirred yoghurts. **Food Hydrocolloids**, v. 67, p. 166-177, 2017.

LLAVERIAS, G. et al. Phytosterols inhibit the tumor growth and lipoprotein oxidizability induced by a high-fat diet in mice with inherited breast cancer. **Journal of Nutricional Biochemistry**, v. 24, n. 1, p. 39-48, 2013.

LEONARD, R.J. et al. Effects of Bolus Rheology on Aspiration in Patients with Dysphagia. **Journal of the Academy of Nutrition and Dietetics**, p. 2212-2672, 2014.

LI, N. et al. Characterization of gum isolated from Camelina seed. **Industrial Crops and Products**, v. 83, p. 268-274, 2016.

LI, Y. et al. An effective strategy to develop active cinnamic acid-directed antioxidants based on elongating the conjugated chains. **Food Chemistry**, v. 158, p. 41–47, 2014.

LI, X.Y. et al. The effect of addition of flaxseed gum on the rheological behavior of mixed flaxseed gum–casein gels. **Carbohydrate Polymers**, v. 88, n. 4, 1214-1220, 2012.

LIU, J. et al. Conlinin inflaxseed (*Linum usitatissimum* L.) gum and its contribution to emulsification properties. **Food Hydrocolloids**, v. 52, p. 963-971, 2016.

LIU, J. et al. Whey protein isolate and flaxseed *(Linum usitatissimum* L.) gum electrostatic coacervates: Turbidity and rheology. **Food Hydrocolloids**, v. 64, p. 18-27, 2017.

LIU, K. et al. Fat droplet characteristics affect rheological, tribological and sensory properties of food gels. **Food Hydrocolloids**, v. 44, p. 244-259, 2015.

LO, W.-L. et al. Dysphagia and risk of aspiration pneumonia: A nonrandomized, pair-matched cohort study. **Journal of Dental Sciences**, available online 22 March 2019, In Press, Corrected Proof.

LUTZ, U. et al. Dose Response for the Stimulation of Cell Division by Caffeic Acid in Forestomach and Kidney of the Male F344 Rat. **Fundamental and Applied Toxicology**, v. 39, n. 2, p. 131-137, 1997.

MACKLEY, M.R. et al. The rheology and processing behavior of starch and gum-based dysphagia thickeners. **Journal of Rheology**, v. 57, n. 6, p. 1533-1553, 2013.

MALLIK, S.B. et al. Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. **Neuroscience Letters**, v. 632, p. 218–223, 2016.

MARTINCHIK, A.N. et al. Nutritional value and functional properties of flaxseed. **Voprosy pitaniia**, v. 81, n. 3, p. 4-10, 2012.

MANDALA, I.G. et al. Influence of preparation and storage conditions on texture of xanthan-starch mixtures. **Journal of Food Engineering**, v. 53, p. 27-38, 2002.

MANDALA I.; PALOGOU E. Effect of Preparation Conditions and Starch/Xanthan Concentration on Gelation Process of Potato Starch Systems. **International Journal of Food Properties**, v. 6, p. 311-328, 2003.

MANDALA, I.G. et al. Xanthan and locust bean gum influence on the rheology and structure of a white model-sauce. **Journal of Food Engineering**, v. 64, n. 3, p. 335-342, 2004.

MANTOVANI, R.A. et al. Stability and *in vitro* digestibility of emulsions containing lecithin and whey protein. **Food and function**, v. 4, p. 1322-1331, 2013.

MARCONATI, M. et al. A review of the approaches to predict the ease of swallowing and post-swallow residues. **Trends in Food Science & Technology**, v. 86, p. 281-297, 2019.

MARTÍNEZ, O. et al. Sensory perception and flow properties of dysphagia thickening formulas with different composition. **Food Hydrocolloids**, v. 90, p. 508-514, 2019.

MAZZA, G.; BILIADERIS, C. Functional properties of flaxseed mucilage. **Journal of Food Science**, v. 54, n. 5, p. 1302–1305, 1989.

MEDINA-TORRES, L. et al. Rheological properties of the mucilage gum (Opuntia ficus indica). **Food Hydrocolloids**, v. 14, p. 417–424, 2000.

MENG, Y. et al. Computer simulation of the pharyngeal bolus transport of Newtonian and Non-Newtonian fluids. **Food and Bioproducts Processing**, v. 83, n. 4, p. 297-305, 2005.

MILLER, J.L; WATKIN, K.L. The influence of bolus volume and viscosity on anterior lingual force during the oral stage of swallowing. **Dysphagia**, v. 11, n. 2, p. 117-124, 1996.

MINEKUS, M. et al. A standardised static in vitro digestion method suitable for food – an international consensus. **Food and Function**, v. 5, p. 1113–1124, 2014.

MIOCHE, L.; PEYRON, M.A. Bite force displayed during assessment of hardness in various texture contexts. **Archives of oral biology**, v. 40, p. 415-423, 1995.

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

MOCZKOWSKA, M. et al. Enzymatic, enzymatic-ultrasonic and alkaline extraction of soluble dietary fibre from flaxseed – A physicochemical approach. **Food Hydrocolloids**, v. 90, p. 105-112, 2019.

MORET-TATAY, A. et al. Commercial thickeners used by patients with dysphagia: Rheological and structural behaviour in different food matrices. **Food Hydrocolloids**, v. 5, p. 318-326, 2014.

MORRIS, D.H.; VAISEY-GENSER, M. FLAXSEED. **Encyclopedia of Food Sciences and Nutrition** (2nd Edition), p. 2525–2531, 2003.

MUIR, A. D. AND WESTCOTT, N. D. Flaxseed constituents and human health. In Muir, A.D. and Westcott, N.D. (eds). Flax: the genus Linum, 243–51. London: **Taylor & Francis**, 2003.

NARAN, R. et al. Novel rhamnogalacturonan I and arabinoxylan polysaccharides of flaxseed mucilage. **Plant Physiology**, v. 148, n. 1, p. 132-141, 2008.

NATIONAL DYSPHAGIA DIET TASK FORCE, 2002. National dysphagia diet: standardization for optimal care. Chicago, IL, **American Dietetic Association**.

NGUYENA, P.T.M. et al. Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. **Food Hydrocolloids**, v. 72, p. 90-104, 2017.

NICOSIA, M.A.; ROBBINS, J. The fluid mechanism of bolus ejection from the oral cavity. **Journal of biomechanics**, v. 34, p. 1537-1544, 2001.

NUNES, C et al. Occurrence of cellobiose residues directly linked to galacturonic acid in pectic polysaccharides, **Carbohydrate Polymers**, v. 87, p. 620–626, 2012.

Oishia, K., Yamamotoa, S., Oikea, H., Ohkurae, N., Taniguchif, M., 2017. Cinnamic acid shortens the period of the circadian clock in mice. Biochemistry and Biophysics Reports, 9, 232-239.

O'LEARY, M. et al. Viscosity and Non-Newtonian Features of Thickened Fluids Used for Dysphagia Therapy. **Journal of Food Science**, v. 75, n. 6, p. 330-338, 2010.

OLIVAS, G.I.; BARBOSA-CÁNOVAS, G.V. Edible Coatings for Fresh-Cut Fruits. **Critical Reviews in Food Science and Nutrition**, v. 45, p. 657-670, 2005.

OLIVEIRA, A.M.F. et al. Total Phenolic Content and Antioxidant Activity of Some Malvaceae Family Species. **Antioxidant**s (Basel*)*, v. 1, n. 1, p. 33–43, 2012.

ONG, J.J.-X. et al. Sensory characteristics of liquids thickened with commercial thickeners to levels specified in the International Dysphagia Diet Standardization Initiative (IDDSI) framework. **Food Hydrocolloids**, v. 79, p. 208-217, 2018.

OOMAH, B.D. et al. Phenolic acids in flaxseed. **Journal of Agricultural and Food Chemistry**, v. 43, n. 8, 2016-2019, 1995.

PARIS, G. et al. Clinical screening of oropharyngeal dysphagia in patients with ALS. **Annals of Physical and Rehabilitation Medicine**, v. 55, n. 9-10, p. 601-608, 2012.

PARK, J.-W. et al. Fork test: A new simple and reliable consistency measurement for the dysphagia diet. **Geriatric Nursing**, v. 37, p. 292-295, 2016.

PAYNE, C. et al. Consistently Inconsistent: Commercially Available Starch-Based Dysphagia Products. **Dysphagia**, v. 26, p. 27–33, 2011.

PATISAUL, H.B.; JEFFERSON, W. The pros and cons of phytoestrogens. **Frontiers in Neuroendocrinology**, v. 31, n. 4, p. 400–419, 2010.

PILUZZA, G.; BULLITTA, S. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. **Pharmaceutical Biology**, v. 49, n. 3, p. 240–247, 2011.

PRAKASH, S. et al. Applications of tribology in studying food oral processing and texture perception. **Food Research International**, v. 54, n. 2, p. 1627-1635, 2013.

PRAKASH, S. Chapter 4 - From Rheology to Tribology: Applications of Tribology in Studying Food Oral Processing and Texture Perception. Advances in Food Rheology and Its Applications; **Woodhead Publishing Series in Food Science, Technology and Nutrition**, 65-86, 2017.

PRIYADARSINI, K.I. et al. Free Radical Studies of Ellagic Acid, a Natural Phenolic Antioxidant. **Journal of Agricultural and Food Chemistry**, v. 50, p. 2200−2206, 2002.

QIAN, K.Y. et al. Structural elucidation of rhamnogalacturonans from flaxseed hulls. **Carbohydrate Research**, v. 362, p. 47-55, 2012a.

QIAN, K.Y., Cui, S.W., Wu, Y., Goff, H.D. Flaxseed gum from flaxseed hulls: extraction, fractionation and characterization. **Food Hydrocolloids**, v. 28, p. 275-283, 2012b.

RAJURKAR, N.S.; HANDE, S. M. Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants. **Indian Journal of Pharmaceutical Sciences**, v. 73, n. 2, p. 146-151, 2011.

RAS, R.T. et al. Intake of phytosterols from natural sources and risk of cardiovascular disease in the European Prospective Investigation into Cancer and Nutrition-the Netherlands (EPIC-NL) population. **European Journal of Preventive Cardiology**, v. 22, n. 8, p. 1067-1075, 2015.

RAY, S. et al. Characterization of mucilage polysaccharides, arabinogalactanproteins and cell-wall hemicellulosic polysaccharides isolated from flax seed meal: A wealth of structural moieties. **Carbohydrate Polymers**, v. 93, p. 651–660, 2013.

RICHARDSON, R. K. et al. Characterization of the perceived texture of thickened systems by dynamic viscosity measurements. **Food Hidrocolloids,** v. 3, p. 175-191, 1989.

RODRIGUES, M.I.; IEMMA, A.F. Experimental Design and Process Optimization. **Taylor & Francis** group (1st Edition), 2015.

ROFES, L. et al. The effects of a xanthan gum-based thickener on the swallowing function of patients with dysphagia. **Alimentary Pharmacology and Therapeutics**, v. 39, p. 1169-1179, 2014.

ROSS, A.I.V. et al. Relationships between shear rheology and sensory attributes of hydrocolloid-thickened fluids designed to compensate for impairments in oral manipulation and swallowing. **Journal of Food Engineering,** v. 263, p. 123-131, 2019.

ROULARD, R. et al. Molecular investigations of flaxseed mucilage polysaccharides. **International Journal of Biological Macromolecules**, v. 86, p. 840-847, 2016.

RUSS, N. et al. Pre-gelatinized tapioca starch and its mixtures with xanthan gum and ι-carrageenan. **Food Hydrocolloids**, v. 56, p. 180-188, 2016.

SABALLY, K. Lipase-catalyzed synthesis of selected phenolic lipid organic solvent media. (Ph.D Thesis) McGill University, Montreal, Canada, 2006.

SANTORO, P.P. et al. Otolaryngology and Speech Therapy evaluation in the assessment of oropharyngeal dysphagia: a combined protocol proposal. **Brazilian Journal of Otorhinolaryngology**, v. 77, n. 2, p. 201-213, 2011.

SCHOLTEN, E, 2017. Composite foods: from structure to sensory perception. **Food and Function**, v. 8, n. 2, 481-497.

SEÇZYK, L. et al. Antioxidant, nutritional and functional characteristics of wheat bread enriched with ground flaxseed hulls. **Food Chemistry**, v. 214, p. 32–38, 2017.

SELWAY, N.; STOKES, J.R. Insights into the dynamics of oral lubrication and mouthfeel using soft tribology: Differentiating semi-fluid foods with similar rheology. **Food Research International,** v. 54, p. 423–431, 2013.

SEPTEMBER, C. et al. Implications of Changing the Amount of Thickener in Thickened Infant Formula for Infants with Dysphagia. **Dysphagia**, v. 29, p. 432–437, 2014.

SHALVIRI et al. Novel modified starch–xanthan gum hydrogels for controlled drug delivery: Synthesis and characterization. **Carbohydrate Polymers**, v. 79, p. 898–907, 2010.

SHAMA, F.; SHERMAN, P. Identification of stimuli controlling the sensory evaluation of viscosity II. Oral methods. **Journal of Texture Studies**, v. 4, n. 1, p. 111-118, 1973.

SHARMA, M. et al. Effect of hydrocolloid type on texture of pureed carrots: Rheological and sensory measures. **Food Hydrocolloids**, v. 63, p. 478-487, 2017.

SILVA, F.G.D et al. Identification of peptides released from flaxseed (*Linum usitatissimum*) protein by Alcalase® hydrolysis: Antioxidant activity. **LWT - Food Science and Technology**, v. 76, Part A, p. 140-146, 2017.

SLUITER, A. et al. Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory, 2008.

SONG, K. et al. Rheology of concentrated xanthan gum solutions: Steady shear flow behaviour. **Fibers and Polymers**, v. 7, n. 2, p. 129-138, 2006.

SOPADE, P.A. et al. Rheological characterization of food thickeners marketed in Australia in various media for the management of dysphagia. II. Milk as a dispersing medium. Journal of Food Engineering, 84, 553–563, 2008a.

SOPADE, P.A. et al. Rheological characterization of food thickeners marketed in Australia in various media for the management of dysphagia. III. Fruit juice as a dispersing medium. **Journal of Food Engineering**, v. 86, p. 604–615, 2008b.

STEFFE, J.F. Rheological Methods in Food Process Engineering. 2nd Edition, Freeman Press, East Lansing, 1996.

STOKES, J. R. et al. Oral processing, texture and mouthfeel: From rheology to tribology and beyond. **Current Opinion in Colloid & Interface Science**, v. 18, p. 349–359, 2013.

STUART, S.; MOTZ, J.M. Viscosity in Infant Dysphagia Management: Comparison of Viscosity of Thickened Liquids Used in Assessment and Thickened Liquids Used in Treatment. **Dysphagi**a, v. 24, p. 412–422, 2009.

SUN, C. et al. Influence of initial protein structures and xanthan gum on the oxidative stability of O/W emulsions stabilized by whey protein**. International Journal of Biological Macromolecule**s, v. 120, p. 34-44, 2018.

SYTAR, O. et al. Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. **Saudi Journal of Biological Sciences**, v. 25, n. 4, p. 631-641, 2018.

SZCZESNIAK, A.S.; FARKAS, E. Objective characterization of the mouth feel of gum solutions. **Journal of Food Science**, v. 27, p. 381-385, 1963.

SZCZESNIAK, A.S. Texture is a sensory property. **Food Quality and Preference**, v. 13, n. 4, 215-225, 2002.

TAKAHASHI, T. et al. Effects of physical properties and oral perception on transit speed and passing time of semiliquid foods from the mid-pharynx to the hypopharynx. **Journal of Texture Studies**, v. 33, p. 585-598, 2003.

TAKO, M. et al. The principles of starch gelatinization and retrogradation. **Food and Nutritional Science**s, v. 5, p. 280-291, 2014.

TESTER, R.F. et al. Starch-composition, fine structure and architecture. **Journal of Cereal Science,** v. 39, n. 2, p. 151-165, 2004.

TORRES, O. et al. Gellan gum: A new member in the dysphagia thickener family. **Biotribology**, v. 17, p. 8-18, 2019.

TROMP, R.H. et al. Confocal scanning light microscopy (CSLM) on mixtures of gelatine and polysaccharides. **Food Research International**, v. 34, n. 10, p. 931-938, 2001.

TURCANU, M., Siegert, N., Secouard, S., Brito-de la Fuente, E., Balan, C., Gallegos, C., 2018. An alternative elongational method to study the effect of saliva on thickened fluids for dysphagia nutritional support. Journal of Food Engineering, 228, 79-83.

UPADHYAY, R.; CHEN, J. Smoothness as a tactile percept: Correlating 'oral' tribology with sensory measurements. **Food Hydrocolloids**, v. 87, p. 38-47, 2019.

VALLONS, K.J.R. et al. Effect of human saliva on the consistency of thickened drinks for individuals with dysphagia. **International Journal of Language & Communication Disorders**, v. 50, n. 2, p. 165–175, 2015.

VAN ECK, A. et al. Oral processing behavior and dynamic sensory perception of composite foods: Toppings assist saliva in bolus formation. **Food Quality and Preference**, v. 71, p. 497-509, 2019.

VEIGA, P.G. et al. Cacracterização química, reológica e aceitação sensorial do queijo petit suisse brasileiro. **Ciência e Tecnologia de Alimentos**, v. 20, n. 3, p. 349-357, 2000.

VIEIRA, J.M. et al. Effect of extraction temperature on rheological behavior and antioxidant capacity of flaxseed gum. **Carbohydrate Polymers**, v. 213, p. 217-227, 2019.

VINOTHIYA, K.; ASHOKKUMAR, N. Modulatory effect of vanillic acid on antioxidant status in high fat diet-induced changes in diabetic hypertensive rats**. Biomedicine & Pharmacotherapy**, v. 87, p. 640–652, 2017.

WANG, E.Q. Dysphagia. **Encyclopedia of Movement Disorders**, p. 365-367, 2010.

WANG, S.T. et al. A food-grade self-nanoemulsifying delivery system for enhancing oral bioavailability of ellagic acid. **Journal of Functional Foods**, v. 34, p. 207–215, 2017.

WANG, X. et al. Topographic study of human tongue in relation to oral tribology. **Food Hydrocolloids**, v. 95, p. 116-121, 2010.

WANG, Y. et al. Effect of flaxseed gum addition on rheological properties of native maize starch. **Journal of Food Engineering**, v. 89, n. 1, p. 87-92, 2008.

WODA, A. et al. Adaptation of healthy mastication to factors pertaining to the individual or to the food. **Physiology and Behavior**, v. 89, p. 28-35, 2006.

WONG-PAZ, J.E. et al. Total phenolic content, in vitro antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. Asian Pacific Journal of Tropical Medicine, 8, 104–111, 2015.

WOOD, G.N. Centric occlusion, centric relation, and the mandibular posture. **The Journal of Prosthetic Dentistry**, v. 20, n. 4, p. 292-306, 1968.

WOODS, C.W. et al. Development of necrotizing enterocolitis in premature infants receiving thickened feeds using SimplyThick. **Journal of Perinatology**, v. 32, p. 150-152, 2012.

WU, Z.M. et al. *In vitro* antiviral efficacy of caffeic acid against canine distemper virus. **Microbial Pathogenesis**, v. 110, p. 240-244, 2017.

YAKUBOV, G.E. et al. Tribology of particle suspensions in rolling-slidding soft contacts. **Biotribology**, v. 3, p. 1-10, 2015

YUAN, B. et al. Extensional and shear rheology of a food hydrocolloid. **Food Hydrocolloids**, v. 74, p. 296-306, 2018.

ZANQUI, A.B. et al. Subcritical extraction of flaxseed oil with n-propane: Composition and purity. **Food Chemistry**, v. 188, n. 1, p. 452–458, 2015.

ZARGARAAN, A. et al. Rheological aspects of dysphagia-oriented food products: A mini review. **Food Science and Human Wellness**, v. 2, p. 173–178, 2013.

ZHAN, D.F. et al. Xanthan-locust bean gum interactions and gelation. **Carbohydrate Polymers,** v. 21, n. 1, p. 53-58, 2003

ZHANG, B. et al. Tribology of swollen starch granule suspensions from maize and potato. **Carbohydrate Polymers**, v. 155, p. 128–135, 2017.

ZIOLKOVSKA, A. Laws of flaxseed mucilage extraction. **Food Hydrocolloids**, v. 26, p. 197-204, 2012.

## *CAPÍTULO IX*

*Appendix A*

*Preliminary tests - Plackett Burman*

*Appendix B*

*Rheological characterization of food thickeners for the management of dysphagia. Orange soy juice and milk as dispersing media*

*Appendix C*

*Tribological characterization of food thickeners for the management of dysphagia. Orange soy juice and milk as dispersing media*

**Appendix A. Preliminary tests –** Plackett Burman

In the initial phase of the experiments, a Plackett-Burman design was used to identify the significant factors ($p < 0.05$) in the main rheological parameters according to the concentration range of the different selected biopolymers. The purpose of this preliminary test was to observe the main effects in order to formulate a complete factorial experiment with the most satisfying optimized response surfaces. As can be seen in Table A-1, this experiment analyzed the impact of 5 biopolymers (factors), flaxseed gum (FG), modified starch (MS), xanthan gum (XG), guar gum (GG) and pectin (P) on viscosity at different rates (10, 50 and 400 $s^{-1}$) having a total of 12 tests with 3 central points (13, 14, 15) (determining the validity of the process). Thus, the number of factors was much smaller than the number of trials.

**Table A-1.** Rheological responses (viscosity at 10, 50 and 400 $s^{-1}$ - $\eta_{ap\,10}$, $\eta_{ap\,50}$ and $\eta_{ap\,400}$- and *n*- flow index) of the thickener formulations constituted by flaxseed gum (FG), modified starch (MS), xanthan gum (XG), guar gum (GG) and pectin (P) in water according to a Placket-Burman experimental design.

| | FG (x1) | MS (*x2*) | XG (*x3*) | GG (*x4*) | P (*x5*) | $\eta_{ap\,10}$ (cP) | $\eta_{ap\,50}$ (cP) | $\eta_{ap\,400}$ (cP) | *n* |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 3 (1) | 0 (-1) | 0.5 (1) | 0 (-1) | 0 (-1) | 2438 | 988 | 308 | 0.44 |
| **2** | 3 (1) | 3 (1) | 0 (-1) | 0.5 (1) | 0 (-1) | 5079 | 2289 | 529 | 0.49 |
| **3** | 0 (-1) | 3 (1) | 0.5 (1) | 0 (-1) | 0.5 (1) | 4037 | 1008 | 201 | 0.29 |
| **4** | 3 (1) | 0 (-1) | 0.5 (1) | 0.5 (1) | 0 (-1) | 4468 | 1571 | 407 | 0.35 |
| **5** | 3 (1) | 3 (1) | 0 (-1) | 0.5 (1) | 0.5 (1) | 3874 | 1407 | 380 | 0.37 |
| **6** | 3 (1) | 3 (1) | 0.5 (1) | 0 (-1) | 0.5 (1) | 16011 | 4380 | 821 | 0.19 |
| **7** | 0 (-1) | 3 (1) | 0.5 (1) | 0.5 (1) | 0 (-1) | 6292 | 1499 | 235 | 0.21 |
| **8** | 0 (-1) | 0 (-1) | 0.5 (1) | 0.5 (1) | 0.5 (1) | 909 | 293 | 68 | 0.30 |
| **9** | 0 (-1) | 0 (-1) | 0 (-1) | 0.5 (1) | 0.5 (1) | 539 | 212 | 63 | 0.42 |
| **10** | 3 (1) | 0 (-1) | 0 (-1) | 0 (-1) | 0.5 (1) | 2882 | 1083 | 307 | 0.44 |
| **11** | 0 (-1) | 3 (1) | 0 (-1) | 0 (-1) | 0 (-1) | 431 | 180 | 45 | 0.69 |
| **12** | 0 (-1) | 0 (-1) | 0 (-1) | 0 (-1) | 0 (-1) | 0 | 0 | 0 | 0.00 |
| **13** | 1.5 (0) | 1.5 (0) | 0.25 (0) | 0.25 (0) | 0.25 (0) | 1968 | 749 | 215 | 0.40 |
| **14** | 1.5 (0) | 1.5 (0) | 0.25 (0) | 0.25 (0) | 0.25 (0) | 2003 | 783 | 219 | 0.40 |
| **15** | 1.5 (0) | 1.5 (0) | 0.25 (0) | 0.25 (0) | 0.25 (0) | 1957 | 742 | 211 | 0.39 |

The large viscosity range obtained ranging from 43 to 16011, 27 to 4380 and 14 to 821 for the shear rates of 10, 50 and 400 $s^{-1}$, respectively. Regarding to the flow index, *n*, all formulations showed a pseudoplastic behavior, since all values were significantly lower

than 1. The generated pareto charts with the biopolymer's effects on the analyzed responses are presented in the Figure A-1.

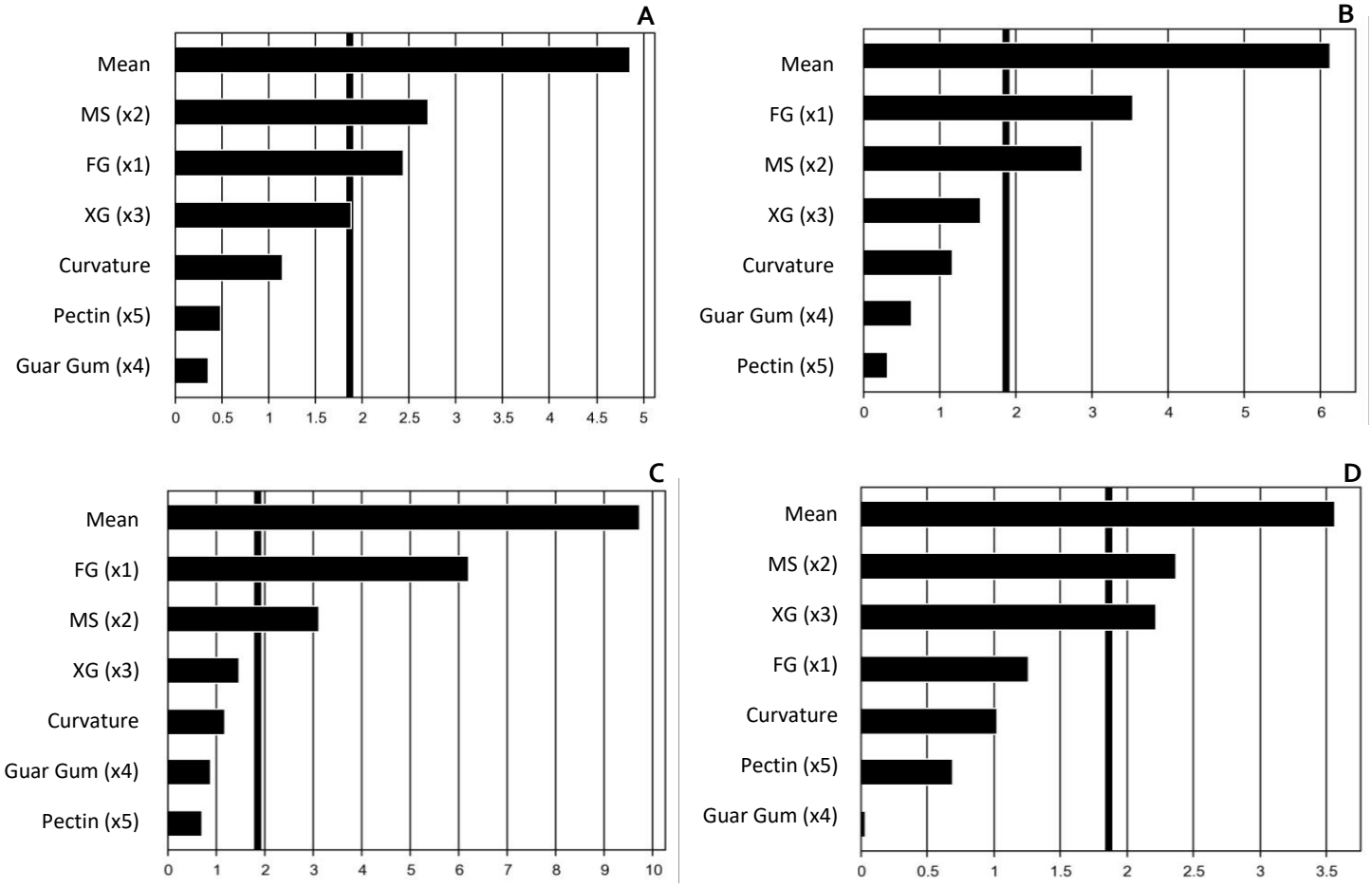



**Figure A-1.** Pareto chart of standardized effects of variables ($p < 0.05$) on viscosity responses at shear rates of 10 $s^{-1}$ (A), 50 $s^{-1}$ (B), 400 $s^{-1}$ (C) and flow index *n* (D), according to Plackett & Burman Design.

According to the Figure A-1, three biopolymers had a significant effect on viscosity at 10 $s^{-1}$ in the MS > FG > XG sequence (Figure A-1A), while only two had a significant effect on the remaining shear rates in the FG > MS sequence (Figure A-1B and A-1C). Regarding to the flow index (Figure A-1D), *n*, only MS and XG had a significant effect on this parameter (MS> XG). It should be noted the important effect of XG on formulations pseudoplasticity, despite its smaller concentration range compared to FG and MS, this fact is mirrored in formulations 6 and 7, where the lowest values of *n* were observed, since in both formulations the XG concentration is maximum.

Therefore, since guar gum and pectin had no significant effects on the analysed rheological parameters, both were withdrawn from the study. While the remaining biopolymers were selected for a complete factorial study, where their concentrations were rearranged within this studied spectrum, given the desired viscosity magnitudes achieved.

## Appendix B. Rheological characterization of food thickeners for the management of dysphagia. Orange soy juice and milk as dispersing media


### Abstract

Dietary viscosity modification is currently used as a strategy to circumvent the problems associated with swallowing by patients with oropharyngeal dysphagia. Flaxseed gum (FG) has been studied as a potential biopolymer to be incorporated in thickener formulations, which allows the safety of consumption by these patients in addition to providing a health benefit. The effect of FG mixed with modified/gelatinized starch (MS) and xanthan gum (XG) (both commonly used in the dysphagic diet) on rheological and colour properties of skim milk and orange-flavoured soy juice were investigated by varying biopolymers concentration according to a central composite rotational design (CCDR). The increase of FG concentration increased significantly the viscosity of the food matrices. All formulations showed a shear time-independent, shear-thinning and elastic behaviour, mainly influenced by XG and MS. However, the increase of FG concentration also resulted in an increase of both $G'$ and $G''$ for all matrices. In general, the thickener formulations incorporated into the different food matrices did not alter their original colour, although the highest concentrations of FG provoked significant colour changes on milk.


**Keywords:** dysphagia, thickeners, flaxseed gum, rheology, colour.

### Introduction

Oropharyngeal dysphagia is a highly prevalent disturbance, mainly a consequence of aging, neurodegenerative diseases and stroke, which can be associated to any dysfunction of the swallowing process (Turcanu *et al.*, 2018; Martinez *et al.*, 2019). As a palliative strategy to overcome these swallowing problems, the following processes can be used: feeding tubes, swallowing therapy and modified texture foods. Thickening liquid foods is the most common strategy, since its efficiency was proven through videofluoroscopy measurements for pharyngeal transit time (Hadde & Chen, 2019). The thickened fluids ability as palliative care of these patients is partly dependent on their rheological properties, that are influenced by thickeners composition and by the food matrix, for example, water, milk and fruit juices (Germain, Dufresne & Ramaswamy, 2006; Sopade *et al.*, 2008a; Martinez *et al.*, 2019). Many national and international guides have been proposed to categorize diet consistency directed to dysphagia patients

with different needs, mainly based on the viscosity measured at 50 $s^{-1}$ (Ong *et al.*, 2018; National Dysphagia Diet Task Force, 2002). For the manipulation of commercially available thickeners, three consistency-groups named nectar-like (50–350 cP), honey-like (350–1750 cP) and pudding/spoon thick (> 1750 cP) were established. The behaviour of the commercially available thickeners (based on xanthan gum or modified starch) shows a significant variability and heterogeneity, depending on the preparation, dispersed liquid food and applied shear rates (Sopade *et al.*, 2008a, Sopade *et al.*, 2008b). Efforts should be made to adapt thickeners and clinical/homemade practice not only according to standardization guidelines, but also to obtain other rheological aspects that return the pleasure in consuming these products.

Therefore, the objective of the present study was to analyse the differences obtained in the rheological parameters of different formulations containing flaxseed gum, modified starch and xanthan gum at different concentrations based on the experimental design (DCCR) presented in Chapter IV for a water matrix, replaced here by milk and orange-flavoured soy juice. Mathematical models were generated to evaluate the rheological parameters prediction, and thus help clinicians and homecare personnel in managing dysphagia thickeners.

## Materials and Methods

### Materials

Golden flaxseeds produced in South of Brazil were kindly provided by CISBRA Ltda (Panambi, RS, Brazil). Xanthan gum (XG) was donated from Danisco (Brazil) and modified starch (MS) 136 from Cargill (Brazil). Orange-flavoured soy juice (Composition: water, soybeans, sugar, concentrated orange juice, maltodextrin, vitamins C, B3, B6 and B12, zinc, pectin, guar gum, citric acid, flavorings, malic acid, urucum, curcuma and sucralose) (Ades, Unilever, Brazil) and skim milk (Composition: sodium citrate, sodium monophosphate and sodium diphosphate) (Shefa, Brazil) were purchased in the local market.

### Preparation of thickeners

Thickener formulations were prepared by dissolving them in milk and orange-flavoured soy juice under mechanical stirring during 30 min at 800 rpm and room temperature (25 °C) to obtain a homogeneous solution. Then, samples were rested for an hour before to be subjected to rheological and colour measurements. Formulations were prepared

according to an experimental design (explained in the next topic) in milk and orange-flavoured soy juice.

**Experimental design**

The composition and concentration of the thickeners were chosen based on preliminary experimental designs. A Central Composite Rotational Design (CCRD), added of three replicates at the central point and six axial point essays, was performed for the three biopolymers concentration. Independent variables *x1* = FG (0 - 3 %, w/v), *x2* = modified starch (0 - 3 %, w/v) and *x3* = xanthan gum (0 - 0.5 %, w/v) were studied based on the importance of each compound for the following responses (dependent variables): viscosity ($\eta$) at different shear rates (10, 50 and 400 $s^{-1}$), flow index ($n$), storage ($G'$) and loss ($G''$) moduli. Analysis of variance (ANOVA) of statistically significant regression coefficients ($p < 0.1$) was used to evaluate predictive models. Then, response surface and contour curves were plotted in order to estimate the concentration range of each formulation component according to the desired viscosity. One formulation for each categorization present in the NDD guidelines (using the parameter $\eta$ at 50 $s^{-1}$) in each food matrix was predicted and experimentally validated aiming to verify the efficiency of the models. All experimental design analyses were performed by *Protimiza software* (www.http://experimentaldesign.protimiza.com.br).

**Rheological behaviour**

Rheological properties of thickeners solutions were obtained using an AR1500ex rheometer (TA Instruments, USA) with a stainless-steel cone-plate geometry (6.0 cm, 2° angle, truncation 67 μm). Flow curves were obtained by an up–down–up step program using different shear stresses range to provide shear rate between 0 to 400 $s^{-1}$ at 25 °C in order to understand the rheological behaviour throughout the swallowing process (Wood *et al.*, 1968, Cutler *et al.*, 1983). Newtonian (Eq. B-1) and power-law equation (Eq. B-2) were fitted to the data to obtain rheological properties.

$$\sigma = \eta . \dot{\gamma} \qquad \text{(Eq. B-1)}$$

$$\sigma = k . \dot{\gamma}^{n} \qquad \text{(Eq. B-2)}$$

where $\sigma$ is the shear stress (Pa), $\eta$ is the viscosity (Pa.s), $k$ is the consistency index ($Pa.s^n$), $\dot{\gamma}$ is the shear rate ($s^{-1}$) and $n$ is the flow index.

The viscoelastic properties were evaluated using a frequency sweep between 0.1 and 10 Hz within the linear viscoelasticity domain. All the measurements were done at 25 °C. The contributions of the elastic and viscous characteristics were analysed from storage ($G'$) and loss ($G''$) moduli, respectively. In order to clarify the prevailing behaviour of the samples, the loss tangent was calculated following Eq. (B-3):

$$tan\ \delta = \frac{G''}{G'} \qquad \text{(Eq. B-3)}$$

where, $\delta$ is the phase angle between the applied strain and the stress response.

**Colorimetry analysis**

The colour of thickeners was measured in triplicate using an Ultra Scan Vis 1043 (Hunter Lab, model Colour Quest II, USA) with reflectance mode, CIELab scale, D65 as illuminant and a 10° observer angle as a reference system. Cylindrical coordinates $C^*$ (chroma, represents the colour intensity) (Eq. B-4) and $\Delta E^*$ (total colour difference) (Eq. B-5) between the samples before and after thickeners addition were evaluated and calculated from $L^*$ (lightness), $a^*$ and $b^*$ (chromaticity parameters), according to:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \qquad \text{(Eq. B-4)}$$

$$\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad \text{(Eq. B-5)}$$

being $\Delta L = (L^* - L_0^*)$, $\Delta a = (a^* - a_0^*)$ and $\Delta b = (b^* - b_0^*)$, where, $L_0^*$, $a_0^*$ and $b_0^*$ are the initial colour values and $L^*$, $a^*$ and $b^*$ are the colour values of matrix (milk or orange-flavoured soy juice) containing thickeners.

**Statistical analyses**

The experimental design was carried out in accordance with the "Protimiza Experimental Design" software ($\alpha = 0.1$). Colour data were subjected to analysis of variance (ANOVA) ($p < 0.05$) and the means were compared using the Tukey's HSD test to examine if differences between formulations were significant ($\alpha = 0.1$).

## Results and discussion

### Thickener formulations

The rheological properties of the formulations were evaluated to quantify and qualify the influence of each component (FG, MS and XG) on viscosity and viscoelastic properties when these biopolymers were added in skim milk and orange-flavoured soy juice. These formulations were prepared based on the same CCRD performed for water as a dispersing medium (Chapter III), in order to evaluate the effect of variables (biopolymers concentration) when incorporated in different matrices. Visual phase separation was not observed for all formulations.

### Rheological properties

Rheological properties of the thickeners added in the milk and juice matrices are shown in Tables B-1 and B-2, respectively. Thickeners were produced from the combination of different concentrations of MS, XG and FG according to a central composite rotational design (CCRD). The evaluated rheological properties were apparent viscosity values at a fixed shear rate of 10 $s^{-1}$ ($\eta_{ap\ 10}$), 50 $s^{-1}$ ($\eta_{ap\ 50}$) and 400 $s^{-1}$ ($\eta_{ap\ 400}$); flow index *n* (as a shear thinning indicator) and viscoelastic properties represented by elastic (*G'*) and viscous (*G''*) moduli at a fixed frequency of 1 Hz.

The level of inability to chew and/or swallow food by dysphagic patients can vary considerably, causing a clinical practice problem, complicating follow-up and increasing the medical errors (Germain *et al.*, 2006). Thus, the effects of thickener formulations and food matrices at different shear rates (simulating different swallowing velocities) were analysed from flow curves. The extreme cases were represented by the highest (Formulation 4: 2.4 % FG, 2.4 % MS, 0.1 % XG) and lowest (Formulation 1: 0.6 % FG, 0.6 % MS, 0.1 % XG) viscosity for different food matrices, which can be seen in Figure B-1. All thickened solutions presented shear-time independent and shear-thinning (*n* values lower than 1) behaviour. Viscosity values of all formulations tended to increase with the change of the food matrix from water to skim milk or orange-flavoured soy juice, which could be related to a stronger network formed from the interactions between the matrix and thickeners' components.

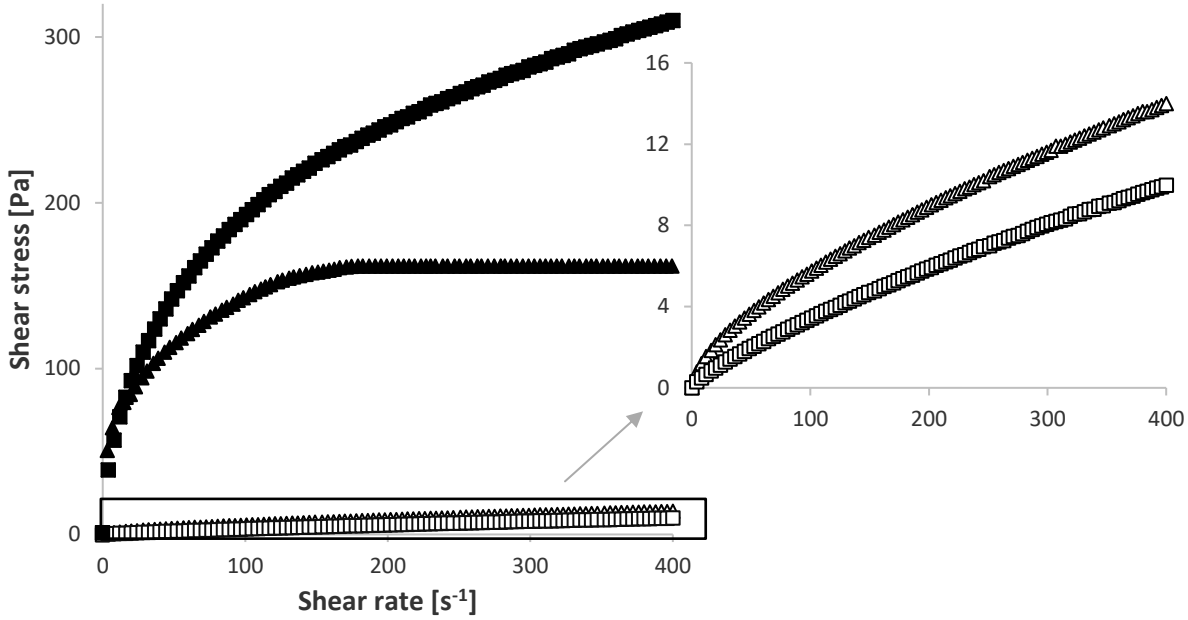



**Figure B-1.** Flow curves of thickener Formulations 1 (open symbols; 0.6 % FG, 0.6 % MS, 0.1 % XG) and 4 (closed symbols; 2.4 % FG, 2.4 % MS, 0.1 % XG) that represent extreme cases regarding viscosity, dissolved in milk (▲) and orange-flavoured soy juice (■).

Viscoelastic properties of the formulations in different food matrices were observed from oscillatory analyses (Figure B-2). Higher *G'* and *G''* values were observed for thickeners incorporated into milk and orange-flavoured soy juice when compared to water (Chapter IV). For most of the formulations, the thickeners solutions presented a predominance of elastic properties within the frequency range 0.1-10 Hz (*G' > G''*). At high biopolymers concentrations, the differences between formulations were small, but at the lowest polysaccharides concentration (Formulation 1) the milk system showed the highest viscoelastic properties.

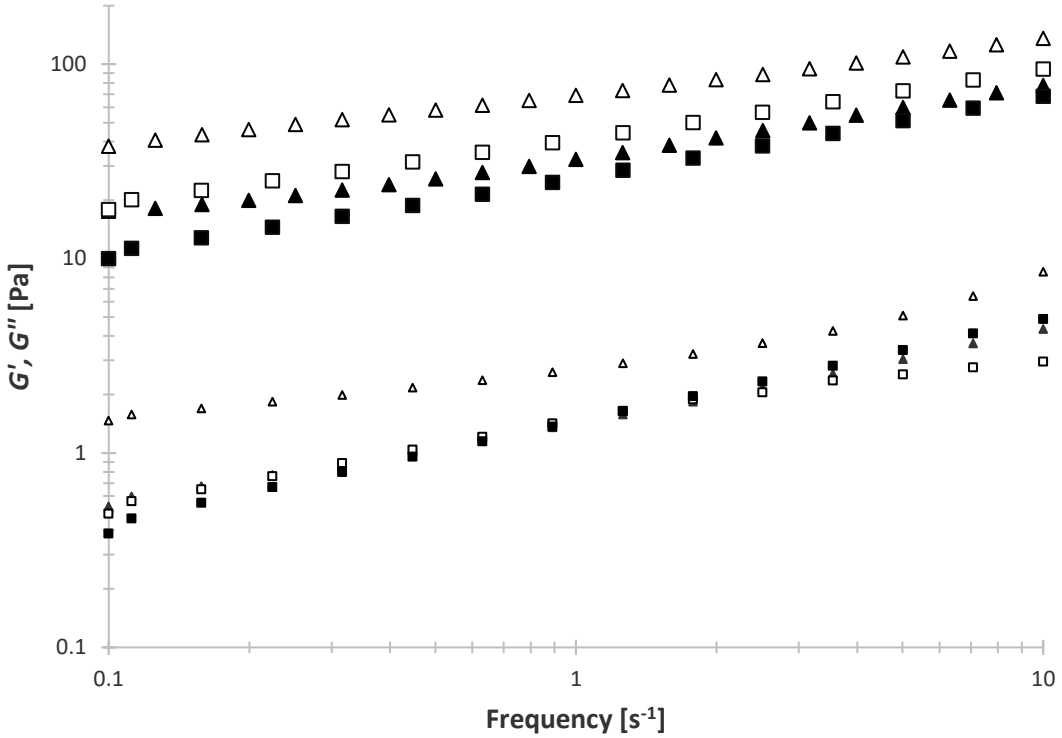



**Figure B-2.** Elastic (open symbols) and viscous moduli (closed symbols) as a function of frequency under isothermal (25 °C) conditions: Formulation 1 (0.6 % FG, 0.6 % MS, 0.1 % XG; smaller symbols) and Formulation 8 (2.4 % FG, 2.4 % MS, 0.4 % XG; larger symbols)) in milk (▲) and orange-flavoured soy juice (■).

**Effect of biopolymers concentration on rheological properties**

In general, an increase in FG, MS and XG concentration promoted an increase in the apparent viscosity and pseudoplasticity of the milk and soy juice matrices as compared to water (Chapter IV). Viscosity values at a shear rate of 10 $s^{-1}$, 50 $s^{-1}$ and 400 $s^{-1}$ ranged from 118 to 9061, 67 to 2394 and 32 to 515 cP for milk and 57 to 7915, 40 to 2888 and 25 to 785 cP for orange-flavoured soy juice, respectively. This broader range of response values in the more complex matrices, resulted in a better fitting by the model to the higher concentration values. Unlike water, the spoon consistency (> 1750 cP) was reached in milk (4, 8, 10 and 12) and orange-flavoured soy juice (4) matrices (shear rate of 50 $s^{-1}$). All these formulations had in common a high concentration of FG and MS.

**Table B-1.** Rheological properties, *G'*, *G''*, *tan δ*, *n* and apparent viscosity at different shear rates of the thickener formulations (TF) (1-17) containing flaxseed gum (FG), modified starch (MS) and xanthan gum (XG) dispersed in milk according to the CCRD matrix.

| TF | FG | MS | XG | $\eta_{ap\ 10}$ | $\eta_{ap\ 50}$ | $\eta_{ap\ 400}$ | *n* | *G'* (1 Hz) | *G''* (1 Hz) | *tan δ* |
|---|---|---|---|---|---|---|---|---|---|---|
| | (%) | (%) | (%) | (cP) | (cP) | (cP) | | (Pa) | (Pa) | |
| 1 | -1 (0.6) | -1 (0.6) | -1 (0.1) | 118 | 67 | 32 | 0.65 | 1.95 | 1.42 | 0.73 |
| 2 | 1 (2.4) | -1 (0.6) | -1 (0.1) | 4484 | 1403 | 313 | 0.28 | 38.32 | 22.02 | 0.57 |
| 3 | -1 (0.6) | 1 (2.4) | -1 (0.1) | 931 | 401 | 135 | 0.48 | 9.14 | 10.02 | 1.10 |
| 4 | 1 (2.4) | 1 (2.4) | -1 (0.1) | 9061 | 2394 | 429 | 0.17 | 22.20 | 18.29 | 0.82 |
| 5 | -1 (0.6) | -1 (0.6) | 1 (0.4) | 1066 | 404 | 115 | 0.40 | 22.93 | 12.53 | 0.55 |
| 6 | 1 (2.4) | -1 (0.6) | 1 (0.4) | 3327 | 1185 | 312 | 0.36 | 17.23 | 15.36 | 0.89 |
| 7 | -1 (0.6) | 1 (2.4) | 1 (0.4) | 2247 | 768 | 192 | 0.33 | 52.65 | 24.28 | 0.46 |
| 8 | 1 (2.4) | 1 (2.4) | 1 (0.4) | 6188 | 1891 | 409 | 0.26 | 67.27 | 31.98 | 0.48 |
| 9 | -1.68 (0) | 0 (1.5) | 0 (0.25) | 357 | 143 | 44 | 0.43 | 6.00 | 3.33 | 0.56 |
| 10 | 1.68 (3) | 0 (1.5) | 0 (0.25) | 5480 | 1805 | 515 | 0.41 | 78.25 | 45.25 | 0.58 |
| 11 | 0 (1.5) | -1.68 (0) | 0 (0.25) | 592 | 281 | 108 | 0.54 | 4.08 | 4.49 | 1.10 |
| 12 | 0 (1.5) | 1.68 (3) | 0 (0.25) | 6977 | 1805 | 315 | 0.17 | 78.60 | 28.95 | 0.37 |
| 13 | 0 (1.5) | 0 (1.5) | -1.68 (0) | 2358 | 956 | 298 | 0.44 | 10.00 | 10.93 | 1.09 |
| 14 | 0 (1.5) | 0 (1.5) | 1.68 (0.5) | 4165 | 1236 | 257 | 0.25 | 44.48 | 19.55 | 0.44 |
| 15 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 2998 | 984 | 191 | 0.26 | 23.57 | 13.47 | 0.57 |
| 16 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 3116 | 1131 | 250 | 0.27 | 28.02 | 15.34 | 0.55 |
| 17 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 3029 | 1001 | 230 | 0.28 | 25.40 | 14.85 | 0.59 |

**Table B-2.** Rheological properties, *G'*, *G''*, *tan δ, n* and apparent viscosity at different shear rates and of the thickener formulations (TF) (1-17) containing flaxseed gum (FG), modified starch (MS) and xanthan gum (XG) dispersed in orange-flavoured soy juice according to a CCRD matrix.

| TF | FG | MS | XG | $\eta_{ap\ 10}$ | $\eta_{ap\ 50}$ | $\eta_{ap\ 400}$ | *n* | *G'* (1 Hz) | *G''* (1 Hz) | *tan δ* |
|---|---|---|---|---|---|---|---|---|---|---|
| | (%) | (%) | (%) | (cP) | (cP) | (cP) | | (Pa) | (Pa) | |
| 1 | -1 (0.6) | -1 (0.6) | -1 (0.1) | 57 | 40 | 25 | 0.78 | 1.02 | 1.05 | 1.03 |
| 2 | 1 (2.4) | -1 (0.6) | -1 (0.1) | 2063 | 966 | 362 | 0.53 | 7.22 | 9.80 | 1.36 |
| 3 | -1 (0.6) | 1 (2.4) | -1 (0.1) | 794 | 307 | 191 | 0.59 | 8.46 | 5.42 | 0.64 |
| 4 | 1 (2.4) | 1 (2.4) | -1 (0.1) | 7915 | 2888 | 785 | 0.37 | 66.13 | 50.63 | 0.77 |
| 5 | -1 (0.6) | -1 (0.6) | 1 (0.4) | 461 | 203 | 71 | 0.49 | 7.72 | 4.28 | 0.55 |
| 6 | 1 (2.4) | -1 (0.6) | 1 (0.4) | 1319 | 596 | 207 | 0.54 | 11.05 | 10.06 | 0.91 |
| 7 | -1 (0.6) | 1 (2.4) | 1 (0.4) | 1553 | 609 | 173 | 0.37 | 32.80 | 13.90 | 0.42 |
| 8 | 1 (2.4) | 1 (2.4) | 1 (0.4) | 4374 | 1677 | 486 | 0.41 | 43.48 | 27.80 | 0.64 |
| 9 | -1.68 (0) | 0 (1.5) | 0 (0.25) | 279 | 120 | 41 | 0.48 | 15.17 | 5.05 | 0.33 |
| 10 | 1.68 (3) | 0 (1.5) | 0 (0.25) | 2752 | 1185 | 399 | 0.48 | 16.70 | 15.80 | 0.95 |
| 11 | 0 (1.5) | -1.68 (0) | 0 (0.25) | 355 | 196 | 91 | 0.63 | 3.75 | 3.69 | 0.98 |
| 12 | 0 (1.5) | 1.68 (3) | 0 (0.25) | 1668 | 747 | 264 | 0.50 | 19.03 | 13.85 | 0.73 |
| 13 | 0 (1.5) | 0 (1.5) | -1.68 (0) | 572 | 310 | 141 | 0.62 | 1.79 | 3.88 | 2.17 |
| 14 | 0 (1.5) | 0 (1.5) | 1.68 (0.5) | 2088 | 714 | 201 | 0.42 | 42.45 | 18.00 | 0.42 |
| 15 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 1172 | 514 | 178 | 0.49 | 11.56 | 7.92 | 0.69 |
| 16 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 999 | 494 | 169 | 0.54 | 8.62 | 7.12 | 0.83 |
| 17 | 0 (1.5) | 0 (1.5) | 0 (0.25) | 1164 | 536 | 197 | 0.52 | 9.29 | 6.34 | 0.68 |

Only Formulation 1 (the lowest concentration of the three biopolymers) may compromise the swallowing safety for patients with dysphagia in both matrices as observed in water, regarding the shear rate of 50 $s^{-1}$, since the viscosity was below or near the lowest recommended limit value (50 cP) (Table B-1 and B-2). This fact can lead to food liquid aspiration into the lungs due to the quick flow of the food bolus from the mouth to the laryngeal airway before its closure (O'Leary *et al.*, 2010, Vallons *et al.*, 2013).

Regarding the viscoelastic nature of the formulations added in the food matrices, a curious fact was observed, since whereas the highest elastic character in the milk was observed for Formulation 12 ($tan\ \delta$ = 0.37) that contained a greater content of MS. On the other hand, in the soy juice the greater elastic character was obtained in the Formulation 9 ($tan\ \delta$ = 0.33), without FG in its composition, which may be related to the antagonism between this gum and XG also present in the soy juice composition (Table B-4).

Once rheological behaviour is critical to be analysed in dysphagia patients' diet, mathematical models were fitted for milk and orange-flavoured soy juice matrices to better visualize the effect of thickener combinations on the matrices. Only statistically significant ($p < 0.1$) coefficients were given and the validity of each model was verified from analysis of variance (ANOVA). It should be noted that central points of these different matrices (15, 16 and 17) showed repeatability for all parameters and, therefore, the study can be considered valid.

**Multivariate analysis: mathematical modelling**

Table B-3 shows the mathematical models fitted to rheological properties of formulations in milk and Figure B-3A exhibits the experimental values of apparent viscosity at a shear rate of 50 $s^{-1}$ *versus* the predicted values by the model as a function of the biopolymers concentrations: FG, MS and XG. Results presented a good fit for all the studied rheological parameters, though the explained variation ($R^2$) was slightly lower than in water (Chapter IV).

**Table B-3.** Regression coefficients for the mathematical model generated (coded model) for formulations dispersed in milk, representing the rheologic behaviour of the formulations ($\eta_{ap\ 10}$, $\eta_{ap\ 50}$, $\eta_{ap\ 400}$, $n$, $G'$ and $G''$) with the respective coefficient of determination ($R^2$). Statistically significant models have $F_{calc}/F_{tab}$ values greater than 1.

| **Response equations** | $R^2$ | $F_{calc}/F_{tab}$ |
|---|---|---|
| $\eta_{ap\ 10}$ **(cP) =** 3323.24 + 2000.04x1 + 1476.96x2 + 680.45x1x2 - 786.67x1x3 | 0.92 | 14.44 |
| $\eta_{ap\ 50}$ **(cP) =** 1050.32 + 587.82x1 + 362.91x2 + 124.91x1x2 - 178.21x1x3 | 0.97 | 32.86 |
| $\eta_{ap\ 400}$ **(cP)** = 214.31 + 130.32x1 + 54.16x2 - 20.14x1x3 | 0.97 | 32.78 |
| $n$ = 0.33 - 0.06x1 - 0.08x2 - 0.04x3 + 0.07x1x3 + 0.03$x1^2$ | 0.80 | 3.55 |
| $G'$ **(1Hz) (Pa) =** 31.18 + 13.17x1 + 14.36x2 + 10.72x3 + 11.09x2x3 | 0.77 | 4.03 |
| $G''$ **(1Hz) (Pa) =** 14.79 + 8.05x1 + 5.45x2 + 3.43x3 + 2.97$x1^2$ | 0.80 | 4.72 |

x1 - FG
x2 - MS
x3 - XG

Regarding to the milk matrix, viscosity was mainly influenced by FG concentration, followed by MS (as in water), while XG, within the range concentration (0-0.5%), did not exert a significant linear effect on viscosity, independently of the applied shear rate. It was noticeable the FG positive interaction with MS (as also observed in water) (Figure B-3B), however, a negative FG-XG interaction was observed at high and low shear rates (Figure B-3D), observing the effect of the greater complexity of the milk matrix compared with water. MS exerted the highest positive effect on the pseudoplastic (shear thinning) character (*n*), followed by FG and XG. The reduced effect of xanthan on the pseudoplasticity was remarkable, suggesting that the milk proteins modified the interactions between this gum and the other biopolymers. In this context, it is clear that the same thickener will work in a different way depending on the composition of the food matrices.

As in water, viscoelastic properties (*G'*, *G''*) were influenced by all biopolymers' concentration, as can be seen in Table B-3. XG showed, again, a relevant contribution to the increase of *G'* and *G''*, but the impact on elastic properties was lower than that of FG and MS. Nevertheless, its contribution was more relevant when interacting with MS, confirming similar positive interactions in water and milk between these polymers, both contributing to safer and easier swallowing (Jo, Bak & Yoo, 2018). Table B-3 shows that FG exhibited a higher viscous contribution rather than elastic one as compared with MS and XG, but this biopolymer also showed a significant positive influence on the elastic properties.

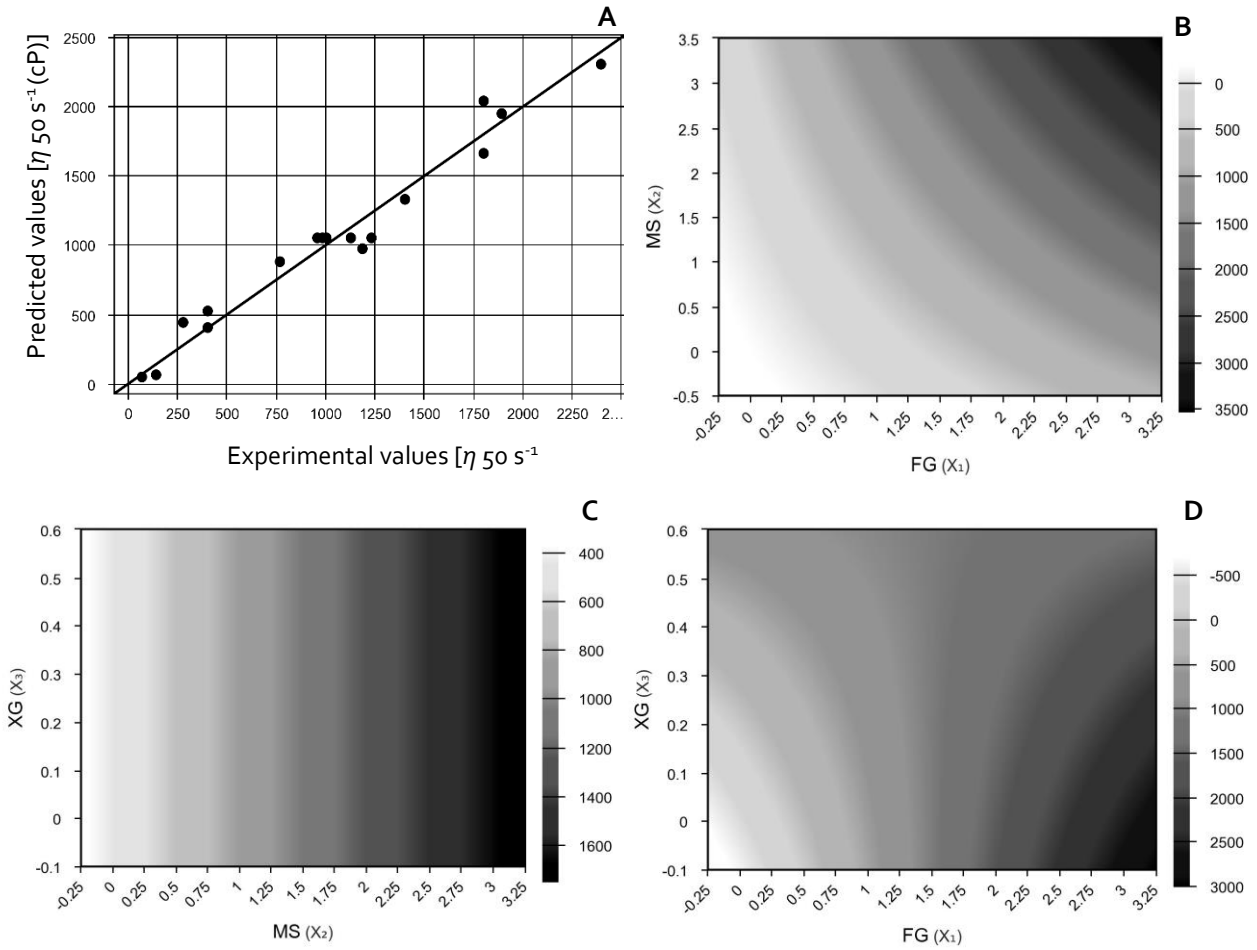



**Figure B-3.** Experimental versus predicted values for response $\eta$ at 50 $s^{-1}$ obtained in milk (A) and the surface response of $\eta$ at 50 $s^{-1}$ as a function of significant interactions of FG, MS and XG concentrations for thickener formulations in milk. XG concentration fixed at 0.25 % (B), FG concentration at 1.5 % (C) and MS concentration at 1.5 % (D).

Table B-4 shows the mathematical model fitted to the rheological data of formulations produced with orange-flavoured soy juice. Figure B-4A shows the experimental values of apparent viscosity at a shear rate of 50 $s^{-1}$ and the values predicted by the model as a function of the concentrations of FG, MS and XG. The explained variation ($R^2$) was significantly lower than in water and milk matrices and results did not present a good fit for all the studied shear rates, despite of the ratio $F_{calc}/F_{tab}$ was always higher than 1.

**Table B-4.** Regression coefficients for the mathematical model generated (coded model) for thickeners dispersed in orange-flavoured soy juice, representing the rheology of the formulations ($\eta_{ap\ 10}$, $\eta_{ap\ 50}$, $\eta_{ap\ 400}$, $n$, $G'$ and $G''$) with the respective coefficient of determination ($R^2$). Statistically significant models show $F_{calc}/F_{tab}$ values greater than 1.

| **Response equations** | $R^2$ | $F_{calc}/F_{tab}$ |
|---|---|---|
| $\eta_{ap\ 10}$ **(cP) =** 1740.19 + 1242.27x1 + 947.84x2 + 884.64x1x2 - 680.99x1x3 | 0.74 | 3.43 |
| $\eta_{ap\ 50}$ **(cP) =** 711.90 + 494.97x1 + 336.99x2 + 291.35x1x2 - 255.64x1x3 | 0.72 | 3.15 |
| $\eta_{ap\ 400}$ **(cP)** = 234.14 + 145.30x1 + 92.35x2 - 60.23x1x3 | 0.77 | 5.59 |
| $n$ = 0.52 – 0.03x1 - 0.03x2 - 0.06x3 + 0.07x1x3 | 0.87 | 7.86 |
| $G'$ **(1Hz) (Pa) =** 17.99 + 5.92x1 + 10.98x2 + 5.93x3 | 0.51 | 1.76 |
| $G''$ **(1Hz) (Pa) =** 12.01 + 6.74x1 + 6.59x2 + 5.53x1x2 - 4.33x1x3 | 0.97 | 16.57 |

x1 - FG
x2 - MS
x3 - XG

In relation to the orange-flavoured soy juice, the viscosity was mainly influenced by the FG concentration followed by MS concentration, regardless of the applied shear rate in the same way of the two previous matrices (water and milk). As in the milk matrix, no significant effect was observed for XG in soy juice matrix, regardless the shear rate. The elastic properties and pseudoplasticity also increased as biopolymers concentration increased, mainly for MS and XG (in the same way as for water). However, unlike the previous matrices, *G"* was no longer influenced by XG concentration. Presence of other biopolymers in this matrix possibly modified the interactions between MS, XG and FG, although the behaviour was intermediate between water and milk. Xanthan was more relevant to pseudoplasticity than in milk; this fact may be related to the network formed between the xanthan gum from the thickening formulation and the xanthan gum present in the soy juice, resulting in a gel with a more pseudoplastic character.

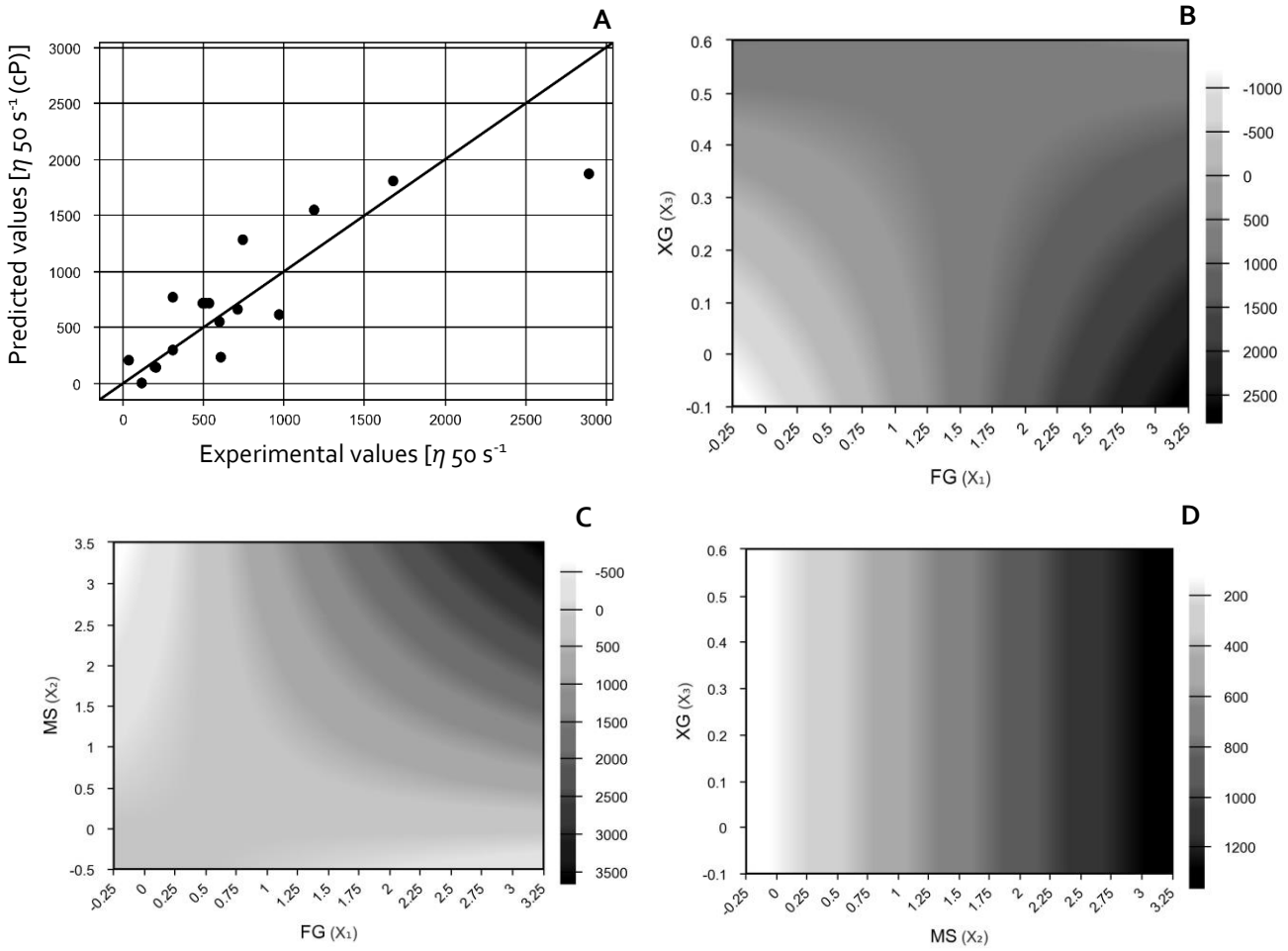



**Figure B-4.** Experimental versus predicted values for response $\eta$ at 50 $s^{-1}$ obtained in orange-flavoured soy juice (A) and the surface response of $\eta$ at 50 $s^{-1}$ as a function of significant interactions between FG and MS concentrations in thickening formulations. XG concentration fixed at 0.25 % (B).

From the analyses of response surfaces and contour curves for viscosity at 50 $s^{-1}$ (Figures B-3 and B-4), six formulations were suggested to confirm the mathematical model generated, aiming to represent each categorization published by NDD (nectar-like, honey-like and spoon thick) (Table B-5). Thus, to validate the predicted values calculated from the mathematical models for the viscosity at 50 $s^{-1}$ (Tables B-3 and B-4), new assays were performed as shown in Table B-5.

**Table B-5.** Predicted and experimental viscosity at a shear rate of 50 $s^{-1}$ obtained from new formulations for validation of the previous results.

| | | Formulation (%) | | | Experimental (cP) | Predicted (cP) | Relative Error (%) |
|---|---|---|---|---|---|---|---|
| **Matrix** | **Categorization** | **FG** | **MS** | **XG** | $\eta_{ap\ 50}$ | $\eta_{ap\ 50}$ | |
| **Milk** | Nectar | 0.2 | 1.5 | 0.25 | 390 ± 8 | 201 ± 66 | 49 |
| | Honey | 1.5 | 0.1 | 0.1 | 437 ± 34 | 486 ± 70 | 11 |
| | Spoon | 2.85 | 1.4 | 0.25 | 1790 ± 78 | 1870 ± 69 | 4 |
| **Soy Juice** | Nectar | 0.9 | 1.5 | 0 | 92 ± 7 | 378 ± 142 | 300 |
| | Honey | 1.5 | 0.9 | 0 | 390 ± 8 | 486 ± 112 | 25 |
| | Spoon | 2 | 3 | 0 | 1783 ± 62 | 1829 ± 318 | 3 |

In milk and soy juice, due to the greater range of viscosity values obtained experimentally, it was foreseeable that the model adjusted better to the highest viscosity values. Therefore, the experimental results obtained for these matrices are within the recommended range in the guidelines generated by the NND for honey-like and spoon-thick.

### Colorimetric analyses

Visually, it was noteworthy that thickeners in general did not present a significant change on the colour of the food matrix, which could play a significant role in the acceptability for consumers. Colour parameters ($L^*$, $C^*$ and $\Delta E^*$) of thickener dispersions were analysed and results are presented in Table B-6.

**Table B-6.** *L**, *C** and Δ*E** values of control (C) and thickener formulations in different food matrices.

| | Milk | | | Orange-flavoured soy juice | | |
|---|---|---|---|---|---|---|
| | ***L**** | ***C**** | **Δ*E**** | ***L**** | ***C**** | **Δ*E**** |
| **C** | $77.7 \pm 0.1^{c}$ | $4.3 \pm 0.1^{h}$ | - | $66.9 \pm 0.1^{b}$ | $27.5 \pm 0.2^{a}$ | - |
| **1** | $64.9 \pm 0.4^{j}$ | $5.5 \pm 0.1^{f}$ | $13.3 \pm 0.6$ | $64.7 \pm 0.1^{f,g}$ | $25.4 \pm 0.1^{d}$ | $3.0 \pm 0.6$ |
| **2** | $72.0 \pm 0.2^{h}$ | $8.2 \pm 0.0^{c}$ | $10.4 \pm 0.1$ | $64.7 \pm 0.0^{g}$ | $26.0 \pm 0.0^{b}$ | $2.6 \pm 0.3$ |
| **3** | $77.8 \pm 0.1^{c}$ | $3.8 \pm 0.1^{i}$ | $4.7 \pm 0.1$ | $64.4 \pm 0.2^{g}$ | $25.4 \pm 0.2^{c,d}$ | $3.3 \pm 0.4$ |
| **4** | $72.3 \pm 0.1^{g}$ | $8.3 \pm 0.1^{c}$ | $10.4 \pm 0.1$ | $65.1 \pm 0.3^{d,e}$ | $25.5 \pm 0.2^{c,d}$ | $2.4 \pm 0.7$ |
| **5** | $78.3 \pm 0.1^{b}$ | $3.9 \pm 0.0^{i}$ | $5.0 \pm 0.1$ | $67.7 \pm 1.0^{b}$ | $25.5 \pm 1.0^{b,c,d}$ | $2.5 \pm 0.6$ |
| **6** | $74.8 \pm 0.2^{e,f}$ | $9.1 \pm 0.3^{a,b}$ | $10.4 \pm 0.1$ | $65.4 \pm 0.4^{c,d}$ | $26.2 \pm 0.3^{b}$ | $1.8 \pm 0.1$ |
| **7** | $74.6 \pm 0.2^{f}$ | $3.8 \pm 0.1^{i}$ | $5.4 \pm 0.3$ | $69.3 \pm 0.2^{a}$ | $25.3 \pm 0.2^{d}$ | $3.2 \pm 0.1$ |
| **8** | $76.4 \pm 0.1^{d}$ | $9.0 \pm 0.1^{b}$ | $9.9 \pm 0.2$ | $65.3 \pm 0.1^{d}$ | $25.9 \pm 0.1^{b}$ | $2.2 \pm 0.3$ |
| **9** | $80.5 \pm 0.1^{a}$ | $2.1 \pm 0.0^{k}$ | $4.4 \pm 0.2$ | $63.3 \pm 0.1^{h}$ | $25.5 \pm 0.1^{c,d}$ | $4.1 \pm 0.1$ |
| **10** | $76.3 \pm 0.3^{d}$ | $9.6 \pm 0.1^{a}$ | $10.6 \pm 0.1$ | $65.3 \pm 0.2^{c,d}$ | $25.2 \pm 0.3^{d}$ | $2.6 \pm 0.4$ |
| **11** | $63.6 \pm 0.2^{j}$ | $3.6 \pm 0.0^{j}$ | $14.2 \pm 0.2$ | $66.7 \pm 0.3^{b}$ | $25.7 \pm 0.0^{c}$ | $1.7 \pm 0.3$ |
| **12** | $72.7 \pm 0.3^{g}$ | $6.2 \pm 0.2^{e}$ | $8.4 \pm 0.6$ | $65.9 \pm 0.3^{c}$ | $25.5 \pm 0.1^{c,d}$ | $2.2 \pm 0.6$ |
| **13** | $68.9 \pm 2.3^{i}$ | $3.6 \pm 0.0^{j}$ | $9.5 \pm 1.9$ | $64.9 \pm 0.1^{e,f}$ | $26.0 \pm 0.1^{b}$ | $2.4 \pm 0.3$ |
| **14** | $75.3 \pm 0.3^{e}$ | $6.9 \pm 0.3^{d}$ | $7.9 \pm 0.4$ | $67.0 \pm 0.3^{b}$ | $25.7 \pm 0.2^{b,c,d}$ | $1.7 \pm 0.5$ |
| **15** | $72.5 \pm 0.2^{g}$ | $4.9 \pm 0.1^{g}$ | $7.5 \pm 0.1$ | $65.6 \pm 0.1^{c}$ | $25.2 \pm 0.3^{d}$ | $2.6 \pm 0.4$ |

a-k Different letters in the same column correspond to statistically different samples for a 95% confidence level.

Reflectance spectrophotometry showed a slight change in the colour of the samples in milk, mainly due to a significant variation ($p < 0.05$) of the lightness (*L**) values for the different formulations (Table B-6). Although less pronounced than in water, a variation between the control (77.7 ± 0.1) and formulations was also observed, mainly in formulations 1 (64.9 ± 0.4), 11 (63.6 ± 0.2) and 13 (68.9 ± 2.3).

Results indicated that high concentrations of FG exerted positive effect on chroma (*C**) of milk formulations, indicating a higher colour intensity as can be seen in formulations 4, 6, 8 and 10. Regarding to soy juice, *C** and *L** parameters were practically unaffected. This fact is confirmed by Δ*E** values, since they were quite low (always Δ*E** < 4.1), concluding that the differently thickened formulations were closer to the control matrix (Olivas & Barbosa-Cánovas, 2005). From Δ*E** values, it was possible to observe that high concentrations of FG significantly affected the initial colour of water and milk matrix. Formulations 3, 5, 7, 9 and 15 presented a Δ*E** designated as "very good" in all matrices according to the American Society for Testing and Materials, since Δ*E** values were always below 8.

Table B-7 shows the mathematical model fitted to the colour data of formulations produced with milk matrix.

**Table B-7.** Percentage of variance explained ($R^2$) for the responses $L^*$ and $C^*$ in milk by analysis of variance (ANOVA). Statistically significant models have $F_{calc}/F_{tab}$ values greater than 1.

| **Response equations** | $R^2$ | $F_{calc}/F_{tab}$ |
|---|---|---|
| $L^* = 70.99 + 2.71x_1^2 + 1.93x_2 + 2.04x_3 - 1.91x_2 x_3$ | 0.73 | |
| $C^* = 5.78 + 2.21x_1$ | 0.77 | |

x1 - FG
x2 - MS
x3 - XG

FG was, as in water (Chapter IV), the component that presented the highest influence on $L^*$ and $C^*$ parameters, being the only component with significant influence on $C^*$, thus contributing to the increase of sample colour intensity. Regarding the $L^*$ parameter, the high influence of XG should be emphasized, since its studied concentration range was considerably lower.

Figure B-5 shows the experimental values of $L^*$ and the values predicted by the model as a function of the concentrations of FG, MS and XG in milk.

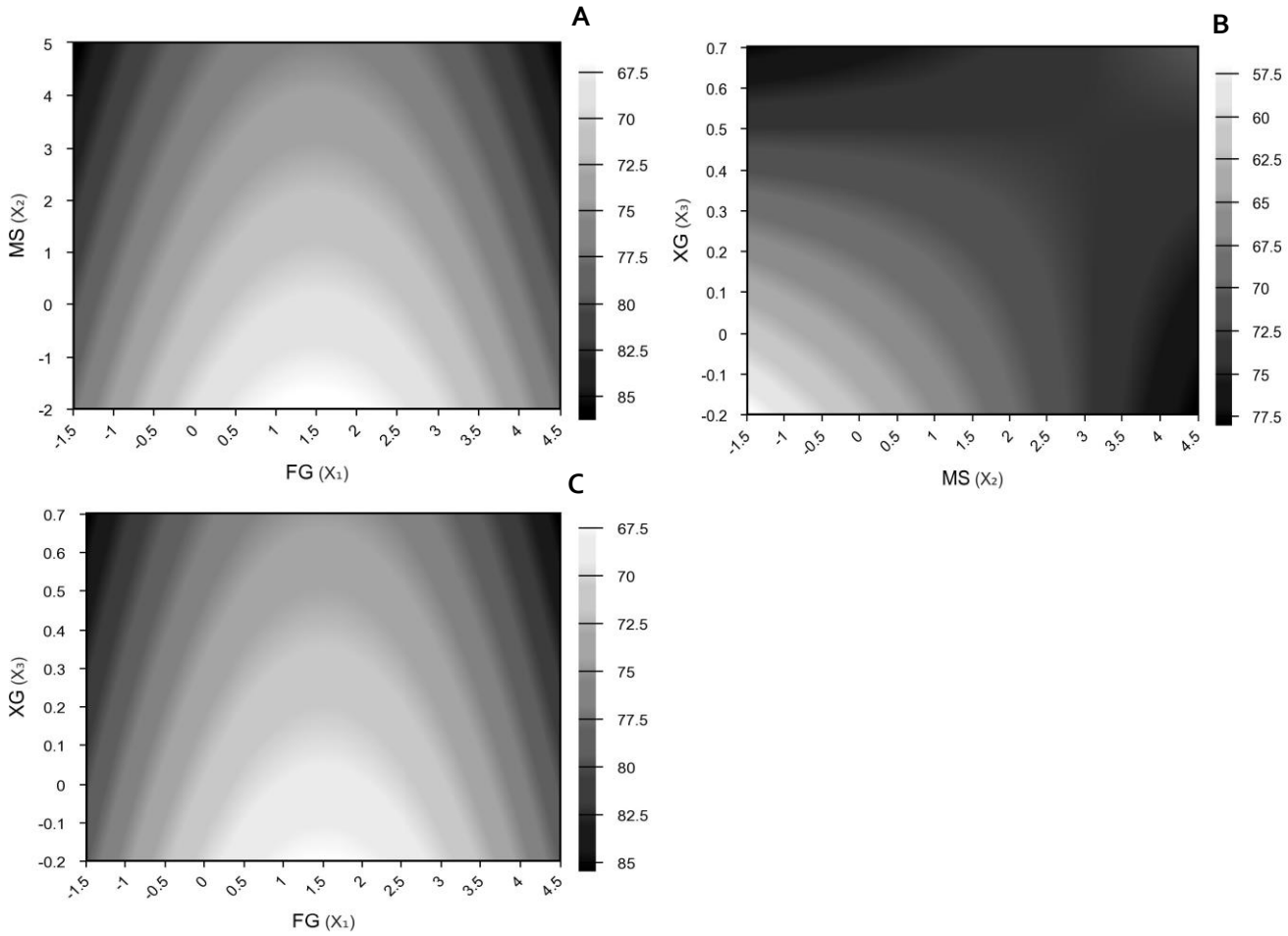



**Figure B-5.** Surface response of *L** (see grey scales on the right side of each graph) as a function of significant interactions of FG, MS and XG concentrations in thickened formulations for: XG concentration fixed at 0.25 % (A), FG concentration fixed at 1.5 % (B) and MS concentration fixed at 1.5 % (C).

Relatively to the orange-flavoured soy juice matrix, no model was generated given the insignificant deviations presented by the thickened samples when compared to the control.

**Conclusions**

Milk and orange soy juice dispersing media differ in their constituents, which caused a distinguished interaction between them and the gum/starch macromolecules. The thickener macromolecules interacted differently with the constituents of the dispersing media, modifying rheological properties, including the viscosity at different shear rates. FG showed the greatest positive influence, followed by MS in all matrices with respect

to the viscosity. The influence of MS was associated to synergistic interactions between starch and other biopolymers. The effect of XG was the most affected by the matrix, since its influence on viscosity was more relevant in water but strongly decreased in the presence of milk and soy proteins. However, XG demonstrated in all matrices a high contribution to the pseudoplasticity of the formulations, even at lower concentrations than FG and MS. All biopolymers concentration exerted a significant impact on viscoelastic properties (*G'*, *G''*), with a higher relevance of FG on the viscous properties and XG to the elastic properties. The colour of the thickened milk and orange-flavoured soy juice with thickeners addition, in general, did not present significant differences.

Therefore, results of the present study demonstrated that a generic labelling information about the food thickener concentrations to be used can be precarious according to the food matrix nature, which can lead to excessive or insufficient thickener content. Thus, generated models could be interesting to better adapt a thickener concentration for different food matrices and/or each patient, according to the necessary viscosity.

**References**

Cutler, A.N., Morris, E.R., Taylor, L.J., 1983. Oral Perception of Viscosity in fluid foods and model systems. Food and Nutrition Press, 14, 377-395.

Germain, I., Dufresne, T. & Ramaswamy, H.S., 2006. Rheological characterization of thickened beverages used in the treatment of dysphagia. Journal of Food Engineering, 73, 64-74.

Hadde, E.K. and Chen, J., 2019. Shear and extensional rheological characterization of thickened fluid for dysphagia management. Journal of Food Engineering, 245, 18-23.

Jo, W., Bak, J.H., Yoo, B., 2018. Rheological characterizations of concentrated binary gum mixtures with xanthan gum and galactomannans. International Journal of Biological Macromolecules, 114, 263-269.

Martínez, O., Vicente, M.S., De Vega, M.C., Salmerón, J., 2019. Sensory perception and flow properties of dysphagia thickening formulas with different composition. Food Hydrocolloids, 90, 508–514.

National Dysphagia Diet Task Force, 2002. National dysphagia diet: standardization for optimal care. Chicago, IL, American Dietetic Association.

O'Leary, M., Hanson, B. and Smith, C., 2010. Viscosity and Non-Newtonian Features of Thickened Fluids Used for Dysphagia Therapy, 2010, Journal of Food Science, 75(6), 330-338.

Olivas, G.I. and Barbosa-Cánovas, G.V., 2005. Edible Coatings for Fresh-Cut Fruits. Critical Reviews in Food Science and Nutrition, (45), 657-670.

Ong, J.J.-X., Steele, C.M., Duizer, L.M., 2018. Sensory characteristics of liquids thickened with commercial thickeners to levels specified in the International Dysphagia Diet Standardization Initiative (IDDSI) framework. Food Hydrocolloids, 79, 208-217.

Sopade, P.A., Halley, P.J., Cichero, J.A.Y., Ward, L.C., Hui, L.S., Teo, K.H., 2008a. Rheological characterization of food thickeners marketed in Australia in various media for the management of dysphagia. II. Milk as a dispersing medium. Journal of Food Engineering, 84, 553–563.

Sopade, P.A., Halley, P.J., Cichero, J.A.Y., Ward, L.C., Liu, J., Varliveli, S., 2008b. Rheological characterization of food thickeners marketed in Australia in various media for the management of dysphagia. III. Fruit juice as a dispersing medium. Journal of Food Engineering, 86, 604–615.

Turcanu, M., Siegert, N., Secouard, S., Brito-de la Fuente, E., Balan, C., Gallegos, C., 2018. An alternative elongational method to study the effect of saliva on thickened fluids for dysphagia nutritional support. Journal of Food Engineering, 228, 79-83.

Vallons, K.J.R., Helmens, H.J. and Oudhuis, A.A.C.M., 2015. Effect of human saliva on the consistency of thickened drinks for individuals with dysphagia. International Journal of Language & Communication Disorders, 50(2), 165-175.

Wood, G.N., 1968. Centric occlusion, centric relation, and the mandibular posture. The Journal of Prosthetic Dentistry, 20 (4), 292-306.

## Appendix C. Tribological characterization of food thickeners for the management of dysphagia: orange soy juice and milk as a dispersing media


### Abstract

Soft tribology was used to obtain the coefficient of friction (*CoF*) of the orange-flavoured soy juice and milk thickened with two commercial food thickeners (based on modified starch- TE and xanthan gum- TU), modified starch (MS), flaxseed gum (FG) or xanthan gum (XG). All thickeners were added at the same concentration (0.75, 1.5, 2.25 and 3 % w/w) and strained between the simulators of the tongue (manufactured from PDMS) and the hard palate (steel ball) using a constant sliding speed (40 mm/s) for 20 s to simulate oral processing. The objective was to conduct a comprehensive study to evaluate the tribological responses of different biopolymer-based thickened solutions when incorporated in different matrices to be compared with water. Coefficient of friction of water was significantly higher than the coefficient of friction obtained in milk or soy juice matrices. At steady state, by increasing the concentration of gum-based thickeners from 0.75 % up to 3 % w/w, the coefficient of friction of the milk matrix decreased in contrast to the addition of starch-based thickeners, which showed an increase in the coefficient of friction (as in water). On the other hand, all biopolymer thickeners decreased the lubrication capacity of orange-flavoured soy juice. The results of the present study indicate that tribology can contribute to estimate the influence of biopolymer thickeners on the lubrication capacity, which may cause a different impact on sensory properties according to the matrix to which they are added.


### Introduction

The increase of dysphagic swallowing disorders that occurs after stroke, neurodegenerative diseases, or treatment for head and neck cancer has led to an intensification in the study related to the palliative care to circumvent the negative consequences caused by this disturbance (Martinez *et al.*, 2019). For this, liquid foods for consumption of patients with dysphagia are commonly thickened with commercial xanthan gum and modified starch-based food thickeners, in order to ensure safe intake, and consequently obtain adequate rehydration, avoiding aspiration and consequently the risk of pneumonia and even death (Ong *et al.*, 2018). Nowadays, thickened fluids by powder thickeners are the most common form because they are cheaper, more stable

and easier to use. However, liquid food can also be thickened to be consumed in ready-to-use products or freshly prepared in different dispersing matrices such as water, fruit juices, and milk, avoiding the generic indications found on labels from the powders packaging (Sopade *et al.*, 2008; Moret-Tatay *et al.*, 2015; Cho & Yoo, 2014).

Rheological properties are influenced by the characteristics of the thickeners and by the food matrices into which they are added (Sopade *et al.*, 2008; Cho & Yoo, 2014), since the macromolecules of each thickener interact differently with the constituents of the diverse food matrices resulting in viscosity changes. This fact was also confirmed in this thesis by comparing the mathematical models generated for the different studied formulations according to the obtained rheological properties in Chapter VI (water) and in Appendix B (milk and soy juice). To ensure the safety of patients with dysphagia, it is important to maintain the adequate viscosity, independently of the food matrix, within the required standards to prevent aspiration and swallowing complications (Prakash, Tan, & Chen, 2013). Although the researchers and clinicians are focused on safety, it is also necessary to understand the changes that concentrations of thickeners for application in water can induce when incorporated into other matrices, which often cause patients to reject consumption. In order to define the mechanical behaviour of thickened fluids, some scientific groups have recently focused on the advances of the study of sensory characteristics to complement/correlate with rheological properties (Wang & Chen, 2017; Torres *et al.*, 2019). According to Szczesniak (2002), food texture (despite of the complexity of the concept) is defined as the sensorial and functional manifestation of the structural, mechanical and surface properties of foods. Food tribology is emerging as a tool to identify not only sensory properties but also interface/surface biopolymer interactions through the lubrication properties of liquid foods or food components such as milk, biopolymer solutions and emulsions (Joyner, Pernell & Daubert, 2014a; Anvari & Joyner, 2018; Martinez *et al.*, 2019).

Thus, in order to understand the impact of the different thickeners in the lubricating capacity of different food matrices, the present study investigated the coefficient of friction of two commercial thickeners with different composition and three possible constituents to be incorporated into a thickening formulation. Varied concentrations of thickeners were added in milk and orange-flavoured soy juice to observe if the matrix prevails over thickeners in a way other than water (Chapter V).

## Materials and Methods

### Material

Golden flaxseeds were kindly provided by CISBRA Ltda (Panambi, RS, Brazil). Xanthan gum was donated by Danisco (Brazil) and modified starch by Cargill (Brazil). Two commercial thickeners acquired in local market were used: one based on xanthan gum (ThickenUP, Nestlé) and another on modified starch (Thicken&Easy, Hormel). The food matrices orange-flavoured soy juice (composition: water, soybean, sugar, concentrated orange juice, maltodextrin, vitamins C, B3, B6 and B12, zinc, pectin, guar gum, citric acid, flavorings, malic acid, urucum, curcuma and sucralose) (Ades, Unilever, Brazil) and skim milk (composition: skim milk, sodium citrate, sodium monophosphate and sodium diphosphate) (Shefa, Brazil) were also purchased in local market. The material used to simulate the tongue surface was polydimethylsiloxane (PDMS) produced from a Sylgard 184 silicone elastomer kit (Dow Corning Corporation, U.S.A.). The base and curing agents were used in a mass ratio of 9:1 to obtain disks with 5 mm thickness and 50 mm diameter. In order to eliminate air bubbles, the PDMS disks were cured for 2 h in the oven at 55 °C.

### Thickened beverages preparation

Dispersions were prepared using the following thickeners: MS = modified starch; XG = xanthan gum; FG = flaxseed gum; TU = XG-based commercial thickener; TE = MS-based commercial thickener at concentrations of 0.75, 1.5, 2.25 and 3 (% w/v). Concentrations were chosen based on preliminary tests (data not shown). All samples were prepared by dissolution of thickeners at room temperature in skim milk and orange-flavoured soy juice using mechanical stirring for 30 min at 800 rpm.

### Tribological measurements

Tribological tests were carried out at 25 °C using a reciprocating ball-on-plane configuration in an UMT-2 (CETR) equipment (USA). The AISI 52100 steel ball (ø = 10 mm) slid without rolling on the PDMS plane surface (disk with 40 mm diameter and 5 mm thickness) during measurements. The ball was used to represent the palate and the PDMS disc was meant to mimic the human tongue surface. PDMS shows a low elastic modulus that could mimic the low-pressure contact typical in bio-lubrication

processes (Prakash *et al.*, 2013; Zhang *et al.*, 2017). The wear track was 10 mm long with 2 Hz of reciprocating frequency (entrainment speed of 40 $mm.s^{-1}$), with a normal load of 5 N during 20 s (swallowing process occurs between milliseconds up to 20 s). The human tongue speeds have been estimated between 10 and 200 $mm.s^{-1}$ and the in-mouth force has been reported between 0.01 N and 10 N (Laiho, Williams, Poelman, Appelqvist, & Logan, 2017). The tribological measurements were performed within this range (40 $mm.s^{-1}$ and 5 N). 1.5 mL of each thickened sample were placed on the PDMS surface and the tribological behaviour of the thickened matrices was evaluated. Milk, orange soy juice and water were evaluated as control systems. The coefficient of friction was measured and reported for each sample as a function of time (s). Results are presented as the average of five replicates.

### Statistical analyses

Data were subjected to analysis of variance (ANOVA) ($p < 0.05$) and the means were compared using Tukey's HSD test to examine if differences between formulations were significant ($\alpha = 0.05$).

## Results and discussion

### Tribological properties

Tribological properties of food matrices containing FG, MS, XG, TU and TE were investigated from the friction coefficient as a function of time (Figure C-1 – highest thickener concentration that presented the most pronounced differences for both matrices) and after achieving steady state (Table C-1). The friction coefficient of formulations reached steady state after approximately 2 s, i.e., 7 s from the beginning of the measurement, when a thin film of lubricant and surface characteristics became dominant.

In relation to the milk matrix, XG and FG showed similar tribological profiles for most concentrations (Table C-1). At steady state, a very significant reduction of the friction coefficient was observed for higher concentrations of FG, XG and TU (2.25 and 3 % w/w) compared to the control milk matrix. This lubrication capacity could be explained by considering that once a threshold concentration is reached, a thicker layer of thickener particles provides a complete barrier to surface contact and a further increase

in the number of particles will probably not have a marked influence on the friction coefficient (Yakubov *et al.*, 2015, Zhang *et al.*, 2017). Nguyena, Kravchuk, Bhandari & Prakash (2017) found that the addition of a small content of XG did not significantly change the lubrication properties of the original matrix (yoghurt); a similar result was observed for lower concentrations (0.75 and 1.5 % w/w) of this biopolymer and also of FG (Table C-1). However, at 2.25 % of both MS-based thickeners the coefficient of friction increased significantly. Therefore, the lubricating properties of the milk formulations varied depending on their composition, being that gum-based formulations have undergone minor changes over time as compared to those based on modified starch. Interactions between milk proteins and polysaccharides (xanthan and flaxseed gum) possibly induced lubricating properties capable of decreasing the friction process (Joyner, Pernell & Daubert, 2014b; Kaushik *et al.*, 2016, Liu *et al.*, 2017, Sun *et al.*, 2018). As noticed in water (Chapter V), the formulations that presented higher friction coefficients over time were those based on modified starch (MS and TE). This result may be attributed to the structure of the starch-thickened formulations, which can show a release of gel particles with larger size that exhibit a higher friction mainly at low entrainment speed (Liu *et al.*, 2015, Nguyen et al, 2017).

In relation to the orange-flavoured soy juice matrix, the lubricating properties of the formulations exhibited a slightly different behavior from water and milk (Figure C-1 and Table C-1). In this matrix, all formulations increased the original soy juice matrix friction coefficient at steady state of the analysis (Table C-1). However, at the highest studied concentration of the xanthan gum-based thickeners, the lubrication of soy juice increased (Table C-1 and Figure C-1A1).

When the coefficients of friction obtained for the different matrices analyzed are compared, it is evident that the coefficient of friction obtained in water was significantly higher than the one obtained in milk and orange-flavored soy juice (Table C-1). Regarding the milk matrix, this fact may be due to the presence of a small amount of fat (skim milk) and proteins, which are considered components with reduced coefficient of friction (Joyner *et al.*, 2014a). On the other hand, the orange-flavored soy juice has proteins, xanthan and other gums in its composition, which are also considered components with high lubricating capacity.

It should be noted that after reaching the constant velocity of 40 $mm.s^{-1}$ and force of 5 N (5 s) the resistance presented by the different thickened solutions to obtain a constant

value of the coefficient of friction was more noticeable (Figure C-1) than in water (Chapter V). This fact may be due to the interactions between the biopolymer thickeners and the complex contents of the matrices analysed, altering their surface characteristics.

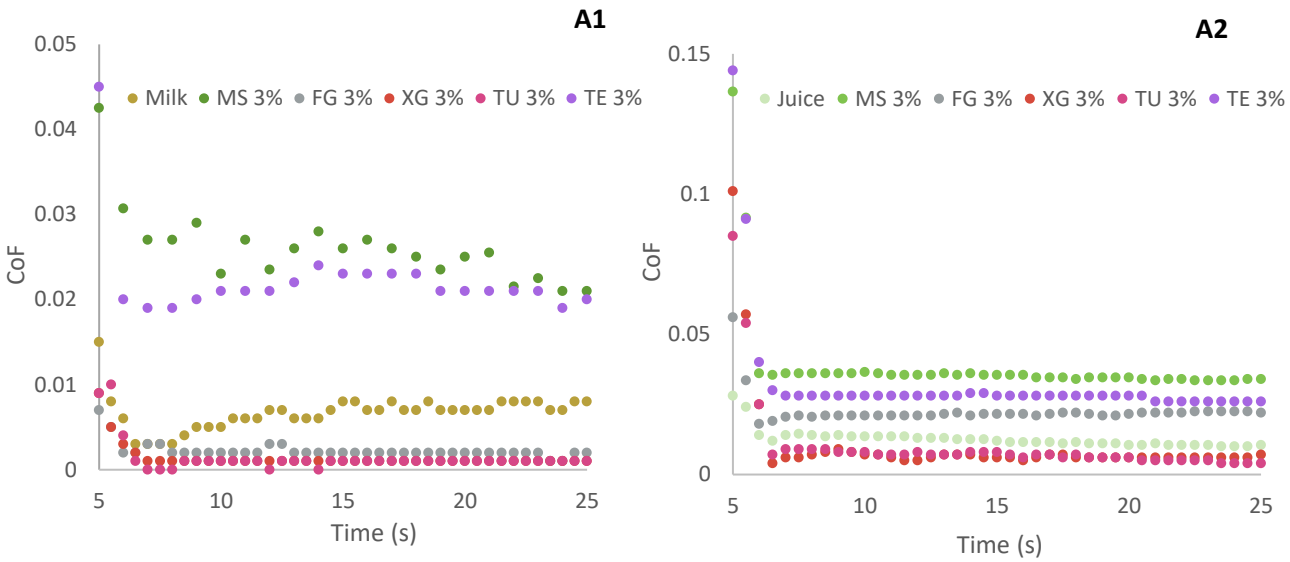



**Figure C-1.** Coefficient of friction as a function of time obtained at a fixed sliding speed (40 mm.s$^{-1}$) for the different formulations produced at the highest concentration of thickener added in milk (3 % w/w (A1)) or orange soy juice matrices (3 % w/w (A2)).

**Table C-1.** Coefficient of friction (*CoF*) for the studied thickeners added in food matrices at different concentrations, at steady state conditions.

| | | $CoF_{steady\ state}$ | | | |
|---|---|---|---|---|---|
| **Water** | | 0.060 | | | |
| **Milk** | | 0.007 | | | |
| **Soy Juice** | | 0.015 | | | |
| | | **0.75% w/w** | **1.5% w/w** | **2.25% w/w** | **3% w/w** |
| **Milk** | **MS** | $0.007^{a}$ | $0.008^{a}$ | $0.016^{b}$ | $0.021^{a}$ |
| | **FG** | $0.008^{a}$ | $0.005^{d}$ | $0.005^{c}$ | $0.004^{c}$ |
| | **XG** | $0.009^{a}$ | $0.010^{c}$ | $0.009^{c}$ | $0.004^{c}$ |
| | **TU** | $0.010^{a}$ | $0.009^{b}$ | $0.005^{c}$ | $0.004^{c}$ |
| | **TE** | $0.006^{a}$ | $0.010^{a}$ | $0.018^{a}$ | $0.020^{b}$ |
| **Soy Juice** | **MS** | $0.019^{a,b}$ | $0.040^{a}$ | $0.035^{b}$ | $0.039^{b}$ |
| | **FG** | $0.017^{b}$ | $0.016^{c}$ | $0.033^{a}$ | $0.025^{a}$ |
| | **XG** | $0.016^{b}$ | $0.018^{c}$ | $0.019^{b}$ | $0.011^{a}$ |
| | **TU** | $0.017^{b}$ | $0.019^{c}$ | $0.019^{b}$ | $0.011^{c}$ |
| | **TE** | $0.020^{a}$ | $0.025^{b}$ | $0.028^{b}$ | $0.032^{c}$ |

a-d Different letters in the same column correspond to statistically different samples for a 95% confidence level.

## Conclusions

The present study provided instrumental evidence of the lubricating characteristics of the studied thickeners, simulating the forces observed in the oral swallowing process according to the food matrices. It was observed a similar lubricating capacity between gum-based formulations regarding the measured friction coefficient, mainly in milk (as in water – Chapter V). The matrices' composition in which the thickeners were incorporated showed a significant impact on the tribological behaviour, not only because they have a significant different lubricating capacity, but also because the coefficient of friction can increase or decrease for a similar thickener content, depending on the matrix. For example, FG reduced the coefficient of friction after addition in water and milk but increased the coefficient of friction in soy juice at higher concentrations. This study shows the relevance of understanding the biopolymers' interactions on the tribological properties and consequently on the swallowing process of thickened food liquids.

## References

Anvari, M. and Joyner, H.S., 2018. Effect of fish gelatin and gum arabic interactions on concentrated emulsion large amplitude oscillatory shear behavior and tribological properties. Food Hydrocolloids, 79, 518-525.

Cho, H.M. and Yoo, B., 2014. Rheological Characteristics of Cold Thickened Beverages Containing Xanthan Gum–Based Food Thickeners Used for Dysphagia Diets. Journal of the Academy of Nutrition and Dietetics, 115(1), 106-111.

Joyner, H.S., Pernell, C.W., Daubert, C.R., 2014. Beyond surface selection: The impact of different methodologies on tribological measurements. Journal of Food Engineering, 134, 45-58.

Joyner, H.S., Pernell, C.W., Daubert, C.R., 2014. Impact of Oil-in-Water Emulsion Composition and Preparation Method on Emulsion Physical Properties and Friction Behaviors. Tribology Letters, 56(1), 143-160.

Kaushik, P., Dowling, K., McKnight, S., Barrow, C.J., Wang, B., Adhikari, B., 2016. Preparation, characterization and functional properties of flax seed protein isolate. Food Chemistry, 197, 212-220.

Laiho, S., Williams, R.P.W., Poelman, A., Appelqvist, I., Logan, A., 2017. Effect of whey protein phase volume on the tribology, rheology and sensory properties of fat-free stirred yoghurts. Food Hydrocolloids, 67, 166-177.

Liu, K., Stieger, M., van der Linden, van de Velde, E, 2015. Fat droplet characteristics affect rheological, tribological and sensory properties of food gels. Food Hydrocolloids, 44: 244-259.

Liu, J., Shim, Y.Y., Shen, J., Wang, Y., Reaney, M.J.T., 2017. Whey protein isolate and flaxseed (Linum usitatissimum L.) gum electrostatic coacervates: Turbidity and rheology. Food Hydrocolloids, 64, 18-27.

Martínez, O., Vicente, M.S., De Vega, M.C., Salmerón, J., 2019. Sensory perception and flow properties of dysphagia thickening formulas with different composition. Food Hydrocolloids, 90, 508–514.

Moret-Tatay, A., Rodríguez-García, J., Martí-Bonmat, E., Hernando, I., Hernández, M.J., 2015. Commercial thickeners used by patients with dysphagia: Rheological and structural behaviour in different food matrices. Food Hydrocolloids, 51, 318-326.

Nguyena, P.T.M., Kravchuk, O., Bhandari, B., Prakash, S., 2017. Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. Food Hydrocolloids, 72, 90-104.

Ong, J.J.-X., Steele, C.M., Duizer, L.M., 2018. Sensory characteristics of liquids thickened with commercial thickeners to levels specified in the International Dysphagia Diet Standardization Initiative (IDDSI) framework. Food Hydrocolloids, 79, 208-217.

Prakash, S., Tan, D.D.Y., Chen, J., 2013. Applications of tribology in studying food oral processing and texture perception. Food Research International, 54, 1627–1635.

Sopade, P.A., Halley, P.J., Cichero, J.A.Y., Ward, L.C., Hui, L.S., Teo, K.H., 2008. Rheological characterisation of food thickeners marketed in Australia in various media for the management of dysphagia. II. Milk as a dispersing medium. Journal of Food Engineering, 84, 553–562.

Sopade, P.A., Halley, P.J., Cichero, J.A.Y., Ward, L.C., Liu, J., Varliveli, S., 2008. Rheological characterization of food thickeners marketed in Australia in various media for the management of dysphagia. III. Fruit juice as a dispersing medium. Journal of Food Engineering, 86, 604–615.

Sun, C., Liang, B., Sheng, H., Wang, R., Zhao, J., Zhang, Z., Zhang, M., 2018. Influence of initial protein structures and xanthan gum on the oxidative stability of O/W emulsions stabilized by whey protein. International Journal of Biological Macromolecules, 120, 34-44.

Szczesniak, A.S., 2002. Texture is a sensory property. Food Quality and Preference, 13(4), 215-225.

Torres, O., Yamada, A., Rigby, N.M., Kawano, Y., Sarkar, A., 2019. Gellan gum: A new member in the dysphagia thickener family. Biotribology, 8-18.

Wang, X. and Chen, J., 2017. Food oral processing: Recent developments and challenges. Current Opinion in Colloid & Interface Science, 28, 22-30.

Yakubov, G.E., Branfield, T.E., Bongaerts, J.H.H., Stokes, J.R., 2015. Tribology of particle suspensions in rolling-slidding soft contacts. Biotribology, 3, 1-10.

Zhang, B., Selwayc, N., Shelat, K.J., Dhital, S., Stokes, J.R., Gidley, M.J., 2017. Tribology of swollen starch granule suspensions from maize and potato. Carbohydrate Polymers 155, 128–135.

# *ANEXO*

*Autorização para a reprodução do artigo*

*Certidão Cadastro nº A825BEA*

*Comprovante de Cadastro de Acesso Cadastro nº A825BEA*

**Ministério do Meio Ambiente**
**CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO**

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

# Certidão

## Cadastro nº A825BEA

Declaramos, nos termos do art. 41 do Decreto nº 8.772/2016, que o cadastro de acesso ao patrimônio genético ou conhecimento tradicional associado, abaixo identificado e resumido, no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado foi submetido ao procedimento administrativo de verificação e não foi objeto de requerimentos admitidos de verificação de indícios de irregularidades ou, caso tenha sido, o requerimento de verificação não foi acatado pelo CGen.

| | |
|---|---|
| Número do cadastro: | **A825BEA** |
| Usuário: | **UNICAMP** |
| CPF/CNPJ: | **46.068.425/0001-33** |
| Objeto do Acesso: | **Patrimônio Genético** |
| Finalidade do Acesso: | **Pesquisa** |

**Espécie**

**Linum usitatissimum**

| | |
|---|---|
| Título da Atividade: | **Relação reologia-funcionalidade em formulações de espessantes para pacientes disfágicos** |

**Equipe**

| | |
|---|---|
| **Rosiane Lopes da Cunha** | **UNICAMP** |
| **Jorge Miguel Magalhães Vieira** | **UNICAMP** |

**Resultados Obtidos**

**Divulgação de resultados em meios científicos ou de comunicação**

| | |
|---|---|
| Identificação do meio onde foi divulgado: | **Tese em andamento- UNICAMP** |

| | |
|---|---|
| Data do Cadastro: | **02/11/2018 12:46:49** |
| Situação do Cadastro: | **Concluído** |

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **10:43** de **23/07/2019**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

**Ministério do Meio Ambiente**
**CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO**

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

## Comprovante de Cadastro de Acesso

## Cadastro nº A825BEA

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

| | |
|---|---|
| Número do cadastro: | **A825BEA** |
| Usuário: | **UNICAMP** |
| CPF/CNPJ: | **46.068.425/0001-33** |
| Objeto do Acesso: | **Patrimônio Genético** |
| Finalidade do Acesso: | **Pesquisa** |

**Espécie**

**Linum usitatissimum**

| | |
|---|---|
| Título da Atividade: | **Relação reologia-funcionalidade em formulações de espessantes para pacientes disfágicos** |

**Equipe**

| | |
|---|---|
| **Rosiane Lopes da Cunha** | **UNICAMP** |
| **Jorge Miguel Magalhães Vieira** | **UNICAMP** |

**Resultados Obtidos**

**Divulgação de resultados em meios científicos ou de comunicação**

| | |
|---|---|
| Identificação do meio onde foi divulgado: | **Tese em andamento- UNICAMP** |

| | |
|---|---|
| Data do Cadastro: | **02/11/2018 12:46:49** |
| Situação do Cadastro: | **Concluído** |

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **10:43** de **23/07/2019**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**